An addendum to this report has been issued and can be found at <https://www.cdpr.ca.gov/docs/registration/reevaluation/chemicals/addendum_neonicotinoid_risk_determination.pdf>

California Department of Pesticide Regulation

Pesticide Registration Branch

California Neonicotinoid Risk Determination

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On the cover: Photo of a bee on honeycomb. Photograph by DPR staff.

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1.0 Executive Summary

California leads the nation in cash farm receipts, and its agricultural production includes more than 400 commodities representing over a third of the country's vegetables and two-thirds of the country's fruits and nuts. Many of these agricultural commodities rely on pollination by bees for optimal production. Today, more than 2.5 million honey bee colonies in the United States pollinate an estimated $15 billion of crops each year, ranging from almonds to zucchini. Of these, approximately 1.8 million colonies are used in the pollination of California's almond crops alone.

Colony losses of these critical natural and managed pollinators have triggered worldwide concern in recent years. Multiple factors may contribute to colony losses and other risks to pollinator and hive health, including possible effects of neonicotinoid pesticides. This risk determination report, prepared by the Department of Pesticide Regulation (DPR) in response to California Food and Agriculture Code Section 12838, assesses those potential effects.

Neonicotinoid insecticides are systemic pesticides that kill insects by attacking their central nervous system. These insecticides are absorbed into plants and distributed throughout their tissues to their stems, leaves, roots, fruits and flowers. Neonicotinoids play an important role in the control of agricultural insect pests. Some examples include:

- Aphids that transmit citrus tristeza virus to citrus affecting the roots, leaves, and fruit causing a rapid decline in tree growth leading to death;
- The glassy-winged sharpshooter that transmits Pierce's disease to grapevines, a bacterium that blocks the movement of water within the plant, killing the vines within 1-3 years; and
- The Asian citrus psyllid that transmits huanglongbing disease to citrus trees causing a yellowing of tree shoots, asymmetrical and bitter fruit, and tree death in 5-8 years.

All of these diseases are known to spread rapidly and have the potential to cause massive destruction to the crops affected.

Neonicotinoids are insecticides developed as alternatives to organophosphates and carbamates that have a greater potential to affect human health (Cimino et al., 2017). Pesticide use reports received by DPR from the County Agricultural Commissioners across the state between 2007 and 2016 show that the use of neonicotinoids (imidacloprid, thiamethoxam, clothianidin, and dinotefuran) increased by 69.6%, while organophosphate and carbamate use decreased by 41.5% and 20.9%, respectively. California requires the monthly reporting of agricultural pesticide use to County Agricultural Commissioners, who in turn, report the data to DPR.

DPR was advised of the potentially harmful effects of neonicotinoids on pollinators in 2008. Studies of imidacloprid on ornamental plants revealed high levels of the insecticide in leaves and blossoms of treated plants, as well as increased imidacloprid residue levels in leaves and blossoms over time, indicating potential threats to pollinator health. In response to the disclosure, DPR placed pesticide products containing imidacloprid and the related neonicotinoid active ingredients thiamethoxam, clothianidin, and dinotefuran, into reevaluation on February 27, 2009 to assess the magnitude of their residues in the pollen and nectar of agricultural crops and the corresponding levels of risk to honey bee colonies. The reevaluation covered 50 registrants and 282 pesticide products with formulations or applications likely to move into plants that bloom or serve as a foraging source for honey bees and other pollinators (Appendix 1). In 2014, the Legislature adopted AB 1789 (Chapter 578, Statutes of 2014) requiring DPR to issue a determination with respect to its reevaluation of neonicotinoids by July 1, 2018, and adopt control measures necessary to protect pollinator health within two years after making the determination (Appendix 2).

This risk determination report documents the results of the DPR's neonicotinoid reevaluation and its first ecologically-based risk assessment. As part of this assessment, the department partnered with scientists at the U.S. Environmental Protection Agency's (U.S. EPA) Office of Pesticide Programs and the Health Canada Pest Management Regulatory Agency to develop the methods and procedures used to conduct ecologically-based studies on the effects of neonicotinoids. DPR followed the methods established by the group to assess the risks of exposure to bee colonies foraging on nectar and pollen in crops treated with the subject neonicotinoids, comparing the levels of neonicotinoid residues to concentrations that cause colony-level effects such as decreased colony strength and decreased stores of honey in honeycombs.

DPR based its risk determination on a series of studies that exposed bee colonies to four types of neonicotinoids (imidacloprid, thiamethoxam, clothianidin and dinotefuran) to establish residue levels in pollen and nectar that produced no observed effects on the colonies (No Observed Effect Concentrations, or NOECs). The department compared those NOEC values to residue levels found on selected agricultural crops in the field. DPR scientists then determined risk levels for combinations of specific crop groups and pesticide application methods (e.g., foliar [applied to leaves] or soil). Crop-application combinations with pollen or nectar residue levels that exceeded the NOEC values were determined to present a risk. Crop-application combinations with residue levels below the NOEC values were determined to be low risk. These risk determinations were based on the maximum allowed annual application rates in California for each agricultural crop group for each of the neonicotinoids listed above, and therefore represent "worst-case" scenarios (Appendix 3). Actual annual application rates may present less risk.

Crop groups considered to present a risk at maximum annual application levels of at least one of the neonicotinoids listed above include fruiting vegetables (e.g., cucumbers, tomatoes), berries, citrus, and tree nuts. Among the crop groups for which maximum application levels are considered a low risk are root and tuber vegetables (e.g., potatoes, turnips), bulb vegetables (e.g., onions, garlic), leafy vegetables and legumes. Again, these are conservative assessments based on maximum allowable application rates, and vary according to the neonicotinoid applied. Additional information on crop group risk may be found in Table 6.

Going forward, DPR will consider mitigation measures for neonicotinoid applications to crops characterized as at risk to reduce residues to levels below the respective NOEC. Such measures could include modifying application rates or the times at which applications may occur. This mitigation process will likely take two years to complete and will include continued research, consultation with experts, other stakeholders, and the use of technology designed to predict measures necessary to ensure bee colony health.

2.0 Background

On February 27, 2009, DPR placed certain pesticide products containing the neonicotinoid active ingredients, imidacloprid, thiamethoxam, clothianidin, and dinotefuran, into reevaluation (Appendix 1). DPR initiated the reevaluation based on submitted adverse effects disclosure data involving the active ingredient imidacloprid. DPR's Ecotoxicology unit evaluated the adverse effects data and noted high concentrations of imidacloprid in leaves and blossoms of treated ornamental plants, with an increase in measured concentrations over time. These observations of residues in treated plants led to a concern over potential exposure of honey bee colonies used for pollination services where hives are purposely placed around agricultural fields. Thiamethoxam, clothianidin, and dinotefuran are in the same chemical class as imidacloprid, known as the nitroguanidine-substituted neonicotinoid insecticides, and have similar physicochemical properties (e.g., soil mobility, half-lives, and toxicity to honey bees; Appendix 5). Thus, DPR included these active ingredients in the reevaluation. The purpose of this reevaluation is to provide DPR with a better understanding of the magnitude of neonicotinoid residues in pollen and nectar of agricultural commodities resulting from legal pesticide applications and the resulting level of risk to honey bee colonies. These data are necessary to provide a credible scientific basis for potential regulatory action to mitigate any significant adverse effects on honey bee health resulting from the use of neonicotinoid insecticides. DPR exempted from the reevaluation products formulated as a gel or impregnated in a strip, termiticides, flea control products combined with rodenticides, pet spot products, ant and roach baits, premise applications for control of nuisance pests, and manufacturing use only products because as formulated or applied, it is unlikely that the neonicotinoid in such products will move into plants that bloom or is a source of forage for honey bees and pollinators.

As part of the reevaluation, DPR required pesticide manufacturers to provide additional data that would allow DPR scientists to conduct a scientific determination of risk. DPR's reevaluation focused on gathering data on residue concentrations in the nectar and pollen of certain neonicotinoid-treated orchard and row crops. On September 15, 2009, DPR issued letters to the registrants of the four pesticide active ingredients describing the objectives and basic design of the studies to be conducted. Sampling was to be conducted in a minimum of three agricultural sites over two consecutive years. When possible, the agricultural sites were selected based on soil texture with three replicates in sandy, coarse-textured soils, three replicates in loamy, medium-textured soils, and three replicates in clayey, fine-textured soils. DPR used the Pesticide Use Reporting database to determine the crops of focus for each active ingredient (DPR, 2018b). On March 12, 2012, DPR modified its residue study strategy to require applications at the highest maximum annual application rate for two consecutive years.

DPR partnered with scientists at the U.S. Environmental Protection Agency's (U.S. EPA) Office of Pesticide Programs and Health Canada Pest Management Regulatory Agency (PMRA) to ensure that required data on the effects of neonicotinoids would provide useful and reliable information across the board for all three agencies to use in guiding their regulatory actions. On June 20, 2014, a Presidential Memorandum creating a federal strategy to promote the health of honey bees and other pollinators was signed. Subsequently, DPR, U.S. EPA, and PMRA published a collaborative document titled, *Guidance for Assessing Pesticide Risks to Bees*

(U.S. EPA, PMRA, and DPR, 2014), which established a tiered approach to data collection and risk assessment.

In January 2016, U.S. EPA, in collaboration with DPR, issued a preliminary pollinator risk assessment for imidacloprid. In January of 2017, U.S. EPA issued preliminary pollinator risk assessments for thiamethoxam, clothianidin, and dinotefuran. U.S. EPA's preliminary pollinator risk assessments include Tier I (acute toxicity) assessments based on model-generated estimates of exposure and laboratory toxicity data at the individual bee level, for all four active ingredients. The Tier I assessments indicate that there is potential risk to honey bees for all crops and application methods where there is a potential for on-field exposure (U.S. EPA and DPR, 2016; U.S. EPA, 2017a; U.S. EPA, 2017b). In accordance with the *Guidance for Assessing Pesticide Risks to Bees* (U.S. EPA, PMRA, and DPR, 2014), U.S. EPA conducted Tier II assessments for imidacloprid, thiamethoxam and clothianidin, and a Tier I assessment on dinotefuran using available data. Tier II assessments compare residue data to colony-level effects data.

A refined Tier II assessment is the focus of DPR's risk determination document. DPR's determination starts with U.S. EPA's preliminary pollinator assessments and includes new data submitted to DPR for all four active ingredients since the issuance of U.S. EPA's preliminary pollinator assessments. This risk determination document meets the requirements of FAC §12838 (a) which states, "On or before July 1, 2018, the department shall issue a determination with respect to its reevaluation of neonicotinoids" (Appendix 2).

3.0 Scope

3.1 Pesticide Type, Class, and Mode of Action

Neonicotinoid insecticides are systemic pesticides that target nicotinic acetylcholine receptors in the central nervous system of insects. DPR's neonicotinoid reevaluation focuses on the nitroguanidine-substituted neonicotinoids (imidacloprid, clothianidin, thiamethoxam, and dinotefuran) as all four active ingredients share similar physicochemical characteristics and toxicity to honey bees. Neonicotinoids are systemic compounds and readily move through the vascular system, xylem and phloem, of plants which then translocate into various plant tissues. Neonicotinoids can be applied using several different application methods including foliar application by aerial or ground spray equipment, soil drench, chemigation, or seed treatment (U.S. EPA and DPR, 2016; U.S. EPA, 2017a; U.S. EPA, 2017b).

3.2 Use Characterization

DPR first registered a pesticide product containing imidacloprid for sale and use in the State of California in 1994. Approximately ten years later, DPR registered the first pesticide products containing dinotefuran, clothianidin, and thiamethoxam (DPR, 2018a). Neonicotinoids are widely used pesticides with a variety of uses ranging from agricultural and residential insecticides, pet products, termiticides, ant and roach baits, and premise application products for nuisance pests. Neonicotinoids are currently registered for use on a diverse array of crops in California such as, but not limited to: citrus fruits, oilseed crops (e.g., cotton), cucurbit vegetables, fruiting vegetables, pome fruits, stone fruits, cereal grains, tree nuts, *Brassica* (Cole) leafy vegetables, root and tuber vegetables, leafy vegetables, legume vegetables, and bulb vegetables. For more information on registered agricultural use sites and specific application rates for each of the neonicotinoid active ingredients, refer to Appendix 3.

Neonicotinoids were developed as alternatives to organophosphates and carbamates (Cimino et al., 2017). Neonicotinoids play an important role in the integrated control of agricultural insect pests such as: aphids that transmit citrus tristeza virus to citrus; the glassy-winged sharpshooter that transmits Pierce's disease to grapevines; and the Asian citrus psyllid that transmits huanglongbing disease to citrus trees.

Pesticide use reports (PUR) between 2007 and 2016 indicate that use of neonicotinoids (imidacloprid, thiamethoxam, clothianidin, and dinotefuran) increased by 69.6% (131,168 lbs. neonicotinoid active ingredients used in 2007; 431,132 lbs. neonicotinoid active ingredients used in 2016) while organophosphate and carbamate use decreased by 41.5% (3,775,011 lbs. organophosphate active ingredients (listed below) used in 2007; 2,209,448 lbs. active ingredients used in 2016) and 20.9% (666,035 lbs. carbamate active ingredients (listed below) used in 2007; 526,677 lbs. active ingredients used in 2016), respectively. In 2016, organophosphates were frequently applied to oranges, almonds, walnuts, lettuce, and cotton while carbamates were frequently applied to oranges, corn, lettuce, tomatoes, and alfalfa. The most frequent neonicotinoid use sites in 2016 include grapes, tomatoes, oranges, tangerines, and pistachios. The inquiry into the PUR database for the organophosphate chemical group included the active ingredients acephate, bensulide, chlorpyrifos, diazinon, DDVP, dimethoate, fosthiazate malathion, ethoprop, naled, phorate, phosmet, tetrachlorvinphos, tribufos, disulfoton, ethoprop, fenamiphos, methamidophos, methidathion, oxydemeton-methyl, and profenofos while the carbamate group included the active ingredients formetanate HCI, methiocarb, methomyl, oxamyl, propoxur, thiodicarb, aldicarb, carbofuran, and carbaryl. Other chemicals that belong within the organophosphate and carbamate chemical group are not currently registered in the State of California.

3.3 Environmental Fate and Transport

Since neonicotinoids are systemic insecticides, they are transported through the vascular system of plants to all tissues, including leaves, nectar and pollen. Both foliar and soil applications of neonicotinoids have resulted in detectable residues in both nectar and pollen following absorption by the foliage, roots, or stems of plants (U.S. EPA and DPR, 2016; U.S. EPA, 2017a; U.S. EPA, 2017b). Physicochemical characteristics consistent among the four neonicotinoid active ingredients include a low organic carbon normalized soil adsorption coefficient (K_{oc}) value, low volatility, longevity in soil after application, and relatively high water solubility (Appendix 5). These properties contribute to the pesticides being highly available for uptake by plant roots. Moreover, neonicotinoids have two main routes of degradation through aquatic photolysis and aerobic soil metabolism (U.S. EPA and DPR, 2016; U.S. EPA, 2017a; U.S. EPA, 2017b). Degradation produces a variety of breakdown products known as metabolites. Refer to Appendix 5 for the specific physicochemical properties and environmental fate of each active ingredient.

This risk determination document includes measurements of metabolite concentrations identified as having similar or greater toxicity to honey bees than the parent compound. For imidacloprid, the evaluation includes the parent and two metabolites, imidacloprid-olefin (IMI-olefin) and imidacloprid-5-hydroxy (5-OH-IMI), since all three compounds have a similar toxicity to honey bees (U.S. EPA and DPR, 2016). Other metabolites do not have a similar toxicity (e.g. 6-chloronicotinic acid, 6-chloro-picolylalcohol, nitrosamine and urea). The risk determination will refer to total imidacloprid, which is the summation of residues of the parent imidacloprid, and the metabolites IMI-olefin and 5-OH-IMI.

The metabolite of concern for thiamethoxam is CGA-322704 (i.e., clothianidin), which itself is an active ingredient in registered pesticide products. As both compounds are toxic to honey bees (U.S. EPA, 2017b), concentrations of total residues for parent (thiamethoxam) and CGA-322704 will be reported and assessed. For clothianidin, the metabolites, N-(2-chloro-5-thiazolylmethyl)-N'-methylurea (TZMU) and N-(2-chloro-5-thizolylmethyl)-N'-nitroguanidine (TZNG) are routinely measured in the plant residue studies. Based on acute toxicity data, TZMU and TZNG are orders of magnitude less toxic to honey bees than the parent clothianidin (U.S. EPA, 2017b). As a result, DPR did not include these metabolites in the risk determination and all references to clothianidin refer to the parent molecule alone.

Dinotefuran metabolites measured in plant tissues include 1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl) guanidine (UF) and 1-methyl-3-(tetrahydro-3-furylmethyl) guanidinium dihydrogen phosphate (DN). Toxicity data submitted to DPR indicate the UF and DN metabolites are less toxic to honeybees, so those metabolites are not included in DPR's risk determination and all references to dinotefuran refer to the parent molecule alone (U.S. EPA, 2017a).

3.4 Potential for Effects on Pollination Activity

This risk determination focuses on potential effects of neonicotinoid exposure on honey bees (*Apis mellifera*) after feeding on nectar and pollen containing neonicotinoid residues. Honey bees are purposefully situated around agricultural sites during bloom to pollinate various crops. As a result, foraging bees could be exposed to residues of these four neonicotinoids from applications made prior to bloom, during flowering, or post-bloom if the residues in bee-attractive matrices (e.g., pollen and nectar) persist for a sufficient duration. DPR's reevaluation required that plant residue studies be conducted using worst-case application scenarios (e.g., maximum application rates, minimum reapplication intervals) found on currently registered pesticide labels. These scenarios generally result in the highest realistic concentrations in the bee-attractive matrices. *Apis* bees serve as a surrogate for other non-*Apis* species of bees (e.g., bumble bees) that may be exposed under agricultural conditions. This surrogate approach is consistent with the *Guidance for Assessing Pesticide Risks to Bees* (U.S. EPA, PMRA, and CDPR, 2014). As described in the guidance document, the husbandry, life cycles, and contribution of pollinator services of honey bees are well-studied.

3.5 Colony Level Exposure and Effects

DPR evaluated both registrant-submitted and open literature (i.e., peer-reviewed research studies published in scientific journals) Tier II semi-field studies for this risk determination. The purpose of Tier II studies is to evaluate possible colony-level effects on hive health through foraging on nectar and pollen. DPR quantitatively evaluated oral consumption (e.g., consumption of contaminated nectar and pollen) as the primary exposure route for honey bees in this determination. In Tier II studies denoted as colony feeding studies, honey bee colonies are exposed to known concentrations of a compound in either surrogate nectar or pollen and measurements are taken that reflect the health of hives. Based on the observed responses from the colony feeding studies, No Observed Effects Concentrations (NOECs) are derived for each active ingredient. In this determination, DPR used the NOEC values to determine each active ingredient's potential to cause effects on hive health. The submitted colony feeding studies measured several response variables including colony survival, the number of cells containing various brood stages (eggs/larvae/pupae), the total population of adult bees per hive, and the number of cells containing food stores (pollen and nectar). Overall, the purpose of these studies is to determine the concentration of each neonicotinoid that honey bees can safely consume over a six-week period with no significant adverse colony-level effects. NOEC values were established for each of the four neonicotinoids in each of the two bee-attractive matrices (pollen and nectar; Table 1 below). DPR scientists compared these values to neonicotinoid concentrations in nectar and pollen collected from representative crops after worst-case scenario applications. DPR also evaluated and considered adverse effects data submitted pursuant to California Food and Agricultural Code (FAC) section 12825.5. However, those data did not provide information pertinent to the scope of this risk determination.

4.0 <u>Risk Characterization Methodology</u>

4.1 Overview of Risk Determination Process

The risk determination process generally follows the methods of a Tier II assessment as detailed in the *Guidance for Assessing Pesticide Risks to Bees* (U.S. EPA, PMRA, and CDPR, 2014). In accordance with the tiered risk assessment process, risks to bees were determined by comparing available exposure data to colony-level effects data. According to Tier I laboratory data, nitroguanidine-substituted neonicotinoids are acutely toxic to individual bees through both contact and oral exposure (Appendix 6). Contact exposure may occur through dermal uptake of residues on plant surfaces or by direct spray deposition onto bees. Oral exposure mainly occurs through the ingestion of contaminated pollen or nectar. Applications can be timed to avoid contact by spray deposition. However, risks to honey bees from oral exposure are more complex to regulate. Upon translocation of the systemic nitroguanidine-substituted neonicotinoids inside plant tissues, concentrations in pollen and nectar may persist, resulting in risks from oral consumption and/or transfer of residues back to the hives.

This risk determination focuses on potential effects posed by oral consumption, so exposure data were determined from measured residue concentrations of nitroguanidine-substituted neonicotinoids and their bee-toxic metabolites in the nectar and pollen of agricultural crops following worst-case scenario applications in compliance with product labels. The exposure data were compared to effects data generated from exposure of honey bee colonies to nectar or pollen spiked with known concentrations of imidacloprid, thiamethoxam, clothianidin, or dinotefuran with various colony-level parameters measured over time. The Tier II data discussed in this risk determination builds upon the preliminary pollinator risk assessments published by the U.S. EPA

(U.S. EPA and DPR, 2016; U.S. EPA, 2017a; U.S. EPA, 2017b) for the four neonicotinoid active ingredients while also incorporating additional California-specific data.

DPR scientists made risk determinations for specific crop groups and application method combinations (e.g., foliar, soil), and characterized them as either having a determination of risk or low risk to honey bee colonies. A determination of risk resulted when residue concentrations in nectar or pollen exceeded the colony-level NOEC for that matrix (e.g., pollen or nectar). Conversely, a determination of low risk resulted when residue concentrations in pollen or nectar did not exceed the respective colony-level effects concentration (e.g., the concentrations were low enough that they would not result in any significant adverse effects to honey bee colonies). The risk determinations are based on oral exposure (e.g., the consumption of contaminated nectar and pollen). Methods used to generate the effects data and exposure data and their utilization in the risk determinations are described in Section 4.2.

Risk determinations were only conducted for foliar and soil applications. Risks from seed treatment applications were evaluated in the preliminary pollinator risk assessments published by U.S. EPA (U.S. EPA and DPR, 2016; U.S. EPA, 2017a; U.S. EPA, 2017b). The preliminary assessment for imidacloprid evaluated multiple seed treatment residue studies conducted on corn, canola, and sunflower. These studies generally reported no residues in pollen and nectar above the limit of detection**.** Values are well below their respective NOEC values, supporting the conclusion that imidacloprid seed treatments pose a low risk to honey bees. The preliminary pollinator risk assessment for clothianidin and thiamethoxam evaluated multiple seed treatment residue studies conducted on corn, sunflower, melon, canola, cotton, and soybean. The resulting residue concentrations are all below the respective NOECs, supporting the conclusion that clothianidin or thiamethoxam seed treatments pose a low risk to honey bee colonies. Dinotefuran is not registered for any seed treatment applications. There have been issues in other states and countries with contact exposure resulting from abraded seed coat dust at planting, but the U.S. EPA has addressed this with best management practices (U.S. EPA and DPR, 2016). DPR has no records of such incidents occurring in California.

4.2 Effects Characterization

Potential effects on honey bees were determined from Tier II studies, which assess effects of exposure at the colony level. The complex nature of assessing hive dynamics and colony-level effects necessitated multiagency collaboration to develop protocols that maximized the regulatory usefulness of such studies. Accordingly, study protocols were developed collaboratively through the efforts of DPR, U.S. EPA, and PMRA scientists, and in consultation with industry experts. This cooperative effort aimed at ensuring reproducibility of results and maximizing statistical power to detect effects while minimizing uncertainties and potential confounding factors, such as diseases, pests, or poor nutrition, which have each been independently associated with declines in colony health.

In comparison to Tier I laboratory studies, which focus on individual bees, Tier II studies focus on colony-level effects and assess a longer period of exposure under conditions that are more representative of exposure in the field. These include semi-field studies such as tunnel studies and colony feeding studies. Tunnel studies typically involve enclosing small bee colonies within a confined area of treated crops on which bees forage. In colony feeding studies, unconfined colonies are provided a food source, such as sucrose solution or pollen patties, that has been spiked with a known and measured concentration of a specific pesticide. Multiple concentrations are tested to produce a dose-response relationship between the concentrations tested and the observed health of the hives. In colony feeding studies, bees are generally exposed to the test feeding substances for six weeks. Measurements of hive health (i.e., Colony Condition Assessments) are taken at multiple time points prior to, during, and after the exposure period. Additionally, an overwintering component is typically included, with at least one additional assessment after the overwintering period. Hive health is determined by measuring parameters such as the population of adult bees (i.e., colony strength), the number of cells containing various brood stages (eggs, larvae, and pupae), and measuring hive resources in terms of honey and bee bread production (U.S. EPA, PMRA, and CDPR, 2014).

The Tier II studies considered in this risk determination document were subject to thorough evaluation for scientific acceptability. As part of this evaluation, DPR, U.S. EPA, and PMRA scientists assessed registrant-submitted study protocols prior to study initiation to ensure that the study designs were scientifically sound. Some examples of the types of requirements necessary for a study design to be deemed scientifically sound include adequate replication and confirmation of exposure by repeated sampling and analysis of spiked sugar solutions or spiked pollen patties to ensure that the honey bee colonies are actually exposed to the neonicotinoid concentrations as planned. Many of the open literature studies reviewed by DPR scientists (Appendix 7) lacked this level of replication and confirmation of exposure. In some cases, study authors were reluctant to provide DPR statisticians with the raw data needed to conduct independent statistical analyses. All colony-level NOEC values used in this assessment are based on mean measured concentrations that resulted from analyses conducted in compliance with rigorous analytical quality control procedures. For scientifically acceptable studies, DPR, U.S. EPA, and PMRA statisticians conducted independent statistical analysis of raw data on pertinent endpoints. In its risk determination process, DPR used the measured concentrations in the sugar solutions or spiked pollen patties associated with these regulatory endpoints. This results in a level of accuracy and certainty that cannot be achieved using nominal concentrations that were never confirmed analytically.

Although DPR considered both open literature and registrant-submitted studies, the registrant-submitted studies were generally found to be more robust and comprehensive when characterizing colony-level effects. These studies had greater replication and confirmation of exposure, and the raw data were available for independent statistical analysis. DPR, U.S. EPA, and PMRA statisticians and biologists independently determined the NOEC values for each active ingredient from studies found to be scientifically acceptable. Refer to Table 1 below for the NOEC concentrations determined for each active ingredient and matrix (i.e., nectar and pollen) combination (e.g. thiamethoxam in nectar). Utilizing only scientifically acceptable studies in the risk determination process produced data gaps in colony-level effects data for pollen. Specifically, acceptable pollen colony feeding studies were not available for thiamethoxam or dinotefuran, necessitating the use of another neonicotinoid as a surrogate. Accordingly, the NOEC value for clothianidin in pollen was bridged to thiamethoxam and dinotefuran. DPR found an acceptable colony feeding study conducted with pollen spiked with imidacloprid in the open literature (Dively et al., 2015). For a review of all the colony feeding studies included in this document, refer to Appendix 8.

As indicated in Table 1, NOEC values are lower for nectar than for pollen. These differences may be explained by the nature of these resources and how they are utilized within the hive. The movement of nectar around the hive is rapid and has been described as a cascade effect where it ultimately encounters most of the hive occupants and matrices. In addition, nectar is added to pollen by hive bees to produce bee bread. In contrast, bees foraging for pollen bring the pollen into the hive and pack it directly into pollen cells themselves. Bees consume less pollen than nectar, based on estimated food consumption rates for honey bees (U.S. EPA, PMRA, and CDPR, 2014). The highest consumption rate of pollen is found in new worker bees that clean and cap cells within the hive. These bees consume only 1.3 – 12 mg/day of pollen compared to approximately 60 mg/day of nectar. After 10 days, the new worker bees move to brood and queen tending. During brood and queen tending, worker consumption of pollen remains the same, whereas, nectar consumption more than doubles to 113 – 167 mg/day (U.S. EPA, PMRA, and CDPR, 2014). All other adult bees consume less pollen per day than nectar. This tendency for immediate exposure of residues in nectar brought back to hives, and the more limited exposure to pollen within the hive, suggests that concentrations of a toxic substance in pollen must be higher than concentrations in nectar to elicit a colony-level effect.

Table 1. Pollen and Nectar NOECs used in the Risk Determinations for Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran.

Active Ingredient	NOEC (µg/Kg)
Nectar – Colony Feeding Studies	
Imidacloprid [a]	23
Thiamethoxam [b]	30
Clothianidin [c]	19
Dinotefuran [d]	71
Pollen – Colony Feeding Studies	
Imidacloprid [e]	97.5
Thiamethoxam [f]	372
Clothianidin [g]	372
Dinotefuran [f]	372

All toxicity values derived from the following colony feeding studies:

[a] Bocksch, 2014.

[b] Bocksch, 2015.

[c] Louque, 2016.

[d] Bocksch, 2016.

[e] Dively et al., 2015.

[f] Bridged from the registrant-submitted colony feeding study with clothianidin.

[g] Bocksch and Werner, 2018.

4.3 Exposure Characterization

To determine the expected on-field exposure, measurements of imidacloprid, thiamethoxam, clothianidin, and dinotefuran were taken in pollen and nectar from previously treated crops. Data were generated for the worst-case scenarios (i.e., highest annual application rates, minimum reapplication intervals, etc.) in compliance with product label directions to provide an estimate of the highest concentrations expected for each active ingredient in nectar and pollen of agricultural crops. The plants were treated under standard agricultural practices (e.g. foliar applications, soil applications, or seed treatments along with irrigation, use of fertilizers, other maintenance chemicals, etc.) as indicated on product labels for crops under investigation. Nectar and pollen samples were not available for all crops. For example, tomato flowers do not produce nectar. In such instances, only pollen samples were available for inclusion in the risk determination. Another exception can be seen with cotton, which produces extra-floral nectar in addition to floral nectar. Cotton extra-floral nectar is known to be a highly attractive resource of forage for honey bees and some beekeepers place their hives near cotton for honey production (McGregor, 1976; USDA, 2017). Accordingly, extra-floral nectar was included in this risk determination for applications to cotton crops. In the rare cases where floral pollen samples were not available for analysis, measured residue concentrations in anthers served as a surrogate.

Statistical analyses were conducted on measured neonicotinoid concentrations in bee-relevant matrices (e.g., pollen and nectar) for each acceptable residue study. DPR did not conduct statistical analysis on seed treatment residue studies, as concentrations were always low, and often below analytically detectable limits. Statistical analysis included the generation of the cumulative empirical distributions of measured concentrations. The cumulative distributions calculate a series of percentile values representing the proportion of samples that are below that value. For estimation of exposure, the concentration chosen at a specified percentage of the sample is the value that represents the exposure value that would be compared to the NOEC value derived from colony feeding studies to characterize potential risk.

For the risk determination, DPR scientists took many factors into consideration when determining which percentile value to use for protection of honey bee colonies. Use of moderate statistics, such as the mean or median, would not reflect the possible danger posed at the higher end of measured distributions, and therefore, would not be protective for two reasons: First, considering the extent of agricultural applications made for each crop in California, the amount of data collected is relatively small compared to the total population (e.g., 27 samples of nectar collected from pumpkins might not be representative of all pumpkins grown in California). Thus, the range in actual concentrations could be much greater and extreme residue values that appear to be statistical outliers might not actually be outliers if more samples were available. Many of the studies used in this risk determination have less than twenty total samples, even when all data were combined from studies spanning two years. Second, concentrations measured in nectar in some of the studies were high enough to be of concern for acute toxicity to honey bees. Use of moderate statistics such as the mean concentration would not reflect the risks from these extreme exposures.

On the other hand, use of maximum measured values in the risk determination could be overly protective because they include outliers. Many samples taken for estimation of exposure represented only one point in time, so it is unknown if the concentrations in pollen and nectar were increasing or decreasing. Since these measured concentrations only provide a snapshot of exposure, direct comparison of colony level NOEC values to maximum values measured in the pollen or nectar samples has a high degree of uncertainty because the duration of exposure to concentrations that exceed the NOEC might be significantly shorter than the six-week duration of the colony feeding studies. This would vary for each crop and would depend on the duration of bloom. Based on the uncertainty associated with the duration of exposure, use of higher percentiles could be unrealistic. Consequently, the 90^{th} percentile value was determined to be a point in the distribution where the value represented a realistic, yet protective approach to determining risk.

DPR based this risk determination document on numerous residue studies submitted by the registrants of neonicotinoid insecticide products. Descriptions of methods, results, and limitations of these studies are available in Appendix 10. In addition, the cumulative empirical distributions of measured concentrations for each residue study included in this document are presented in Appendix 11. In many cases, residue data was only available for one or two crops within a specific crop group [as defined in Title 40 Code of Federal Regulations (40 CFR) § 180.41; Appendix 4]. In cases where residue data was lacking for a specific crop, the worst-case scenario within the same crop group was used to represent the missing crop. Additionally, there were cases in which there was no residue data available for an entire crop group. In such instances, data from an appropriate surrogate was used, such as the same crop group from a study utilizing a different nitroguanidine-substituted neonicotinoid active ingredient. In its reevaluation letter, DPR notified registrants of its intention to bridge data from one active ingredient to any of the other three active ingredients if no residue data were available for a given crop or crop group (Appendix 12).

4.4 Risk Determination Categories

DPR conducted risk determinations for agricultural uses registered in California with expected worst-case on-field exposure to honey bees. Applications of neonicotinoid insecticides may result in on-field exposure to honey bees when the crop is bee-attractive and harvested after bloom. Crop groups with limited on-field exposure to honey bees are considered low-risk. According to the United States Department of Agriculture document, *Attractiveness of Agricultural Crops to Pollinating Bees for the Collection of Nectar and/or Pollen* (USDA, 2017), certain crops and crop groups, such as bulb vegetables, leafy vegetables (including *Brassica* vegetables), and globe artichokes, are generally harvested before bloom, except when grown for seed. Thus, the risk determinations for these crops and crop groups are classified as low risk, except when grown for seed.

For crops associated with expected on-field exposure to honey bees, the 90th percentile was calculated using residue data conducted at the maximum annual application rate and compared to colony-level NOEC values to determine risk. Risk determinations were categorized as either having a determination of risk, or low risk to honey bee colonies. Determinations of risk were made for those crops or crop groups with 90th percentile pollen or nectar residue values that exceed the appropriate NOEC value for the specific active ingredient and matrix. Conversely, low risk determinations are those crops or crop groups with 90th percentile pollen or nectar residue values that do not exceed the appropriate NOEC value. For more details, including exceptions, refer to Tables 2-5**.**

It is important to note that determinations of risk in Table 6 were derived from worst-case application scenarios. Crops with determinations of risk may be able to be mitigated by modifying label use directions in a manner that will result in residues that are below the respective NOEC values. By definition, if the residues in pollen or nectar are below the respective colony-level NOEC values, then no significant colony effects are expected to occur. The footnotes in Table 6 provide guidance on some potential adjustments to management practices and label directions based on submitted data that could result in a determination of low risks to honey bee colonies.

5.0 Risk Characterization

DPR made risk determinations for specific crops where crop-specific data was available (Tables 2, 3, 4, and 5 below). In most cases, residue data were only available for one or two crops within a specific crop group. In cases where there were no residue data for other crops in the group, the worst-case scenario (i.e., specific crop data that resulted in the highest residues in pollen and nectar) within the same crop group was used to represent all other crops in that crop group. For example, for imidacloprid, the Berries Crop Group (Crop Group 13) includes both a strawberry and blueberry residue study (Table 2). The strawberry study resulted in higher residues than the blueberry study. Thus, DPR used the risk determination based on the strawberry residue data to represent all crops within Crop Group 13, with the exception of blueberries. The submission of additional data can change these determinations on a crop by crop basis.

Similarly, if a given crop and active ingredient had more than one acceptable residue study, the study that resulted in the higher residues was used to represent that crop in the final risk determination for that crop. One case in which this occurred was with thiamethoxam and cucumber. There are two acceptable cucumber residue studies. DPR used the study with the highest residues in pollen and nectar to represent cucumbers in the overall risk determination. This conservative approach is appropriate given the limitations of the residue data in terms of relatively small sample sizes, environmental variability, and the various other factors (e.g., soil texture, irrigation practices, use of fertilizers, temperature, etc.) that can influence how representative these data sets are of the crops grown in various microclimates of California. If no acceptable residue data was available for a crop group, data from an appropriate surrogate was used, such as data on the same crop group using a different nitroguanidine-substituted neonicotinoid active ingredient.

Tables 2, 3, 4, and 5 below, show which crops had available residue data at the maximum application rate. These tables compare the resulting residue concentrations to appropriate NOEC values, state if the residues exceed the respective NOEC values, and make determinations of risk based on 90^{th} percentile residue values:

Table 2. Imidacloprid 90th percentile residue values and NOEC exceedances.

Imidacloprid								
Crop Group	**Crop**	**Residue Study Lab ID**	**Application**	**Residue Matrix**	**Residue (µg/Kg)**	**NOEC (µg/Kg)**	**Exceedance (Y/N)**	**Risk**
Crop Group 8. Fruiting Vegetables Group	Tomato	EBNTN012	1 Soil + 2 Foliar	Pollen	476.9	97.5	Y	
Crop Group 10. Citrus Fruit Group	Orange	EBNTY007	2 Foliar (Pre-Bloom)	Pollen	3257.9	97.5	Y	
				Nectar	267.1	23	Y	
	Citrus [a]	EBNTL056-7	1 Soil	Nectar	25.0	23	Y	
Crop Group 11. Pome Fruits Group	Apple	EBNTN014	1 Soil + 2 Foliar	Pollen	58.5	97.5	N	
				Nectar	3.5	23	N	
Crop Group 12. Stone Fruits Group	Stone Fruit [b]	EBNTN013	1 Soil + 2 Foliar	Pollen	136.2	97.5	Y	
				Nectar	9.5	23	N	
	Cherry	EBNTY008	5 Foliar	Pollen	393.8	97.5	Y	
				Nectar	5.1	23	N	
Crop Group 13. Berries Group	Blueberry	EBNTY006	1 Soil	Pollen	17.5	97.5	N	
				Nectar	4.6	23	N	
	Strawberry	EBNTL056-04	1 Soil	Pollen	247.0	97.5	Y	
Crop Group 20. Oilseed Group	Cotton	EBNTN011	1 Soil + 3 Foliar (At Bloom)	Pollen	182.2	97.5	Y	
				Floral Nectar	107.0	23	Y	
				Extrafloral Nectar	578.6	23	Y	
		EBNTY010	5 Foliar (Pre-Bloom)	Pollen	6.6	97.5	N	
				Floral Nectar	18.4	23	N	
				Extrafloral Nectar	13.3	23	N	

Notes:

Red shading indicates soil or foliar applications that result in pollen or nectar residues that exceed the NOEC.

Green shading indicates soil or foliar applications that do not result in pollen or nectar residues that exceed the NOEC.

[a] Residue study was conducted on multiple crops within the crop group, including orange, tangerine, grapefruit, tangelo, and lemon. However, data was not analyzed by individual crop due to limited replication.

[b] Residue study was conducted on multiple crops within the crop group, including cherry, plum, apricot, and peach. However, data was not analyzed by individual crop due to limited replication.

Table 3. Thiamethoxam 90th percentile residue values and NOEC exceedances.

Thiamethoxam								
Crop Group	**Crop**	**Residue Study Lab ID**	**Application**	**Residue Matrix**	**Residue (µg/Kg)**	**NOEC (µg/Kg)**	**Exceedance (Y/N)**	**Risk**
Crop Group 6. Legume Vegetables (Succulent or Dried) Group	Soybean	TK0250070	2 Foliar	Anthers	41.2	372	N	
				Nectar	4.7	30	N	
Crop Group 8. Fruiting Vegetables Group	Tomato	TK0222531	2 Foliar	Pollen	6519.7	372	Y	
		TK0242072	1 Soil	Pollen	157.2	372	N	
	Pepper	TK0236306	1 Soil	Pollen	259.9	372	N	
				Nectar	180.9	30	Y	
Crop Group 9. Cucurbit Vegetables Group	Cucumber	TK0024668	1 Soil	Pollen	10.8	372	N	
				Nectar	13.2	30	N	
		TK0222532	2 Foliar	Pollen	1079.9	372	Y	
				Nectar	288.6	30	Y	
	Muskmelon	TK0222530	1 Soil	Pollen	119.7	372	N	
				Nectar	27.9	30	N	
	Pumpkin	TK0222530	1 Soil	Pollen	8.1	372	N	
				Nectar	12.2	30	N	
		TK0242074	2 Foliar	Pollen	18.0	372	N	
				Nectar	15.0	30	N	
	Summer Squash	TK0222530	1 Soil	Pollen	16.1	372	N	
				Nectar	31.7	30	Y	
Crop Group 10. Citrus Fruit Group	Citrus [a]	TK0177221	1 Soil	Pollen	62.3	372	N	
				Nectar	10.2	30	N	
	Sweet Orange	TK0250069	2 Foliar	Pollen	126.7	372	N	
				Nectar	2.1	30	N	
Crop Group 11. Pome Fruits Group	Apple [b]	TK0250071	1 Foliar	Pollen	1954.7	372	Y	
				Nectar	225.4	30	Y	
Crop Group 12. Stone Fruits Group	Stone Fruit [c]	TK0177222	2 Foliar	Pollen	1.6	372	N	
				Nectar	133.2	30	Y	
Crop Group 13. Berries Group	Blueberry	TK0250072	3 Foliar	Pollen	836.4	372	Y	
				Nectar	613.0	30	Y	
	Strawberry	TK0177224	3 Foliar	Pollen	7411.0	372	Y	
				Nectar	301.0	30	Y	
		TK0250068	1 Soil	Pollen	541.0	372	Y	
				Nectar	52.3	30	Y	
	Cranberry	TK0236307	3 Foliar	Pollen	1226.4	372	Y	
				Nectar	921.9	30	Y	
Crop Group 15. Cereal Grains Group	Corn	TK0258214	Seed + 2 Foliar	Pollen	538.9	372	Y	
Crop Group 20. Oilseed Group	Cotton	TK0177223	2 Foliar	Pollen	102.5	372	N	
				Nectar	5.8	30	N	
				Extrafloral Nectar	125.9	30	Y	

Notes:

Red shading indicates soil or foliar applications that result in pollen or nectar residues that exceed the NOEC.

Green shading indicates soil or foliar applications that do not result in pollen or nectar residues that exceed the NOEC.

[a] Residue study was conducted on multiple crops within the crop group, including orange and lemon. However, data was not analyzed by individual crop due to limited replication.

[b] The residue study for this crop was not conducted at the maximum application rate allowed by the product label, therefore worst-case residues are expected to be higher than reported in this table.

[c] Residue study was conducted on multiple crops within the crop group, including peach, plum, cherry, and prune. However, data was not analyzed by individual crop due to limited replication.

Table 4. Clothianidin 90th percentile residue values and NOEC exceedances.

Clothianidin								
Crop Group	**Crop**	**Residue Study Lab ID**	**Application**	**Residue Matrix**	**Residue (µg/Kg)**	**NOEC (µg/Kg)**	**Exceedance (Y/N)**	**Risk**
Crop Group 1. Root and Tuber Vegetables Group	Potato	VP-38985	1 Soil	Pollen	113.9	372	N	Green
Crop Group 9. Cucurbit Vegetables Group	Cucumber	VP-38938	1 Soil	Anthers	32	372	N	Red
				Nectar	39.6	19	Y	
	Melon	VP-38938	1 Soil	Anthers	18.7	372	N	Green
				Nectar	14.6	19	N	
	Squash	VP-38938	1 Soil	Pollen	10.7	372	N	Green
				Nectar	4.4	19	N	
	Pumpkin	VP-38938	1 Soil	Pollen	21	372	N	Green
				Nectar	6.6	19	N	
		VP-38263	1 Soil (At Planting)	Pollen	17	372	N	Green
				Nectar	6.3	19	N	
		VP-38313	2 Foliar	Pollen	71	372	N	Green
				Nectar	5	19	N	
		VP-38971	1 Soil (Post-Emergence)	Pollen	20.3	372	N	Green
				Nectar	9.9	19	N	
Crop Group 11. Pome Fruits Group	Apple [a]	VP-38552	1 Foliar (Post-Bloom)	Pollen	57.4	372	N	Green
				Nectar	0.71	19	N	
Crop Group 12. Stone Fruits Group	Peach [a]	VP-38563	2 Foliar (Post-Bloom)	Pollen	10	372	N	Green
				Nectar	0.3	19	N	
Crop Group 13. Berries Group	Grape	VP-38992	1 Soil	Pollen	157.3	372	N	Green
			1 Foliar (Pre-Bloom)	Pollen	1229.8	372	Y	Red
Crop Group 14. Tree Nuts Group	Almond [a]	VP-38473	2 Foliar (Post-Bloom)	Pollen	12.7	372	N	Green
				Nectar	0.8	19	N	
Crop Group 20. Oilseed Group	Cotton	VP-38259	2 Foliar	Pollen	246	372	N	Red
				Nectar	79.4	19	Y	
				Extrafloral Nectar	647	19	Y	

Notes:

Red shading indicates soil or foliar applications that result in pollen or nectar residues that exceed the NOEC.

Green shading indicates soil or foliar applications that do not result in pollen or nectar residues that exceed the NOEC.

[a] Data indicate that post-bloom applications on these crops are not associated with a determination of risk to honey bees; however, these studies do not represent the worst-case scenario for the crop group.

Table 5. Dinotefuran 90th percentile residue values and NOEC exceedances.

Dinotefuran								
Crop Group	**Crop**	**Residue Study Lab ID**	**Application**	**Residue Matrix**	**Residue (µg/Kg)**	**NOEC (µg/Kg)**	**Exceedance (Y/N)**	**Risk**
Crop Group 1. Root and Tuber Vegetables Group	Potato	10934.4100	1 Soil	Anthers	56.9	372	N	
Crop Group 8. Fruiting Vegetables Group	Bell Pepper	S16-01167	2 Soil	Pollen	183	372	N	
				Nectar	4.46	71	N	
	Tomato	10934.4103	2 Foliar	Pollen	10438.6	372	Y	
			2 Soil	Pollen	5532.4	372	Y	
Crop Group 9. Cucurbit Vegetables Group	Pumpkin	10934.4104	2 Soil	Pollen	88.3	372	N	
				Nectar	39.0	71	N	
Crop Group 12. Stone Fruits Group	Cherry	10934.4105	2 Foliar	Pollen	130.5	372	N	
				Nectar	12.5	71	N	
Crop Group 13. Berries Group	Blueberry	10934.4107	2 Foliar	Pollen	468.9	372	Y	
				Nectar	470.8	71	Y	
	Cranberry	10934.4101	2 Foliar	Pollen	763.5	372	Y	
				Nectar	780.9	71	Y	
Crop Group 20. Oilseed Group	Cotton	43411B104	2 Foliar	Pollen	6968	372	Y	
				Floral Nectar	81.6	71	Y	
				Extrafloral Nectar	1660	71	Y	

Notes:

Red shading indicates soil or foliar applications that result in pollen or nectar residues that exceed the NOEC.

Green shading indicates soil or foliar applications that do not result in pollen or nectar residues that exceed the NOEC.

6.0 CONCLUSIONS

6.1. Overview by Crop Grouping

In summary, this risk determination document is based upon colony-level risks to honey bees resulting from the consumption of nectar or pollen containing neonicotinoid residues that exceed the colony-level NOEC values. DPR conducted risk determinations for the maximum annual application rate of each agricultural crop group as found on currently registered imidacloprid, clothianidin, thiamethoxam, and dinotefuran product labels (Appendix 3). DPR's risk determinations for soil and foliar applications on registered agricultural crop groupings for imidacloprid, thiamethoxam, clothianidin, and dinotefuran are detailed below and in Table 6. The risk determination process discussed previously states that crop groups are categorized as either having a determination of risk or low risk to honey bee colonies. In Table 6 below, red shading indicates a determination of risk for all crops in the crop group. Green shading indicates a determination of low risk for the entire crop group. Yellow shading is to be considered as having a determination of risk for the crop group, with some crop-specific exceptions. Only crop groups currently registered for agricultural use in California are included in this risk determination, with crop-specific exceptions noted in Table 6.

For imidacloprid, using the 90th percentile as the expected exposure to honey bees, the following crop groups have a determination of low risk: Root and Tuber Vegetables (Crop Group 1), Bulb Vegetables (Crop Group 3), Leafy Vegetables (Except *Brassica* Vegetables) (Crop Group 4), *Brassica* (Cole) Leafy Vegetables (Crop Group 5), Legume Vegetables (Succulent or Dried) (Crop Group 6), Pome Fruits (Crop Group 11), Herbs and Spices (Crop Group 19), and Globe Artichoke. The following crop groups have a determination of risk for imidacloprid: Fruiting Vegetables (Crop Group 8), Cucurbit Vegetables (Crop Group 9), Citrus Fruit (Crop Group 10), Stone Fruits (Crop Group 12), Berries (Crop Group 13), Tree Nuts (Crop Group 14), Oilseed Crops (Crop Group 20), Tropical and Subtropical Fruits with Inedible Peels (Crop Group 24), Hops, Tobacco, and Coffee.

For thiamethoxam, using the 90^{th} percentile as the expected exposure to honey bees, the following crop groups have a determination of low risk: Root and Tuber Vegetables (Crop Group 1), Bulb Vegetables (Crop Group 3), Leafy Vegetables (Except *Brassica* Vegetables) (Crop Group 4), *Brassica* (Cole) Leafy Vegetables (Crop Group 5), Legume Vegetables (Succulent or Dried) (Crop Group 6), Citrus Fruit (Crop Group 10), Globe Artichoke, and Mint. The following crop groups have a determination of risk for thiamethoxam: Fruiting Vegetables (Crop Group 8), Cucurbit Vegetables (Crop Group 9), Pome Fruits (Crop Group 11), Stone Fruits (Crop Group 12), Berries (Crop Group 13), Cereal Grains (Crop Group 15), Oilseed Crops (Crop Group 20), Tropical and Subtropical Fruits with Inedible Peels (Crop Group 24), Hops, and Tobacco.

For clothianidin, using the 90^{th} percentile as the expected exposure to honey bees, the following crop groups have a determination of low risk: Root and Tuber Vegetables (Crop Group 1), Leafy Vegetables (Except *Brassica* Vegetables) (Crop Group 4), *Brassica* (Cole) Leafy Vegetables (Crop Group 5), and Legume Vegetables (Succulent or Dried) (Crop Group 6). The following crop groups have a determination of risk for clothianidin: Cucurbit Vegetables (Crop Group 9), Citrus Fruit (Crop Group 10), Pome Fruits (Crop Group 11), Stone Fruits (Crop Group 12), Berries (Crop Group 13), Tree Nuts (Crop Group 14), Cereal Grains (Crop Group 15), Oilseed Crops (Crop Group 20), Tropical and Subtropical Fruits with Inedible Peels (Crop Group 24), and Tobacco.

For dinotefuran, using the 90^{th} percentile as the expected exposure to honey bees, the following crop groups have a determination of low risk: Root and Tuber Vegetables (Crop Group 1), Bulb Vegetables (Crop Group 3), Leafy Vegetables (Except *Brassica* Vegetables) (Crop Group 4), *Brassica* (Cole) Leafy Vegetables (Crop Group 5), Cucurbit Vegetables (Crop Group 9), and Stone Fruits (Crop Group 12). The following crop groups have a determination of risk for dinotefuran: Fruiting Vegetables (Crop Group 8), Berries (Crop Group 13), and Oilseed Crops (Crop Group 20).

There are crop- and application-specific exceptions for the risk determinations mentioned above. Please refer to Tables 2-6 for more detail on exceptions.

Table 6. Risk determinations for foliar or soil applications of imidacloprid, thiamethoxam, clothianidin, and dinotefuran at the maximum allowed annual application rate based on 90th percentile residue values.

Crop Group	Imidacloprid		Thiamethoxam		Clothianidin		Dinotefuran	
	Risk	Notes	Risk	Notes	Risk	Notes	Risk	Notes
Crop Group 1. Root and Tuber Vegetables Group		d		d				
Crop Group 3. Bulb Vegetables Group		c		c				c
Crop Group 4. Leafy Vegetables (Except *Brassica* Vegetables) Group		c		c		c		c
Crop Group 5. *Brassica* (Cole) Leafy Vegetables		c		c		c		c
Crop Group 6. Legume Vegetables (Succulent or Dried) Group		b				b		
Crop Group 8. Fruiting Vegetables Group								k
Crop Group 9. Cucurbit Vegetables Group		b		h		j		
Crop Group 10. Citrus Fruit Group								
Crop Group 11. Pome Fruits Group						b, e		
Crop Group 12. Stone Fruits Group						b, e		
Crop Group 13. Berries Group		i						
Crop Group 14. Tree Nuts Group		g				e, g		
Crop Group 15. Cereal Grains Group						b		
Crop Group 19. Herbs and Spices		c						
Crop Group 20. Oilseed Group								
Crop Group 24. Tropical and Subtropical Fruit, Inedible Peel Group		f		f		f		
Globe Artichoke [a]		c		c				
Hops [a]		f		f				
Mint [a]				c				
Tobacco [a]		f		f		f		
Coffee [a]		f						

Notes:

Patterned gray shading indicates that the active ingredient is not currently registered for foliar or soil applications on the crop group.

Red shading indicates a determination of risk for all crops in the crop group based on evaluated data.

Yellow shading indicates a determination of risk for the crop group; however, there were crop-specific or application-specific exceptions indicating low risk.

Green shading indicates a determination of low risk for the crop group based on evaluated data.

For additional detail on residue values, please see tables 3, 4, 5, and 6.

[a] Not categorized into a general crop group, according to 40 CFR 180.41 crop group tables.
[b] Risk determination category bridged from thiamethoxam.
[c] No on-field exposure to honey bees expected unless grown for seed.
[d] Risk determination category bridged from clothianidin.
[e] Risk except for post-bloom, pre-harvest applications.
[f] In absence of tier II data and no similar crop groups from which to bridge, the crop group determination defaults to risk to honey bees.
[g] Risk determination category bridged from thiamethoxam stone fruit, as tree nuts and stone fruits are taxonomically related.
[h] Risk except certain applications to pumpkin, muskmelon, and cucumber.
[i] Risk except certain applications to blueberry.
[j] Risk except certain applications to melon, pumpkin, and squash.
[k] Risk except certain applications to bell pepper.

6.2 Seed Treatments and Tree Injection Applications

Risk determinations were only conducted for foliar and soil applications. Risks from seed treatment applications were evaluated in the preliminary pollinator risk assessments published by U.S. EPA (U.S. EPA and DPR, 2016; U.S. EPA, 2017a; U.S. EPA, 2017b). The preliminary assessment for imidacloprid evaluated multiple seed treatment residue studies conducted on corn, canola, and sunflower. These studies generally reported no residues in pollen and nectar above the limit of detection**.** Values are well below their respective NOEC values, supporting the conclusion that imidacloprid seed treatments pose a low risk to honey bees. The preliminary pollinator risk assessment for clothianidin and thiamethoxam evaluated multiple seed treatment residue studies conducted on corn, sunflower, melon, canola, cotton, and soybean. The resulting residue concentrations are all below the respective NOECs, supporting the conclusion that clothianidin or thiamethoxam seed treatments pose a low risk to honey bee colonies. Dinotefuran is not registered for any seed treatment applications. There have been issues in other states and countries with contact exposure resulting from abraded seed coat dust at planting, but the U.S. EPA has addressed this with best management practices (U.S. EPA and DPR, 2016). DPR has no records of such incidents occurring in California.

DPR considered a single residue study testing a tree injection application in this risk determination. This study measured residues of dinotefuran in pollen and nectar following tree injection applications to cherry trees. Dinotefuran 20SG, EPA Reg. No. 86203-12, was injected into the trunks of cherry trees late in the season (September), before leaf drop, at a rate of 2 grams of product per inch of trunk diameter either at breast height or right below the first trunk bifurcation. Samples of pollen and nectar were collected 165-243 days after the last application. The maximum measured dinotefuran residues resulting from tree injection applications were 31,688 µg/Kg in pollen (201 days after application) and 17,484 µg/Kg in nectar (237 days after application); the corresponding 90th percentile measured residues were 24,894 µg/Kg in pollen and 16,241 µg/Kg in nectar (Lab Study ID 10934.4105; Louque, 2016). These are some of the highest residues noted in pollen and nectar from any application methods on any crops. Currently, no products containing the four neonicotinoids are registered with DPR that allow tree injections to stone fruits or any other agricultural crops in California.

7.0 Considerations for Mitigation

The focus of this document is to identify risks to honey bees at the colony level following applications of imidacloprid, clothianidin, thiamethoxam, and dinotefuran. As stated previously, only worst-case application scenarios, as allowed by currently registered labels in California, were included for analysis. Studies involving less frequent application intervals or lower application rates were excluded from consideration in this document. However, these studies contain valuable data to help inform future mitigation options. For instance, clothianidin residue studies included data on post-bloom applications to several crops, such as peach, almond, and apple. Though these studies were not considered worst-case, and thus not included in the overall risk determination, the resultant residues did not exceed the NOEC and would be categorized as low risk. The information from the additional studies provides potential directions for development of management practices based on the number of applications, frequency of reapplication, soil texture, timing of applications in relation to bloom, and application site.

Though outside the scope of this document, additional analysis of the submitted data would likely provide further science-based mitigation options to reduce risks to honey bees from agricultural applications of nitroguanidine-substituted neonicotinoid pesticides.

8.0 Risk Appraisal

The comparison of neonicotinoid concentrations measured in nectar and pollen of treated crops to NOEC concentrations developed from colony feeding studies is not straightforward for several reasons. First, the duration of exposure in the colony feeding studies was set at six weeks based on bloom duration. Calculating a realistic duration of exposure for pollinating bees is difficult because of differences in blooming periods of crops and commercial beekeeping management practices. The flowering intervals for different crops can be relatively short, such as for early flowering fruit and nut trees, or long, such as for cotton plants, where plants continuously flower throughout the growing season. In addition, during the growing season, managed honey bee colonies are often transported from one flowering crop to another, which extends the duration of exposure. In colony feeding studies, the spiked sugar solutions or pollen patties were regularly replenished throughout the 6-week exposure to ensure the colonies were exposed to a consistent concentration. As indicated by the data, concentrations measured in flowers can be variable, so pollinating honey bee colonies are likely to be exposed to a range of concentrations. In addition, the data presented in this document shows that concentrations measured in the nectar and pollen of certain plants could be orders of magnitude higher than the highest dose levels used in the colony feeding studies. This could result in exposure to residues that are acutely toxic to worker honey bees.

If distributional statistics at the lower to middle portion (i.e. 25th or 50th percentiles) of the measured range in concentrations of treated crops are compared to the NOEC values derived from the colony feeding studies, they could underestimate the potential risk to pollinating honey bee colonies. Conversely, if statistics at the upper end of the distribution are used, they could be overly conservative. Tables 7 to 10 present a visual comparison for the range in potential exceedances that would result from using the 50th, 75th, 90th or 100th (maximum) percentile residue values for each crop and application scenario reviewed for imidacloprid, thiamethoxam, clothianidin, and dinotefuran. The comparison between the 50th and 100th percentile to the NOEC conforms to the observation that the 50th percentile would likely not be protective, whereas, the 100th percentile is potentially overprotective.

The concentrations calculated for each of the percentiles are presented in Appendix 11. Of significance is the rather large range in values that was measured in some of the treated crops. Both nectar and pollen values at the highest percentiles were measured in the parts per million, values that would cause acute toxicity.

In summary, the 50th percentile concentration would likely not be protective of honey bee colonies, especially in light of extremely high values that were measured for certain combinations of crop and application methods. On the other hand, the maximum concentration value would likely be overly protective because of complications in the comparison of total exposure to NOEC values generated from the colony feeding studies. The uncertainty is caused by difficulties in calculating the total magnitude and duration of exposure, as there are potentially large differences in exposure durations between the bees in the colony feeding study and those bees foraging on the flowers of the crops they are pollinating. Use of the 90^{th} percentile residue values indicate either a determination of risk or a determination of low risk for the studies evaluated and appears to be a realistic, yet protective approach.

Table 7. Imidacloprid comparison of NOEC exceedances based on maximum and 50th, 75th, and 90th percentile values for each crop and application scenario, based on acceptable data.

Imidacloprid						
Crop Group	**Crop**	**Application Type**	**Exceedance Category**			
			50%	**75%**	**90%**	**Max**
Crop Group 8. Fruiting Vegetables Group	Tomato	1 Soil + 2 Foliar				
Crop Group 10. Citrus Fruit Group	Orange	2 Foliar (Pre-Bloom)				
	Citrus [a]	1 Soil				
Crop Group 11. Pome Fruits Group	Apple	1 Soil + 2 Foliar				
Crop Group 12. Stone Fruits Group	Stone Fruit [b]	1 Soil + 2 Foliar				
	Cherry	5 Foliar				
Crop Group 13. Berries Group	Blueberry	1 Soil				
	Strawberry	1 Soil				
Crop Group 20. Oilseed Group	Cotton	1 Soil + 3 Foliar (At Bloom)				
		5 Foliar (Pre-Bloom)				
Crop Groups With Data Gaps						
Crop Group 1. Root and Tuber Vegetables Group	N/A	N/A				
Crop Group 3. Bulb Vegetables Group	N/A	N/A				
Crop Group 4. Leafy Vegetables (Except *Brassica* Vegetables) Group	N/A	N/A				
Crop Group 5. *Brassica* (Cole) Leafy Vegetables	N/A	N/A				
Crop Group 6. Legume Vegetables (Succulent or Dried) Group	N/A	N/A				
Crop Group 9. Cucurbit Vegetables Group	N/A	N/A				
Crop Group 14. Tree Nuts Group	N/A	N/A				
Crop Group 19. Herbs and Spices	N/A	N/A				
Crop Group 24. Tropical and Subtropical Fruit, Inedible Peel Group	N/A	N/A				
Globe Artichoke	N/A	N/A				
Hops	N/A	N/A				
Tobacco	N/A	N/A				
Coffee	N/A	N/A				

Notes:

Red shading indicates soil or foliar applications that result in pollen or nectar residues that exceed the NOEC.

Green shading indicates soil or foliar applications that do not result in pollen or nectar residues that exceed the NOEC.

[a] Residue study was conducted on multiple crops within the crop group, including orange, tangerine, grapefruit, tangelos, and lemon. However, data was not analyzed by individual crop due to limited replication.

[b] Residue study was conducted on multiple crops within the crop group, including cherry, plum, apricot, and peach. However, data was not analyzed by individual crop due to limited replication.

Table 8. Thiamethoxam comparison of NOEC exceedances based on maximum and 50th, 75th, and 90th percentile values for each crop and application scenario, based on acceptable data.

Thiamethoxam						
Crop Group	Crop	Application Type	Exceedance Category			
			50%	75%	90%	Max
Crop Group 6. Legume Vegetables (Succulent or Dried) Group	Soybean	2 Foliar				
Crop Group 8. Fruiting Vegetables Group	Tomato	2 Foliar				
		1 Soil				
	Pepper	1 Soil				
Crop Group 9. Cucurbit Vegetables Group	Cucumber	1 Soil				
		2 Foliar				
	Muskmelon	1 Soil				
	Pumpkin	1 Soil				
		2 Foliar				
	Summer Squash	1 Soil				
Crop Group 10. Citrus Fruit Group	Citrus [a]	1 Soil				
	Sweet Orange	2 Foliar				
Crop Group 11. Pome Fruits Group	Apple	1 Foliar				
Crop Group 12. Stone Fruits Group	Stone Fruit [b]	2 Foliar				
Crop Group 13. Berries Group	Blueberry	3 Foliar				
	Strawberry	3 Foliar				
		1 Soil				
	Cranberry	3 Foliar				
Crop Group 15. Cereal Grains Group	Corn	Seed + 2 Foliar				
Crop Group 20. Oilseed Group	Cotton	2 Foliar				
Crop Groups With Data Gaps						
Crop Group 1. Root and Tuber Vegetables Group	N/A	N/A				
Crop Group 3. Bulb Vegetables Group	N/A	N/A				
Crop Group 4. Leafy Vegetables (Except *Brassica* Vegetables) Group	N/A	N/A				
Crop Group 5. *Brassica* (Cole) Leafy Vegetables	N/A	N/A				
Crop Group 24. Tropical and Subtropical Fruit, Inedible Peel Group	N/A	N/A				
Globe Artichoke	N/A	N/A				
Hops	N/A	N/A				
Mint	N/A	N/A				
Tobacco	N/A	N/A				

Notes:

Red shading indicates soil or foliar applications that result in pollen or nectar residues that exceed the NOEC.

Green shading indicates soil or foliar applications that do not result in pollen or nectar residues that exceed the NOEC.

[a] Residue study was conducted on multiple crops within the crop group, including orange and lemon. However, data was not analyzed by individual crop due to limited replication.

[b] Residue study was conducted on multiple crops within the crop group, including peach, plum, cherry, and prune. However, data was not analyzed by individual crop due to limited replication.

Table 9. Clothianidin comparison of NOEC exceedances based on maximum and 50th, 75th, and 90th percentile values for each crop and application scenario, based on acceptable data.

Clothianidin						
Crop Group	**Crop**	**Application Type**	**Exceedance Category**			
			50%	**75%**	**90%**	**Max**
Crop Group 1. Root and Tuber Vegetables Group	Potato	1 Soil				
Crop Group 9. Cucurbit Vegetables Group	Cucumber	1 Soil				
	Melon	1 Soil				
	Squash	1 Soil				
	Pumpkin	1 Soil				
		1 Soil (At Planting)				
		2 Foliar				
		1 Soil (Post-Emergence)				
Crop Group 13. Berries Group	Grape	1 Soil				
		1 Foliar (Pre-Bloom)				
Crop Group 20. Oilseed Group	Cotton	2 Foliar				
Crop Groups With Data Gaps						
Crop Group 4. Leafy Vegetables (Except *Brassica* Vegetables) Group	N/A	N/A				
Crop Group 5. *Brassica* (Cole) Leafy Vegetables	N/A	N/A				
Crop Group 6. Legume Vegetables (Succulent or Dried) Group	N/A	N/A				
Crop Group 11. Pome Fruits Group [a]	N/A	N/A				
Crop Group 12. Stone Fruits Group [b]	N/A	N/A				
Crop Group 14. Tree Nuts Group [c]	N/A	N/A				
Crop Group 15. Cereal Grains Group	N/A	N/A				
Crop Group 24. Tropical and Subtropical Fruit, Inedible Peel Group	N/A	N/A				
Tobacco	N/A	N/A				

Notes:

Red shading indicates soil or foliar applications that result in pollen or nectar residues that exceed the NOEC.

Green shading indicates soil or foliar applications that do not result in pollen or nectar residues that exceed the NOEC.

[a] Risk except post-bloom foliar applications to apple.

[b] Risk except post-bloom foliar applications to peach.

[c] Risk except post-bloom foliar applications to almond.

Table 10. Dinotefuran comparison of NOEC exceedances based on maximum and 50th, 75th, and 90th percentile values for each crop and application scenario, based on acceptable data.

Dinotefuran						
Crop Group	**Crop**	**Application Type**	**Exceedance Category**			
			50%	**75%**	**90%**	**Max**
Crop Group 1. Root and Tuber Vegetables Group	Potato	1 Soil				
Crop Group 8. Fruiting Vegetables Group	Bell Pepper	2 Soil				
	Tomato	2 Soil				
		2 Foliar				
Crop Group 9. Cucurbit Vegetables Group	Pumpkin	2 Soil				
Crop Group 12. Stone Fruits Group	Cherry	2 Foliar				
Crop Group 13. Berries Group	Blueberry	2 Foliar				
	Cranberry	2 Foliar				
Crop Group 20. Oilseed Group	Cotton	2 Foliar				
Crop Groups With Data Gaps						
Crop Group 3. Bulb Vegetables Group	N/A	N/A				
Crop Group 4. Leafy Vegetables (Except *Brassica* Vegetables) Group	N/A	N/A				
Crop Group 5. *Brassica* (Cole) Leafy Vegetables	N/A	N/A				

Notes:

Red shading indicates soil or foliar applications that result in pollen or nectar residues that exceed the NOEC.

Green shading indicates soil or foliar applications that do not result in pollen or nectar residues that exceed the NOEC.

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Department of Pesticide Regulation

Mary-Ann Warmerdam
Director

Arnold Schwarzenegger
Governor

California Notice 2009-02

POST UNTIL March 31, 2009

NOTICE OF DECISION TO INITIATE REEVALUATION OF CHEMICALS IN THE NITROGUANIDINE INSECTICIDE CLASS OF NEONICOTINOIDS.

Pursuant to Section 6220, et seq., Title 3. California Code of Regulations, the Director of the Department of Pesticide Regulation (DPR) notices her decision to initiate a reevaluation of certain pesticide products within the nitroguanidine insecticide class of neonicotinoids and containing the following active ingredients: imidacloprid, clothianidin, dinotefuran, and thiamethoxam. Interested persons may comment on this decision up to and including the date shown on the top-right corner of this notice to the Department of Pesticide Regulation, Pesticide Registration Branch, 1001 I Street, P.O. Box 4015, Sacramento, California 95812-4015.

REEVALUATION

DPR is hereby commencing a reevaluation of chemicals in the nitroguanidine insecticide class of neonicotinoids and containing the following active ingredients: imidacloprid, clothianidin, dinotefuran, and thiamethoxam. This reevaluation involves 50 registrants and 282 pesticide products. DPR determined that the number of products included in this reevaluation were too numerous to list within this notice. A list of products included in the reevaluation is available upon written request to the address listed above or on DPR's Web site at: <http://www.cdpr.ca.gov/docs/registration/reevaluation/chemicals/neonicotinoids.htm>.

BASIS OF REEVALUATION

In 2008, DPR received an adverse effects disclosure pursuant to Federal Insecticide Fungicide and Rodenticide Act (FIFRA) section 6(a)(2) and Food and Agricultural Code section 12825.5 regarding the active ingredient imidacloprid. The disclosure included twelve residue and two combination residue, honey, bumble bee studies of imidacloprid use on a number of ornamental plants. DPR's evaluation of the data noted two critical findings. One, high levels of imidacloprid in leaves and blossoms of treated plants, and two, increases in residue levels over time.

Imidacloprid levels in leaves and blossoms varied depending on the application rate and the type of plant, but the data indicate that residues in some plants measured higher than 4 parts per million (ppm). The data also indicate that when using soil application methods, imidacloprid residues remained relatively low for the first six months after application, followed by a dramatic increase that remained stable in some cases for more than 500 days after treatment. Where imidacloprid was applied to the soil, no significant decline in residue levels was observed in any of the studies, even in studies where residues were tested at 540 days after treatment. DPR found that the treatment rates used in the studies where high imidacloprid residue levels were found in

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leaves and blossoms, were comparable to application rates found on currently registered labels for orchards, assuming the orchards were planted at a density of 200 trees per acre or fewer. The data indicate that use of imidacloprid on an annual basis may be additive, in that significant residues from the previous use season appear to be available to the treated plant. DPR also received preliminary information from a University of California at Riverside researcher who is investigating imidacloprid residues in eucalyptus nectar and pollen. The researcher's preliminary results indicate imidacloprid residues in eucalyptus nectar at levels of up to 550 parts per billion (ppb).

Based upon data on file, DPR estimates the lethal concentration of imidacloprid needed to kill 50 percent of a test population (LC_{50}) of honey bees is 185 ppb[1]. In their everyday foraging and pollination activities, honey bees collect both nectar and pollen from flowering plants. If the imidacloprid residue levels in a plant's nectar and pollen are similar to those found in the leaves and blossoms of the plants described in the adverse effects data, the levels are well above the estimated LC_{50} for honey bees. The levels found in some of the plants were more than twenty times the estimated honey bee LC_{50} of 185 ppb.

All of the neonicotinoids share many of the same characteristics as imidacloprid. However, the three other neonicotinoids included in this reevaluation, clothianidin, dinotefuran, and thiamethoxam, are in the same chemical family (nitroguanidines) as imidacloprid. These three other active ingredients, in particular, have soil mobility characteristics and half-lives that are very similar to imidacloprid. Based on available data, DPR scientists believe these active ingredients would have the same potential residue concerns as imidacloprid. Data also indicate that these active ingredients are similar to imidacloprid in toxicity to honey bees. Due to the chemical and toxicological similarities between imidacloprid and the other neonicotinoids, DPR is providing those registrants with the option of generating data on their own chemicals or providing/relying upon data generated using a surrogate nitroguanidine.

DPR exempted the following formulation categories and product types from the reevaluation:

1. Formulated as a gel or impregnated in a strip;
2. Termiticide;
3. Flea control products combined with rodenticide;
4. Pet spot applications;
5. Ant and roach baits;
6. Premise application for control of nuisance pests; or,
7. Manufacturing use only products.

[1] The LC_{50} was estimated by converting the acute oral LD_{50} (the amount of a material that causes the death of 50 percent of a test population) to a concentration in nectar using the standard consumption model used in bee feeding studies.

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DPR exempted the above types of products from the reevaluation because the manner in which the products are formulated or applied makes it unlikely that the neonicotinoid will move into plants that bloom or be a source of forage for honey bees and pollinators.

DPR has not yet made a final decision as to the data it will require registrants to conduct pursuant to this reevaluation. In general, DPR intends to require registrants to analyze residues from the nectar and pollen of a representative number of crops grown in California in order to better understand the impact of neonicotinoids on honey bees. In addition, DPR plans to require acute toxicity studies on various honey bee life stages.

DPR plans to work closely with the United States Environmental Protection Agency's (U.S. EPA's) Office of Pesticide Programs throughout the reevaluation process. U.S. EPA's registration review docket for imidacloprid <http://www.epa.gov/oppsrrd1/registration_review/imidacloprid/index.htm> opened in December 17, 2008, and the docket for nithiazine is scheduled to be opened in March 2009. In order to better ensure a "level playing field" for the neonicotinoid class as a whole, and to best take advantage of new research as it becomes available, U.S. EPA has scheduled the docket openings for the remaining neonicotinoids (acetamiprid, clothianidin, dinotefuran, thiacloprid, and thiamethoxam) for fiscal year 2012.

For information regarding the reevaluation process, please contact either Ms. Denise Webster, by e-mail at <dwebster@cdpr.ca.gov> or by telephone at (916) 324-3522, or Ms. Alveena Prasad, by e-mail at <aprasad@cdpr.ca.gov> or by telephone at (916) 324-3905.

Original signed by
Ann M. Prichard, Chief
Pesticide Registration Branch
(916) 324-3931

February 27, 2009
Date

cc: Ms. Denise Webster, Program Specialist
Ms. Alveena Prasad, Environmental Scientist

Appendix 2. California Food and Agricultural Code (FAC) section (§) 12838

Assembly Bill No. 1789

CHAPTER 578

An act to add Section 12838 to the Food and Agricultural Code, relating to pesticides.

[Approved by Governor September 26, 2014. Filed with Secretary of State September 26, 2014.]

Legislative counsel's digest

AB 1789, Williams. Pesticides: neonicotinoids: reevaluation: determination: control measures.

Existing law requires pesticides to be registered by the Department of Pesticide Regulation. Existing law requires that a pesticide be thoroughly evaluated prior to registration, and provides for the continued evaluation of registered pesticides.

This bill would require the department, by July 1, 2018, to issue a determination with respect to its reevaluation of neonicotinoids. The bill would require the department, on or before 2 years after making this determination, to adopt any control measures necessary to protect pollinator health.

The bill would require the department to submit a report to the appropriate committees of the Legislature if the department is unable to adopt those control measures and to update the report annually until the department adopts those control measures.

The people of the State of California do enact as follows:

SECTION 1. (a) The Legislature finds and declares all of the following:

(1) Honey bees are vital to the pollination of many of California's crops, which are critical to our national food system and essential to the economy of the state.

(2) Annual colony losses from 2006 to 2011, inclusive, averaged about 33 percent each year, which is more than double what is considered sustainable according to the United States Department of Food and Agriculture.

(3) Scientists now largely agree that a combination of factors is to blame for declining pollinator health, including lack of varied forage and nutrition, pathogens and pests such as the Varroa mite, and chronic and acute exposure to a variety of pesticides.

(4) Based on data submitted to the Department of Pesticide Regulation showing a potential hazard to honey bees, the department initiated a

Appendix 2. California Food and Agricultural Code (FAC) section (§) 12838

reevaluation process for four neonicotinoid compounds in 2009: imidacloprid, thiamethoxam, clothianidin, and dinotefuran.

(b) It is the intent of the Legislature to set a timeline for completion of the reevaluation of neonicotinoid compounds to ensure that the Department of Pesticide Regulation completes a thorough, scientifically sound, and timely analysis of the effects of neonicotinoids on pollinator health.

SEC. 2. Section 12838 is added to the Food and Agricultural Code, to read:

12838. (a) On or before July 1, 2018, the department shall issue a determination with respect to its reevaluation of neonicotinoids.

(b) (1) Within two years after making the determination specified in subdivision (a), the department shall adopt any control measures necessary to protect pollinator health.

(2) If the department is unable to adopt necessary control measures within two years as required in paragraph (1), the department shall submit a report to the appropriate committees of the Legislature setting forth the reasons the requirement of paragraph (1) has not been met.

(3) The department shall update the report submitted to the appropriate committees of the Legislature pursuant to paragraph (2) every year until the department adopts the necessary control measures specified in paragraph (1).

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

The neonicotinoid informational use tables include crop groups that have been defined in Title 40 of the Code of Federal Regulations (40 CFR) Part 180.41. In accordance with the risk determination, a single crop or a subset of a crop group could represent an entire crop group listed in the tables. Crop groups and use rates in the tables are representative of agricultural commodities that are currently registered for use in California. 40 CFR Part 180.41 does not categorize hops, globe artichoke, and peanuts into crop groups as these are seen as miscellaneous commodities.

Imidacloprid					
Crop groups listed		**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Berry and Small Fruit	Low Growing Berry	0.047 lbs ai/A (foliar) 0.50 lbs ai/A (soil)	0.14 lbs ai/A (foliar) 0.50 lbs ai/A (soil)	5 days	• When applied as a soil post-harvest treatment, the maximum single application rate the maximum annual seasonal application rate is 0.38 lb ai/A. • Do not use both soil application methods on the same crop in the same season. • Do not apply during bloom or within 10 days prior to bloom or when bees are foraging.
	Bushberry	0.1 lbs ai/A (foliar) 0.5 lbs ai/A (soil)	0.5 lbs ai/A (foliar) 0.5 lbs ai/A (foliar)	7 days	• Do not apply pre-bloom or during bloom or when bees are foraging.
	Caneberry	0.1 lbs ai/A (foliar) 0.5 lbs ai/A (soil)	0.3 lbs ai/A (foliar) 0.5 lbs ai/A (soil)	7 days	• Do not apply pre-bloom or during bloom or when bees are foraging.
	Small fruit vine climbing subgroup except fuzzy kiwifruit	0.05 lbs ai/A (foliar) 0.5 lbs ai/A (soil)	0.1 lbs ai/A (foliar) 0.5 lbs ai/A (soil)	14 days	• Apply with ground application equipment only.

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Imidacloprid				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Citrus Fruit	0.25 lbs ai/A (foliar) 0.50 lbs ai/A (soil) 0.0013 lbs ai/ft^3 (soil; containerized)	0.50 lbs ai/A (foliar) 0.50 lbs ai/A (soil) 0.0037 lbs ai/plant (soil; containerized)	10 days	• Do not apply during bloom or within 10 days prior to bloom or when bees are foraging.
Oilseed	0.063 lbs ai/A (foliar) 0.33 lbs ai/A (soil) 0.5 lbs ai/100 lb seed (seed treatment; cotton) 1 lbs ai/100 lbs seed (seed treatment; canola, rapeseed, mustard seed, flax, crambe, borage) 0.5 mg ai/seed (seed treatment; safflower, sunflower)	0.31 lbs ai/A (foliar) 0.33 lbs ai/A (soil)	7 days	• Regardless of formulation or method of application, apply no more than 0.5 lb. active ingredient per acre per year, including seed treatment, soil, and foliar uses. • Do not graze treated fields after any application imidacloprid
Cucurbit Vegetable	0.38 lbs ai/A (soil)	0.38 lbs ai/A (soil)		• Not for use on crops grown for seed unless allowed by state-specific 24(c) labeling.

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Imidacloprid				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Fruiting Vegetable	0.075 lbs ai/A (foliar) 0.38 lbs ai/A (soil) 0.5 lbs ai/A (soil; okra and peppers)	0.24 lbs ai/A (foliar) 0.38 lbs ai/A (soil) 0.5 lbs ai/A (soil; okra and peppers)	5 days	• Not for use on crops grown for seed unless allowed by state-specific 24(c) labeling.
Pome Fruit	0.1 lbs ai/A (foliar) 0.25 lbs ai/A (foliar; pear) 0.38 lbs ai/A (soil)	0.5 lbs ai/A (foliar) 0.5 lbs ai/A (foliar; pear) 0.38 lbs ai/A (soil)	10 days	• Do not apply pre-bloom or during bloom or when bees are foraging.
Stone Fruit	0.1 lbs ai/A (foliar) 0.1 lbs ai/A (foliar; apricot, nectarine, and peach) 0.38 lbs ai/A (soil)	0.5 lbs ai/A (foliar) 0.3 lbs ai/A (foliar; apricot, nectarine, and peach) 0.38 lbs ai/A (soil)	10 days 7 days (apricot, nectarine, and peach)	• The maximum annual foliar rate allowed per year for apricot, nectarine, and peach: 0.3 lb ai/A • Do not apply pre-bloom or during bloom or when bees are foraging

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Imidacloprid				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Cereal Grains	0.53 lbs ai/100 lbs seed (seed treatment; field corn) 0.94 lbs ai/100 lbs seed (seed treatment; wheat, barley, oats, rye, triticale) 0.25 lbs ai/100 lbs seed (seed treatment; sorghum, millet) 0.2 lbs ai/100,000 of pelleted seed (seed treatment; sugar beet) 0.094 lbs ai/100,000 of raw seed (seed treatment; sugar beet) 0.25 lbs ai/100 lbs seed (seed treatment; popcorn) 0.25 lbs ai/100 lbs seed (seed treatment; sweet corn)			• Corn: Do not graze or feed livestock on treated areas for 45 days after planting. • Wheat, barley, oats, rye, triticale, sorghum, and millet: Do not graze or feed livestock on treated areas for 45 days after planting. • The maximum application rate for imidacloprid (including seed treatments, foliar applications, and soil applications) is 0.5 pound active ingredient per acre per calendar year.

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Imidacloprid				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Tree Nut Group	0.1 lbs ai/A (foliar) 0.50 lbs ai/A (soil)	0.36 lbs ai/A (foliar) 0.50 lbs ai/A (soil)	6 days	• Do not apply to almonds • Do not apply pre-bloom or during bloom or when bees are foraging.
Brassica (Cole) Leafy Vegetable	0.047 lbs ai/A (foliar) 0.38 lbs ai/A (soil)	0.24 lbs ai/A (foliar) 0.38 lbs ai/A (soli)	5 days	• Not for use on crops grown for seed unless allowed by state-specific 24(c) labeling.
Tropical and Subtropical Fruit, Inedible Peel Group	0.1 lbs/A (foliar) 0.1lbs ai/A (foliar; pomegranate) 0.5 lbs ai/A (soil)	0.5 lbs ai/A (foliar) 0.3 lbs ai/A (foliar; pomegranate) 0.5 lbs ai/A (soil)	7 days 14 days (banana and plantain)	• Do not apply pre-bloom or during bloom or when bees are foraging.
Root and Tuber Vegetables	0.047 lbs ai/A (foliar; potato) 0.044 lbs ai/A (foliar) 0.044 lbs ai/A (foliar; radish) 0.31 lbs ai/A (soil; potato) 0.38 lbs ai/A (soil) 0.18 lbs ai/A (soil; sugar beet)	0.2 lbs ai/A (foliar; potato) 0.13 lbs ai/A (foliar) 0.044 lbs ai/A (foliar; radish) 0.31 lbs ai/A (soil; potato) 0.38 lbs ai/A (soil) 0.18 lbs ai/A (soil; sugar beet)	7 days (potato) 5 days	• Not for use on crops grown for seed unless allowed by state-specific 24(c) labeling. • Side-dress no more than 0.3 fl oz/1000 row feet no later than 45 days after planting. • Sugar beet: No not apply immediately prior to bud opening or during bloom or when bees are foraging.

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Imidacloprid				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Root and Tuber Vegetables, *continued*	0.26 lbs ai/A (seed treatment; potato) 0.25 lbs ai/100 lbs seed (seed treatment; carrot)			
Leafy Vegetable (Except *Brassica* Vegetable)	0.047 lbs ai/A (foliar) 0.38 lbs ai/A (soil)	0.24 lbs ai/A (foliar) 0.38 lbs ai/A (soil)	5 days	• Not for use on crops grown for seed unless allowed by state-specific 24(c) labeling.
Legume Vegetables (Succulent or Dried)	0.044 lbs ai/A (foliar) 0.047 lb ai/A (foliar; soybean) 0.38 lbs ai/A (soil) 0.125 lbs ai/100 lbs seed (seed treatment; soybean)	0.13 lbs ai/A (foliar) 0.14 lbs ai/A (foliar; soybean) 0.38 lbs ai/A (soil)	7 days	• Not for use on crops grown for seed unless allowed by state-specific 24(c) labeling. • Foliar and soil application on Soybean not permitted in California unless otherwise directed by state specific 24(c) labeling. • Soybean: Do not graze or feed livestock on soybean forage or hay.
Herbs and Spices	0.044 lbs ai/A (foliar) 0.38 lbs ai/A (soil)	0.13 lbs ai/A (foliar) 0.38 lbs ai/A (soil)	5 days	
Bulb Vegetables	0.5 lbs ai/A (soil)	0.5 lbs ai/A (soil)		• Not for use on crops grown for seed unless by state-specific 24(c) labeling.

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Imidacloprid				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Tobacco	0.05 lbs ai/A (foliar) 0.016 lbs ai/1,000 plants (soil)	0.28 lbs ai/A (foliar) 0.5 lbs ai/A (soil)	7 days	
Coffee	0.1 lbs ai/A (foliar) 0.5 lbs ai/A (soil)	0.5 lbs ai/A (foliar) 0.5 lbs ai/A (soil)	7 days	• Do not apply pre-bloom or during bloom or when bees are foraging.
Hops	0.1 lbs ai/A (foliar) 0.3 lbs ai/A (soil)	0.3 lbs ai/A (foliar) 0.3 lbs ai/A (soil)	21 days	
Globe Artichoke	0.125 lbs ai/A (foliar)	0.5 lbs ai/A (foliar)	14 days	

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Thiamethoxam				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Cucurbit Vegetables	0.086 lbs ai/A (foliar) 0.172lbs ai/A (soil) 0.75 mg ai/seed, Do not exceed 0.164 lbs ai/A (seed treatment)	0.172 lbs ai/A (foliar) 0.172lbs ai/A (soil)	5 days	• Refer to Pollinator Precautions section. • Refer to Resistance Management section.
Citrus Fruit	0.086 lbs ai/A (foliar) 0.172lbs ai/A (soil)	0.172 lbs ai/A (foliar) 0.172lbs ai/A (soil)	7 days	• Thiamethoxam is highly toxic to bees exposed to direct treatment on blooming crops. Do not apply during pre-bloom or during bloom when bees are actively foraging. Do not apply thiamethoxam or allow it to drift to blooming crops or weeds if bees are foraging in for adjacent to the treatment area. This is especially critical if there are adjacent orchards that are blooming. After a thiamethoxam application, wait at least 5 days before placing beehives in the treated field. If bees are foraging in the ground cover and it contains any blooming plants or weeds, always remove flowers before making an application. This may be accomplished by mowing, disking, mulching, flailing, or applying a labeled herbicide.

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Thiamethoxam					
Crop groups listed		**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Oilseed		0.063 lbs ai/A (foliar) 0.375 mg ai/seed (Seed treatment; Cotton) 0.25 mg ai/seed (seed treatment; Sunflower) 0.039 lbs ai/100 lbs seed (seed treatment; safflower)	0.125 lbs ai/A (foliar) 0.075 lbs ai/A (seed treatment; cotton) 0.14 lbs ai/A (seed treatment; sunflower) 0.14 lbs ai/A (seed treatment; safflower)	5 days Do not apply a neonicotinoid insecticide within 45 days of planting seed treated cotton seeds	• To protect the Preble's Meadow Jumping Mouse, sunflower seed treated with Cruiser 5FS Alfalfa may not be planted in Elbert or Weld Counties in Colorado. • Treated sunflower seed must be planted at a minimum depth of one inch.
Stone Fruit		0.086 lbs ai/A (foliar)	0.172 lbs ai/A (foliar)	7 days	• Refer to Pollinator Precautions section. • Refer to Resistance Management section.
Berry and Small Fruit	Small fruit vine climbing subgroup except fuzzy kiwifruit	0.055 lbs ai/A (foliar) 0.266 lbs ai/A (soil)	0.109 lbs ai/A (foliar) 0.266 lbs ai/A (soil)	14 days	• Refer to Pollinator Precautions section. • Refer to Resistance Management section.

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Thiamethoxam					
Crop groups listed		**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Berry and Small Fruit, *continued*	Low growing berry subgroup	0.063 lbs ai/A (foliar) 0.188 lbs ai/A (soil)	0.188 lbs ai/A (foliar) 0.188 lbs ai/A (soil)	10 days	• Do not apply by air • Refer to Pollinator Precautions section. • Refer to Resistance Management section.
	Bushberry	0.063 lbs ai/A (foliar) 0.188 lbs ai/A (soil)	0.188 lbs ai/A (foliar) 0.188 lbs ai/A (soil)	7 days	• Apply after bud-break, but prior to the beginning of bloom (first open blooms) • Refer to Pollinator Precautions section. • Refer to Resistance Management section.
Fruiting Vegetables		0.086 lbs ai/A (foliar) 0.172lbs ai/A (soil)	0.172 lbs ai/A (foliar) 0.172 lbs ai/A (soil)	5 days	• Refer to Pollinator Precautions section. • Refer to Resistance Management section.

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Thiamethoxam				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Legume Vegetables (Succulent or Dried)	0.031 lbs ai/A (foliar) 0.05 lbs ai/100 lbs seed, Do not exceed 0.075 lbs ai/A (seed treatment) 0.05 lbs ai/100 lbs seed, Do not exceed 0.083 lbs ai/A (seed treatment; soybean)	0.125 lbs ai/A (foliar)	7 days Do not apply a neonicotinoid insecticide within 45 days of planting seed treated with Cruiser 5FS.	• Refer to Pollinator Precautionary section • Refer to Resistance Management section
Leafy Vegetables (Except Brassica Vegetables)	0.086 lbs ai/A (foliar) 0.172lbs ai/A (soil)	0.172 lbs ai/A (foliar) 0.172lbs ai/A (soil)	7 days	• Refer to Pollinator Precautions section. • Refer to Resistance Management section.
Bulb Vegetables	0.266 lbs ai/A (seed treatment)			
Brassica (Cole) Leafy Vegetables	0.086 lbs ai/A (foliar) 0.172 lbs ai/A (soil)	0.172 lbs ai/A (foliar) 0.172 lbs ai/A (soil)	7 days	• Refer to Pollinator Precautions section. • Refer to Resistance Management section.

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Thiamethoxam					
Crop groups listed		**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Root and Tuber Vegetables	Tuberous and Corm	0.047 lbs ai/A (foliar) 0.125 lbs ai/A (soil) 0.125 lbs ai/A (seed treatment)	0.094 lbs ai/A (foliar) 0.125 lbs ai/A (soil)	7 days	• Refer to Pollinator Precautions section. • Refer to Resistance Management section. • Do not use this thiamethoxam on potato seed in Nassau or Suffolk County, New York.
	Root Vegetables	0.063 lbs ai/A (foliar) 0.188 lbs ai/A (soil) 0.63 lbs ai/A (foliar; radish) 0.102 lbs ai/A (soil; radish) 70 gram ai/100,000 seeds; Do not exceed 0.206 lbs ai/A (seed treatment; Sugar Beets)	0.125 lbs ai/A (foliar) 0.188 lbs ai/A (soil) 0.063 lbs ai/A (foliar; radish) 0.102 lbs ai/A (soil; radish)	7 days	

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Thiamethoxam				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Pome Fruit	0.086 lbs ai/A (foliar)	0.258 lbs ai/A (foliar)	10 days	• Refer to Pollinator Precautionary Section • Refer to resistance management section
Tropical and Subtropical Fruit, Inedible Peel Group	0.063 lbs ai/A (foliar)	0.188 lbs ai/A (foliar)	7 days	• Refer to Pollinator Precautionary Section • Refer to resistance management section
Globe Artichoke	0.047 lbs ai/A (foliar)	0.094 lbs ai/A (foliar)	7 days	• Refer to Pollinator Precautions section. • Refer to Resistance Management section.
Peanuts	0.29 mg ai/seed; Do not exceed 0.08 lbs ai/A (seed treatment)			• Do not use a thiamethoxam rate that will result in more than 0.08 lbs ai/A (35.0 grams ai/A) per season, based on a maximum seeding rate of 120,700 seeds/acre. • Do not use in hopper box, planter box, slurry box, or other farmer applied applications. Apply thiamethoxam seed treatment in commercial seed treatment facilities only.

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Thiamethoxam				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Cereal Grains	0.0625 lbs ai/A (foliar; barley) 0.052 lbs ai/100 lbs seeds, Do not exceed 0.52 lbs ai/A (seed treatment; barley) 0.80 mg ai/kernel, Do not exceed 0.165 lbs ai/A (seed treatment; corn) 0.03 mg ai/seed, Do not exceed 0.17 lb ai/A (seed treatment; rice) 0.093 mg ai/seed, Do not exceed 0.03 lbs ai/A (seed treatment; sorghum) 0.052 lbs ai/100 lbs seeds, Do not exceed	0.125 lbs ai/A (foliar; barely)	7 days	• Refer to Pollinator Precautionary Section • Refer to resistance management section • For field, pop, seed and sweet corn, do not use a cruiser rate that will result in more than 0.21 lb ai/A based on a maximum seeding rate for sweet corn of 75,000 seeds/acre. • Do not apply more than 215 gallons per 8 hour day for seed treatments utilizing a closed system. • Do not apply more than 38 gallons of thiamethoxam per 8 hour day for seed treatments utilizing an open system. If it is necessary to apply more than 28 gallons of cruiser per 8 hour day, a closed system must be used • A closed system must be used for commercial treatment of sorghum seed

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Thiamethoxam				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Cereal Grains, *continued*	0.08 lbs ai/A (seed treatment; wheat) 0.052 lbs ai/100 lbs seeds, Do not exceed 0.04 lbs ai/A (seed treatment; buckwheat, pearl millet, proso millet, oats, rye, tesinte, triticale, and wild rice)			• Not for use in water seeded rice production. Do not plant or sow thiamethoxam treated rice seed by aerial application equipment. Do not use treated fields for the aquaculture of edible fish and crustacean.
Hops	0.125 lbs ai/A (soil)	0.125 lbs ai/A (soil)		
Tobacco	0.047 lbs ai/A (foliar) 0.43 oz/1,000 plants (soil)	0.047 lbs ai/A (foliar) 0.125 lbs ai/A (soil)		• Refer to Pollinator Precautionary Section • Refer to resistance management section
Mint	0.063 lbs ai/A (foliar)	0.188 lbs ai/A (foliar)	14 days	• Refer to Pollinator Precautionary Section • Refer to resistance management section

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Clothianidin				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Tree Nuts	0.1 lbs ai/A (foliar)	0.2 lbs ai/A (foliar)	Do not apply treatments less than 10 days apart	• Insecticide must not be applied during bloom or when bees are foraging. • Do not feed or allow livestock to graze on cover crops from treated orchards. • Regardless of the application method, do not apply more than 0.2 lb active ingredient clothianidin per acre per year.
Root and Tuber Vegetables	0.05 lbs ai/A (foliar) 0.2 lbs ai/A (soil)	0.2 lbs ai/A (foliar) 0.2 lbs ai/A (soil)	Do not apply treatments less than 7 days apart	• Do not apply treatment between 50% row closure and petal fall. • Do not make more than one application per year prior to 50% row closure. • Regardless of the application method, do not apply more than 0.2 lb active ingredient clothianidin per acre per year. • Do not apply by air except for potato.
Cereal Grains	0.075 lbs ai/A (foliar; rice)			• Regardless of application method (seed treatment, soil, or foliar), do not apply more than 0.2 lb active ingredient clothianidin per acre per year.

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Clothianidin				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Cereal Grains, *continued*	0.023 mg ai/seed (seed treatment; except corn) 1.25 mg ai/seed (seed treatment; corn)			• For use only in commercial seed treatment facilities. Not for use in hopper box, planter box, slurry box, or other on-farm seed treatment applications except for cereal grains and potato seed pieces • Regardless of application method (seed treatment, soil, or foliar), do not apply more than 0.2 lb active ingredient clothianidin per acre per year. • Rice: Do not apply Insecticide after third tillering has initiated. • Rice: Do not apply Insecticide following a clothianidin seed treatment application. • Rice: Do not use Insecticide treated rice fields for the aquaculture of edible fish and crustaceans. • Rice: Insecticide is not to be used on rice crops that contain or support crawfish or any form of aquaculture operation. • Rice: Insecticide is not to be used on rice crops near fish farm, shrimp, prawn or crab pond (or nursery) operations -

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Clothianidin				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Cereal Grains, *continued*				particularly when weather conditions are conducive to drift. Exercise caution with air and ground applications near those operations to avoid product drift.
Legume Vegetables (Succulent or Dried)	0.1 lbs ai/A (foliar) 0.13 mg ai/seed (seed treatment)	0.2 lbs ai/A (foliar)	Do not apply foliar treatments less than 7 days apart	• Do not make foliar applications of clothianidin in fields treated with a neonicotinoid insecticide seed treatment(s) within 45 days after planting. • Regardless of formulation or type of application method, do not apply more than 0.2 lb ai of clothianidin per acre per year. • Do not graze or feed soybean forage and hay to livestock.
Oilseed	0.083 lbs ai/A (foliar) 0.018 mg ai/seed (seed treatment; canola, rapeseed)	0.02 lbs ai/A (foliar)	One year	• Do not make application after pinhead square formation.

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Clothianidin				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Cucurbit Vegetables	0.067 lbs ai/A (foliar) 0.2 lbs ai/A (soil)	0.2 lbs ai/A (foliar) 0.2 lbs ai/A (soil)	Do not apply treatments less than 10 days apart	• Insecticide must not be applied during bloom or when bees are foraging. • Do not make application after 4th true leaf on main stem is unfolded
Brassica (Cole) Leafy Vegetables	0.067 lbs ai/A (foliar) 0.2 lbs ai/A (soil)	0.2 lbs ai/A (foliar) 0.2 lbs ai/A (soil)	Do not apply treatments less than 10 days apart	• Insecticide must not be applied during bloom or when bees are foraging. • Do not use on crops grown for seed production
Leafy Vegetables (Except Brassica Vegetables)	0.067 lbs ai/A (foliar) 0.2 lbs ai/A (soil)	0.2 lbs ai/A (foliar) 0.2 lbs ai/A (soil)	10 days	• Do not use on crops grown for seed production. • Insecticide must not be applied during bloom or when bees are foraging.
Tropical and Subtropical Fruit, Inedible Peel	0.1 lb ai/A (foliar; pomegranate)	0.2 lbs ai/A (foliar; pomegranate)	Do not apply treatments less than 14 days apart	• Do not feed or allow livestock to graze on cover crops from treated orchards. • Insecticide must not be applied during bloom or when bees are foraging. • Post bloom applications only

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Clothianidin					
Crop groups listed		**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Berry and Small Fruit	Small fruit vine climbing subgroup except fuzzy kiwifruit	0.1 lbs ai/A (foliar) 0.2 lbs ai/A (soil)	0.2 lbs ai/A (foliar) 0.2 lbs ai/A (soil)	One year for foliar For soil: do not apply treatments less than 14 days apart	
Tropical and Subtropical Fruit, Edible Peel Group		0.1 lbs ai/A (foliar; fig)	0.2 lbs ai/A (foliar; fig)	Do not apply treatments less than 14 days apart.	• Do not feed or allow livestock to graze on cover crops from treated orchards.
Stone Fruit		0.1 lbs ai/A (foliar; peach)	0.2 lbs ai/A (foliar; peach)	Do not apply treatments less than 10 days apart.	• Do not feed or allow livestock to graze on cover crops from treated orchards. • Insecticide must not be applied during bloom or when bees are foraging.
Tobacco		0.067 lbs ai/A (foliar)	0.2 lbs ai/A (foliar)	Do not apply treatments less than 7 days apart.	

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Clothianidin				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Pome Fruit	0.1 lbs ai/A (foliar)	0.2 lbs ai/A (foliar)		• Do not feed or allow livestock to graze on cover crops from treated orchards. • Insecticide must not be applied during bloom or when bees are foraging.

Dinotefuran				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Oilseed	0.134 lbs ai/A (foliar)	0.268 lbs ai/A (foliar)	7 days	• Follow application instructions as indicated in the Bee Hazard Direction for Use.
Cucurbit Vegetable	0.179 lbs ai/A (foliar) 0.33 lbs ai/A (soil)	0.268 lbs ai/A (foliar) 0.536 lbs ai/A (soil)	7 days	• Follow application instructions as indicated in Bee Hazard Direction for Use. • Do not combine foliar applications with soil applications, or vice versa. Only use one application method.

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

Dinotefuran				
Crop groups listed	**Maximum single application rate (soil or foliar)**	**Maximum annual or seasonal application rate (soil or foliar)**	**Minimum reapplication interval**	**Restrictions**
Fruiting Vegetable	0.179 lbs ai/A (foliar) 0.33 lbs ai/A (soil)	0.268 lbs ai/A (foliar) 0.536 lbs ai/A (soil)	7 days	• Follow application instructions as indicated in Bee Hazard Direction for Use. • Do not combine foliar applications with soil applications, or vice versa. Only use one application method. • Do not apply to vegetables grown for seed.
Root and Tuber Vegetables	0.068 lbs ai/A (foliar) 0.338 lbs ai/A (soil)	0.203 lbs ai/A (foliar) 0.338 lbs ai/A (soil)	14 days	• Follow application instructions as indicated in Bee Hazard Direction for Use. • Do not combine foliar applications with soil applications, or vice versa. Only use one application method.
Brassica Head & Stem Vegetables	0.179 lbs ai/A (foliar) 0.33 lbs ai/A (soil)	0.268 lbs ai/A (foliar) 0.536 lbs ai/A (soil)	7 days	• Do not combine foliar applications with soil applications, or vice versa. Only use one application method. • Do not apply to vegetables grown for seed.

Appendix 3. California Registered Agricultural Uses of Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran

<table>
<tr><th colspan="6">Dinotefuran</th></tr>
<tr><th colspan="2">Crop groups listed</th><th>Maximum single application rate (soil or foliar)</th><th>Maximum annual or seasonal application rate (soil or foliar)</th><th>Minimum reapplication interval</th><th>Restrictions</th></tr>
<tr><td colspan="2">Leafy Vegetables (Except Brassica Vegetables)</td><td>0.134 lbs ai/A (foliar)
0.180 lbs ai/A (foliar; watercress)
0.33 lbs ai/A (soil)</td><td>0.268 lbs ai/A (foliar)
0.360 lbs ai/A (foliar; watercress)
0.536 lbs ai/A (soil)</td><td>7 days</td><td>• Do not combine foliar applications with soil applications, or vice versa. Only use one application method.
• Do not apply to vegetables grown for seed.</td></tr>
<tr><td colspan="2">Bulb Vegetables</td><td>0.180 lbs ai/A (foliar)
0.270 lbs ai/A (soil)</td><td>0.270 lbs ai/A (foliar)
0.270 lbs ai/A (soil)</td><td>7 days</td><td>• Regardless of application method, do not exceed 0.383 lbs ai/A per crop season.</td></tr>
<tr><td rowspan="2">Berry and Small Fruit</td><td>Small fruit vine climbing subgroup except fuzzy kiwifruit</td><td>0.135 lbs ai/A (foliar)
0.338 lb ai/A (soil)</td><td>0.270 lbs ai/A (foliar)
0.338 lb ai/A (soil)</td><td>14 days</td><td>• Follow application instructions as indicated in Bee Hazard Direction for Use.
• Regardless of application method, do not apply more than a total of 0.540 lbs ai/A per season of Dinoteufran 20 SG.</td></tr>
<tr><td>Low Growing Berry Subgroup, except strawberry</td><td>0.180 lbs ai/A (foliar)</td><td>0.360 lbs ai/A (foliar)</td><td>14 days</td><td></td></tr>
</table>

Appendix 4. 40 CFR 180.41 Crop Group Tables

the pre-existing crop group; however, the revised crop group number will be followed by a hyphen and the final two digits of the year in which it was established (e.g., if Crop Group 1 is amended in 2007, the revised group will be designated as Crop Group 1-07). If the pre-existing crop group had crop subgroups, these subgroups will be numbered in a similar fashion in the revised crop group. The name of the revised crop group will not be changed from the pre-existing crop group unless the revision so changes the composition of the crop group that the pre-existing name is no longer accurate. Once a revised crop group is established, EPA will no longer establish tolerances under the pre-existing crop group. At appropriate times, EPA will amend tolerances for crop groups that have been superseded by revised crop groups to conform the pre-existing crop group to the revised crop group. Once all of the tolerances for the pre-existing crop group have been updated, the pre-existing crop group will be removed from the CFR.

(k) Establishment of a tolerance does not substitute for the additional need to register the pesticide under a companion law, the Federal Insecticide, Fungicide, and Rodenticide Act. The Registration Division of the Office of Pesticide Programs should be contacted concerning procedures for registration of new uses of a pesticide.

[60 FR 26635, May 17, 1995, as amended at 70 FR 33363, June 8, 2005; 72 FR 69155, Dec. 7, 2007; 75 FR 56014, Sept. 15, 2010; 81 FR 26476, May 3, 2016]

§ 180.41 Crop group tables.

(a) The tables in this section are to be used in conjunction with § 180.40 to establish crop group tolerances.

(b) Commodities not listed are not considered as included in the groups for the purposes of paragraph (b), and individual tolerances must be established. Miscellaneous commodities intentionally not included in any group include globe artichoke, hops, peanut, and water chestnut.

(c) Each group is identified by a group name and consists of a list of representative commodities followed by a list of all commodity members for the group. If the group includes subgroups, each subgroup lists the subgroup name, the representative commodity or commodities, and the member commodities for the subgroup. Subgroups, which are a subset of their associated crop group, are established for some but not all crops groups.

(1) *Crop Group 1:* Root and Tuber Vegetables Group.

(i) *Representative commodities.* Carrot, potato, radish, and sugar beet.

(ii) *Table.* The following table 1 lists all the commodities included in Crop Group 1 and identifies the related crop subgroups.

TABLE 1—CROP GROUP 1: ROOT AND TUBER VEGETABLES

Commodities	Related crop subgroups
Arracacha (*Arracacia xanthorrhiza*)	1C, 1D
Arrowroot (*Maranta arundinacea*)	1C, 1D
Artichoke, Chinese (*Stachys affinis*)	1C, 1D
Artichoke, Jerusalem (*Helianthus tuberosus*)	1C, 1D
Beet, garden (*Beta vulgaris*)	1A, 1B
Beet, sugar (*Beta vulgaris*)	1A
Burdock, edible (*Arctium lappa*)	1A, 1B
Canna, edible (Queensland arrowroot) (*Canna indica*)	1C, 1D
Carrot (*Daucus carota*)	1A, 1B
Cassava, bitter and sweet (*Manihot esculenta*)	1C, 1D
Celeriac (celery root) (*Apium graveolens* var. *rapaceum*)	1A, 1B
Chayote (root) (*Sechium edule*)	1C, 1D
Chervil, turnip-rooted (*Chaerophyllum bulbosum*)	1A, 1B
Chicory (*Cichorium intybus*)	1A, 1B
Chufa (*Cyperus esculentus*)	1C, 1D
Dasheen (taro) (*Colocasia esculenta*)	1C, 1D
Ginger (*Zingiber officinale*)	1C, 1D
Ginseng (*Panax quinquefolius*)	1A, 1B
Horseradish (*Armoracia rusticana*)	1A, 1B
Leren (*Calathea allouia*)	1C, 1D

Appendix 4. 40 CFR 180.41 Crop Group Tables

TABLE 1—CROP GROUP 1: ROOT AND TUBER VEGETABLES—Continued

Commodities	Related crop subgroups
Parsley, turnip-rooted (*Petroselinum crispum* var. *tuberosum*)	1A, 1B
Parsnip (*Pastinaca sativa*)	1A, 1B
Potato (*Solanum tuberosum*)	1C
Radish (*Raphanus sativus*)	1A, 1B
Radish, oriental (daikon) (*Raphanus sativus* subvar. *longipinnatus*)	1A, 1B
Rutabaga (*Brassica campestris* var. *napobrassica*)	1A, 1B
Salsify (oyster plant) (*Tragopogon porrifolius*).	1A, 1B
Salsify, black (*Scorzonera hispanica*)	1A, 1B
Salsify, Spanish (*Scolymus hispanicus*)	1A, 1B
Skirret (*Sium sisarum*)	1A, 1B
Sweet potato (*Ipomoea batatas*)	1C, 1D
Tanier (cocoyam) (*Xanthosoma sagittifolium*)	1C, 1D
Turmeric (*Curcuma longa*)	1C, 1D
Turnip (*Brassica rapa* var. *rapa*)	1A, 1B
Yam bean (jicama, manoic pea) (*Pachyrhizus* spp.)	1C, 1D
Yam, true (*Dioscorea* spp.)	1C, 1D

(iii) *Table*. The following table 2 identifies the crop subgroups for Crop Group 1, specifies the representative commodity(ies) for each subgroup, and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 1 SUBGROUP LISTING

Representative commodities	Commodities
Crop Subgroup 1A. Root vegetables subgroup.	
Carrot, radish, and sugar beet.	Beet, garden; beet, sugar; burdock, edible; carrot; celeriac; chervil, turnip-rooted; chicory; ginseng; horseradish; parsley, turnip-rooted; parsnip; radish; radish, oriental; rutabaga; salsify; salsify, black; salsify, Spanish; skirret; turnip.
Crop Subgroup 1B. Root vegetables (except sugar beet) subgroup.	
Carrot and radish.	Beet, garden; burdock, edible; carrot; celeriac; chervil, turnip-rooted; chicory; ginseng; horseradish; parsley, turnip-rooted; parsnip; radish; radish, oriental; rutabaga; salsify; salsify, black; salsify, Spanish; skirret; turnip.
Crop Subgroup 1C. Tuberous and corm vegetables subgroup.	
Potato.	Arracacha; arrowroot; artichoke, Chinese; artichoke, Jerusalem; canna, edible; cassava, bitter and sweet; chayote (root); chufa; dasheen; ginger; leren; potato; sweet potato; tanier; turmeric; yam bean; yam, true.
Crop Subgroup 1D. Tuberous and corm vegetables (except potato) subgroup.	
Sweet potato.	Arracacha; arrowroot; artichoke, Chinese; artichoke, Jerusalem; canna, edible; cassava, bitter and sweet; chayote (root); chufa; dasheen; ginger; leren; sweet potato; tanier; turmeric; yam bean; yam, true.

(2) *Crop Group 2.* Leaves of Root and Tuber Vegetables (Human Food or Animal Feed) Group (Human Food or Animal Feed) Group.

(i) *Representative commodities.* Turnip and garden beet or sugar beet.

(ii) *Commodities.* The following is a list of all the commodities included in Crop Group 2:

CROP GROUP 2: LEAVES OF ROOT AND TUBER VEGETABLES (HUMAN FOOD OR ANIMAL FEED) GROUP—COMMODITIES

Beet, garden (*Beta vulgaris*)
Beet, sugar (*Beta vulgaris*)
Burdock, edible (*Arctium lappa*)
Carrot (*Daucus carota*)
Cassava, bitter and sweet (*Manihot esculenta*)
Celeriac (celery root) (*Apium graveolens* var. *rapaceum*)
Chervil, turnip-rooted (*Chaerophyllum bulbosum*)
Chicory (*Cichorium intybus*)
Dasheen (taro) (*Colocasia esculenta*)
Parsnip (*Pastinaca sativa*)
Radish (*Raphanus sativus*)
Radish, oriental (daikon) (*Raphanus sativus* subvar. *longipinnatus*)
Rutabaga (*Brassica campestris* var. *napobrassica*)
Salsify, black (*Scorzonera hispanica*)
Sweet potato (*Ipomoea batatas*)
Tanier (cocoyam) (*Xanthosoma sagittifolium*)

Appendix 4. 40 CFR 180.41 Crop Group Tables

Turnip (*Brassica rapa* var. *rapa*)
Yam, true (*Dioscorea* spp.)

(3) *Crop Group 3*. Bulb Vegetables (*Allium* spp.) Group.

(i) *Representative commodities*. Onion, green; and onion, dry bulb.

(ii) *Commodities*. The following is a list of all the commodities in Crop Group 3.

CROP GROUP 3: BULB VEGETABLE (*Allium* SPP.) GROUP—COMMODITIES

Garlic, bulb (*Allium sativum*)
Garlic, great headed, (elephant) (*Allium ampeloprasum* var. *ampeloprasum*)

CROP GROUP 3: BULB VEGETABLE (*Allium* SPP.) GROUP—COMMODITIES—Continued

Leek (*Allium ampeloprasum, A. porrum, A. tricoccum*)
Onion, dry bulb and green (*Allium cepa, A. fistulosum*)
Onion, Welsh, (*Allium fistulosum*)
Shallot (*Allium cepa* var. *cepa*)

(4) *Crop Group 3-07*. Bulb Vegetable Group.

(i) *Representative Commodities*. Onion, bulb and onion, green.

(ii) *Table*. The following Table 1 lists all the commodities listed in Crop Group 3-07 and identifies the related crop subgroups.

TABLE 1—CROP GROUP 3-07: BULB VEGETABLE GROUP

Commodities	Related crop subgroups
Chive, fresh leaves (*Allium schoenoprasum L.*)	3-07B
Chive, Chinese, fresh leaves (*Allium tuberosum* Rottler ex Spreng)	3-07B
Daylily, bulb (*Hemerocallis fulva* (L.) L. var. *fulva*)	3-07A
Elegans hosta (*Hosta Sieboldiana (Hook.) Engl*)	3-07B
Fritillaria, bulb (*Fritillaria L. fritillary*)	3-07A
Fritillaria, leaves (*Fritillaria L. fritillary*)	3-07B
Garlic, bulb (*Allium sativum* L. var. *sativum*) (*A. sativum* Common Garlic Group)	3-07A
Garlic, great headed, bulb (*Allium ampeloprasum* L. var. *ampeloprasum*) (*A. ampeloprasum* Great Headed Garlic Group)	3-07A
Garlic, Serpent, bulb (*Allium sativum* var. *ophioscorodon* or *A. sativum* Ophioscorodon Group)	3-07A
Kurrat (*Allium kurrat Schweinf. Ex. K. Krause* or *A. ampeloprasum* Kurrat Group)	3-07B
Lady's leek (*Allium cernuum* Roth)	3-07B
Leek *Allium porrum* L. (syn: *A. ampeloprasum L.* var. *porrum* (L.) J. Gay) (*A.ampeloprasum* Leek Group)	3-07B
Leek, wild (*Allium tricoccum* Aiton)	3-07B
Lily, bulb (*Lilium* spp. (*Lilium Leichtlinii* var. *maximowiczii, Lilium lancifolium*))	3-07A
Onion, Beltsville bunching (*Allium* x *proliferum* (Moench) Schrad.) (syn: *Allium fistulosum* L. x *A. cepa* L.)	3-07B
Onion, bulb (*Allium cepa* L. var. *cepa*) (*A. cepa* Common Onion Group)	3-07A
Onion, Chinese, bulb (*Allium chinense* G. Don.) (syn: *A. bakeri* Regel)	3-07A
Onion, fresh (*Allium fistulosum* L. var. *caespitosum* Makino)	3-07B
Onion, green (*Allium cepa* L. var. *cepa*) (*A. cepa* Common Onion Group)	3-07B
Onion, macrostem (*Allium macrostemom* Bunge)	3-07B
Onion, pearl (*Allium porrum* var. *sectivum* or *A. ampeloprasum* Pearl Onion Group)	3-07A
Onion, potato, bulb (*Allium cepa* L. var. *aggregatum* G. Don.) (*A. cepa* Aggregatum Group)	3-07A
Onion, tree, tops (*Allium x proliferum* (Moench) Schrad. ex Willd.) (syn: *A. cepa* var. *proliferum* (Moench) Regel; *A. cepa* L. var. *bulbiferum* L.H. Bailey; *A. cepa* L. var. *viviparum* (Metz.) Alef.)	3-07B
Onion, Welsh, tops (*Allium fistulosum* L.)	3-07B
Shallot, bulb (*Allium cepa* var. *aggregatum* G. Don.)	3-07A
Shallot, fresh leaves (*Allium cepa* var. *aggregatum* G. Don.)	3-07B
Cultivars, varieties, and/or hybrids of these.	

(iii) *Table*. The following Table 2 identifies the crop subgroups for Crop Group 3-07, specifies the representative commodities for each subgroup and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 3-07: SUBGROUP LISTING

Representative commodities	Commodities
Crop subgroup 3-07A. Onion, bulb, subgroup. Onion, bulb.	Daylily, bulb; fritillaria, bulb; garlic, bulb; garlic, great-headed, bulb; garlic, serpent, bulb; lily, bulb; onion, bulb; onion, Chinese, bulb; onion, pearl; onion, potato, bulb; shallot, bulb; cultivars, varieties, and/or hybrids of these.
Crop subgroup 3-07B. Onion, green, subgroup. Onion, green.	Chive, fresh leaves; chive, Chinese, fresh leaves; elegans hosta; fritillaria, leaves; kurrat; lady's leek; leek; leek, wild; Onion, Beltsville bunching; onion, fresh; onion, green; onion, macrostem; onion, tree, tops; onion, Welsh, tops; shallot, fresh leaves; cultivars, varieties, and/or hybrids of these.

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(5) *Crop Group 4.* Leafy Vegetables (Except *Brassica* Vegetables) Group.

(i) *Representative commodities.* Celery, head lettuce, leaf lettuce, and spinach (*Spinacia oleracea*).

(ii) *Table.* The following table 1 lists all the commodities included in Crop Group 4 and identifies the related crop subgroups.

TABLE 1—CROP GROUP 4: LEAFY VEGETABLES (EXCEPT BRASSICA VEGETABLES) GROUP

Commodities	Related crop subgroups
Amaranth (leafy amaranth, Chinese spinach, tampala) (*Amaranthus* spp.)	4A
Arugula (Roquette) (*Eruca sativa*)	4A
Cardoon (*Cynara cardunculus*)	4B
Celery (*Apium graveolens* var. *dulce*)	4B
Celery, Chinese (*Apium graveolens* var. *secalinum*)	4B
Celtuce (*Lactuca sativa* var. *angustana*)	4B
Chervil (*Anthriscus cerefolium*)	4A
Chrysanthemum, edible-leaved (*Chrysanthemum coronarium* var. *coronarium*)	4A
Chrysanthemum, garland (*Chrysanthemum coronarium* var. *spatiosum*)	4A
Corn salad (*Valerianella locusta*)	4A
Cress, garden (*Lepidium sativum*)	4A
Cress, upland (yellow rocket, winter cress) (*Barbarea vulgaris*)	4A
Dandelion (*Taraxacum officinale*)	4A
Dock (sorrel) (*Rumex* spp.)	4A
Endive (escarole) (*Cichorium endivia*)	4A
Fennel, Florence (finochio) (*Foeniculum vulgare* Azoricum Group)	4B
Lettuce, head and leaf (*Lactuca sativa*)	4A
Orach (*Atriplex hortensis*)	4A
Parsley (*Petroselinum crispum*)	4A
Purslane, garden (*Portulaca oleracea*)	4A
Purslane, winter (*Montia perfoliata*)	4A
Radicchio (red chicory) (*Cichorium intybus*)	4A
Rhubarb (*Rheum rhabarbarum*)	4B
Spinach (*Spinacia oleracea*)	4A
Spinach, New Zealand (*Tetragonia tetragonioides, T. expansa*)	4A
Spinach, vine (Malabar spinach, Indian spinach) (*Basella alba*)	4A
Swiss chard (*Beta vulgaris* var. *cicla*)	4B

(iii) *Table.* The following table 2 identifies the crop subgroups for Crop Group 4, specifies the representative commodities for each subgroup, and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 4 SUBGROUP LISTING

Representative commodities	Commodities
Crop Subgroup 4A. Leafy greens subgroup.	
Head lettuce and leaf lettuce, and spinach (*Spinacia oleracea*).	Amaranth; arugula; chervil; chrysanthemum, edible-leaved; chrysanthemum, garland; corn salad; cress, garden; cress, upland; dandelion; dock; endive; lettuce; orach; parsley; purslane, garden; purslane, winter; radicchio (red chicory); spinach; spinach, New Zealand; spinach, vine.
Crop Subgroup 4B. Leaf petioles subgroup.	
Celery.	Cardoon; celery; celery, Chinese; celtuce; fennel, Florence; rhubarb; Swiss chard.

(6) *Crop Group 4–16.* Leafy Vegetable Group.

(i) *Representative commodities.* Head lettuce, leaf lettuce, mustard greens, and spinach.

(ii) *Commodities.* The following Table 1 lists all commodities included in Crop Group 4–16.

TABLE 1—CROP GROUP 4–16: LEAFY VEGETABLE GROUP

Commodities	Related crop subgroups
Amaranth, Chinese (*Amaranthus tricolor* L.)	4–16A

Appendix 4. 40 CFR 180.41 Crop Group Tables

TABLE 1—CROP GROUP 4–16: LEAFY VEGETABLE GROUP—Continued

Commodities	Related crop sub-groups
Amaranth, leafy (*Amaranthus* spp.)	4–16A
Arugula (*Eruca sativa* Mill.)	4–16B
Aster, Indian (*Kalimeris indica* (L.) Sch. Bip.)	4–16A
Blackjack (*Bidens pilosa* L.)	4–16A
Broccoli, Chinese (*Brassica oleracea* var. *alboglabra* (L.H. Bailey) Musil)	4–16B
Broccoli raab (*Brassica ruvo* L.H. Bailey)	4–16B
Cabbage, abyssinian (*Brassica carinata* A. Braun)	4–16B
Cabbage, Chinese, bok choy (*Brassica rapa* subsp. *chinensis* (L.) Hanelt)	4–16B
Cabbage, seakale (*Brassica oleracea* L. var. *costata* DC.)	4–16B
Cat's whiskers (*Cleome gynandra* L.)	4–16A
Cham-chwi (*Doellingeria scabra* (Thunb.) Nees)	4–16A
Cham-na-mul (*Pimpinella calycina* Maxim)	4–16A
Chervil, fresh leaves (*Anthriscus cerefolium* (L.) Hoffm.)	4–16A
Chipilin (*Crotalaria longirostrata* Hook & Arn)	4–16A
Chrysanthemum, garland (*Glebionis coronaria* (L.) Cass. ex Spach. *Glebionis* spp.)	4–16A
Cilantro, fresh leaves (*Coriandrum sativum* L.)	4–16A
Collards (*Brassica oleracea L.* var. *viridis* L.)	4–16B
Corn salad (*Valerianella* spp.)	4–16A
Cosmos (*Cosmos caudatus* Kunth)	4–16A
Cress, garden (*Lepidium sativum* L.)	4–16B
Cress, upland (*Barbarea vulgaris* W.T. Aiton)	4–16B
Dandelion, leaves (*Taraxacum officinale* F.H. Wigg. Aggr.)	4–16A
Dang-gwi, leaves (*Angelica gigas Nakai)*	4–16A
Dillweed (*Anethum graveolens* L.)	4–16A
Dock (*Rumex patientia* L.)	4–16A
Dol-nam-mul (*Sedum sarmentosum* Bunge)	4–16A
Ebolo (*Crassocephalum crepidioides* (Benth.) S. Moore)	4–16A
Endive (*Cichorium endivia* L.)	4–16A
Escarole (*Cichorium endivia* L.)	4–16A
Fameflower (*Talinum fruticosum* (L.) Juss.)	4–16A
Feather cockscomb (*Glinus oppositifolius* (L.) Aug. DC.)	4–16A
Good King Henry (*Chenopodium bonus-henricus* L.)	4–16A
Hanover salad (*Brassica napus* var. *pabularia* (DC.) Rchb.)	4–16B
Huauzontle (*Chenopodium berlandieri* Moq.)	4–16A
Jute, leaves (*Corchorus spp.*)	4–16A
Kale (*Brassica oleracea* L. var. *Sabellica* L.)	4–16B
Lettuce, bitter (*Launaea cornuta* (Hochst. ex Oliv. & Hiern) C. Jeffrey)	4–16A
Lettuce, head (*Lactuca sativa* L.; including *Lactuca sativa* var. *capitata* L.)	4–16A
Lettuce, leaf (*Lactuca sativa* L.; including *Lactuca sativa* var. *longifolia* Lam.; *Lactuca sativa* var. *crispa* L.)	4–16A
Maca, leaves (*Lepidium meyenii* Walp.)	4–16B
Mizuna (*Brassica rapa* L. subsp. *nipposinica* (L.H. Bailey) Hanelt)	4–16B
Mustard greens (*Brassica juncea* subsp., including *Brassica juncea* (L.) Czern. subsp. *integrifolia* (H. West) Thell., *Brassica juncea* (L.) Czern. var. *tsatsai* (T.L. Mao) Gladis)	4–16B
Orach (*Atriplex hortensis* L.)	4–16A
Parsley, fresh leaves (*Petroselinum crispum* (Mill.) Fuss; *Petroselinum crispum* var. *neapolitanum* Danert)	4–16A
Plantain, buckthorn (*Plantago lanceolata* L.)	4–16A
Primrose, English (*Primula vulgaris* Huds.)	4–16A
Purslane, garden (*Portulaca oleracea* L.)	4–16A
Purslane, winter (*Claytonia perfoliata* Donn ex Willd.)	4–16A
Radicchio (*Cichorium intybus* L.)	4–16A
Radish, leaves (*Raphanus sativus* L. var *sativus,* including *Raphanus sativus* L. var. *mougri* H. W. J. Helm (*Raphanus sativus* L. var. *oleiformis* Pers)	4–16B
Rape greens (*Brassica napus* L. var. *napus,* including *Brassica rapa* subsp. *trilocularis* (Roxb.) Hanelt; *Brassica rapa* subsp. *dichotoma* (Roxb.) Hanelt; *Brassica rapa* subsp. *oleifera* Met)	4–16B
Rocket, wild (*Diplotaxis tenuifolia* (L.) DC.)	4–16B
Shepherd's purse (*Capsella bursa-pastoris* (L.) Medik)	4–16B
Spinach (*Spinacia oleracea* L.)	4–16A
Spinach, Malabar (*Basella alba* L.)	4–16A
Spinach, New Zealand (*Tetragonia tetragonioides* (Pall.) Kuntze)	4–16A
Spinach, tanier (*Xanthosoma brasiliense* (Desf.) Engl.)	4–16A
Swiss chard (*Beta vulgaris* L. ssp. *vulgaris*)	4–16A
Turnip greens (*Brassica rapa* L. ssp. *rapa*)	4–16B
Violet, Chinese, leaves (*Asystasia gangetica* (L.) T. Anderson)	4–16A
Watercress (*Nasturtium officinale* W.T. Aiton)	4–16B
Cultivars, varieties, and hybrids of these commodities.	

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(iii) *Crop subgroups.* The following Table 2 identifies the crop subgroups for Crop Group 4–16, specifies the representative commodities for each subgroup, and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 4–16: SUBGROUP LISTING

Representative commodities	Commodities
Crop Subgroup 4–16A. Leafy greens subgroup	
Head lettuce, leaf lettuce, and spinach	Amaranth, Chinese; amaranth, leafy; aster, Indian; blackjack; cat's whiskers; cham-chwi; cham-na-mul; chervil, fresh leaves; chipilin; chrysanthemum, garland; cilantro, fresh leaves; corn salad; cosmos; dandelion, leaves; dang-gwi, leaves; dillweed; dock; dol-nam-mul; ebolo; endive; escarole; fameflower; feather cockscomb; Good King Henry; huauzontle; jute, leaves; lettuce, bitter; lettuce, head; lettuce, leaf; orach; parsley, fresh leaves; plantain, buckhorn; primrose, English; purslane, garden; purslane, winter; radicchio; spinach; spinach, Malabar; spinach, New Zealand; spinach, tanier; Swiss chard; violet, Chinese, leaves; cultivars, varieties, and hybrids of these commodities.
Crop Subgroup 4–16B. *Brassica* leafy greens subgroup	
Mustard greens	Arugula; broccoli, Chinese; broccoli raab; cabbage, abyssinian; cabbage, Chinese, bok choy; cabbage, seakale; collards; cress, garden; cress, upland; hanover salad; kale; maca, leaves; mizuna; mustard greens; radish, leaves; rape greens; rocket, wild; shepherd's purse; turnip greens; watercress; cultivars, varieties, and hybrids of these commodities.

(7) *Crop Group 5. Brassica* (Cole) Leafy Vegetables Group.

(i) *Representative commodities.* Broccoli or cauliflower; cabbage; and mustard greens.

(ii) *Table.* The following table 1 lists all the commodities included in Crop Group 5 and identifies the related crop subgroups.

TABLE 1—CROP GROUP 5: *Brassica* (COLE) LEAFY VEGETABLES

Commodities	Related crop subgroups
Broccoli (*Brassica oleracea* var. *botrytis*)	5A
Broccoli, Chinese (gai lon) (*Brassica alboglabra*)	5A
Broccoli raab (rapini) (*Brassica campestris*)	5B
Brussels sprouts (*Brassica oleracea* var. *gemmifera*)	5A
Cabbage (*Brassica oleracea*)	5A
Cabbage, Chinese (bok choy) (*Brassica chinensis*)	5B
Cabbage, Chinese (napa) (*Brassica pekinensis*)	5A
Cabbage, Chinese mustard (gai choy) (*Brassica campestris*)	5A
Cauliflower (*Brassica oleracea* var. *botrytis*)	5A
Cavalo broccolo (*Brassica oleracea* var. *botrytis*)	5A
Collards (*Brassica oleracea* var. *acephala*)	5B
Kale (*Brassica oleracea* var. *acephala*)	5B
Kohlrabi (*Brassica oleracea* var. *gongylodes*)	5A
Mizuna (*Brassica rapa* Japonica Group)	5B
Mustard greens (*Brassica juncea*)	5B
Mustard spinach (*Brassica rapa* Perviridis Group)	5B
Rape greens (*Brassica napus*)	5B

(iii) *Table.* The following table 2 identifies the crop subgroups for Crop Group 5, specifies the representative commodity(ies) for each subgroup, and lists all the commodities included in each subgroup.

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TABLE 2—CROP GROUP 5 SUBGROUP LISTING

Representative commodities	Commodities
Crop Subgroup 5A. Head and stem *Brassica* subgroup	
Broccoli or cauliflower; and cabbage	Broccoli; broccoli, Chinese; brussels sprouts; cabbage; cabbage, Chinese (napa); cabbage, Chinese mustard; cauliflower; cavalo broccolo; kohlrabi
Crop Subgroup 5B. Leafy *Brassica* greens subgroup.	
Mustard greens	Broccoli raab; cabbage, Chinese (bok choy); collards; kale; mizuna; mustard greens; mustard spinach; rape greens

(8) *Crop Group 5–16. Brassica* Head and Stem Vegetable Group.

(i) *Representative commodities.* Broccoli or cauliflower and cabbage.

(ii) *Commodities.* The following List 1 contains all commodities included in Crop Group 5–16.

LIST 1—CROP GROUP 5–16: BRASSICA HEAD AND STEM VEGETABLE GROUP

Commodities
Broccoli (*Brassica oleracea* L. var. *italica* Plenck)
Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* (DC.) Zenker)
Cabbage (*Brassica oleracea* L. var. *capitata* L.)
Cabbage, Chinese, napa (*Brassica rapa* L. subsp. *pekinensis* (Lour.) Hanelt)

LIST 1—CROP GROUP 5–16: BRASSICA HEAD AND STEM VEGETABLE GROUP—Continued

Commodities
Cauliflower (*Brassica oleracea* L. var. *capitata* L)
Cultivars, varieties, and hybrids of these commodities.

(9) *Crop Group 6.* Legume Vegetables (Succulent or Dried) Group.

(i) *Representative commodities.* Bean (*Phaseolus* spp.; one succulent cultivar and one dried cultivar); pea (*Pisum* spp.; one succulent cultivar and one dried cultivar); and soybean.

(ii) *Table.* The following table 1 lists all the commodities included in Crop Group 6 and identifies the related crop subgroups.

TABLE 1—CROP GROUP 6: LEGUME VEGETABLES (SUCCULENT OR DRIED)

Commodities	Related crop subgroups
Bean (*Lupinus* spp.) (includes grain lupin, sweet lupin, white lupin, and white sweet lupin)	6C
Bean (*Phaseolus* spp.) (includes field bean, kidney bean, lima bean, navy bean, pinto bean, runner bean, snap bean, tepary bean, wax bean)	6A, 6B, 6C
Bean (*Vigna* spp.) (includes adzuki bean, asparagus bean, blackeyed pea, catjang, Chinese longbean, cowpea, Crowder pea, moth bean, mung bean, rice bean, southern pea, urd bean, yardlong bean)	6A, 6B, 6C
Broad bean (fava bean) (*Vicia faba*)	6B, 6C
Chickpea (garbanzo bean) (*Cicer arietinum*)	6C
Guar (*Cyamopsis tetragonoloba*)	6C
Jackbean (*Canavalia ensiformis*)	6A
Lablab bean (hyacinth bean) (*Lablab purpureus*)	6C
Lentil (*Lens esculenta*)	6C
Pea (*Pisum* spp.) (includes dwarf pea, edible-pod pea, En glish pea, field pea, garden pea, green pea, snow pea, sugar snap pea)	6A, 6B, 6C
Pigeon pea (*Cajanus cajan*)	6A, 6B, 6C
Soybean (*Glycine max*)	N/A
Soybean (immature seed) (*Glycine max*)	6A
Sword bean (*Canavalia gladiata*)	6A

(iii) *Table.* The following table 2 identifies the crop subgroups for Crop Group 6, specifies the representative commodities for each subgroup, and lists all the commodities included in each subgroup.

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TABLE 2—CROP GROUP 6 SUBGROUP LISTING

Representative commodities	Commodities
Crop Subgroup 6A. Edible-podded legume vegetables subgroup. Any one succulent cultivar of edible-podded bean (*Phaseolus* spp.) and any one succulent cultivar of edible-podded pea (*Pisum* spp.)..	Bean (*Phaseolus* spp.) (includes runner bean, snap bean, wax bean); bean (*Vigna* spp.) (includes asparagus bean, Chinese longbean, moth bean, yardlong bean); jackbean; pea (*Pisum* spp.) (includes dwarf pea, edible-pod pea, snow pea, sugar snap pea); pigeon pea; soybean (immature seed); sword bean.
Crop Subgroup 6B. Succulent shelled pea and bean subgroup. Any succulent shelled cultivar of bean (*Phaseolus* spp.) and garden pea (*Pisum* spp.)..	Bean (*Phaseolus* spp.) (includes lima bean (green)); broad bean (succulent); bean (*Vigna* spp.) (includes blackeyed pea, cowpea, southern pea); pea (*Pisum* spp.) (includes English pea, garden pea, green pea); pigeon pea.
Crop Subgroup 6C. Dried shelled pea and bean (except soybean) subgroup Any one dried cultivar of bean (*Phaseolus* spp.); and any one dried cultivar of pea (*Pisum* spp.).	Dried cultivars of bean (*Lupinus* spp.) (includes grain lupin, sweet lupin, white lupin, and white sweet lupin); (*Phaseolus* spp.) (includes field bean, kidney bean, lima bean (dry), navy bean, pinto bean; tepary bean; bean (*Vigna* spp.) (includes adzuki bean, blackeyed pea, catjang, cowpea, Crowder pea, moth bean, mung bean, rice bean, southern pea, urd bean); broad bean (dry); chickpea; guar; lablab bean; lentil; pea (*Pisum* spp.) (includes field pea); pigeon pea.

(10) *Crop Group 7.* Foliage of Legume Vegetables Group.

(i) *Representative commodities.* Any cultivar of bean (*Phaseolus* spp.), field pea (*Pisum* spp.), and soybean.

(ii) *Table.* The following table 1 lists the commodities included in Crop Group 7.

TABLE 1—CROP GROUP 7: FOLIAGE OF LEGUME VEGETABLES GROUP

Representative commodities	Commodities
Any cultivar of bean (*Phaseolus* spp.) and field pea (*Pisum* spp.), and soybean (*Glycine max*).	Plant parts of any legume vegetable included in the legume vegetables that will be used as animal feed.

(iii) *Table.* The following table 2 identifies the crop subgroup for Crop Group 7 and specifies the representative commodities for the subgroup, and lists all the commodities included in the subgroup.

TABLE 2—CROP GROUP 7 SUBGROUP LISTING

Representative commodities	Commodities
Crop Subgroup 7A. Foliage of legume vegetables (except soybeans) subgroup Any cultivar of bean (*Phaseolus* spp.), and field pea (*Pisum* spp.).	Plant parts of any legume vegetable (except soybeans) included in the legume vegetables group that will be used as animal feed.

(11) *Crop Group 8.* Fruiting Vegetables Group.

(i) *Representative commodities.* Tomato, bell pepper, and one cultivar of non-bell pepper.

(ii) *Commodities.* The following is a list of all the commodities included in Crop Group 8:

CROP GROUP 8: FRUITING VEGETABLES (EXCEPT CUCURBITS)—COMMODITIES

Eggplant (*Solanum melongena*)
Groundcherry (*Physalis* spp.)
Pepino (*Solanum muricatum*)
Pepper (*Capsicum* spp.) (includes bell pepper, chili pepper, cooking pepper, pimento, sweet pepper)
Tomatillo (*Physalis ixocarpa*)
Tomato (*Lycopersicon esculentum*)

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(12) *Crop Group 8–10.* Fruiting Vegetable Group.

(i) *Representative commodities.* Tomato, standard size, and one cultivar of small tomato; bell pepper and one cultivar of small nonbell pepper.

(ii) *Commodities.* The following is a list of all commodities included in the Crop group 8–10.

TABLE 1—CROP GROUP 8–10: FRUITING VEGETABLE GROUP

Commodities	Related crop subgroups
African eggplant, *Solanum macrocarpon* L	8–10B, 8–10C
Bush tomato, *Solanum centrale* J.M. Black	8–10A
Cocona, *Solanum sessiliflorum* Dunal	8–10A
Currant tomato, *Lycopersicon pimpinellifolium* L	8–10A
Eggplant, *Solanum melongena* L	8–10B, 8–10C
Garden huckleberry, *Solanum scabrum* Mill	8–10A
Goji berry, *Lycium barbarum* L	8–10A
Groundcherry, *Physalis alkekengi* L., *P. grisea* (Waterf.) M. Martinez, *P. peruviana* L., *P. pubescens* L	8–10A
Martynia, *Proboscidea louisianica* (Mill.) Thell	8–10B, 8–10C
Naranjilla, *Solanum quitoense* Lam	8–10A
Okra, *Abelmoschus esculentus* (L.) Moench	8–10B, 8–10C
Pea eggplant, *Solanum torvum* Sw.	8–10B, 8–10C
Pepino, *Solanum muricatum* Aiton	8–10B, 8–10C
Pepper, bell, *Capsicum annuum* L. var. *annuum, Capsicum* spp	8–10B
Pepper, nonbell, *Capsicum chinese* Jacq., *C. annuum* L. var. *annuum, C. frutescens* L., *C. baccatum* L., *C. pubescens* Ruiz & Pav., *Capsicum spp.*	8–10B, 8–10C
Roselle, *Hibiscus sabdariffa* L	8–10B, 8–10C
Scarlet eggplant, *Solanum aethiopicum* L	8–10B, 8–10C
Sunberry, *Solanum retroflexum* Dunal	8–10A
Tomatillo, *Physalis philadelphica* Lam	8–10A
Tomato, *Solanum lycopersicum* L., *Solanum lycopersicum* L. var. *lycopersicum*	8–10A
Tree tomato, *Solanum betaceum* Cav	8–10A
Cultivars, varieties and/or hybrids of these	

(iii) *Table.* The following Table 2 identifies the crop subgroups for Crop Group 8–10, specifies the representative commodities for each subgroup and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 8–10. SUBGROUP LISTING

Representative commodities	Commodities
Crop subgroup 8–10A. Tomato subgroup	
Tomato, standard size, and one cultivar of small tomato	Bush tomato; cocona; currant tomato; garden huckleberry; goji berry; groundcherry; naranjilla; sunberry; tomatillo; tomato; tree tomato; cultivars, varieties, and/or hybrids of these.
Crop subgroup 8–10B. Pepper/Eggplant subgroup	
Bell pepper and one cultivar of small nonbell pepper	African eggplant; bell pepper; eggplant; Martynia; nonbell pepper; okra; pea eggplant; pepino; roselle; scarlet eggplant; cultivars, varieties, and/or hybrids of these.
Crop subgroup 8–10C. Nonbell pepper/Eggplant subgroup	
One cultivar of small nonbell pepper or one cultivar of small eggplant.	African eggplant; eggplant; martynia; nonbell pepper; okra; pea eggplant; pepino; roselle; scarlet eggplant; cultivars, varieties, and/or hybrids of these.

(13) *Crop Group 9.* Cucurbit Vegetables Group.

(i) *Representative commodities.* Cucumber, muskmelon, and summer squash.

(ii) *Table.* The following table 1 lists all the commodities included in Crop Group 9 and identifies the related subgroups.

TABLE 1—CROP GROUP 9: CUCURBIT VEGETABLES

Commodities	Related crop subgroups
Chayote (fruit) (*Sechium edule*)	9B

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TABLE 1—CROP GROUP 9: CUCURBIT VEGETABLES—Continued

Commodities	Related crop subgroups
Chinese waxgourd (Chinese preserving melon) (*Benincasa hispida*)	9B
Citron melon (*Citrullus lanatus* var. *citroides*)	9A
Cucumber (*Cucumis sativus*)	9B
Gherkin (*Cucumis anguria*)	9B
Gourd, edible (*Lagenaria* spp.) (includes hyotan, cucuzza); (*Luffa acutangula, L. cylindrica*) (includes hechima, Chinese okra)	9B
Momordica spp. (includes balsam apple, balsam pear, bitter melon, Chinese cucumber)	9B
Muskmelon (hybrids and/or cultivars of *Cucumis melo*) (includes true cantaloupe, cantaloupe, casaba, crenshaw melon, golden pershaw melon, honeydew melon, honey balls, mango melon, Persian melon, pineapple melon, Santa Claus melon, and snake melon)	9A
Pumpkin (*Cucurbita* spp.)	9B
Squash, summer (*Cucurbita pepo* var. *melopepo*) (includes crookneck squash, scallop squash, straightneck squash, vegetable marrow, zucchini)	9B
Squash, winter (*Cucurbita maxima; C. moschata*) (includes butternut squash, calabaza, hubbard squash); (*C. mixta; C. pepo*) (includes acorn squash, spaghetti squash)	9B
Watermelon (includes hybrids and/or varieties of *Citrullus lanatus*)	9A

(iii) *Table.* The following table 2 identifies the crop subgroups for Crop Group 9, specifies the representative commodities for each subgroup, and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 9 SUBGROUP LISTING

Representative commodities	Commodities
Crop Subgroup 9A. Melon subgroup	
Cantaloupes	Citron melon; muskmelon; watermelon
Crop Subgroup 9B. Squash/cucumber subgroup	
One cultivar of summer squash and cucumber.	Chayote (fruit); Chinese waxgourd; cucumber; gherkin; gourd, edible; *Momordica* spp.; pumpkin; squash, summer; squash, winter.

(14) *Crop Group 10.* Citrus Fruit Group.

(i) *Representative commodities.* Sweet orange; lemon and grapefruit.

(ii) *Commodities.* The following is a list of all the commodities in Crop Group 10:

CROP GROUP 10: CITRUS FRUITS (CITRUS SPP., FORTUNELLA SPP.) GROUP—COMMODITIES

Calamondin (*Citrus mitis* × *Citrofortunella mitis*)
Citrus citron (*Citrus medica*)
Citrus hybrids (*Citrus* spp.) (includes chironja, tangelo, tangor)
Grapefruit (*Citrus paradisi*)
Kumquat (*Fortunella* spp.)
Lemon (*Citrus jambhiri, Citrus limon*)
Lime (*Citrus aurantiifolia*)
Mandarin (tangerine) (*Citrus reticulata*)
Orange, sour (*Citrus aurantium*)
Orange, sweet (*Citrus sinensis*)
Pummelo (*Citrus grandis, Citrus maxima*)
Satsuma mandarin (*Citrus unshiu*)

(15) *Crop Group 10–10.* Citrus Fruit Group.

(i) *Representative commodities.* Orange or Tangerine/Mandarin, Lemon or Lime, and Grapefruit.

(ii) *Commodities.* The following is a list of all the commodities in Crop Group 10–10.

TABLE 1—CROP GROUP 10–10: CITRUS FRUIT GROUP

Commodities	Related crop subgroups
Australian desert lime, *Eremocitrus glauca* (Lindl.) Swingle	10–10B
Australian finger lime, *Microcitrus australasica* (F. Muell.) Swingle	10–10B
Australian round lime, *Microcitrus australis* (A. Cunn. Ex Mudie) Swingle	10–10B
Brown River finger lime, *Microcitrus papuana* Winters	10–10B
Calamondin, *Citrofortunella microcarpa* (Bunge) Wijnands	10–10A
Citron, *Citrus medica* L	10–10A
Citrus hybrids, *Citrus spp. Eremocitrus* spp., *Fortunella* spp., *Microcitrus* spp., and *Poncirus* spp	10–10A
Grapefruit, *Citrus paradisi* Macfad	10–10C

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TABLE 1—CROP GROUP 10–10: CITRUS FRUIT GROUP—Continued

Commodities	Related crop subgroups
Japanese summer grapefruit, *Citrus natsudaidai* Hayata	10–10C
Kumquat, *Fortunella* spp	10–10B
Lemon, *Citrus limon* (L.) Burm. f	10–10B
Lime, *Citrus aurantiifolia* (Christm.) Swingle	10–10B
Mediterranean mandarin, *Citrus deliciosa* Ten	10–10A
Mount White lime, *Microcitrus garrowayae* (F.M. Bailey) Swingle	10–10B
New Guinea wild lime, *Microcitrus warburgiana* (F.M. Bailey) Tanaka	10–10B
Orange, sour, *Citrus aurantium* L	10–10A
Orange, sweet, *Citrus sinensis* (L.) Osbeck	10–10A
Pummelo, *Citrus maxima* (Burm.) Merr	10–10C
Russell River lime, *Microcitrus inodora* (F.M. Bailey) Swingle	10–10B
Satsuma mandarin, *Citrus unshiu* Marcow	10–10A
Sweet lime, *Citrus limetta* Risso	10–10B
Tachibana orange, *Citrus tachibana* (Makino) Tanaka	10–10A
Tahiti lime, *Citrus latifolia* (Yu. Tanaka) Tanaka	10–10B
Tangelo, *Citrus xtangelo* J.W. Ingram & H.E. Moore	10–10A, 10–10C
Tangerine (Mandarin), *Citrus reticulata* Blanco	10–10A
Tangor, *Citrus nobilis* Lour	10–10A
Trifoliate orange, *Poncirus trifoliata* (L.) Raf	10–10A
Uniq fruit, *Citrus aurantium* Tangelo group	10–10C
Cultivars, varieties and/or hybrids of these.	

(iii) *Table.* The following Table 2 identifies the crop subgroups for Crop Group 10–10, specifies the representative commodities for each subgroup and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 10–10: SUBGROUP LISTING

Representative commodities	Commodities
Crop Subgroup 10–10A. Orange subgroup	
Orange or tangerine/mandarin	Calamondin; citron; citrus hybrids; mediterranean mandarin; orange, sour; orange, sweet; satsuma mandarin; tachibana orange; tangerine (mandarin); tangelo; tangor; trifoliate orange; cultivars, varieties, and/or hybrids of these.
Crop Subgroup 10–10B. Lemon/Lime subgroup	
Lemon or lime	Australian desert lime; Australian finger lime; Australian round lime; brown river finger lime; kumquat; lemon; lime; mount white lime; New Guinea wild lime; Russell River lime; sweet lime; Tahiti lime; cultivars , varieties, and/or hybrids of these.
Crop Subgroup 10–10C. Grapefruit subgroup	
Grapefruit	Grapefruit; Japanese summer grapefruit; pummelo; tangelo; uniq fruit; cultivars, varieties, and/or hybrids of these.

(16) *Crop Group 11:* Pome Fruits Group.

(i) *Representative commodities.* Apple and pear.

(ii) *Commodities.* The following is a list of all the commodities included in Crop Group 11:

CROP GROUP 11: POME FRUITS GROUP—COMMODITIES

Apple (*Malus domestica*)
Crabapple (*Malus* spp.)
Loquat (*Eriobotrya japonica*)
Mayhaw (*Crataegus aestivalis, C. opaca*, and *C. rufula*)
Pear (*Pyrus communis*)
Pear, oriental (*Pyrus pyrifolia*)
Quince (*Cydonia oblonga*)

(17) *Crop group 11–10.* Pome Fruit Group.

(i) *Representative commodities.* Apple and Pear

(ii) *Commodities.* The following is a list of all the commodities in Crop Group 11–10.

CROP GROUP 11–10: POME FRUIT GROUP—COMMODITIES

Apple, *Malus domestica* Borkh.
Azarole, *Crataegus azarolus* L.
Crabapple, *Malus sylvestris* (L.) Mill., *M. prunifolia* (Willd.) Borkh.
Loquat, *Eriobotrya japonica* (Thunb.) Lindl.

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Mayhaw, *Crataegus aestivalis* (Walter) Torr. & A. Gray, C. *opaca*
Hook. & Arn., and *C. rufula* Sarg.
Medlar, *Mespilus germanica* L.
Pear, *Pyrus communis* L.
Pear, Asian, *Pyrus pyrifolia* (Burm. f.) Nakai var. *culta* (Makino) Nakai
Pseudocydonia sinensis (Thouin) C.K. Schneid.
Quince, *Cydonia oblonga* Mill.
Quince, Chinese, *Chaenomeles speciosa* (Sweet) Nakai,
Quince, Japanese, *Chaenomeles japonica* (Thunb.) Lindl. ex Spach
Tejocote, *Crataegus mexicana* DC.
Cultivars, varieties and/or hybrids of these.

(18) *Crop Group 12.* Stone Fruits Group.

(i) *Representative commodities.* Sweet cherry or tart cherry; peach; and plum or fresh prune (*Prunus domestica, Prunus* spp.)

(ii) *Commodities.* The following is a list of all the commodities included in Crop Group 12:

CROP GROUP 12: STONE FRUITS GROUP—COMMODITIES

Apricot (*Prunus armeniaca*)
Cherry, sweet (*Prunus avium*),
Cherry, tart (*Prunus cerasus*)
Nectarine (*Prunus persica*)
Peach (*Prunus persica*)
Plum (*Prunus domestica, Prunus* spp.)
Plum, Chickasaw (*Prunus angustifolia*)
Plum, Damson (*Prunus domestica* spp. *insititia*)
Plum, Japanese (*Prunus salicina*)
Plumcot (*Prunus. armeniaca* × *P. domestica*)
Prune (fresh) (*Prunus domestica, Prunus* spp.)

(19) *Crop Group 12–12:* Stone Fruit Group.

(i) *Representative commodities.* Sweet cherry or Tart cherry; Peach; and Plum or Prune plum.

(ii) *Commodities.* The following Table 1 is a list of all commodities included in Crop Group 12–12.

TABLE 1—CROP GROUP 12–12: STONE FRUIT GROUP

Commodities	Related crop subgroup
Apricot (*Prunus armeniaca* L.)	12–12C
Apricot, Japanese (*Prunus mume* Siebold & Zucc.)	12–12C
Capulin (*Prunus serotina* Ehrh. var. *salicifolia* (Kunth) Koehne)	12–12A
Cherry, black (*Prunus serotina* Ehrh.)	12–12A
Cherry, Nanking (*Prunus tomentosa* Thunb.)	12–12A
Cherry, sweet (*Prunus avium* (L.) L.)	12–12A
Cherry, tart (*Prunus cerasus* L.)	12–12A
Jujube, Chinese (*Ziziphus jujuba* Mill.)	12–12C
Nectarine (*Prunuspersica* (L.) Batsch var. *nucipersica* (Suckow) C.K. Schneid)	12–12B
Peach (*Prunus persica* (L.) Batsch var. *persica*)	12–12B
Plum (*Prunus domestica* L. subsp. *domestica*)	12–12C
Plum, American (*Prunus americana* Marshall)	12–12C
Plum, beach (*Prunus maritima* Marshall)	12–12C
Plum, Canada (*Prunus nigra* Aiton)	12–12C
Plum, cherry (*Prunus cerasifera* Ehrh.)	12–12C
Plum, Chickasaw (*Prunus angustifolia* Marshall)	12–12C
Plum, Damson (*Prunus domestica* L. subsp. *insititia* (L.) C.K. Schneid.)	12–12C
Plum, Japanese (*Prunus salicina* Lindl.; *P. salicina* Lindl. var. *salicina*)	12–12C
Plum, Klamath (*Prunus subcordata* Benth.)	12–12C
Plum, prune (*Prunus domestica* L. subsp. *domestica*)	12–12C
Plumcot (*Prunus* hybr.)	12–12C
Sloe (*Prunus spinosa* L.)	12–12C
Cultivars, varieties, and/or hybrids of these.	

(iii) *Crop subgroups.* The following Table 2 identifies the crop subgroups for Crop Group 12–12, specifies the representative commodities for each subgroup, and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 12–12: SUBGROUP LISTING

Representative commodities	Commodities
Crop subgroup 12–12A. Cherry subgroup	
Cherry, sweet or Cherry, tart	Capulin; Cherry, black; Cherry, Nanking; Cherry, sweet; Cherry, tart; cultivars, varieties, and/or hybrids of these.

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TABLE 2—CROP GROUP 12–12: SUBGROUP LISTING—Continued

Representative commodities	Commodities
	Crop subgroup 12–12B. Peach subgroup
Peach	Peach; Nectarine; cultivars, varieties, and/or hybrids of these.
	Crop subgroup 12–12C. Plum subgroup
Plum or Prune plum	Apricot; Apricot, Japanese; Jujube, Chinese; Plum; Plum, American; Plum, beach; Plum, Canada; Plum, cherry; Plum, Chickasaw; Plum, Damson; Plum, Japanese; Plum, Klamath; Plumcot; Plum, prune; Sloe; cultivars, varieties, and/or hybrids of these.

(20) *Crop Group 13.* Berries Group.

(i) *Representative commodities.* Any one blackberry or any one raspberry; and blueberry.

(ii) *Table.* The following table 1 lists all the commodities included in Crop Group 13 and identifies the related subgroups.

TABLE 1—CROP GROUP 13: BERRIES GROUP

Commodities	Related crop subgroups
Blackberry (*Rubus eubatus*) (including bingleberry, black satin berry, boysenberry, Cherokee blackberry, Chesterberry, Cheyenne blackberry, coryberry, darrowberry, dewberry, Dirksen thornless berry, Himalayaberry, hullberry, Lavacaberry, lowberry, Lucretiaberry, mammoth blackberry, marionberry, nectarberry, olallieberry, Oregon evergreen berry, phenomenalberry, rangeberry, ravenberry, rossberry, Shawnee blackberry, youngberry, and varieties and/or hybrids of these)	13A
Blueberry (*Vaccinium* spp.)	13B
Currant (*Ribes* spp.)	13B
Elderberry (*Sambucus* spp.)	13B
Gooseberry (*Ribes* spp.)	13B
Huckleberry (*Gaylussacia* spp.)	13B
Loganberry (*Rubus loganobaccus*)	13A
Raspberry, black and red (*Rubus occidentalis, Rubus strigosus, Rubus idaeus*)	13A

(iii) *Table.* The following table 2 identifies the crop subgroups for Crop Group 13, specifies the representative commodities for each subgroup, and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 13 SUBGROUPS LISTING

Representative commodities	Commodities
Crop Subgroup 13A. Caneberry (blackberry and raspberry) subgroup.	
Any one blackberry or any one raspberry.	Blackberry; loganberry; red and black raspberry; cultivars and/or hybrids of these.
Crop Subgroup 13B. Bushberry subgroup..	
Blueberry, highbush.	Blueberry, highbush and lowbush; currant; elderberry; gooseberry; huckleberry.

(21) *Crop Group 13-07.* Berry and Small Fruit Crop Group

(i) *Representative commodities.* Any one blackberry or any one raspberry; highbush blueberry; elderberry or mulberry; grape; fuzzy kiwifruit, and strawberry.

(ii) *Table.* The following Table 1 lists all the commodities listed in Crop Group 13-07 and identifies the related crop subgroups.

TABLE 1—CROP GROUP 13-07: BERRY AND SMALL FRUIT CROP GROUP

Commodities	Related crop subgroups
Amur river grape (*Vitis amurensis Rupr*)	13-07D, 13-07E, 13-07F
Aronia berry (*Aronia* spp.)	13-07B
Bayberry (*Myrica* spp.)	13-07C

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TABLE 1—CROP GROUP 13-07: BERRY AND SMALL FRUIT CROP GROUP—Continued

Commodities	Related crop subgroups
Bearberry (*Arctostaphylos uva-ursi*)	13-07G, 13-07H
Bilberry (*Vaccinium myrtillus L.)*	13-07G, 13-07H
Blackberry (*Rubus* spp.) (including Andean blackberry, arctic blackberry, bingleberry, black satin berry, boysenberry, brombeere, California blackberry, Chesterberry, Cherokee blackberry, Cheyenne blackberry, common blackberry, coryberry, darrowberry, dewberry, Dirksen thornless berry, evergreen blackberry, Himalayaberry, hullberry, lavacaberry, loganberry, lowberry, Lucretiaberry, mammoth blackberry, marionberry, mora, mures deronce, nectarberry, Northern dewberry, olallieberry, Oregon evergreen berry, phenomenalberry, rangeberry, ravenberry, rossberry, Shawnee blackberry, Southern dewberry, tayberry, youngberry, zarzamora, and cultivars, varieties and/or hybrids of these.)	13-07A
Blueberry, highbush (*Vaccinium* spp.)	13-07B
Blueberry, lowbush (*Vaccinium angustifolium* Aiton)	13-07B
Buffalo currant (*Ribes aureum* Pursh)	13-07B
Buffaloberry (*Shepherdia argentea* (Pursh) Nutt.)	13-07C
Che (*Cudrania tricuspidata* Bur. Ex Lavallee)	13-07C
Chilean guava (*Myrtus ugni* Mol.)	13-07B
Chokecherry (*Prunus virginiana* L.)	13-07C
Cloudberry (*Rubus chamaemorus* L.)	13-07G, 13-07H
Cranberry (*Vaccinium macrocarpon* Aiton)	13-07G, 13-07H
Currant, black (*Ribes nigrum* L.)	13-07B
Currant, red (*Ribes rubrum* L.)	13-07B
Elderberry (*Sambucus* spp.)	13-07B, 13-07C
European barberry (*Berberis vulgaris* L.)	13-07B
Gooseberry (*Ribes* spp.)	13-07B, 13-07D, 13-07E, 13-07F
Grape (*Vitis* spp.)	13-07D, 13-07F
Highbush cranberry (*Viburnum opulus* L. var. *Americanum* Aiton)	13-07B
Honeysuckle, edible (*Lonicera caerula L.* var. *emphyllocalyx* Nakai, *Lonicera caerula L* var . *edulis Turcz. ex herder*)	13-07B
Huckleberry (*Gaylussacia* spp.)	13-07B
Jostaberry (*Ribes x nidigrolaria* Rud. Bauer and A. Bauer)	13-07B
Juneberry (Saskatoon berry) (*Amelanchier* spp.)	13-07B, 13-07C
Kiwifruit, fuzzy (*Actinidia deliciosa* A. Chev.) (C.F. Liang and A.R. Fergusons, *Actinida chinensis* Planch.)	13-07D, 13-07E
Kiwifruit, hardy (*Actinidia arguta* (Siebold and Zucc.) Planch. ex Miq)	13-07D, 13-07E, 13-07F
Lingonberry (*Vaccinium vitis-idaea* L.)	13-07B, 13-07G 13-07H
Maypop (*Passiflora incamata* L.)	13-07E, 13-07F
Mountain pepper berries (*Tasmannia lanceolata*)(Poir.) A.C.Sm.	13-07C
Mulberry (*Morus* spp.)	13-07C
Muntries (*Kunzea pomifera* F. Muell.)	13-07G, 13-07H
Native currant (*Acrotriche depressa* R. BR.)	13-07B
Partridgeberry (*Mitchella repens* L.)	13-07G, 13-07H
Phalsa (*Grewia subinaequalis* DC.)	13-07C
Pincherry (*Prunus pensylvanica* L.f.)	13-07C
Raspberry, black and red (*Rubus* spp.)	13-07A
Riberry (*Syzygium luehmannii*)	13-07C
Salal (*Gaultheria shallon* Pursh.)	13-07B, 13-07C
Schisandra berry (Schisandra chinensis (Turcz.) Baill.)	13-07D, 13-07E, 13-07F
Sea buckthorn (*Hippophae rhamnoides* L.)	13-07B
Serviceberry (*Sorbus* spp.)	13-07C
Strawberry (*Fragaria x ananassa* Duchesne)	13-07G
Wild raspberry (*Rubus muelleri* Lefevre ex P.J. Mull)	13-07A
Cultivars, varieties, and/or hybrids of these.	

(iii) *Table.* The following Table 2 identifies the crop subgroups for Crop Group 13-07, specifies the representative commodities for each subgroup and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 13-07: SUBGROUP LISTING

Representative commodities	Commodities
Crop Subgroup 13-07A. Caneberry subgroup	
Any one blackberry or any one raspberry..	Blackberry; loganberry; raspberry, red and black; wild raspberry; cultivars, varieties, and/or hybrids of these.
Crop Subgroup 13-07B. Bushberry subgroup.	

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TABLE 2—CROP GROUP 13-07: SUBGROUP LISTING—Continued

Representative commodities	Commodities
Blueberry, highbush.	Aronia berry; blueberry, highbush; blueberry, lowbush; buffalo currant; Chilean guava; currant, black; currant, red; elderberry; European, barberry; gooseberry; cranberry, highbush; honeysuckle, edible; huckleberry; jostaberry; Juneberry; lingonberry; native currant; salal; sea buckthorn; cultivars, varieties; and/or hybrids of these.
Crop Subgroup 13-07C. Large shrub/tree berry subgroup.	
Elderberry or mulberry.	Bayberry; buffaloberry; che; chokecherry; elderberry; Juneberry; mountain pepper berries; mulberry; phalsa; pincherry; riberry; salal; serviceberry; cultivars, varieties, and/or hybrids of these.
Crop Subgroup 13-07D. Small fruit vine climbing subgroup.	
Grape and fuzzy kiwifruit.	Amur river grape; gooseberry; grape; kiwifruit, fuzzy; kiwifruit, hardy; Maypop; schisandra berry; cultivars, varieties, and /or hybrids of these.
Crop Subgroup 13-07E. Small fruit vine climbing subgroup, except grape.	
Fuzzy kiwifruit.	Amur river grape; gooseberry; kiwifruit, fuzzy; kiwifruit, hardy; Maypop; schisandra berry; cultivars, varieties, and/or hybrids of these.
Crop Subgroup 13-07F. Small fruit vine climbing subgroup except fuzzy kiwifruit.	
Grape.	Amur river grape; gooseberry; grape; kiwifruit, hardy; Maypop; schisandra berry; cultivars varieties, and/or hybrids of these.
Crop Subgroup 13-07G. Low growing berry subgroup.	
Strawberry.	Bearberry; bilberry; blueberry, lowbush; cloudberry; cranberry; lingonberry; muntries; partridgeberry; strawberry; cultivars, varieties, and/or hybrids of these.
Crop Subgroup 13-07H. Low growing berry subgroup, except strawberry.	
Cranberry	Bearberry; bilberry; blueberry, lowbush; cloudberry; cranberry; lingonberry; muntries; partridgeberry; cultivars, varieties, and/or cultivars of these.

(22) *Crop Group 14.* Tree Nuts Group.

(i) *Representative commodities.* Almond and pecan.

(ii) *Commodities.* The following is a list of all the commodities included in Crop Group 14:

CROP GROUP 14: TREE NUTS—COMMODITIES

Almond (*Prunus dulcis*)
Beech nut (*Fagus* spp.)
Brazil nut (*Bertholletia excelsa*)
Butternut (*Juglans cinerea*)
Cashew (*Anacardium occidentale*)
Chestnut (*Castanea* spp.)
Chinquapin (*Castanea pumila*)
Filbert (hazelnut) (*Corylus* spp.)
Hickory nut (*Carya* spp.)
Macadamia nut (bush nut) (*Macadamia* spp.)
Pecan (*Carya illinoensis*)
Walnut, black and English (Persian) (*Juglans* spp.)

(23) *Crop Group 14–12.* Tree Nut Group.

(i) *Representative commodities.* Almond and Pecan.

(ii) *Commodities.* The following is a list of all commodities included in Crop Group 14–12.

CROP GROUP 14–12: TREE NUT GROUP

African nut-tree (*Ricinodendron heudelotii* (Baill.) Heckel)
Almond (*Prunus dulcis* (Mill.) D.A. Webb)
Beechnut (*Fagus grandifolia* Ehrh.; *F. sylvatica* L.)
Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.)
Brazilian pine (*Araucaria angustifolia* (Bertol.) Kuntze)
Bunya (*Araucaria bidwillii* Hook.)
Bur oak (*Quercus macrocarpa* Michx.)
Butternut (*Juglans cinerea* L.)
Cajou nut (*Anacardium giganteum* Hance ex Engl.)
Candlenut (*Aleurites moluccanus* (L.) Willd.)
Cashew (*Anacardium occidentale* L.)
Chestnut (*Castanea crenata* Siebold & Zucc.; *C. dentata* (Marshall) Borkh.; *C. mollissima* Blume; *C. sativa* Mill.)
Chinquapin (*Castaneapumila* (L.) Mill.)
Coconut (*Cocos nucifera* L.)
Coquito nut (*Jubaea chilensis* (Molina) Baill.)
Dika nut (*Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill.)
Ginkgo (*Ginkgo biloba* L.)
Guiana chestnut (*Pachira aquatica* Aubl.)
Hazelnut (Filbert) (*Corylus americana* Marshall; *C. avellana* L.; *C. californica* (A. DC.) Rose; *C. chinensis* Franch.)
Heartnut (*Juglans ailantifolia* Carrière var. *cordiformis* (Makino) Rehder)

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Hickory nut (*Carya cathayensis* Sarg.; *C. glabra* (Mill.) Sweet; *C. laciniosa* (F. Michx.) W. P. C. Barton; *C. myristiciformis* (F. Michx.) Elliott; *C. ovata* (Mill.) K. Koch; *C. tomentosa* (Lam.) Nutt.)
Japanese horse-chestnut (*Aesculus turbinate* Blume)
Macadamia nut (*Macadamia integrifolia* Maiden & Betche; *M. tetraphylla* L.A.S. Johnson)
Mongongo nut (*Schinziophyton rautanenii* (Schinz) Radcl.-Sm.)
Monkey-pot (*Lecythis pisonis* Cambess.)
Monkey puzzle nut (*Araucaria araucana* (Molina) K. Koch)
Okari nut (*Terminalia kaernbachii* Warb.)
Pachira nut (*Pachira insignis* (Sw.) Savigny)
Peach palm nut (*Bactris gasipaes* Kunth var. *gasipaes*)
Pecan (*Carya illinoinensis* (Wangenh.) K. Koch)
Pequi (*Caryocar brasiliense* Cambess.; *C. villosum* (Aubl.) Pers; *C. nuciferum* L.)
Pili nut (*Canarium ovatum* Engl.; *C. vulgare* Leenh.)
Pine nut (*Pinus edulis* Engelm.; *P. koraiensis* Siebold & Zucc.; *P. sibirica* Du Tour; *P. pumila* (Pall.) Regel; *P. gerardiana* Wall. ex D. Don; *P. monophylla* Torr. & Frém.; *P. quadrifolia* Parl. ex Sudw.; *P. pinea* L.)
Pistachio (*Pistacia vera* L.)
Sapucaia nut (*Lecythis zabucaja* Aubl.)
Tropical almond (*Terminalia catappa* L.)
Walnut, black (*Juglans nigra* L.; *J. hindsii* Jeps. ex R. E. Sm.; *J. microcarpa* Berland.)
Walnut, English (*Juglans regia* L.)
Yellowhorn (*Xanthoceras sorbifolium* Bunge)
Cultivars, varieties, and/or hybrids of these

(24) *Crop Group 15.* Cereal Grains Group.

(i) *Representative commodities.* Corn (fresh sweet corn and dried field corn), rice, sorghum, and wheat.

(ii) *Commodities.* The following is a list of all the commodities included in Crop Group 15:

CROP GROUP 15: CEREAL GRAINS—COMMODITIES

Barley (*Hordeum* spp.)
Buckwheat (*Fagopyrum esculentum*)
Corn (*Zea mays*)
Millet, pearl (*Pennisetum glaucum*)
Millet, proso (*Panicum milliaceum*)
Oats (*Avena* spp.)
Popcorn (*Zea mays* var. *everta*)
Rice (*Oryza sativa*)
Rye (*Secale cereale*)
Sorghum (milo) (*Sorghum* spp.)
Teosinte (*Euchlaena mexicana*)
Triticale (*Triticum-Secale* hybrids)
Wheat (*Triticum* spp.)
Wild rice (*Zizania aquatica*)

(25) *Crop Group 16.* Forage, Fodder and Straw of Cereal Grains Group.

(i) *Representative commodities.* Corn, wheat, and any other cereal grain crop.

(ii) *Commodities.* The commodities included in Crop Group 16 are: Forage, fodder, stover, and straw of all commodities included in the group cereal grains group. EPA may establish separate group tolerances on forage, fodder, hay, stover, or straw, if data on the representative commodities indicate differences in the levels of residues on forage, fodder, stover, or straw.

(26) *Crop Group 17.* Grass Forage, Fodder, and Hay Group.

(i) *Representative commodities.* Bermuda grass; bluegrass; and bromegrass or fescue.

(ii) *Commodities.* The commodities included in Crop Group 17 are: Forage, fodder, stover, and hay of any grass, *Gramineae/Poaceae* family (either green or cured) except sugarcane and those included in the cereal grains group, that will be fed to or grazed by livestock, all pasture and range grasses and grasses grown for hay or silage. EPA may establish separate group tolerances on forage, fodder, stover, or hay, if data on the representative commodities indicate differences in the levels of residues on forage, fodder, stover, or hay.

(27) *Crop Group 18.* Nongrass Animal Feeds (Forage, Fodder, Straw, and Hay) Group.

(i) *Representative commodities.* Alfalfa and clover (*Trifolium* spp.)

(ii) *Commodities.* EPA may establish separate group tolerances on forage, fodder, straw, or hay, if data on the representative commodities indicate differences in the levels of residues on forage, fodder, straw, or hay. The following is a list of all the commodities included in Crop Group 18:

CROP GROUP 18: NONGRASS ANIMAL FEEDS (FORAGE, FODDER, STRAW, AND HAY) GROUP—COMMODITIES

Alfalfa (*Medicago sativa* subsp. *sativa*)
Bean, velvet (*Mucuna pruriens* var. *utilis*)
Clover (*Trifolium* spp., *Melilotus* spp.)
Kudzu (*Pueraria lobata*)
Lespedeza (*Lespedeza* spp.)
Lupin (*Lupinus* spp.)
Sainfoin (*Onobrychis viciifolia*);
Trefoil (*Lotus* spp.)
Vetch (*Vicia* spp.)
Vetch, crown (*Coronilla varia*)
Vetch, milk (*Astragalus* spp).

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(28) *Crop Group 19.* Herbs and Spices Group.

(i) *Representative commodities.* Basil (fresh and dried); black pepper; chive; and celery seed or dill seed.

(ii) *Table.* The following table 1 lists all the commodities included in Crop Group 19 and identifies the related subgroups.

TABLE 1—CROP GROUP 19: HERBS AND SPICES GROUP

Commodities	Related crop sub-groups
Allspice (*Pimenta dioica*)	19B
Angelica (*Angelica archangelica*)	19A
Anise (anise seed) (*Pimpinella anisum*)	19B
Anise, star (*Illicium verum*)	19B
Annatto (seed)	19B
Balm (lemon balm) (*Melissa officinalis*)	19A
Basil (*Ocimum basilicum*)	19A
Borage (*Borago officinalis*)	19A
Burnet (*Sanguisorba minor*)	19A
Camomile (*Anthemis nobilis*)	19A
Caper buds (*Capparis spinosa*)	19B
Caraway (*Carum carvi*)	19B
Caraway, black (*Nigella sativa*)	19B
Cardamom (*Elettaria cardamomum*)	19B
Cassia bark (*Cinnamomum aromaticum*)	19B
Cassia buds (*Cinnamomum aromaticum*)	19B
Catnip (*Nepeta cataria*)	19A
Celery seed (*Apicum graveolens*)	19B
Chervil (dried) (*Anthriscus cerefolium*)	19A
Chive (*Allium schoenoprasum*)	19A
Chive, Chinese (*Allium tuberosum*)	19A
Cinnamon (*Cinnamomum verum*)	19B
Clary (*Salvia sclarea*)	19A
Clove buds (*Eugenia caryophyllata*)	19B
Coriander (cilantro or Chinese parsley) (leaf) (*Coriandrum sativum*)	19A
Coriander (cilantro) (seed) (*Coriandrum sativum*)	19B
Costmary (*Chrysanthemum balsamita*)	19A
Culantro (leaf) (*Eryngium foetidum*)	19A
Culantro (seed) (*Eryngium foetidum*)	19B
Cumin (*Cuminum cyminum*)	19B
Curry (leaf) (*Murraya koenigii*)	19A
Dill (dillweed) (*Anethum graveolens*)	19A
Dill (seed) (*Anethum graveolens*)	19B
Fennel (common) (*Foeniculum vulgare*)	19B
Fennel, Florence (seed) (*Foeniculum vulgare* Azoricum Group)	19B
Fenugreek (*Trigonella foenumgraecum*)	19B
Grains of paradise (*Aframomum melegueta*)	19B
Horehound (*Marrubium vulgare*)	19A
Hyssop (*Hyssopus officinalis*)	19A
Juniper berry (*Juniperus communis*)	19B
Lavender (*Lavandula officinalis*)	19A
Lemongrass (*Cymbopogon citratus*)	19A
Lovage (leaf) (*Levisticum officinale*)	19A
Lovage (seed) (*Levisticum officinale*)	19B
Mace (*Myristica fragrans*)	19B
Marigold (*Calendula officinalis*)	19A
Marjoram (*Origanum spp.*) (includes sweet or annual marjoram, wild marjoram or oregano, and pot marjoram)	19A
Mustard (seed) (*Brassica* juncea, *B.* hirta, *B.* nigra)	19B
Nasturtium (*Tropaeolum majus*)	19A
Nutmeg (*Myristica fragrans*)	19B
Parsley (dried) (*Petroselinum crispum*)	19A
Pennyroyal (*Mentha pulegium*)	19A
Pepper, black (*Piper nigrum*)	19B
Pepper, white	19B
Poppy (seed) (*Papaver somniferum*)	19B
Rosemary (*Rosemarinus officinalis*)	19A
Rue (*Ruta graveolens*)	19A
Saffron (*Crocus sativus*)	19B
Sage (*Salvia officinalis*)	19A
Savory, summer and winter (*Satureja spp.*)	19A
Sweet bay (bay leaf) (*Laurus nobilis*)	19A
Tansy (*Tanacetum vulgare*)	19A
Tarragon (*Artemisia dracunculus*)	19A
Thyme (*Thymus spp.*)	19A
Vanilla (*Vanilla planifolia*)	19B
Wintergreen (*Gaultheria procumbens*)	19A
Woodruff (*Galium odorata*)	19A
Wormwood (*Artemisia absinthium*)	19A

(iii) *Table.* The following table 2 identifies the crop subgroups for Crop Group 19, specifies the representative commodities for each subgroup, and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 19 SUBGROUPS

Representative commodities	Commodities
Crop Subgroup 19A. Herb subgroup.	
Basil (fresh and dried) and chive.	Angelica; balm; basil; borage; burnet; camomile; catnip; chervil (dried); chive; chive, Chinese, clary; coriander (leaf); costmary; culantro (leaf); curry (leaf); dillweed; horehound; hyssop; lavender; lemongrass; lovage (leaf); marigold; marjoram (*Origanum* spp.); nasturtium; parsley (dried); pennyroyal; rosemary; rue; sage; savory, summer and winter; sweet bay; tansy; tarragon; thyme; wintergreen; woodruff; and wormwood.
Crop Subgroup 19B. Spice subgroup.	
Black pepper; and celery seed or dill seed.	Allspice; anise (seed); anise, star; annatto (seed); caper (buds); caraway; caraway, black; cardamom; cassia (buds); celery (seed); cinnamon; clove (buds); coriander (seed); culantro (seed); cumin; dill (seed); fennel, common; fennel, Florence (seed); fenugreek; grains of paradise; juniper (berry); lovage (seed); mace; mustard (seed); nutmeg; pepper, black; pepper, white; poppy (seed); saffron; and vanilla.

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(29) *Crop Group 20*. Oilseed Group.

(i) *Representative commodities*. Rapeseed (canola varieties only); sunflower, seed and cottonseed.

(ii) *Table*. The following Table 1 lists all the commodities listed in Crop Group 20 and identifies the related crop subgroups and includes cultivars and/or varieties of these commodities.

TABLE 1—CROP GROUP 20: OILSEED GROUP

Commodities	Related crop subgroups
Borage, *Borago officinalis* L	20A
Calendula, *Calendula officinalis* L	20B
Castor oil plant, *Ricinus communis* L	20B
Chinese tallowtree, *Triadica sebifera* (L.) Small	20B
Cottonseed, *Gossypium hirsutum* L. *Gossypium* spp	20C
Crambe, *Crambe hispanica* L.; *C. abyssinica* Hochst. ex R.E. Fr	20A
Cuphea, *Cuphea hyssopifolia* Kunth	20A
Echium, *Echium plantagineum* L	20A
Euphorbia, *Euphorbia esula* L	20B
Evening primrose, *Oenothera biennis* L	20B
Flax seed, *Linum usitatissimum* L	20A
Gold of pleasure, *Camelina sativa* (L.) Crantz	20A
Hare's ear mustard, *Conringia orientalis* (L.) Dumort	20A
Jojoba, *Simmondsia chinensis* (Link) C.K. Schneid	20B
Lesquerella, *Lesquerella recurvata* (Engelm. ex A. Gray) S. Watson	20A
Lunaria, *Lunaria annua* L	20A
Meadowfoam, *Limnanthes alba* Hartw. ex Benth	20A
Milkweed, *Asclepias* spp	20A
Mustard seed, *Brassica hirta* Moench, *Sinapis alba* L. subsp. *Alba*.	20A
Niger seed, *Guizotia abyssinica* (L.f.) Cass	20B
Oil radish, *Raphanus sativus* L. var. *oleiformis* Pers	20A
Poppy seed, *Papaver somniferum* L. subsp. *Somniferum*	20A
Rapeseed, *Brassica* spp.; *B. napus* L	20A
Rose hip, *Rosa rubiginosa* L	20B
Safflower, *Carthamus tinctorious* L	20B
Sesame, *Sesamum indicum* L., *S. radiatum* Schumach. & honn	20A
Stokes aster, *Stokesia laevis* (Hill) Greene	20B
Sunflower, *Helianthus annuus* L	20B
Sweet rocket, *Hesperis matronalis* L	20A
Tallowwood, *Ximenia americana* L	20B
Tea oil plant, *Camellia oleifera* C. Abel	20B
Vernonia, *Vernonia galamensis* (Cass.) Less	20B
Cultivars, varieties, and/or hybrids of these.	

(iii) *Table*. The following Table 2 identifies the crop subgroups for Crop Group 20, specifies the representative commodities for each subgroup and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 20: SUBGROUP LISTING

Representative commodities	Commodities
Crop subgroup 20A. Rapeseed subgroup	
Rapeseed, canola varieties only	Borage; crambe; cuphea; echium; flax seed; gold of pleasure; hare's ear mustard; lesquerella; lunaria; meadowfoam; milkweed; mustard seed; oil radish; poppy seed; rapeseed; sesame; sweet rocket cultivars, varieties, and/or hybrids of these.
Crop subgroup 20B. Sunflower subgroup	
Sunflower, seed	Calendula; castor oil plant; chinese tallowtree; euphorbia; evening primrose; jojoba; niger seed; rose hip; safflower; stokes aster; sunflower; tallowwood; tea oil plant; vernonia; cultivars, varieties, and/or hybrids of these.
Crop subgroup 20C. Cottonseed subgroup	
Cottonseed	Cottonseed; cultivars, varieties, and/or hybrids of these.

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(30) *Crop Group 21.* Edible fungi Group.

(i) *Representative commodities.* White button mushroom and any one oyster mushroom or any Shiitake mushroom.

(ii) *Table.* The following is a list of all the commodities in Crop Group 21. There are no related subgroups.

CROP GROUP 21—EDIBLE FUNGI GROUP—COMMODITIES

Blewitt (*Lepista nuda*)
Bunashimeji (*Hypsizygus marmoreus*)
Chinese mushroom (*Volvariella volvacea*) (Bull.) Singer
Enoki (*Flammulina velutipes*) (Curt.) Singer
Hime-Matsutake (*Agaricus blazei*) Murill
Hirmeola (*Auricularia auricular*)
Maitake (*Grifola frondosa*)
Morel (*Morchella spp.*)
Nameko (*Pholiota nameko*)
Net Bearing (*Dictyophora*)
Oyster mushroom (*Pleurotus spp.*)
Pom Pom (*Hericium erinaceus*)
Reishi mushroom (*Ganoderma lucidum* (Leyss. Fr.) Karst.)
Rodman's agaricus (*Agaricus bitorquis*) (Quel.) Saccardo
Shiitake mushroom (*Lentinula edodes* (Berk.) Pegl.)
Shimeji (*Tricholoma conglobatum*)
Stropharia (*Stropharia spp.*)
Truffle (*Tuber spp.*)
White button mushroom (*Agaricus bisporous* (Lange) Imbach)
White Jelly Fungi (*Tremella fuciformis*)

(31) *Crop Group 22.* Stalk, Stem and Leaf Petiole Vegetable Group.

(i) *Representative commodities.* Asparagus and celery.

(ii) *Commodities.* The following Table 1 lists all commodities included in Crop Group 22.

TABLE 1—CROP GROUP 22: STALK, STEM AND LEAF PETIOLE VEGETABLE GROUP

Commodities	Related crop subgroups
Agave (*Agave* spp.)	22A
Aloe vera (*Aloe vera* (L.) Burm.f.)	22A
Asparagus (*Asparagus officinalis* L.)	22A
Bamboo, shoots (*Arundinaria* spp.; *Bambusa* spp., *Chimonobambusa* spp.; *Dendrocalamus* spp., *Fargesia* spp.; *Gigantochloa* spp., *Nastus elatus; Phyllostachys* spp.; *Thyrsostachys* spp.)	22A
Cardoon (*Cynara cardunculus* L.)	22B
Celery (*Apium graveolens* var. *dulce* (Mill.) Pers.)	22B
Celery, Chinese (*Apium graveolens* L. var. *secalinum* (Alef.) Mansf.)	22B
Celtuce (*Lactuca sativa* var. *angustana* L.H. Bailey)	22A
Fennel, Florence, fresh leaves and stalk (*Foeniculum vulgare* subsp. *vulgare* var. *azoricum* (Mill.) Thell.)	22A
Fern, edible, fiddlehead	22A
Fuki (*Petasites japonicus* (Siebold & Zucc.) Maxim.)	22B
Kale, sea (*Crambe maritima* L.)	22A
Kohlrabi (*Brassica oleracea* L. var *gongylodes* L.)	22A
Palm hearts (various species)	22A
Prickly pear, pads (*Opuntia ficus-indica* (L.) Mill., *Opuntia* spp.)	22A
Prickly pear, Texas, pads (*Opuntia engelmannii* Salm-Dyck ex Engelm. var. *lindheimeri* (Engelm.) B.D. Parfitt & Pinkav)	22A
Rhubarb (*Rheum x rhabarbarum* L.)	22B
Udo (*Aralia cordata* Thunb.)	22B
Zuiki (*Colocasia gigantea* (Blume) Hook. f.)	22B
Cultivars, varieties, and hybrids of these commodities.	

(iii) *Crop subgroups.* The following Table 2 identifies the crop subgroups for Crop Group 22, specifies the representative commodities for each subgroup, and lists all the commodities included in each subgroup.

Appendix 4. 40 CFR 180.41 Crop Group Tables

TABLE 2—CROP GROUP 22: SUBGROUP LISTING

Representative commodities	Commodities
Crop Subgroup 22A. Stalk and stem vegetable subgroup	
Asparagus	Agave; aloe vera; asparagus; bamboo, shoots; celtuce; fennel, florence, fresh leaves and stalk; fern, edible, fiddlehead; kale, sea; kohlrabi; palm hearts; prickly pear, pads; prickly pear, Texas, pads; cultivars, varieties, and hybrids of these commodities.
Crop Subgroup 22B. Leaf petiole vegetable subgroup	
Celery	Cardoon; celery; celery, Chinese; fuki; rhubarb; udo; zuiki; cultivars, varieties, and hybrids of these commodities.

(32) *Crop Group 23.* Tropical and Subtropical Fruit, Edible Peel Group.

(i) *Representative commodities.* Date, fig, guava, and olive.

(ii) *Commodities.* The following Table 1 lists all commodities included in Crop Group 23.

TABLE 1—CROP GROUP 23: TROPICAL AND SUBTROPICAL FRUIT, EDIBLE PEEL GROUP

Commodities	Related crop subgroups
Açaí (*Euterpe oleracea* Mart.)	23C
Acerola (*Malpighia emarginata* DC.)	23A
Achachairú (*Garcinia gardneriana* (Planch. & Triana) Zappi)	23B
African plum (*Vitex doniana* Sweet)	23A
Agritos (*Berberis trifoliolata* Moric.)	23A
Almondette (*Buchanania lanzan* Spreng.)	23A
Ambarella (*Spondias dulcis* Sol. ex Parkinson)	23B
Apak palm (*Brahea dulcis* (Kunth) Mart.)	23C
Appleberry (*Billardiera scandens* Sm.)	23A
Arazá (*Eugenia stipitata* McVaugh)	23B
Arbutus berry (*Arbutus unedo* L.)	23A
Babaco (*Vasconcellea* x *heilbornii* (V.M. Badillo) V.M. Badillo)	23B
Bacaba palm (*Oenocarpus bacaba* Mart.)	23C
Bacaba-de-leque (*Oenocarpus distichus* Mart.)	23C
Bayberry, red (*Morella rubra* Lour.)	23A
Bignay (*Antidesma bunius* (L.) Spreng.)	23A
Bilimbi (*Averrhoa bilimbi* L.)	23B
Borojó (*Borojoa patinoi* Cuatrec.)	23B
Breadnut (*Brosimum alicastrum* Sw.)	23A
Cabeluda (*Plinia glomerata* (O. Berg) Amshoff)	23A
Cajou, fruit (*Anacardium giganteum* Hance ex Engl.)	23B
Cambucá (*Marlierea edulis* Nied.)	23B
Carandas-plum (*Carissa edulis* Vahl)	23A
Carob (*Ceratonia siliqua* L.)	23B
Cashew apple (*Anacardium occidentale* L.)	23B
Ceylon iron wood (*Manilkara hexandra* (Roxb.) Dubard)	23A
Ceylon olive (*Elaeocarpus serratus* L.)	23A
Cherry-of-the-Rio-Grande (*Eugenia aggregata* (Vell.) Kiaersk.)	23A
Chinese olive, black (*Canarium tramdenum* C.D. Dai & Yakovlev)	23A
Chinese olive, white (*Canarium album* (Lour.) Raeusch.)	23A
Chirauli-nut (*Buchanania latifolia* Roxb.)	23A
Ciruela verde (*Bunchosia armeniaca* (Cav.) DC.)	23B
Cocoplum (*Chrysobalanus icaco* L.)	23A
Date (*Phoenix dactylifera* L.)	23C
Davidson's plum (*Davidsonia pruriens* F. Muell.)	23B
Desert-date (*Balanltes aegyptiacus* (L.) Delile)	23A
Doum palm coconut (*Hyphaene thebaica* (L.) Mart.)	23C
False sandalwood (*Ximenia americana* L.)	23A
Feijoa (*Acca sellowiana* (O. Berg) Burret)	23B
Fig (*Ficus carica* L.)	23B
Fragrant manjack (*Cordia dichotoma* G. Forst.)	23A
Gooseberry, abyssinian (*Dovyalis abyssinica* (A. Rich.) Warb.)	23A
Gooseberry, Ceylon (*Dovyalis hebecarpa* (Gardner) Warb.)	23A
Gooseberry, Indian (*Phyllanthus emblica* L.)	23B
Gooseberry, otaheite (*Phyllanthus acidus* (L.) Skeels)	23A
Governor's plum (*Flacourtia indica* (Burm. F.) Merr.)	23A
Grumichama (*Eugenia brasiliensis* Lam)	23A

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TABLE 1—CROP GROUP 23: TROPICAL AND SUBTROPICAL FRUIT, EDIBLE PEEL GROUP—Continued

Commodities	Related crop subgroups
Guabiroba (*Campomanesia xanthocarpa* O. Berg)	23A
Guava (*Psidium guajava* L.)	23B
Guava berry (*Myrciaria floribunda* (H. West ex Willd.) O. Berg)	23A
Guava, Brazilian (*Psidium guineense* Sw.)	23A
Guava, cattley (*Psidium cattleyanum* Sabine)	23B
Guava, Costa Rican (*Psidium friedrichsthalianum* (O. Berg) Nied.)	23A
Guava, Para (*Psidium acutangulum* DC.)	23B
Guava, purple strawberry (*Psidium cattleyanum* Sabine var. *cattleyanum*)	23B
Guava, strawberry (*Psidium cattleyanum* Sabine var. *littorale* (Raddi) Fosberg)	23B
Guava, yellow strawberry (*Psidium cattleyanum* Sabine var. *cattleyanum* forma *lucidum* O. Deg.)	23B
Guayabillo (*Psidium sartorianum* (O. Berg) Nied.)	23A
Illawarra plum (*Podocarpus elatus* R. Br. Ex Endl.)	23A
Imbé (*Garcinia livingstonei* T. Anderson)	23B
Imbu (*Spondias tuberosa* Arruda ex Kost.)	23B
Indian-plum (*Flacourtia jangomas* (Lour.). basionym)	23A
Jaboticaba (*Myrciaria cauliflora* (Mart.) O. Berg)	23B
Jamaica-cherry (*Muntingia calabura* L.)	23A
Jambolan (*Syzygium cumini* (L.) Skeels)	23A
Jelly palm (*Butia capitata* (Mart.) Becc.)	23C
Jujube, Indian (*Ziziphus mauritiana* Lam.)	23B
Kaffir-plum (*Harpephyllum caffrum* Bernh. Ex C. Krauss)	23A
Kakadu plum (*Terminalia latipes* Benth. subsp. *psilocarpa* Pedley)	23A
Kapundung (*Baccaurea racemosa* (Reinw.) Mull. Arg.)	23A
Karanda (*Carissa carandas* L.)	23A
Kwai muk (*Artocarpus hypargyreus* Hance ex Benth.)	23B
Lemon aspen (*Acronychia acidula* F. Muell)	23A
Mangaba (*Hancomia speciosa* Gomes)	23B
Marian plum (*Bouea macrophylla* Griff.)	23B
Mombin, malayan (*Spondias pinnata* (J. Koenig ex L. f.) Kurz)	23B
Mombin, purple (*Spondias purpurea* L.)	23B
Mombin, yellow (*Spondias mombin* L.)	23A
Monkeyfruit (*Artocarpus lacucha* Buch. Ham.)	23B
Monos plum (*Pseudanamomis umbellulifera* (Kunth) Kausel)	23A
Mountain cherry (*Bunchosia cornifolia* Kunth)	23A
Nance (*Byrsonima crassifolia* (L.) Kunth)	23B
Natal plum (*Carissa macrocarpa* (Eckl.) A. DC.)	23B
Noni (*Morinda citrifolia* L.)	23B
Olive (*Olea europaea* L. subsp. *europaea*)	23A
Papaya, mountain (*Vasconcellea pubescens* A. DC.)	23B
Pataua (*Oenocarpus bataua* Mart.)	23C
Peach palm, fruit (*Bactris gasipaes* Kunth var. *gasipaes*)	23C
Persimmon, black (*Diospyros texana* Scheele)	23A
Persimmon, Japanese (*Diospyros kaki* Thunb.)	23B
Pitomba (*Eugenia luschnathiana* Klotzsch ex O. Berg)	23A
Plum-of-Martinique (*Flacourtia inermis* Roxb.)	23A
Pomerac (*Syzygium malaccense* (L.) Merr. & L.M. Perry)	23B
Rambai (*Baccaurea motleyana* (Mull. Arg.) Mull. Arg.)	23B
Rose apple (*Syzygium jambos* (L.) Alston)	23B
Rukam (*Flacourtia rukam* Zoll. & Moritizi)	23A
Rumberry (*Myrciaria dubia* (Kunth) McVaugh *Myrtaceae*)	23A
Sea grape (*Coccoloba uvifera* (L.) L.)	23A
Sentul (*Sandoricum koetjape* (Burm. F.) Merr.)	23B
Sete-capotes (*Campomanesia guazumifolia* (Cambess.) O. Berg)	23A
Silver aspen (*Acronychia wilcoxian* (F. Muell.) T.G. Hartley)	23A
Starfruit (*Averrhoa carambola* L.)	23B
Surinam cherry (*Eugenia uniflora* L.)	23B
Tamarind (*Tamarindus indica* L.)	23B
Uvalha (*Eugenia pyriformis* Cambess)	23B
Water apple (*Syzygium aqueum* (Burm. F.) Alston)	23A
Water pear (*Syzygium guineense* (Willd.) DC)	23A
Water berry (*Syzygium cordatum* Hochst. Ex C. Krauss)	23A
Wax jambu (*Syzygium samarangense* (Blume) Merr. & L.M. Perry)	23A
Cultivars, varieties, and hybrids of these commodities.	

(iii) *Table*. The following Table 2 identifies the crop subgroups for Crop Group 23, specifies the representative commodities for each subgroup, and lists all the commodities included in each subgroup.

Appendix 4. 40 CFR 180.41 Crop Group Tables

TABLE 2—CROP GROUP 23: SUBGROUP LISTING

Representative commodities	Commodities
Crop Subgroup 23A. Tropical and Subtropical, Small fruit, edible peel subgroup	
Olive	Acerola; African plum; agritos; almondette; appleberry; arbutus berry; bayberry, red; bignay; breadnut; cabeluda; carandas-plum; Ceylon iron wood; Ceylon olive; cherry-of-the-Rio-Grande; Chinese olive, black; Chinese olive, white; chirauli-nut; cocoplum; desert-date; false sandalwood; fragant manjack; gooseberry, abyssinian; gooseberry, Ceylon; gooseberry, otaheite; governor's plum; grumichama; guabiroba; guava berry; guava, Brazilian; guava, Costa Rican; guayabillo; illawarra plum; Indian-plum; Jamaica-cherry; jambolan; kaffir-plum; kakadu plum; kapundung; karanda; lemon aspen; mombin, yellow; monos plum; mountain cherry; olive; persimmon, black; pitomba; plum-of-Martinique; rukam; rumberry; sea grape; sete-capotes; silver aspen; water apple; water pear; water berry; wax jambu; cultivars, varieties, and hybrids of these commodities.
Crop Subgroup 23B. Tropical and Subtropical, Medium to large fruit, edible peel subgroup	
Fig and guava	Achachairú; ambarella; arazá; babaco; bilimbi; borojó; cajou, fruit; cambucá; carob; cashew apple; ciruela verde; davidson's plum; feijoa; fig; gooseberry, Indian; guava; guava, cattley; guava, Para; guava, purple strawberry; guava, strawberry; guava, yellow strawberry; imbé; imbu; jaboticaba; jujube, Indian; kwai muk; mangaba; Marian plum; mombin, Malayan; mombin, purple; monkeyfruit; nance; natal plum; noni; papaya, mountain; persimmon, Japanese; pomerac; rambai; rose apple; sentul; starfruit; Surinam cherry; tamarind; uvalha; cultivars, varieties, and hybrids of these commodities.
Crop Subgroup 23C. Tropical and Subtropical, Palm fruit, edible peel subgroup	
Date	Açaí; apak palm; bacaba palm; bacaba-de-leque; date; doum palm coconut; jelly palm; patauá; peach palm, fruit; cultivars, varieties, and hybrids of these commodities.

(33) *Crop Group 24*. Tropical and Subtropical Fruit, Inedible Peel Group.

(i) *Representative commodities*. Atemoya or sugar apple, avocado, banana or pomegranate, dragon fruit, lychee, passionfruit, pineapple, and prickly pear, fruit.

(ii) *Commodities*. The following Table 1 lists all commodities included in Crop Group 24.

TABLE 1—CROP GROUP 24: TROPICAL AND SUBTROPICAL FRUIT, INEDIBLE PEEL GROUP

Commodities	Related crop subgroups
Abiu (*Pouteria caimito* (Ruiz & Pav.) Radlk)	24B
Aisen (*Boscia senegalensis* (Pers.) Lam.)	24A
Akee apple (*Blighia sapida* K.D. Koenig)	24B
Atemoya (*Annona cherimola* Mill. X A. squamosa L.)	24C
Avocado (*Persea americana* Mill.)	24B
Avocado, Guatemalan (*Persea americana* Mill. var. *guatemalensis*)	24B
Avocado, Mexican (*Persea americana* Mill. var. *drymifolia* (Schltdl. & Cham.) S.F. Blak)	24B
Avocado, West Indian (*Persea americana* var. *americana*)	24B
Bacury (*Platonia insignis* Mart.)	24B
Bael fruit (*Aegle marmelos* (L.) Corrêa)	24A
Banana (*Musa* spp.)	24B
Banana, dwarf (*Musa* hybrids; *Musa acuminata* Colla)	24B
Binjai (*Mangifera caesia* Jack)	24B
Biriba (*Annona mucosa* Jacq.)	24C
Breadfruit (*Artocarpus altilis* (Parkinson) Fosberg)	24C
Burmese grape (*Baccaurea ramiflora* Lour.)	24A
Canistel (*Pouteria campechiana* (Kunth) Baehni)	24B
Cat's-eyes (*Dimocarpus longan* Lour. subsp. *malesianus* Leenh.)	24A
Champedak (*Artocarpus integer* (Thunb.) Merr.)	24C
Cherimoya (*Annona cherimola* Mill.)	24C
Cupuacú (*Theobroma grandiflorum* (Willd. Ex Spreng.) K. Schum.)	24B
Custard apple (*Annona reticulata* L.)	24C
Dragon fruit (*Hylocereus undatus* (Haw.) Britton & Rose)	24D
Durian (*Durio zibethinus* L.)	24C

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TABLE 1—CROP GROUP 24: TROPICAL AND SUBTROPICAL FRUIT, INEDIBLE PEEL GROUP—Continued

Commodities	Related crop subgroups
Elephant-apple (*Limonia acidissima* L.)	24C
Etambe (*Mangifera zeylanica* (Blume) Hook. F.)	24B
Granadilla (*Passiflora ligularis* Juss.)	24E
Granadilla, giant (*Passiflora quadrangularis* L.)	24E
Ilama (*Annona macroprophyllata* Donn. Sm.)	24C
Ingá (*Inga vera* Willd. subsp. *affinis* (DC.) T.D. Penn.)	24A
Jackfruit (*Artocarpus heterophyllus* Lam.)	24C
Jatobá (*Hymenaea courbaril* L.)	24B
Karuka (*Pandanus julianettii* Martelli)	24C
Kei apple (*Dovyalis caffra* (Hook. F. & Harv.) Warb.)	24B
Langsat (*Lansium domesticum* Corrêa)	24B
Lanjut (*Mangifera lagenifera* Griff.)	24B
Longan (*Dimocarpus longan* Lour.)	24A
Lucuma (*Pouteria lucuma* (Ruiz & Pav.) Kuntze)	24B
Lychee (*Litchi chinensis* Sonn.)	24A
Mabolo (*Diospyros blancoi* A. DC.)	24B
Madras-thorn (*Pithecellobium dulce* (Roxb.) Benth.)	24A
Mammy-apple (*Mammea americana* L.)	24C
Manduro (*Balanites maughamii* Sprague)	24A
Mango (*Mangifera indica* L.)	24B
Mango, horse (*Mangifera foetida* Lour.)	24B
Mango, Saipan (*Mangifera odorata* Griff.)	24B
Mangosteen (*Garcinia mangostana* L.)	24B
Marang (*Artocarpus odoratissimus* Blanco)	24C
Marmaladebox (*Genipa americana* L.)	24C
Matisia (*Matisia cordata* Humb. & Bonpl.)	24A
Mesquite (*Prosopis juliflora* (Sw.) DC.)	24A
Mongongo, fruit (*Schinziophyton rautanenii* (Schlnz) Radcl.-Sm)	24A
Monkey-bread-tree (*Adansonia digitata* L.)	24C
Monstera (*Monstera deliciosa* Liebm.)	24E
Nicobar-breadfruit (*Pandanus leram* Jones ex Fontana)	24C
Paho (*Mangifera altissima* Blanco)	24B
Pandanus (*Pandanus utilis* Bory)	24C
Papaya (*Carica papaya* L.)	24B
Passionflower, winged-stem (*Passiflora alata* Curtis)	24E
Passlonfruit (*Passiflora edulis* Sims)	24E
Passionfruit, banana (*Passiflora tripartita* var. *mollissima* (Kunth) Holm-Niels. & P. Jorg.)	24E
Passionfruit, purple (*Passiflora edulis* Sims forma *edulis*)	24E
Passlonfruit, yellow (*Passiflora edulis* Sims forma *flavicarpa* O. Deg.)	24E
Pawpaw, common (*Asimina triloba* (L.) Dunal)	24B
Pawpaw, small-flower (*Asimina parviflora* (Michx.) Dunal)	24A
Pelipisan (*Mangifera casturi* Kosterm.)	24B
Pequi (*Caryocar brasiliense* Cambess)	24B
Pequia (*Caryocar villosum* (Aubl.) Pers.)	24B
Persimmon, American (*Diospyros virginiana* L.)	24B
Pineapple (*Ananas comosus* (L.) Merr.)	24C
Pitahaya (*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose)	24D
Pitaya (*Hylocereus* sp. including *H. megalanthus* (*H. ocamponis* and *H. polychizus*)	24D
Pitaya, amarilla (*Hylocereus triangularis* Britton & Rose)	24D
Pitaya, roja (*Hylocereus ocamponis* (Salm-Dyck) Britton & Rose)	24D
Pitaya, yellow (*Hylocereus megalanthus* (K. Schum. ex Vaupel) Ralf Bauer)	24D
Plantain (*Musa paradisiaca* L.)	24B
Pomegranate (*Punica granatum* L.)	24B
Poshte (*Annona liebmanniana* Baill.)	24B
Prickly pear, fruit (*Opuntia ficus-indica* (L.) Mill.)	24D
Prickly pear, Texas, fruit (*Opuntia engelmannii* Salm-Dyck ex Engelm. var. *lindheimeri* (Engelm.) B.D. Parfitt & Pinkav)	24D
Pulasan (*Nephelium ramboutan-ake* (Labill.) Leenh.)	24C
Quandong (*Santalum acuminatum* (R. Br.) DC.)	24B
Rambutan (*Nephelium lappaceum* L.)	24C
Saguaro (*Carnegiea gigantea* (Engelm.) Britton & Rose)	24D
Sapodilla (*Manilkara zapota* (L.) P. Royen)	24C
Sapote, black (*Diospyros digyna* Jacq.)	24B
Sapote, green (*Pouteria viridis* (Pittier) Cronquist)	24B
Sapote, mamey (*Pouteria sapota* (Jacq.) H.E. Moore & Steam)	24C
Sapote, white (*Casimiroa edulis* La Llave & Lex)	24B
Sataw (*Parkia speciosa* Hassk.)	24B
Satinleaf (*Chrysophyllum oliviforme* L.)	24A
Screw-pine (*Pandanus tectorius* Parkinson)	24B
Sierra Leone-tamarind (*Dialium guineense* Willd.)	24A
Soncoya (*Annona purpurea* Moc. & Sessé ex Dunal)	24C

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TABLE 1—CROP GROUP 24: TROPICAL AND SUBTROPICAL FRUIT, INEDIBLE PEEL GROUP—Continued

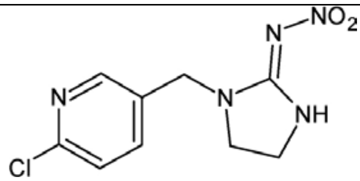
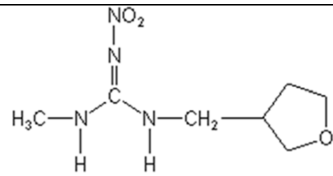
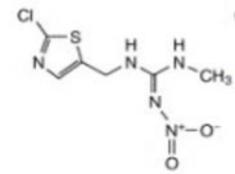
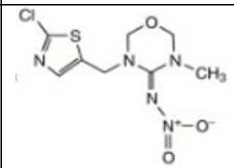
Commodities	Related crop subgroups
Soursop (*Annona muricata* L.)	24C
Spanish lime (*Melicoccus bijugatus* Jacq.)	24A
Star apple (*Chrysophyllum cainito* L.)	24B
Sugar apple (*Annona squamosa* L.)	24C
Sun sapote (*Licania platypus* (Hemsl.) Fritsch)	24C
Tamarind-of-the-Indies (*Vangueria madagascariensis* J.F. Gmel.)	24B
Velvet tamarind (*Dialium indum* L.)	24A
Wampi (*Clausena lansium* (Lour.) Skeels)	24A
White star apple (*Chrysophyllum albidum* G. Don)	24A
Wild loquat (*Uapaca kirkiana* Müll. Arg.)	24B
Cultivars, varieties, and hybrids of these commodities.	

(iii) *Table*. The following Table 2 identifies the crop subgroups for Crop Group 24, specifies the representative commodities for each subgroup, and lists all the commodities included in each subgroup.

TABLE 2—CROP GROUP 24: SUBGROUP LISTING

Representative commodities	Commodities
Crop Subgroup 24A. Tropical and Subtropical, Small fruit, inedible peel subgroup	
Lychee	Aisen; baei fruit; Burmese grape; cat's-eyes; ingá; longan; lychee; madras-thorn; manduro; matisia; mesquite; mongongo, fruit; pawpaw, small-flower; satinleaf; Sierra Leone-tamarind; Spanish lime; velvet tamarind; wampi; white star apple; cultivars, varieties, and hybrids of these commodities.
Crop Subgroup 24B. Tropical and Subtropical, Medium to large fruit, smooth, inedible peel subgroup	
Avocado, plus pomegranate or banana	Abiu; akee apple; avocado; avocado, Guatemalan; avocado, Mexican; avocado, West Indian; bacury; banana; banana, dwarf; binjai; canistel; cupuacú; etambe; jatobá; kei apple; langsat; lanjut; lucuma; mabolo; mango; mango, horse; mango, Saipan; mangosteen; paho; papaya; pawpaw, common; pelipisan; pequi; pequia; persimmon, American; plantain; pomegranate; poshte; quandong; sapote, black; sapote, green; sapote, white; sataw; screw-pine; star apple; tamarind-of-the-Indies; wild loquat; cultivars, varieties, and hybrids of these commodities.
Crop Subgroup 24C. Tropical and Subtropical, Medium to large fruit, rough or hairy, inedible peel subgroup	
Pineapple, plus atemoya or sugar apple	Atemoya; biriba; breadfruit; champedak; cherimoya; custard apple; durian; elephant-apple; ilama; jackfruit; karuka; mammy-apple; marang; marmaladebox; monkey-bread tree; nicobar-breadfruit; pandanus; pineapple; pulasan; rambutan; sapodilla; sapote, mamey; soncoya; soursop; sugar apple; sun sapote; cultivars, varieties, and hybrids of these commodities.
Crop Subgroup 24D. Tropical and Subtropical, Cactus, inedible peel subgroup	
Dragon fruit and Prickly pear fruit	Dragon fruit; pitahaya; pitaya; pitaya, amarilla; pitaya, roja; pitaya, yellow; prickly pear, fruit; prickly pear, Texas, fruit; saguaro; cultivars, varieties, and hybrids of these commodities.
Crop Subgroup 24E. Tropical and Subtropical, Vine, inedible peel subgroup	
Passionfruit	Granadilla; granadilla, giant; monstera; passionflower, winged-stem; passionfruit, passionfruit, banana; passionfruit, purple; passionfruit, yellow cultivars varieties, and hybrids of these commodities.

Appendix 5. Physicochemical Properties of Imidacloprid, Clothianidin, Thiamethoxam, and Dinotefuran

Property	Imidacloprid	Dinotefuran	Clothianidin	Thiamethoxam
Chemical Structure: IUPAC Name[a]	 N-{1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl}nitramide	 N-methyl-N'-nitro-N''-[(tetrahydro-3-furanyl)methyl)]guanidine	 (E)-1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine	 3-(2-Chloro-thiazolyl-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidene-N-nitroamine
Molecular Formula[a]	$C_9H_{10}ClN_5O_2$	$C_7H_{14}N_4O_3$	$C_6H_8ClN_5O_2S$	$C_8H_{10}ClN_5O_3S$
Molecular Weight (CAS No.)[a]	255.7 g/mole (13826-41-3)	202.2 g/mol (165252-70-0)	249.7 g/mole (210880-92-5)	291.7 g/mole (153719-23-4)
Water Solubility (WS)[a]	580 mg/L@ 20 °C	39,830 mg/L@ 20 °C	327 mg/L@ 20 °C	4100 mg/L @ 25 °C
Octanol: Water Coefficient (K_{ow})[a]	3.7 @ 21 °C	0.283 @ 25 °C	4.4 (EPISuite v4.11)	0.74 @ 25 °C
Soil Adsorption Coefficient (K_{oc})[a]	277 – 411 mL/g	6 – 45 mL/g	84 – 345 mL/g	33 – 177 mL/g
Henry's Law Constant (H)[a]	9.9 x 10^{-13} atm m^3 mol^{-1}	8.63 x 10-14 atm m ^{3} mol^{-1}	2.9 x 10^{-16} atm m^3 mol^{-1}	4.63 x 10^{-15} atm m^3 mol^{-1}
Vapor Pressure (VP)[b]	1 x 10-7 mmHg @ 20 ^{o}C	1.28 x 10-8 mmHg @ 30 ^{o}C	1 x 10-7 mmHg @ 20 ^{o}C	5 x 10-11 Hg @ 25 ^{o}C
Terrestrial Field Dissipation Half-Life (TFD)[b]	27 - 146 Days	23 - 77 Days	282 - >982 Days	83-91 Days

a. Data obtained from the following U.S. EPA preliminary pollinator assessments:
- U.S. EPA, & DPR. (2015). Preliminary pollinator assessment to support the registration review of imidacloprid. Report Number EPA-HQ-OPP-2008-0844-0140. Washington, D.C.: U.S. EPA.
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b. DPR, 2018. DPR Pesticide Chemistry Database. Department of Pesticide Regulation Environmental Monitoring Branch Internal Website. Accessed: April 30, 2018.

Appendix 6. Tier I Toxicity Values for Imidacloprid, Clothianidin, Thiamethoxam, and Dinotefuran

Endpoints Used in Tier I Risk Determinations

	Adult Acute Contact LD_{50}	Adult Acute Oral LD_{50}	Adult Chronic Oral NOEL / LOEL	Larval Acute LD_{50}	Larval Chronic NOEL / LOEL
Imidacloprid	0.043 µg ai/bee (96 hours) [a]	0.0039 µg ai/bee (48 hours) [a]	0.00016 / 0.00024 µg ai/bee (10 Days) [a]	> 0.0018 µg ai/larva/day (7 Days) [b]	0.0018 / > 0.0018 µg ai/larva/day (21 Days) [a]
Clothianidin	0.0275 µg ai/bee (48 hours) [c]	0.00368 µg ai/bee (48 hours) [c]	0.00036 / 0.00072 µg ai/bee/day (10 Days) [c]	> 0.0018 µg ai/larva/day (7 Days) [c]	0.0009 / 0.0018 µg ai/larva/day (22 Days) [c]
Thiamethoxam	0.024 µg ai/bee (96 hours) [d]	0.0044 µg ai/bee (48 hours) [d]	0.00245 / (unknown) µg ai/bee/day (10 Days) [d]	Unknown	0.0157 / (unknown) µg ai/larva /day (22 Days) [d]
Dinotefuran	0.024 µg ai/bee (96 hours) [e]	0.0076 µg ai/bee (48 hours) [e]	0.0015 / 0.0035 µg ai/bee/day (10 Days) [e]	> 3.75 µg ai/larva/day (8 Days) [f]	3.75 / > 3.75 µg ai/larva/day (22 Days) [e]

Notes:

- *Adult acute contact and oral toxicity tests are single exposure tests followed by observation periods. A 48 hour observation period is standard, but the observation period must be extended if there is an increase in mortality from 24 to 48 hours (OCSPP 850.3020; OECD 213).*
- *In the adult chronic oral tests bees are fed a diet containing the test chemical continuously for ten days (OECD 245).*
- *The larval acute and larval chronic toxicity endpoints are derived from a single toxicity test in which larvae are exposed to the test chemical on Days 4, 5, and 6 (three days of exposure), and then observed until emergence (Day 22). Mortality is assessed at 8 days to determine an 8-day LD_{50}. Emergence (the inverse of mortality) is assessed at 22 days to determine a 22-day NOEL (OECD 239).*

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[a] 2016, U.S. Environmental Protection Agency, California Department of Pesticide Regulation, Preliminary Pollinator Assessment to Support the Registration Review of Imidacloprid, EPA-HQ-OPP-2008-0844-0140.

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[c] 2017, Pest Management Regulatory Agency, Health Canada, Proposed Re-evaluation Decision, Clothianidin and Its Associated End-use Products: Pollinator Re-evaluation, PRVD2017-23.

[d] 2017, Pest Management Regulatory Agency, Health Canada, Proposed Re-evaluation Decision, Thiamethoxam and Its Associated End-use Products: Pollinator Re-evaluation, PRVD2017-24.

[e] 2017, U.S. Environmental Protection Agency, Draft Assessment of the Potential Effects of Dinotefuran on Bees, EPA-HQ-OPP-2011-0920-0014.

[f] 2016, Patnaude, M.R., Dinotefuran Technical Grade: 22-Day Survival of Honey Bee Larvae, Apis mellifera L., during an In Vitro Exposure; Unpublished study prepared by Smithers Viscient; Laboratory Report ID: 10934.6161; MRID: 49860001.

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Data Evaluation Report

Study Titles:

Bocksch, S. (2014): Honey bee brood and colony level effects following imidacloprid intake via treated artificial diet in a field study in North Carolina

Final Report Source:
Eurofins Agroscience Services Inc., unpublished report No: S13-03176, 06 Nov 2014

Year of study: 2013-2014

PMRA#: 2474495
PMRA DACO#: 9.2.4.9
MRID: 49510001

Study Type:
Tier II colony feeding study conducted in an open field

Review Date (final): January 28, 2016

PMRA reviewer:
Primary Evaluator: Wayne Hou
Data Statistical Analysis: Keith O'Rourke

EPA reviewer:
Primary Evaluators: Justin Housenger, Biologist
Keith G. Sappington, Senior Science Advisor
Data Statistical Analysis: Christine Hartless, Wildlife Biologist

CDPR reviewer:
Primary Evaluators: Richard Bireley, Sr. Environmental Scientist (Specialist)
Alexander Kolosovich, Environmental Scientist
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Data Statistical Analysis: John Troiano, Ph.D., Research Scientist III

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

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Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Executive Summary

A colony feeding study was conducted with honey bees to assess the potential for long-term effects, including overwintering survival, resulting from exposure to imidacloprid. The study was conducted in twelve test areas of low agricultural cultivation (Apiaries A – L) in North Carolina from June 21, 2013 to March 24, 2014. Eighty-four hives were divided according to hive strength (number of brood frames) with the strongest 7 hives ssigned to Apiary A and the weakest 7 hives assigned to Apiary L. Within each apiary, the 7 hives were randomly assigned to treatment groups.

At each apiary, five test hives were artificially fed with 50% sugar solution spiked with imidacloprid at 12.5, 25, 50, 100 or 200 µg ai/L for six weeks continuously in the field, with two hives at each apiary serving as controls. Assuming the density of a 50% sugar solution is 1.2296 g/ml, the reviewer calculated that the test concentrations at 12.5, 25, 50, 100, and 200 µg/L are equivalent to 10.2, 20.3, 40.7, 81.3, and 162.7 ppb (µg/kg), respectively. The average measured ppb (µg/kg) concentrations in dosing solutions was confirmed to be 11.0, 23.3, 46.7, 96.3, 189.6 ppb (µg/kg), respectively.

Eight Colony Condition Assessments (CCAs) were conducted during the study. Three CCAs (CCA1 - 3) were conducted prior to feeding to determine hive strength and initial hive conditions. A CCA was conducted during exposure with another one conducted one week after termination of exposure (CCA4 and CCA5, respectively) which characterize hive conditions during exposure. Two more CCAs were conducted at 5 and 10 weeks after exposure (CCA6 and CCA7, respectively) to assess the chronic effect following exposure to imidacloprid and to characterize pre-overwintering hive conditions. A final CCA was conducted after overwintering in March 2014 (CCA8) to assess potential exposure impact on survival and chronic colony level effects. Multiple parameters, such as hive weight, number of individuals at different life stages in the hive, hive honey and pollen stores, and hive overwintering survival, were measured during the course of the study.

Levels of imidacloprid residues in hives were measured before, during and after the feeding exposure. Potential contamination of pesticides from other food sources was monitored using pollen collected in additional hives at each apiary that served as monitoring hives. The results showed that while there were a few instances of imidacloprid detected in the pollen and nectar of the control hives, the frequency and magnitude of these detections is not expected to confound the results of this study. Residues measured in hive matrices demonstrated that higher treatment exposures corresponded well to higher residues in hive matrices. There were individual hive variations in measured residues, with some overlap in measured hive concentrations, particularly at the lower doses. This variability likely originates from the limited spatial and temporal sampling methodology (*i.e.* one sample from one side of the comb on one frame to represent a hive, and only at 3 CCAs) employed for this study. Overall during the exposure period, imidacloprid concentration in hive nectar averaged 63.7% of the nominal concentration in the feeding solution, whereas imidacloprid concentration in hive pollen averaged 30.2% of the nominal concentration in feeding solution. This dilution is expected since bees could forage on outside pollen and nectar sources, and hive pollen (bee bread) includes only some nectar. See **Section 3.7** for more details regarding the residues of imidacloprid in the dosing solutions and hive matrices.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

During the review of the study, a joint review effort was conducted by the United States Environmental Protection Agency (EPA), Health Canada's Pest Management Regulatory Agency (PMRA), and the California Department of Pesticide Regulation (CDPR). As part of that effort, a separate statistical analysis was conducted by each regulatory entity as an independent verification of the results from the analysis provided by the registrant. These analyses (described in detail in **Appendices A, B,** and **C** for EPA's, PMRA's and CDPR's approaches, respectively) were distinct in approach but generally yielded similar statistical results. When weighing these results as well as biological concerns, particularly as they relate to honey bee biology at the colony level, EPA, PMRA, and CDPR arrived at the same conclusions and are therefore harmonized in terms of the determination of an overall No Observed Adverse Effect Concentration (NOAEC) and Lowest Observed Adverse Effect Concentration (LOAEC) for this study.

As will be discussed in **Section 3 (Results)** the PMRA, EPA, and CDPR analyses determined statistically significant imidacloprid dose-related effects in the 50, 100, and 200 µg/L treatment groups across multiple CCAs for the majority of response variables. Indeed, for the 100 and 200 µg/L treatment groups, significant effects ($p<0.05$) were determined for every response variable, persisted across multiple CCAs and eventually resulted in loss of nearly all hives in those treatment groups after the overwintering period. The 50 µg/L treatment group also showed significant effects for multiple response variables across multiple CCAs, and poorer colony condition in surviving hives after overwintering in comparison to controls.

Conversely, there was not a strong indication from the PMRA, EPA, and CDPR analyses of an impact at the colony level for the 12.5 and 25 µg/L treatment groups for individual life stages. This is evidenced not only by a general lack of statistically significant effects ($p>0.1$) at these treatment levels but in cases where significant effects were determined, they either did not show strong dose-responsiveness, did not persist across multiple CCAs, or were considered potential transient effects which did not persist after overwintering. This latter point was the case for the statistically significant effects noted at CCA6, which included pupal cell and total individual effects for which the PMRA analyses determined significant effects at all treatment levels, and EPA determined significant effects for pupae at 12.5, 100 and 200 µg/L (but not at 25 or 50 µg a.i/L). As well at CCA6, PMRA determined significant effects with eggs and larvae at 25 µg/L treatment (but not at the 50 µg/L). For the two lowest treatment groups (12.5 and 25 µg/L), the colony condition of surviving hives at CCA8 following overwintering was similar to controls, indicating the effects observed at CCA6 were likely transient and the colony was able to compensate for these effects.

When examining the effects on food stores (pollen and nectar), the PMRA, EPA, and CDPR analyses did not determine any consistent and significant reductions in pollen and nectar stores at the 12.5 and 25 µg/L treatment groups. This is distinguished from the 50 µg/L group where effects on nectar in particular were very apparent, when compared alongside the response of the control, in terms of the level of nectar buildup before hive preparation for overwintering at CCA7. This finding was also confirmed statistically in all three analyses with significant reductions in honey stores at CCAs 6, 7, and 8 (CCA8 data excluded from the EPA analysis for the 100 and 200 µg/L groups). Significant reductions in pollen stores were also confirmed at CCAs 4 and 5 (*i.e.* during the exposure period) at the 50 µg/L treatment group.

Specifically, when considering the proportion of adults as well as honey and pollen stores response variables, the differences from control were apparent both visually and statistically, particularly in the three highest treatment groups. For the proportion of adults, the onset of a decline in numbers occurred one CCA earlier in these groups than in the control, 12.5 and 25 µg/L treatment groups. For honey stores, the buildup that occurred starting at CCA5 in the 50 µg/L treatment group, reached only half the level reached in the control, 12.5, and 25 µg/L treatment groups by CCA7. Pollen stores were also reduced at CCA4 and CCA5 compared to controls for the three highest treatment groups, as well as at CCA6 and CCA7 at the highest treatment group. These effects were statistically significant ($p<0.05$) and indicate that the 50 µg/L treatment group was associated with trends and proportions of abundance for life stages and food stores not observed in the control, 12.5, and 25 µg/L treatment groups.

The study is considered to be informative and will be used as a line of evidence in the pollinator risk assessment. While there were uncertainties that were generally related to inherent aspects of any semi-field or full field study design (described in the section below) this study still provides information on a number of colony health parameters about the long term (including overwintering) exposure to imidacloprid at the colony level. **When weighing biological significance and the natural seasonal changes of honey bee colonies, as well as supporting conclusions from the statistical approaches used by PMRA, EPA, and CDPR, the NOAEC and LOAEC for this study are determined to be 25 and 50 µg/L, respectively.**

Consideration of Study Strengths, Limitations and Interpretation

It is important to recognize the inherent strengths and limitations of this study as results are interpreted and potentially considered in risk assessment.

In the context of available field studies involving honey bees and imidacloprid, this study contains a number of strengths including:

- Use of a high degree of replication (n=12) to achieve a reasonable level of statistical power
- Demonstration of a generalized concentration-response relationship with respect to the concentration of imidacloprid in sucrose solution and the magnitude and duration of adverse effects
- Quantification of exposure to parent (imidacloprid) and toxicologically-relevant metabolites in diet and in hive matrices (uncapped nectar, pollen, honey, bee bread)
- Use of a 6-week exposure duration to represent a "high end" exposure scenario
- Inclusion of multiple colony-level endpoints reflecting hive strength, brood development and food stores
- Detailed QA/QC results regarding quantification of chemical residues in various matrices
- Availability of raw data for conducting statistical analysis.

A number of limitations are also noted with this study, including:

- Exposure of bees to imidacloprid occurred through nectar (sucrose) alone, whereas bees in the field are likely exposed through both pollen and nectar routes. Therefore, the design of this study may not reflect a "worst case" exposure scenario in which bees are experiencing prolonged exposure to both contaminated nectar and pollen. While exclusion of the pollen route is expected to reduce overall exposure, the impact of this exclusion on the study results is uncertain and will likely depend on the life stage/caste of bee.

- It is noted that imidacloprid was found in both hive nectar and hive pollen (beebread), at concentrations lower than the feeding solutions. Dilution compared to the treatment feeding solution is expected since bees could also forage on outside nectar and pollen sources. Additionally, hive pollen contains only some hive nectar, thus would not be expected to have a concentration equivalent to nectar alone, and it is mixed with pollen which will come from outside sources. Therefore exposure through both hive pollen and nectar occurred via exposure to the sucrose feeding solution, but how this compares to exposure through contaminated pollen directly is not known. A recent paper by Dively (2015)[1] showed that higher residues throughout the hive resulted from feeding pollen treatments compared to feeding sucrose solution treatments. It is also noted that while nectar is considered the dominant exposure route for forager bees; other hive bees and larvae consume both nectar and pollen. In addition, since bees were forced to forage for pollen in this study, the potential impact of imidacloprid exposure on reducing pollen foraging efficiency of bees could be incorporated into the overall expression of adverse effects, as suggested by published literature. Had contaminated pollen been provided to bees, it is not known if the potential impact on pollen foraging efficiency would have been masked.

- The quantity of nectar provided to hives (2 L per week per hive) likely did not fulfill the complete carbohydrate needs of the colony, as indicated by colony bioenergetics and the lack of remaining sucrose solution upon their renewal. This suggests that bees could be exposed to a greater dose of imidacloprid in nectar had a greater volume of spiked sucrose been provided. Although one can infer that the dosing regimen may have underestimated exposure through sucrose relative to 100% contaminated diet, it is also noted that bees had to supplement their spiked sucrose by foraging on their own for other sources of nectar. As with the previous discussion of pollen, it is noted that had 100% of the carbohydrate needs of the colony been provided via feeders, the potential impact of purported reductions in nectar foraging efficiency may have been masked to some degree.

- Overwintering success of controls was impacted (36% hive mortality). This may have reduced the ability to detect adverse effects related to hive loss following overwintering. Although comparable to overwintering losses of commercial beekeepers, it is possible that elements of the study design may have contributed to this loss (e.g., lack of supers to allow for colony growth, delayed supplemental feeding during fall).

- Hive detections with pesticides from food sources other than the artificial feeding was detected during the exposure period and post-exposure periods through collection of pollen from pollen traps. Although the study was deliberately conducted in a low agricultural area in order to minimize the potential for pesticide contamination from other sources, the bees still appeared to be foraging on contaminated pollen and possibly nectar. During both

[1] Dively GP, Embrey MS, Kamel A, Hawthorne DJ, Pettis JS (2015) Assessment of Chronic Sublethal Effects of Imidacloprid on Honey Bee Colony Health. PLoS ONE 10(4): e0126043. doi: 10.1371/journal.pone.0126043

exposure and post-exposure periods, a high level of multiple pesticides that may cause concern for bees were detected in most monitoring hives, such as spiromesifen (maximum at 961 ppb) and piperonyl butoxide (maximum at 591 ppb). Trace amounts of other bee-toxic pesticides, such as chlorpyifos (LOD = 1.0 ppb) and malathion (LOD = 4.0 ppb) were also detected. The test chemical imidacloprid was found at 12.1 ppb in pollen from one (apiary L) of the total of six sites analysed. This level is similar to one of the test concentrations.

- Residues of imidacloprid in uncapped nectar and bee bread within the hives at CCAs 4, 5, and 8 represent a single sample per hive on a single frame rather than a composite sample from multiple portions of the comb within a hive. This means that residue results may reflect "hit or miss" scenario with respect to detecting residues in nectar laid down from contaminated (fed) vs. outside sources.

- The exposure, based on residues measured in the hive (hive nectar and hive pollen) indicated that, overall, higher measured hive residues correlated with higher nominal residues in feeding solutions. However, individual hive residue values varied, and there was some overlap in measured values, particularly among the three lowest doses. Given the limited spatial and temporal sampling methodology (as mentiond above), there is uncertainy in whether these residues represent actual in hive residues across all portions of the frame. Specifically, one sample of one area of the comb on one side of the frame to represent the nectar or pollen residues of an entire hive may not reflect the true nature of the residues across all portions of a given hive.

- Exposure dilution during the study was evident. Pollen storage was observed consistently in the control hives and hives exposed to lower test concentrations during the exposure period, indicating that test bees were foraging on food sources other than the spiked sugar solution. Remarkably lower residue concentrations detected in bee bread and hive nectar in some test hives compared to the feeding concentrations may also indicate foraging on other food sources. This uncertainty is inherent in any semi-field or full-field study design.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

1. Study Objective

To determine the potential long term effects on the honey bee (*Apis mellifera* L.) colony health during and after dietary intake of imidacloprid, including the potential effects on overwintering. The long term exposure allows for the characterization and distinction of short-term versus a persistent nature of effects.

2. Study Methods

2.1. Test crop

Not applied. The study was conducted in an open field where multiple field flowers were available and may serve as food sources for the test bees, in addition to the artificial feeding of spiked sugar solution.

2.2. Test chemical

The test substance was technical imidacloprid. Further details are provided in **Table 1** below.

Table 1. Details about the test substance

Test Item			
Name	Imidacloprid TC	**Batch number:**	EDE0015669
Test item code:	NC-0116	**Appearance / colour:**	Solid / beige, light
Formulation type:	Technical compound	**Intended Usage:**	Insecticide
Active ingredient:	imidacloprid	**Content of a.i. analysed:**	98.7 %
CAS number:	138261-41-3		
Density (20 °C) analysed:	Not applicable	**Risk symbol(s):**	Not available
Date of analysis:	17 July 2014	**Expiry date:**	17 July 2016
Stability in solution:	sufficient for the test purpose (at least 1h)	**Storage conditions:**	ambient

2.3. Test sites

The field and sampling phases of this study were conducted by Eurofins Agroscience Services Inc., Cedar Grove Research Station Mebane, NC, USA; the analytical phase was conducted by Bayer CropScience in Durham, NC, USA. The apiary sites were located in the vicinity of the EASI Cedar Grove Research Station in Orange, Caswell, Person and Alamance counties, North Carolina.

There were 12 apiaries separated by more than 1 mile. Land use surveys in 1- mile radius and 3-mile radius were conducted. Pollen species identification and multiple pesticide analysis were conducted using pollen samples collected from the monitoring hives to characterize outside food sources of the test bees and contamination. Pollen samples were collected for a period of 24-48 hours using pollen traps at 5 times during the feeding exposure period (Jun 28, July 3, 12, and 19, and Aug 2, 2013) and 1 time after the exposure (Oct 17, 2013).

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

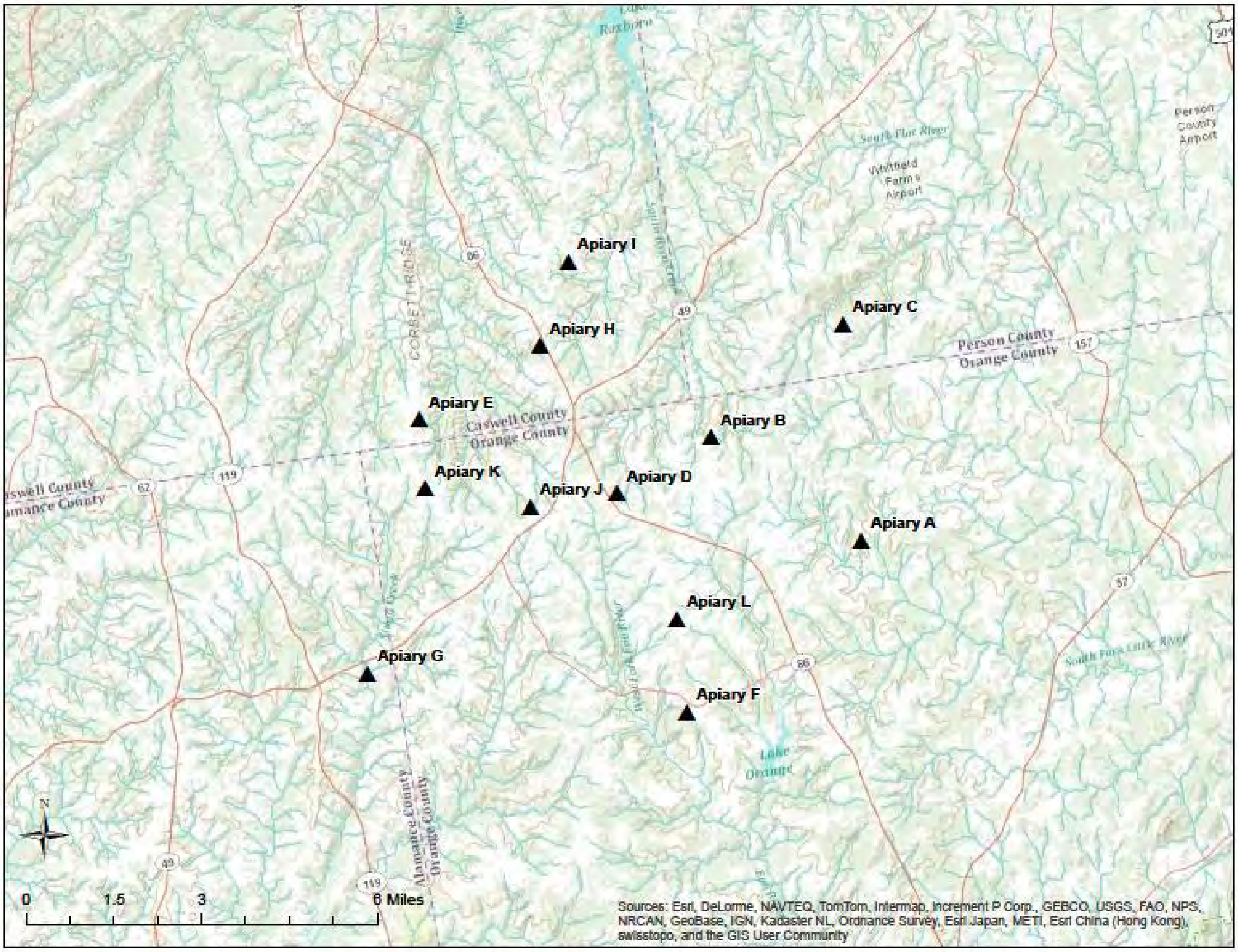

Figure 1: Location of test apiary sites

Table 2: GPS-coordinates of the test apiary sites

Apiary	GPS-coordinates
Apiary A	36°12’01.33” N, 79°06’33.76” W
Apiary B	36°13’55.12” N, 79°08’58.15” W
Apiary C	36°15’12.77” N, 79°06’11.58” W
Apiary D	36°13’20.79” N, 79°10’51.85” W
Apiary E	36°14’55.69” N, 79°14’13.95” W
Apiary F	36°9’59.15” N, 79°10’18.26” W
Apiary G	36°11’22.53” N, 79°15’59.81” W
Apiary H	36°15’41.51” N, 79°11’47.16” W
Apiary I	36°16’50.40” N, 79°11’00.11” W
Apiary J	36°13’22.39” N, 79°12’29.23” W
Apiary K	36°13’55.09” N, 79°14’21.00” W
Apiary L	36°11’22.12” N, 79°10’10.64” W

From Table 2, page 19 of the study report.

2.4. Test organisms

The test species was the honey bee (*Apis mellifera*), Italian race (*Apis mellifera ligustica*). Hives were established from package bees bought from the commercial bee supplier (J J's Honey, 5748 Chancey Road, Patterson, GA 31557, USA), typical of the bee stock used in commercial beekeeping operations. A new queen was introduced into each colony. All queens were purchased from the package supplier. The colonies were maintained in 10-frame Langstroth boxes with an empty deep super on top as a feeder box. In the test field, hives were raised above ground level.

Eighty-four hives that met the conditions provided below at the third Colony Condition Assessments (CCA3) were selected for the study. More than 100 inspected hives were screened based on the outcome of CCA2. Hives were checked for the "appearance" of a healthy colony with no visible symptoms of *Varroa* or *Nosema*, as well as having all stages of brood, a queen, and some food stores.

- 4-8 brood combs containing eggs, larvae or capped cells (except one colony in the control which contained only 2 brood combs at test start);
- 6-10 food combs containing honey and pollen;

Reviewer note: Although the number of adult bees was not considered here as a criteria, it was estimated to be 7000-8000 bees per hive according to the study report.

Eighty-four hives were blocked into 12 apiary sites by brood strength of the colony, starting with Apiary A as the strongest group of hives, and Apiary L as the weakest group of hives. Assignment of apiaries to the geographic locations was done randomly.

Hives were moved on 18/19 Jun 2013 to the Cedar Grove site temporarily from their original apiary locations. On the night of 19 Jun 2013, hives for Apiary sites A-F were moved to their study locations and had their CCA3 on 21 Jun 2013. Hives for Apiary sites G-L were moved on 21 Jun 2013 and had their CCA3 on 23 Jun 2013. After evaluating the assessments, 6 hives were deemed unsuitable and were replaced the morning of 26 Jun 2013, just before exposure began. CCA3 on the 6 replaced hives were conducted. The replaced hives were A1, B8, G1, F2, I4, and J1.

There were eight hives at each site (7 hives for biological assessments and one as the monitoring hive for pollen sample collection). Each hive was spatially isolated from other treatment rates by 30 feet (9 m) spacing at each apiary site (**Figure 2**). Hives were arranged in a semi-circular pattern, facing east to west, with 125 feet (38 m) spacing between the two end hives.

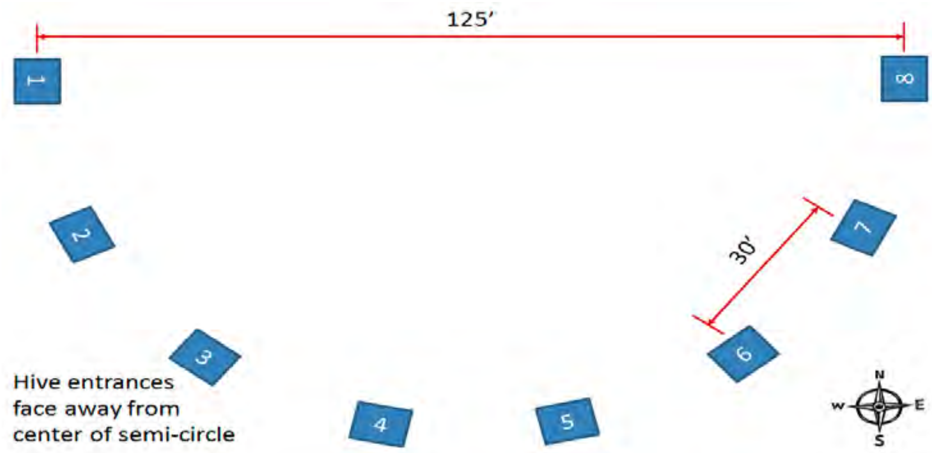

Figure 2. Layout of test hives in a test site

During the study, all hives were treated with one application of Apiguard® (active ingredient: thymol) following typical apicultural practice for the region. The initial application occurred immediately after the CCA6 (17 Sep, 2013) to prevent high mite loads. No treatments for any other hive pests, predators or diseases were administered to any hives.

To minimize the potential for robbing amongst test hives, hives at 100 and 200 ppb treatments were removed from all test apiaries in week 10 (5-6 Sep, 2013) immediately before CCA6. The hives were placed at a separate apiary. Information on the separate apiary was not provided. For over wintering, the surviving colonies were fed with 1 L of 2:1 sugar syrup on 13 Dec 2013, 19 Dec 2013, 13 Jan 2014, 20 Jan 2014, 27 Jan 2014, 07 Feb 2014, 18 Feb 2014, 02 Mar 2014 and 11 Mar 2014.

The monitoring hives were used for outsource pollen sample collection. In addition, test solutions were sealed and placed in monitoring hives in order to assess imidacloprid stability under field test conditions. These stability solutions were not available as a food source to the monitoring hives.

2.5. Treatments

There were:

- 6 treatment groups (5 test concentrations and control): 0, 12.5, 25, 50, 100, or 200 µg/L. At each site, there were 2 control hives, and one hive for each test concentration.
- 12 replicates per treatment group (apiaries),

The individual treatment groups, the respective feeding rates and the respective feeding volumes are summarized in **Table 3.**

Table 3. Treatment groups, feeding rates and feeding volume

Treatment Group	Code	Feeding Timing	Concentration a.i.	Feeding Volume
1 : UTC	UTC (T1+T2)	Twice a week	---	1000 mL
2 : Lowest Rate	T3	Twice a week	12.5 ppb	1000 mL
3 : Low rate	T4	Twice a week	25 ppb	1000 mL
4 : Moderate rate	T5	Twice a week	50 ppb	1000 mL
5: High rate	T6	Twice a week	100 ppb	1000 mL
6: Highest rate	T7	Twice a week	200 ppb	1000 mL

From Table 3, page 21 of the study report.

The assignment of each test hive at 12 apiaries is summarized in **Table 4.**

Table 4. Hive assignment to test apiaries

Treatment group	Apiary											
	A	B	C	D	E	F	G	H	I	J	K	L
UTC	A1	B2	C7	D4	E1	F4	G6	H5	I5	J1	K3	L7
UTC	A2	B8	C6	D6	E8	F7	G5	H3	I8	J7	K4	L5
12.5 ppb	A4	B3	C3	D8	E5	F5	G4	H7	I7	J3	K2	L6
25 ppb	A6	B4	C1	D1	E4	F3	G8	H6	I6	J4	K6	L2
50 ppb	A8	B7	C5	D5	E7	F2	G1	H2	I3	J2	K5	L3
100 ppb	A5	B6	C2	D7	E2	F8	G3	H8	I4	J5	K1	L4
200 ppb	A7	B1	C8	D3	E6	F1	G7	H1	I2	J6	K8	L8
Monitoring	A3	B5	C4	D2	E3	F6	G2	H4	I1	J8	K7	L1

From Table 4, page 22 of the study report.

2.5.1. Preparation of stock solution

Stock solution was created by combining 0.051 g of Imidacloprid Technical Compound, dissolved in approx. 20 mL of acetone, in 1000 mL of distilled water. After preparation, the stock solution was re-stored in a refrigerator until use or replacement. Stock solution was replaced once during feeding on 16 Jul 2013.

2.5.2. Preparation of sugar solution

Sugar syrup was created by combining 10, 100 mL tap water with 10,100 g of sugar in a 5-gallon (19 L) container to make approximately 17 L of sugar syrup.

2.5.3. Preparation of feeding solution

- 12.5 µg/L: mixing 4.25 mL of stock solution into the 17 L of sugar solution.
- 25 µg/L: mixing 8.5 mL of stock solution into the 17 L of sugar solution

- 50 µg/L: mixing 17 mL of stock solution into the 17 L of sugar solution
- 100 µg/L: mixing 34 mL of stock solution into the 17 L of sugar solution
- 200 µg/L: mixing 68 mL of stock solution into the 17 L of sugar solution.

The test concentrations were reported as "ppb" in the study report. However, the values are in fact in the unit of µg/L, not ppb (ug/kg). For example, 12.5 µg/L: can be calculated by 4.25 ml * 0.051 g /1020 ml)/17 L.

The test solution density was not provided. Assuming the density of a 50% sugar solution is 1.2296 g/ml[2], the reviewer calculated that the test concentrations at 12.5, 25, 50, 100, and 200 µg/L are equivalent to 10.2, 20.3, 40.7, 81.3, and 162.7 µg/L (ug/kg), respectively.

2.5.4. Artificial Feeding

Each hive had an empty deep super on top, between the lid and the inner cover to allow dark space to place the feeder inside the hive. This allowed the feeder to be placed on the inner cover so that the bees had easy access without allowing the feeder to come into constant contact with light.

The treated sugar syrup was prepared one day in advance for each feeding event and stored overnight at room temperature. The feeding started on 26 Jun, 2013 and continued for 6 weeks. All of the hives were artificially fed with 1 liter of 50% sugar solution, two times per week. The remaining feeding syrup was removed from the feeder and weighed to determine the consumed amount. The study observation period was 21 Jun, 2013 – 24 Mar, 2014, which includes the overwintering period.

2.6. Meteorological Data

Temperature, humidity and rainfall data were obtained from two apiary sites (from the EASI weather stations located at Apiaries K and J; distance to the other apiaries between 0.1 to 7.5 miles).

A total of 11.93 inches (303 mm) of rainfall accumulated throughout the exposure period – including CCA3 (from 21 Jun 2013 until 08 Aug 2013), with 2.63 inches (67 mm) in June, 7.96 inches (202 mm) in July and 1.34 inches (34 mm) in August. For this period the on-site temperature minimum was 13 °C (55.4 °F) and the temperature maximum was 34 °C (93.2 ° F). The humidity ranged from 38-100 %.

2.7. Observations

Important activity and dates are summarized in **Table 5**.

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[2] Cell Biology Laboratory Manual, http://homepages.gac.edu/~cellab/chpts/chpt3/table3-2.html, accessed on Dec 12, 2014

Table 5. Chronological list of key dates and activities

Week	Date	Activity	Week	Date	Activity
-7	12 May 2013	CCA1	6	07 Aug 2013	1st sample shipment
-4	30 May 2013	Hive samples (uncapped nectar, bee bread)	6	08 Aug 2013	Measurement of remaining food
-4	30 May – 13 Jun 2013	CCA2	7	12 Aug 2013	Recording of hive weights
-1	21 Jun 2013	Recording of hive weights	7	14 Aug 2013	Hive samples (uncapped nectar, bee bread)
-1	21 – 25 Jun 2013	CCA3	7	14/15 Aug 2013	CCA5
-1	21 – 23 Jun 2013	Hive bee sampling for *Varroa* and *Nosema* assessment	7	14/15 Aug 2013	Hive bee sampling for *Varroa* and *Nosema* assessment
0	26 Jun 2013	Feeding	8	21 Aug 2013	Varroa counts CCA5
0	28 Jun 2013	Feeding; Measurement of remaining food	10	05/06 Sep 2013	Removal of 100 and 200 ppb hives to separate apiary
0	28 Jun 2013	Pollen samples from pollen trap	11	10/11 Sep 2013	CCA6 (UTC, 12.5 ppb, 25 ppb, 50 ppb)
1	01 Jul 2013	Feeding; Measurement of remaining food	12	17 Sep 2013	Recording of hive weights
1	03 Jul 2013	Stability samples	12	18/20 Sep 2013	CCA6 (100 ppb, 200 ppb)
1	03 Jul 2013	Feeding; Measurement of remaining food	15	08 Oct 2013	Recording of hive weights
1	03 Jul 2013	Pollen samples from pollen trap	16	16/17 Oct 2013	CCA7 (UTC, 12.5 ppb, 25 ppb, 50 ppb)
2	09 Jul 2013	Feeding; Measurement of remaining food	16	17 Oct 2013	Pollen samples from pollen trap
2	12 Jul 2013	Feeding; Measurement of remaining food	16/17	18/23 Oct 2013	CCA7 (100 ppb, 200 ppb)
2	12 Jul 2013	Stability samples	--	13 Dec 2013	Feeding 1 L 2:1 sugar syrup per hive
2	12 Jul 2013	Pollen samples from pollen trap	--	19 Dec 2013	Feeding 1 L 2:1 sugar syrup per hive
3	16 Jul 2013	New stock solution	--	13 Jan 2014	Feeding 1 L 2:1 sugar syrup per hive
3	17 Jul 2013	Feeding; Measurement of remaining food	--	20 Jan 2014	Feeding 1 L 2:1 sugar syrup per hive
3	17/18 Jul 2013	CCA4	--	27 Jan 2014	Feeding 1 L 2:1 sugar syrup per hive
3	18 Jul 2013	Hive samples (uncapped nectar, bee bread)	--	07 Feb 2014	Feeding 1 L 2:1 sugar syrup per hive
3	19 Jul 2013	Feeding; Measurement of remaining food	--	18 Feb 2014	Feeding 1 L 2:1 sugar syrup per hive
3	19 Jul 2013	Pollen samples from pollen trap	--	02 Mar 2014	Feeding 1 L 2:1 sugar syrup per hive
4	22 Jul 2013	Recording of hive weights	--	11 Mar 2014	Feeding 1 L 2:1 sugar syrup per hive
4	24 Jul 2013	Feeding; Measurement of remaining food	After over-wintering		
4	26 Jul 2013	Feeding; Measurement of remaining food	--	22 Mar 2014	CCA8
5	27/28 Jul 2013	Apiary C vandalized	--	22 Mar 2014	Hive bee sampling for *Varroa* and *Nosema* assessment
5	31 Jul 2013	Feeding; Measurement of remaining food	--	22 Mar 2014	Hive samples (capped honey, bee bread)
5	02 Aug 2013	Feeding; Measurement of remaining food	--	24 Mar 2014	Recording of hive weights
5	02 Aug 2013	Stability samples	--	15 Apr 2014	2nd sample shipment
5	02 Aug 2013	Pollen samples from pollen trap			

2.7.1. Colony mortality

Any colony (hive) that did not show the presence of a queen and had no open brood or eggs, or was devoid of worker (female) bees was considered "dead". If a hive was considered "dead" at the time of assessment, it was no longer used in the analysis of endpoints (e.g., adult bee numbers, hive weight). The number of individual dead bees was not recorded.

2.7.2. Colony Condition Assessments (CCA)

Observations were blocked by the observer, with the same person always observing the same set of hives to avoid viewer discrepancies in the data, Apiary A, B, C, G, J, and L were inspected by one inspector and apiaries D, E, F, H, I, and K by another inspector.

Eight CCAs were conducted during the entire study. CCA1 (day -45), and CCA2 (day -13 to -27) were conducted during the hive establishment. CCA3 (day -1 to -5 days) was conducted 1 week prior to the feeding exposure which served as initial hive conditions prior to the feeding exposure. CCA4 (17/18 Jul 2013) was conducted 3 weeks after the start of feeding exposure. After the end of feeding exposure (Week 6), the following additional CCAs were conducted: CCA5 (week 7), CCA6 (week 11), CCA7 (week 16) and after overwintering CCA8 on 22-24 Mar 2014. Each CCA period in 2013 took two or more days to complete. For summary statistics, the first day is used to characterize any given CCA.

The time schedule of CCAs is summarized in **Table 6** .

Table 6. Schedule for colony assessment and beekeeper checks

Date	Timing	Evaluation/Activity
12 May 2013	45 DBE	CCA1 (non-GLP)
30, 31 May, and 3, 4, 5, 9, 10, 12 and 13 Jun 2013	27 to 13 DBE	CCA2 (non-GLP)
21, 23 and 25 Jun 2013	5 to 1 DBE	CCA3 (before start of feeding; feeding began 26 June)
17 and 18 Jul 2013	21/22 DAE	CCA4 (during exposure, 3 weeks after start of exposure)
14 and 15 Aug 2013	49/50 DAE	CCA5 (1 week after exposure ended)
10, 11, 18 and 20 Sep 2013	76/77/86 DAE	CCA6 (5 weeks after exposure ended)*
16, 17, 18 and 23 Oct 2013	112 to 119 DAE	CCA7 (prior to over-wintering)
22 Mar 2014	269 DAE	CCA8 (after over-wintering)

DBE: Days before start of exposure; DAE: Days after start of exposure

*CCA6 timing allows all bee individuals (eggs, larvae, pupae) present during the exposure period to complete their development cycle to adults.

During the colony condition assessments, each frame was removed and inspected one at a time, with measurements for endpoints taken as percent of total frame area covered by honey / nectar, bee bread / pollen, eggs, open brood (larvae), capped brood (pupae), and adult bees.

The estimation was made by:

- Each hive consisted of 20 observed panels (10 frames with two sides of each frame), with an area of 860 cm^2 per side, or a total area of 17,200 cm^2 for all 10 frames.
- There were 130 bees per 100 cm^2.
- The total number of cells per frame is 3440. Estimated to be 4 cells/cm^2.

2.7.3. Evaluation of Disease or Pests in the Hive

Colonies were also checked for visible symptoms of disease or pests, such as *Nosema*, foulbrood, *Varroa* mites or small hive beetle.

To assess the presence of Varroa in the hive, bee samples were taken at the CCA3, CCA5 and CCA8. Bees were washed in alcohol to remove mites. The number of mites per 100 bees was calculated.

2.7.4. Hive weights

Hive weights were recorded after 10 a.m. once a month from June to October, as well as after over-wintering, on 21 Jun 2013 (week -1), 22 Jul 2013 (week 4), 12 Aug 2013 (week 7), 17 Sep 2013 (week 12), 08 Oct 2013 (week 15) and 24 Mar 2014 (week 39).

2.8. Residue analysis

All residue and stability samples collected from feeding solution, pollen traps, and test hives were analysed for imidacloprid, olefin- and 5-hydroxy imidacloprid. Samples from pollen traps in the monitoring hives were also analysed for residues of multiple pesticides from outside sources at the National Science Laboratories of USDA in Gastonia (non-GLP). The residue results were reported as ng per g of sample matrix (ppb), which is different from the test solution that was reported in µg/L,

The LOQ was 5 ppb for imidacloprid, olefin- and 5-hydroxy imidacloprid in feeding solution and hive nectar samples, and the LOQ was 2 ppb for pollen samples. The LODs are listed in **Table 7**.

Table 7. LOD for imidacloprid and its metabolites

Matrix	Imidacloprid Olefin	Imidacloprid 5-hydroxy	Imidacloprid
Dosing/Stability Solutions	2.07 ppb	2.22 ppb	0.38 ppb
Hive Collected Nectar	1.38 ppb	1.43 ppb	1.43 ppb
Pollen	0.74 ppb	0.18 ppb	0.36 ppb

Taken from page 175 of the study report

Olefin- and 5-hydroxy imidacloprid were not detected in any samples except for two samples. The reported average residue results included only imidacloprid. For the values <LOD, a half of the LOD value was used in order to calculate the means. Multiple pesticide analysis was conducted in order to monitor pesticide contamination from outside food sources using pollen collected from pollen traps on the monitoring hives.

All samples for residue analysis were protected from sunlight by using amber vials and transported to freezer storage after field collection. All samples were placed in frozen storage upon receipt at the test facility. Samples were maintained frozen (≤ -15° C) (up to -13.9° C for a short period of time) at the test facility until shipment under frozen conditions to the test site for residue analysis. Daily minimum/maximum temperatures were recorded for the duration of the storage period at the test facility.

2.8.1. Pollen from outside sources

Pollen samples were collected from pollen traps attached for 24-48 hours to the monitoring hives at each site to assess the potential contaminant exposure from outside sources. Pollen amounts collected from each hive were variable and samples were not available from every site each time. Pollen samples from the monitoring hives were taken at weeks 0 (CCA3), 1, 2, 3 (CCA4), 5, and 16 (17 Oct 2013).

2.8.2. Stored pollen and nectar in test hives

Stored bee bread and bee-collected nectar were collected within the study hives for imidacloprid residue analysis. Samples weighed at least 500 mg each. Bee bread and uncapped nectar were collected at weeks -3 (CCA4), 4, and 7 (CCA5). Bee bread and capped honey were collected at CCA8 (after overwintering). All test hives were sampled at CCA4, but only part of them were sampled at the other sampling times. However, bee bread and honey were not available from every colony each time.

2.8.3. Feeding solution and stability of test item

The monitoring hives were used for dose verification and to evaluate stability of the test item in a hive environment. Monitoring hives were set up in the same manner as test hives except the colony was denied access to the spiked or unspiked sucrose. Residue samples comprising approx. 5 g each from the sugar syrup were taken on week 1 (3 July 2013), week 2 (12 July 2013) and week 5 (2 August 2013).

Table 8. Sampling schedule for feeding solution and stability of test chemical.

Timing	**Week 1**	**Week 2**	**Week 5**
Apiary / replicate	**03 Jul 2013**	**12 Jul 2013**	**02 Aug 2013**
UTC	X	X	X
12.5 ppb	X	X	X
25 ppb	X	X	X
50 ppb	X	X	X
100 ppb	X	X	X
200 ppb	X	X	X

X = samples taken but no sample ID available

3. Results

3.1. Land use near test hives

Land use pattern within a 1-mile and 3-mile radius around the 12 apiaries are summarized in **Table 9**. The cultivated crop area occupied 0.2-5.5% of the total land within 1 mile radius, and 1.1-2.7% within a 3 mile radius range from the test apiaries. Using the raw data provided, the reviewer calculated the area of cultivated crops as summarised in **Table 10**. The mean area of cultivated cropping land was 19 and 168 ha within 1 mile and 3 miles, respectively, of the radius from each apiary.

Table 9: Percent (%) land use pattern

1 Mile Radius	Apiary											
Land Use Category	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
Open Water	0.7	0.8	0.2	1.0	0.7	6.1	0.5	0.6	0.9	0.7	0.8	1.3
Developed, Open Space	5.7	6.4	2.6	6.9	1.6	5.0	3.5	1.9	1.4	6.5	4.3	7.1
Developed, Low Intensity	1.1	1.5	0.6	1.8	0.8	0.1	1.0	2.9	1.9	2.9	0.8	1.7
Developed, Medium Intensity	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.0
Developed, high Intensity	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Barren Land (Rock/Sand/Clay)	0.0	0.1	2.4	0.2	0.0	0.2	0.0	0.0	0.1	0.0	0.0	0.1
Deciduous Forest	44.5	38.7	52.2	33.7	49.9	48.3	39.1	46.5	35.4	32.6	40.1	28.8
Evergreen Forest	5.9	4.2	2.5	8.5	9.6	3.3	7.7	5.8	5.5	4.4	6.8	4.7
Mixed Forest	2.3	2.3	3.6	4.0	4.2	2.1	3.5	3.8	4.6	2.7	4.2	2.4
Shrub/Scrub	1.2	1.8	2.1	0.1	1.1	3.7	2.6	0.9	2.7	0.7	2.9	0.8
Grassland/Herbaceous	4.5	3.2	4.7	3.4	3.6	2.0	5.1	4.0	7.3	3.0	3.2	2.1
Pasture/Hay	31.6	39.1	27.1	36.7	25.7	29.0	35.7	31.1	34.7	41.1	33.9	48.3
Cultivated Crops	0.7	1.9	1.8	2.9	1.4	0.2	0.6	2.4	5.5	5.0	2.8	2.4
Woody Wetlands	1.8	0.1	0.3	0.7	1.3	0.0	0.4	0.1	0.0	0.0	0.2	0.0
Emergent Herbaceous Wetland	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
3 Mile Radius	**Apiary**											
Land Use Category	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
Open Water	0.6	0.6	0.5	0.8	0.5	2.3	0.6	0.4	0.6	0.8	0.5	2.5
Developed, Open Space	5.6	4.8	3.5	5.6	3.3	5.1	4.9	2.6	2.1	5.2	4.2	6.1
Developed, Low Intensity	1.0	1.6	1.2	2.0	1.6	0.8	1.2	1.7	1.6	1.8	1.4	1.4
Developed, Medium Intensity	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1
Developed, High Intensity	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Barren Land (Rock/Sand/Clay)	0.1	0.4	0.3	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.1
Deciduous Forest	41.2	41.2	44.4	39.4	44.0	45.8	40.2	43.4	44.0	39.2	42.6	38.3
Evergreen Forest	6.3	5.6	4.8	5.5	6.5	5.0	8.1	6.7	7.2	5.9	6.3	4.9
Mixed Forest	2.2	2.7	2.7	2.7	3.1	2.2	2.8	3.8	3.8	3.1	3.1	2.2
Shrub/Scrub	1.6	1.6	2.0	1.4	2.6	2.2	1.9	2.6	3.1	1.5	1.8	1.5
Grassland/Herbaceous	3.5	4.6	4.4	4.7	7.1	3.0	4.2	7.9	9.8	5.2	5.3	3.3
Pasture/Hay	34.9	34.1	33.2	34.7	28.4	31.7	33.9	27.8	24.7	33.9	31.5	36.8

Table 10: Cultivated cropping area near each test apiary

	Apiary												Mean
	A	B	C	D	E	F	G	H	I	J	K	L	
1 mile radius (813 ha)													
Cultivated Crops (%)	0.7	1.9	1.8	2.9	1.4	0.2	0.6	2.4	5.5	5.0	2.8	2.4	2.3
Area of cultivated crop (ha)	5.7	15.4	14.6	23.6	11.4	1.6	4.9	19.5	44.7	40.7	22.8	19.5	18.7
3 mile radius (7323 ha)													
Cultivated Crops (%)	1.7	2.4	2.5	2.5	2.6	1.1	1.7	2.7	2.7	2.6	2.7	2.0	2.3
Area of cultivated crop (ha)	124.5	175.8	183.1	183.1	190.4	80.6	124.5	197.7	197.7	190.4	197.7	175.8	168.4

3.2. Pollen sources of test hives

Monitoring hives were used at each test apiary to collect pollen for assessment of the local pollen flora (non-GLP). Pollen trap samples from the monitoring hives were taken at CCA3 (28 Jun 2013; week 0) and CCA4 (03 Jul 2013; week 1), as well as on 12 Jul 2013 (week 2), 19 Jul 2013 (week 3) and 02 Aug 2013 (week 5), and at CCA5 (week 7) and after CCA7 (week 16).

The major pollen was from non-cultivated crops, such as Parthenocissus, Melilotus, Plantago, Rhus, and Asteraceae. Cultivated crops such as *Zea mays* (maize) and *Fagopyrum esculentum* (Buckwheat) were identified occasionally, and took up the maximum of 13% and 21% of the total pollen particles, respectively. Full results can be found in Table 49 of the study report (pages 128-129).

3.3. Consumption of spiked sucrose

Hive consumption rates for the feeding solution (sugar syrup) ranged from 10,290 mL to 12,000 mL of the total 12,000 mL per hive provided during a 6-week period (*i.e* 1 litre per colony 2 times a week for a total of 12,000 mL per colony during the exposure period). All colonies consumed most or all of the sugar solution (see **Figure 3**) with a slightly lower consumption in 100 µg/L and 200 µg/L treatment.

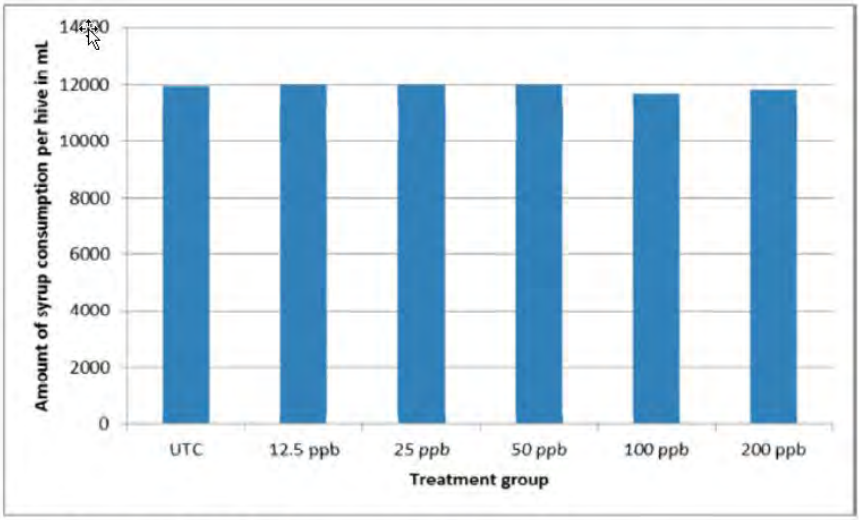

Figure 3 : Mean total food consumption [mL] per colony

Figure 3. Mean total food consumption (mL) per colony during the 6-week exposure period

3.4. Examination of pesticides from other sources

Monitoring hives were used to assess the potential contaminant exposure from outside sources (non-GLP) at each site. Pollen trap samples from the monitoring hives were taken after CCA3 (28 Jun 2013; week 0) and CCA4 (03 Jul 2013; week 1), as well as on 12 Jul 2013 (week 2), 19 Jul 2013 (week 3) and 02 Aug 2013 (week 5), and at CCA5 (week 7) and after CCA7 (week 16). The amount of pollen collected from traps on the monitoring hives varied. A large portion of the pollen samples collected on Aug 2, and Oct 17 were reported as either "No sample" or "no sample sent to USDA". For these, samples with no pollen collected were indicated as "No sample", while those samples without enough to meet the mass requirement for pesticide analysis were indicated as 'No sample sent to USDA'. It is noted that out of 16 weeks from the beginning of feeding exposure to the last CCA before overwintering, pollen samples were collected 6 times with each collection over a period of 1-2 days and a few of them were not analyzed for residue analysis due an insufficient amount of pollen for analysis. Pesticide contamination was unknown for those intervals when pollen samples were not collected.

Dimethenamid (maximum at 87 ppb), Fenamidone (maximum at 345 ppb), Spiromesifen (maximum at 961 ppb) were the major pesticides detected in the pollen samples originating from outside food sources (**Table 11**). High levels of piperonyl butoxide (maximum at 591 ppb) were detected in the monitoring hive at Apiary A along with several other pesticides. Out of 6 test sites where the pollen samples were collected on 02 Aug 2013, imidacloprid was detected in one pollen sample at Apiary L at 12.1 ppb. The detection frequency by the test sites was 1/6. It was found that the contaminated pollen sample at Apiary L consisted of 92.4% of *Rhus*, 5.2% *plantago*, 2.3% of *Lagerstroemia indica,* none of which are major cultivated crops.

Table 11. Residues from outside sources in pollen samples from pollen trap (non-GLP)

Apiary	Sampling Date					
	6/28/13	7/3/13	7/12/13	7/19/13	8/2/13	10/17/13
A	No detects	Chlorpyrifos (trace)	No detects	DDD p,p' (14.4) Dimethenamid (87.0) Fenamidone (345) Fenhexamid (trace) Metalaxyl (trace) Piperonyl butoxide (591) Quinoxyfen (474) Spiromesifen (961)	No detects	No sample sent to USDA
B	No detects	No detects	No sample sent to USDA	No sample sent to USDA	THPI (299)	No sample sent to USDA
C	No detects	No detects	No detects	Metalaxyl (6.0) Methamidophos (62.3)	No sample	No sample
D	Hydroprene (trace)	No detects	No detects	Dimethenamid (71.7) Fenamidone (215) Spiromesifen (584)	No detects	No detects
E	No sample	No sample sent to USDA	No sample	No sample	No sample	No sample
F	No detects	No detects	No sample sent to USDA	Dimethenamid (47.0) Fenamidone (90.5) Spiromesifen (362)	No sample sent to USDA	No sample
G	No detects	No detects	No detects	Dimethenamid (14.6) Spiromesifen (148)	No detects	No sample sent to USDA
H	No detects	No detects	Dimethenamid (44.9) Fenamidone (134)	No sample sent to USDA	No sample sent to USDA	No sample
I	No detects	No detects	Fenhexamid (trace)	No detects	No sample sent to USDA	No sample sent to USDA
J	No detects	No detects	Dimethenamid (26.5)	No sample sent to USDA	No sample sent to USDA	Thymol (193)
K	No detects	No detects	Dimethenamid (10.3) Spiromesifen (80.1)	Trifluralin (trace)	No detects	No sample
L	No detects	No sample	MGK-326 (trace)	No detects	Imidacloprid (12.1) Malathion (trace)	No detects

THPI = tetrahydrophthalimide
Residue values in parentheses are ppb.
From Table 64, page 142 of study report.

3.5. Confirmation of test concentrations

Imidacloprid and its major transformation products were analyzed from feeding solutions sampled after they were prepared before start of feeding, three times on 2 July (week1), 12 July (week 2) and 2 August 2013 (week 5). The averages of measured concentrations were <LOD, 11.0, 23.3, 46.7, 96.3, and 189.6 ppb for the nominal concentrations of control, 12.5, 25, 50, 100, and 200 µg/L, respectively. It is noted that imidacloprid and 5-OH-imidacloprid were detected in one control feeding solution at 0.45 and 4.22 ppb respectively, sampled at week 2 on 12 July 2013. It is unknown which control hives were fed with the contaminated feeding solution. The data are tabulated below in **Table 12**.

Table 12. Dosing solution residue data from 2 July 2013 (Week 1), 12 July 2013 (Week 2) and 2 August 2013 (Week 5)

Nominal concentrations		Average of measured concentrations (ppb)	Measured imidacloprid concentrations (ppb) (n=6)					
(µg/L)	(ppb)							
0 (Control)	0	<LOD†	<LOD	<LOD	<LOD	**0.45***	<LOD	<LOD
12.5	10.2	11.0	11.3	11.96	10.62	10.2	10.73	11.22
25	20.3	23.3	24.24	23.45	22.91	22.14	23.45	23.75
50	40.7	46.7	44.34	46.71	48.99	43.5	48.66	48.11
100	81.3	96.3	99.15	99.23	91.04	96.1	96.87	95.12
200	162.7	189.6	193.51	195.06	186.9	190.07	189.04	182.94

-Regenerated from Table section 5.5, on page 189-190 in the study report
†: LOD=0.38 ppb for imidacloprid;
*: In the same control sample, 5-OH-imidacloprid was also detected at 4.22 ppb

3.6. Stability of the test item in feeding solution

Stability of imidacloprid in the sugar solution during the feeding period was examined from diet collected from closed-off feeding solutions placed in the monitoring hives, sampled three times on 3 July 12 July, and 2 August 2013. No reduction of test concentrations in the feeding solution was noticed during the feeding period. The stability of imidacloprid at 200 µg/L in the feeding solution was not provided, but a significant reduction is not expected based on the reported data for all other concentrations. It is noted that imidacloprid was detected at 0.56 ppb in one of the control solution for the control hive H4 sampled on 12 July 2014. No imidacloprid olefin or imidacloprid 5-hyrdoxy was detected in any of the samples (LOD of 2.07 ppb and 2.22 ppb, respectively). Average imidacloprid residue data for the stability solution are presented in **Table 13.**

Table 13. The stability of imidacloprid in feeding solution on 3 Jul, 12 Jul, and 2 Aug, 2013.

Nominal concentration (µg/L)	Average of measured concentrations (ppb)	Number of samples measured	Measured imidacloprid concentrations (ppb)		
			03 Jul, 2013	12 Jul, 2013	02 Aug, 2013
Control	<LOD†	20	0.56 ppb in one out of 20 samples. <LOD in 19 samples;		
12.5	11.4	12	11.74	11.86	10.65
25	23.2	10	23.65	23.40	22.89
50	47.4	10	46.62	46.09	51.78
100	93.6	12	95.77	92.09	92.98
200	N/A*	N/A	N/A		

- Regenerated from Section 5.6, on page 191-193 in the study report
†: LOD=0.38 ppb for imidacloprid;
*: N/A: data not available.

3.7. Residues in hive matrices

It is noted here as it was in the uncertainties section that the residue samples from the different hive matrices represent a single sample from a single hive. Therefore there is variation in the residues that likely stems from the sampling procedure employed for this study (single sample, one side of the comb).

3.7.1. Background imidacloprid contamination in hives prior to the feeding exposure

The background imidacloprid contamination in test hives was examined using hive bee bread (hive pollen) and nectar collected about a month (30 May 2013) prior to the beginning of feeding exposure. Imidacloprid was detected in two out of a total of six hive pollen samples at 0.43 ppb and 1.19 ppb, respectively (**Table 14**), with a mean of 0.81 ppb and a detection frequency of 33% (2 hives out of total 6 hives). It is noted that the limit of detection for imidacloprid in pollen for this study was 0.36 ppb. Imidacloprid was not detected in any hive nectar samples collected prior to the feeding exposure (**Table 15**). Residue analysis for other pesticides was not conducted prior to exposure.

Table 14. Detailed hive pollen residue data, pre-study collection (30 May 2013)

Location	Imidacloprid Olefin (ppb)	Imidacloprid 5 Hydroxy (ppb)	Imidacloprid (ppb)
LODs	**0.74**	**0.18**	**0.36**
Pope	<LOD	<LOD	<LOD
Maple	<LOD	<LOD	<LOD
Greenhouse	<LOD	<LOD	0.43
Corbett Ridge	<LOD	<LOD	<LOD
Cedar Grove	<LOD	<LOD	<LOD
Prospect Hill	<LOD	<LOD	1.19

Residue values were not corrected for recovery or moisture content of the sample.
Residues below are LOD are reported as <LOD.
From page 183 of the study report.

Table 15. Detailed hive nectar residue data, pre-study sample collection (30 May 2013)

Location	Imidacloprid Olefin (ppb)	Imidacloprid 5 Hydroxy (ppb)	Imidacloprid (ppb)	Brix (%)*
LODs	**1.38**	**1.43**	**1.43**	
Pope	<LOD	<LOD	<LOD	>80
Maple	<LOD	<LOD	<LOD	79
Greenhouse	<LOD	<LOD	<LOD	78
Corbett Ridge	<LOD	<LOD	<LOD	80
Cedar Grove	<LOD	<LOD	<LOD	80
Prospect Hill	<LOD	<LOD	<LOD	80

Taken from page 194 of the study report. *Brix % is the percentage of sugar content in honey by mass, measured by a refractometer.

3.7.2. Residues in hive matrices during and after feeding exposure

Imidacloprid and its two major transformation products in hives (imidacloprid olefin and 5-hydroxy imidacloprid) were examined three times after the feeding started using hive bee bread and hive nectar. All test hives were sampled at 1st batch of sampling (CCA4, 18 July 2013) during the exposure phase, but only part of test hives were sampled at the other two sampling times, CCA5 which was one week after the feeding exposure, and CCA8 after the overwintering).

3.7.2.1. *Residues in hive matrices at CCA4 (after 3 weeks of exposure)*

The level of imidacloprid in hive bee bread and uncapped nectar after 3 weeks of feeding (CCA4) was summarized in **Table 16** and **17**. All test hives were sampled at CCA4 (18 July 2013). A dose-response correlation was observed between the imidacloprid concentrations in the feeding solution and measured concentrations in both bee bread and uncapped hive nectar. However, the imidacloprid concentration in hive uncapped nectar and pollen was 64% and 26% of the mean concentrations in feeding solution, respectively. It is possible that dilution of stored pollen and nectar from other food sources occurred during the exposure period since, as indicated in the study, a significant degradation of imidacloprid in test solution was not detected in the study.

Imidacloprid in bee bread at CCA4: The level of imidacloprid in hive bee bread after 3 weeks of feeding (CCA4) was summarized in the **Table 16.** Imidacloprid was detected in all measured treatment samples. No imidacloprid metabolites were detected. It was noted that not all residue information in pollen was available. No residue information for treatment at 200 ug/L in bee bread was provided. Out of 12 hives, four hives at 100 ug/L and eight hives at 50 ug/L were measured, respectively.

The results showed a dose-response correlation between the average concentrations measured in hive bee bread and the concentrations in the feeding solution. However, the concentrations varied within each treatment group (see **Table 16**). The mean of the measured concentrations in bee bread within each treatment group of 12.5, 25, 50 and 100 ug/L was 2.86 (range: 0.77-5.34), 5.37 (range: 1.45-9.41), 10.84 (range: 4.2-19.41), and 17.89 ppb (range: 2.66-35.1), respectively. By average, the measured concentration was 25.8% (range 22-28.1%) of the concentration in feeding solution, and 27.8% (range 24.9-31.8 %) of the measured concentrations in uncapped hive nectar (data not shown in the table). The results showed that after 3 weeks of feeding, imidacloprid concentrations in hive bee bread appeared remarkably lower than that in the feeding solutions and in hive nectar. The lower concentration in bee bread is expected due to the dilution since bee bread is a mixture of nectar and pollen from various sources.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 16. Imidacloprid concentrations (ppb) in hive pollen sampled three weeks after the start of artificial feeding on 18 July 2013 (CCA4).

Apiary	Measured imidacloprid concentrations (ppb) (LOD = 0.38 ppb)*						
	Nominal concentration (ug/L)						
	Control 1	**Control 2**	**12.5**	**25**	**50**	**100**	**200**
	Nominal concentration (ppb) ‡						
	0	**0**	**10.2**	**20.3**	**40.7**	**81.3**	**162.7**
A	<LOD	<LOD	3.02	4.01	7.17	9.84	-
B	<LOD	1.05	3.33	2.34	4.2	-	-
C	<LOD	<LOD	3.47	7.17	9.56	23.97	-
D	<LOD	<LOD	0.77	2.32	5.79	2.66	-
E	<LOD	<LOD	1.59	3.03	10.1	-	-
F	<LOD	<LOD	2.09	5.19	-	-	-
G	<LOD	<LOD	1.54	6.38	11.19	35.1	-
H	1.24	-	5.34	1.45	-	-	-
I	<LOD	<LOD	4.15	9.41	19.31	-	-
J	<LOD	<LOD	3.51	7.81	-	-	-
K	<LOD	<LOD	2.4	8.05	-	-	-
L	<LOD	<LOD	3.17	7.31	19.41	-	-
Number of samples measured	12	12	12	12	8	4	0
Average concentration	<LOD		2.86	5.37	10.84	17.89	-
% of the feeding concentration[††]	Not applicable		28.1	26.4	26.7	22.0	-
% of the average detection in hive Nectar[†††]	Not applicable		31.8	27.3	27.4	24.9	-

* regenerated from the additional residue information (email forwarded by Keith Sappington (EPA) to Tina Singal (PMRA) on March 10, 2015) ; "-" indicates that data are not available

‡Nominal concentration in ppb is estimated from the concentration in µg/L by assuming the volume density of the test solution to be 1.2296 g/ml.

†† % Feeding concentration: the average of measured concentration compared with the nominal feeding concentrations in ppb.

††† % of the average detection in hive Nectar**:** the average of measured concentration in pollen compared with the average measured concentration in nectar ppb without corrections for sugar.

Imidacloprid in hive uncapped nectar at CCA4: The level of imidacloprid in hive uncapped nectar during the feeding exposure (CCA4) was summarized in **Table 17**. All twelve test hives were measured. Imidacloprid was detected in the majority of the measured treatment samples. Out of 12 hives measured for each concentration, <LOD was reported in two hives at 12.5 ug/L (Apiary E and H) and two hives at 200 ug/L (Apiary D and F). No imidacloprid metabolites were detected.

The results showed a dose-response correlation between the average concentrations measured in uncapped hive nectar and the concentrations in the feeding solution. However, the concentrations varied remarkably within each treatment group (see **Table 17**). Remarkably lower concentrations were detected in nine test hives, including all 5 treatment hives at apiary D, E5, E7, F1, and H7. <LOD was reported for treatment at 200 µg/L in hive D3 and F1. After correction with Brix values to 50% sugar concentration, the mean of the measured concentrations in uncapped hive nectar within each treatment group of 12.5, 25, 50, 100, and 200 ug/L was 6.31 (range: 0.88-9.42), 13.24 (range: 1.19-20.53), 27.66 (range: 2.31-40.59), 46.87 (range: 2.1-

80.15), and 109.14 ppb (range: 0.89-152.94) respectively. By average, the measured concentration in hive nectar was 64% (range 62.0-68.0%) of the concentration in feeding solution. The results showed that after 3 weeks of feeding, imidacloprid concentrations in hive nectar appeared lower than that in the feeding solutions, which indicated that the foraging bees also foraged on nectar sources other than the provided sugar sources which diluted the level of treatment. It is noted that this result is expected, as bees were allowed to freely forage, and also, under natural conditions bees typically forage on multiple plant pollen and nectar sources.

Table 17. Imidacloprid concentrations (ppb) in uncapped hive nectar sampled three weeks after the start of artificial feeding on 18 Jul, 2013 (CCA4).

Apiary	Measured imidacloprid concentrations (ppb) (LOD=1.43 ppb)*						
	Nominal concentration (ug/L)						
	Control 1†	Control 2†	12.5	25	50	100	200
	Nominal concentration (ppb) ‡						
	0	0	10.2	20.3	40.7	81.3	162.7
A	<LOD	<LOD	9.42	14.06	40.36	48.85	96.98
B	<LOD	2.98	7.91	20.53	31.12	57.45	124.53
C	<LOD	<LOD	6.29	7.97	38.61	66.88	134.77
D	<LOD	<LOD	3.37	1.19	2.91	22.58	0.89
E	<LOD	<LOD	0.89	7.12	2.31	80.15	90.19
F	<LOD	<LOD	8.04	13.89	34.71	62.15	0.89
G	<LOD	<LOD	7.6	14.82	16.71	58.9	150.79
H	<LOD	<LOD	0.88	10.47	32.32	26.17	137.27
I	<LOD	<LOD	8.02	13.69	18.15	23.55	136.91
J	<LOD	<LOD	7.66	15.51	40.59	47.7	139.32
K	<LOD	<LOD	7.01	19.67	38.83	2.1	144.27
L	<LOD	<LOD	8.57	20	35.25	65.95	152.94
Number of samples	12	12	12	12	12	12	12
Average concentration	<LOD		6.31	13.24	27.66	46.87	109.14
% Feeding concentration††			62.0	65.1	68.0	57.6	67.1

* Concentrations in all treatments except for the controls are corrected to 50% sugar using Brix values that are not listed in the table, but were in the table section 5.9 on page 195-197 of the study report

† Concentrations in the controls are measured concentrations in hive uncapped without corrections for sugar concentrations.

‡Nominal concentration in ppb is estimated from the concentration in µg/L by assuming the volume density of the test solution to be 1.2296 g/ml.

†† % Feeding concentration: the average of measured concentration compared with the nominal feeding concentration in ppb.

3.7.2.2. Residues in Hive Matrices at CCA5 (1 week after end of exposure)

The level of imidacloprid in hive bee bread and uncapped nectar one week after the end of feeding exposure (CCA5, 14-15 Aug 2013) was summarized in **Table 18**. Only three apiaries were sampled (Apiaries A, B, and L). Again, the level of imidacloprid residues in hive nectar was reported for all treatment concentrations but not for all the bee bread, especially for 100 and 200 µg/L. In summary, similar to CCA4, a dose-response correlation was observed between the average concentrations of imidacloprid measured in both bee bread and uncapped hive nectar and the concentrations in the feeding solution. However, the imidacloprid concentration in hive uncapped nectar and beebread was lower than what was in the feeding solutions, indicating dilution of stored bee bread and nectar from other food sources.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Shown in **Table 18,** a dose-response correlation was shown between the average concentrations of imidacloprid measured in both bee bread and uncapped hive nectar and the concentrations in the feeding solution. However, the concentrations varied remarkably within some treatment groups despite the low number of samples measured. In bee bread, the mean of the measured concentrations for 12.5, 25, 50 and 100 ug/L was 4.22 (range: 3.26-5.25), 5.74 (range: 4.89-6.4), 16.44 (range: 14.37-18.00), and 22.89 ppb (no range, only one measurement), respectively. By average, the measured concentration was 28.1% (range 22.9-33.8%) of the concentration in feeding solution, and 40.3% (range 26.0-51.5 %) of the measured concentrations in uncapped hive nectar without correction for sugar content (data not shown in the table). In uncapped hive nectar, after correction with Brix values (amount of sugar dissolved in solution) to 50% sugar concentration, the mean of the measured concentrations within each treatment group of 12.5, 25, 50, 100, and 200 ug/L was 5.88 (range: 3.36- 7.28), 7.18 (range: 0.89-10.68), 27.46 (range: 22.93-33.39), 54.98 (range:5.79-79.79), and 127.93 ppb (range:103.32-144.27) respectively. By average, the measured concentration in hive nectar was 61.4% (range 35.3-78.7%) of the concentration in feeding solution.

Table 18. Imidacloprid concentrations (ppb) in beebread and uncapped hive nectar sampled one week after the end of artificial feeding on 14 Aug, 2013 (CCA5).

Matrix	Apiary	Measured imidacloprid concentrations (ppb) (LOD=0.38 ppb pollen; 1.43 ppb nectar)*						
		Nominal concentration (ug/L)						
		Control 1	Control 2	12.5	25	50	100	200
		Nominal concentration (ppb) ‡						
		0	0	10.2	20.3	40.7	81.3	162.7
Residues in bee bread	A	<LOD	<LOD	5.25	4.89	14.37	22.89	-
	B	<LOD	<LOD	4.16	5.94	18.00	-	-
	L	<LOD	0.55	3.26	6.4	16.96	-	-
	Number of samples measured	3	3	3	3	3	1	0
	Average	<LOD		4.22	5.74	16.44	22.89	-
	% Feeding Solution (ppb) ††	Not applicable		41.5	28.2	40.4	28.1	
	% Nectar †††	Not applicable		45.6	51.5	38.1	26.0	-
Residues in uncapped nectar after correction to 50% sugar	A	<LOD	<LOD	7.28	0.89	22.93	5.79	136.19
	B	<LOD	<LOD	7.01	10.68	33.39	79.79	144.27
	L	<LOD	<LOD	3.36	9.97	26.06	79.36	103.32
	Number of samples measured	3	3	3	3	3	3	3
	Average	<LOD		5.88	7.18	27.46	54.98	127.93
	% of the Feeding Solution††	Not applicable		57.8	35.3	67.5	67.6	78.7

* Concentration in all treatments except for the controls are corrected to 50% sugar using Brix values. "-" indicates that data are not available

‡Nominal concentration in ppb is estimated from the concentration in µg/L by assuming the volume density of the test solution to be 1.2296 g/ml.

†† % Feeding solution: the average of measured concentration compared with the nominal feeding concentrations in ppb.

††† % Nectar: percent of the average of measured concentration in bee bread (hive pollen) compared with the average measured concentration in nectar (ppb) without corrections for sugar.

3.7.2.3. *Residues in hive matrices at CCA8 (after overwintering)*

The level of imidacloprid in hive bee bread and capped honey after overwintering (CCA8, 22 March 2014) was summarized in **Table 19**. Only surviving hives in four apiaries were sampled (Apiaries E, I, J, and L). Again, imidacloprid residue was not reported for all hives sampled. In bee bread, imidacloprid was not detected in treatments of control, 12.5, 25 and 100 ug/L, but was detected at 0.52 ppb (E7), 0.52 ppb (I3), and 0.40 ppb (J2 in three 50 ug/L treatment hives. No measurement was provided for treatment at 200 ug/L.

In honey, no imidacloprid residues were detected in all measured hives except for one at 13.53 ppb (L4) in the 100 ug/L treatment group.

The average concentration of imidacloprid in hives after overwintering (CCA8) is considered to be uncertain, especially for hives at 100 and 200 ug/L. After overwintering, residues were analyzed only from surviving hives, not from the dead hives. In the study, a high number of hives was reported dead after overwintering. At 100 and 200 ug/L treatments, only one and two hives survived, respectively. The unmeasured level of residues in dead hives presents an uncertainty as to the average of residues that might represent the level of treatments at CCA8.

Table 19: Imidacloprid concentrations (ppb) in bee bread and honey sampled after overwintering on March 24, 2014 (CCA8).

Matrix	Apiary	Measured imidacloprid concentrations (ppb) (LOD=0.38 ppb pollen; 1.43 ppb nectar)*						
		Nominal concentration (ug/L)						
		Control 1	**Control 2**	**12.5**	**25**	**50**	**100**	**200**
		Nominal concentration (ppb) ‡						
		0	**0**	**10.2**	**20.3**	**40.7**	**81.3**	**162.7**
Residues in bee bread	E	<LOD	<LOD	<LOD	<LOD	0.52	-	-
	I	<LOD	<LOD	<LOD	<LOD	0.52	-	-
	J	<LOD	<LOD	-	<LOD	0.4	-	-
	L	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-
	Number of samples measured	4	4	3	4	4	1	0
	Average	<LOD		<LOD	<LOD	0.48	<LOD	-
	% Feeding Solution ††	Not applicable				1.2	Not applicable	
Residues in uncapped nectar after correction to 50% sugar	E	<LOD	<LOD	<LOD	<LOD	<LOD	-	-
	I	<LOD	<LOD	<LOD	<LOD	<LOD	-	-
	J	<LOD	<LOD	-	<LOD	<LOD	-	-
	L	<LOD	<LOD	<LOD	<LOD	<LOD	13.53	-
	Number of samples measured	4	4	3	4	4	1	0
	Average	<LOD		<LOD	<LOD	<LOD	54.98	-
	% Feeding Solution††	Not applicable					16.7	Not applicable

* "-" indicates that data are not available

‡Nominal concentration in ppb is estimated from the concentration in μg/L by assuming the volume density of the test solution to be 1.2296 g/ml.

†† % Feeding concentration: the average of measured concentration compared with the nominal feeding concentrations in ppb.

3.7.2.4. Detection of imidacloprid in test hives at CCA2, CCA4, CCA5, and CCA8

Imidacloprid was detected during the entire course of the study in test hives before the feeding exposure (CCA2) and in the control hives during the exposure (CCA4) and post exposure (CCA5). However, it was not detected in the live control hives after overwintering (CCA8). The magnitude and the frequency of detection of imidacloprid in control hives are summarized in **Table 20**. The detection in control hives was relatively low and was <LOD in the majority of control hives. In a total of 35 bee bread samples from control hives, imidacloprid was detected in 6 samples with a maximum of 1.24 ppb. In a total of 36 uncapped nectar samples, imidacloprid was detected in only one control hive at 2.98 ppb. 33% of the hives had been exposed to the test chemicals before the start of the test (CCA2), and <20% of control hives were contaminated with the test chemical during (CCA4), and one week after, the exposure (CCA5).

Before the start of the artificial feeding exposure (CCA2), test hives had been contaminated with a low level of imidacloprid. Out of the total six hives measured, imidacloprid was detected in bee bread of two hives (33% of the measured hives) at 0.43 and 1.19 ppb respectively (LOD= 0.36 ppb). However, it was not detected in uncapped nectar (LOD= 1.43 ppb) in all six measured hives.

During the exposure period (CCA4), the control hives were contaminated with a low level of imidacloprid. Out of 23 control hives measured, imidacloprid was detected in hive bee bread in three control hives at 1.05, 1.24, and 0.68 ppb (LOD= 0.36 ppb), respectively. Out of 24 control hives measured, imidacloprid was detected in uncapped nectar in one control hive at 2.98 ppb (LOD=1.43 ppb). The frequency of detections in the control hives was 3/21 in bee bread and 1/24 in nectar.

One week after the end of the feeding exposure (CCA5), a low level of imidacloprid was also detected in the control hives. Out of five control hives measured, imidacloprid was detected in one control hives in bee bread at 0.55 ppb (LOD=0.36 ppb). Imidacloprid was not detected in any of the six control hives in nectar (LOD = 1.43 ppb). Out of 8 control hives, no imidacloprid were detected in hive bee bread and honey samples after overwintering (CCA8). It was noted that the frequency of detection of imidacloprid was lower in hive nectar than in the bee bread during CCA2, CCA4 and CCA5. This likely resulted from the less sensitive LOD in nectar as the maximum detection in bee bread was even lower than the LOD for nectar.

In order to consider the potential impact of the detected imidacloprid contamination, the maximum detections in the control hives were compared with the average residues detected in the treatment hives and expressed as a percentage in **Table 21**. The maximum residues in the controls counted for 1.8-41.6% of the average detections in treatment hives. The percentage varied by the test concentrations, low in treatments with high concentrations but high in treatments with low concentrations. It appeared that the level of imidacloprid contamination might have a greater impact to the treatments with low concentrations than the higher ones. Therefore impact of the contamination should not be ignored especially for treatment at low concentrations. However, due to the low detection frequency in control hives, this impact is likely to be only on a few individual hives.

Imidacloprid detected in uncapped nectar in the control hives indicated that a slight level of cross foraging among test hives might have occurred during the exposure period. Overall: (1) imidacloprid (2.98 ppb) was detected in one control hive nectar during the exposure period at CCA4, but no detection (<LOD =1.43 ppb) in all hives prior to the feeding exposure (**Table 20**); (2) Although a low level of imidacloprid was detected in one of the control feeding solutions at 0.45 ppb (Table 14), this level was so low it would unlikely result in such a high level of the detection in hive nectar (about 6X increase); (3) No other sources of imidacloprid were detected at CCA4 and earlier. Imidacloprid was not detected (LOD=0.36 ppb) in any

pollen samples trapped in the monitoring hives at CCA4 and earlier (**Table 21**). Considering the dose-response relationship of the residues detected in the treatment hives, the imidacloprid contamination in control hives is expected to have minimal impact to the colony level effect, especially for the treatments with high concentrations.

Some individual hives with measured residues had exposure levels more similar to exposure levels measured in higher or lower doses. While the residue data provides a good indication of exposure, there is some uncertainty regarding the extent of the variability in exposure since residues in all hives were not measured, residues were measured only at certain timepoints, and as discussed earlier, residue samples were taken only from one location in the hive. Therefore, there is some uncertainty regarding the true extent of variability in measured hive residues and exposure. The residue data does clearly indicate that a dose response-relationship is expected since higher treatment levels had higher mean measured residues in the hive pollen and nectar.

Table 20. Imidacloprid (ppb) detected in test hives before the exposure (CCA2), and in control hives during the exposure (CCA4) and after the exposure (CCA5 and CCA8).

	Pre-exposure (background)		**During exposure (26 Jun –8 Aug, 2013)**		**Post exposure (8 Aug 2013 – 22 Mar, 2014)**			
	CCA2		**CCA4**		**CCA5**		**CCA8**	
Sampling dates	30 May, 2013		18 Jul, 2013		14 Aug, 2013		22 Mar, 2014	
Sample matrix	**Bee bread**	**Uncapped nectar**	**Bee bread**	**Uncapped nectar**	**Bee bread**	**Uncapped nectar**	**Bee bread**	**Honey**
LOD (ppb)	0.36	1.43	0.36	1.43	0.36	1.43	0.36	1.43
Total number of samples measured	6	6	23	24	6	6	8	8
Number of samples with quantifiable level of residues (Residues in ppb)	2 (1.19, 0.43)	0	3 (1.05, 1.24, 0.68)	1 (2.98)	1 (0.55)	0	0	0
% of samples with detected residue (95% confidence limit, low-upper)	33.3 (4.3-77.7)	0.0 (0.0-45.9)	13.0 (2.8-33.6)	4.2 (0.1-21.1)	16.7 (0.4-64.1)	0.0 (0.0-45.9)	0.0 (0.0-36.9)	0.0 (0.0-36.9)

Table 21. Comparison between the maximum detections of imidacloprid in control hives and the average residues detected in the same hive matrices in the treatment hives fed at different concentrations of imidacloprid for three weeks (CCA4).

Test concentration in feeding solution		**Average residue measured in bee bread) at CCA4 (ppb**	**Average residue in hive nectar at CCA4 (ppb)**	**% of the maximum detection in control hives in comparison to the average of measured concentrations in treatment hives at CCA4**			
				Bee bread			**Hive nectar**
ug/L	**ppb**			**Pre-exposure (CCA2) (Max=1.19 ppb)**	**During exposure (CCA4) (max=1.25 ppb)**	**Post exposure (CCA5) (max=0.55 ppb)**	**During exposure (CCA4) (max=2.98 ppb)**
12.5	10.2	2.86	9	41.6	43.7	19.2	33.1
25	20.3	5.37	19.7	22.2	23.3	10.2	15.1

Test concentration in feeding solution		Average residue measured in bee bread) at CCA4 (ppb	Average residue in hive nectar at CCA4 (ppb)	% of the maximum detection in control hives in comparison to the average of measured concentrations in treatment hives at CCA4			
				Bee bread			Hive nectar
ug/L	ppb			Pre-exposure (CCA2) (Max=1.19 ppb)	During exposure (CCA4) (max=1.25 ppb)	Post exposure (CCA5) (max=0.55 ppb)	During exposure (CCA4) (max=2.98 ppb)
50	40.7	10.84	39.5	11	11.5	5.1	7.5
100	81.3	17.89	71.8	6.7	7	3.1	4.2
200	162.7	NA	162.4	Not applicable	Not applicable	Not applicable	1.8

-Imidacloprid was not detected in hive nectar (LOD=1.43 ppb) in any control hives in CCA2 and CCA5
-NA: data was not available.
-Measured concentration in hive nectar was not corrected for sugar content.

3.7.2.5. *Comparison of concentration in feeding solution and hive matrices*

A correlation between the imidacloprid concentrations in the feeding solution and the concentrations measured in hive beebread and uncapped nectar was observed during the exposure period (CCA4) and one week after the end of exposure (CCA5). However, imidacloprid measured concentrations in hive matrices were lower than that in the feeding solution. The average concentrations in hive uncapped nectar and hive bee bread were 62.7% and 30.2%, respectively, of the concentration in the feeding solution (**Table 22**).

Table 22. Imidacloprid concentration measured in hive uncapped nectar and hive bee bread

Nominal concentration in test feeding solution	µg/L	12.5	25	50	100	200	Average
	ppb	10.2	20.3	40.7	81.3	162.7	
Imidacloprid concentration in hive uncapped nectar in % of the concentration of	CCA 4 (n=12)	62.0 (6.31)	65.1 (13.24)	68.0 (27.66)	57.6 (46.87)	67.1 (109.14)	64.0

Nominal concentration in test feeding solution	µg/L	12.5	25	50	100	200	Average
	ppb	10.2	20.3	40.7	81.3	162.7	
nominal feeding solution (average measured concentration in ppb)¥	CCA 5 (n=3)	57.8 (5.88)	35.3 (7.18)	67.5 (27.46)	67.6 (54.98)	78.7 (127.93)	61.4
Imidacloprid concentration in hive beebread in % of the concentration of nominal feeding solution (average measured concentration in ppb)	CCA 4 (n=12)	28.1 (2.86)	26.4 (5.37)	26.7* (10.84)	22.0** (17.89)	NA	25.8
	CCA 5 (n=3)	41.5 (4.22)	28.2 (5.74)	40.4 (16.44)	28.1*** (22.89)	NA	34.6

¥ Measured concentrations in uncapped nectar were corrected for sugar concentration equivalence.
* n=8; **n=4. *** n=1;

The study did not find a significant degradation of imidacloprid in the test solution. No imidacloprid transformation products (olefin imidacloprid and 5-hydroxy imidacloprid) were detected in almost all the samples of test solution and hives matrices.

Considering the stability of imidacloprid in the test solution, the reduced concentrations of imidacloprid in hive matrices likely indicates that test bees were also foraging for pollen and nectar from sources other than the feeding solution.

3.8. Pathogens

Besides a standard treatment for *Varroa* mites, no treatments for any other hive pests, predators or diseases were administered to any hives.

3.8.1. Varroa Presence

Varroa mite occurrence in the colonies was assessed the week before and after the feeding period, as well as after over-wintering (CCA3, CCA5 and CCA8). The number of mites per 100 bees was calculated (**Figure4**). Hives were treated with one application of Apiguard® (active ingredient: thymol) following typical apicultural practice for the region immediately after the September CCA's to prevent high mite loads. After over-wintering, the colonies of all treatment groups, except the 100 µg/L group, had similar *Varroa* infestation levels.

The study showed no correlation between the treatments and the level of *Varroa* infestation.

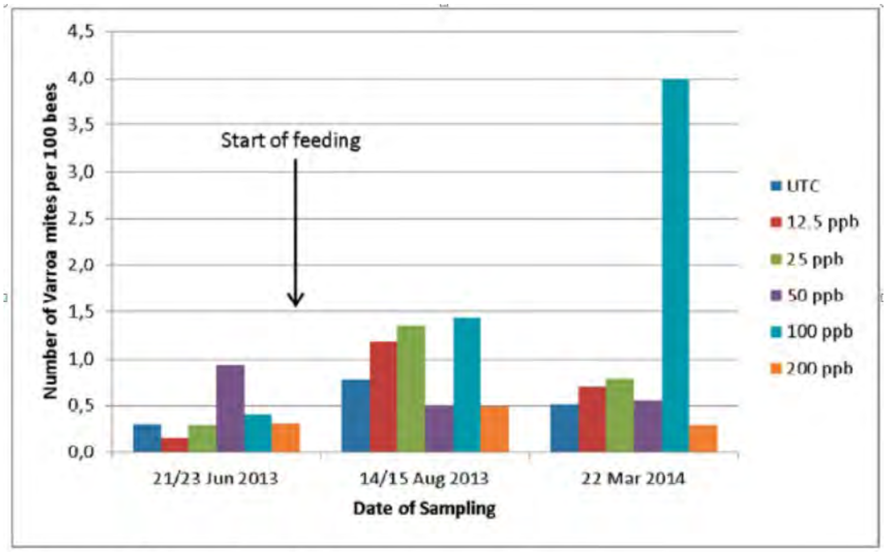

Figure 14 : Mean number of *Varroa* mites per 100 bees

Figure 4. Mean number of *Varroa* mites per 100 bees

3.8.2. *Nosema* presence

The number of *Nosema* spores per bee was determined at three time points at CCA3, CCA5 and CCA8. The study showed no correlation between the treatments and the level of *Nosema* infestation.

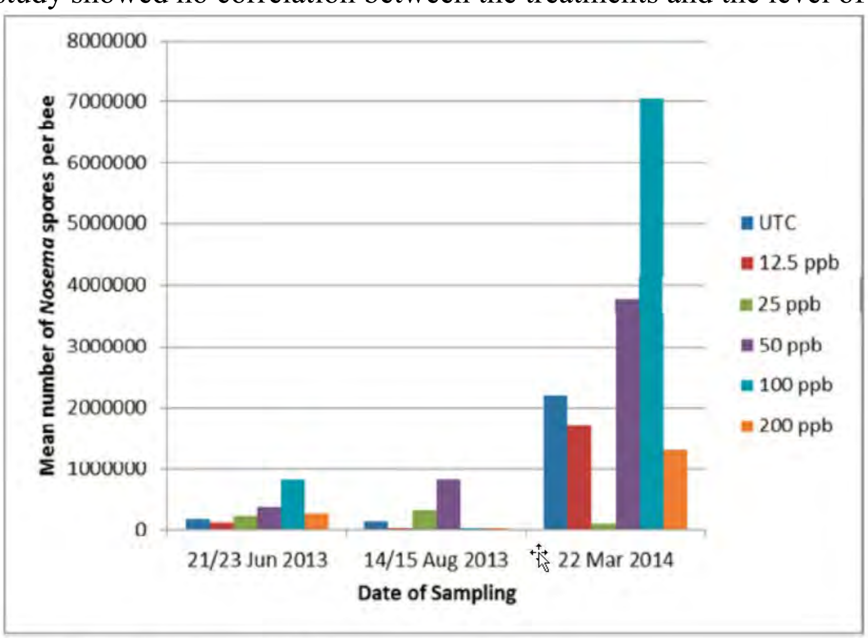

Figure 15 : Mean number of *Nosema* spores per bee

Figure 5. Mean number of *Nosema* spores per bee

3.9. Statistical Analysis

What follows are brief summaries of each of the statistical analyses employed for the review of this study. It is noted here, and later, when discussing the results that the PMRA, EPA, and CDPR statistical approaches, and when weighing statistical and biological lines of evidence, that the three Agencies are harmonized in the determination of the overall NOAEC and LOAEC.

3.9.1. Study Authors Analysis

The study author conducted statistical analysis using SAS (version 9.3). The analysis included colony strength (as indicated by mean number of adults), brood stages (as indicated by the mean number of eggs, larval cells, and pupal cells) and food stores (as indicated by the mean number of pollen and nectar/honey cells). For the pre-test data, all tests were done in a two tailed approach, whereas for the data assessed after application, one tailed (lower) tests were conducted. According to the study author, procedure GLM was used for ANOVA analysis. Williams' Trend Test was used to test data that passed statistical tests that assessed the assumptions of normality, variance homogeneity, and monotonicity. Dunnett's t-Test was used to test data that are non-monotonic, but pass tests of normality and variance homogeneity. Dunnett's T3 Test was used to test data that satisfy the criteria for normality, but fail the criteria for homogeneity of variance. For hive mortality, Cochran Armitage Exact Trend Test was used.

3.9.2. Study Reviewer Analysis

During the review of the study, a separate statistical analysis was conducted using the raw data submitted by the study author. As part of the collaborative review effort of the study by EPA, PMRA, and CDPR, a variety of statistical analyses were conducted for the evaluation of the data. The detailed methods of these analyses including statistical model selection and parameterization are presented in **Appendices A, B, and C** for the EPA, PMRA, and CDPR analyses, respectively. What follows is a brief summarization of each method. It is noted that while each method was distinct in the manner in which the data were analyzed, all three methods produced similar statistical results, that is, similar findings of significance for a given response variable at a given treatment level and CCA at a specified alpha level.

3.9.3. EPA Analysis

The general experimental design was a randomized complete block (apiary) with repeated measures (CCA). Since hives were not randomly assigned and placed in the study apiaries until shortly before CCA3, the data for the statistical analysis only included CCA3 through CCA8. For the two highest treatment levels (100 and 200 µg/L), data obtained from CCA8 was deleted from the analysis as only one and two hives (respectively) were surviving at the CCA8 measurement time. Temporal correlations were evaluated for each response variable; compound symmetry with heterogeneous variance was selected as the best fitting covariance structure. PROC MIXED in SAS was used for the data analysis, and the TREATMENT*CCA interaction was statistically significant for all evaluated response variables. This interaction was explored by 1) at each CCA, treatment means were compared to the control using a one-sided Dunnett's test; and 2) for each treatment level, CCAs 4 through 8 were compared to CCA3 using a two-sided Dunnett's test. Further details of the EPA statistical analysis can be found in **Appendix A.**

The EPA approach controlled for multiplicities by using a Dunnett's test which holds the family wise error rate (*i.e* the probability of make Type I errors or false discoveries of significance) at or below the level of α.

3.9.4. PMRA Analysis

The differences of each measurement parameter between the treatment and control at the same apiary were calculated for each apiary site. The means of the differences among 11 sites (Apiary C excluded due to vandalism) were plotted. A formal comparison from the highest to lowest concentration with the control was carried out using an often used conventional analysis of the block randomised experiments using the raw data with adjustment for baseline measurements: linear modeling (or ANOVA) stratified on Apiary (block) and adjusted for baseline measurements at CCA3 with one-side testing for effect. In the remainder of the document where PMRA analysis results are referred to as "raw data" it is noted that it is actually the model estimates using the raw data with adjustment for baseline measurements. Taking into consideration a limited detection power in a typical field level study, alpha levels of both 0.1 and 0.05 were considered as statistically significant. A list of statistical P values for each measuring parameter is summarized and included in this report.

Prior to the data analysis, for the purposes of controlling for multiplicity in the statistical analysis, a primary parameter for detection of treatment effects was defined as the total individuals in hives (sum of eggs, larvae, pupa and adults) at CCA6. However, when determining an overall NOAEC all response variables were examined with equal weight in considering treatment related effects, but were considered against statistical results for the primary parameter. Further details of the PMRA statistical approach can be found in **Appendix B.**

3.9.5. CDPR Analysis

A multivariate mixed repeated measures model approach was employed and is distinguished from the univariate approaches above in that all bee life stages or hive food storage variables are simultaneously analyzed as a single model. Multivariate analyses of variance for fixed effects models are conducted, using Statistical Analysis System (SAS) software, through implementing the MANOVA option in the PROC GLM procedure. Recently, multivariate analyses have been extended to mixed models using the PROC MIXED procedure. The MIXED procedure is designed to conduct a mixed model analysis of variance where fixed and random effects can be specified. Inclusion of random effects in a model provides a broader application of results. For this study, locations were denoted as apiaries with individual hives as test subjects. Use of a mixed model with apiaries identified as a random variable provides some assurance that the results can be generalized to other locations and hives. Further details of the CDPR analysis can be found in **Appendix C.**

The CDPR approach controlled for multiplicities by way of a Bonferroni adjustment to fix the family wise error rate at α.

3.10. Hive mortality

The study author reported that 72 out of 84 colonies for biological observations were maintained over the 6-week exposure period. Apiary C was vandalized after 8 feedings during the weekend of 27/28th Jul 2013 during which hives were left open. This allowed all colonies in this apiary access to treated sugar solution and treated food stores in the hives. Since all hives were compromised, they were no longer used to collect data for the study and this apiary was thereafter removed from the study and subsequent data analysis.

From the 11 remaining sites with a combined 77 hives, 34 hives were considered dead by 22 March 2014 (end of test), as summarized by **Table 23** and **Figure 6**. The author reported that based on an one-sided Cochran-Armitage exact trend test, mortality in the 100 ppb and 200 µg/L treatments are significantly different from the control mortality ($p = 0.01$ and $p<0.01$ respectively).

After overwintering, only one hive survived (Apiary L) at the treatment 100 µg/L, and two hives survived (Apiary D and F) at the treatment 200 µg/L. The hive mortality showed a U-shaped response to the treatments (**Figure 6**). The percent mortality decreased from control (36%) to 25 µg/L (9%), and then increased from 25 µg/L to 50 ug/L (36%) to 100 µg/L (91%) and 200 µg/L (82%). It is noted from the results presented in **Table 23a and b** and **Figure 6** below show that control mortality after overwintering was higher than it was for the 12.5 and 25 µg/L groups. For this reason, the ability to detect treatment related decreases in overwintering colony survival may be masked by the magnitude of control colony loss.

Table 23a. Hive survival at CCA8 (after overwintering)

Treatment group	Apiary											
	A	B	C	D	E	F	G	H	I	J	K	L
UTC	A1	-	-	-	E1	-	G6	-	I5	J1	-	L7
UTC	A2	B8	-	-	E8	F7	-	H3	I8	J7	-	L5
12.5 ppb	A4	B3	-	D8	E5	F5	G4	H7	I7	J3	K2	L6
25 ppb	-	B4	-	D1	E4	F3	G8	H6	I6	J4	K6	L2
50 ppb	A8	B7	-	-	E7	-	-	H2	I3	J2	K5	L3
100 ppb	-	-	-	-	-	-	-	-	-	-	-	L4
200 ppb	-	-	-	D3	-	F1	-	-	-	-	-	-

Table 23b. Hive mortality after overwintering measure at CCA8

Treatment (µg/L)	Control	12.5	25	50	100	200
Number of deceased colonies /total colonies	8/22	2/11	1/11	4/11	10/11	9/11
Colony mortality (%)	36	18	9	36	91	82
Colony survival (%)	64	82	91	64	9	18

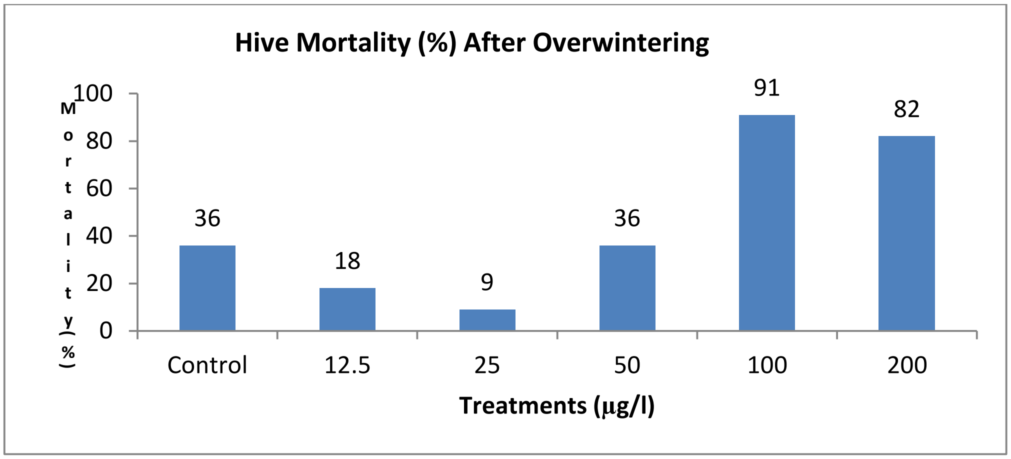

Figure 6. Hive mortality after overwintering**.**

3.11. Colony Condition Assessment Response Variables

What follows is a breakdown of each response variable assessed and the significant effects that were determined at each CCA. A couple of general points are made below when examining the results of the response variables:

- Unless explicitly stated otherwise, all discussion of statistical findings refer to shared determinations from the PMRA, EPA, and CDPR analyses.
- All analyses considered effects at both the 0.05 and 0.1 alpha levels when weighing statistically significant effects with biological considerations.
- The tables are the percent differences from control based on raw counts of the data (model estimations using the raw count data with adjustment for baseline measurements at CCA3) and generated by PMRA. The figures with significance "dots" were based off of the proportions of frame coverage for each hive for each response variable (with the exception of hive weight) and were generated by EPA.
- In the EPA analysis, the data in the 100 and 200 µg/L treatment groups were excluded from the analysis at CCA8. This was done primarily to facilitate the statistical model converging. Excluding these two treatment groups from the analysis at CCA8 is not expected to have an impact on the interpretation of results as there was a clear effect at these two treatment groups by the time of CCA8 indicated primarily by hive mortality.
- Even though data from CCA8 at the 100 and 200 µg/L groups were included in the PMRA analysis, the lack of statistical difference from control in these two groups is considered unreliable as there were only one and two remaining hives in these groups at CCA8, respectively.
- The PMRA approach used raw counts of the each response variable while the EPA and CDPR approaches converted these data into proportion of frame coverage (using methods described in **Appendix A**) to facilitate convergence of the statistical model. The tables of percent differences from control are based off of the raw counts of the data (model estimates using raw counts adjusted for baseline measurements) while the figures present the trends of the proportions of life stages and food stores with significant findings indicated. When differences in statistical findings are discussed, the findings of PMRA were based off of the raw counts (model estimates using raw counts

adjusted for baseline measurements) while the findings of the EPA and CDPR approaches were based off proportions.

- CCA3 was the baseline covariate and therefore is not presented in the tables for each response variable with percent reductions.
- CDPR did not include the "total individuals" endpoint in its analysis so those results will only pertain to EPA and PMRA findings.
- For its simplicity in visualizing the trends and findings of statistical significance simultaneously, the EPA-generated figures are presented below. The figures generated by PMRA can be found in **Appendix B** and those generated by CDPR can be found in **Appendix C.** It is noted here as well as above that in the discussion of each response variable, the results of all approaches will be discussed and noted where divergent from each other.
- The figures below indicate significance with black and red "dots" denoting a significant finding at the 0.1 and 0.05 alpha levels, respectively. Although these figures refer to EPA's analysis, as mentioned previously, the PMRA, EPA, and CDPR statistical results were generally in agreement and it is noted below where there were differences. As also mentioned previously, although there were different statistical findings in a few cases depending on the method employed, the interpretation of the results leads to a shared overall NOAEC/LOAEC of the study.
- While the EPA and PMRA analyses looked at each response variable across CCAs, the CDPR is distinguished from this approach by looking at all life stages or food store variables simultaneously within a single CCA across treatments.
- While it is not depicted in the figures below, it is acknowledged (and addressed in a variety of ways through the various statistical approaches) that there was considerable variability for some response variables at certain treatment groups and CCAs. Please refer to **Appendix A** for summary statistics tables (*i.e.* max, min, standard deviation values) of the proportions of each response variable for further information.

Life Stages

3.11.1. Adults

Figure 7 below shows the effects on adult honey bees across CCAs and treatment groups. Compared with the control, no differences in the number of adults in hives ($p>0.1$) during the CCA4 exposure period were apparent in any of the treatments with the exception of the CDPR analysis where a marginally significant ($0.05< p< 0.1$) reduction was determined at the 200 µg/L group. Additionally, the total number of adults in the 12.5 and 25 µg/L treatments was not reduced in any of the CCAs. However, the numbers of the adults in the 100 and 200 µg/L treatments were consistently reduced ($p<0.05$) at CCA5, CCA6, and CCA7 with reductions ranging from 24.4 – 59.4% (data from CCA8 excluded from EPA analysis in the 100 and 200 µg/L group due to clear effects on hive mortality). An exception is that the EPA and CDPR analyses did not determine a significant reductions ($p>0.05$) at the 200 µg/L treatment group at CCA5 while the PMRA analysis did. However, it is apparent from all analyses that there were impacts to adults at the 200 µg/L group during the course of the study. The number of adults in the 50 µg/L treatment was also reduced with marginal statistical significance at CCA5 ($0.05< p<0.1$) but at $p<0.05$ at CCA6 and CCA8 with percent reductions from control of 21.7%, 19.8% and 78%, respectively based off raw counts (**Table 24**). The PMRA analysis also determined a significant reduction from control at CCA7 while the EPA and CDPR analyses did not, but like the 100 and 200 µg/L treatment groups, the persistent nature of significant reductions to adults at the 50 µg/L group is evident.

Table 24. Estimated percent reduction from control for number of adults

Test concentration (µg/L)	Estimated reduction from control (%)[1]				
	CCA4	CCA5	CCA6	CCA7	CCA8[2]
12.5	2.2	-5.4	2.3	-12.6	-11
25	2.7	8.7	7.7	-4.1	-4.3
50	-23.3	21.7*	19.8*	18.6*	78**
100	5.2	34**	28.7**	51**	172.5**
200	-29.2	24.4**	52.8**	59.4**	7.3

Note: Negative value indicates increased number of adults in comparison to control.
*0.05<p<0.1
p<0.05[1]Percent differences from control are based on the raw counts of adults, not proportions of the adults as **Figure 7 below shows.
[2]At CCA8, comparisons made to the 100 and 200 treatment groups are with uncertainty as 1 and 2 hives survived in these groups, respectively. These data are included in the PMRA and CDPR analyses but excluded from the EPA analysis.

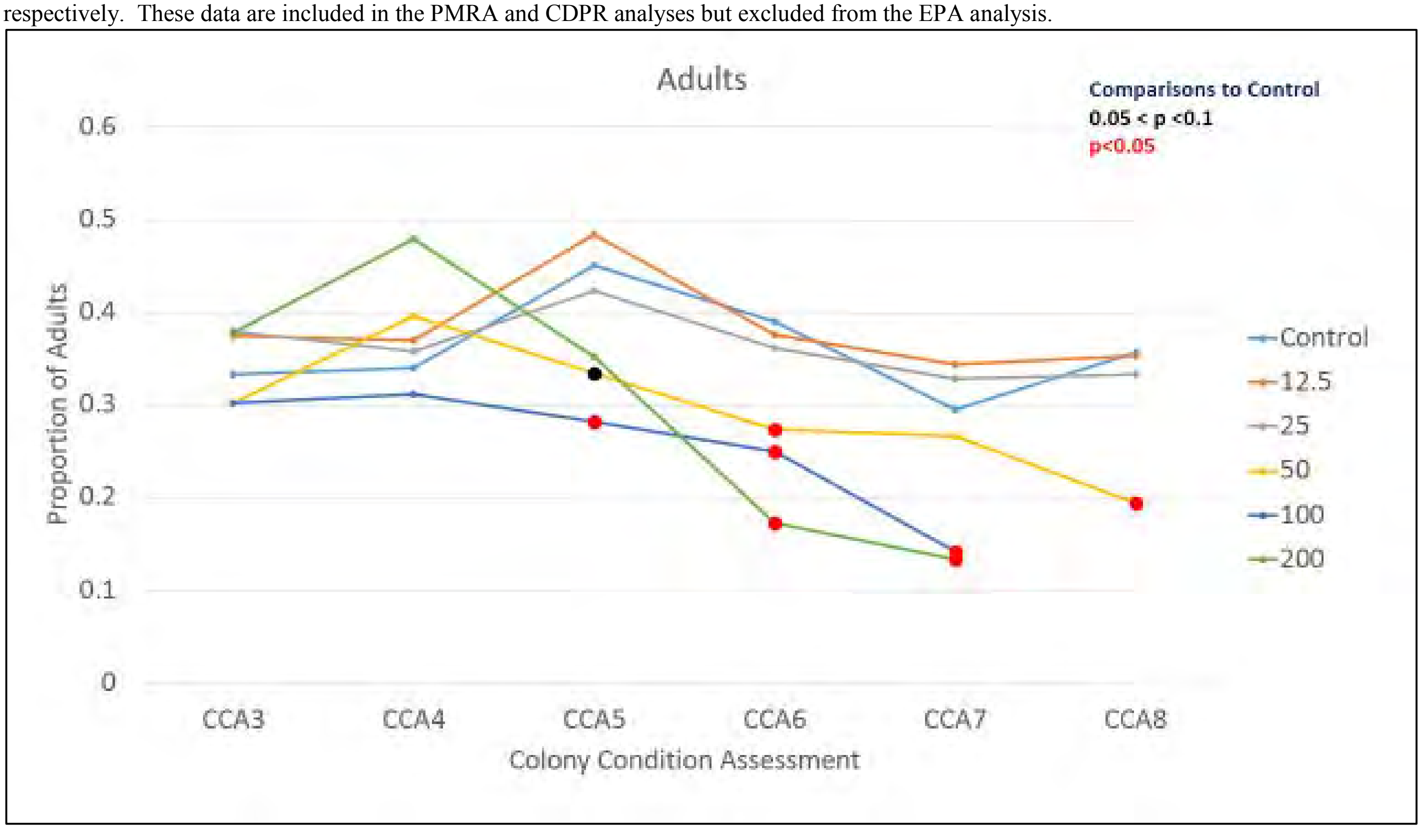

Figure 7. Proportion of adults following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8.

Figure 8 below shows the trends proportions of adults across the CCAs for the control and three lowest treatment groups only as the impact at the two highest groups was evident, especially when considering overwintering mortality. Removing the two highest treatment concentrations adjusts the scale of the figures to see the trends more clearly at the lower treatment groups. There is a clear divergence in the trends at the

50 µg/L treatment group not only in the decline in numbers beginning one CCA earlier (CCA4 as compared to CCA5 in the control, 12.5, and 25 µg/L groups) but also the average proportion of adults after overwintering at CCA8 (approximately 20% frame coverage as opposed to 33-35% for control and the lower groups).

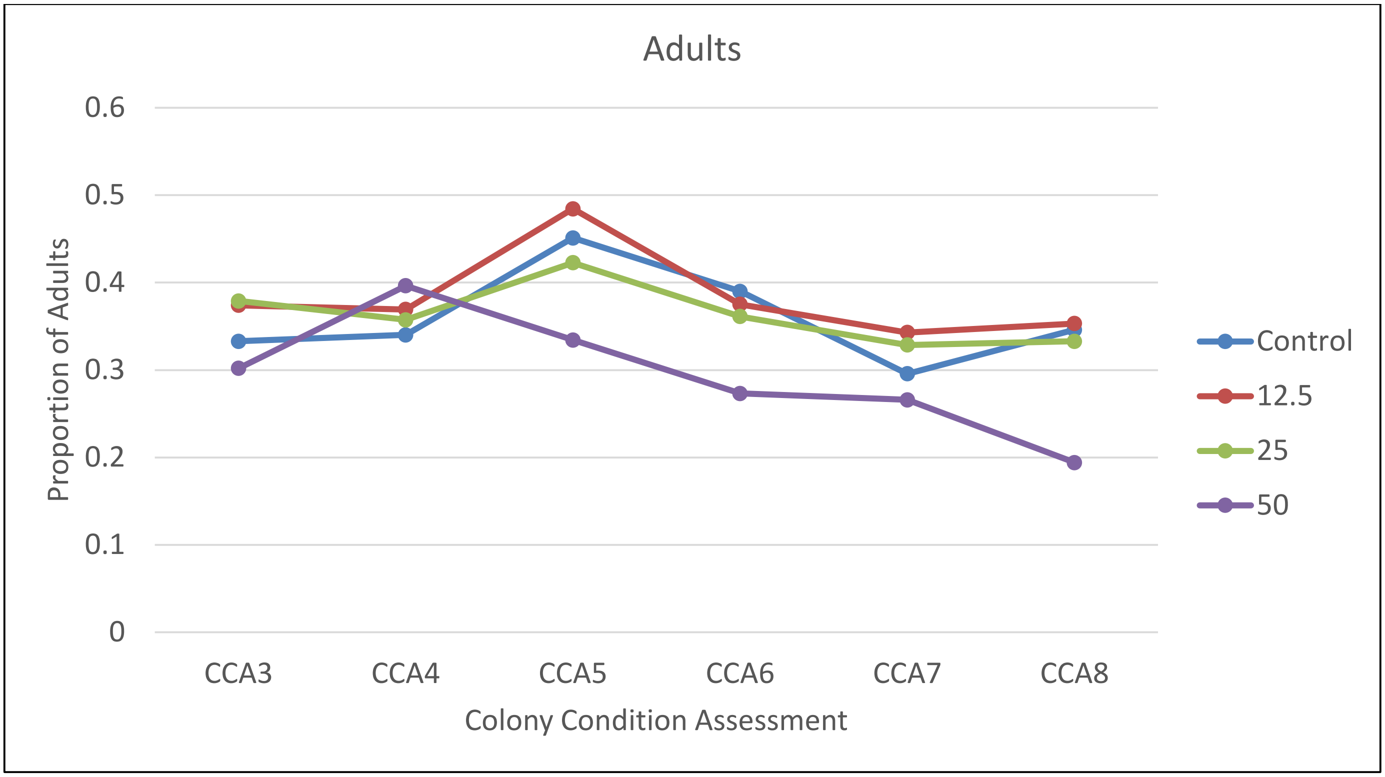

Figure 8. Proportion of adults following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8 in the control, 12.5, 25, and 50 µg/L groups only.

In an examination of the trends of adults in the control group, by CCA6, the average number of adults began to decline to 39% based off the proportions of adults covering the hive frames (**Figure 9** below). CCA6 represents the time when the colony as a whole starts to prepare for overwintering and therefore starts to begin a "shut-down" phase where the numbers of adults and other life stages are clearly decreased by the time of CCA7. During this pre-overwintering phase, adult proportions decline due to natural die off of worker bees and reduced rates of replenishment from reduced egg laying by the queen. It is noted that the average proportion of comb area as adults is similar after overwintering at CCA8 (35%) as compared to before exposure at CCA3 (33%) when the hives were initially placed in the test sites.

Also notably, as distinguished from the control and 12.5 and 25 µg/L groups, while the proportions of adults for those groups generally increased through CCA5 before beginning to decline, the numbers of adults at the 50 µg/L began to decline as early as CCA4, where these numbers were being built up in the control and lower treatment groups to support the foraging worker bee force for nectar and pollen collection. This again is evidenced by the average proportion of adults at CCA5 in the 50 µg/L group which was 33% as compared to 45, 48, and 42% for the control, 12.5 and 25 µg/L groups, respectively (percent reductions ranging from 24 – 37% based off the proportions of frame coverage).

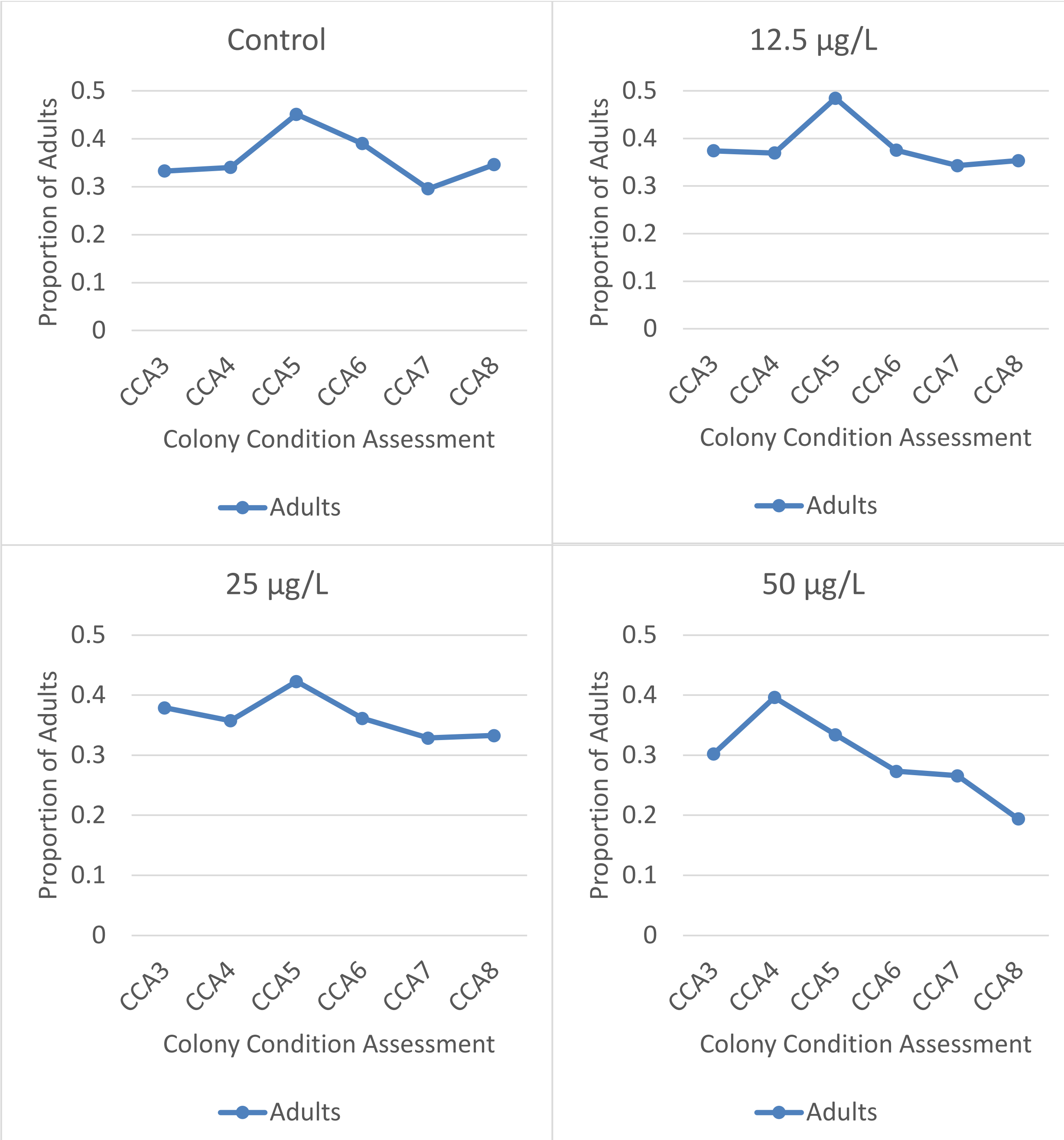

Figure 9. Proportion of adults following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8 in the control, 12.5, 25, and 50 µg/L groups.

When weighing statistical and biological significance, the overall NOAEC and LOAEC for adults is determined to be 25 and 50 µg/L, respectively.

3.11.2. Eggs

There were consistently lower numbers of eggs in treatments at 100 and 200 µg/L ($p<0.05$) during the course of the CCAs following exposure. There were minor differences in the statistical findings of the three analyses not only at a given CCA and treatment group but also at what alpha level an effect was significant at, but these differences do not have an impact on the determination that there was not only an early onset but a persistent nature of a reduction in the numbers of eggs that was not confined to one CCA. At the 100 and 200 µu/L treatment groups, all analyses determined significant reductions (p<0.1) in eggs for the 100 and 200 µg/L treatment groups for at least two CCAs. The sole finding of significance at the 50 µg/L group was at CCA8 at the 0.05 alpha level.

For the 12.5 µg/L the PMRA and EPA analyses only determined a significant reduction in the number of eggs at CCA8 (p<0.1). A similar finding was made at CCA4 in the PMRA analysis only. However, there were no significant reductions (p>0.1) at both CCA4 and CCA8 in the 25 µg/L group, indicating a lack of dose responsiveness within these time points. The biological significance of this finding at 12.5 µg/L is therefore considered to be low. Similarly, there was a significant reduction in eggs determined at CCA6 in the 25 µg/L group (PMRA analysis only). However, similar findings of statistical significance at this CCA was not determined for the 50 µg/L group. Finally, this effect was not observed before CCA6 or in the subsequent CCAs (CCAs 7 and 8) indicating this effect was isolated to this time point.

At the 50 µg/L treatment level, all analyses determined a significant reduction (p<0.05) at CCA8. While this effect was isolated to just CCA8 for this treatment group, there is uncertainty as to whether hives could have compensated for this reduction as could or may not have been shown by an additional CCA.

Table 25. Estimated percent reduction from control for number of eggs

Test concentration (µg/L)	Estimated reduction from control (%)[1]				
	CCA4	**CCA5**	**CCA6**	**CCA7**	**CCA8[2]**
12.5	22.8*	11.9	5.9	0.1	37.7*
25	-1.1	8.3	26.3*	31.3	5.8
50	-9.6	1.8	11.7	2.6	78.2**
100	37.9**	39.4**	70.8**	46.6**	138**
200	14.5	32.2**	60.1**	77.8**	153.2**

Note: Negative value indicates increased number of eggs in comparison to control.
*0.05<p<0.1
**p<0.05
[1]Percent differences from control are based on the raw counts of eggs, not proportions of the eggs as **Figure 10** below shows.
[2]At CCA8, comparisons made to the 100 and 200 treatment groups are with uncertainty as 1 and 2 hives survived in these groups, respectively. These data are included in the PMRA and CDPR analyses analysis but excluded from the EPA analysis.

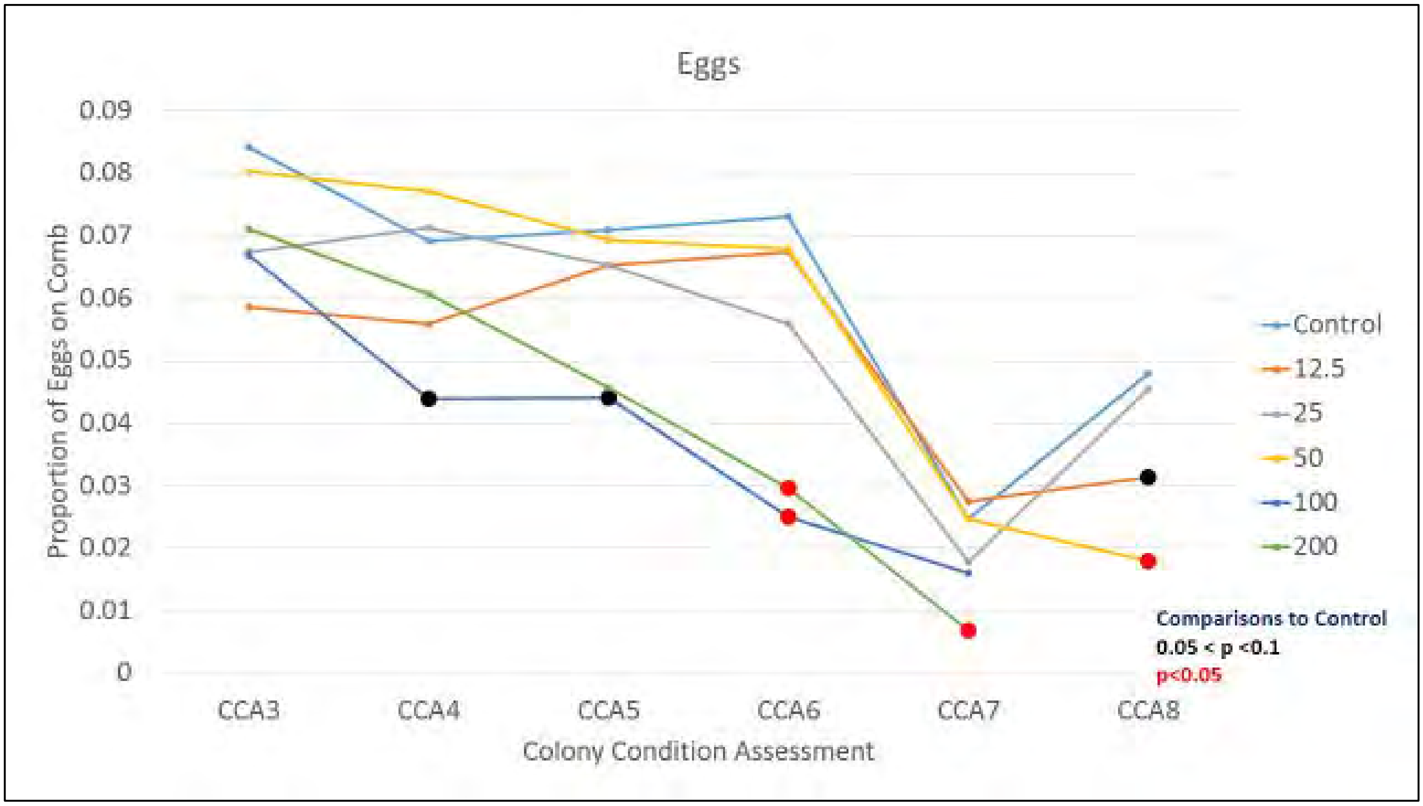

Figure 10. Proportion of eggs following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8.

Figure 11 below shows the responses for the control, 12.5, 25, and 50 µg/L treatment groups. Removing the two highest treatment concentrations adjusts the scale of the figures to see the trends more clearly. It is noted from this graph the variability present in the groups at CC3 before exposure had started. Particularly, the variation in egg coverage of the frame at the early CCAs is noted which may have contributed to some of these findings at CCA4.

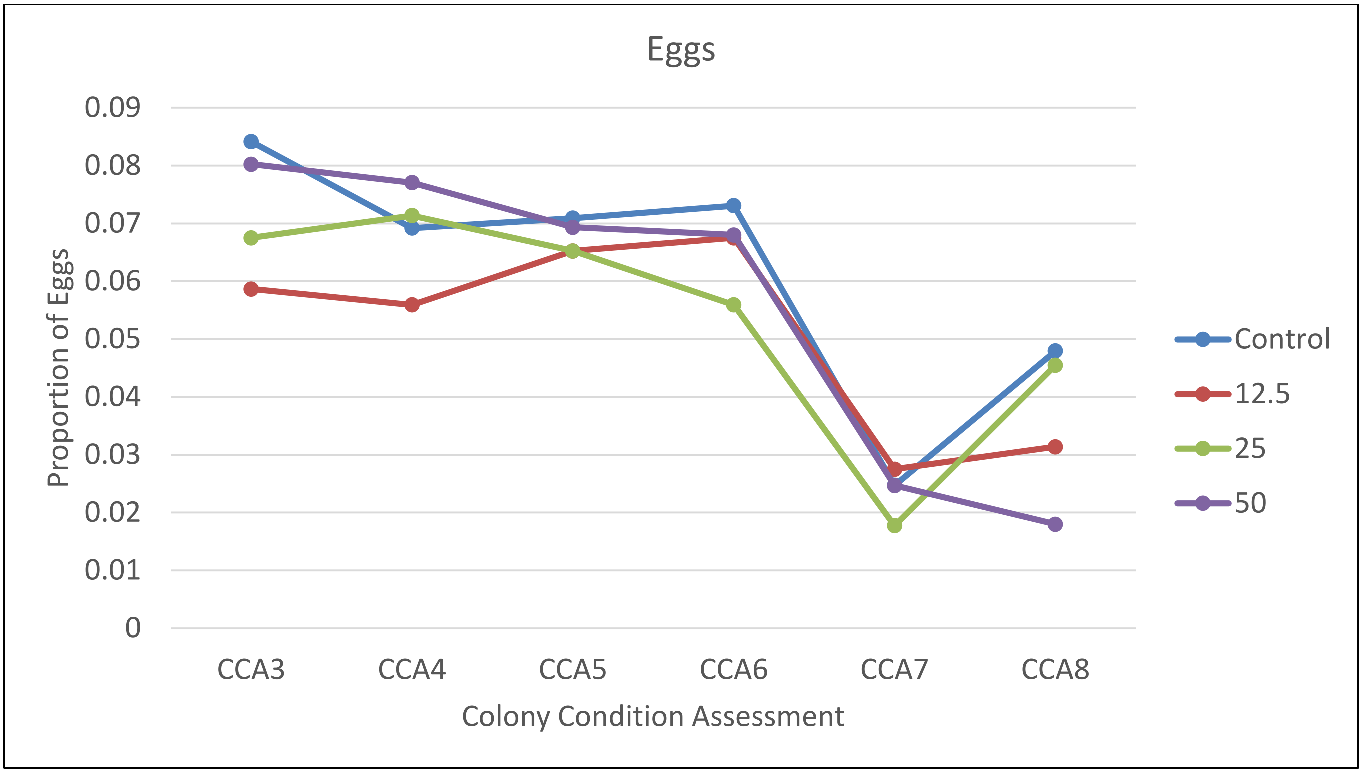

Figure 11. Proportion of eggs following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8 in the control, 12.5, 25, and 50 µg/L groups only.

By CCA8, the average of eggs in the control group increased relative to CCA7, but the average proportion at CCA8 (4.8%) was approximately half the proportion initially recorded at CCA3 (8.4%). It is noted that the 50 µg/L group was the only group of the control and three lowest treatment levels that underwent a downward trend from CCA7 to CCA8. Additionally, the average proportions of egg cells at CCA8 for the 50 µg/L group are approximately half of the proportion for the control, 12.5, and 25 µg/L groups (**Figure 12**).

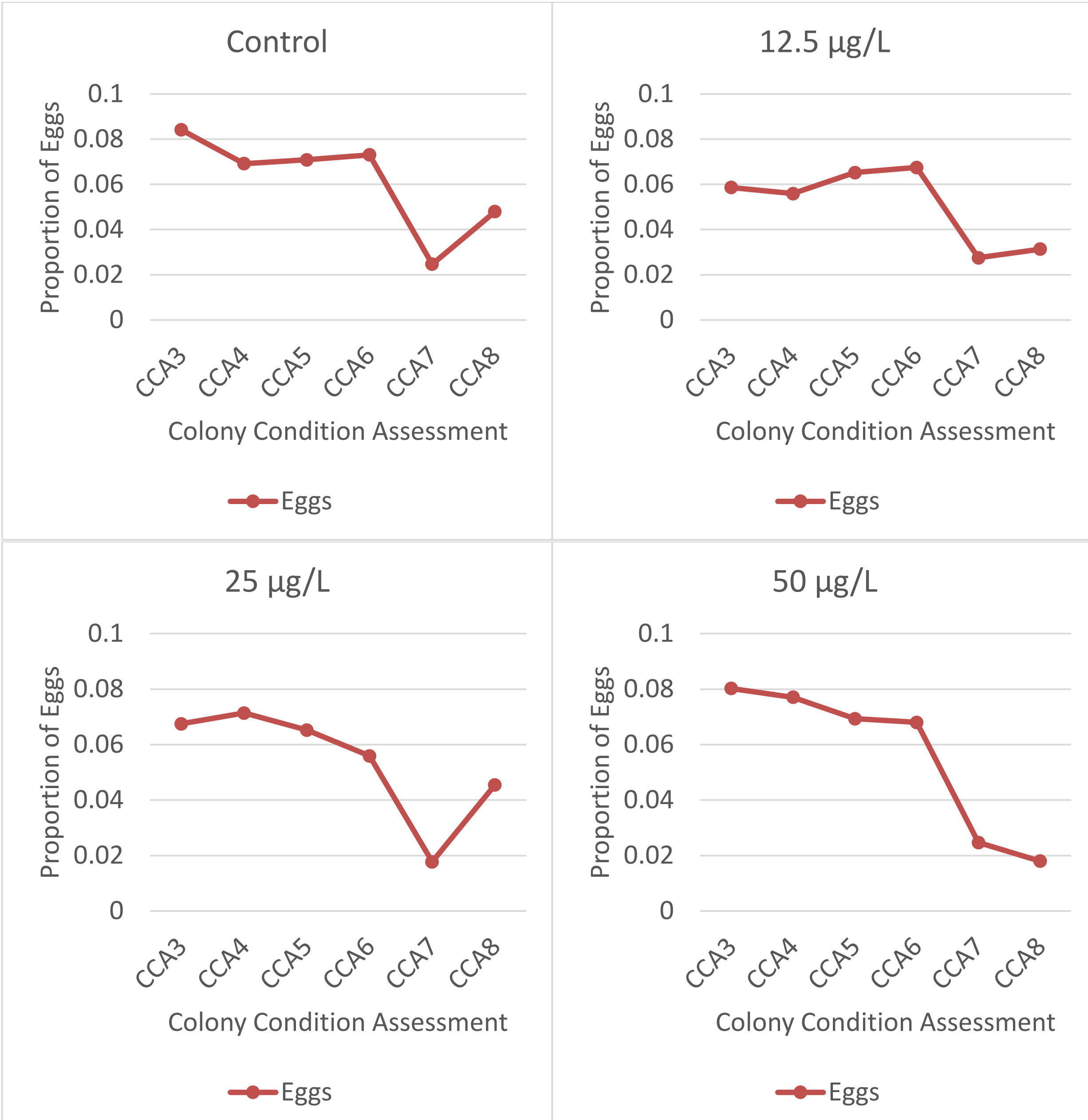

Figure 12. Proportion of eggs following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8 in the control, 12.5, 25, and 50 µg/L groups.

When weighing statistical and biological significance, the overall NOAEC and LOAEC for eggs is determined to be 25 and 50 µg/L, respectively, based on a significant reduction in eggs at CCA8 that represented a 78.2% reduction based on raw counts as well as being clearly divergent in its response at CCA8 (based off the proportions) from the control and 12.5 and 25 µg/L groups.

3.11.3. Larvae (Open/Uncapped brood)

There were consistently and significantly lower numbers of larvae in the 100 and 200 µg/L groups as compared to control ($p<0.05$) beginning at CCA4 and persisting throughout the entirety of the study up to and including after overwintering at CCA8 (data from CCA8 in these groups excluded from EPA analysis). There were no significant reductions from control at any CCA for the 12.5, 25, and 50 µg/L treatment groups in the EPA analysis.

In the PMRA analysis at CCA6, a significant reduction from control was determined at 25 µg/L ($p<0.1$), but not at 12.5 µg/L and 50 µg/L ($p>0.1$ for both treatments). Additionally, the effect was not determined at CCA4, CCA5, and CCA7. Similarly, the CDPR analysis determined a significant difference ($p<0.05$) at CCA7 at 12.5 µg/L, but this finding was not determined at the 25 and 50 µg/L treatment groups ($p>0.1$). Also in the PMRA analysis only, at CCA8, a statistical reduction was determined at 50 µg/L treatment group ($p<0.05$). This effect was not determined to be significantly reduced from control from CCA4 to CCA7 ($p>0.1$). Although this difference was not detected in the EPA and CDPR analyses, the percent reduction in larval cells at CCA8 for this group was 43% (based on raw counts).

Table 26. Estimated percent reduction from control for number of larvae (open/uncapped brood)

Test concentration (µg/L)	Estimated reduction from control (%)[1]				
	CCA4	CCA5	CCA6	CCA7	CCA8[2]
12.5	12.3	10.7	-15.5	-62.6	0.8
25	8.1	16.2	23*	-25.5	-9.9
50	12.1	15.3	-2.1	-32.4	42.6**
100	37.1**	30.9**	52.3**	64.4**	159.9**
200	64**	65**	57.2**	78.3**	54.1

Note: Negative value indicates increased number of larvae in comparison to control.
*0.05<p<0.1
**p<0.05
[1]Percent differences from control are based on the raw counts of larvae (open) brood, not proportions of the larvae (open) brood as **Figure 13** below shows.
[2]At CCA8, comparisons made to the 100 and 200 treatment groups are with uncertainty as 1 and 2 hives survived in these groups, respectively. These data are included in the PMRA and CDPR analyses but excluded from the EPA analysis.

Figure 13 below shows the trends of the control and all treatment groups for larval cells across all CCAs assessed. A clear divergence in the 100 and 200 µg/L groups is evident beginning at CCA4 where the numbers of larvae in these groups undergo a marked decline while the other treatment groups generally trend with control.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

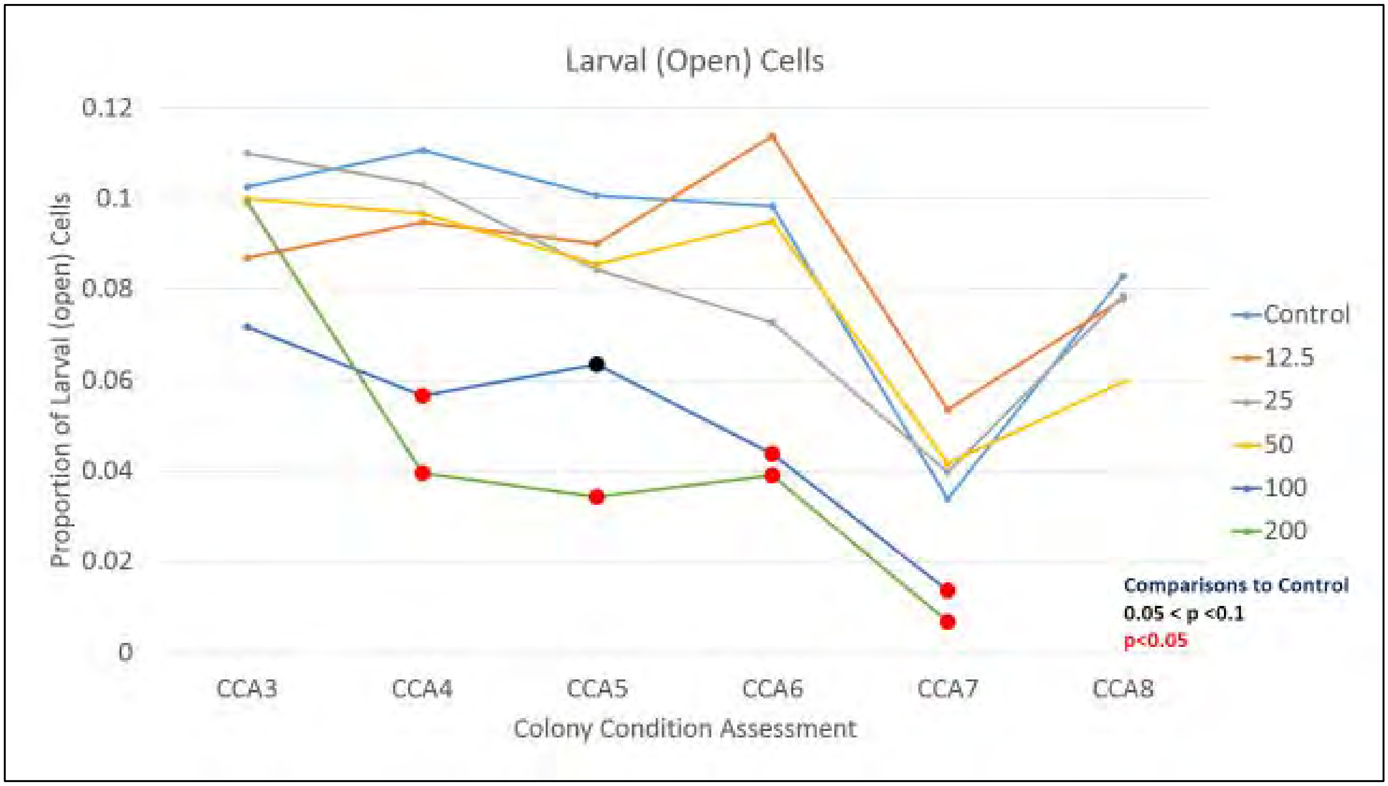

Figure 13. Proportion of larval cells following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8.

When examining the trends with the control and three lowest treatment groups alone, differences are less apparent than when the 100 and 200 µg/L groups depicted alongside with the exception of the separation of the 50 µg/L treatment group at CCA8 (**Figure 14**)

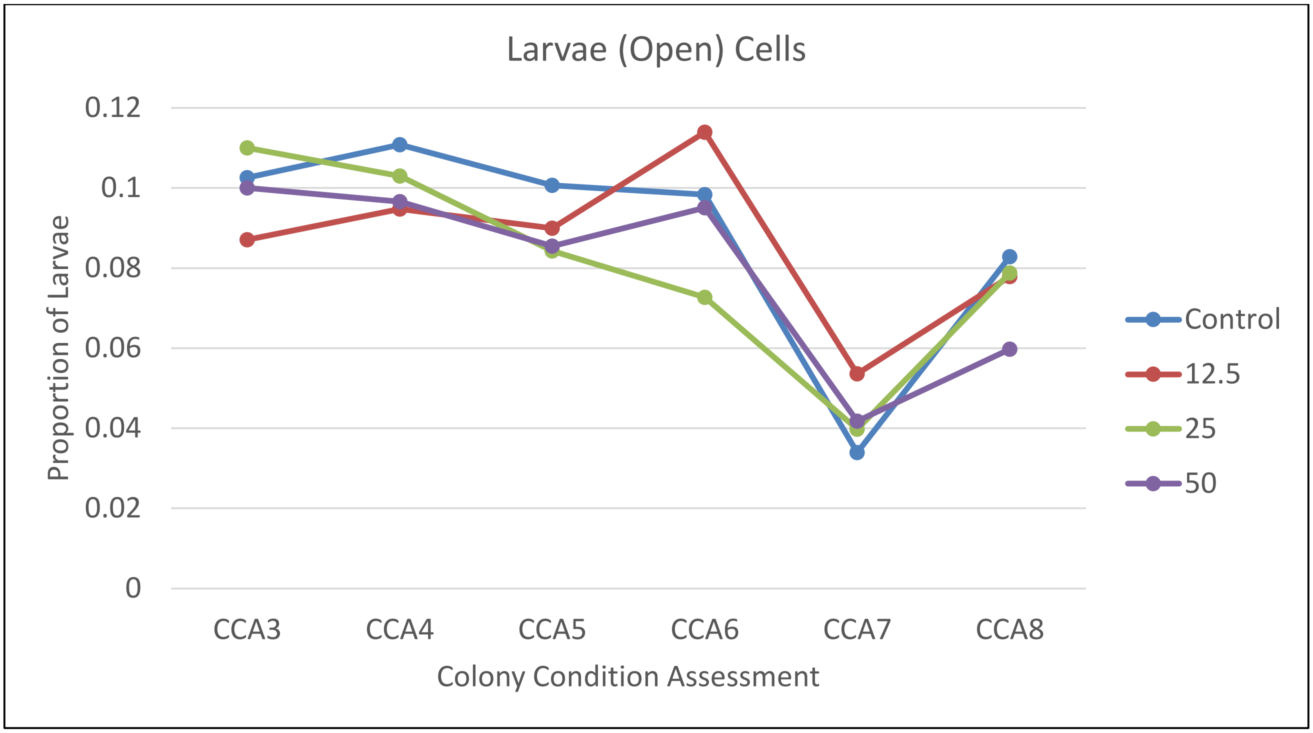

Figure 14. Proportion of larval cells following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8 in the control, 12.5, 25, and 50 µg/L groups only.

When examining the trends in the control group, the average proportion of larval cells increased from CCA7 to CCA8, and at CCA8 had a similar level (8.2%) that was recorded for CCA3 (10%) (**Figure 15**). With the 12.5 µg/L group, the starting average proportions of life stages at CCA3, prior to exposure, were similar to those in the control group with respect to the proportions of larval cells being approximately 5-8% of the comb area. The average proportion of larval cells trended down beginning as early as CCA4 (7%) before experiencing a more marked decline ahead of CCA7 (2%) as with the other life stages, which is anticipated ahead of overwintering. The proportions of larval cells were again relatively stable from the time of CCA3 (10%) to CCA6 (10%). This was preceded by a marked decline at CCA7 to an average proportions of 4%.

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Figure 15. Proportion of larval cells following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8 in the control, 12.5, 25, and 50 µg/L groups.

It is also noted from the treatment by treatment comparisons below for larval cells across CCAs that although the trends for the control, 12.5, 25, and 50 µg/L groups are similar, that the 50 µg/L group was the only group of the 4 that did not end up at approximately 8% coverage of the frame, but instead was approximately 6% of frame coverage. The 25 ppb group also look to undergo a more marked decline beginning at CCA4 as compared to the control, 12.5, and 50 µg/L group but as stated earlier, the difference was only marginally significant in the PMRA analysis, similar findings were not determined in the 50 µg/L group, and the effect at 25 µg/L was not significant at CCA7 and CCA8

When weighing statistical and biological significance, the overall NOAEC and LOAEC for larval cells is determined to be 25 and 50 µg/L, respectively. This is based on a significant reduction at the 50 µg/L at CCA8. While this finding is isolated to CCA8, it is an uncertainty what the response would have been at an additional CCA (*i.e.*, whether the hives could have compensated for this potential effect). It is also noted, as has been above, that the 50 µg/L group showed a reduced percentage of frame coverage of larval cells at CCA8 as compared to the control and two lower treatment groups.

3.11.4. Pupae (Capped brood)

In the 50, 100 and 200 µg/L treatment groups, there were significant reductions from control ($p<0.05$) that persisted through most of the study (EPA findings at the 50 µg/L were significant at two CCAs, CDPR at 3 CCAs and the PMRA analysis determined significant reductions from control at 5 CCAs). The percent reductions from control based on the raw counts of pupal cells in the 100 and 200 µg/L groups ranged from 49.7 – 93.5% during CCA4 – CCA7.

At the 12.5 µg/L treatment group, there were significant reductions determined at CCA6 for the EPA and PMRA analyses. While the findings were not determined at the 25 µg/L treatment group at CCA6 for the EPA and CDPR analyses, they were for the approach used by PMRA. It is noted that significant reductions in pupal cells were not determined by any analysis at 12.5 and 25 µg/L in any CCA preceding or subsequent to CCA6, thus the significant effect for the 12.5 and 25 µg/L treatments was isolated to the CCA6 timepoint. Additionally, although PMRA determined significant reductions at CCA6 for all treatment groups, the effects did not demonstrate a dose response at the lowest three doses with the percent reductions from control (based on raw counts of the data) at 22.3, 18.3, 12.5, 49.7 and 75.5% for the 12.5, 25, 50, 100 and 200 µg/L groups, respectively. However, responses at the lower three doses were all similarly reduced compared to controls (12.5 -22.3%), and some overlap in dose-response might be expected at the lower doses, given the variability and overlap in exposure among individual hives. It is also noted that the confidence intervals among the three lowest doses are similar and overlapping (see Bees 8, PMRA analysis Appendix B). Finally, after overwintering at CCA8, the levels of pupae in the surviving hives at the 12.5 and 25 µg/L treatment groups were actually above the level of control (based on raw counts of the data) by 1.3 and 10.8%, respectively. The percent reduction from control at CCA8 in the 50 µg/L group was 70.6%.

Table 27. Estimated percent reduction from control for number of pupae

Test concentration (µg/L)	Estimated reduction from control (%)[1]				
	CCA4	CCA5	CCA6	CCA7	CCA8[2]
12.5	2.8	-3.7	22.2**	-8.4	-1.3
25	17.7	5.8	18.3**	18.1	-10.8
50	28.1*	34.6**	12.5*	9.7	70.6**
100	51.7**	56.6**	49.7**	75.6**	150.9*
200	83.3**	79.5**	75.5**	93.5**	42

Note: Negative value indicates increased number of pupae in comparison to control.
*$0.05<p<0.1$
**$p<0.05$
[1]Percent differences from control are based on the raw counts of pupae (capped) brood, not proportions of the pupae (capped) brood as **Figure 16** below shows.
[2]At CCA8, comparisons made to the 100 and 200 treatment groups are with uncertainty as 1 and 2 hives survived in these groups, respectively. These data are included in the PMRA and CDPR analyses but excluded from the EPA analysis.

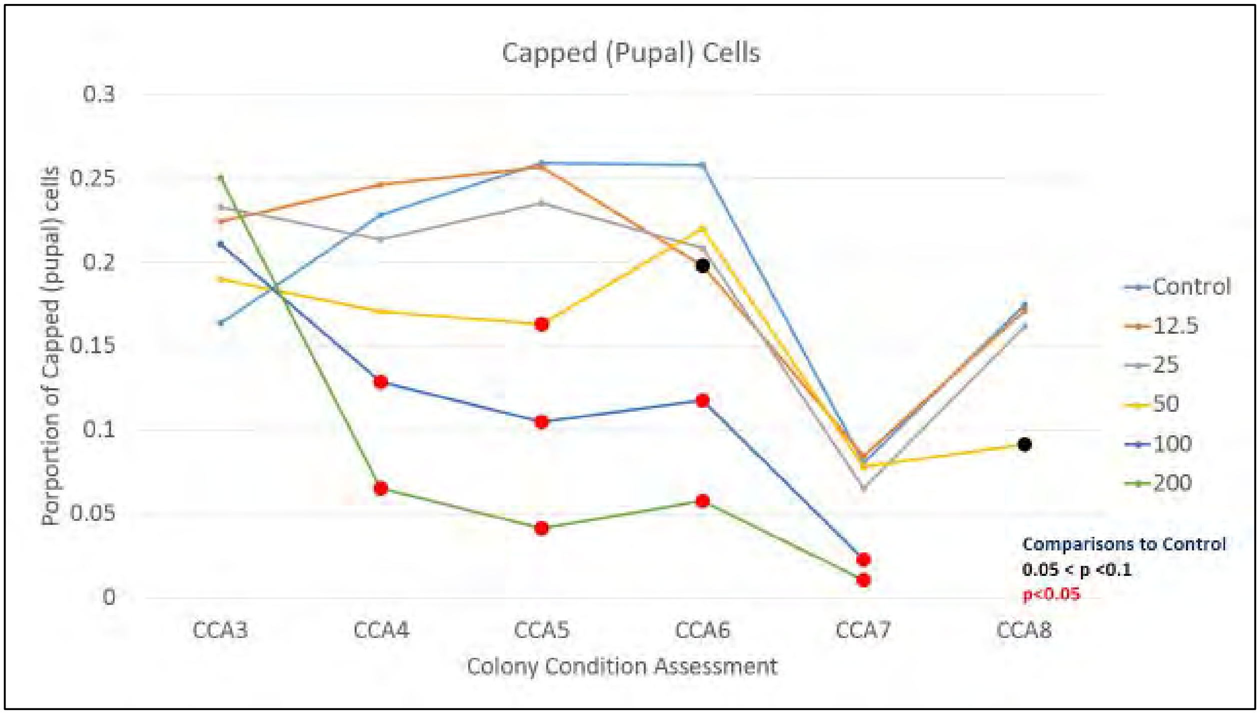

Figure 16. Proportion of pupal (capped) cells following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8.

In summarizing the information provided by the different analyses for this response variable, a few points can be made:

- All analyses find significant differences at the 100 and 200 µg/L levels starting at CCA4 and persisting until CCA7 (data from CCA8 excluded from EPA analysis at these two treatment groups)
- PMRA analysis determined significant difference at the 50 µg/L group (at either 0.1 or 0.05 alpha level) for all CCAs assessed except CCA7 (for CDPR, same findings except no significant findings at CCA6 and CCA7)
- EPA analysis determined significant differences at 50 µg/L group (at either 0.1 or 0.05 alpha level) at CCA5 and CCA8
- PMRA determined significant differences at all treatment groups at CCA6 while EPA determined significant effects for the 12.5, 100 and 200 µg/L groups, and CDPR only at 100 and 200 µg/L.

In further exploring this last point, the difference in findings can potentially be explained by the statistical model selections employed for each analyses. The discussion below focuses on additional lines of evidence to further characterize the findings.

Figure 17 shows the trends in pupal cells over the course of the study in the control and three lowest treatment groups only. As indicated in both the PMRA and EPA analyses, the impacts to pupal cells occurs early as there is divergence from the control as early as CCA4.

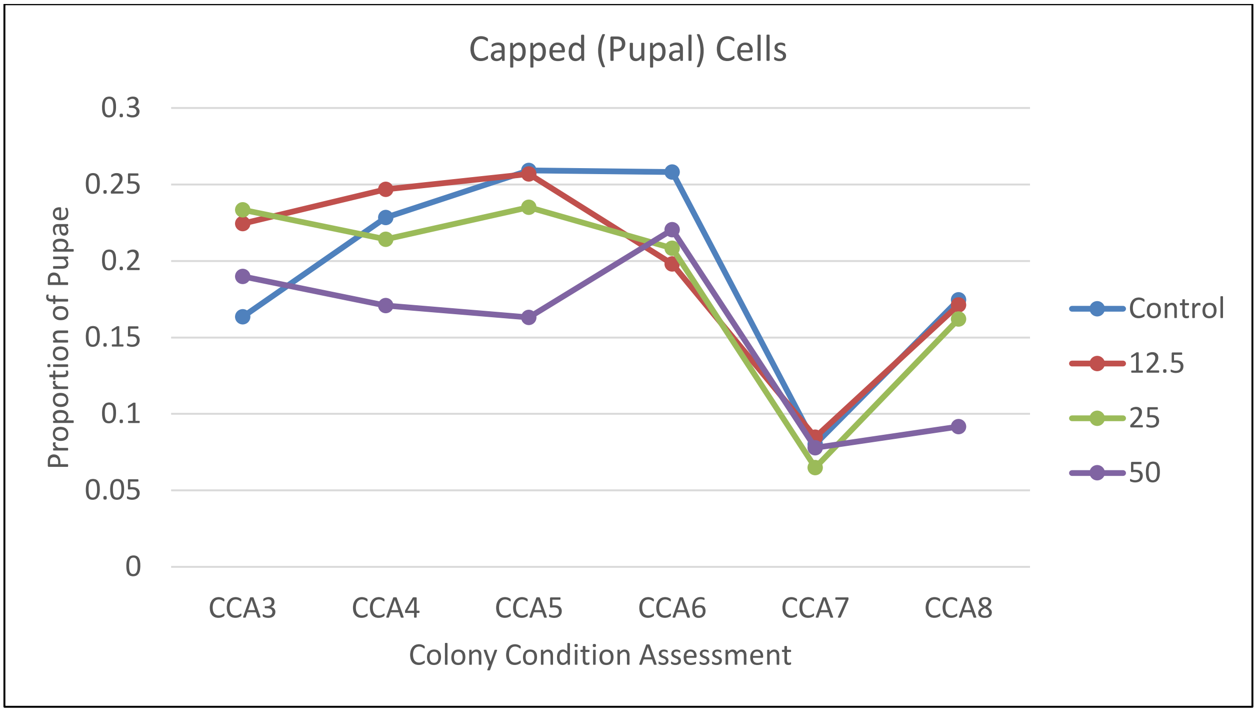

Figure 17. Proportion of pupal (capped) cells following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8 in the control, 12.5, 25, and 50 µg/L groups only.

It is noted that the average proportion of comb area as pupal cells in controls is similar after overwintering at CCA8 (17%) as compared to CCA3 (16%) when the hives were initially placed in the test sites. Also notably, the average proportion of pupal cells in the 12.5 and 25 µg/L groups (22%) is higher than it was for the control group (16%) but this is obviously not a finding related to imidacloprid treatment, given that exposure had not occurred yet.

In the 12.5 µg/L group, unlike the control group, where the average proportion of pupal cells remained stable between the time of CCA5 and CCA6 (26%), there was an apparent decrease in the 12.5 µg/L group from CCA5 (26%) to CCA6 (20%) based on average proportions. This decrease continued for the CCA6 to CCA7 interval to an equivalent level as controls (approximately 8%). After the overwintering period in the 12.5 µg/L group, the proportions of life stages were similar to CCA8 for the control group in that the proportion of pupal cells 17% at CCA8 (as compared to 17% in the control).

In the 25 µg/L treatment group, there were again no significant differences in the proportions of all life stages at CCA3 before the start of exposure ($p>0.05$). As opposed to the steady buildup that was observed in the control and 12.5 µg/L treatment groups from CCA3 to CCA5, the numbers of pupal cells remained similar from CCA3 to CCA5; they were decreased slightly at CCA4 (21%) as compared to CCA3 (23%) but at CCA5 (24%) were again to the level of CCA3. As with the other life stages, a decline in numbers was observed between CCA6 and CCA7 as the hives prepared for overwintering. The average proportion of pupal cells at CCA8 for the 25 µg/L treatment group were similar to the proportions in CCA8 of the control group, that is, 16% and 17% frame coverage at CCA8 for the 25 µg/L group and control group, respectively.

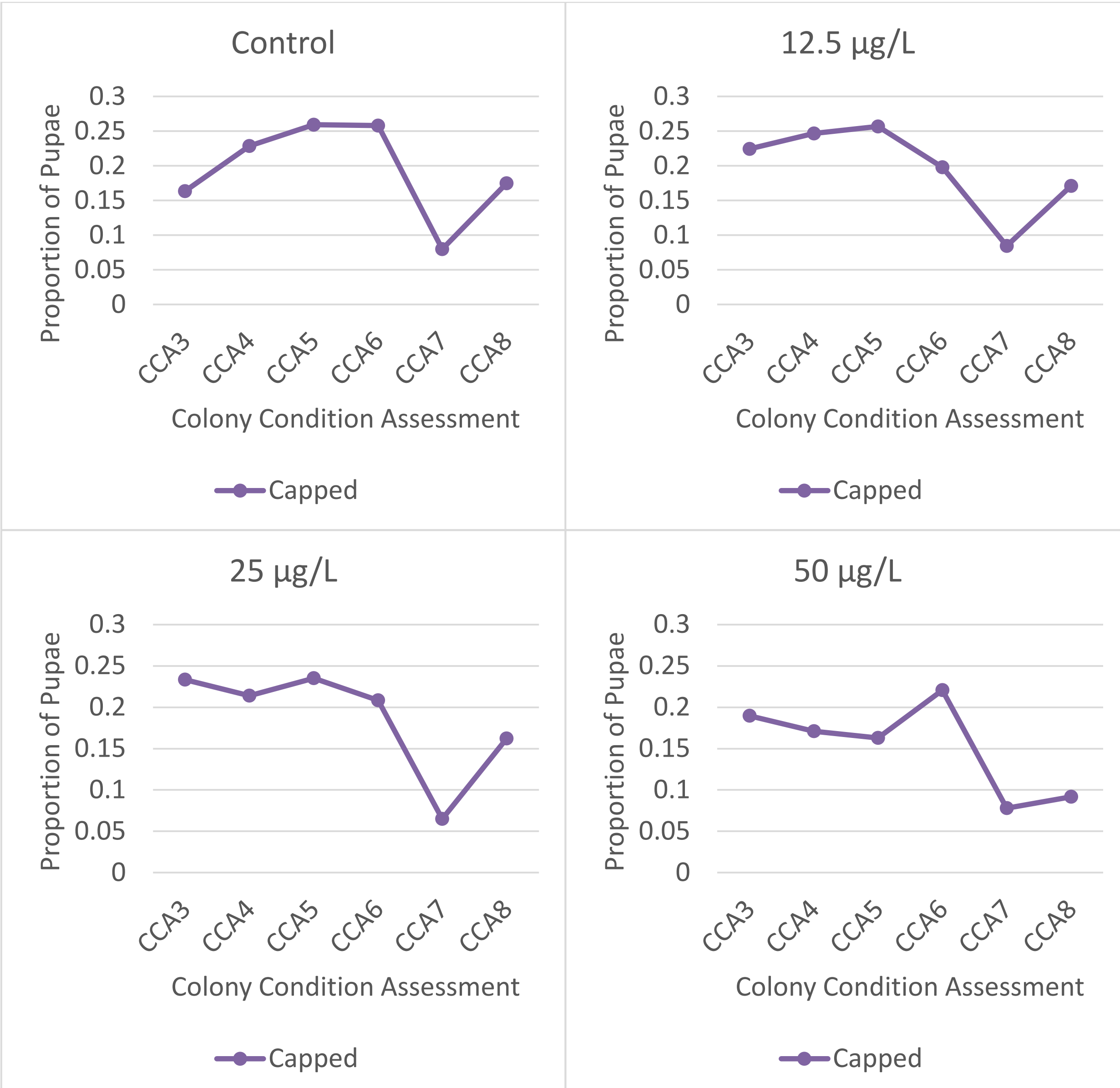

Figure 18. Proportion of pupal (capped) cells following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8 in the control, 12.5, 25, and 50 µg/L groups.

As distinguished from the control and 12.5 and 25 µg/L groups, the number of pupal cells at the 50 µg/L group underwent a steady decline beginning at CCA4 and continuing through CCA5 (average proportion at CCA3 was 19% compared to 16% at CCA5). This is also evidenced by the average proportion of pupal cells at CCA5 at the 50 µg/L group which was 16% of the comb areas as compared to 26, 26, and 24% at CCA5 for the control, 12.5, and 25 µg/L groups, respectively. This finding is also statistically significant for all analyses conducted as indicated above. An examination of the proportions at CCA8 also suggest the persistent nature of these effects and their lasting impact at this treatment group. The average proportion of pupal cells at CCA8 for the 50 µg/L group was 9% as compared to 17, 17, and 16% for the control, 12.5, and 25 µg/L groups, respectively. This finding was significant at the $\alpha = 0.1$ in the EPA analysis and a $\alpha = 0.05$ for the PMRA analysis (with 70.6 % reduction compared to control).

Regarding the statistical analyses, all methods found significant differences at 100 and 200 µg/L that were apparent at early CCAs and persisted throughout the study. Additionally, effects were noted at multiple CCAs for 50 µg/L, and the effects continued following overwintering. While all analyses found a significant effect at the 12.5 µg/L treatment level at CCA6, and the PMRA analysis also found significant effect at the 25 µg/L treatment level, these effects were considered transient. This is because effects at 12.5 and 25µg/L were isolated to CCA6 with levels returning to those similar to control after overwintering, and at CCA6 the effects lacked a clear dose-response relationship and were similar among all three lower treatment levels (12.5, 25, and 50 µg/L; 22.2, 18.3, and 12.5 % reductions compared to control based on raw data, respectively). Additionally, the discussion presented above indicates that the average proportions of pupal cells in the 12.5 and 25 µg/L group at different CCAs resemble the responses found in the control group in terms of their level before, during, and after exposure and overwintering. The effects at the 50 µg/L however, appear earlier, persist longer, and have a clear impact, especially after overwintering, when compared to the control.

When weighing statistical and biological significance, the overall NOAEC and LOAEC for pupal cells is determined to be 25 and 50 µg/L, respectively.

3.11.5. Total individuals in hives

When evaluating the proportion of frame coverage of total individuals, the pattern of effects has some similarity to the proportion of frame coverage of adults and pupae, as these two life stages make up the largest components of the hive population throughout the course of the study. In the 100 and 200 µg/L treatment groups, total individuals were significantly reduced from the level of control from CCA4 to CCA7 ($p<0.05$). The EPA analysis did not find a significant difference ($p>0.1$) at the 200 µg/L group at CCA4 but the impact at this treatment level is evident at other CCAs. The CDPR analysis did not assess this response variable.

Table 28. Estimated percent reduction from control for total number of individuals

Test concentration (µg/L)	Estimated reduction from control (%)[1]				
	CCA4	CCA5	CCA6	CCA7	CCA8[2]
12.5	4	1	8.7*	-16.9	-2.6
25	10.8	10.9	17.4**	2.2	-11.4
50	10.3	25.4*	12.1**	8.1	49.1**
100	35.3**	46**	48.2**	60.9**	145.1**
200	48.6**	60.5**	65.9**	74.6**	54.1

Note: Negative value indicates increased number of total individuals in comparison to control.
*$0.05<p<0.1$
**$p<0.05$
[1]Percent differences from control are based on the raw counts of total individuals, not proportions of the total individuals as **Figure 19** below shows.
[2]At CCA8, comparisons made to the 100 and 200 treatment groups are with uncertainty as 1 and 2 hives survived in these groups, respectively. These data are included in the PMRA analysis but excluded from the EPA analysis

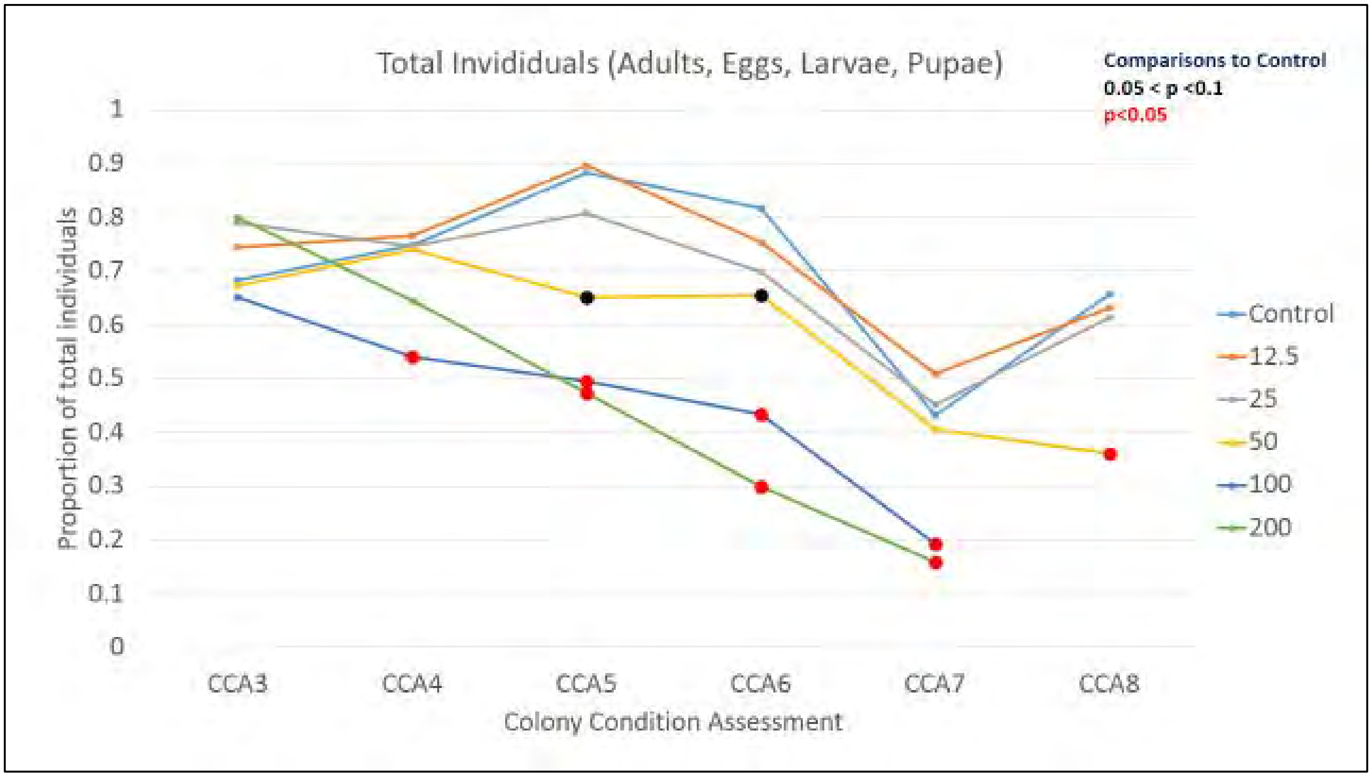

Figure 19. Proportion of total individuals (adult, eggs, larvae, pupae) following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8.

For the 50 ppb treatment group, reductions relative to the control at CCA5 and CCA6 were significant at α=0.10 and reductions relative to the control at CCA8 were significant for α=0.05 (CCA6 result was significant at 0.05 in the PMRA analysis). For the EPA analysis, there were no further findings of statistical significance which includes all CCAs at the 12.5 and 25 µg/L treatment groups. In the PMRA analysis, total individuals were significantly reduced at 12.5 ($p<0.1$) and at the 25 µg/L treatment groups ($p<0.05$). It is noted here, as it has been previously for other response variables, that the effects determined at 12.5 and 25 µg/L are isolated to CCA6, with no determinations of significance before and after this CCA, indicating this may be a transient effect. After overwintering at CCA8, the surviving hives in the 12.5 and 25 µg/L groups were actually above the level of control by 2.6 and 11.4% respectively (based on the raw counts of total individuals) while the 49% reduction from control at CCA8 in the 50 µg/L group was significantly reduced at a 0.05 alpha level.

When weighing statistical and biological significance, the overall NOAEC and LOAEC for total individuals is determined to be 25 and 50 µg/L, respectively.

Figures 20-22 below provide another visual representation of the effects across CCAs variables within a response variable for the various life stages of bees during the course of the study. The bar charts represent the percent differences from control with negative percent differences from control indicating an increase in a given response variable above the level of control. These figures provide further evidence of the general lack of dose responsiveness in effects at the three lowest treatment groups. Furthermore these charts are effective in indicating how the percent differences with a given response variable, changed over the course of the study within a treatment group. The 50 µg/L group chart (**Figure 22**) in particular shows a progression of a continuous impact throughout the study for certain response variables that is still present to a higher degree after overwintering at CCA8. It is also noted here that the scale for percent difference from control (y-axis) has been standardized across all charts. It is noted here also that negative ("-") responses refer to a percent increase above the level of control. Charts are not shown for the 100 and 200µg/L groups given the clear impacts on those hives across multiple response variables. Additional charts of the data represented in a slightly different way, across response variables within a CCA, are provided in **Appendix B**.

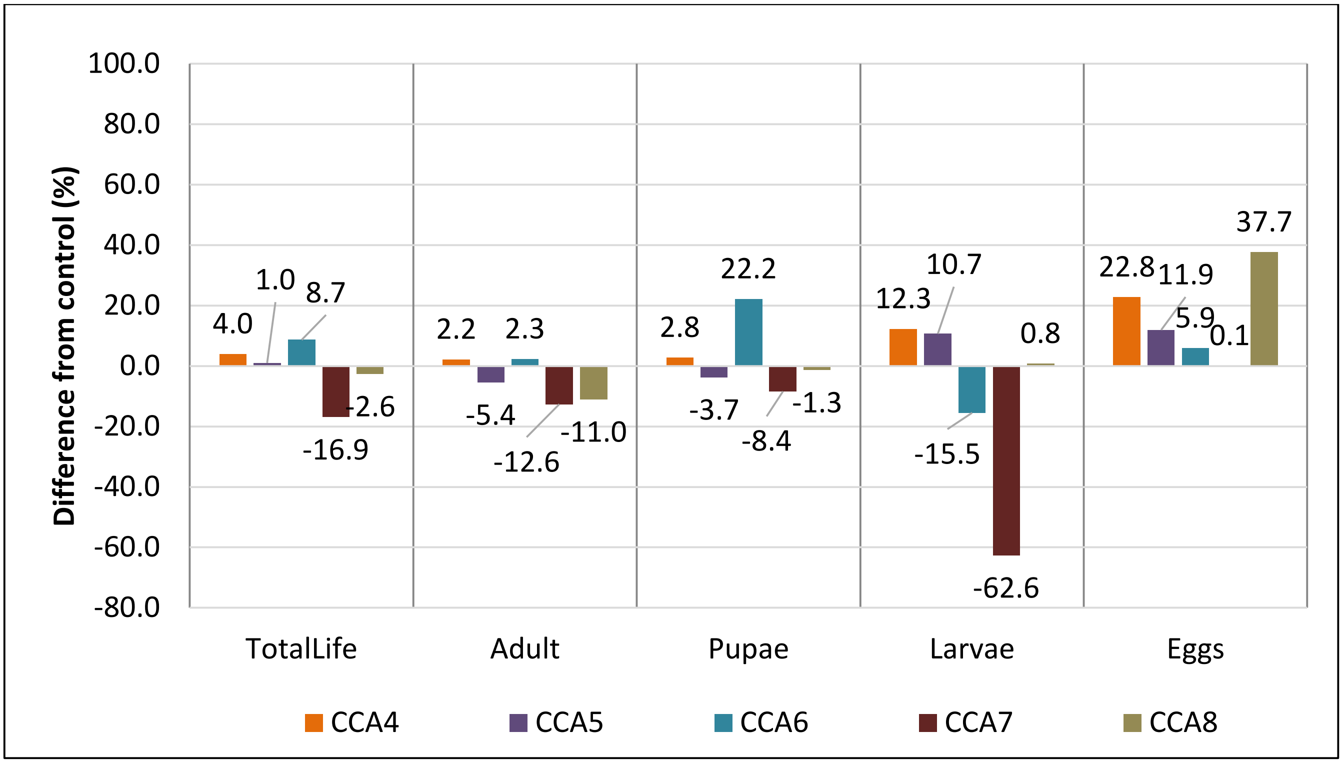

Figure 20. Summary of living organism parameters at the 12.5 µg/L treatment group

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

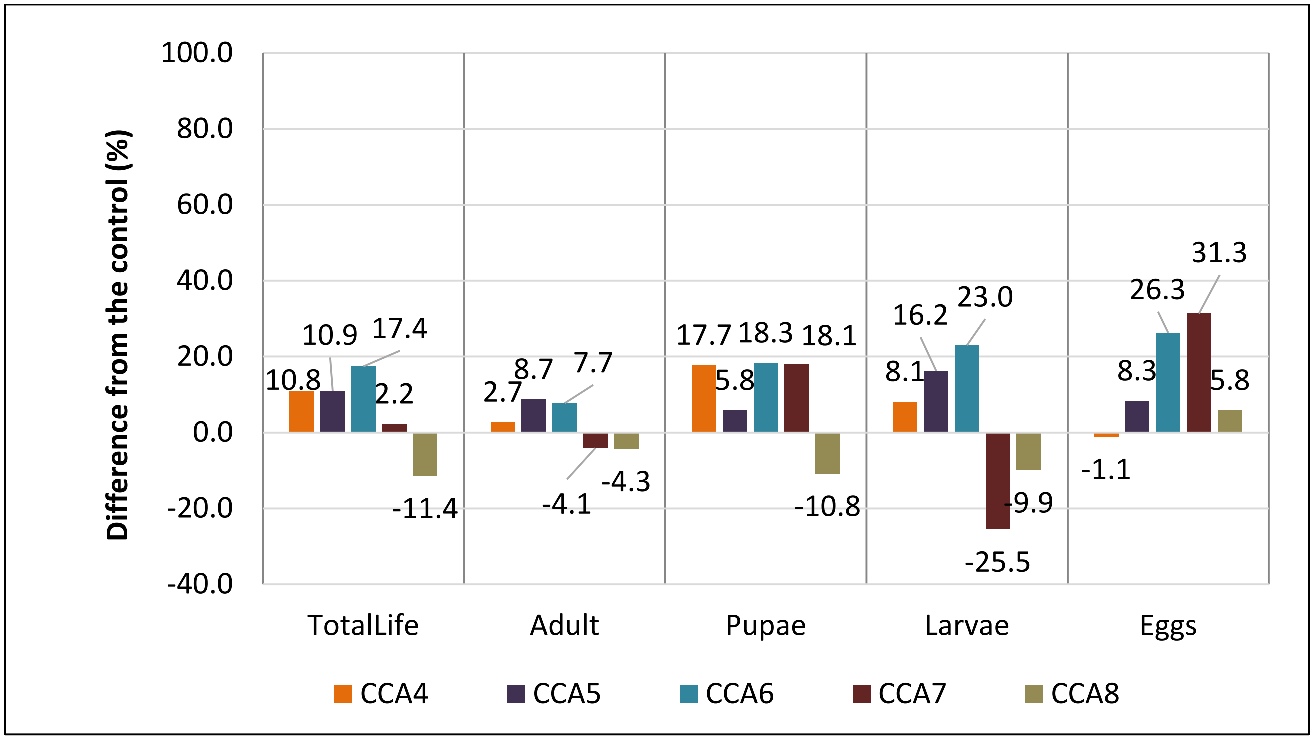

Figure 21. Summary of living organism parameters at the 25 µg/L treatment group

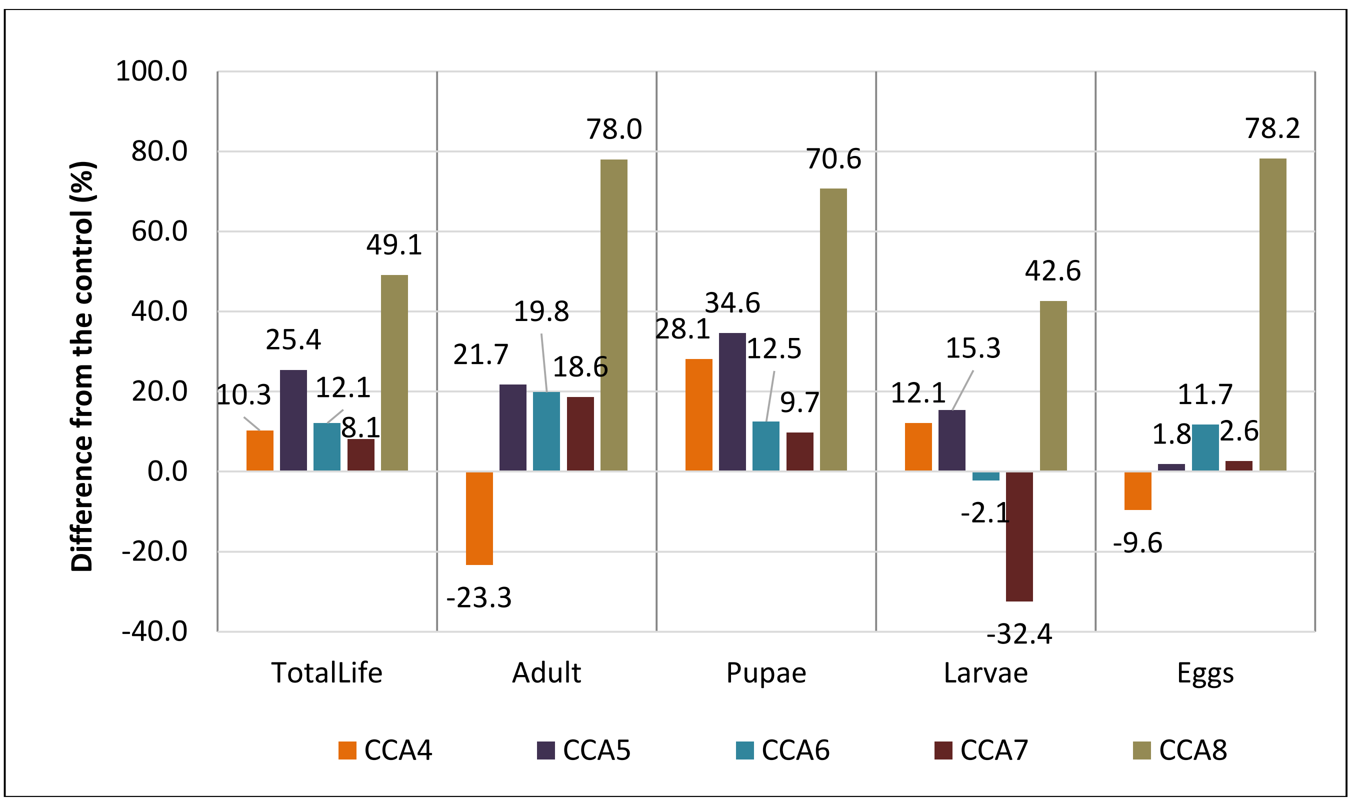

Figure 22. Summary of living organism parameters at the 50 µg/L treatment group

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Figures 23-27 provide an additional visual representation of the effects on life stages during the course of the study. This representation is distinguished from the figures previously presented for the life stage response variables in that the trends for each variable are presented within a CCA. It is noted for these figures that the scale has been adjusted to match for the y-axis for all CCAs. This helps visualize the trends of the response variables particularly in the later CCAs as compared to the earlier ones as well as to highlight the fact that the control hives themselves experience seasonal reductions and increases in certain life stages through time.

Although the assessment of hive health at CCA4 was taken only 3 weeks after the exposure period began, decreasing numbers of pupal and larval cells are indicated with increase in imidacloprid dose, particularly at the 100 and 200 µg/L groups (**Figure 23)**. These effects were also confirmed statistically by the three analyses. Effects on pupal and larval cells numbers were persistent throughout the subsequent CCAs as was discussed above.

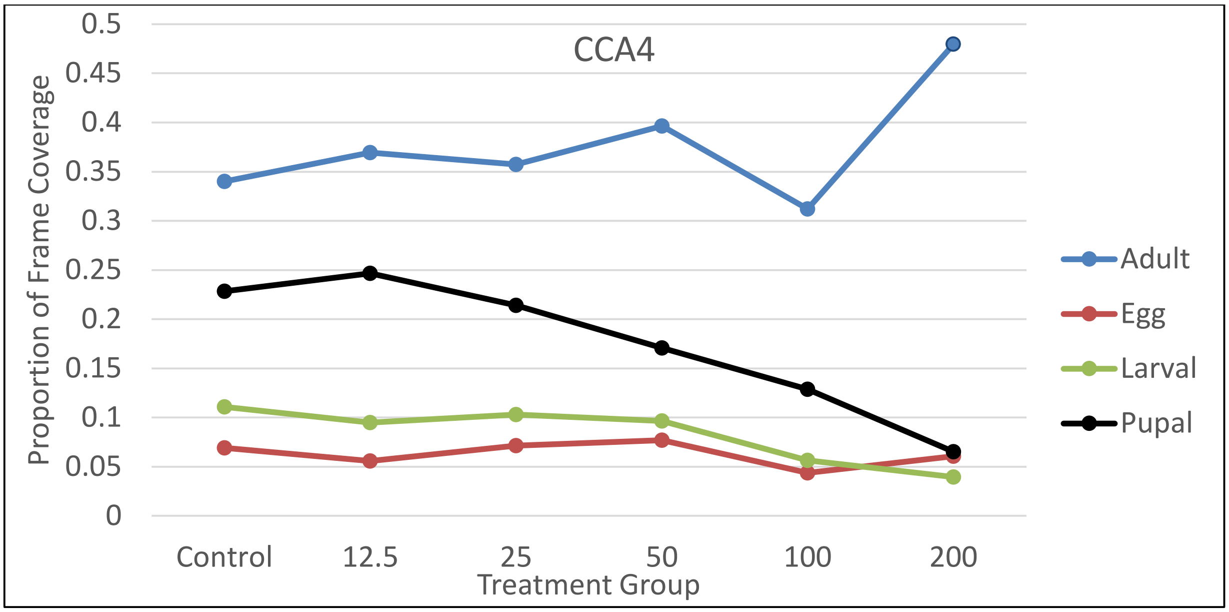


Figure 23. Trends of life stages across treatment groups within CCA4

At CCA5 (**Figure 24**), the effects become more readily apparent in the 50 µg/L group for adults and pupal cells especially. In the 100 and 200 µg/L groups, adults, pupal cells, and egg cells continue to be repressed as they were from CCA4. This is also visualized by the sharp dip in the trend lines for adult and pupal cells in particular at the 50 µg/L groups and above in comparison to the level of control while the responses in the 12.5 and 25 µg/L groups remain generally at the level of the control.

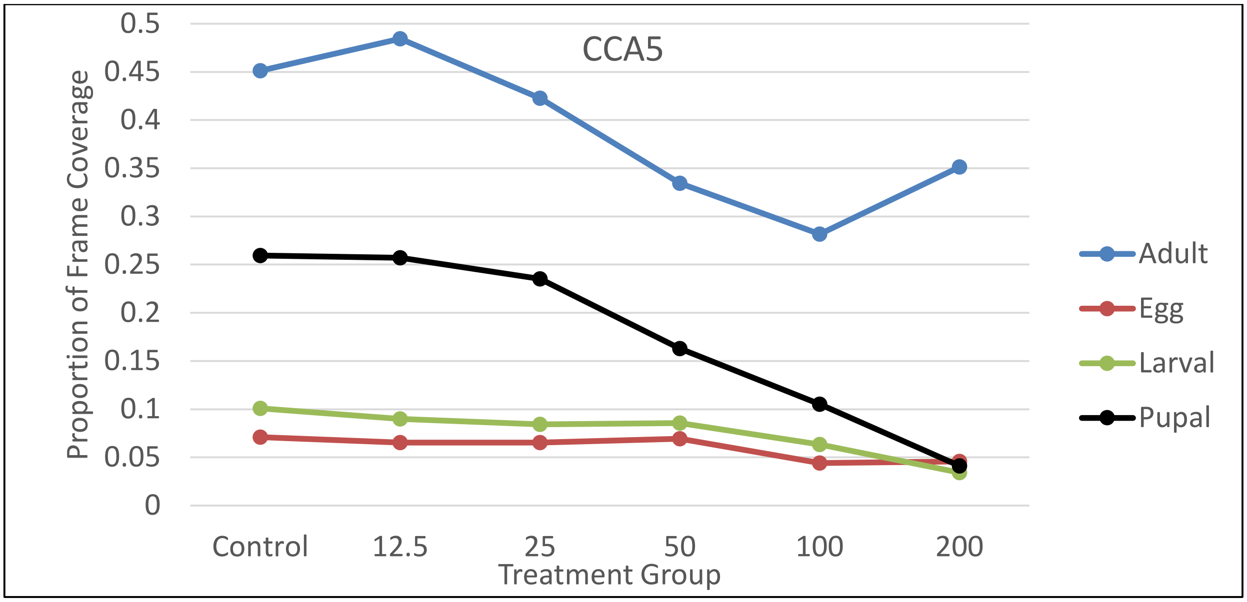


Figure 24. Trends of life stages across treatment groups within CCA5

At CCA6 (**Figure 25**), reductions that were determined in the three analyses for all life stage response variables at the two highest treatment groups continue to be repressed from the level of control. At the 50 µg/L group, the reductions in adults and pupal cells are also evident. Most noteworthy is the reduction in pupal cells that was confirmed as statistically significant ($p<0.05$) by the EPA and PMRA analyses at the 12.5 µg/L (the PMRA analysis also determined a significant reduction at the 25 µg/L treatment group). This is also evidenced visually by the dip in the proportion of pupal cells of frame coverage at these two groups (12.5 and 25 µg/L) while other response variables in this group (12.5 µg/L) at this CCA are in line with the level of the control.

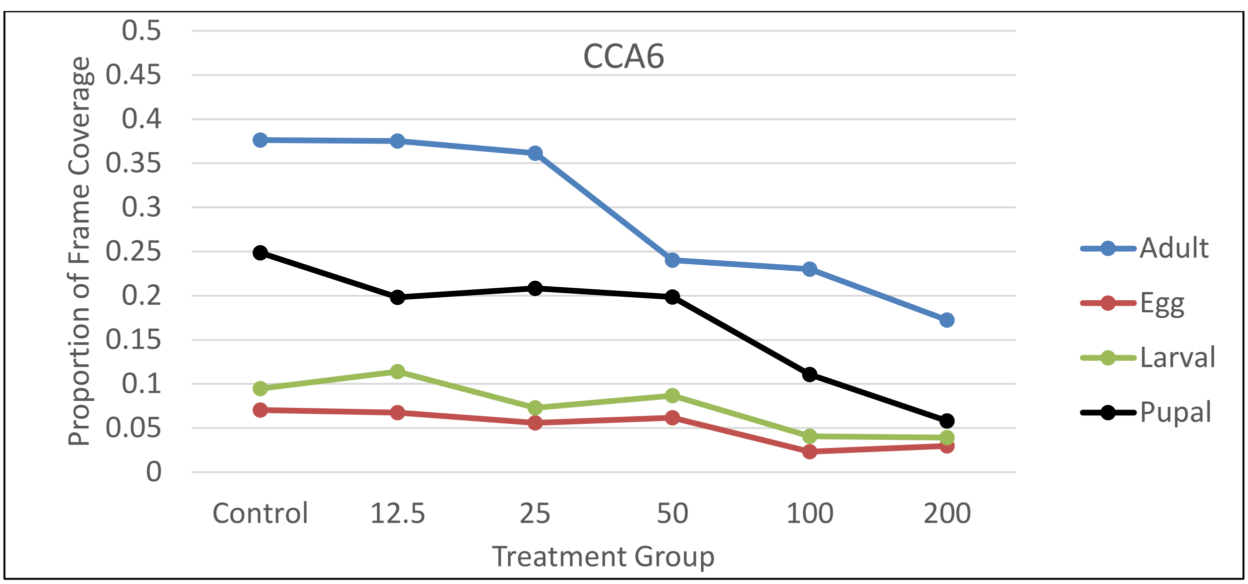


Figure 25. Trends of life stages across treatment groups within CCA

As mentioned previously, CCA7 represented the time during the study when hives are in a “shut down” mode and preparing for overwintering. As a result, the proportions of all life stages are in a natural decline, independent of imidacloprid exposure. The significant effects for adults, eggs, larvae, and pupal cells at the 100 and 200 µg/L treatment groups that were identified in previous CCAs were again determined by all three analyses which are also visually evident in **Figure 26** with the proportions of life stages in these two groups clearly being below the level of that in the control. Effects at the 50 µg/L for adults (no other significant effects at this group for other life stages) were determined as statistically significant by the PMRA analysis but the level at CCA7 is reduced from control as well as in previous CCAs.

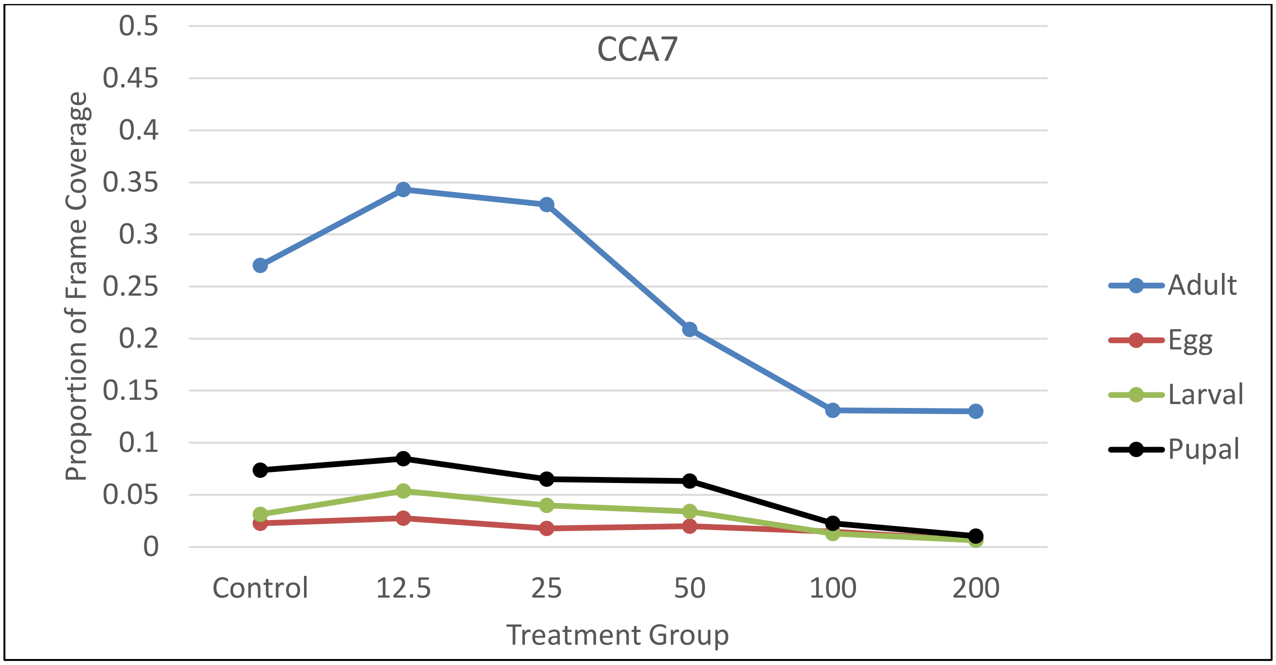


Figure 26. Trends of life stages across treatment groups within CCA7

After overwintering at CCA8 (**Figure 27a and 27 b**), there were a number of hives that were lost due to hive mortality. Despite this, colony health response variables of surviving hives are still considered informative in examining the success of certain treatment groups over others (see **Section 5 – Reviewer’s Comments** for more details regarding this). As evidenced by the hive mortality data previously discussed as well as the trends shown below at the 100 and 200 µg/L group, there was a clear impact at these groups. It is worth noting that significant reductions in these life stage response variables were evident well before the time of CCA8, with reductions being determined as early as CCA4. What is noteworthy is the levels of proportions at the 50 µg/L group as compared to the control and 12.5 and 25 µg/L groups. In addition to the statistically significant findings of all analyses, the levels of proportions in all life stages is shown below to be reduced from the level of control (EPA and CDPR analyses did not determine significance for larval cells). This is distinguished from the 12.5 and 25 µg/L groups whose levels are generally in line or above the level of control.

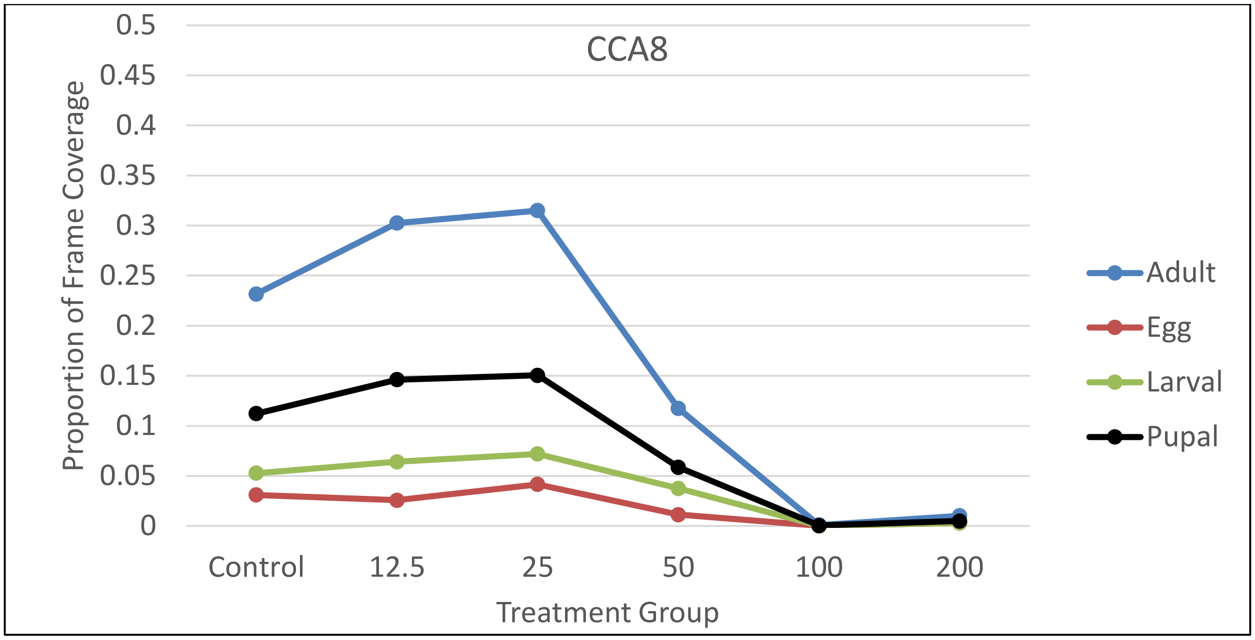


Figure 27. Trends of life stages across treatment groups within CCA8

Colony Condition Assessments – Food Store Response Variables

3.11.6. Pollen

Pollen stores were significantly reduced in the 200 µg/L treatment group during from CCA4 to CCA7 ($p<0.05$), with the CDPR analysis not finding a significant difference at CCA7 only. Pollen stores were reduced at 100 µg/L treatment group at CCA4 andCCA5 ($p<0.05$). Similarly, pollen stores were significantly reduced at the 50 µg/L treatment group at the 0.05 alpha level at CCA4 and CCA5, but not CCA6 and CCA7 ($p>0.1$). A marginal reduction at CCA7 ($0.05 < p < 0.1$) for the 50 µg/L was determined in the PMRA analysis but not in the EPA or CDPR analyses. The reduction of pollen stores was not determined in the 12.5 and 25 µg/L treatment groups for any CCA assessed. The reduced pollen store was most prevalent during and just after the exposure phase (CCA4 and CCA5) of the treatment levels exhibiting effects.

Table 29. Estimated percent reduction from control for pollen stores

Test concentration (µg/L)	Estimated reduction from control (%)[1]				
	CCA4	CCA5	CCA6	CCA7	CCA8[2]
12.5	11.8	-6.8	-53.3	-26.1	-12.2
25	5.2	2.1	-25.2	-20.7	0.7
50	56.1**	62.5**	15.5	29.2*	63.8**
100	83.7**	83.6**	15.3	12.9	100.6**
200	94.5**	90.4**	54.7**	50.6**	12.3

Note: Negative value indicates increased pollen stores in comparison to control.
*0.05<p<0.1

**p<0.05

[1]Percent differences from control are based on the raw counts of pollen stores, not proportions of the pollen stores as **Figure 28** below shows.
[2]At CCA8, comparisons made to the 100 and 200 treatment groups are with uncertainty as 1 and 2 hives survived in these groups, respectively. These data are included in the PMRA and CDPR analyses but excluded from the EPA analysis.

At CCA8, in the hives that survived overwintering, the total amount of pollen store was reduced in in the 50 µg/L group (↓64% based off raw counts). The PMRA analysis also determined a significant reduction in the 100 µg/L group ($p<0.05$) but not in the 200 µg/L group ($p>0.1$) (this data excluded from EPA's analysis). It is noted however, that the lack of statistical difference of pollen stores in the 200 µg/L group is considered to be uncertain as there were only two hives surviving overwinter and a large confidence interval.

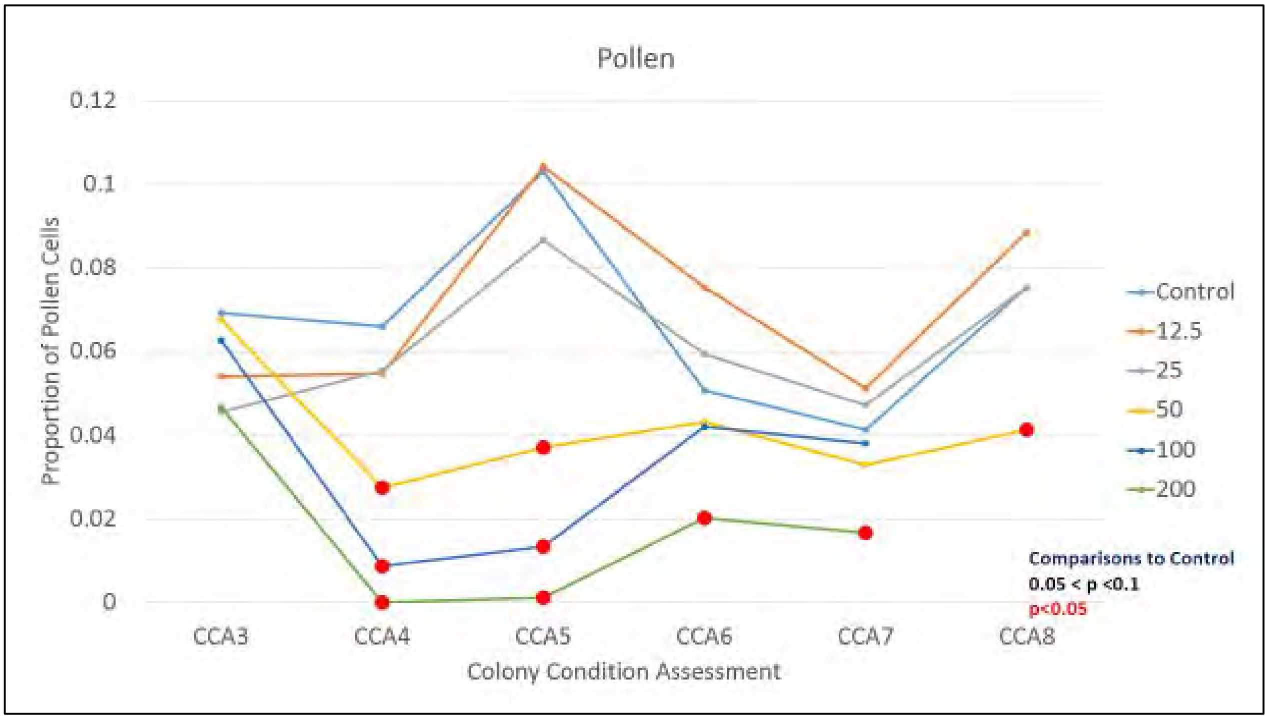


Figure 28. Proportion of pollen stores following exposure of honey bees to varying concentrations of imidacloprid in the diet across CCA3 – CCA8.

Figure 29 below shows the clear divergence of pollen stores in the 50 µg/L group as compared to the control, 12.5, and 25 µg/L groups where stores begin to decline immediately following exposure and continue to be repressed throughout the study including after overwintering.

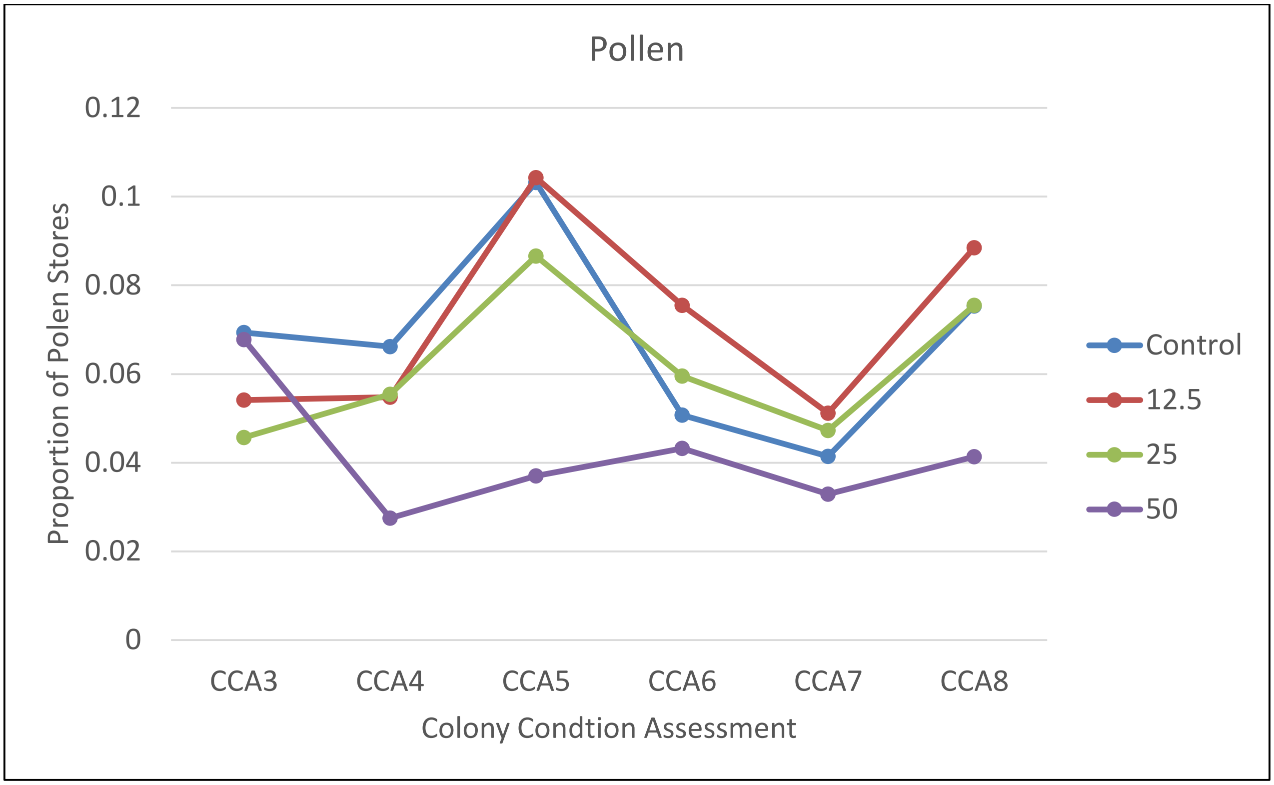


Figure 29. Proportion of pollen stores following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8 in the control, 12.5, 25, and 50 µg/L groups only.

In examining the trends of pollen stores in the control (**Figure 30** below), there was a buildup that occurred from CCA3 (7% of frame coverage area) to CCA5 (10%). This increase supports the queen in her effort to build up brood during the late spring and early summer months. Pollen stores experienced a decline in numbers from CCA5 (10%) to CCA7 (4%) before showing an upward trend from CCA7 to CCA8 (8%). This downward trend reflects that the fact that up to overwintering, brood production will slow as the hive prepares for winter and therefore there is a reduced need for pollen within the hive.

As depicted in the **Figure 30** below, the trends of the proportions in the 12.5 and 25 µg/L groups tracks very similarly with the control. Pollen stores at the 50 µg/L group, in contrast to the control, 12.5 and 25 µg/L groups, began a decline in stores earlier than the other groups as well as having an average proportion of approximately 50% of the stores after wintering in CCA8 among the surviving hives.

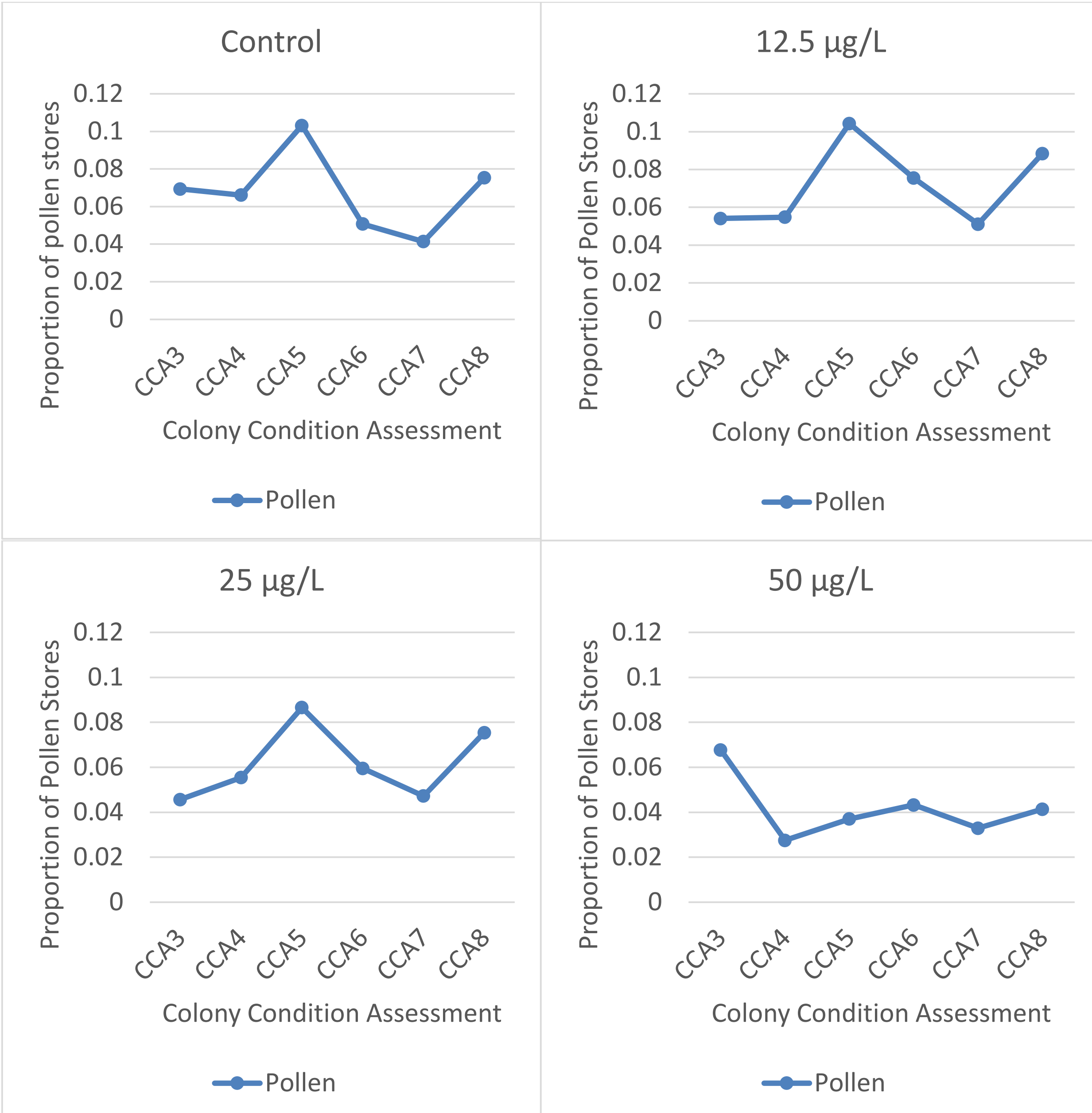


Figure 30. Proportion of pollen stores following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8 in the control, 12.5, 25, and 50 µg/L groups.

When weighing statistical and biological significance, the overall NOAEC and LOAEC for pollen stores is determined to be 25 and 50 µg/L, respectively.

3.11.7. Nectar / Honey

There was consistently and significantly ($p<0.1$) a lower amount of honey stored in treatment hives at 50, 100 and 200 µg/L than in the control at CCA6 and thereafter (**Figure 31** below). One exception is the absence of a determination of significance at CCA6 for the 100 µg/L group which PMRA determined as significant but EPA did not. All other findings after CCA6 at the 50, 100 and 200 µg/L (EPA excluded data at CCA8 for the 100 and 200 µg/L groups) were in general agreement with slight variations of the alpha level that the effect was significant. No reduction of the honey stores was determined at the 12.5 and 25 µg/L during the study, with the exception of the PMRA finding of a reduction at CCA6 at the 12.5 µg/L treatment group ($p<0.1$) at CCA6. This statistical difference at 12.5 µg/L was unlikely to be treatment related, as there were no reductions before or after the CCA6 at the same concentration, nor at the higher concentration of 25 µg/L at CCA6. While there were no significant findings of impact at CCA8 in the 200 µg/L group (EPA excluded this data) it is noted that this lack of finding is considered to be uncertain as there were only 2 surviving hives at this treatment group.

Table 30. Estimated percent reduction from control for nectar/honey stores

Test concentration (µg/L)	Estimated reduction from control (%)				
	CCA4	CCA5	CCA6	CCA7	CCA8
12.5	-7.5	1.3	15.8*	12.5	-10.9
25	-10.4	-15.3	-2.4	10.6	13.3
50	-6.2	21.6*	36**	41.2**	60.4**
100	-8	7.1	21.8*	52.9**	156.6**
200	-21.1	-84.1	70.5**	80**	5.1

Note: Negative value indicates increased nectar/honey stores in comparison to control.
*$0.05<p<0.1$
**$p<0.05$
[1]Percent differences from control are based on the raw counts of nectar/honey stores, not proportions of the nectar/honey stores as **Figure 31** below shows.
[2]At CCA8, comparisons made to the 100 and 200 treatment groups are with uncertainty as 1 and 2 hives survived in these groups, respectively. These data are included in the PMRA analysis but excluded from the EPA analysis

Figure 32 below for the honey store trends in the control, 12.5, 25, and 50 µg/L groups only show a marked divergence at the 50 µg/L treatment group beginning at CCA6 and persisting up to and after overwintering at CCA8.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

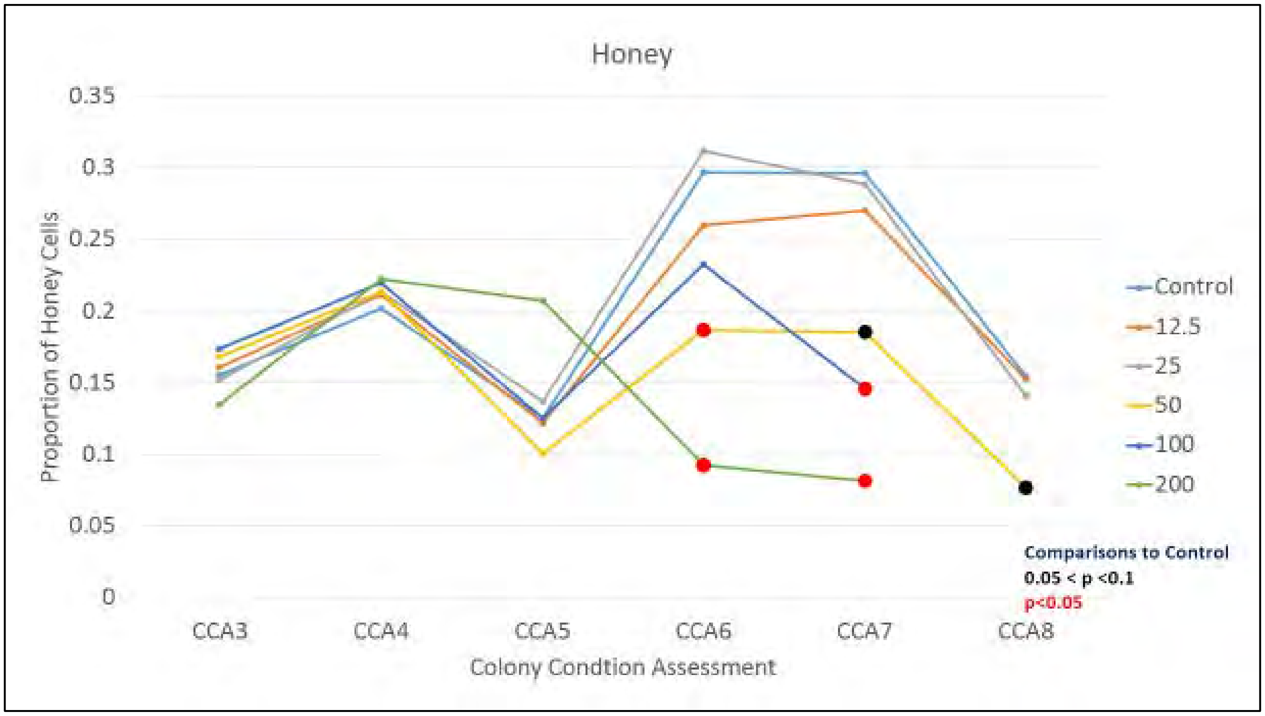


Figure 31. Proportion of honey stores following exposure of honey bees to varying concentrations of imidacloprid in the diet across CCA3 – CCA8.

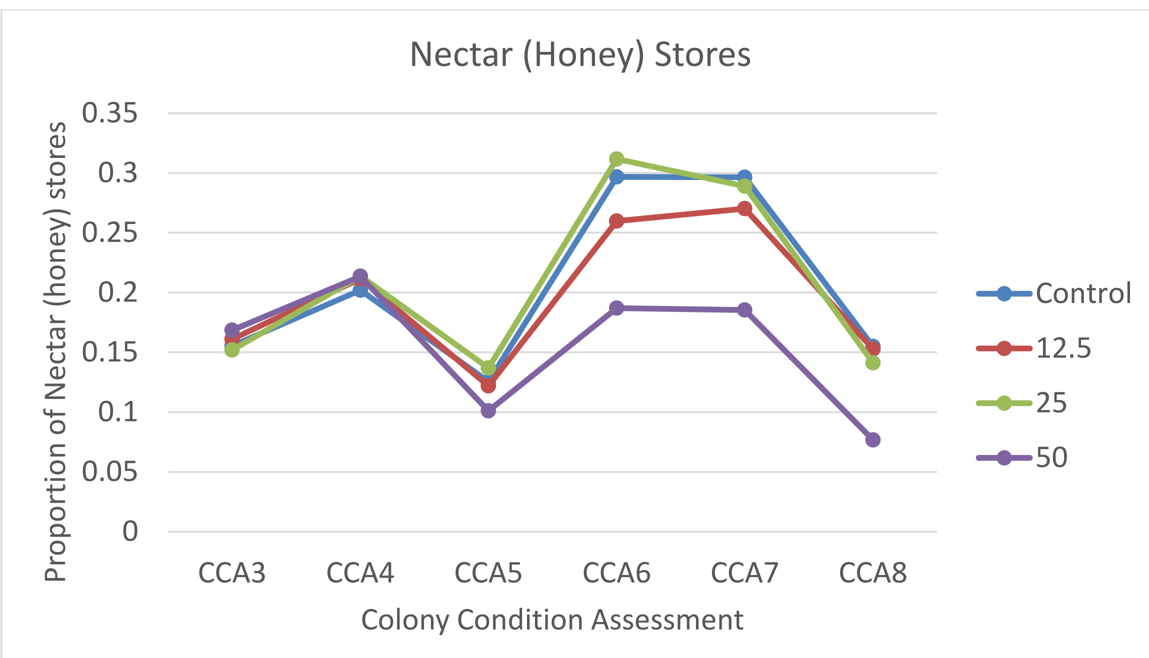


Figure 32. Proportion of honey stores following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8 in the control, 12.5, 25, and 50 µg/L groups only.

For the control group, honey stores underwent an upward trend from CCA3 (16% of the frame coverage) to CCA4 (20%), before a subsequent decline in average proportion from CCA4 to CCA5 (13%). This was followed by an approximately 140% increase in honey cells from CCA5 to CCA6 (average proportion of 30%) that remained stable until CCA7 (30%). This buildup of honey stores took place ahead of CCA7 that represented the last time point before overwintering. The honey stores declined markedly from CCA7 to CCA8 (16%) which is expected given lack of foraging and utilization of these reserves during the overwintering period. It is noted that the proportion of comb cells containing honey stores at CCA3 and at CCA8 were approximately the same at 16%.

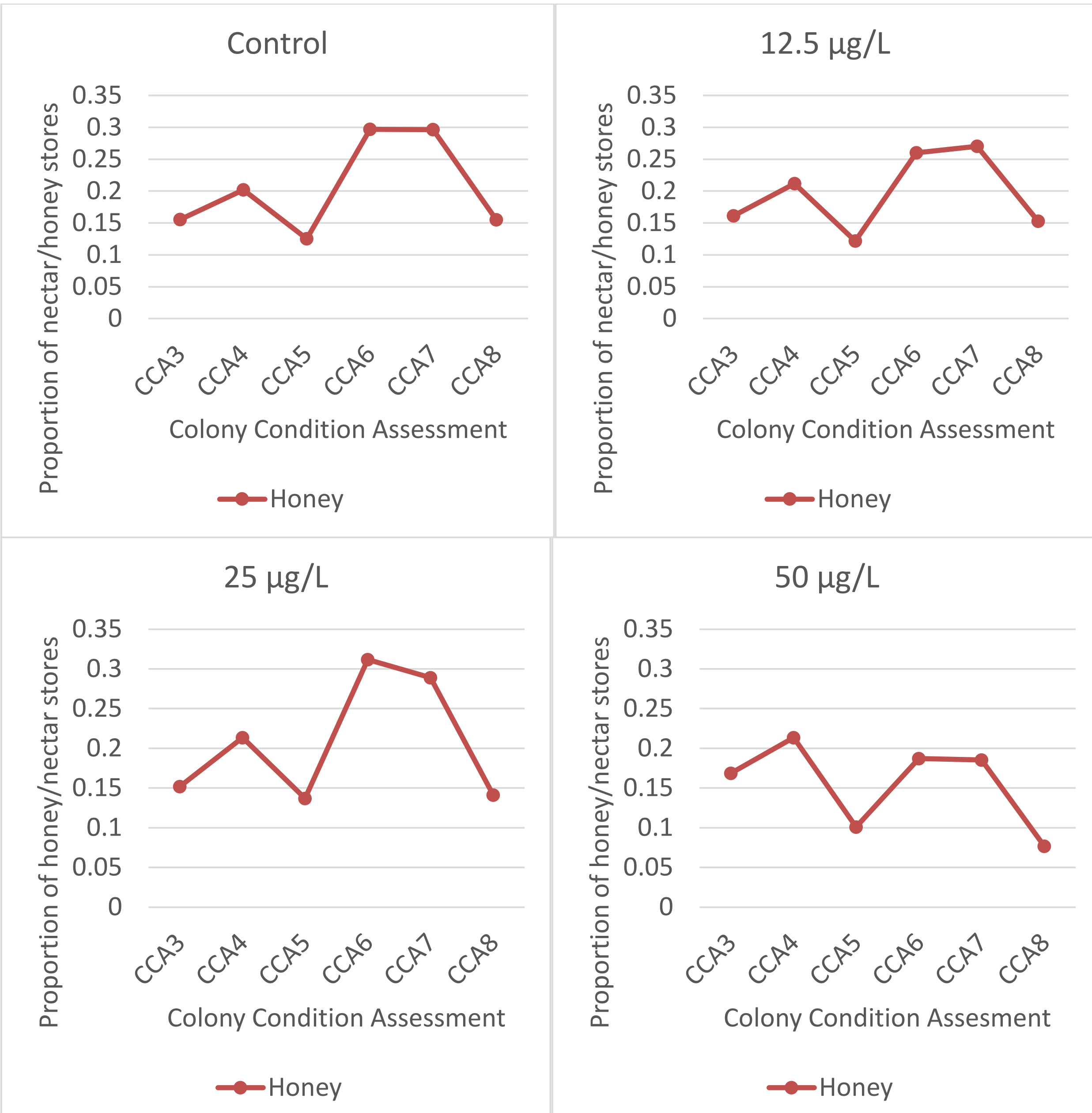


Figure 33. Proportion of honey stores following exposure to varying concentrations of imidacloprid in the diet across CCA3 – CCA8 in the control, 12.5, 25, and 50 µg/L groups.

In the 12.5 µg/L treatment group, honey stores also underwent an initial build up from CCA3 (16%) to CCA4 (21%) before a subsequent decline from CCA4 to CCA5 (12%), similar to that of the control. This was followed by a large buildup of honey stores from CCA5 to CCA7 (27%). Honey stores declined during overwintering and represented 15% of the brood area at CCA8, as compared to 16% at CCA3, which a similar finding to that in the control group. The proportion of honey stores at CCA8 was comparable to that of the control group (15% for both 12.5 µg/L and control). A similar picture was found for the 25 µg/L treatment group, in that honey stores underwent an initial build from CCA3 (15%) to CCA4 (21%) before a decline at CCA5 (14%). The subsequent build up reached 29% at CCA7 before a decline through overwintering to CCA8 (14%). CCA8 concentrations of nectar and pollen were comparable to that of the control at CCA8 (honey: 14% and 15%; 25 µg/L and control groups, respectively).

These trends in the control and two lower treatment groups are distinguished from the response at the 50 µg/L group. While honey stores underwent an initial buildup and then decline from CCA 3 (17%) to CCA4 (21%), the subsequent larger buildup leading up to CCA7 that took place in the control and lower treatment groups was much less pronounced with the 50 µg/L group. Specifically, the proportion of honey stores from CCA3 to CCA7 roughly double in numbers from 15 to 30% of the brood comb in the control, 12.5 and 25 µg/L treatment groups. This is distinguished from the 50 µg/L group were the buildup that occurred from CCA5 to CCA7 reached a marginally higher level that the starting proportion at CCA3 (18% and 17% respectively). That is to say, that the amount of honey stores at CCA7 (before the overwintering period) in the 50 µg/L group was approximately half of that in the control, 12.5, and 25 µg/L treatment groups (18% for 50 µg/L as compared to 30, 27, and 29% at CCA7 for the control, 12.5 and 25 µg/L treatment groups, respectively). Notably, it is also the only group out of these 4 in which the proportion of honey stores at CCA8 was markedly lower than that of CCA3 (8% at CCA8 and 17% at CCA3).

It is noted that the feeding solutions (sugar solutions) provided during the exposure period might have affected natural honey storage patterns; however, effects on honey storage are still able to be considered as all treatments were compared to control hives (which also received feeding solutions).

When weighing statistical and biological significance, the overall NOAEC and LOAEC for honey stores is determined to be 25 and 50 µg/L, respectively.

Figures 34-36 below show an additional visual representation of the impacts on food stores across the CCAs for each treatment group (Figures not shown for the 100 and 200 µg/L treatment groups due to the clear impacts on the hives at that level, primarily on hive mortality). It is noted that the scale for percent difference from the control (y-axis) was standardized to the level of the 50 µg/L chart. When visualized this way, the impact of the early on and persistent nature of effects (particularly with pollen) at the 50 µg/L is clearly divergent from the responses at the 12.5 and 25 µg/L, which generally show a buildup in food stores that was also observed in the control group. It is noted here that negative ("-") responses refer to a percent increase above the level of control.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

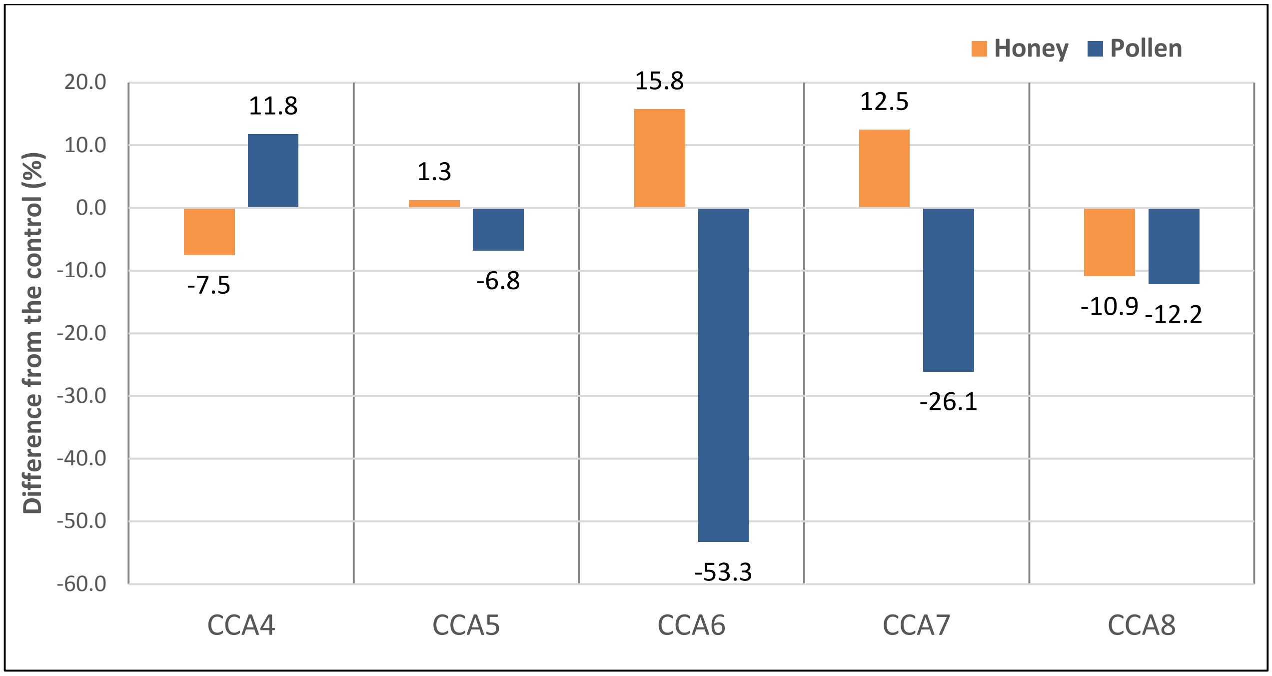


Figure 34. Summary of hive food supply parameters at the 12.5 µg/L treatment group

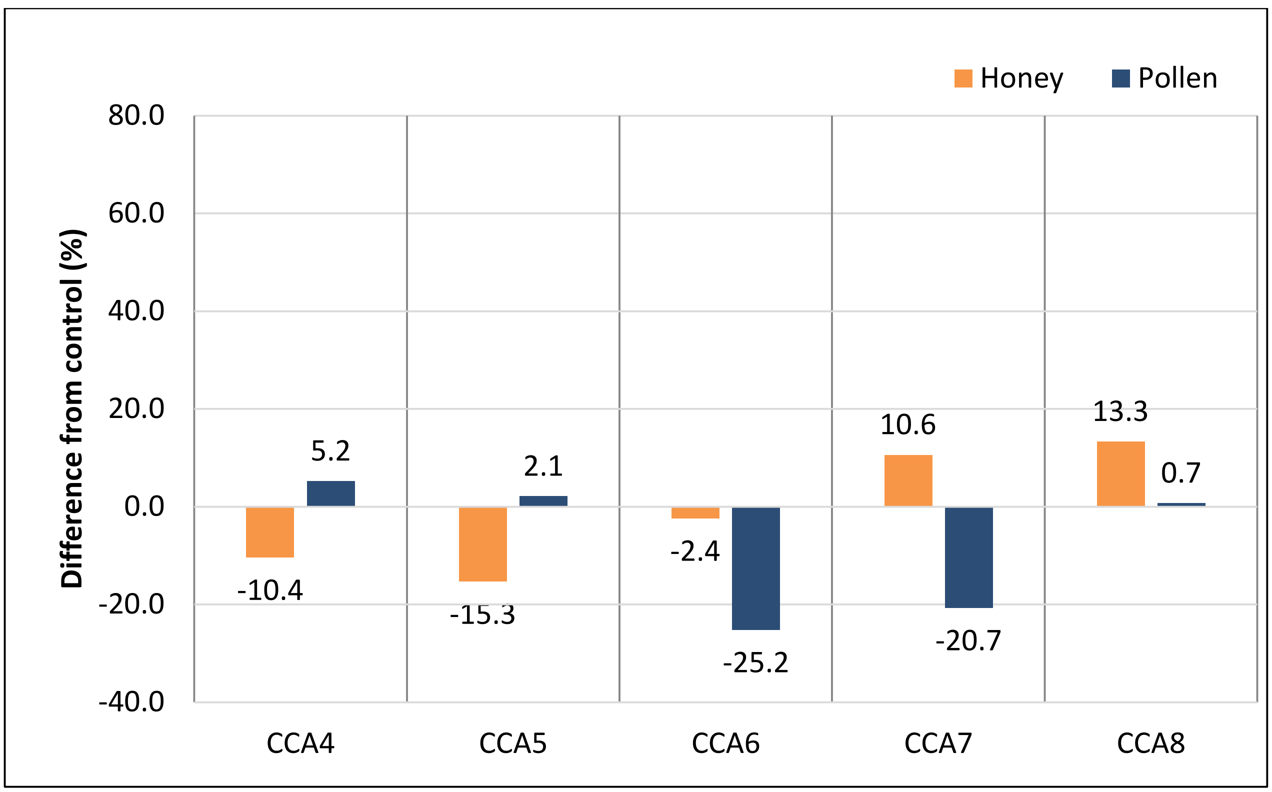


Figure 35. Summary of hive food supply parameters at the 25 µg/L treatment group

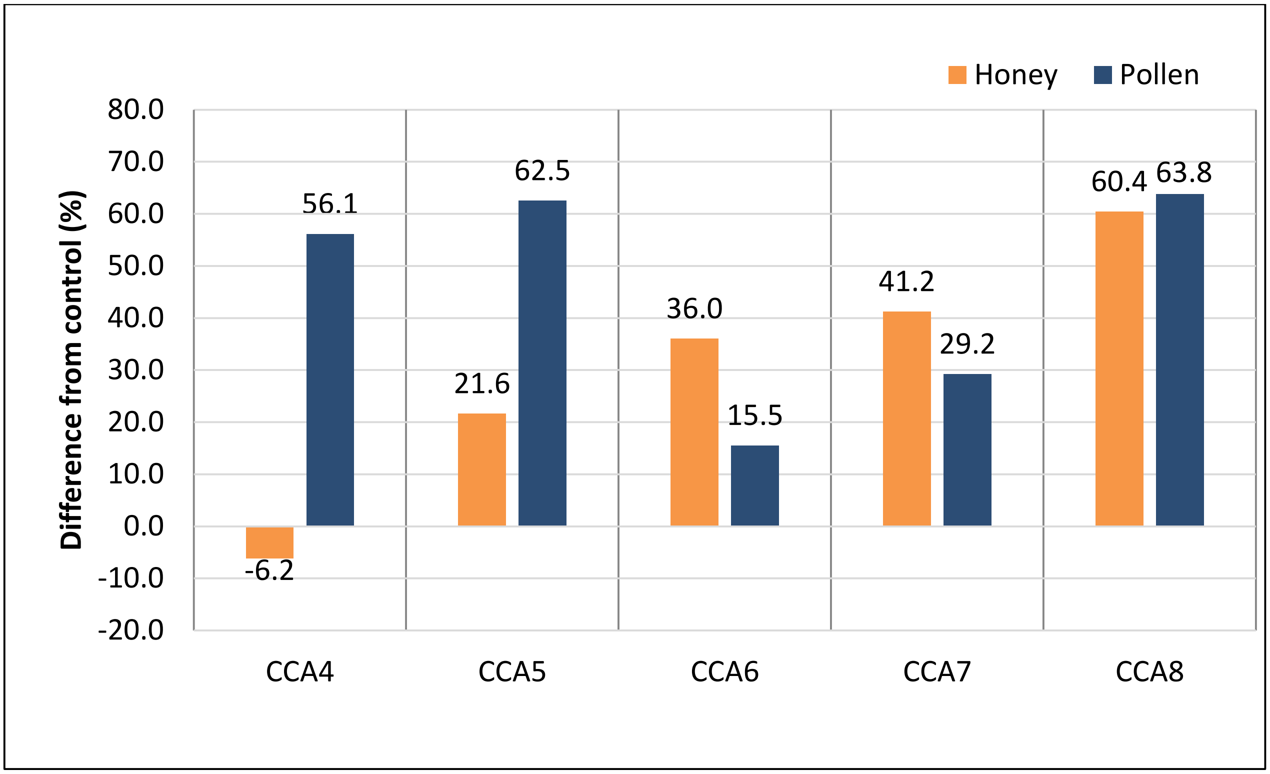


Figure 36. Summary of hive food supply parameters at the 50 µg/L treatment group

Figures 37-41 provide an additional visual representation of the effects on food store parameters during the course of the study. This representation is distinguished from the figures previously presented for the food store response variables in that the trends for each variable are presented within a CCA8 across treatment groups. It is noted for these figures that the scale has been adjusted to match for the y-axis for all CCAs. This helps visualize the trends of the response variables particularly when examining the level of control at each CCA as honey stores for example initially fall then build up across multiple CCAs in preparation for overwintering heading into CCA7.

At CCA4, 3 weeks into the exposure period, honey stores across all treatment groups remained at the level of the control, as shown below, and this was also confirmed statistically by all analyses. Pollen stores however, began to undergo an immediate reduction at the 50, 100, and 200 µg/L groups while the 12.5 and 25 µg/L remain generally at the level of control.

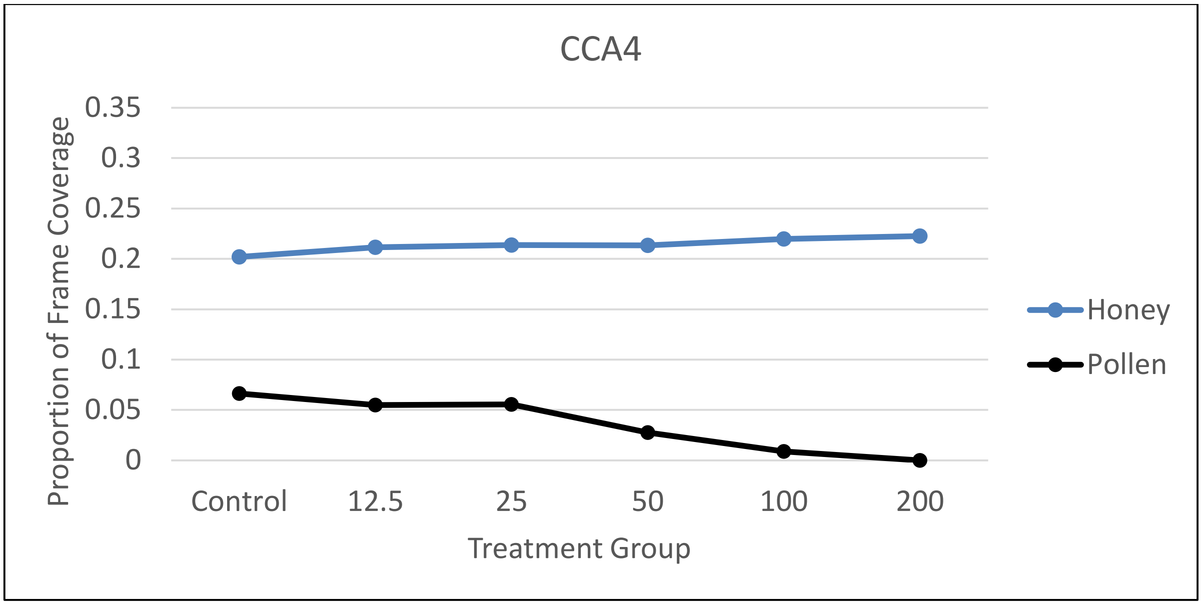


Figure 37. Trends of food store parameters across treatment groups within CCA4

The responses at CCA5 are the same as those at CCA4 in terms of honey responses although the trend at the 200 µg/L group rises above the level of the control. Pollen stores continue to be suppressed at the 50, 100 and 200 µg/L, while the responses at 12.5 and 25 µg/L are generally at the level of the negative control.

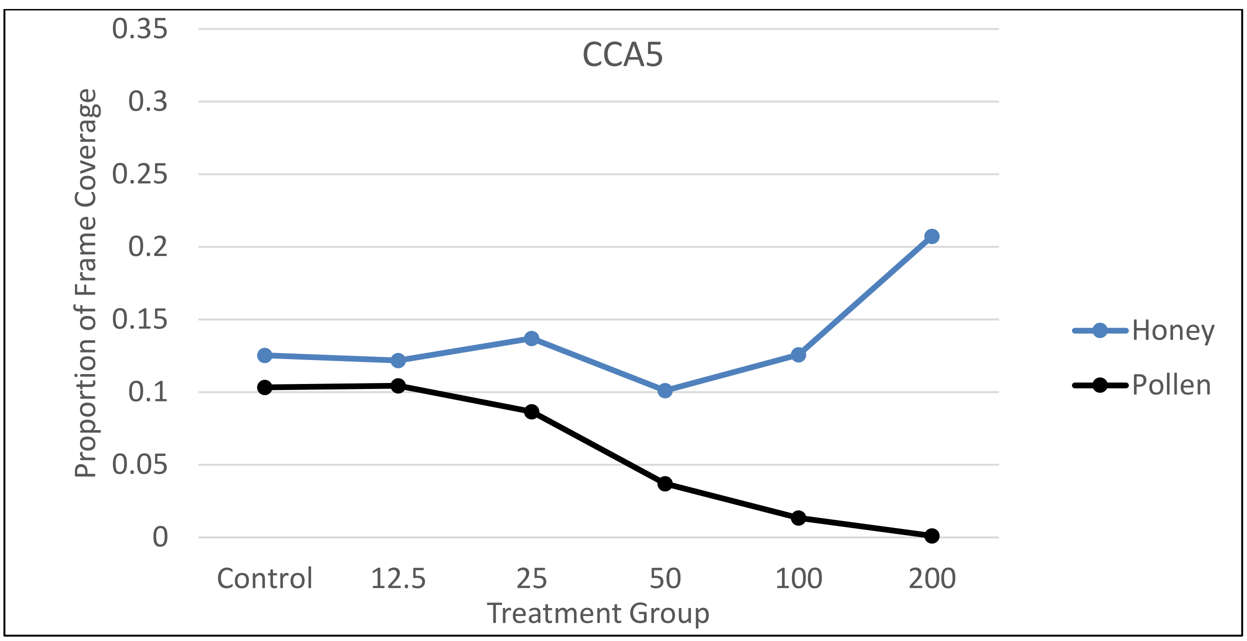


Figure 38. Trends of food store parameters across treatment groups at CCA5

At CCA6, significant reductions in honey stores began to become apparent and were confirmed statistically at the 50 and 200 µg/L levels. These groups also appear visually reduced from the level of control. Of note is that the proportions of honey in the control are above that of the level of CCA5 by approximately 2.5 fold, indicating a buildup in stores in the weeks before overwintering.

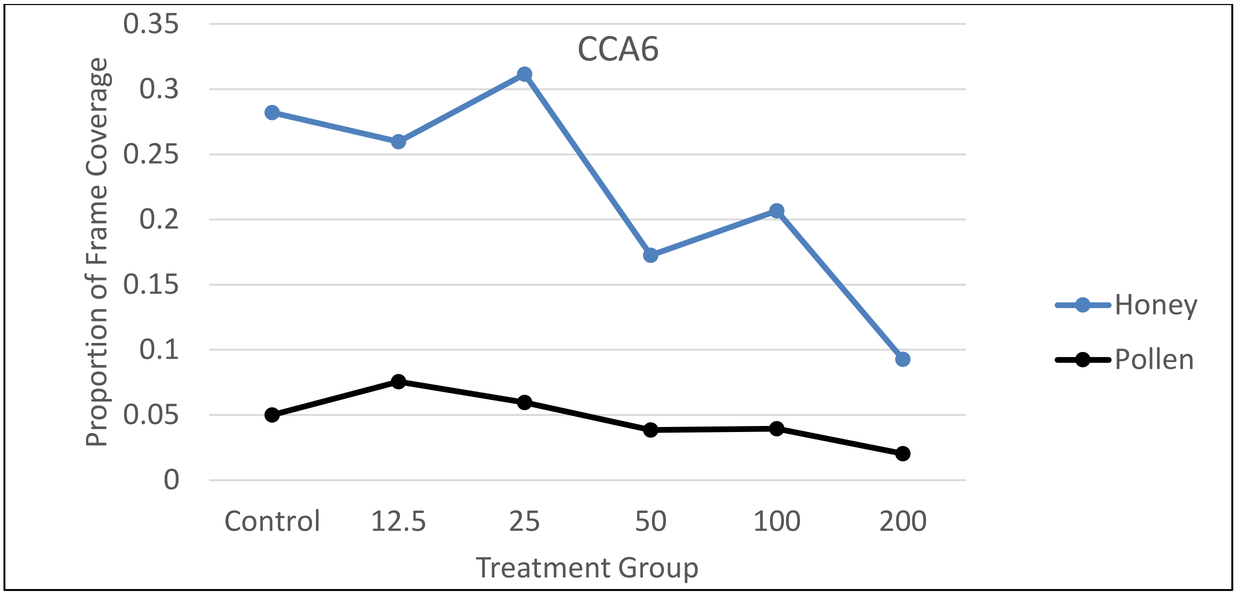


Figure 39. Trends of food store parameters across treatment groups at CCA6

At CCA7, just before the overwintering phase begins, there is a marked decline in honey stores in the 50, 100 and 200 µg/L groups below that of the level of control. This is noteworthy as the reduced stores provide an indication that these hives will have reduced success after overwintering. In the case of the 100 and 200 µg/L groups, these hives largely did not survive overwintering.

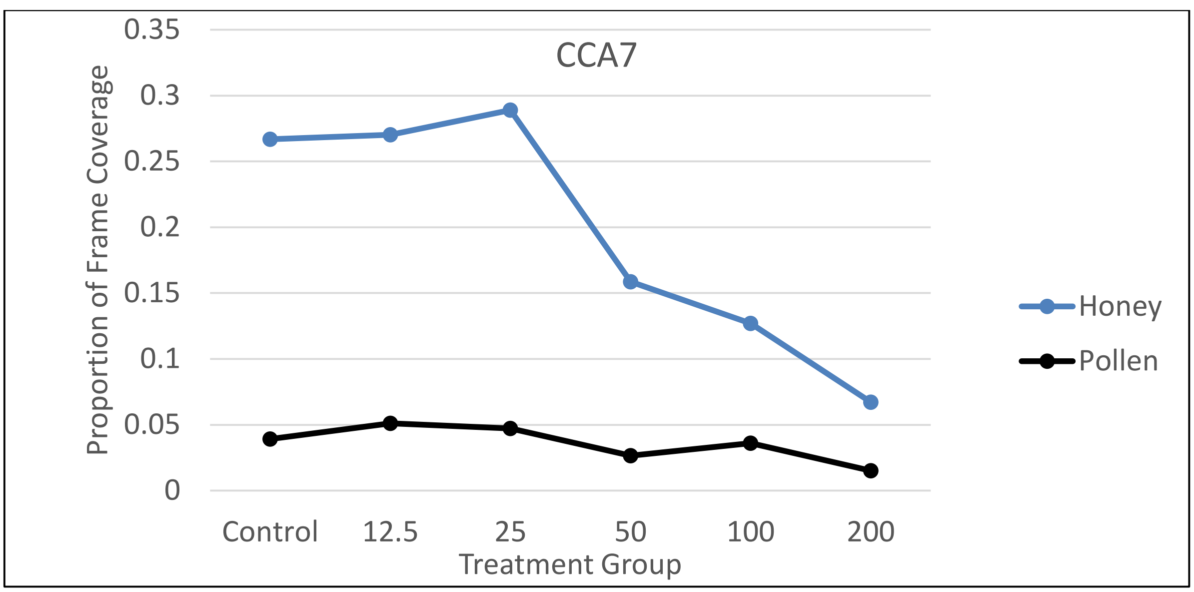


Figure 40. Trends of food store parameters across treatment groups at CCA7

Finally, at CCA8 after overwintering, honey stores were markedly decreased in the control group from their level heading into overwintering at CCAs 6 and 7. The levels at the 100 and 200 µg/L groups are clearly suppressed as these groups had one and two hives surviving, respectively. The response at the 50 µg/L level was also reduced from control while the response at the 12.5 and 50 groups again are in line with or above the level of control.

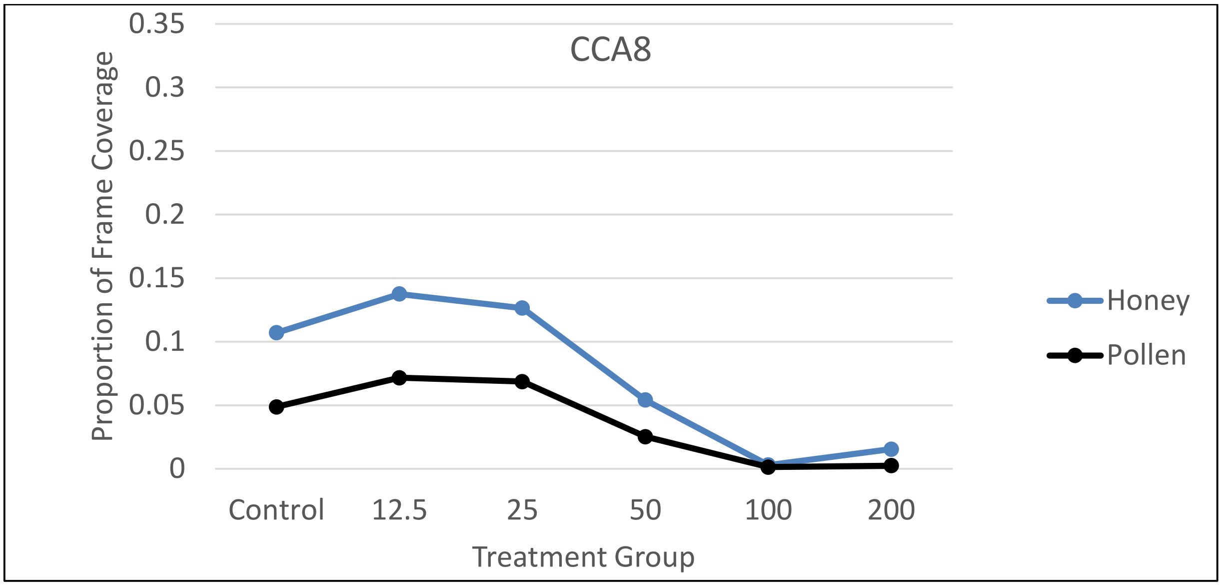


Figure 41. Trends of food store parameters across treatment groups at CCA8

3.11.8. Hive Weight

There were significant reductions from control observed at the two highest dose levels (100 and 200 µg/L) beginning at CCA4 and persisting until CCA8 ($p<0.05$, data from CCA8 excluded from EPA analysis). Additionally, there were no significant reductions from control in the 12.5 and 25 µg/L treatment groups determined for all CCAs assessed ($p>0.1$). For the 50 µg/L group, there were significant reductions at both the 0.05 and 0.1 alpha level starting as early as CCA4 (CDPR and PMRA analyses). The PMRA analysis determined significant reductions at the 0.1 alpha level for CCA4 and CCA6 and at the 0.05 alpha level for CCAs 5, 7, and 8. The EPA analysis determined significant reductions from the control at the 0.1 alpha level at CCAs 7 and 8 only. Despite these differences in statistical findings, there is an apparent effect on hive weight at the 50 µg/L level that is supported by both analyses indicating significant reductions at multiple CCAs.

While there was no difference ($p>0.1$) in the hive weight at the 200 µg/L treatment groups (data not included in EPA analysis), the lack of statistical difference is considered to be uncertain as there were only two hives surviving overwintering.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 31. Estimated percent reduction from control for hive weights

Test concentration (µg/L)	Estimated reduction from control (%)				
	CCA4	CCA5	CCA6	CCA7	CCA8[1]
12.5	-0.5	2.4	0.2	-1.6	-1.1
25	2.7	-0.4	-1.5	0.6	1.2
50	4.1*	11**	12.3*	15**	20.9**
100	7.1**	14.9**	15**	18.9**	67.7**
200	10.1**	10.4**	30.4**	33.3**	-25.5

Note: Negative value indicates increased hive weight in comparison to control.
*0.05<p<0.1
**p<0.05
[2]At CCA8, comparisons made to the 100 and 200 treatment groups are with uncertainty as 1 and 2 hives survived in these groups, respectively. These data are included in the PMRA analysis but excluded from the EPA analysis

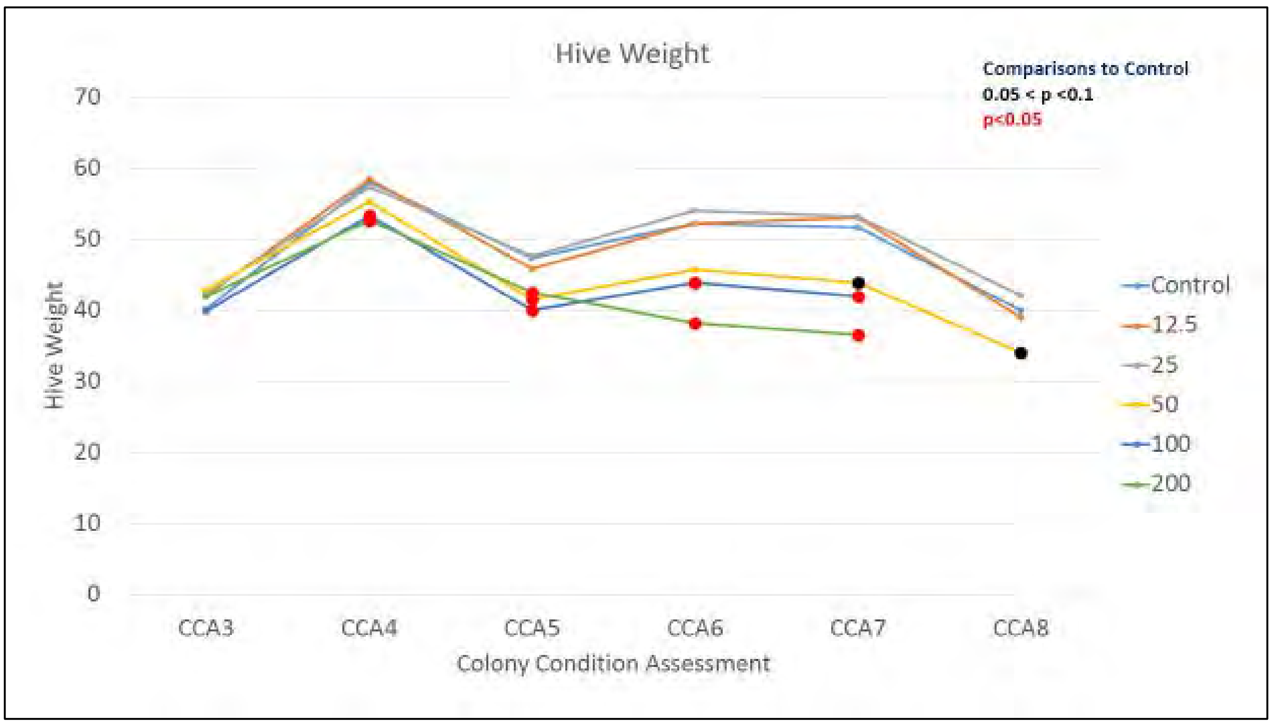


Figure 42. Proportion of hive weight following exposure of honey bees to varying concentrations of imidacloprid in the diet across CCA3 – CCA8

When weighing statistical and biological significance, the overall NOAEC and LOAEC for hive weight is determined to be 25 and 50 µg/L, respectively.

4. Reviewer comments:

What follows is brief discussion of some of the elements taken into consideration when evaluating the results of this study.

General Considerations for Biological Interpretation

While the hive mortality is considered as the most relevant measurement of survival at the colony level, sublethal effects at the colony level were estimated by measuring multiple parameters during the course of study. Each measured parameter is expected to reflect only part of the colony conditions, and all measurements have to be integrated for a better understanding of the hive status at the colony level. A honey bee colony is a super-organism in which live individuals and food supply are the two major components in maintaining the proper function of the colony. There are interactions between the two components and even within each component.

Bee individuals are present in the colony as eggs, larvae, pupa and adults and they develop from one stage to another and interact with each other to perform a variety of tasks to maintain the integrity of the colony. The measurement of each stage of the bees is expected to provide information on the potential treatment effect on a specific life stage of bees during their development.

Hive food supplies including hive pollen and nectar are collected and processed by adults and are expected to have a large impact on the development of all stages of bees in hives. However, the amount of hive food storage is dependent on not only the power/number of foragers available for food collection, but also the number of individuals that consume the food. In addition, the seasonal availability of outside pollen and nectar sources also affects the amount of storage, thus impacting hive development. As well, sucrose feeding solutions were provided to the hives as a means of treatment and as a supplement for hive overwintering, which may have affected foraging and food storage during those time periods.

Hive weight was measured during the study. However, it is largely affected by the honey storage and number of bees that consume the food. A strong colony with a high number of bees likely consumes a high amount of stored honey and may result in a reduced hive weight. In this study, additional sugar solution was provided as the means of treatment and a supplement for hive overwintering in the study. Such feeding likely further confounds the relevance of the hive weight to the treatment effect. In addition, hive weights were taken after 10 a.m. However, weighing hives at different time periods of the day may result in an increased variation of the measurement due to the fact that foragers may not be present in the hive when the weight is measured. Hive weights may be artificially lower in hives which contain a high number of forager bees that may be out collecting food during a different time of the day.

Considerations regarding the measurement time points:

- CCA3 represents the background hive conditions as the first colony assessment after the hives were placed in the test fields prior to the exposure.
- CCA4 and CCA5 represent the hive conditions during the exposure phase. It was noted that the CCA5 was conducted a week after the end of the 6-week exposure period, but is expected to represent effects during the exposure period.

- CCA6 was measured at 5 weeks after the end of exposure. It allows all bee individuals, including eggs, larvae and pupa that were exposed to treatment to finish their development cycle and become adults.
- CCA7 represents the hive conditions prior to overwintering. It is considered that hives were physiologically preparing for overwintering by reducing the production of immature bee individuals. Treatment effects may be masked by the natural decline of hive individuals.
- CCA8 represents hive conditions of surviving hives after overwintering. Additionally, hive overwintering mortality at CCA8 is expected to be directly relevant to the treatment effect at the colony level.

Control Performance

Control mortality:

The level of colony loss after overwintering in controls (36%), though not desirable, is consistent with that historically experienced by beekeepers on average across the United States[3]; the 2014-2015 preliminary results estimate US overwintering loss on average to be 23.1%. There are, however, differences in bee management practices associated with the test hives compared to commercial hives, making a direct comparison of expected overwintering success challenging. Commercial hives could experience very different beekeeping practices than test hives, including being transported for pollination services, being harvested for honey production, given additional feedings, receiving different pest and disease control treatments, having different sized hives and different preparation for overwintering. The test hives, while closely monitored, may also experience invasive disruptions during the colony condition assessments, have different/less supplemental feeding or mite treatments than commercial hives, have a lower colony size or be prepared differently going into overwintering as was the case with hives in this study which were not given supers to allow for growth as well as having supplemental feeding delayed.

Sublethal effects on life stages and food stores

As described with discussion of the response variables in **Section 3** above, the parameters measured indicate that control hives behaved as would be expected through the seasonal changes that a honey bee colony undergoes. Although a large variation among apiaries was detected for each parameter in all control hives, the average of the total number of individuals in hives, as well as eggs, larvae, pupae and adults increased or remained at similar levels during the exposure period from CCA3 to CCA5. After the end of the exposure, all these biological parameters also increased or remained at a similar level at CCA5 and CCA6, and then all decreased sharply from CCA6 to CCA7.

The similarity in the dynamics of all parameters for the individual living organisms at various stages indicates that control hives were well developed during CCA3 to CCA6, and the hives were preparing for overwintering at CCA7 as expected in the late fall in October. The increase of hive food supply (pollen and honey store) and hive weight also confirms that hives were actively developing during the exposure period from CCA3 to CCA5. The increased level of honey store from CCA5 to CCA6 and CCA7 indicates that there were plenty of outside nectar sources in the test area after the end of feeding exposure period.

[3] http://beeinformed.org/2015/05/colony-loss-2014-2015-preliminary-results/

However, the pollen store reduction observed during CCA5 to CCA7 might have resulted from increased pollen consumption due to the increase of live individuals and/or limited availability of outside pollen sources. If the outside pollen source were limited, it may have impacted the further development of control hives after CCA5. However, the decline of pollen stores in the control hives after CCA5 is likely normal for the local region of the study, and control hives did not appear to be impacted, therefore, this is not expected to have biased the study.

Consideration of CCA8

Control overwintering mortality

While the level of control mortality after overwintering is generally in line with historical findings from North American beekeepers as described above, it is noted that the use of data from CCA8 to distinguish treatment-related effects on colony survival (*i.e.* dead or alive) is compromised due to the level of control mortality observed. That is to say, the ability to detect treatment-related decreases in overwintering colony survival may be masked by the magnitude of control hive loss.

Response Variables from Surviving Hives

While the measure of overwintering success may be compromised by the control hive loss, the evaluation of life stage and food store metrics at CCA8 for surviving hives is considered to be useful and important to interpreting the study results. Specifically, the data suggest that the weaker control hives were not able to survive overwintering because they had disproportionately fewer numbers of adults and honey stores compared to control hives that survived overwintering. (**Figure 43**).

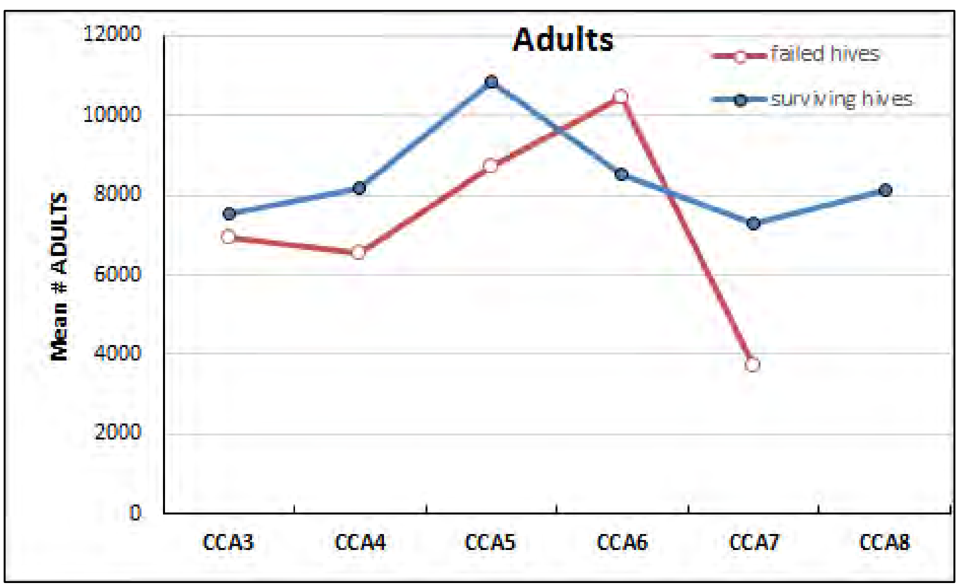


Figure 43. Fate of control hives as compared to number of adults across CCAs

Control hives that survived overwintering had 2.5X more honey on average at CCA7 than those that did not survive overwintering (**Figure 44**)

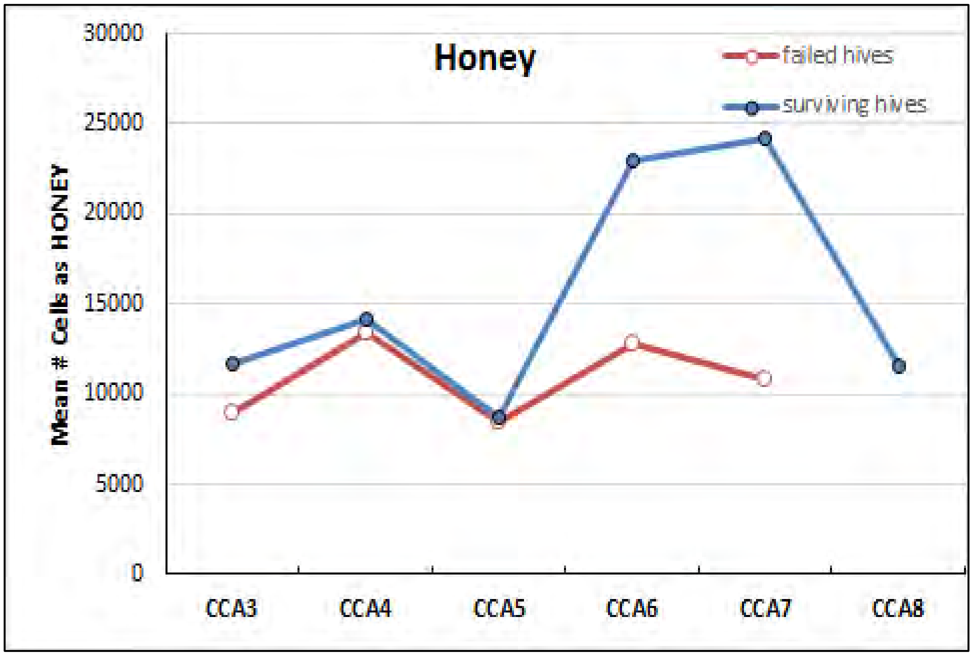


Figure 44. Fate of control hives as compared to the honey stores across CCAs

This, coupled with the experimental design which limited colony development due to lack of supers, provides a plausible explanation for the loss of control hives. Assuming this hypothesis, the bias introduced by this "culling" of weaker hives would theoretically render the remaining control hives at CCA8 stronger (on average) than the initial population of control hives. This could conceivably improve the ability to detect treatment-related colony condition effects at CCA8, since the actual values for controls would be weaker (on average) if the data from the dead hives were available for inclusion, and because there were more non-survived hives among controls than the two lowest treatments.

Consideration of Study Strengths, Limitations and Interpretation

It is important to recognize the inherent strengths and limitations of this study as results are interpreted and potentially considered in risk assessment.

In the context of available field studies involving honey bees and imidacloprid, this study contains a number of strengths including:

- Use of a high degree of replication (n=12) to achieve a reasonable level of statistical power
- Demonstration of a generalized concentration-response relationship with respect to the concentration of imidacloprid in sucrose solution and the magnitude and duration of adverse effects
- Quantification of exposure to parent (imidacloprid) and toxicologically-relevant metabolites in diet and in hive matrices (uncapped nectar, pollen, honey, bee bread)
- Use of a 6-week exposure duration to represent a "high end" exposure scenario
- Inclusion of multiple colony-level endpoints reflecting hive strength, brood development and food stores

- Detailed QA/QC results regarding quantification of chemical residues in various matrices
- Availability of raw data for conducting statistical analysis.

A number of limitations are also noted with this study, including:

- Exposure of bees through nectar (sucrose) alone, whereas bees in the field are likely exposed through both pollen and nectar routes. Therefore, the design of this study may not reflect a "worst case" exposure scenario in which bees are experiencing prolonged exposure to both contaminated nectar and pollen. While exclusion of the pollen route is expected to reduce overall exposure, the impact of this exclusion on the study results is uncertain and will likely depend on the life stage/caste of bee.
-

  It is noted that imidacloprid was found in both hive nectar and hive pollen (beebread), at concentrations lower than the feeding solutions. Dilution compared to the treatment feeding solution is expected since bees could also forage on outside nectar and pollen sources. As well, hive pollen contains only some hive nectar, thus would not be expected to have a concentration equivalent to nectar alone, and it is mixed with pollen which will come from outside sources. Therefore exposure through both hive pollen and nectar occurred via exposure to the sucrose feeding solution, but how this compares to exposure through contaminated pollen directly is not known. A recent paper by Dively (2014) showed that higher residues throughout the hive resulted from feeding pollen treatments compared to feeding sucrose solution treatments. It is also noted that nectar is considered the dominant exposure route for forager bees; other hive bees and larvae consume both nectar and pollen. In addition, since bees were forced to forage for pollen in this study, the potential impact of imidacloprid exposure on reducing pollen foraging efficiency of bees could be incorporated into the overall expression of adverse effects, as suggested by published literature. Had contaminated pollen been provided to bees, it is not known if the potential impact on pollen foraging efficiency would have been masked.

- The quantity of nectar provided to hives (2 L per week per hive) likely did not fulfill the complete carbohydrate needs of the colony, as indicated by colony bioenergetics and the lack of remaining sucrose solution upon their renewal. This suggests that bees could be exposed to a greater dose of imidacloprid in nectar had a greater volume of spiked sucrose been provided. Although one can infer that the dosing regimen may have underestimated exposure through sucrose relative to 100% contaminated diet, it is also noted that bees had to supplement their spiked sucrose by foraging on their own for other sources of nectar. As with the previous discussion of pollen, it is noted that had 100% of the carbohydrate needs of the colony been provided via feeders, the potential impact of purported reductions in nectar foraging efficiency may have been masked to some degree.

- Overwintering success of controls was impacted (36% hive mortality). This may have reduced the ability to detect adverse effects related to hive loss following overwintering. Although comparable to overwintering losses of commercial beekeepers, it is possible that elements of the study design may have contributed to this loss (e.g., lack of supers to allow for colony growth, delayed supplemental feeding during fall).

- Hive contamination with pesticides from food sources other than the artificial feeding was detected during the exposure period and post-exposure periods through collection of pollen from pollen traps.

Although the study was deliberately conducted in a low agricultural area in order to minimize the potential for pesticide contamination from other sources, the bees still appeared to be foraging on contaminated pollen and possibly nectar. During both exposure and post-exposure periods, high levels of multiple pesticides that may cause concern for bees were detected in most monitoring hives, such as spiromesifen (maximum at 961 ppb) and piperonyl butoxide (maximum at 591 ppb). Trace amounts of other bee-toxic pesticides, such as chlorpyifos (LOD = 1.0 ppb) and malathion (LOD = 4.0 ppb) were also detected. The test chemical imidacloprid was found at 12.1 ppb in pollen from one (apiary L) of the total of six sites analysed. This level is similar to one of the test concentrations.

- Residues of imidacloprid in uncapped nectar and bee bread within the hives at CCAs 4, 5, and 8 represent a single sample per hive on a single frame rather than a composite sample from multiple portions of the comb within a hive. This means that residue results may reflect “hit or miss” scenario with respect to detecting residues in nectar laid down from contaminated (fed) vs. outside sources.

- The exposure, based on residues measured in the hive (hive nectar and hive pollen) indicated that, overall, higher measured hive residues correlated with higher nominal residues in feeding solutions. However, individual hive residue values varied, and there was some overlap in measured values, particularly among the three lowest doses.

- Exposure dilution during the study was evident. Pollen storage was observed consistently in the control hives and hives exposed to lower test concentrations during the exposure period, indicating that test bees were foraging on food sources other than the spiked sugar solution. Remarkably lower residue concentrations detected in bee bread and hive nectar in some test hives compared to the feeding concentrations may also indicate foraging on other food sources. This uncertainty is inherent in any semi-field or full-field study design.

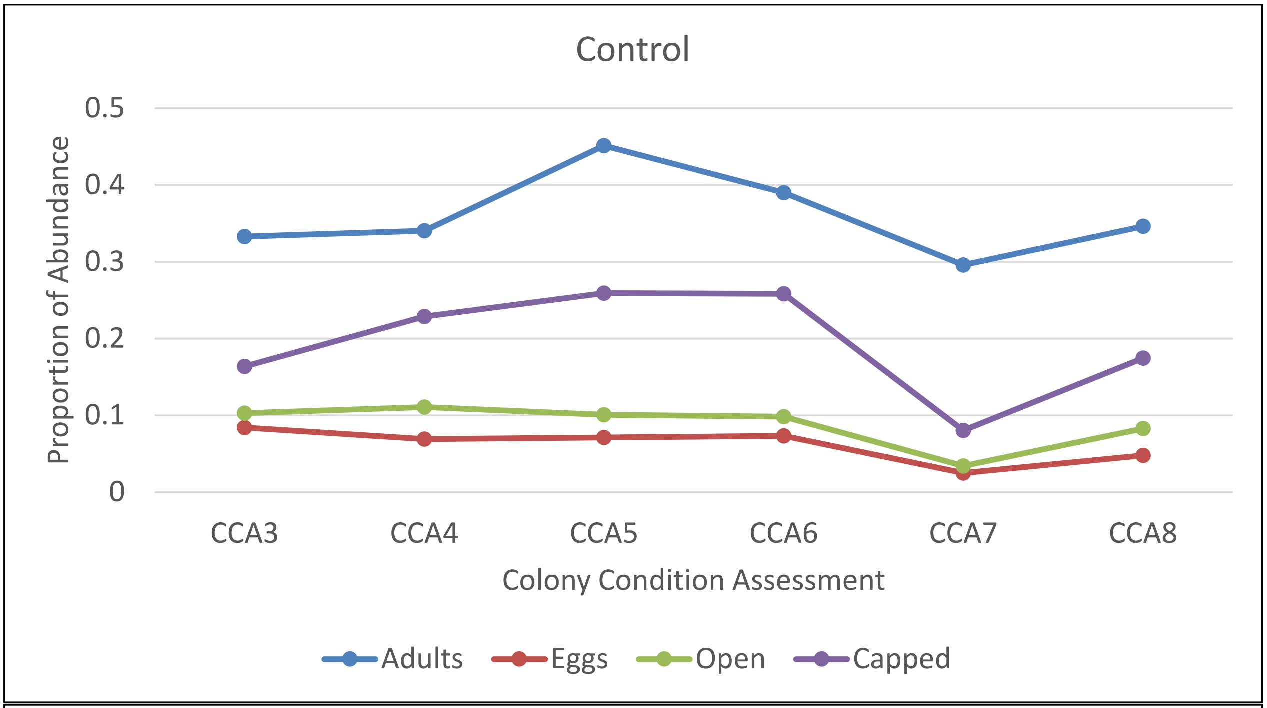
5. Conclusions

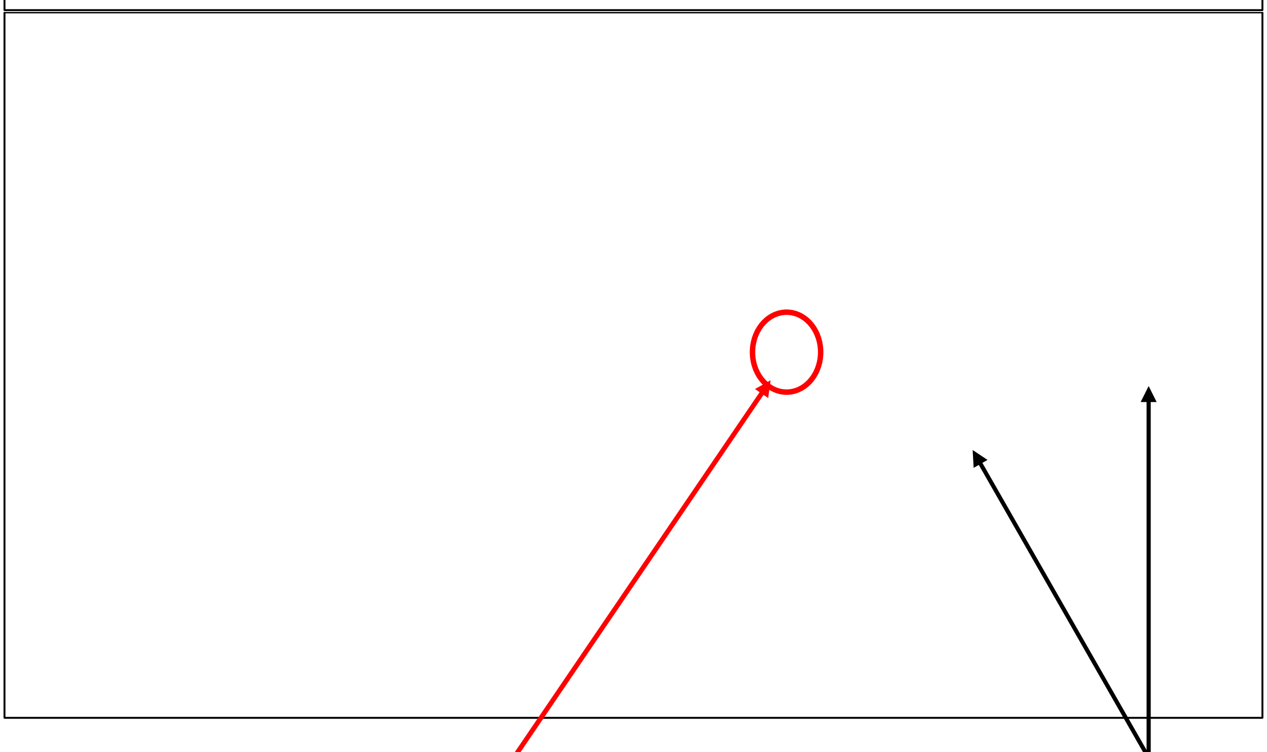
The study is considered to be informative and will be used as a line of evidence in the pollinator risk assessment. While there were uncertainties that were generally related to inherent aspects of any semi-field or full field study design (such as dilution of the test chemical through alternative sources of forage, detection of other chemicals in the monitoring hives), this study still provides information on a number of colony health parameters about the long term (including overwintering) exposure to imidacloprid at the colony level.

As indicated in the results section above, the PMRA, EPA, and CDPR analyses determined significant effects (at both the 0.05 and 0.1 alpha levels) in the 50, 100, and 200 µg/L groups across multiple CCAs for the majority of response variables. Specifically, for the 100 and 200 µg/L treatment groups, significant effects ($p<0.05$) were determined for every response variable and persisted across at least 2 CCAs, along with very high overwintering mortality. While the 50 µg/L group had overwintering mortality similar to the controls, colony condition effects were different from controls with an early onset of effects which tended to persist, and notably poorer colony condition in surviving hives after overwintering in comparison to controls.

Conversely, there was not a strong indication from the PMRA, EPA, and CDPR analyses of an impact at the colony level at the 12.5 and 25 µg/L treatment groups. This is evidenced not only by a general lack of statistical findings ($p>0.1$) at these treatment levels but in cases where significant effects were determined, they either did not show strong dose-responsiveness, did not persist across multiple CCAs, or were considered potential transient effects (e.g. at CCA6) which did not persist after overwintering. This latter point was the case for the total life and pupal cell findings in which the PMRA analysis determined significant effects at all treatment levels at CCA6 (EPA also determined a significant reduction in pupal cells at the lowest treatment group of 12.5 µg/L at CCA6). As well at CCA6, PMRA determined significant effects with eggs and larvae at 25 µg/L treatment (but not at the 50 µg/L). For these two lowest treatment groups (12.5 and 25 µg/L), the colony condition of surviving hives at CCA8 following overwintering was similar to controls, indicating the effects observed at CCA6 were likely transient and the colony was able to compensate for these effects.

The figures below present the trends of life stages across all CCAs in the 12.5, 25, and 50 µg/L treatment groups compared alongside the response of that in the control.

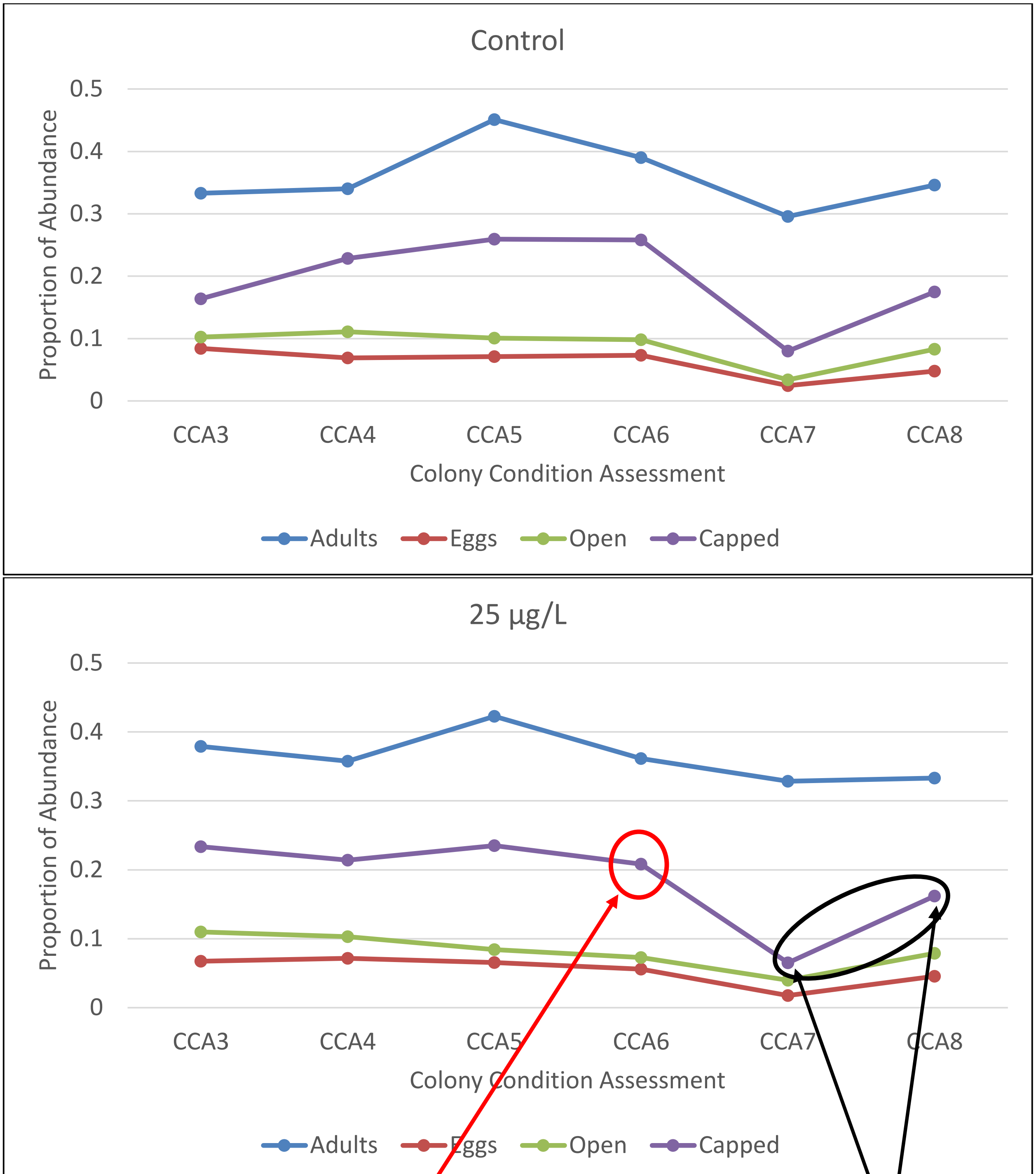




Significantly reduced from control in the PMRA, EPA, and CDPR analyses (either 0.1 or 0.05 level depending on the analysis)

Proportions at CCA7 and CCA8 are approximately the same as control (8% and 17% of frame coverage, respectively)

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document



Significantly reduced from control in the PMRA, EPA, and CDPR analyses (either 0.1 or 0.05 level depending on the analysis)

Proportions at CCA7 and CCA8 are approximately the same as control (7% in treatment vs 8% in control and 16% in treatment vs 18% in control, respectively

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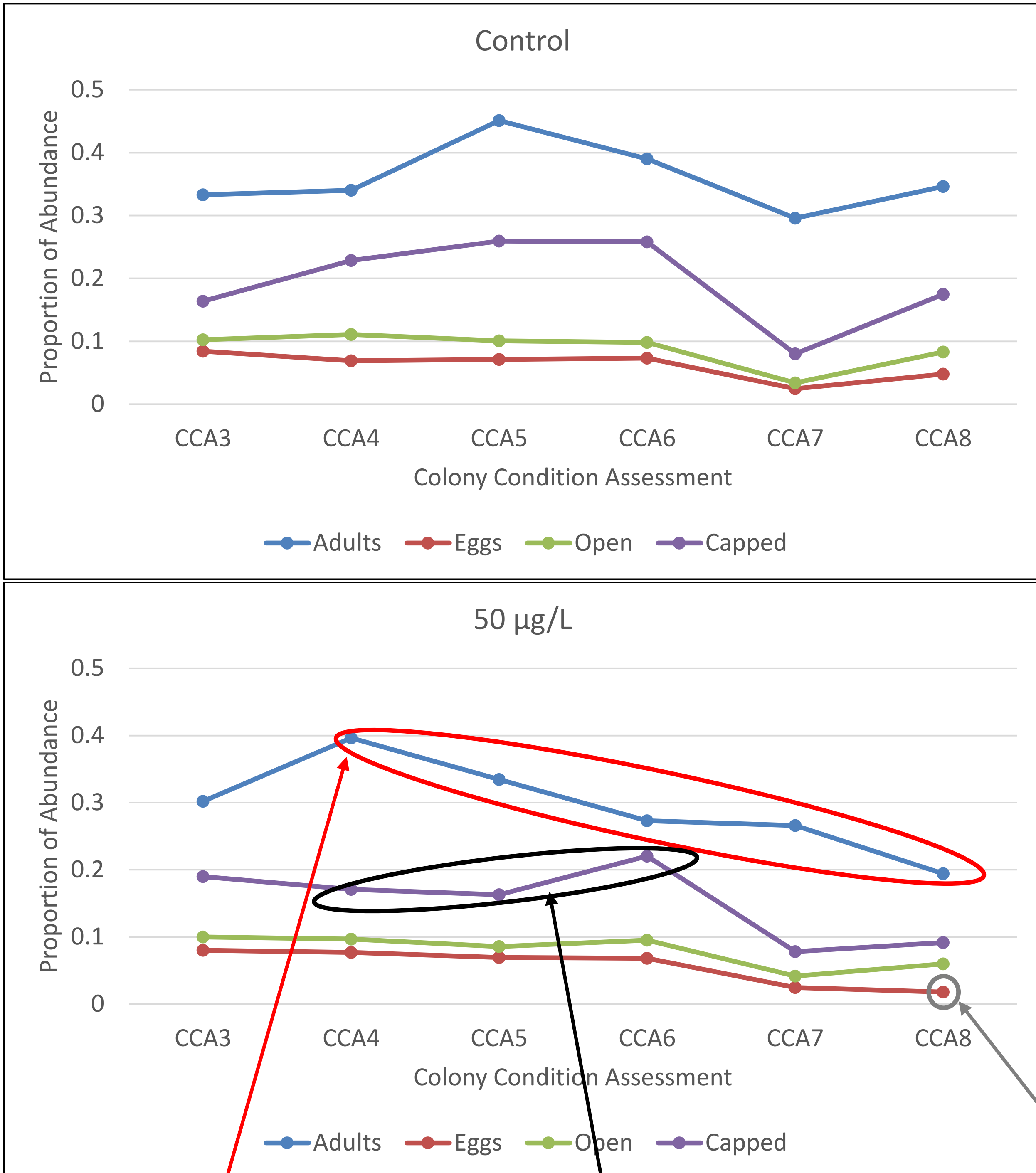
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

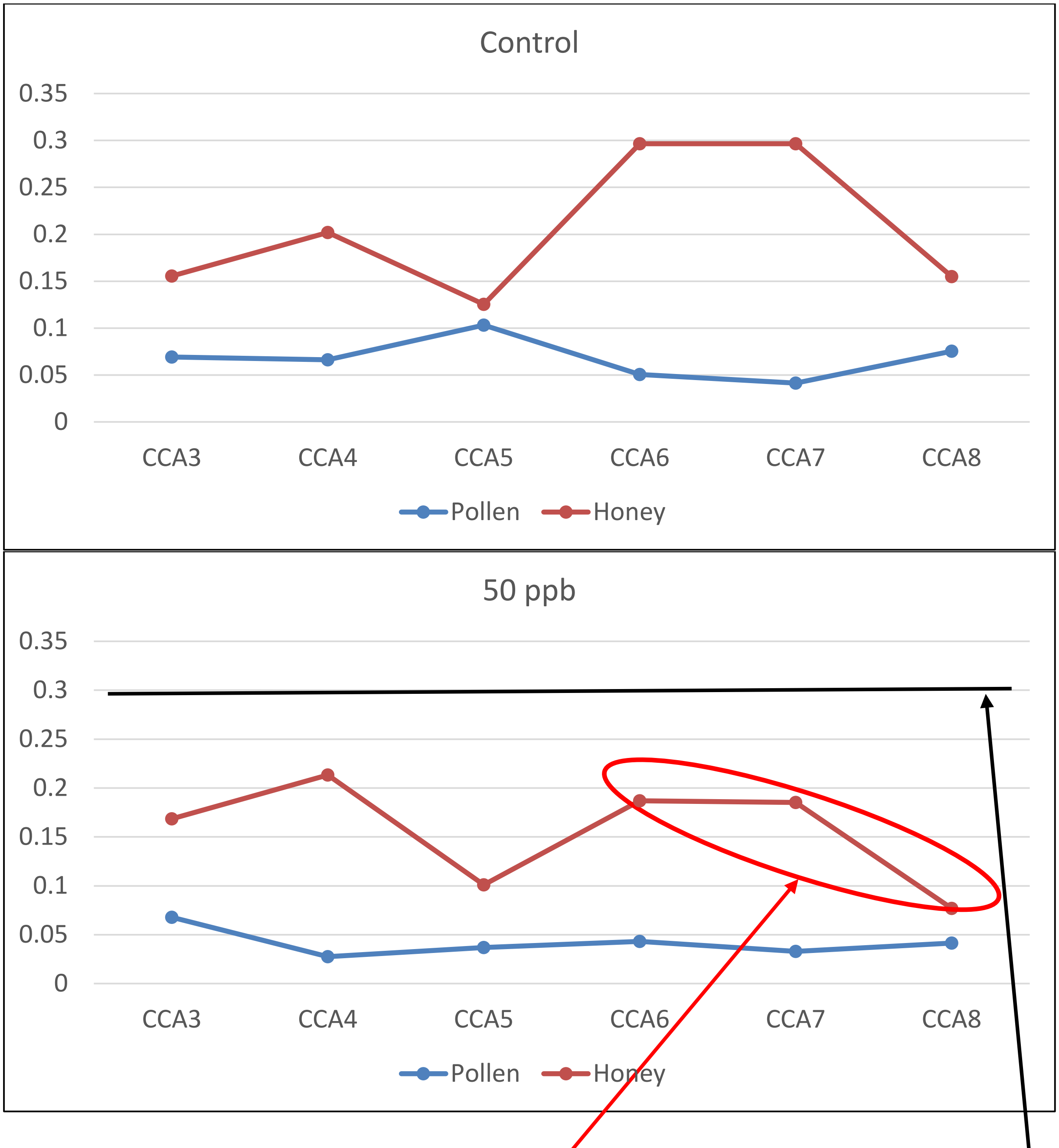


Significantly reduced from control in the PMRA, EPA, and CDPR analyses (either 0.1 or 0.05 level depending on the analysis). Effects begin at a time when buildup of adults is still taking place in controls and two lower treatment groups and repression continues up to and including after overwintering.

Significantly reduced from control in the PMRA and EPA (at 0.05 level). The 50 µg/L is the only group of the three lower treatment groups that does not show an upward trend from CCA7 to CCA8.

Significantly reduced from control in EPA, CDPR and PMRA analyses ($p<0.05$ or $p<0.01$, depending on the analysis, CCA6 not determined in EPA analysis). Effects are clearly divergent from control in which a buildup in pupal cells occurs in the control from CCA3 to CCA6 whereas the 50 µg/L group undergoes an initial decline from CCA4 to CCA5

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document



Significantly reduced from control in the PMRA, EPA, and CDPR analyses (either 0.1 or 0.05 level depending on the analysis). The level of honey stores at CCA8 in the 50 µg/L group is approximately half of that of the control at CCA8.

Level of honey stores at their peak in control group heading into overwintering at CCA7. The control group had approximately 30% of frame coverage of honey stores at CCA7 while the 50µg/L group had 19%

When examining the effects on food stores (pollen and nectar), the PMRA, EPA, and CDPR analyses did not determine any consistent and significant reductions in pollen and nectar stores at the 12.5 and 25 µg/L treatment groups. This is distinguished from the 50 µg/L group where effects on nectar in particular were very apparent when compared alongside the response of the control in terms of the level of nectar buildup before the hive preparation for overwintering at CCA7. This finding was also confirmed statistically in all three analyses with significant reductions in honey stores at CCAs 6, 7, and 8 (CCA8 data excluded from the EPA analysis for the 100 and 200 µg/L groups). Significant reductions in pollen stores were also confirmed at CCAs 4 and 5 at the 50 µg/L treatment during the exposure period.

Specifically, when considering the adult and honey and pollen stores response variables, the differences from control were apparent both visually and statistically, particularly in the three highest treatment groups. For the proportion of adults, the onset of a decline in numbers occurred one CCA earlier in these groups than in the control, 12.5 and 25 µg/L treatment groups. For honey stores, the buildup that occurred starting at CCA5 in the 50 µg/L treatment group, reached only half the level reached in the control, 12.5, and 25 µg/L treatment groups by CCA7. Pollen stores were also reduced at CCA4 and CCA5 compared to controls for the three highest treatment groups, as well as at CCA6 and CCA7 at the highest treatment group. These effects were statistically significant ($p<0.05$) and indicate that the 50 µg/L treatment group was associated with trends and proportions of abundance for life stages and food stores not observed in the control, 12.5, and 25 µg/L treatment groups.

Therefore, when weighing biological significance and the natural seasonal changes of honey bees colonies, as well as supporting conclusions from the statistical approaches used in PMRA, EPA, and CDPR, the NOAEC and LOAEC for this study is determined to be 25 and 50 µg/L, respectively.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Appendix A: Details of EPA Statistical Analysis

In the statistical analysis of the Tier II colony feeding study for imidacloprid and honey bees, there were three main questions that were investigated:

1. For given CCA (colony condition assessment) and treatment level, what treatments were having an effect on the measures of hive health, as determined relative to control?
2. Were the observed treatment effects consistent over time?
3. What was nature of the onset of effects over time for various treatments?

To answer these questions, a variety of statistical approaches were considered and a repeated measure mixed model analysis was deemed best to address the above questions.

For this analysis the time by treatment interaction was evaluated across all CCAs and treatment groups for each response variable. In this way, the trends for each response variable (*i.e.* adult, eggs, etc.) across all CCAs for a given treatment group could be examined. The details of the parameterization of the repeated measure mixed model along with the statistical results (p-values) are provided below.

Background on data manipulation

- Data utilized for the statistical analysis were provided in an Excel file by the study author. This file was made available to the Agency on May 15, 2015. Additional QA of the entire data file was completed by the study author, as some transcription errors were found in an earlier electronic file submitted by the study author.
- Data to be included in the data analysis were data that were collected on or after CCA3. CCA3 represents the first time hive parameters were measured after the hives were placed in their treatment apiaries/locations.
- Zeros in the data set represent instances when no frame contained >5% coverage of a given matrix. Missing values for all matrices during a given timepoint indicate the hive was dead and no measurements were taken. Definition of a dead hive is provided in the study report. For these data analyses, all entered zeros were maintained in the data set. Missing values were kept as 'missing'. These parameters were not replaced with zeros.
- Time (days) between measurements was roughly even for CCA3 through CCA7, while CCA8 was measured during the following spring (to evaluate any impacts on hive overwintering). Number of days was estimated using the median number of days between CCAs. Assuming that CCA3 was on day 0, CCA4 through CCA8 occurred on the following days: 25, 53, 84, 119, and 272.
- In the initial analyses, there were difficulties with convergence of the PROC MIXED algorithm for many of the more complicated temporal covariance structures. John Troiano (California Department of Pesticide Regulation) provided an article from SAS suggesting that by re-scaling the response variable, the likelihood of convergence would be improved. Given the nature of the response variable (most were count cells of a given matrix within in a hive), a re-scaling was straight-forward. For eggs, open, capped, pollen, and honey cells, the count (the variable recorded in the excel spreadsheet) was divided by 68800 (total number of cells in the hive). This resulted in the proportion of cells occupied by a given matrix (values between zero and one). Number of adult bees, as recorded in the spreadsheet, was derived from the percent coverage, the density estimate of 1.3 bees/cell, and the density estimate of 4 cells/cm^2. Therefore, the conversion from the data stored in the spreadsheet

was to divide by 68800, divide by 1.3, and multiply by 4. The resulting number is the proportion of cells occupied by adult bees. Following the re-scaling of the response variables, the model convergence improved dramatically.

- The variables to be analyzed included:
    - Proportion of hive covered in adults
    - proportion of hive covered in eggs
    - proportion of hive covered in open cells (larvae)
    - proportion of hive covered in capped cells (pupae)
    - Proportion of hive covered in live bees (at any life stage). This was obtained by summing the proportions of the four life stages (described above). Note that it is possible for the proportion of a hive covered in live bees to be greater than 1.0, as adult bees will be observed on top of cells containing eggs, larvae, or pupae.
    - Proportion of hive covered in pollen cells
    - proportion of hive covered in honey cells, and
    - Hive weight.

Examination of the associations among the variables

- Based on physical hive constructs and the nature of the honey bees, it is generally accepted that the colony condition assessment (CCA) variables may be correlated over time and may also be correlated within a time point (sampling time). Given this background, a series of scatterplots, correlation matrices, and principal component analyses for this bee study was prepared (Further details are provided in the section entitled "**Scatterplot and Principal Component Discussion**" and the full SAS output is included as **Attachment 3**).
- Some of the general summary points are:
    - Variables tended to have stronger correlations at adjacent time points than at farther time points (i.e., correlations for CCA3 and CCA4 tended to be stronger than correlations for CCA3 and CCA7 or for CCA4 and CCA7).
    - CCA8 tended to have weaker pairwise correlations with all the other time points than CCA3 through CCA7 had with each other.
    - The first principal component for each of the CCAs explained 20-50% of the total variation. The lowest was capped, and the highest were adults, honey, and pollen.
    - The general interpretation of the first principal component was a weighted average over all time points (weights varied depending on variable).

Analysis Approach and Model Setup

The general experimental design was a randomized complete block with repeated measures. Apiary was the block effect and the repeated measures were the CCAs. Within each block, the control treatment was replicated 2x and each treatment occurred one time. Since hives were not placed till shortly before CCA3, the data for the statistical analysis only included CCA3 through CCA8. Exploring the interaction between treatment and CCA will address the first two questions above.

Once the design component of the analysis was established, the next part of the analysis was to determine which correlation structure (across time) was the best fitting for these data. One of the challenges was that many hives died before the end of the study (especially the 100 and 200 ppb treatments in CCA8), creating censored data or an imbalanced design. PROC MIXED in SAS can handle an unbalanced design; however, convergence may not be attained for some correlation structures. In this case the imbalance was due to treatment (the higher treatments had a higher rate of hive death) as well as random mortality (control hive mortality rate during overwintering was similar to background mortalities of commercial hives). After exploring several options (which are detailed in the section entitled **"Options for Addressing the Data Imbalance"**), the data were analyzed after removing data from the few remaining hives in treatment groups 100 and 200 ppb from CCA8. Data from hives treated with 100 or 200 ppb imidacloprid remain in the data set and analysis for CCA3 through CCA7 permitting utilization of a majority of the data. These deletions create a 5x6 factorial design matrix with two of the cells (CCA8, treatment=100) and (CCA8, treatment=200) missing all data. CCA*treatment interaction means can still be calculated. Least square means on the main effects (treatment and CCA) cannot be estimated for Concentration=100, Concentration=200, and CCA8. Since the expectation was that the interaction would be significant and all further statistical tests would be exploring the interaction, this was not considered a significant hindrance.

To address the primary research questions above, the "treatment * CCA" interaction was evaluated in two ways:

- At each CCA, was there a reduction in the response relative to the control? This was evaluated using a one-sided Dunnett's test.
- At each treatment level, was there a difference in the response relative to the baseline? This was evaluated using a two-sided Dunnett's test comparing CCA4 through CCA8 against CCA3.

Scatterplot and Principal Component Discussion

Based on physical hive constructs and the nature of the honey bees, it is generally accepted that the colony condition assessment (CCA) variables may be correlated over time and may also be correlated within a time point (sampling time). Given this background, a series of scatterplots, correlation matrices, and principal component analyses (PCA) for this bee study were prepared. The full printout is included as **Attachment 3**. Some of the general summary points are:

The first series looked at: for a given response variable, what were the pairwise correlations over time, and how would a principal components analysis best explain the observed variation. Data were plotted and subjected to a PCA without accounting for treatment (i.e., all data were included in a single series of plots and PCAs; separate assessments were not done for each treatment). Some general interpretations are:

- Scatterplot and correlation matrices indicated that variables tended to have stronger correlations at adjacent time points than at farther time points.
- CCA8 tended to have weaker pairwise correlations with all the other time points than CCA3 through CCA7 had with each other.
- The first principal component for each of the variables explained 26-53% of the total variation. The lowest was proportion frame coverage of eggs, and the highest were adults, honey, and hive weight. The general interpretation of the first principal component was a weighted average over all time points (weights varied depending on variable). For most variables, CCA3 tended to carry the least weight in the weighted average.
- The second principle component explained an additional 16 to 27 percent of the total variation. For most endpoints, a general interpretation of the principle component eigenvector was a difference between measured taken early in the study and measure recorded later in the study (*e.g.*, average of CCA3 and CCA4 minus the average of CCA7 and CCA8).

These scatterplots, correlation matrices, and principle component analyses were used to inform the choice of covariance structure used in the repeated measure analysis. Some summary points are:

- Variance for a given response variable was not consistent across all CCAs. This may indicate that the correlation structures with a constant variance for all CCAs (*e.g.*, CS, SP(pow)(1), and AR(1)) may not fit as well as those that allow for heterogenous variance (*e.g.*, CSH, ToepH(1), and ToepH(2)).
- There did not appear to be a consistent decrease in correlation if the paired CCAs were farther apart (i.e., correlation between CCA4 and CCA8 (three time steps apart) was not consistently less than correlation between measurements 2 time steps apart, e.g., (CCA4, CCA6), (CCA5, CCA7), and (CCA6, CCA8). This may indicate that the AR(1) and SP(pow)(1) covariance structures may not fit the data as well as other structures.

The second series of scatterplots and PCAs looked at: for a given CCA, what were the pairwise correlations across matrices, and how would a principal components analysis best explain the observed variation. Data were plotted and subjected to a PCA without accounting for treatment (*i.e.*, all data were included in a single series of plots and PCAs; separate assessments were not done for each treatment). Some of the general summary points are:

- o Honey had the weakest correlations (honey and any of the other measured matrices) amongst all the pairwise correlations.
- o The first principal component explained 36-66% of the total variation. The CCAs with the lowest percent of variation explained were CCA3 and CCA4. The percent variation explained tended to increase over time. At each time point the first principle component tended to be interpreted as a weighted average, with honey receiving the least weight.
- o The second principal component explained an additional 15 to 24% of the total variation. The interpretations of the eigenvectors from the second principal component were less clear and consistent. They tended to be an average of a measure of hive food stores (pollen, honey, and/or hive weight) or an average of the hive food stores contrasted with a subset of the population matrices (e.g., eggs, open, capped, adult, and/or total).

Options for Addressing the Data Imbalance

Once the design component of the analysis was established, the next part of the analysis was to determine which correlation structure (across time) was the best fitting for these data. One of the challenges was that many hives died before the end of the study (especially the 100 and 200 ppb treatments in CCA8), creating censored data or an imbalanced design. PROC MIXED in SAS can handle an unbalanced design; however, convergence may not be attained for some correlation structures. In this case the imbalance was due to treatment (the higher treatments had a higher rate of hive death) as well as random mortality (control hive mortality rate during overwintering was similar to background mortalities of commercial hives). Several options were explored for addressing the data imbalance and convergence issues: [4]

[4] These analyses were conducted utilizing an earlier version of the data set that was provided by the study author. It was later determined that there were some data entry errors. The errors were corrected and the main analyses were re-run. These exploratory analyses discussed in Appendix B were not re-run utilizing the corrected data set; however, the scope of the data errors was such that it was unlikely that the analysis choices would have changed.

- Analyze the data with treatment groups 100 and 200 deleted for all CCAs. The reasoning behind this path forward are:
    - There was consensus that treatments 100 and 200 impacted hive health with no recovery amongst all evaluators.
    - Removing these treatment groups greatly improves the percentage of covariance structures that were able to be fitted in PROC MIXED.
    - During the study, hives treated with 100 and 200 concentrations of test material were physically moved away from the initial placements in the field to minimize the potential impact these hives may have on the other nearby hives. Thus data obtained on these hives after moving may not be comparable to data obtained before moving.
- Analyze the data with CCA8 removed and conduct separate analysis for CCA8. The justifications are:
    - The majority of hive deaths occurred between CCA7 and CCA8; therefore, data are better balanced from CCA3 to CCA7 and convergence success will be much higher for all responses.
    - Number of days between CCAs was very similar from CCA3 to CCA8; therefore, the AR(1) covariance structure which requires equal spacing between time points could be evaluated.
- Analyze the data analyzed after removing data for the few remaining hives in treatment groups 100 and 200 ppb from CCA8. The justifications are:
    - The vast majority of the data can still be included in the analysis.
    - Data on some hives after overwintering can be included in the full analysis.
    - Pulled error terms (and standard errors) will utilize the majority of the data set, thus increasing confidence in the estimates.

After exploring these options, the data were analyzed after removing data from the few remaining hives in treatment groups 100 and 200 ppb from CCA8 (third option above). Data from hives treated with 100 or 200 ppb imidacloprid remain in the data set and analysis for CCA3 through CCA7 permitting utilization of a majority of the data. These deletions create a 5x6 factorial design matrix with two of the cells (CCA8, treatment=100) and (CCA8, treatment=200) missing all data. CCA*treatment interaction means can still be calculated. Least square means on the main effects (treatment and CCA) cannot be estimated for Concentration=100, Concentration=200, and CCA8. Since the expectation was that the interaction would be significant and all further statistical tests would be exploring the interaction, this was not considered a significant hindrance.

Determining the temporal covariance structure

Before conducting Dunnett's test, several different correlation structures to best fit the temporal correlation were evaluated. The structures that were fitted included:

- **Compound symmetry (CS)**: assumes equal correlation for all pairwise correlations (regardless of distance of timepoint).
- **Compound symmetry with heterogeneous variance (CSH):** Estimates a unique variance at each time point, but assumes equal correlation for all pairwise correlations (regardless of distance of time point).
- **Sp(pow)(1):** this is a correlation structure that fits an AR(1) but it adjusts for unequal spacing between time points.

- **AR(1): autoregressive correlation**. Assumes equal correlation between adjacent timepoints. Time points further apart have a lesser correlation.
- **Heterogeneous Toeplitz TOEPH(1):** models a unique variance for each timepoint. Correlation between timepoints was zero.
- **Heterogeneous Toeplitz TOEPH(2):** models a unique variance for each timepoint and a common correlation for adjacent timepoints. Correlation for timepoints not immediately adjacent was assumed to be zero.

More information about each of the correlation structures can be found here: http://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#statug_mixed_sect019.htm . The full SAS output is provided in **Attachment 4**.

To compare structure fit, Bayesian Information Criterion (BIC) was utilized. The BIC is similar to the AIC and both are functions of the log likelihood with a penalty for an increase in the number of covariance parameters to be fitted. The BIC value for each fitted model for all eight response variables is reported in **Table A-1; s**maller values of the BIC indicate a better fit (bolded). For many of the endpoints, heterogeneity of variance at different time points was indicated as CSH, ToepH(1), or ToepH(2) were the covariance structures providing the best fits. This is not surprising as unequal variances were observed in the exploratory multivariate/principle component analysis. Although the AR(1) model was fit here, it may not be appropriate as the spacing between measurement times is not consistent (minor deviations as between CCA3, CCA4, CCA5, CCA6, and CCA7 are acceptable, but CCA8 is clearly not equidistant from CCA7).

For the variables that represent the individual life stages (adults, eggs, open cells, and capped cells), ToepH(2) is the one covariance structure that provides a good fit for all four matrices of the evaluated structures. In addition, the BIC statistics suggests that CSH, AR(1) and ToepH(1) provide adequate fits for at least one of the life stages. For proportion frame coverage of total individuals, ToepH(2) provides the best fit to the covariance structure of all the evaluated models. For pollen, CSH, ToepH(1) and ToepH(2) provided the best fits to the data. For honey, SP(pow)(1) and AR(1) provided the best fits to the covariance structure; ToepH(2) did not converge (infinite likelihood). For hive weight, SP(pow)(1), AR(1), and ToepH(1) were the best fitting covariance structures. Compound symmetry (CS) was not identified as quality fit to the data for any of the eight evaluated response variables.

Residual plots were also evaluated for each of the response variables and covariance structures.
Patterns indicative of heterogeneous variance of the residuals were evident for many of the response variables and models where an assumption of equal variance at each time point was made. It was recognized that many of the response variables were proportions, hence the distribution of the response variable and the residuals may not meet assumptions of normality. Review of the residual plots indicated that estimating unique variances at each CCA (e.g., CSH, ToepH(1), or ToepH(2) covariance structures) appears to address the concern of unequal variance.

The varying strength and pattern of correlation seen in the pairwise scatterplots (CCA*x* vs. CCA*y* for any given response variable for *x* and *y* equal to 3, 4, 5, 6, 7, and 8), the BIC comparisons, and the residual plot review indicate that there is not a single covariance structure that is clearly best for all eight response variables. General conclusions that can be made from the scatter plots and covariance analysis are that within a response variable, variance varies over time and that there is correlation in response variable over time; however, the pattern of correlation is not particularly strong nor is it consistent. Given these interpretations along with the BIC analysis and residual plots, the CSH covariance was chosen for the mixed model structure.

Table A-1. BIC values for fitted models. CCA3 - CCA8; concentrations 100 and 200 ppb deleted from CCA8

Variable → Model ↓	Adults	Eggs	Open	Capped	Total indiv.	Pollen	Honey	Hive weight
SP(pow)(1)	-389	-1457	-1268	-735	1.95	-1518	**-724**	2502
CS	-374	-1459	-1265	-724	33.3	-1518	-679	2588
CSH	-385	**-1549**	**-1321**	**-816**	-5.85	**-1534**	-709	2533
AR(1)	**-401*@**	-1456	-1268@	-738@	-2.27@	-1522	**-729**	**2492@**
ToepH(1)	-381	**-1552**	**-1320**	**-815**	1.99	**-1532**	-711	2547
ToepH(2)	**-406**	**-1550**	**-1319**	**-824**	**-35.5**	**-1535**	Inf. lklhd	**2484**

*Within a response variable, bolded BIC values indicate better covariance model fit. Kass and Raferty (1995) suggested that differences of greater than 10 in BIC values provides very strong evidence that model fits are not equivalent.
@Convergence was attained, but estimated G matrix was not positive definite.

Treatment by time interaction and follow-up contrasts

The text box provides the SAS code for the mixed model that was used for the Dunnett's comparisons. **Table A-2** provides the results from the Dunnett's comparisons in which treatment means were tested to see if significantly less than control at each CCA. For these analyses, the CSH covariance matrix was used for each of the variables.

```
title 'concentration 100 and 200 deleted for CCA=8, covariance=csh';
proc mixed data=cca3_8  ;
  title2 "Dunnett's tests - adult";
  class apiary cca concval replicate;
  model adult_p = concval|cca /DDFM=SATTERTHWAITE;
  random apiary ;
  repeated cca/ subject=replicate*concval(apiary) type=csh ;
  lsmeans concval*cca;
  slice concval*cca /sliceby=cca diff=controll adjust=dunnett;
  slice concval*cca /sliceby=concval diff=control adjust=dunnett;
  run;
```

Treatment effects within a CCA

The table of p-values resulting from the Dunnett's tests for evaluating whether within a CCA, the treatment mean are significantly less than control means) are summarized in **Table A-2. Figures 1-7** below show the results for each response variable across all CCAs analyzed (CCA3-CCA8) and all treatment levels. It is noted as discussed previously that the 100 and 200 ppb treatment groups were excluded from CCA8 due to high hive mortality. For all figures presented below, significant reductions from the negative control with p-values below the 0.05 alpha level are denoted by a red dot at a given treatment level and CCA and those reductions with p-values between 0.05 and 0.1 are denoted by a black dot. Statistical NOAECs and LOAECs will be determined using an alpha-level of 0.05. Additional comparisons using and alpha-level of 0.10 are included for additional characterization. The tables of p-values resulting from the Dunnett's test are summarized in **Table A-2**. The associated SAS output containing the full results of the Dunnett's comparisons can be found in **Attachment 5.**

Table A-2. Results of one-sided Dunnett's test (comparing control to each treatment group) with 100 and 200 concentrations deleted from CCA8, correlations modeled using CSH.*

	Adults	Eggs	Open	Capped	Total indiv.	Pollen	Honey	Hive weight
CCA3	NS	NS	NS	NS	NS	NS	NS	NS
CCA4	NS	*100*	**100** **200**	**100** **200**	*100*	**50** **100** **200**	NS	**100** **200**
CCA5	*50* **100**	*100*	*100* **200**	**50** **100** **200**	*50* **100** **200**	**50** **100** **200**	NS	**50** **100** **200**
CCA6	**50** **100** **200**	**100** **200**	**100** **200**	*12.5* **100** **200**	*50* **100** **200**	**200**	**50** **200**	**100** **200**
CCA7	**100** **200**	**200**	**100** **200**	**100** **200**	**100** **200**	**200**	*50* **100** **200**	*50* **100** **200**
CCA8	**50**	*12.5* **50**	NS	*50*	**50**	**50**	*50*	*50*

*Listed concentrations are those that were significantly less than the control following the results of Dunnett's test. NS indicates that there were no test concentrations with means significantly less than the control (p>0.10).
Bolded concentration = significantly less than control (p< 0.05)
Italicized concentration = less than control (0.05< P < 0.10)

Temporal trends within a treatment level

A second component to evaluating the "treatment x CCA" interaction is to look at the temporal changes within a treatment group. This was accomplished by comparing each CCA (CCA4 through CCA8) to CCA3 by use of a two-sided Dunnett's test (**Table A-3** and **Table A-4**). This suite of comparisons is not as informative as the contrasts of control against the treatment group within a CCA for establishing a NOAEC and LOAEC. However, it may aid in interpretations and further biological understanding of temporal shifts in the life stages and food components present in the hive. Differences in patterns of temporal shifts between the control and various treatment groups can provide further understanding of the potential impacts of imidacloprid on beehive population dynamics.

Table A-3. Results of two-sided Dunnett's test (comparing CCA3 to each following CCA) with 100 and 200 concentrations deleted from CCA8, correlations modeled using CSH.*

Trt Group	**Response Variable**				
	Adults	**Eggs**	**Open**	**Capped**	**Total indiv**
Control	CCA5>CCA3	CCA7 and CCA8 < CCA3	CCA7 < CCA3	CCA4-6 > CCA3 CCA7 < CCA3	CCA7 < CCA3
12.5	NS	NS	NS	CCA7 < CCA3	CCA7 < CCA3
25	NS	CCA7 < CCA3	CCA7 < CCA3	CCA7 < CCA3	CCA7 < CCA3

50	NS	CCA7 and CCA8 < CCA3	CCA7<CCA3	CCA7 < CCA3	CCA7-8 < CCA3
100*	CCA7<CCA3	CCA6 and CCA7 <CCA3	CCA7<CCA3	CCA5-7 < CCA3	CCA6-7<CCA3
200*	CCA6 and CCA7<CCA3	CCA6 and CCA7 <CCA3	CCA4-7 < CCA3	CCA4-7 < CCA3	CCA4-7<CCA3

* CCA8 not included for test concentrations 100 and 200
NS – No significant differences from control (p>0.05)

Table A-4. Results of two-sided Dunnett's test (comparing CCA3 to each following CCA) with 100 and 200 concentrations deleted from CCA8, correlations modeled using CSH.*

Trt Group	Response Variable		
	Pollen	**Honey**	**Hive weight**
Control	CCA5>CCA3 CCA7 < CCA3	CCA6-7 > CCA3	CCA4-7> CCA3
12.5	CCA5 and CCA8>CCA3	CCA6-7 > CCA3	CCA4, 6-7 >CCA3
25	CCA5>CCA3	CCA6-7 > CCA3	CCA4-7> CCA3
50	CCA4 and CCA7<CCA3	CCA5 and CCA8< CCA3	CCA4>CCA3 CCA8<CCA3
100*	CCA4-5<CCA3	NS	CCA4>CCA3
200*	CCA4-5 and CCA7<CCA3	CCA4-5> CCA3	CCA4>CCA3

* CCA8 not included for test concentrations 100 and 200
NS – No significant differences from control (p>0.05)

Tables A-5 – A-12 tabulate the summary statistics (including mean and standard deviation) of each response variable for all treatment levels across CCAs 3-8.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table A-5. Summary statistics for adults

Treatment Group µg/L	Parameter	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
0	N	21	22	22	21	20	14
0	MIN	0.157513	0.127504	0.077504	0.155009	0.130009	0.047496
0	MAX	0.489982	0.632513	0.742487	0.875	0.532513	0.605009
0	MEAN	0.328452	0.34023	0.451138	0.394288	0.29725	0.36411
0	STD	0.099238	0.15064	0.170406	0.170447	0.117737	0.169285
12	N	11	11	11	11	11	9
12	MIN	0.15	0.130009	0.212522	0.194991	0.130009	0.180009
12	MAX	0.727504	0.680009	0.687522	0.555009	0.485018	0.577504
12	MEAN	0.374093	0.36932	0.484327	0.375224	0.342958	0.369728
12	STD	0.177567	0.183432	0.157566	0.124439	0.09466	0.13788
25	N	11	11	11	11	11	10
25	MIN	0.155009	0.219991	0.175	0.139982	0.117487	0.077504
25	MAX	0.705009	0.614982	0.672496	0.597496	0.6	0.632513
25	MEAN	0.379094	0.357501	0.422731	0.361364	0.328639	0.346498
25	STD	0.16288	0.141233	0.173179	0.169438	0.13711	0.191584
50	N	11	11	11	10	9	7
50	MIN	0.175	0.175	0.155009	0.147496	0.172496	0.039982
50	MAX	0.535018	0.707513	0.562522	0.407513	0.432513	0.305501
50	MEAN	0.302053	0.396361	0.334323	0.264244	0.255282	0.184718
50	STD	0.098915	0.174624	0.148201	0.086622	0.077175	0.10027
100	N	11	11	11	10	10	1
100	MIN	0.155009	0.117487	0.027504	0.037522	0.042487	0.010018
100	MAX	0.560018	0.664982	0.647496	0.597496	0.397496	0.010018
100	MEAN	0.301374	0.312047	0.281591	0.253001	0.144253	0.010018
100	STD	0.132728	0.157151	0.21117	0.21868	0.137779	
200	N	11	11	11	11	10	2
200	MIN	0.214982	0.202504	0.225	0.112522	0.037522	0.035018
200	MAX	0.589982	0.787522	0.632513	0.302504	0.260018	0.077504
200	MEAN	0.378175	0.479541	0.351374	0.172508	0.143256	0.056261
200	STD	0.120328	0.180595	0.133209	0.059981	0.069188	0.030043

Table A-6. Summary statistics for eggs

Treatment Group µg/L	Parameter	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
0	N	21	22	22	21	20	14
0	MIN	0	0.025	0.0075	0.03	0	0.0175
0	MAX	0.165	0.1525	0.12	0.1225	0.0675	0.08
0	MEAN	0.083929	0.069205	0.070909	0.07369	0.025	0.04875
0	STD	0.052331	0.026574	0.028301	0.027552	0.015749	0.019728

12	N	11	11	11	11	11	9
12	MIN	0	0	0	0.03	0.0125	0
12	MAX	0.15	0.0875	0.1125	0.175	0.05	0.0925
12	MEAN	0.058636	0.055909	0.065227	0.0675	0.0275	0.031389
12	STD	0.041418	0.026369	0.030485	0.039655	0.01199	0.027475
25	N	11	11	11	11	11	10
25	MIN	0	0	0	0	0	0.0275
25	MAX	0.105	0.12	0.1175	0.1175	0.035	0.1
25	MEAN	0.0675	0.071364	0.065227	0.055909	0.017727	0.04575
25	STD	0.034605	0.030951	0.034414	0.034229	0.010214	0.021572
50	N	11	11	11	10	9	7
50	MIN	0	0.0125	0.0125	0.0375	0.0125	0.0075
50	MAX	0.1425	0.1475	0.14	0.1125	0.0475	0.0375
50	MEAN	0.080227	0.077045	0.069318	0.068	0.024444	0.017857
50	STD	0.039851	0.037213	0.038342	0.023682	0.01191	0.011586
100	N	11	11	11	10	10	1
100	MIN	0	0	0	0	0	0.0025
100	MAX	0.15	0.105	0.1125	0.0675	0.0425	0.0025
100	MEAN	0.066818	0.043864	0.044091	0.0255	0.01625	0.0025
100	STD	0.053445	0.039376	0.046088	0.026557	0.014589	
200	N	11	11	11	11	10	2
200	MIN	0	0	0	0	0	0.015
200	MAX	0.1225	0.1175	0.1	0.065	0.015	0.0175
200	MEAN	0.071136	0.060682	0.045682	0.029545	0.007	0.01625
200	STD	0.036372	0.037167	0.035019	0.021961	0.006433	0.001768

Table A-7. Summary statistics for larval (open) cells

Treatment Group µg/L	Parameter	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
0	N	21	22	22	21	20	14
0	MIN	0	0.015	0	0.0425	0	0
0	MAX	0.215	0.185	0.1725	0.18	0.0675	0.1425
0	MEAN	0.101905	0.110795	0.100682	0.099048	0.03425	0.083036
0	STD	0.055778	0.037736	0.044207	0.033001	0.017698	0.042508
12	N	11	11	11	11	11	9
12	MIN	0	0.005	0	0.0425	0.0275	0
12	MAX	0.15	0.1525	0.1425	0.2275	0.085	0.15
12	MEAN	0.087045	0.094773	0.09	0.113864	0.053636	0.078333
12	STD	0.05073	0.041132	0.040481	0.055389	0.017189	0.049418
25	N	11	11	11	11	11	10
25	MIN	0	0.075	0	0	0	0.0125
25	MAX	0.1775	0.16	0.1575	0.1125	0.1075	0.1475

25	MEAN	0.11	0.102955	0.084318	0.072727	0.039773	0.07925
25	STD	0.049422	0.025637	0.047593	0.036408	0.027075	0.04026
50	N	11	11	11	10	9	7
50	MIN	0	0.0225	0	0.0575	0.02	0.01
50	MAX	0.2075	0.155	0.1775	0.1475	0.0725	0.125
50	MEAN	0.1	0.096591	0.085455	0.0955	0.041667	0.058929
50	STD	0.054118	0.040316	0.058372	0.029411	0.018028	0.042127
100	N	11	11	11	10	10	1
100	MIN	0	0	0	0	0	0.0025
100	MAX	0.12	0.1325	0.15	0.09	0.045	0.0025
100	MEAN	0.071818	0.056591	0.063409	0.0445	0.01425	0.0025
100	STD	0.041399	0.049816	0.063946	0.03704	0.014484	
200	N	11	11	11	11	10	2
200	MIN	0.0425	0	0	0	0	0.005
200	MAX	0.1375	0.1125	0.15	0.085	0.0225	0.0275
200	MEAN	0.099318	0.039545	0.034318	0.039091	0.007	0.01625
200	STD	0.03406	0.042849	0.043288	0.030522	0.0084	0.01591

Table A-8. Summary statistics for pupal (capped) cells

Treatment Group µg/L	Parameter	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
0	N	21	22	22	21	20	14
0	MIN	0	0	0	0.19	0.04	0
0	MAX	0.345	0.36	0.4075	0.3675	0.1525	0.31
0	MEAN	0.16381	0.228523	0.259318	0.260595	0.080875	0.17625
0	STD	0.123803	0.093985	0.119229	0.053003	0.031843	0.093627
12	N	11	11	11	11	11	9
12	MIN	0.0625	0.195	0.13	0	0.0325	0
12	MAX	0.285	0.3025	0.3575	0.315	0.16	0.2875
12	MEAN	0.224545	0.246818	0.257045	0.197955	0.084773	0.178611
12	STD	0.068628	0.037518	0.062897	0.104228	0.038705	0.085961
25	N	11	11	11	11	11	10
25	MIN	0.18	0.04	0	0	0.0175	0.0325
25	MAX	0.3375	0.3275	0.35	0.3275	0.115	0.2725
25	MEAN	0.233409	0.214091	0.235227	0.208409	0.065	0.1655
25	STD	0.042519	0.086373	0.120931	0.084656	0.034893	0.085965
50	N	11	11	11	10	9	7
50	MIN	0	0	0	0.145	0.05	0.02
50	MAX	0.3325	0.3475	0.325	0.2975	0.115	0.1625
50	MEAN	0.189773	0.170909	0.162955	0.2185	0.077222	0.092143
50	STD	0.108975	0.114511	0.112704	0.051763	0.024253	0.058531
100	N	11	11	11	10	10	1

100	MIN	0	0	0	0	0	0.005
100	MAX	0.355	0.2425	0.3175	0.255	0.0825	0.005
100	MEAN	0.211136	0.128864	0.105	0.1215	0.025	0.005
100	STD	0.097477	0.082016	0.128826	0.110001	0.024438	
200	N	11	11	11	11	10	2
200	MIN	0.1425	0	0	0	0	0.01
200	MAX	0.33	0.3025	0.195	0.165	0.03	0.045
200	MEAN	0.250682	0.065227	0.041136	0.057955	0.0115	0.0275
200	STD	0.053934	0.095796	0.059091	0.062077	0.012315	0.024749

Table A-9. Summary statistics for total individuals

Treatment Group µg/L	Parameter	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
0	N	21	22	22	21	20	14
0	MIN	0.242513	0.210013	0.102504	0.515009	0.212509	0.064996
0	MAX	1.012491	1.142513	1.242487	1.4775	0.664996	1.107509
0	MEAN	0.678095	0.748753	0.882047	0.827622	0.437375	0.672146
0	STD	0.237041	0.254076	0.312739	0.233682	0.147805	0.30578
12	N	11	11	11	11	11	9
12	MIN	0.2125	0.450004	0.617522	0.5175	0.205009	0.180009
12	MAX	1.267504	1.132504	1.160013	0.972487	0.714991	1.067496
12	MEAN	0.744321	0.76682	0.896599	0.754542	0.508867	0.658061
12	STD	0.264013	0.236201	0.212789	0.132926	0.140911	0.277451
25	N	11	11	11	11	11	10
25	MIN	0.477509	0.479991	0.175	0.15	0.149987	0.152504
25	MAX	1.112509	1.087482	1.184996	1.049996	0.735	1.107513
25	MEAN	0.790003	0.74591	0.807504	0.698409	0.451139	0.636998
25	STD	0.197306	0.219977	0.332487	0.246998	0.151117	0.325348
50	N	11	11	11	10	9	7
50	MIN	0.47	0.45	0.219996	0.462491	0.270004	0.092482
50	MAX	1.062518	1.150013	1.080022	0.787496	0.580013	0.575018
50	MEAN	0.672053	0.740907	0.65205	0.646244	0.398616	0.353646
50	STD	0.187031	0.261104	0.317216	0.122113	0.094003	0.204817
100	N	11	11	11	10	10	1
100	MIN	0.347509	0.124991	0.027504	0.064982	0.059996	0.020018
100	MAX	1.152518	0.860018	1.199996	0.9525	0.492496	0.020018
100	MEAN	0.651147	0.541365	0.494091	0.444501	0.199753	0.020018
100	STD	0.235144	0.251096	0.431212	0.370248	0.179209	
200	N	11	11	11	11	10	2
200	MIN	0.519996	0.212504	0.3	0.117487	0.050022	0.065018
200	MAX	1.042522	1.262522	0.952513	0.610004	0.260018	0.167504
200	MEAN	0.799312	0.644996	0.472511	0.299099	0.168756	0.116261

200	STD	0.175532	0.29121	0.196226	0.155814	0.070029	0.072469

Table A-10. Summary statistics for nectar (honey) cells

Treatment Group µg/L	Parameter	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
0	N	21	22	22	21	20	14
0	MIN	0.0375	0.0625	0.0075	0.0975	0.0325	0.055
0	MAX	0.305	0.345	0.25	0.4875	0.5325	0.2925
0	MEAN	0.156667	0.202045	0.125341	0.295476	0.293625	0.168214
0	STD	0.069211	0.07982	0.059833	0.12647	0.156801	0.086937
12	N	11	11	11	11	11	9
12	MIN	0.0625	0.06	0.0125	0.115	0.105	0.0325
12	MAX	0.4275	0.415	0.285	0.4475	0.55	0.3375
12	MEAN	0.160909	0.211591	0.121818	0.259773	0.270227	0.168056
12	STD	0.098503	0.110478	0.09819	0.121431	0.135906	0.080833
25	N	11	11	11	11	11	10
25	MIN	0.0125	0.0125	0	0.1075	0.075	0.065
25	MAX	0.2725	0.3575	0.2675	0.55	0.49	0.29
25	MEAN	0.152045	0.213636	0.137045	0.311591	0.288864	0.139
25	STD	0.09124	0.120781	0.093252	0.138912	0.153913	0.067063
50	N	11	11	11	10	9	7
50	MIN	0.08	0.1	0.02	0.0475	0.06	0
50	MAX	0.2775	0.405	0.195	0.3275	0.445	0.2325
50	MEAN	0.168636	0.213409	0.101136	0.18975	0.193889	0.085
50	STD	0.066533	0.08095	0.05968	0.099467	0.122226	0.072414
100	N	11	11	11	10	10	1
100	MIN	0.055	0.085	0.0175	0.03	0.0175	0.0325
100	MAX	0.2775	0.3225	0.3	0.4675	0.355	0.0325
100	MEAN	0.173864	0.219773	0.125682	0.22725	0.13975	0.0325
100	STD	0.080602	0.084915	0.085089	0.162235	0.112023	
200	N	11	11	11	11	10	2
200	MIN	0.035	0.0975	0.055	0.0175	0.0025	0.08
200	MAX	0.25	0.33	0.3575	0.17	0.16	0.09
200	MEAN	0.135227	0.2225	0.207273	0.092727	0.07375	0.085
200	STD	0.06755	0.076722	0.10878	0.053074	0.048265	0.007071

Table A-11. Summary statistics for pollen cells

Treatment Group µg/L	Parameter	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
0	N	21	22	22	21	20	14
0	MIN	0.0175	0	0.0025	0.02	0.0025	0.0225
0	MAX	0.18	0.1375	0.205	0.1	0.09	0.12
0	MEAN	0.069286	0.066136	0.103182	0.0525	0.043125	0.076607
0	STD	0.043805	0.031156	0.048936	0.022389	0.02484	0.027185
12	N	11	11	11	11	11	9
12	MIN	0.0225	0.005	0.05	0.0225	0.0275	0.03
12	MAX	0.105	0.1025	0.205	0.1475	0.1125	0.13
12	MEAN	0.054091	0.054773	0.104318	0.075455	0.051136	0.0875
12	STD	0.024425	0.035151	0.045772	0.039351	0.024555	0.032089
25	N	11	11	11	11	11	10
25	MIN	0.01	0.0325	0.0275	0.0075	0.0125	0.035
25	MAX	0.115	0.095	0.17	0.1325	0.1025	0.1475
25	MEAN	0.045682	0.055455	0.086591	0.059545	0.047273	0.0755
25	STD	0.026671	0.023044	0.053083	0.039605	0.02425	0.032783
50	N	11	11	11	10	9	7
50	MIN	0.0125	0	0	0.0075	0.0025	0.015
50	MAX	0.16	0.0625	0.11	0.09	0.0825	0.08
50	MEAN	0.067727	0.0275	0.037045	0.04225	0.0325	0.039643
50	STD	0.040488	0.025471	0.035686	0.026574	0.026071	0.026826
100	N	11	11	11	10	10	1
100	MIN	0.0275	0	0	0.0175	0.01	0.015
100	MAX	0.125	0.07	0.0775	0.0725	0.0925	0.015
100	MEAN	0.062727	0.008636	0.013409	0.0435	0.0395	0.015
100	STD	0.024886	0.021429	0.023218	0.018379	0.024743	
200	N	11	11	11	11	10	2
200	MIN	0.025	0	0	0	0	0.0125
200	MAX	0.085	0	0.0125	0.08	0.0875	0.015
200	MEAN	0.046818	0	0.001136	0.020227	0.0165	0.01375
200	STD	0.019464	0	0.003769	0.027916	0.026358	0.001768

Table A-11. Summary statistics for hive weight

Treatment Group µg/L	Parameter	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
0	N	21	22	22	21	21	14
0	MIN	32	49	31	30	31.5	25.5
0	MAX	50	66	56.5	68	66.5	53.5
0	MEAN	40.19524	58.06818	47.43182	52.59524	52.14286	41.71429
0	STD	4.284913	5.005462	5.910635	10.71286	10.99675	7.392081
12	N	11	11	11	11	11	9
12	MIN	33.5	50	37	39	41	27.5
12	MAX	49	71.5	56.5	68	74	49
12	MEAN	42.13636	58.5	45.86364	52.27273	53	40.11111
12	STD	5.822761	6.492303	5.263511	8.866689	9.721111	6.436118
25	N	11	11	11	11	11	10
25	MIN	33.5	48.5	42	37.5	41	34
25	MAX	51	64	54.5	69.5	64.5	48.5
25	MEAN	42.36364	57.31818	47.59091	54.09091	53.18182	42.1
25	STD	5.186959	5.891828	4.597924	9.194613	9.453234	4.629615
50	N	11	11	11	10	10	7
50	MIN	33.5	47.5	36	30	22.5	30
50	MAX	55.5	65	47	55.5	57.5	43
50	MEAN	42.95455	55.31818	41.54545	45.85	44	35
50	STD	6.254816	6.108489	4.143999	8.131045	9.436925	4.481443
100	N	11	11	11	10	10	1
100	MIN	35.5	47	32.5	33	32.5	26.5
100	MAX	50	65	50.5	67	62.5	26.5
100	MEAN	39.90909	53.31818	40	44.05	42.15	26.5
100	STD	4.559705	6.569904	6.160357	11.48296	10.78592	
200	N	11	11	11	11	11	2
200	MIN	35.5	47	38	34.5	33.5	32
200	MAX	52	60	49.5	43.5	42	32
200	MEAN	41.90909	52.63636	42.54545	38.18182	36.5	32
200	STD	5.337688	4.080998	3.173756	3.356134	3.138471	0

Appendix B: Details of PMRA Statistical Analysis

During the review of the study, a separate statistical analysis was conducted with the program R (version 3.1.2)[5]using the raw data submitted by the study author.

Statistical analysis

Analysis Strategy
Hive condition data:
To analyze colony condition data which contains many components over many assessments at different times, a primary analysis was set out to effectively prevent multiplicities from interfering with the interpretation of p_values and confidence intervals. These multiplicities arise from having multiple dose levels, multiple outcomes and multiple time points, and are dealt with as follows:

- The multiplicities from having multiple dose levels was dealt with by using step down testing, the highest dose group's data was compared directly to the control group's data, if statistically significant at a chosen alpha level the next lowest dose group's data was compared to the control group's data and this was continued down to the dose where statistical significance was no longer achieved. A technical reference for this step down testing would be Multiple Comparison Procedures in Dose Response Studies. Tamhane, Ajit C. and Logan, Brent R., in Dose Finding in Drug Development edited by Ting, Naitee. Springer New York 2006. This step down procedure (referred to as the SD2PC procedure in the technical reference) was chosen as it provides good power for detecting the minimum effective dose (lowest does where effect is present) when monotonic dose effects are *expected* while providing stringent control of type one error, *regardless of the true pattern of dose effects*. That is, with minimal assumptions, the procedure strongly controls family wise type one error rate while maintaining good power for effect patterns that are expected.
  The applicant's choice of multiplicity adjustment procedure, which was William's trend test (Williams 1972), presumably chosen to be in accord with OECD. 2003. Draft guidance document for the statistical analysis of ecotoxicity data. They are both step down procedures but ours differs from William's in that it uses only within dose group data based estimates of means rather than maximum likelihood estimates of dose group means using all group's data simultaneously - under monotonicity assumption (i.e. order restricted or isotonic means) additionally assuming homogeneous variances . Although these additional assumptions may not be problematic and are within the OECD guidelines, we simply chose not to rely on them (and by doing so, exceed the OECD guidelines.)
- The multiplicities from having multiple outcomes, was dealt with by choosing to focus on the assessment of total life in the hive – simply the number of viable life forms at any stage in the hive. It is considered that the total number of individuals includes all live individuals in hives and is expected to be a better indicator of the hive status at the colony level than any single stage of bees alone. This outcome would provide good power when background knowledge is lacking on the stage most likely to be affected (i.e. it cannot be well anticipated) and it is not expected that there

[5] R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

will be simultaneous trade-offs effects between the stages. That is, when it is not expected that a toxic effect on one stage would have a beneficial effect for another stage at the same point in time.

- The multiplicities from having multiple time points was dealt with by choosing to focus on the time when the effects were believed most pronounced both in terms of having an impact on total life and having a high powered assessment of that. In this case CCA6 was selected for the following reasons.
    - CCA6 (five weeks after the end of feeding exposure) was selected as it maximises the time period for detecting a potential latent effect from exposure and occurs before the start of hive decline prior to overwintering.
    - CCA7 was not chosen simply due to the natural decline of hive size in the late fall that may mask the effect of treatment.
    - CCA8 was not selected because of the higher hive mortality observed in the controls in comparison to lower dose groups, and because data was available only for surviving hives. The hive mortality in the control was higher than the two lowest test concentrations (12.5 and 25 ug/l) and equal to the 50 ug/l treatment. The uncertainty regarding the cause of dead hives, (i.e. whether mortality resulted from the treatment effects or random background effect or both), is considered to reduce the confidence of using CCA8 data as the primary time point. Additionally, no hive condition data were available on hives that died during the overwintering, meaning only the condition of hives that survived overwintering (informatively selected on ability to survive) were able to be compared. The lack of data on dead hives poses difficulties in the statistical analysis for hive condition at CCA8.

While the total individuals at CCA6 is considered as a primary parameter to control multiplicity for statistical analysis, all parameters including eggs, open brood and capped brood, adults, hive weight, pollen and nectar store, that were observed during the entire study including CCA4, CCA5, CCA6, CCA7 and CCA8 were also considered in the review. Given that the primary analysis has prevented multiplicities from interfering with the interpretation of p_values and confidence intervals, if statistical significance has been achieved (at given dose levels), further analysis with all other outcomes is undertaken "with prejudice" for the assessment of similar effects as being significant. More formally, re-allowance for multiplicities is not required and less stringent alpha levels are allowed. Essentially the price has been paid for searching for the pattern in the primary analysis (measures taken to prevent multiplicities) and it need not be re-paid evaluating the same pattern elsewhere. On the other hand, if statistical significance has not been achieved (at given dose levels), further analysis with all other outcomes is undertaken "with prejudice" for assessment of other effects as likely being just noise. Here though dramatic effects should not be ignored but carefully considered and noted.

With the primary focus on CCA6 to discern treatment effects, the later assessment of recovery from detected effects at CCA7 and CCA8 was subsequently addressed. Here the use of confidence intervals was chosen to provide assurance that important underlying differences at the later period had been reliably ruled out – that is the upper confidence limit did not include worrisome differences. Given the arguable need to make important bias adjustment for confidence intervals in CCA8 but little to no background information to accurately make these bias adjustments, the assessment of recovery was limited to CCA7. There was no formal analysis of whether and when treatment effects detected at CCA6 were present before CCA6. The consideration of recovery at CCA7, while considered, was not formally presented because of concern regarding relying on parameters at CCA7 which are all decreasing as colonies prepare for overwintering.
Hive mortality data:

The analysis of Mortality at CCA8 had been anticipated as the primary or key assessment for this study design and the only multiplicities to be dealt with there were from the multiple dose levels (for which the step down method was used). Unfortunately, the observed hive mortality in the control was higher than the two lowest test concentrations (12.5 and 25 ug/l) and equal to the 50 ug/l treatment which is at odds with underlying biological understanding and thus greatly reduced the confidence of using CCA8 mortality as the primary assessment.

Analysis methods for hive conditions

For all hive conditions total life, eggs, open brood and capped brood, adults, hive weight, pollen and nectar store at CCA4, CCA5, CCA6, CCA7 and CCA8 a conventional analysis of block randomised experiments with a baseline measurements was undertaken. In line with the statistical strategy discussed above, the focus was on total life at CCA6 (with step down adjustment for multiplicities applied) but identical analysis was carried out (less the step down adjustment) on all other hive conditions assessed at the given assessment points (with CCA8 considered biased and problematic). This analysis comprised of linear modeling (or ANOVA) stratified on Apiary (block) and adjusted for baseline measurements at CCA3 with one-side testing for harm using only the control group data and the data from a single dose group at a time, starting with the highest and then through lowest dose groups. It is a series of robust "t.test like" analyses that conservatively implement the step down testing procedure. Under the assumption of no effect in the single dose group being tested (relevant to type one error control), the means and variances and covariate effects should be identical in both the control group and the single dose group being tested. (In an analysis that includes all dose group data together e.g. William's procedure, an impact of a treatment effect on the variance and covariate effects at a higher dose, in addition to an effect on the mean, would invalidate the assumptions needed to control type one error rate in the lower doses.) The results of these analyses are presented in tables of unadjusted p_values (adjusted p_values can be simply read off as the maximum of all p_values in higher doses), effect estimates and upper and lower confidence intervals (currently labelled as Table A-2) as well as plots of the confidence intervals (pdf file Bees8.pdf).
The code snippet to implement these analyses in R was:

```
glm(outcome~Apiary + baseline + exposed, data= x[x$exposed == " control " | x$exposed == dose,])
```

Sensitivity analysis was undertaken on the primary analysis (total life) by taking logs. The resulting p_values were 0.089, 0.043, 0.042, 0.000 and 0.000 for dose of 13, 25, 50, 100 and 200 the largest difference for the original scale being .013.

Analysis methods for hive mortality

The analysis of hive mortality at CCA8, like all other outcomes was also blocked by Apiary, but unlike other outcomes, it was not adjusted for a baseline measurement at CCA3. Additionally, given the sparsity of the outcome when blocked, common methods of analysis for binary data like Cochran–Armitage test for trend, Pearson's chi-square test or logistic regression should not be used as they are well known to be biased. Instead, conditional logistic regression or exact tests are required. The code snippet to implement conditional logistic regression in R was:

```
clogit(Mortality ~ Conc + strata(Apiary), data=filter(mm,Conc =="CON" | Conc== dose)).
```

As with other outcomes, step down testing from highest dose, and then in turn lower doses was used to control for multiplicity.

Transcript/program of analyses carried out

The file RunJune25.2015 contains the transcript of the final run of the R program used to carry out the analysis and generate the tables and plots.

Supporting graphs

The following graphs were produced as part of the analysis.

Bees1.pdf – Plots of individual hive condition assessments over-time group by Apiary.

Bees2.pdf – Plots of control versus exposed condition assessments over-time group by Apiary.

Bees3a.pdf – Plots of mean and Apiary mean control condition assessments over time.

Bees5.pdf – Plots of model estimated differences and confidence intervals (title revised).

Bees6.pdf – Plots of model estimated difference versus observed mean for total life.

Bees7.pdf – Plots of individual exposed hive versus control condition assessments for all parameters.

Bees8.pdf – Plots of effect estimates and confidence intervals for all parameters.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table B-1: Summary of the differences between treatment and controls on the basis of observations and model estimations, and P values

Parameter	Time (CCA) [1]	Test conc. (µg/l)[2]	Observed mean difference from control[3]	Standard error observed mean	n	Model estimate mean difference from control[4]	Standard error of estimated mean	P value for comparison with the control	90% confidence upper limit	90% confidence lower limit	Estimated reduction from control (%)[5]	Estimated reduction from control (number)	Obeserve d means in control	Ttest confidence limit
TotalLife	3	200	-5284	3194	11	0							32147	-1.729
TotalLife	3	100	1344	3438	11	0							32147	-1.729
TotalLife	3	50	-63	4496	11	0							32147	-1.729
TotalLife	3	25	-4600	3028	11	0							32147	-1.729
TotalLife	3	13	-1690	5092	11	0							32147	-1.729
TotalLife	4	200	13608	3399	11	17344.8	3970.078	0	0.678	0.293	0.486	24210	35714	-1.729
TotalLife	4	100	12959	3492	11	12608	3851.589	0.002	0.54	0.167	0.353	19268	35714	-1.729
TotalLife	4	50	3147	3246	11	3667.244	3939.317	0.182	0.293	-0.088	0.103	10479	35714	-1.729
TotalLife	4	25	998	2684	11	3863.666	3887.011	0.166	0.296	-0.08	0.108	10585	35714	-1.729
TotalLife	4	13	108	2566	11	1412.154	3701.529	0.354	0.219	-0.14	0.04	7813	35714	-1.729
TotalLife	5	200	23543	3240	11	24026.8	4838.476	0	0.815	0.394	0.605	32393	39734	-1.729
TotalLife	5	100	18818	4838	11	18258.98	5313.62	0.001	0.691	0.228	0.46	27447	39734	-1.729
TotalLife	5	50	10399	6533	11	10080.39	6256.939	0.062	0.526	-0.019	0.254	20899	39734	-1.729
TotalLife	5	25	3809	6432	11	4333.576	6380.06	0.253	0.387	-0.169	0.109	15366	39734	-1.729
TotalLife	5	13	540	3894	11	385.681	4958.292	0.469	0.225	-0.206	0.01	8959	39734	-1.729
TotalLife	6	200	25992	1870	11	25425.35	2545.607	0	0.774	0.545	0.659	29840	38559	-1.734
TotalLife	6	100	19875	4884	10	18601.92	3977.831	0	0.662	0.303	0.482	25522	38559	-1.74
TotalLife	6	50	6104	2836	10	4674.351	2657.457	0.048	0.241	0.001	0.121	9297	38559	-1.74
TotalLife	6	25	7290	3261	11	6715.559	3326.913	0.029	0.324	0.025	0.174	12485	38559	-1.734
TotalLife	6	13	4072	1865	11	3357.78	2424.369	0.091	0.196	-0.022	0.087	7562	38559	-1.734
TotalLife	7	200	11178	1781	10	11783.5	1864.738	0	0.952	0.54	0.746	15039	15794	-1.746
TotalLife	7	100	9015	2385	10	9612.191	2061.392	0	0.836	0.381	0.609	13211	15794	-1.746
TotalLife	7	50	1212	779	9	1275.1	1411.714	0.19	0.237	-0.076	0.081	3750	15794	-1.753
TotalLife	7	25	18	2073	11	353.425	1937.944	0.429	0.236	-0.191	0.022	3725	15794	-1.74

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)[2]	Observed mean difference from control[3]	Standard error observed mean	n	Model estimate mean difference from control[4]	Standard error of estimated mean	P value for comparison with the control	90% confidence upper limit	90% confidence lower limit	Estimated reduction from control (%)[5]	Estimated reduction from control (number)	Obeserve d means in control	Ttest confidence limit
TotalLife	7	13	-3289	2471	11	-2666.66	1985.142	0.902	0.05	-0.387	-0.169	787	15794	-1.74
TotalLife	8	200	14495	NA	1	15094.09	19208.88	0.238	2.009	-0.927	0.541	56044	27899	-2.132
TotalLife	8	100	42178	NA	1	40478.37	16670.41	0.036	2.725	0.177	1.451	76017	27899	-2.132
TotalLife	8	50	15852	5456	7	13690.55	6342.992	0.028	0.903	0.079	0.491	25187	27899	-1.812
TotalLife	8	25	-2246	6876	8	-3173.88	7643.389	0.657	0.378	-0.606	-0.114	10553	27899	-1.796
TotalLife	8	13	936	2165	8	-724.634	4642.539	0.561	0.273	-0.325	-0.026	7613	27899	-1.796
Eggs	3	200	1134	976	11	0							6028	-1.729
Eggs	3	100	1431	1163	11	0							6028	-1.729
Eggs	3	50	508	1151	11	0							6028	-1.729
Eggs	3	25	1384	1043	11	0							6028	-1.729
Eggs	3	13	1994	1280	11	0							6028	-1.729
Eggs	4	200	586	907	11	691.939	847.57	0.212	0.453	-0.162	0.145	2157	4761	-1.729
Eggs	4	100	1743	628	11	1805.239	706.582	0.01	0.636	0.123	0.379	3027	4761	-1.729
Eggs	4	50	-539	1014	11	-457.571	898.501	0.692	0.23	-0.422	-0.096	1096	4761	-1.729
Eggs	4	25	-149	737	11	-54.052	763.511	0.528	0.266	-0.289	-0.011	1266	4761	-1.729
Eggs	4	13	915	573	11	1087.188	680.722	0.063	0.476	-0.019	0.228	2264	4761	-1.729
Eggs	5	200	1736	1002	11	1571.261	864.911	0.043	0.629	0.016	0.322	3067	4879	-1.729
Eggs	5	100	1845	1193	11	1920.253	1006.424	0.036	0.75	0.037	0.394	3660	4879	-1.729
Eggs	5	50	109	794	11	88.23	736.676	0.453	0.279	-0.243	0.018	1362	4879	-1.729
Eggs	5	25	391	1094	11	405.731	941.939	0.336	0.417	-0.251	0.083	2034	4879	-1.729
Eggs	5	13	391	840	11	578.592	788.155	0.236	0.398	-0.161	0.119	1941	4879	-1.729
Eggs	6	200	2916	744	11	2973.088	707.187	0	0.849	0.353	0.601	4199	4949	-1.734
Eggs	6	100	3449	721	10	3505.81	669.65	0	0.944	0.473	0.708	4671	4949	-1.74
Eggs	6	50	396	497	10	578.756	531.111	0.146	0.304	-0.07	0.117	1503	4949	-1.74
Eggs	6	25	1102	860	11	1302.118	787.695	0.058	0.539	-0.013	0.263	2668	4949	-1.734
Eggs	6	13	305	1045	11	291.007	940.826	0.38	0.388	-0.271	0.059	1922	4949	-1.734

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)[2]	Observed mean difference from control[3]	Standard error observed mean	n	Model estimate mean difference from control[4]	Standard error of estimated mean	P value for comparison with the control	90% confidence upper limit	90% confidence lower limit	Estimated reduction from control (%)[5]	Estimated reduction from control (number)	Obeserve d means in control	Ttest confidence limit
Eggs	7	200	1170	322	10	1259.78	371.642	0.002	1.179	0.378	0.778	1909	1618	-1.746
Eggs	7	100	662	405	10	754.486	410.884	0.042	0.909	0.023	0.466	1472	1618	-1.746
Eggs	7	50	-38	242	9	41.97	335.082	0.451	0.389	-0.337	0.026	629	1618	-1.753
Eggs	7	25	399	376	11	506.208	404.398	0.114	0.747	-0.122	0.313	1210	1618	-1.74
Eggs	7	13	-274	437	11	2.106	429.799	0.498	0.463	-0.461	0.001	750	1618	-1.74
Eggs	8	200	3956	NA	1	4993.274	916.963	0.003	2.132	0.932	1.532	6948	3258	-2.132
Eggs	8	100	3612	NA	1	4496.733	793.551	0.002	1.899	0.861	1.38	6188	3258	-2.132
Eggs	8	50	2027	387	7	2548.007	434.937	0	1.024	0.54	0.782	3336	3258	-1.812
Eggs	8	25	-54	428	8	189.139	542.807	0.367	0.357	-0.241	0.058	1164	3258	-1.796
Eggs	8	13	1032	723	8	1227.919	719.885	0.058	0.774	-0.02	0.377	2521	3258	-1.796
Open brood	3	200	235	1230	11	0							7068	-1.729
Open brood	3	100	2127	990	11	0							7068	-1.729
Open brood	3	50	188	1511	11	0							7068	-1.729
Open brood	3	25	-500	1105	11	0							7068	-1.729
Open brood	3	13	1079	1612	11	0							7068	-1.729
Open brood	4	200	4902	1036	11	4876.767	1033.897	0	0.874	0.405	0.64	6665	7623	-1.729
Open brood	4	100	3729	1093	11	2825.234	1049.719	0.007	0.609	0.133	0.371	4640	7623	-1.729
Open brood	4	50	977	1014	11	918.781	1063.42	0.199	0.362	-0.121	0.121	2758	7623	-1.729
Open brood	4	25	539	663	11	618.534	861.531	0.241	0.277	-0.114	0.081	2108	7623	-1.729
Open brood	4	13	1102	717	11	935.28	920.463	0.161	0.331	-0.086	0.123	2527	7623	-1.729
Open brood	5	200	4566	1188	11	4505.546	1123.44	0	0.931	0.37	0.65	6448	6927	-1.729

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA) [1]	Test conc. (µg/l)[2]	Observed mean difference from control[3]	Standard error observed mean	n	Model estimate mean difference from control[4]	Standard error of estimated mean	P value for comparison with the control	90% confidence upper limit	90% confidence lower limit	Estimated reduction from control (%)[5]	Estimated reduction from control (number)	Obeserve d means in control	Ttest confidence limit
Open brood	5	100	2564	931	11	2137.769	1035.374	0.026	0.567	0.05	0.309	3928	6927	-1.729
Open brood	5	50	1048	1563	11	1060.273	1359.971	0.223	0.493	-0.186	0.153	3412	6927	-1.729
Open brood	5	25	1126	1360	11	1125.489	1237.741	0.187	0.471	-0.146	0.162	3266	6927	-1.729
Open brood	5	13	735	991	11	743.713	1041.675	0.242	0.367	-0.153	0.107	2545	6927	-1.729
Open brood	6	200	4042	491	11	3848.744	692.846	0	0.75	0.393	0.572	5050	6731	-1.734
Open brood	6	100	3844	797	10	3519.266	851.435	0	0.743	0.303	0.523	5000	6731	-1.74
Open brood	6	50	189	652	10	-144.032	726.133	0.577	0.166	-0.209	-0.021	1119	6731	-1.74
Open brood	6	25	1728	989	11	1548.347	905.214	0.052	0.463	-0.003	0.23	3118	6731	-1.734
Open brood	6	13	-1102	1420	11	-1046.67	1215.331	0.8	0.158	-0.469	-0.155	1061	6731	-1.734
Open brood	7	200	1737	385	10	1738.782	405.588	0	1.102	0.464	0.783	2447	2220	-1.746
Open brood	7	100	1462	421	10	1431.022	433.195	0.002	0.985	0.304	0.644	2187	2220	-1.746
Open brood	7	50	-698	370	9	-719.181	396.111	0.955	-0.011	-0.637	-0.324	-25	2220	-1.753
Open brood	7	25	-516	681	11	-565.735	573.919	0.831	0.195	-0.704	-0.255	433	2220	-1.74
Open brood	7	13	-1470	476	11	-1389.37	456.941	0.996	-0.268	-0.984	-0.626	-594	2220	-1.74
Open brood	8	200	3096	NA	1	2958.927	3590.736	0.228	1.942	-0.859	0.541	10614	5466	-2.132
Open brood	8	100	8944	NA	1	8738.39	3111.431	0.024	2.812	0.385	1.599	15371	5466	-2.132
Open brood	8	50	2138	1418	7	2328.118	1194.927	0.04	0.822	0.03	0.426	4494	5466	-1.812
Open brood	8	25	-677	1406	8	-543.73	1466.604	0.641	0.382	-0.581	-0.099	2090	5466	-1.796

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)[2]	Observed mean difference from control[3]	Standard error observed mean	n	Model estimate mean difference from control[4]	Standard error of estimated mean	P value for comparison with the control	90% confidence upper limit	90% confidence lower limit	Estimated reduction from control (%)[5]	Estimated reduction from control (number)	Obeserve d means in control	Ttest confidence limit
Open brood	8	13	43	585	8	45.576	856.197	0.479	0.29	-0.273	0.008	1583	5466	-1.796
Capped brood	3	200	-5684	2040	11	0							11563	-1.729
Capped brood	3	100	-2963	2161	11	0							11563	-1.729
Capped brood	3	50	-1493	3468	11	0							11563	-1.729
Capped brood	3	25	-4495	1327	11	0							11563	-1.729
Capped brood	3	13	-3886	2448	11	0							11563	-1.729
Capped brood	4	200	11235	1859	11	13101.38	2806.568	0	1.142	0.525	0.833	17954	15722	-1.729
Capped brood	4	100	6857	1666	11	8125.651	2490.725	0.002	0.791	0.243	0.517	12432	15722	-1.729
Capped brood	4	50	3964	2316	11	4411.824	2826.411	0.068	0.591	-0.03	0.281	9299	15722	-1.729
Capped brood	4	25	993	1631	11	2778.589	2653.562	0.154	0.469	-0.115	0.177	7367	15722	-1.729
Capped brood	4	13	-1259	1275	11	433.306	2337.261	0.427	0.285	-0.229	0.028	4475	15722	-1.729
Capped brood	5	200	15011	1722	11	14191.99	2739.248	0	1.061	0.53	0.795	18929	17841	-1.729
Capped brood	5	100	10617	2093	11	10099.86	2683.038	0.001	0.826	0.306	0.566	14739	17841	-1.729
Capped brood	5	50	6630	3684	11	6173.291	3431.445	0.044	0.679	0.013	0.346	12107	17841	-1.729
Capped brood	5	25	1657	3361	11	1028.506	3529.735	0.387	0.4	-0.284	0.058	7132	17841	-1.729
Capped brood	5	13	156	2183	11	-665.442	2729.606	0.595	0.227	-0.302	-0.037	4054	17841	-1.729
Capped brood	6	200	14026	1220	11	13595.89	1412.206	0	0.891	0.619	0.755	16045	18013	-1.734
Capped brood	6	100	9477	2550	10	8945.157	2165.524	0	0.706	0.287	0.497	12712	18013	-1.74

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA) [1]	Test conc. (µg/l)[2]	Observed mean difference from control[3]	Standard error observed mean	n	Model estimate mean difference from control[4]	Standard error of estimated mean	P value for comparison with the control	90% confidence upper limit	90% confidence lower limit	Estimated reduction from control (%)[5]	Estimated reduction from control (number)	Obeserve d means in control	Ttest confidence limit
Capped brood	6	50	2761	1515	10	2244.065	1367.303	0.06	0.257	-0.007	0.125	4623	18013	-1.74
Capped brood	6	25	3675	1774	11	3304.405	1729.37	0.036	0.35	0.017	0.183	6303	18013	-1.734
Capped brood	6	13	4394	2297	11	3994.561	2028.775	0.032	0.417	0.026	0.222	7513	18013	-1.734
Capped brood	7	200	4730	610	10	5046.549	808.018	0	1.197	0.674	0.935	6457	5395	-1.746
Capped brood	7	100	3750	848	10	4078.307	875.563	0	1.039	0.473	0.756	5607	5395	-1.746
Capped brood	7	50	506	353	9	523.439	697.699	0.232	0.324	-0.13	0.097	1747	5395	-1.753
Capped brood	7	25	923	755	11	974.261	846.077	0.133	0.453	-0.092	0.181	2446	5395	-1.74
Capped brood	7	13	-438	1038	11	-454.128	917.895	0.686	0.212	-0.38	-0.084	1143	5395	-1.74
Capped brood	8	200	4816	NA	1	4816	12138.26	0.356	2.674	-1.835	0.42	30693	11476	-2.132
Capped brood	8	100	17716	NA	1	17320.16	10822.64	0.092	3.52	-0.501	1.509	40392	11476	-2.132
Capped brood	8	50	6917	2328	7	8097.621	4134.314	0.039	1.359	0.053	0.706	15591	11476	-1.812
Capped brood	8	25	-785	2926	8	-1239.48	3942.057	0.62	0.509	-0.725	-0.108	5840	11476	-1.796
Capped brood	8	13	172	1273	8	-148.408	2879.268	0.52	0.438	-0.464	-0.013	5022	11476	-1.796
Adults	3	200	-968	577	11	0							7488	-1.729
Adults	3	100	749	896	11	0							7488	-1.729
Adults	3	50	734	638	11	0							7488	-1.729
Adults	3	25	-988	904	11	0							7488	-1.729
Adults	3	13	-877	926	11	0							7488	-1.729
Adults	4	200	-3115	980	11	-2220.6	1189.671	0.961	-0.021	-0.562	-0.292	-164	7608	-1.729
Adults	4	100	630	1345	11	391.889	1414.562	0.392	0.373	-0.27	0.052	2838	7608	-1.729

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA) [1]	Test conc. (µg/l)[2]	Observed mean difference from control[3]	Standard error observed mean	n	Model estimate mean difference from control[4]	Standard error of estimated mean	P value for comparison with the control	90% confidence upper limit	90% confidence lower limit	Estimated reduction from control (%)[5]	Estimated reduction from control (number)	Obeserve d means in control	Ttest confidence limit
Adults	4	50	-1255	1159	11	-1771.83	1144.327	0.931	0.027	-0.493	-0.233	207	7608	-1.729
Adults	4	25	-386	741	11	205.329	1175.032	0.432	0.294	-0.24	0.027	2237	7608	-1.729
Adults	4	13	-650	1085	11	168.16	1163.063	0.443	0.286	-0.242	0.022	2179	7608	-1.729
Adults	5	200	2231	598	11	2466.328	1208.532	0.028	0.452	0.037	0.244	4556	10087	-1.729
Adults	5	100	3791	1342	11	3425.479	1451.632	0.015	0.588	0.091	0.34	5936	10087	-1.729
Adults	5	50	2612	1430	11	2191.531	1483.044	0.078	0.471	-0.037	0.217	4756	10087	-1.729
Adults	5	25	635	1120	11	874.801	1378.486	0.267	0.323	-0.15	0.087	3258	10087	-1.729
Adults	5	13	-742	1148	11	-544.515	1369.016	0.652	0.181	-0.289	-0.054	1823	10087	-1.729
Adults	6	200	5008	728	11	4677.835	932.194	0	0.71	0.345	0.528	6294	8865	-1.734
Adults	6	100	3105	1397	10	2544.891	1196.879	0.024	0.522	0.052	0.287	4627	8865	-1.74
Adults	6	50	2759	1078	10	1754.005	1053.102	0.057	0.404	-0.009	0.198	3586	8865	-1.74
Adults	6	25	785	642	11	686.452	950.173	0.24	0.263	-0.108	0.077	2334	8865	-1.734
Adults	6	13	475	953	11	199.536	910.841	0.415	0.201	-0.156	0.023	1779	8865	-1.734
Adults	7	200	3541	1095	10	3895.534	993.559	0.001	0.858	0.329	0.594	5630	6561	-1.746
Adults	7	100	3142	965	10	3347.475	917.098	0.001	0.754	0.266	0.51	4949	6561	-1.746
Adults	7	50	1441	650	9	1222.715	799.636	0.074	0.4	-0.027	0.186	2625	6561	-1.753
Adults	7	25	-788	1243	11	-272.138	1019.519	0.604	0.229	-0.312	-0.041	1501	6561	-1.74
Adults	7	13	-1108	959	11	-826.715	874.128	0.821	0.106	-0.358	-0.126	694	6561	-1.74
Adults	8	200	2627	NA	1	564.155	2791.126	0.425	0.846	-0.7	0.073	6514	7699	-2.132
Adults	8	100	11906	NA	1	13280.14	2402.712	0.003	2.39	1.06	1.725	18402	7699	-2.132
Adults	8	50	4770	1434	7	6008.543	1473.09	0.001	1.127	0.434	0.78	8678	7699	-1.812
Adults	8	25	-730	2337	8	-329.695	2346.527	0.555	0.505	-0.59	-0.043	3884	7699	-1.796
Adults	8	13	-311	695	8	-845.547	1275.142	0.74	0.188	-0.407	-0.11	1444	7699	-1.796
Honey	3	200	1407	1690	11	0							10711	-1.729
Honey	3	100	-1251	1594	11	0							10711	-1.729
Honey	3	50	-891	1300	11	0							10711	-1.729

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA) [1]	Test conc. (µg/l)[2]	Observed mean difference from control[3]	Standard error observed mean	n	Model estimate mean difference from control[4]	Standard error of estimated mean	P value for comparison with the control	90% confidence upper limit	90% confidence lower limit	Estimated reduction from control (%)[5]	Estimated reduction from control (number)	Obeserve d means in control	Ttest confidence limit
Honey	3	25	250	1801	11	0							10711	-1.729
Honey	3	13	-360	2276	11	0							10711	-1.729
Honey	4	200	-1407	2205	11	-2934.5	1555.065	0.963	-0.018	-0.405	-0.211	-246	13901	-1.729
Honey	4	100	-1220	1573	11	-1111.34	1055.672	0.847	0.051	-0.211	-0.08	714	13901	-1.729
Honey	4	50	-782	2012	11	-864.527	1431.841	0.723	0.116	-0.24	-0.062	1611	13901	-1.729
Honey	4	25	-797	1894	11	-1438.89	1134.467	0.89	0.038	-0.245	-0.104	523	13901	-1.729
Honey	4	13	-657	1710	11	-1036.17	1160.622	0.808	0.07	-0.219	-0.075	971	13901	-1.729
Honey	5	200	-5637	2476	11	-7251.97	1690.564	1	-0.502	-1.18	-0.841	-4329	8623	-1.729
Honey	5	100	-23	1814	11	609.048	1392.03	0.333	0.35	-0.208	0.071	3016	8623	-1.729
Honey	5	50	1665	1294	11	1862.689	1342.59	0.091	0.485	-0.053	0.216	4184	8623	-1.729
Honey	5	25	-805	2119	11	-1323.61	1297.1	0.84	0.107	-0.414	-0.153	919	8623	-1.729
Honey	5	13	242	2130	11	112.248	1320.202	0.467	0.278	-0.252	0.013	2395	8623	-1.729
Honey	6	200	14526	2808	11	14734.61	2475.396	0	0.91	0.499	0.705	19027	20906	-1.734
Honey	6	100	4059	3930	10	4547.091	3224.44	0.088	0.486	-0.051	0.218	10156	20906	-1.74
Honey	6	50	7327	3279	10	7524.125	2905.267	0.01	0.602	0.118	0.36	12578	20906	-1.74
Honey	6	25	-532	2597	11	-507.008	2427.394	0.582	0.177	-0.226	-0.024	3702	20906	-1.734
Honey	6	13	3033	2428	11	3297.32	2397.491	0.093	0.357	-0.041	0.158	7455	20906	-1.734
Honey	7	200	16194	3296	10	16768.37	3080.791	0	1.057	0.543	0.8	22147	20961	-1.746
Honey	7	100	10243	3273	10	11091.16	3101.539	0.001	0.787	0.271	0.529	16506	20961	-1.746
Honey	7	50	8485	4632	9	8636.163	3907.923	0.022	0.739	0.085	0.412	15487	20961	-1.753
Honey	7	25	1087	3820	11	2226.627	3127.457	0.243	0.366	-0.153	0.106	7667	20961	-1.74
Honey	7	13	2369	3644	11	2614.224	3427.576	0.228	0.409	-0.16	0.125	8577	20961	-1.74
Honey	8	200	516	NA	1	559.044	7145.141	0.471	1.447	-1.345	0.051	15791	10912	-2.132
Honey	8	100	17114	NA	1	17084.95	6067.538	0.024	2.751	0.38	1.566	30020	10912	-2.132
Honey	8	50	6217	2261	7	6589.059	2225.801	0.007	0.973	0.234	0.604	10623	10912	-1.812
Honey	8	25	1333	3139	8	1450.279	2523.066	0.288	0.548	-0.282	0.133	5981	10912	-1.796

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)[2]	Observed mean difference from control[3]	Standard error observed mean	n	Model estimate mean difference from control[4]	Standard error of estimated mean	P value for comparison with the control	90% confidence upper limit	90% confidence lower limit	Estimated reduction from control (%)[5]	Estimated reduction from control (number)	Obeserve d means in control	Ttest confidence limit
Honey	8	13	-1161	1972	8	-1185.77	2059.405	0.712	0.23	-0.448	-0.109	2513	10912	-1.796
Pollen	3	200	1392	645	11	0							4613	-1.729
Pollen	3	100	297	717	11	0							4613	-1.729
Pollen	3	50	-47	1057	11	0							4613	-1.729
Pollen	3	25	1470	764	11	0							4613	-1.729
Pollen	3	13	891	833	11	0							4613	-1.729
Pollen	4	200	4550	478	11	4297.751	690.417	0	1.207	0.682	0.945	5492	4550	-1.729
Pollen	4	100	3956	651	11	3806.351	713.408	0	1.108	0.565	0.837	5040	4550	-1.729
Pollen	4	50	2658	385	11	2551.377	594.965	0	0.787	0.335	0.561	3580	4550	-1.729
Pollen	4	25	735	615	11	236.8	695.034	0.369	0.316	-0.212	0.052	1439	4550	-1.729
Pollen	4	13	782	541	11	536.343	685.609	0.222	0.378	-0.143	0.118	1722	4550	-1.729
Pollen	5	200	7021	642	11	6419.711	1061.603	0	1.163	0.646	0.904	8255	7099	-1.729
Pollen	5	100	6176	899	11	5935.685	1078.422	0	1.099	0.573	0.836	7800	7099	-1.729
Pollen	5	50	4550	704	11	4438.326	1047.955	0	0.88	0.37	0.625	6250	7099	-1.729
Pollen	5	25	1141	1327	11	145.606	1282.746	0.455	0.333	-0.292	0.021	2364	7099	-1.729
Pollen	5	13	-78	1005	11	-485.925	1177.574	0.658	0.218	-0.355	-0.068	1550	7099	-1.729
Pollen	6	200	2119	493	11	1921.621	506.636	0.001	0.798	0.297	0.547	2800	3510	-1.734
Pollen	6	100	731	610	10	537.423	519.822	0.158	0.411	-0.105	0.153	1442	3510	-1.74
Pollen	6	50	628	595	10	544.13	502.694	0.147	0.404	-0.094	0.155	1419	3510	-1.74
Pollen	6	25	-586	699	11	-885.905	606.289	0.919	0.047	-0.552	-0.252	165	3510	-1.734
Pollen	6	13	-1681	584	11	-1872.63	550.502	0.998	-0.262	-0.805	-0.533	-918	3510	-1.734
Pollen	7	200	1531	748	10	1400.095	688.615	0.029	0.94	0.071	0.506	2602	2768	-1.746
Pollen	7	100	292	660	10	357.835	605.124	0.281	0.511	-0.252	0.129	1414	2768	-1.746
Pollen	7	50	860	506	9	807.288	538.712	0.077	0.633	-0.05	0.292	1752	2768	-1.753
Pollen	7	25	-485	568	11	-573.884	549.941	0.844	0.138	-0.553	-0.207	383	2768	-1.74
Pollen	7	13	-751	776	11	-723.548	633.978	0.865	0.137	-0.66	-0.261	379	2768	-1.74

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)[2]	Observed mean difference from control[3]	Standard error observed mean	n	Model estimate mean difference from control[4]	Standard error of estimated mean	P value for comparison with the control	90% confidence upper limit	90% confidence lower limit	Estimated reduction from control (%)[5]	Estimated reduction from control (number)	Obeserve d means in control	Ttest confidence limit
Pollen	8	200	688	NA	1	611.922	2401.029	0.406	1.153	-0.907	0.123	5731	4969	-2.132
Pollen	8	100	4902	NA	1	4997.097	2082.223	0.037	1.899	0.112	1.006	9436	4969	-2.132
Pollen	8	50	2949	672	7	3170.529	780.622	0.001	0.923	0.353	0.638	4585	4969	-1.812
Pollen	8	25	-172	1125	8	34.473	1041.741	0.487	0.383	-0.37	0.007	1905	4969	-1.796
Pollen	8	13	-580	787	8	-605.432	809.164	0.765	0.171	-0.414	-0.122	848	4969	-1.796
Weight	3	200	-2	2	11	0							40	-1.729
Weight	3	100	0	2	11	0							40	-1.729
Weight	3	50	-3	2	11	0							40	-1.729
Weight	3	25	-2	2	11	0							40	-1.729
Weight	3	13	-2	2	11	0							40	-1.729
Weight	4	200	5	1	11	5.863	1.472	0	0.145	0.057	0.101	8	58	-1.729
Weight	4	100	5	2	11	4.147	1.436	0.005	0.114	0.029	0.071	7	58	-1.729
Weight	4	50	3	2	11	2.382	1.532	0.068	0.087	-0.005	0.041	5	58	-1.729
Weight	4	25	1	2	11	1.569	1.495	0.154	0.072	-0.017	0.027	4	58	-1.729
Weight	4	13	0	2	11	-0.271	1.798	0.559	0.049	-0.058	-0.005	3	58	-1.729
Weight	5	200	5	2	11	4.929	1.972	0.011	0.176	0.032	0.104	8	47	-1.729
Weight	5	100	7	2	11	7.088	2.034	0.001	0.224	0.075	0.149	11	47	-1.729
Weight	5	50	6	2	11	5.241	2.189	0.014	0.19	0.031	0.11	9	47	-1.729
Weight	5	25	0	2	11	-0.188	2.015	0.537	0.069	-0.077	-0.004	3	47	-1.729
Weight	5	13	2	2	11	1.146	2.222	0.306	0.105	-0.057	0.024	5	47	-1.729
Weight	6	200	15	3	11	16.153	3.421	0	0.415	0.192	0.304	22	53	-1.734
Weight	6	100	8	4	10	7.991	3.955	0.03	0.28	0.021	0.15	15	53	-1.74
Weight	6	50	8	3	10	6.564	3.966	0.058	0.253	-0.006	0.123	13	53	-1.74
Weight	6	25	-1	3	11	-0.802	3.677	0.585	0.105	-0.135	-0.015	6	53	-1.734
Weight	6	13	1	3	11	0.088	3.5	0.49	0.116	-0.112	0.002	6	53	-1.734
Weight	7	200	16	3	11	17.512	3.434	0	0.446	0.22	0.333	23	53	-1.734

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA) [1]	Test conc. (µg/l)[2]	Observed mean difference from control[3]	Standard error observed mean	n	Model estimate mean difference from control[4]	Standard error of estimated mean	P value for comparison with the control	90% confidence upper limit	90% confidence lower limit	Estimated reduction from control (%)[5]	Estimated reduction from control (number)	Obeserve d means in control	Ttest confidence limit
Weight	7	100	9	4	10	9.946	3.841	0.01	0.316	0.062	0.189	17	53	-1.74
Weight	7	50	9	4	10	7.903	4.104	0.036	0.286	0.015	0.15	15	53	-1.74
Weight	7	25	-1	4	11	0.306	3.853	0.469	0.133	-0.121	0.006	7	53	-1.734
Weight	7	13	0	3	11	-0.825	3.756	0.586	0.108	-0.139	-0.016	6	53	-1.734
Weight	8	200	-6	NA	1	-10.331	5.62	0.93	0.041	-0.551	-0.255	2	41	-2.132
Weight	8	100	26	NA	1	27.447	3.867	0.001	0.881	0.474	0.677	36	41	-2.132
Weight	8	50	8	2	7	8.475	2.777	0.006	0.333	0.085	0.209	14	41	-1.812
Weight	8	25	-1	4	8	0.503	3.511	0.444	0.168	-0.143	0.012	7	41	-1.796
Weight	8	13	-1	1	8	-0.454	1.484	0.617	0.055	-0.077	-0.011	2	41	-1.796

Notes:

1, Observation dates for hive weight measurement were slight different from the dates when colony assessments were conducted.

2, The test concentration labelled as "13" in the table was originated from the raw data submitted by the study author. The actual concentration is expected to be 12.5 µg/l .

3, Mean of observations in controls minus the observation in the treatment.

4, Difference between the mean of observation in controls and estimated number in treatment after adjustment for covariance for CCA3 to be a 0 baseline.

5, The percentage of the estimated difference between the treatment and control divided by the number in the control.[Value in column must be multiplied by 100 to be a %]

Results

The following tables and graphs present results for individual measurement endpoints (total individuals, eggs, larvae, pupae, pollen stores, nectar stores, hive weight). The percent reductions are the means of the differences between each treatment and control at the same apiary, based on observations and expected values estimated by the statistical model that adjusted baseline measurement for CCA3, using raw count data.

Table B-2. Estimated percent reduction from control for total number of individuals

Test concentration (µg/L)	Estimated reduction from control (%) (P value)				
	CCA4	CCA5	CCA6	CCA7	CCA8
12.5	4 (0.354)	1 (0.469)	8.7 (0.091)*	-16.9 (0.902)	-2.6 (0.561)
25	10.8 (0.166)	10.9 (0.253)	17.4 (0.029)**	2.2 (0.429)	-11.4 (0.657)
50	10.3 (0.182)	25.4 (0.062)*	12.1 (0.048)**	8.1 (0.19)	49.1 (0.028)**
100	35.3 (0.002)**	46 (0.001)**	48.2 (<0.001)**	60.9 (<0.001)**	145.1 (0.036)**
200	48.6 (<0.001)**	60.5 (<0.001)**	65.9 (<0.001)**	74.6 (<0.001)**	54.1 (0.238)

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<P<0.1
**P<0.05
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level. At CCA8, the step-down approach was not applied to the 200 or 100 treatment levels where very few hives survived.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure B-1: Estimates and 90% CIs for Total Life with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

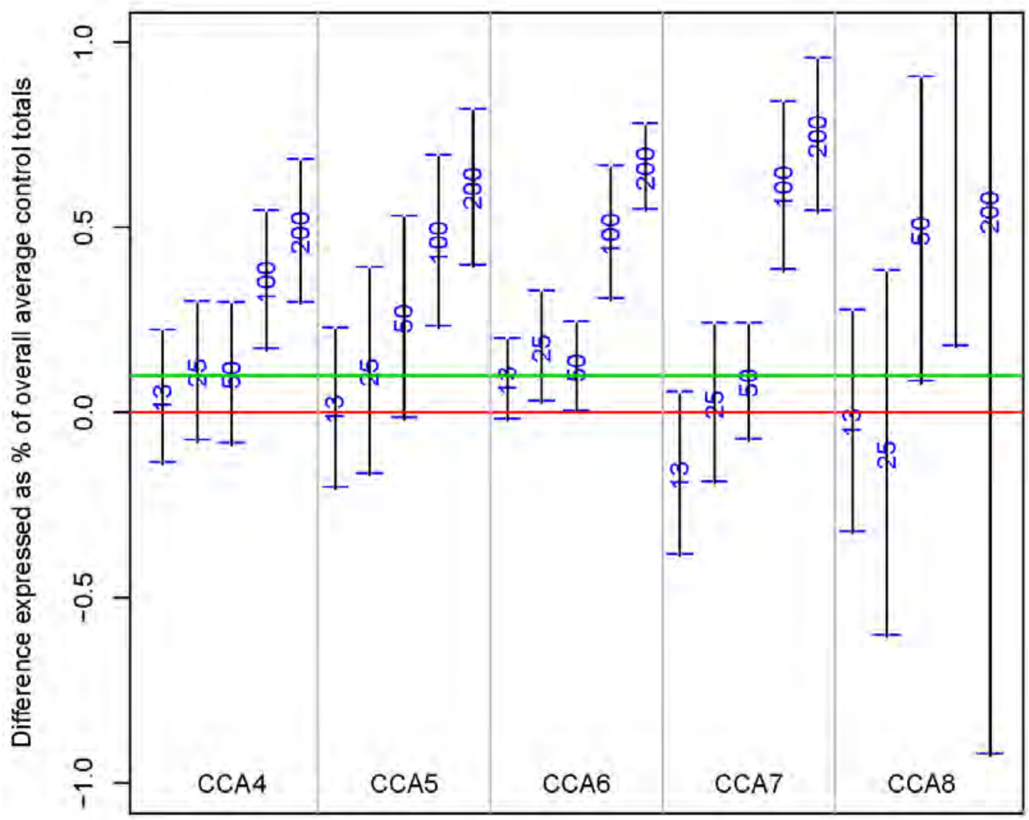


Figure B-2: Mean of the differences of the <u>total individuals in hives</u> between treatments and control at the same apiary.

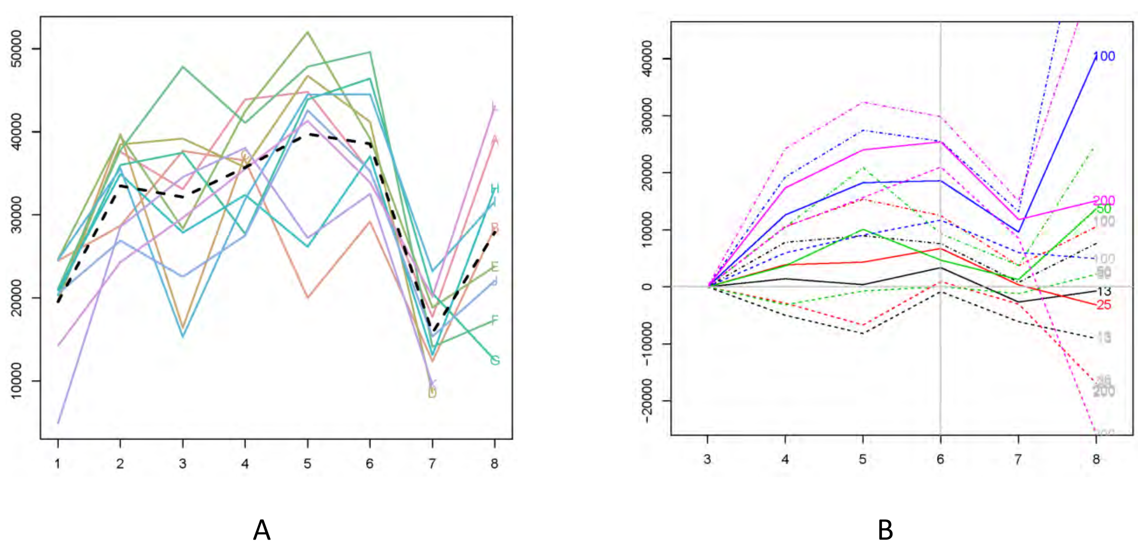


Figure A: Total number of individuals in control hives at each apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the total individuals in control hives.
Apiaries are coded with various colors.
Black dash line represents the mean of all apiaries.

Figure B: Mean of the differences of the total individuals in hives between the treatments and control at the same apiary (n=11) with 90% upper and lower confidence interval.
X-axis represents the number of CCA; Y-axis represents the mean of the differences of the total individuals in hives between the control and treatment at the same apiary (control minus treatment).
The line of "0" indicates no effect from the control within the same Apiary and it was adjusted for baseline covariate at CCA3 for each CCAs.
Labels at the end of each line are the treatment concentrations (µg/L).
A positive Y value indicates there are more live individuals in hives in the control than in the treatment, and a negative value indicates reversely.
A dash line indicates the 90% confidence interval estimated by a GLM model for a solid line with the same color.

Table B-3: Estimated percent reduction from control for number of adults

Test concentration (µg/L)	Estimated reduction from control (%) (P value)				
	CCA4	**CCA5**	**CCA6**	**CCA7**	**CCA8**
12.5	2.2 (0.443)	-5.4 (0.652)	2.3 (0.415)	-12.6 (0.821)	-11 (0.74)
25	2.7 (0.432)	8.7 (0.267)	7.7 (0.24)	-4.1 (0.604)	-4.3 (0.555)
50	-23.3 (0.931)	21.7 (0.078)*	19.8 (0.057)*	18.6 (0.074)*	78** (0.001)
100	5.2 (0.392)	34 (0.015)**	28.7 (0.024)**	51 (0.001)**	172.5 (0.003)**
200	-29.2 (0.961)	24.4** (0.028)	52.8** (<0.001)	59.4** (0.001)	7.3 (0.425)

Note: Negative value indicates increased number of adults in comparison to control.
*0.05<P<0.1
**P<0.05
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level. At CCA8, the step-down approach was not applied to the 200 or 100 treatment levels where very few hives survived.

Figure B-3: Estimates and 90% CIs for adult bees with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

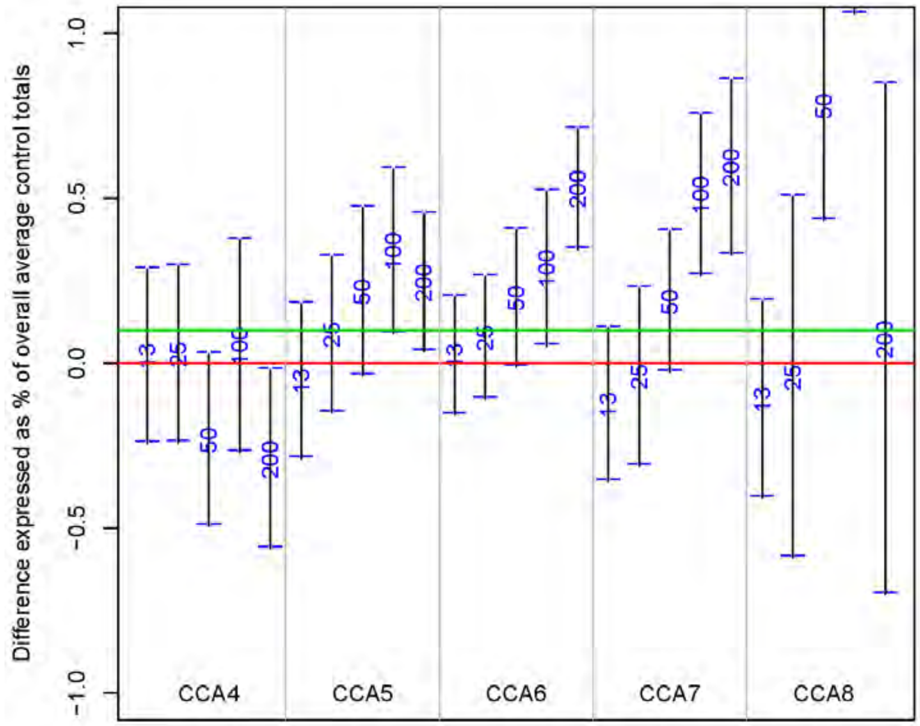
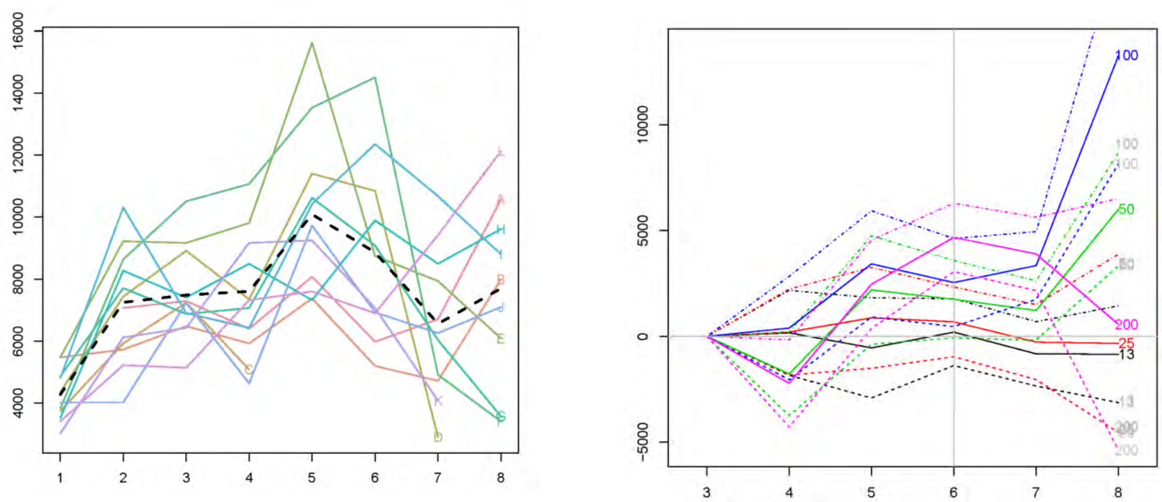


Figure B-4: Mean of the differences of adult bees between treatments and control at the same apiary.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B

Figure A: Total member of adults in control hives at each apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the number of adults in control hives.
Apiaries are coded with various colors.
Black dash line represents the mean of all apiaries.

Figure B: Mean of the differences of the number of adults in hives between the treatments and control at the same apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the mean of the differences of the number of adults in hives between the control and treatment at the same apiary (control minus treatment).
The line of "0" indicates no effect from the control within the same Apiary and it was adjusted for baseline covariate at CCA3 for each CCAs.
Labels at the end of each line are the treatment concentrations (µg/L).
A positive Y value indicates there are more adult bee in hives in the control than in the treatment, and a negative value indicates reversely.
A dash line indicates the 90% confidence interval estimated by a GLM model for a solid line with the same color.

Table B-3: Estimated percent reduction from control for number of eggs

Test concentration (µg/L)	Estimated reduction from control (%) (P value)				
	CCA4	**CCA5**	**CCA6**	**CCA7**	**CCA8**
12.5	22.8 (0.063*/0.69)	11.9 (0.236)	5.9 (0.38)	0.1 (0.498)	37.7 (0.058*/0.36)
25	-1.1 (0.528)	8.3 (0.336)	26.3 (0.058*/0.14)	31.3 (0.114)	5.8 (0.367)
50	-9.6 (0.692)	1.8 (0.453)	11.7 (0.146)	2.6 (0.451)	78.2 (<0.001)**
100	37.9 (0.01**/0.21)	39.4 (0.036)**	70.8 (<0.001)**	46.6 (0.042)**	138 (0.002)**
200	14.5 (0.212)	32.2 (0.043)**	60.1 (<0.001)**	77.8 (0.002)**	153.2 (0.003)**

Note: Negative value indicates increased number of eggs in comparison to control.
*0.05<P<0.1
**P<0.05
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level. At CCA8, the step-down approach was not applied to the 200 or 100 treatment levels where very few hives survived.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure B-5: Estimates and 90% CIs for eggs with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

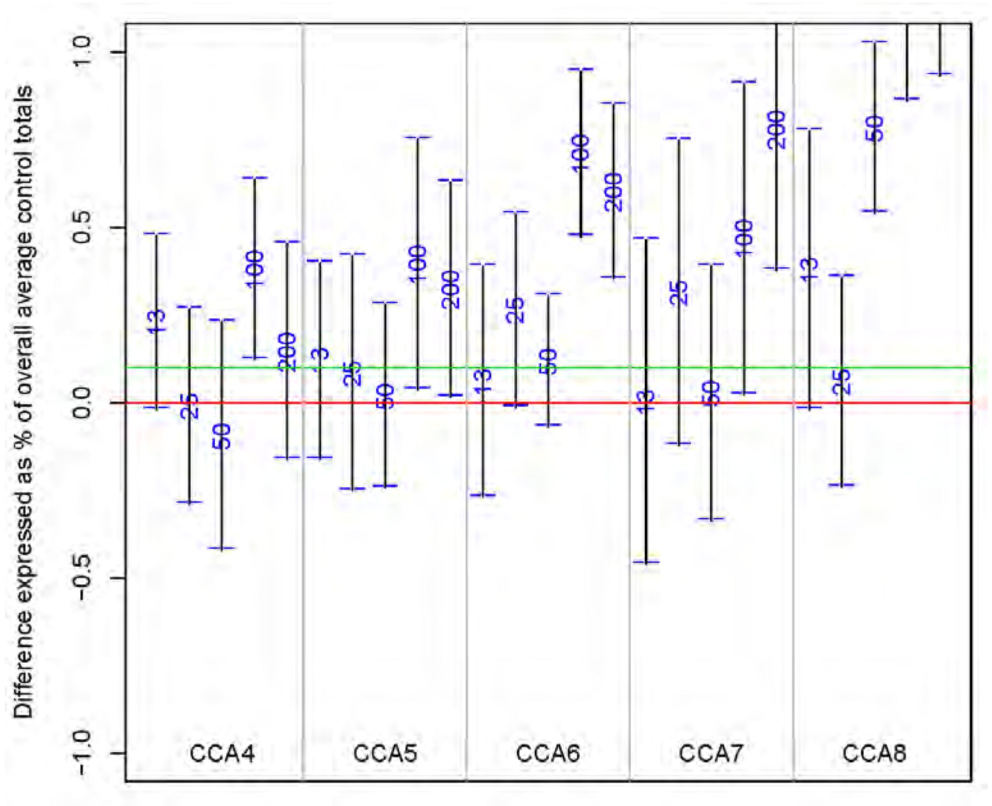
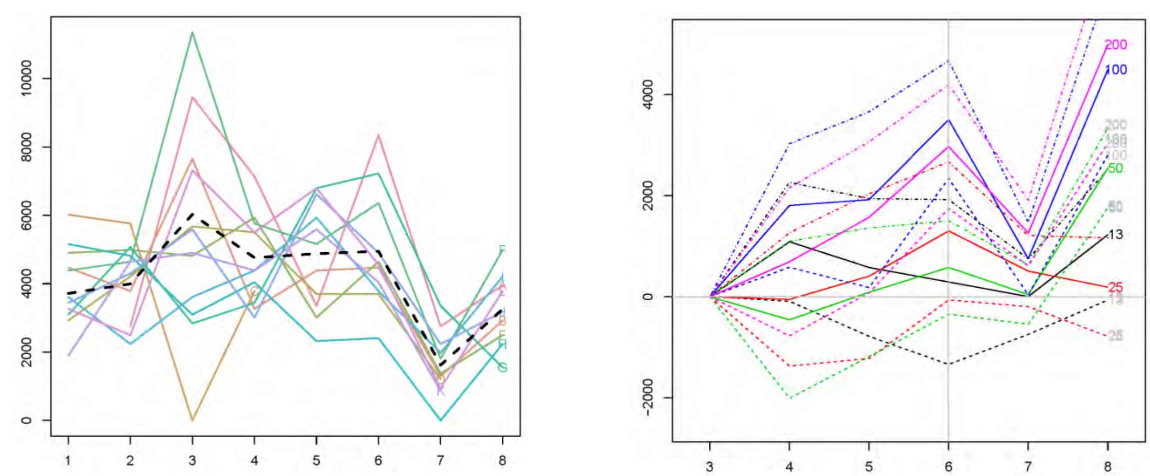


Figure B-6: Mean of the differences of <u>number of eggs</u> in hives between treatments and control at the same apiary.



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B

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure A: Total number of eggs in control hives at each apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the number of eggs in control hives.
Apiaries are coded with various colors.
Black dash line represents the mean of all apiaries.

Figure B: : Mean of the differences of the number of eggs in hives between the treatments and control at the same apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the mean of the differences of the number of eggs in hives between the control and treatment at the same apiary (control minus treatment).
The line of "0" indicates no effect from the control within the same Apiary and it was adjusted for baseline covariate at CCA3 for each CCAs.
Labels at the end of each line are the treatment concentrations (µg/L).
A positive Y value indicates there are more eggs in hives in the control than in the treatment, and a negative value indicates reversely.
A dash line indicates the 90% confidence interval estimated by a GLM model for a solid line with the same color.

Table B-4: Estimated percent reduction from control for number of larvae

Test concentration (µg/L)	Estimated reduction from control (%) (P value)				
	CCA4	**CCA5**	**CCA6**	**CCA7**	**CCA8**
12.5	12.3 (0.161)	10.7 (0.242)	-15.5 (0.8)	-62.6 (0.996)	0.8 (0.479)
25	8.1 (0.241)	16.2 (0.187)	23 (0.052*/0.57)	-25.5 (0.831)	-9.9 (0.641)
50	12.1 (0.199)	15.3 (0.223)	-2.1 (0.577)	-32.4 (0.955)	42.6 (0.04)**
100	37.1 (0.007)**	30.9 (0.026)**	52.3 (<0.001)**	64.4 (0.002)**	159.9 (0.024)**
200	64 (<0.001)**	65 (<0.001)**	57.2 (<0.001)**	78.3 (<0.001)**	54.1 (0.228)

Note: Negative value indicates increased number of larvae in comparison to control.
*0.05<P<0.1
**P<0.05
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level. At CCA8, the step-down approach was not applied to the 200 or 100 treatment levels where very few hives survived.

Figure B-7 Estimates and 90% CIs for larvae with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

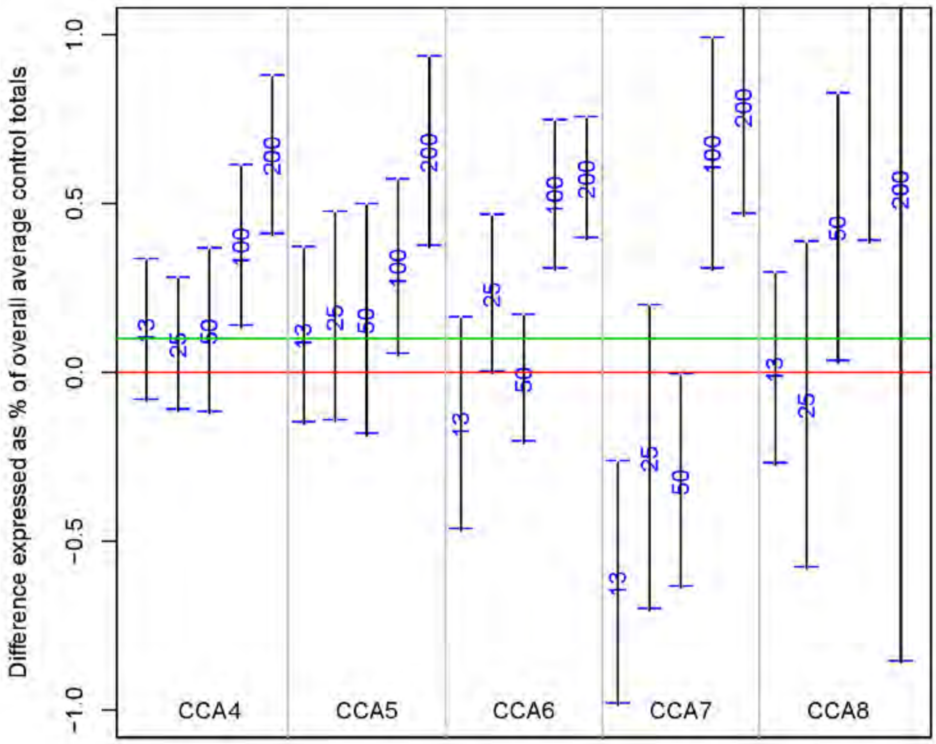
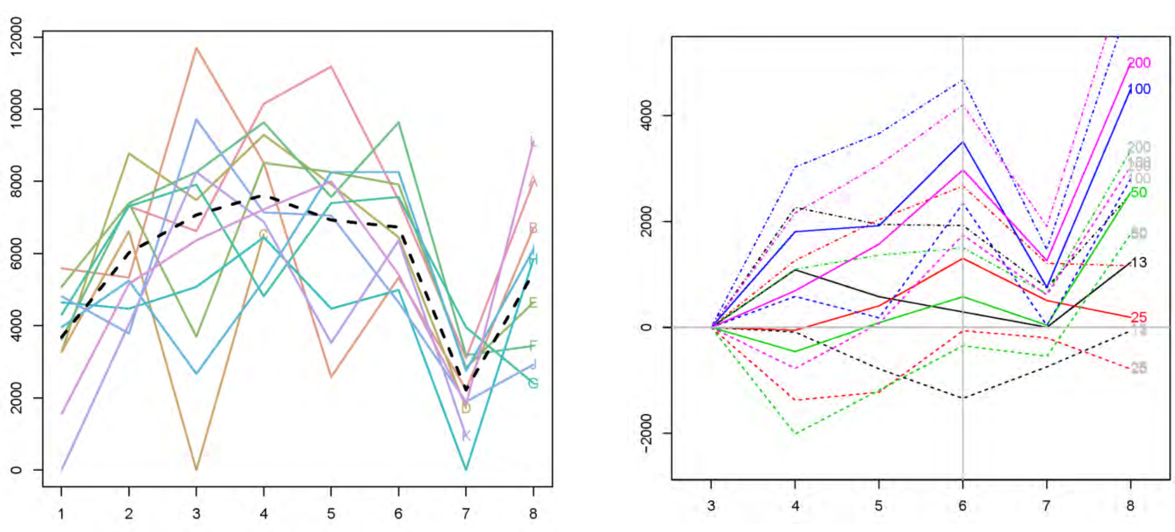


Figure B-8: Mean of the differences of <u>number of larvae</u> in hives between treatments and control at the same apiary.



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Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure A: Total number of larvae in control hives at each apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the number of larvae in control hives.
Apiaries are coded with various colors.
Black dash line represents the mean of all apiaries.

Figure B: : Mean of the differences of the number of larvae in hives between the treatments and control at the same apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the mean of the differences of the number of larvae in hives between the control and treatment at the same apiary (control minus treatment).
The line of "0" indicates no effect from the control within the same Apiary and it was adjusted for baseline covariate at CCA3 for each CCAs.
Labels at the end of each line are the treatment concentrations (µg/L).
A positive Y value indicates there are more larvae in hives in the control than in the treatment, and a negative value indicates reversely.
A dash line indicates the 90% confidence interval estimated by a GLM model for a solid line with the same color.

Table B-5: Estimated percent reduction from control for number of pupae

Test concentration (µg/L)	Estimated reduction from control (%) (P value)				
	CCA4	CCA5	CCA6	CCA7	CCA8
12.5	2.8 (0.427)	-3.7 (0.595)	22.2 (0.032**/ 0.06*)	-8.4 (0.686)	-1.3 (0.52)
25	17.7 (0.154)	5.8 (0.387)	18.3 (0.036**/ 0.06*)	18.1 (0.133)	-10.8 (0.62)
50	28.1 (0.068)*	34.6 (0.044)**	12.5 (0.06)*	9.7 (0.232)	70.6 (0.039)**
100	51.7 (0.002)**	56.6 (0.001)**	49.7 (<0.001)**	75.6 (<0.001)**	150.9 (0.092)*
200	83.3 (<0.001)**	79.5 (<0.001)**	75.5 (<0.001)**	93.5 (<0.001)**	42 (0.356)

Note: Negative value indicates increased number of pupae in comparison to control.
*0.05<P<0.1
**P<0.05
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level. At CCA8, the step-down approach was not applied to the 200 or 100 treatment levels where very few hives survived.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure B-9: Estimates and 90% CIs for pupae with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

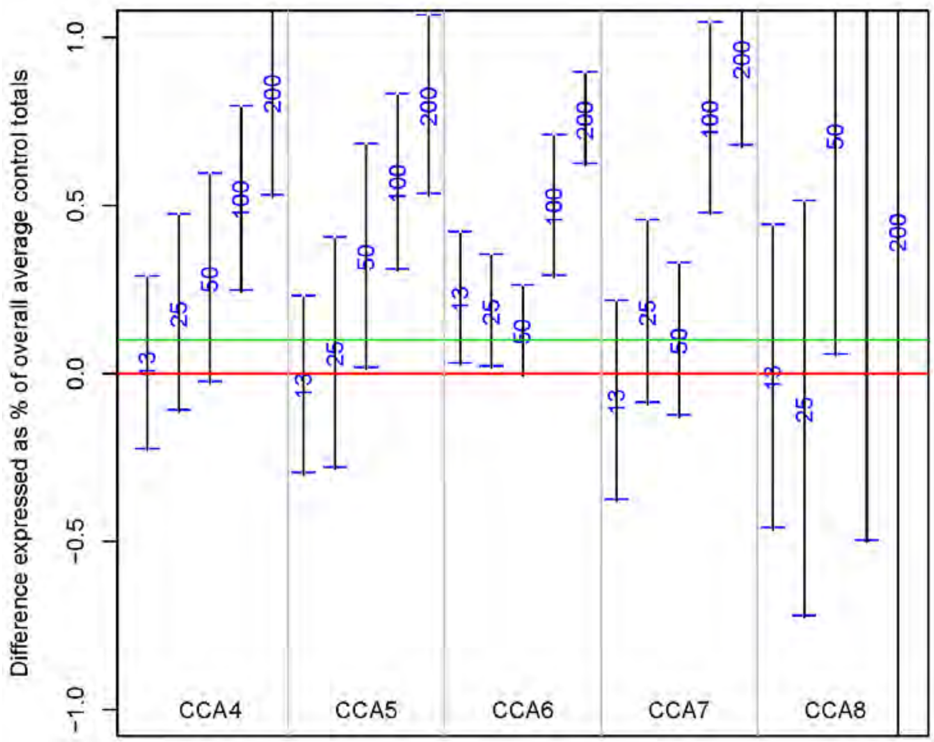

Figure B-10: Mean of the differences of <u>number of pupae</u> in hives between treatments and control at the same apiary

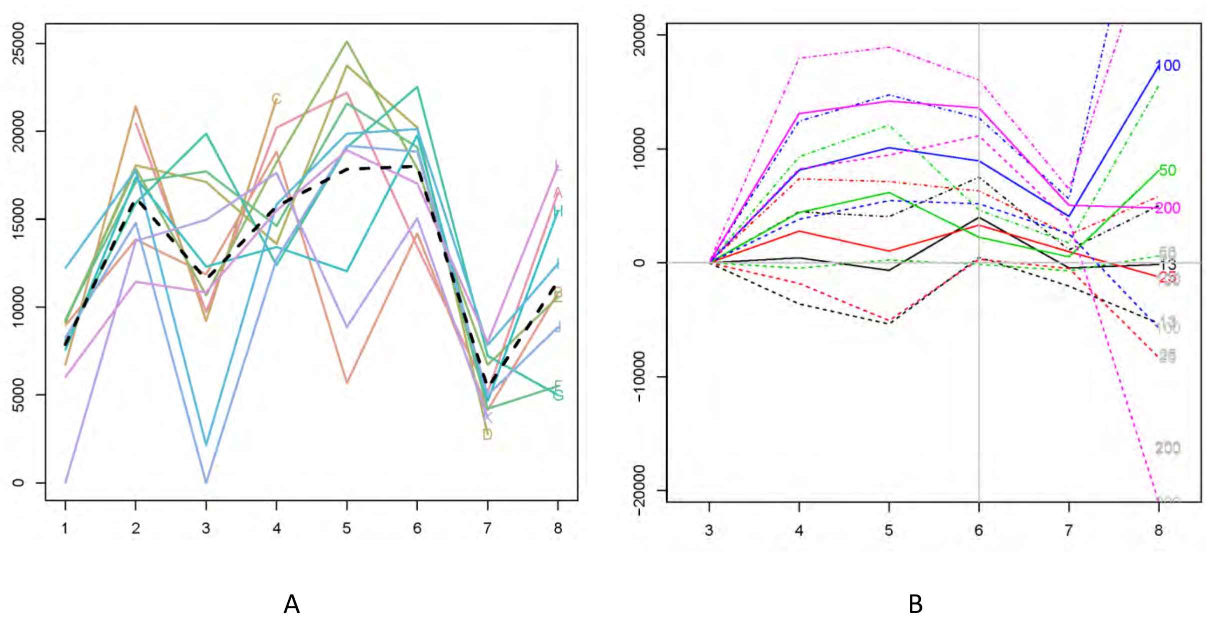

Figure A: Total number of capped brood (pupae) in control hives at each apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the number of pupae in control hives.
Apiaries are coded with various colors.
Black dash line represents the mean of all apiaries.

Figure B: Mean of the differences of the number of capped brood (pupae) in hives between the treatments and control at the same apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the mean of the differences of the number of pupae in hives between the control and treatment at the same apiary (control minus treatment).
The line of "0" indicates no effect from the control within the same Apiary and it was adjusted for baseline covariate at CCA3 for each CCAs.
Labels at the end of each line are the treatment concentrations (μg/L).
A positive Y value indicates there are more pupae in hives in the control than in the treatment, and a negative value indicates reversely.
A dash line indicates the 90% confidence interval estimated by a GLM model for a solid line with the same color.

Table B-6: Estimated percent reduction from control for pollen store

Test concentration (μg/L)	Estimated reduction from control (%) (P value)				
	CCA4	**CCA5**	**CCA6**	**CCA7**	**CCA8**
12.5	11.8 (0.222)	-6.8 (0.658)	-53.3 (0.998)	-26.1 (0.865)	-12.2 (0.765)
25	5.2 (0.369)	2.1 (0.455)	-25.2 (0.919)	-20.7 (0.844)	0.7 (0.487)
50	56.1 (<0.001)**	62.5 (<0.001)**	15.5 (0.147)	29.2 (0.077*/0.28)	63.8 (0.001)**
100	83.7 (<0.001)**	83.6 (<0.001)**	15.3 (0.158)	12.9 (0.281)	100.6 (0.037)**
200	94.5 (<0.001)**	90.4 (<0.001)**	54.7 (0.001)**	50.6 (0.029)**	12.3 (0.406)

Note: Negative value indicates increased pollen store in comparison to control.
*0.05<P<0.1
**P<0.05
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level. At CCA8, the step-down approach was not applied to the 200 or 100 treatment levels where very few hives survived.

Figure B-11: Estimates and 90% CIs for pollen store with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

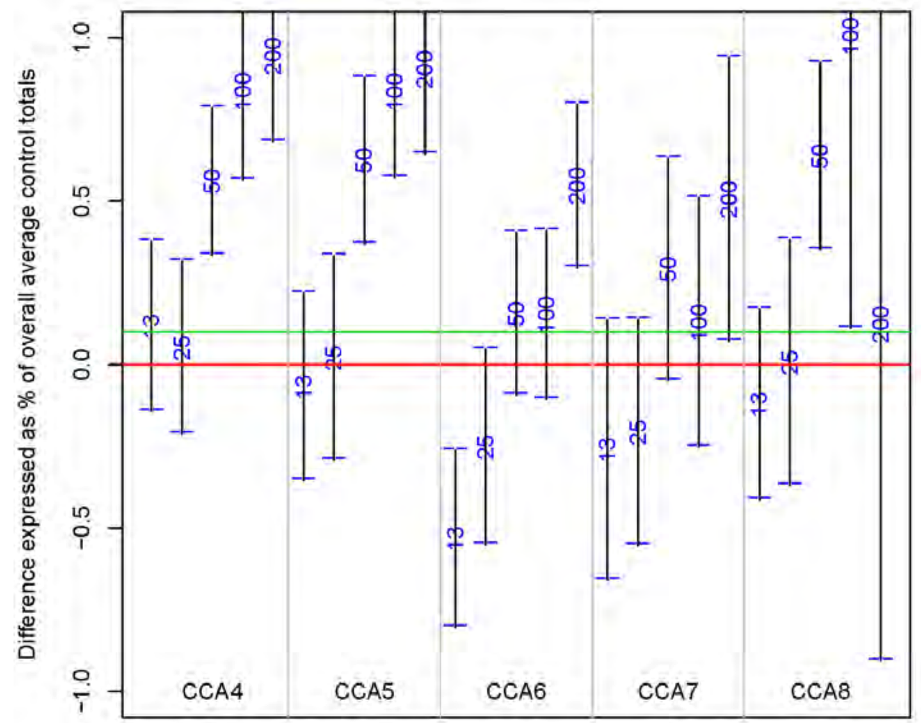

Figure B-12: Mean of the differences of the <u>amount of pollen store</u> in hives between treatments and control at the same apiary.

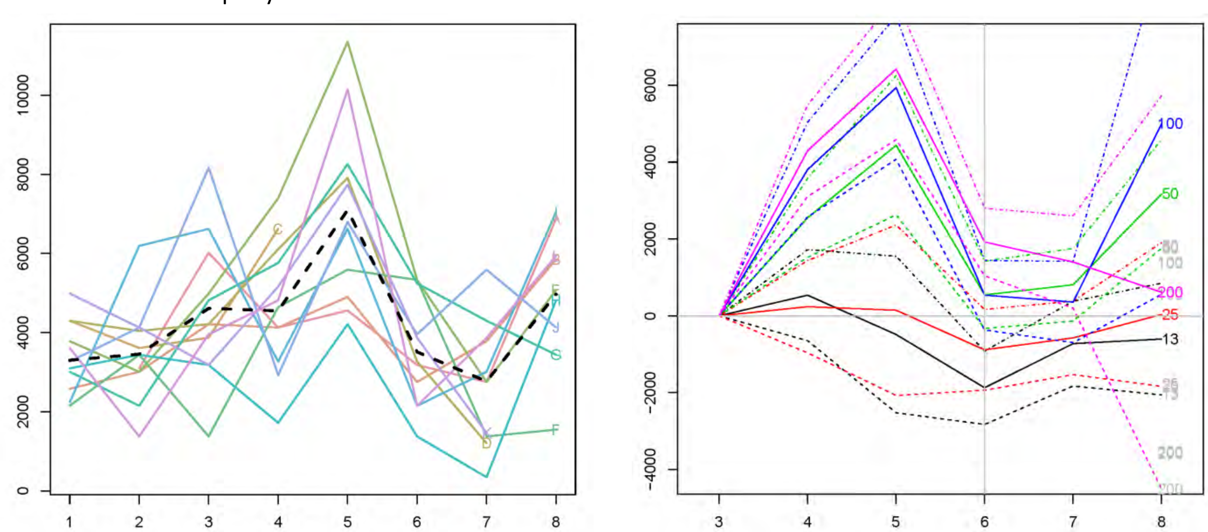

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Figure A: Total amount of pollen store in control hives at each apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the amount of pollen store in control hives.
Apiaries are coded with various colors.
Black dash line represents the mean of all apiaries.

Figure B: Mean of the differences of the amount of pollen store in hives between the treatments and control at the same apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the mean of the differences of the amount of pollen store in hives between the control and treatment at the same apiary (control minus treatment).
The line of "0" indicates no effect from the control within the same Apiary and it was adjusted for baseline covariate at CCA3 for each CCAs.
Labels at the end of each line are the treatment concentrations (µg/L).
A positive Y value indicates there are more pollen store in hives in the control than in the treatment, and a negative value indicates reversely.
A dash line indicates the 90% confidence interval estimated by a GLM model for a solid line with the same color.

Table B-7: Estimated percent reduction from control for honey store

Test concentration (µg/L)	Estimated reduction from control (%) (P value)				
	CCA4	CCA5	CCA6	CCA7	CCA8
12.5	-7.5 (0.808)	1.3 (0.467)	15.8 (0.093*/0.58)	12.5 (0.228)	-10.9 (0.712)
25	-10.4 (0.89)	-15.3 (0.84)	-2.4 (0.582)	10.6 (0.243)	13.3 (0.288)
50	-6.2 (0.723)	21.6 (0.091*/0.33)	36 (0.01)**	41.2 (0.022)**	60.4 (0.007)**
100	-8 (0.847)	7.1 (0.333)	21.8 (0.088)*	52.9 (0.001)**	156.6 (0.024)**
200	-21.1 (0.963)	-84.1 (1)	70.5 (<0.001)**	80 (<0.001)**	5.1 (0.471)

Note: Negative value indicates increased honey store in comparison to control.
*0.05<P<0.1
**P<0.05
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level. At CCA8, the step-down approach was not applied to the 200 or 100 treatment levels where very few hives survived.

Figure B-13: Estimates and 90% CIs for honey store with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

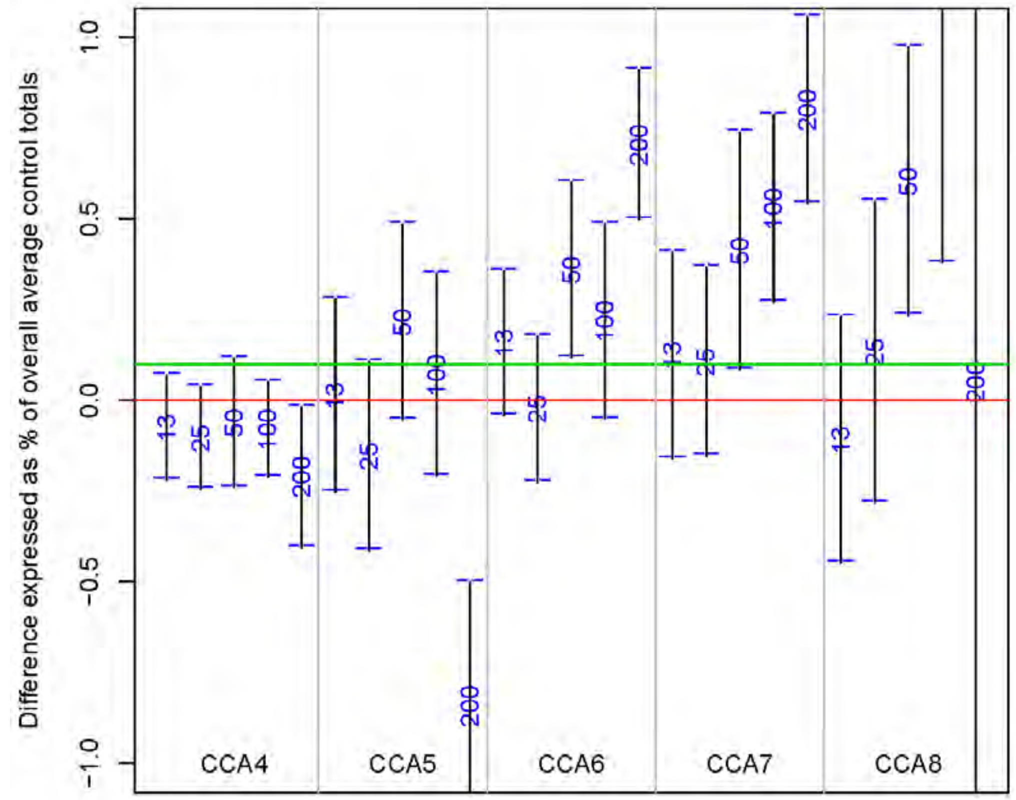
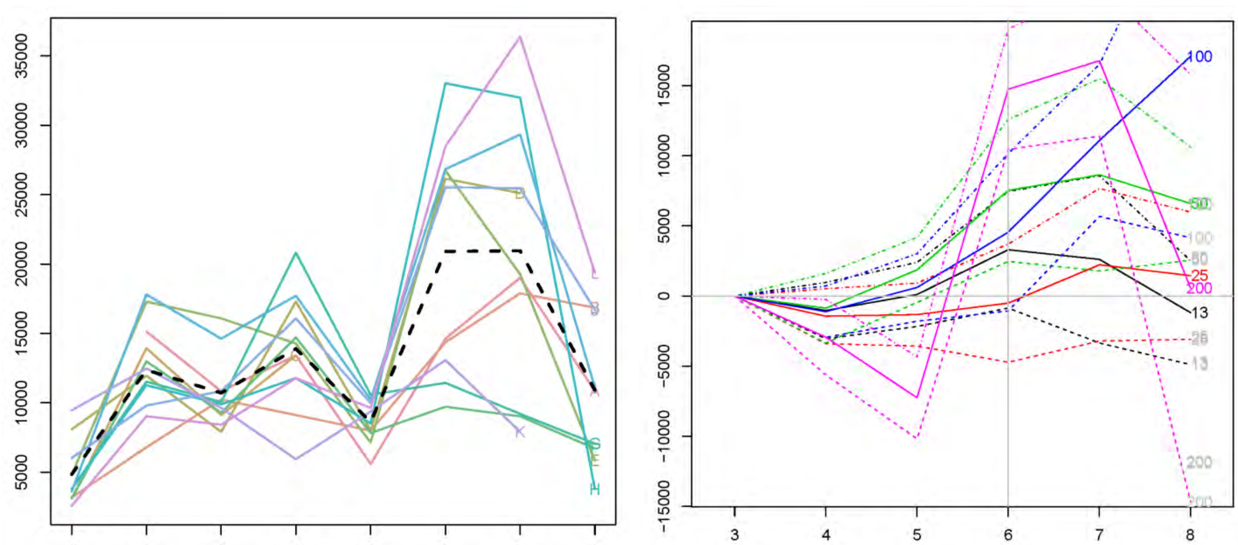


Figure B-14: Mean of the differences of the <u>amount of honey store</u> in hives between the treatments and control at the same apiary.



Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

A

B

Figure A: Total amount of honey store in control hives at each apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the amount of honey store in control hives.
Apiaries are coded with various colors.
Black dash line represents the mean of all apiaries.

Figure B: : Mean of the differences of the amount of honey store in hives between the treatments and control at the same apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the mean of the differences of the amount of honey store in hives between the control and treatment at the same apiary (control minus treatment).
The line of "0" indicates no effect from the control within the same Apiary and it was adjusted for baseline covariate at CCA3 for each CCAs.
Labels at the end of each line are the treatment concentrations (µg/L).
A positive Y value indicates there is more honey store in hives in the control than in the treatment, and a negative value indicates reversely.
A dash line indicates the 90% confidence interval estimated by a GLM model for a solid line with the same color.

Table B-8: Estimated percent reduction from control for hive weights

Test concentration (µg/L)	Estimated reduction from control (%) (P value)				
	CCA4	CCA5	CCA6	CCA7	CCA8
12.5	-0.5 (0.559)	2.4 (0.306)	0.2 (0.49)	-1.6 (0.586)	-1.1 (0.617)
25	2.7 (0.154)	-0.4 (0.537)	-1.5 (0.585)	0.6 (0.469)	1.2 (0.444)
50	4.1 (0.068)*	11 (0.014)**	12.3 (0.058)*	15 (0.036)**	20.9 (0.006)**
100	7.1 (0.005)**	14.9 (0.001)**	15 (0.03)**	18.9 (0.01)**	67.7 (0.001)**
200	10.1 (<0.001)**	10.4 (0.011)**	30.4 (<0.001)**	33.3 (<0.001)**	-25.5 (0.93)

Note: Negative value indicates increased hive weight in comparison to control.
*0.05<P<0.1
**P<0.05
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level. At CCA8, the step-down approach was not applied to the 200 or 100 treatment levels where very few hives survived.

Figure B-15: Estimates and 90% CIs for hive weight with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

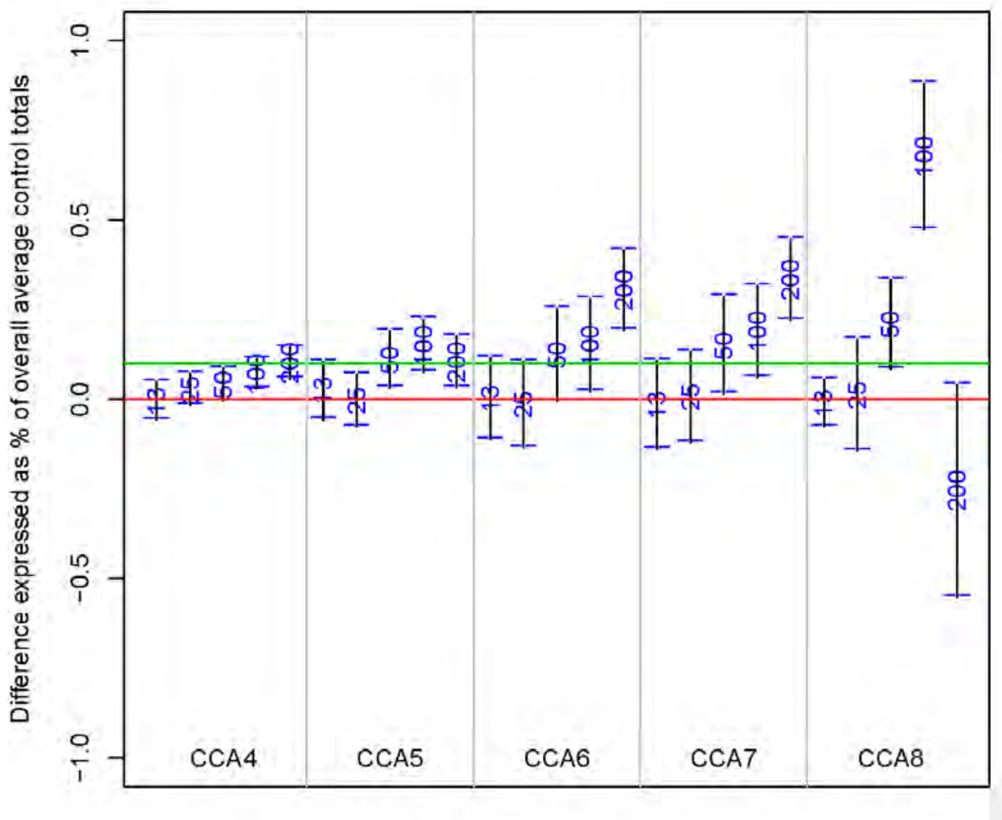


Figure B-16 Mean of the differences of the <u>hive weight</u> between the treatments and control at the same apiary.

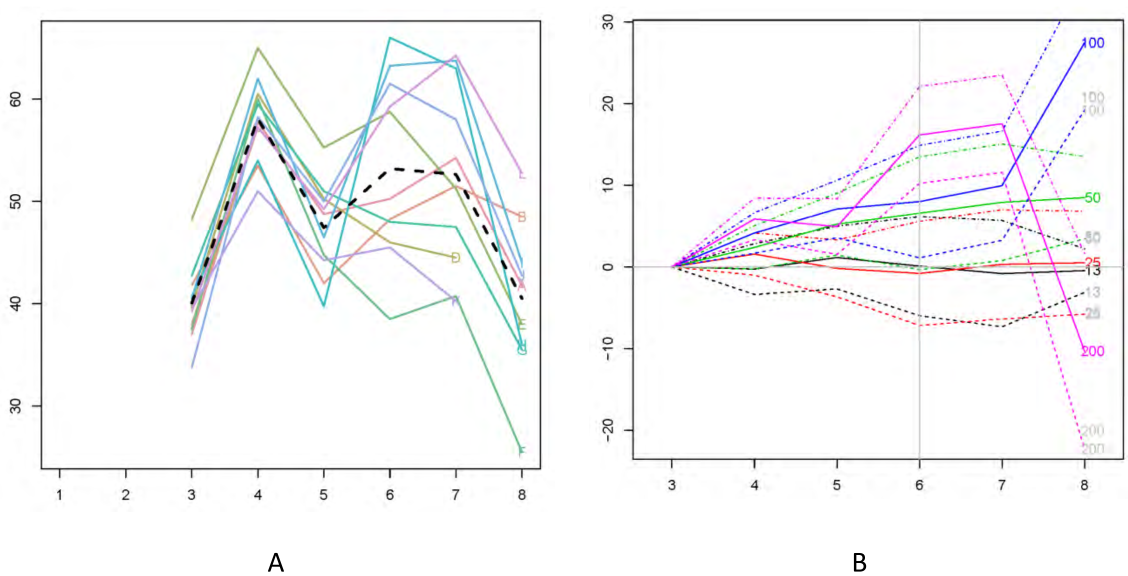
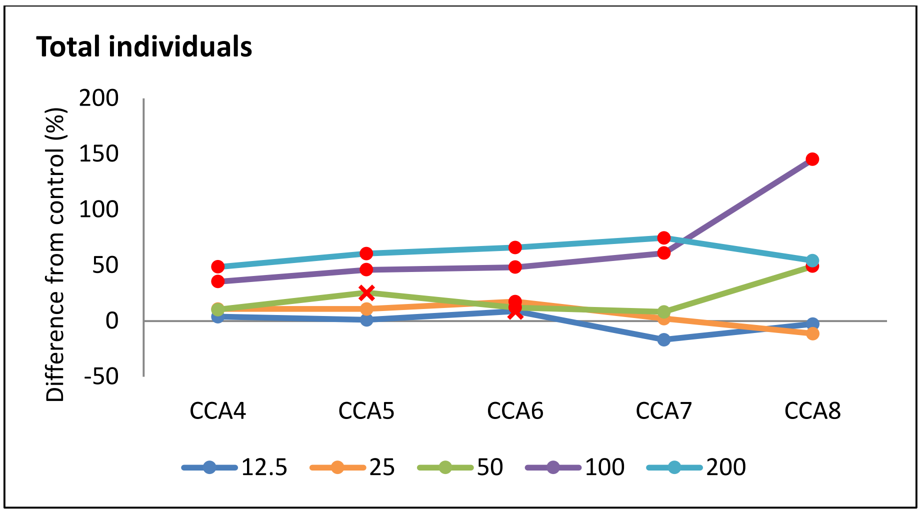


Figure A: Total hive weight in control hives at each apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the hive weight in control hives.
Apiaries are coded with various colors.
Black dash line represents the mean of all apiaries.

Figure B: Mean of the differences of the hive weight between the treatments and control at the same apiary (n=11).
X-axis represents the number of CCA; Y-axis represents the mean of the differences of the hive weight between the control and treatment at the same apiary (control minus treatment).
The line of "0" indicates no effect from the control within the same Apiary and it was adjusted for baseline covariate at CCA3 for each CCAs.
Labels at the end of each line are the treatment concentrations (μg/L).
A positive Y value indicates that the hive weight was more in the control than in the treatment, and a negative value indicates reversely.
A dash line indicates the 90% confidence interval estimated by a GLM model for a solid line with the same color.



X: 0.1>P>0.05, ●: 0.05>P

Figure B-17. Total Individuals (percent difference from control)

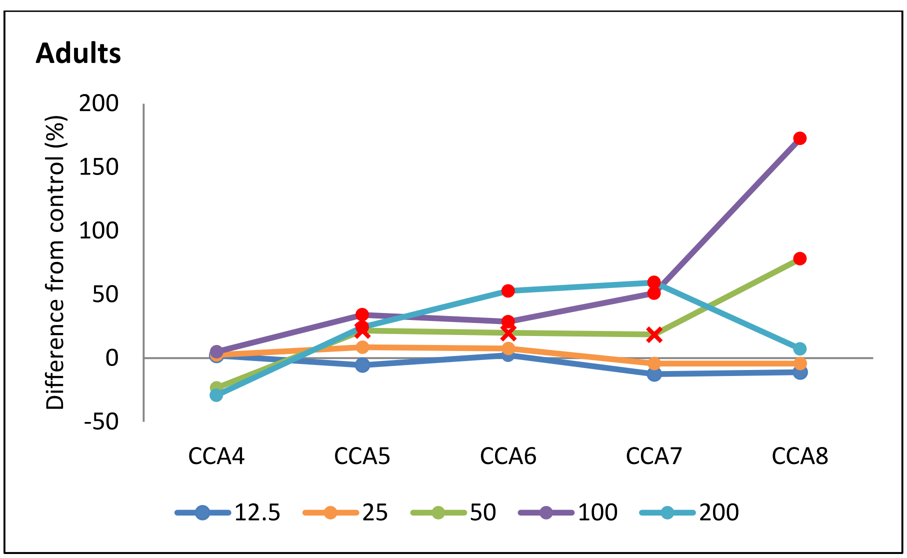


Figure B-18. Adults (percent difference from control)

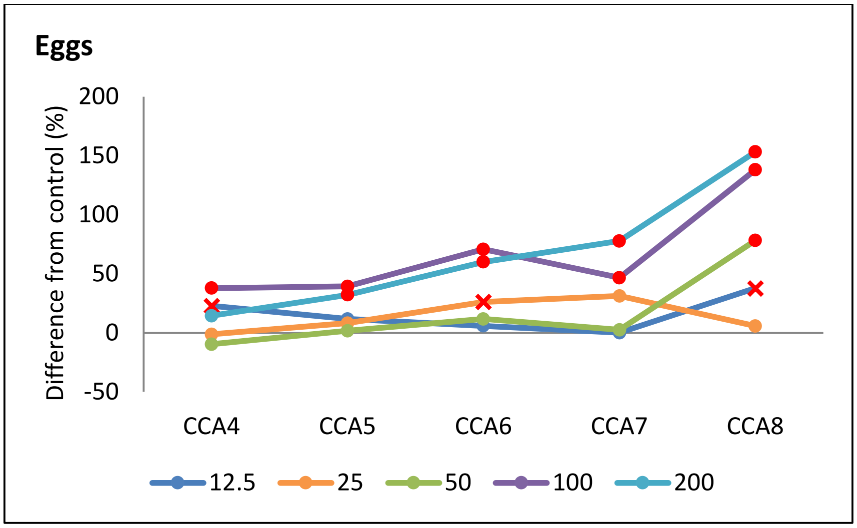


Figure B-19. Eggs (percent difference from control)

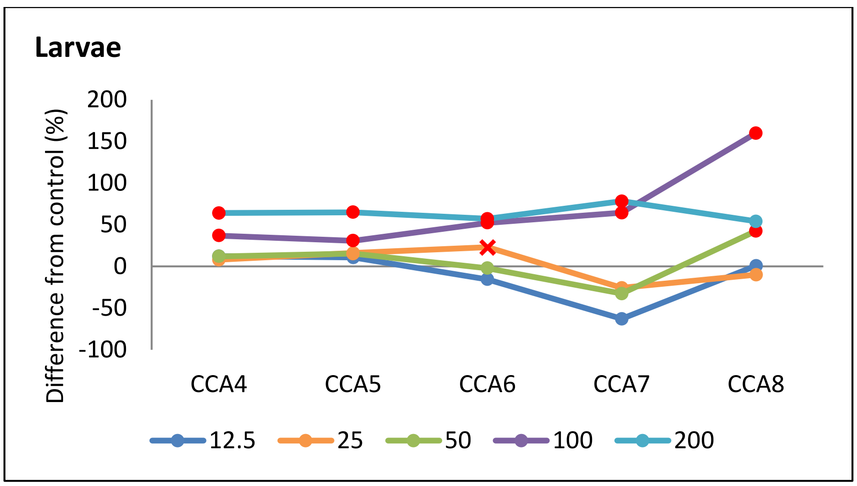


Figure B-20. Larvae (percent difference from control)

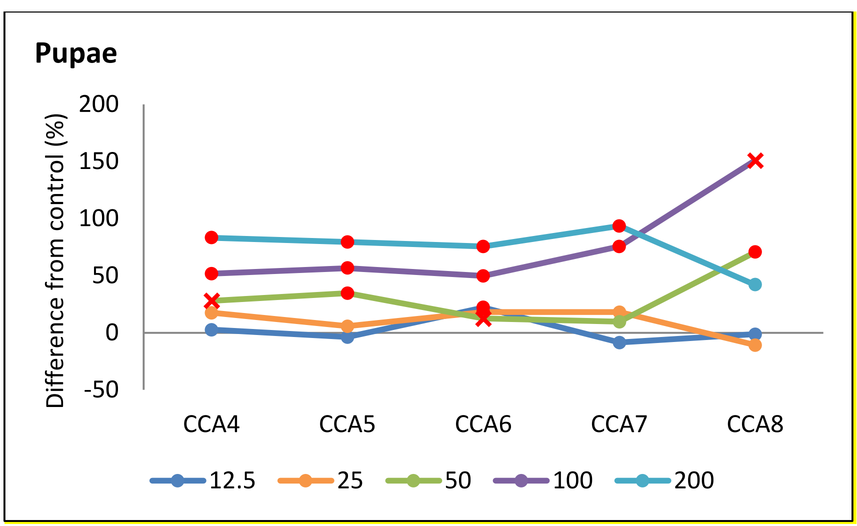


Figure B-21. Pupae (percent difference from control)

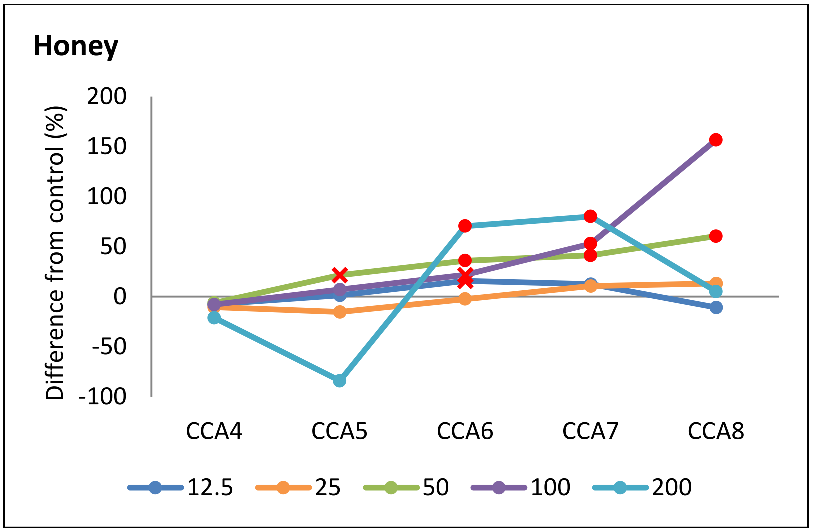


Figure B-22. Honey (percent difference from control)

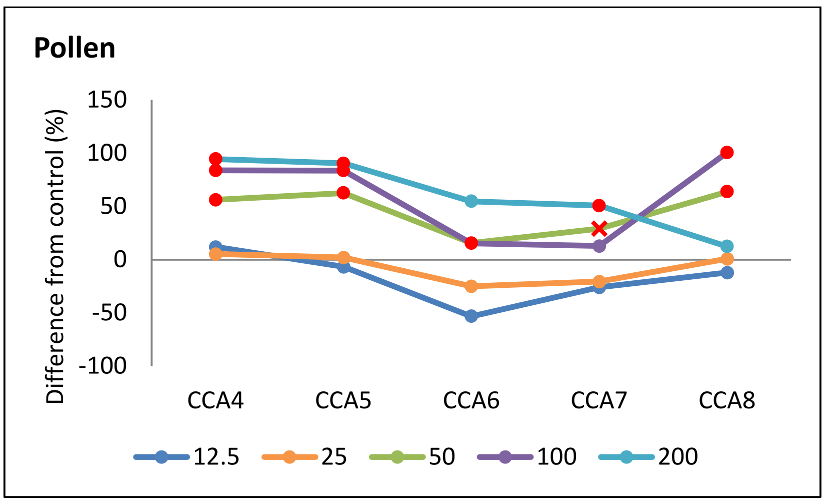


Figure B-23. Pollen (percent difference from control)

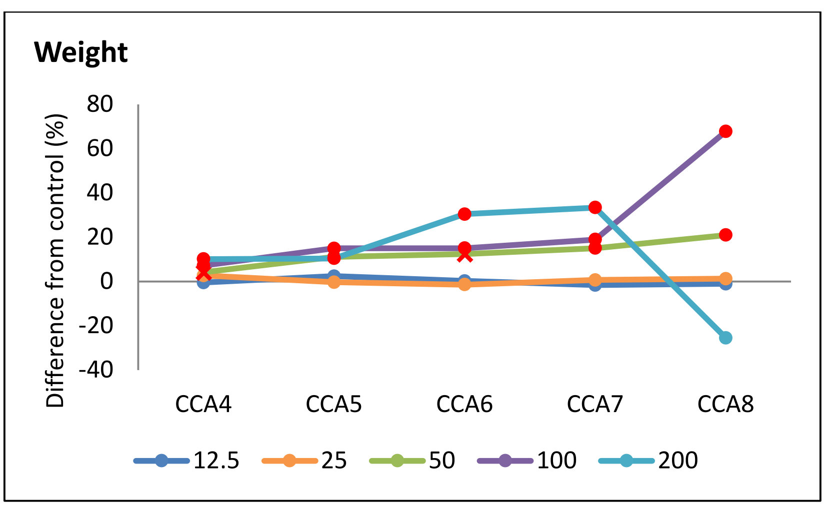


Figure B-24. Weight (percent difference from control)

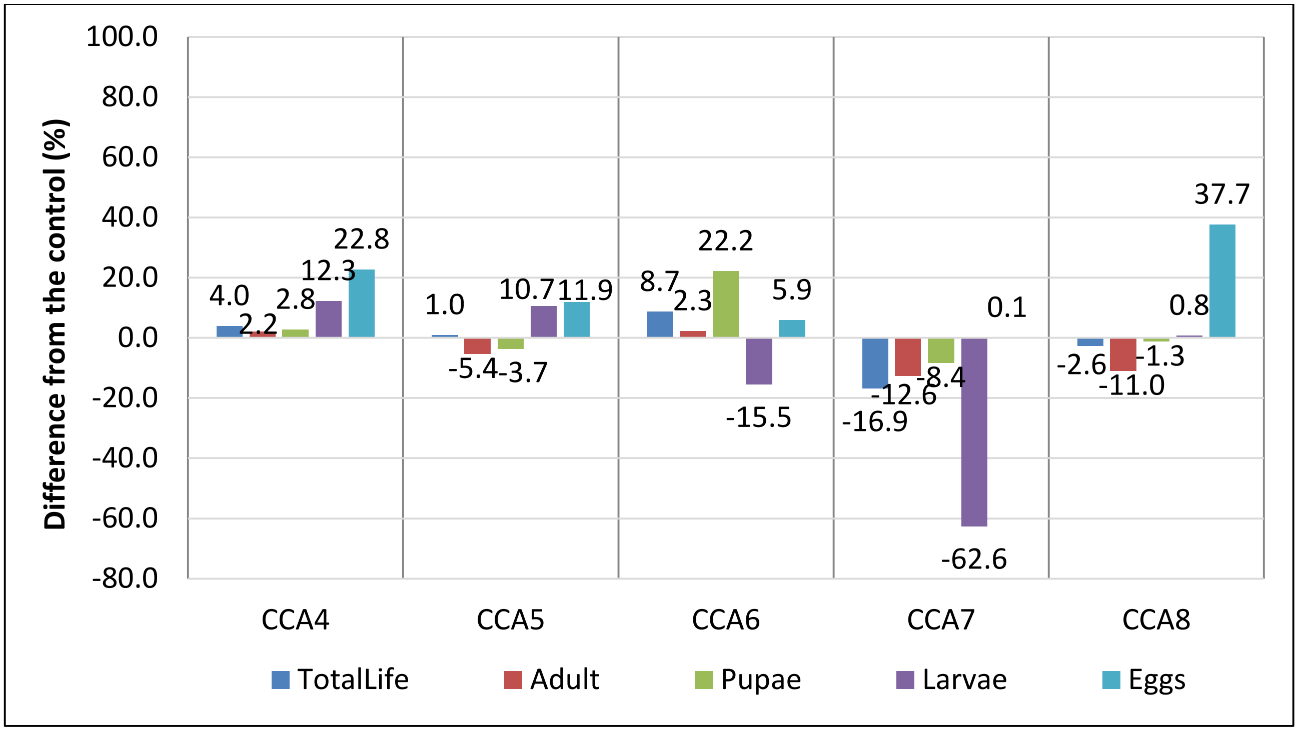


Figure B-25 Summary of living organism parameters at 12.5 µg/L

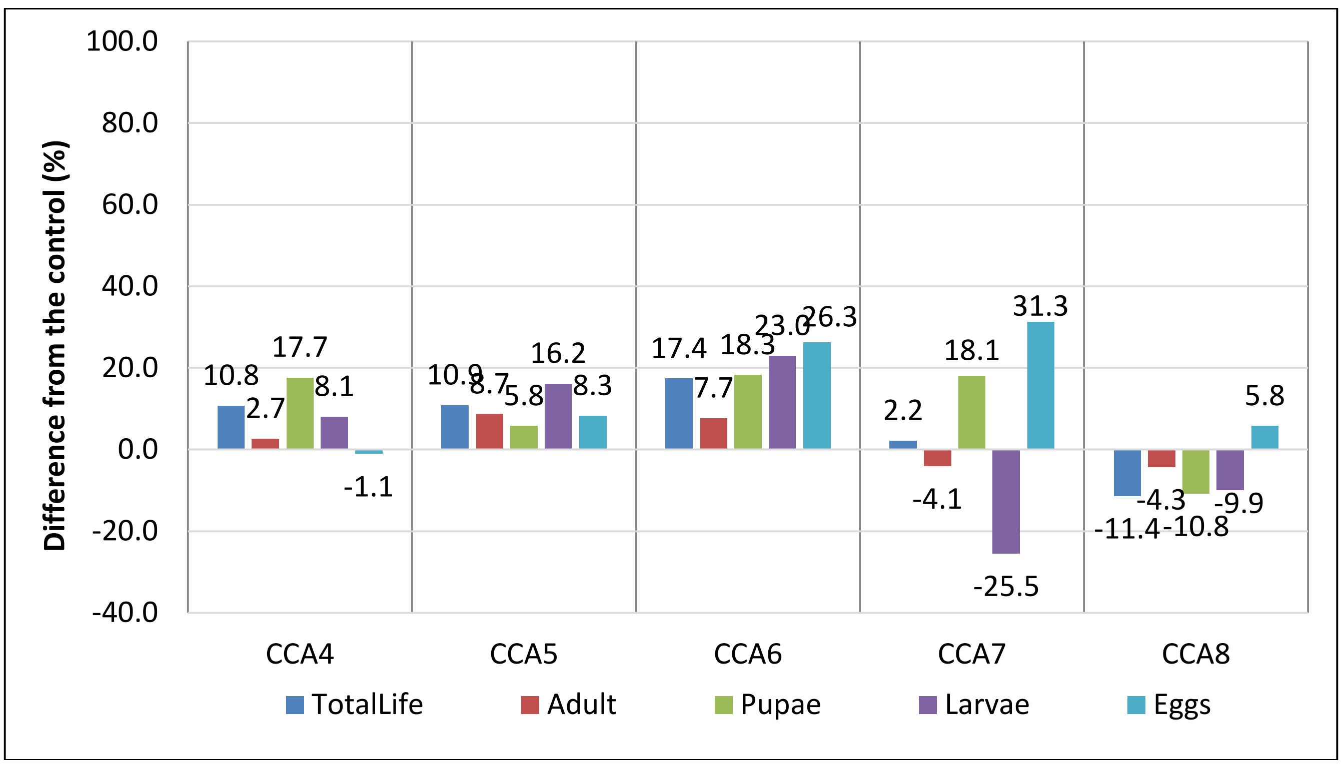


Figure B-26. Summary of living organism parameters at 25 µg/L

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

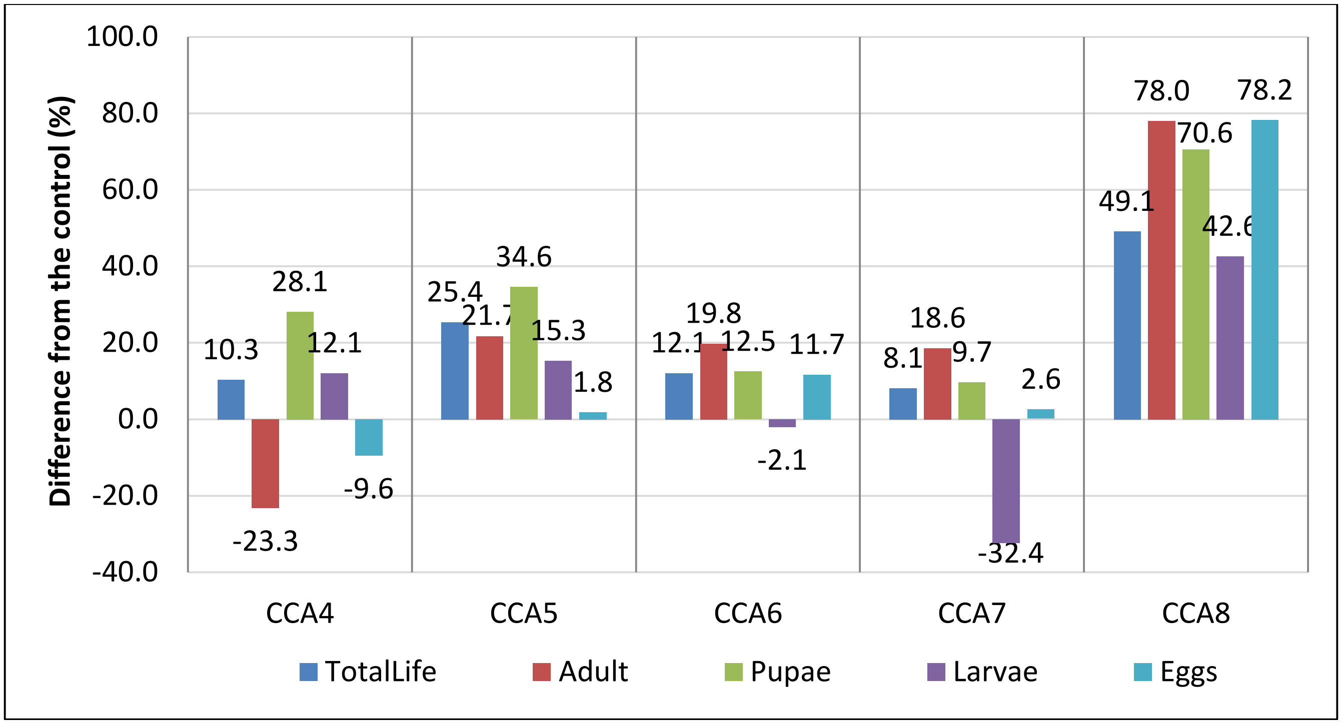


Figure B-27. Summary of living organism parameters at 50 µg/L

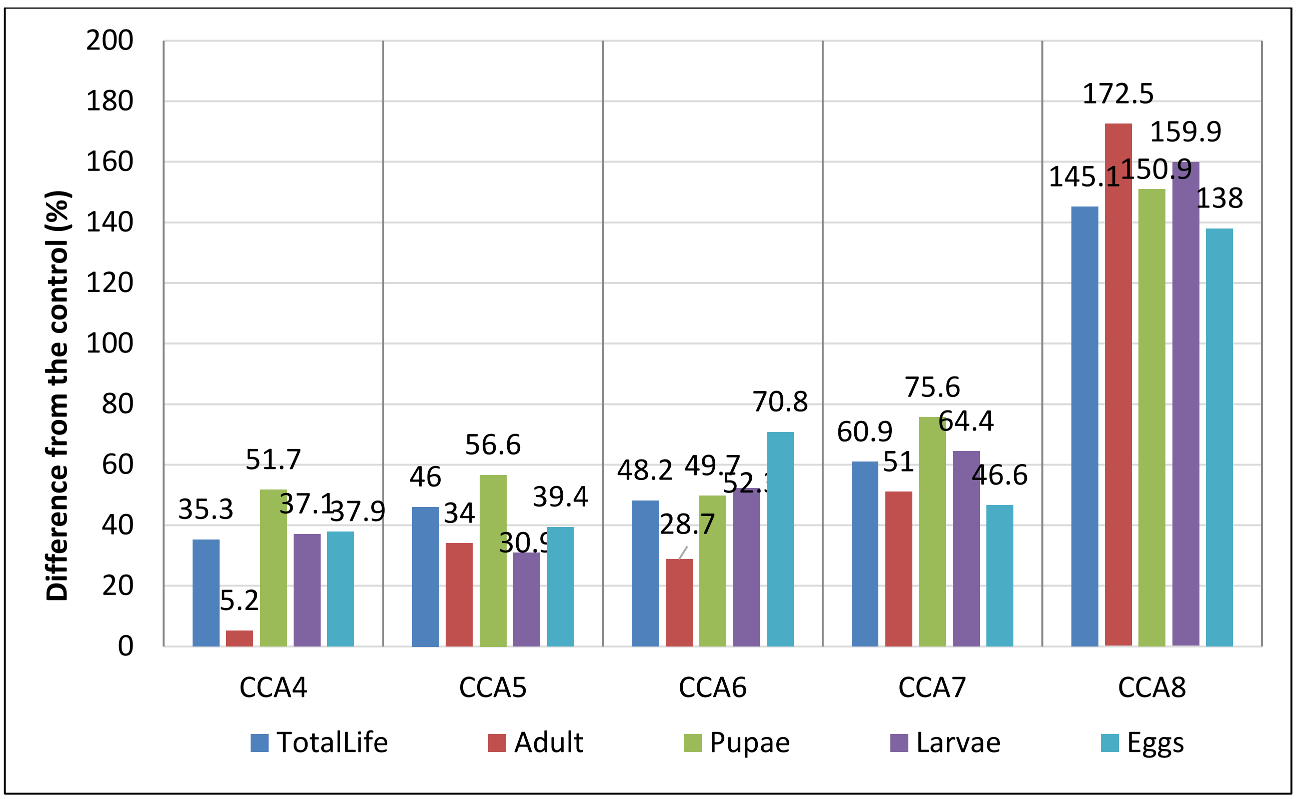


Figure B-28. Summary of living organism parameters at 100 µg/L

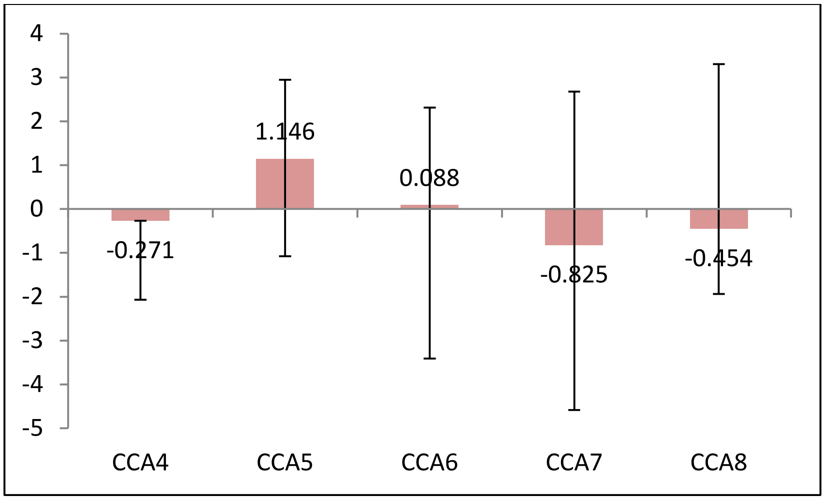


Figure B-29. Summary of hive weight at 12.5 µg/L

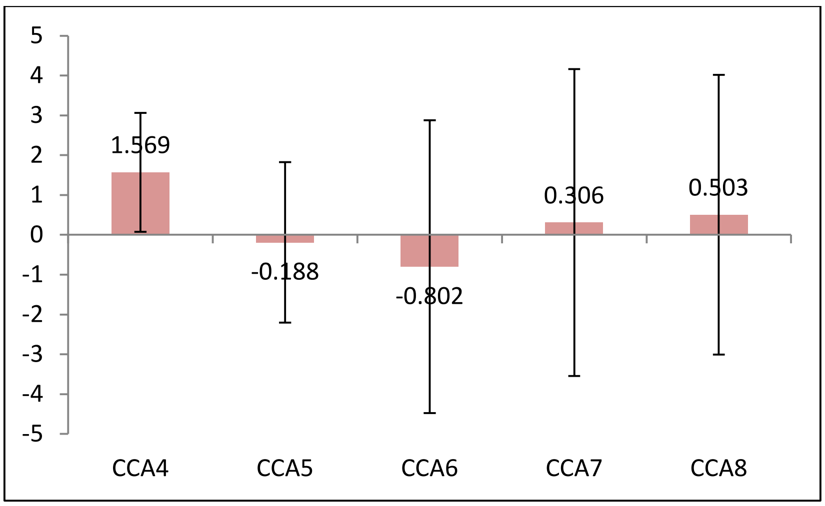


Figure B-30. Summary of hive weight at 25 µg/L

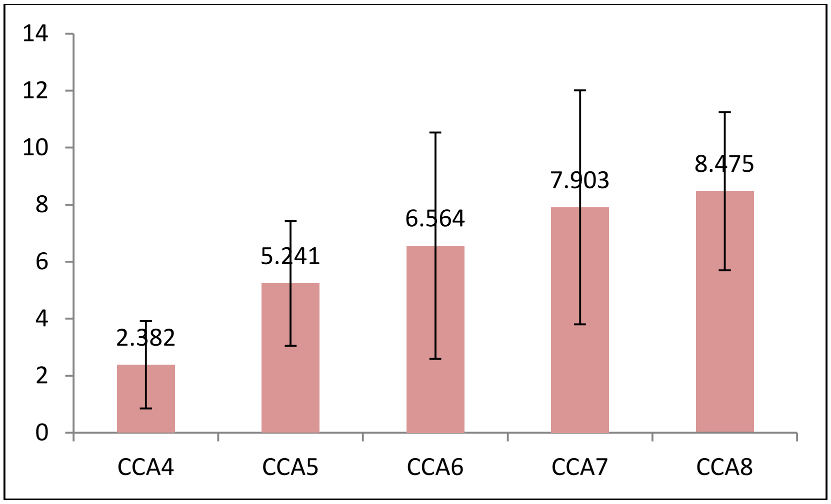


Figure B-31. Summary of hive weight at 50 µg/L

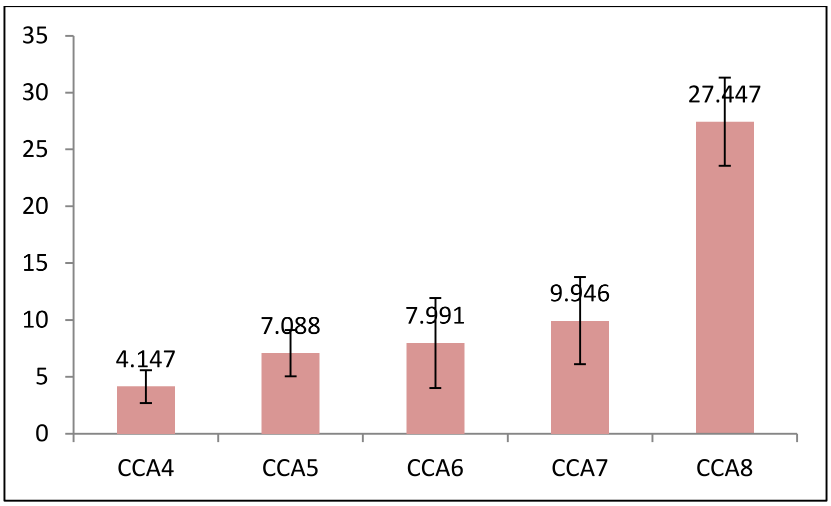


Figure B-32. Summary of hive weight at 100 µg/L

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table B-9. Summary of observed effects at each treatment level (Note: Values reported in the table are the % reduction compared to control, based on model estimated raw numbers corrected for baseline measurements).

Treatment (µg/l)	Observations
12.5	• Decreased total number of individuals in hive at CCA6 (8.7%, P<0.1) • Decreased number of pupae at CCA6 (22.2%, P<0.05) • Decreased honey store at CCA6 (15.8%, P<0.1) • At CCA8, two out of 11 colonies did not survive overwintering (better survival than control) • At CCA8 among surviving hives, hive condition similar to control • The potential colony effects at CCA6 were considered short-term, colony able to compensate
25	• Decreased total number of individuals in hive at CCA6 (17.4%, P<0.05) • Decreased number of pupae at CCA6 (18.3%, P<0.05) • Decreased number of eggs at CCA6 (26.3%, P<0.1) • Decreased number of larvae at CCA6 (23%, P<0.1) • At CCA8, one out of 11 colonies did not survive overwintering (better survival than control) • At CCA8 among surviving hives, hive condition similar to control • The potential colony effects at CCA6 were considered short-term, colony able to compensate
50	• Decreased total number of individuals in hive at CCA5 (25.4%, P<0.1) and CCA6 (12.1%, P<0.05) • Decreased pupae at CCA4, CCA5, CCA6 (28.1, 34.6, 12.5%, P<0.1, <0.05, <0.1) • Decreased number of adults at CCA5 - CCA7 (18.6 - 21.7%, P<0.1) • No effect on eggs or larvae observed • Decreased pollen store at CCA4, CCA5, CCA7 • Decreased honey store at CCA5 – CCA7 • Decreased hive weight at CCA4 – CCA7 • At CCA8, four out of 11 colonies did not survive overwintering, comparable to control • At CCA8 among surviving hives, poorer hive condition compared control
100 and 200	• Decreased total number of individuals in hive at CCA4-CCA7 • Decreased number of adults at CCA5 - CCA7 • Decreased number of eggs at CCA4 – CCA7 • Decreased number of larvae at CCA4 – CCA7 • Decreased number of pupae at CCA4 – CCA7 • Decreased pollen store at CCA4 and CCA5 • Decreased honey store at CCA6-CCA7 • Decreased hive weight at CCA4 – CCA7 • High overwintering mortality (only 1 survived at 100; only 2 survived at 200)
OVERALL ENDPOINT	• NOAEL: 25 µg/L sucrose solution (nominal 20.3 ppb; measured 23.3 ppb) • LOAEL: 50 µg/L sucrose solution (nominal 40.7 ppb; measured 46.7 ppb)

Appendix C: Details of CDPR Statistical Analysis

Statistical Summary

A clear progression of effects on hive health in response to imidacloprid dose was indicated by the results of the multivariate mixed model analysis.

- After only **3 weeks** into the exposure period **pupal and larval** numbers decreased in response to higher dose levels with effects initiated on pupal cells at the 50 ppb treatment. **Pollen** food stores also exhibited decreased numbers as a dose response relationship at the higher treatment levels with effects initiated at the 50 ppb dose level. Overall **hive weight** was decreased at 100 and 200 ppb doses.

- At **7 weeks** after initiation of exposure (1 week after end of treatments), numbers of **adult and egg** cells were then decreased at the higher treatments with effects initiated on adult cells at the 50 ppb dose level. A clear dose response was also observed for hive weight at the higher doses with effects now measured at the 50 ppb treatment.

- Later at **11 weeks** after initiation of exposure (5 weeks after end of treatments), number of **honey cells** exhibited a dose response relationship at the higher treatment levels with effects initiated at the 50 ppb dose level.

- At the final assessment before overwintering at 16 weeks after initiation of exposure (10 weeks after end of treatments) decreases in number of honey cells and hive weight were measured at the 50 ppb dose level. Numbers of adults and pupal and larval cells were decreased at the 100 and 200 ppb dose levels. Note that some effects previously measured between control and 50 ppb treatment levels were not apparent at this assessment.

- Assessment of the hives after overwintering indicated that dose related effects noted at CCA7 were expressed in CCA8 where extreme loss of hives was observed at the 100 and 200 ppb. Decreased vigor of hives at the 50 ppb dose level was indicated due to decreased numbers of adults and pupal and larval cells as compared to control hives.

The general conclusion is that the data indicate a NOEC value at 25 ppb and a LOEC value at 50 ppb.

Background

The multivariate mixed repeated measures model approach is distinguished from the univariate approach previously in that all bee life stages or hive food storage variables are simultaneously analyzed as a single model. Multivariate analyses of variance for fixed effects models are conducted, using Statistical Analysis System (SAS) software, through implementing the MANOVA option in the PROC GLM procedure. Recently, multivariate analyses have been extended to mixed models using the PROC MIXED procedure. The MIXED procedure is designed to conduct a mixed model analysis of variance where fixed and random effects can be specified. Inclusion of random effects in a model provides a broader application of results. For this study, locations were denoted as apiaries with individual hives as test subjects. Use of a mixed model with apiaries identified as a random variable provides some assurance that the results can be generalized to other locations and hives.

Model Setup

- The Multivariate Analysis of Variance in PROC MIXED is conducted by combining the data for response variables to be analyzed into two columns of data. One column contains the list of variables to be analyzed, denoted as 'RESPONSE' in this analysis, and the other variable contains the measured value for that variable, denoted 'VALUE' in this analysis.
- Factors on the effect side of the equation for the multivariate response were dose of imidacloprid and date sampled, which was denoted as day for each CCA assessment. .
- Technically, the RESPONSE variable is listed as a class variable in the multivariate analysis and then included in the effects side of the model.

The SAS code below illustrates the syntax for one of the programs used to determine the appropriate covariate model to use:

```
proc mixed data=a7;
title4 'Overall Multivariate analysis';
title5 'EPA scaling - Apiary random - CV = un@ar(1)';
class day dose response hivenum apiary;
model epavaluet=response|day|dose/noint;
random apiary;

run;
```

Features in this syntax are:

1. Class Statement: The RESPONSE variable is included in the list of effects variables and contains the variables to be analyzed for the multivariate analysis.
2. Model Statement:
   a. VALUE is the respective measure for the RESPONSE variable. For example, VALUE contains the value for number of adult bees taken for hive number 2 located in Apiary A and taken at CCA3 at the 12.5 ppb treatment. For the statistical analysis, the original values were scaled as in the univariate analyses: Raw values for pupal, larval, and egg cells were dived by 68800 with adult cells divided by 68800/1.3 then times by 4.
   b. The RESPONSE variable is tested for interactive effects with day and dose on the effects side of the model.
3. Random Statement: Apiary is treated as a random variable because effects are to be generalized to other locations.
4. Repeated Statement:
   a. Provides for a repeated measures analysis of variance.
   b. The subject= indicates the hive where the repeated measures were taken.
   c. Type=UN@AR(1) indicates the covariance model used where the symbols represent a Compound Symmetry model applied to the response variable and a first-order autoregressive model applied to day. Various covariance models were tested

to determine the model which provided the best fit. The best model was chosen by comparing values of informational criteria for -2 Res Log Likelihood, a criteria that provided an overall estimate of the amount of variance explained by the model, and the BIC criteria that adjusts the previous criteria based on the number of additional parameters added to the model for each structured model. .

The sequence of statistical analyses conducted was:

1. Conduct a full model analysis of variance as reflected in syntax above. Owing to the large number of dead hives at CCA8 for the 100 and 200 ppb treatments, data from CCA3 through CCA7 were used in this overall analysis to test for interactive effects. Three covariance models relevant to this design were tested. For bee life stages the UN*AR(1) model provided the best fit, whereas, UN@CS provided the best fit for analysis of food stores .
2. Upon observation of a significant interactive term between dose, day, and response, further analyses were conducted at each CCA to determine the differential responses among variables over time. Data for CCA8 were included in this analysis. Seven covariance models were tested for each CCA. The autoregressive-first-order model with heterogeneity model fit best at CCA3, the compound symmetry model with heterogeneity fit best at CCA4, and the unstructured model fit best for CCA5 through CCA8.

Multivariate Analysis of Variance

- Two sets of analyses were conducted. One focused on the counts for the various life stages of bees within the hive, contrasting the numbers of adults, pupal, larval, and eggs over time.
- A second analysis explored the relationship between nectar and pollen cells. These values indicate the level of food stores in the hive over. Analysis on hive weight was conducted separately.

Results

Life Stages

All effects in the multivariate full model for adult, pupal, larval, and eggs cells were highly significant (**Table C-1**). Notably, the triple interactive effect for Day*Dose*Response indicated that the various bee stages responded differently over time to imidacloprid dosage. Analyses were then conducted by CCA to determine the sequence of effects over time.

In order to determine the pattern of response for life stages at each CCA, a regression analysis was first conducted to measure potential linear and curvilinear effects of dose at each CCA (**Table C-2**). Quadratic and cubic dose effects were included to indicate potential curvature in response. The second analysis provided a LSMEANS test for each pairwise comparison between levels of dose for each response (**Table C-3**). These contrasts provide a basis for estimating potential no observed effect concentration (NOAEC) and lowest observed effect concentration (LOAEC) values.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table C-1. Results for multivariate mixed model analysis of variance for potential interactive effects of imidacloprid dose over time on counts of bee stages for adult, pupal, larval and egg cells.

Overall Model Effect	Num DF	Den DF	PR>F
Response	3	213	<.0001
Day	4	283	<.0001
Day*Response	12	849	<.0001
Dose	5	61	<.0001
Dose*Response	15	213	<.0001
Day*Dose	20	283	0.0001
Day*Dose*Response	60	849	<.0001

Comparison of the pattern of significant regression results between CCAs provided evidence for the differential responses in bee life stages over time (**Table C-2**). In the figures for effects, the response for each variable over dose and is plotted. In addition, oversized dots and colors indicate levels of significant difference between the control value and the value at each treatment level as indicated from **Table C-3**.

- **CCA3:** Prior to dietary administration of imidacloprid at CCA3, baselines for life stages assigned to each treatment level were essentially not significantly different ($p<0.05$) from control, with the exception of pupal cells (**Table C-3**). For pupal cells, the initial number of cells tended to be lower for the control group. The reason for this finding is not known but differences caused by imidacloprid treatments were measured in subsequent analyses. At this time, adults were in greatest number followed by pupal cells and then larval and egg cells (**Figure C-1**).

- **CCA4:** Although this assessment of hive health was taken only 3 weeks into the exposure period, significant regression indicated decreasing numbers of **pupal and larval** cells with increase in imidacloprid dose (**Figure C-2**). For pupal cells the effect was first measured at the 50 ppb treatment ($p<.1$)) and then at progressively increasing probability levels for 100 and 200 ppb treatments. For larval cells the effects were significant for the 100 ppb treatment and higher. Effects on pupal and larval cells numbers were persistent throughout the subsequent CCAs (**Figures C-2 to C-6**).

- **CCA5:** Decreases in numbers of **adult and egg cells** were now indicated at the higher 100 and 200 ppb dose levels (**Figure C-3).**

- **CCA6:** Decreases noted in the previous CCAs at the 100 and 200 pbb levels of dose were measured for all life stages. For adult cells, decreased numbers of adults were also measured for the 50 ppb treatment, indicating a dose response to treatments starting at this level (**Figure C-4**).

- **CCA7:** At CCA7 higher order regression coefficients were significant for counts of adults and larval cells (**Table C-2**). The curvilinear nature of the response reflected the extreme effects on reductions in numbers measured for the 100 and 200 ppb levels of imidacloprid dose **(Figure C-5)**.

- __**CCA8:**__ The pattern noted at CCA7 was reflected at the final assessment at CCA8 where curvature in response measured for all life stages reflected a grouping of treatment levels: Results were similar for 0, 12.5, and 25 ppb treatments and with 50, 100, and 200 ppb treatments reflecting detrimental effects due to imidacloprid treatment (**Table C-2, Figure C-6**). Loss of hives at 100 and 200 ppb treatments was an obvious effect resulting in essentially loss of all life stages at these treatments. But additional decreases in numbers of adults, pupal, and egg cells were measured at the 50 ppb treatment compared to the control, indicating lower vigor of hives at this treatment.

Table C-2. Regression for regression effects conducted within each CCA and for each bee life stage.

Bee Life Stage	Regression Effect	Regression Results for Each CCA					
		CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
		Pr>t	Pr>t	Pr>t	Pr>t	Pr>t	Pr>t
Adult	Dose Linear	0.6945	0.0622	0.0341	0.0003	<.0001	<.0001
	Dose Quadratic	0.1763	0.1761	0.017	0.2027	0.0938	0.034
	Dose Cubic	0.3279	0.143	0.5933	0.8789	0.0501	0.0417
Pupal	Dose Linear	0.0923	<.0001	<.0001	<.0001	<.0001	<.0001
	Dose Quadratic	0.9986	0.4165	0.1366	0.2841	0.1758	0.0437
	Dose Cubic	0.2244	0.6987	0.9101	0.7008	0.1117	0.0511
Larval	Dose Linear	0.626	<.0001	0.0003	<.0001	<.0001	<.0001
	Dose Quadratic	0.1992	0.3551	0.8878	0.1037	0.5789	0.0968
	Dose Cubic	0.2381	0.3424	0.9101	0.2008	0.0055	0.0275
Egg	Dose Linear	0.7645	0.2451	0.021	<.0001	0.0003	<.0001
	Dose Quadratic	0.6786	0.2995	0.4279	0.0421	0.9673	0.0244
	Dose Cubic	0.7019	0.0996	0.4022	0.1842	0.9576	0.1589

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table C-3. Mean comparison for the response between each level of dose at each CCA for each bee life stage. The difference is the percent frame coverage of dose1 minus dose2 where a positive value indicates a higher value for the lower dosage and a negative value indicates a lower value for the lower dosage.

Significant Pairwise Comparisons Testing Differences Between Each Level of Imidicloprid Dose at Each CCA and Each Life Stage														
CCA3					CCA4					CCA5				
Life Stage	Dose1	Dose2	PR>t	Difference	Life Stage	Dose1	Dose2	PR>t	Difference	Life Stage	Dose1	Dose2	PR>t	Difference
Pupal	0	12.5	0.0969	-0.06	Adult	0	200	0.0225	-0.13	Adult	0	50	0.0636	0.12
	0	25	0.0577	-0.07		25	200	0.0812	-0.12		0	100	0.0079	0.17
	0	200	0.0185	-0.09		100	200	0.0177	-0.17		12.5	50	0.0397	0.15
Larval	0	100	0.0972	0.03	Pupal	0	50	0.0822	0.06		12.5	100	0.006	0.21
	25	100	0.0709	0.04		0	100	0.0032	0.1		12.5	200	0.0674	0.13
						0	200	<0.0001	0.16		25	100	0.0525	0.14
						12.5	50	0.048	0.08	Pupal	0	50	0.0172	0.1
						12.5	100	0.0026	0.12		0	100	0.0002	0.15
						12.5	200	<0.0001	0.18		0	200	<0.0001	0.02
						25	100	0.027	0.09		12.5	50	0.0428	0.09
						25	200	0.0002	0.15		12.5	100	0.0014	0.15
						50	200	0.0065	0.11		12.5	200	<0.0001	0.22
						100	200	0.0961	0.06		25	100	0.0056	0.13
					Larval	0	100	0.0003	0.05		25	200	<0.0001	0.19
						0	200	<0.0001	0.07		50	200	0.0094	0.12
						12.5	200	0.024	0.04	Larval	0	100	0.045	0.04
						25	100	0.0065	0.05		0	200	0.0005	0.07
						25	200	0.0003	0.06		12.5	200	0.0102	0.06
						50	100	0.0182	0.04		25	200	0.0205	0.05
						50	200	0.001	0.06		50	200	0.0179	0.05
					Egg	0	100	0.0367	0.03	Egg	0	100	0.0407	0.03
						25	100	0.0491	0.03		0	200	0.0538	0.03
						50	100	0.0184	0.03					

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table C-3 Continued. Mean life stage comparisons continued for CCA6 through CCA8:

Significant Pairwise Comparisons Testing Differences Between Each Level of Imidicloprid Dose at Each CCA and Each Life Stage														
CCA6					CCA7					CCA8				
Life Stage	Dose1	Dose2	PR>t	Difference	Life Stage	Dose1	Dose2	PR>t	Difference	Life Stage	Dose1	Dose2	PR>t	Difference
Adult	0	50	0.0246	0.14	Adult	0	100	0.0036	0.14	Adult	0	50	0.00707	0.11
	0	100	0.016	0.15		0	200	0.0034	0.14		0	100	0.0004	0.23
	0	200	0.001	0.21		12.5	50	0.0142	0.13		0	200	0.0007	0.22
	12.5	50	0.0525	0.14		12.5	100	0.0002	0.21		12.5	50	0.0121	0.19
	12.5	100	0.0374	0.15		12.5	200	0.0002	0.21		12.5	100	<0.0001	0.3
	12.5	200	0.0042	0.21		25	50	0.0278	0.12		12.5	200	0.0001	0.3
	25	50	0.0811	0.12		25	100	0.0004	0.2		25	50	0.0076	0.2
	25	100	0.059	0.13		25	200	0.0004	0.2		25	100	<0.0001	0.31
	25	200	0.0074	0.19	Pupal	0	100	0.0001	0.05		25	200	<0.0001	0.3
Pupal	0	100	<0.0001	0.14		0	200	<0.0001	0.06	Pupal	0	50	0.0942	0.05
	0	200	<0.0001	0.19		12.5	100	<0.0001	0.06		0	100	0.0007	0.11
	12.5	100	0.02	0.09		12.5	200	<0.0001	0.07		0	200	0.0011	0.11
	12.5	200	0.0003	0.14		25	100	0.0044	0.04		12.5	50	0.0189	0.09
	25	100	0.0096	0.1		25	200	0.0003	0.05		12.5	100	0.0002	0.15
	25	200	0.0001	0.15		50	100	0.0063	0.04		12.5	200	0.0002	0.14
	50	100	0.0191	0.09		50	200	0.0005	0.05		25	50	0.014	0.09
	50	200	0.0003	0.14	Larval	0	12.5	0.0023	-0.02		25	100	0.0001	0.15
Larval	0	100	0.0005	0.05		0	100	0.0127	0.02		25	200	0.0002	0.15
	0	200	0.0003	0.06		0	200	0.0009	0.02	Larval	0	100	0.0013	0.05
	12.5	25	0.0178	0.04		12.5	25	0.0958	0.01		0	200	0.0022	0.05
	12.5	100	<0.0001	0.07		12.5	50	0.02	0.02		12.5	100	0.0008	0.06
	12.5	200	<0.0001	0.07		12.5	100	<0.0001	0.04		12.5	200	0.0012	0.06
	25	100	0.0609	0.03		12.5	200	<0.0001	0.05		25	50	0.0609	0.03
	25	200	0.0511	0.03		25	100	0.0017	0.03		25	100	0.0002	0.07
	50	100	0.0079	0.05		25	200	0.0001	0.03		25	200	0.0003	0.07
	50	200	0.0063	0.05		50	100	0.0122	0.02		50	100	0.0435	0.04
Egg	0	100	<0.0001	0.05	Egg	50	200	0.0012	0.03		50	200	0.0609	0.03
	0	200	0.0006	0.04		0	200	0.0014	0.02	Egg	0	50	0.0158	0.02
	12.5	100	0.0012	0.04		12.5	100	0.0284	0.01		0	100	0.0002	0.03
	12.5	200	0.005	0.04		12.5	200	0.0004	0.02		0	200	0.0007	0.03
	25	100	0.0148	0.03		25	200	0.0495	0.01		12.5	25	0.0871	-0.02
	25	200	0.0477	0.03		50	200	0.0192	0.01		12.5	100	0.0071	0.03
	50	100	0.0043	0.04							12.5	200	0.0156	0.02
	50	200	0.0162	0.03							25	50	0.0016	0.03
											25	100	<0.0001	0.04
											25	200	<0.0001	0.04

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

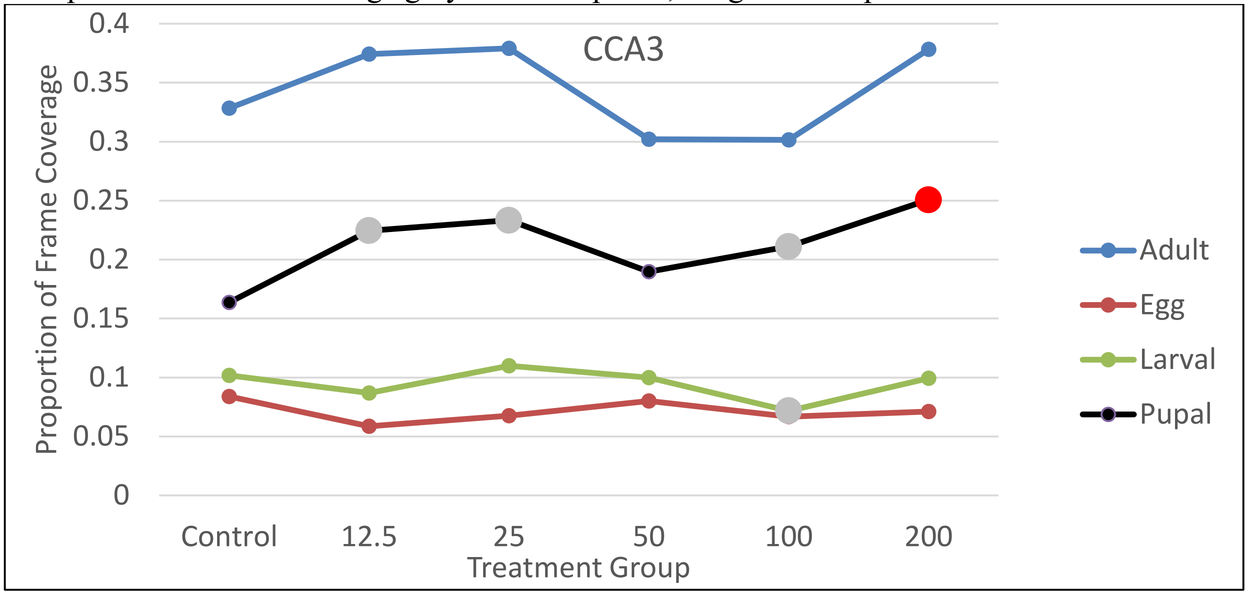


Figure C-1. Response of each life stage measured prior to the initiation of imidacloprid treatments at CCA3. Except for pupal cells, the baseline for each group was not significantly different (p<0.05) from control. For pupal cells, significant differences from the control were determined for treatment locations except for the 50 ppb group. The reason for these differences is not known.

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

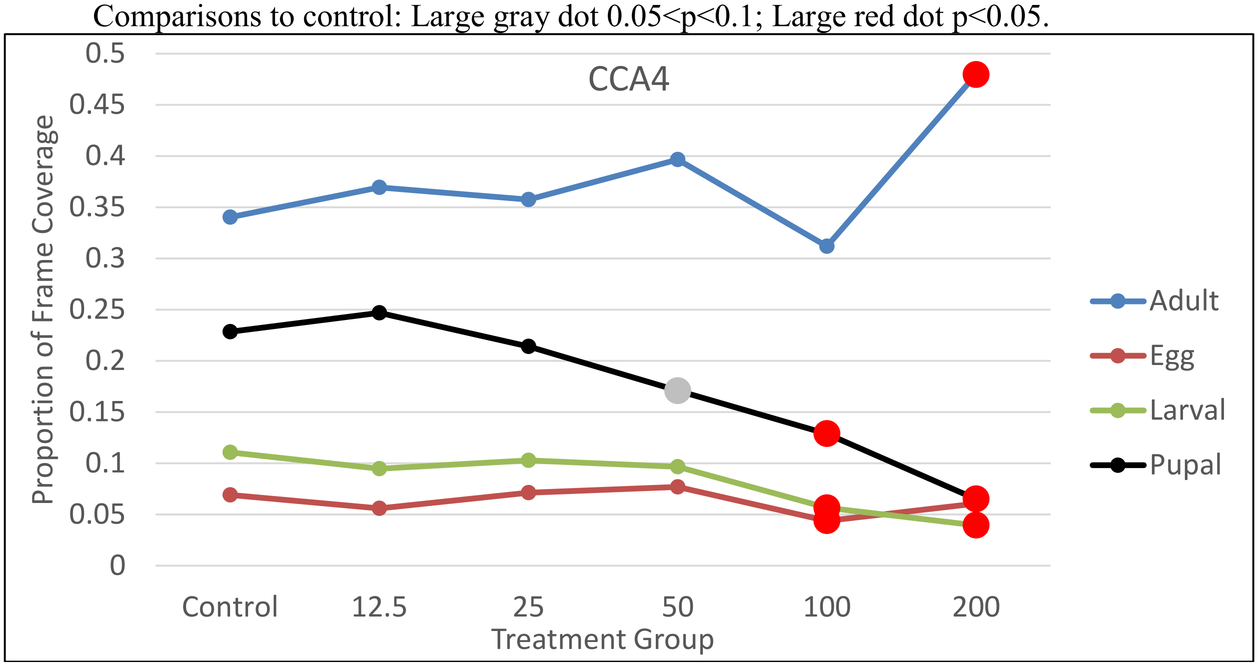


Figure C-2. At CCA4, significant dose related effects were measured for larval and pupal life stages. Sporadic effects were noted for egg and adult cells, which were most likely not related to dosing level.

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

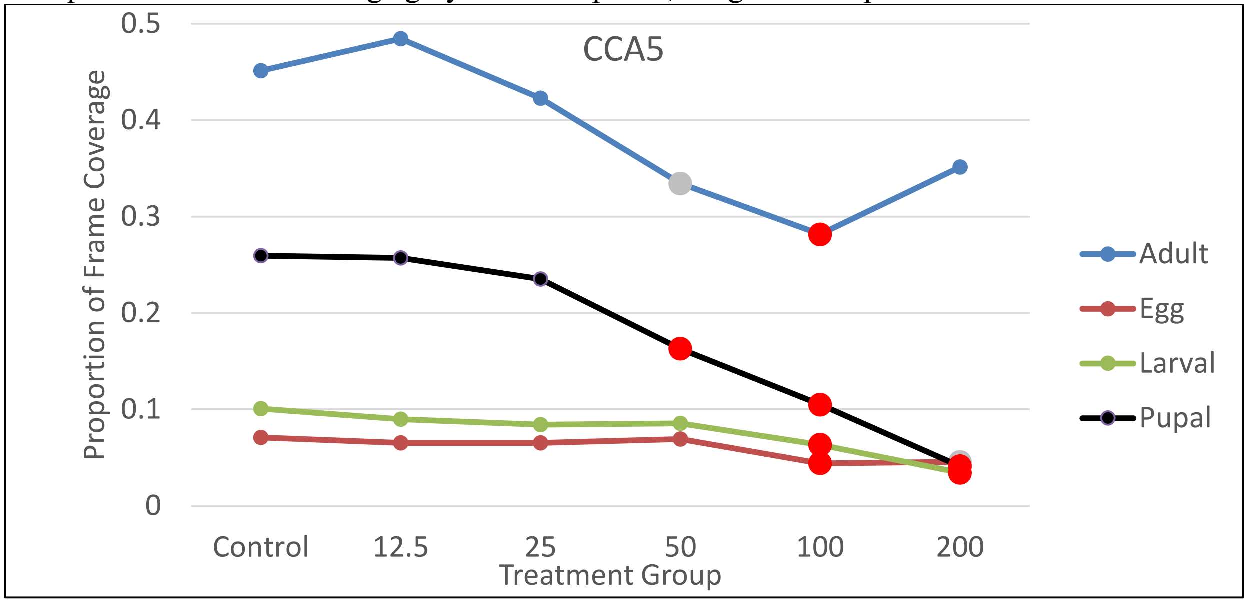


Figure C-3. Effects on larval and pupal cells measured at CCA4 were sustained in CCA5 with significant reductions from control (p<0.05) at the 50, 100, and 200 ppb levels for pupal cells and at the 100 and 200 level for larval cells.

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

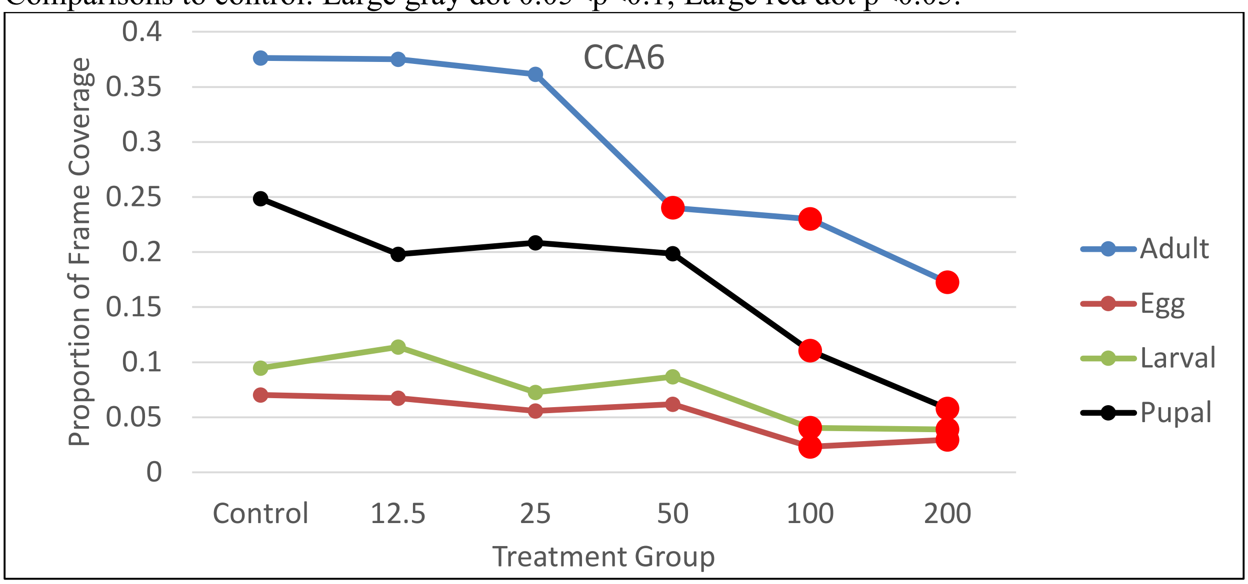


Figure C-4. At CCA 6 effects on pupal and larval cells were again sustained with onset of dose related effects measured for egg and adult cells. Specifically, significant reductions (p<0.05) in the number of adults and eggs were determined, along with effects to larval and pupal cells that were determined in CCAs 4 and 5.

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

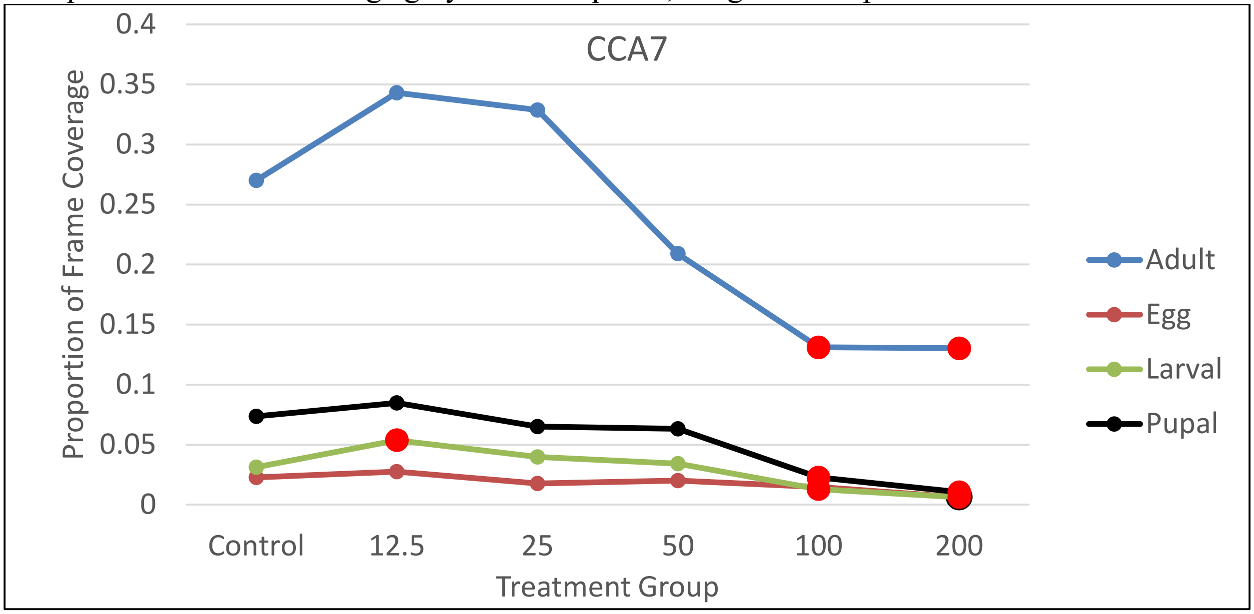


Figure C-5. At CCA7, decreases in all life stages from control group were measured for 100 and 200 ppb levels of imidacloprid.

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

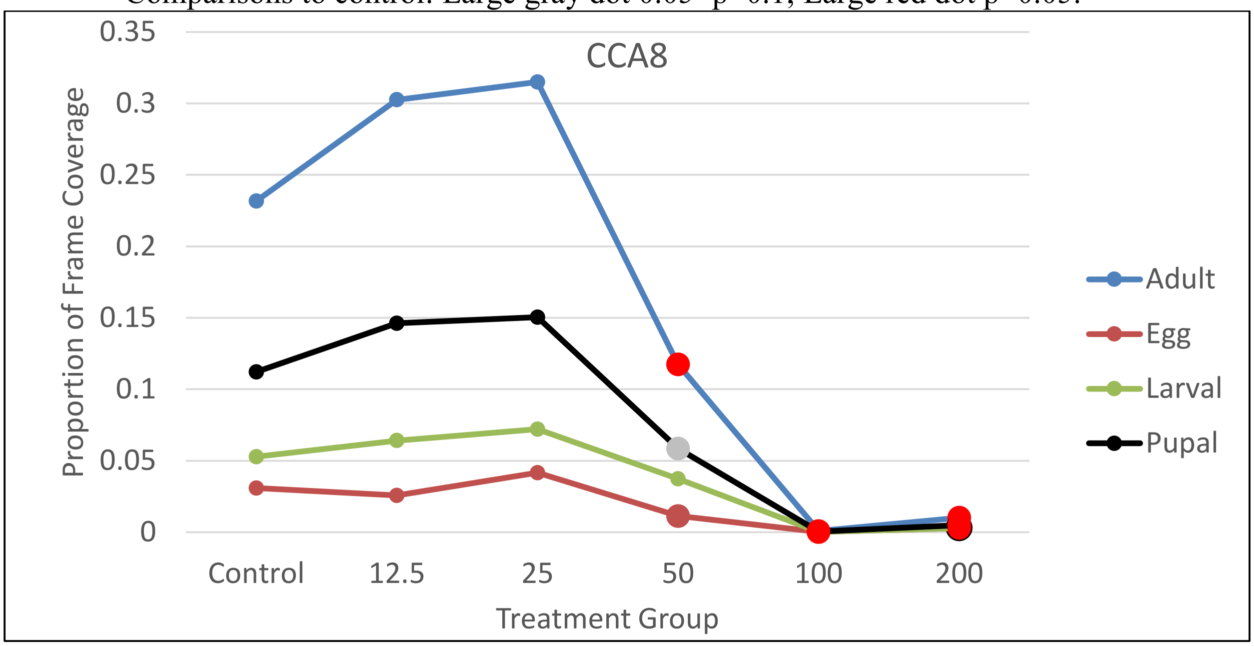


Figure C-6. Clear effects of hive death were measured at CCA8 at the 100 and 200 ppb treatments. Decreased numbers of adults, pupal, and egg cells were also measured at the 50 ppb treatment.

Food Stores

A second multivariate analysis was conducted to determine potential effects on honey and pollen cells. Univariate mixed model analyses were conducted for hive weight. The triple interaction of Day*Dose*Response was again significant in the combined analysis of pollen and honey cells (**Table C-4**).

Table C-4. Analysis of Food Stores (Pollen and honey cells, and hive weight

Overall Model Effect	Num DF	Den DF	Pr > F
Response	1	116	<.0001
Day	4	294	<.0001
Day*Response	4	306	<.0001
Dose	5	118	0.0002
Dose*Response	5	116	0.8633
Day*Dose	20	294	<.0001
Day*Dose*Response	20	306	<.0001

In light of the significant three way interaction, the approach used for bee life stages was followed where further multivariate analyses were conducted to determine the pattern of response at each CCA. Results from the regression analysis for each CCA indicated that there was a differential response over time (**Table C-5**).

- **CCA3:** As indicated in the results for bee life stages initial values for pollen and honey cells and hive weight were essentially similar between all treatment levels. Some slight differences between the controls and a few treatment levels for pollen cells were measured but these appeared to be sporadic and not related to dose levels (**Table C-6; Figure C-6 and Figure C-12**).

- **CCA4:** The number of **pollen cells and hive weight** were first affected at CCA4 with decreases measured in relation to increasing imidacloprid concentration (**Figure C-7 and Figure C-13**). Dose related effects on pollen cells were evident at the 50 ppb treatment with progressive decreases in numbers in relation to increases in level of dose. The pattern for hive weight mimicked that observed for pollen cells but significant effects were measured at the 100 ppb treatment and higher. Again, these decreases are rather significant because this assessment was made only 3 weeks into the exposure period.

- **CCA5:** The pattern in response for pollen cells and hive weight was similar to that observed at CCA4 (**Figure C-8 and Figure C-13**). An additional effect was observed for hive weight in that a significant effect was also observed at the 50 ppb level of dose. Together the effects on honey cells and hive weight indicated two groupings of effects for the levels of dose: One where the numbers were similar between 0, 12.5, and 25 ppb treatments and a second where effects of imidacloprid dose were measured for 50, 100, and 200 ppb treatments There was one significant effect indicated for honey cells but it was an isolated effect where higher numbers were recorded for the 200 ppb treatment versus the rest of the treatments.

- **CCA6:** At CCA6 decreased numbers of pollen cells were only measured at the 200 ppb level of imidacloprid dose (**Figure C-9**). The response of **honey cells**, on the other hand, now mimicked the initial response for pollen cells in that decreased numbers compared to the control were measured at 50 ppb and higher dose levels. The two grouping pattern noted at CCA5 was now reflected in the responses for number of honey cells and hive weight (**Figure C-9 and Figure C-14).**

- **CCA7:** The response at CCA7 was very similar to that measured at CCA6 with the same noted grouping of effects for honey cells and hive weight (**Figure C-10 and Figure C-15**). Numbers of pollen cells were not as plentiful as for honey cells throughout the study. By this assessment all treatment means were low with values either at or below 0.05% frame coverage. The lower coverage at this CCA is most likely the cause for diminishing effects of imidacloprid treatment at this CCA and perhaps noted at the previous CCA6. Effects though were still measured at the 200 ppb level when compared to the control.

- **CCA8:** Similar to the effects measured for life stages, the pattern noted at CCA7 was reflected at the final assessment at CCA8 where curvature in response was measured for all life stages, reflecting the extreme loss of hives at the 100 and 200 ppb levels of imidacloprid dose (**Table C-5**). Lowered counts for honey and pollen cells were also measured for the 50 ppb treatment when compared to control values, indicating lower hive vigor (**Figure C-11 and Figure C-16**). These results provide evidence for the sustained influence of effects noted at CCA7 on the over wintering heath of hives.

Table C-5. Regression for linear and quadratic effect conducted within each CCA and for pollen or nectar cells and hive weight.

Food Storage Variable	Regression Effect	Regression Results for Each CCA					
		CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
		Pr>t	Pr>t	Pr>t	Pr>t	Pr>t	Pr>t
Honey Cells	Dose Linear	0.5037	0.5842	0.0056	0.0001	<.0001	<.0001
	Dose Quadratic	0.2801	0.7943	0.0821	0.9244	0.2545	0.0272
	Dose Cubic	0.7263	0.8815	0.9365	0.6355	0.644	0.1049
Pollen Cells	Dose Linear	0.2855	<0.0001	<0.0001	<0.0001	0.002	<.0001
	Dose Quadratic	0.5849	0.0007	0.0006	0.9176	0.6843	0.0279
	Dose Cubic	0.222	0.8135	0.7822	0.4604	0.8962	0.0215
Hive Weight	Dose Linear	0.9952	0.0027	0.0011	0.0014	0.0007	<.0001
	Dose Quadratic	0.9164	0.2306	0.0023	0.2494	0.1424	0.0541
	Dose Cubic	0.0581	0.7024	0.5848	0.4948	0.4931	0.002

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table C-6. Mean comparisons for the response between each level of dose for number of honey and pollen cells and hive weight conducted at each CCA. For pollen and honey cells the difference is the percent frame coverage of dose1 minus dose2 where a positive value indicates a higher value for the lower dosage and a negative value indicates a lower value for the lower dosage. For hive cells the difference represents measurements made in pounds (lbs).

Significant Pairwise Comparisons Testing Differences Between Each Level of Imidicloprid Dose at Each CCA for Food Stores														
CCA3					CCA4					CCA5				
Effect	Dose1	Dose2	PR>t	Difference	Effect	Dose1	Dose2	PR>t	Difference	Effect	Dose1	Dose2	PR>t	Difference
Pollen	0	25	0.0582	0.02	Pollen	0	50	<.0001	0.04	Pollen	0	50	<0.0001	0.07
	0	200	0.071	0.02		0	100	<.0001	0.06		0	100	<0.0001	0.09
Hive Weight	0	12.5	0.0149	-4		0	200	<.0001	0.07		0	200	<0.0001	0.1
	12.5	100	0.0081	5.2		12.5	50	0.0067	0.03		12.5	50	0.0001	0.07
	12.5	200	0.0261	4.1		12.5	100	<.0001	0.05		12.5	100	<0.0001	0.09
						12.5	200	<.0001	0.05		12.5	200	<0.0001	0.1
						25	50	0.0055	0.03		25	50	0.0038	0.05
						25	100	<.0001	0.05		25	100	<0.0001	0.07
						25	200	<.0001	0.06		25	200	<0.0001	0.09
						50	100	0.0568	0.02		50	200	0.033	0.04
						50	200	0.0063	0.03	Honey	0	200	0.009	-0.08
					Hive Weight	0	100	0.0208	4.3		12.5	200	0.0177	-0.08
						0	200	0.0278	4.1		25	200	0.0498	-0.07
						12.5	50	0.0391	4.4		50	200	0.0036	-0.11
						12.5	100	0.0056	6.1		100	200	0.0233	-0.08
						12.5	200	0.0074	5.9	Hive Weight	0	12.5	0.0305	3.6
						25	100	0.0273	4.8		0	50	<0.0001	7.4
						25	200	0.0348	4.5		0	100	<0.0001	10.7
											0	200	0.0015	5.5
											12.5	50	0.0464	3.8
											12.5	100	0.0005	7.1
											25	50	0.0024	6
											25	100	<0.0001	9.3
											25	200	0.0311	4.1
											50	100	0.0843	3.3
											100	200	0.0081	-5.2

Table C-6 Continued. Mean food storage comparisons continued for CCA6 through CCA8:

Significant Pairwise Comparisons Testing Differences Between Each Level of Imidicloprid Dose at Each CCA for Food Stores														
CCA6					CCA7					CCA8				
Effect	Dose1	Dose2	PR>t	Difference	Effect	Dose1	Dose2	PR>t	Difference	Effect	Dose1	Dose2	PR>t	Difference
Pollen	0	12.5	0.0086	-0.03	Pollen	0	200	0.0087	0.02	Pollen	0	12.5	0.0677	-0.02
	0	200	0.0022	0.03		12.5	50	0.0203	0.02		0	50	0.0601	0.02
	12.5	50	0.0011	0.04		12.5	200	0.0009	0.04		0	100	0.0003	0.05
	12.5	100	0.0015	0.04		25	50	0.0492	0.02		0	200	0.0004	0.05
	12.5	200	<0.0001	0.06		25	200	0.0027	0.03		12.5	50	0.017	0.05
	25	50	0.0545	0.02		100	200	0.0468	0.02		12.5	100	<0.0001	0.07
	25	100	0.0683	0.04	Honey	0	50	0.0392	-0.003		12.5	200	<0.0001	0.07
	25	200	0.0006	-0.001		0	100	0.0083	0.11		50	100	0.0975	0.02
	50	200	0.0967	0.02		0	200	0.0002	0.14	Hoiney	0	50	0.08	0.05
	100	200	0.078	0.02		12.5	50	0.0649	0.11		0	100	0.0008	0.1
Honey	0	50	0.0255	0.11		12.5	100	0.0188	0.14		0	200	0.003	0.09
	0	200	0.0002	0.19		12.5	200	0.0011	0.2		12.5	50	0.018	0.08
	12.5	200	0.0036	0.17		25	50	0.032	0.13		12.5	100	0.0002	0.13
	25	50	0.0144	0.14		25	100	0.0082	0.16		12.5	200	0.0007	0.12
	25	100	0.0623	0.11		25	200	0.0004	0.22		25	50	0.0394	0.07
	25	200	0.0002	0.22	Hive	0	50	0.0471	9.8		25	100	0.0006	0.12
	100	200	0.0436	0.11	Weight	0	100	0.0206	11.5		25	200	0.0019	0.11
Hive	0	50	0.0818	8.5		0	200	0.0077	13.3	Hive	0	25	0.0618	-11.7
Weight	0	100	0.0389	10.2		12.5	50	0.0228	13	Weight	0	100	0.0002	24
	0	200	0.0151	12		12.5	100	0.0105	14.7		0	200	0.0013	20.7
	12.5	50	0.0615	10.6		12.5	200	0.0042	16.5		12.5	100	<0.0001	30.4
	12.5	100	0.0316	12.2		25	50	0.021	13.2		12.5	200	0.0003	27
	12.5	200	0.0137	14.1		25	100	0.0096	14.9		25	50	0.0281	16
	25	50	0.0292	12.4		25	200	0.0039	16.7		25	100	<0.0001	35.9
	25	100	0.014	14							25	200	<0.0001	32.5
	25	200	0.0057	15.9							50	100	0.0069	19.9
											50	200	0.0241	16.5

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

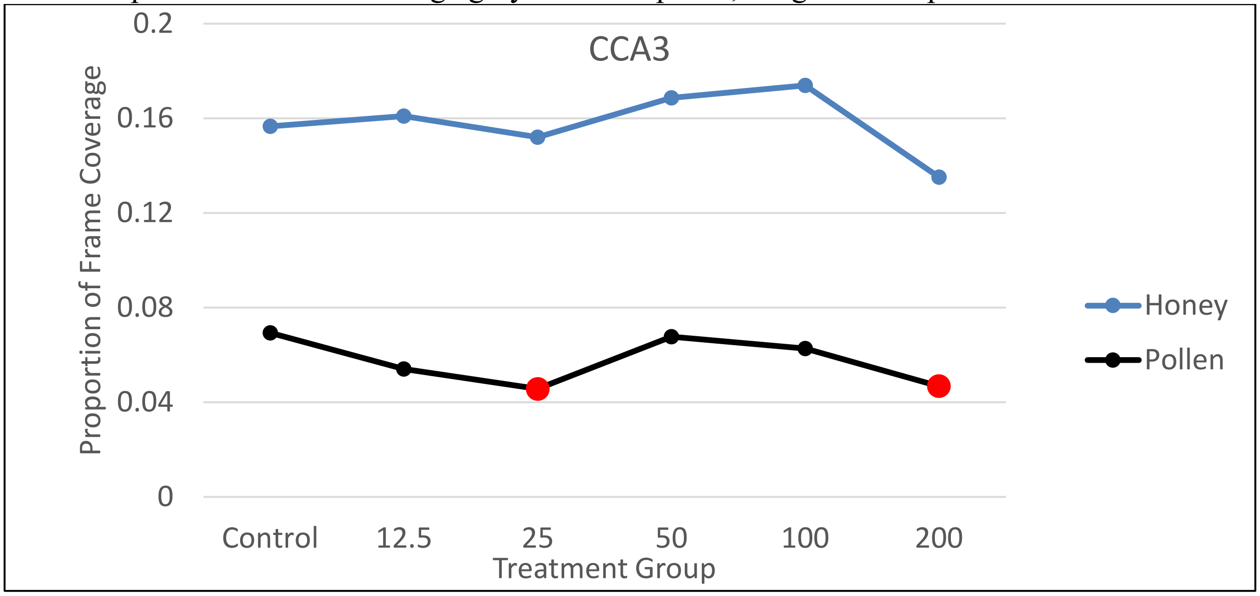


Figure C-6. Response of pollen and honey cells in relation to treatment group at CCA3.

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

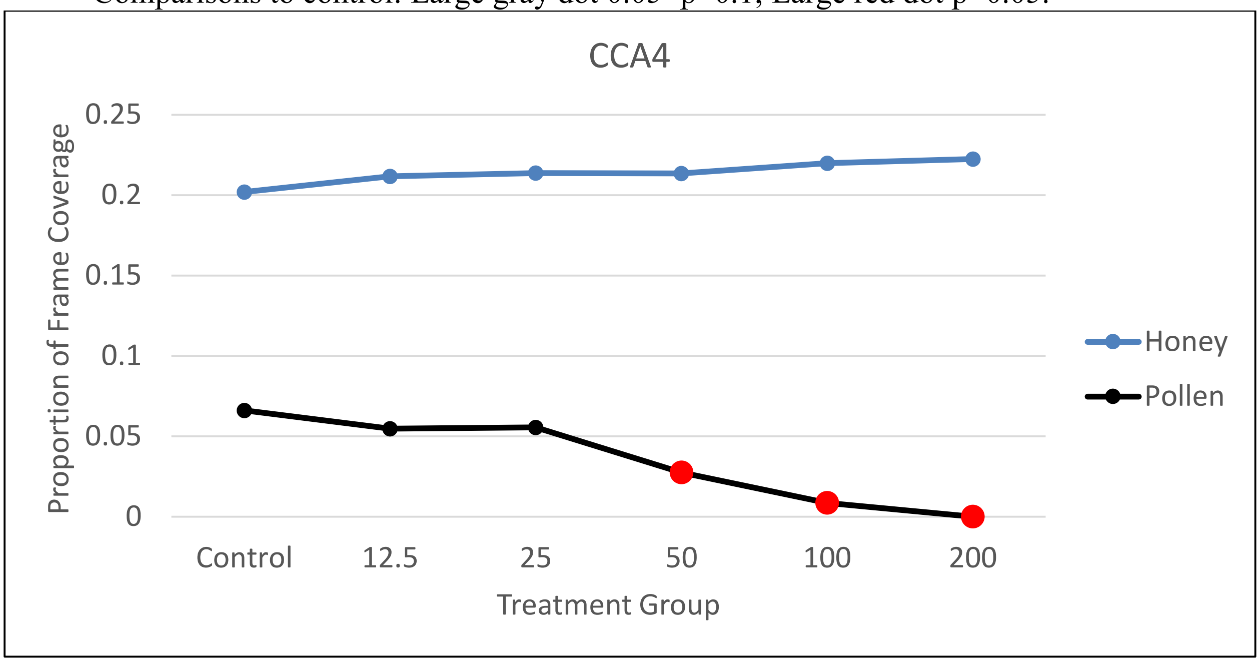


Figure C-7. At CCA4, onset of dosage related effects were measured for pupal cells and hive weight (see Figure C-13).

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

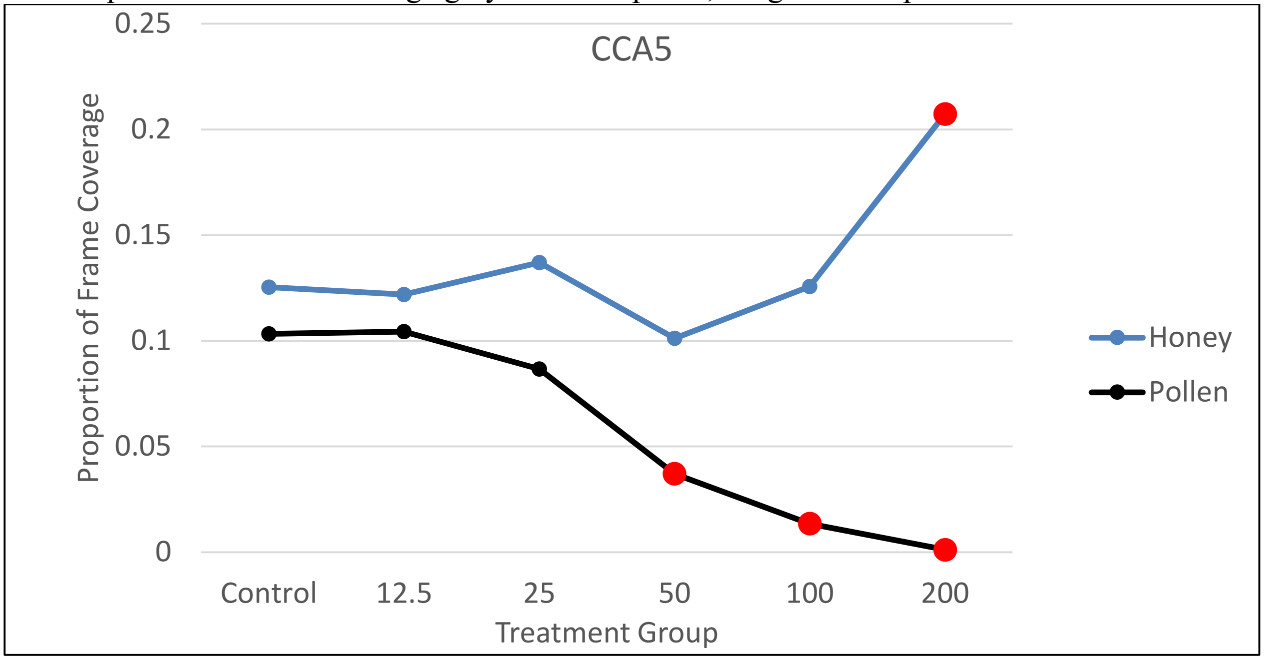


Figure C-8. At CCA5, the effects on pollen cells and hive weight (See Figure C-14) measured previously at CCA4 were sustained at the 50, 100, and 200 ppb treatment groups (p<0.05) for pollen cells with additional significant effects at the 50 ppb level (p<0.05) measured for hive weight. An isolated significant effect for honey was noted at the 200 ppb treatment.

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

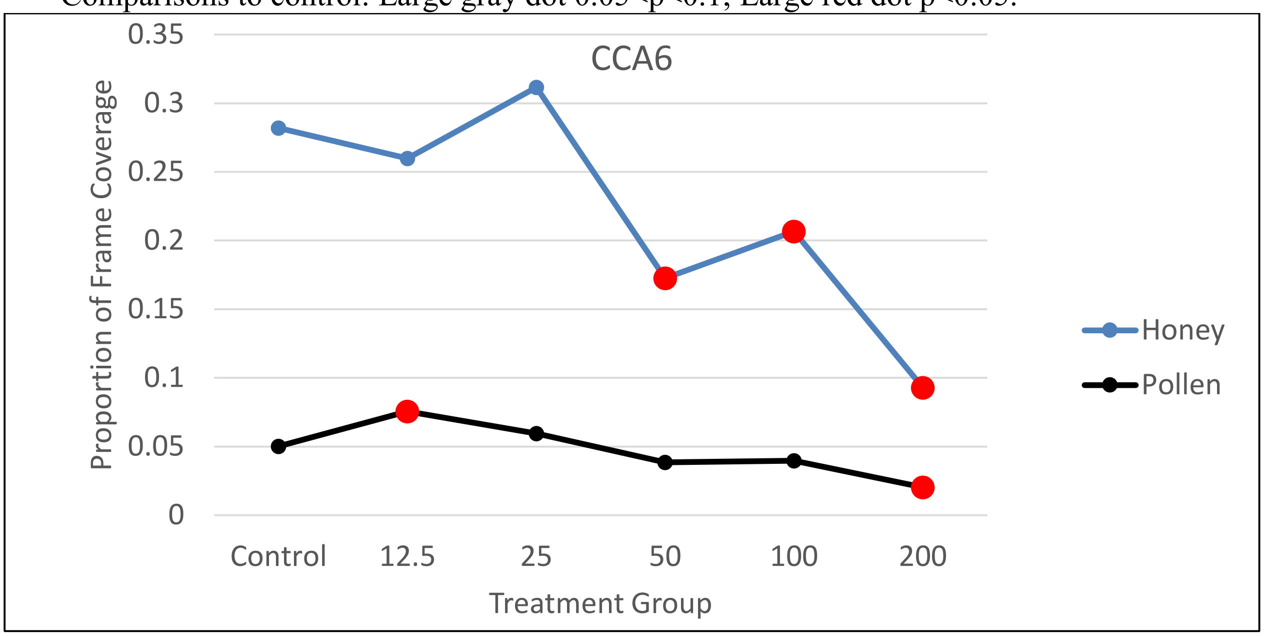


Figure C-9. At CCA6, significant treatment related effects were also measured for the number of honey cells with decreases at the 50, 100 and 200 ppb dose levels. Effects measured on hive weight at CCA5 were sustained at CCA6 (See Figure C-15).

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

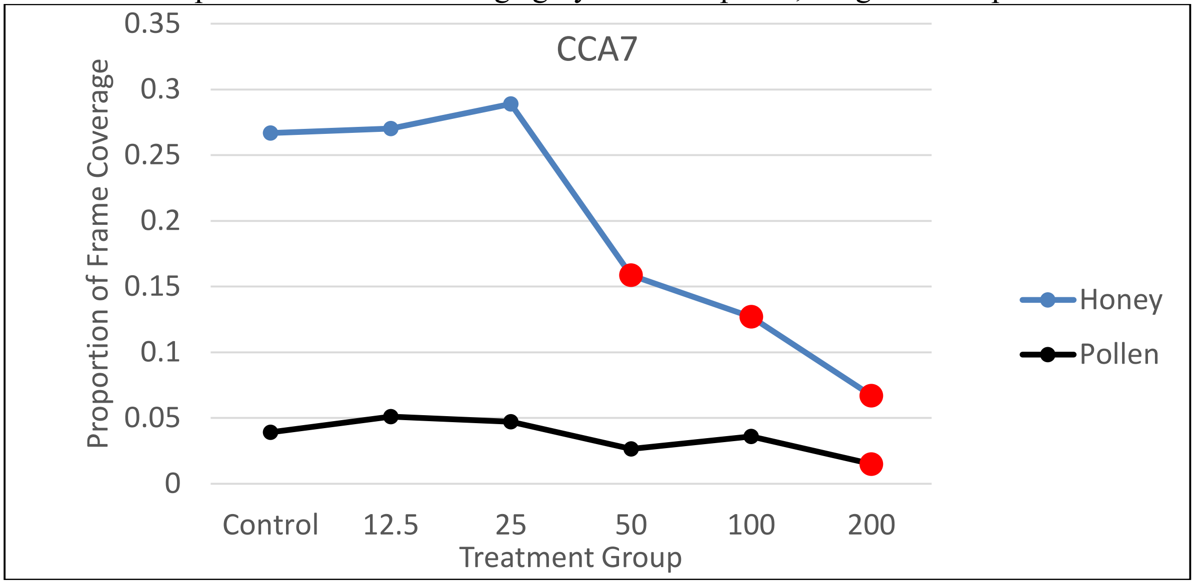


Figure C-10. At CCA7 the effects on number of honey cells and hive weight (see Figure C-16) appear to form two groups where numbers are similar between 0, 12.5 and 25 ppb treatments and then imidacloprid dose-related effects were measured for the 50, 100, and 200 ppb treatments (See Figure C-x for hive weight).

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

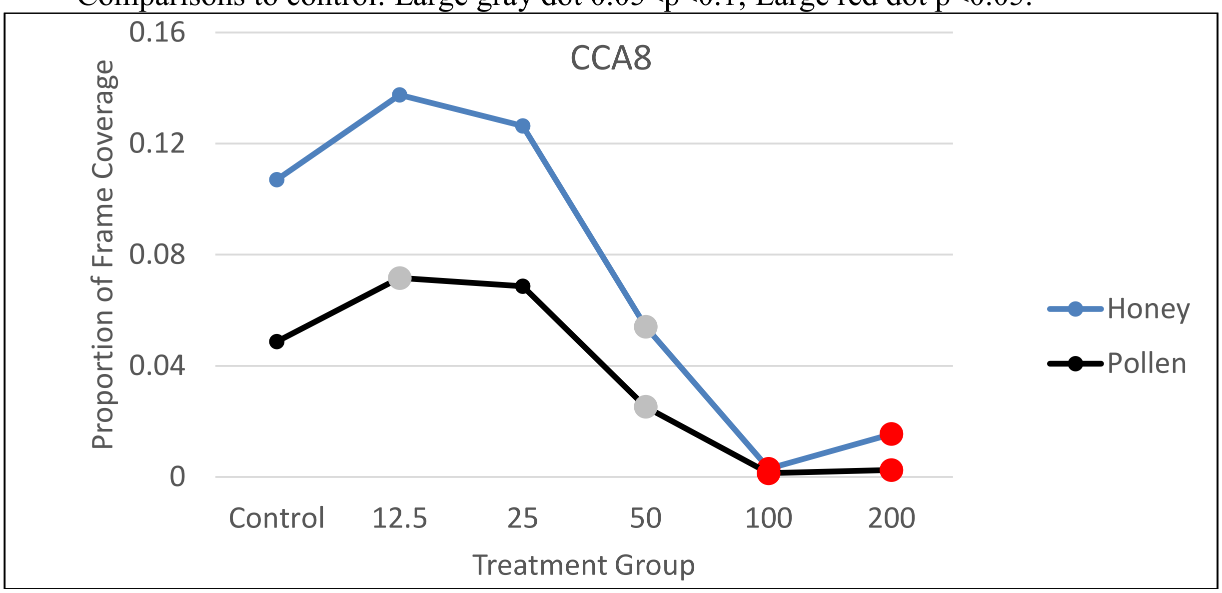


Figure C-11. At CCA8 effects measured at the 100 and 200 ppb treatments resulted in loss of colonies after overwintering (see Figure C-17for hive weight graph). Lower vigor at 50 ppb treatment was also indicated by the lower numbers of adults, pupal, and egg cells when compared to the control hives.

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

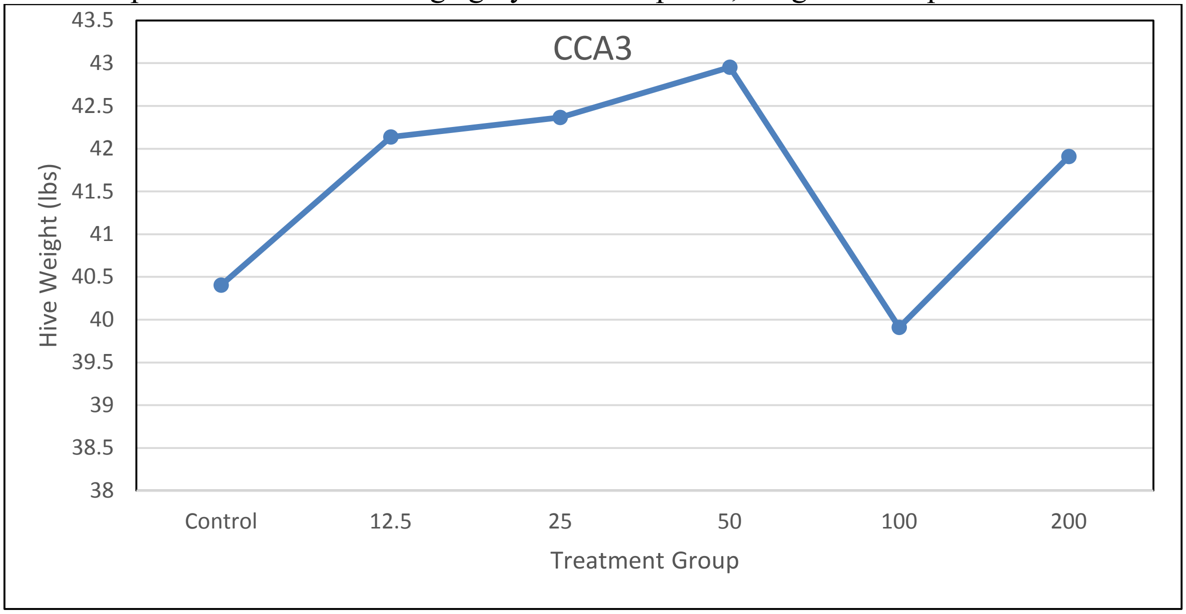


Figure C-12. Response of hive weight in relation to treatment group at CCA3

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

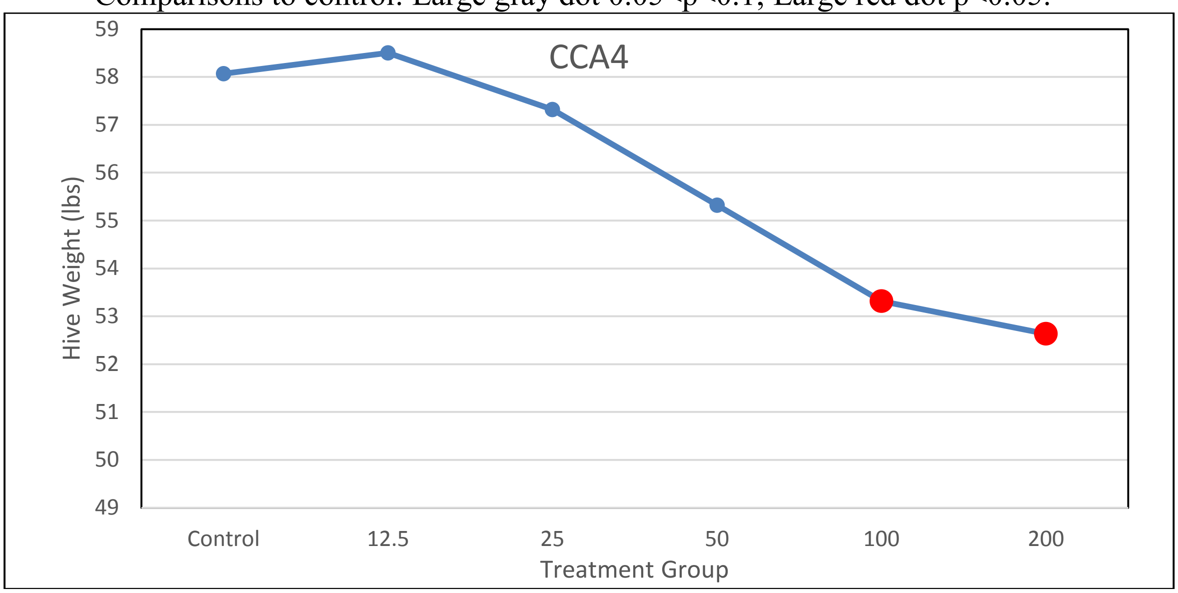


Figure C-13. Response of hive weight in relation to treatment group at CCA4.

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

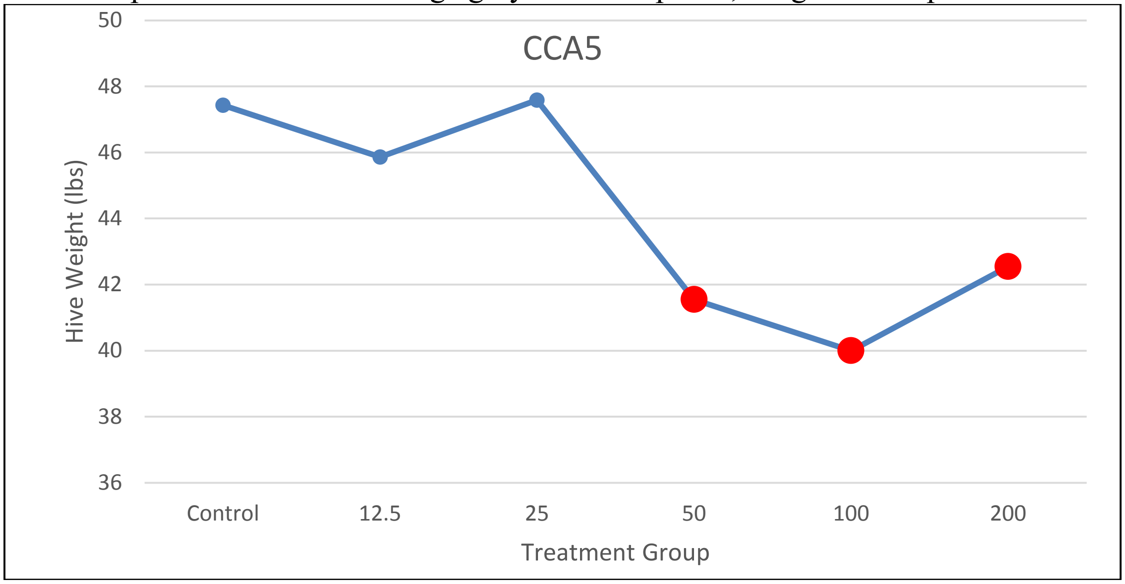


Figure C-14. Response of hive weight in relation to treatment group at CCA5

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

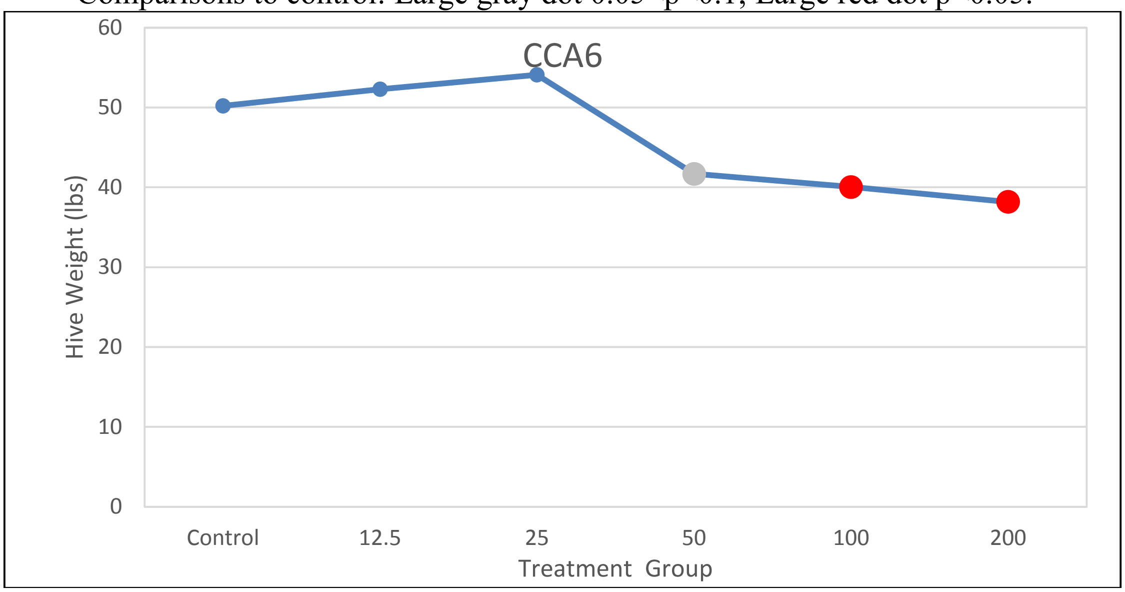


Figure C-15. Response of hive weight in relation to treatment group at CCA6

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

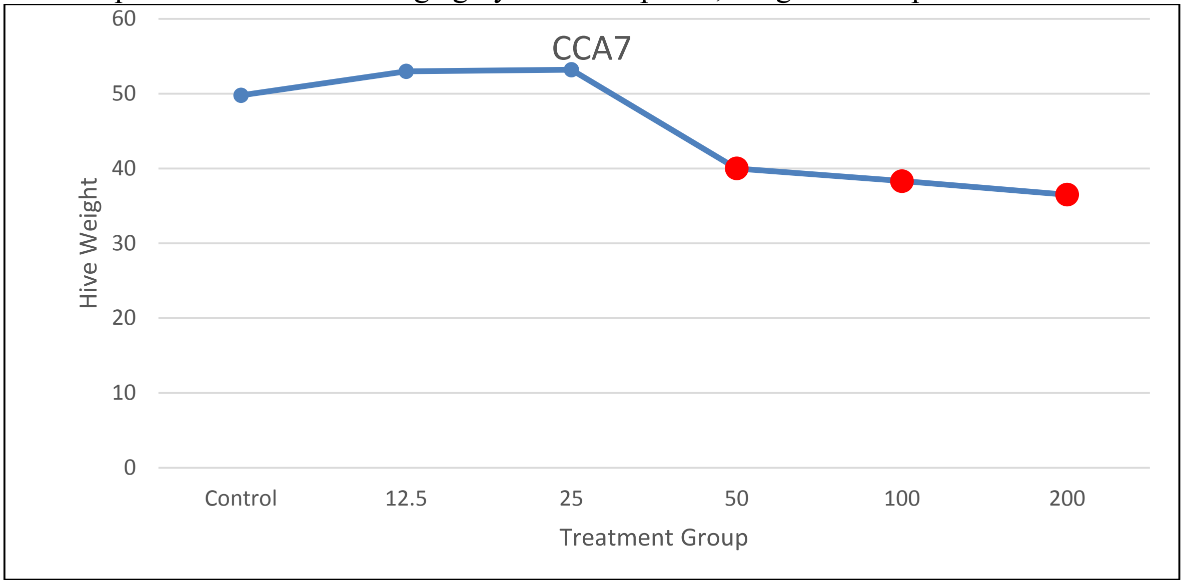


Figure C-16. Response of hive weight in relation to treatment group at CCA7

Comparisons to control: Large gray dot 0.05<p<0.1; Large red dot p<0.05.

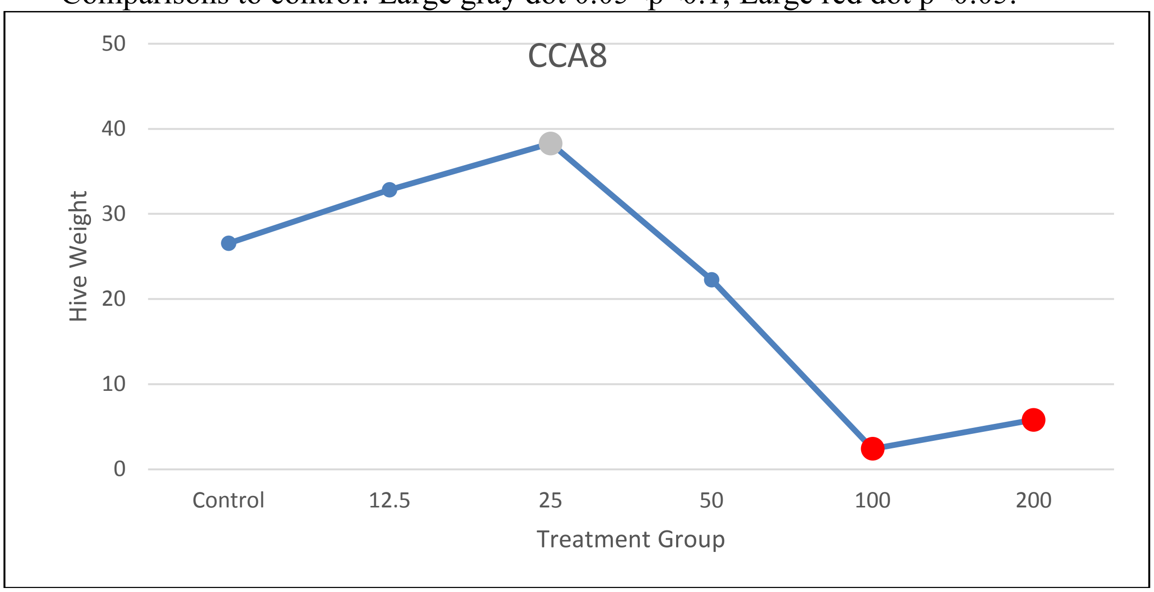


Figure C-17. Response of hive weight in relation to treatment group at CCA8

EVALUATION REPORT - Imidacloprid Pollen Colony Feeding Study

John Troiano, Research Scientist III
Alexander Kolosovich, Senior Environmental Scientist (Specialist)

June, 2018

A review of:- Dively, G.P., Embrey, M.S., Kamel, A., Hawthorne, D.J., & J.S. Pettis. (2015). Assessment of chronic sublethal effects of imidacloprid on honey bee colony health. PLoS ONE, 10(3), e011874. DOI:10.1371/journal.pone.0118748.

Introduction

The objective of the study was to determine sublethal effects on bee colony health as a result of exposure to beebread fortified at 0, 5, 20, or 100 ug/kg of imidacloprid. The study was replicated in two years in 2009 and 2010. The study design differed between the two years. In 2009, 2 replicate hives at each treatment level were located in 5 separate apriaries whereas in 2010 there was only replicate hive in each treatment placed in 7 separate apriaries. Spacing between apiaries was not specified but within an apiary the hives were spaced 10 meters apart.

Colony health was assessed by measuring the percentage of frame area covered with drawn cells, adult bees, capped bees, cells with older larvae, cells packed with beebread and honey, the number of hives that survived overwintering, and measures of foraging activity. No significant effect of imidacloprid treatment was measured in either year on coverage of drawn cells, beebread, capped brood, and adult bees where measurements made at the end of exposure and prior to overwintering of hives. No dose effects indicating decreases in numbers were indicated at the individual time intervals where measurements were taken to follow development throughout the summer and fall. No significant consistent effects were indicated for measurements made on foraging activity. The area of frame coverage for honey was consistently greater at the 100 ug/kg treatment in both years. Inconsistent effects between years were noted for measurements made on frequency of queen events, on number of supesedual cells, and in analyses conducted on cumulative area under curves.

Statistical Analysis

Conclusions on the effect of treatment on overwintering survival of colonies also indicated inconsistent effects when a statistical analysis for effect of dose was measured within each year. In 2009 the statistical test used to measure effect of treatment on survival rate indicated a significant effect of treatment with less hives surviving in imidacloprid treatments, whereas, in 2010 survival percentages were similar between all treatment levels and no associated statistical significance was measured. A further manipulation of the data that combined survival data obtained prior to wintering and then after wintering produced an overall significant effect of treatment, indicating reduction in survival rate in response to imidacloprid dose. A potential oddity in the data was the measure of perfect survival of all hives in the control treatment in 2009 where 10 of 10 hives successfully overwintered. A boxplot of all 8 values composed of the rate measured at each treatment level in both years indicates that this may be an extreme value (Figure 1). The proportional mean of the 8 values is 0.62 with a standard deviation of 0.21. All values except for the for the control value in 2009 fall within 1 standard deviation of the mean where the range in measured values is 0.43 to 0.8 and the range for one standard deviation is from 0.41 to 0.83. The measurement of an overall significant effect is most likely driven by this one value, especially when the months are combined and this value receives even greater weight. The validity of measuring this extreme event in this study is not at question. But survival of 100% hives from overwintering in actual practice is an event with an extremely low probability of occurrence. The problem statement for investigating effects of stressors on rate of overwintering of beehives was based on an elevated rate of decreased survival from a normal occurrence of around 15% to an increased rate of 25 to 30% of hives not surviving overwintering. The veracity of results obtained from the study is not questioned. The noted inconsistencies in effects between years, the lack of an imidacloprid effect on bee life stages, and potential for skewing of results due to the survival rate for the control in 2009 indicate that verification of effect of exposure by beebread equires more study. Consequently, derivation of a LOEC or NOEC from this study would have a large uncertainty associated with it.

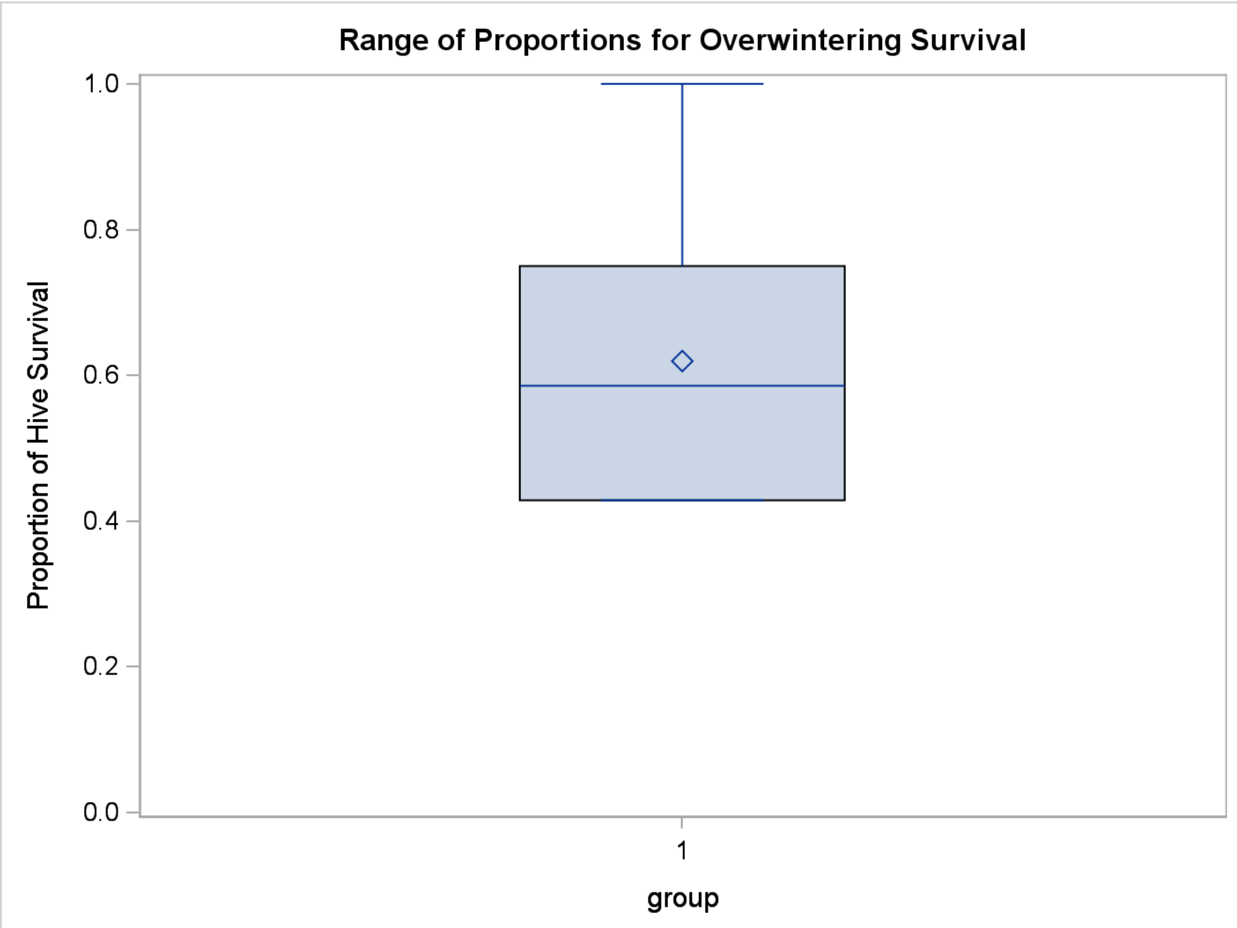
Conclusion

Investigation of the effect of exposure of beehives from ingestion of imidacloprid from pollen is of critical importance to determining the relevant endpoints for assuring healthy bee colonies. However, derivation of a LOEC or NOEC from this study would have large uncertainty associated with it. Even though the authors provide a basis for continued investigation on effect of ingestion of beebread dosed with imidacloprid, causes for uncertainty are the inconsistencies measured for survival rate between years and lack of effects on bee life stages. Effects on bee life stages prior to overwintering would be indicators for weakening of hives prior to overwintering.

Inspection of the data indicated that complete survival of hives in the control treatment in 2009 is a result that is odd compared to the rest of the treatments. Furthermore, complete survival is not an expected biological event. Consequently, this one value most likely exerts extraordinary influence on the significance measured when data were combined between months and subject to further statistical analysis. Replication of the experiment is required in order to verify a consistent effect of ingestion of beebread dosed with imidacloprid on health and survival of bee colonies.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 1. Boxplot for distributional statistics for proportion of overwintering survival of hives. Note that except for the control value proportion at 1.0 (all survived) measured for the control in 2009, the remaining 7 values are captured within the range for a distribution based on the mean plus/minus 1 standard deviation.



Data Evaluation Report

Study Titles:

Louque, J. (2016): Colony feeding study evaluating the chronic effects of clothianidin-fortified sugar diet on honey bee (*Apis mellifera*) colony health under free foraging conditions.

Final Report Source:
Smithers Viscient, unpublished report No: 13798.4143 Activity ID EBTIN114, February, 2016

Years of study: 2014-2015

PMRA#: 2610259
PMRA DACO#: 9.2.4.3
MRID: 49836101

Study Type:
Tier II colony feeding study conducted in an open field

Review Date (final): February 7, 2017

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Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

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Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Executive Summary

A colony feeding study was conducted with honey bees to assess the potential for long-term effects, including overwintering survival, resulting from exposure to clothianidin in artificial nectar (i.e. spiked sugar) diet. The study was conducted in twelve test areas of low agricultural cultivation (Apiaries A – L) in North Carolina from June 17, 2014 (when hives were moved to the study apiaries) to April 27, 2015 (final colony condition assessment). Eighty-four hives were divided according to hive strength (number of brood frames) with the strongest 7 hives ssigned to Apiary A and the weakest 7 hives assigned to Apiary L. Within each apiary, the 7 hives were randomly assigned to control and treatment groups.

At each apiary, five test hives were artificially fed with 50% sugar solution spiked with clothianidin at 10, 20, 40, 80 or 160 µg ai/L for six weeks continuously in the field, with two hives at each apiary serving as controls. Assuming the density of a 50% sugar solution is 1.2296 g/ml, the reviewer calculated that the test concentrations at 10, 20, 40, 80 or 160 µg/L are equivalent to 8.1, 16.3, 32.5, 65.1, and 130.1 ppb (µg/kg), respectively. Residue analysis of the dosing solutions on 7/3/14 and 7/28/2014 provided mean measured ppb (µg/kg) concentrations of <LOD (0.5 ppb), 9.5, 19.0, 35.6, 71.8 and 140.0 ppb (µg/kg), respectively with stability samples from hive feeders indicating 93—105% recoveries in the dosing solutions.

Nine Colony Condition Assessments (CCAs) were conducted during the study. Three CCAs (CCA1 – 3; May 12, June 2 and 18, respectively) were conducted prior to feeding to determine hive strength and initial hive conditions. A CCA was conducted during exposure (CCA4; July 15) with another one conducted within one week after termination of exposure (CCA5; August 5) which characterize hive conditions during exposure. Two more CCAs were conducted at 5 (CCA6; Sept. 8) and 10 (CCA7; Oct. 14) weeks after exposure (or 11 for hives in the 80 and 160 ppb treatment groups, only) to assess the chronic effect following exposure to clothianidin and to characterize pre-overwintering hive conditions. Two final CCAs were conducted after overwintering in mid-March 2015 (CCA8; Mar 17-19 for all treatment groups except for the 80 ppb treatment group whose CCA was delayed to April 2) and mid-late April (CCA9; April 22-27) to assess potential exposure impact on survival and chronic colony level effects. Multiple parameters, such as hive weight, number of individuals at different life stages in the hive, hive honey and pollen stores, and hive overwintering survival, were measured during the course of the study.

Levels of clothianidin residues were measured before (in pollen and nectar collected from hives' at CCA2), during (uncapped nectar at CCA4), immediately post-exposure (uncapped nectar and bee bread at CCA5), 10 weeks after the feeding exposure (capped honey at CCA7) and following overwintering (capped honey at CCA9). Potential contamination of colonies by pesticides from other food sources was monitored using pollen and uncapped nectar collected in additional hives at each apiary that served as monitoring hives. The results showed that while there were a few instances of clothianidin detected in the pollen (bee bread) and nectar (uncapped and capped) of the control hives, the frequency and magnitude of these detections is not expected to confound the results of this study. The residue samples collected at CCA2 were from four hives, while residue samples at CCAs 4, 5, 7 and 9 were from all available hives with sufficient material for analysis. Mean residues measured in hive matrices generally demonstrated that higher treatment exposures corresponded well to higher residues in hive matrices. There were individual hive variations in measured residues, with some overlap in measured hive concentrations, particularly at the lower doses. This variability likely originates from the limited spatial and temporal sampling methodology (*i.e.* one sample from one side of the comb on one frame to represent a hive, and only at 4 CCAs) employed for this study. Mean measured residues at CCA5 (end of exposure) in uncapped nectar were 68% (5.5 ppb), 62% (10.2 ppb), 61% (19.9 ppb), 57% (37.0 ppb) and 51% (65.7 ppb) and in bee bread were 43% (3.5 ppb), 41% (6.7 ppb), 37% (12.2 ppb), and 55% (35.8 ppb) compared to the nominal concentrations from the feeding solutions (10, 20, 40, 80 and 160 µg/L or 8.1, 16.3, 32.5, 65.1 and 130.1 µg/kg; insufficient bee bread was available for sampling in the nominal 160 µg/L treatment). This dilution is expected since bees could forage on outside pollen and nectar sources, and hive pollen (bee bread) includes nectar (both from the supplied sucrose solution and untreated foraged) and pollen (untreated). See **Section 3.7** for more details regarding the residues of clothianidin in the dosing solutions and hive matrices.

Study Endpoint Conclusions:

Colony Survival:

Overwintering mortality was 65%, 75%, 33%, 50%, 17% and 100% in the control, 10, 20, 40, 80 and 160 µg/L treatment groups, respectively. As overwintering losses were so high in the controls that statistical differences would not be able to be detected, no statistical analysis was conducted on these colonies for the CCAs following overwintering and no NOAEC or LOAEC could therefore be determined for this endpoint.

Life Stage Endpoints:

Specifically, when considering the number of adults, pupae, total brood and total live bees, the differences from control were apparent both visually and statistically, particularly in the three highest treatment groups. For the number of adults, the onset of a decline in numbers occurred at least one CCA earlier (CCA5) in the three highest treatment groups than in the control, 10 and 20 µg/L treatment groups. Consistent significant effects were observed at multiple CCAs, showing a dose-response relationship beginning at the 40 µg/L treatment group for adults, pupae, total brood (pupae, larvae and eggs combined) and total live (all life stages combined).

Food Stores

When examining the effects on food stores (pollen and nectar), the analyses did not determine any consistent and significant reductions in pollen and nectar stores at the 10 and 20 µg/L treatment groups. This is distinguished from the 40 µg/L group where effects on pollen in particular were very apparent during and immediately after exposure, when compared alongside the response of the control (though these effects had lessened by the last two CCAs prior to overwintering). Similarly, significant dose-repsonse decreases in pollen stores were observed in the 80 and 160 µg/L treatment groups at all CCAs following exposure. No significant reductions from the control were observed in the nectar and total food cells, but higher treatments generally had greater numbers of cells with nectar than the lower treatments and the control.

Overall Study Conclusions

As will be discussed more fully in **Section 3.9 (Results)** the analyses determined statistically significant clothianidin dose-related effects in the 40, 80, and 160 µg/L treatment groups across multiple CCAs for the majority of response variables. Indeed, for the 80 and 160 µg/L treatment groups, significant effects ($p<0.05$) were determined for every response variable, except for honey and total food stores and persisted across multiple CCAs. The 40 µg/L treatment group also showed significant effects for multiple response variables (adults, pupae, total live, total brood and pollen storage) across multiple CCAs.

Conversely, there was not a strong indication of an impact at the colony level for the 10 and 20 µg/L treatment groups for individual life stages or food storage. This is evidenced not only by a general lack of statistically significant effects ($p>0.1$) at these treatment levels but in cases where significant effects were determined, they either did not show strong dose-responsiveness and/or did not persist across multiple CCAs. This was the case for the statistically significant effects noted by EPA in pollen storage at CCA5 at 10 and 20 µg/L (effects did not persist at subsequent CCAs), in the number of eggs at CCA5 at 20 µg/L (but no statistically significant effects at 40 µg/L and the effect did not persist at subsequent CCAs) and in the number of adults at CCA6 in the 10 µg/L treatment group (but no statistically significant effects at 20 µg/L and the effect did not persist to CCA7). The PMRA statistical results were slightly different from EPAs for eggs and adults, but resulted in the same conclusions. PMRA determined significant effects on the number of eggs at CCA4 and CCA5 at all test concentrations, but not in subsequent CCAs in the 10, 20 and 40 µg/L treatment groups, suggesting this effect did not persist following exposure. PMRA also determined significant reductions in the number of adults at CCA6 at all test concentrations, but not in subsequent CCAs in the 10, 20 and 40 µg/L treatment groups, suggesting this effect did not persist.

The study is considered to be informative and will be used as a line of evidence in the pollinator risk assessment. While there were uncertainties that were generally related to inherent aspects of any semi-field or full field study design (described in the section below) this study still provides information on a number of colony health parameters about the long term (excluding overwintering) exposure to clothianidin at the colony level. As control survival was only 35% after the overwintering period, results from the overwintering period are not considered valid for assessing the potential chronic risks of clothianidin. **When weighing biological and statistical significance, the NOAEC and LOAEC for this study are determined to be 20 and 40 µg/L, respectively based on effects to number of adults, pupae, total brood, total live bees and pollen storage at the 40 µg/L treatment group. These effect levels include the understanding that evaluation of overwintering was not possible which limits the ability to fully evaluate potential long-term effects in the two lower treatments groups, and therefore, remains a major source of uncertainty.**

Consideration of Study Strengths, Limitations and Interpretation

It is important to recognize the inherent strengths and limitations of this study as results are interpreted and potentially considered in risk assessment.

In the context of available field studies involving honey bees and clothianidin, this study contains a number of strengths including:

- Use of a high degree of replication (n=12) to achieve a reasonable level of statistical power
- Demonstration of a generalized concentration-response relationship with respect to the concentration of clothianidin in sucrose solution and the magnitude and duration of adverse effects
- Quantification of exposure to clothianidin in diet and in hive matrices (uncapped nectar, pollen, capped honey, bee bread)

- Use of a 6-week exposure duration to represent a "high end" exposure scenario
- Inclusion of multiple colony-level endpoints reflecting hive strength, brood development and food stores
- Detailed QA/QC results regarding quantification of clothianidin residues in various matrices
- Availability of raw data for conducting statistical analysis.

A number of limitations are also noted with this study, including:

- Dosing of bees by clothianidin occurred through sucrose (nectar-substitute) alone, whereas bees in the field are likely exposed through both pollen and nectar routes. Therefore, the design of this study may not reflect a "worst case" exposure scenario in which bees are experiencing prolonged exposure to both contaminated nectar and pollen. While exclusion of the pollen route is expected to reduce overall exposure, the impact of this exclusion on the study results is uncertain and will likely depend on the life stage/caste of bee. However, it is notable that in addition to the nectar exposure route and subsequently through honey storage, bees would also be exposed (albeit in lower doses) in bee bread, as bee bread would incorporate both supplied and foraged nectar/sucrose and foraged pollen.
- Residues in hive matrices were only analyzed for parent clothianidin. Metabolites of clothianidin were not considered. Clothianidin degradates (*e.g.* TZNG) have been demonstrated in laboratory studies to have much less acute toxicity to adult honey bees, though data is not available for their chronic effects to adult bees or potential effects to other honey bee life stages.
- Clothianidin was found in both hive nectar and hive pollen (beebread), at concentrations lower than the feeding solutions. Dilution compared to the treatment feeding solution is expected since bees could also forage on outside nectar and pollen sources. As well, hive pollen contains only some hive nectar, thus would not be expected to have a concentration equivalent to nectar alone, and it is mixed with pollen which will come from outside sources. Therefore exposure through both hive pollen and nectar occurred via exposure to the sucrose feeding solution, but how this compares to exposure through contaminated pollen directly is not known. It is also noted that nectar is considered the dominant exposure route for forager bees; other hive bees and larvae consume both nectar and pollen. A recent paper by Sandrock (2014)[1] indicated that consuming contaminated pollen containing low levels of both clothianidin and thiamethoxam had effects on many hive parameters. In addition, since bees were forced to forage for pollen in this study, the potential impact of clothianidin exposure on reducing pollen foraging efficiency of bees could be incorporated into the overall expression of adverse effects, as suggested by published literature. Had contaminated pollen been provided to bees, it is not known if the potential impact on pollen foraging efficiency would have been masked.
- The quantity of nectar provided to hives (4 L per week per hive) likely did not fulfill the complete carbohydrate needs of the colony, as indicated by colony bioenergetics and the lack of remaining sucrose solution upon their renewal at some of the test concentrations.

[1] Sandrock C, Tanadini M, Tanadini LG, Fauser-Misslin A, Potts SG, et al. (2014) Impact of Chronic Neonicotinoid Exposure on Honeybee Colony Performance and Queen Supersedure. PLoS ONE 9(8): e103592. doi:10.1371/journal.pone.0103592

This suggests that bees could be exposed to a greater dose of clothianidin in nectar had a greater volume of spiked sucrose been provided. Although one can infer that the dosing regimen may have underestimated exposure through sucrose relative to 100% contaminated diet, it is also noted that bees had to supplement their spiked sucrose by foraging on their own for other sources of nectar. As with the previous discussion of pollen it is noted that had 100% of the carbohydrate needs of the colony been provided via feeders, the potential impact of purported reductions in nectar foraging efficiency may have been masked to some degree.

- Overwintering success of controls was severely impacted (65% hive mortality). This prevents the ability to detect adverse effects related to hive loss following overwintering. The lack of control hive overwintering may reflect the study design that prevented earlier supplemental feeding in the fall (in order to ensure that treatment hives were consuming their exposed food stores), while typical beekeeping practice would have permitted additional feeding earlier in the fall.
- Pesticides from food sources other than the artificial feeding were also detected during the exposure period and post-exposure periods through collection of pollen from pollen traps from monitoring hives. This contributes to exposure uncertainty and can add confounding effects when interpreting results. However, it is noted that detections occurred in <10% of samples from monitoring hives and that the only pesticides detected (propiconazole, chlorothalonil and carbaryl) had relatively low toxicity compared to parent clothianidin (ranging from practically non-toxic for chlorothalonil to moderately toxic for cararyl).
- Residues of clothianidin in uncapped nectar and bee bread within the hives at CCAs 4, 5, 7 and 9 represent a single sample per hive on a single frame rather than a composite sample from multiple portions of the comb within a hive. This means that residue results may reflect a “hit or miss” scenario with respect to detecting residues in nectar laid down from contaminated (fed) vs. outside sources.
- The exposure, based on residues measured in the hive (hive nectar and hive pollen) indicated that, overall, higher measured hive residues correlated with higher nominal residues in feeding solutions. However, individual hive residue values varied, and there was some overlap in measured values, particularly among the three lowest doses.
- Exposure dilution during the study was evident. Remarkably lower residue concentrations detected in bee bread and hive nectar in some test hives compared to the feeding concentrations indicate foraging on other food sources. This uncertainty is inherent in any semi-field or full-field study design.
- Following standard beekeeping practice, supers (additional hive bodies) were added or removed on a case-by-case basis from each hive to support growth or restrictions in the size of the bee colony. Since each hive was treated differently, this may have added variability and uncertainty into the study design. Additionally, because of this difference between hives, no analysis could be conducted on the proportion of each hive devoted to different life stages and/or food storage.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

1. Study Objective

To determine the potential long term effects on the honey bee (*Apis mellifera* L.) colony health during and after dietary intake of clothianidin, including the potential effects on overwintering. The long term exposure allows for the characterization and distinction of short-term versus a persistent nature of effects.

2. Study Methods

2.1. Test crop

Not applied. The study was conducted in an open field where multiple field flowers were available and may serve as food sources for the test bees, in addition to the artificial feeding of spiked sugar solution.

2.2. Test chemical

The test substance was technical clothianidin. Further details are provided in **Table 1** below.

Table 1. Details about the test substance

Test Item			
Name	Clothianidin	**Batch number:**	AE1283742-01-10
Test item code:	TMC 14-63	**Appearance / colour:**	White solid
Formulation type:	Technical compound	**Intended Usage:**	Insecticide
Active ingredient:	Clothianidin	**Content of a.i. analysed:**	98.6 %
CAS number:	210880-92-5		
Density (20 °C) analysed:	Not applicable	**Risk symbol(s):**	Not available
Date of analysis:	14 Jan 2015	**Expiry date:**	14 Apr 2016
Stability in solution:	sufficient for the test purpose (at least 1h)	**Storage conditions:**	+10 to +30^{0}C

2.3. Test sites

The field and sampling phases of this study were conducted by Smithers Viscient, CRC, Carolina Research Station, Snow Camp, NC, USA; the analytical phase was conducted by Bayer CropScience in Research Triangle Park, NC, USA. The apiary sites were located in the vicinity of the Smithers Viscient CRC site in Guilford, Randolf, Alamance, and Chatham counties, North Carolina.

There were 12 apiaries separated by at least 1 mile. Land use surveys in 1-, 3- and 5-mile radii were conducted. The land use pattern based on National Land Cover Database (NLCD) coverage (2011 dataset layer) indicates that the surrounding area of the apiaries is dominated by forests and hay/pasture with only ~0.5% cultivated crops, while the more contemporary Cropland Data Layer (CDL) coverage (2014 dataset layer) indicated that corn and soybeans were the predominant crop types with approximately 8% coverage in the surrounding area of the apiaries. Pollen species identification and multiple pesticide analysis were conducted using pollen samples collected from the monitoring hives to characterize outside food sources of the test bees and contamination. Pollen samples were collected for a period of 24-48 hours using pollen traps once prior to exposure (June 18-20, 2014), two times during the feeding exposure period (July 1 and 18, 2014), once immediately after exposure (Aug 13, 2014) and 3 additional post-exposure times (September 5-13, Sep 26, and Oct 20 2014). The study authors noted that pollen amounts from these hives were variable and sufficient sample material were not available from every site at each measurement time point.

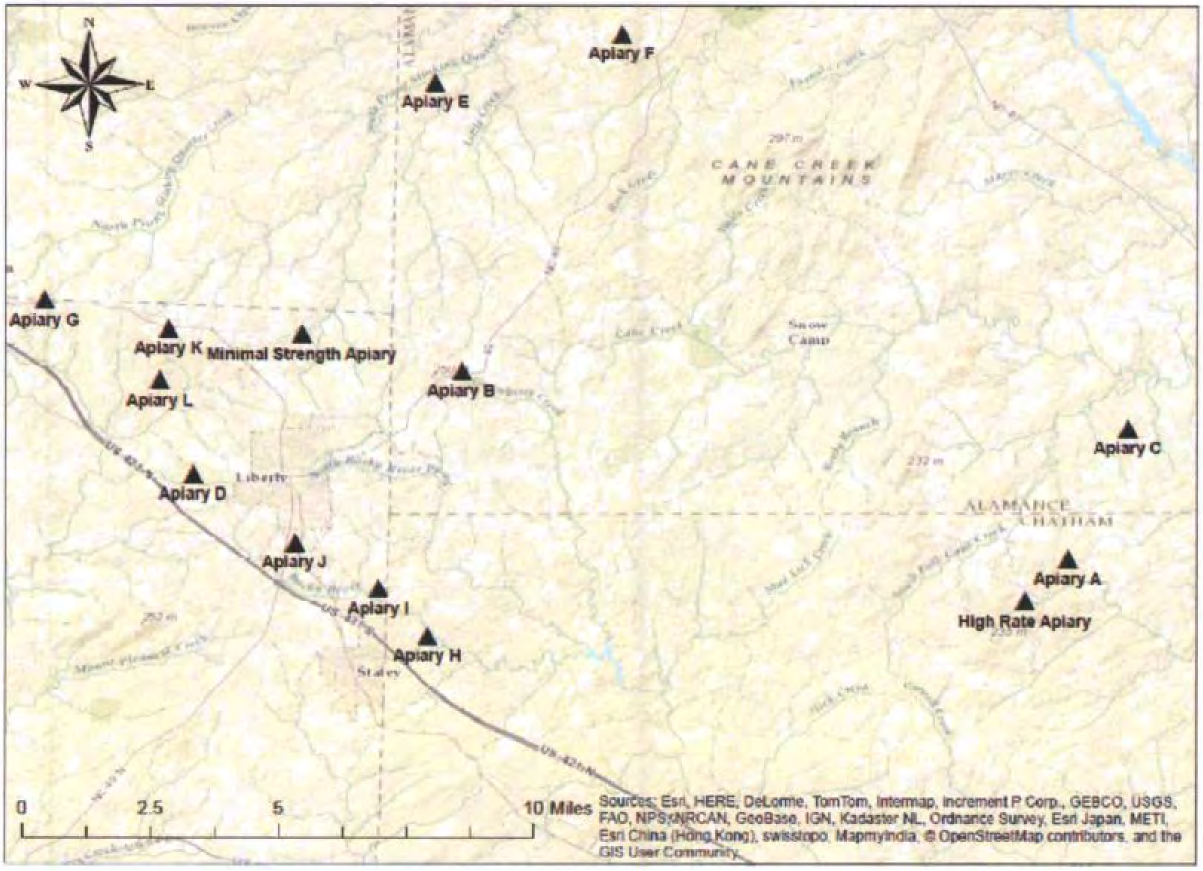


Figure 1: Location of test apiary sites

Table 2: GPS-coordinates of the test apiary sites

Apiary	GPS-coordinates
New Package Apiary	35°51'48.0"N,79°22'24.0"W
Apiary A	35°49'50.0"N,79°21'03.0"W
Apiary B	35°53'01.0"N,79°31'20.5"W
Apiary C	35°52'04.0" N, 79°20'02.0" W
Apiary D	35°51'16.0"N,79°35'54.0"W
Apiary E	35°57'55.0"N,79°31'48.0"W
Apiary F	35°58'45.0" N, 79°28'38.0" W
Apiary G	35°54'13.0"N,79°38'25.0"W
Apiary H	35°48'31.0"N,79°31'55.0"W
Apiary I	35°49'19.0"N,79°32'45.0"W
Apiary J	35°50'06.0"N,79°34'10.0"W

Apiary	GPS-coordinates
Apiary K	35°53'44.0"N,79°36'19.0"W
Apiary L	35°52'52.0" N, 79°36'28.0" W
High Rate Apiary	35°49'06.9"N, 79^{0}21'46.5" W
Minimal Strength Apiary	35^{0}53'38.4" N, 79°34'03.4" W

From Table 2, page 18 of the study report.

2.4. Test organisms

The test species was the honey bee (*Apis mellifera*), Italian race (*Apis mellifera ligustica*). Hives were established from package bees bought from the commercial bee supplier The Carolina Honey Bee Company (10 South Main Street, Travelers Rest, SC 29690, USA) typical of the bee stock used in commercial beekeeping operations. A new queen was introduced into each colony. Four breeder queens which were sister queens were used to generate all the queens used in the study. All queens were purchased from the package supplier. The colonies were maintained in 10-frame Langstroth boxes with an empty deep super on top as a feeder box. In the test field, hives were raised above ground level.

Eighty-four hives that met the study author's criteria (presence of all stages of brood, laying queen and stored pollen and nectar) at the second Colony Condition Assessments (CCA2) were selected for the study. More than 100 inspected hives were screened based on the outcome of CCA2. Hives were checked for the "appearance" of a healthy colony with no visible symptoms of *Varroa* or *Nosema*, as well as having all stages of brood, a queen, and some food stores.

Eighty-four hives were blocked into 12 apiary sites (8 hives/apiary) by brood strength of the colony, starting with Apiary A as the strongest group of hives, and Apiary L as the weakest group of hives. Assignment of apiaries to the geographic locations was done randomly.

Hives were moved from the new package apiary on 17/18 Jun 2014 to their study apiaries. CCA3 was initiated on 18 Jun 2014. After evaluating CCA3, 7 hives were deemed unsuitable due to moving stress that caused swarming or loss of queen and were replaced just before exposure initiation. The replaced hives were A7, B2, H3, H8, J5, J6 and L4.

There were eight hives at each site (7 hives for biological assessments and one as the monitoring hive for pollen sample collection). Each hive was spatially isolated from other treatment rates by 30 feet (9 m) spacing at each apiary site (**Figure 2**). Hives were arranged in a semi-circular pattern, facing east to west, with 125 feet (38 m) spacing between the two end hives.

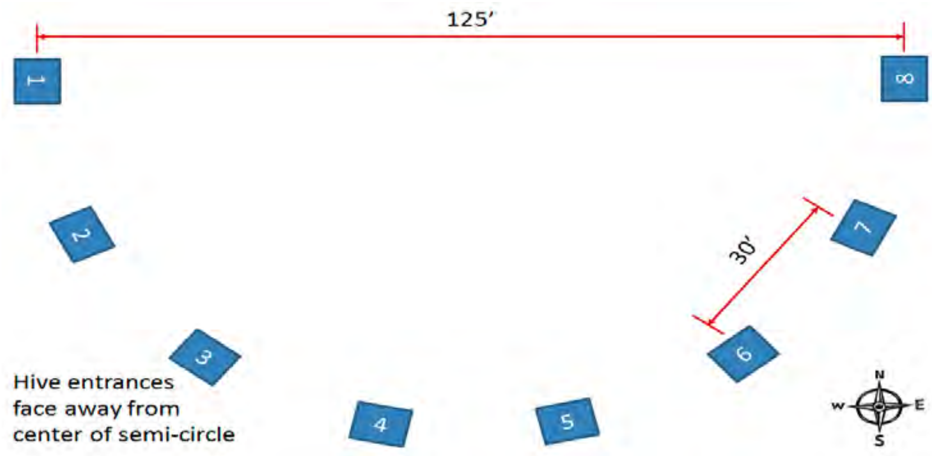


Figure 2. Layout of test hives in a test site

During the study, all hives were treated for *Varroa* with one application of Apiguard® (active ingredient: thymol) following typical apicultural practice for the region. The initial application occurred immediately after CCA6 (8-12 Sep, 2014) to prevent high mite loads. No treatments for any other hive pests, predators or diseases were administered to any hives. To evaluate *Varroa* mite infestations, hive bees were sampled to obtain specific mite counts the week before and after the exposure period, as well as after over-wintering (3rd, 5th and 9th CCAs)

To minimize the potential for robbing amongst test hives, hives at 80 and 160 ppb treatments were removed from all test apiaries in week 8 (12 Aug, 2014) following CCA5. The hives were placed at a separate "high treatment" apiary. For over wintering, the surviving colonies were fed with 1 L of 2:1 sugar syrup on 30 Oct 2014, 06 Nov 2014, 17 Nov 2014, 24 Nov 2014, 01 Dec 2014, 29 Jan 2015, 04 Feb 2015, and 11 Feb 2015

The monitoring hives were used for outsource pollen sample collection. In addition, test solutions were sealed and placed in monitoring hives in order to assess clothianidin stability under field test conditions. These stability solutions were not available as a food source to the monitoring hives.

2.5. Treatments

There were:

- 6 treatment groups (5 test concentrations and control): 0, 10, 20, 40, 80, and 160 µg/L. At each site, there were 2 control hives, and one hive for each test concentration.
- 12 replicates per treatment group (apiaries), with 24 replicates in the control group.

The individual treatment groups, the respective feeding rates and the respective feeding volumes are summarized in **Table 3** below.

Table 3. Treatment groups, feeding rates and feeding volume

Treatment Group	Code	Feeding Timing	Concentration a.i.	Feeding Volume

1 : UTC	UTC (C1+C2)	Twice a week	---	2000 mL
2 : Lowest Rate	T1	Twice a week	10 ppb	2000 mL
3 : Low rate	T2	Twice a week	20 ppb	2000 mL
4 : Moderate rate	T3	Twice a week	40 ppb	2000 mL
5: High rate	T4	Twice a week	80 ppb	2000 mL
6: Effect rate	T5	Twice a week	160 ppb	2000 mL

From Table 3, page 21 of the study report.
UTC = untreated control

The assignment of each test hive at 12 apiaries is summarized in **Table 4.**

Table 4. Hive assignment to test apiaries

Treatment group	Apiary											
	A	B	C	D	E	F	G	H	I	J	K	L
UTC	A3	B2	C1	D4	E1	F2	G2	H1	I6	J7	K5	L4
UTC	A5	B3	C3	D8	E4	F5	G8	H6	I7	J8	K7	L6
10 ppb	A7	B8	C5	D3	E2	F6	G6	H2	I1	J4	K4	L1
20 ppb	A1	B5	C4	D6	E7	F7	G3	H9	I4	J5	K3	L2
40 ppb	A4	B1	C2	D7	E8	F3	G5	H7	I8	J6	K2	L5
80 ppb	A2	B6	C8	D5	E6	F1	G4	H4	I5	J3	K1	L7
160 ppb	A8	B4	C7	D1	E5	F8	G1	H3	I2	J2	K6	L3
Monitoring	A6	B7	C6	D2	E3	F4	G7	H5	I3	J1	K8	L8

From Table 4, page 21 of the study report.

2.5.1. Preparation of stock solution

Stock solution was created by combining 0.051 g of clothianidin dissolved in 20 mL of acetone and added to 1000 mL of distilled water. After preparation, the stock solution was re-stored in a refrigerator until use or replacement. New stock solution was prepared on 09 Jul 2014, 26 July 2014 and 31 July 2014. The study author did not report whether the control sucrose solution contained any acetone.

2.5.2. Preparation of sugar solution

Sugar syrup was created by combining 3 gallons of water for every 25 pound bag of sugar to produce approximately 200 gallons (757L) of sugar syrup.

2.5.3. Preparation of feeding solution

- 10 µg/L: mixing 3.0 mL of stock solution into the 15 L of sugar solution.
- 20 µg/L: mixing 6.0 mL of stock solution into the 15 L of sugar solution
- 40 µg/L: mixing 12.0 mL of stock solution into the 15 L of sugar solution
- 80 µg/L: mixing 24.0 mL of stock solution into the 15 L of sugar solution
- 160 µg/L: mixing 48.0 mL of stock solution into the 15 L of sugar solution.

The test concentrations were reported as "ppb" in the study report. However, the values are in fact in the unit of µg/L, not ppb (ug/kg). For example, 10 µg/L: can be calculated by 3.0 ml * (0.051 g /1020 ml)/15,000 ml.

The test solution density was not provided. Assuming the density of a 50% sugar solution is 1.2296 g/ml[2], the reviewer calculated that the nominal test concentrations at 10, 20, 40, 80 and µg/L are equivalent to 8.1, 16.3, 32.5, 65.1, and 130.1 µg/L (ug/kg), respectively.

2.5.4. Artificial Feeding

A hive top feeder was placed on the top box (either original hive box or an added super) and covered with a telescoping lid. This allowed easy access only to those bees within the hive and minimized light exposure of the test material.

The treated sugar syrup was prepared one day in advance for each feeding event. The feeding started on 26 Jun, 2014 and continued for 6 weeks until the last feeding on August 4. All of the hives were artificially fed with 2 liters of 50% sugar solution, two times per week. Prior to each feeding, any remaining feeding syrup was removed from the feeder and weighed to determine the consumed amount. The study observation (commencing when hives were moved to their study apiaries) period was 17 Jun, 2014 – 27 Apr, 2015, which includes the overwintering period.

2.6. Meteorological Data

Temperature, humidity and precipitation data were monitored at each study apiary. An average total of 6.32 inches (161 mm) of rainfall accumulated throughout the exposure period (from 26 Jun 2014 until 11 Aug 2014) across the 12 apiaries. However, Apiary I did not record any data on 9 days during the exposure period and only recorded a total of 1.1 mm precipitation throughout the exposure period. Removal of Apiary I's rainfall data would result in an average of 6.90 inches (175 mm) across the 11 apiaries during the exposure period. The minimum and maximum weekly average temperatures are shown in **Figure 3.**

[2] Cell Biology Laboratory Manual, http://homepages.gac.edu/~cellab/chpts/chpt3/table3-2.html, accessed on Dec 12, 2014

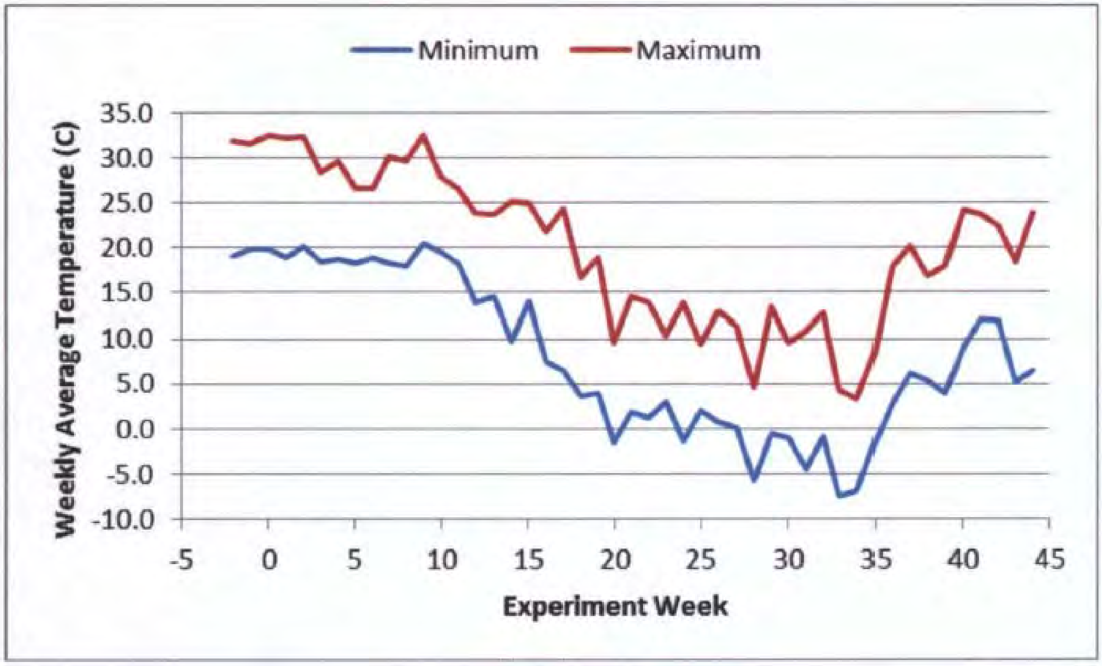


Figure 3. Average minimum and maximum temperatures across all apiaries
From Figure 4, page 32 of the study report

2.7. Observations

Important activity and dates are summarized in **Table 5.**

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 5. Chronological list of key dates and activities

Week	Date	Activity	Week	Date	Activity
-6	12-21 May 2014	**CCA1**	6	05-11 Aug 2014	Hive samples (uncapped nectar, bee bread)
-3	02-13 June 2014	**CCA2**; Hive samples (uncapped nectar, bee bread)	6	05-11 Aug 2014	**CCA5**
-2	17 – 18 Jun 2014	Hives moved to study apiaries	6	05-11 Aug 2014	Hive bee sampling for *Varroa* and *Nosema* assessment
-1	18-23 Jun 2014	**CCA3**	6	12 Aug 2014	Removal of 80 and 160 ppb hives to high treatment apiary
-1	18-20 Jun 2014	Hive bee sampling for *Varroa* and *Nosema* assessment	6	13 Aug 2014	Monitoring hive sampling (uncapped nectar, pollen)
-1	18-20 Jun 2014	Sampling of Monitoring Hives (uncapped nectar, pollen)	8	25 Aug 2014	Hive C2 was removed from the study
-1	24 Jun 2014	Seven hives replaced based on CCA3 results	10	05 Sep 2014	Monitoring hive sampling (uncapped nectar, pollen)
0	26 Jun 2014	1st Feeding	10	8-12 Sep 2014	**CCA6***
0	30 Jun 2014	2nd Feeding; measurement of remaining food	13	23 Sep 2014	Monitoring hive sampling (uncapped nectar, pollen)
0	01-02 Jul 2014	Monitoring hive sampling (uncapped nectar, pollen)	13	25 Sep 2014	Weakest hives moved to minimal strength apiary
1	03 Jul 2014	3rd Feeding; measurement of remaining food	15	14-16 Oct 2014	**CCA7** (UTC, 10 ppb, 20 ppb, 40 ppb) capped honey sample
1	07 Jul 2013	Stability samples	16	21-22 Oct 2014	**CCA7** (high treatment apiary)
1	07 Jul 2014	4th Feeding; measurement of remaining food	17	28 Oct 2014	Additional hive moved to minimal strength apiary
1	09 Jul 2014	New stock solution prepared	18	30 Oct 2014	Feeding 1 L 2:1 sugar syrup per hive
2	10 Jul 2014	5th Feeding; measurement of remaining food	18	04 Nov 2014	All remaining 160 ppb treatment hives destroyed
2	10 Jul 2014	Hive I7 removed from study	19	06 Nov 2014	Feeding 1 L 2:1 sugar syrup per hive
2	14 Jul 2014	6th Feeding; measurement of remaining food	20	17 Nov 2014	Feeding 1 L 2:1 sugar syrup per hive
3	18 Jul 2014	7th Feeding; measurement of remaining food	21	24 Nov 2014	Feeding 1 L 2:1 sugar syrup per hive
3	15-18 Jul 2014	**CCA4**	22	01 Dec 2014	Feeding 1 L 2:1 sugar syrup per hive
3	16-18 Jul 2014	Hive sample (uncapped nectar)	31	29 Jan 2015	Feeding 1 L 2:1 sugar syrup per hive
3	18 Jul 2014	Monitoring hive sampling (uncapped nectar, pollen)	31	04 Feb 2015	Feeding 1 L 2:1 sugar syrup per hive
3	21 Jul 2014	8th Feeding; measurement of remaining food	32	11 Feb 2015	Feeding 1 L 2:1 sugar syrup per hive
4	24 Jul 2014	9th Feeding: measurement of remaining food	After over-wintering		
4	26 Jul 2014	New stock solution prepared	37	17-19 Mar 2015	**CCA8** (UTC, 10 ppb, 20 ppb, 40 ppb)

Week	Date	Activity	Week	Date	Activity
4	28 Jul 2014	10th Feeding; measurement of remaining food	40	02 Apr 2015	**CCA8** (80 ppb)
5	31 Jul 2014	New stock solution prepared	43	22-23 Mar 2015	Hive bee sampling for *Varroa* and *Nosema assessment*
5	01 Aug 2014	11th Feeding; measurement of remaining food	43	22-23 Apr 2015	**CCA9** (UTC, 10 ppb, 20 ppb, 40 ppb)
5	01 Aug 2014	Stability samples, monitoring hive sampling (uncapped nectar, pollen)	43	22-23 Apr 2015	Hive samples (capped honey)
5	04 Aug 2014	12th (final) Feeding; measurement of remaining food	43	27 Apr 2015	**CCA9** (80 ppb)
6	07 Aug 2014	Measurement of remaining food			

*CCA6 timing allows all bee individuals (eggs, larvae, pupae) present during the exposure period to complete their development cycle to adults.

2.7.1. Colony mortality

The study author did not report what defined a "dead hive". However, the reviewer has assumed that any colony (hive) that did not show the presence of a queen and had no open brood or eggs, or was devoid of worker (female) bees was considered "dead". If a hive was considered "dead" at the time of assessment, it was no longer used in the analysis of endpoints (e.g., adult bee numbers, hive weight). The number of individual dead bees was not recorded.

2.7.2. Colony Condition Assessments (CCA)

Beginning with CCA 3, observations were blocked by the observer, with the same person always observing the same set of hives to avoid viewer discrepancies in the data. Hives at apiaries A, B, E, G, I, and J were inspected by the study author and those at apiaries C, D, F, H, K and L by another inspector.

Nine CCAs were conducted during the entire study. CCA1 (day -45 to -36), and CCA2 (day -24 to -13) were conducted during the hive establishment. CCA3 (day -10 to -3 days) was conducted 1 week prior to the feeding exposure which served as initial hive conditions prior to the feeding exposure. CCA4 was conducted 3 weeks (19—22 days) after the start of feeding exposure. After the end of feeding exposure (Week 6), the following additional CCAs were conducted: CCA5 (week 6, 40-47 days post exposure), CCA6 (days 74—78), CCA7 (days 110—118) and after overwintering CCA8 in Mar 2015 (days 264—280) and CCA9 in April 2015 (300—305 days post-exposure). Each CCA period took multiple days to complete. For summary statistics, the average day is used to characterize any given CCA. The time schedule of CCAs in relation to other study activities is summarized in **Table 5.**

During the colony condition assessments, each frame was removed and inspected one at a time (observations recorded for each side), with measurements for endpoints taken as percent of total frame area covered by honey / nectar, bee bread / pollen, eggs, open brood (larvae), capped brood (pupae), and adult bees.

The estimation was made by:

- Each hive consisted of 20 observed panels (10 frames with two sides of each frame), with an area of 929 cm^2 per side, or a total area of 18,580 cm^2 for all 10 frames.
- A frame with 100% coverage of adult bees was assumed to have an adult bee density of 1.30 bees/cm^2.
- Each cell is a regular hexagon with a flat-to-flat distance of 5.2cm and an area of 0.234 cm^2
- Each frame was considered to have 3970 cells/frame side (929 cm^2/cell area of 0.234 cm^2)

For adult bees, therefore, a frame side with 100% coverage of adult bees would contain 1208 bees (929 cm^2 * 1.30 bees/cm^2). For the number of cells containing honey/nectar, pollen, capped or open brood or eggs, the following equation was used:

$$\text{Number of cells (for a given hive matrix)} = \frac{\Sigma \text{ \%frame coverage}}{100\%} * \text{cells per frame side (3970)}$$

2.7.3. Evaluation of Disease or Pests in the Hive

At each CCA, colonies were also checked for visible symptoms of disease or pests, such as *Nosema*, foulbrood, *Varroa* mites or small hive beetle. To quantify the presence of Varroa in the hive, bee samples were taken at CCA3, CCA5 and CCA9. Bees were washed in alcohol to remove mites. The number of mites per 100 bees was calculated.

2.7.4. Hive weights

Each hive had a dedicated scale beneath it that was placed just before exposure initiation and remained until the end of the study. Each scale was programmed to record the hive weight every hour.

2.8. Residue analysis

All residue and stability samples collected from feeding solution, pollen traps, and test hives were analysed for clothianidin residues at Bayer CropScience in Durham, NC. Samples from pollen traps in the monitoring hives were also analysed for residues of multiple pesticides from outside sources at the National Science Laboratories of USDA in Gastonia, NC (non-GLP). The residue results were reported as µg per kg of sample matrix (ppb), which is different from the test solution that was reported in µg/L. Samples were not analyzed for residues of clothianidin metabolites (*e.g.* TZNG).

The LOQ was 1 ppb for clothianidin in feeding solution and hive nectar samples for pollen samples. The LODs are listed in **Table 6**.

Table 6. LOD for clothianidin

Matrix	Clothianidin	
	LOD	LOQ
Dosing/Stability Solutions	0.5 ppb	1 ppb
Hive Collected Nectar	0.1 ppb	1 ppb
Pollen	0.4 ppb	1 ppb

Taken from page 373-374 of the study report

For the values <LOD, half of the LOD value was used in order to calculate the means. For values between the LOD and LOQ, half of the LOQ value was used to calculate means. Multiple pesticide analysis was conducted in order to monitor pesticide contamination from outside food sources using pollen collected from pollen traps on the monitoring hives.

All samples for residue analysis were protected from sunlight by using amber vials and transported to freezer storage after field collection. All samples were placed in frozen storage upon receipt at the test facility. Samples were maintained frozen (≤ -15° C) at the test facility until shipment under frozen conditions to the test site for residue analysis. Daily minimum/maximum temperatures were recorded for the duration of the storage period at the test facility.

2.8.1. Pollen from outside sources

Pollen samples were collected from pollen traps attached for 24-48 hours to the monitoring hives at each site to assess the potential contaminant exposure from outside sources. Pollen traps were only activated at seven time points during the study and were occasionally left open longer than 48 hours if sufficient pollen sample was not available at the expected date. Pollen amounts collected from each hive were variable and samples were not available from every site each time. Pollen samples from the monitoring hives were taken at weeks -1 (CCA3), 0, 3 (CCA 4), 6 (CCA5), 10 (CCA6), 13, and 16 (CCA7).

2.8.2. Stored pollen and nectar in test hives

Stored bee bread and bee-collected nectar were collected within the study hives for clothianidin residue analysis. Samples weighed at least 500 mg each and were not available from every colony each time. Bee bread was collected at week 6 (CCA5). Uncapped nectar was collected at weeks 3 and 6 (CCAs 4 and 5) and capped honey was collected at weeks 15 and 43 (CCAs 7 and 9).

2.8.3. Feeding solution and stability of test item

The monitoring hives were used for dose verification and to evaluate stability of the test item in a hive environment. Monitoring hives were set up in the same manner as test hives except the colony was denied access to the spiked or unspiked sucrose. Residue samples comprising approx. 5 g each from the sugar syrup were taken on week 1 (7 July 2014), and week 5 (1 August 2014). Stability of the test material was evaluated by placing treated feeding solutions in closed-off vials in the feeding compartments of hives at representative apiaries.

Table 7. Number of samples and sampling schedule for feeding solution and stability of test chemical.

Timing	**Week 1**	**Week 5**
Apiary / replicate	**07 Jul 2014**	**01 Aug 2014**
UTC	--	8
10 ppb	4	4
20 ppb	4	4
40 ppb	4	4
80 ppb	4	4
160 ppb	4	4

-- = no samples taken

3. Results

3.1. Land use near test hives

Land use pattern within a 1-mile, 3-mile and 5-mile radii around the 12 apiaries are summarized in **Table 8**. Generally, the results indicate that the area around the apiaries during the year the study was conducted was dominated by forest and grassland/pasture/hay and that corn and soybean were the predominant crop types. The cultivated crop area occupied 6.7% of the total land within 1 mile radius, 8.3% within a 3 mile radius range, and 7.7% within a 5 mile radius range from the test apiaries. Data from the 2011 cropland data layer also indicate forests (particulary deciduous) and hay and pasture land were dominant in the study area prior to test initiation, but that cultivated crop acreage was lower (**Table 9**).

Table 1. Average percent (%) land use pattern across the 12 study apiaries (based on 2014 Cropland Data Layers (CDL))

Land Use Category	Average of 12 Study Apiaries		
	1 mile radius	3 mile radius	5 mile radius
Corn	2.5%	3.1%	2.7%
Soybean	3.3%	4.5%	4.4%
Other Crops	0.9%	0.7%	0.6%
Developed, Open Space	6.0%	5.7%	5.3%
Developed, Low-High Intensity	3.0%	2.7%	2.3%
Forest	44.4%	45.7%	47.8%
Grassland/Pasture/Hay	38.8%	36.1%	35.2%
Water/Barren/Shrub/Wetland	1.6%	1.5%	1.6%

Table 2. Average percent (%) land use pattern across the 12 study apiaries (based on 2011 National Land Cover Database (NLCD))

Land Use Category	Average of 12 Study Apiaries		
	1 mile radius	3 mile radius	5 mile radius
Open Water	0.4%	0.6%	0.7%
Developed, Open Space	5.8%	5.6%	5.3%
Developed, Low Intensity	2.6%	2.2%	1.8%
Developed, Medium Intensity	0.4%	0.5%	0.4%
Developed, High Intensity	0.1%	0.2%	0.1%
Barren Land	0.1%	0.1%	0.1%
Deciduous Forest	32.5%	34.0%	35.5%
Evergreen Forest	5.5%	5.2%	5.6%
Mixed Forest	2.5%	2.2%	2.4%
Shrub/Scrub	2.0%	2.7%	2.9%
Herbaceous	4.0%	4.3%	4.5%
Hay/Pasture	42.9%	41.3%	39.5%
Cultivated Crops	0.5%	0.5%	0.5%
Woody Wetlands	0.6%	0.7%	0.6%

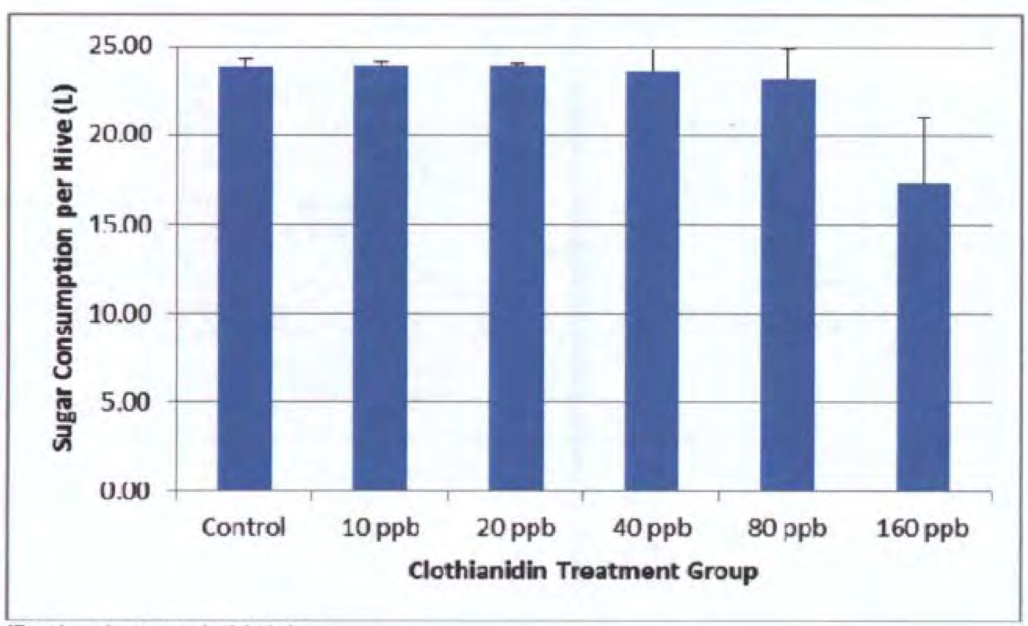
3.2. Pollen sources of test hives

Monitoring hives were used at each test apiary to collect pollen for assessment of the local pollen flora (non-GLP). Pollen trap samples from the monitoring hives were taken at seven times: CCA3 (18-20 Jun 2014; week -1), July 1-2 (week 0) and CCA4 (18 Jul 2014; week 3), as well as at CCA 5 (13 Aug 2014; week 6), on September 5-13 (week 10) and 26 (week 13), at prior to overwintering at CCA7 (20 Oct 2014; week 16).

Major sources (>10%) of pollen at any measured time point were from clovers, crepe myrtle, plaintain, vitis, corn, virginia creeper, chickory, pigweed, ragweed, goldenrod/tickseed, and grass. Corn was the only cultivated crop with significant levels of pollen in monitoring hives (average of 18—23% in July). In the fall, pollen in monitoring hives was dominated by asters (goldenrod, tickseed and ragweed) with average proportions of 55—88% in September and October) and grasses (mean 37% in late September). Full results can be found in Table 9 (p. 44) and Tables 64-70 (p. 319-324) of the study report.

3.3. Consumption of spiked sucrose

Individual hive consumption rates (determined by the remaining food in the feeder added up throughout the entire exposure period) for the feeding solution (sugar syrup) ranged from 11,636 mL to 24,000 mL of the total 24,000 mL per hive provided during a 6-week period (*i.e.* 2 litres per colony 2 times a week for a total of 24,000 mL per colony during the exposure period). All colonies in the control, 10, 20, 40 and 80 ppb treatment groups consumed most or all of the sugar solution (see **Figure 4**) with some colonies in the 160 ppb treatment having substantially lower consumption. Mean total consumption in control hives was 99.5% of provided sugar syrup (minimum control hive consumption of 93%).



*Error bars denote standard deviations.

Figure 4. Mean total food consumption (L) per colony during the 6-week exposure period

3.4. Examination of pesticides from other sources

Monitoring hives were used to assess the potential contaminant exposure from outside sources (non-GLP) at each site. Pollen trap samples from the monitoring hives were taken at seven times: CCA3 (18-20 Jun 2014; week -1), July 1-2 (week 0) and CCA4 (18 Jul 2014; week 3), as well as at CCA 5 (13 Aug 2014; week 6), on September 5 (week 10) and 23 (week 13), at prior to overwintering at CCA7 (20 Oct 2014; week 16). The study author reported that the amount of pollen collected from traps on the monitoring hives varied and that not all hives had samples collected at each measurement time point. However, the hives and measurement times where there was insufficient material were not identified in the study report and no list of samples that were sent to the USDA National Science Laboratories in Gastonia, NC was provided.

Out of the 42 pollen samples that were analyzed for pesticide residues, only three found any residues higher than the LOD. The 01 Jul 2014 sample (week 0) from Apiary E has measured residues of 203 µg/kg chlorothalonil, the 13 Aug 2014 (week 6) sample from Apiary I had measured residues of 119 µg/kg carbaryl and the 20 Oct 2014 (week 16) sample from Apiary B had measured residues of 2010 µg/kg propiconazole. No residues of clothianidin, thiamethoxam or imidacloprid (LODs all 1.0 ppb) were detected in any pollen sample from any monitoring hive. The LODs for each pesticide in the residue analysis can be found in Appendix I on p. 346 of the original study report.

59 uncapped nectar samples from monitoring hives were analyzed and none had any detectable pesticide residues.

Pesticide contamination is unknown for those intervals when pollen or nectar samples were not collected.

3.5. Confirmation of test concentrations

Clothianidin was analyzed from feeding solutions sampled after they were prepared on 03 July 2014 (week 1) and 28 Jul 2014 (week 4). The averages of measured concentrations were <LOD, 9.5, 19.1, 35.7, 71.9, and 140.0 µg/kg for the nominal concentrations of control, 10, 20, 40, 80, and 160 µg/L, respectively. The data are tabulated below in **Table 10**.

Table 10. Dosing solution residue data from 03 July 2014 (Week 1) and 28 July 2014 (Week 4).

Nominal concentrations		Average of measured concentrations (ppb)	Measured clothianidin Concentrations (n=2)	
µg/L	ppb (µg/kg)			
0 (Control)	0	<LOD†	<LOD	<LOD
10	8.1	9.5	9.13	9.89
20	16.3	19.1	18.3	19.8
40	32.5	35.7	34.9	36.4
80	65.1	71.9	68.5	75.2
160	130.1	140.0	129	151

†LOD = 0.5 ppb in feeding solutions

3.6. Stability of the test item in feeding solution

Stability of clothianidin in the sugar solution during the feeding period was examined from diet collected from closed-off feeding solutions placed in the monitoring hives, placed two times, on 3 July and 28 July, 2014 (n=4 for all treatment hives at each sampling event) and removed four days after placement. No reduction of test concentrations in the feeding solution was noticed during these two samplings. No clothianidin residues were detected (LOD of 0.5 ppb) in any of the control samples (n=8) taken on from test materials placed in closed-off hive feeders on 28 July 2014 and removed on 01 Aug 2014. Control samples were not placed in closed-off feeding solutions on 03 July 2014. Average clothianidin residue data for the stability solution are presented in **Table 11.**

Table 11. The stability of clothianidin in feeding solution on 3 Jul and 28 Jul, 2014.

Nominal concentration (µg/L)	Average of measured concentrations across sampling dates (ppb)	Number of samples measured	Range of measured clothianidin concentrations (ppb)	
			03 Jul, 2014	28 Jul, 2014
Control	<LOD†	8*	N/A*	<LOD
10	9.59	8	8.31—12.3	9.45—9.82
20	19.98	8	18.9—21.7	19.5—20.1
40	36.48	8	31.6—39.4	36.9—37.8
80	70.88	8	62—75.5	73.3—75.3
160	131.0	8	106—128	145—149

- Regenerated from Section 5.2, on page 382-383 in the study report
†: LOD=0.5 ppb for clothianidin;
*All control samples were from the samples placed on 28 July 2014.

3.7. Residues in hive matrices

It is noted here as it was in the uncertainties section that the residue samples from the different hive matrices represent a single sample from a single frame. Therefore there is variation in the residues that likely stems from the sampling procedure employed for this study (single sample, one side of the comb).

3.7.1. Clothianidin residues in hives prior to the feeding exposure

Potential background clothianidin contamination in test hives was examined using hive bee bread (hive pollen) and nectar collected about three weeks (03 June 2014) prior to the beginning of feeding exposure from two hives at each of the initial installation apiaries (2 apiaries). Clothianidin was not detected in any of four hive pollen samples (LOD = 0.4 ppb) or four nectar samples (LOD = 0.1 ppb). Residue analysis for other pesticides was not conducted prior to exposure beyond that reported from the monitoring hives in **Section 3.4**

3.7.2. Residues in hive matrices during and after feeding exposure

Clothianidin residues in hives were examined five times after the feeding started using hive bee bread and hive nectar. All test hives were sampled during or immediately following the exposure phase, for uncapped nectar at at CCA4, 16-18 July 2014 and uncapped nectar and bee bread at

CCA5 (5-11 Aug, 2014), and post exposure in capped honey at CCA7 (14-16 Oct, 2014) and at CCA9 (after overwintering on 22-23 Apr, 2015).

3.7.2.1. Residues in hive matrices at CCA4 (after 3 weeks of exposure)

The level of clothianidin in uncapped nectar after 3 weeks of feeding (CCA4) is summarized in **Table 12**. All test hives were sampled at CCA4 (16-18 July 2014). Average clothianidin concentrations were calculated assuming that values below the LOD contained one-half the LOD (0.05 µg/L) and values below the LOQ contained one-half the LOQ (0.5 µg/L). A dose-response correlation was observed between the clothianidin concentrations in the feeding solution and the mean-measured concentrations in uncapped hive nectar. However, the clothianidin concentration in hive uncapped nectar ranged from 16.6—38.3% of the mean concentrations in feeding solution, after accounting for brix content of the uncapped nectar compared to the original 50% sugar solution. It is possible that dilution of nectar from other food sources occurred during the exposure period since, as indicated in the study, a significant degradation of clothianidin in test solution was not detected in the study.

Clothianidin in hive uncapped nectar at CCA4: The level of clothianidin in hive uncapped nectar during the feeding exposure (CCA4) was summarized in **Table 12**. All but one control hive and one treatment hive were measured (these were removed from the study due to either technical issues or vandalism of the test hive). Out of 12 hives measured for each concentration, levels below either the LOD or LOQ were reported in three hives at 10 µg/L, one hive at 20 µg/L, one hive at 40 µg/L, three hives at 80 µg/L and one hive at 160 µg/L, while the remaining treatment hives had quantifiable levels of clothianidin. Clothianidin was undetected in the majority (18/23) of control samples and was detected at levels below the LOQ in four additional samples. Only one control sample had a level of clothianidin above the LOQ (1.36 ppb)

The results showed a dose-response correlation between the average concentrations measured in uncapped hive nectar and the concentrations in the feeding solution. However, the concentrations varied remarkably within each treatment group (see **Table 12**). After correction with Brix values to 50% sugar concentration, the mean of the measured concentrations in uncapped hive nectar within each treatment group of 10, 20, 40, 80 and 160 ug/L (8.1, 16.3, 32.5, 65.1, and 130.1 ppb) was 2.97 (range: <LOQ-8.04), 6.01 (range: <LOD-13.29), 12.44 (range: <LOQ-35.83), 15.67 (range: <LOD-45.4), and 21.61 ppb (range: <LOD-84.18), respectively. By average, the measured concentration in hive nectar was 30.5% (range 16.6-38.3%) of the concentration in feeding solution. The results showed that after 3 weeks of feeding, clothianidin concentrations in uncapped hive nectar appeared lower than that in the feeding solutions, which indicated that the foraging bees also foraged on nectar sources other than the provided sugar sources which diluted the level of treatment. It is noted that this result is expected, as bees were allowed to freely forage, and also, under natural conditions bees typically forage on multiple plant pollen and nectar sources.

Table 5. Clothianidin concentrations (ppb) in uncapped hive nectar sampled 27 days after the start of artificial feeding on 23 Jul, 2014 (CCA4).

Apiary	**Measured Clothianidin concentrations (ppb) (LOD=0.1 ppb)***						
	Nominal concentration (ug/L)						
	Control 1†	**Control 2†**	**10**	**20**	**40**	**80**	**160**
	Nominal concentration (ppb) ‡						
	0	**0**	**8.1**	**16.3**	**32.5**	**65.1**	**130.1**
A	<LOD	<LOQ	1.84	8.42	5.94	4.89	26.69
B	<LOD	<LOD	<LOQ	1.18	7.34	<LOD	4.67
C	<LOD	<LOD	3.08	7.50	16.81	17.94	16.50
D	<LOQ	<LOD	1.42	6.96	17.34	<LOD	<LOD
E	<LOD	<LOQ	<LOQ	5.18	5.69	11.56	25.63
F	<LOD	<LOD	0.96	4.60	***	13.06	1.18
G	<LOD	<LOD	5.57	<LOD	8.23	32.09	19.38
H	<LOD	<LOD	4.69	9.62	10.06	9.69	28.44
I	<LOD	**	<LOQ	2.44	<LOQ	<LOQ	16.63
J	<LOQ	<LOD	4.59	10.63	22.56	36.81	34.94
K	<LOD	1.36	8.04	2.27	6.69	16.19	84.18
L	<LOD	<LOD	4.57	13.29	35.83	45.40	1.08
Number of samples	12	11	12	12	11	12	12
Average concentration	<LOQ		2.97	6.01	12.44	15.67	21.61
% Feeding concentration††	Not applicable		36.7	36.9	38.3	24.1	16.6

* Concentrations in all treatments except for the controls are corrected to 50% sugar using Brix values that are not listed in the table, but were in the table section 6 on page 384-387 of the study report (brix values reported as >80, were assumed to be 80% for the purpose of this calculation). The brix corrected residue value reported here is determined using the formula that the brix corrected concentration = measured concentration * (feeding solution brix {50%}/hive nectar measured brix)
** Hive was removed from the study due to a technical error and no sample collected.
***Hive was removed from study after it was found knocked over and no sample collected.
† Concentrations in the controls are measured concentrations in hive uncapped without corrections for sugar concentrations (brix).
‡Nominal concentration in ppb is estimated from the concentration in µg/L by assuming the volume density of the test solution to be 1.2296 g/ml.
†† % Feeding concentration: the average of measured concentration compared with the nominal feeding concentration in ppb.

3.7.2.2. Residues in Hive Matrices at CCA5 (1 week after end of exposure)

The level of clothianidin in hive bee bread and uncapped nectar one week after the end of feeding exposure (CCA5, 5-11 Aug 2014) is summarized in **Tables 13-14**. As with the uncapped nectar results from CCA4, these measurements indicate a dose-response correlation between the average concentrations of clothianidin measured in both bee bread and uncapped hive nectar and the concentrations in the feeding solution. However, the concentrations varied remarkably within some treatments.

Clothianidin in bee bread at CCA5: The level of clothianidin in bee bread (hive pollen) following exposure (6 weeks of feeding) at CCA5 was summarized in the **Table 13.** Clothianidin was detected in all measured treatment samples. It was noted that not all residue information in pollen was available. No residue information for treatment at 160 µg/L in bee bread was provided, primarily due to insufficient pollen stores. Out of 12 hives, two hives at 80 µg/L, nine hives at 40 µg/L, eleven hives at 20 µg/L and ten hives at 10 µg/L were measured, respectively. For the remaining hives that did not have bee bread measurements, the vast majority were due to insufficient pollen stores for sample collection, especially in the 80 and 160 µg/L treatment groups.

The results showed a dose-response correlation between the average concentrations measured in hive bee bread and the concentrations in the feeding solution. However, the concentrations varied within each treatment group (see **Table 13**). The mean of the measured concentrations in bee bread within each treatment group of 10, 20, 40, and 80 µg/L (8.1, 16.3, 32.5, and 65.1 ppb) was 3.52 (range: 2.32-5.26), 6.68 (range: 3.54-9.41), 12.16 (range: 2.19-19.2), and 35.8 ppb (range: 30.9-40.6), respectively. By average, the measured concentration was 44.2% (range 37.4-54.9%) of the concentration in feeding solution, and 45.9% (range 38.9-61.7 %) of the measured concentrations in uncapped hive nectar (data not shown in the table). The results showed that after 3 weeks of feeding, clothianidin concentrations in hive bee bread appeared remarkably lower than that in the feeding solutions and in hive nectar. The lower concentration in bee bread is expected due to the dilution since bee bread is a mixture of nectar and pollen from various sources.

Table 6. Clothianidin concentrations (ppb) in hive pollen (bee bread) sampled six weeks after the start of artificial feeding on 5-11 August 2014 (CCA5).

Apiary	Measured clothianidin concentrations (ppb) (LOD = 0.4 ppb)						
	Nominal concentration (ug/L)						
	Control 1	Control 2	10	20	40	80	160
	Nominal concentration (ppb) ‡						
	0	0	8.1	16.3	32.5	65.1	130.1
A	--	<LOD	-	-	9.79	-	-
B	<LOD	<LOD	2.91	9.41	-	-	-
C	<LOD	<LOQ	2.58	6.4	15.8	-	-
D	<LOQ	<LOQ	2.4	4.65	6.15	-	-
E	<LOQ	<LOQ	4.58	3.54	13	-	-
F	<LOD	<LOD	2.73	9.03	-	-	-
G	<LOQ	--	5.26	6.93	12.2	30.9	-
H	<LOD	<LOD	2.32	5.6	2.19	-	-
I	<LOD	--	-	3.78	13.3	-	-
J	<LOD	<LOD	3.02	6.67	-	-	-
K	<LOQ	<LOQ	4.79	8.3	19.2	40.6	-
L	<LOD	<LOD	4.56	9.2	17.8	-	-
Number of samples measured	11	10	10	11	9	2	0
Average concentration	<LOD		3.52	6.68	12.16	35.75	-
% of the feeding concentration††	Not applicable		43.5%	41.0%	37.4%	54.9%	-
% of the average detection in hive Nectar†††	Not applicable		41.0%	41.9%	38.9%	61.7%	-

"-" indicates that data are not available

‡Nominal concentration in ppb is estimated from the concentration in µg/L by assuming the volume density of the test solution to be 1.2296 g/ml.

†† % Feeding concentration: the average of measured concentration compared with the nominal feeding concentrations in ppb.

††† % of the average detection in hive Nectar**:** the average of measured concentration in bee bread compared with the average measured concentration in nectar ppb without corrections for sugar (see Section 7 pp 388-391 in the study report).

Clothianidin in uncapped hive nectar at CCA5: Similar to CCA4, a dose-response correlation was observed between the average concentrations of clothianidin measured in uncapped hive nectar and the concentrations in the feeding solution. However, the clothianidin concentration in hive uncapped nectar was lower than what was in the feeding solutions, indicating dilution of stored nectar from other food sources. The level of clothianidin in hive uncapped nectar following feeding exposure (CCA5) is summarized in **Table 14.** All but one control hive and one treatment hive were measured (these were removed from the study due to either technical issues or vandalism of the test hive). Clothianidin was detected above the LOQ (0.5 ppb) in all of the measured treatment samples. Clothianidin was undetected in the majority (19/23) of control samples and was detected at levels below the LOQ in three additional samples. Only one control sample had a level of clothianidin above the LOQ (1.4 ppb).

The results showed a dose-response correlation between the average concentrations measured in uncapped hive nectar and the concentrations in the feeding solution. Additionally, these measured concentrations had much less overlap in ranges than the uncapped nectar concentrations indicated at CCA 4 (**Table 12**). After correction with Brix values to 50% sugar concentration, the mean of the measured concentrations in uncapped hive nectar within each treatment group of 10, 20, 40, 80 and 160 ug/L (8.1, 16.3, 32.5, 65.1, and 130.1 ppb) was 5.51 (range: 3.09-7.06), 10.17 (range: 8.45-13.25), 19.94 (range: 12.66-26.86), 36.99 (range: 25-48.97), and 65.65 ppb (range: 31.4-107.69), respectively. By average, the measured concentration in hive nectar was 59.8% (range 50.5-68%) of the concentration in feeding solution. The results showed that after 6 weeks of feeding, although clothianidin concentrations in uncapped hive nectar still appeared lower than that in the feeding solutions (indicating that the foraging bees also foraged on nectar sources other than the provided sugar sources which diluted the level of treatment), they appeared to utilize the feeding solutions much more than they had after only 3 weeks of feeding. As the level of clothianidin in the uncapped nectar compared to the feeding solution appeared similar across treatment groups, this may indicate that there was less available alternate forage during the final 3 weeks of exposure compared with the initial 3 weeks represented by the nectar stores at CCA4.

Table 7. Clothianidin concentrations (ppb) in uncapped hive nectar sampled 40-46 days after the start of artificial feeding on 5-11 Aug, 2014 (CCA5).

Apiary	Measured Clothianidin concentrations (ppb) (LOD=0.1 ppb)*						
	Nominal concentration (ug/L)						
	Control 1†	Control 2†	10	20	40	80	160
	Nominal concentration (ppb) ‡						
	0	0	8.1	16.3	32.5	65.1	130.1
A	<LOD	<LOQ	5.72	10.39	23.13	31.88	31.4
B	<LOD	<LOD	5.79	10.44	16.17	27.17	61.58
C	<LOD	<LOD	5.98	9.87	22.66	38.86	72.60
D	<LOQ	<LOD	6.63	9.94	21.32	29.55	61.82
E	<LOD	<LOQ	3.79	9.87	20.45	28.40	69.23
F	<LOD	1.4	5.06	12.04	-	32.78	107.69
G	<LOD	<LOD	5.35	10.06	19.56	47.53	41.92
H	<LOQ	<LOD	4.81	8.45	12.66	42.57	40.83
I	<LOD	**	3.09	8.56	12.97	25	63.90
J	<LOD	<LOD	5.97	8.94	23.94	45	77.5
K	<LOD	<LOD	7.06	10.06	19.69	46.20	68.99
L	<LOD	<LOD	6.82	13.25	26.86	48.97	90.38

Number of samples	12	11	12	12	11	12	12
Average concentration	<LOQ		5.51	10.17	19.94	36.99	65.65
% Feeding concentration††	Not applicable		68.0	62.4	61.4	56.8	50.5

* Concentrations in all treatments except for the controls are corrected to 50% sugar using Brix values that are not listed in the table, but were in the table section 7 on page 388-391 of the study report (brix values reported as >80, were assumed to be 80% for the purpose of this calculation). The brix corrected residue value reported here is determined using the formula that the brix corrected concentration = measured concentration * (feeding solution brix {50%}/hive nectar measured brix)
** Hive was removed from the study due to a technical error and no sample collected.
***Hive was removed from study after it was found knocked over and no sample collected.
† Concentrations in the controls are measured concentrations in hive uncapped without corrections for sugar concentrations.
‡Nominal concentration in ppb is estimated from the concentration in µg/L by assuming the volume density of the test solution to be 1.2296 g/ml.
†† % Feeding concentration: the average of measured concentration compared with the nominal feeding concentration in ppb.

3.7.2.3. *Residues in hive matrices at CCA7 (prior to overwintering)*

The level of clothianidin in capped honey at the last CCA prior to overwintering (CCA7, 14-22 Oct, 2014) is summarized in **Table 15**. As with the uncapped nectar results from CCAs 4 & 5, these measurements generally indicated a dose-response correlation between the average concentrations of clothianidin measured in the hive matrix (capped honey) and the concentrations in the feeding solution. However, the concentrations varied remarkably within some treatments and the mean concentrations in capped honey were very similar between the nominal 20 and 40 µg/L concentrations (means of 6.15 and 6.38 ppb, respectively). The concentration of clothianidin in the capped honey was lower than in the uncapped nectar at CCA5, indicating that either bees were continuing to consume the clothianidin-exposed food stores, there was continued dilution of the stores from other nectar sources and/or there was potential degradation of clothianidin in the capped honey (which seems unlikely given the storage stability data).

At this point in the study there were more hives that did not have measurements taken (compared with the measurements at CCAs 4 and 5), often due to insufficient capped honey stores for samples to be taken. Five control hives and six treatment hives were not measured. Of the treatment hives with measurements, levels below either the LOD or LOQ were reported in three hives at 10 µg/L, one hive at 20 µg/L, two hives at 40 µg/L, three hives at 80 µg/L and one hive at 160 µg/L, while the remaining treatment hives had quantifiable levels of clothianidin. Clothianidin was undetected in all but one control samples (18/19) and was detected at levels below the LOQ in the remaining sample. After correction with Brix values to 50% sugar concentration, the mean of the measured concentrations in capped honey within each treatment group of 10, 20, 40, 80 and 160 ug/L (8.1, 16.3, 32.5, 65.1, and 130.1 ppb) was 2.14 (range: <LOD-6.81), 6.15 (range: <LOQ-10.88), 6.38 (range: <LOD-21.38), 22.32 (range: <LOD-44.31), and 27.23 ppb (range: <LOD-66.25), respectively. By average, the measured concentration in capped honey was 27.8% (range 19.6-37.7%) of the concentration in feeding solution. It is notable that the mean residues in uncapped nectar in the 20 and 40 µg/L treatment groups were highly similar.

Table 8. Clothianidin concentrations (ppb) in capped honey sampled 97--103 days after the start of artificial feeding during CCA7 on either 14-16 Oct, 2014 (all but the highest treatment groups) or 21-22 Oct, 2014 (80 and 160 µg/L groups only).

Apiary	**Measured Clothianidin concentrations (ppb) (LOD=0.1 ppb)***						
	Nominal concentration (µg/L)						
	Control 1†	**Control 2†**	**10**	**20**	**40**	**80**	**160**
	Nominal concentration (ppb) ‡						
	0	**0**	**8.1**	**16.3**	**32.5**	**65.1**	**130.1**
A	<LOD	<LOD	--	6.63	6.44	<LOD	39.81
B	--	<LOD	<LOD	10.88	6.56	32.63	24.29
C	<LOD	<LOD	1.29	3.35	--	25.19	<LOD
D	<LOQ	--	6.81	5.72	<LOD	<LOQ	22.06
E	<LOD	<LOD	<LOD	3.02	2.28	<LOQ	17.97
F	<LOD	<LOD	2.16	3.06	--	16	49.5
G	<LOD	<LOD	<LOD	8.19	5.04	30.13	29.88
H	--	<LOD	0.78	7.19	3.7	36.5	45.94
I	<LOD	--	3.52	8.94	3.49	39.75	14.69
J	<LOD	<LOD	4.59	10.38	21.38	44.31	5.59
K	<LOD	--	--	<LOQ	<LOD	30.56	66.25
L	<LOD	<LOD	--	--	14.88	12.09	10.75
Number of samples	10	9	9	11	10	12	12
Average concentration	<LOD		2.14	6.15	6.38	22.32	27.23
% Feeding concentration††	Not applicable		26.4	37.7	19.6	34.3	20.9

* Concentrations in all treatments except for the controls are corrected to 50% sugar using Brix values that are not listed in the table, but were in the table section 8 on page 392-395 of the study report (brix values reported as >80, were assumed to be 80% for the purpose of this calculation). The brix corrected residue value reported here is determined using the formula that the brix corrected concentration = measured concentration * (feeding solution brix {50%}/hive nectar measured brix)
"-" indicates that no data are available (either no sample was taken {due to either minimal capped honey stores or the hive had already been removed due to technical errors or vandalism} or no sample was received by the analytical lab {only 1 instance})
† Concentrations in the controls are measured concentrations in hive uncapped without corrections for sugar concentrations.
‡Nominal concentration in ppb is estimated from the concentration in µg/L by assuming the volume density of the test solution to be 1.2296 g/ml.
†† % Feeding concentration: the average of measured concentration compared with the nominal feeding concentration in ppb.

3.7.2.4. *Residues in hive matrices at CCA9 (following overwintering)*

The level of clothianidin in capped honey at the final CCA following overwintering (CCA9, 22-23 Apr, 2015 for UTC, 10, 20, and 40 ppb; 27 Apr 2015 for 80 ppb treatment group) is summarized in **Table 16**. As with the uncapped nectar results from CCAs 4 & 5 and capped honey from CCA 7, these measurements generally indicated a dose-response correlation between the average concentrations of clothianidin measured in capped honey and the concentrations in the feeding solution. However, the concentrations varied remarkably within some treatments and as survival in several treatments was very poor (including in controls), and samples were only taken from surviving hives, there were a low number of hive samples in all but the 20 and 80 µg/L treatment groups.

Of the treatment hives with measurements, levels below the LOQ were reported in two (out of three remaining) hives at 10 µg/L, three hives (out of eight) at 20 µg/L, one hive (out of five) at 40 µg/L, and two hives (out of ten) at 80 µg/L, while the remaining surviving treatment hives had quantifiable levels of clothianidin. Out of eight surviving control hives, clothianidin was undetected in six samples and was detected at levels below the LOQ in the remaining two samples. After correction with Brix values to 50% sugar concentration, the mean of the measured concentrations in capped honey within each treatment group of 10, 20, 40, and 80 ug/L (8.1, 16.3, 32.5, and 65.1 ppb) was 1.17 (range: <LOQ-2.89), 1.82 (range: <LOQ-6.81), 5.24 (range: <LOQ-12.88), and 13.41 ppb (range: <LOQ-31.5), respectively. The average measured concentration in capped honey at CCA9 was 15.6% (range 11.2-20.6%) of the concentration in feeding solution. No measurement was provided for treatment at 160 ug/L as all colonies in this treatment group were destroyed after CCA7 (Dec, 2014). The unmeasured level of residues in dead hives presents an additional uncertainty as to the average residues that might represent the level of treatments at following overwintering.

Table 9. Clothianidin concentrations (ppb) in capped honey sampled 305 days after the start of artificial feeding on 22-27 Apr (CCA9).

Apiary	Measured Clothianidin concentrations (ppb) (LOD=0.1 ppb)*						
	Nominal concentration (ug/L)						
	Control 1†	Control 2†	10	20	40	80	160
	Nominal concentration (ppb) ‡						
	0	0	8.1	16.3	32.5	65.1	130.1
A	--	--	--	2.43	12.88	5.89	--
B	--	--	<LOQ	--	2.49	1.65	--
C	<LOD	--	--	<LOQ	--	31.5	--
D	--	<LOD	--	--	<LOQ	<LOQ	--
E	--	<LOD	--	1.91	--	<LOQ	--
F	--	--	2.89	--	***	12.06	--
G	--	<LOQ	--	0.68	--	23.63	--
H	--	<LOD	<LOQ	--	--	26.28	--
I	<LOD	**	--	1.78	3.69	--	--
J	--	<LOD	--	<LOQ	6.81	31.5	--
K	<LOQ	--	--	<LOQ	--	0.98	--
L	--	--	--	6.81	--	--	--
Number of samples	3	5	3	8	5	10	0
Average concentration	<LOQ		1.17	1.82	5.24	13.41	N/A
% Feeding concentration††	Not applicable		14.4	11.2	16.1	20.6	N/A

* Concentrations in all treatments except for the controls are corrected to 50% sugar using Brix values that are not listed in the table, but were in the table section 9 on page 396-398 of the study report (brix values reported as >80, were assumed to be 80% for the purpose of this calculation). The brix corrected residue value reported here is determined using the formula that the brix corrected concentration = measured concentration * (feeding solution brix {50%}/hive nectar measured brix)

"-" indicates that no data are available (either no sample was taken {due to either a dead hive, minimal capped honey stores or the hive had already been removed due to technical errors or vandalism} or no sample was received by the analytical lab {only 1 instance})

† Concentrations in the controls are measured concentrations in hive uncapped without corrections for sugar concentrations.

‡Nominal concentration in ppb is estimated from the concentration in µg/L by assuming the volume density of the test solution to be 1.2296 g/ml.

†† % Feeding concentration: the average of measured concentration compared with the nominal feeding concentration in ppb.

3.7.2.5. *Comparison of concentration in feeding solution and hive matrices*

A correlation between the clothianidin concentrations in the feeding solution and the concentrations measured in hive beebread, uncapped nectar and capped honey was observed in the middle of the exposure period (CCA4), one week after the end of exposure (CCA5) and continued through succeeding CSAs (CCAs 7 and 9). However, clothianidin measured concentrations in all hive matrices were lower than that in the feeding solutions (**Table 17**).

Table 10. Clothianidin concentration measured in hive uncapped nectar, capped honey and hive bee bread compared to nominal concentrations in the test feeding solutions

Nominal concentration in test feeding solution	**µg/L**	**10**	**20**	**40**	**80**	**160**	**Average**
	ppb	**8.1**	**16.3**	**32.5**	**65.1**	**130.1**	
Clothianidin concentration in hive uncapped nectar (CCAs 4 and 5) or capped honey (CCAs 7 and 9) in % of the concentration of nominal feeding solution (average measured concentration in ppb)[¥]	CCA 4	36.7 (2.97)	36.9 (6.01)	38.3 (12.44)	24.1 (15.67)	16.6 (21.61)	30.5
	CCA 5	68.0 (5.51)	62.4 (10.17)	61.4 (19.94)	56.8 (36.99)	50.5 (65.5)	59.8
	CCA 7	26.4 (2.14)	37.7 (6.15)	19.6 (6.38)	34.3 (22.32)	20.9 (27.23)	27.8
	CCA 9	14.4 (1.17)	11.2 (1.82)	16.1 (5.24)	20.6 (13.41)	N/A	15.6
Clothianidin concentration in hive beebread in % of the concentration of nominal feeding solution (average measured concentration in ppb)	CCA 5	43.4 (3.52)	41.0 (6.68)	37.4 (12.16)	54.9 (35.75)	N/A	44.2

The study did not test for clothianidin degradation products (*e.g.* TZNG) in the test solution. Considering the stability of clothianidin in the test solution, the reduced concentrations of clothianidin in hive matrices likely indicates that test bees were also foraging for pollen and nectar from sources other than the feeding solution.

3.8. Pathogens

Besides a standard treatment for *Varroa* mites, no treatments for any other hive pests, predators or diseases were administered to any hives.

3.8.1. *Varroa* Presence

Varroa mite occurrence in the colonies was assessed the week before and after the feeding period, as well as after over-wintering (CCA3, CCA5 and CCA9). The number of mites per 100 bees was counted following washing bees in alcohol to remove the mites. Hives were treated with one application of Apiguard® (active ingredient: thymol) following typical apicultural practice for the region immediately after the September CCA's to prevent high mite loads.
Prior to exposure at CCA3, the hives had similar mite loads (mean ranges of 0.28—0.44 mites/100 bees). Immediately following exposure (CCA5), mite loads were more variable (mean ranges of 0.71-2.40 mites/100 bees), but generally appeared to be positively correlated with treatment dose, though the 160 µg/L treatment group had lower infestation levels compared with the 80 µg/L treatment group (Error! Reference source not found. **5**). After over-wintering, *Varroa* levels were

highly variable (mean ranges of 0.67-2.61mites/100 bees) and did not appear to follow a dose-response relationship, though this may be confounded by the low number of remaining hives with measurements in both contols and treatment groups.

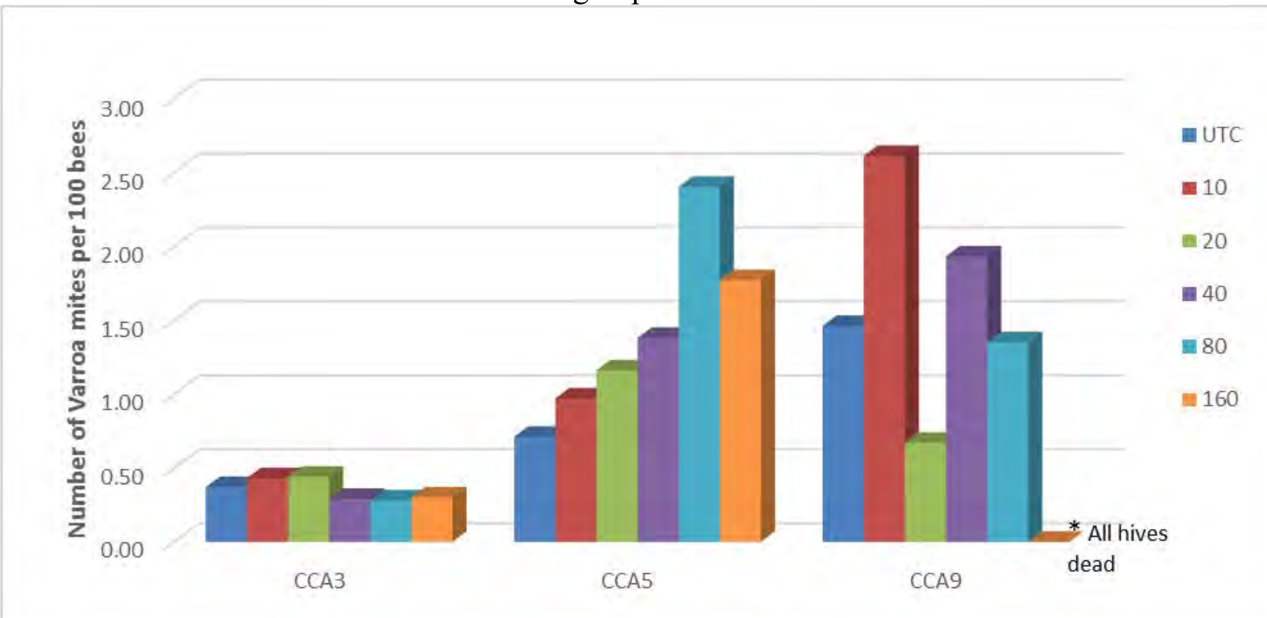


Figure 5. Varroa infestation levels in control and treatment groups prior to exposure (CCA 3), immediately following the termination of exposure (CCA5) and after over-wintering (CCA9).

3.8.2. *Nosema* presence

The number of *Nosema* spores per bee was determined at three time points at CCA3, CCA5 and CCA9. At CCA3, there were 2-3 measurements per hive, while at CCA9 there were 2 measurements and at CCA5 there was only 1 sample measurement. It was unclear from the study report why the number of samples were different between the CCAs or why different numbers of samples were taken at some hives during CCA3. There generally appeared to be no trend between *Nosema* infestation and treatment dose, though there were generally more *Nosema* spores in the higher treatments following overwintering, although the 10 µg/L treatment group had lower levels than controls at this measurement time (**Figure 6)** and the 20 µg/L treatment group was only slightly elevated compared to the controls (2.29 million spores/bee comapred to 2.15 million spores/bee). As with Varroa, above, the CCA9 numbers may also be confounded by the low number of remaining surviving hives in both control and treatment groups.

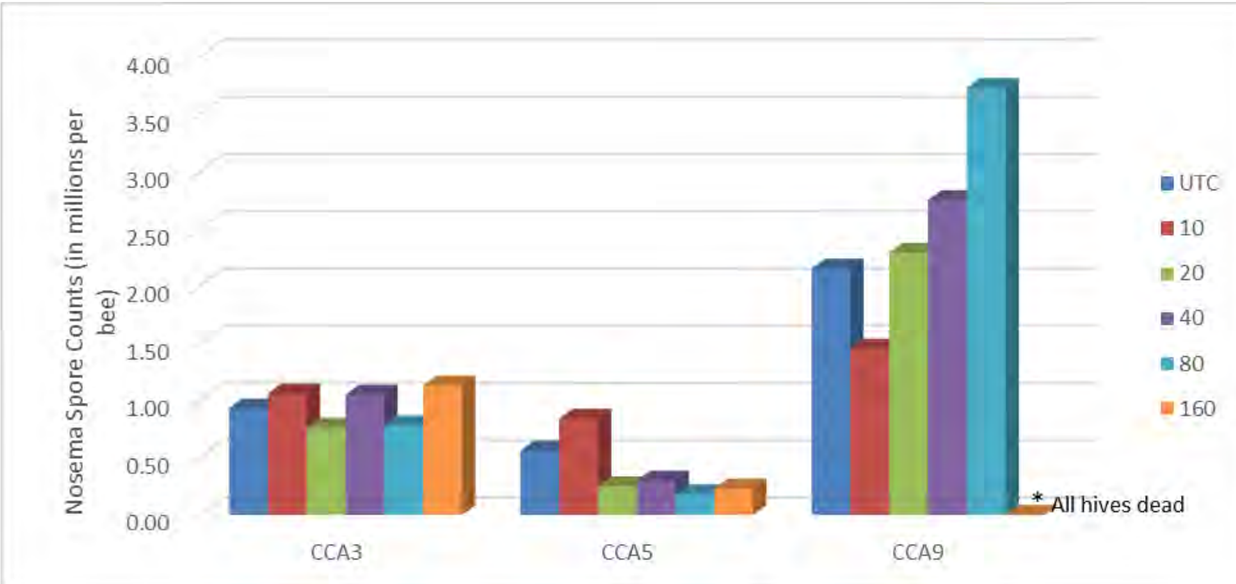


Figure 6. Nosema spore loads in control and treatment groups prior to exposure (CCA 3), immediately following the termination of exposure (CCA5) and after over-wintering (CCA9).

3.9. Statistical Analysis

What follows are brief summaries of the study author's and reviewer's statistical analyses employed for the review of this study.

3.9.1. Study Author's Analysis

The study author conducted statistical analysis using SAS (version 9.3). The analysis included colony strength (as indicated by mean number of adults), brood stages (as indicated by the mean number of eggs, larval cells, and pupal cells) and food stores (as indicated by the mean number of pollen and nectar/honey cells). For the pre-test data, all tests were done in a two tailed approach, whereas for the data assessed after exposure, one tailed (lower) tests were conducted. According to the study author, after Shapiro-Wilks and Levene's were used to test assumptions of normality and equal variances, respectively, procedure GLM was used for the ANOVA analysis. Williams' Trend Test was used to test data that passed the assumptions of normality, variance homogeneity, and monotonicity. Dunnett's t-Test was used to test data that were non-monotonic, but passed tests of normality and variance homogeneity. Dunnett's T3 Test with Rank Transformed (within blocks) data was used to test data that were normally distributed, but failed the criteria for equal variance.

3.9.2. Study Reviewer's Statistical Analysis Approach

As part of the collaborative review effort of the study, separate statistical analyses were conducted by EPA and PMRA using the raw data submitted by the study author. A description of EPA's statistical methodology is provided here while PMRA's methodology is presented in **Appendix A.** However, the discussions below in the Colony Condition Assessment section (**Section 3.9.5)** presents the results of both analyses. It is noted that while the Agencies utilized different statistical analysis approaches, interpretations based on the PMRA analysis tended to be similar to interpretations from the EPA analysis. Although the PRMA analysis resulted in some differences in statistically significant endpoints and time periods, these differences do not significantly alter the ultimate biological interpretation of the study regarding colony level effects leading to a clearly defined, highly-confident protective endpoint.

The general experimental design was a randomized complete block (apiary) with repeated measures (CCA) and data will be analyzed in SAS (v9.4) using the PROC MIXED procedure. Since hives were not assigned and placed in the study apiaries until shortly before CCA3, the data for the statistical analysis only included data collected from CCA3 and the following CCAs. Shortly before CCA3, hives were ranked by strength and the 'strongest' hives were placed in the one apiary. The next eight strongest hives were then placed in an empty apiary. This process continued until hives were placed in all apiaries. Within each apiary, the control treatment was replicated two times and each treatment occurred one time (total of 8 hives in each apiary: seven hives were randomly assigned as control or treatment group and the eighth hive was used for additional sampling during the study). Given this design, the blocking factor 'apiary,' represents variation due to geographic location and initial hive strength.

As a large percentage of hives did not survive overwintering, data collected the following spring will not be included in the statistical analyses. Other than the three hives (removed from the study and noted in **Table 18**), no hive mortality occurred prior to overwintering.

Table 11. Timeline including major milestones of study

Date	Study action*	Comments
12 May 2014	Initiate CCA1 (non-GLP)	Not included in statistical analysis.
2 Jun 2014	Initiate CCA2 (non-GLP)	Not included in statistical analysis.
17-18 Jun 2014	Hives moved to study locations	none
18 Jun 2014	Initiate CCA3 (non-GLP)	First CCA to be included in the statistical analyses.
26 Jun 2014	Initiate clothianidin exposure through sucrose solution.	none
15 Jul 2014	Initiate CCA4 (GLP)	Hive I7 (control) removed from study; possible contamination. Hive F3 (40 ppb) removed from study; found knocked over.
5 Aug 2014	Initiate CCA5 (GLP)	none
7 Aug 2014	End clothianidin exposure through sucrose solution	none
8 Sep 2014	Initiate CCA6 (GLP)	Hive C2 (40 ppb) removed from study; found knocked over.
14 Oct 2014	Initiate CCA7 (GLP)	Final CCA to be included in the statistical analyses.
December 2014	All remaining hives in the 160 ppb treatment group were destroyed as colony strength was low and they were not expected to survive the winter.	
17 Mar 2015	Initiate CCA8 (GLP)	Overwintering survival was 35, 27, 67, 50, and 85% for control, 10 ppb, 20 ppb, 40 ppb, and 80 ppb treatment groups, respectively. Therefore, CCA8 and CCA9 were not included in statistical analyses.
22 Apr 2015	Initiate CCA9 (GLP)	
*each CCA took three or more days to complete.		

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Variables recorded at each CCA included number of adult bees in the hive and number of cells containing each of the following life stages or food stores: eggs, larvae (open cells), pupae (closed cells), pollen, and honey. Following standard bee keeping practices, supers were added or removed from each hive to best support growth or reductions in the size of the bee colony. Timing for addition and removal of supers is provided in **Appendix B**. A queen excluder was placed between the initial hive box and added super boxes; this limited the summed number of egg, pupae, and larvae cells to the number of cells in the initial box (3970 cells). All adult bees, with the exception of the queen, could move to any added supers, and honey and pollen could be stored in those additional supers as well. The suite of variables that were subjected to data analysis were:

- Number of adults
- Number of egg cells
- Number of open (larvae) cells
- Number of capped (pupae) cells
- Number of pollen cells
- Number of honey cells
- Total number of individuals (adults + eggs + larvae + pupae)
- Total brood (eggs + larvae + pupae), and
- Total food (pollen + honey).

To facilitate computation and algorithm convergence in the SAS Procedures, all data was divided by 1000 prior to any statistical analysis. Since all response variables were divided by the same constant, there was no effect on any of the test statistics or p-values. No adjustments for addition or removal of supers were conducted for the statistical analysis.

Total brood and total food are new summary variables; EPA's Environmental Fate and Effects Division (EFED) is still evaluating their utility in providing additional information on biological effects beyond the initial set of variables. PMRA did not analyze these summary variables in their statistical analysis.

Prior to the repeated measures analysis, the data were evaluated for patterns in temporal correlation and correlations across hive components within each of the evaluated CCAs. This analysis was accomplished through a series of pairwise scatterplots and principle components analyses (PCA).

3.9.2.1 *Scatterplot and Principle Component Analysis*

Based on physical hive constructs and the nature of honey bees, it is generally accepted that the colony condition assessment (CCA) variables may be correlated over time and may also be correlated within a time point (sampling time). Given this background, a series of scatterplots, correlation matrices, and principle component analyses was prepared; the full SAS output is included as **Attachments 1-3**. For these analyses, there was no adjustment for treatment effects, only correlation over time was evaluated.

For the single hive components, adults, eggs, larvae, pupae, pollen, and honey, some of the general summary points are:

- Honey had the strongest and most consistent pairwise correlations across all the time points.
- CCA3 tended to have the lowest pairwise correlations with the other CCAs for all components.
- For each of the hive components, the first principle component explained 46 to 85% of the total variation across all CCAs; the lowest percent of explained variation was for larvae and the highest was for honey.
- For each of the hive components except honey, the general interpretation of the first principle component was a weighted average with CCA4, CCA5, CCA6, and CCA7 carrying approximately equal weights and CCA3 carrying much less weight. Note that for pupae, CCA7 carried slightly less weight than CCA4, CCA5, and CCA6. For honey, the general interpretation of the first principle component was a weighted average with all CCAs carrying approximately equal weight.

For the three composite hive variables (live, brood, and food), general summary points are:

- For live, all possible pairwise correlations between CCA4, CCA5, CCA6, and CCA7 ranged from 0.61 to 0.87, while the pairwise correlations between CCA3 and the following CCAs ranged from 0.15 to 0.30. For brood, the pairwise correlations between CCA4, CCA5, and CCA6 were highest; pairwise correlations with CCA7 were lower, and pairwise correlations with CCA3 were the lowest.
- For live and brood, the first principle component explained 67% and 57%, respectively, of the total variation. As with the individual components, the general interpretation of the first principle component was a weighted average over all time points with CCA3 carrying the least weight. For brood, as with pupae (above), CCA7 carried slightly less weight than CCA4, CCA5, and CCA6.
- For food, all pairwise correlations were strong (ranged from 0.67 to 0.92), and the general interpretation of the first principle component was a weighted average with all CCAs carrying approximately equal weight.

In addition to exploring correlations among CCAs for each of the response variables, correlations among response variables within a CCA were explored. For this exploratory analysis, only the individual hive components were evaluated. No adjustment was made for treatment effects (*i.e.*, all data were included in a single series of plots and PCAs; separate assessments were not done for each treatment). Some general interpretations are:

- For all of the CCAs, honey had the weakest pairwise correlations (honey with any of the other measured matrices) amongst all the pairwise correlations. For many of the CCAs, honey was negatively correlated with some of the other variables.
- For each of the CCAs, the percent of the total variation explained by the first principle component ranged from 40 to 52%. At each time point the first principle component tended to be interpreted as a weighted average. The weights and interpretations for the first principle component were not consistent when compared across CCAs.

3.9.2.2 Analysis Approach and Model Setup

As discussed above, the experimental design was a randomized complete block (apiary) with repeated measures (CCAs). Exploring the interaction between treatment and CCA can address these two questions:

- At each CCA, was there a reduction in the response relative to the control?
- At each treatment level, was there a difference in the response relative to the baseline time point (CCA3)?

With the experimental design component of the analysis established, the next part of the analysis was to determine which correlation structure (across time) was the best fitting for these data. The scatterplots, correlation matrices, and principle component analyses were used to inform the choice of covariance structure used in the repeated measure analysis. Some summary points from the above exploratory analyses are that temporal correlations within a response variable tended to be stronger than correlations among response variables within a time point; variance for a given response variable was not homogenous among the CCAs; and that the pairwise correlations did not consistently decrease as the distance between the temporal pairs increased.

Before conducting any comparisons among treatments or CCAs, several different correlation structures to best fit the temporal correlation were evaluated. The structures that were fitted included:

- **Compound symmetry (CS)**: assumes equal correlation for all pairwise correlations (regardless of distance of time point).
- **Compound symmetry with heterogeneous variance (CSH):** Estimates a unique variance at each time point, but assumes equal correlation for all pairwise correlations (regardless of distance of time point).
- **Autoregressive correlation (AR(1))**. Assumes equal correlation between adjacent time points. Time points further apart have a lesser correlation.
- **Heterogeneous Toeplitz (ToepH):** models a unique variance for each time point and separate correlations for equidistant time points (*e.g.*, correlation between CCA3 and CCA5 is the same as the correlation between CCA4 and CCA6).

More information about each of the covariance structures available in the REPEATED statement in SAS can be found here: https://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#statug_mixed_sect019.htm . The full SAS output is provided in **Attachment 1.**

To compare covariance structure fits, Bayesian Information Criterion (BIC) was utilized[3]. The BIC is a function of the log likelihood with a penalty for an increase in the number of covariance parameters to be fitted. The BIC value for each fitted model for all response variables is reported in **Table 19; s**maller values of the BIC indicate a better fit (bolded). For many of the endpoints, heterogeneity of variance at different time points was indicated as compound symmetry with heterogeneous variance (CSH) and heterogeneous Toeplitz (ToepH) were the covariance structures providing the best fits. This is not surprising as unequal variances were observed in the exploratory multivariate/principle component analysis.

---

[3] Schwarz, Gideon. Estimating the Dimension of a Model. Ann. Statist. 6 (1978), no. 2, 461--464. doi:10.1214/aos/1176344136. http://projecteuclid.org/euclid.aos/1176344136.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 12. BIC values for fitted models. CCA3 – CCA7 -clothianidin

Variable → Model ↓	Adults	Eggs	Larvae (open)	Pupae (capped)	Pollen	Honey	Live	Brood	Food
CS	2216	1671	2005	2281	2118	2950	2852	2640	3018
CSH	2216	**1614**	**1990**	**2248**	**2062**	2938	2829	**2619**	3012
AR(1)	**2189**	1670	1998@	2277	2118	2905	2833	2632	**2974**
ToepH	**2197**	**1609**	**1983@**	**2247@**	**2059@**	**2893@**	**2810@**	**2614@**	**2972@**
*Within a response variable, smaller BIC values (bolded) indicate better covariance model fit. Kass and Raferty (1995) suggested that differences of greater than 10 in BIC values provides very strong evidence that model fits are not equivalent. @Convergence was attained, but estimated G matrix was not positive definite and not all covariance parameters could be estimated.									

For all the evaluated response variables, ToepH was identified as one of the 'best fitting' covariance structures; however, all covariance parameters could not be estimated for majority of the endpoints. CSH was identified as one of the best fitting covariance structures for four of the six single hive components and one of the three composite hive variable. Compound symmetry (CS) was not identified as quality fit to the data for any of the eight evaluated response variables. AR(1) was identified as a quality fit for two of the evaluated endpoints.

Residual plots were also evaluated for each of the response variables and covariance structures. Patterns indicative of heterogeneous variance of the residuals were evident for many of the response variables and models where an assumption of equal variance at each time point was made. For many of the residual plots when CS or AR(1) covariance structure was modeled, the vertical spread of the residuals around increased as the predicted mean increased (indicating larger variances as the mean increased; see **Figure 7**, for example). These response variables are counts, hence the distribution of the response variable and the residuals may not meet assumptions of normality and/or equal variance. More specifically, review of the residual plots indicates that estimating utilizing a covariance structure that estimated unique variances for each CCA (*e.g.*, CSH, ToepH covariance structures) appears to improve overall model fit.

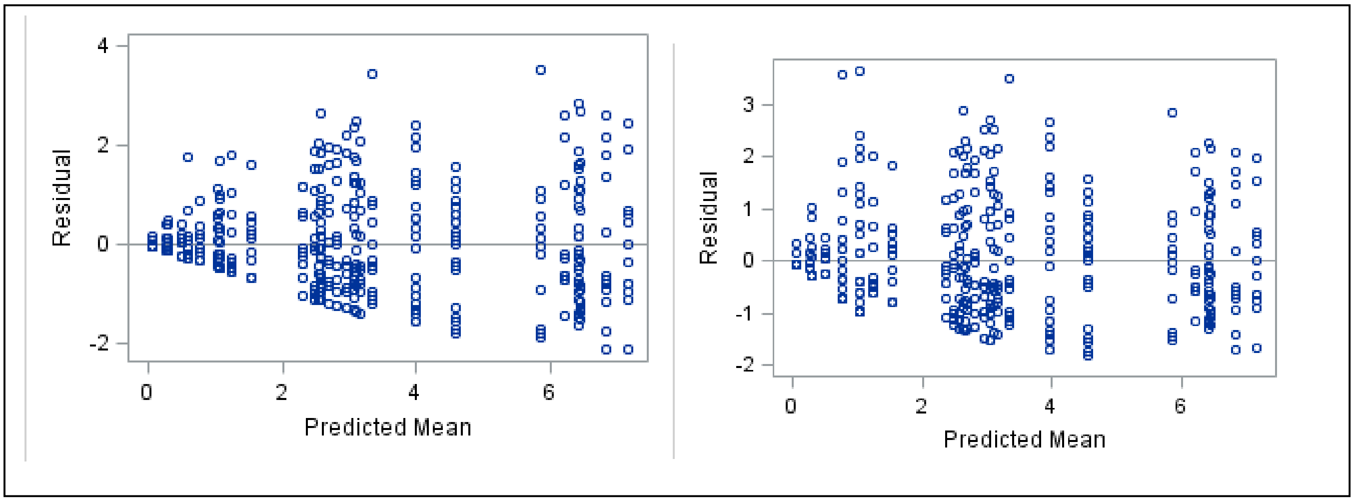


Figure 7. Studentized residual plots for eggs with covariance structures of (left) compound symmetry (CS) and (right) compound symmetry with heterogeneous variance (CSH). Distribution of the residuals indicates a better fitting model for the CSH covariance structure.

Of the evaluated models, either CSH or ToepH should be selected as the covariance structure for the repeated measure of CCA as they provided better fitted models for multiple endpoints. The additional covariance parameters could not always be estimated in the ToepH model suggesting that the increase in the number of parameters relative to CSH that were to be estimated is an overparameterization of the model based on the available data. Therefore, the review team elected to move forward with the heterogeneous compound symmetry (CSH) covariance structure for the final analyses.

3.9.2.3 Treatment by Time Interaction and Follow-up Contrasts

The text box below provides the SAS code for the mixed model that was used for follow-up statistical contrasts to address the following questions:

- At each CCA, was there a reduction in the response relative to the control?
- At each treatment level, was there a difference in the response relative to the baseline time point (CCA3)?

- The contrasts that were utilized for this analysis were Dunnett's test. Dunnett's test is a set of pairwise contrasts in which each treatment mean is compared to the control mean; the tests can be one- or two-sided. For a given set of contrasts, the experiment-wise error-rate is controlled as the specified alpha-level. In this case, a 'set of contrasts' is either (1) comparisons of treatment means to the control for a specific endpoint at a specific CCA or (2) comparison of time-points CCA4, CCA5, CCA6, and CCA7 to the baseline CCA3 for a given endpoint. For all analyses, the CSH covariance matrix was used for each of the variables.

Text Box 1. SAS Code for the mixed model used to run the statistical analysis

```
title 'Clothianidin - ColonyFeedingStudy(2015) data analysis';
proc mixed data=cca3_7  ;
      title2 "Dunnett's tests - adult_scale";
      class apiary cca conc hive;
                model adult_scale = conc|cca /DDFM=SATTERTHWAITE;
                random apiary  ;
                repeated cca/ subject=hive*conc(apiary) type=csh ;
                lsmeans  conc*cca/cl;
                slice conc*cca /sliceby=cca diff=controll adjust=dunnett;
                slice conc*cca /sliceby=conc diff=control adjust=dunnett;
                run;
```

Williams' test was also considered for use for one set of the follow-up contrasts - comparisons of treatment means to the control for a specific endpoint at a specific CCA. Williams' test has been shown to be more powerful than Dunnett's test when the assumption of monotonicity is met. Williams' requires the assumption that if there is an effect of the chemical, it follows the classic dose-response relationship (*i.e.*, assuming there test material has a negative effect on the response variable, then as the test concentration increases, mean response is equal to or less than the mean response of the next lower dose concentration). The test procedure then determines the lowest dose level for which the mean is significantly less than the control mean. This concentration is identified as the LOAEC and the next lower concentration is identified as the NOAEC. Williams' test was not utilized for this analysis for several reasons:

- Review of the treatment means identified several instances when the underlying assumption of monotonicity does not appear to be met. Given the large variation in the measured responses in general, it could not be determined if the observed deviations from monotonicity were due to large background variation or to a non-monotone treatment response.
- For any one response variable, the data are combined across CCAs into one mixed model analysis. Incorporating data from all CCAs improves the variance/covariance estimates and increases the degrees of freedom for hypothesis testing. As the degrees of freedom for hypothesis testing increases, differences in power between Dunnett's test and Williams' test become small.
- It has not been codified in the PROC MIXED procedure in SAS, and the level of effort to code and QA the test would be significant.

An analysis approach where data from each CCA was analyzed separately as a randomized complete block design was also considered as SAS has options for use of Williams' test for simpler experimental designs. This approach was not selected for several reasons:

- Equality of variance would still need to be evaluated. If the assumption of homogenous variances was not met for some CCAs, then transforming the response or non-parametric analyses would need to be considered. Incorporating the heterogeneous variances into the error matrices of the general linear model (GLM) would increase the complexity of the model such that the Williams' options in SAS could no longer be utilized.
- A statistical analysis approach that does not utilize the strength of the correlations among time points to improve estimates of error variance would not be as powerful as one that does incorporate that additional information about the nature of the responses.

3.9.3 Treatment Effects Within a CCA

The table of p-values resulting from the Dunnett's tests (for evaluating whether within a CCA, the treatment mean are significantly less than control means) are summarized in **Table 20. Figures 8-17** below show the results for each response variable across all CCAs analyzed (CCA3-CCA7) and all treatment levels. For all the figures presented below, significant reductions from the negative control with p-values below the 0.05 alpha level are denoted by a red dot at a given treatment level and CCA and those reductions with p-values between 0.05 and 0.1 are denoted by a black dot. Statistical NOAECs and LOAECs within a CCA will be determined using an alpha-level of 0.05. Additional comparisons using and alpha-level of 0.10 are included for additional characterization. Error bars represent one standard error from the mean calculated from the model residual mean squares estimate. The associated SAS output containing the full results of the Dunnett's comparisons can be found in **Attachment 1.**

Table 13. Results of one-sided Dunnett's test (comparing control to each treatment group), correlations modeled using CSH. Cells include the treatment groups that were significantly lower than control.

	Adults	Eggs	Larvae (Open)	Pupae (Capped)	Pollen	Honey	Live	Brood	Food
CCA3	NS	NS	NS	NS	NS	NS	NS	NS	NS
CCA4	**160**	*80* **160**	**160**	**40** **80** **160**	*40* **80** **160**	NS	**40** **80** **160**	**40** **80** **160**	NS
CCA5	**40** **80** **160**	*20* **80** **160**	**80** **160**	**40** **80** **160**	**10** **20** **40** **80** **160**	NS	**40** **80** **160**	**40** **80** **160**	NS
CCA6	**10** **40** **80** **160**	*80* **160**	**80** **160**	**40** **80** **160**	**80** **160**	NS	**40** **80** **160**	**40** **80** **160**	NS
CCA7	**80** **160**	**80** **160**	**80** **160**	**160**	**10** **80** **160**	NS	**80** **160**	**80** **160**	NS

	Adults	Eggs	Larvae (Open)	Pupae (Capped)	Pollen	Honey	Live	Brood	Food
* NS indicates that there were no test concentrations with means significantly less than the control (p>0.10). **Bolded concentration = significantly less than control (p< 0.05)** *Italicized concentration = less than control (0.05< p < 0.10)*									

3.9.4 Temporal Trends Within a Treatment Level

A second component to evaluating the "treatment x CCA" interaction is to look at the temporal changes within a treatment group. This was accomplished by comparing each CCA (CCA4 through CCA7) to CCA3 by use of a two-sided Dunnett's test (**Table 21** and **Table 22**). This suite of comparisons is not as informative as the contrasts of control against the treatment group within a CCA for establishing a statistical NOAEC and LOAEC. However, it may aid in interpretations and further biological understanding of temporal shifts in the life stages and food components present in the hive. Differences in patterns of temporal shifts between the control and various treatment groups can provide further understanding of the potential impacts of clothianidin on beehive population dynamics.

Table 14. Results of two-sided Dunnett's test (comparing CCA3 to each following CCA), correlations modeled using CSH.

Trt Group	Response Variable				
	Adults	**Eggs**	**Open**	**Capped**	**Pollen**
Control	CCA5-6>CCA3	CCA4-7<CCA3	CCA7<CCA3	CCA7<CCA3	CCA4-5>CCA3
10	CCA7<CCA3	CCA4-7<CCA3	CCA7<CCA3	CCA7<CCA3	CCA4>CCA3 CCA7<CCA3
20	CCA5>CCA3	CCA4-7<CCA3	CCA7<CCA3	CCA7<CCA3	CCA4>CCA3
40	CCA7<CCA3	CCA4-7<CCA3	CCA7<CCA3	CCA5-7<CCA3	CCA4>CCA3
80	CCA5-7<CCA3	CCA4-7<CCA3	CCA4-7<CCA3	CCA4-7<CCA3	CCA5-7<CCA3
160	CCA4-7<CCA3	CCA4-7<CCA3	CCA4-7<CCA3	CCA4-7<CCA3	CCA4-7<CCA3

Table 15. Results of two-sided Dunnett's test (comparing CCA3 to each following CCA), correlations modeled using CSH.

Trt Group	Response Variable			
	Honey	**Live**	**Brood**	**Food**
Control	CCA4-5>CCA3 CCA6-7<CCA3	CCA7<CCA3	CCA7<CCA3	CCA4-5>CCA3 CCA6-7<CCA3
10	CCA4-5>CCA3 CCA7<CCA3	CCA7<CCA3	CCA4,5,7<CCA3	CCA4-5>CCA3 CCA7<CCA3
20	CCA4-5>CCA3 CCA7<CCA3	CCA7<CCA3	CCA7<CCA4	CCA4-5>CCA3 CCA7<CCA3
40	CCA4-5>CCA3	CCA5-7<CCA3	CCA4-7<CCA3	CCA4-5>CCA3
80	CCA4-6>CCA3	CCA4-7<CCA3	CCA4-7<CCA3	CCA4-5>CCA3
160	CCA4-6>CCA3	CCA4-7<CCA3	CCA4-7<CCA3	CCA4-6>CCA3

3.9.5 Colony Condition Assessment Response Variables

What follows is a breakdown of each response variable assessed and the significant effects that were determined at each CCA (after set up and prior to overwintering; i.e., CCAs 3-7). A couple of general points are made below when examining the results data analysis:

- Unless explicitly stated otherwise, all discussion of statistical findings refer to shared determinations from the PMRA and EPA analyses.
- All analyses considered effects at both the 0.05 and 0.1 alpha levels when weighing statistically significant effects with biological considerations. All analyses considered effects at both the 0.05 and 0.1 alpha levels when weighing statistically significant effects with biological considerations.
- For simplicity and consistency in visualizing the trends and findings of statistical significance simultaneously, the EPA-generated tables and figures are presented below while PMRA-generated tables and figures reflect PMRA's statistical analysis and are presented in **Appendix A** (and as such, estimated values and significance in EPA tables presented below may differ in some instances from the PMRA generated tables in this appendix).
- As noted above, the EPA-generated tables below indicate the percent differences from control based on raw counts of the data which have been scaled (divided by 1000) for each response variable to facilitate convergence of the statistical model.
- The EPA-generated table values are the percent reductions of the response model-based mean for a given treatment relative to the control model-based mean. The model-based means are the Least Square means based on the randomized complete block, repeated-measures design and model fit using SAS PROC MIXED algorithms. These Least Square means may differ from arithmetic means due to missing values in the raw data (this also accounts for some of the differences between calculations of mean percent inhibitions between EPA and PMRA's analyses).
- The figures with colored significance "dots" representing p-values of <0.05 or <0.10 were based on the results of the mixed model analyses conducted by EPA. off of these counts for each hive for each response variable (with the exception of hive weight) and were generated by EPA. The figures indicate statistical significance (reduction in treatment mean relative to control within a CCA) with black and red "dots" denoting a significant reduction at the 0.10- and 0.05-alpha levels, respectively.
- CCA3 was the baseline covariate and therefore is not presented in the tables generated by PMRA (in **Appendix A)** for each response variable with percent reductions.
- Even though data from CCA8 and CCA9 were included in the PMRA analysis and presented in the tables in **Appendix A**, the evaluation of effects at these time points is considered unreliable (by both EPA and PMRA) due to the high hive mortality observed in the controls (65% mortality at these CCAs).
- PMRA did not include "total brood in hives" and "total food storage" in its analysis so those results pertain solely to EPA findings.
- It is acknowledged that there was considerable variability for some response variables at certain treatment groups and CCAs. In order to better understand the variability of treatment groups and make comparisons with controls, for certain variables the reviewer has provided additional graphs focusing on the controls and lower treatment groups (10,

20, and 40 µg/L) that includes error bars around the means. Please refer to **Appendix C** for summary statistics tables (*i.e.* 95% lower and upper confidence intervals, means and standard error values) of the proportions of each response variable for further information.

3.9.6 Life Stage Results

The tables and figures below present results from CCA3 thru CCA7 across the different life stages. As discussed previously, CCA3 is the final assessment just prior to placing the clothianidin-treated sucrose solutions (or untreated control) in the hives. CCA4 occurs during the 6-week treatment period and CCA5 is just after the treatment period. CCA6 represents the time of year when the colony as a whole starts to prepare for overwintering and therefore starts to begin a "shut-down" phase where the numbers of adults and other life stages are clearly decreased which is noted at CCA7. During this pre-overwintering phase, adult proportions decline due to natural die off of worker bees and reduced rates of replenishment from reduced egg laying by the queen.

3.9.6.1 Adults

Table 23 and Figure 8 below show the effects on adult honey bees across CCAs and treatment groups. Compared with the control, no differences in the number of adults in hives ($p>0.1$) during the CCA4 exposure period were apparent in any of the treatments with the exception of a significant ($p<0.05$) reduction determined for the 160 µg/L group, which was also reduced in all subsequent CCAs (percent inhibtitions ranging from 30.3—96.2% in EPA's analysis and 32.3-98.4% in PMRA's analysis). The number of adults in the 80 µg/L treatment group, though not significantly reduced at CCA4, was significantly reduced ($p<0.05$) compared to controls at all subsequent CCAs (percent inhibitions ranging from 8.5% [non-statistically significant at CCA 4] to 56.5%) in EPA's analysis. For PMRA the number of adults in the 80 µg/L treatment group was significantly reduced ($p<0.05$) at CCA4 (13.9% estimated reduction) and in all subsequent CCAs (estimated percent reduction ranging from 41.2-64.1%). The number of adults in the 40 µg/L treatment group was also significantly reduced ($p<0.05$) at CCAs 5 and 6 (inhibitions of 23.7% and 30.4% for EPA and 23.1% and 29.9% for PMRA), but was not significantly reduced at CCA7 (13.3% fewer adults at CCA7, compared with controls). From CCA3 through CCA7, no significant reductions relative to controls were observed in the 10 and 20 µg/L treatment groups, except for at CCA6 where a significant reduction was observed in the 10 µg/L group (EPA: 21.9% reduction; PMRA: 19.6% reduction), but not for the 20 µg/L treatment group (non-statistically significant 16.5% reduction compared to controls). While the findings were not determined to be statistically significant at 20 µg/L treatment group at CCA6 for the EPA analysis, they were for the analysis used by PMRA (17% reduction compared to control, $p<0.05$).

Table 16. Estimated percent reduction from control for mean number of adults

Test concentration (µg/L)	Reduction relative to the control mean				
	CCA3	CCA4	CCA5	CCA6	CCA7
10	4.4	-1.0	9.0	21.9*	18.9
20	-0.6	0.4	1.4	16.5	6.1
40	-2.0	-2.1	23.7*	30.4*	13.3

Test concentration (µg/L)	Reduction relative to the control mean				
	CCA3	CCA4	CCA5	CCA6	CCA7
80	-10.0	8.5	37.1*	56.5*	47.6*
160	-4.3	30.3*	68.9*	87.7*	96.2*

Note: Negative value indicates increased number of adults in comparison to control.
*p<0.05

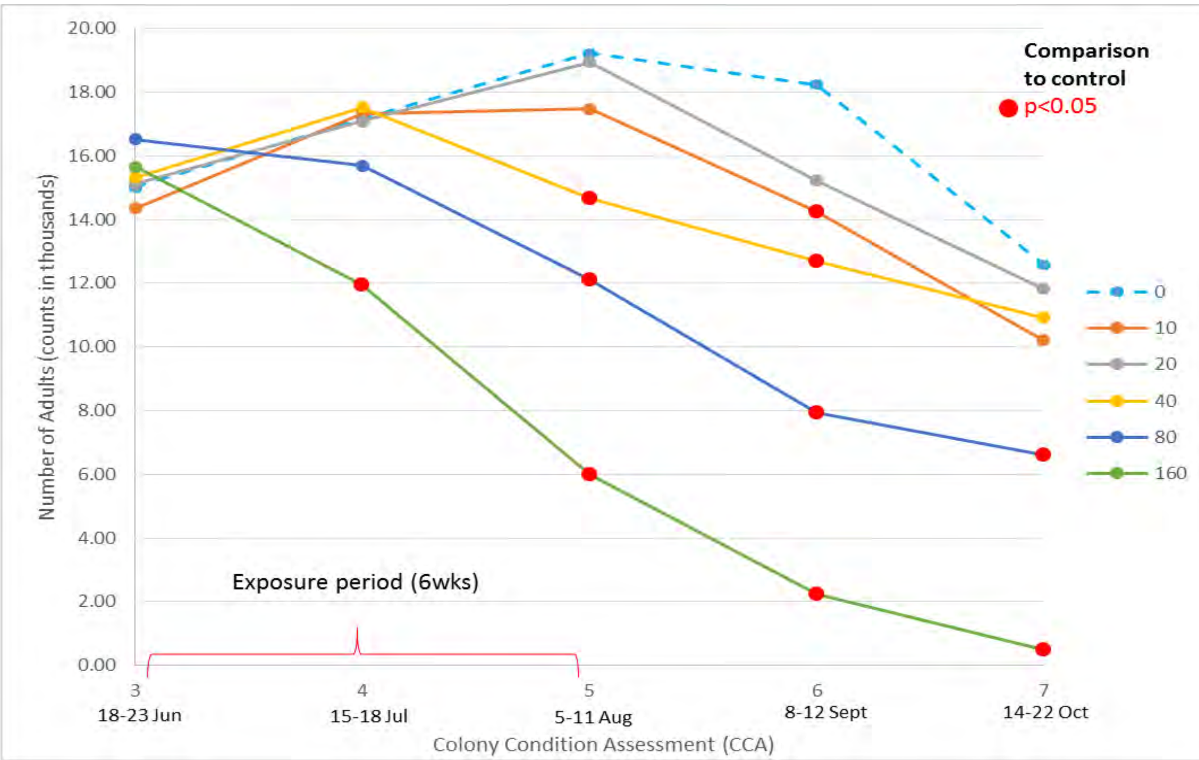


Figure 8. Number of adult honeybees at colony condition assessments (CCA) 3 thru 7 for each treatment group (based on model residual mean squares estimates).

Figure 9 below shows the trends in mean numbers of adults across the CCAs for the control and three lowest treatment groups only as the impact at the two highest groups was evident, and adds standard error bars in order to better compare differences in the populations. Removing the two highest treatment concentrations adjusts the scale of the figures to see the trends more clearly at the lower treatment groups. There is a clear divergence in the trends at the 40 µg/L treatment group in comparison to the control group at CCAs 5 and 6, though by CCA7 there appears to be substantial overlap in the error bars (one standard error from the mean). The 10 and 20 µg/L treatment groups, which appear similar compared to the control group at CCAs 4 and 5, appear to have low overlap with the control group at CCA6 (when statistically significant reductions were observed in the 10 µg/L, but not the 20 µg/L treatment group), but by CCA7, the control and lowest treatment groups are much closer together with a distinct lack of dose-response. Also notably, as distinguished from the control and 10 and 20 µg/L groups, while the proportions of adults for those groups generally increased or remained stable through CCA5 before beginning to decline, the numbers of adults at 40 µg/L began to decline as early as CCA4, where these numbers were being built up or remained constant in the control and lower treatment groups to support the foraging worker bee force for nectar and pollen collection.

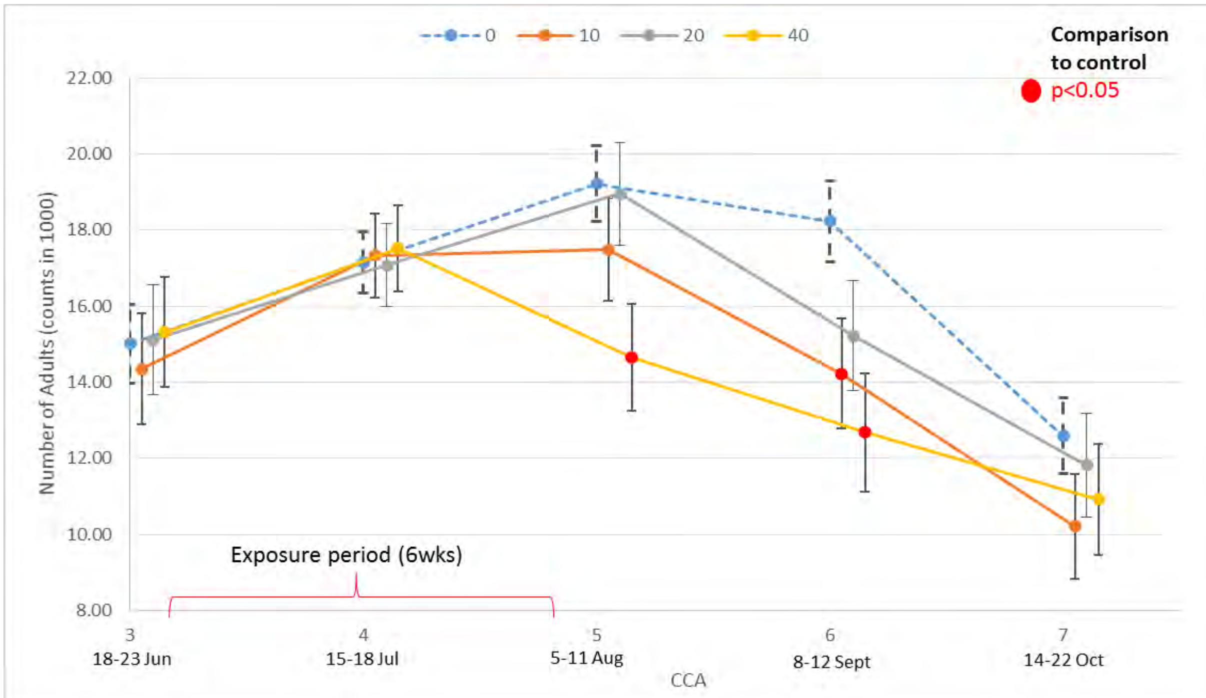


Figure 9. Number of adult honeybees at colony condition assessments (CCA) 3 thru 7 for the control and three lowest treatment groups. Error bars represent one standard error from the mean calculated from the model residual mean squares estimate.

Although treatment means were significantly reduced from the control at the lowest treatment group (10 µg/L) at CCA6 in both EPA and PMRA analyses and were also significantly reduced from control in the 20 µg/L treatment group at CCA6 (PMRA's analysis only), these effects were considered to be potentially transient, while the early onset and persistence of significant effects in the highest treatment groups (40, 80 and 160 µg/L) supports the **conclusion that the overall NOAEC and LOAEC for adults is 20 and 40 µg/L, respectively.** It is also notable that no obvious dose-response relationship is observable in the two lowest doses at CCA6, though this may also be a function of the overlap in exposure among individual hives, based on residue analysis of hive matrices.

3.9.6.2 Eggs

Table 24 and **Figure 10** below show the effects on eggs across CCAs and treatment groups. For the EPA analysis at CCA4, compared with the controls, significant differences were observed in the 40 µg/L ($p<0.05$) and 80 µg/L ($0.05<p<0.1$) treatment groups, but no dose-response relationship was observed across any dosage and a lack of significant inhibition was observed even at the highest treatment dose at this CCA. However, at CCA5 there were clear significant reductions ($p<0.05$) at the 80 µg/L and 160 µg/L treatment groups (68.7% and 92.9% reduction of eggs relative to controls), which persisted at the subsequent CCAs. At CCA5 there were also marginaly significant reductions ($p<0.1$) at the 20 µg/L treatment group (37% reduction), but not at either the 10 and 40 µg/L treatment groups (32 and 34% reductions, respectively). Further, there were no significant reductions (p>0.1) for the three lowest treatments at any of the subsequent CCAs.

For the PMRA analysis treatment means were significantly reduced (p>0.05) from the control at the lowest three doses (10, 20 and 40 µg/L) during and immediately after the exposure period (CCA4 and CCA5) but not in subsequent CCAs after the exposure period and were significantly reduced (p<0.05) from the control at the two highest treatment groups (80 and 160 µg/L) at all CCAs. Similar to the EPA analysis, there was no dose response evident at CCA4. A general dose response was evident starting at CCA5 which became more pronounced over subsequent CCAs up to CCA7.

Table 17. Estimated percent reduction from control for number of eggs.

Test concentration (µg/L)	Reduction relative to the control mean				
	CCA3	CCA4	CCA5	CCA6	CCA7
10	-6.6	30.1	32.0	3.1	0.9
20	-0.6	26.4	37.0**	15.1	26.5
40	-11.8	48.0*	33.9	12.9	25.6
80	8.4	38.1**	68.7*	49.4**	71.2*
160	3.0	31.9	92.9*	83.1*	95.2*

Note: Negative value indicates increased number of eggs in comparison to control.
*p<0.05
**0.05<p<0.1

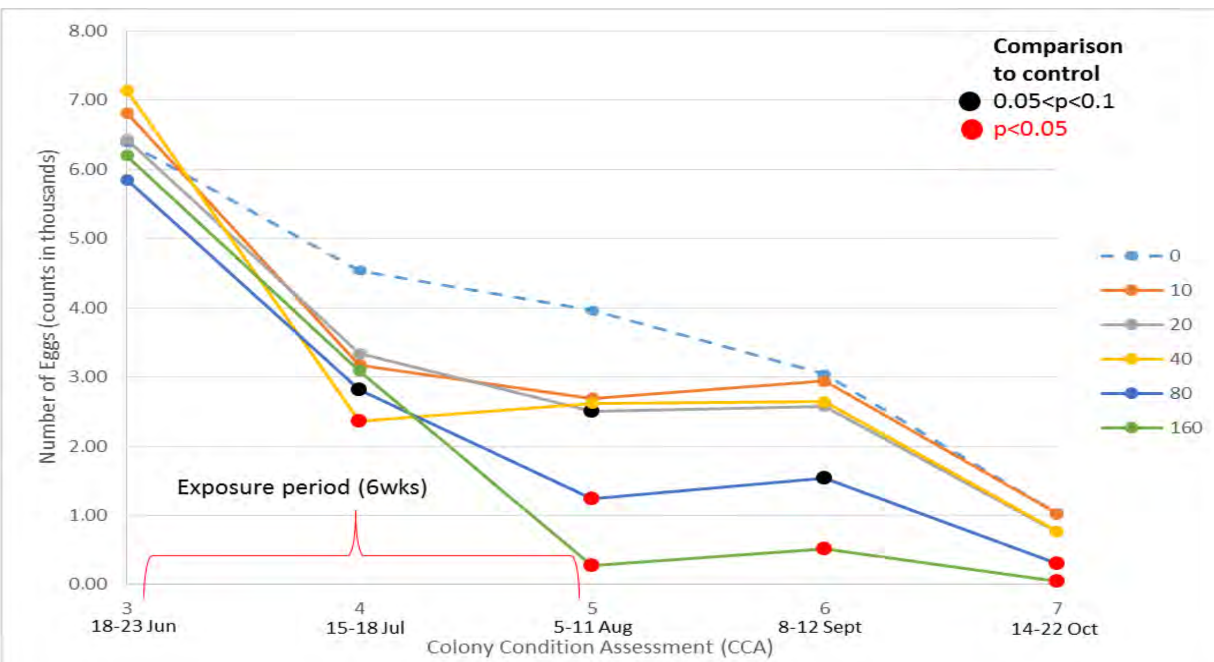


Figure 10. Number of eggs (cells) following exposure to varying concentrations of clothianidin in the diet across CCA3 – CCA7 (based on model residual mean squares estimate).

Figure 11 below shows the responses for the mean number of eggs for the control, 10, 20 and 40 µg/L treatment groups. Removing the two highest treatment concentrations adjusts the scale of the figures to see the trends more clearly. It is noted from this graph that at CCA4, while significant differences ($p<0.05$) were observed for the 40 µg/L treatment group, as well as lower (though non-significant) numbers at CCA5, where the 20 µg/L treatment group was significantly ($p<0.1$) different from controls, at both of these CCAs there were no obvious dose-response trends within a CCA and across CCAs for these treatment groups (*i.e.* substantial overlap of these populations based on their standard errors). Additionally, at subsequent CCAs the mean number of eggs in all three treatments appears to have reverted close to control means. However, it is noted that the mean and standard error values for the lower treatment groups are lower than the control.

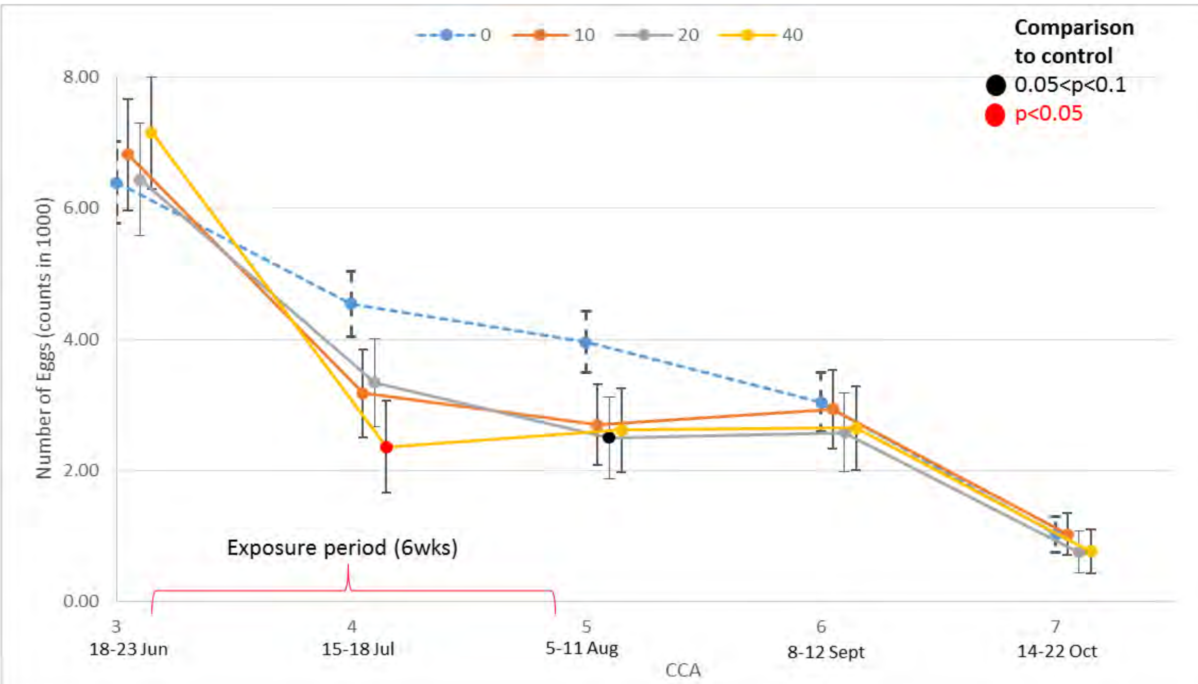


Figure 11. Number of egg cells following exposure to varying concentrations of clothianidin in the diet across CCA3—CCA7 in the control, 10, 20, and 40 µg/L groups only. Error bars represent one standard error from the mean calculated from the model residual mean square estimate.

When weighing statistical and biological significance, the overall NOAEC and LOAEC for eggs is determined to be 40 and 80 µg/L, respectively, based on a significant reduction in eggs consistent at all CCAs following the end of exposure. Although there is some uncertainty regarding this endpoint as the means of the three lowest treatments were lower than the control means at CCAs 4 and 5, the lack of dose-response surrounding these doses and the reversion of these three treatments back to control means by CCA6 indicates a potential transient effect and supports the use of 40 µg/L as the NOAEC and 80 µg/L as the LOAEC for effects from clothianidin on egg production following a six-week exposure period.

3.9.6.3 Larvae (Open/Uncapped brood)

Table 25 and **Figure 12** below show the effects on larvae (open/uncapped brood) across CCAs and treatment groups. In the EPA analysis, compared with the control, no differences in the number of larvae (open/uncapped cells) in hives (p>0.1) during the CCA4 exposure period were apparent in any of the treatments with the exception of a significant (p<0.05) dramatic reduction determined for the 160 µg/L group, which was also reduced in all subsequent CCAs (percent inhibtitions ranging from 86.8—100%). The number of larval cells in the 80 µg/L treatment group, though not significantly reduced at CCA4, was significantly reduced (p<0.05) compared to controls at CCAs 5 and 6 (percent inhibitions ranging from 9.1% [non-statistically significant at CCA 4] to 81.7%), but was not statistically significantly inhibited at CCA7, despite a reduction of 46.2% relative to controls. From CCA3 through CCA7, no significant (p>0.1) reductions were observed in any of the lowest treatment groups.

In the PMRA analysis treatment means were significantly reduced (0.05< p<0.1) from the control at the lowest three doses (10, 20 and 40 µg/L) during a single CCA during the exposure period (CCA4) but not in subsequent CCAs and were significantly reduced (p<0.05, 0.05< p<0.1) at all CCAs at the two highest treatment groups (80 and 160 µg/L). A clear dose response (increase in the reduction from the control as the dose increases) was evident over all CCAs except at CCA4.

Table 18. Estimated percent reduction from control for number of larvae (open/uncapped brood)

Test concentration (µg/L)	Reduction relative to the control mean				
	CCA3	CCA4	CCA5	CCA6	CCA7
10	-0.9	14.8	1.6	-5.0	33.2
20	9.4	12.7	14.5	4.1	36.8
40	5.1	22.4	19.8	24.0	33.0
80	-15.3	9.1	81.7*	48.7*	46.2
160	3.5	86.8*	100*	93.2*	98.6*

Note: Negative value indicates increased number of larvae in comparison to control.
*p<0.05

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

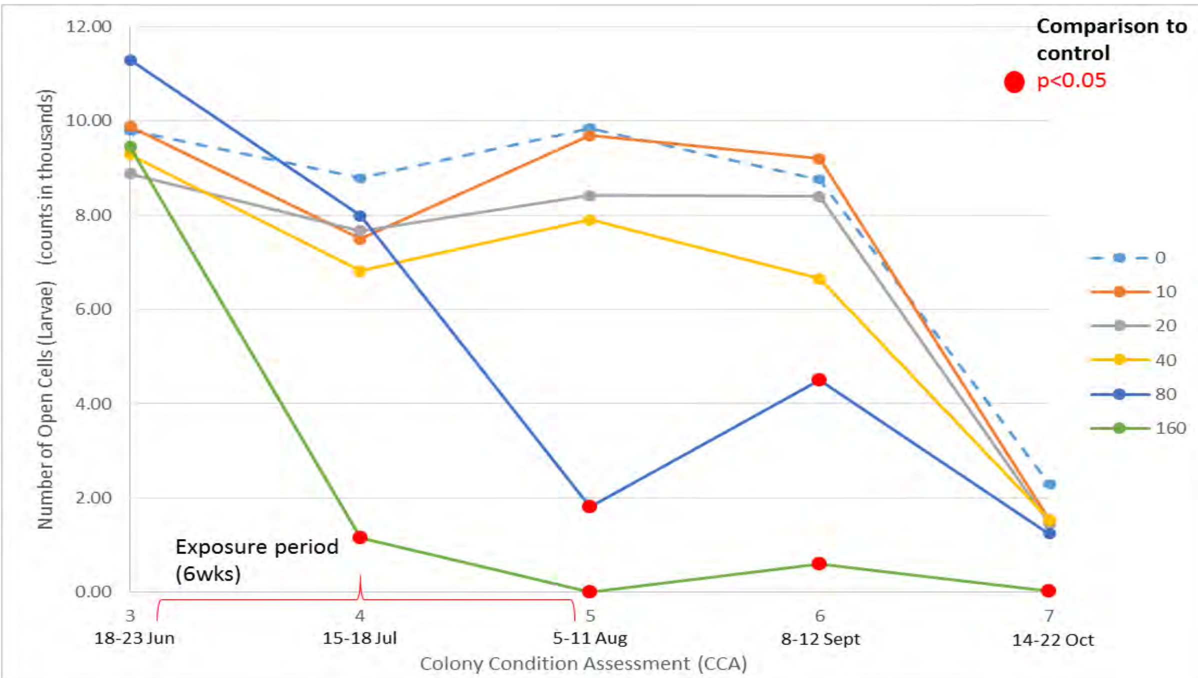


Figure 12. Number of open cells (larvae) at colony condition assessments (CCA) 3 thru 7 for each treatment group (based on model residual mean squares estimates).

Figure 13 below shows the responses for the control, 10, 20 and 40 µg/L treatment groups. Removing the two highest treatment concentrations adjusts the scale of the figures to see the trends more clearly. It is noted from this graph that although error bars surrounding the control group generally have some overlap with those surrounding the 10 and 20 µg/L treatment groups, they just overlap with the 40 µg/L treatment group from CCAs 5 through 6 and a general dose-response relationship is observed across these doses during CCAs 5 and 6.

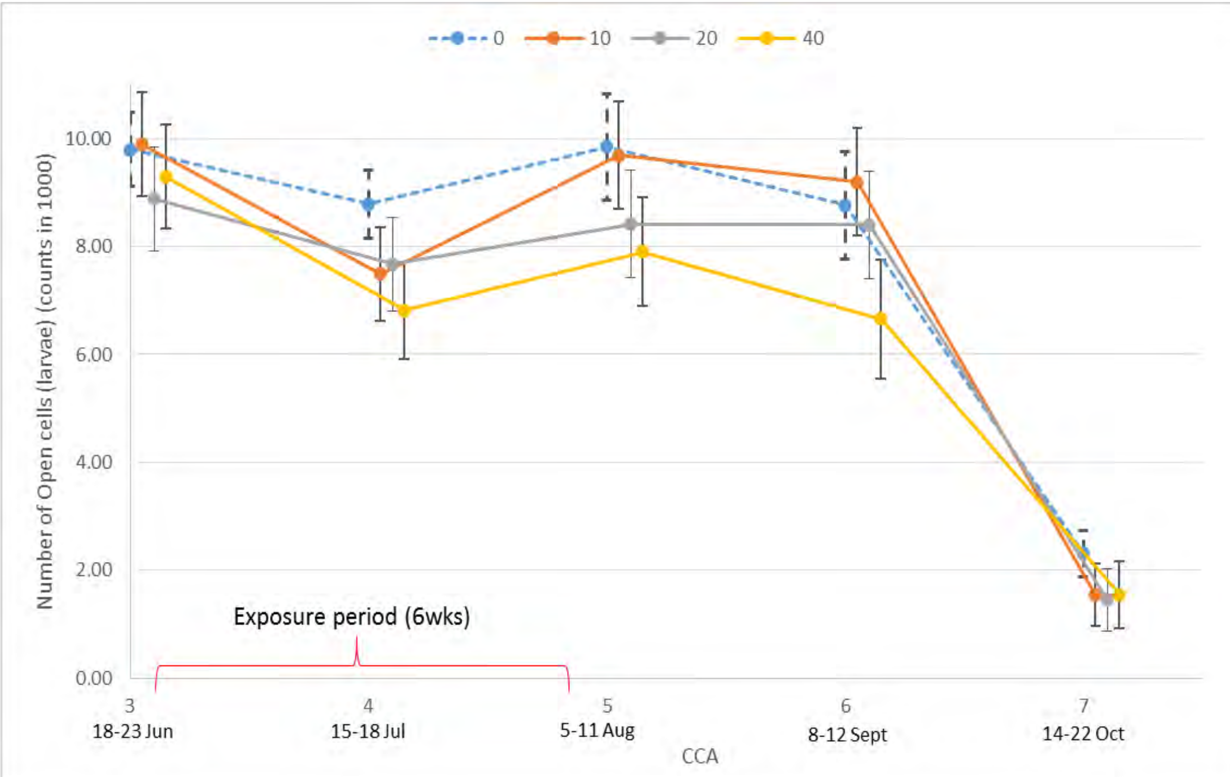


Figure 13. Number of open cells (larvae) at colony condition assessments (CCA) 3 thru 7 for the control and three lowest treatment groups. Error bars represent one standard error from the mean calculated from the model residual mean squares estimate.

When weighing statistical and biological significance, the overall NOAEC and LOAEC for larval cells is determined to be 40 and 80 µg/L, respectively. This is based on persistent significant effects at the 80 µg/L and 160 µg/L treatment groups and lack of persistent effects at the three lowest treatment groups (10, 20 and 40 µg/L). However, there is some uncertainty in this endpoint, given the lack of overlap of the 40 µg/L and control group populations at all CCAs from the beginning of exposure through CCA6 and the appearance of a dose-response relationship beginning at 40 µg/L.

3.9.6.4 Pupae (Capped Brood)

In the 40, 80 and 160 µg/L treatment groups in the EPA analysis, there were significant reductions from the control in pupae (capped brood) ($p<0.05$) that persisted through multiple measurement points (CCA's 4-6). The percent reductions from control based on the raw counts of pupal cells ranged from 16.4—47.1%, 26.1—83.4% and 46.3—100% in the 40, 80 and 160 µg/L treatment groups, respectively during CCAs 4-6 (**Table 26** and **Figure 14**, below).

In the PMRA analysis, the number of pupal cells was significantly reduced ($p<0.05$) compared to the control at the three highest treatment groups (40, 80 and 160 µg/L) which persisted over multiple CCAs (16.3—47.0%, 30.9—87.7% and 46.9—99.6% reduction in the 40, 80 and 160 µg/L treatment groups, respectively during CCAs 4-6 and 98.2% reduction at CCA7 in the 160 µg/L treatment group). No significant reduction ($p>0.1$) in the number of pupae was observed at the lowest two treatment groups (10 and 20 µg/L) during any of the CCAs with the exception of CCA5 where the number of pupae was significantly reduced by 13.1% in the 20 µg/L treatment group (p=0.039) and by 19.9% in the 10 µg/L treatment group (p=0.05). This analysis considers that the overlap in dose-response at the lower doses is not unexpected since the dose levels are similar and measured exposures indicate overlap in exposure among individual hives, particularly at the lower two doses.

Table 19. Estimated percent reduction from control for number of capped (pupal) cells.

Test concentration (µg/L)	Reduction relative to the control mean				
	CCA3	CCA4	CCA5	CCA6	CCA7
10	-8.3	0.4	17.2	10.3	27.0
20	-7.8	3.9	9.7	3.7	4.7
40	1.1	16.4*	47.1*	36.7*	19.6
80	-7.7	26.1*	83.4*	59.3*	7.9
160	-0.9	46.3*	100*	98.1*	97.3*

Note: Negative value indicates increased number of pupae in comparison to control.
*p<0.05

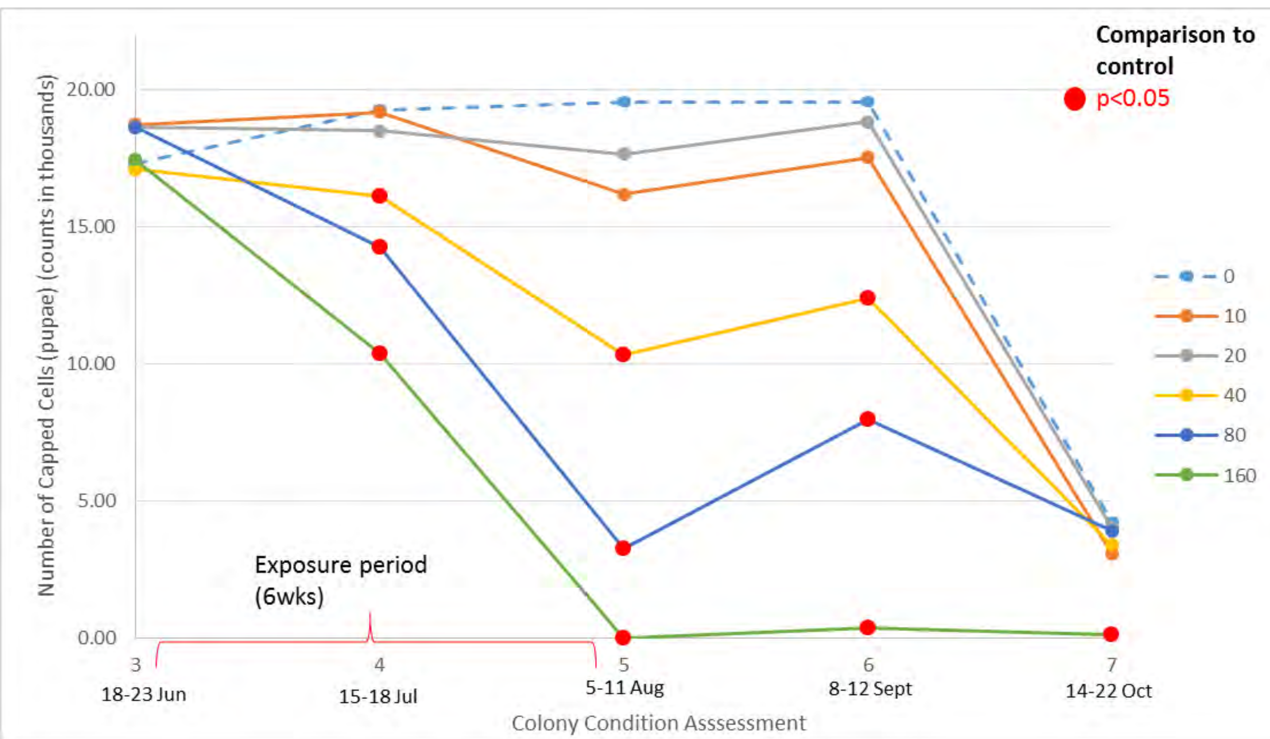


Figure 14. Number of capped cells (pupae) at colony condition assessments (CCA) 3 thru 7 for each treatment group (based on model residual mean squares estimate).

Figure 15 below shows the responses for pupae for the control, 10, 20 and 40 µg/L treatment groups. Removing the two highest treatment concentrations adjusts the scale of the figures to see the trends more clearly. It is noted from this graph that although error bars surrounding the control group, and the 10 and 20 µg/L treatment groups generally overlap with each other, they do not show any overlap with the 40 µg/L treatment group from CCAs 4 through 6.

When weighing statistical and biological significance, the overall NOAEC and LOAEC for pupal cells is determined to be 20 and 40 µg/L, respectively.

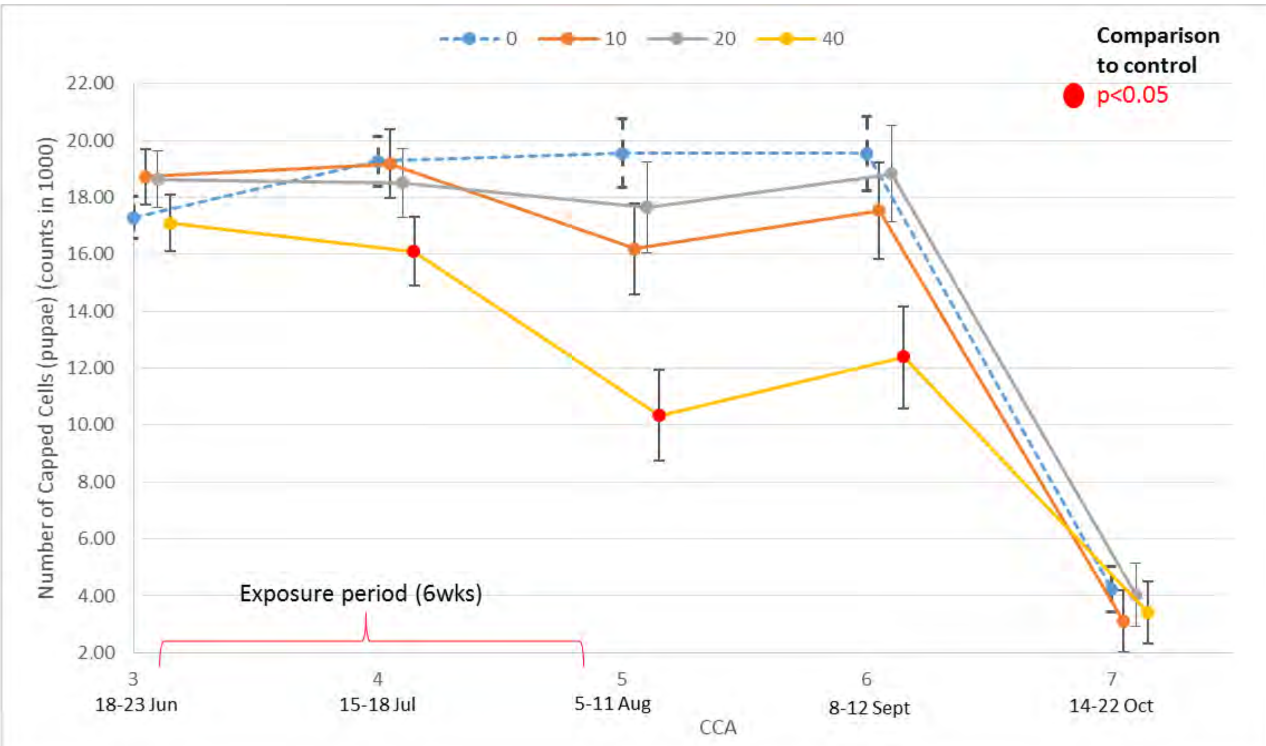


Figure 15. Number of capped cells (pupae) at colony condition assessments (CCA) 3 thru 7 for the control and three lowest treatment groups. Error bars represent one standard error from the mean calculated from the model residual mean squares estimate.

3.9.6.5 Total Individuals in Hives

When evaluating the total number of live individuals (total adults + combined number of cells of eggs, larvae and pupae), significant effects (p<0.05) were observed at the three highest treatments for CCAs 4-6 and in the two highest treatments at CCA7 (**Table 27**), generally following the pattern observed earlier for total adults and pupae, the two life stages that made up the largest components of the hive population throughout the course of the study (**Figures 8**, **14** and **16**). No significant differences (p>0.1) were observed for the 10 and 20 µg/L treatment groups relative to the controls in the EPA analysis.

In the PMRA analysis, significant reductions were observed for the 20 µg/L treatment group at CCA4 and for the 10 and 20 µg/L treatment groups at CCA5 (0.05< p<0.1) A general dose response (increase in the reduction from the control as the dose increases) at the three highest treatment groups (40, 80 and 160 µg/L) was evident over all CCAs. For the two lowest treatment groups, the reduction in total individuals in the 10 µg/L treatment group was consistently higher than or equivalent to the 20 µg/L treatment group over all CCAs (6.1-23.7% and 6.7-11.1% reduction in the 10 and 20 µg/L treatment groups, respectively) and in particular, CCA7. This analysis considers that the overlap in dose-response at the lower doses is not unexpected since the dose levels are similar and measured exposures indicate overlap in exposure among individual hives, particularly at the lower two doses.

Table 20. Estimated percent reduction from control for total individuals

Test concentration (µg/L)	Reduction relative to the control mean				
	CCA3	CCA4	CCA5	CCA6	CCA7
10	-2.6	5.1	12.4	11.4	21.3
20	-1.2	6.3	9.6	9.1	10.4
40	-0.8	14.0*	32.5*	30.6*	17.9
80	-7.8	18.1*	65.0*	55.8*	40.3*
160	-0.6	46.6*	88.1*	92.5*	96.6*

Note: Negative value indicates increased number of total individuals in comparison to control.
*p<0.05

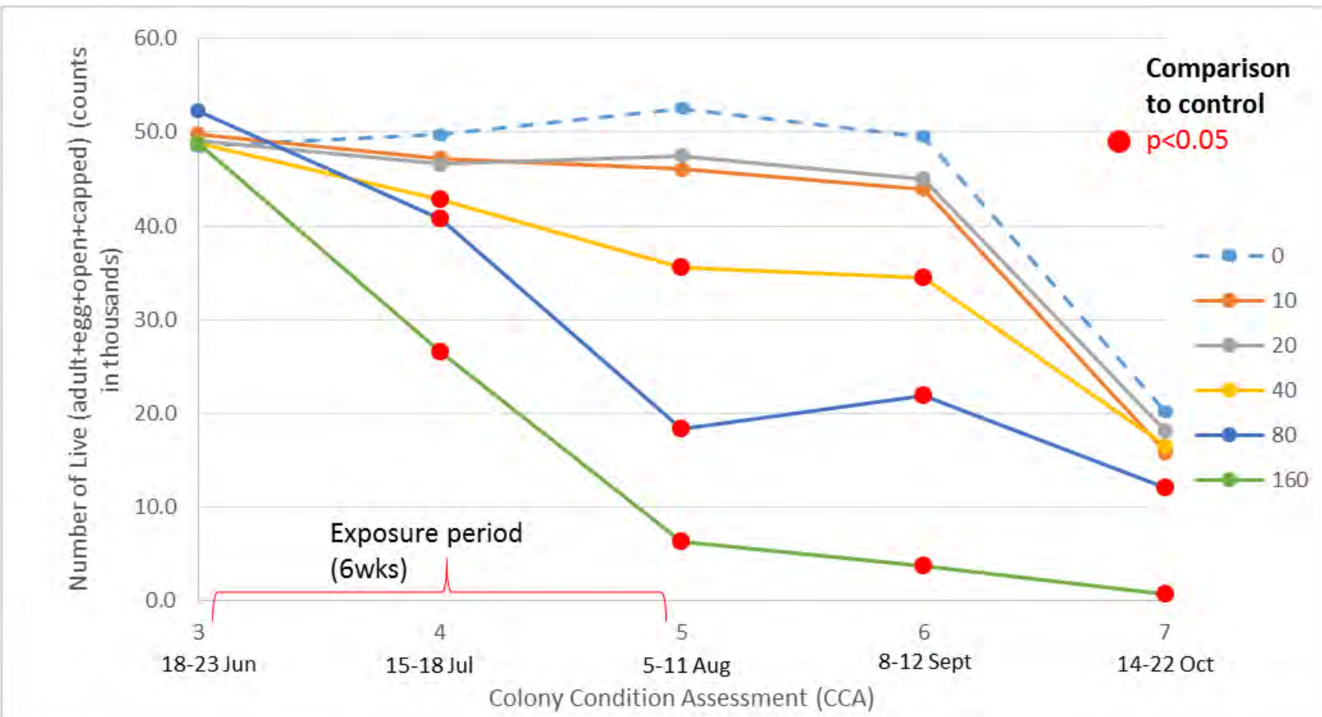


Figure 16. Number of live (adult numbers+cells of brood) at colony condition assessments (CCA) 3 thru 7 for each treatment group (based on model residual mean squares estimate).

Figure 17 below shows the responses for total live for the control, 10, 20 and 40 µg/L treatment groups. Removing the two highest treatment concentrations adjusts the scale of the figures to see the trends more clearly. It is noted from this graph that although error bars surrounding the control group, and the 10 and 20 µg/L treatment groups generally overlap with each other, they do not show any overlap with the 40 µg/L treatment group from CCAs 4 through 6. **When weighing statistical and biological significance, the overall NOAEC and LOAEC for total individuals is determined to be 20 and 40 µg/L, respectively.**

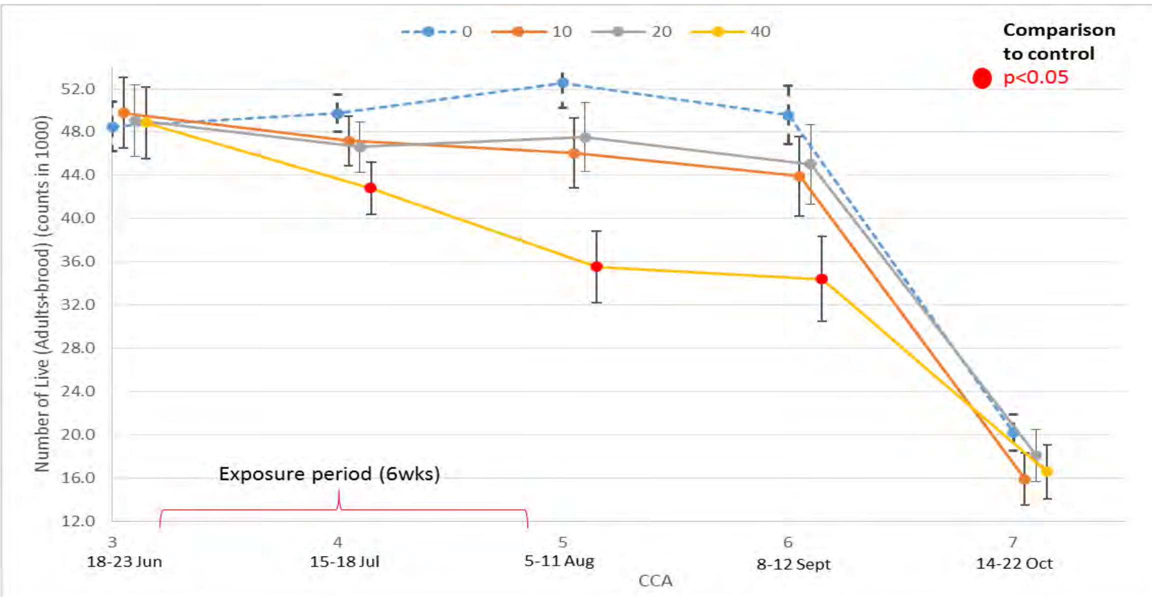


Figure 17. Number of total live (adult+brood) at colony condition assessments (CCA) 3 thru 7 for the control and three lowest treatment groups. Error bars represent one standard error from the mean calculated from the model residual mean squares estimate.

3.9.6.6 Total Brood in Hives

When evaluating the total number of brood cells (eggs, open and capped), significant effects ($p<0.05$) were consistently observed at the three highest treatments for CCAs 4-6 and in the highest treatment at CCA7 (**Table 28** and **Figure 18**). No significant differences ($p>0.1$) were observed for the 10 and 20 µg/L treatment groups relative to the controls.

Table 21. Estimated percent reduction from control for total brood cells

Test concentration (µg/L)	Percent reduction relative to the control mean (%)				
	CCA3	CCA4	CCA5	CCA6	CCA7
10	-5.8	8.4	14.4	5.4	25.5
20	-1.4	9.5	14.4	5.0	17.6
40	-0.2	22.5*	37.5*	31.0*	25.4
80	-6.8	23.2*	81.2*	55.4*	28.3
160	1.1	55.2*	99.2*	95.3*	97.4*

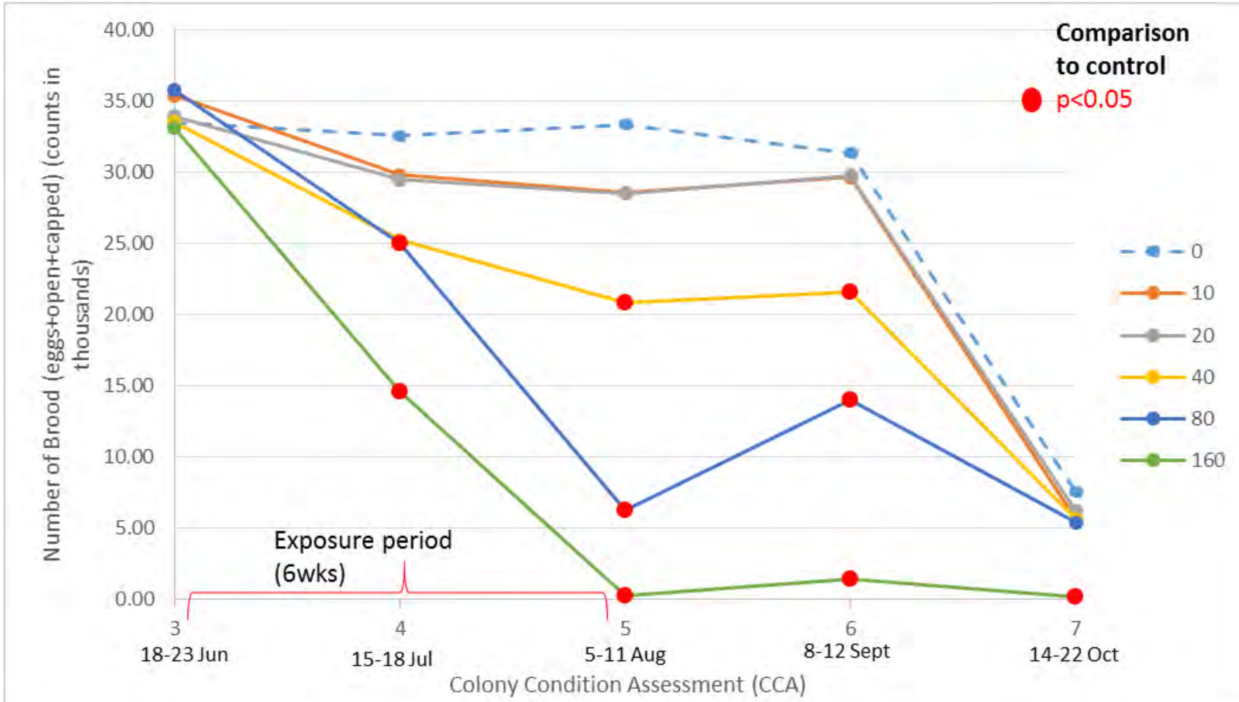


Figure 18. Number of brood at colony condition assessments (CCA) 3 thru 7 for each treatment group (based on model residual mean squares estimate).

Figure 19 below shows the responses for total brood for the control, 10, 20 and 40 µg/L treatment groups. Removing the two highest treatment concentrations adjusts the scale of the figures to see the trends more clearly. It is noted from this graph that although error bars surrounding the control group, and the 10 and 20 µg/L treatment groups generally show some overlap with each other, there is clear and consistent divergence of the 40 µg/L treatment group compared to controls and the lower treatment groups. **Therefore, when weighing statistical and biological significance, the overall NOAEC and LOAEC for total brood is determined to be 20 and 40 µg/L, respectively.**

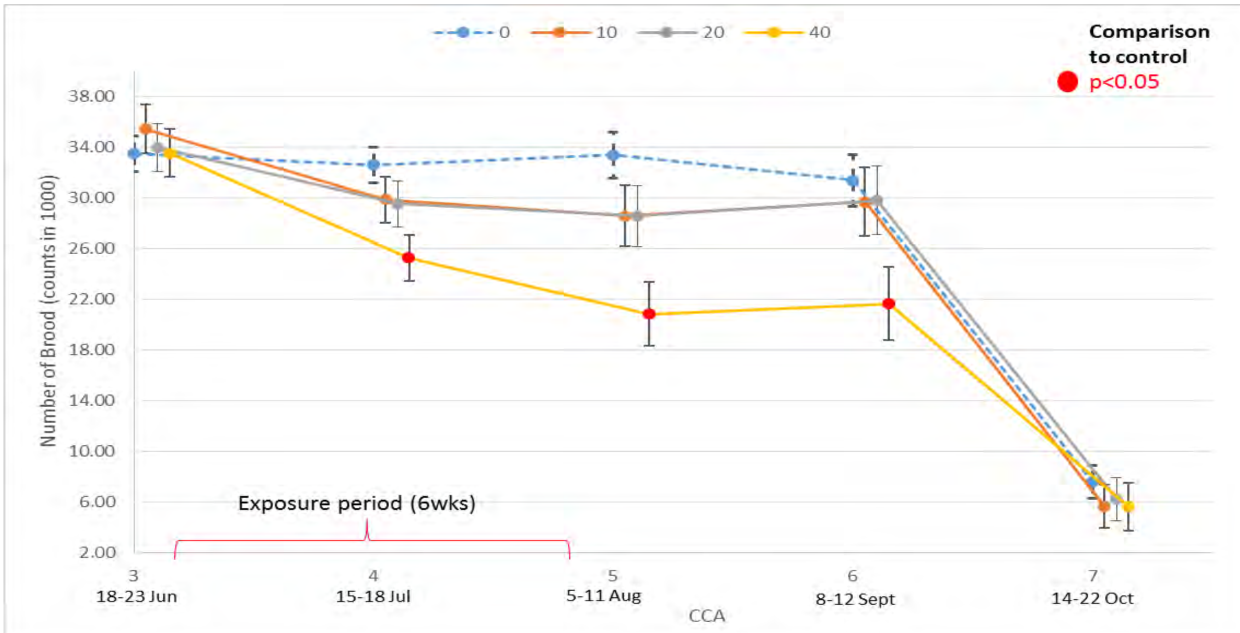


Figure 19. Number of brood at colony condition assessments (CCA) 3 thru 7 for the control and three lowest treatment groups. Error bars represent one standard error from the mean calculated from the model residual mean squares estimate.

Figures 20-24 below provide another visual representation of the effects across CCAs variables within a response variable for the various life stages of bees during the course of the study for the three lowest treatments. The bar charts represent the percent differences from control with negative percent differences from control indicating an increase in a given response variable above the level of control. Although these figures show what appear to be substantial decreases compared to controls for some endpoints at select CCAs (*e.g.* larvae at CCA7), these figures provide further evidence of the general lack of dose responsiveness in effects at the lowest treatment groups, while a clear dose-response relationship is observed between the 40, 80 and 160 µg/L treatment groups. Furthermore these charts are effective in indicating how the percent differences with a given response variable, changed over the course of the study within a treatment group. It is also noted here that the scale for percent difference from control (y-axis) has been standardized across all charts and that negative ("-") responses refer to a percent increase above the level of control.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 20. Summary of living organism parameters at the 10 µg/L treatment group

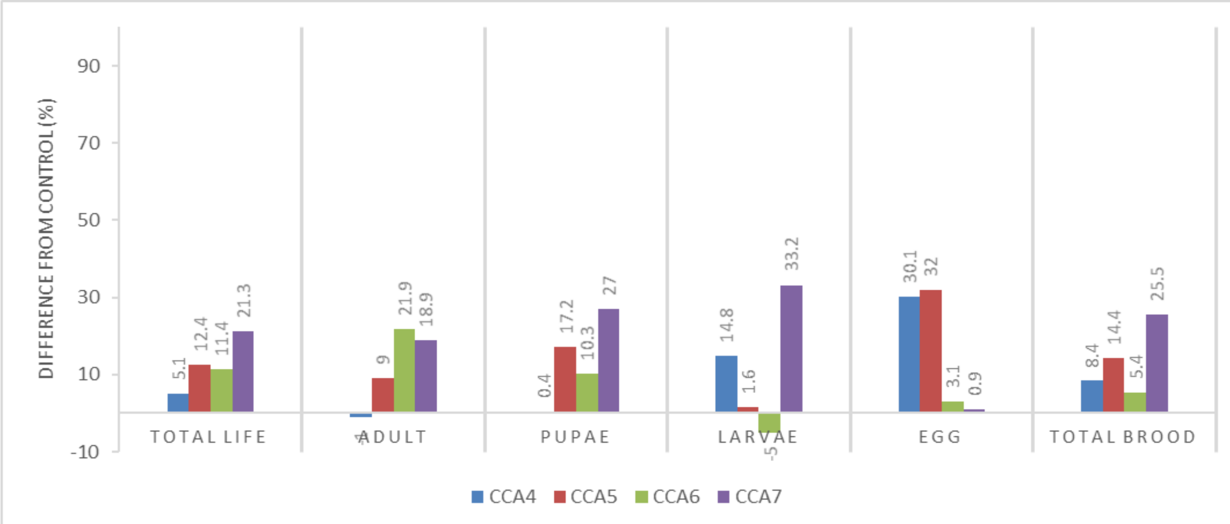


Figure 21. Summary of living organism parameters at the 20 µg/L treatment group

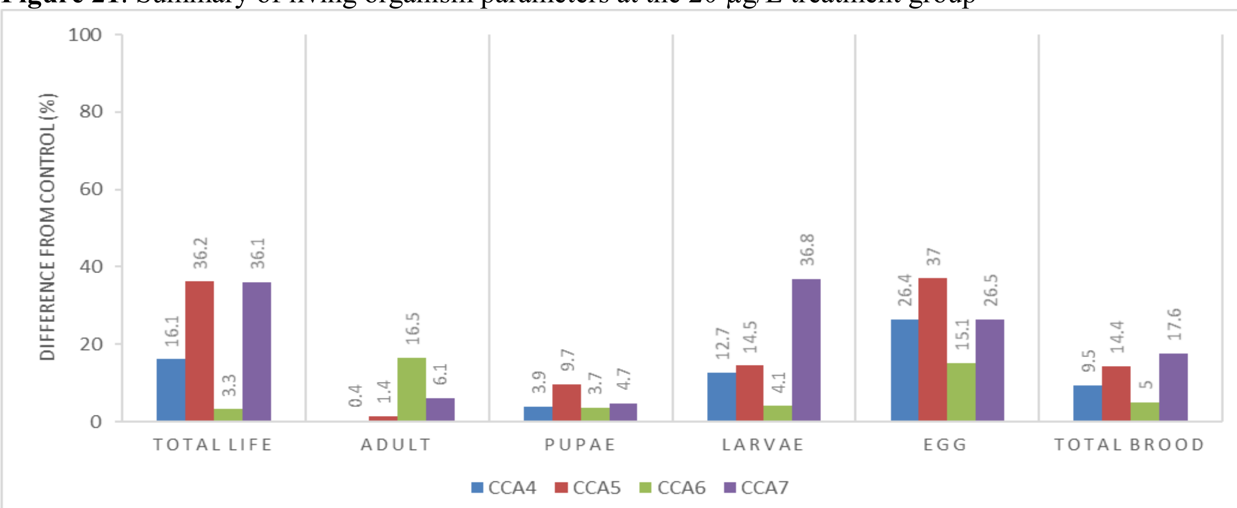


Figure 22. Summary of living organism parameters at the 40 µg/L treatment group

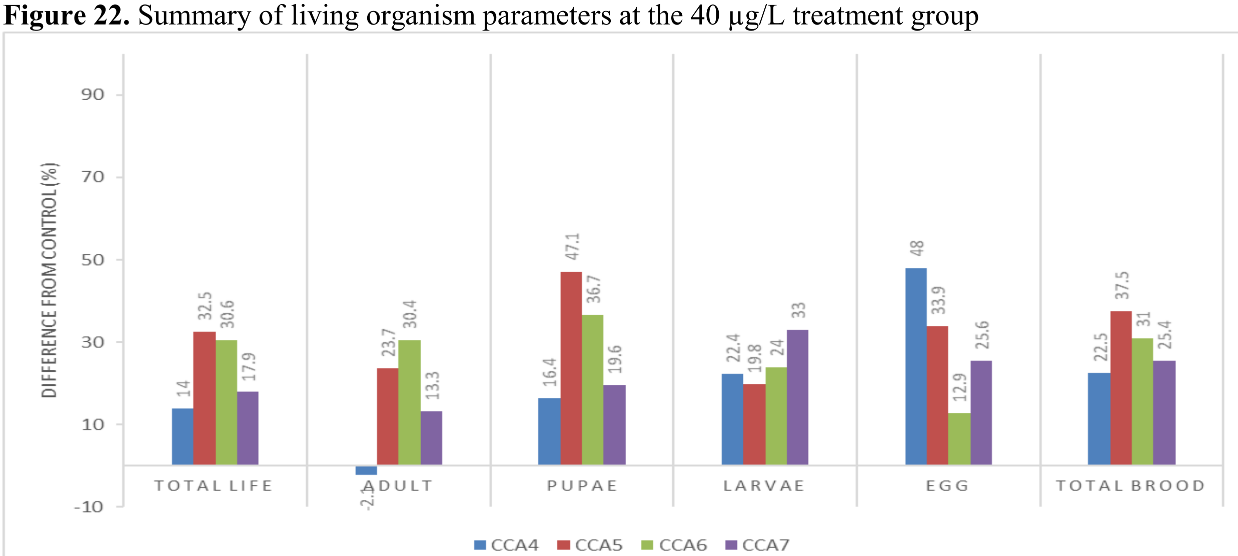


Figure 23. Summary of living organism parameters at the 80 µg/L treatment group

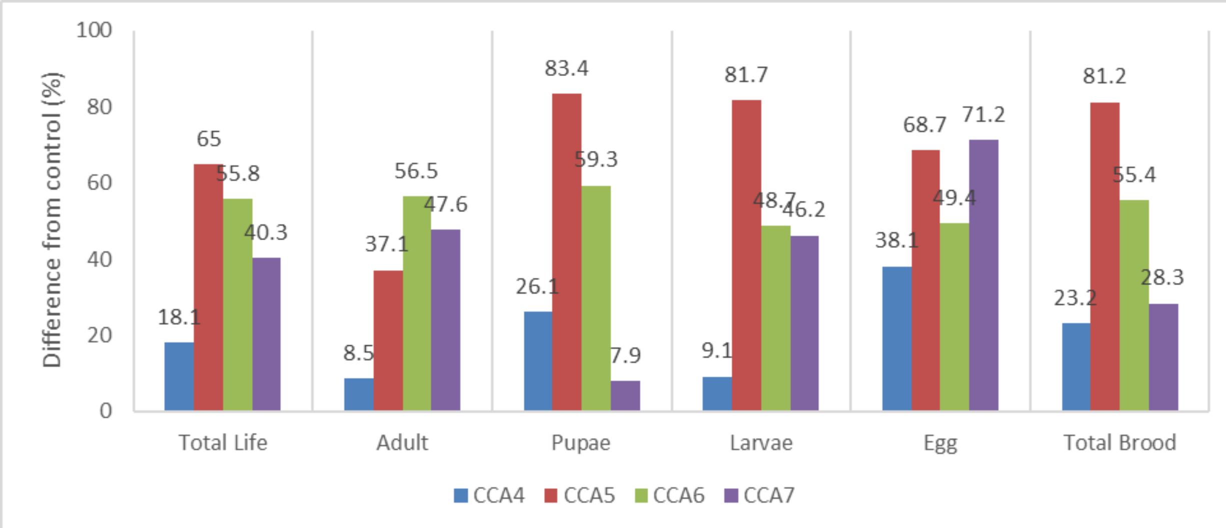
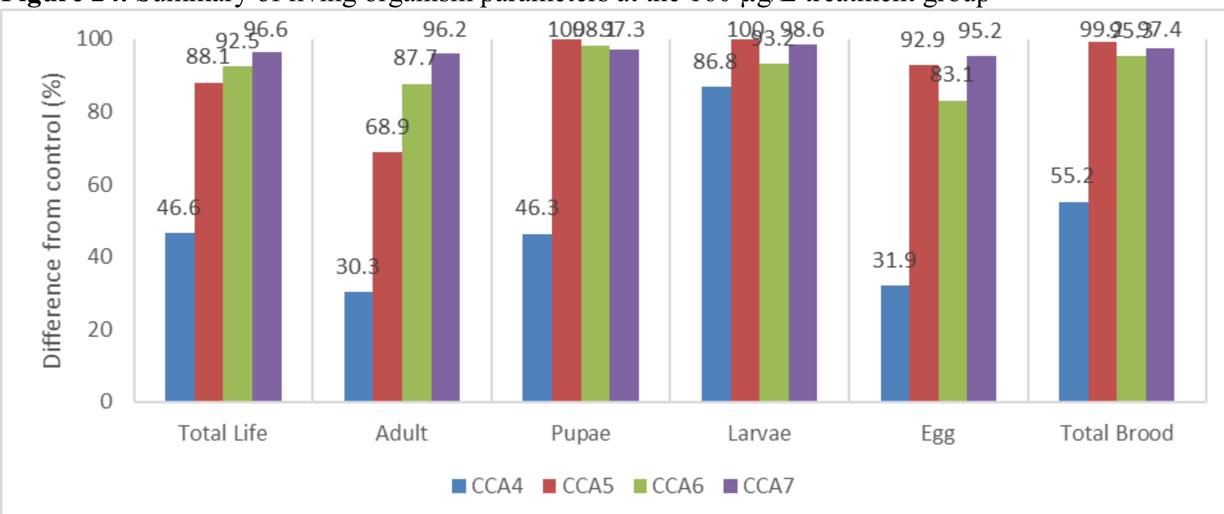


Figure 24. Summary of living organism parameters at the 160 µg/L treatment group



3.9.7 Colony Condition Assessments – Food Store Response Variables

3.9.7.1 Pollen

Pollen stores were significantly reduced ($p<0.05$) in the 80 and 160 µg/L treatment groups from CCA4 to CCA7 (inhibitions of 46.8—98% and 96.7—100%, respectively in the 80 and 160 µg/L treatment groups in the EPA analysis and 47.5-99.4% and 94.3-100%, respectively in the PMRA analysis). Pollen stores were also significantly reduced in the 40 µg/L treatment group during CCAs 4 and 5 (inhibitions of 29.3 and 64.7%, respectively in the EPA anlaysis and 21.9 and 55.3% in the PMRA analysis), though at CCA4 this difference was more marginal in the EPA analysis ($0.05<p<0.1$; **Figure 25**). In the two lower treatment groups, pollen stores were significantly reduced ($p<0.05$) at CCA5 and for EPA's analysis only, at CCA7, though at CCA7 these were not observed to follow a dose-response trend (**Table 29**).

Table 22. Estimated percent reduction from control for pollen stores

Test concentration (µg/L)	Reduction relative to the control mean				
	CCA3	CCA4	CCA5	CCA6	CCA7
10	1.2	6.9	32.7*	26.1	62.2
20	9.3	16.1	36.2*	3.3	36.1*
40	11.3	29.3**	64.7*	34.1	19.0
80	-2.3	46.8*	98.0*	60.4*	70.9*
160	2.9	96.7*	100*	99.8*	100*

*p<0.05
**0.05<p<0.1

Figure 25. Number of pollen cells at colony condition assessments (CCA) 3 through 7 for each treatment group.

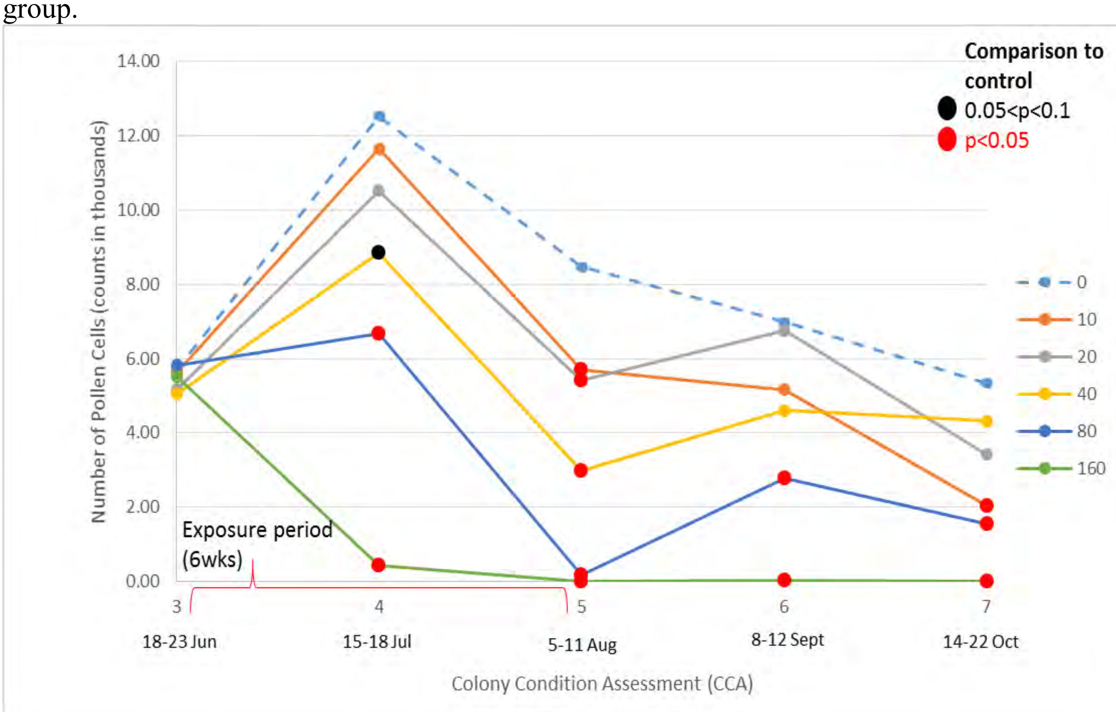


Figure 26 below shows the clear divergence of pollen stores in all three lower treatment groups at CCA5 as compared to the control, but pollen stores than appear to overlap at CCAs 6 through 7, except for in the 10 µg/L treatment group which does not appear to have any dose-response relationship with the other doses at CCA7. The data indicate clear and consistent effects during (and immediately after) the feeding exposure on the 40 µg/L group, however are approaching levels near the control following exposure. There is more uncertainty surrounding the two lower treatment groups, for which a significant decrease in pollen storage is observed at a single measurement (CCA5) without any statistical significance or dose-response at CCA6 and 7, with the 10 µg/L treatment having reduced pollen storage compared to controls while the 20 µg/L treatment group more closely tracks the control pollen storage.

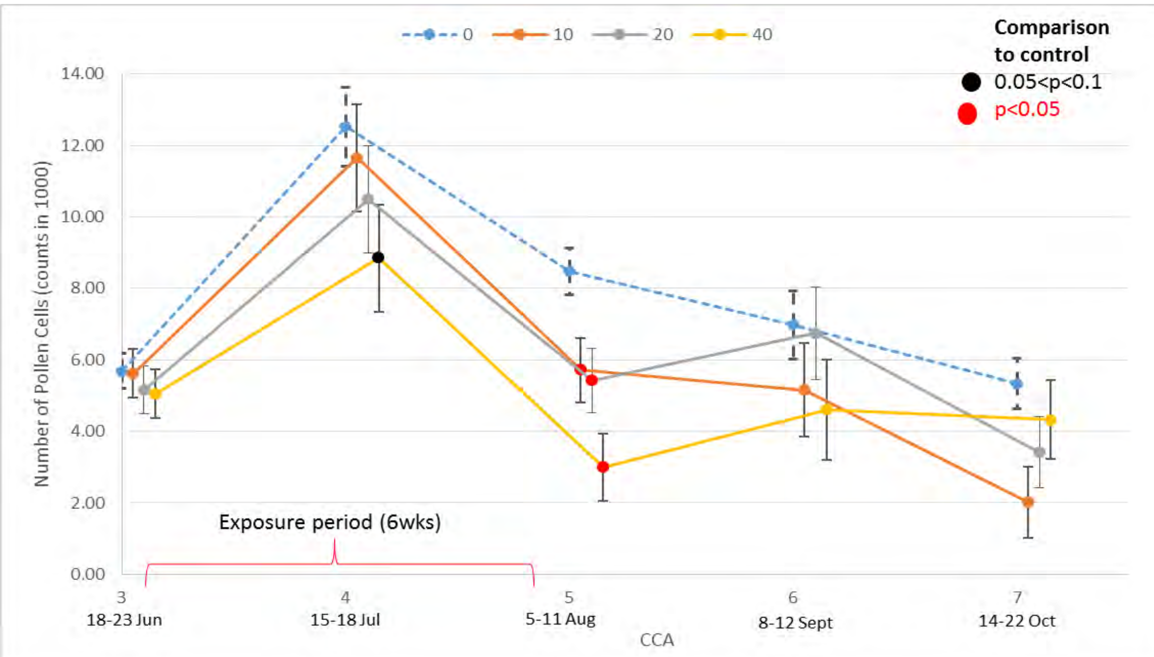


Figure 26. Number of pollen cells at colony condition assessments (CCA) 3 thru 7 for the control and three lowest treatment groups. Error bars represent one standard error from the mean calculated from the model residual mean squares estimate.

Due to consistent effects in the 40 µg/L treatment group at CCAs 4 and 5, the overall LOAEC for pollen stores is determined to be 40 µg/L. There is some uncertainty surrounding this endpoint given the significant reduction at all treatment doses at one measurement point (CCA5), that generally follows a dose-response relationship. However, as this only occurs at one CCA and thereafter no significant effects or dose-response are observed, the NOAEC is therefore considered to be 20 µg/L.

3.9.7.2 Nectar / Honey

There were no significant decreases (p>0.1) for honey/nectar storage at any CCA for any treatment dose (**Table 30** and **Figure 27**). However, there was a general trend of more honey storage in higher treatments compared to the controls and lower treatment groups. It is noted that honey storage in the 10 and 20 µg/L treatment groups did not differ appreciably from contols (<20% difference at all CCAs), while in the two highest treatments there was substantially more honey stored (>50%) by CCAs 6 and 7. Given the lack of statistical significance for honey, a second graph focusing on the control and three lowest treatment groups was not generated for this endpoint. It is noted that the feeding solutions (sugar solutions) provided during the exposure period might have affected natural honey storage patterns; however, effects on honey storage are still able to be considered as all treatments were compared to control hives (which also received feeding solutions).

Table 23. Estimated percent reduction from control for nectar/honey stores

Test concentration (µg/L)	Reduction relative to the control mean				
	CCA3	CCA4	CCA5	CCA6	CCA7
10	15.7	4.0	1.1	1.8	12.6
20	-4.3	-7.2	-13.1	-19.4	-6.8
40	5.3	-7.6	-17.2	-39.5	-46.7
80	-4.8	-15.8	-29.7	-75.4	-86.5
160	-3.4	-20.0	-21.3	-93.6	-114.1

Note: Negative value indicates increased nectar/honey stores in comparison to control.

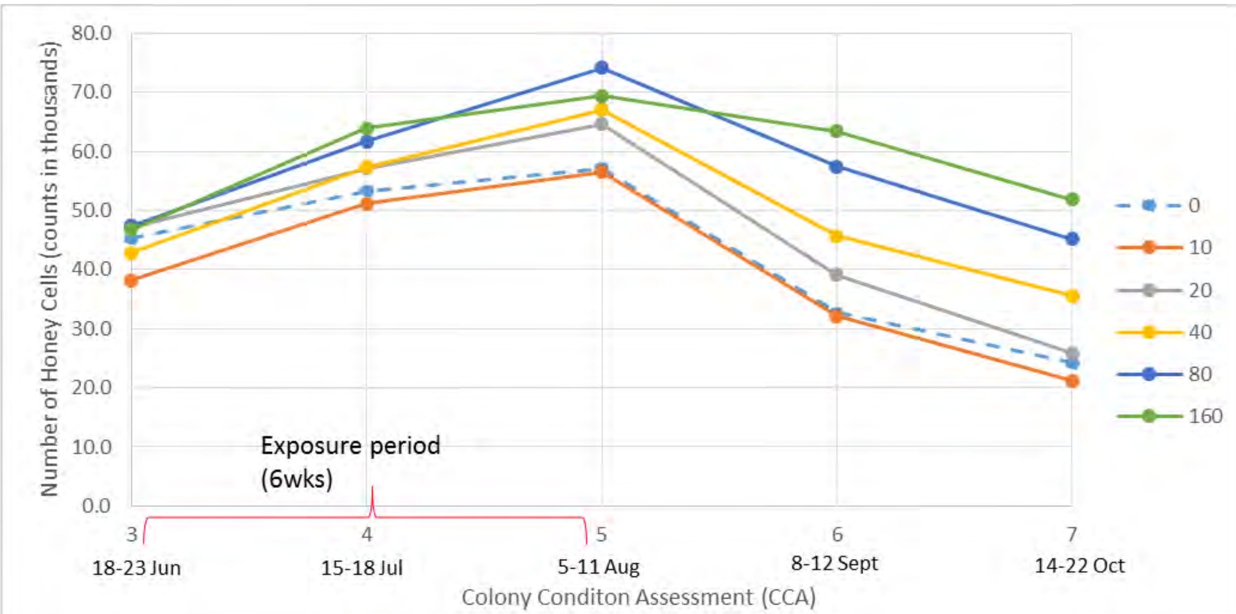


Figure 27. Number of honey cells at colony condition assessments (CCA) 3 thru 7 for each treatment group.

As no significant effects were observed and there were no evidence of adverse effects on honey storage, the overall NOAEC and LOAEC for honey stores is determined to be 160 and >160 µg/L, respectively.

3.9.7.3 Total Food Storage

There were no significant decreases (p>0.1) for total food storage at any CCA for any treatment dose (**Table 31** and **Figure 28**). However, similar to the honey storage above, there was a general trend of more food cells in higher treatments compared to the controls and lower treatment groups. It is noted that food storage in the 10 and 20 µg/L treatment groups did not differ appreciably from contols (<20% difference at all CCAs except for a 21.4% decrease at CCA7 in the 10 µg/L treatment), while in the two highest treatments there was substantially more food stored (>50%) by CCAs 6 and 7. Given the lack of statistical significance for food storage, a second graph focusing on the control and three lowest treatment groups was not generated for this endpoint. It is noted that the feeding solutions (sugar solutions) provided during the exposure period might have affected natural food storage patterns; however, effects on food cells are still able to be considered as all treatments were compared to control hives (which also received feeding solutions).

Table 24. Estimated percent reduction from control for food (pollen + nectar) storage

Test concentration (µg/L)	Reduction relative to the control mean				
	CCA3	CCA4	CCA5	CCA6	CCA7
10	14.0	4.4	5.2	6.0	21.4
20	-2.7	-2.9	-6.8	-15.5	0.9
40	6.0	-0.7	-6.7	-26.7	-35.1
80	-4.5	-4.0	-13.2	-51.5	-58.2
160	-2.7	2.1	-5.6	-59.7	-75.6

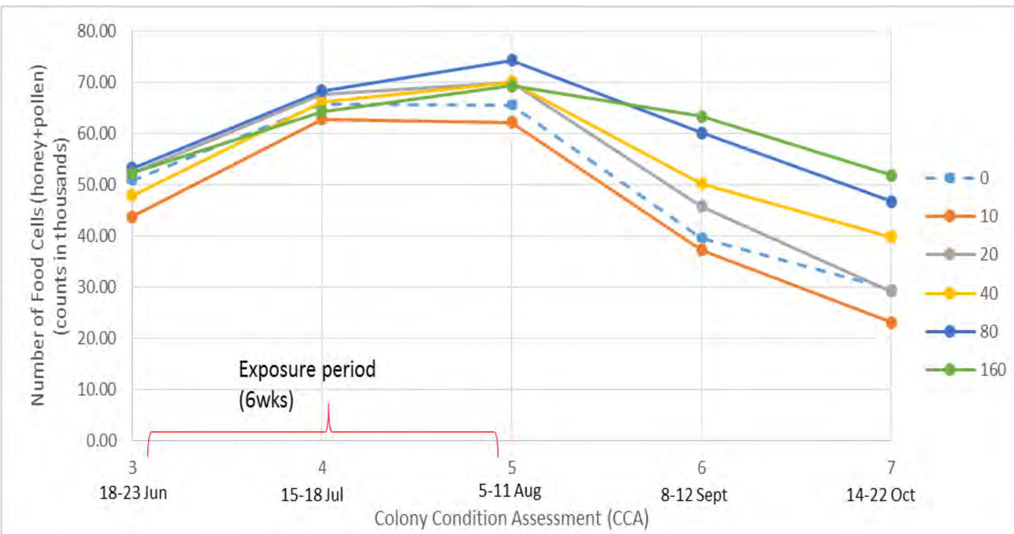
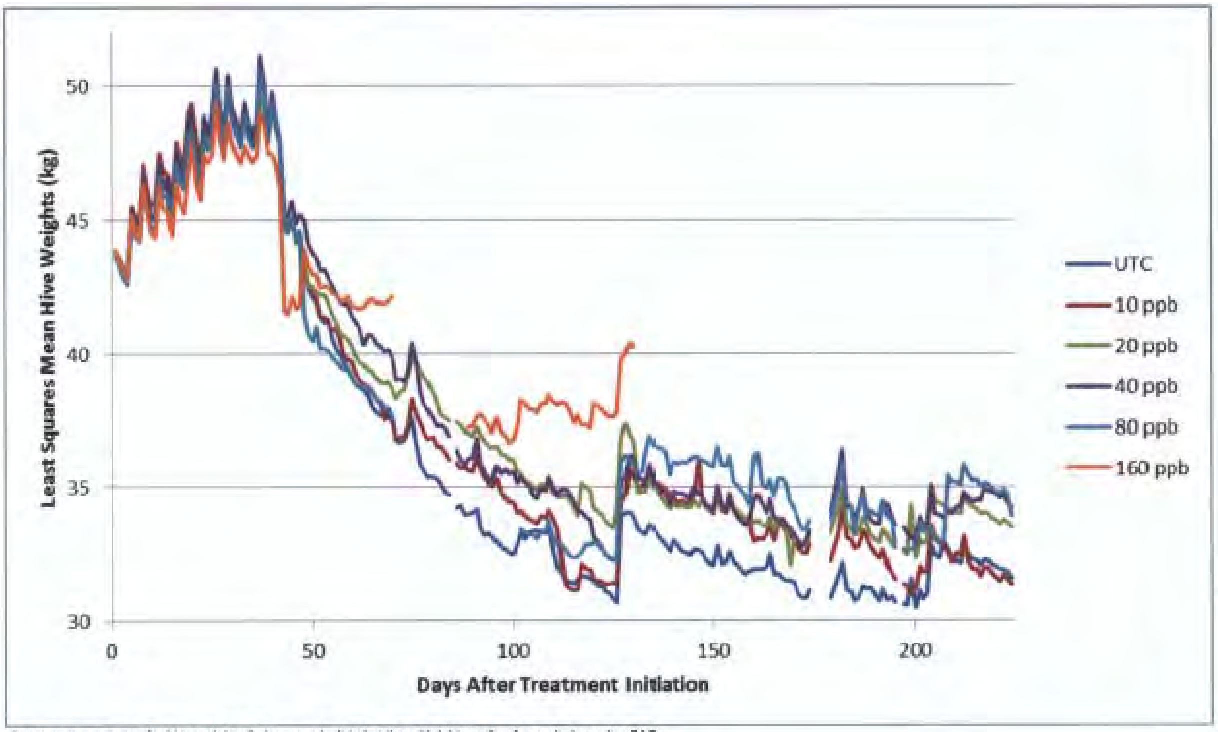


Figure 28. Number of food cells (honey+pollen) at colony condition assessments (CCA) 3 thru 7 for each treatment group.

As no significant effects were observed and there were no evidence of adverse effects on total food storage, the overall NOAEC and LOAEC for this endpoint is determined to be 160 and >160 µg/L, respectively.

3.9.8 Hive Weight

As supers were added and removed based on the study author's considerations to best support growth or reductions in the size of the bee colony and the weights of individual (empty) hive bodies were not reported in the study report, no statistical analysis was conducted by either EPA or PMRA on the hive weight parameters. Daily hive weight data can be found in Appendix E of the study report on pages 211—319. The figure below is taken directly from the author's study report. Hive weights generally oscillated similarly.



- Least squares means for hive weights (kg) were calculated at the midnight reading for each day using SAS

Figure 29. Proportion of hive weight following exposure of honey bees to varying concentrations of clothianidin in the diet for six weeks.

3.9.9 Hive mortality

Hive survival following overwintering is described below in **Tables 32-33** and **Figure 29.** The study author reported that 81 out of 84 colonies for biological observations were maintained over the 6-week exposure period and survived until the last CCA before overwintering (CCA7). Three colonies were removed due to technical issues between treatment initiation and CCA7. One hive in the control (I7) was removed due to potential contamination of the feeder following a technical error during feeding on 10 Jul 2014 and two hives in the 40 ppb treatment were removed after being knocked over (F3 on 07 Jul 2014 and C2 on 25 Aug 2014). All colonies in the 160 ppb treatment group had greatly reduced adult bee strength that was determined to be insufficient to survive overwintering and were subsequently destroyed following CCA7 in December 2014.

As 65% of control hives did not survive overwintering, the study lacks the capability to reliably determine differences in treatments compared to controls regarding colony survival.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 25. Proportion of hive weight following exposure of honey bees to varying concentrations of clothianidin in the diet for six weeks.

Treatment group	Apiary											
	A	B	C	D	E	F	G	H	I	J	K	L
UTC	-	-	C1	-	-	-	-	-	I6	-	K5	-
UTC	-	-	-	D8	E4	-	G8	H6	--[1]	J8	-	-
10 ppb	-	B8	-	-	-	F6	-	H2	-	-	-	-
20 ppb	A1	-	C4	-	E7	-	G3	-	I4	J5	K3	L2
40 ppb	A4	B1	--[1]	D7	-	--[1]	-	-	I8	J6	-	-
80 ppb	A2	B6	C8	D5	E6	F1	G4	H4	-	J3	K1	-
160 ppb	-	-	-	-	-	-	-	-	-	-	-	-

- = hive dead

--[1] = hive was removed from study due to technical error or vandalism prior to overwintering.

Table 26. Hive mortality statistics after overwintering measure at CCA8

Treatment (µg/L)	**Control**	**10**	**20**	**40**	**80**	**160[1]**
Number of deceased colonies /total colonies	15/23	9/12	4/12	5/10	2/12	11/11
Colony mortality (%)	65%	75%	33%	50%	17%	100%
Colony survival (%)	35%	25%	67%	50%	83%	0%
Treatment (µg/L)	**Control**	**10**	**20**	**40**	**80**	**160[1]**

[1] All colonies in the 160 ppb treatment group were destroyed after CCA7 (Dec 2014)

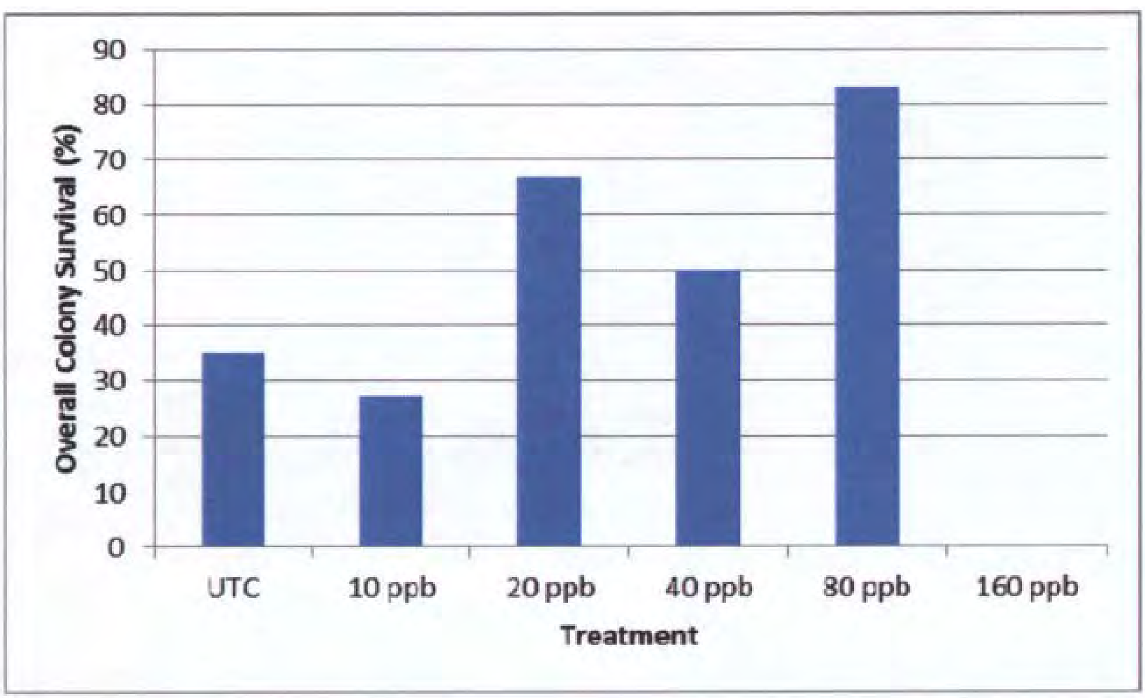


Figure 30. Overall hive survival after overwintering (reproduced from study report, p. 34).

4.0 Reviewer comments

What follows is brief discussion of some of the elements taken into consideration when evaluating the results of this study.

4.1 General Considerations for Biological Interpretation

While the hive mortality is considered as the most relevant measurement of survival at the colony level, sublethal effects at the colony level were estimated by measuring multiple parameters during the course of study. Each measured parameter is expected to reflect only part of the colony conditions, and all measurements have to be integrated for a better understanding of the hive status at the colony level. A honey bee colony is a super-organism in which live individuals and food supply are the two major components in maintaining the proper function of the colony. There are interactions between the two components and even within each component.

Individual bees are present in the colony as eggs, larvae, pupa and adults and they develop from one stage to another and interact with each other to perform a variety of tasks to maintain the integrity of the colony. The measurement of each stage of the bees is expected to provide information on the potential treatment effect on a specific life stage of bees during their development.

Hive food supplies including hive pollen and nectar are collected and processed by adults and are expected to have a large impact on the development of all stages of bees in hives. However, the amount of hive food storage is dependent on not only the number of foragers available for food collection, but also the number of individuals that consume the food. In addition, the seasonal availability of outside pollen and nectar sources also affects the amount of storage, thus impacting hive development. As well, sucrose feeding solutions were provided to the hives as a means of treatment and as a supplement for hive overwintering, which may have affected foraging and food storage during those time periods.

Hive weight was measured during the study. However, it is largely affected by the honey storage and number of bees that consume the food. A strong colony with a high number of bees likely consumes a high amount of stored honey and may result in a reduced hive weight. Weighing hives at different time periods of the day may result in an increased variation of the measurement due to the fact that foragers may not be present in the hive when the weight is measured. Hive weights may be artificially lower in hives which contain a high number of forager bees that may be out collecting food during a different time of the day.

Considerations regarding the measurement time points:

- CCA3 represents the background hive conditions as the first colony assessment after the hives were placed in the test fields prior to the exposure.
- CCA4 and CCA5 represent the hive conditions during the exposure phase. It was noted that the CCA5 was conducted a week after the end of the 6-week exposure period, but is expected to represent effects during the exposure period.

- CCA6 was measured at 4 weeks after the end of exposure. It allows all bee individuals, including eggs, larvae and pupa that were exposed to treatment to finish their development cycle and become adults.
- CCA7 represent the hive conditions immediately prior to overwintering. It is considered that hives were physiologically preparing for overwintering by reducing the production of immature bee individuals. Treatment effects may be masked by the natural decline of hive individuals.
- CCA8 and CCA9 represents hive conditions of surviving hives after overwintering. High mortality in the control hives excluded these assessments from analysis.

4.2 Control Performance

Control mortality and sublethal effects on life stages and food stores

The control performance in this study offers some challenges relating to the interpretation of the results. The level of colony loss after overwintering in controls (65%), adds a great deal of uncertainty when considering the results of individual measurements. The fact that many of the hives in the lower treatment groups performed/trended similarly to the control hives for these measurements could be indicative of either a lack of treatment effects or potentially that the control hives were suboptimal to begin the study. Because so few hives survived overwintering and trended relatively closely to the lower level treatment hives during exposure, the overwintering component would be extra important to determine if the lack of significant reductions compared to the control in most treatment groups is biologically significant. Almost every parameter for life stages decreased after exposure ended (endpoints generally reached their apex at CCA5) which could have been a factor of either the time of year or of treatment. The fact this also happened in the control groups suggest a performance issue is possible, or at the very least an uncertainty with respect to if the exposure measurements were taken too late in the year to be able to reliably discern treatment effects.

The similarity in the dynamics of all parameters for the individual living organisms at various stage across the control and lower (10-20 μg/L treatments may indicate that control hives were stressed prior to overwintering. For most parameters in the lower treatment groups the means converged to those of the control at CCA 6 through CCA 7 (and through CCA8, for those hives that survived overwintering). The time of year likely influenced control hive performance as colonies are normally producing far fewer bees at this time of year, but it is still considered uncertain if the hives were developing normally. There was no apparent spike of honey collection or pollen stores from the control hives indicating they may not have been developing and storing enough food to survive the winter. Pollen stores were decreasing at the same time other biological parameters were indicating consumption of resources but not replenishment for the hive.

4.3 Consideration of Study Strengths, Limitations and Interpretation

It is important to recognize the inherent strengths and limitations of this study as results are interpreted and potentially considered in risk assessment.

In the context of available field studies involving honey bees and clothianidin, this study contains a number of strengths including:

- Use of a high degree of replication (n=12) to achieve a reasonable level of statistical power
- Demonstration of a generalized concentration-response relationship with respect to the concentration of clothianidin in sucrose solution and the magnitude and duration of adverse effects
- Quantification of exposure to clothianidin in diet and in hive matrices (uncapped nectar, pollen, capped honey, bee bread)
- Use of a 6-week exposure duration to represent a "high end" exposure scenario
- Inclusion of multiple colony-level endpoints reflecting hive strength, brood development and food stores
- Detailed QA/QC results regarding quantification of clothianidin residues in various matrices
- Availability of raw data for conducting statistical analysis.

A number of limitations are also noted with this study, including:

- Exposure of bees to clothianidin occurred through nectar (sucrose) alone, whereas bees in the field are likely exposed through both pollen and nectar routes. Therefore, the design of this study may not reflect a "worst case" exposure scenario in which bees are experiencing prolonged exposure to both contaminated nectar and pollen. While exclusion of the pollen route is expected to reduce overall exposure, the impact of this exclusion on the study results is uncertain and will likely depend on the life stage/caste of bee.

- Residues in hive matrices were only analyzed for parent clothianidin. Metabolites of clothianidin were not considered. Clothianidin degradates (*e.g.* TZNG) have been demonstrated in laboratory studies to have much less acute toxicity to adult honey bees, though data is not available for their chronic effects to adult bees or potential effects to other honey bee life stages.

- Clothianidin was found in both hive nectar and hive pollen (beebread), at concentrations lower than the feeding solutions. Dilution compared to the treatment feeding solution is expected since bees could also forage on outside nectar and pollen sources. As well, hive pollen contains only some hive nectar, thus would not be expected to have a concentration equivalent to nectar alone, and it is mixed with pollen which will come from outside sources. Therefore exposure through both hive pollen and nectar occurred via exposure to the sucrose feeding solution, but how this compares to exposure through contaminated pollen directly is not known. It is also noted that nectar is considered the dominant exposure route for forager bees; other hive bees and larvae consume both nectar and pollen. A recent paper by Sandrock (2014)[4] indicated that consuming contaminated pollen containing low levels of both clothianidin and thiamethoxam had effects on many hive parameters. In addition, since bees were forced to forage for pollen in this study, the potential impact of clothianidin exposure on reducing pollen foraging efficiency of bees could be incorporated

[4] Sandrock C, Tanadini M, Tanadini LG, Fauser-Misslin A, Potts SG, et al. (2014) Impact of Chronic Neonicotinoid Exposure on Honeybee Colony Performance and Queen Supersedure. PLoS ONE 9(8): e103592. doi:10.1371/journal.pone.0103592

into the overall expression of adverse effects, as suggested by published literature. Had contaminated pollen been provided to bees, it is not known if the potential impact on pollen foraging efficiency would have been masked.

- The quantity of nectar provided to hives (4 L per week per hive) likely did not fulfill the complete carbohydrate needs of the colony, as indicated by colony bioenergetics and the lack of remaining sucrose solution upon their renewal at some of the test concentrations. This suggests that bees could be exposed to a greater dose of clothianidin in nectar had a greater volume of spiked sucrose been provided. Although one can infer that the dosing regimen may have underestimated exposure through sucrose relative to 100% contaminated diet, it is also noted that bees had to supplement their spiked sucrose by foraging on their own for other sources of nectar. As with the previous discussion of pollen it is noted that had 100% of the carbohydrate needs of the colony been provided via feeders, the potential impact of purported reductions in nectar foraging efficiency may have been masked to some degree.

- Overwintering success of controls was severly impacted (65% hive mortality). This prevents the ability to detect adverse effects related to hive loss following overwintering. The lack of control hive overwintering may reflect the study design that prevented earlier supplemental feeding in the fall (in order to ensure that treatment hives were consuming their exposed food stores), while typical beekeeping practice would have permitted additional feeding earlier in the fall.

- Pesticides from food sources other than the artificial feeding were also detected during the exposure period and post-exposure periods through collection of pollen from pollen traps from monitoring hives. This contributes to exposure uncertainty and can add confounding effects when interpreting results. However, it is noted that detections occurred in <10% of samples from monitoring hives and that the only pesticides detected (propiconazole, chlorothalonil and carbaryl) had relatively low toxicity compared to parent clothianidin (ranging from practically non-toxic for chlorothalonil to moderately toxic for cararyl).

- Residues of clothianidin in uncapped nectar and bee bread within the hives at CCAs 4, 5, 7 and 9 represent a single sample per hive on a single frame rather than a composite sample from multiple portions of the comb within a hive. This means that residue results may reflect a "hit or miss" scenario with respect to detecting residues in nectar laid down from contaminated (fed) vs. outside sources.

- The exposure, based on residues measured in the hive (hive nectar and hive pollen) indicated that, overall, higher measured hive residues correlated with higher nominal residues in feeding solutions. However, individual hive residue values varied, and there was some overlap in measured values, particularly among the three lowest doses.

- Exposure dilution during the study was evident. Remarkably lower residue concentrations detected in bee bread and hive nectar in some test hives compared to the feeding concentrations indicate foraging on other food sources. This uncertainty is inherent in any semi-field or full-field study design.

5.0 Overall Study Conclusions

The study is considered to be informative and will be used as a line of evidence in the pollinator risk assessment. While there were uncertainties that were generally related to inherent aspects of any semi-field or full field study design (such as dilution of the test chemical through alternative sources of forage, detection of other chemicals in the monitoring hives), this study still provides information on a number of colony health parameters about the long term (however excluding overwintering) exposure to clothianidin at the colony level.

An evaluation of the observed effects was conducted considering statistical reductions relative to the control, trends within each treatment and in comparison to the control, recognition of the natural trends honey bee colonies follow during the course of the year, and finally, the fact that successful overwintering in the controls was not observed. With regard to the top two test treatments (160 and 80 ug/L), statistical reductions relative to the control ($p<0.05$) were observed across several different endpoints and at many CCAs within an endpoint. Statistically significant decreases in the number of adults (30-96% reductions in EPA's analysis, 14—98% reduction in PMRA's analysis) and brood (eggs (38-95% in EPA's analysis, 31-94% in PMRA's analysis), larvae (49-100% in EPA's analyis, 12-99% in PMRA's analysis), and pupae (26-100% in EPA's analysis and 31-100% in PMRA's analysis) were observed compared to the control, starting at CCA4 with effects being sustained through CCA7, particularly for number of adults and eggs. At these top two test concentrations, decreases in pollen storage compared to the control was observed with significant decreases at CCA4 thru 7. At 40 ug/L, significant decreases in pollen compared to the control were observed at CCA 4 ($0.05<p<0.1$ in EPA's analysis, $p<0.05$ in PMRA's analysis) and CCA5 ($p<0.05$, both analyses). Also at this concentration, significant decreases ($p<0.05$, both analyses) in the number of adults was observed at CCA5 and 6, and in the number of pupae at CCA4 thru 6, though these responses were at levels similar to the control for CCA7. In addition, PMRA determined significant reductions at this concentration in the total number of individuals at CCA4 thru 6 ($p<0.05$), number of eggs at CCA4 and 5 ($p<0.05$) and the number of larvae at CCA4 ($p<0.05$).

With regards to the lower two test treatments (10 and 20 ug/L), most endpoint responses were not significantly different from the control ($p>0.1$). For the EPA analysis there were two endpoints for which a statistical reduction for one or both of these treatments was observed. First, the number of adults was statistically reduced ($p<0.05$) at CCA6 at the lowest treatment (10 ug/L), but was not significant at 20 ug/L (mean number of adults was slightly greater at 20 ug/L compared to 10 ug/L, both at this CCA and consistently following feeding exposure) and was not significant at any other CCA. Second, significant decreases ($p<0.05$) in pollen were observed at all test concentrations at CCA5. However, at other CCAs for pollen, only the 160 and 80 ug/L treatment groups were significantly decreased, except for an observed (non-dose responsive) decrease at the lowest test concentration (10 ug/L) at CCA7.

The PMRA results were slightly different from the EPAs for the lower two test treatments but resulted in the same conclusion. While most endpoint responses were not significantly different from the control at various timepoints throughout the study ($p<0.1$), significant reductions were observed at both test concentrations in the number of adults (CCA6), eggs (CCA4 and 5), larvae (CCA4), pupae (CCA5), total number of individuals (CCA5 at 10 ug/L and CCA4 and 5 at 20 ug/L) and pollen stores (CCA5). These effects were considered to be potentially transient with numbers returning to control levels in subsequent CCAs.

Hive mortality is considered the most relevant measurement of survival at the colony level. The level of colony loss after overwintering experienced by controls in this study (65%), precludes the use of this endpoint in evaluating chronic exposures of colonies to clothianidin. The lack of control overwintering success also has significant implications in evaluating effects on other measured parameters in the study. The potential for observed effects (in the ≥ 40 µg/L treatments) to be ameliorated following exposure and subsequent recovery cannot be assessed. Furthermore, there is potential that additional chronic effects, not observed prior to overwintering, may subsequently manifest themselves at the lower doses (*e.g.* 10 and 20 µg/L) which could not be adequately captured from this study.

Therefore, the overall quantitative NOAEC and LOAEC for this study is 20 and 40 µg/L, respectively, based on impacts on pollen storage, number of adults, number of pupae and total brood and total live bees in the ≥40 µg/L treatment groups that were sustained across multiple CCAs prior to overwintering (effects on larvae, though not significant at 40 µg/L may also have been suggestive of an impact from this dose, as they consistently did not track well with the control and lower treatment doses). These effect levels include the understanding that evaluation of overwintering was not possible which limits the ability to fully evaluate potential long-term effects in the two lower treatments groups, and therefore, remains a major source of uncertainty.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Appendix A: Details of PMRA Statistical Analysis

During the review of the study, a separate statistical analysis was conducted with the program R (version 3.1.2)[5] using the raw data submitted by the study author.

Statistical analysis

<u>*Analysis Strategy*</u>

Hive condition data:

To analyze colony condition data which contains many components over many assessments at different times, a primary analysis was set out to effectively prevent multiplicities from interfering with the interpretation of p_values and confidence intervals. These multiplicities arise from having multiple dose levels, multiple outcomes and multiple time points, and are dealt with as follows:

- The multiplicities from having multiple dose levels was dealt with by using step down testing, the highest dose group's data was compared directly to the control group's data, if statistically significant at a chosen alpha level the next lowest dose group's data was compared to the control group's data and this was continued down to the dose where statistical significance was no longer achieved. A technical reference for this step down testing would be Multiple Comparison Procedures in Dose Response Studies. Tamhane, Ajit C. and Logan, Brent R., in Dose Finding in Drug Development edited by Ting, Naitee. Springer New York 2006. This step down procedure (referred to as the SD2PC procedure in the technical reference) was chosen as it provides good power for detecting the minimum effective dose (lowest does where effect is present) when monotonic dose effects are expected while providing stringent control of type one error, regardless of the true pattern of dose effects. That is, with minimal assumptions, the procedure strongly controls family wise type one error rate while maintaining good power for effect patterns that are expected.

  This step down procedure is implemented by PMRA using only data from the control group and the dose group being tested in that step which alleviates any concern about heterogeneity of variance across dose groups. Especially with outcome data that involves estimates of underlying counts, it is expected that effects at a given dose necessarily involves both the mean and variance. When this is the case - the use of data from a higher dose with a putative effect in the comparison of a lower dose would thus be inappropriate and would invalidate the control of type one error.
  The applicant's choice of multiplicity adjustment procedure, which was William's trend test (Williams 1972), was presumably chosen to be in accord with *OECD, 2003. Draft guidance document for the statistical analysis of ecotoxicity data.* They are both step down procedures but ours differs from William's in that it uses only within dose group data based estimates of means rather than maximum likelihood estimates of dose group means using

[5] R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

all group's data simultaneously - under monotonicity assumption (i.e. order restricted or isotonic means) additionally assuming homogeneous variances. Although these additional assumptions may not be problematic and are within the OECD guidelines, we simply chose not to rely on them (and by doing so, exceed the OECD guidelines.)

- The multiplicities from having multiple outcomes, was dealt with by choosing to focus on the assessment of total life in the hive – simply the number of viable life forms at any stage in the hive. It is considered that the total number of individuals includes all live individuals in hives (eggs larvae, pupae and adults) and is expected to be a better indicator of the hive status at the colony level than any single stage of bees alone. This outcome would provide good power when background knowledge is lacking on the stage most likely to be affected (i.e. it cannot be well anticipated) and it is not expected that there will be simultaneous trade-offs effects between the stages. That is, when it is not expected that a toxic effect on one stage would have a beneficial effect for another stage at the same point in time.

- The multiplicities from having multiple time points was dealt with by choosing to focus on the time when the effects were believed to be most pronounced both in terms of having an impact on total life and having a high powered assessment of that. In this case CCA6 was selected for the following reasons.
  - CCA4 and CCA5 were not selected as they represent the hive conditions during the 6-week exposure phase. It is noted that CCA5 was conducted a week after the end of the 6-week exposure period, but it is expected to represent effects during the exposure period.
  - CCA6 (4 weeks after the end of feeding exposure) was selected as it maximises the time period for detecting a potential latent effect from exposure and occurs before the start of hive decline prior to overwintering at most apiaries.
  - CCA7 (9 weeks after the end of feeding exposure) was not selected simply due to the natural decline of hive size in the late fall that may mask the effect of treatments.
  - CCA8 and CCA9 (after over-wintering period) were not selected because of the high hive mortality observed in the controls.

While the total individuals at CCA6 is considered as a primary parameter to control multiplicity for statistical analysis, all parameters including eggs, open brood and capped brood, adults, pollen and nectar store, that were observed during the entire study including CCA4, CCA5, CCA6 and CCA7 were also considered in the review. Hive weight was also measured throughout the study however, given the inherent variability of this parameter it was not further considered in the statistical review. Given that the primary analysis has prevented multiplicities from interfering with the interpretation of p_values and confidence intervals, if statistical significance has been achieved (at given dose levels), further analysis with all other outcomes is undertaken "with prejudice" for the assessment of similar effects as being significant. More formally, re-allowance for multiplicities is not required and less stringent alpha levels are allowed. Essentially the price has been paid for searching for the pattern in the primary analysis (measures taken to prevent multiplicities) and it need not be re-paid evaluating the same pattern elsewhere. On the other hand, if statistical significance has not been achieved (at given dose levels), further analysis with all other outcomes is undertaken "with prejudice" for assessment of other effects as likely being just noise. Here though dramatic effects should not be ignored but carefully considered and noted.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Analysis methods for hive conditions

For all hive conditions total life, eggs, open brood and capped brood, adults, pollen and nectar store at CCA4, CCA5, CCA6 and CCA7 a conventional analysis of block randomised experiments with a baseline measurements was undertaken. In line with the statistical strategy discussed above, the focus was on total life at CCA6 (with step down adjustment for multiplicities applied) but identical analysis was carried out on all other hive conditions assessed at the given assessment points. This analysis comprised of linear modeling (or ANOVA) stratified on Apiary (block) and adjusted for baseline measurements at CCA3 with one-side testing for harm using only the control group data and the data from a single dose group at a time, starting with the highest and then through lowest dose groups. It is a series of robust "t.test like" analyses that conservatively implement the step down testing procedure. Under the assumption of no effect in the single dose group being tested (relevant to type one error control), the means and variances and covariate effects should be identical in both the control group and the single dose group being tested. (In an analysis that includes all dose group data together e.g. William's procedure, an impact of a treatment effect on the variance and covariate effects at a higher dose, in addition to an effect on the mean, would invalidate the assumptions needed to control type one error rate in the lower doses.) The results of all analyses are presented in tables of unadjusted p_values (adjusted p_values can be simply read off as the maximum of all p_values in any higher dose), effect estimates and upper and lower confidence intervals (in file Clot_summariesF) as well as plots of the confidence intervals (pdf file Bees8.pdf).

The code snippet to implement these analyses in R was:

```
glm(outcome~Apiary + baseline + exposed, data= x[x$exposed == " control " | x$exposed == dose,])
```

Sensitivity analysis was undertaken by extensive graphical analyses sometimes using the square root transformation as well as calculating non-parametric randomisation (permutation) tests on the differences between high dose group and control group average within Apiary. These are given in the column named PermP_value in Clot_summariesF.

Transcript/program of analyses carried out

The file ClothianidinBees2.R contains the transcript of the final run of the R program used to carry out the analysis and generate the tables and plots.

Supporting graphs

The following graphs were produced as part of the analysis.

Bees1a.pdf – Plots of individual hive condition assessments over-CCAs by Apiary.

Bees1b.pdf – Plots of individual hive condition assessments over-CCAs up to CCA7 by Apiary.

Bees2.pdf – Plots of control versus exposed condition assessments over-time group by Apiary.

Bees3.pdf – Plots of overall mean and Apiary mean control condition assessments over time.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Bees3S.pdf – Plots of overall mean and Apiary mean of the square root of control condition assessments over time.

Bees7.pdf – Plots of individual exposed hive versus control condition assessments for "everything".

Bees7S.pdf – Plots of individual exposed hive versus square root of control condition assessments for "everything".

Bees7d.pdf – Plots of individual exposed hive versus control condition assessments for "everything" by dose group.

Bees7dS.pdf – Plots of individual exposed hive versus square root of control condition assessments for "everything" by dose group.

Bees8.pdf – Plots of effect estimates and confidence intervals for "everything"

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table A-1. Summary of the differences between treatment and controls on the basis of observations and model estimations, and p values.

Parameter	Time (CCA)[1]	Test conc. (µg/l)	Observed mean difference from control[2]	Standard error observed mean	n	Model estimate mean difference from control[3,4]	Standard error of estimated mean[4]	p_value for comparison with the control[4]	90% confidence upper limit[4]	90% confidence lower limit[4]	Estimated reduction from control (%)[4,5]	Estimated reduction from control (number)[4]	Observed means in control	T-test confidence limit
Adults	3	160	-644	1014	12	0	0	0	0	0	0	0	15014	-1.717
Adults	3	80	-1500	683	12	0	0	0	0	0	0	0	15014	-1.717
Adults	3	40	-307	1331	12	0	0	0	0	0	0	0	15014	-1.717
Adults	3	20	-96	1422	12	0	0	0	0	0	0	0	15014	-1.717
Adults	3	10	664	1746	12	0	0	0	0	0	0	0	15014	-1.717
Adults	4	160	5386	1216	12	5604.866	1164.551	0	0.439	0.208	0.323	7609	17340	-1.721
Adults	4	80	1641	1294	12	2413.834	1201.969	0.029	0.258	0.02	0.139	4482	17340	-1.721
Adults	4	40	-63	961	11	-229.053	1262.953	0.571	0.112	-0.139	-0.013	1949	17340	-1.725
Adults	4	20	262	1248	12	108.853	1240.316	0.465	0.129	-0.117	0.006	2243	17340	-1.721
Adults	4	10	10	1124	12	-346.826	1325.985	0.602	0.112	-0.152	-0.02	1935	17340	-1.721
Adults	5	160	13331	1112	12	13491.43	1567.828	0	0.838	0.559	0.699	16189	19310	-1.721
Adults	5	80	7215	1451	12	7947.582	1683.568	0	0.562	0.262	0.412	10845	19310	-1.721
Adults	5	40	4577	1383	11	4463.445	1799.951	0.011	0.392	0.07	0.231	7568	19310	-1.725
Adults	5	20	360	1250	12	315.422	1643.241	0.425	0.163	-0.13	0.016	3143	19310	-1.721
Adults	5	10	1830	1967	12	1480.459	1949.511	0.228	0.25	-0.097	0.077	4835	19310	-1.721
Adults	6	160	16182	825	12	16521.8	1312.766	0	1.019	0.774	0.897	18781	18427	-1.721
Adults	6	80	10505	1396	12	11810.21	1449.897	0	0.776	0.506	0.641	14305	18427	-1.721
Adults	6	40	5786	2450	10	5501.19	2254.948	0.012	0.51	0.087	0.299	9400	18427	-1.729
Adults	6	20	3210	1561	12	3133.999	1664.1	0.037	0.325	0.015	0.17	5997	18427	-1.721
Adults	6	10	4198	1360	12	3611.868	1537.387	0.014	0.34	0.052	0.196	6257	18427	-1.721
Adults	7	160	12279	656	12	12552.58	1663.736	0	1.208	0.76	0.984	15415	12757	-1.721
Adults	7	80	6158	1215	12	7107.94	1837.448	0	0.805	0.309	0.557	10270	12757	-1.721
Adults	7	40	1984	2082	10	1684.602	2381.384	0.244	0.455	-0.191	0.132	5802	12757	-1.729
Adults	7	20	934	1167	12	932.356	1855.67	0.31	0.323	-0.177	0.073	4125	12757	-1.721
Adults	7	10	2544	1531	12	1893.609	1756.561	0.147	0.385	-0.088	0.148	4916	12757	-1.721

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	Observed mean difference from control[2]	Standard error observed mean	n	Model estimate mean difference from control[3,4]	Standard error of estimated mean[4]	p_value for comparison with the control[4]	90% confidence upper limit[4]	90% confidence lower limit[4]	Estimated reduction from control (%)[4,5]	Estimated reduction from control (number)[4]	Observed means in control	T-test confidence limit
Adults	8	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Adults	8	80	-2252	3112	7	-2474.47	3577.028	0.74	0.817	-1.672	-0.427	4733	5791	-2.015
Adults	8	40	-4047	3416	3	-3178.64	5618.408	0.664	5.577	-6.675	-0.549	32295	5791	-6.314
Adults	8	20	-4077	1453	6	-4081.79	1628.141	0.967	-0.105	-1.304	-0.705	-611	5791	-2.132
Adults	8	10	3020	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Adults	9	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Adults	9	80	-915	4716	7	-1218.75	5424.588	0.584	0.661	-0.827	-0.083	9712	14685	-2.015
Adults	9	40	-1107	6047	3	2664.342	7809.261	0.395	3.539	-3.176	0.181	51970	14685	-6.314
Adults	9	20	-4510	3073	6	-4280.88	3056.584	0.883	0.152	-0.735	-0.292	2235	14685	-2.132
Adults	9	10	23798	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Honey	3	160	-1530	6052	12	0	0	0	0	0	0	0	45266	-1.717
Honey	3	80	-2175	6139	12	0	0	0	0	0	0	0	45266	-1.717
Honey	3	40	2407	7070	12	0	0	0	0	0	0	0	45266	-1.717
Honey	3	20	-1927	8031	12	0	0	0	0	0	0	0	45266	-1.717
Honey	3	10	7088	6550	12	0	0	0	0	0	0	0	45266	-1.717
Honey	4	160	-9511	3598	12	-9672.55	2608.755	0.999	-0.095	-0.26	-0.178	-5184	54439	-1.721
Honey	4	80	-7262	4172	12	-7006.23	2471.091	0.995	-0.051	-0.207	-0.129	-2754	54439	-1.721
Honey	4	40	-2851	5334	11	-5838.4	3036.367	0.966	-0.011	-0.203	-0.107	-602	54439	-1.725
Honey	4	20	-2696	5969	12	-2623.74	2657.341	0.833	0.036	-0.132	-0.048	1949	54439	-1.721
Honey	4	10	3275	6129	12	-3203.22	2969.458	0.854	0.035	-0.153	-0.059	1906	54439	-1.721
Honey	5	160	-11025	5173	12	-11140.3	2971.193	0.999	-0.103	-0.279	-0.191	-6028	58334	-1.721
Honey	5	80	-15822	4384	12	-15678.4	2659.207	1	-0.19	-0.347	-0.269	-11103	58334	-1.721
Honey	5	40	-9068	6034	11	-12035.1	3210.807	0.999	-0.111	-0.301	-0.206	-6497	58334	-1.725
Honey	5	20	-6344	5649	12	-6197.9	2677.684	0.985	-0.027	-0.185	-0.106	-1590	58334	-1.721
Honey	5	10	1795	5304	12	-3832.55	2535.103	0.927	0.009	-0.14	-0.066	530	58334	-1.721
Honey	6	160	-29353	4792	12	-29434.8	3572.762	1	-0.685	-1.046	-0.865	-23287	34018	-1.721

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Parameter	Time (CCA)[1]	Test conc. (µg/l)	Observed mean difference from control[2]	Standard error observed mean	n	Model estimate mean difference from control[3,4]	Standard error of estimated mean[4]	p_value for comparison with the control[4]	90% confidence upper limit[4]	90% confidence lower limit[4]	Estimated reduction from control (%)[4,5]	Estimated reduction from control (number)[4]	Observed means in control	T-test confidence limit
Honey	6	80	-23382	5113	12	-23289.3	3606.386	1	-0.502	-0.867	-0.685	-17084	34018	-1.721
Honey	6	40	-12615	6754	10	-15217.6	4513.887	0.998	-0.218	-0.677	-0.447	-7413	34018	-1.729
Honey	6	20	-5070	6073	12	-5105.39	3524.015	0.919	0.028	-0.328	-0.15	959	34018	-1.721
Honey	6	10	1861	7227	12	-3921.35	4673.865	0.795	0.121	-0.352	-0.115	4121	34018	-1.721
Honey	7	160	-26814	4512	12	-26591.2	3857.038	1	-0.796	-1.325	-1.06	-19954	25077	-1.721
Honey	7	80	-20115	4172	12	-20117.7	3402.106	1	-0.569	-1.036	-0.802	-14264	25077	-1.721
Honey	7	40	-11136	4562	10	-12889.2	3628.178	0.999	-0.264	-0.764	-0.514	-6616	25077	-1.729
Honey	7	20	-794	4928	12	-703.192	3778.982	0.573	0.231	-0.287	-0.028	5799	25077	-1.721
Honey	7	10	3887	6456	12	-691.527	4370.679	0.562	0.272	-0.327	-0.028	6829	25077	-1.721
Honey	8	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Honey	8	80	-4537	9702	7	-496.727	8503.231	0.522	0.757	-0.802	-0.023	16638	21984	-2.015
Honey	8	40	5029	18842	3	-2400.74	5396.559	0.633	1.441	-1.659	-0.109	31672	21984	-6.314
Honey	8	20	-16773	4392	6	-14240	5284.67	0.973	-0.135	-1.16	-0.648	-2974	21984	-2.132
Honey	8	10	18064	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Honey	9	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Honey	9	80	-2184	6379	7	1156.033	4362.47	0.401	0.37	-0.284	0.043	9947	26847	-2.015
Honey	9	40	2580	8835	3	-861.119	3209.364	0.583	0.723	-0.787	-0.032	19402	26847	-6.314
Honey	9	20	-25772	9308	6	-16502.4	8654.203	0.935	0.073	-1.302	-0.615	1947	26847	-2.132
Honey	9	10	-397	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pollen	3	160	165	737	12	0	0	0	0	0	0	0	5690	-1.717
Pollen	3	80	-132	816	12	0	0	0	0	0	0	0	5690	-1.717
Pollen	3	40	645	654	12	0	0	0	0	0	0	0	5690	-1.717
Pollen	3	20	529	533	12	0	0	0	0	0	0	0	5690	-1.717
Pollen	3	10	66	754	12	0	0	0	0	0	0	0	5690	-1.717
Pollen	4	160	12423	992	12	12105.42	1546.157	0	1.15	0.736	0.943	14766	12836	-1.721
Pollen	4	80	6170	959	12	6098.508	1421.854	0	0.666	0.284	0.475	8545	12836	-1.721

Parameter	Time (CCA)[1]	Test conc. (µg/l)	Observed mean difference from control[2]	Standard error observed mean	n	Model estimate mean difference from control[3,4]	Standard error of estimated mean[4]	p_value for comparison with the control[4]	90% confidence upper limit[4]	90% confidence lower limit[4]	Estimated reduction from control (%)[4,5]	Estimated reduction from control (number)[4]	Observed means in control	T-test confidence limit
Pollen	4	40	3844	1207	11	2816.395	1541.659	0.041	0.427	0.012	0.219	5475	12836	-1.725
Pollen	4	20	2332	1500	12	1396.809	1612.023	0.198	0.325	-0.107	0.109	4171	12836	-1.721
Pollen	4	10	1174	1708	12	921.97	1781.651	0.305	0.311	-0.167	0.072	3988	12836	-1.721
Pollen	5	160	8263	986	12	8269.915	1171.897	0	1.245	0.757	1.001	10286	8263	-1.721
Pollen	5	80	8097	945	12	8216.444	997.742	0	1.202	0.787	0.994	9933	8263	-1.721
Pollen	5	40	5341	1846	11	4572.426	1468.524	0.003	0.86	0.247	0.553	7105	8263	-1.725
Pollen	5	20	2853	1011	12	2290.263	1038.79	0.019	0.494	0.061	0.277	4078	8263	-1.721
Pollen	5	10	2556	1173	12	2494.555	1271.055	0.032	0.567	0.037	0.302	4682	8263	-1.721
Pollen	6	160	7014	1359	12	6744.362	1805.483	0.001	1.401	0.517	0.959	9851	7030	-1.721
Pollen	6	80	4268	1394	12	4238.004	1667.556	0.009	1.011	0.195	0.603	7107	7030	-1.721
Pollen	6	40	1449	1340	10	537.407	1814.263	0.385	0.523	-0.37	0.076	3675	7030	-1.729
Pollen	6	20	281	1176	12	-784.589	1635.662	0.682	0.289	-0.512	-0.112	2030	7030	-1.721
Pollen	6	10	1869	1882	12	1623.023	1908.998	0.202	0.698	-0.236	0.231	4908	7030	-1.721
Pollen	7	160	5450	1225	12	5214.728	1290.707	0	1.364	0.549	0.957	7436	5450	-1.721
Pollen	7	80	3896	1252	12	3983.153	1176.532	0.001	1.102	0.359	0.731	6008	5450	-1.721
Pollen	7	40	1310	1855	10	590.312	1621.575	0.36	0.623	-0.406	0.108	3394	5450	-1.729
Pollen	7	20	2043	1187	12	1277.085	1228.752	0.155	0.622	-0.154	0.234	3391	5450	-1.721
Pollen	7	10	3432	1528	12	3257.764	1434.03	0.017	1.05	0.145	0.598	5725	5450	-1.721
Pollen	8	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pollen	8	80	-3233	2437	7	-5068.17	2502.893	0.951	-0.003	-1.327	-0.665	-25	7617	-2.015
Pollen	8	40	-5360	5566	3	-2304.84	3426.556	0.688	2.538	-3.143	-0.303	19330	7617	-6.314
Pollen	8	20	-6352	2592	6	-5298.69	3324.068	0.907	0.235	-1.626	-0.696	1788	7617	-2.132
Pollen	8	10	-18858	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pollen	9	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pollen	9	80	-4339	3524	7	-6755.88	3762.322	0.934	0.076	-1.328	-0.626	825	10793	-2.015
Pollen	9	40	-2978	2066	3	-2037.76	2064.719	0.748	1.019	-1.397	-0.189	10998	10793	-6.314

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	Observed mean difference from control[2]	Standard error observed mean	n	Model estimate mean difference from control[3,4]	Standard error of estimated mean[4]	p_value for comparison with the control[4]	90% confidence upper limit[4]	90% confidence lower limit[4]	Estimated reduction from control (%)[4,5]	Estimated reduction from control (number)[4]	Observed means in control	T-test confidence limit
Pollen	9	20	-3011	2453	6	77.583	1658.125	0.482	0.335	-0.32	0.007	3612	10793	-2.132
Pollen	9	10	-11910	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Capped	3	160	-157	730	12	0	0	0	0	0	0	0	17294	-1.717
Capped	3	80	-1332	868	12	0	0	0	0	0	0	0	17294	-1.717
Capped	3	40	190	874	12	0	0	0	0	0	0	0	17294	-1.717
Capped	3	20	-1348	904	12	0	0	0	0	0	0	0	17294	-1.717
Capped	3	10	-1431	978	12	0	0	0	0	0	0	0	17294	-1.717
Capped	4	160	9007	1797	12	9089.959	1665.078	0	0.617	0.321	0.469	11955	19362	-1.721
Capped	4	80	5120	1770	12	5980.513	1592.068	0.001	0.45	0.167	0.309	8720	19362	-1.721
Capped	4	40	3266	1263	11	3161.897	1410.491	0.018	0.289	0.038	0.163	5595	19362	-1.725
Capped	4	20	852	547	12	1215.668	1112.801	0.144	0.162	-0.036	0.063	3131	19362	-1.721
Capped	4	10	174	849	12	390.583	1260.73	0.38	0.132	-0.092	0.02	2560	19362	-1.721
Capped	5	160	19726	886	12	19653.92	1278.542	0	1.108	0.885	0.996	21854	19726	-1.721
Capped	5	80	16484	1496	12	17301.22	1564.406	0	1.014	0.741	0.877	19993	19726	-1.721
Capped	5	40	9429	2607	11	9269.012	2196.511	0	0.662	0.278	0.47	13057	19726	-1.725
Capped	5	20	2076	994	12	2591.968	1395.063	0.039	0.253	0.01	0.131	4993	19726	-1.721
Capped	5	10	3532	2541	12	3930.858	2280.717	0.05	0.398	0	0.199	7855	19726	-1.721
Capped	6	160	19478	1271	12	19351.6	1908.239	0	1.141	0.81	0.975	22635	19842	-1.721
Capped	6	80	11885	1902	12	13436.24	2020.548	0	0.852	0.502	0.677	16913	19842	-1.721
Capped	6	40	6908	3851	10	6126.323	3155.141	0.034	0.584	0.034	0.309	11582	19842	-1.729
Capped	6	20	1017	1574	12	2215.388	2014.878	0.142	0.286	-0.063	0.112	5682	19842	-1.721
Capped	6	10	2308	1916	12	3332.882	2306.77	0.082	0.368	-0.032	0.168	7302	19842	-1.721
Capped	7	160	4061	1211	12	4102.683	1106.04	0.001	1.438	0.527	0.982	6006	4177	-1.721
Capped	7	80	273	1481	12	845.123	1306.775	0.262	0.741	-0.336	0.202	3094	4177	-1.721
Capped	7	40	1092	1641	10	913.244	1230.771	0.234	0.728	-0.291	0.219	3041	4177	-1.729
Capped	7	20	141	1264	12	448.692	1206.418	0.357	0.604	-0.39	0.107	2525	4177	-1.721

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	Observed mean difference from control[2]	Standard error observed mean	n	Model estimate mean difference from control[3,4]	Standard error of estimated mean[4]	p_value for comparison with the control[4]	90% confidence upper limit[4]	90% confidence lower limit[4]	Estimated reduction from control (%)[4,5]	Estimated reduction from control (number)[4]	Observed means in control	T-test confidence limit
Capped	7	10	1083	840	12	1554.033	920.202	0.053	0.751	-0.007	0.372	3137	4177	-1.721
Capped	8	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Capped	8	80	-3913	3384	7	-555.205	4282.752	0.549	1.379	-1.569	-0.095	8075	5856	-2.015
Capped	8	40	-7609	6002	3	-3432.66	48.886	0.995	-0.533	-0.639	-0.586	-3124	5856	-6.314
Capped	8	20	-6782	2295	6	-5455.82	2497.38	0.953	-0.023	-1.841	-0.932	-132	5856	-2.132
Capped	8	10	-1985	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Capped	9	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Capped	9	80	-2552	5953	7	7620.467	4910.237	0.091	1.211	-0.157	0.527	17515	14466	-2.015
Capped	9	40	-7212	3853	3	-4539.09	478.388	0.967	-0.105	-0.523	-0.314	-1519	14466	-6.314
Capped	9	20	-5326	2185	6	-5617.14	2729.671	0.946	0.014	-0.791	-0.388	202	14466	-2.132
Capped	9	10	12108	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Open	3	160	339	708	12	0	0	0	0	0	0	0	9801	-1.717
Open	3	80	-1497	1258	12	0	0	0	0	0	0	0	9801	-1.717
Open	3	40	505	602	12	0	0	0	0	0	0	0	9801	-1.717
Open	3	20	918	530	12	0	0	0	0	0	0	0	9801	-1.717
Open	3	10	-91	793	12	0	0	0	0	0	0	0	9801	-1.717
Open	4	160	7642	812	12	7723.364	715.022	0	1.017	0.738	0.878	8954	8800	-1.721
Open	4	80	811	996	12	1084.371	794.465	0.093	0.279	-0.032	0.123	2451	8800	-1.721
Open	4	40	1949	978	11	1882.119	823.751	0.017	0.375	0.052	0.214	3303	8800	-1.725
Open	4	20	1125	734	12	1077.189	691.449	0.067	0.258	-0.013	0.122	2267	8800	-1.721
Open	4	10	1307	936	12	1322.625	783.507	0.053	0.303	-0.003	0.15	2671	8800	-1.721
Open	5	160	9900	589	12	9725.764	1006.838	0	1.157	0.807	0.982	11458	9900	-1.721
Open	5	80	8097	923	12	8402.752	1204.924	0	1.058	0.639	0.849	10476	9900	-1.721
Open	5	40	2111	1848	11	1943.583	1622.327	0.122	0.479	-0.086	0.196	4742	9900	-1.725
Open	5	20	1480	1089	12	1063.085	1260.766	0.204	0.327	-0.112	0.107	3233	9900	-1.721
Open	5	10	207	1493	12	280.262	1449.109	0.424	0.28	-0.224	0.028	2774	9900	-1.721

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	Observed mean difference from control[2]	Standard error observed mean	n	Model estimate mean difference from control[3,4]	Standard error of estimated mean[4]	p_value for comparison with the control[4]	90% confidence upper limit[4]	90% confidence lower limit[4]	Estimated reduction from control (%)[4,5]	Estimated reduction from control (number)[4]	Observed means in control	T-test confidence limit
Open	6	160	8122	572	12	8294.56	1398.848	0	1.228	0.675	0.951	10702	8717	-1.721
Open	6	80	4218	947	12	4177.953	1558.807	0.007	0.787	0.172	0.479	6860	8717	-1.721
Open	6	40	1925	1979	10	1958.857	2088.029	0.18	0.639	-0.189	0.225	5569	8717	-1.729
Open	6	20	314	680	12	555.968	1473.638	0.355	0.355	-0.227	0.064	3092	8717	-1.721
Open	6	10	-480	952	12	-489.901	1528.829	0.624	0.246	-0.358	-0.056	2141	8717	-1.721
Open	7	160	2274	513	12	2279.459	729.205	0.003	1.532	0.444	0.988	3534	2308	-1.721
Open	7	80	1067	723	12	1274.387	825.177	0.069	1.168	-0.063	0.552	2694	2308	-1.721
Open	7	40	983	761	10	953.73	887.858	0.148	1.079	-0.252	0.413	2489	2308	-1.729
Open	7	20	852	655	12	937.724	801.426	0.128	1.004	-0.191	0.406	2317	2308	-1.721
Open	7	10	769	691	12	761.399	801.139	0.176	0.927	-0.267	0.33	2140	2308	-1.721
Open	8	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Open	8	80	-170	2340	7	2026.873	2522.734	0.229	1.365	-0.587	0.389	7110	5211	-2.015
Open	8	40	-5426	3617	3	-1805.65	3635.901	0.647	4.059	-4.752	-0.347	21151	5211	-6.314
Open	8	20	-4102	1098	6	-4157.35	1198.747	0.987	-0.307	-1.288	-0.798	-1602	5211	-2.132
Open	8	10	4367	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Open	9	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Open	9	80	-2297	3193	7	2146.953	2206.34	0.188	1.03	-0.359	0.335	6593	6402	-2.015
Open	9	40	-265	1610	3	-201.982	2976.105	0.522	2.904	-2.967	-0.032	18588	6402	-6.314
Open	9	20	-1621	1662	6	-1802.36	1607.44	0.838	0.254	-0.817	-0.282	1624	6402	-2.132
Open	9	10	13895	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Eggs	3	160	190	717	12	0	0	0	0	0	0	0	6393	-1.717
Eggs	3	80	538	892	12	0	0	0	0	0	0	0	6393	-1.717
Eggs	3	40	-753	841	12	0	0	0	0	0	0	0	6393	-1.717
Eggs	3	20	-41	602	12	0	0	0	0	0	0	0	6393	-1.717
Eggs	3	10	-422	976	12	0	0	0	0	0	0	0	6393	-1.717
Eggs	4	160	1489	972	12	1429.084	795.466	0.043	0.611	0.013	0.312	2798	4582	-1.721

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	Observed mean difference from control[2]	Standard error observed mean	n	Model estimate mean difference from control[3,4]	Standard error of estimated mean[4]	p_value for comparison with the control[4]	90% confidence upper limit[4]	90% confidence lower limit[4]	Estimated reduction from control (%)[4,5]	Estimated reduction from control (number)[4]	Observed means in control	T-test confidence limit
Eggs	4	80	1770	620	12	1661.358	573.629	0.004	0.578	0.147	0.363	2648	4582	-1.721
Eggs	4	40	2445	525	11	2447.167	556.634	0	0.744	0.325	0.534	3407	4582	-1.725
Eggs	4	20	1241	786	12	1242.122	677.598	0.04	0.526	0.017	0.271	2408	4582	-1.721
Eggs	4	10	1406	646	12	1380.358	600.909	0.016	0.527	0.076	0.301	2414	4582	-1.721
Eggs	5	160	3755	804	12	3674.178	726.848	0	1.22	0.6	0.91	4925	4036	-1.721
Eggs	5	80	2796	659	12	2811.043	662.938	0	0.979	0.414	0.696	3952	4036	-1.721
Eggs	5	40	1660	931	11	1681.656	826.62	0.028	0.77	0.063	0.417	3107	4036	-1.725
Eggs	5	20	1538	550	12	1507.223	592.724	0.009	0.626	0.121	0.373	2527	4036	-1.721
Eggs	5	10	1340	669	12	1416.205	631.272	0.018	0.62	0.082	0.351	2502	4036	-1.721
Eggs	6	160	2614	575	12	2516.76	671.964	0.001	1.175	0.435	0.805	3673	3126	-1.721
Eggs	6	80	1588	469	12	1546.563	668.294	0.015	0.863	0.127	0.495	2697	3126	-1.721
Eggs	6	40	635	829	10	932.125	903.77	0.158	0.798	-0.202	0.298	2495	3126	-1.729
Eggs	6	20	546	406	12	488.995	614.952	0.218	0.495	-0.182	0.156	1547	3126	-1.721
Eggs	6	10	182	697	12	202.448	771.323	0.398	0.489	-0.36	0.065	1530	3126	-1.721
Eggs	7	160	976	312	12	963.467	354.762	0.006	1.535	0.344	0.939	1574	1026	-1.721
Eggs	7	80	728	259	12	729.072	336.527	0.021	1.276	0.146	0.711	1308	1026	-1.721
Eggs	7	40	218	299	10	428.311	367.232	0.129	1.037	-0.202	0.418	1063	1026	-1.729
Eggs	7	20	265	313	12	261.589	355.17	0.235	0.851	-0.341	0.255	873	1026	-1.721
Eggs	7	10	0	353	12	17.235	378.245	0.482	0.651	-0.618	0.017	668	1026	-1.721
Eggs	8	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Eggs	8	80	425	988	7	558.045	1089.023	0.315	1.233	-0.733	0.25	2752	2233	-2.015
Eggs	8	40	-1456	919	3	-461.442	644.484	0.698	1.616	-2.029	-0.207	3608	2233	-6.314
Eggs	8	20	-1654	509	6	-1729.75	345.134	0.996	-0.445	-1.104	-0.775	-994	2233	-2.132
Eggs	8	10	0	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Eggs	9	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
Eggs	9	80	284	740	7	316.162	833.592	0.36	1.087	-0.743	0.172	1996	1836	-2.015

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Parameter	Time (CCA)[1]	Test conc. (µg/l)	Observed mean difference from control[2]	Standard error observed mean	n	Model estimate mean difference from control[3,4]	Standard error of estimated mean[4]	p_value for comparison with the control[4]	90% confidence upper limit[4]	90% confidence lower limit[4]	Estimated reduction from control (%)[4,5]	Estimated reduction from control (number)[4]	Observed means in control	T-test confidence limit
Eggs	9	40	397	1050	3	-482.609	1346.899	0.61	4.369	-4.894	-0.263	8021	1836	-6.314
Eggs	9	20	959	790	6	1069.694	586.629	0.071	1.264	-0.099	0.583	2320	1836	-2.132
Eggs	9	10	2580	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
TotalLife	3	160	-272	1957	12	0	0	0	0	0	0	0	48503	-1.717
TotalLife	3	80	-3791	1920	12	0	0	0	0	0	0	0	48503	-1.717
TotalLife	3	40	-365	2729	12	0	0	0	0	0	0	0	48503	-1.717
TotalLife	3	20	-567	2619	12	0	0	0	0	0	0	0	48503	-1.717
TotalLife	3	10	-1279	3075	12	0	0	0	0	0	0	0	48503	-1.717
TotalLife	4	160	23524	3038	12	23503.64	2987.178	0	0.572	0.367	0.469	28644	50084	-1.721
TotalLife	4	80	9341	3109	12	11199.39	2704.951	0	0.317	0.131	0.224	15854	50084	-1.721
TotalLife	4	40	7597	2447	11	7599.152	2884.741	0.008	0.251	0.052	0.152	12575	50084	-1.725
TotalLife	4	20	3479	1395	12	3345.871	2225.651	0.074	0.143	-0.01	0.067	7176	50084	-1.721
TotalLife	4	10	2897	2634	12	3041.294	2823.896	0.147	0.158	-0.036	0.061	7900	50084	-1.721
TotalLife	5	160	46712	2502	12	46430.78	3249.602	0	0.982	0.771	0.877	52023	52973	-1.721
TotalLife	5	80	34592	2824	12	37199.69	3451.789	0	0.814	0.59	0.702	43139	52973	-1.721
TotalLife	5	40	17777	6001	11	17789.99	5455.377	0.002	0.513	0.158	0.336	27199	52973	-1.725
TotalLife	5	20	5455	2440	12	5324.279	3631.571	0.079	0.218	-0.017	0.101	11573	52973	-1.721
TotalLife	5	10	6908	4323	12	7674.205	4250.837	0.043	0.283	0.007	0.145	14989	52973	-1.721
TotalLife	6	160	46396	2367	12	46099.07	4247.171	0	1.066	0.774	0.92	53407	50113	-1.721
TotalLife	6	80	28196	3656	12	32832.62	4383.394	0	0.806	0.505	0.655	40375	50113	-1.721
TotalLife	6	40	15255	8144	10	15272.1	7556.661	0.029	0.565	0.044	0.305	28339	50113	-1.729
TotalLife	6	20	5088	3185	12	4992.65	4951.257	0.162	0.27	-0.07	0.1	13512	50113	-1.721
TotalLife	6	10	6208	3272	12	7047.944	4953.287	0.085	0.311	-0.029	0.141	15571	50113	-1.721
TotalLife	7	160	19590	1983	12	19560.29	2938.276	0	1.215	0.716	0.965	24616	20267	-1.721
TotalLife	7	80	8226	2257	12	10750.1	2990.428	0.001	0.784	0.277	0.53	15896	20267	-1.721
TotalLife	7	40	4277	4304	10	4546.944	3947.73	0.132	0.561	-0.112	0.224	11373	20267	-1.729

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	Observed mean difference from control[2]	Standard error observed mean	n	Model estimate mean difference from control[3,4]	Standard error of estimated mean[4]	p_value for comparison with the control[4]	90% confidence upper limit[4]	90% confidence lower limit[4]	Estimated reduction from control (%)[4,5]	Estimated reduction from control (number)[4]	Observed means in control	T-test confidence limit
TotalLife	7	20	2191	1961	12	2241.1	3020.135	0.233	0.367	-0.146	0.111	7438	20267	-1.721
TotalLife	7	10	4397	2452	12	4802.467	3065.589	0.066	0.497	-0.023	0.237	10078	20267	-1.721
TotalLife	8	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
TotalLife	8	80	-5910	8862	7	5535.519	11348.39	0.323	1.488	-0.908	0.29	28403	19090	-2.015
TotalLife	8	40	-18537	13728	3	-21082.6	27248.42	0.71	7.908	-10.116	-1.104	150957	19090	-6.314
TotalLife	8	20	-16616	4430	6	-16421	5116.892	0.984	-0.289	-1.432	-0.86	-5513	19090	-2.132
TotalLife	8	10	5402	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
TotalLife	9	160	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
TotalLife	9	80	-5480	13625	7	20926.94	12019.68	0.071	1.208	-0.088	0.56	45147	37388	-2.015
TotalLife	9	40	-8187	10550	3	-219.332	17872.92	0.504	3.012	-3.024	-0.006	112626	37388	-6.314
TotalLife	9	20	-10498	5444	6	-11621.1	5925.836	0.939	0.027	-0.649	-0.311	1012	37388	-2.132
TotalLife	9	10	52382	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA

Notes:

1. Colony Condition Assessment (CCA) Observation dates.
2. Mean of observations in controls minus the observation in the treatment.
3. Difference between the mean of observation in controls and estimated number in treatment after adjustment for covariance for CCA3 to be a 0 baseline.
4. 'NA' indicates there was not enough data to do the test (n = number of Apiaries is small) and '0' means rounded to 0 (except for CCA3 where p_values are constrained to be 0 as the exposure has not occurred.)
5. The percentage of the estimated difference between the treatment and control divided by the number in the control. [Value in column must be multiplied by 100 to be a %]

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Results

The following tables and graphs present results for individual measurement endpoints (total individuals, adults, eggs, larvae, pupae, pollen stores, honey stores). The percent reductions are the means of the differences between each treatment and control at the same apiary, based on observations and expected values estimated by the statistical model that adjusted baseline measurement for CCA3, using raw count data.

Control Trends

For a comparison between the numbers of live bees in the three different neonicotinoid colony feeding studies (clothianidin, imidacloprid, thiamethoxam), refer to the thiamethoxam statistical analysis.

Colony Condition Assessments – Life stages in the hive

Total number of individuals (total life)

Table A-2. and **Figure A-1.** show the estimated effects on total number of individual bees (total life) across CCAs and treatment groups. Compared to the control, a significant reduction ($p<0.05$) in total life was observed at the three highest treatment groups (40, 80 and 160 µg/L) at CCAs 4-6 (15.2-33.6%, 22.4-70.2% and 46.9-92.0% reduction at 40, 80 and 160 µg/L, respectively) and in the two highest treatment groups at CCA7 (53% reduction at 80 µg/L, p=0.001 and 96.5% reduction at 160 µg/L, $p<<0.05$). A significant reduction in total life was also observed at CCA4 for the 20 µg/L treatment group (6.7% reduction, p=0.074) and CCA5 for the 10 µg/L (14.5% reduction, p=0.043) and 20 µg/L (10.1% reduction, p=0.079) treatment groups. While significant effects were observed at CCA4 and/or CCA5 at the lowest two treatment groups, no significant effects ($p>0.1$) were observed at subsequent CCAs (CCAs 6-7) indicating a potential transient effect on total life at 10 and 20 µg/L.

Figure A-1. shows a general dose responsiveness (increase in the reduction from the control as the dose increases) at the three highest treatment groups (40, 80 and 160 µg/L) over all CCAs. For the two lowest treatment groups, the reduction in total individuals in the 10 µg/L treatment group was consistently higher than or equivalent to the 20 µg/L treatment group over all CCAs (6.1-23.7% and 6.7-11.1% reduction in the 10 and 20 µg/L treatment groups, respectively) and in particular, CCA7. The overlap in dose-response at the lower doses is not unexpected since the dose levels are similar and measured exposures indicate overlap in exposure among individual hives, particularly at the lower two doses. In general, the width of the confidence intervals increased with increasing CCAs. As seen in **Figure A-2.** the total number of individuals at CCA6 was reduced from the control at 58% (7/12), 58% (7/12), 60% (6/10), 100% (12/12) and 100% (12/12) of the apiaries in the 10, 20 , 40, 80 and 160 µg /L dose groups, respectively.

When weighing statistical and biological significance, the overall NOAEC and LOAEC for total life is determined to be 20 and 40 µg/L, respectively. This is based on significant and persistent reductions in total life throughout the study from CCA4-CCA7 at the highest three treatment groups (40, 80 and 160 µg/L) and potential transient effects at ≤ 20 µg/L at CCAs 4-5.

Table A-2. Estimated percent reduction from control for total number of individuals.

Test concentration (µg/L)	Estimated reduction from control (%) (p value)					
	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9
10	6.1 (0.147)	14.5 (0.043**/0.079*)	14.1 (0.085*/0.162)	23.7 (0.066*/0.233)	NA	NA
20	6.7 (0.074*)	10.1 (0.079*)	10.0 (0.162)	11.1 (0.233)	-86.0 (0.984)	-31.1 (0.939)
40	15.2 (0.008**)	33.6 (0.002**)	30.5 (0.029**)	22.4 (0.132)	-110 (0.71)	-0.6 (0.504)
80	22.4 (0**)	70.2 (0**)	65.5 (0**)	53.0 (0.001**)	29.0 (0.323)	56.0 (0.071*)
160	46.9 (0**)	87.7 (0**)	92.0 (0**)	96.5 (0**)	NA	NA

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<p<0.1, **p<0.05
NA indicates there was not enough data to do the test (n = number of Apiaries is small) and '0' means rounded to 0
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level.
At CCA8 and CCA9, the step-down approach was not applied to the 160 or 10 treatment levels where very few hives survived.

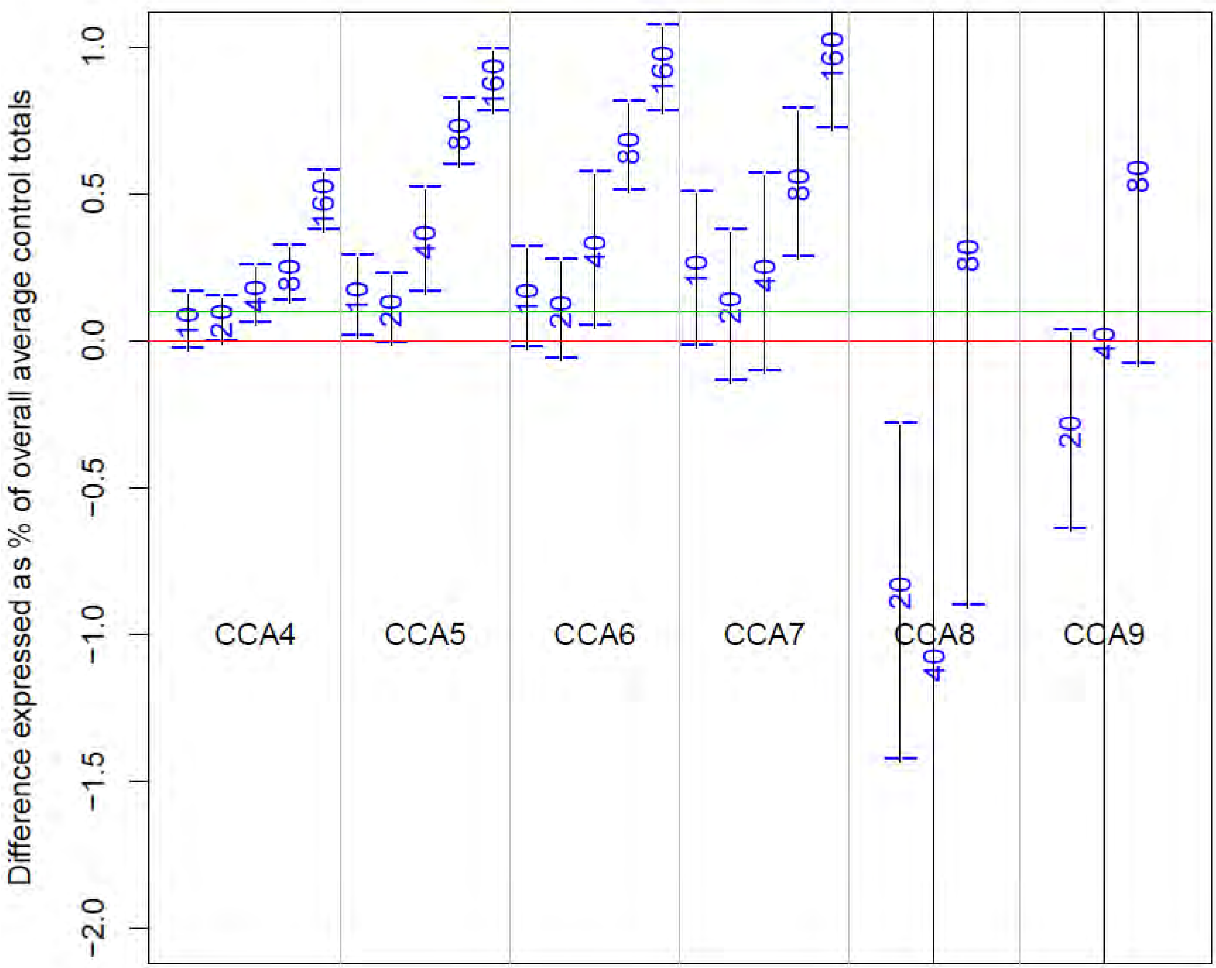


Figure A-1. Estimates and 90% CIs for Total Life with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

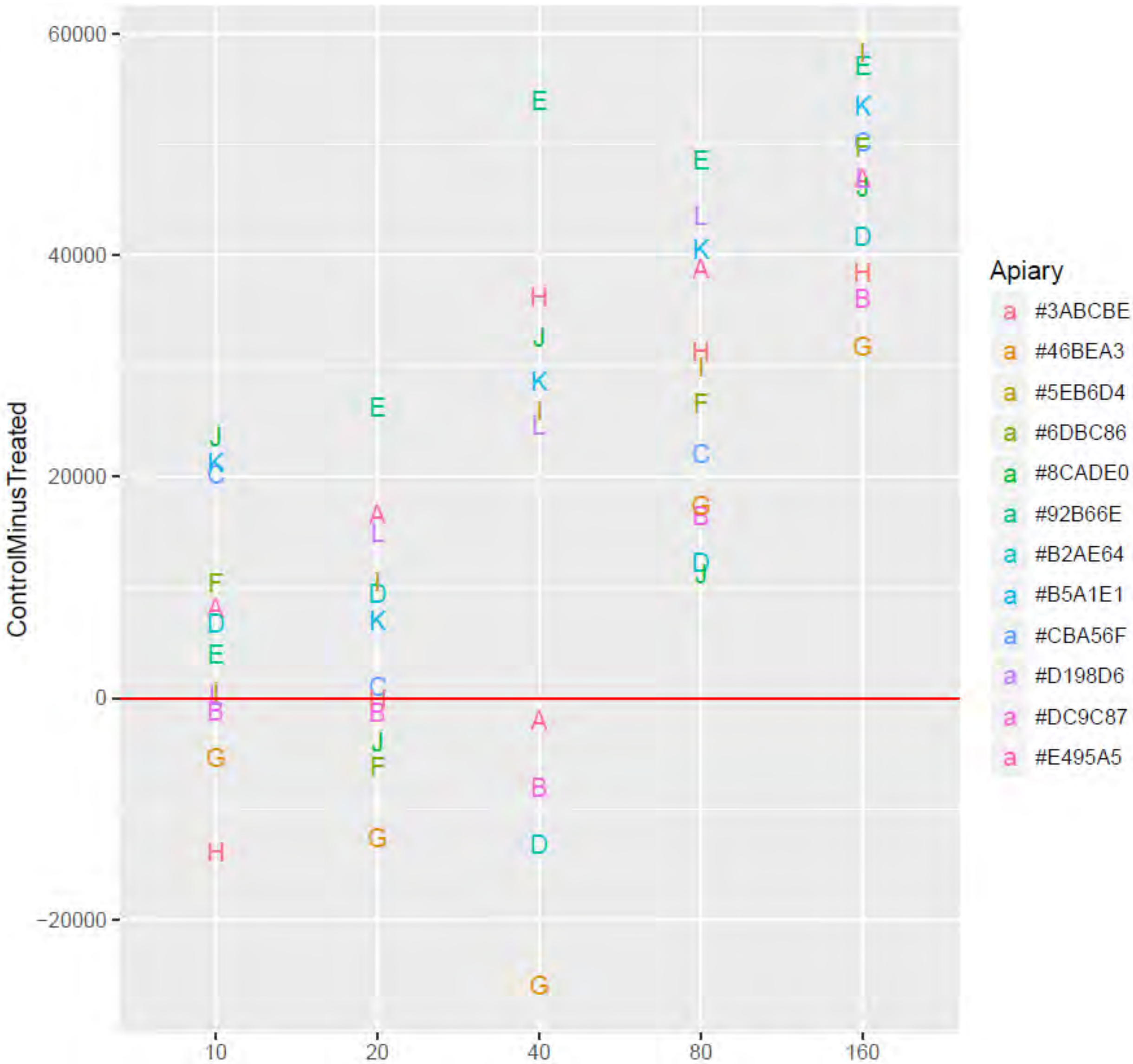


Figure A-2. Difference from control for all treatments and apiaries at CCA6 for total life. Apiaries shown above the zero line had better control outcomes in comparison to the treatment.

Adults

Table A-3 and **Figure A-3** show the effects on adult honey bees across CCAs and treatment groups. Compared with the control group, a significant reduction ($p<0.05$) in the number of adults was observed at the two highest treatment groups (80 and 160 µg/L) starting at CCA4 and persisting over multiple CCAs from CCAs 4-7 (13.9-55.7% and 32.3-98.4% reduction at 80 and 160 µg/L, respectively). The number of adults in the 40 µg/L treatment group was also significantly reduced starting at CCA5 (23.1% reduction, $p=0.011$) and persisting through to CCA6 (29.9% reduction, $p=0.012$), but was not significantly reduced ($p>0.1$) at CCAs 4 and 7 (reductions of -1.3 and 13.2%, respectively). No significant reduction ($p>0.1$) in the number of adults was observed in the two lowest treatment groups (10 and 20 µg/L) from CCA4 through to CCA7, except at CCA6 where the number of adults was significantly reduced from the control by 19.6% (p=0.014) in the 10 µg/L treatment group and 17% (p=0.037) in the 20 µg/L treatment group.

Figure A-3 shows a general dose response (increase in the reduction from the control as the dose increases) at the three highest treatment groups (40, 80 and 160 µg/L) over all CCAs. For the two lowest treatment groups, the reduction in the number of adults from the control in the 10 µg/L treatment group was consistently higher than or equivalent to the 20 µg/L treatment group over all CCAs although generally similar (-2.0-19.6% and 0.6-17.0% reduction in the 10 and 20 µg/L treatment groups, respectively). The overlap in dose-response at the lower doses is not unexpected since the dose levels are similar and measured exposures indicate overlap in exposure among individual hives, particularly at the lower two doses. In general, the width of the confidence intervals increased with increasing CCAs. As seen in **Figure A-4** the number of adults at CCA6 was reduced from the control in 75% (9/12), 75% (7/12), 60% (6/10), 100% (12/12) and 100% (12/12) of the apiaries in the 10, 20, 40, 80 and 160 µg /L dose groups, respectively.

Significant reductions in the number of adults were found at the highest two treatment levels (80 and 160 µg/L) that were apparent from the beginning of exposure (CCA4) and persisted through to after exposure (CCA7) when populations were in natural decline. Additionally, persistent significant effects were noted at 40 µg/L over multiple CCAs after exposure. While treatment means were significantly reduced from the control at the lowest two doses (10 and 20 µg/L) at a single CCA (CCA6), these effects were considered to be isolated and potentially transient. This finding supports the use of 20 µg/L as the NOAEC as determined through the primary analysis for total life at CCA6. The early onset and persistence of significant effects during and/or after the end of exposure at the highest treatment groups (40, 80 and 160 µg/L) supports the use of 40 µg/L as the LOAEC.

Table A-3. Estimated percent reduction from control for number of adults.

Test concentration (µg/L)	Estimated reduction from control (%) (p value)					
	CCA4	**CCA5**	**CCA6**	**CCA7**	**CCA8**	**CCA9**
10	-2.0 (0.602)	7.7 (0.228)	19.6 (0.014**)	14.8 (0.147)	NA	NA
20	0.6 (0.465)	1.6 (0.425)	17.0 (0.037**)	7.3 (0.31)	-70.5 (0.967)	-29.2 (0.883)
40	-1.3 (0.571)	23.1 (0.011**)	29.9 (0.012**)	13.2 (0.244)	-54.9 (0.664)	18.1 (0.395)
80	13.9 (0.029**)	41.2 (0**)	64.1 (0**)	55.7 (0**)	-42.7 (0.74)	-8.3 (0.584)
160	32.3 (0**)	69.9 (0**)	89.7 (0**)	98.4 (0**)	NA	NA

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<p<0.1, **p<0.05
NA' indicates there was not enough data to do the test (n = number of Apiaries is small) and '0' means rounded to 0
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level.
At CCA8 and CCA9, the step-down approach was not applied to the 160 or 10 treatment levels where very few hives survived.

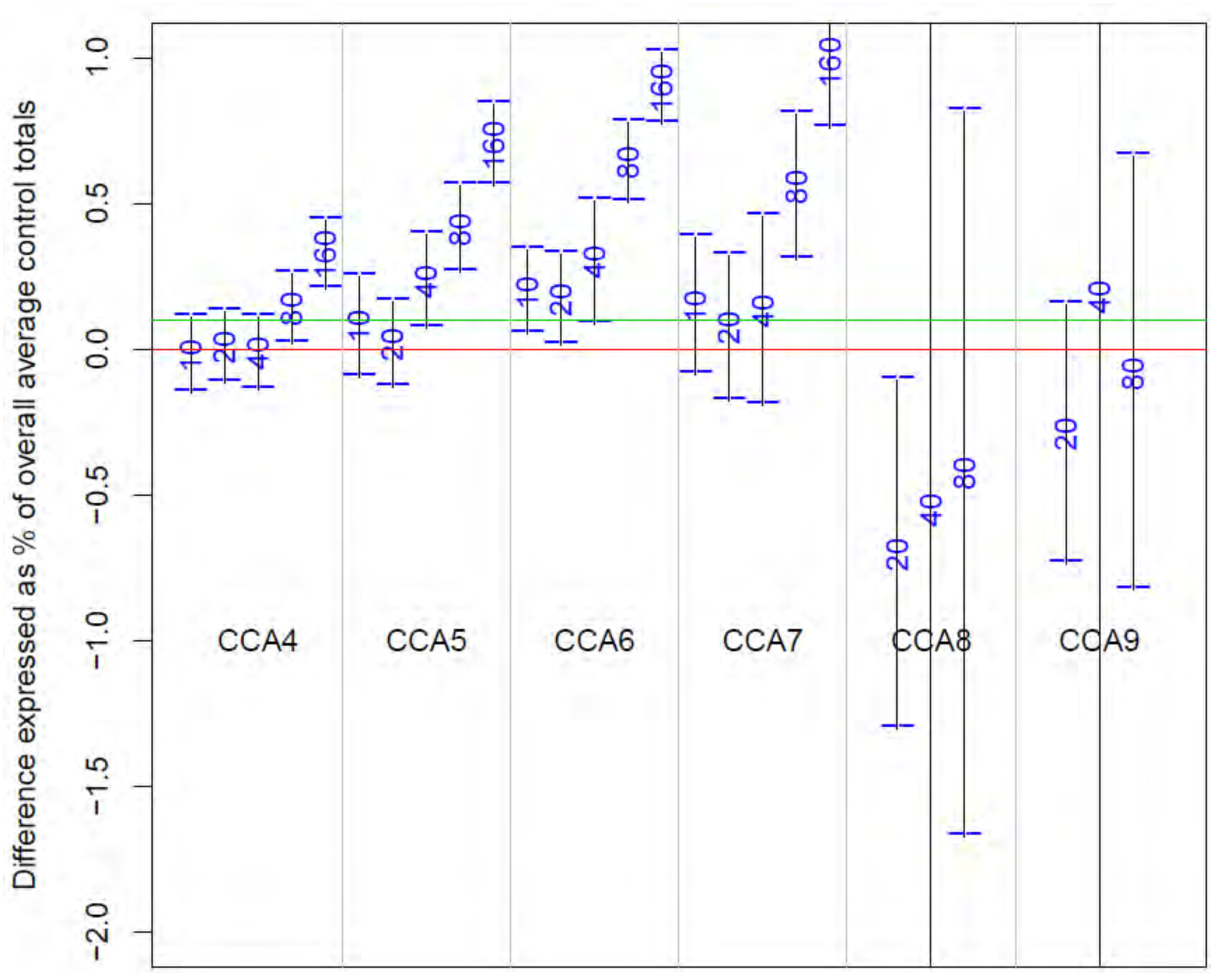


Figure A-3. Estimates and 90% CIs for adults with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

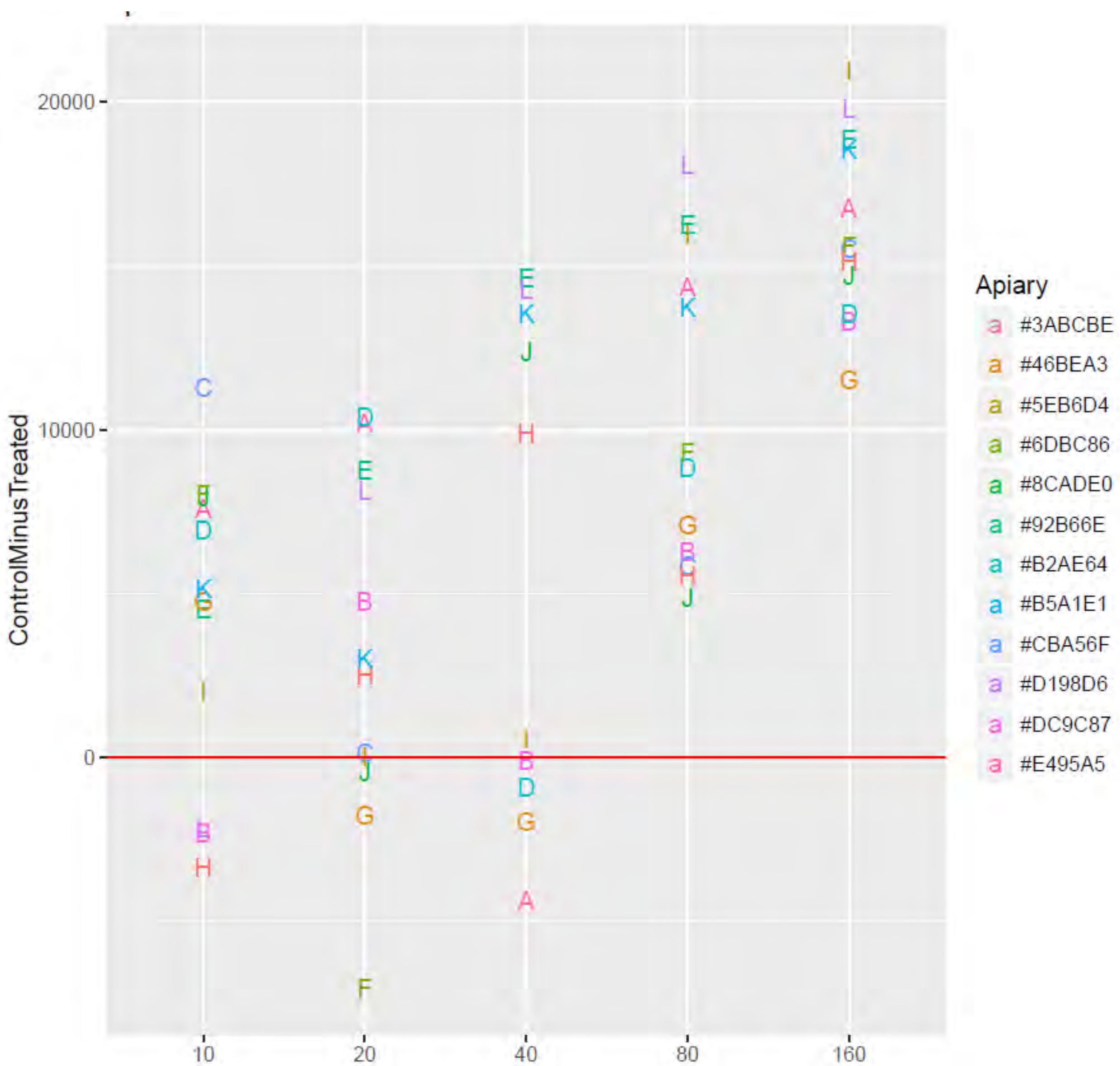


Figure A-4. Difference from control for all treatments and apiaries at CCA6 for adults. Apiaries shown above the zero line had better control outcomes in comparison to the treatment.

Eggs

Table A-4 and **Figure A-5** show the effects on the number of honey bee eggs across CCAs and treatment groups. A significant reduction ($p<0.05$) in the number of eggs relative to the control group was observed for all treatment groups (10, 20, 40, 80 and 160 µg/L) during CCA4 (27.1-53.4% reduction) and CCA5 (35.1-91.0% reduction). The number of eggs was also significantly reduced ($p<0.05$) from the control during subsequent CCAs (6 and 7) at the two highest treatment groups (49.5-93.9% reduction) but not at the three lowest treatment groups (1.7-41.8% reduction).

Figure A-5 shows a general dose response (increase in the reduction from the control as the dose increases) starting at CCA5 which becomes more pronounced over subsequent CCAs up to CCA7. There was no clear dose response observed at CCA4. In general, the width of the confidence intervals increased with increasing CCAs. As seen in **Figure A-6**, egg cells at CCA6 were reduced from the control in 42% (5/12), 67% (8/12), 60% (6/10), 92% (11/12) and 92% (11/12) apiaries in the 10, 20, 40, 80 and 160 µg /L dose groups, respectively.

Treatment means were significantly reduced from the control at the lowest three doses (10, 20 and 40 µg/L) during and immediately after the exposure period (CCA4 and CCA5) but not in subsequent CCAs after the exposure period and were significantly reduced from the control at the two highest treatment groups (80 and 160 µg/L) at all CCAs (CCA4-CCA7). While the width of the confidence intervals increased with increasing CCAs, the observed reversion back to control levels at the three lowest treatment groups during subsequent CCAs indicates a potential transient effect on eggs and supports the use of 40 µg/L as the NOAEC and 80 µg/L as the LOAEC which are less sensitive than the endpoints determined through the primary analysis for total life at CCA6 (NOAEC of 20 µg/L, LOAEC of 40 µg/L).

Table A-4. Estimated percent reduction from control for number of eggs.

Test concentration (µg/L)	Estimated reduction from control (%) (P value)					
	CCA4	**CCA5**	**CCA6**	**CCA7**	**CCA8**	**CCA9**
10	30.1 (0.016**)	35.1 (0.018**)	6.5 (0.398)	1.7 (0.482)	NA	NA
20	27.1 (0.04**)	37.3 (0.009**)	15.6 (0.218)	25.5 (0.235)	-77.5 (0.996)	58.3 (0.071*)
40	53.4 (0**)	41.7 (0.028**)	29.8 (0.158)	41.8 (0.129)	-20.7 (0.698)	-26.3 (0.61)
80	36.3 (0.004**)	69.6 (0**)	49.5 (0.015**)	71.1 (0.021**)	25.0 (0.315)	17.2 (0.36)
160	31.2 (0.043**)	91.0 (0**)	80.5 (0.001**)	93.9 (0.006**)	NA	NA

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<P<0.1, **P<0.05
NA' indicates there was not enough data to do the test (n = number of Apiaries is small) and '0' means rounded to 0
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level.
At CCA8 and CCA9, the step-down approach was not applied to the 160 or 10 treatment levels where very few hives survived.

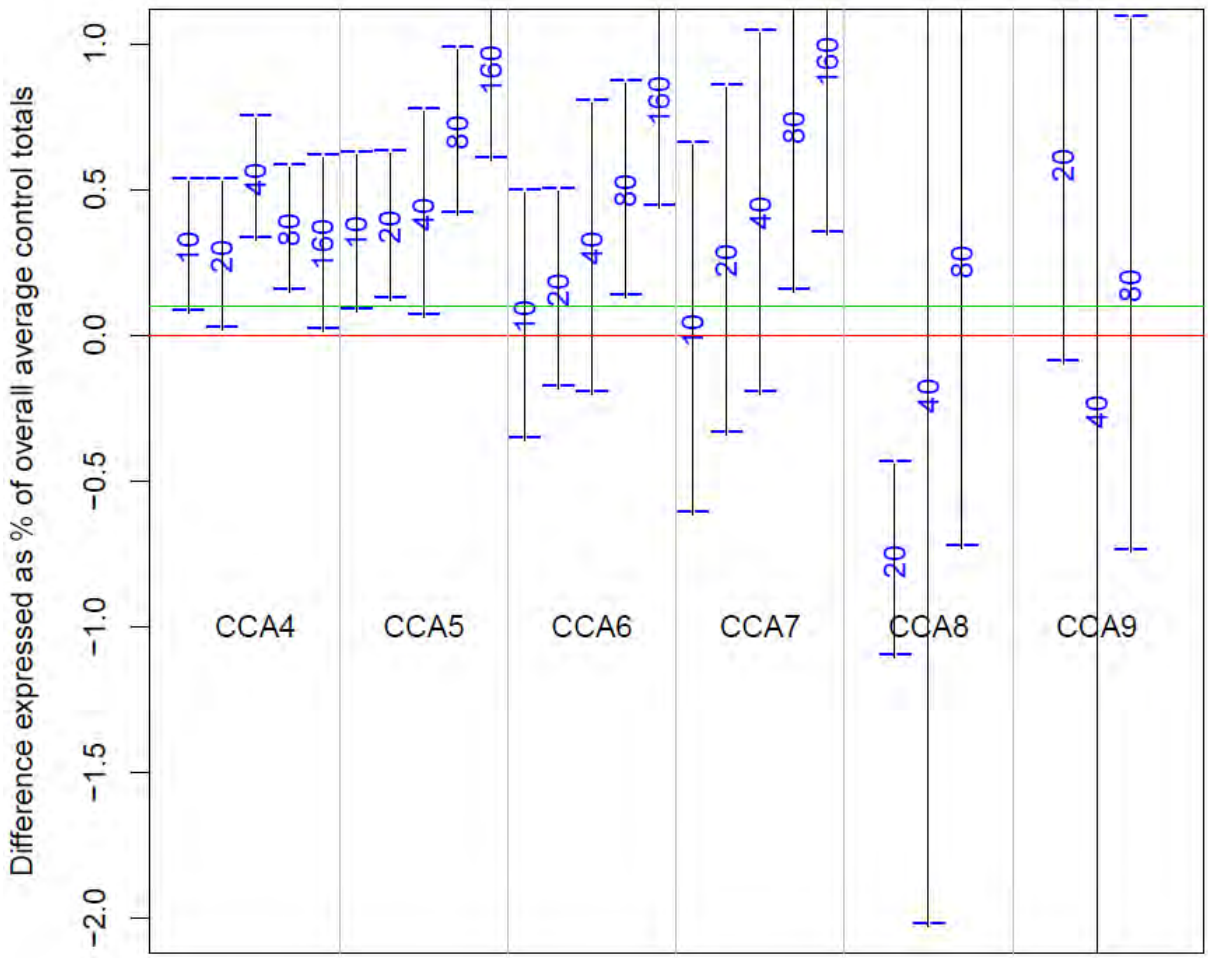


Figure A-5. Estimates and 90% CIs for eggs with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

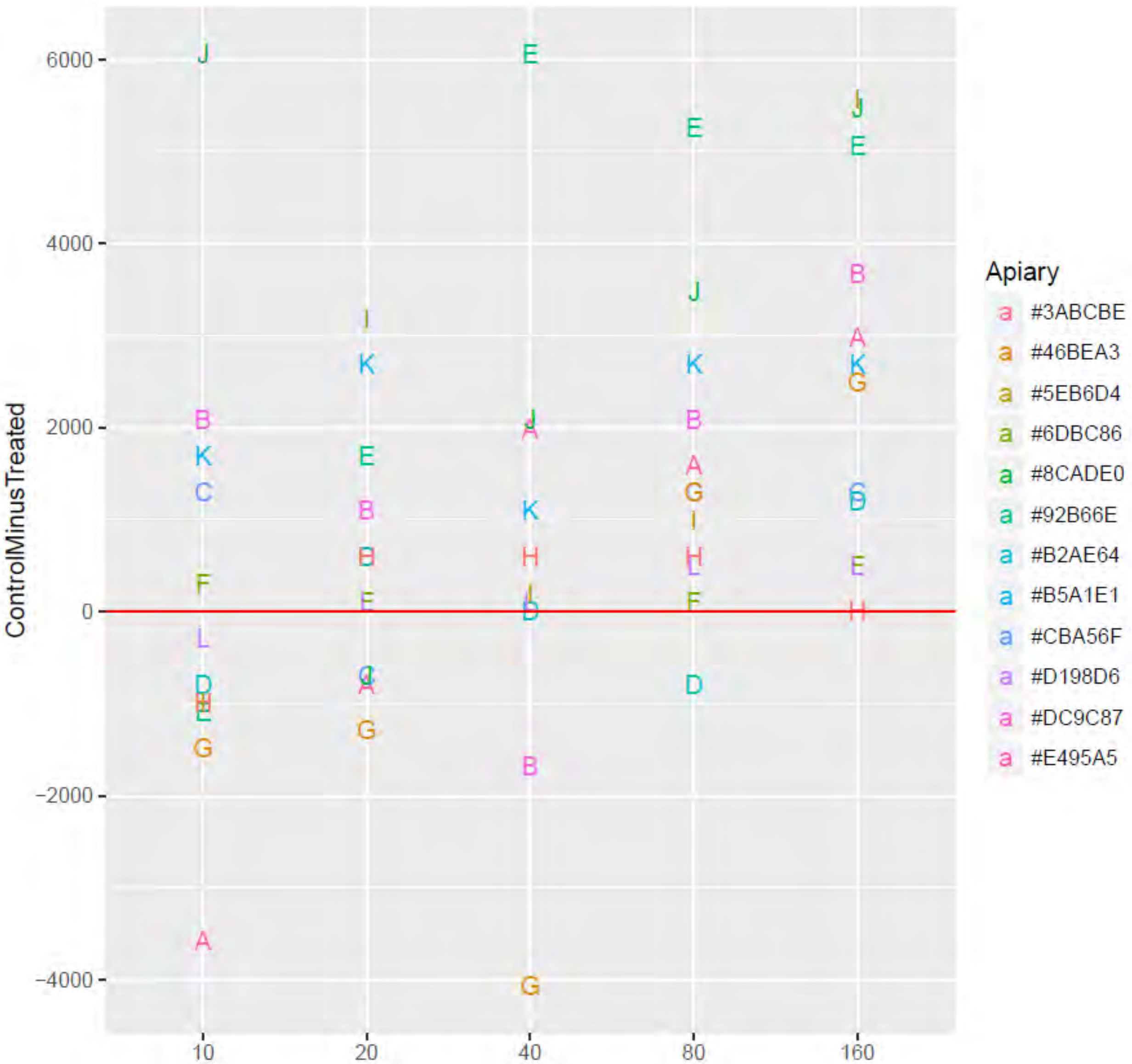


Figure A-6. Difference from control for all treatments and apiaries at CCA6 for eggs. Apiaries shown above the zero line had better control outcomes in comparison to the treatment.

<u>Larvae (Open/uncapped brood)</u>

Table A-5 and **Figure A-7** below show the effects on larvae (open/uncapped brood) across CCAs and treatment groups. Compared with the control group, a significant reduction ($p<0.05$) in the number of larvae was observed at the highest treatment group (160 µg/L) which persisted over multiple CCAs from CCA4 to CCA7 (87.8-98.8% reduction). The number of larvae was also significantly reduced from the control in the 80 µg/L treatment group over multiple CCAs including CCA4 (12.3% reduction, $p=0.093$), CCAs 5-6 (47.9-84.9% reduction, $p<<0.05$) and CCA7 (55.2% reduction, $p=0.069$). No significant reduction ($p>0.1$) in the number of larvae was observed at the lowest three treatment groups (10, 20 and 40 µg/L) during any of the CCAs with the exception of CCA4, where the number of larvae was significantly reduced from the control by

15.0% (p=0.053), 12.2% (p=0.067) and 21.4% (p=0.017) at 10, 20 and 40 µg/L treatment groups, respectively. The effects were observed during a single CCA indicating a potential transient effect at the lowest three doses for this endpoint.

Figure A-7 shows a clear dose response (increase in the reduction from the control as the dose increases) over all CCAs except at CCA4. In general, the width of the confidence intervals increased with increasing CCAs. As seen in **Figure A-8**, larval cells at CCA6 were reduced from the control at 33% (4/12), 75% (7/12), 70% (7/10), 83% (10/12) and 100% (12/12) of apiaries in the 10, 20, 40, 80 and 160 µg /L dose groups, respectively.

Significant reductions in the number of larvae were found at the highest two treatment levels (80 and 160 µg/L) that were apparent from the beginning of exposure (CCA4) and persisted through to after exposure (CCA7) when populations were in natural decline. Treatment means were significantly reduced from the control at the lowest three doses (10, 20 and 40 µg/L) during a single CCA during the exposure period (CCA4) but not in subsequent CCAs. While the width of the confidence intervals increased with increasing CCAs, the observed reversion back to control levels at the three lowest treatment groups during subsequent CCAs indicates a potential transient effect on larvae at these doses and supports the use of 40 µg/L as the NOAEC and 80 µg/L as the LOAEC. These endpoints are less sensitive than the endpoints determined through the primary analysis for total life at CCA6 (NOAEC of 20 µg/L, LOAEC of 40 µg/L).

Table A-5. Estimated percent reduction from control for number of larvae.

Test concentration (µg/L)	**Estimated reduction from control (%) (P value)**					
	CCA4	**CCA5**	**CCA6**	**CCA7**	**CCA8**	**CCA9**
10	15.0 (0.053*)	2.80 (0.424)	-5.6 (0.624)	33.0 (0.176)	NA	NA
20	12.2 (0.067*)	10.7 (0.204)	6.4 (0.355)	40.6 (0.128)	-79.8 (0.987)	-28.2 (0.838)
40	21.4 (0.017**/0.093*)	19.6 (0.122)	22.5 (0.18)	41.3 (0.148)	-34.7 (0.647)	-3.2 (0.522)
80	12.3 (0.093*)	84.9 (0**)	47.9 (0.007**)	55.2 (0.069*)	38.9 (0.229)	33.5 (0.229)
160	87.8 (0**)	98.2 (0**)	95.1 (0**)	98.8 (0**)	NA	NA

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<P<0.1, **P<0.05
NA' indicates there was not enough data to do the test (n = number of Apiaries is small) and '0' means rounded to 0
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level.
At CCA8 and CCA9, the step-down approach was not applied to the 160 or 10 treatment levels where very few hives survived.

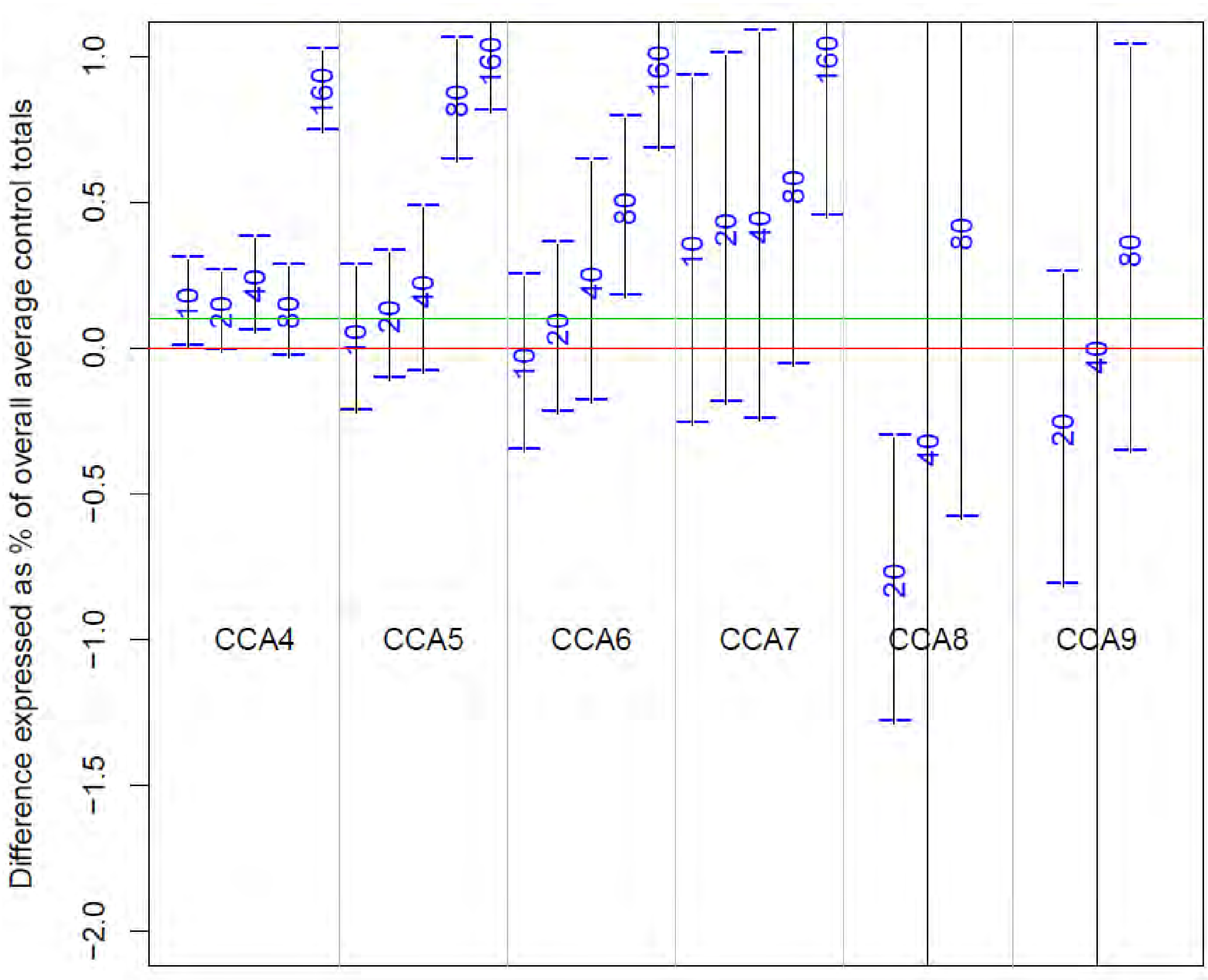


Figure A-7. Estimates and 90% CIs for larvae with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

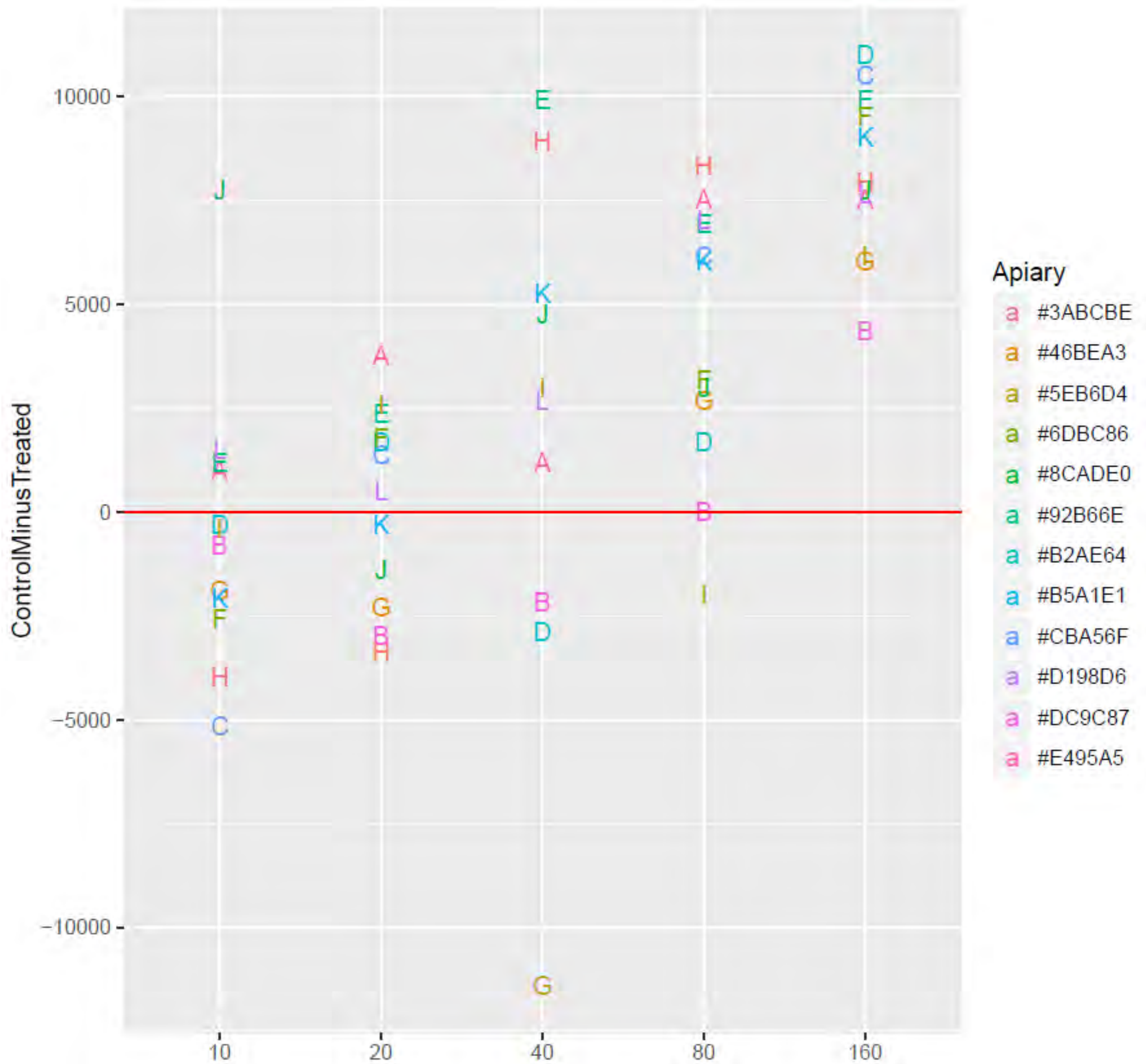


Figure A-8. Difference from control for all treatments and apiaries at CCA6 for larvae. Apiaries shown above the zero line had better control outcomes in comparison to the treatment.

Pupae (Capped brood)

Table A-6 and **Figure A-9** below show the effects on pupae (capped brood) across CCAs and treatment groups. Compared to the control, a significant reduction ($p<0.05$) in the number of pupal cells was observed at the three highest treatment groups (40, 80 and 160 µg/L) which persisted over multiple CCAs (16.3—47.0%, 30.9—87.7% and 46.9—99.6% reduction in the 40, 80 and 160 µg/L treatment groups, respectively during CCAs 4-6 and 98.2% reduction at CCA7 in the 160 µg/L treatment group). No significant reduction ($p>0.1$) in the number of pupae was observed at the lowest two treatment groups (10 and 20 µg/L) during any of the CCAs with the exception of CCA5 where the number of pupae was significantly reduced by 13.1% in the 20 µg/L treatment group (p=0.039) and by 19.9% in the 10 µg/L treatment group (p=0.05). As the effect was only observed during a single CCA immediately after the exposure period a potential transient effect at the lowest two doses is indicated.

Figure A-9 shows a clear dose response (increase in the reduction from the control as the dose increases), at the first CCA during exposure (CCA4). In CCA5 and CCA6, the 10 and 20 µg/L treatment groups showed about the same level of effect with the 10 µg/L treatment group overlapping the 20 µg/L treatment group (16.8-19.9% and 11.2-13.2% reduction from the control in the 10 and 20 µg/L treatment groups, respectively). The overlap in dose-response at the lower doses is not unexpected since the dose levels are similar and measured exposures indicate overlap in exposure among individual hives, particularly at the lower two doses. No clear dose response was observed at CCA7 for all doses when all hives were in decline. In general, the width of the confidence intervals increased with increasing CCAs. As seen in **Figure A-10**, pupal cells at CCA6 were reduced from the control in 75% (7/12), 42% (5/12), 60% (6/10), 92% (11/12) and 100% (12/12) of apiaries in the 10, 20, 40, 80 and 160 µg /L dose groups, respectively.

Significant reductions in the number of pupae were found at the highest three treatment levels (40, 80 and 160 µg/L) that were apparent from the beginning of exposure (CCA4) and persisted throughout the study until hives would begin to naturally decline before overwintering (CCA7). While treatment means were significantly reduced from the control at the lowest two doses (10 and 20 µg/L) at a single CCA immediately after exposure (CCA5), these effects were considered transient. This is because effects at 10 and 20 µg/L were isolated to CCA5 with levels returning to those similar to the control in subsequent CCAs. This finding supports the use of 20 µg/L as the NOAEC as determined through the primary analysis for total life at CCA6. The early onset and persistence of significant effects at the highest three treatment groups (40, 80 and 160 µg/L) supports the use of 40 µg/L as the LOAEC.

Table A-6. Estimated percent reduction from control for number of pupae.

Test concentration (µg/L)	Estimated reduction from control (%) (P value)					
	CCA4	**CCA5**	**CCA6**	**CCA7**	**CCA8**	**CCA9**
10	2.0 (0.38)	19.9 (0.05*)	16.8 (0.082*/0.142)	37.2 (0.053*/0.357)	NA	NA
20	6.3 (0.144)	13.1 (0.039**)	11.2 (0.142)	10.7 (0.357)	-93.2 (0.953)	-38.8 (0.946)
40	16.3 (0.018**)	47.0 (0**)	30.9 (0.034**)	21.9 (0.234)	-58.6 (0.995)	-31.4 (0.967)
80	30.9 (0.001**)	87.7 (0**)	67.7 (0**)	20.2 (0.262)	-9.5 (0.549)	52.7 (0.091*)
160	46.9 (0**)	99.6 (0**)	97.5 (0**)	98.2 (0.001**)	NA	NA

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<P<0.1, **P<0.05
NA' indicates there was not enough data to do the test (n = number of Apiaries is small) and '0' means rounded to 0
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level.
At CCA8 and CCA9, the step-down approach was not applied to the 160 or 10 treatment levels where very few hives survived.

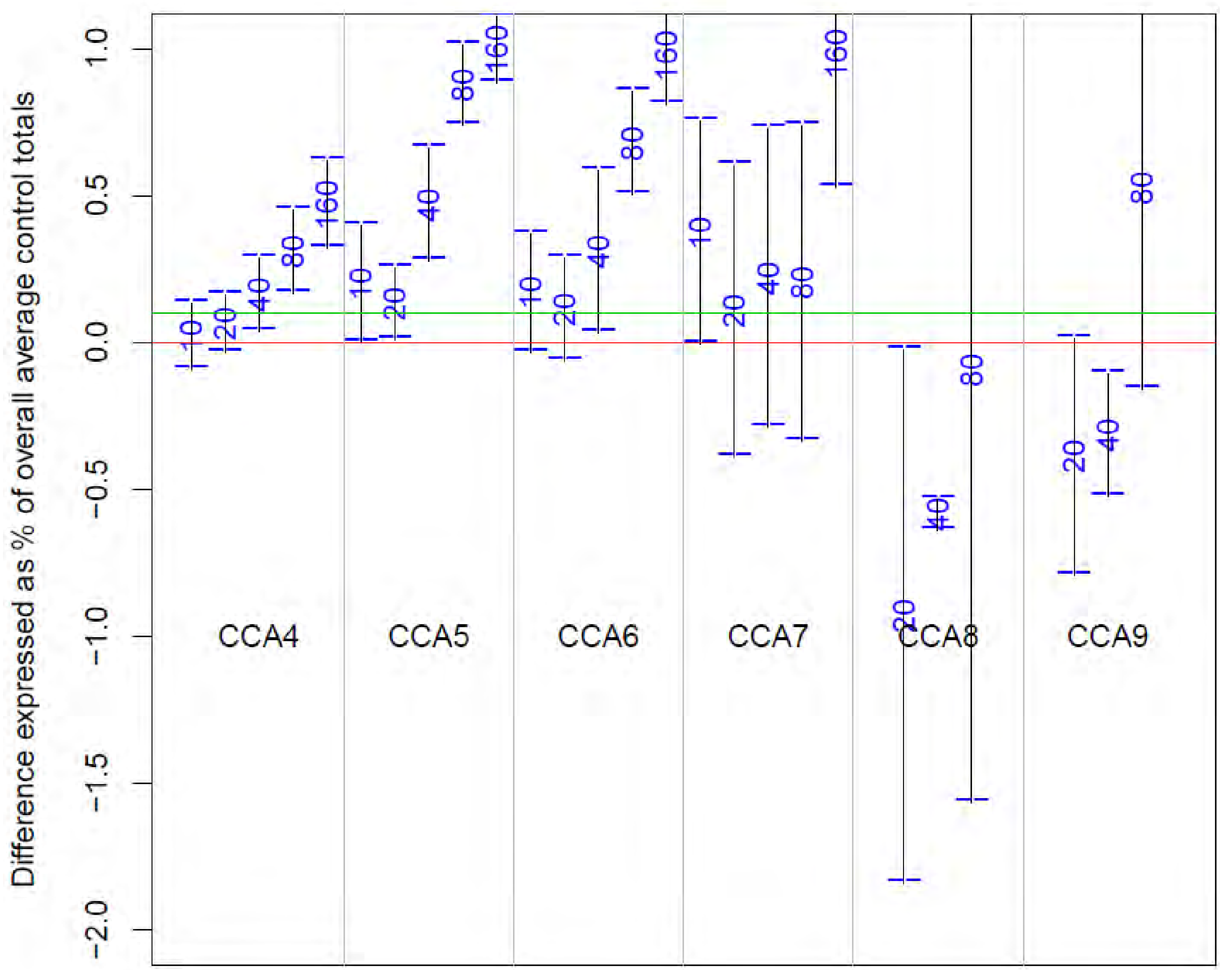


Figure A-9. Estimates and 90% CIs for pupae with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

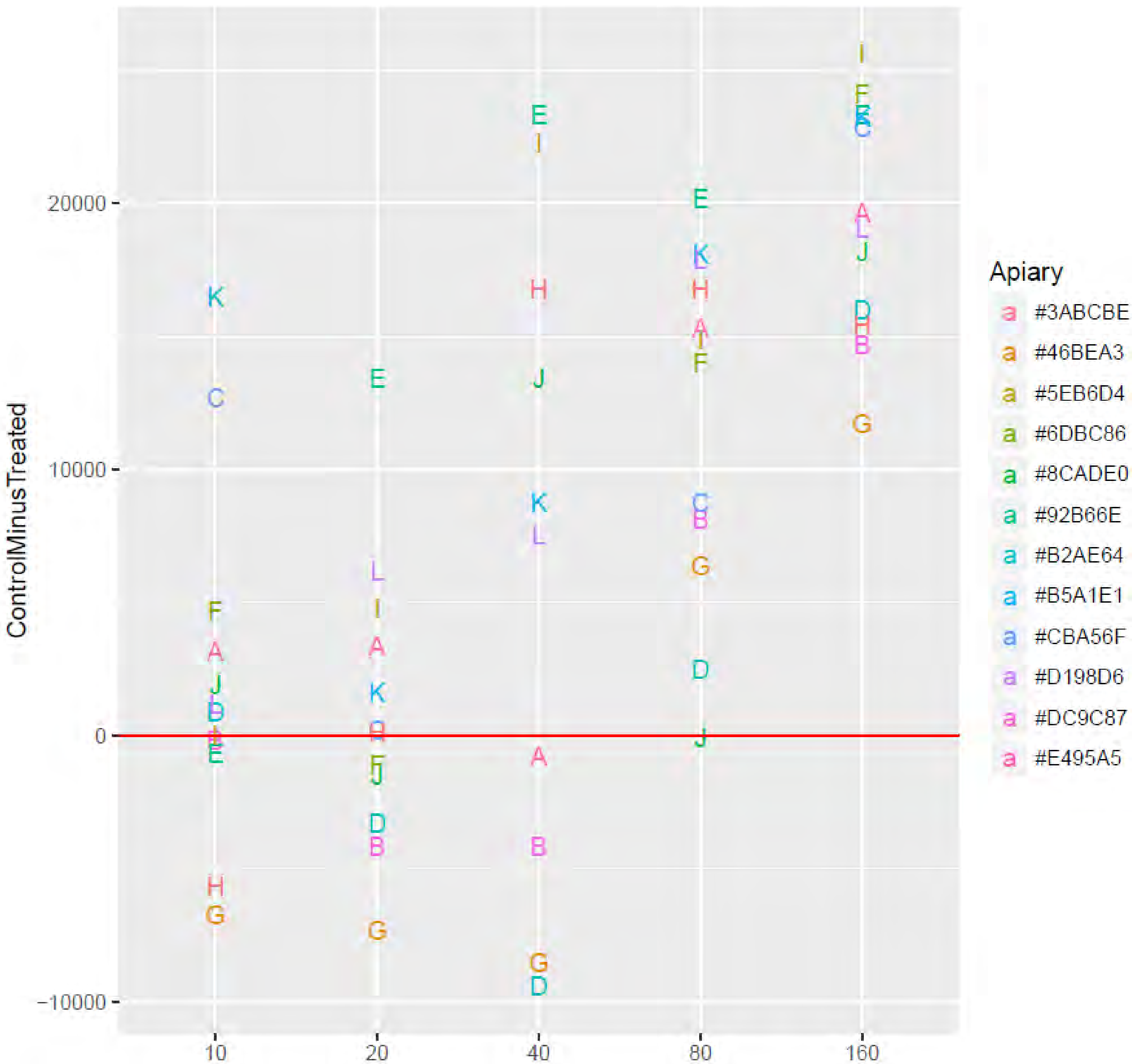


Figure A-10. Difference from control for all treatments and apiaries at CCA6 for pupae. Apiaries shown above the zero line had better control outcomes in comparison to the treatment.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Colony Condition Assessments - Food Stores

Pollen

Table A-7 and **Figure A-11** below show the effects on pollen storage across CCAs and treatment groups. Pollen stores were significantly reduced ($p<0.05$) from the control at the highest two treatment groups (80 and 160 µg/L) from CCA4 through to CCA7 (47.5-99.4% and 94.3- 100% reduction at 80 and 160 µg/L, respectively). Pollen stores were also significantly reduced in the 40 µg/L treatment groups during the exposure period at CCA4 (21.9% reduction, p= 0.041) and CCA5 (55.3% reduction, p= 0.003) but not during subsequent CCAs after the exposure period (7.6 and 10.8% reduction at CCA6 and CCA7, respectively, $p>0.1$). No significant reduction in pollen stores was observed at the two lowest treatment groups (10 and 20 µg/L) from CCA4 through to CCA7 except at CCA5 where pollen stores were significantly reduced from the control by 30.2% (p=0.032) in the 10 µg/L treatment group and 27.7% (p=0.019) in the 20 µg/L treatment group and at CCA7 where pollen stores were significantly reduced from the control by 59.8% (p=0.017).

Figure A-11 shows a general dose response where the reduction in pollen stores from the control increases with increasing dose, during the exposure period at CCA4 and CCA5. There was no clear dose response observed after the exposure period at CCA6 and CCA7 for the three lowest doses (10, 20 and 40 µg/L). For the 10 µg/L treatment group, in particular, there was a significant reduction in pollen stores at CCA7 but no significant reductions at the 20 and 40 µg/L treatment group for the same time period. Therefore the biological significance of this finding is considered to be low. In general, the width of the confidence intervals increased with increasing CCAs. As seen in **Figure A-12**, pollen stores at CCA6 were reduced from the control in 58% (7/12), 50% (6/12), 70% (7/10), 75% (9/12) and 100% (12/12) of apiaries in the 10, 20, 40, 80 and 160 µg /L dose groups, respectively.

Treatment means were significantly reduced from the control at the lowest three doses during and immediately after the exposure period at CCA4 (40 µg/L) and CCA5 (10, 20 and 40 µg/L) but not in subsequent CCAs after the exposure except at CCA7 (10 µg/L) and were significantly reduced from the control at the two highest treatment groups (80 and 160 µg/L) at all CCAs. While the width of the confidence intervals increased with increasing CCAs, the observed reversion back to control levels at the three lowest treatment groups during subsequent CCAs indicates a potential transient effect on pollen storage at ≤ 40 µg/L and supports the use of 40 µg/L as the NOAEC and 80 µg/L as the LOAEC. These endpoints are less sensitive than the endpoints determined through the primary analysis for total life at CCA6 (NOAEC of 20 µg/L, LOAEC of 40 µg/L).

Table A-7. Estimated percent reduction from control for pollen store.

Test concentration (µg/L)	Estimated reduction from control (%) (P value)					
	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9
10	7.2 (0.305)	30.2 (0.032**)	23.1 (0.202)	59.8 (0.017**/0.36)	NA	NA
20	10.9 (0.198)	27.7 (0.019**)	-11.2 (0.682)	23.4 (0.155)	-69.6 (0.907)	0.7 (0.482)
40	21.9	55.3	7.6	10.8	-30.3	-18.9

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Test concentration (µg/L)	Estimated reduction from control (%) (P value)					
	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9
	(0.041**)	(0.003**)	(0.385)	(0.36)	(0.688)	(0.189)
80	47.5 (0**)	99.4 (0**)	60.3 (0.009**)	73.1 (0.001**)	-66.5 (0.951)	-62.6 (0.934)
160	94.3 (0**)	100 (0**)	95.9 (0.001**)	95.7 (0**)	NA	NA

Note: Negative value indicates increased pollen stores in comparison to control.
*0.05<P<0.1, **P<0.05
NA' indicates there was not enough data to do the test (n = number of Apiaries is small) and '0' means rounded to 0
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level.
At CCA8 and CCA9, the step-down approach was not applied to the 160 or 10 treatment levels where very few hives survived.

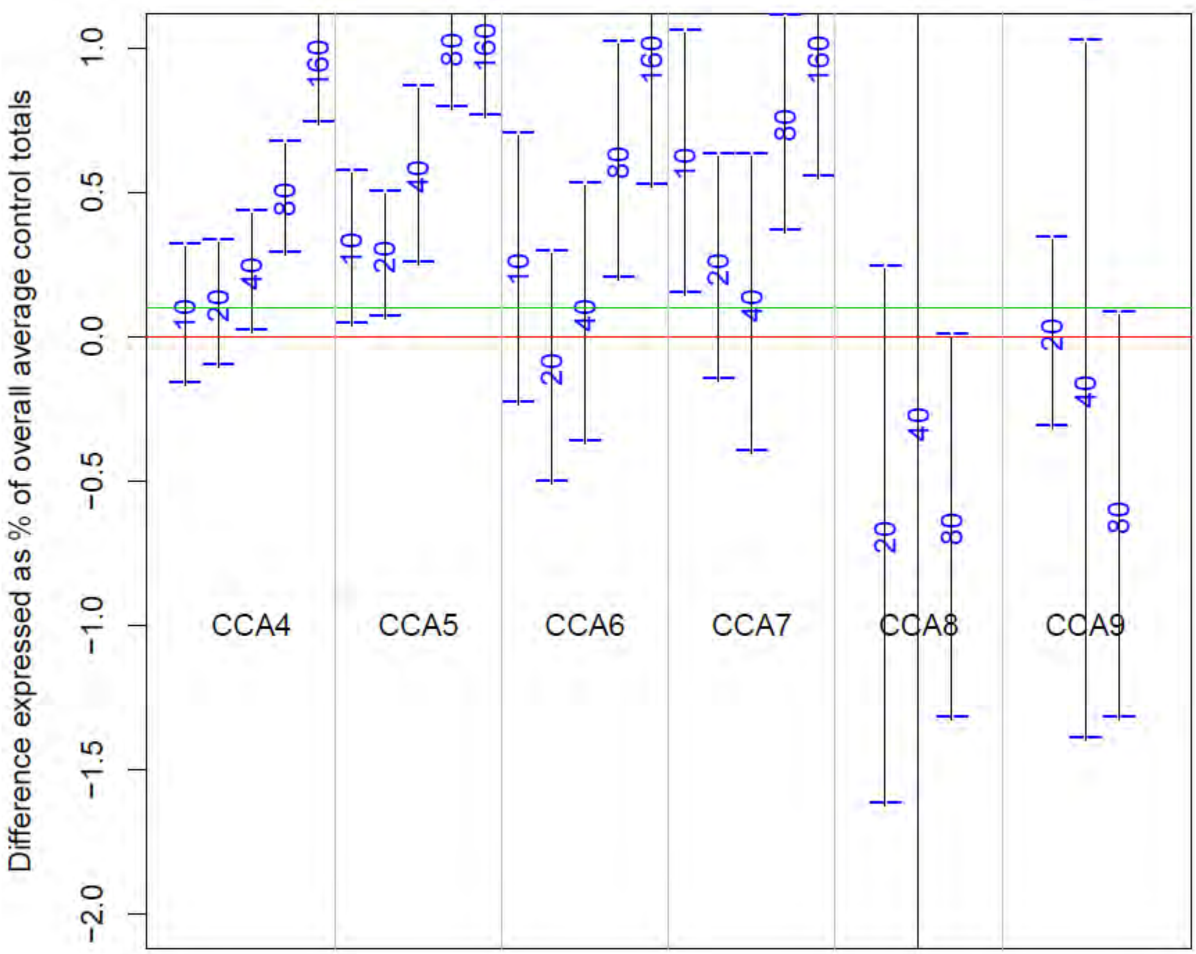


Figure A-11. Estimates and 90% CIs for pollen stores with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

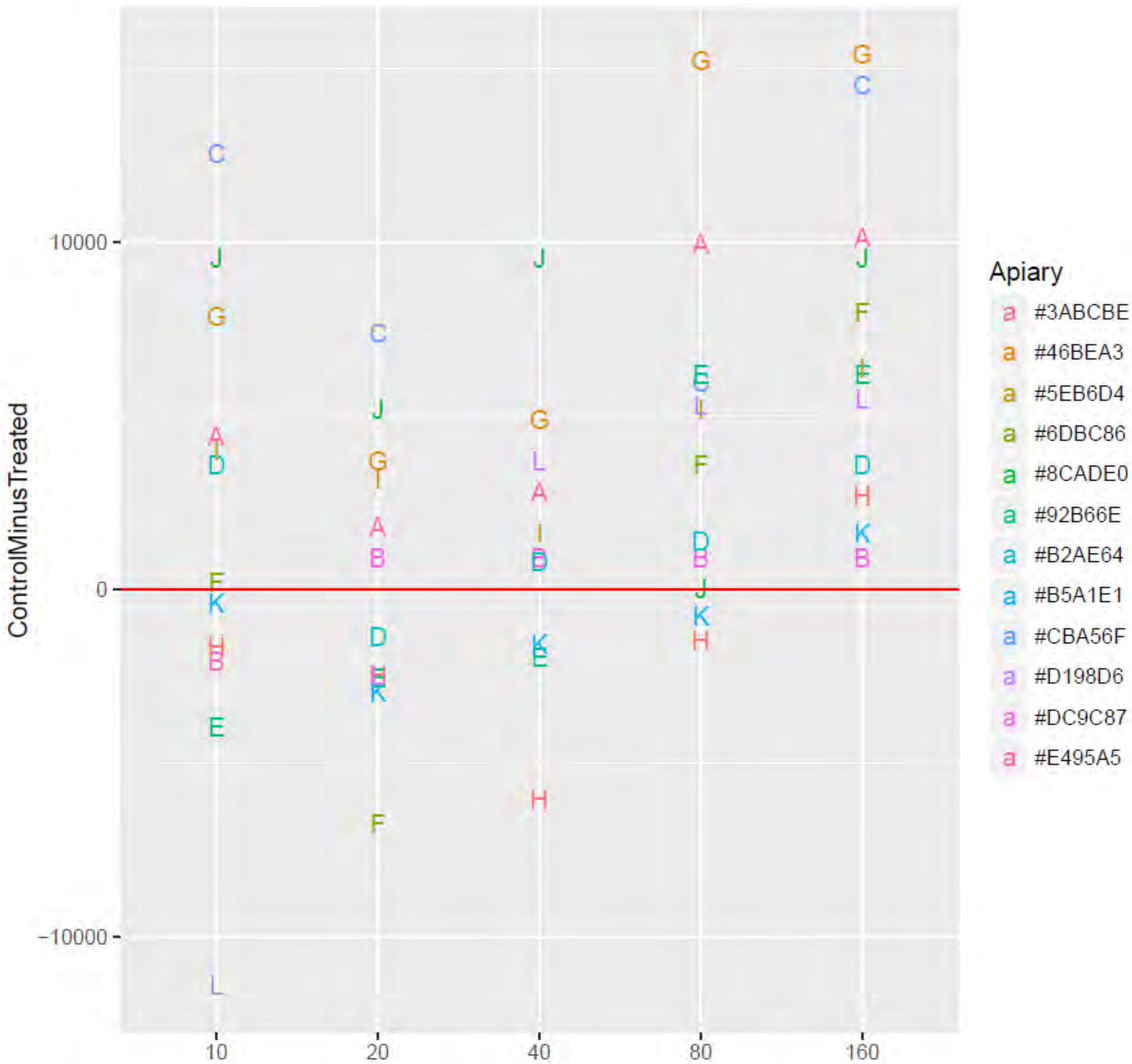


Figure A-12. Difference from control for all treatments and apiaries at CCA6 for pollen stores. Apiaries shown above the zero line had better control outcomes in comparison to the treatment.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Honey

Table A-8 and **Figure A-13** below show the effects on honey storage across CCAs and treatment groups. No significant adverse effects were observed on honey storage at any test concentration. Honey stores increased in all treatment groups over all CCAs compared to control hives. In general honey stores increased at each successive CCA for all treatment groups except at CCA7 where honey stores were lower than the proceeding CCAs for the two lowest treatment groups (10 and 20 µg/L). **Figure A-13** shows a dose response at CCA4 through to CCA7, with honey stores increasing with increasing dose. In general, the width of the confidence intervals increased with increasing CCAs. As seen in **Figure A-14**, honey stores at CCA6 were reduced from the control in 33% (4/12), 50% (6/12), 60% (6/10), 100% (12/12) and 100% (12/12) of apiaries in the 10, 20, 40, 80 and 160 µg /L dose groups, respectively.

Table A-8. Estimated percent reduction from control for honey store.

Test concentration (µg/L)	Estimated reduction from control (%) (P value)					
	CCA4	**CCA5**	**CCA6**	**CCA7**	**CCA8**	**CCA9**
10	-5.9 (0.854)	-6.6 (0.927)	-11.5 (0.795)	-2.8 (0.562)	NA	NA
20	-4.8 (0.833)	-10.6 (0.985)	-15.0 (0.919)	-2.8 (0.573)	-64.8 (0.973)	-61.5 (0.935)
40	-10.7 (0.966)	-20.6 (0.999)	-44.7 (0.998)	-51.4 (0.999)	-10.9 (0.633)	-3.2 (0.583)
80	-12.9 (0.995)	-26.9 (1.0)	-68.5 (1.0)	-80.2 (1.0)	-2.3 (0.522)	-4.3 (0.401)
160	-17.8 (0.999)	-19.1 (0.999)	-86.5 (1.0)	-100 (1.0)	NA	NA

Note: Negative value indicates increased honey stores in comparison to control.
*0.05<P<0.1, **P<0.05
NA' indicates there was not enough data to do the test (n = number of Apiaries is small) and '0' means rounded to 0
Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level.
At CCA8 and CCA9, the step-down approach was not applied to the 160 or 10 treatment levels where very few hives survived.

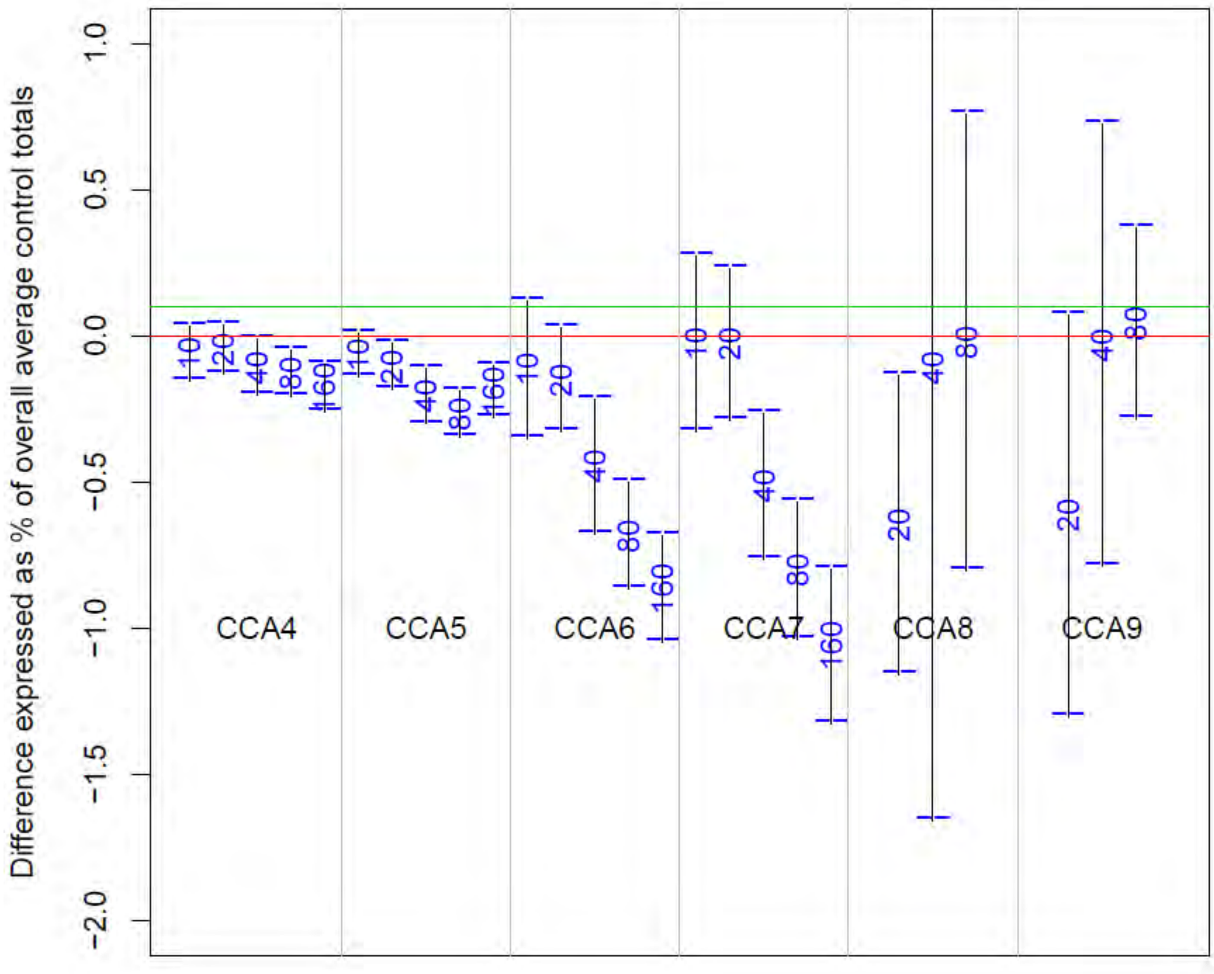


Figure A-13. Estimates and 90% CIs for larvae with thresholds of statistical (red) significance shown. The green line shows 10% difference from control.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

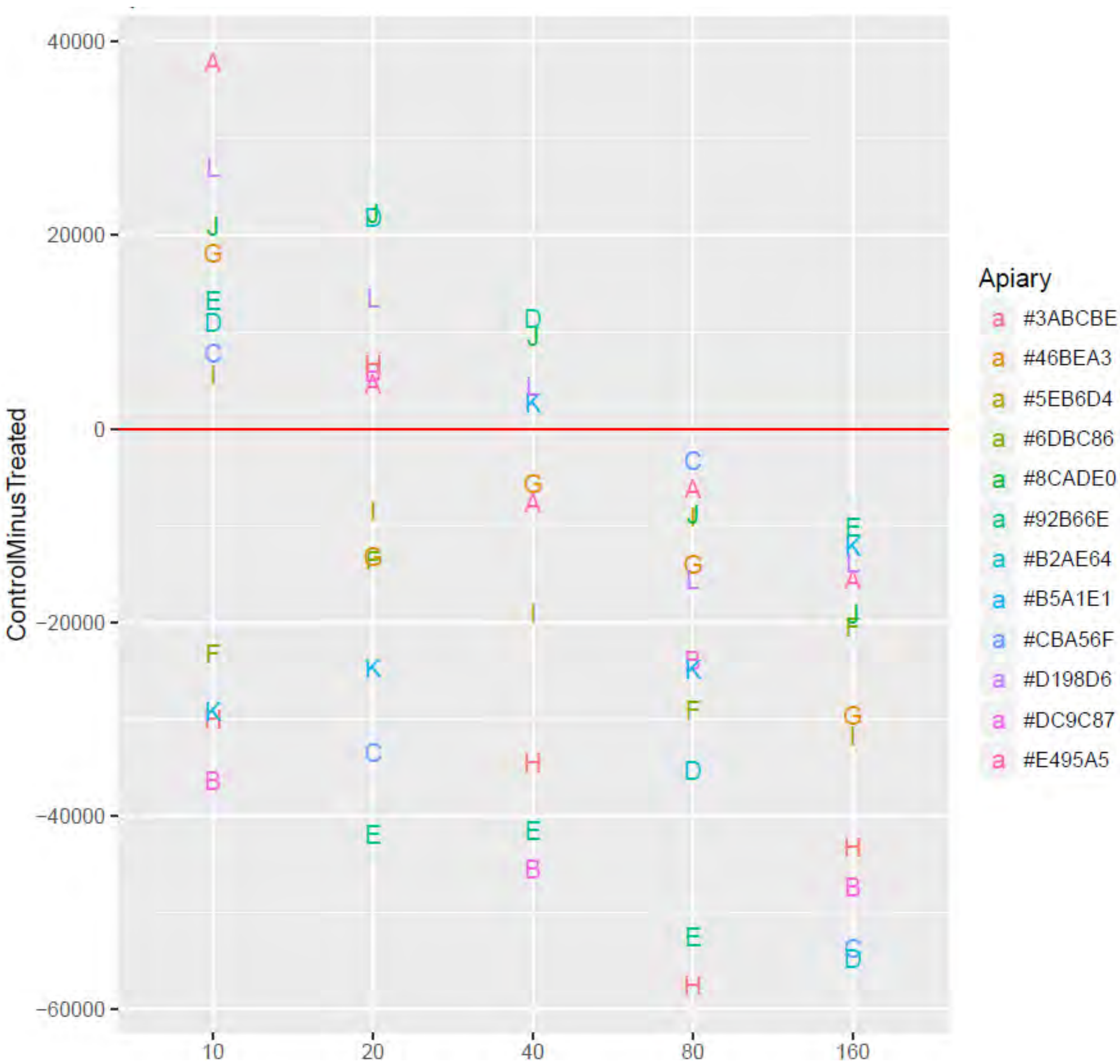


Figure A-14. Difference from control for all treatments and apiaries at CCA6 for honey stores. Apiaries shown above the zero line had better control outcomes in comparison to the treatment.

Table A-9. Summary of statistically significant (at 0.05 and 0.10) observed effects at each treatment level (Note: Values reported in the table are the % reduction compared to control, based on model estimated raw numbers corrected for baseline measurements). Where two p values are listed, the first is the non-adjusted p value, the second is the p value adjusted for the step-down approach. The step-down adjustment was shown only if it changed the significance level.

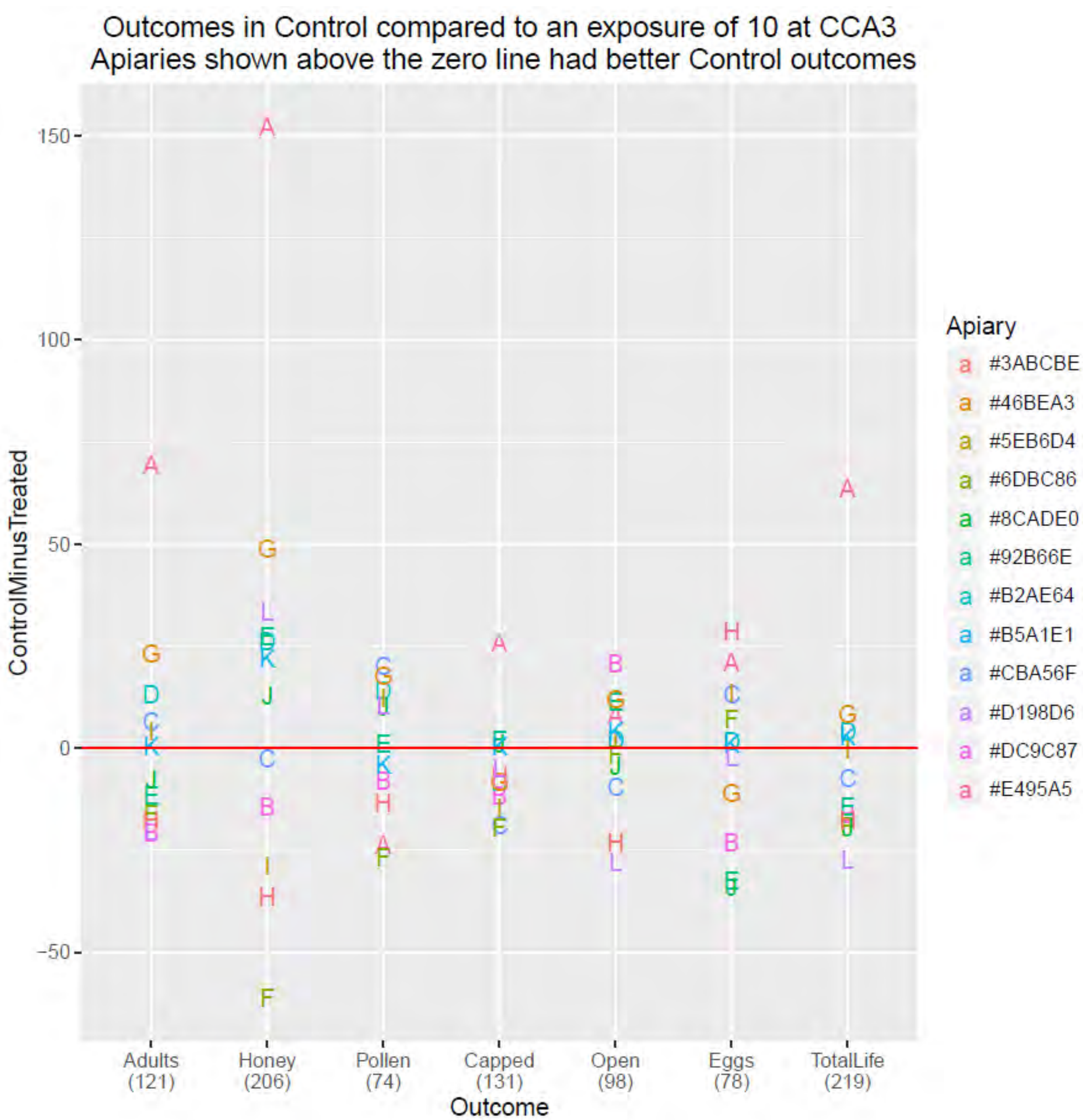
Treatment (µg/l)	Observations
10	• Decreased **total number of individuals** in hive at **CCA5** (14.5%, p=0.043/0.079) • Decreased number of **adults** at **CCA6** (19.6%, p=0.014) • Decreased number of **eggs** at **CCA4** (30.1%, p=0.016) and at **CCA5** (35.1%, p=0.018) • Decreased number of **larvae** at **CCA4** (15.0%, p=0.053)

	• Decreased number of **pupae** at **CCA5** (19.9%, p=0.05) • Decreased **pollen stores** at **CCA5** (30.2%, p=0.032) • The potential colony effects were not consistently sustained through to when colonies were in natural decline. Most endpoint responses were not significantly different from the control (p>0.1) • At CCA8 and CCA9, 75% of hives (9 out of 12 colonies) did not survive **overwintering** compared to 65% of control hives
20	• Decreased **total number of individuals** in hive at **CCA4** (6.7%, p=0.074) and **CCA5** (10.1% (p=0.079) • Decreased number of **adults** at **CCA6** (17%, p=0.037) • Decreased number of **eggs** at **CCA4** (27.1%, p=0.04) and a t **CCA5** (37.3%, p=0.009) • Decreased number of **larvae** at **CCA4** (12.2%, p=0.067) • Decreased number of **pupae** at **CCA5** (13.1%, p=0.039) • Decreased **pollen store** at **CCA5** (27.7%, p=0.019) • The potential colony effects were not consistently sustained through to when colonies were in natural decline. Most endpoint responses were not significantly different from the control (p>0.1) • At CCA8 and CCA9, 33% of hives (4 out of 12 colonies) did not survive **overwintering** compared to 65% of control hives
40	• Decreased **total number of individuals** in hive at **CCA4** (15.2%, p=0.008), **CCA5** (33.6%, p=0.002) and **CCA6** (30.5%, p=0.029) • Decreased number of **adults** at **CCA5** (23.1%, p=0.011) and at **CCA6** (29.9%, p=0.012) • Decreased number of **eggs** at **CCA4** (53.4%, p<<0.05) and at **CCA5** (41.7%, p=0.028) • Decreased number of **larvae** at **CCA4** (21.4%, p=0.017/0.093) • Decreased number of **pupae** at **CCA4** (16.3%, p=0.018) and a **CCA5** (47.0%, p<<0.05) and **CCA6** (30.9% p=0.034) • Decreased **pollen store** at **CCA4** (21.9%, p=0.041) and at **CCA5** (55.3%, p=0.003) • The potential colony effects were more consistently sustained across multiple CCAs prior to when colonies were in natural decline (CCA7). • At CCA8 and CCA9, 50% of hives (5 out of 10 colonies) did not survive **overwintering** compared to 65% of control hives
80	• Decreased **total number of individuals** in hive at **CCA4-CCA7** (22.4-70.2%, p<<0.05) and **CCA9** (56%, p=0.071) • Decreased number of **adults CCA4-CCA7** (13.9-64.1%, p<0.05) • Decreased number of **eggs** at **CCA4-CCA7** (36.3-69.6% p<0.05) • Decreased number of **larvae** at **CCA4** (12.3%, p=0.093), **CCA5-CCA6** (47.9-84.9%, p<0.05) and at **CCA7** (55.2%, p=0.069) • Decreased number of **pupae** at **CCA4-CCA6** (30.9-87.7%, p<0.05) • Decreased **pollen store** at **CCA4-CCA7** (47.5-99.4%, p<<0.05) • The potential colony effects were consistently sustained across multiple CCAs through to when colonies were in natural decline (CCA7). • At CCA8 and CCA9, 17% of hives (2 out of 12 colonies) did not survive **overwintering** compared to 65% of control hives
160	• Decreased **total number of individuals** in hive at **CCA4-CCA7** (46.9-96.5%, p<<0.05) • Decreased number of **adults CCA4-CCA7** (32.3-98.4%, p<<0.05) • Decreased number of **eggs CCA4-CCA7** (31.2-93.9%, p<0.05) • Decreased number of **larvae CCA4-CCA7** (87.8-98.8%, p<<0.05) • Decreased number of **pupae CCA4-CCA7** (46.9-99.6%, p<0.05) • Decreased **pollen store CCA4-CCA7** (94.3-100%, p<<0.05) • The potential colony effects were consistently sustained across multiple CCAs through to when colonies were in natural decline (CCA7). • At CCA8 and CCA9, 100% of hives (11 out of 11 colonies) did not survive **overwintering** compared to 65% of control hives

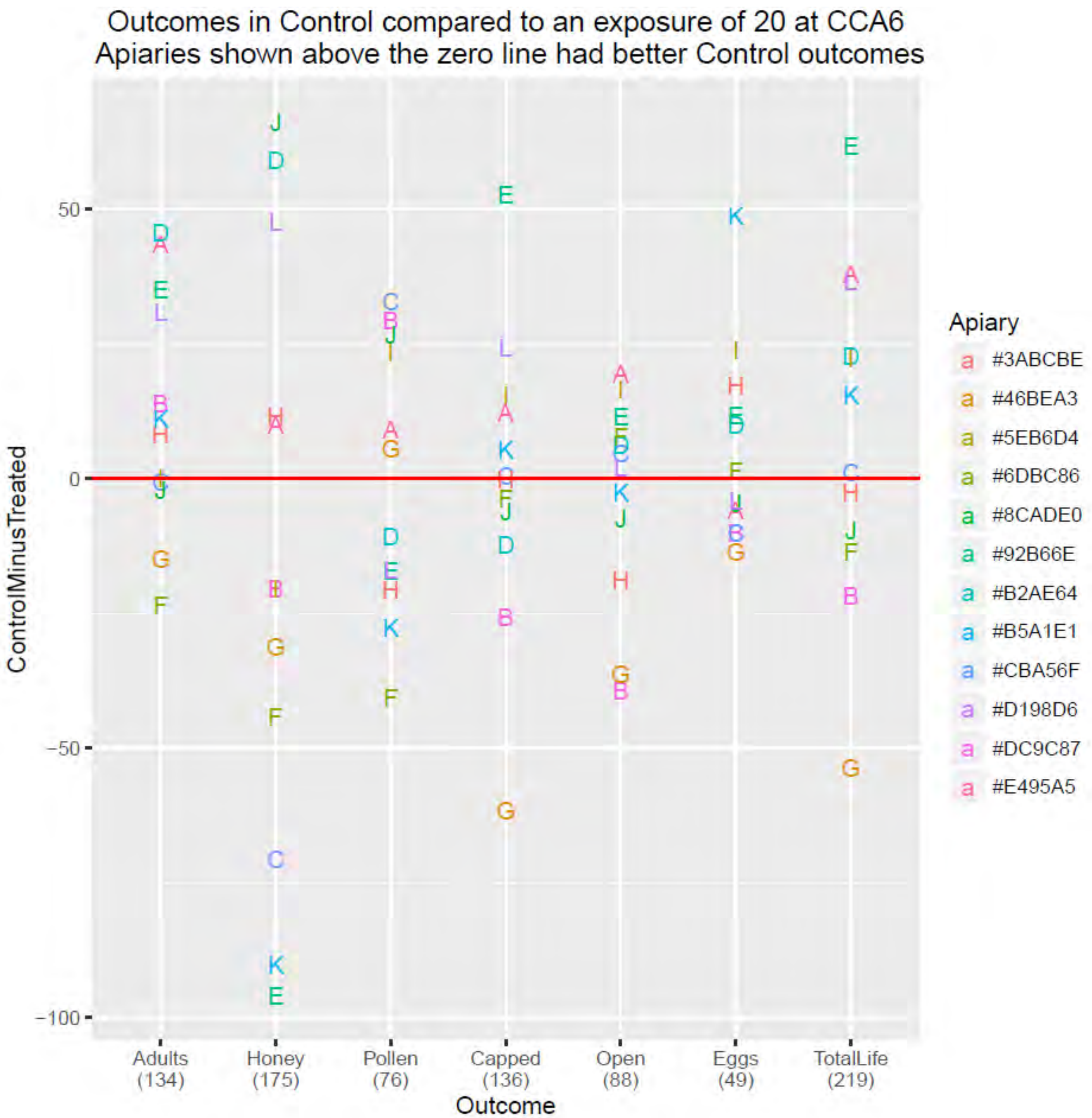
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

OVERALL ENDPOINT	**Potential colony effects were more consistently sustained across multiple CCAs prior to when colonies were in natural decline (CCA7) at ≥40 µg/L. The high overwintering mortality in the control hives limits the ability to fully assess recovery in the treatment hives, in particular for the lowest two test doses. In the interim the overall quantitative NOAEC and LOAEC for this study are considered to be:** • **NOAEC: 20 µg/L sucrose solution** • **LOAEC: 40 µg/L sucrose solution** **The LOAEC is based on significant effects on the number of adults, pupae and total number of individuals.**

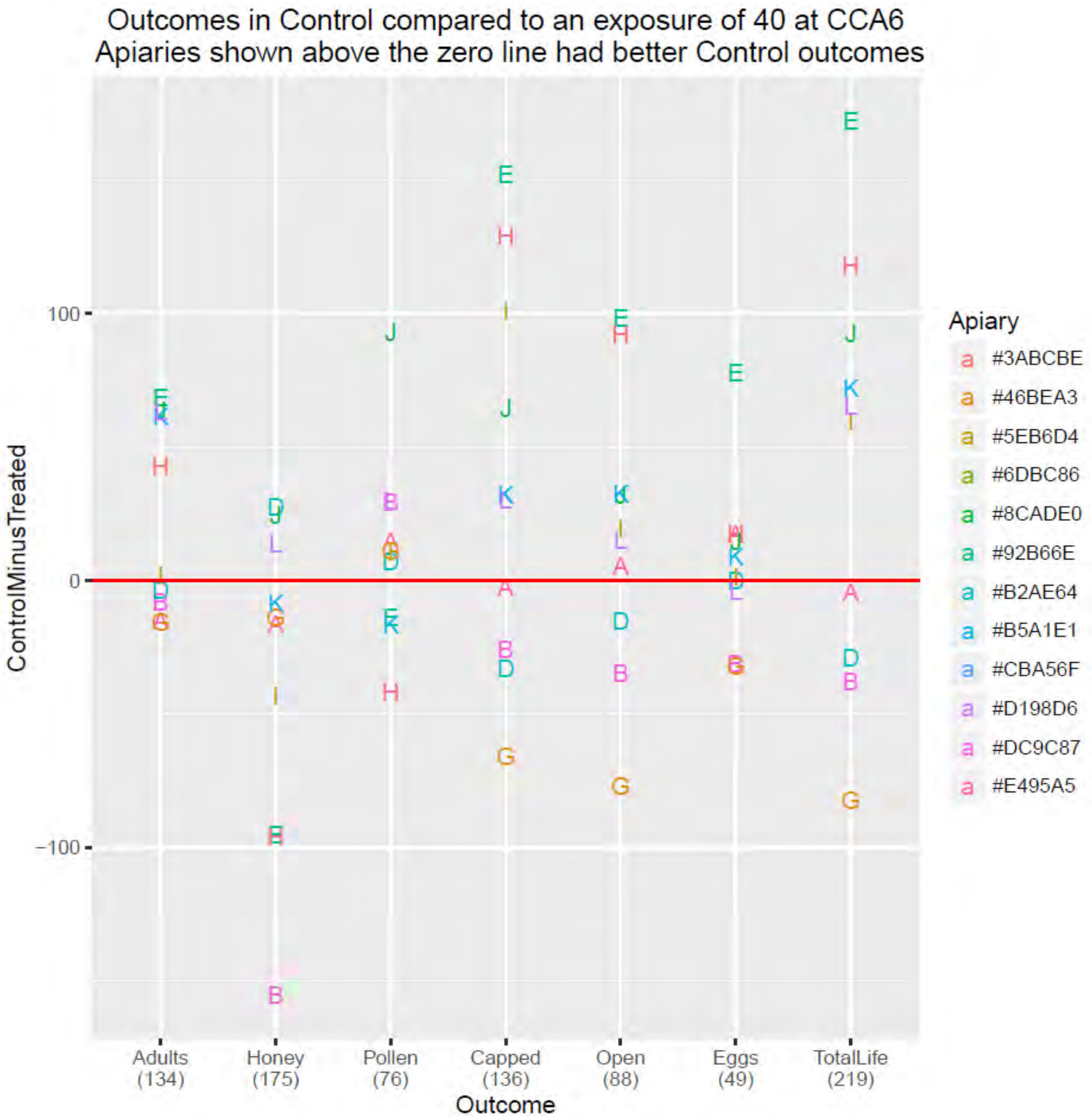
<u>Graphical representation of all parameters at CCA6</u>



Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document



Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document



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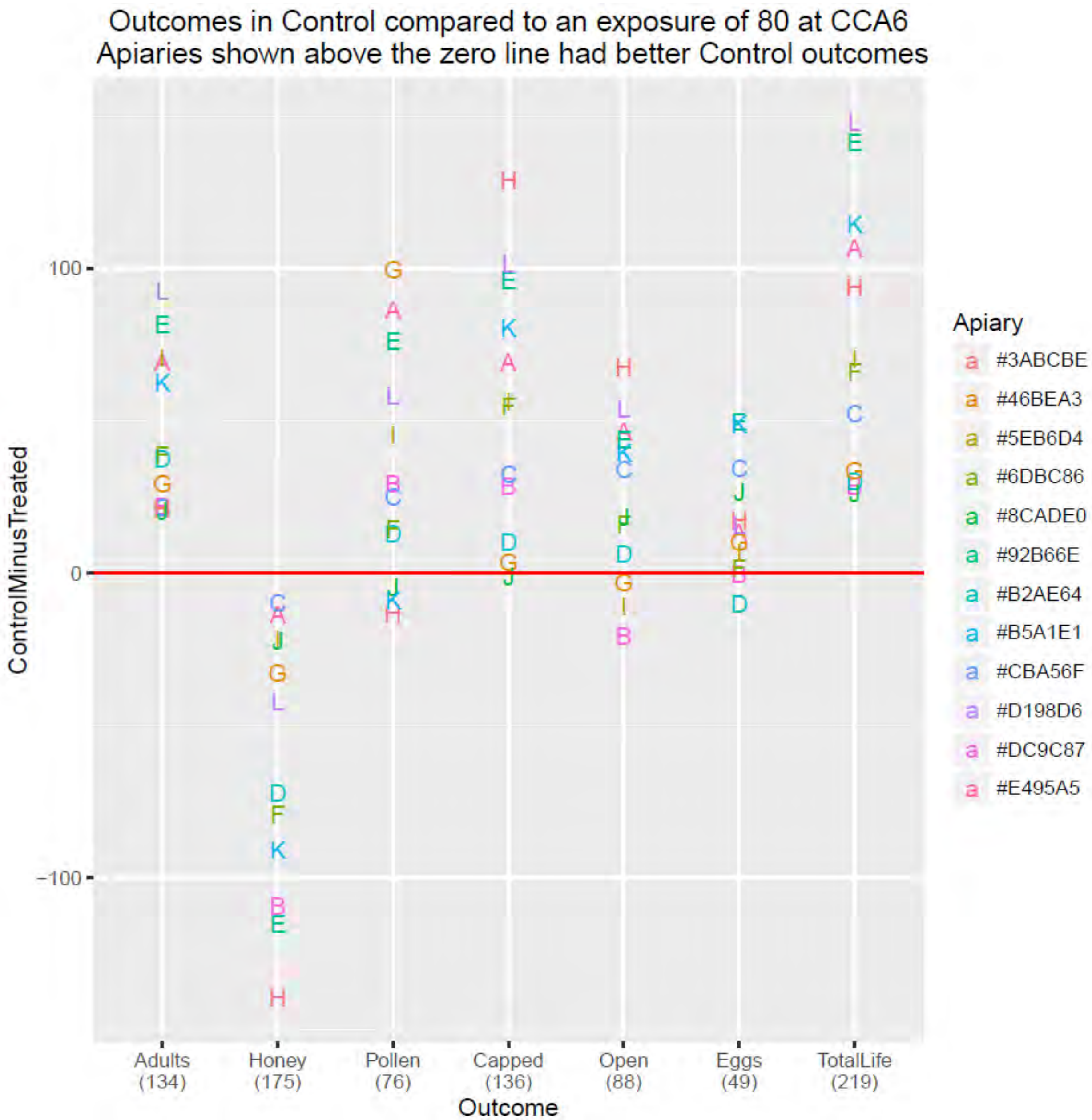
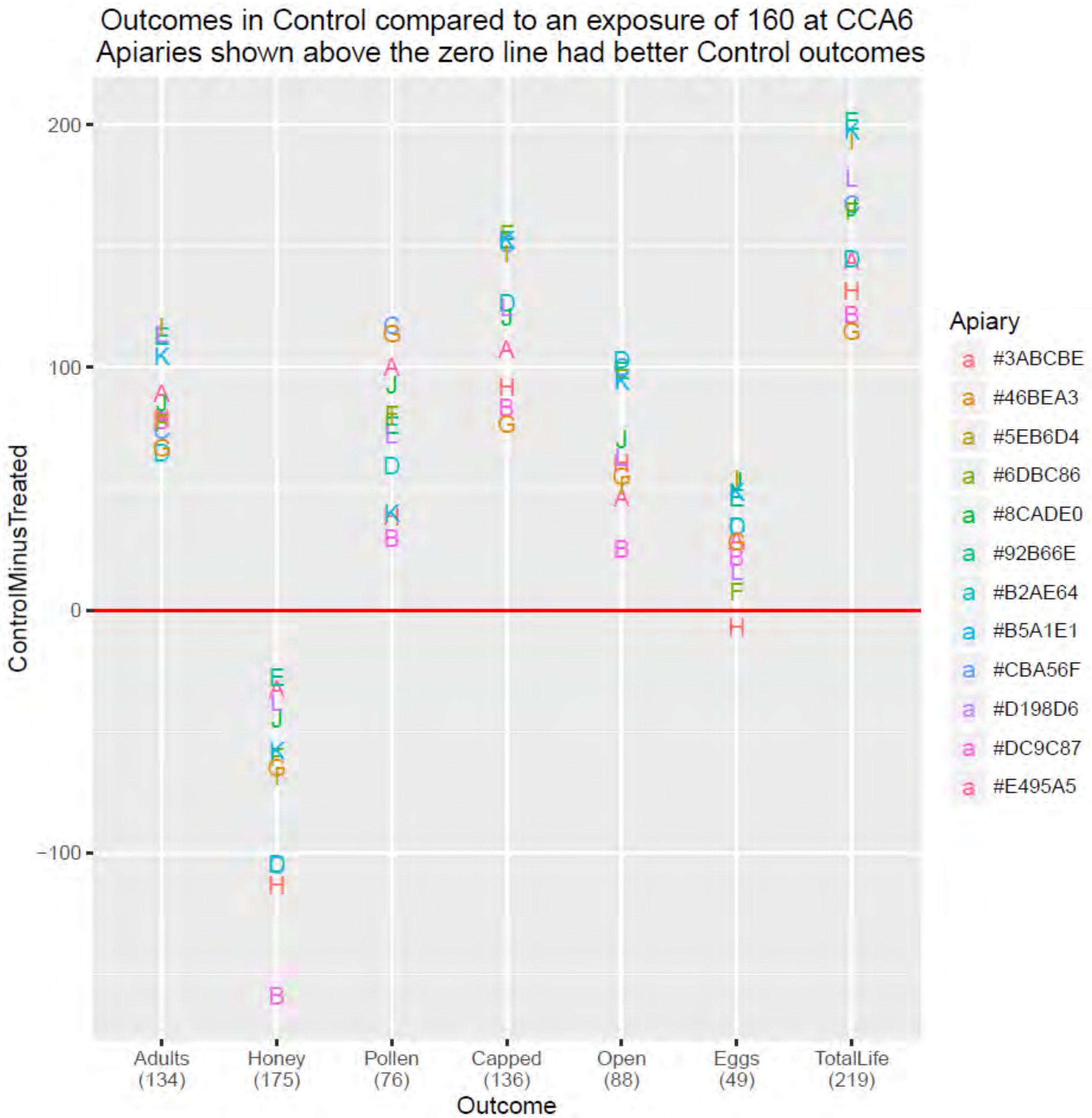

Outcomes in Control compared to an exposure of 80 at CCA6
Apiaries shown above the zero line had better Control outcomes
ControlMinusTreated
100
0
−100
Adults (134)
Honey (175)
Pollen (76)
Capped (136)
Open (88)
Eggs (49)
TotalLife (219)
Outcome
Apiary
#3ABCBE
#46BEA3
#5EB6D4
#6DBC86
#8CADE0
#92B66E
#B2AE64
#B5A1E1
#CBA56F
#D198D6
#DC9C87
#E495A5


Outcomes in Control compared to an exposure of 160 at CCA6
Apiaries shown above the zero line had better Control outcomes
ControlMinusTreated
200
100
0
-100
Adults (134)
Honey (175)
Pollen (76)
Capped (136)
Open (88)
Eggs (49)
TotalLife (219)
Outcome
Apiary
#3ABCBE
#46BEA3
#5EB6D4
#6DBC86
#8CADE0
#92B66E
#B2AE64
#B5A1E1
#CBA56F
#D198D6
#DC9C87
#E495A5

Appendix B. Timeline of addition and removal of supers during the clothianidin field trial

Hive	Apiary	Tmt	Number of hive boxes at each CCA measurement								
			CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9
A3	A	C	1	2	2	2	2	2	2	.	.
A5	A	C	1	3	3	3	3	3	3	.	.
B2	B	C	1	.	2	2	2	2	2	.	.
B3	B	C	1	2	2	2	3	3	2	.	.
C1	C	C	1	2	2	2	2	2	2	2	2
C3	C	C	1	2	2	2	2	2	2	.	.
D4	D	C	1	2	2	2	2	2	2	.	.
D8	D	C	1	2	2	2	2	2	2	2	2
E1	E	C	1	2	2	2	2	2	2	.	.
E4	E	C	1	2	2	2	2	2	2	2	2
F2	F	C	1	1	2	2	2	2	2	.	.
F5	F	C	1	2	2	2	2	2	2	.	.
G2	G	C	1	2	2	2	3	2	2	.	.
G8	G	C	1	2	2	2	3	2	2	2	2
H1	H	C	1	1	2	2	2	2	2	.	.
H6	H	C	1	2	2	2	2	2	2	2	2
I6	I	C	1	2	2	2	2	2	2	2	2
I7	I	C	1	1	1	.	.	.	.	.	.
J7	J	C	1	1	2	2	2	2	2	.	.
J8	J	C	1	1	1	2	2	2	2	2	2
K5	K	C	1	1	2	2	2	2	2	2	2
K7	K	C	1	1	1	1	2	2	2	.	.
L4	L	C	.	.	1	2	2	2	2	.	.
L6	L	C	1	2	2	2	2	2	2	.	.
A7	A	T1	1	.	1	1	1	1	1	.	.
B8	B	T1	1	2	2	2	2	2	2	2	2
C5	C	T1	1	2	2	2	2	2	2	.	.
D3	D	T1	1	2	2	2	2	2	2	.	.
E2	E	T1	1	1	2	2	2	2	2	.	.
F6	F	T1	1	2	2	2	2	2	2	2	2
G6	G	T1	.	1	2	2	2	2	2	.	.
H2	H	T1	.	2	2	2	2	2	2	2	2
I1	I	T1	1	1	2	2	2	2	2	.	.
J4	J	T1	1	1	2	2	2	2	2	.	.
K4	K	T1	1	1	2	2	2	2	2	.	.
L1	L	T1	1	1	2	2	2	2	2	.	.
A1	A	T2	1	2	2	2	2	2	2	2	2
B5	B	T2	1	2	2	2	2	2	2	.	.
C4	C	T2	1	2	3	3	3	3	3	2	2
D6	D	T2	1	2	3	3	3	3	2	.	.

E7	E	T2	1	2	2	2	2	2	2	2	2
F7	F	T2	1	2	2	2	2	2	2	.	.
G3	G	T2	1	1	1	2	2	2	2	2	2
H8	H	T2	1	.	1	2	2	2	2	.	.
I4	I	T2	1	2	2	2	2	2	2	2	2
J5	J	T2	1	.	1	2	2	2	2	2	2
K3	K	T2	1	2	2	2	2	2	2	2	2
L2	L	T2	1	2	1	1	1	1	1	1	1
A4	A	T3	1	2	2	2	2	2	2	2	2
B1	B	T3	1	2	2	2	2	2	2	2	2
C2	C	T3	1	1	2	2	2	.	.	.	.
D7	D	T3	1	2	2	2	2	2	2	2	2
E8	E	T3	1	2	2	2	2	2	2	.	.
F3	F	T3	1	2	2	.	.	.	.	.	.
G5	G	T3	1	2	2	2	2	2	2	.	.
H7	H	T3	.	2	2	2	2	2	2	.	.
I8	I	T3	1	2	2	2	2	2	2	2	2
J6	J	T3	1	.	1	1	1	1	1	1	1
K2	K	T3	1	2	2	2	2	2	1	.	.
L5	L	T3	1	1	1	1	1	1	1	.	.
A2	A	T4	1	2	2	2	2	2	2	2	2
B6	B	T4	1	2	2	2	2	2	2	2	2
C8	C	T4	1	2	2	2	2	2	2	2	2
D5	D	T4	2	2	3	3	3	3	2	2	2
E6	E	T4	1	2	2	2	2	2	2	2	2
F1	F	T4	1	2	2	2	2	2	2	2	2
G4	G	T4	1	2	2	2	2	2	2	2	2
H4	H	T4	1	2	2	2	2	2	2	2	2
I5	I	T4	1	1	2	2	2	2	2	.	.
J3	J	T4	1	1	2	2	2	2	2	2	2
K1	K	T4	1	2	2	2	2	2	2	2	2
L7	L	T4	1	2	2	2	2	1	1	.	.
A8	A	T5	1	2	2	2	2	2	2	.	.
B4	B	T5	1	2	2	2	2	2	2	.	.
C7	C	T5	1	2	2	2	2	2	2	.	.
D1	D	T5	1	2	2	2	2	2	2	.	.
E5	E	T5	1	1	2	2	1	1	1	.	.
F8	F	T5	1	1	1	1	2	2	1	.	.
G1	G	T5	1	2	2	2	2	2	2	.	.
H3	H	T5	1	1	2	2	2	2	2	.	.
I2	I	T5	1	2	2	2	2	2	2	.	.
J2	J	T5	1	2	2	2	2	2	2	.	.
K6	K	T5	1	1	1	1	1	1	1	.	.
L3	L	T5	1	1	2	2	2	1	1	.	.

Appendix C. Summary Statistics for Each Response Variable for All Clothianidin Treatment Levels Across CCAs 3—7

Table C-1. Summary Statistics for Adults

CCA	Treatment Group µg/L	Mean	SE	DF	Lower Bound	Upper Bound
3	0	15.0143	1.0393	70.4	12.9418	17.0869
4	10	14.3501	1.4500	72.8	11.4602	17.2400
5	20	15.1103	1.4500	72.8	12.2204	18.0001
6	40	15.3216	1.4500	72.8	12.4317	18.2115
7	80	16.5143	1.4500	72.8	13.6244	19.4041
3	160	15.6587	1.4500	72.8	12.7688	18.5485
4	0	17.1515	0.8078	66.9	15.5390	18.7640
5	10	17.3297	1.0991	79.6	15.1423	19.5172
6	20	17.0782	1.0991	79.6	14.8907	19.2656
7	40	17.5195	1.1361	81.7	15.2594	19.7796
3	80	15.6988	1.0991	79.6	13.5114	17.8863
4	160	11.9543	1.0991	79.6	9.7668	14.1417
5	0	19.2155	0.9860	74.5	17.2511	21.1800
6	10	17.4807	1.3515	80.8	14.7916	20.1699
7	20	18.9505	1.3515	80.8	16.2613	21.6397
3	40	14.6602	1.3972	82.8	11.8810	17.4393
4	80	12.0951	1.3515	80.8	9.4059	14.7842
5	160	5.9797	1.3515	80.8	3.2905	8.6688
6	0	18.2291	1.0561	70.6	16.1232	20.3350
7	10	14.2293	1.4503	78.7	11.3423	17.1163
3	20	15.2168	1.4503	78.7	12.3297	18.1038
4	40	12.6790	1.5474	83	9.6012	15.7568
5	80	7.9227	1.4503	78.7	5.0357	10.8097
6	160	2.2449	1.4503	78.7	0	5.1319
7	0	12.5891	0.9990	68.7	10.5959	14.5822
3	10	10.2127	1.3699	77.7	7.4854	12.9401
4	20	11.8231	1.3699	77.7	9.0957	14.5505
5	40	10.9166	1.4614	82	8.0095	13.8238
6	80	6.5987	1.3699	77.7	3.8713	9.3261
7	160	0.4782	1.3699	77.7	0	3.2056

Table C-2. Summary statistics for eggs

CCA	Treatment Group µg/L	Mean	SE	DF	Lower Bound	Upper Bound
3	0	6.3936	0.6202	83.1	5.1601	7.6272
3	10	6.8154	0.8541	76.5	5.1145	8.5163
3	20	6.4351	0.8541	76.5	4.7342	8.1360
3	40	7.1463	0.8541	76.5	5.4453	8.8472
3	80	5.8560	0.8541	76.5	4.1551	7.5569

CCA	Treatment Group µg/L	Mean	SE	DF	Lower Bound	Upper Bound
3	160	6.2034	0.8541	76.5	4.5025	7.9043
4	0	4.5425	0.5033	80.6	3.5409	5.5440
4	10	3.1762	0.6712	77.8	1.8399	4.5124
4	20	3.3417	0.6712	77.8	2.0054	4.6779
4	40	2.3641	0.6962	78.5	0.9782	3.7500
4	80	2.8123	0.6712	77.8	1.4761	4.1486
4	160	3.0934	0.6712	77.8	1.7572	4.4296
5	0	3.9624	0.4661	85.8	3.0359	4.8889
5	10	2.6964	0.6172	89.6	1.4702	3.9226
5	20	2.4980	0.6172	89.6	1.2718	3.7242
5	40	2.6200	0.6399	90.4	1.3489	3.8912
5	80	1.2408	0.6172	89.6	0.01461	2.4671
5	160	0.2814	0.6172	89.6	0	1.5076
6	0	3.0384	0.4526	81.3	2.1379	3.9389
6	10	2.9446	0.5976	87.2	1.7568	4.1324
6	20	2.5806	0.5976	87.2	1.3928	3.7684
6	40	2.6460	0.6423	89.3	1.3699	3.9221
6	80	1.5385	0.5976	87.2	0.3507	2.7263
6	160	0.5131	0.5976	87.2	0	1.7009
7	0	1.0349	0.2694	17.7	0.4682	1.6016
7	10	1.0258	0.3202	30.7	0.3724	1.6791
7	20	0.7610	0.3202	30.7	0.1077	1.4143
7	40	0.7701	0.3383	34.8	0.08317	1.4571
7	80	0.2978	0.3202	30.7	0	0.9512
7	160	0.04967	0.3202	30.7	0	0.7030

Table C-3. Summary statistics for larval (open) cells

CCA	Treatment Group µg/L	Mean	SE	DF	Lower Bound	Upper Bound
3	0	9.8012	0.6855	75.8	8.4359	11.1666
3	10	9.8922	0.9585	67.1	7.9791	11.8053
3	20	8.8833	0.9585	67.1	6.9702	10.7963
3	40	9.2967	0.9585	67.1	7.3836	11.2098
3	80	11.2982	0.9585	67.1	9.3851	13.2113
3	160	9.4621	0.9585	67.1	7.5490	11.3752
4	0	8.7909	0.6329	78.2	7.5310	10.0507
4	10	7.4937	0.8651	72	5.7691	9.2182
4	20	7.6755	0.8651	72	5.9509	9.4001
4	40	6.8202	0.9023	71.9	5.0214	8.6190
4	80	7.9899	0.8651	72	6.2653	9.7145
4	160	1.1580	0.8651	72	0	2.8826
5	0	9.8509	0.7199	77.6	8.4176	11.2841
5	10	9.6935	0.9870	79.9	7.7293	11.6577
5	20	8.4199	0.9870	79.9	6.4557	10.3842

CCA	Treatment Group µg/L	Mean	SE	DF	Lower Bound	Upper Bound
5	40	7.9044	1.0296	80	5.8555	9.9533
5	80	1.8032	0.9870	79.9	0	3.7674
5	160	1.94E-14	0.9870	79.9	0	1.9642
6	0	8.7630	0.7459	79.1	7.2783	10.2478
6	10	9.1975	1.0235	79.5	7.1605	11.2345
6	20	8.4033	1.0235	79.5	6.3663	10.4403
6	40	6.6587	1.1169	79.6	4.4358	8.8816
6	80	4.4997	1.0235	79.5	2.4627	6.5367
6	160	0.5956	1.0235	79.5	0	2.6326
7	0	2.3045	0.4287	33.4	1.4327	3.1762
7	10	1.5387	0.5768	47.1	0.3783	2.6990
7	20	1.4560	0.5768	47.1	0.2957	2.6163
7	40	1.5447	0.6283	48.8	0.2818	2.8075
7	80	1.2408	0.5768	47.1	0.08042	2.4011
7	160	0.03317	0.5768	47.1	0	1.1935

Table C-4. Summary statistics for pupal (capped) cells

CCA	Treatment Group µg/L	Mean	SE	DF	Lower Bound	Upper Bound
3	0	17.2946	0.7332	63.2	15.8295	18.7597
3	10	18.7254	0.9755	72.5	16.7811	20.6697
3	20	18.6427	0.9755	72.5	16.6983	20.5870
3	40	17.1043	0.9755	72.5	15.1600	19.0487
3	80	18.6263	0.9755	72.5	16.6819	20.5706
3	160	17.4516	0.9755	72.5	15.5073	19.3959
4	0	19.2671	0.8811	64.8	17.5072	21.0269
4	10	19.1887	1.1730	78	16.8535	21.5239
4	20	18.5104	1.1730	78	16.1752	20.8456
4	40	16.1024	1.2195	79.2	13.6750	18.5297
4	80	14.2426	1.1730	78	11.9074	16.5778
4	160	10.3553	1.1730	78	8.0201	12.6905
5	0	19.5472	1.1589	76	17.2392	21.8553
5	10	16.1944	1.5697	80.1	13.0707	19.3181
5	20	17.6503	1.5697	80.1	14.5266	20.7741
5	40	10.3399	1.6339	80.6	7.0887	13.5911
5	80	3.2423	1.5697	80.1	0.1186	6.3661
5	160	-955E-16	1.5697	80.1	0	3.1237
6	0	19.5516	1.2522	80	17.0596	22.0435
6	10	17.5344	1.7018	81.9	14.1489	20.9200
6	20	18.8246	1.7018	81.9	15.4390	22.2101
6	40	12.3767	1.8482	82.7	8.7004	16.0530
6	80	7.9568	1.7018	81.9	4.5713	11.3424
6	160	0.3642	1.7018	81.9	0	3.7497
7	0	4.2369	0.7993	40.6	2.6220	5.8517
7	10	3.0935	1.0545	52.4	0.9779	5.2091
7	20	4.0363	1.0545	52.4	1.9207	6.1520

CCA	Treatment Group µg/L	Mean	SE	DF	Lower Bound	Upper Bound
7	40	3.4065	1.1405	55	1.1210	5.6920
7	80	3.9040	1.0545	52.4	1.7884	6.0196
7	160	0.1158	1.0545	52.4	0	2.2315

Table C-5. Summary statistics for pollen cells

CCA	Treatment Group µg/L	Mean	SE	DF	Lower Bound	Upper Bound
3	0	5.6906	0.4794	69.7	4.7344	6.6468
3	10	5.6243	0.6771	77.7	4.2762	6.9725
3	20	5.1612	0.6771	77.7	3.8131	6.5094
3	40	5.0455	0.6771	77.7	3.6973	6.3937
3	80	5.8229	0.6771	77.7	4.4747	7.1711
3	160	5.5253	0.6771	77.7	4.1771	6.8734
4	0	12.5242	1.0534	77.6	10.4268	14.6217
4	10	11.6622	1.4627	77.7	8.7499	14.5744
4	20	10.5043	1.4627	77.7	7.5920	13.4165
4	40	8.8542	1.5180	78.9	5.8327	11.8758
4	80	6.6666	1.4627	77.7	3.7544	9.5788
4	160	0.4136	1.4627	77.7	0	3.3258
5	0	8.4747	0.6483	74.6	7.1830	9.7663
5	10	5.7072	0.8999	79.6	3.9162	7.4981
5	20	5.4094	0.8999	79.6	3.6185	7.2003
5	40	2.9918	0.9339	81	1.1337	4.8499
5	80	0.1655	0.8999	79.6	0	1.9564
5	160	8.35E-15	0.8999	79.6	0	1.7909
6	0	6.9830	0.9529	81.8	5.0873	8.8787
6	10	5.1613	1.3231	81.2	2.5289	7.7936
6	20	6.7492	1.3231	81.2	4.1168	9.3815
6	40	4.6042	1.4238	84.2	1.7729	7.4355
6	80	2.7627	1.3231	81.2	0.1303	5.3950
6	160	0.01658	1.3231	81.2	0	2.6489
7	0	5.3361	0.7206	77.2	3.9011	6.7710
7	10	2.0183	1.0003	77.4	0.02659	4.0101
7	20	3.4078	1.0003	77.4	1.4161	5.3996
7	40	4.3212	1.0765	80.3	2.1791	6.4634
7	80	1.5551	1.0003	77.4	0	3.5468
7	160	-957E-16	1.0003	77.4	0	1.9917

Table C-6. Summary statistics for nectar (honey) cells

CCA	Treatment Group µg/L	Mean	SE	DF	Lower Bound	Upper Bound
3	0	45.2665	4.6463	54.7	35.9541	54.5790
3	10	38.1785	6.1934	77.5	25.8471	50.5099
3	20	47.1937	6.1934	77.5	34.8623	59.5250
3	40	42.8597	6.1934	77.5	30.5283	55.1910
3	80	47.4417	6.1934	77.5	35.1104	59.7731

3	**160**	46.7966	6.1934	77.5	34.4652	59.1279
4	**0**	53.2940	3.9580	41.4	45.3028	61.2851
4	**10**	51.1635	5.1172	69.7	40.9567	61.3703
4	**20**	57.1352	5.1172	69.7	46.9285	67.3420
4	**40**	57.3690	5.1836	72.4	47.0366	67.7014
4	**80**	61.7007	5.1172	69.7	51.4940	71.9075
4	**160**	63.9503	5.1172	69.7	53.7436	74.1571
5	**0**	57.1814	4.0722	47.9	48.9933	65.3695
5	**10**	56.5398	5.2911	74.1	45.9975	67.0822
5	**20**	64.6782	5.2911	74.1	54.1358	75.2205
5	**40**	67.0401	5.3603	76.9	56.3661	77.7140
5	**80**	74.1566	5.2911	74.1	63.6142	84.6990
5	**160**	69.3595	5.2911	74.1	58.8171	79.9019
6	**0**	32.7332	4.1171	40	24.4122	41.0543
6	**10**	32.1572	5.3591	65.7	21.4566	42.8577
6	**20**	39.0882	5.3591	65.7	28.3876	49.7887
6	**40**	45.6546	5.4886	70.3	34.7086	56.6006
6	**80**	57.3998	5.3591	65.7	46.6993	68.1004
6	**160**	63.3712	5.3591	65.7	52.6706	74.0717
7	**0**	24.2344	3.7648	28.5	16.5281	31.9406
7	**10**	21.1900	4.8212	50.1	11.5069	30.8731
7	**20**	25.8712	4.8212	50.1	16.1882	35.5543
7	**40**	35.5606	4.9342	53.4	25.6653	45.4559
7	**80**	45.1922	4.8212	50.1	35.5091	54.8752
7	**160**	51.8913	4.8212	50.1	42.2083	61.5744

Table C-7. Summary statistics for total live individuals

CCA	Treatment Group µg/L	Mean	SE	DF	Lower Bound	Upper Bound
3	**0**	48.5038	2.3430	73.1	43.8343	53.1732
3	**10**	49.7831	3.2626	68.3	43.2732	56.2929
3	**20**	49.0713	3.2626	68.3	42.5614	55.5811
3	**40**	48.8688	3.2626	68.3	42.3590	55.3787
3	**80**	52.2947	3.2626	68.3	45.7848	58.8045
3	**160**	48.7758	3.2626	68.3	42.2659	55.2856
4	**0**	49.7408	1.7287	67.9	46.2912	53.1904
4	**10**	47.1883	2.3355	78.6	42.5391	51.8374
4	**20**	46.6058	2.3355	78.6	41.9566	51.2549
4	**40**	42.7860	2.4179	80.2	37.9745	47.5976
4	**80**	40.7437	2.3355	78.6	36.0945	45.3928
4	**160**	26.5610	2.3355	78.6	21.9119	31.2101
5	**0**	52.5617	2.3162	82.3	47.9542	57.1692
5	**10**	46.0651	3.1694	85.4	39.7639	52.3663
5	**20**	47.5188	3.1694	85.4	41.2176	53.8199
5	**40**	35.5021	3.2828	87.1	28.9774	42.0269
5	**80**	18.3814	3.1694	85.4	12.0802	24.6826
5	**160**	6.2611	3.1694	85.4	0	12.5623
6	**0**	49.5587	2.6826	83	44.2232	54.8942

6	**10**	43.9058	3.6854	84	36.5770	51.2347
6	**20**	45.0253	3.6854	84	37.6964	52.3541
6	**40**	34.4159	3.9498	87.4	26.5658	42.2660
6	**80**	21.9177	3.6854	84	14.5888	29.2465
6	**160**	3.7178	3.6854	84	0	11.0466
7	**0**	20.1691	1.7497	49.9	16.6546	23.6836
7	**10**	15.8707	2.3656	67.1	11.1492	20.5922
7	**20**	18.0764	2.3656	67.1	13.3549	22.7979
7	**40**	16.5686	2.5316	70.4	11.5200	21.6171
7	**80**	12.0413	2.3656	67.1	7.3197	16.7628
7	**160**	0.6768	2.3656	67.1	0	5.3983

Table C-8. Summary statistics for Total Brood

CCA	Treatment Group µg/L	Mean	SE	DF	Lower Bound	Upper Bound
3	**0**	33.4894	1.4304	68.5	30.6356	36.3433
3	**10**	35.4330	1.9250	70.4	31.5940	39.2720
3	**20**	33.9610	1.9250	70.4	30.1220	37.8000
3	**40**	33.5473	1.9250	70.4	29.7083	37.3862
3	**80**	35.7804	1.9250	70.4	31.9415	39.6194
3	**160**	33.1171	1.9250	70.4	29.2781	36.9560
4	**0**	32.6109	1.3514	57.3	29.9050	35.3168
4	**10**	29.8585	1.7744	74.4	26.3232	33.3938
4	**20**	29.5276	1.7744	74.4	25.9923	33.0629
4	**40**	25.2779	1.8427	75.9	21.6077	28.9480
4	**80**	25.0448	1.7744	74.4	21.5095	28.5802
4	**160**	14.6067	1.7744	74.4	11.0714	18.1421
5	**0**	33.3740	1.8064	80.1	29.7792	36.9688
5	**10**	28.5843	2.4308	85.5	23.7517	33.4169
5	**20**	28.5682	2.4308	85.5	23.7357	33.4008
5	**40**	20.8444	2.5284	86	15.8180	25.8707
5	**80**	6.2863	2.4308	85.5	1.4537	11.1189
5	**160**	0.2814	2.4308	85.5	0	5.1140
6	**0**	31.3669	1.9832	81.4	27.4213	35.3124
6	**10**	29.6765	2.6824	84.5	24.3427	35.0103
6	**20**	29.8085	2.6824	84.5	24.4747	35.1423
6	**40**	21.6505	2.9087	85.2	15.8673	27.4336
6	**80**	13.9950	2.6824	84.5	8.6612	19.3288
6	**160**	1.4728	2.6824	84.5	0	6.8066
7	**0**	7.5922	1.3183	41.4	4.9306	10.2538
7	**10**	5.6579	1.7259	55.4	2.1997	9.1162
7	**20**	6.2533	1.7259	55.4	2.7951	9.7116
7	**40**	5.6646	1.8630	58.1	1.9356	9.3937
7	**80**	5.4426	1.7259	55.4	1.9843	8.9008
7	**160**	0.1987	1.7259	55.4	0	3.6569

Table C-9. Summary statistics for Total Food

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

CCA	Treatment Group µg/L	Mean	SE	DF	Lower Bound	Upper Bound
3	0	50.9571	4.8783	55.6	41.1832	60.7311
3	10	43.8028	6.5220	77.1	30.8162	56.7895
3	20	52.3549	6.5220	77.1	39.3683	65.3416
3	40	47.9052	6.5220	77.1	34.9185	60.8918
3	80	53.2647	6.5220	77.1	40.2780	66.2513
3	160	52.3218	6.5220	77.1	39.3352	65.3085
4	0	65.7439	4.4303	49.1	56.8411	74.6466
4	10	62.8257	5.8092	73.2	51.2485	74.4028
4	20	67.6395	5.8092	73.2	56.0623	79.2167
4	40	66.1805	5.8898	76	54.4501	77.9109
4	80	68.3673	5.8092	73.2	56.7902	79.9445
4	160	64.3639	5.8092	73.2	52.7867	75.9411
5	0	65.6537	4.2353	46.9	57.1330	74.1744
5	10	62.2470	5.5152	72.5	51.2538	73.2402
5	20	70.0876	5.5152	72.5	59.0944	81.0807
5	40	70.0359	5.5908	75.2	58.8991	81.1728
5	80	74.3221	5.5152	72.5	63.3289	85.3152
5	160	69.3595	5.5152	72.5	58.3663	80.3527
6	0	39.6986	4.4005	43.4	30.8264	48.5708
6	10	37.3184	5.7644	68	25.8156	48.8212
6	20	45.8373	5.7644	68	34.3346	57.3401
6	40	50.2935	5.9118	72.8	38.5108	62.0763
6	80	60.1625	5.7644	68	48.6597	71.6653
6	160	63.3877	5.7644	68	51.8850	74.8905
7	0	29.5429	4.0102	31.5	21.3694	37.7165
7	10	23.2083	5.1729	54.2	12.8382	33.5785
7	20	29.2791	5.1729	54.2	18.9090	39.6492
7	40	39.9037	5.3015	57.9	29.2911	50.5164
7	80	46.7472	5.1729	54.2	36.3771	57.1174
7	160	51.8913	5.1729	54.2	41.5212	62.2615

EVALUATION REPORT - Clothianidin Nectar Colony Feeding Study: Repeat Study Conducted in 2016-2017

John Troiano, Research Scientist III

June, 2018

A review of: - Louque, J. (2017). Colony feeding study evaluating the chronic effects of chothianidin-fortified sugar diet on honey bee (Apis mellifera) colony performance under free foraging conditions. Unpublished study prepared by Smithers Viscient. 609p., Laboratory Report Number 13798.4162. MRID 49836101.

Introduction

A colony feeding study was conducted to determine the effects of graded levels of clothianidin on the health of honey bee hives where doses mimicked exposure from foraging on nectar. Clothianidin was dosed directly to hives, supplied in a sugar solution that mimicked a nectar source for food supply. Hive health was determined by Colony Condition Assessments (CCAs) where measurements were made over time on the number of individuals in each bee life stage in the hive, the storage of honey and pollen food supplies in the hives, and the weight of hives. This study, conducted in 2016-2017, was a repeat of a study conducted in 2014-2015 (Louque, J., 2016). The second study was conducted in the same area as the first study where locations of apiary sites were distributed throughout a forested area of North Carolina. Not all sites were in the exact location as in the previous study. The distance between each apiary site was approximately 3 miles apart. The majority of land near the apiaries was non-intensively managed pasture and forest with low potential exposure of bees to pesticides applied for agricultural purposes.

Measurements made over time were indicated by sequential numbering of the colony condition assessments (CCAs), which were conducted at approximately monthly intervals. CCAs were made at similar time intervals for the two studies. The exposure period for both studies was initiated in early July with the treatment period lasting approximately 6 weeks. The CCAs included in this analysis are:

- Just prior to initiation of treatments in early July, denoted CCA2 in this analysis
- End of July around 3 weeks into the exposure period, denoted CCA3 in this analysis
- End of August around 6 weeks after initiation of exposure, denoted CCA4 in this analysis
- End of September around 10 weeks after initiation of exposure, denoted CCA5 in this analysis

- End of September around 13 weeks after initiation of exposure, denoted CCA6 in this analysis

These CCAs were chosen because this was the time period used to determine No Observed Effects Concentrations (NOECs) and Lowest Observed Effects Concentrations (LOECs) in the previous neonicotinoid nectar colony feeding studies. The range in levels of dose differed in this second repeat study. In the first study dose levels were 0, 10, 20, 40, 80, and 160 ng clothianidin per g of solution. In the repeat study the highest level was not included where levels of dose were at 0, 10, 20, 30, 40, and 80 ng/g. Data from the previous study was analyzed jointly by DPR, U.S. EPA, and Canada's PMRA staff scientists (U.S. EPA, PMRA, & DPR, 2017).

Statistical Analysis

Evaluation of the data followed the statistical approach used by DPR and EPA scientists to analyze previously reviewed neonicotinoid colony feeding studies. Since measurements for each variable were made in each hive over time, the statistical analysis was conducted as a repeated measures over time. Additionally, a mixed model was used where apiary location was identified as a random variable and clothianidin levels of dose as a fixed effect. The mixed model was chosen because the results of the analysis were to be applied to the larger population of bee hives. For data collected in 2016, statistical analysis was conducted on data collected for CCAs numbered 2-6. As indicated previously, results of statistical analyses collected from the timing of these assessments made from July through September were the basis for development of NOEC and LOEC values derived from data generated in previous neonicotinoid colony feeding studies. Normality tests were conducted for each CCA within each year as indicated by Shapiro-Wilk and Kolmogorov-Smirnov test statistics produced by the PROC CAPABILITY procedure in Statistical Analysis System (SAS, version 9.4). For comparison, data were also transformed to natural logarithms to determine if transformation provided better results. The majority of results indicated that the distributions were normal, with the logarithm transformation indicating many instances of non-normality. Based on these results the raw data were used in the analyses. The mixed model approach used to analyze the data included tests to determine the appropriate covariance model that describes the covariance structure reflected by the data. Inclusion of a covariance model in the analysis accounts for heterogeneity of variances that often are measured between treatment levels.

The PROC MIXED procedure in the Statistical Analysis System (SAS, version 9.4) was used to run the repeated measures effects mixed model. Measurements of colony health and hive weight were conducted approximately 1 month apart so CCAs were treated as equally spaced intervals. The effects side of the single year model statement included testing differences in the response between CCAs indicating changes in response over the monthly measurements, between the levels of clothianidin dose, and the potential interaction for effects of dose over time as indicated by the CCA factor. SAS Program 1 below reflects the structure of the program used to analyze the data for single years. Statistical options were included in the 'Slice' statement to protect against falsely discovering a significant multiple comparisons for paired mean values between the value at the control and each level of dose. The 'Simulated' option is a Monte Carlo approach that computes adjusted p-values from simulated distributions based on distributional statistics generated during the analysis (Edwards and Berry, 1987). In addition, the 'Stepdown' option was invoked because it tends to increase the power of the multi-comparison tests (SAS, version 9.4). SAS Program 2 indicates the structure of the program used to conduct the analyses for each year.

There were two statements in the mixed model used to analyze the data, where a covariance model could be specified. One was in the 'Random' statement with apiary indicated as a random variable. The second was in the 'Repeated' measures statement where each hive was indicated as the subject for the repeated measure. For the random statement only the Variance Component (VC) model successfully paired with the covariance model specified in the repeated statement: Specifying more complex covariance models in the random statement resulted in indications of converge problems for that model. As observed in the previous colony feeding studies the correlation structure indicated greater correlation between samples taken at close time intervals and, conversely, decreased correlation the further apart the samples were taken in time. Since this structure is normally represented by autoregressive covariance models, the covariance structure for the repeated statement was tested using variance component (VC), compound symmetry (CS), compound symmetry with heterogeneity (CSH), autoregressive first order (AR(1)), autoregressive first order with heterogeneity (ARH(1)), and unstructured (UN) models. Covariance model selection was based on the statistic generated for the Bayesian Information Criteria (BIC) where a lower value of the criterion indicated a better fit of the covariance model. A statistical basis for choosing the appropriate model was determined from Chi-square tests conducted on the difference of the value of the BIC criteria between the two models tested with the number of degrees of freedom determined as the difference between the number of parameters in the model and where the significance level of probability was at 0.01 (Hammer, 2000; Littell et al., 2006). With the VC covariance model specified in the random statement, the best fit covariance model in the repeated statement for analysis of data collected in 2016 was ARH(1) for number of adult bees and number of pupal, larval, egg, and pollen cells and AR(1) for number of nectar cells (Table 1). Values for numbers of cells measured for each bee life stage and food supply were divided by 1000 prior to statistical analysis to minimize potential convergence problems due to magnitude of values.

SAS Program 1

```
proc mixed data=a4 order=data;by year;
class apiary dose cca hive rep ;
model transvalue =cca dose dose*cca /ddfm=sat htype=1;
random apiary/type=vc;
repeated cca/ subject=hive*rep(dose) type=ar(1);
slice dose*cca /sliceby=cca diff=controll stepdown(report)   adjust=simulate adjdfe=row;
run;
```

Results

Data Combined for Years: Means and standard deviation for each response variable measured at each dose and each CCA are the same as presented in this and the previous report of study results so they are not reprinted in this analysis (Louque, J., 2016). Results from the data collected in 2016 indicated numerous effects due to dose of clothianidin (Table 2). The significant interactive effect of dose with CCA reflects the varying magnitude in the level of differences for significant effects over the sampling interval. For example, there were no differences between the levels of dose for the first CCA2 samples as these were taken prior to exposure and indicate no bias in treatments at the start of the study (Figures 1 to 6). In later CCAs, the number of adult bees at the 80 ng/g treatment indicates a downward trend where the magnitude in difference compared to the controls becomes progressively greater at later CCAs (Figure 1). Results from the pairwise comparisons between values for control and each dose level indicated numerous instances of significant effects (Table 3 and Figures 1 through 6). Effects were first indicated for measurements taken at CCA3 where pollens cells were affected even at the 30 ng/g level of dose. Initial effects were indicated for numbers of adults which were significant throughout the remaining CCAs. With respect to determining the LOEC value, there were indications of significant effects at the 30 ng/g level of dose whereas the next lowest treatment at 20 ng/g had only one indication for a potential trend for decreased pollen cells at CCA3. This pattern of effects indicates that the 20 ng/g dose is the NOEC value and the 30 ng/g dose is the LOEC value. Actual mean measured values as reported by the authors of the report were 28 ng/g for the LOEC value and 19 ng/g for the NOEC value.

Conclusion

Results of this statistical analysis are in agreement with the conclusions drawn by the authors of the report. The nominal LOEC value was the 30 ng/g level of dose and the NOEC value was the 20 ng/g level of clothianidin dose. The mean measured concentrations of clothianidin in the treated solutions in the nominal 30 and 20 ng/g treatment groups were 28 and 19 ng/g, which are the actual LOEC and NOEC values, respectively. This study has been determined to be scientifically sound and can be used quantitatively to assess risks to honey bee colonies.

References

Edwards, D., and Berry, J. J. (1987). “The Efficiency of Simulation-Based Multiple Comparisons.” Biometrics 43:913–928

Hammer,R.M. 2000. Mixed-Up Mixed Models: Things That Look Like They Should Work But Don't, and Things That Look Like They Shouldn't Work But Do. Proceedings of the twenty-fifth annual SAS users group International Conference, April 9-12, 2000, Indianapolis, Indiana. Available at: http://www2.sas.com/proceedings/sugi25/25/aa/25p020.pdf. (Verified, Feb 12,2018).

Littell, R.C., G.A. Milliken, W.W. Stroup, R.D Wolfinger, and O. Schabenberger. 2006. SAS System for Mixed Model, Second Edition. SAS Institute Inc., Cary NC.

Louque, J. (2016). Colony feeding study evaluating the chronic effects of clothianidin-fortified sugar diet on honey bee {Apis mellifera) colony health under free foraging conditions: Final Report. Unpublished study prepared by Smithers Viscient. 550p., Laboratory Report Number 13798.4143. MRID 49836101 CDPR Study ID TBD.

SAS Institute Inc 2013. SAS/ACCESS® 9.4.. Cary, NC: SAS Institute Inc.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

U.S. EPA, PMRA, & DPR. (2017). Data evaluation report: Louque, J. (2016) - Colony feeding study evaluating the chronic effects of clothianidin-fortified sugar diet on honey bee (Apis mellifera) colony health under free foraging conditions. Washington, D.C.: U.S. EPA.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 1. Mixed Model Analysis of Variance: BIC goodness-of-fit values generated for each covariance model structure tested in the repeated measures analysis of variance program. Shaded cells indicate the covariance structure used for the analysis. DNC indicates that the model failed to converge to solution.

CV Model Tested	Number of Parameters	Model BIC Value for Data Collected in 2016:					
		Adults	Pupae	Larvae	Eggs	Nectar	Pollen
VC	2	2349.5	2259.7	2048.7	1637	3011.6	2302.9
CS	3	2262.1	2230.3	2043.1	1638.5	2904.3	2205.7
AR(1)	3	2197.5	2208.5	2032.9	1637.9	2858.8	2198.8
CSH	7	2251.4	2239.9	2020.4	1585.2	2913.3	2135.4
ARH(1)	7	2168.9	2217.3	2006.1	1583.8	2865.4	2123.6
UN	16	2170.4	2225.4	2013.6	1552.8	2878.6	2122.2

Table 2. Combined Years: Results of the repeated measures mixed model testing the response of each variable to clothianidin dosed surrogate honey.

Mixed Model Results for Repeated Measures Analysis of Variance All Doses					
Variable	Effect	DF	Den DF	F Value	Pr > F
Adult Bees	cca	4	185	94.79	<.0001
AR(1)	dose	5	73.1	2.87	0.0204
	dose*cca	20	190	1.22	0.2391
Pupal Cells	cca	4	170	104.01	<.0001
	dose	5	85.6	5.98	<.0001
	dose*cca	20	170	4.37	<.0001
Larval Cells	cca	4	120	73.06	<.0001
	dose	5	86.3	2.8	0.0217
	dose*cca	20	122	1.93	0.0160
Egg Cells	cca	4	135	42.43	<.0001
	dose	5	106	1.23	0.2999
	dose*cca	20	142	0.65	0.8654
Nectar Cells	cca	4	269	32.72	<.0001
	dose	5	76	1.5	0.2009
	dose*cca	20	271	2.25	0.0020
Pollen Cells	cca	4	166	57.07	<.0001
	dose	5	71.6	5.44	0.0003
	dose*cca	20	169	4.56	<.0001

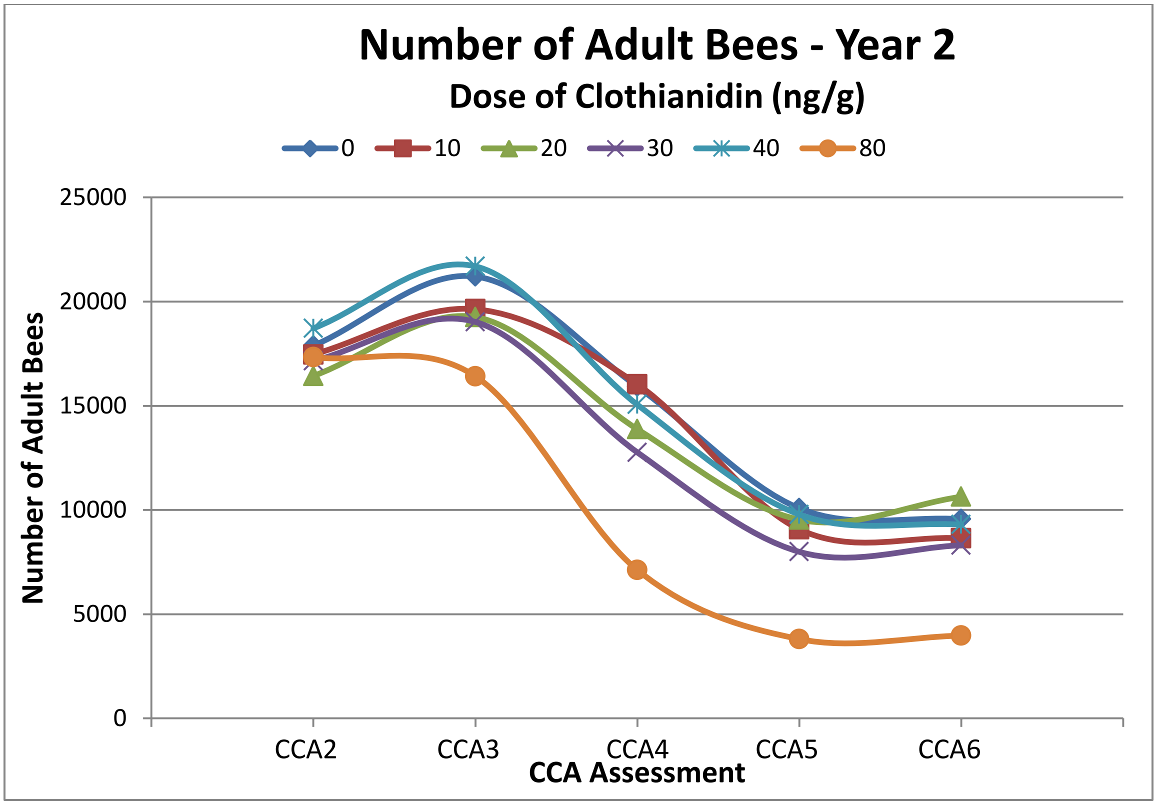
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 3. Data from 2016 Study: Probability value for the contrast of the control to each clothianidin dose at each CCA and for each variable measured. Dark shaded cells indicate significance at P<0.01 and lighter shaded cells at 0.1>P>0.05.

Response and Dose (ng/g)		Probability Value for Contrast of the Control to Each Clothianidin Dose at Each CCA				
		CCA2	CCA3	CCA4	CCA5	CCA6
Adult Bees	10	0.721	0.410	0.519	0.384	0.433
	20	0.611	0.410	0.363	0.551	0.720
	30	0.721	0.410	0.208	0.222	0.325
	40	0.721	0.589	0.519	0.551	0.630
	80	0.721	0.063	0.000	<.0001	0.000
Pupal Cells	10	0.709	0.348	0.069	0.850	0.362
	20	0.709	0.778	0.263	0.850	0.482
	30	0.332	0.444	0.005	0.675	0.482
	40	0.709	0.778	0.015	0.718	0.379
	80	0.745	0.474	<.0001	0.005	0.000
Larval Cells	10	0.668	0.559	0.415	0.819	0.761
	20	0.758	0.881	0.236	0.819	0.761
	30	0.758	0.881	0.052	0.819	0.761
	40	0.451	0.881	0.087	0.632	0.097
	80	0.949	0.542	<.0001	0.013	0.005
Egg Cells	10	0.581	0.865	0.960	0.538	0.924
	20	0.545	0.865	0.861	0.538	0.792
	30	0.573	0.855	0.555	0.537	0.924
	40	0.581	0.702	0.555	0.392	0.499
	80	0.545	0.640	0.312	0.067	0.320
Nectar Cells	10	0.930	0.757	0.902	0.547	0.168
	20	0.654	0.637	0.464	0.756	0.168
	30	0.811	0.890	0.902	0.462	0.168
	40	0.930	0.890	0.902	0.678	0.121
	80	0.930	0.999	1.000	0.756	0.168
Pollen Cells	10	0.387	0.099	0.789	0.744	0.566
	20	0.491	0.099	0.789	0.744	0.900
	30	0.446	0.031	0.086	0.244	0.514
	40	0.510	0.022	0.022	0.099	0.514
	80	0.841	<.0001	<.0001	<.0001	<.0001

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 1. Data from 2016 Study: Mean number of adult bee in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of of clothianidin.



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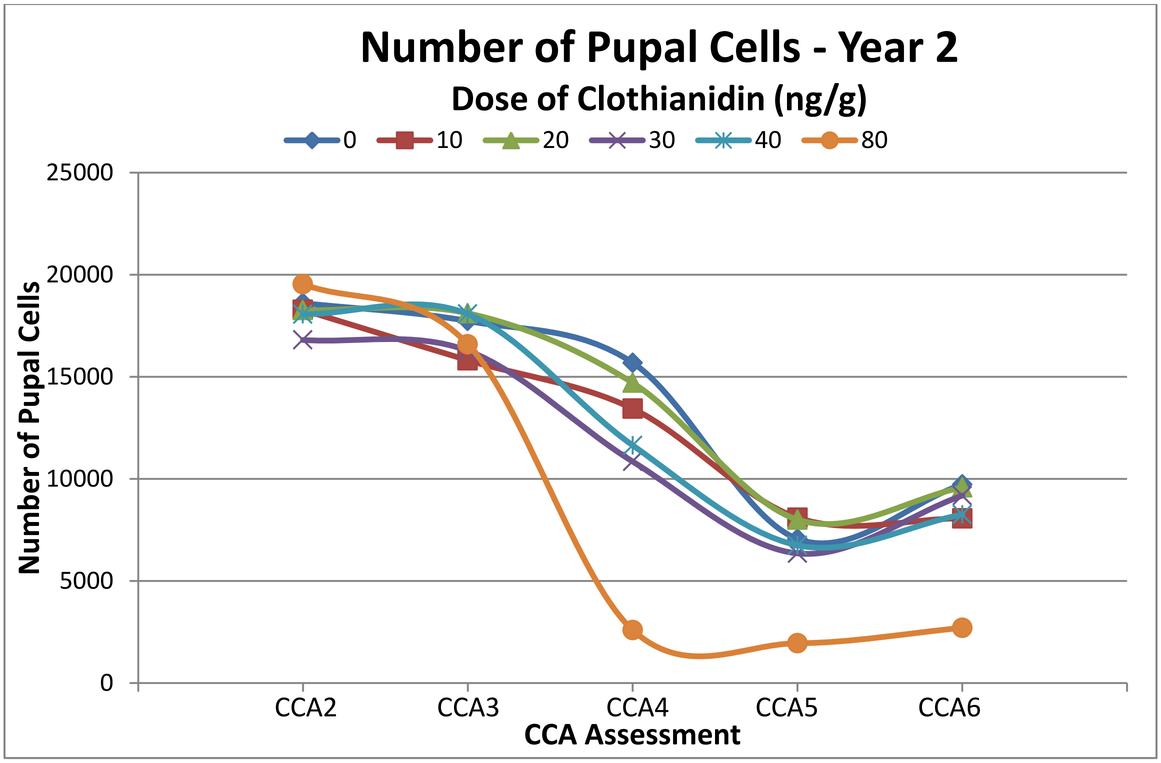
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 2. Data from 2016 Study: Mean number of pupal cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of of clothianidin.



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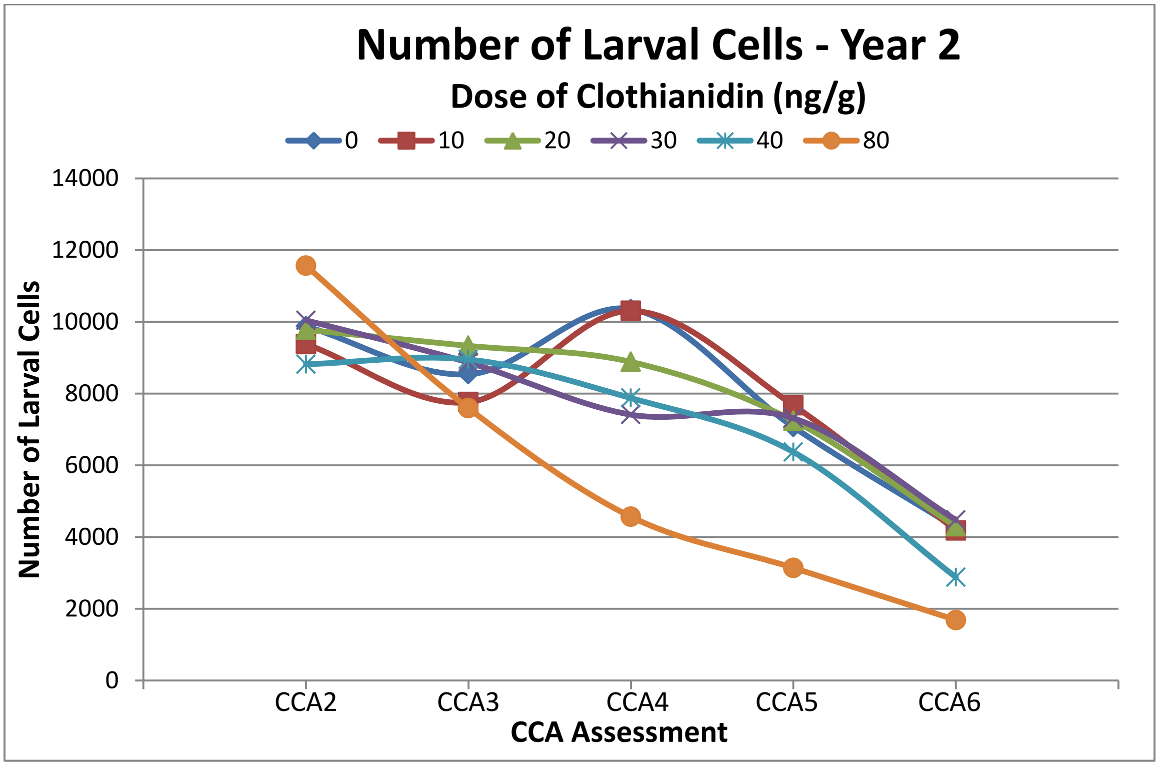
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 3. Data from 2016 Study: Mean number of larval cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of clothianidin.



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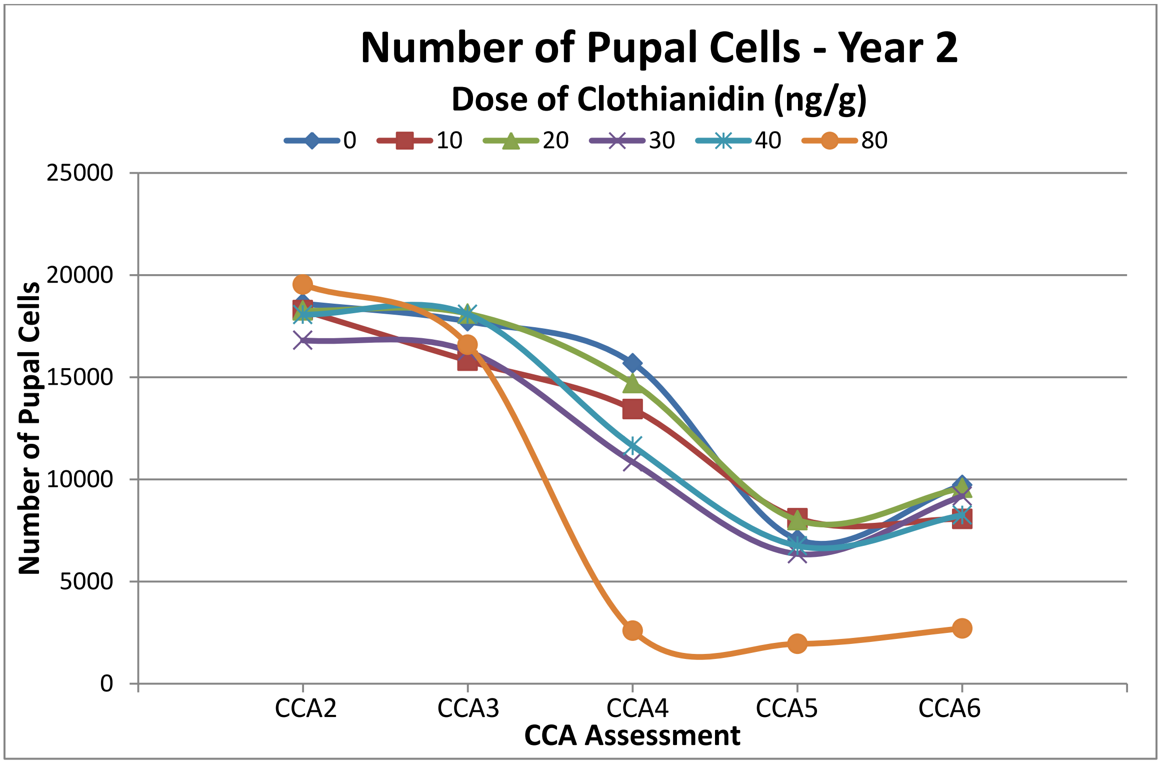
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 4. Data from 2016 Study: Mean number of pupal cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of clothianidin.



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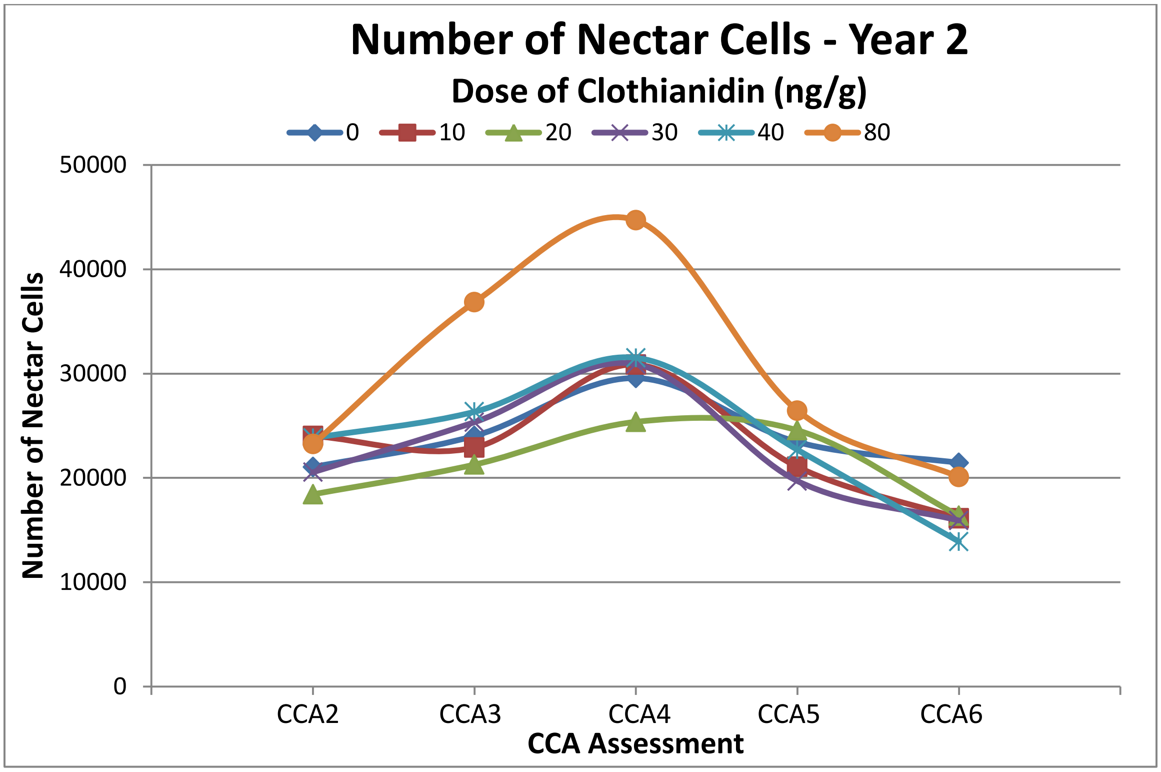
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 5. Data from 2016 Study: Mean number of nectar cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of clothianidin.



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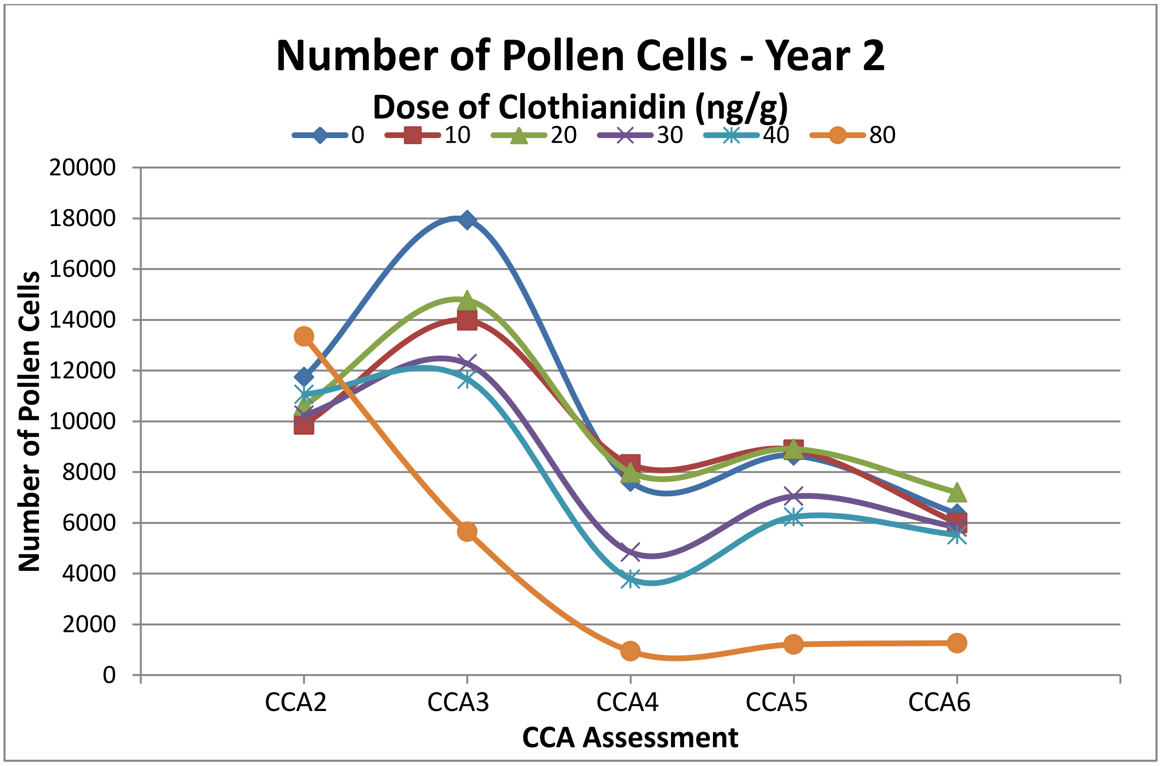
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 6. Data from 2016 Study: Mean number of pollen cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of clothianidin.



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Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

State of California **Department of Pesticide Regulation**

EVALUATION REPORT - Clothianidin Pollen Colony Feeding Study

John Troiano, Research Scientist III

June, 2018

A review of: - Bocksch, S., & Werner, S. (2018). Clothianidin Technical - Honey Bee Brood and Colony Level Effects Following Clothianidin Intake via Treated Pollen in a Field Study in North Carolina - USA 2017. Unpublished study prepared by Eurofins Agroscience Services EcoChem CmbH. 192p., Laboratory Report Number S17-02137. MRID 50478501.
CDPR Study ID 305901.

Introduction

A colony feeding study was conducted to determine the effects of graded levels of clothianidin on the health of honey bee hives where doses mimicked exposure from foraging on pollen. The dose of clothianidin was supplied in a pollen patty, which was a mixture of pollen powder, sugar solution, and additives to stimulate feeding. The patties were placed inside the hives and replenished on a set schedule. The health of hives was determined by Colony Condition Assessments (CCAs) in which measurements were made over time on the number of individuals in each bee life stage in the hive, the storage of honey and pollen food supplies in the hives, and the weight of hives. The design of the study was similar to ones employed in previous feeding studies on potential effects of nectar feeding of imidacloprid, thiamethoxam, and clothianidin on bee hive health. Major differences were:

- A smaller number of replicate apiaries were used: There were 4 instead of 12 replicate apiaries.
- Two replicates of each treatment were located at each apiary: Previous designs included replicates for only the control treatment at each apiary.
- A smaller number of dose levels were used but covered a greater range: Dose levels were 0, 100, 400, and 1600 ng clothianidin/g of patty.

The location of the apiary sites was similar to the sites used for the nectar colony feeding studies where they were located throughout a forested area of North Carolina. The distance between each apiary site was approximately 1 mile apart. The majority of land near the apiaries is non-intensively managed pasture and forest with low potential exposure of bees to pesticides applied for agricultural purposes.

Measurements made over time were indicated by sequential numbering of the colony condition assessments (CCAs). Assessments were made at approximately monthly intervals. For this analysis data obtained from CCAs 2 through 5 were included. Observations at CCA2 were taken one week prior to initiation of clothianidin pollen feeding treatments, which was during the week of July 3, 2017. Observations at CCA3 were taken during the middle of the six week feeding period with CCA4 occurring 6 weeks after the initiation of treatments and CCA5 occurring 10 weeks after the initiation of treatments. Observation of bee and colony health taken during this period was the basis for the determination of No Observed Effects Concentrations (NOECs) and Lowest Observed Effects Concentrations (LOECs) in previous nectar colony feeding studies.

Statistical Analysis

Evaluation of the data followed the statistical approach used by DPR and EPA scientists to analyze previously reviewed neonicotinoid colony feeding studies. Since measurements for each variable were made in each hive over time, the statistical analysis was conducted as a repeated measures over time. Additionally, a mixed model was used where apiary location was identified as a random variable and dinotefuran levels of dose as a fixed effect. The mixed model was chosen because the results of the analysis were to be applied to the larger population of bee hives. Normality tests were conducted for each CCA as indicated by Shapiro-Wilk and Kolmogorov-Smirnov test statistics produced by the PROC CAPABILITY procedure in Statistical Analysis System (SAS, version 9.4). For comparison, data were also transformed to natural logarithms to determine if transformation provided better results. The majority of results indicated that the distributions were normal with the logarithm transformation indicating many instances of non-normality. Based on these results the raw data were used in the analyses. The mixed model approach used to analyze the data included tests to determine the appropriate covariance model that describes the covariance structure reflected by the data. Inclusion of a covariance model in the analysis accounts for heterogeneity of variances that often are measured between treatment levels.

A repeated measures analysis of variance was conducted to determine potential effects of dinotefuran dose on each measurement of hive health over time. Data collected from colony condition assessments (CCAs) numbered CCA2 to CCA5 were included because these are the time intervals (July through September) where effects were observed in the previous neonicotinoid colony feeding studies. Data obtained from CCA6 was excluded because of extreme loss of hives at the 1600 ng/g treatment. Also, hive labeled T1a in the report was excluded from the analysis because the hive was lost and no data was generated after the CCA2 assessment. The PROC MIXED procedure in the Statistical Analysis System (SAS, version 9.4) was used to run the repeated measures effects mixed model. Since measurements of colony health and hive weight were conducted approximately 1 month apart, CCAs were treated as equally spaced intervals. The effects side of the model statement included effects for testing differences in CCAs over time (CCA), differences in response between the levels of dose (Dose), and the potential interaction for effects of dose over time (SAS Program 1 below). The regression model was run twice. First, all dose levels were included which were 0, 100, 400, and 1600 ng clothianidin/g of patty. Based on these results, a reduced model was run that included the concentrations that appeared to define LOEC and NOEC concentrations. The second run was intended to remove extraneous variance produced from treatments that were not contributing information to the model. In order to protect against falsely discovering a significant comparison between mean values, the 'Simulated' option was used to generate comparisons between the control and each dose level (Edwards, D., and Berry, 1987). The 'Simulated' option is a Monte Carlo approach that computes adjusted p-values from simulated distributions based on distributional statistics generated during the analysis.

There were two statements in the mixed model used to analyze the data, where a covariance model could be specified. One was in the 'Random' statement with apiary indicated as a random variable. The second was in the 'Repeated' measures statement where each hive was indicated as the subject for the repeated measure. For the random statement only the Variance Component (VC) model successfully paired with the covariance model specified in the repeated statement: Specifying more complex covariance models in the random statement resulted in indications of converge problems for that model. As observed in the previous colony feeding studies the correlation structure indicated greater correlation between samples taken at close time intervals and, conversely, decreased correlation the further apart the samples were taken in time. Since this structure is normally represented by autoregressive covariance models, the covariance structure for the repeated statement was tested using variance component (VC), compound symmetry (CS), compound symmetry with heterogeneity (CSH), autoregressive first order (AR(1)), autoregressive first order with heterogeneity (ARH(1)), and unstructured (UN) models. Covariance model selection was based on the statistic generated for the Bayesian Information Criteria (BIC) where a lower value of the criterion indicated a better fit of the covariance model. A statistical basis for choosing the appropriate model was determined from Chi-square tests conducted on the difference of the value of the BIC criteria between the two models tested with the number of degrees of freedom determined as the difference between the number of parameters in the model and where the significance level of probability was at 0.01 (Hammer, 2000; Littell et al., 2006). With the VC covariance model specified in the random statement, the best fit covariance models in the repeated statement were AR(1) for adult bees, pupae, larvae, and egg, CS for pollen, ARH(1) for honey and nectar, and UN for hive weight (Table 1). The greater number of parameters for hive weight was due to more sampling intervals included in the analysis. Values for numbers of cells measured for each bee life stage and food supply were divided by 1000 prior to statistical analysis to minimize potential convergence problems due to magnitude of values.

SAS Program 1

```
proc mixed data=a3 order=data;
class apiary dose cca hive rep;
model transvalue =cca dose dose*cca /ddfm=sat htype=1;
random apiary/type=vc;
repeated cca/ subject=hive*rep(dose) type=ar(1);
slice dose*cca /sliceby=cca diff=controll adjust=simulate adjdfe=row;
run;
```

Results

Means and standard deviation for each response variable measured at each dose and each CCA are presented in Appendix A. Data for CCA2 through CCA5 were included in the repeated measures analysis. For the repeated measures regression model that included all levels of dose there were numerous indications of effects due to CCA, dose and dose by CCA interactions (Table 2). The Significant CCA effect indicated changes in overall abundance of the numbers of bee life stages or food supplies over time. Except for egg cells all other variables had an indication of a significant effect due to dose or a dose by CCA interaction. From the pattern observed for the pairwise comparisons, the obvious effect was a sustained and incremental decrease in all measures due to exposure at the highest level at 1600 ng/g of spiked pollen patty as compared to the control values (Table 3). The first measurement of statistical significance occurred first for adult bees at CCA3, which was taken in the middle of the 6 week exposure (Figures 1 through 8). The early onset of loss in numbers of adult bees appeared to have a cascading effect on reduction in the numbers of the other bee life stages and food stores because significant effects for these measures appeared at the later CCAs, starting at CCA4 (Table 3). These results indicated that the 400 ng/g dose level was potentially an NOEC value and 1600 ng/g an LOEC value. Results from the reduced analyses, using data for 0, 400, and 1600 ng/g dose levels, confirmed the proposed NOEC and LOEC values (Tables 4). The same timing of effects was indicated where reduction in the number of adult bees was observed first at CCA3 at the 1600 ng/g treatment followed by reductions in numbers for the other measures at later CCAs. For hive weight, significant reduction was measured at the September 1 sampling interval for the 1600 ng/g dose level, though there was indication in a graphical downward trend in the previous sampling interval (Table 5 and Figure 8). Sampling at September 1 occurred around 2 weeks after the sampling for CCA4 when most of the measures of bee health indicated decreases at the highest dose.

Conclusion

Statistical analyses indicated significant effects of clothianidin dosed in pollen patties on nearly all measures of bee life stages and food stores. The effect was obviously due to the highest treatment at 1600 ng/g. Adults bees were first affected when sampled at CCA3 which was taken mid-way through the dosing period of six weeks. The effect on adult bees was sustained throughout the remainder of the sampling intervals and apparently caused a cascading effect within the hives: Reduction in essentially all other measures of bee and hive health occurred in subsequent CCAs. Ultimately 75% of the hives at the 1600 ng/g dose were lost by the last assessment at CCA6. Reduction in bee life stages and loss of hives provide ample evidence of the detrimental effects that feeding pollen laced patties at 1600 ng/g had on health of bees and the hives. Lack of effects at the next highest dose at 400 ng/g indicated that this is a NOEC value for this set of treatments. Therefore, nominal concentration for the Lowest Observed Effect Concentration (LOEC) was determined to be 1600 ng/g and for the No Observed Effect Concentration (NOEC) was 400ng/g. The study authors reached the same conclusion, stating that the NOEC was established at 400 ng/g. The measured value of clothianidin in the pollen patties in the nominal 400 ng/g treatment group was 372 ng/g, which is the actual NOEC value. This study has been determined to be scientifically sound and can be used quantitatively to assess risks to honey bee colonies.

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Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 1. Mixed Model Analysis of Variance: BIC goodness-of-fit values generated for each covariance model structure tested in the repeated measures analysis of variance program. Shaded cells indicate the covariance structure used for the analysis.

CV Model Tested	Number of Parameters	Model BIC Value for:								
		Adults	Pupae	Larvae	Eggs	Honey	Nectar	Pollen	Number of Parameters	Hive Weight
VC	2	1100.1	1114.6	988.2	941.7	1318.5	1404.5	1012	2	1082.2
CS	3	1087.3	1111.6	984.1	942.2	1256.8	1405.5	1006.3	3	1028.9
AR(1)	3	1078.9	1101.5	984.6	935.8	1227.7	1402.4	1007.5	3	972.4
CSH	6	1085.5	1113.1	985.7	945.3	1245.4	1364.4	996.4	8	1017.8
ARH(1)	6	1075.8	1104.1	986.3	939.4	1213	1362.9	997.3	8	970.4
UN	11	1078.8	1096.3	991	929.5	1197.6	1377.2	992.3	22	930.5

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Table 2. All Dose Levels: Results of the repeated measures mixed model testing the response of each variable to clothianidin dosed surrogate honey.

Mixed Model Results for Repeated Measures Analysis of Variance All Doses of Clothianidin					
Variable	Effect	DF	Den DF	F Value	Pr > F
Adult Bees	cca	3	74	20.65	<.0001
	dose	3	30.1	16.79	<.0001
	dose*cca	9	74	8.81	<.0001
Pupae	cca	3	71.1	36.38	<.0001
	dose	3	30.3	5.73	0.0031
	dose*cca	9	71.1	2.43	0.0179
Larvae	cca	3	76.9	18.09	<.0001
	dose	3	33.4	5.85	0.0025
	dose*cca	9	76.9	1.44	0.19
Eggs	cca	3	74	6.23	0.0008
	dose	3	32.8	1.37	0.27
	dose*cca	9	74	1.7	0.10
Honey	cca	3	47.5	36.04	<.0001
	dose	3	24.6	0.51	0.6759
	dose*cca	9	47.5	2.51	0.02
Nectar	cca	3	42.1	6.83	0.0007
	dose	3	31.4	6.9	0.0011
	dose*cca	9	42.1	3.85	0.0013
Pollen	cca	3	81	66.42	<.0001
	dose	3	24.3	2.12	0.1244
	dose*cca	9	81	3.14	0.0027
Total Brood	cca	3	73.6	35.01	<.0001
	dose	3	33.1	7.03	0.00
	dose*cca	9	73.6	2.43	0.02
Honey + Nectar	cca	3	82.8	3.39	0.0219
	dose	3	38.3	4.36	0.0097
	dose*cca	9	82.8	2.17	0.03
Hive Weight	monthnum	5	27	46.59	<.0001
	dose	3	27	1.92	0.15
	dose*monthnum	15	27	5.26	<.0001

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Table 3. All Dose Levels: Probability value for the contrast of the control to each clothianidin dose at each CCA and for each variable measured.

Response and Dose (ng/g)		Probability Value for Contrast of the Control to Each Clothianidin Dose at Each CCA			
		CCA2	CCA3	CCA4	CCA5
Bees	100	0.710	0.220	0.477	0.709
	400	0.733	0.362	0.515	0.906
	1,600	0.948	<.0001	<.0001	<.0001
Pupae	100	0.830	0.834	0.707	0.628
	400	0.821	0.965	0.583	0.860
	1,600	0.628	0.668	<.0001	0.001
Larvae	100	0.790	1.000	0.996	0.935
	400	0.902	0.999	0.998	0.992
	1,600	0.925	0.828	0.234	0.071
Eggs	100	0.746	0.998	0.705	0.917
	400	0.876	0.998	0.438	0.246
	1,600	0.634	0.995	0.133	0.016
Honey	100	0.870	0.966	0.915	0.510
	400	0.968	0.985	0.977	0.950
	1,600	0.965	0.998	0.980	0.301
Nectar	100	0.935	0.592	0.381	0.916
	400	0.874	0.351	0.085	0.364
	1,600	0.824	0.251	0.001	<.0001
Pollen	10040	0.755	0.907	0.839	1.000
	400	0.952	0.867	0.862	0.942
	1,600	0.977	0.827	0.062	0.083
Total Brood	100	0.855	0.993	0.896	0.867
	400	0.893	0.999	0.806	0.871
	1,600	0.787	0.863	0.0002	0.0004
Honey + Nectar	100	0.888	0.785	0.510	0.741
	400	0.935	0.552	0.189	0.796
	1,600	0.923	0.555	0.001	0.0002

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Table 4. Reduced Dose Levels: Probability value for the contrast of the control to each clothianidin dose at each CCA and for each variable measured.

Response and Dose (ng/g)		Probability Value for Contrast of the Control to Each Clothianidin Dose at Each CCA			
		CCA2	CCA3	CCA4	CCA5
Bees	400	0.639	0.278	0.422	0.854
	1,600	0.905	<.0001	<.0001	<.0001
Pupae	400	0.748	0.940	0.490	0.794
	1,600	0.531	0.577	<.0001	0.0002
Larvae	400	0.850	0.998	0.996	0.983
	1,600	0.881	0.755	0.168	0.045
Eggs	400	0.819	0.996	0.341	0.167
	1,600	0.540	0.992	0.087	0.009
Honey	400	0.957	0.976	0.952	0.909
	1,600	0.952	0.996	0.955	0.239
Nectar	400	0.815	0.292	0.013	0.317
	1,600	0.749	0.205	<.0001	0.0002
Pollen	400	0.922	0.800	0.800	0.904
	1,600	0.962	0.750	0.037	0.051
Total Brood	400	0.841	0.996	0.730	0.810
	1,600	0.716	0.792	0.001	0.0002
Honey + Nectar	400	0.893	0.455	0.142	0.719
	1,600	0.876	0.459	0.001	0.0002

Table 5. Reduced Dose Levels-HiveWeight: Probability value for the contrast of the control to each clothianidin dose at each CCA for hive weight

Response and Dose (ng/g)	Probability Value for Contrast of the Control to Each Clothianidin Dose at Each Weighing Interval					
	Jul 4	Jul 21	Aug 4	Aug 18	Sep 1	Sep 15
Hive Weight						
100	0.880	0.937	0.918	0.896	0.2902	0.2674
400	0.966	0.917	0.805	0.703	0.3928	0.645
1,600	0.974	0.949	0.560	0.196	<.0001	<.0001

Figure 1. Mean number of adult bee in each treatment group measured at every CCA. Treatment levels are 0, 100 , 400, and 1600 ng Clothianidin per g of pollen patty.

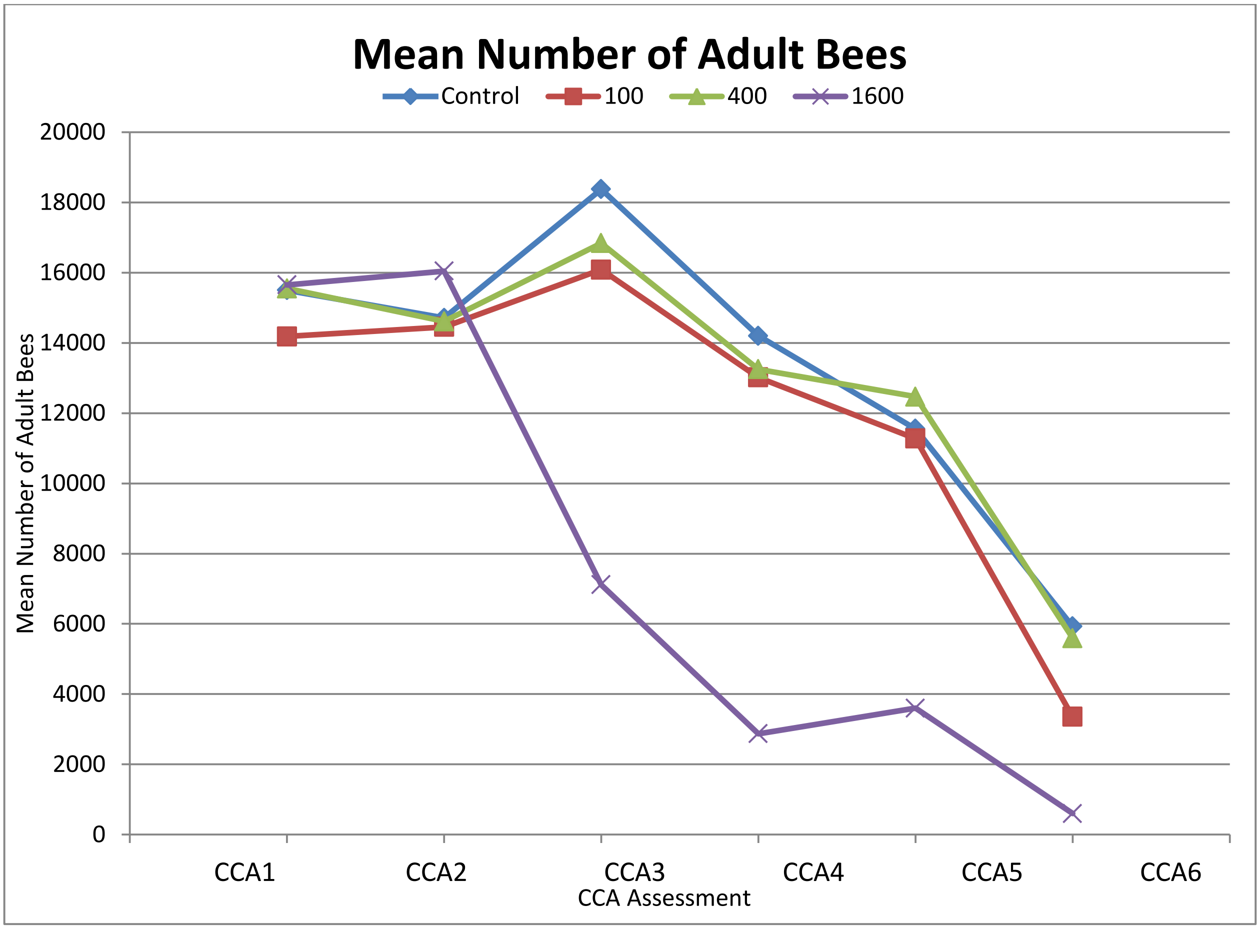

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Figure 2. Mean number of pupal cells in each treatment group measured at every CCA. Treatment levels are 0, 100 , 400, and 1600 ng Clothianidin per g of pollen patty.

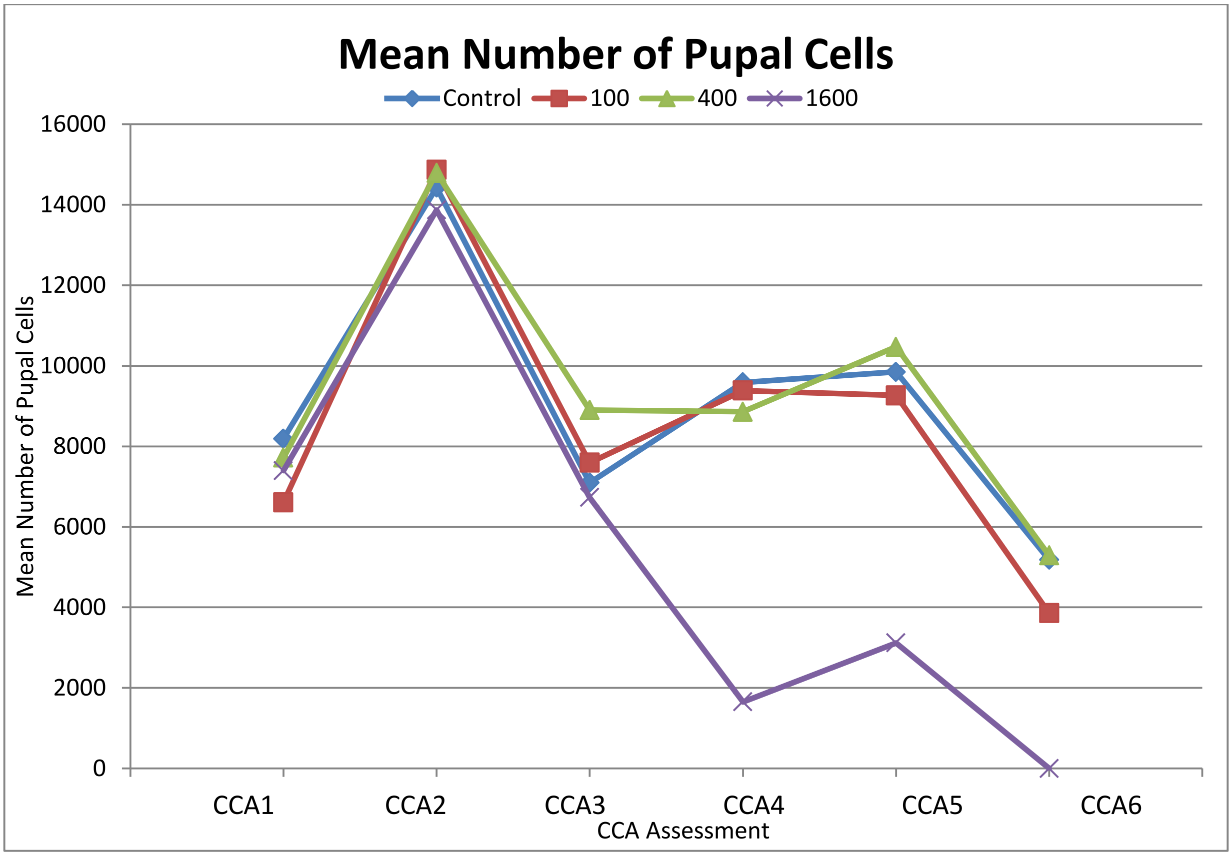

Figure 3. Mean number of larval cells in each treatment group measured at every CCA. Treatment levels are 0, 100 , 400, and 1600 ng Clothianidin per g of pollen patty.

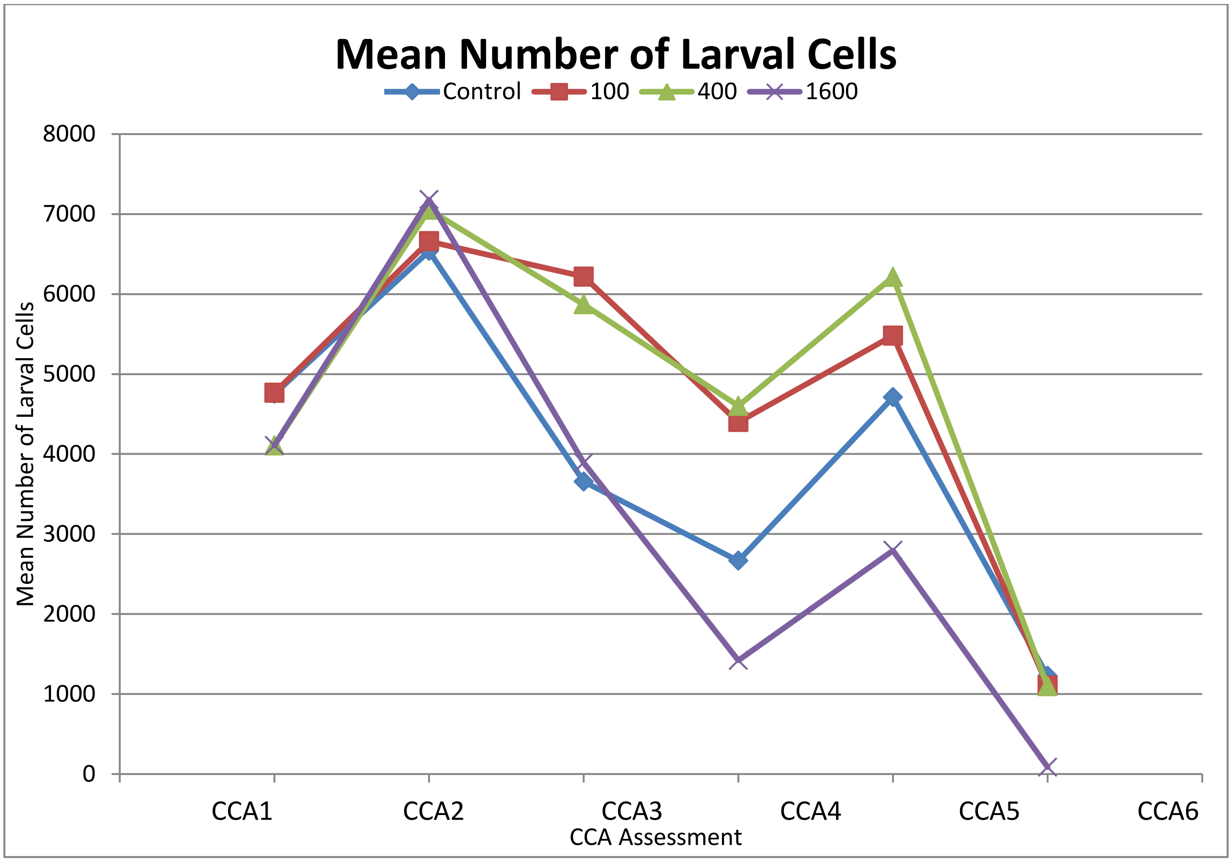

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Figure 4. Mean number of egg cells in each treatment group measured at every CCA. Treatment levels are 0, 100 , 400, and 1600 ng Clothianidin per g of pollen patty.

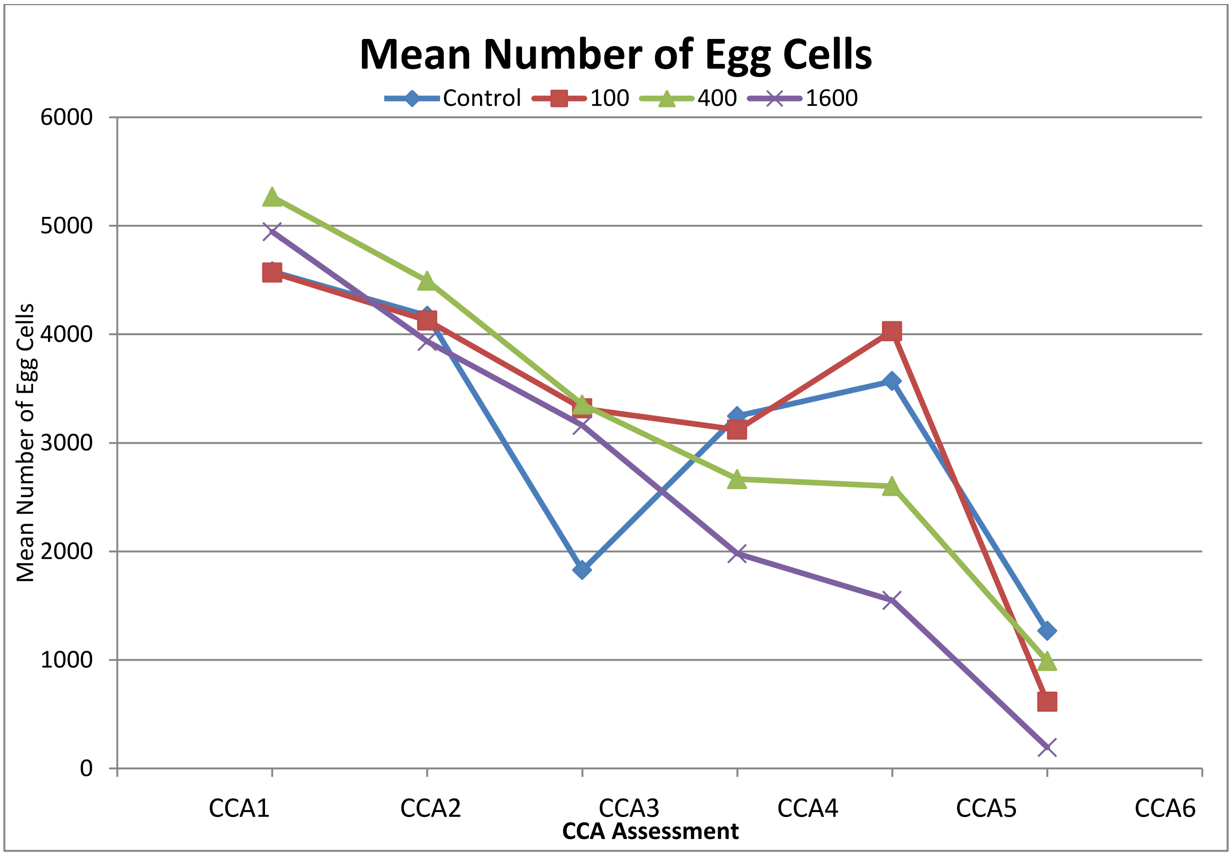

Figure 5. Mean number of honey cells in each treatment group measured at every CCA. Treatment levels are 0, 100 , 400, and 1600 ng Clothianidin per g of pollen patty.

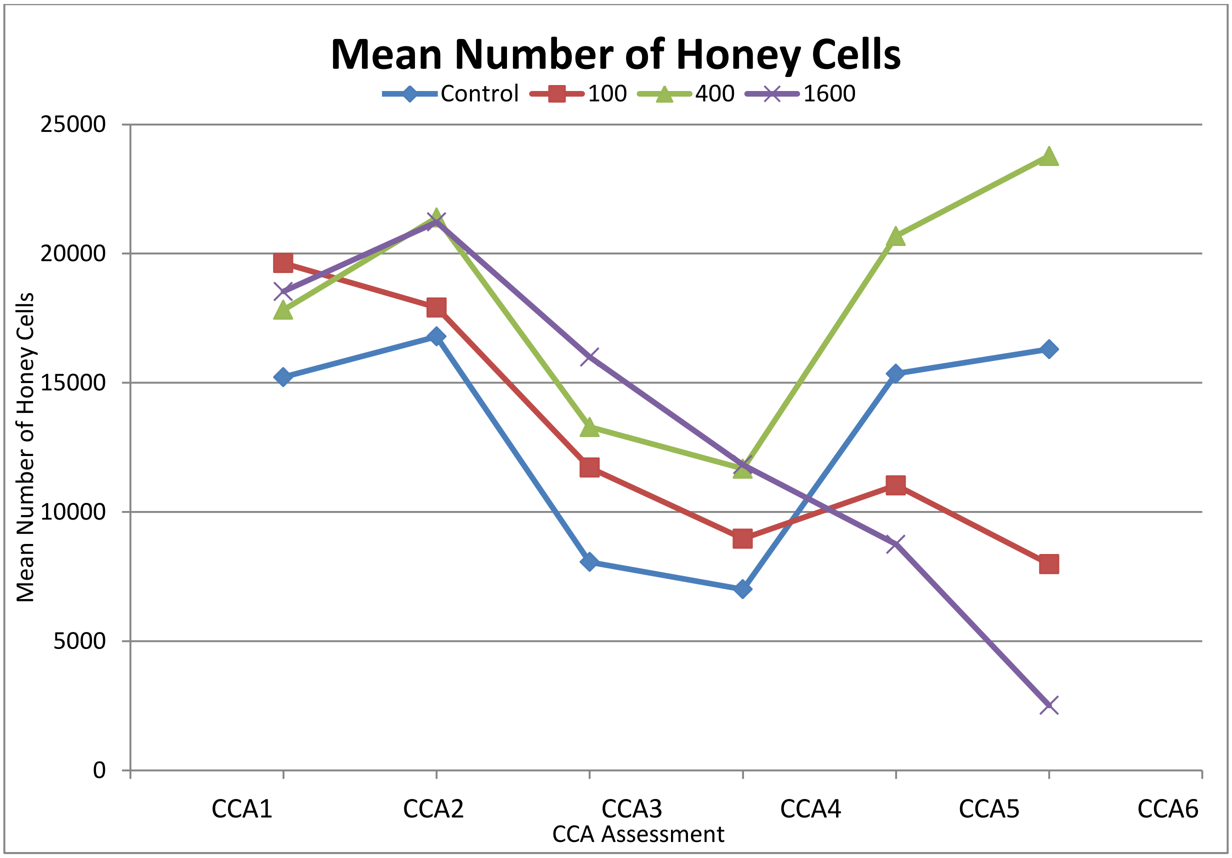

Figure 6. Mean number of nectar cells in each treatment group measured at every CCA. Treatment levels are 0, 100 , 400, and 1600 ng Clothianidin per g of pollen patty.

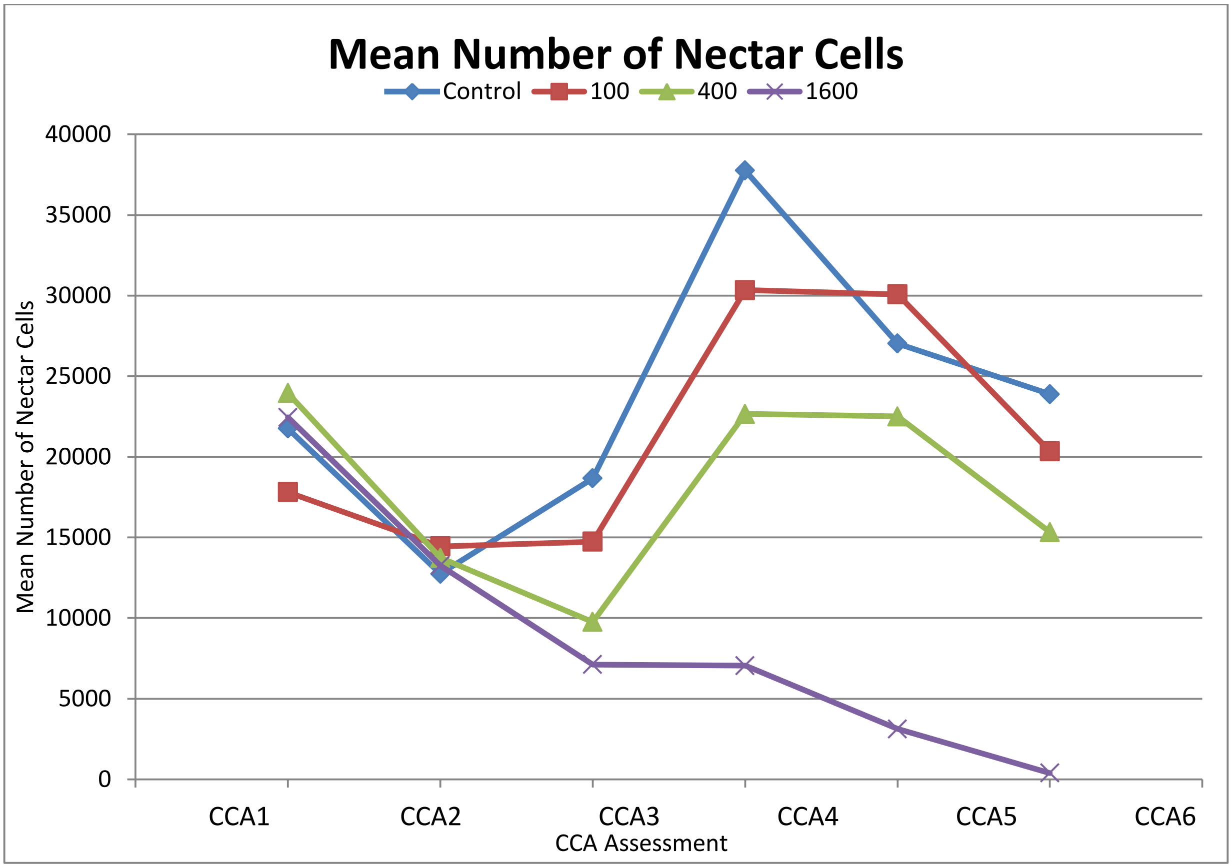

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Figure 7. Mean number of pollen cells in each treatment group measured at every CCA. Treatment levels are 0, 100 , 400, and 1600 ng Clothianidin per g of pollen patty.

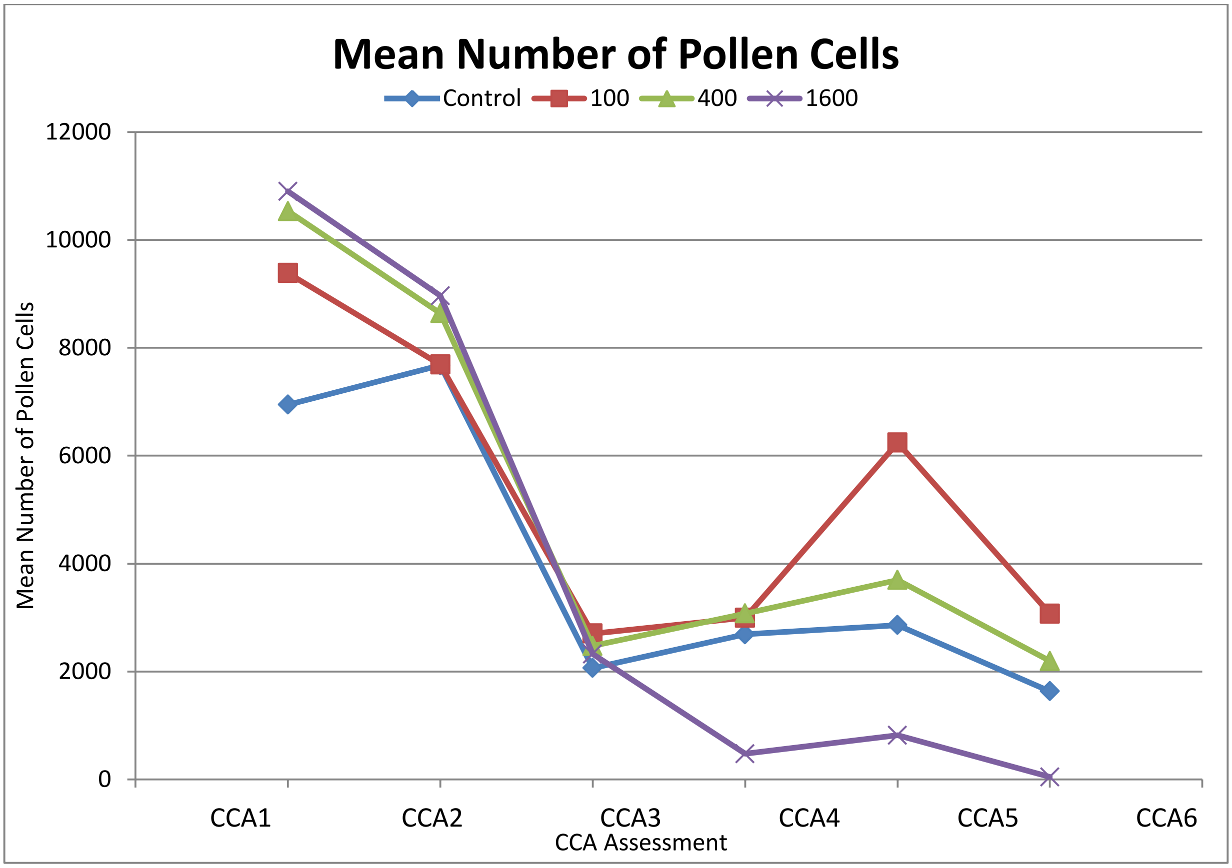

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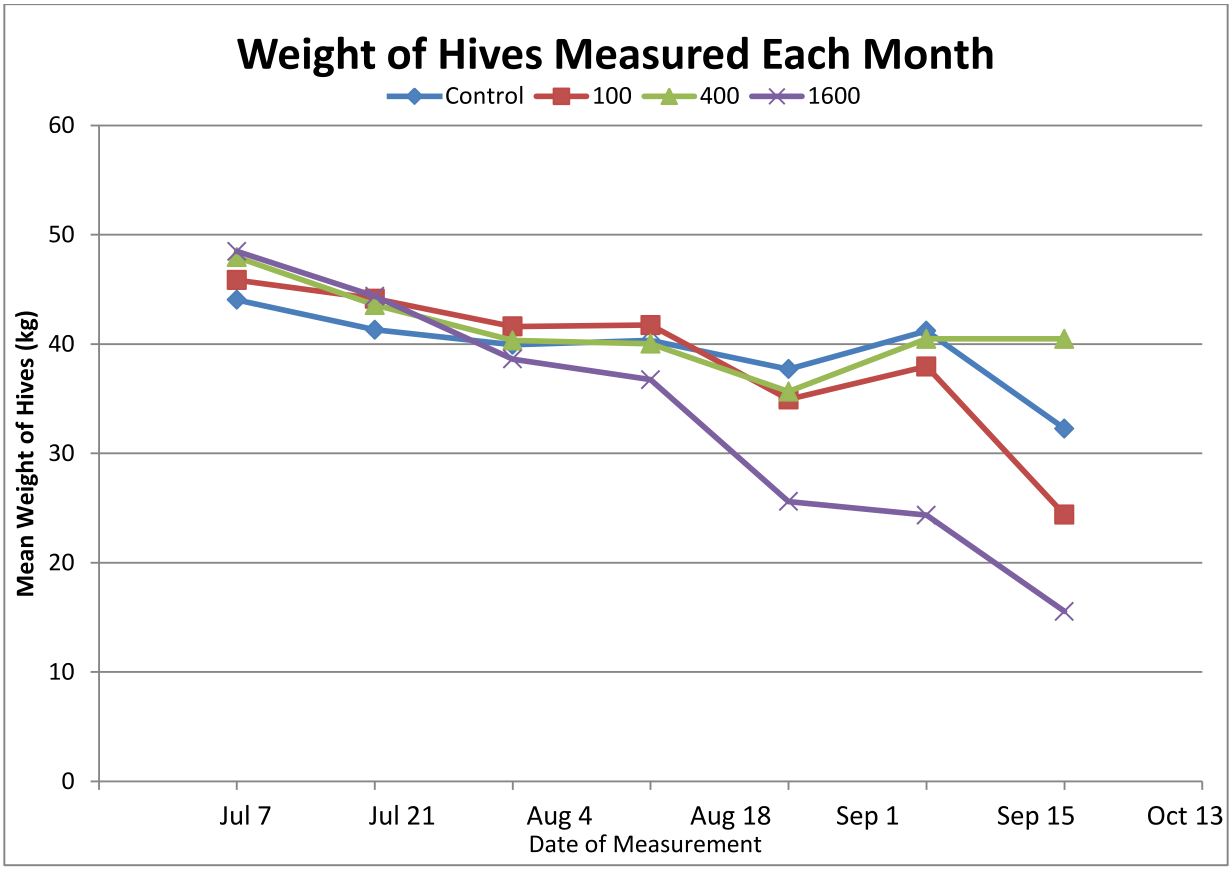
Figure 8. Weight of hives in each treatment group measured at every month. Treatment levels are 0, 100 , 400, and 1600 ng Clothianidin per g of pollen patty.



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APPENDIX A

Clothianidin Pollen Feeding Study

Mean Statistics for Response Variables Measured at Each CCA

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table A-1. Adult Bees: Number of replicate hives (N), mean number of adult bees in hives (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the clothianidin pollen feeding study.

Clothianidin Dose	Counts for Adult Bees Measured at Each CCA						
	Statistic	CCA1	CCA2	CCA3	CCA4	CCA5	CCA6
0 ng/g	N	8	8	8	8	8	8
	Mean	15498.25	14708.88	18377.13	14198.75	11550.38	5925.5
	SD	3056.124	3812.937	3184.335	4187.525	5276.044	2638.949
100 ng/g	N	7	7	7	7	7	7
	Mean	14182.71	14454.14	16083.29	13016.71	11275.86	3354.143
	SD	2096.892	2334.947	2548.907	4044.477	4030.799	3660.29
400 ng/g	N	8	8	8	8	8	8
	Mean	15547.25	14610.88	16839.75	13248.25	12465.88	5589.875
	SD	3078.215	1956.657	4615.185	4432.031	3316.341	3165.588
1600 ng/g	N	8	8	8	8	8	8
	Mean	15652	16043.5	7113.25	2872	3598.625	601
	SD	2824.109	1982.498	1496.583	1369.262	1706.061	1160.374

Table A-2. Pupal Cells: Number of replicate hives (N), mean number of cells in each hive with pupae (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the clothianidin pollen feeding study.

Clothianidin Dose	Counts for Pupal Cells Measured at Each CCA						
	Statistic	CCA1	CCA2	CCA3	CCA4	CCA5	CCA6
0 ng/g	N	8	8	8	8	8	8
	Mean	8191.5	14426.5	7095	9589	9847	5181.5
	SD	4753.23	3790.068	3784.838	5359.867	4219.888	2729.545
100 ng/g	N	7	7	7	7	7	7
	Mean	6609.714	14865.71	7592.571	9386.286	9263.429	3857.714
	SD	1920.086	3745.833	3837.603	3367.362	4801.058	4542.946
400 ng/g	N	8	8	8	8	8	8
	Mean	7718.5	14792	8901	8858	10470.5	5289
	SD	2695.266	3719.793	1992.635	2818.762	4153.962	3402.633
1600 ng/g	N	8	8	8	8	8	8
	Mean	7396	13867.5	6729.5	1655.5	3117.5	0
	SD	4421.628	2519.395	2123.421	1843.205	3297.53	0

Table A-3. Larval Cells: Number of replicate hives (N), mean number of cells in each hive with lavae (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the clothianidin pollen feeding study

Clothianidin Dose	Counts for Larval Cells Measured at Each CCA						
	Statistic	CCA1	CCA2	CCA3	CCA4	CCA5	CCA6
0 ng/g	N	8	8	8	8	8	8
	Mean	4751.5	6536	3655	2666	4708.5	1225.5
	SD	2719.462	1341.789	1960.563	1710.143	2463.416	883.9313
100 ng/g	N	7	7	7	7	7	7
	Mean	4766.857	6658.857	6216.571	4398.286	5479.429	1105.714
	SD	2540.936	1126.63	2958.248	1328.07	2971.552	1263.379
400 ng/g	N	8	8	8	8	8	8
	Mean	4106.5	7052	5869.5	4601	6213.5	1096.5
	SD	1627.683	2150.246	1743.035	2275.81	2639.814	1019.381
1600 ng/g	N	8	8	8	8	8	8
	Mean	4106.5	7181	3891.5	1419	2795	86
	SD	1593.571	2445.876	1083.688	936.4584	1055.285	243.2447

Table A-4. Egg Cells: Number of replicate hives (N), mean number of cells in each hive with eggs (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the clothianidin pollen feeding study

Clothianidin Dose	Counts for Eggs Cells Measured at Each CCA						
	Statistic	CCA1	CCA2	CCA3	CCA4	CCA5	CCA6
0 ng/g	N	8	8	8	8	8	8
	Mean	4579.5	4171	1827.5	3246.5	3569	1268.5
	SD	886.3187	2285.077	1479.421	2017.272	2253.416	1140.687
100 ng/g	N	7	7	7	7	7	7
	Mean	4570.286	4128	3317.143	3120.571	4029.714	614.2857
	SD	1118.472	1263.937	2029.586	1972.205	1943.06	665.0949
400 ng/g	N	8	8	8	8	8	8
	Mean	5267.5	4493.5	3354	2666	2601.5	989
	SD	1290.205	1767.115	1019.64	1184.537	1231.547	654.9565
1600 ng/g	N	8	8	8	8	8	8
	Mean	4945	3934.5	3160.5	1978	1548	193.5
	SD	1239.457	1314.543	985.6558	1488.144	675.6026	425.6783

Table A-5. Honey Cells: Number of replicate hives (N), mean number of cells in each hive with honey (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the clothianidin pollen feeding study

Clothianidin Dose	Statistic	Counts for Honey Cells Measured in Hives at Each CCA					
		CCA1	CCA2	CCA3	CCA4	CCA5	CCA6
0 ng/g	N	8	8	8	8	8	8
	Mean	15222	16791.5	8062.5	7009	15351	16297
	SD	9074.412	11612.93	8879.236	7028.739	11526.66	11892.63
100 ng/g	N	7	7	7	7	7	7
	Mean	19632.57	17912.57	11720.57	8968.571	11032.57	7985.714
	SD	8172.22	8463.862	7816.341	6431.381	8956.356	13603.54
400 ng/g	N	8	8	8	8	8	8
	Mean	17823.5	21392.5	13287	11674.5	20683	23779
	SD	7853.527	10342.88	7605.74	7453.455	14040.25	17802.68
1600 ng/g	N	8	8	8	8	8	8
	Mean	18533	21220.5	15996	11825	8750.5	2515.5
	SD	10138.52	10908.47	12197.28	10357.51	9078.806	6260.515

Table A-6. Nectar Cells: Number of replicate hives (N), mean number of cells in each hive with honey (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the clothianidin pollen feeding study.

Clothianidin Dose	Statistic	Counts for Nectar Cells Measured in Hives at Each CCA					
		CCA1	CCA2	CCA3	CCA4	CCA5	CCA6
0 ng/g	N	8	8	8	8	8	8
	Mean	21758	12749.5	18662	37754	27025.5	23886.5
	SD	10917.79	4640.301	31103.68	14616.1	15436.38	13867.22
100 ng/g	N	7	7	7	7	7	7
	Mean	17814.29	14448	14742.86	30345.71	30075.43	20345.14
	SD	7591.975	6572.861	16093.64	26333.02	8076.939	20059.63
400 ng/g	N	8	8	8	8	8	8
	Mean	23951	13738.5	9761	22661	22510.5	15329.5
	SD	8446.158	5284.978	13910.83	12146.15	9625.169	9003.547
1600 ng/g	N	8	8	8	8	8	8
	Mean	22446	13265.5	7116.5	7052	3117.5	387
	SD	10251.79	3587.271	6270.633	4870.104	1902.997	722.4585

Table A-7. Pollen Cells: Number of replicate hives (N), mean number of cells in each hive with pollen (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the clothianidin pollen feeding study

Clothianidin Dose	Statistic	Counts for Pollen Cells Measured in Hives at Each CCA					
		CCA1	CCA2	CCA3	CCA4	CCA5	CCA6
0 ng/g	N	8	8	8	8	8	8
	Mean	6944.5	7675.5	2064	2687.5	2859.5	1634
	SD	1796.762	3352.818	1332.306	1140.687	1366.561	1129.751
100 ng/g	N	7	7	7	7	7	7
	Mean	9386.286	7690.857	2702.857	2997.714	6241.143	3071.429
	SD	3770.76	2372.341	1478.648	2136.443	4035.852	3560.343
400 ng/g	N	8	8	8	8	8	8
	Mean	10535	8643	2472.5	3074.5	3698	2193
	SD	4232.888	4032.448	2276.159	2775.606	1129.751	3075.112
1600 ng/g	N	8	8	8	8	8	8
	Mean	10900.5	8965.5	2322	473	817	43
	SD	3511.662	1809.653	1418.348	438.5157	534.1107	121.6224

Table A-8. Hive Weight: Number of replicate hives (N), mean number of cells in each hive with pollen (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the clothianidin pollen feeding study

Clothianidin Dose		Weight of Hives Measured at Each Month						
	Statistic	Jul 4	Jul 21	Aug 4	Aug 18	Sep 1	Sep 15	Oct 1
0 ng/g	N	8	8	8	8	8	8	8
	Mean (kg)	44.05	41.3	39.925	40.35	37.7	41.2	32.275
	SD (kg)	7.328808	6.55526	3.365264	4.648502	3.732483	5.267691	14.57785
100 ng/g	N	7	7	7	7	7	7	7
	Mean (kg)	45.85714	44.14286	41.6	41.74286	34.94286	37.94286	24.4
	SD (kg)	6.489699	6.307365	2.620433	4.11779	4.287523	3.279663	23.54768
400 ng/g	N	8	8	8	8	8	8	8
	Mean (kg)	47.95	43.575	40.325	40.025	35.65	40.475	40.475
	SD (kg)	8.75557	8.930486	4.032635	4.396671	4.275846	5.65376	5.65376
1600 ng/g	N	8	8	8	8	8	8	8
	Mean (kg)	48.475	44.375	38.625	36.75	25.6	24.35	15.55
	SD (kg)	7.660614	7.350364	8.75406	6.989584	6.731589	6.811545	12.19871

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Data Evaluation Record

Study Titles:

Bocksch, S. (2015): Thiamethoxam Technical - Honey Bee Brood and Colony Level Effects Following Thiamethoxam Intake via Treated Sucrose Solution in a Field Study in North Carolina

Final Report Source:
Eurofins Agroscience Services, unpublished report No: S14-02633, 30 Oct 2015

Year of study: 2014-2015

PMRA#:
PMRA DACO#:
MRID: 49757201

Study Type:
Tier II colony feeding study conducted in an open field

Review Date (final): February 10, 2017

Health Canada Pest Management Regulatory Agency (PMRA) reviewer:
Primary Evaluator: Barbara Martinovic Barrett, Sr. Senior Evaluation Officer
Secondary Evaluator: Connie Hart, Senior Science Advisor
Data Statistical Analysis: Keith O'Rourke, Senior Epidemiologist/Bio-statistician

United States Environmental Protection Agency (EPA) reviewer:
Primary Evaluator**:** Ryan Mroz, Biologist
Secondary Evaluator: Kristina Garber, Senior Science Advisor
Data Statistical Analysis: Christine Hartless, Wildlife Biologist

CDPR reviewer:
Primary Evaluators: Richard Bireley, Sr. Environmental Scientist (Specialist)
Alexander Kolosovich, Environmental Scientist
Russel Darling, Environmental Scientist
Brigitte Tafarella, Environmental Scientist
Denise Alder, Sr. Environmental Scientist (Specialist)
Data Statistical Analysis: John Troiano, Ph.D., Research Scientist III

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

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Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Executive Summary

A colony feeding study was conducted with honey bees (*Apis mellifera* L.) to assess the potential for long-term effects, including overwintering survival, resulting from exposure to thiamethoxam. The study was conducted in twelve rural test areas lacking extensive acreages of crops treated with pesticides (Apiaries A – L) in North Carolina from June 27, 2014 (hive installation) to April 28, 2015 (last colony assessment). Colonies were divided into 12 groups of 7 colonies placed at the different apiaries. These 84 hives were used for biological assessments and an additional hive was placed in each apiary for residue analysis and pollen source identification (96 total hives). Hives were divided according to hive strength (total brood coverage) with the strongest 7 hives assigned to Apiary A and the weakest 7 hives assigned to Apiary L. Within each apiary, the 7 hives were randomly assigned to treatment groups.

At each apiary, five test hives were artificially fed with 50% sugar solution spiked with thiamethoxam at 12.5, 25, 37.5, 50 or 100 µg a.i/L for six weeks continuously in the field, with two hives serving as controls and one hive for pesticide residues and pollen source identification (8 hives/Apiary). Assuming the density of a 50% sugar solution is 1.2296 g/ml, the reviewer calculated that the test concentrations at 12.5, 25, 37.5, 50, and 100 µg/L are equivalent to 10.2, 20.3, 30.5, 40.7, and 81.3 ppb (µg a.i/kg-solution), respectively. Residues of thiamethoxam in the dose verification samples were 9.3 ppb (Tl; 12.5 ppb), 24.1 ppb (T2; 25 ppb), 29.5 ppb (T3; 37.5 ppb), 39.7 ppb (T4; 50 ppb) and 73.7 ppb (T5; 100 ppb). Relative to nominal this is equivalent to 91.4% (Tl), 118.7 % (T2), 96.8 % (T3), 97.5 % (T4) and 90.7 % (T5). Clothianidin was not detected in any of the test solutions (Level of Detection = 0.25 ppb).

Ten Colony Condition Assessments (CCAs) were conducted during the study. The two initial CCAs (CCA1 and CCA2) were conducted prior to exposure to thiamethoxam through feeding. The objectives of CCAs 1 and 2 were to determine hive strength and initial hive conditions and to select the 84 hives subject to the treatment and to be used as controls. The third CCA was conducted just prior (one week) to dosing with thiamethoxam. A CCA was conducted during the feeding period with another one conducted shortly after the feeding period (CCA4 and CCA5, respectively) which characterize hive conditions during exposure. Three more CCAs were conducted at 10, 13, and 16 weeks after exposure (CCA6, CCA7, and CCA8 respectively) to assess the chronic effect following exposure to thiamethoxam and to characterize pre-overwintering hive conditions. Two final CCAs were conducted after overwintering in March 2014 (CCA9) and April 2015 (CCA10) to assess potential exposure impact on survival and chronic colony level effects. Due to substantial overwinter failure in control hives (79%), the biological results from CCA9 and CCA10 are not considered scientifically valid and are not considered in this DER.

Levels of thiamethoxam and its major metabolite (CGA322704 – clothianidin) residues in hives were measured before, during and after the feeding exposure. Multiple parameters, such as hive weight, number of individuals at different life stages in the hive, hive honey and pollen stores, and hive overwintering survival, were measured during the course of the study. Pollen was collected from monitoring hives at each site for assessment of forage availability and the local pollen flora was also analysed for 174 active ingredients using multi residue methods for assessment of potential exposure to pesticides from sources other than the dosing solution. Varroa and Nosema was also measured throughout the study.

Potential test colony contamination with pesticides from other food sources was monitored using pollen collected from the additional hive at each apiary that served as a monitoring hive. The results showed that while there were a few instances of thiamethoxam detected in the pollen of the control hives, the frequency and magnitude of these detections is not expected to confound the results of this study. Maximum residues measured at CCA5 (end of exposure) in hive matrices demonstrated 80, 68, 64, 70, and 43% of nominal concentrations in pollen and in honey 142, 110, 75, 124, and 102% of nominal concentrations in the 12.5, 25, 37.5, 50, and 100 µg/L treatment groups respectively. Variability was apparent, as many of the hives had minimum measured residue levels in pollen of 21, 0, 28, 22, and 25%; and in honey of 0, <LOD (0.25 µg a.i./kg), 0, <LOD-, and 0% of nominal concentrations in the 12.5, 25, 37.5, 50, and 100 µg/L respectively. Dilution is expected since bees forage on outside pollen and nectar sources, and hive pollen (bee bread) includes only a small amount of nectar. Clothianidin (CGA322704) residues ranged from <LOD (0.25 µg a.i./kg) - <LOQ (0.5 µg a.i./kg) in nectar/honey of biological hives, dose verification and stability samples. In one treatment (T3) CGA32274 was detected at the end of the exposure period with a maximum residue value of 1.362 µg a.i./kg. See **Section 3.7** for more details regarding the residues of thiamethoxam and CGA32274 in the dosing solutions and hive matrices.

In the control colonies, the number of adults increased until CCA 5 (august) and then started to decline. This trend was also observed in open cells and pollen, and was very evident with capped cells, whereby a dramatic decline was observed following CCA 5. The number of eggs appeared to drop slightly at CCA 5, but then increased by CCA 6 before a drop in numbers at CCA 7. The decline in the number of live bees after CCA 5 (August) is likely the result of a later start date for the thiamethoxam study, which resulted in the 6th colony condition assessment being taken in late September, which is closer to the period of time in which the colony is preparing for overwintering. As a result, any effects observed in the thiamethoxam study at CCA 5 were difficult to follow to CCA 6 (or thereafter) for potential recovery (or reversion) of effects, since the control colony was declining in numbers at this time, resulting in less sensitivity in the analysis.

In the highest test treatment (100 µg/L), statistical reductions relative to the control ($p<0.05$) were observed across several different endpoints and at several CCAs within an endpoint. Relative to the control, decreases were observed in the number of adults, brood (eggs, larvae, pupae), and food stores (pollen). Significant decreases started at CCA4 (mid exposure period) for pollen and pupae and total number of individuals, and at CCA5 (end of exposure period) for the remaining measured endpoints. Significant reductions were seen through CCA6 for number of eggs and larvae while through CCA7, number of individuals, pupae, and pollen. At CCA 8 (late October), most of the response variables were decreased from CCA7; however, by this time all hives should have been winding down for winter. Preparation for overwintering could have begun as early as CCA6, which would comparisons to controls for most endpoints relative to control hives were are also in decline. Only the number of adults in the 100 µg/µg/L treatment ($p<0.05$) showed significant reductions compared to the control past CCA6 for non-food response variables.

Several other significant statistical differences relative to control hives were observed in the next to highest treatment group (50 µg/L) at CCA5. Pupae, larvae, and total individuals showed statistically significant reductions ($p<0.05$), while adults and pollen showed marginal significant reductions ($0.05<p<0.1$) at this CCA (the end of the exposure period) compared to the control.

Since the control colonies were in decline by CCA 6 (possibly in preparation for overwintering), it is not possible to discern effects from the control at any time point following CCA 5.

With regards to all other treatments (12.5, 25, and 37.5 µg/µg/L), most endpoint responses were not significantly different from the control ($p>0.05$). However, there were two endpoints for which a statistical reduction in these treatments was observed after exposure. The number of pupae were reduced ($p<0.05$) in the 37.5 µg/µg/L treatment group at CCA5. Also at CCA 4 ($0.05<p<0.1$), 5 and again at CCA7, the number of pollen cells were reduced ($p<0.05$) in the 25 µg/µg/L treatment group (no difference at CCA6). Additionally, prior to exposure, hives in the 25 µg/µg/L treatment group were reduced compared to the controls for Eggs ($0.05<p<0.1$), as well as larvae and pupae ($p<0.05$). There were also transient reductions of marginal significance ($0.05<p<0.1$) in the 12.5 treatment group at CCA6 for eggs and CCA5 for honey cells.

Other combination parameters such as total life (adults, eggs, larvae, and pupae), brood, and food stores (honey and pollen) showed similar patterns of significant reductions *i.e.* in the highest treatment group (100 µg/µg/L) relative to the control.

Honey cell counts were not significantly ($p<0.05$) reduced for any treatment level relative to the controls.

In summary, clear decreases in multiple endpoints (relative to controls) and declining trends were observed over several CCAs in colonies exposed to 100 µg/L thiamethoxam. In addition, larvae, pupae, pollen and adults were declining in the 50 µg a.i/L group shortly after exposure. Some effects were also observed in some endpoints at some CCAs in bees exposed to 25, and 37.5 µg/L thiamethoxam. At the lowest test level, i.e., 12.5 µg/µg/L, except for eggs at CCA6 and honey at CCA5 (both $0.05<p<0.1$), numbers of bees and food stores were similar in numbers and trends compared to controls (i.e., no significant differences noted).

It is important to recognize the additional inherent strengths and limitations of this study as results are interpreted and potentially considered in risk assessment. In the context of available field studies involving honey bees and thiamethoxam, this study contains a number of strengths including:

- Use of a high degree of replication (n=12) to achieve a reasonable level of statistical power
- Demonstration of a generalized concentration-response relationship with respect to the concentration of thiamethoxam in sucrose solution and the magnitude and duration of adverse effects (at least for the 50 and 100 **µ**g/L treatment groups**),**
- Quantification of exposure to parent (thiamethoxam) and toxicologically-relevant metabolites (i.e., clothianidin) in diet and in hive matrices (uncapped nectar, pollen, honey, bee bread)
- Use of a 6-week exposure duration to represent a “high end” exposure scenario,
- Inclusion of multiple colony-level endpoints reflecting hive strength, brood development and food stores
- Detailed QA/QC results regarding quantification of chemical residues in various matrices (screen for 174 pesticides/degradates) to better understand other chemical exposures bees encounter.

- Availability of raw data for conducting statistical analysis.

A number of limitations are also noted with this study, including:

- Exposure of bees to thiamethoxam occurred through nectar (sucrose) alone, whereas bees in the field are likely exposed through both pollen and nectar routes. Therefore, the design of this study may not reflect a "worst case" exposure scenario in which bees are experiencing prolonged exposure to both contaminated nectar and pollen as may occur through exposure to a treated crop. While exclusion of the pollen route is expected to underestimate overall exposure, the impact of this exclusion on the study results is uncertain and will likely depend on the life stage/caste of bee.

- Overwintering success of controls was severely impacted (79% hive mortality). In fact, no hives survived overwintering in any significant proportion. This makes interpretation of results difficult. If control hives had survived, comparing trends or lack thereof with the treatment hives would be more meaningful.

- The later date of initiation for the study resulted in CCA 6 (and onward) occurring at the time of overwintering preparation. Measurements at CCA 5 were taken in August, and CCA 6 was taken in September. As observed in the control colony, by CCA 6, the number of total individuals, open cells, capped cells, adults and pollen were all in decline following CCA 5. This resulted in difficulty assessing potential "reversion of effects" (in comparison to the control).

- Thiamethoxam was found in both hive nectar and hive pollen (bee bread), at concentrations ranging from <LOQ to greater than the specific colony feeding solution. Dilution compared to the treatment feeding solution is possible since bees foraged on outside nectar and pollen sources. Additionally, bee bread contains only a small quantity of hive nectar, thus would not be expected to have a concentration equivalent to nectar alone. Therefore, exposure through both bee bread and nectar occurred via exposure to the sucrose feeding solution. Since bees were forced to forage for pollen, the potential impact of thiamethoxam exposure on reducing pollen foraging efficiency of bees could be incorporated into the overall expression of adverse effects. Had contaminated pollen been provided to bees, it is not known if the potential impact on pollen foraging efficiency would have been masked.

- Hive detections of pesticides from food sources other than thiamethoxam and metabolite was detected during the exposure period and post-exposure periods through analysis of pollen from pollen traps. Although the study was deliberately conducted in a low agricultural area in order to minimize the potential for pesticide contamination from other sources, the bees still appeared to be foraging on contaminated pollen and possibly nectar. During both exposure and post-exposure periods, a high level of multiple pesticides that may cause concern for bees were detected in most monitoring hives. Acephate (1 sample, 1600 ppb), Carbaryl (1 sample, 214 ppb), Carbendazim (5 samples, traces - 1300 ppb), Imidacloprid (2 samples, 4.3 and 6.1 ppb), Methamidophos (1 sample, 109 ppb), Thiamethoxam (2 samples, 8.8 ppb and 11.3 ppb), and Thymol (23 samples, 405 ppb to

12300 ppb; during period of Varroa treatment) accounted for the highest residue levels found in pollen samples.

- Exposure dilution during the study was evident. The exposure, based on residues measured in the hive (hive nectar and hive pollen) indicated that, overall, higher measured hive residues correlated with higher nominal residues in feeding solutions. However, individual hive residue values varied, and there was some overlap in measured values. Pollen storage was observed consistently in the control hives and hives exposed to lower test concentrations during the exposure period, indicating that test bees were foraging on food sources other than the spiked sugar solution. Remarkably lower residue concentrations detected in bee bread and hive nectar in some test hives compared to the feeding concentrations may also indicate foraging on other food sources.

This study is considered scientifically valid; however, it is classified Supplemental due to the key limitations listed below:

- late timing of exposure that coincides with ramping down trends of colony endpoints,
- lower than expected performance of controls, and
- lack of overwintering success.

Based on the limitations of this study, A NOAEC derived from this study is considered highly uncertain. Effects were observed to multiple endpoints and multiple CCAs at the highest test level (i.e., 100 µg a.i./L thiamethoxam; 86 µg a.i./L clothianidin-equivalents). Effects to several endpoints (number of adults, amount of brood) were also observed at CCA5 of the second highest test level (i.e., 50 µg a.i./L thiamethoxam; 43 µg a.i./L clothianidin-equivalents[1]). It is uncertain whether or not effects at 50 µg a.i./L are meaningful to the colony since these effects were only observed at CCA5, which is the conclusion of the exposure window, however, potential recovery could not be determined due to the limitations of CCA6, 7, and 8 (during downward trend of colonies) and a lack of overwintering data. Also, the utility of CCAs 6, 7 and 8 in showing treatment related effects are questionable because of the downward trend in endpoints that is consistent with preparation of colonies for winter. If effects observed at 50 µg a.i./L are biologically significant to the colonies, the NOAEC from this study would appear to be 37.5 µg a.i./L (32 µg a.i./L-clothianidin equivalents). There is uncertainty in whether or not this value is conservative. Since the hives did not perform as expected, and given the late timing of the exposure window, it is uncertain whether or not effects due to thiamethoxam could be detected.

A comparison can be made between the effects of this study and those in the CFS with clothianidin (MRID 49836101) which was conducted in a similar location and in the same year. In the clothianidin CFS, clear effects were observed at 40 µg a.i./L over multiple endpoints and multiple CCAs, leading to a NOEC of 20 µg a.i./L (clothianidin). At the NOEC of 20 µg/L, some effects were observed at CCA5, but these effects did not manifest at later CCAs. This suggests that effects were observed at lower levels in the clothianidin study compared to the thiamethoxam study.

[1] Clothianidin equivalents are used in the preliminary pollinator risk assessment, and concentrations may be expressed in terms of either thiamethoxam or these clothianidin equivalents (c.e.) in this document.

Therefore, when considering the uncertainties described above, the apparent NOAEC for the thiamethoxam CFS is tentatively determined to be 37.5 µg a.i./L (32 µg a.i./L-clothianidin equivalents), noting that this may value may not be conservative. The apparent NOAEC of 37.5 µg a.i./L (thiamethoxam; 32 µg a.i./L- clothianidin equivalent) along with the effects levels of 50 and 100 µg a.i./L (thiamethoxam) will be used to characterize the risk of thiamethoxam to honey bee colonies. Additional information from the clothianidin CFS may also be used to characterize the risk of thiamethoxam to honey bees.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

1. Study Objective

To determine the potential long term effects on the honey bee (*Apis mellifera* L.) colony health during and after dietary intake of thiamethoxam, including the potential effects on overwintering. The long term exposure allows for the characterization and distinction of short-term versus chronic or sub-lethal effects.

2. Study Methods

2.1. Test crop

This study did not include a test crop. The study was conducted in an open field where flowers from various wild and cropped areas were available, serving as potential pollen and nectar sources for the test bees.

2.2. Test chemical

The test substance was technical thiamethoxam. Further details are provided in **Table 1** below.

Table 1. Details about the test substance

Test Item			
Name	Thiamethoxam technical	**Batch number:**	WRS 1239/3;623769
Test item code:	NC-0421	**Appearance / colour:**	Powder / beige
Formulation type:	Technical compound	**Intended Usage:**	Insecticide
Active ingredient:	Thiamethoxam	**Content of a.i. analysed:**	98.9 %
CAS number:	153719-23-4	**Molecular Weight:**	291.7 g/mol
Density (20 °C) analysed:	Not applicable	**Risk symbol(s):**	Xn
Certificate of analysis:	19 March 2012	**Expiry date:**	31 March 2016
Stability in solution:	sufficient for the test purpose	**Storage conditions:**	ambient

2.3. Test sites

The field and sampling phases of this study were conducted by Eurofins Agroscience Services Inc. (EASI), at Cedar Grove Research Station, located in Mebane, NC, USA. Analysis of samples for residues of thiamethoxam in nectar and bee bread/pollen collected from hives was performed by EPL Analytical Laboratory, IL, USA. The apiary sites were located in the vicinity of the EASI Cedar Grove Research Station in Orange, Caswell, Person and Alamance counties, North Carolina.

There were 12 apiaries separated by at least 1 mile. Land use surveys in 1- mile radius and 3-mile radius were conducted. Pollen species identification and multiple pesticide analysis (174 active ingredients) were conducted using pollen samples collected from the monitoring hives to characterize outside food sources of the test bees and contamination. These pollen samples were collected for a period of 24-48 hours using pollen traps for 8 sampling events according to the schedule below (**Table 2**).

Table 2: Pollen Trap Sample Dates for Residue Analysis and Pollen Source Identification

Pollen sample Event	Date	Timing*
S1	7-Jul-2014	CCA3 (1 BE)
S2	16-18 Jul 2014	1 WAE
S3	28-30 Jul 2014	CCA 4 (3 WAE)
S4	15-Aug-2014	5 WAE
S5	20-21, 25 Aug 2015	CCA5(6 WAE)
S6	16-Sep-2014	CCA6 (10 WAE)
S7	7-Oct-2014	CCA7 (13 WAE)
S8	28-Oct-2014	16 WAE

*If at a specific CCA that is noted otherwise timing is based in weeks after exposure
WBE = Week before exposure; WAE = Week after exposure

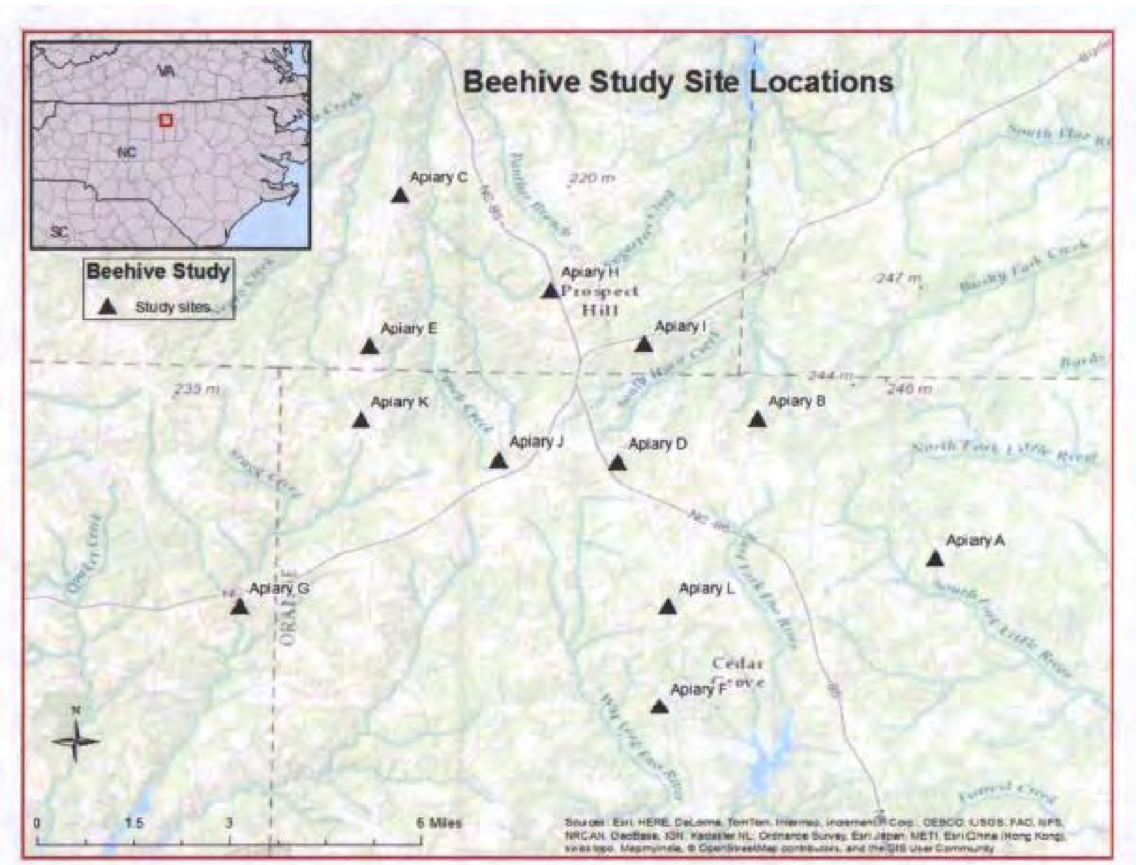


Figure 1: Locations of test apiary sites (figure taken directly from study report)

Table 3. GPS-coordinates of the test apiary sites

Apiary	GPS-coordinates
Apiary A	36° 12.025'N, 79° 6.560' W
Apiary B	36° 13.921'N, 79° 8.971' W
Apiary C	36° 17.013'N, 79° 13.828'W
Apiary D	36° 13.344'N, 79° 10.861' W

Apiary	GPS-coordinates
Apiary E	36° 14.923'N, 79° 14.244'W
Apiary F	36° 9.880'N, 79° 10.286' W
Apiary G	36° 11.376' N, 79° 15.993'W
Apiary H	36° 15.681'N, 79° 11.787'W
Apiary I	36° 14.961'N, 79° 10.517'W
Apiary J	36° 13.372' N, 79° 12.486' W
Apiary K	36° 13.937' N, 79° 14.364' W
Apiary L	36° 11.367'N, 79° 10.161' W

From Table 2, page 19 of the study report.

2.4. Test organisms

The test species was the honey bee (*Apis mellifera*), Italian race (*A. mellifera ligustica*). Hives were established from package bees bought from the commercial bee supplier (J J's Honey, 5748 Chancey Road, Patterson, GA 31557, USA), typical of the bee stock used in commercial beekeeping operations. A new queen was introduced into each colony. All queens were purchased from the package supplier. The colonies were maintained in 10-frame Langstroth boxes with an empty deep super on top as a feeder box. In the test field, hives were placed on a pallet. More than 100 inspected hives were screened based on the outcome of the second Colony Condition Assessment (CCA2). Hives were checked for the "appearance" of a healthy colony with no visible symptoms of *Varroa* or *Nosema*, as well as having all stages of brood, a queen, and some food stores.

Eighty-four hives that met the following conditions by the third Colony Condition Assessment (CCA3) were used in the biological evaluations in the study:

- At least 3 brood combs containing brood (actual: 3-12 brood combs with all brood stages; exceptions apiary B T2: 0 frames, apiary H T2: 1 frame, apiary D T3: 1 frame, due to previous swarming)
- At least 3 combs containing honey or pollen (actual: 4-16 combs containing food)
- Honey bees were free of Nosema and Varroa disease symptoms and other bee diseases.

In addition, a monitoring hive was placed at each apiary for collection of pollen and nectar which were analyzed for pesticide residues and pollen source identification (total 96 hives included in study). At CCA3, the start of the dosing period, each colony consisted of one to two brood boxes with 10 frames in each box and an mean estimated 8264 (T2) to 11040 (UTC) adult honey bees per colony.

The eighty-four hives were blocked into 12 apiary sites by colony strength (measured by coverage of brood), starting with Apiary A as the strongest group of hives, and Apiary L as the weakest group of hives with an additional hive at each site for pollen sample collection. Assignment of apiaries to the geographic locations was done randomly. Hives were moved from the holding yards to the study apiary site locations during the period from 27 Jun 2014 to 01 Jul 2014.

Each hive was spatially isolated from other treatment rates by 30 feet (9 m) spacing at each apiary site (**Figure 2**). Hives were arranged in a semi-circular pattern, facing east to west, with 125 feet (38 m) spacing between the two end hives.

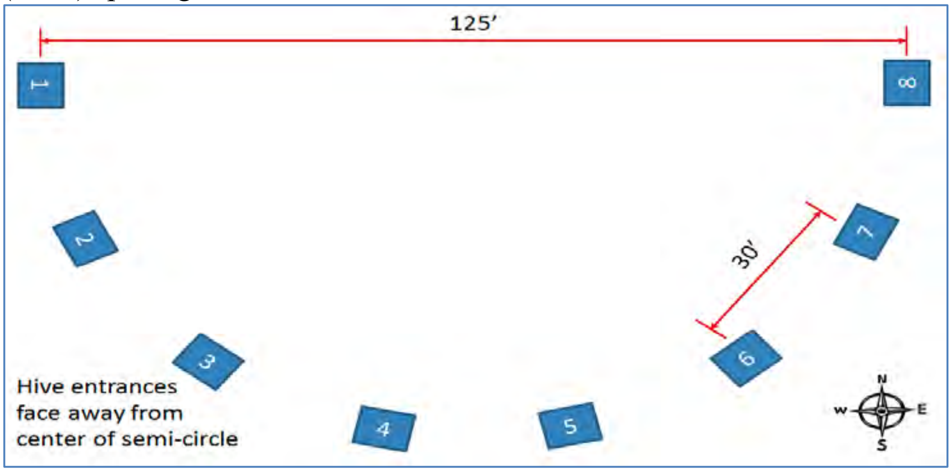


Figure 2. Layout of test hives in a test site (figure taken directly from study report).

During the study, all hives were treated for *Varroa* with two applications of Apiguard® (active ingredient: thymol) following typical apicultural practice for the region. The initial application occurred immediately after CCA5 (5/6 Sep, 2014) and the second application took place on 22/23 Sep 2014 to attempt to prevent high mite loads. No treatments for any other hive pests, predators or diseases were administered to any hives.

The monitoring hives were used for pollen sample collection. In addition, one test item dosing solution in a sealed container was placed in the monitoring hive at each apiary at 2 feeding events (1 and 5 weeks after exposure initiation), resulting in 2 stability samples for each test item concentration or controlin order to assess thiamethoxam stability under field test conditions. These stability solutions were not available as a food source to the monitoring hives.

2.5. Treatments

There were:

- 12 replicates per treatment group (apiaries),
- 6 treatment groups (5 test concentrations and control): 0, 12.5, 25, 37.5, 50, or 100 µg/L.
- At each site, there were 2 control hives, one hive for each test concentration, and one hive for pollen source/contamination monitoring.
- The monitoring hive had different treatment group concentrations (unavailable to bees) to measure stability.

The individual treatment groups, the respective feeding rates and the respective feeding volumes are summarized in **Table 4.** The assignment of each test hive at 12 apiaries is summarized in **Table 5.**

Table 4. Treatment groups, feeding rates and feeding volume

Treatment Group	Code	Feeding Timing	Nominal Concentration (µg a.i./L solution)	Feeding Volume	Total dose (mg a.i./hive; nominal)
1: UTC	UTC	Twice a week, over a duration of six weeks (= 12feeding events) from 1 week after CCA3 to CCA5 (=A1-A12)	---	8 X 1000 mL+ 4x 1500mL	0
2: Lowest test item rate	T1		12.5	8 X 1000 mL+ 4x 1500mL	0.6
3: Low test item rate	T2		25	8 X 1000 mL+ 4x 1500mL	1.2
4: Moderate test item rate	T3		37.5	8 X 1000 mL+ 4x 1500mL	1.8
5: High test item rate	T4		50	8 X 1000 mL+ 4x 1500mL	2.4
6: Effect test item rate	T5		100	8 X 1000 mL+ 4x 1500mL	4.8

From page 23 of the study report.

Table 5. Hive assignment to test apiaries

Treatment group	Apiary											
	A	B	C	D	E	F	G	H	I	J	K	L
UTC	A1	B7	C7	D1	E1	F2	G1	H1	I6	J6	K2	L1
UTC	A4	B4	C2	D7	E7	F4	G8	H6	I8	J7	K8	L7
12.5 ppb	A5	B6	C8	D5	E2	F8	G4	H4	I4	J5	K6	L2
25 ppb	A3	B3	C5	D2	E3	F7	G5	H2	I1	J2	K5	L6
37.5 ppb	A6	B1	C3	D3	E6	F5	G6	H5	I5	J8	K7	L8
50 ppb	A2	B5	C4	D6	E8	F6	G7	H3	I7	J1	K3	L3
100 ppb	A7	B8	C1	D8	E5	F1	G3	H7	I3	J4	K1	L4
Monitoring	A8	B2	C6	D4	E4	F3	G2	H8	I2	J3	K4	L7

From Table 3, page 54 of the study report.
Grey highlighting indicates the treatment level the monitoring hive received in sealed sucrose solution to test stability. For example, the monitoring hive (position 8) in Apiary A received a 50 ppb (T4) sealed bottle for storage stability.

2.5.1. Preparation of stock solution

Stock solution was created by combining 0.050 g of technical Thiamethoxam, dissolved in approx. 20 mL of acetone, and diluted to 1000 mL with tap water (2% solvent). After preparation, the stock solution was stored in a refrigerator until use or replacement. Stock solution was replaced twice during feeding on 21 Jul 2014 and 01 Aug 2014.

2.5.2. Preparation of sugar solution

For feedings prior to 4 Aug 2014 (1000 mL), sugar solutions were prepared by combining 10.9 kg tap water with 10.9 kg of sugar in a 5-gallon (19 L) container to make approximately 17 L of sugar solution. For feeding from 4 Aug 2014 onward (1500 mL), sugar solution was created by combining 5.8 kg tap water with 5.8 kg of sugar to make approximately 9 L of sugar syrup.

2.5.3. Preparation of feeding solution

For the 1000 mL feeding volume 08 Jul 2014 – 03 Aug 2014):

- 12.5 µg/L: mixing 4.25 mL of stock solution into the 17 L of sugar solution
- 25 µg/L: mixing 8.5 mL of stock solution into the 17 L of sugar solution
- 37.5 µg/L: mixing 12.75 mL of stock solution into the 17 L of sugar solution
- 50 µg/L: mixing 17 mL of stock solution into the 17 L of sugar solution
- 100 µg/L: mixing 34 mL of stock solution into the 17 L of sugar solution

For the 1500 mL feeding volume (04 Aug 2014 onward):

- 12.5 µg/L: mixing 2.25 mL of stock solution into the 9 L of sugar solution
- 25 µg/L: mixing 4.5 mL of stock solution into the 9 L of sugar solution
- 37.5 µg/L: mixing 6.75 mL of stock solution into the 9 L of sugar solution
- 50 µg/L: mixing 9 mL of stock solution into the 9 L of sugar solution
- 100 µg/L: mixing 18 mL of stock solution into the 9 L of sugar solution

As noted previously, acetone was used in the stock solutions. It appears that no acetone was added to the control and no attempt was made to equalize the concentration of acetone in the treatment groups. Given the small volume of stock solution added to the 17 and 9 L sugar solutions, the concentration of acetone in the treatment solutions was 0.0005-0.004%. Although having an unequal concentration of acetone in the treatment groups and control represents an additional variable in this study, the low concentration of acetone suggests that this aspect of the study design should not have a substantial impact on the study results.

The test concentrations were reported as "ppb" in the study report. However, the values are in fact in the unit of µg/L, not ppb (µg/kg). For example, 12.5 µg/L: can be calculated by 4.25 ml * (0.051 g /1020 ml)/17 L.

The test solution density was not provided. Assuming the density of a 50% sugar solution is 1.2296 g/ml,[2] the reviewer calculated that the test concentrations (**Table 6**) at 12.5, 25, 37.5, 50, and 100 µg/L are equivalent to 10.2, 20.3, 30.5, 40.7, and 81.3 (µg/kg), respectively. Residues (mean of two measurements from week 1 and week 5) of thiamethoxam in the dose verification samples were 9.327 ppb (Tl; 12.5 **µg a.i./L**), 24.089 ppb (T2; 25 **µg a.i./L**), 29.528 ppb (T3; 37.5 **µg a.i./L**), 39.693 ppb (T4; 50 **µg a.i./L**) and 73.748 ppb (T5; 100 **µg a.i./L**). This is equivalent to 91.4% (Tl), 118.7 % (T2), 96.8 % (T3), 97.5 % (T4) and 90.7 % (T5).

Table 6. Nominal and measured test concentrations.

Treatment Group	Code	µg a.i./L-solution		µg a.i./kg-solution		
		Nominal	Measured*	Nominal**	Measured	Percent Nominal
1: UTC	UTC	---	<LOD***	---	<LOD***	--
2: Lowest test item rate	T1	12.5	11.5	10.2	9.327	91.4

[2] Cell Biology Laboratory Manual, http://homepages.gac.edu/~cellab/chpts/chpt3/table3-2.html, accessed on Dec 12, 2014

Treatment Group	Code	µg a.i./L-solution		µg a.i./kg-solution		
		Nominal	Measured*	Nominal**	Measured	Percent Nominal
3: Low test item rate	T2	25.0	29.6	20.3	24.089	118.7
4: Moderate test item rate	T3	37.5	36.3	30.5	29.528	96.8
5: High test item rate	T4	50.0	48.8	40.7	39.693	97.5
6: Effect test item rate	T5	100	90.7	81.3	73.748	90.7

*Estimated by multiplying measured test concentration (in µg a.i./kg-solution) by assumed solution density of 1.2296 g/mL
**Estimated by dividing nominal test concentration (in µg a.i./L-solution) by assumed solution density of 1.2296 g/mL
***Level of Detection (LOD) = 0.25 µg a.i./kg-solution, assumed to be 0.31 µg a.i./L-solution.

2.5.4. Artificial Feeding

Each hive had an empty deep super on top, between the lid and the inner cover to allow dark space to place the Boardman feeder inside the hive. This allowed the feeder to be placed on the inner cover so that the bees had easy access without allowing the feeder to come into constant contact with light. Control and treatment solutions were sealed inside each hive and were not accessible outside of the hive.

The treated sugar syrup was prepared one day in advance for each feeding event and stored overnight at room temperature. The spiked sugar solution was provided to the treated hives twice a week for six weeks, starting on 08 Jul 2014 and ending with last retrieval on 19 Aug 2014. At each renewal, 1000 or 1500 mL of freshly prepared sugar solution was provided to each colony and any solution remaining from the previous feeding was removed and measured. Renewal of the sugar solution was separated by at least one day. The study observation period was 20 May, 2014 (CCA1) – 28 Apr, 2015 (CCA10), which includes the overwintering period. In fall and over-winter the surviving colonies were fed with 1 L of 2:1 sugar solution.

2.6. Meteorological Data

Temperature, humidity and rainfall data were obtained from two apiary sites (from the EASI weather stations located at Apiaries K and J; distance to the other apiaries between 0.1 to 7.5 miles). Data from Apiary K were the only data reported as the study authors stated data from the weather station at apiary J (Pope Farm) are nearly identical to the data from the weather station at Apiary K.

According to weather station at apiary K, a total of 8.3 inches (211 mm) of rainfall accumulated throughout the exposure period - including CCA3 (from 02 Jul 2014 to 15 Aug 2015), with 4.4 inches (112 mm; 02 - 31 Jul 2014) in July and 3.9 inches in August (99 mm; 01-15 Aug 2014). The on-site temperature minimum during the exposure period was 14 °C (57.2 °F) and the temperature maximum was 36 °C (96.8 °F). The humidity ranged from 32-100%

2.7. Observations

2.7.1. Dates of observation.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

The important activities and date are below in **Table 7**.

Table 7. Chronological list of key dates and activities

Activity code[a]	Study week	Timing[b]	Description
EA	-9 03 May 2014	9(±1)WBE	Installation of bee packages (before study initiation, non-GLP)
CCA1	-7 20-23 May 2014	7(±l)WBE	1st visual colony condition assessment (CCA1; before study initiation, non-GLP)
CCA2	-4 09-13 Jun 2014	4(±1)WBE	2nd visual colony condition assessment (CCA2; before study initiation, non-GLP); Assignment of hive locations
EA	-2 27 Jun -01 Jul 2014	2(±1)WBE	Move hives to the test locations *I* apiaries
CCA3, S1	-1(±1) 02 -06 Jul 2014	l(±l)WBE	3nd visual colony condition assessment (CCA3); Assessment of colony strength with digital images; Sampling of nectar and bee bread (pollen) from combs from all biological hives; Sampling of nectar and bee bread (pollen) from combs from monitoring hives; Sampling of pollen with pollen traps from monitoring hives; Sampling of adult bees for *Varroa* testing from all biological hives; Start of hive weight recording
Al + A2	0 08 + 10 Jul 2014	0WAE	Start of exposure = 1st application via feeding spiked sugar syrup (two feedings per week = Al and A2)
-	12 07 2014	0WAE	Installation of hive scales underneath each hive
A3 + A4, S2	+1 14 + 17 Jul 2014	1WAE	Two feedings (A3 and A4); Sampling of feeding solution before and after feeding from monitoring hives; Sampling of nectar and bee bread (pollen) from combs from monitoring hives; Sampling of pollen with pollen traps from monitoring hives
A5 + A6	+2 22 + 25 Jul 2014	2WAE	Two feedings (A5 and A6)
CCA4, S3	+3(±1) 28 - 31 Jul 2014	3(±1)WAE	4th visual colony condition assessment (CCA4); Assessment of colony strength with digital images; Sampling of nectar and bee bread (pollen) from combs from monitoring hives; Sampling of pollen with pollen traps from monitoring hives
A7 + A8	+3 29 + 31 Jul 2014	3WAE	Two feedings (A7 and A8)
A9 + A10	+4 04 + 07 Aug 2014	4WAE	Two feedings (A9 and A10)
Al l + A12, S4	5 11 + 15 Aug 2014	5WAE	Two feedings (Al l and Al2); Sampling of feeding solution before and after feeding from monitoring hives; Sampling of nectar and bee bread (pollen) from combs from monitoring hives; Sampling of pollen with pollen traps from monitoring hives

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Activity code[a]	Study week	Timing[b]	Description
CCA5, S5	+6(±1) 20 - 28 Aug 2014	6(±1)WAE	5th visual colony condition assessment (CCA5); Assessment of colony strength with digital images; Sampling of nectar and bee bread (pollen) from combs from all biological hives; Sampling of nectar and bee bread (pollen) from combs from monitoring hives; Sampling of pollen with pollen traps from monitoring hives; Sampling of adult bees for *Varroa* testing from all biological hives (19 -22 Aug 2014)
CCA6, S6	+10(±1) 17 - 23 Sep 2014	10(±1) WAE	6th visual colony condition assessment (CCA6); Assessment of colony strength with digital images; Sampling of nectar and bee bread (pollen) from combs from monitoring hives; Sampling of pollen with pollen traps from monitoring hives
CCA7, S7	+13(±1) 06 – 10 Oct 2014	13(±1) WAE	7th visual colony condition assessment (CCA7); Assessment of colony strength with digital images; Sampling of nectar and bee bread (pollen) from combs from monitoring hives; Sampling of pollen with pollen traps from monitoring hives
CCA8, S8	+ 16(±1) 27 -29 Oct 2014	16(±1) WAE	8th visual colony condition assessment (CCA8); Assessment of colony strength with digital images; Sampling of nectar and bee bread (pollen) from combs from monitoring hives; Sampling of pollen with pollen traps from monitoring hives;
CCA9, S9	31 Mar 2015	After over-wintering	9th visual colony condition assessment (CCA9); Assessment of colony strength with digital images; Sampling of adult bees for *Varroa* and *Nosema* testing from all biological hives; Sampling of nectar and pollen / bee bread from combs from all biological hives;
CCA10	28 Apr 2015	After over-wintering	10th visual colony condition assessment (CCA10); Assessment of colony strength with digital images;

[a] Activity code corresponds with EASSM system: A= application, S= sampling, CCA = colony condition assessment
[b] WBE / WAE= weeks before / after start of exposure

2.7.2. Colony mortality

Any colony that did not show the presence of a queen and had no open brood or eggs, or was devoid of worker (female) bees was considered "dead". If a hive was considered "dead" at the time of assessment, it was no longer used in the analysis of endpoints (e.g., adult bee numbers, hive weight).

2.7.3. Colony Condition Assessments (CCA)

Hive assessments were made by two trained experts. Each expert was assigned a set of apiaries and this person made all assessments for the hives at those apiaries, Apiaries A, F, G, J, K and inspected by one inspector and Apiaries B, C, D, E, H and I by another inspector. Ten CCAs were conducted during the entire study according to the schedule in **Table 8**. For summary statistics, the first day is used to characterize any given CCA.

Table 8. Schedule for colony assessment and beekeeper checks

Assessment	Timing[a]	Comments
CCA1	7WBE 20-23 May 2014	First colony assessment for hive selection
CCA2	4WBE 09-13 Jun 2014	Final assessment for hive selection and assignment of hives to apiaries and treatments (stratification)
CCA3	1WBE 02-06 Jul 2014	Assessment before start of feeding
CCA4	3WAE 28-31Jul 2014	Assessment during feeding period
CCA5	6-7WAE 20-28 Aug 2014	Assessment shortly after feeding period
CCA6	10-11 WAE 17-23 Sep 2014	-
CCA7	13 WAE 06-10 Oct 2014	-
CCA8	16 WAE 27-29 Oct 2014	Last assessment before over-wintering
CCA9	After over-wintering 31 Mar 2015	First assessment after over-wintering
CCA10	After over-wintering 28-29 Apr 2015	Last assessment of the study

[a] WBE / WAE = weeks before / after start of exposure (feeding); CCA = colony condition assessment

During the colony condition assessments, each frame was removed and inspected, with measurements for endpoints taken as percent of total frame area covered by honey/nectar, bee bread /pollen, capped brood, larvae, eggs and adult bees. For CCA3 through CCA 10 adult bees was assessed using digital imaging.

The estimation was made by:

- Each hive consisted of 20 observed panels (10 frames with two sides of each frame), with an area of 860 cm^2 per side, or a total area of 17,200 cm^2 for all 10 frames.
- The observed percentage of each matrix was converted to this area ratio for the estimated area covered by honey, pollen or brood types.
- Density was assumed to be 130 bees per 100 cm^2

- The area in cm^2 covered by bees multiplied by 1.30 gives the approximate number of bees in a colony
- The total number of cells per frame is 3440. To calculate total number of cells of brood or food stores the percentage coverage was multiplied by 3440 for one side of a frame.

The digital estimation of adult bees was made by transferring digital photographs to a computer for analysis using software Irfanview (version 4.38) and Mousetron (Blacksun Software (2001-2015); versions 5.0 and 9.1, 10.0). The data were transferred to Report Number: S14-02633 Page 28 of 467 Excel (2010, versions 14.0.6 and 14.0.7) and SAS (version 9.3) for statistical analysis calculations.

2.7.4. Evaluation of Disease or Pests in the Hive

Colonies were also checked for visible symptoms of disease or pests, such as *Nosema*, foulbrood, *Varroa* mites or small hive beetle.

The number of Nosema spores per bee was determined once during the study after over-wintering (CCA9). To assess the presence of Varroa in the hive, bee samples were taken at the CCA3, CCA5 and CCA9. Bees were washed in alcohol to remove mites. The number of mites per 100 bees was calculated.

2.7.5. Hive weights

The weight of each hive and temperature (recorded hourly) was monitored continuously using a digital balance (B-ware™ Beehive Monitoring System from Solutionbee LLC). The balances were installed under the hives one week after the third CCA and remained until the final CCA after overwintering. During the week of CCA3 the hive weights were recorded manually. Several balances failed sporadically during the study due to technical problems so the hive weights were recorded manually (during malfunction) with a calibrated balance (note: the study authors provide no additional information on timing of balance failure or manual recording).

2.8. Residue analysis

All residue and stability samples collected from feeding solution, pollen traps, and test hives were analysed for thiamethoxam and its major degradate CGA322704 (clothianidin) at the EPL Bio Analytical Services (Niantic, IL). Samples from pollen traps in the monitoring hives were also analysed for residues of multiple pesticides from outside sources at USDA Laboratory in Gastonia NC. The residue results were reported as ng per g of sample matrix (ppb), which is different from the test solution that was reported in µg/L. The LOQ and LOD for thiamethoxam and its metabolite CGA322704 in each matrix are listed below:

- Bee Bread: LOQ - 1 ppb; LOD - 0.5 ppb
- Nectar/Honey: LOQ - 0.5 ppb; LOD - 0.25 ppb
- Dose verification/Stability: LOQ - 0.5 ppb; LOD - 0.25 ppb

CGA322704 was only detected above the LOQ in one hive's bee bread (T3) sample at CCA5. Multiple pesticide analysis was conducted in order to monitor pesticide contamination from outside food sources using pollen collected from pollen traps on the monitoring hives.

All samples collected for residue analysis and pollen identification were placed in freezer storage as soon as possible after collection. The samples for residue analysis were shipped from EASI in NC to the laboratory of EASI in NJ on 10 Sep 2014 and 04 Nov 2014. The samples were then shipped to EPL in IL on 02 Mar 2015. The samples for pesticide screening were shipped from EPL to the USDA Laboratory in Gastonia NC on 22 Apr 2015.

2.8.1. Pollen from outside sources

Pollen samples were collected from pollen traps attached for 24-48 hours to the monitoring hives at each site to assess the potential contaminant exposure from outside sources. The amount of pollen collected from each hive was variable and samples were not available from every site each time. Pollen samples from the monitoring hives were taken at CCA3 (7 Jul 2014), study week +1 (16-18 Jul 2014), CCA4 (28-30 Jul 2014) study week +5 (15 Aug 2014) CCA5, (20-21, 25 Aug 2014), CCA6 (16 Sep 2014), CCA7 (7 Oct 2014), and CCA8 (28 Oct 2014).

2.8.2. Stored pollen and nectar in test apiaries

Bee bread (pollen) samples were collected during and after the exposure phase with pollen corers. A sample consisted of bee bread collected from at least 3 different frames if possible. Samples were analyzed for residues of the test item. After start of the exposure phase, bee bread samples were collected twice from all biological hives. The samples were taken 6 weeks after start of exposure during CCA5 and after overwintering at CCAl0. After start of the exposure phase, bee bread samples were collected at seven time points from the monitoring hives (1, 3, 5, 6, 10, 13 and 16 weeks after start of exposure).

Honey (nectar) samples were collected during and after exposure with a single use plastic spoon or other suitable tool. A sample consisted of nectar collected from at least 3 different frames (if available) per colony. After the start of the exposure phase, nectar samples were collected twice from all biological hives. Samples were collected at 6 weeks after start of exposure during the CCA5 and after over-wintering at CCA10. After start of the exposure phase, nectar samples were collected at seven time points from the monitoring hives (1, 3, 5, 6, 10, 13 and 16 weeks after start of exposure).

2.8.3. Feeding solution and stability of test item

Dosing solution concentration and solution stability in hives was evaluated by collection of samples before and after placement of dosing solution in monitoring hives (**Table 9**). Monitoring hives were set up in the same manner as test hives except the colony was denied access to the spiked or un-spiked sucrose. Residue samples for dose verification were taken on week +1 (14 July 2014), week 2 (12 July 2013) and week +5 (11 August 2013).

Table 9. Sampling schedule dose verification and storage stability of test chemical.

Timing	Week +1 (1WAE)	Week 5 (5 WAE)
Apiary / replicate	**03 Jul 2013**	**02 Aug 2013**
UTC	361A	475A
12.5 ppb	362A	476A
25 ppb	363A	477A
37.5 ppb	364A	478A
50 ppb	365A	479A
100 ppb	365A	480A

WAE = Week after exposure

3. Results

3.1. Land use near test hives

Land use pattern within a 1-mile and 3-mile radius around the 12 apiaries are summarized in **Table 10**. The majority (approximately 70%) of areas near the apiaries is represented by deciduous forest and pasture/hay land covers. The cultivated crop area occupied 0.22-4.76% of the total land within 1-mile radius, and 1.71-2.69% within a 3-mile radius range from the test apiaries. Using the raw data provided, the reviewer calculated the area of cultivated crops as summarised in **Table 11**. The mean area of cultivated cropping land was 15 and 161 ha within 1 mile and 3 miles, respectively, of the radius from each apiary.

Table 10. Percent (%) land use pattern

1 Mile Radius	Apiary											
Land Use Category	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
Open Water	0.7	0.7	0.9	1.0	0.7	6.1	0.5	0.6	0.1	0.7	0.8	1.2
Developed, Open Space	5.3	5.9	0.8	6.4	1.5	4.6	3.2	2.3	3.4	6.4	3.9	11.2
Developed, Low Intensity	1.5	2.1	0.8	2.3	0.9	0.4	1.2	2.4	2.2	3.0	1.2	2.2
Developed, Medium Intensity	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.2	0.1	0.2	0.0	0.2
Developed, High Intensity	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Barren Land (Rock/Sand/Clay)	0.0	0.1	0.0	0.2	0.0	0.2	0.0	0.0	0.3	0.0	0.0	0.1
Deciduous Forest	**43.6**	**38.7**	**39.1**	**33.4**	**47.8**	**46.3**	**37.5**	**45.4**	**42.8**	**31.6**	**39.4**	**26.9**
Evergreen Forest	5.6	4.5	3.8	8.1	9.0	3.3	6.6	5.5	7.0	4.5	6.5	4.7
Mixed Forest	2.3	2.4	2.5	3.9	3.8	1.9	3.3	3.5	3.6	2.6	4.0	2.2
Shrub/Scrub	2.0	2.1	7.6	0.7	3.9	5.0	5.3	2.6	8.6	2.8	4.6	1.8
Grassland/Herbaceous	5.0	3.0	13.4	3.8	4.0	2.8	5.5	4.2	6.5	2.5	3.1	3.0
Pasture/Hay	**31.7**	**38.7**	**28.0**	**36.7**	**25.7**	**29.1**	**35.6**	**30.9**	**24.7**	**40.8**	**33.5**	**43.9**
Cultivated Crops	0.7	1.8	2.3	2.9	1.4	0.2	0.6	2.4	0.8	4.8	2.8	2.4
Woody Wetlands	1.8	0.8	1.1	0.7	1.3	0.1	0.4	0.1	0.0	0.2	0.2	0.4
Emergent Herbaceous Wetland	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0

3 Mile Radius	Apiary											
Land Use Category	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
Open Water	0.6	0.6	0.4	0.8	0.5	2.4	0.6	0.4	0.5	0.7	0.5	2.5
Developed, Open Space	5.9	5.1	2.3	5.9	3.2	5.6	4.6	2.6	3.4	4.9	4.0	6.6
Developed, Low Intensity	1.3	1.8	1.4	2.2	1.6	1.1	1.4	1.7	1.9	2.1	1.6	1.8
Developed, Medium Intensity	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Developed, High Intensity	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Barren Land (Rock/Sand/Clay)	0.1	0.3	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.1
Deciduous Forest	**40.1**	**39.7**	**41.9**	**38.4**	**41.6**	**44.3**	**37.8**	**41.9**	**41.8**	**37.9**	**40.4**	**37.2**
Evergreen Forest	6.1	5.4	6.8	5.3	5.9	4.7	7.3	6.6	6.0	5.5	5.7	4.7
Mixed Forest	2.1	2.6	2.9	2.6	2.8	2.1	2.6	3.5	3.3	2.9	2.9	2.1
Shrub/Scrub	2.4	3.2	5.5	3.4	6.2	3.3	4.3	5.2	4.5	4.7	5.4	2.7
Grassland/Herbaceous	4.1	4.9	10.8	4.2	7.1	3.5	5.3	7.3	6.0	4.1	5.1	3.3
Pasture/Hay	**34.3**	**33.5**	**24.7**	**34.1**	**28.1**	**31.0**	**33.7**	**27.7**	**29.7**	**33.8**	**31.3**	**36.1**
Cultivated Crops	1.7	2.4	2.1	2.5	2.5	1.1	1.7	2.7	2.5	2.5	2.7	2.0
Woody Wetlands	1.2	0.6	1.0	0.6	0.4	0.8	0.6	0.3	0.3	0.7	0.4	0.8
Emergent Herbaceous Wetland	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 11: Cultivated cropping area near each test apiary

	Apiary												
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**Mean**
1-mile radius (813 ha)													
Cultivated Crops (%)	0.7	1.8	2.3	2.9	1.4	0.2	0.6	2.4	0.8	4.8	2.8	2.4	1.9
Area of cultivated crop (ha)	5.7	14.6	18.7	23.6	11.4	1.6	4.9	19.5	6.5	39.0	22.8	19.5	15.7
3-mile radius (7323 ha)													
Cultivated Crops (%)	1.7	2.4	2.1	2.5	2.5	1.1	1.7	2.7	2.5	2.5	2.7	2.0	2.2
Area of cultivated crop (ha)	125.2	172.8	153.8	181.6	183.8	77.6	125.2	195.5	186.0	183.8	197.0	148.7	161.1

3.2. Sources of pollen from monitoring hives

Pollen samples from the monitoring hives were taken at CCA3 (7 Jul 2014), study week +1 (16-18 Jul 2014), CCA4 (28-30 Jul 2014) study week +5 (15 Aug 2014) CCA5, (20-21, 25 Aug 2014), CCA6 (16 Sep 2014), CCA7 (7 Oct 2014), and CCA8 (28 Oct 2014).

The majority of the pollen originated from local sources, bees clearly favored five types: *Chenopodium, Plantago, Rhus, Ambrosia, Helianthus* and Asteraceae-type. Bees also foraged on plants such as *Parthenocissus*, *Zea mays* and *Lagerstroemia*. Nearby agricultural fields, included mostly tobacco (*Nicotiana*) and corn (*Zea mays*) fields. These were identified occasionally, and took up the maximum of 78.0% and 40.0% of the total pollen particles, respectively. Soybean pollen was also found in high proportions (83.2%) in hives sampled on CCA4. Maximum values found during an assessment are presented below in **Table 12. Note these are not necessarily from the same apiary/hive, just the time of assessment.** (Full results can be found in Tables 52-59 of the study report (pages 150-157).

Table 12. Maximum pollen percentages for agricultural crops found in a hive during any assessment.

	-1 WAE	1WAE	3WAE	5WAE	6WAE	10WAE	13WAE	16WAE
Soybean	--	--	83.2 Hive G2 (71.8% in F3)	7 Hive F3	--	--	--	--
Corn	30 Hive C6	40 Hive G2	22 Hive B2	1.4 Hive K4	--	--	--	--
Tobacco	7.8 Hive H8	78 Hive H8	17.8 Hive H8	--	--	--	--	66.8 Hive E4 (33% in H8)
Sunflower				4.2 Hive D4	-	77 Hives D4 and B2 (50 and 54% in A8 and D4)	8.2 Hive J3	-

3.3. Consumption of spiked sucrose

Hive sugar solution consumption rates ranged from 6910 mL to 14000 mL of the total 14000 mL provided per hive during 6-week dosing phase. The sugar solution for most hives in all treatments was consumed completely, excluding the 100 µg a.i/L dose group (see **Figure 3**). The total food consumption of H7 in the 100 ppb treatment was 6910 mL; while all the other hives in the 100 µg a.i/L consumed between 12320 and 14000 ml of sucrose solution. In the other treatment groups, the least amount or sucrose solution consumed was 11970 mL (T2 hive J2).

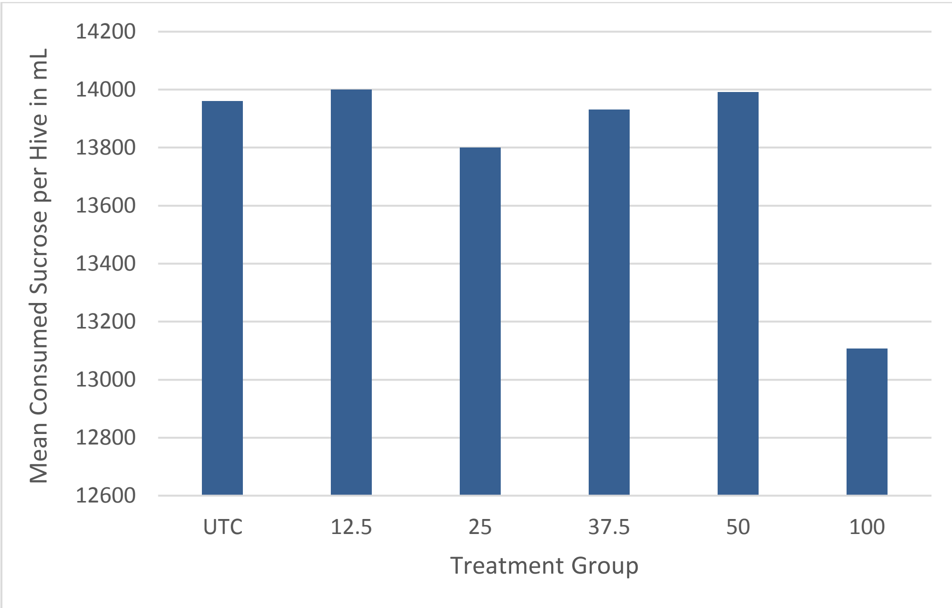


Figure 3. Mean total food consumption (mL) per colony during the 6-week exposure period

3.4. Examination of pesticides from other sources

Pollen samples were collected from monitoring hives at each apiary by means of a pollen trap eight times during the study for subsequent multiple pesticide screening. Acephate (1 sample, 1600 ppb), Carbaryl (1 sample, 214 ppb), Carbendazim (5 samples, traces - 1300 ppb), Imidacloprid (2 samples, 4.3 and 6.1 ppb), Methamidophos (1 sample, 109 ppb), Thiamethoxam (2 samples, 8.8 ppb and 11.3 ppb), and Thymol (23 samples, 405 ppb to 12300 ppb; during period of Varroa treatment) accounted for the highest residue levels found in these pollen samples.

3.5. Confirmation of test concentrations

Thiamethoxam and CGA 322704 (clothianidin) were analyzed from feeding solutions sampled before and after placement of dosing solution in monitoring hives. Dosing solution was placed in monitoring hives 1 week and 5 weeks after initiation of the exposure period. Duplicate samples (A for residue analysis and R for retained or backup sample) of at least 5 g dosing solution were collected at each sampling event. Dose verification samples were collected from just before placement of solutions in hives, one sample per dose = total of 6 samples). The data are tabulated below in **Table 13**.

Table 13. Dosing solution residue data from 14-17 July 2014 (Week 1) and 11-15 August 2014 (Week 5)

Nominal concentrations		Average of measured concentrations (ppb)^	Measured thiamethoxam concentrations(ppb*) (n=2)		Measured CGA322704 concentrations(ppb*) (n=2)	
(µg/L)	(ppb*)		17 Jul, 2014	15 Aug, 2014	17 Jul, 2014	15 Aug, 2014
0 (Control)	0	<LOD†	0	0	0	0
12.5	10.2	9.327	10.715	7.939	0	<LOD
25	20.3	24.089	26.503	21.674	0	0
37.5	30.5	29.528	33.703	25.352	<LOD†	<LOD
50	40.7	39.693	42.024	37.362	<LOD	0
100	81.3	73.748	71.941	75.554	0	<LOD

†: LOD=0.25 ppb for thiamethoxam; LOQ=0.5ppb
^ Equivalent to 91.4 % (Tl), 118.7 % (T2), 96.8 % (T3), 97.5 % (T4) and 90.7 % (T5)
*ppb = µg a.i./kg-solution (calculated by dividing nominal **µg/L concentration by assumed 50% sugar solution density of 1.2296**

3.6. Stability of the test item in feeding solution

Stability of thiamethoxam in the sugar solution during the feeding period was examined from diet collected from closed-off feeding solutions placed in the monitoring hives, sampled twice on 17 July 15 August 2014. One test item dosing solution was placed in the monitoring hive at each apiary at 2 feeding events, resulting in 2 stability samples for each test item concentration or control (two samples per dosing solution concentration = total of 12 samples). The solutions were contained and sealed in 15 mL Falcon tubes and were placed inside the monitoring hives. The samples remained in the hives until the next dosing solution change and feeding event. The stability samples were separated from other samples during storage and shipping. Average thiamethoxam residue data for the stability solution are presented in **Table 14.**

Table 14. The stability of thiamethoxam in feeding solution on 14-17 July 2014 (Week 1) and 11-15 August 2014 (Week 5)

Nominal concentration (µg/L)		Average of measured concentrations (ppb)^	Number of samples measured	Measured thiamethoxam concentrations (ppb)** †			
(µg/L)	(ppb*)			17 Jul, 2014***		15 Aug, 2014***	
Control	0	0	4	0	0	0	0
12.5	10.2	10.834	4	13.577	8.768	10.279	10.711
25	20.3	20.627	4	22.334	16.142	22.006	21.527
37.5	30.5	34.186	4	28.238	40.537	38.168	31.800
50	40.7	43.049	4	46.135	42.975	38.744	44.340
100	81.3	82.314	4	95.777	71.585	81.152	80.740

ppb = µg a.i./kg-solution (calculated by dividing nominal **µg/L concentration by assumed 50% sugar solution density of 1.2296
** All CGA322704 concentrations were reported as 0.0*** Samples were collected 17 Jul 2014 (week 1; placed on 14 Jul 2014 = duration inside hives 3 days) and 15 Aug 2014 (week 5; placed 11 Aug 2014 = duration inside hive 4 days)
†: LOD=0.25 ppb for thiamethoxam; LOQ=0.5ppb
^ Equivalent to 106.2 % (Tl), 101.6 % (T2), 112.1 % (T3), 105.8 % (T4) and 101.2 % (T5).

3.7. Residues in hive matrices

3.7.1. Thiamethoxam residues in hives prior to the feeding exposure

Bee bread (pollen) and nectar (honey) samples were collected at CCA3 from all hives (biological and monitoring) before the exposure phase. Samples were collected using pollen corers and spoons, and were analyzed for pesticide residues (174 active ingredients or metabolites). A pooled sample was collected from bee bread at least 3 different areas of frames per colony. The limit of detection for thiamethoxam in pollen for this study was 0.5 ppb, and for honey was 0.25 ppb. Prior to exposure neither thiamethoxam nor CGA322704 were detected in nectar/honey.

3.7.2. Residues in hive matrices during and after feeding exposure

After start of the exposure phase, nectar/bee bread samples were collected twice from all biological hives. The samples were taken 6 weeks after start of exposure during CCA5 and after overwintering at CCA9. After start of the exposure phase, bee bread samples were also collected at seven time points from the monitoring hives (1, 3, 5, 6, 10, 13 and 16 weeks after start of exposure). Ranges in the biological hives are presented in **Table 15** below. Details from all residue analyses is presented in Appendix 4 of the study report.

Table 15. Residues of thiamethoxam and CGA322704 in Biological Hives[1,2]

Treatment (µg/L)	Bee Bread		Honey		
	CCA5	CCA9	CCA3	CCA5	CCA9
UTC	<LOD-1.303	<LOD-<LOQ	<LOD	<LOD-<LOQ	<LOD
12.5	2.599-10.020	NS	<LOD	<LOD-17.675	NS
25	<LOD-17.038	<LOD-<LOQ	<LOD	<LOD-27.554	<LOD-2.746
37.5	10.368-24.012	<LOD-2.42	<LOD	<LOD-28.060	<LOD-5.136
50	10.984-34.960	<LOQ-4.084	<LOD	<LOD-61.806	<LOQ-21.899
100	25.085-43.005	NS	<LOD	<LOD-102.030	NS

Pollen – LOQ = 1ppb, LOD=0.5; Honey - LOQ=0.5 ppb, LOD = 0.25 ppb
NS = None surviving
[1] – CGA322704 was detected at CCA5 in the 37.5 treatment group in one sample (hive D3) at 1.362 in bee bread
[2] – CGA322704 was reported as < LOQ or < LOD in all honey samples

There were some detections of thiamethoxam in bee bread of the biological control hives. A CCA5 hives in Apiary K (hives K2 – 1.269 ppb and K8 – 1.303 ppb) had detects above the LOD. Additionally, two monitoring hives that thiamethoxam detects above the LOQ (F3 – 0.888 ppb and L7 – 0.752 ppb). These are considered not to impact the results of this study. At CCA5 (after exposure) Thiamethoxam concentrations in bee bread ranged from 21-80, 0-68, 28-64, 22-70, and 25-43% of nominal concentrations in the 12.5, 25, 37.5, 50, and 100 µg/L treatment groups respectively. In honey at CCA5 the thiamethoxam concentrations ranged from 0-142, <LOD-110, 0-75, <LOD-124, and 0-102% of nominal concentrations in the 12.5, 25, 37.5, 50, and 100 µg/L treatment groups respectively.

Considering the stability of thiamethoxam in the test solution, the reduced concentrations of thiamethoxam in hive matrices likely indicates that test bees were also foraging for pollen and nectar from outside floral sources. However, the residue concentrations and consumption of the sugar solutions confirms bees were being exposed to thiamethoxam.

3.8. Pathogens

The colonies were managed as typical for good practice in the region, including applying miticide treatments *Varroa* mites and *Nosema* were quantified in test hives. Besides a standard treatment for *Varroa* mites, no treatments for any other hive pests, predators or diseases were administered to any hives. Applications of *Varroa* treatments were made based on the assessments of control hives only and were performed after consultation with the study sponsor. All hives were treated equally when such practices were employed.

3.8.1. Varroa Presence

Varroa mite occurrence in the colonies was assessed once before start of exposure – CCA3, once after exposure (before overwintering) – CCA5 and once after overwintering – CCA9. To remove and count mites, bees were washed in alcohol and the number of mites per 100 bees was calculated.

Varroa infestation levels were similar for control, 12.5 µg/L group, lower for 25 µg/L and 37.5 µg/L treatment groups, increased at 50 µg/L treatment and was highest at 100 µg/L The highest mite load was found in samples collected at CCA5 (August 2014) ranging from a mean of 1.5 mites per 100 bees (37.5 ppb) to 2.6 mites per 100 bees (100 ppb). After over-wintering, the colonies of all treatment groups were on a similar infestation level ranging from 0.3 mites per 100 bees (37.5 ppb) to 0.6 mites per 100 bees (25 ppb)

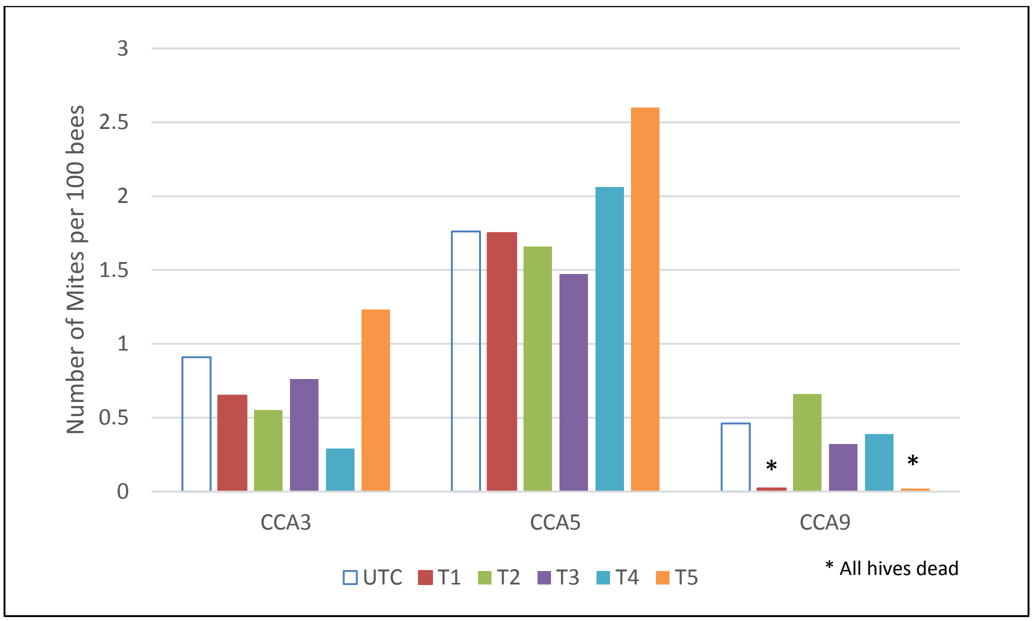


Figure 4. Mean number of *Varroa* mites per 100 bee

3.8.2. *Nosema* presence

The number of *Nosema* spores per bee was determined once during the study at CCA9. The number of spores per bee was 1,500,000 (UTC), 750,000 (25 ppb), 3,966,667 (37.5 ppb) and 2,637,500 (T4). This measurement was taken at a time when all the hives in T1 and T5 were dead. There was no correlation between test item treatment and *Nosema* infestation level.

3.9. Hive mortality

The study authors reported that 69 out of 84 colonies were considered deceased by 26 April 2015 (**Table 16**). Colonies were considered dead if they did not show presence of a queen and had no open brood or eggs or was devoid of worker (female) bees. The study authors maintain that the late start of maintenance (supplemental) feeding after the dosing phase (before winter) led to low bee populations going into winter and in combination with a cold spring, many colonies did not survive the over-wintering period, and consequently data were not used for interpretation and conclusion related to over-wintering success between treatments. Once a hive was declared dead, it was physically removed from the study site and from subsequent data analysis. Since successful overwintering in the controls was not achieved, it is difficult to discern treatment related effects. Control mortality after overwintering was higher (79%) than mortality in the 25 (67%) and 50 (67%) µg/L groups.

Table 16. Hive survival at CCA9 (after overwintering)

Treatment group	Apiary												Deceased Colonies (%Mortality)
	A	B	C	D	E	F	G	H	I	J	K	L	
UTC	A1	-	-	-	E1	F4	G8	-	-	-	-	L5	19/24 (79%)
UTC	-	-	-	-	-	-	-	-	-	-	-	-	
12.5 ppb	-	-	-	-	-	-	-	-	-	-	-	-	12/12 (100%)
25 ppb	A3	-	C5	D2	-	-	-	-	-	-	-	L6	8/12 (67%)
37.5 ppb	-	-	C3	D3	-	F5	-	-	-	-	-	-	10/12 (83%)
50 ppb	A2	-	-	-	-	F6	G7	-	-	-	-	L3	8/12 (67%)
100 ppb	-	-	-	-	-	-	-	-	-	-	-	-	12/12 (100%)

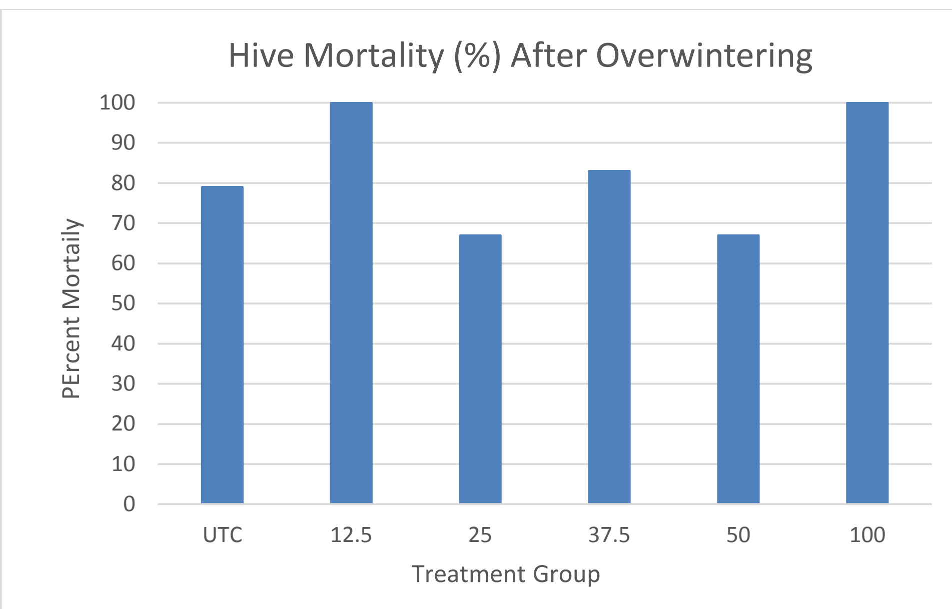


Figure 5. Hive mortality after overwintering.

3.10. Hive Weight

The figure below is taken directly from the author's study report. Hive weights oscillated similarly with the exception of the 37.5 (higher) and 100 µg/L (lower) treatment groups in September of 2014.

FIGURE 11 Mean Weight Change per Month during the Observation Period in kg

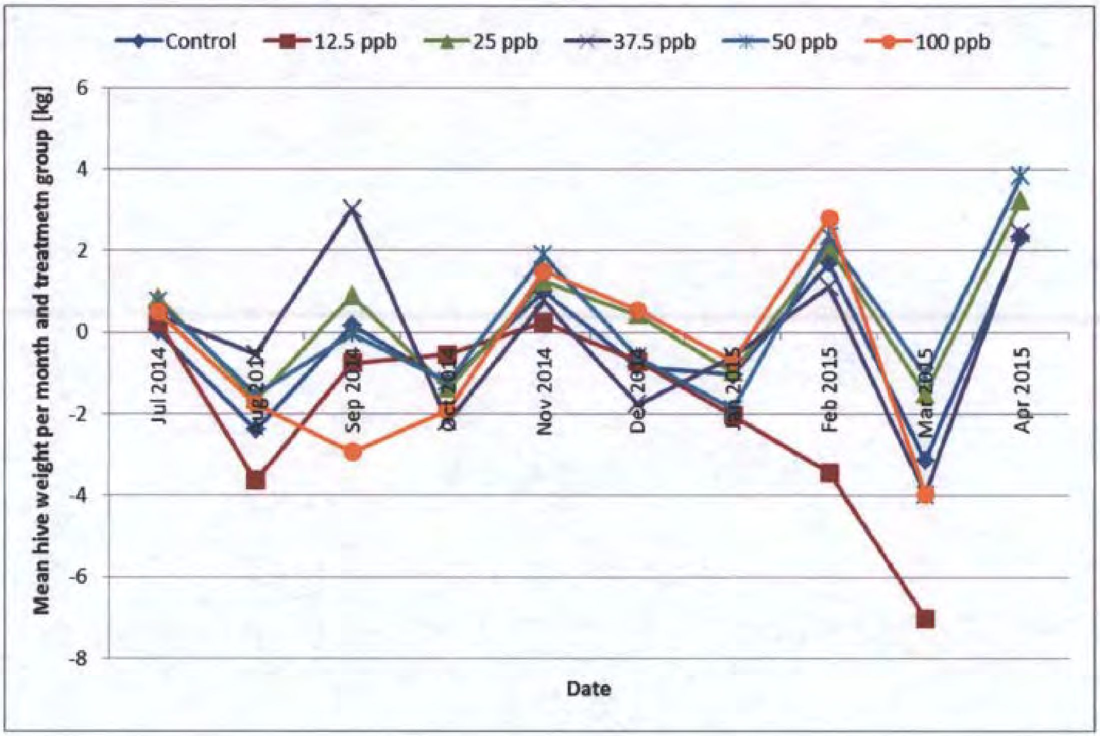


Figure 6. Proportion of hive weight following exposure of honey bees to varying concentrations of thiamethoxam in the diet across July through the hives that survived overwintering (taken directly from the study report).

3.11. Colony Condition Assessment Response Variables

What follows is a breakdown of each response variable assessed and the significant effects that were determined at each CCA (after set up and prior to overwintering; i.e., CCAs 3-8). A couple of general points are made below when examining the results data analysis:

- Unless explicitly stated otherwise, all discussion of statistical findings refers to the EPA statistical analysis. All analyses considered effects at both the 0.05 and 0.1 alpha levels when weighing statistically significant effects with biological considerations. For simplicity and consistency in visualizing the trends and findings of statistical significance simultaneously, the EPA-generated figures are presented below.

- As noted above, the data counts of individuals (adult bees) or number of occupied cells (immature life stages and food stores) which have been scaled (divided by 1000) of each response variable to facilitate convergence of the statistical model.
- The table values are the percent reductions of the response model-based mean for a given treatment relative to the control model-based mean. The model-based means are the Least Square means based on the randomized complete block, repeated-measures design and model fit using SAS PROC MIXED algorithms. These Least Square means may differ from arithmetic means due to missing values in the raw data.
- The figures with colored significance "dots" representing p-values of <0.05 or <0.10 were based on the results of the mixed model analyses conducted by EPA. off of these counts for each hive for each response variable (with the exception of hive weight) and were generated by EPA. The figures indicate statistical significance (reduction in treatment mean relative to control within a CCA) with black and red "dots" denoting a significant reduction at the 0.10- and 0.05-alpha levels, respectively.
-
- While it is not depicted in the figures below, it is acknowledged (and addressed in a variety of ways through the various statistical approaches and discussion) that there was considerable variability for some response variables at certain treatment groups and CCAs. Please refer to **Appendix A** or **Appendix B** for summary statistics tables (*i.e.* max, min, standard deviation values) of the proportions of each response variable for further information.

3.11.1. Statistical Analysis

What follows are brief summaries of each of the statistical analyses employed for the review of this study. When discussing the results both statistical and biological lines of evidence are weighted in the final evaluation.

3.11.1.1. Study Authors Analysis

The study author conducted statistical analysis using SAS (version 9.3) including brood and hive weight data as well as number of bees. For the exposure data, all tests were done in a two tailed approach, whereas for the data assessed after application, one tailed (lower) tests were conducted.

Data for the test item treatments (Tl, T2, T3, T4, and T5) and the untreated control UTC were checked for normality using Shapiro-Wilks Test (p 0.05). Data were analyzed for homoscedasticity using Bartlett Test for data with well proven normality (p-value according Shapiro-Wilks test above p=0.2) and Levene Test for data with poor fit to normal distribution. If Box-Cox transformation of the data improved normality or homoscedasticity in a way that leads to the option to use statistical tests with higher power, Box-Cox-transformed data were used for statistical analysis. For data where normality and homoscedasticity were proven, Dunnett's t-Test was used to check for possible statistically significant differences of each measured subject in Tl, T2, T3, T4 and T5 compared to the control. If homoscedasticity was disturbed but normality was given, Bonferroni-Holms corrected Satterthwaite t-Test (same as Welch-Test) was used for analysis. In case of disturbed normality, Bonferroni-U Test was conducted. Each of the tests were conducted with $p < 0.05$ as indicator of statistically significant difference.

An analysis of covariance (ANCOVA) was performed on the Box-Cox transformed data for the data generated at the colony condition assessments. To eliminate factors with possible impact on the data other than effects of treatment, the different apiaries as well as the last values of each observed subject were used as covariates for ANCOVA models. Three models were tested: ANCOVA with apiaries (blocks) as covariates, ANCOVA with apiaries and pre-treatment values as covariates, and a one-way ANOVA. The decision for which parameter was used for ANCOVA or if a simple ANOVA was conducted instead of the

ANCOVA was made based on the significance of the impact of the parameter for the analysis. Impacts of covariates on the model were analysed using significance tests (in particular, F-tests) to determine whether the pre-treatment values or apiary influence the posttreatment values of each parameter. If the covariate was found to be significant an analysis of covariance was selected, whereas if the covariate is found to be non-significant an analysis of variance was selected. For each assessed subject, the pooled estimate of residual error variance obtained from the selected form of analysis (ANOVA or ANCOVA) were used to compare each treatment to the control using a two-sided Dunnett's t-test at the 5 % significance level.

If an analysis of covariance was selected, the transformed means (and therefore the de-transformed means) were adjusted for the effect of the covariate. Adjusting the means involves removing all differences between the treatment groups that can be accounted for by the covariate.

3.11.1.2. Study Reviewer Analysis

During the review of the study, a separate statistical analysis was conducted using the raw data submitted by the study author. As part of the collaborative review effort of the study EPA, PMRA, and CDPR, will all be reviewing the study; however, the discussion in the Colony Condition Assessment section is mostly based on the statistical approach completed by EPA. A detailed description of the methods, including statistical model selection and parameterization, are presented in **Appendix A,** what follows is a brief summarization of the EPA's method.

3.11.1.2.1. EPA Analysis

The same statistical analysis approach was used for all the analyzed endpoints. The evaluated endpoints included:

- Number of adults divided by 1000
- Number of egg cells divided by 1000
- Number of open (larvae) cells divided by 1000
- Number of capped (pupae) cells divided by 1000
- Number of pollen cells divided by 1000
- Number of honey cells divided by 1000
- Total number of individuals (adults + eggs + larvae + pupae) divided by 1000
- Total brood (eggs + larvae + pupae) divided by 1000, and
- Total food (pollen + honey) divided by 1000.

Total brood and total food are new summary variables; EPA's Environmental Fate and Effects Division (EFED) is still evaluating their utility in providing additional information on biological effects beyond the initial set of variables.

No adjustments for addition or removal of supers were included in the statistical analysis. The data were analyzed as a repeated measures design (multiple CCAs) with a randomized complete block (apiary) and only data obtained prior to overwintering were included. The covariance structure used for the repeated measures component was compound symmetry with heterogeneous variances at each time point. The time*treatment interaction was explored two ways:

- At each post-treatment CCA, one-sided Dunnett's test to identify treatments with mean response significantly less than control.
- For each treatment (including control), two-sided Dunnett's test to identify change in the response over time relative to the initial CCA3 measurement.

The interpretations below focus on the one-sided Dunnett's tests identifying treatment effects at each CCA. Significant statistical results at two alpha-levels (0.05 and 0.10) were identified.

3.11.1.2.2. PMRA Analysis

In addition to the statistical approach presented by EPA. PMRA has completed an additional statistical analysis. It is noted that while this method utilized a different statistical analysis approach, interpretations based on the PMRA analysis tended to be similar to interpretations from the EPA analysis. Although the PRMA analysis resulted in some differences in statistically significant endpoints and time periods, these differences do not significantly alter the ultimate biological interpretation of the study regarding colony level effects leading to a clearly defined, highly-confident protective endpoint. Differences from EPA statistical significance findings are noted (footnotes) in the specific life stages section of the DER and the detailed method is provided in **Appendix B**.

3.11.2. Colony Condition Assessment Variables - Life Stages

3.11.2.1. Adults[3]

Figure 6 below shows the effects on adult honey bees across CCAs and treatment groups. After the exposure period at CCA5 the number of adults was marginally significant ($0.05<p<0.1$) in the 50 µg/L treatment group (24.5% reduction) and significant ($p<0.05$) at CCA5 through CCA8 in the highest treatment group (44.9-57.2% reduction). By CCA8 adult numbers in all but the highest treatment group had converged to near the number of adults in the control. The mean number of adults in the control colonies was actually lower in than the treatment groups, again with the exception of the 100 µg/L treatment group.

It is apparent from all analyses that there were impacts to adults at the 100 µg/L group during the course of the study. The number of adults in the 50 µg/L treatment was also reduced with marginal statistical significance at CCA5 ($0.05< p<0.1$) (**Table 17**). However, the mean number of adults in the subsequent CCA was not significantly different from the control adding uncertainty to interpreting the effect of this treatment group on the colony. Qualitatively, after CCA4 the trends of the 37.5 and 50 µg/L group tend to trend closer to the 100 µg/L group (decline), while the 12.5 and 25 µg/L groups tend to trend with the

[3] PMRA significant difference $p<0.05$ at CCA4 for 100 µg/L and at CCA 5 for 50 µg/L treatment group.

controls (no clear dose trend) as an increase in adults with the decline beginning after CCA 5. From CCA6 onward no significant differences were seen outside of the 100 µg/L group and the mean number of adults tended to cluster similarly at each assessment. This was a later time in the year in which hives would begin to wind down for the winter. As the colony as a whole starts to prepare for overwintering the numbers of adults and other life stages are clearly decreased by the time of CCA6 and CCA7. During this pre-overwintering phase, adult proportions decline due to natural die off of worker bees and reduced rates of replenishment from reduced egg laying by the queen.

Table 17. Percent reduction from control for mean number of adults

Test concentration (µg/L)	Reduction from control (%)					
	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
12.5	18.8	18.5	15.1	15.6	1.2	-4.9
25	25.1	18.4	7.7	3.8	-3.5	-16.9
37.5	4.3	5.6	13.5	14.3	-2.3	-21.1
50	9.4	7.6	24.5*	15.3	11.3	-16.7
100	-5.8	11.0	44.9**	57.2**	56.7**	53.8**

Note: Negative value indicates increased number of adults in comparison to control.
*0.05<p<0.1
**p<0.05

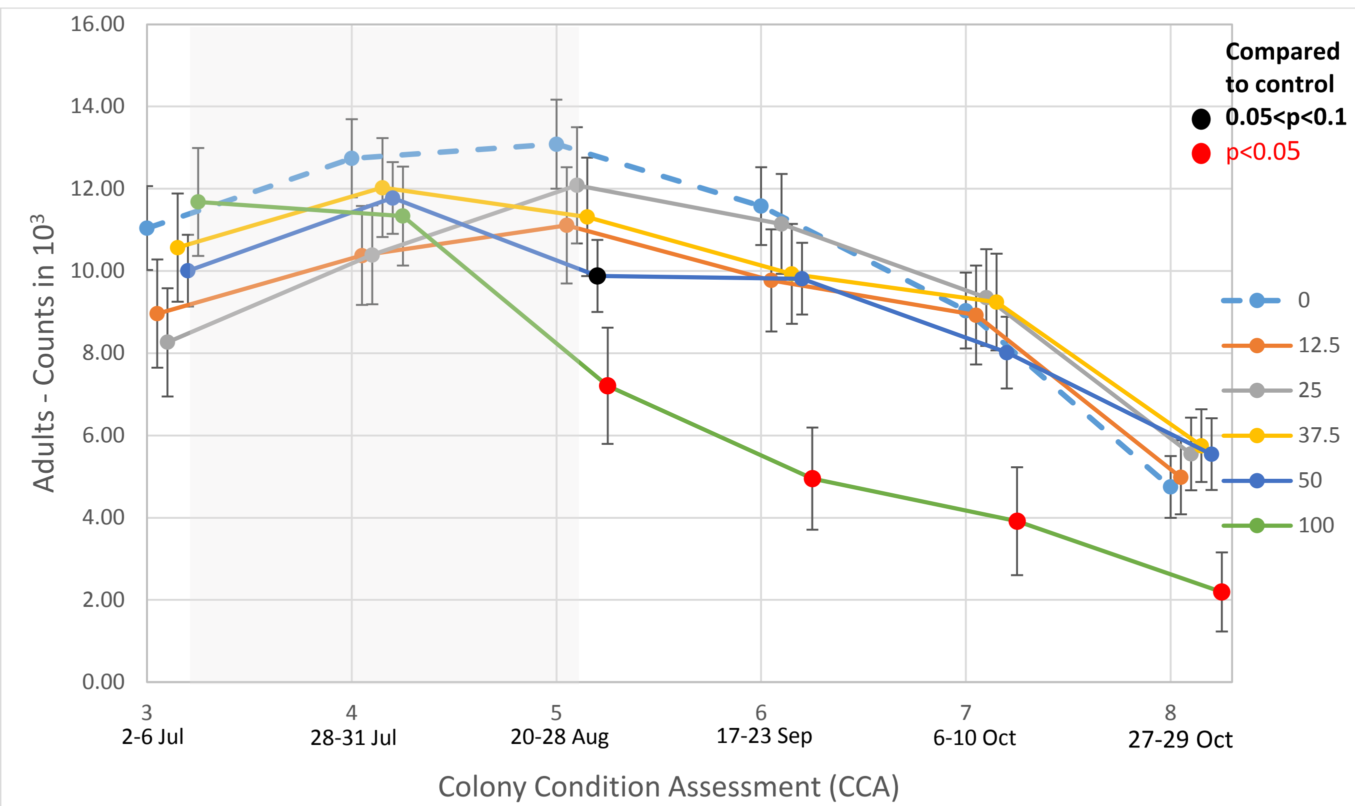


Figure 7. Number of adults (10^3) following exposure to varying concentrations of thiamethoxam in the diet across CCA3 – CCA8. Error bars represent standard error and the shaded box represents the exposure window.

3.11.2.2. Eggs[4]

There were similar numbers of eggs in all treatments through CCAs 3 and 4 and again in CCAs 7 and 8. There was marginal significance ($0.05<p<0.1$) in the 25 µg/L treatment group at CCA 3 prior to exposure. Eggs at CCA4 began a sharp decline in the 100 µg/L treatment group and reduction compared to the control was statistically significant ($p <0.05$) at CCAs 5 and 6. By CC7 means of egg numbers had converged to similar levels of controls in all treatment groups. In general, all treatment groups began a decline in the number or eggs after CCA 4 with the exception of the 12.5 ppb treatment group which did not begin its [sharp] decline until after CCA 5 (and appeared variable with number of eggs more similar to the control by CCA 7). At CCA 6 the 12.5 ppb treatment group was marginally significantly different ($0.05<p<0.1$) than the control before all the data converged at CCA7 for all treatment groups. The marginal significance CCA3 for the 25µg/L group is not considered biologically relevant, as the hives trended similarly with the remaining treatments, including the controls and this was prior to thiamethoxam exposure. By CCAs 7 and 8, the average of number eggs converged for all treatments, with the only apparent biological effects at the 100 µg/L treatment level.

Table 18. Percent reduction from control for mean number of eggs

Test concentration (µg/L)	Estimated reduction from control (%)					
	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
12.5	11.3	11.0	-16.5	38.6*	5.3	-12.8
25	32.2*	-1.0	6.2	4.9	26.6	-11.9
37.5	20.8	-0.2	-8.1	6.9	-21.0	5.1
50	7.8	-5.7	16.0	13.2	23.2	36.1
100	-7.8	8.6	44.4**	56.0**	37.8	-48.8

Note: Negative value indicates increased number of eggs in comparison to control.
*$0.05<p<0.1$
**$p<0.05$

[4] PMRA significant difference ($p<0.05$) at CCA 6 for 12.5 µg/L treatment group

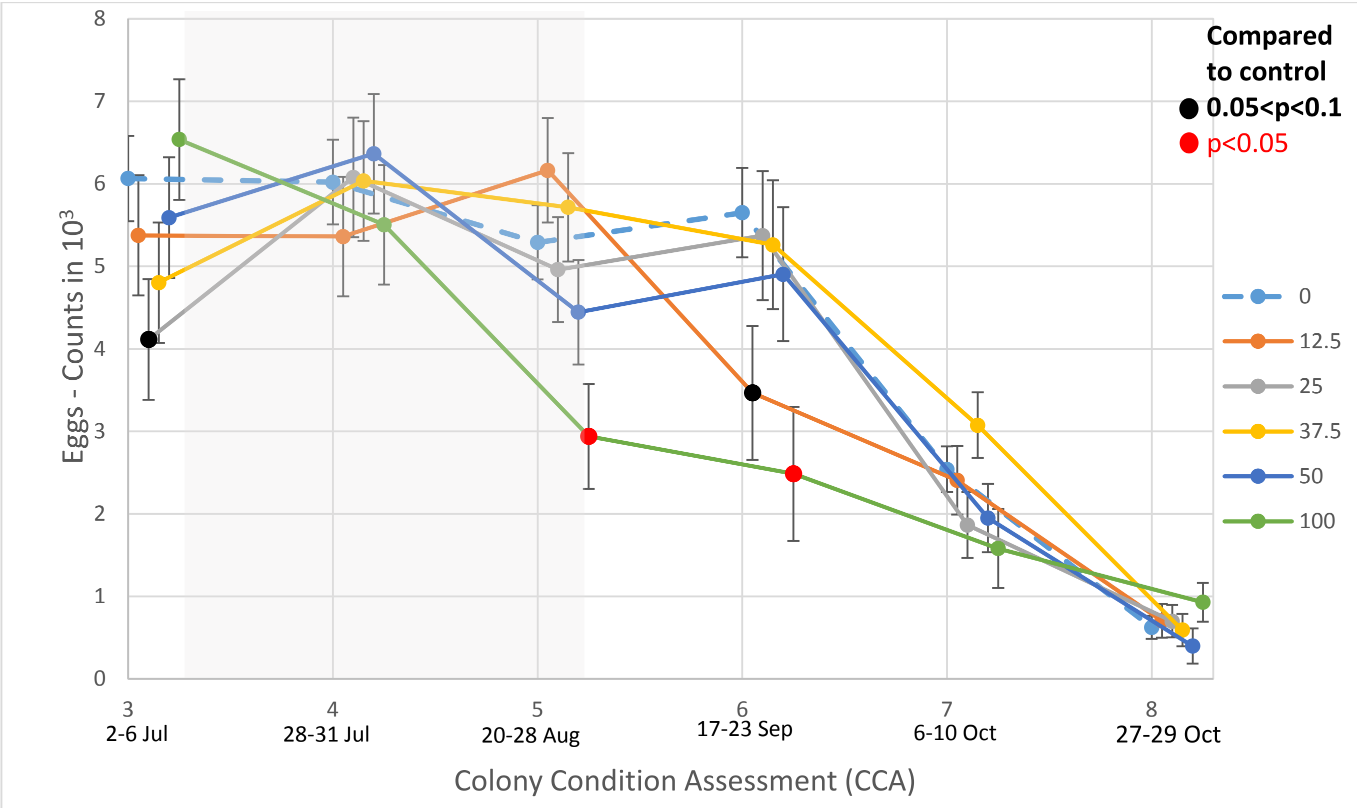


Figure 8. Number of eggs (10^3) following exposure to varying concentrations of thiamethoxam in the diet across CCA3 – CCA8. Error bars represent standard error and the shaded box represents the exposure window.

3.11.2.3. Larvae (Open/Uncapped brood)[5]

There were significantly lower numbers of larvae in the 100 µg/L treatment group as compared to control ($p<0.05$) beginning at CCA5 and continuing onto CCA6. There was also a significant reduction compared to the controls in the 50 µg/L treatment group at CCA 5 right after exposure ended. There was also a significant reduction compared to the controls in the 25 µg/L treatment group before exposure. By CCA6, excluding the 100 µg/L treatment group, and after a slight increase in the 50 µg/L treatment group, numbers of larva had converged to similar to the control numbers with no significant differences in any treatment group. It is uncertain if the significance after CCA5 is isolated or treatment related. The effects were not sustained at CCA6 indicating some level or recovery; however, there was a decrease in numbers after CCA 4 while the other groups generally sustained numbers or increased.

Table 19. Percent reduction from control for mean number of larvae (open/uncapped brood)

[5] PMRA significant difference $p<0.05$ at CCA7 and marginal significant difference $0.05<p<0.1$ at CCA 8 for 25 µg/L treatment group

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Test concentration (µg/L)	Estimated reduction from control (%)					
	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
12.5	17.2	-3.1	8.7	3.7	-24.3	-5.1
25	41.9**	3.3	10.0	-1.6	58.4	42.7
37.5	16.5	-4.5	7.2	-2.7	-8.8	-25.2
50	15.7	2.6	35.2**	16.5	11.5	78.8*
100	16.5	32.3	60.3**	59.6**	51.0	33.8

Note: Negative value indicates increased number of larvae in comparison to control.
*0.05<p<0.1
**p<0.05

Figure 9 below shows the trends of the control and all treatment groups for larval cells across all CCAs assessed. A clear divergence in the 100 µg/L groups is evident beginning at CCA4 where the numbers of larvae in these groups undergo a marked decline while the other treatment groups generally (except 50 µg/L) trend with control.

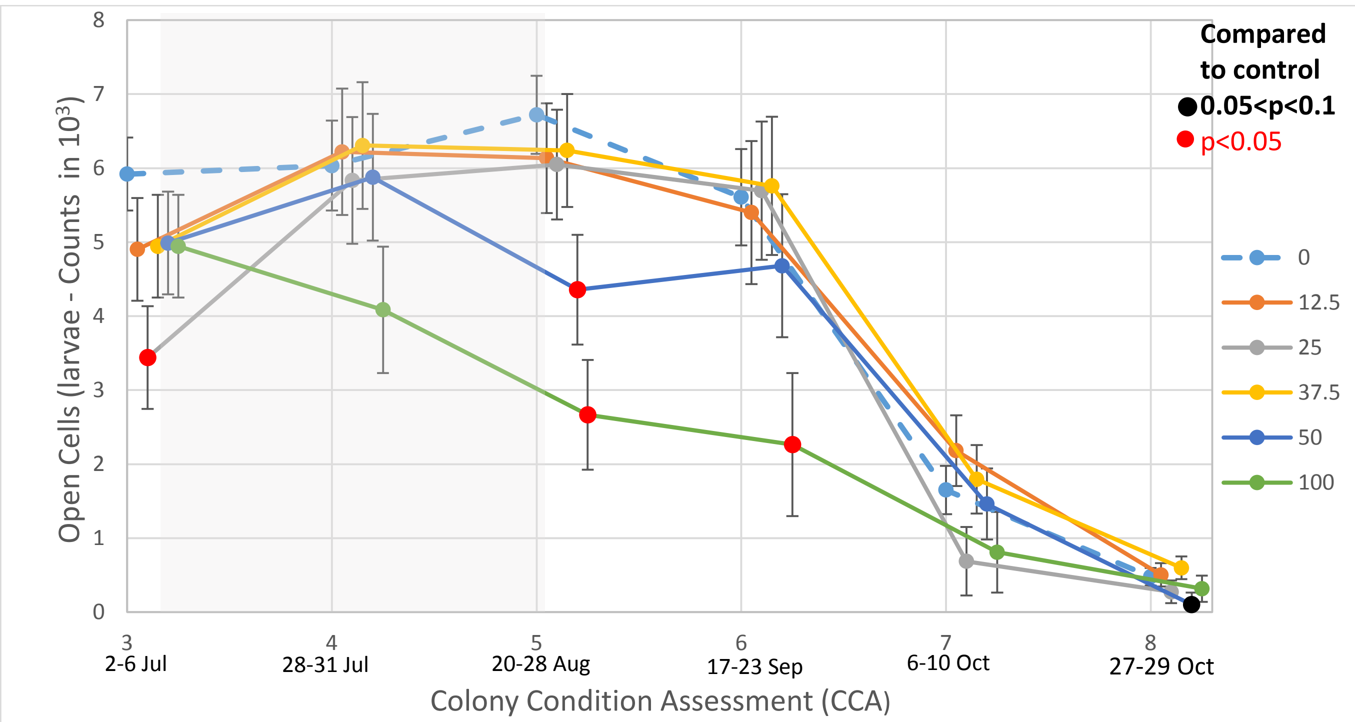


Figure 9. Number of larval cells (10^3) following exposure to varying concentrations of thiamethoxam in the diet across CCA3 – CCA8. Error bars represent standard error and the shaded box represents the exposure window.

3.11.2.4. Pupae (Capped brood)[6]

[6] PMRA significant difference (p<0.05) and marginal significant difference (0.05<p<0.1) at CCA 7 for 50 and 25 µg/L treatment groups respectively

In the 37.5, 50 and 100 µg/L treatment groups, there were significant reductions from control ($p<0.05$) at CCA5. However, the only significant differences beyond CCA5 relative to the control were in the 100 µg/L treatment group. There was a significant reduction relative to the control in the 25 µg/L treatment group prior to exposure. The two lowest treatment groups (12.5 and 25 µg/L) trended most similar to the control (peak at CCA5 then sharp drop), while 37.5 and 50 µg/L treatment groups trended similar to each other (slight to no drop CCA3-5 then sharp drop after CCA6) and 100 µg/L trending uniquely (consistent decline over all CCAs) before CCA8. By CCA6, all treatments, except for 100 µg/L, had converged to numbers where no significant differences were detected. As with the other variables, there is uncertainty in discerning treatment related effects beyond CCA5 as the values converge and decline potentially due to preparation for overwintering. By CCA8 all numbers were similar for all treatment groups. Worth noting, the controls, 12.5 and 25 groups all show an increase in the number of pupae in CCA5 relative to the number of pupae in CCA4, and that the temporal increase in the number of pupae is not seen in the 37.5, 50 and 100 groups. This trend is prominent here (**Figure 10**) and in **Figure 12**.

Table 20. Percent reduction from control for mean number of pupae

Test concentration (µg/L)	Estimated reduction from control (%)					
	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
12.5	13.4	11.5	9.3	-0.6	-39.1	-56.3
25	25.3**	11.9	16.9	0.6	53.8	35.0
37.5	12.1	5.4	26.7**	2.1	-1.8	-32.8
50	9.0	20.8	42.0**	15.9	45.6	-0.7
100	6.6	47.3**	75.5**	58.1**	92.5**	14.3

Note: Negative value indicates increased number of pupae in comparison to control.
**p<0.05

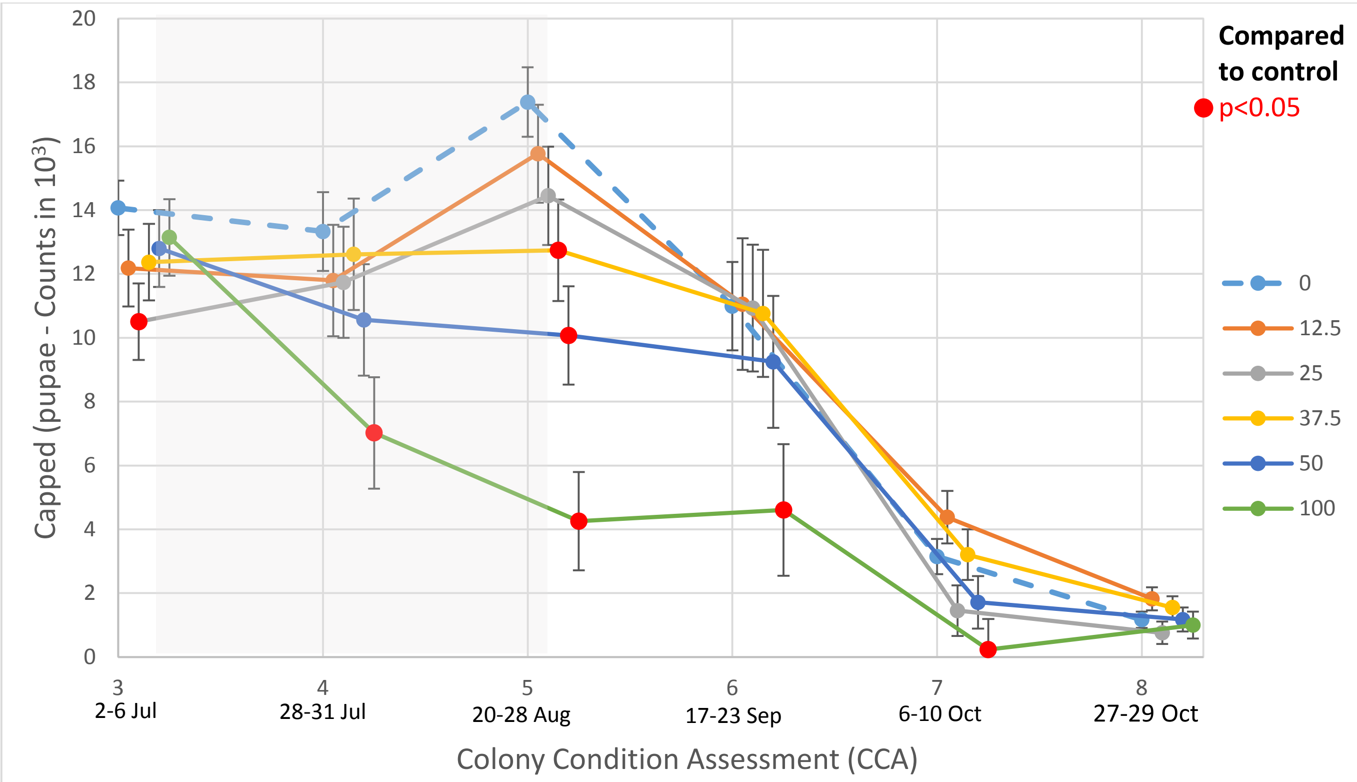


Figure 10. Number of pupal (capped) cells (10^3) following exposure to varying concentrations of thiamethoxam in the diet across CCA3 – CCA8. Error bars represent standard error and the shaded box represents the exposure window.

3.11.2.5. Total individuals in hives

Similar to the individual (adults, larvae, pupae) parameters, the total number of individuals was consistently significantly reduced compared to the control in the 100 µg/L treatment group. Again, directly after exposure (CCA5) there was a significant difference in the 50 µg/L treatment in which the numbers begin to decline after CCA4. There was also a decline in total individuals in the 37.5 µg/L treatment group although not as dramatic as in the two highest treatment groups. There was a continual decline throughout the remaining CCAs (CCA 6-8). Similar to pupae, the two lowest treatment groups (12.5 and 25 µg/L) trended most similar to the control (peak at CCA 5 then sharp drop), while 37.5 and 50 µg/L treatment groups trended similar to each other (slight to no drop CCA3-5 then sharp drop after CCA6) and 100 µg/L trending uniquely (consistent decline over all CCAs). By CCA6 all treatments (with the exception of 100 µg/L) had converged to numbers similar to that of the controls and that trend was consistent for the remaining of the assessments. From CCA4-7, numbers of individuals in the 100 µg/µg/L group were significantly lower than in controls.

Table 21. Percent reduction from control for mean total number of individuals

Test concentration (µg/L)	Estimated reduction from control (%)[1]					
	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
12.5	15.3	11.5	7.8	12.5	-9.2	-12.2
25	29.0**	10.7	11.6	2.3	18.8	-2.1

Test concentration (µg/L)	Estimated reduction from control (%)[1]					
	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
37.5	11.9	3.0	15.5	6.0	-5.6	-19.3
50	10.0	9.3	32.3**	16.3	19.9	-2.6
100	2.1	26.7**	59.8**	58.3**	61.3**	36.6

Note: Negative value indicates increased number of total individuals in comparison to control.
*0.05<p<0.1
**p<0.05

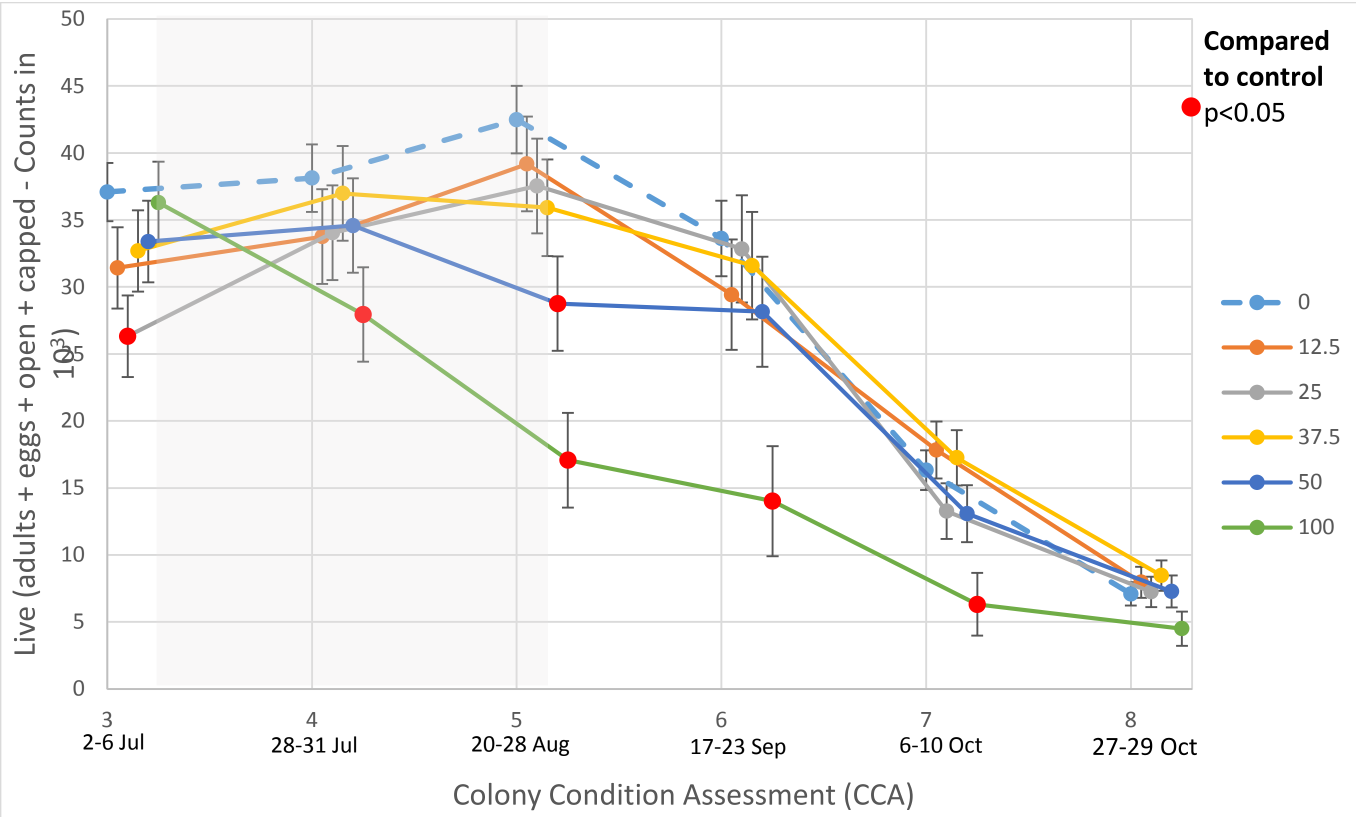


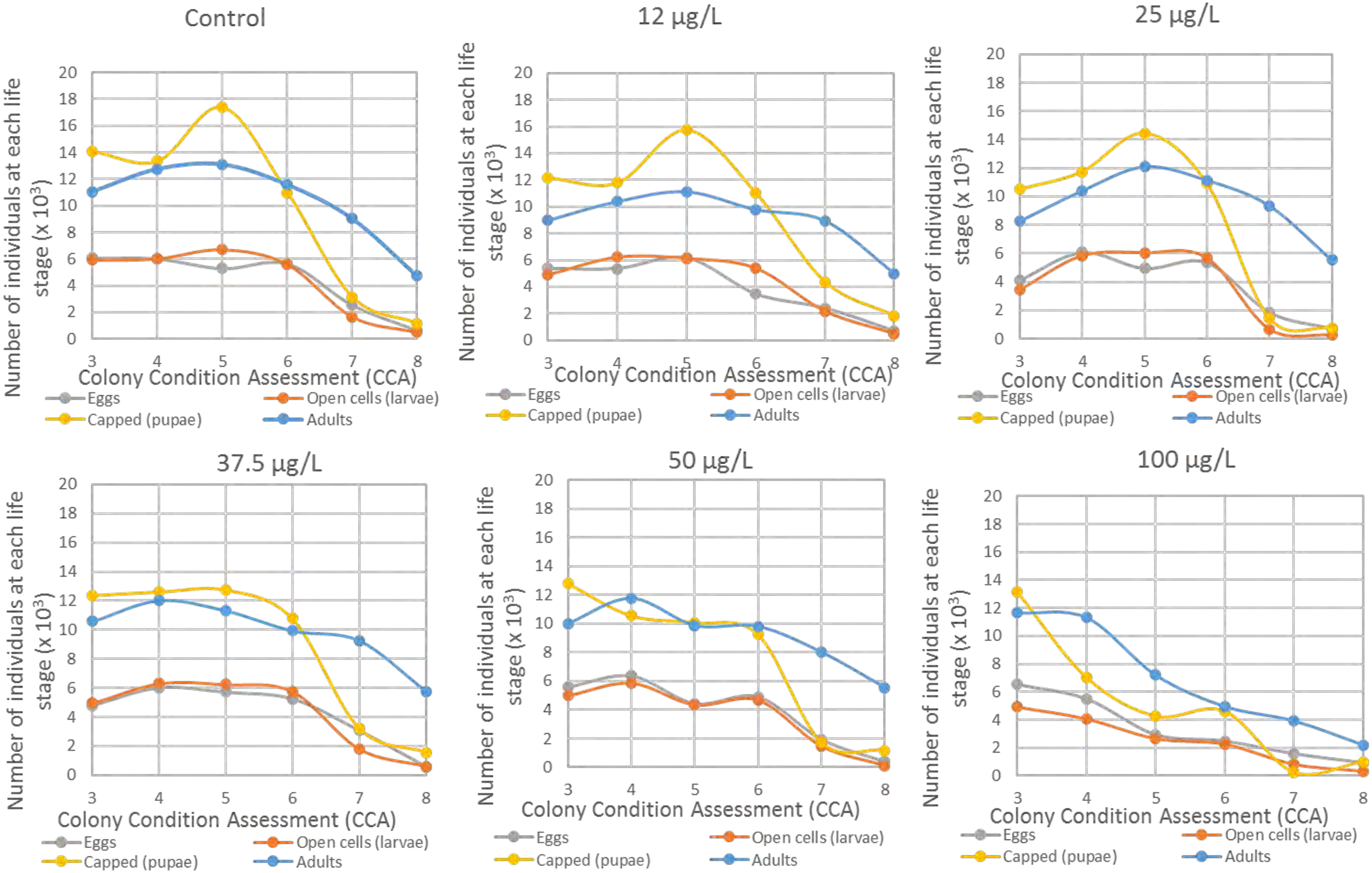
Figure 11. Number of total (adult, eggs, larvae, pupae) individuals (10^3) following exposure to varying concentrations of thiamethoxam in the diet across CCA3 – CCA8. Error bars represent standard error and the shaded box represents the exposure window.

Similar to pupae after CCA3, there is an upward trend in the total number of individuals in the control, 12.5 and **25 µg/L** groups while the trend is downward in the 37.5, 50 and **100 µg/L** groups. Without successful overwintering it is hard **to understand the significance (biological if any) of this trend if the colonies had made it through** winter.

Figure 12 below provides another visual representation of the effects across CCAs variables within a response variable for the various life stages of bees during the course of the study. The graphs are meant to show the general trends in the life stages for each treatment group.

The figure illustrates clear treatment effects for all life stages in the 100 µg/L treatment group. In that group, all life stages begin an immediate decline from CCA3 (which is one week before exposure) to CCA4 (which is 1 week after exposure) and continue to decline throughout the course of the assessments. Control, 12, and 25 µg/L treatment groups appeared to have similar trends across life stages. In the three highest treatment groups the trends begin to diverge at 37.5 and 50 µg/µg/L as the spike in pupae is not observed and the decline in the number of adults starts sooner. Additionally, the larvae and eggs both begin to decline together in the highest three treatment groups. The effect is again clearest in the 100 µg/µg/L where the sharp decline begins immediately after CCA 3 for pupae and adults, rather than peaking around CCA5 for these two life stages. These are meant to qualitatively illustrate the trends. Variability in the estimates may contribute to the lack of statistical differences which may point to control performance and uncertainty of the treatment effects.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document



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The inability to achieve overwintering limits the utility of these results. Additionally, there were also uncertainties with respect to the health of the hives used particularly the controls and timing of exposure. Mostly, other than in the highest treatment group, significant reductions compared to the control hives of biological parameters were only observed in the other treatment groups at CCA5 (the end of exposure). Other differences occurred transiently prior to exposure concluding but were not consistent enough to be suggestive of colony level effects at other treatment levels. After CCA5 (end of exposure) there was also a general decline in all parameters in all treatment groups (including controls), suggesting all hives were in preparation for overwintering and limiting the ability of the study to detect treatment related effects Healthier control colonies and an earlier exposure window may further refine the effects seen for some measurements.

3.11.3. Colony Condition Assessments – Food Store Response Variables

3.11.3.1. Pollen[7]

Pollen stores were significantly reduced in the 100 µg/L treatment group from CCA4 to CCA7 ($p<0.05$). Pollen stores were also significantly reduced compared to the control in the 25 µg/L group at CCAs 5 and 7. At CCA4 in the 25 µg/L treatment groups and at CCA5 in the 50 µg/L dose group pollen stores were marginally significant ($0.05<p<0.1$). With the exception of a slight increase in the 12.5 µg/L treatment group, all treatment groups had a drop in pollen stores after exposure began (between CCA 3 and CCA 4). However, to some degree they increased between CCA 4 and 5 with the exception of the 100 µg/L treatment. There was no significant reduction (relative to controls) of pollen stores in the 12.5 and 37.5 µg/L treatment groups for any CCA assessed. The stored pollen numbers converged at the last CCA where no differences were detected.

Table 22. Percent reduction from control for mean number of pollen cells

Test concentration (µg/L)	Estimated reduction from control (%)[1]					
	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
12.5	17.6	2.0	21.0	6.2	4.8	7.1
25	16.4	51.0*	43.7**	28.3	46.3**	22.7
37.5	-9.9	10.0	22.4	11.6	13.9	-14.1
50	21.0	42.1	37.8*	28.3	27.4	9.5
100	-11.1	56.8**	80.7**	74.8**	66.6**	38.6

Note: Negative value indicates increased pollen stores in comparison to control.
*$0.05<p<0.1$
**$p<0.05$

[7] PMRA significant reductions $p<0.05$ at CCAs 4 and 5 for 50 for µg/L treatment group and CCA 4 for for 25 µg/L treatment group

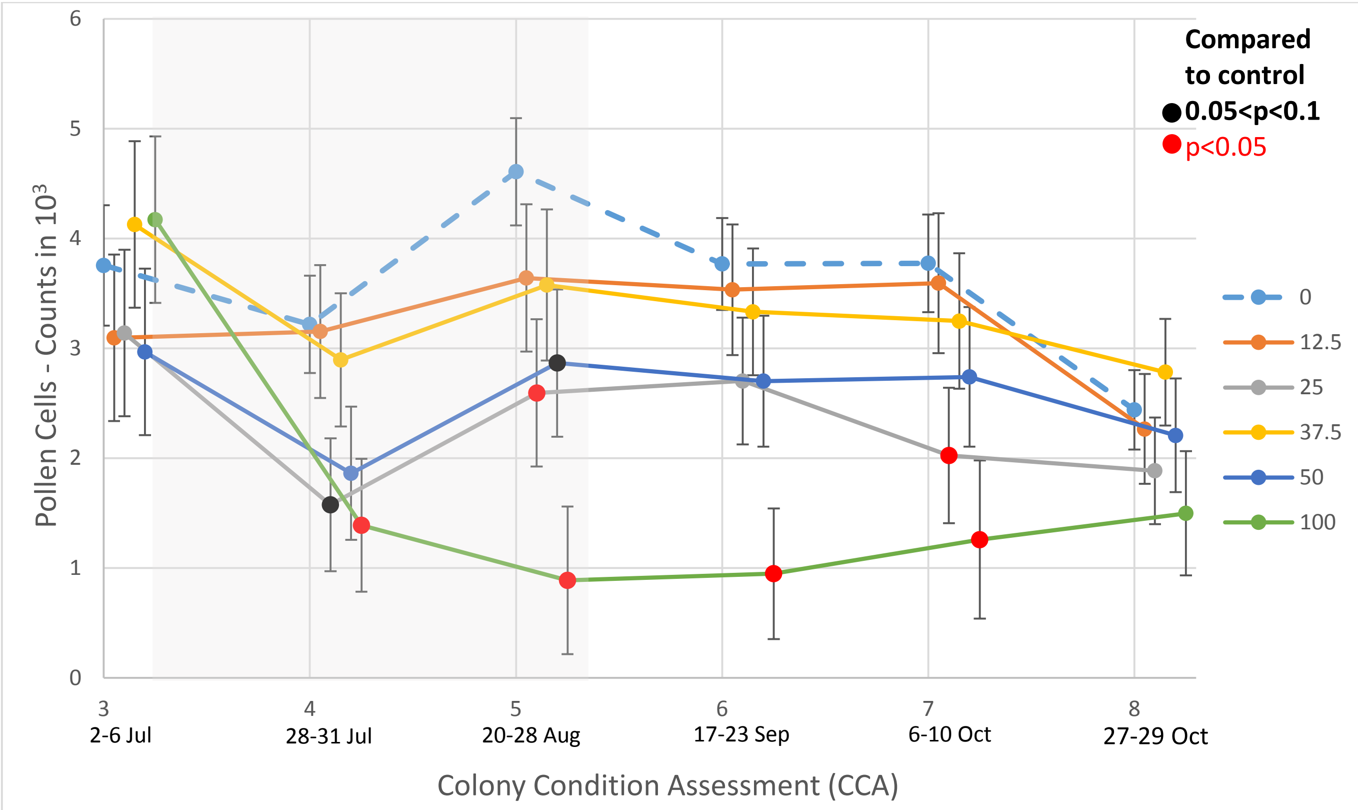


Figure 13. Number of pollen cells (10^3) following exposure of honey bees to varying concentrations of thiamethoxam in the diet across CCA3 – CCA8. Error bars represent standard error and the shaded box represents the exposure window.

In examining the trends of pollen stores in the control, there was only a slight buildup between CCA 4 and CCA 5. All treatment groups (excluding 100 µg/L) had the same buildup trend between CCA4 and CCA5 before generally decreasing or leveling off by CCA6 and decreasing thereafter. Pollen stores actually increased (slightly) in the 12.5 µg/L treatment group until CCA 7. While there were no differences between these two groups a similar increase would be expected in the control. This increase would support the queen in her effort to build up brood. Pollen stores experienced (in general) a slight decline in numbers from CCA 5 to CCA 8. This downward trend reflects that the fact that up to overwintering, brood production will slow as the hive prepares for winter and therefore there is a reduced need for pollen within the hive. The lack of continual increase in the pollen stores of the control add some uncertainty if the control colonies were functioning well enough to collect enough food for overwintering.

3.11.3.2. Nectar / Honey[8]

There were no significant reductions in honey relative to the control. There was one marginal significance ($0.05<p<0.1$) for a lower amount of honey stored in treatment hives at 12.5µg/L than in the control at CCA6 and thereafter (**Figure 14** below). All hives generally increased in honey stores until CCA 6 with a drop-off between CCA 6 and CCA 7 before increasing again. Trends were similar for all the treatment groups.

[8] PMRA marginal statically significant difference 0.05<p<0.1 at CCA 8 for 100 for µg/L treatment group.

The 100 actually increased more quickly than the controls which could be an indication of decreased activity in the hive due to treatment effects. All other treatments trended similar to the control.

Table 23. Percent reduction from control for mean nectar/honey stores

Test concentration (µg/L)	Estimated reduction from control (%)[1]					
	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
12.5	19.3	24.6	31.6*	14.1	10.1	5.9
25	7.7	8.6	17.5	14.0	-3.0	-5.6
37.5	-14.1	-7.2	-1.8	-11.5	-18.6	-11.8
50	6.3	3.4	4.2	0.8	-15.4	-6.0
100	-3.0	-33.4	-47.5	1.2	-17.2	24.5

Note: Negative value indicates increased nectar/honey stores in comparison to control.
*0.05<p<0.1
**p<0.05

Figure 14 below for the honey store trends in the control, 12.5, 25, and 50 µg/L groups only show a marked divergence at the 50 µg/L treatment group beginning at CCA6 and persisting up to and after overwintering at CCA8.

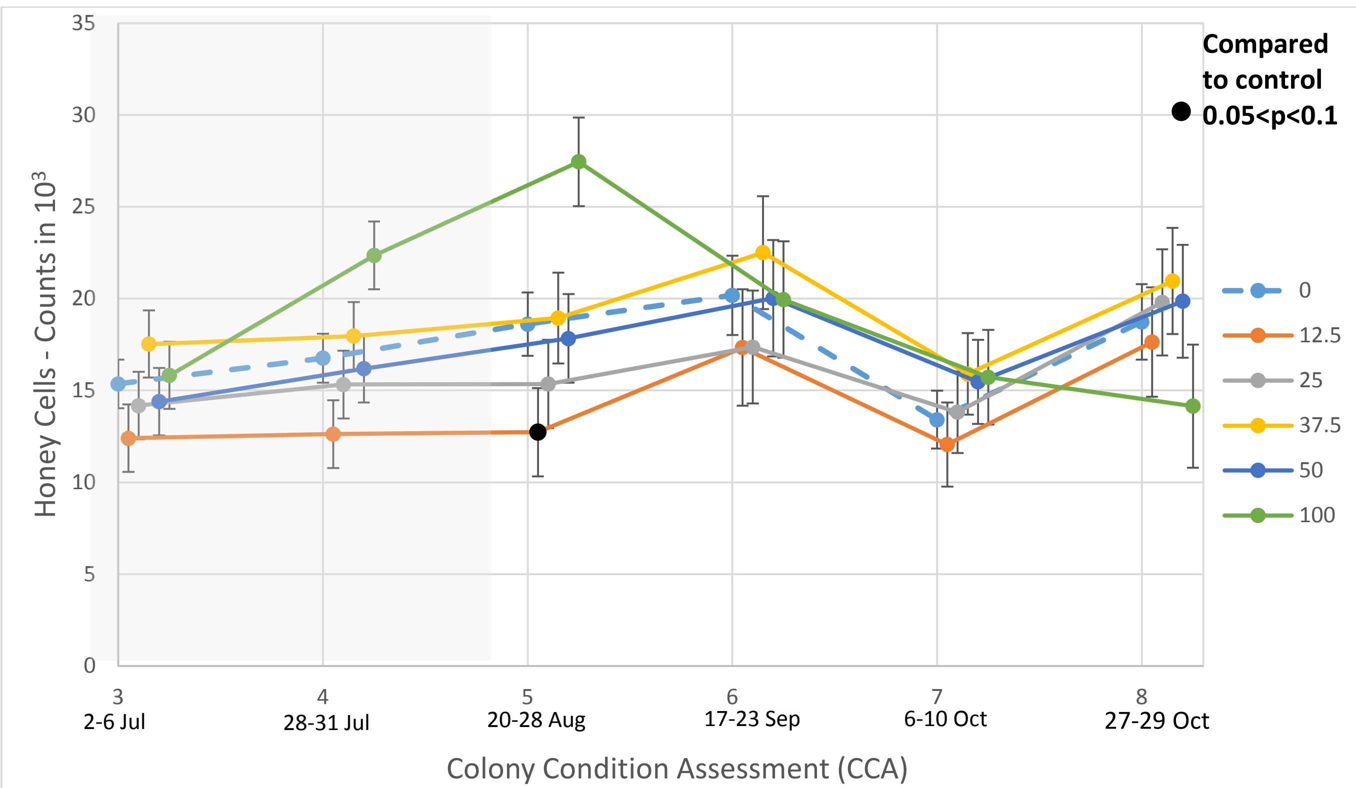


Figure 14. Number of honey cells (10^3) following exposure of honey bees to varying concentrations of thiamethoxam in the diet across CCA3 – CCA8. Error bars represent standard error and the shaded box represents the exposure window.

It is noted that the feeding solutions (sugar solutions) provided during the exposure period might have affected natural honey storage patterns (because a decline in foraging outside of the hive may not impact the storage of honey); however, effects on honey storage are still able to be considered as all treatments were compared to control hives (which also received feeding solutions).

Figure 15 below shows food stores (pollen and honey). The trends are similar to those for honey. With the only a marginal significant reduction in the 12.5 µg/L treatment group at CCA 5. The higher food stores in the 100 µg/L could be an indication of decreased activity (higher morality) and increased utilization of the sucrose (rather than outside forage sources and sucrose) in the hive or an uncertainty of how the control hives were performing. Trends for all the remaining treatment groups were similar to the controls**.**

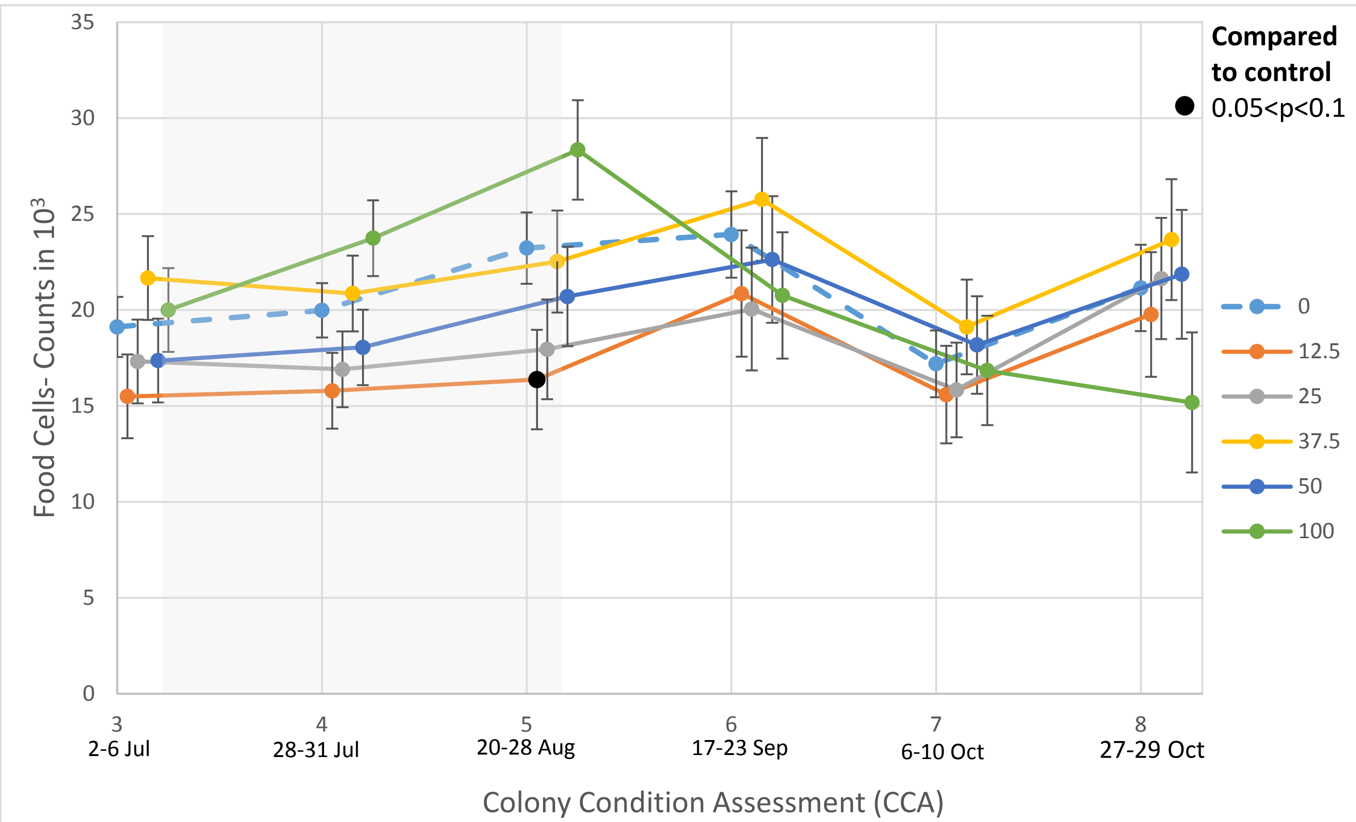


Figure 15. Number of food cells (10^3) following exposure of honey bees to varying concentrations of thiamethoxam in the diet across CCA3 – CCA8. Error bars represent standard error and the shaded box represents the exposure window

4. Reviewer comments:

What follows is brief discussion of some of the elements taken into consideration when evaluating the results of this study.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

4.1. General Considerations for Biological Interpretation

While the hive mortality is considered as the most relevant measurement of survival at the colony level, sub-lethal effects at the colony level were estimated by measuring multiple parameters during the course of study. Each measured parameter is expected to reflect only part of the colony conditions, and all measurements have to be integrated for a better understanding of the hive status at the colony level. A honey bee colony is a super-organism in which live individuals and food supply are the two major components in maintaining the proper function of the colony. There are interactions between the two components and even within each component.

Individual bees are present in the colony as eggs, larvae, pupa and adults and they develop from one stage to another and interact with each other to perform a variety of tasks to maintain the integrity of the colony. The measurement of each stage of the bees is expected to provide information on the potential treatment effect on a specific life stage of bees during their development.

Hive food supplies including hive pollen and nectar are collected and processed by adults and are expected to have a large impact on the development of all stages of bees in hives. However, the amount of hive food storage is dependent on not only the number of foragers available for food collection, but also the number of individuals that consume the food. In addition, the seasonal availability of outside pollen and nectar sources also affects the amount of storage, thus impacting hive development. As well, sucrose feeding solutions were provided to the hives as a means of treatment and as a supplement for hive overwintering, which may have affected foraging and food storage during those time periods.

Hive weight was measured during the study. However, it is largely affected by the honey storage and number of bees that consume the food. A strong colony with a high number of bees likely consumes a high amount of stored honey and may result in a reduced hive weight. Weighing hives at different time periods of the day may result in an increased variation of the measurement due to the fact that foragers may not be present in the hive when the weight is measured. Hive weights may be artificially lower in hives which contain a high number of forager bees that may be out collecting food during a different time of the day.

Considerations regarding the measurement time points:

- CCA3 represents the background hive conditions as the first colony assessment after the hives were placed in the test fields prior to the exposure.
- CCA4 and CCA5 represent the hive conditions during the exposure phase. It was noted that the CCA5 was conducted a week after the end of the 6-week exposure period, but is expected to represent effects during the exposure period.
- CCA6 was measured at 4-5 weeks after the end of exposure. It allows all bee individuals, including eggs, larvae and pupa that were exposed to treatment to finish their development cycle and become adults. However, the thiamethoxam study began later in the season, and therefore, by CCA 6, all colonies, including the controls were in decline.
- CCA6, 7 and CCA8 represent the hive conditions prior to overwintering. It is considered that hives were physiologically preparing for overwintering by reducing the production of immature bee individuals. Treatment effects may be masked by the natural decline of hive individuals.
- CCA9 and CCA10 represents hive conditions of surviving hives after overwintering. Mortality in the control and treatment hives excluded these assessments from analysis.

4.2. Control Performance

Control mortality and Sub-lethal effects on life stages and food stores

The control performance in this study offers some challenges relating to the interpretation of the results. The level of colony loss after overwintering in controls (79%) adds a great deal of uncertainty when considering the results of individual measurements. The fact that many of the treatment hives performed/trended similarly to the control hives could be indicative of either a lack of treatment effects or simply the control hives were not optimal to begin the study. Because so few control hives survived overwintering and trended closely to the treatment hives during exposure, the overwintering component would be important to determine if the lack of significant reductions compared to the control in most treatment groups is biologically significant. Almost every parameter for life stages decreased after exposure ended which could have been a factor of the time of year or treatment. The fact this also happened in the control groups suggest a performance issue is possible, or at the very least an uncertainty with respect to if the exposure measurements were taken too late in the year to discern treatment effects.

Additionally, the number of adult bees in the control hives is something to consider. Trends in the adult bees generally increased during the early CCAs, but no sharp increase was observed. We would expect hives to build during this time when the packages were first set up in May as there would have been pollen and nectar flows in North Carolina[9]. The hive would be expected to ramp up numbers of adults to some degree to achieve the appropriate number of bees to keep the hive alive throughout the winter. As the high loss in overwintering colonies in the control suggests, this number of adults (while not necessarily the only factor, but could be considered a potential contributing factor) was not achieved. In subsequent CCAs after exposure the control measurements trended similarly to the lowest treatment groups in most cases; although a comparison was also made difficult by the later initiation date for feeding and all hives were in decline. A stronger performing group of control hives (potentially with a higher starting number of adults) would increase the certainty of conclusions based on effects observed relative to these hives.

The similarity in the dynamics of all parameters for the individual living organisms at various stage across the treatments indicates that control hives were may have been stressed. For most parameters and treatment groups the means converged to those of the control at CCA 6 through CCA 8. The time of year could have influenced control hive performance, but it is still uncertain if the hives were developing normally. There was no apparent spike of honey collection or pollen stores from the control hives indicating they may not have been developing and storing enough food to survive the winter. Pollen stores were decreasing at the same time other biological parameters were indicating consumption of resources but not replenishment for the hive.

4.3. Consideration of Study Strengths, Limitations and Interpretation

[9] https://growingsmallfarms.ces.ncsu.edu/wp-content/uploads/2015/02/CALENDAR-FOR-BEEKEEPING-IN-CENTRAL-NORTH-CAROLINA.pdf?fwd=no
https://www.ncbeekeepers.org/honey/floral-sourceswhats-blooming

It is important to recognize the inherent strengths and limitations of this study as results are interpreted and potentially considered in risk assessment.

In the context of available field studies involving honey bees and thiamethoxam, this study contains a number of strengths. A high degree of replication, demonstration of sustained effects at the highest treatment level, quantification (and method validation) of both thiamethoxam and CGA322704 (clothianidin), high end exposure scenario, multiple measured endpoints in detailed CCAs, and the available raw data of these endpoints for analysis all add to the strength of the study.

However, there are limitations with this study including: Exposure through nectar (sucrose) alone may not reflect a likely field scenario where bees are probably exposed through both pollen and nectar routes. Thus the potential effects due to prolonged exposure to both contaminated nectar and/or pollen may be underrepresented, although, the impact of this exclusion on the study results is uncertain and will likely depend on the life stage/caste of bee. Additionally, because exposure was channeled through both hive pollen and nectar via exposure to the sucrose feeding solution, there is uncertainty how this compares to exposure through contaminated pollen directly (a potential for foraging bees). Pesticides from food sources other than the artificial feeding were also detected during the exposure period and post-exposure periods through collection of pollen from pollen traps. This contributes to exposure uncertainty and can add confounding effects when interpreting results.

Finally, a key component of this study was achieving overwintering survival. Overwintering success of controls was severely impacted (79% hive mortality). In fact, no hives survived overwintering in any significant proportion. If control hives had survived, comparing trends or lack thereof with the treatment hives would be more meaningful. Since no sustained significant effects were seen except in the 100 µg/L treatment it is uncertain if the control hives were performing normally during the test.

5. Conclusions

Based on the limitations of this study, a NOAEC derived from this study is considered highly uncertain. The key limitations include: 1) late timing of exposure that coincides with ramping down trends of colony endpoints, 2) lower than expected performance of controls, and 3) lack of overwintering success. Effects were observed to multiple endpoints and multiple CCAs at the highest test level (i.e., 100 µg a.i./L thiamethoxam; 86 µg a.i./L clothianidin-equivalents). Effects to several endpoints (number of adults, amount of brood) were also observed at CCA5 of the second highest test level (i.e., 50 µg a.i./L thiamethoxam; 43 µg a.i./L clothianidin-equivalents). It is uncertain whether or not effects at 50 µg a.i./L are meaningful to the colony since these effects were only observed at CCA5, which is the conclusion of the exposure window, however, potential recovery could not be determined due to the limitations of CCA6, 7, and 8 (during downward trend of colonies) and a lack of overwintering data. Also, the utility of CCAs 6, 7 and 8 in showing treatment related effects are questionable because of the downward trend in endpoints that is consistent with preparation of colonies for winter. If effects observed at 50 µg a.i./L are biologically significant to the colonies, the NOAEC from this study would appear to be 37.5 µg a.i./L (32 µg a.i./L-clothianidin equivalents). There is uncertainty in whether or not this value is conservative. Since the hives did not perform as expected, and given the late timing of the exposure window, it is uncertain whether or not effects due to thiamethoxam could be detected.

A comparison can be made between the effects of this study and those in the CFS with clothianidin (MRID 49836101; PMRA#), which was conducted in a similar location and in the same year. In the clothianidin CFS, clear effects were observed at 40 µg a.i./L over multiple endpoints and multiple CCAs, leading to a NOEC of 20 µg a.i./L (clothianidin). At the NOEC of 20 µg/L, some effects were observed at CCA5, but these effects did not manifest at later CCAs. This suggests that effects were observed at lower levels in the clothianidin study compared to the thiamethoxam study. Therefore, when considering the uncertainties described above, the apparent NOAEC for the thiamethoxam CFS is tentatively determined to be 37.5 µg a.i./L (32 µg a.i./L-clothianidin equivalents), noting that this may value may not be conservative. The apparent NOAEC of 37.5 µg a.i./L (thiamethoxam; 32 µg a.i./L- clothianidin equivalent) along with the effects levels of 50 and 100 µg a.i./L (thiamethoxam) will be used to characterize the risk of thiamethoxam to honey bee colonies. Additional information from the clothianidin CFS may also be used to characterize the risk of thiamethoxam to honey bees. **This study is considered scientifically valid; however, it is classified supplemental due to the limitations summarized above and throughout this DER.**

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Appendix A: Details of EPA Statistical Analysis

Study Design and Overview of Statistical Analysis Approach

The general experimental design was a randomized complete block (apiary) with repeated measures (CCA) and data will be analyzed in SAS (v9.4) using the PROC MIXED procedure. Since hives were not assigned and placed in the study apiaries until shortly before CCA3, the data for the statistical analysis only included data collected from CCA3 and the following CCAs. Shortly before CCA3, hives were ranked by strength and the 'strongest' hives were placed in the one apiary. The next eight strongest hives were then placed in an empty apiary. This process continued until hives were placed in all apiaries. Within each apiary, the control treatment was replicated two times and each treatment occurred one time (total of 8 hives in each apiary: seven hives were randomly assigned as control or treatment group and the eighth hive was used for additional sampling during the study). Given this design, the blocking factor 'apiary,' represents variation due to geographic location and initial hive strength.

As a large percentage of hives did not survive overwintering, data collected the following spring will not be included in the statistical analyses (**Table 23**).

Table A.1 Timeline including major milestones of study

Date	Study action*	Comments
20 May 2014	Initiate CCA1 (non-GLP)	Not included in statistical analysis.
9 Jun 2014	Initiate CCA2 (non-GLP)	Not included in statistical analysis.
27 Jun-1 Jul 2014	Hives moved to study locations	none
2 Jul 2014	Initiate CCA3 (GLP)	First CCA to be included in the statistical analyses.
8 Jul 2014	Initiate thiamethoxam exposure through sucrose solution.	none
28 Jul 2014	Initiate CCA4 (GLP)	none
19 Aug 2014	End thiamethoxam exposure through sucrose solution	none
20 Aug 2014	Initiate CCA5 (GLP)	None
17 Sep 2014	Initiate CCA6 (GLP)	None
6 Oct 2014	Initiate CCA7 (GLP)	None
28 Oct 2014	Initiate CCA8 (GLP)	Final CCA to be included in the statistical analyses.
31 Mar 2015	Initiate CCA9 (GLP)	Overwintering survival was 21, 0, 33, 17, 33, and 0% in the control, 12.5 ppb, 25 ppb, 37.5 ppb, 50 ppb, and 100 ppb treatment groups, respectively. Therefore, CCA9 and CCA10 were not included in statistical analyses.
28 Apr 2015	Initiate CCA10 (GLP)	
*each CCA took two or more days to complete.		

Variables recorded at each CCA included number of adult bees in the hive and number of cells containing each of the following life stages or food stores: eggs, larvae (open cells), pupae (closed cells), pollen, and honey. Following standard bee keeping practices, supers were added or removed from each hive to best support growth or reductions in the size of the bee colony. A queen excluder was placed between the initial hive box and added super boxes; this limited the summed number of egg, pupae, and larvae cells to the number of cells in the initial box (68,880 cells: 3440 cells/frame X 2sides/frame X 10 frames/box). All adult bees, with the exception of the queen, could move to any added supers, and honey and pollen could be stored in those additional supers as well. The suite of variables that were subjected to data analysis were:

- Number of adults
- Number of egg cells
- Number of open (larvae) cells
- Number of capped (pupae) cells
- Number of pollen cells
- Number of honey cells
- Total number of individuals (adults + eggs + larvae + pupae)
- Total brood (eggs + larvae + pupae), and
- Total food (pollen + honey).

To facilitate computation and algorithm convergence in the SAS Procedures, all data was divided by 1000 prior to any statistical analysis. Since all response variables were divided by the same constant, there was no effect on any of the test statistics or p-values. No adjustments for addition or removal of supers were conducted for the statistical analysis.

Prior to the repeated measures analysis, the data were evaluated for patterns in temporal correlation and correlations across hive components within each of the evaluated CCAs. This analysis was accomplished through a series of pairwise scatterplots and principle components analyses (PCA).

Scatterplot and Principle Component Analysis

Based on physical hive constructs and the nature of honey bees, it is generally accepted that the colony condition assessment (CCA) variables may be correlated over time and may also be correlated within a time point (sampling time). Given this background, a series of scatterplots, correlation matrices, and principle component analyses was prepared; the full SAS output is included as **Attachment 1**. For these analyses, there was no adjustment for treatment effects, only correlation over time was evaluated.

For the single hive components, adults, eggs, larvae, pupae, pollen, and honey, some of the general summary points are:

- With the exceptions of adults and honey, CCA8 tended to have the lowest pairwise correlations with the other CCAs for all components. For adults, there was no CCA that tended to be less (or more highly) correlated with other CCAs. For honey, CCA3 tended to have the lowest pairwise correlations with the other CCAs.
- For each of the hive components, the first principle component explained 39 to 65% of the total variation across all CCAs; the lowest percent of explained variation was for larvae and the highest was for adults.
- For all the hive components, the first principle component was a weighted average of all the evaluated time points; however, The weights and varied depending on the endpoint. For adults, pollen, and honey, all CCAs carried approximately equal weights (CCA3 and CCA8 had slightly smaller weights). For eggs and larvae, CCA3, CCA4, CCA5, CCA6, and CCA7 carried approximately equal weight and CCA8 carried much less weight. For pupae, CCA3, CCA4, CCA5 andCCA6 had approximately equal weights while CCA7 and CCA8 had smaller weights.

For the three composite hive variables (live, brood, and food), general summary points are:

- For live, the first principle component explained 59% of the total variation and could be described as a weighted average of all CCAs with CCA3 and CCA8 carrying slightly less weight.
- For brood, the first principle component explained 44% of the total variation. The first principle component could be described as a weighted average with CCA3, CCA4, CCA5 and CCA6 carrying approximately equal weights while CCA7 carried less weight and CCA8 had the least weight.
- For food, the first principle component explained 61% of the total variation, and the general interpretation of the first principle component was a weighted average with CCA3, CCA4, CCA5, CCA6 and CCA7 carrying approximately equal weights while CCA8 carried less weight.

In addition to exploring correlations among CCAs for each of the response variables, correlations among response variables within a CCA were explored. For this exploratory analysis, only the individual hive components were evaluated. No adjustment was made for treatment effects (*i.e.*, all data were included in a single series of plots and PCAs; separate assessments were not done for each treatment). For each of the CCAs, the percent of the total variation explained by the first principle component ranged from 35 to 52%. At each time point the first principle component tended to be interpreted as a weighted average. Honey had the lowest weight for all of the CCAs was negatively weighted for CCA4 and CCA5.

Analysis Approach and Model Setup

As discussed above, the experimental design was a randomized complete block (apiary) with repeated measures (CCAs). Exploring the interaction between treatment and CCA can address these two questions:

- At each CCA, was there a reduction in the response relative to the control?
- At each treatment level, was there a difference in the response relative to the baseline time point (CCA3)?

With the experimental design component of the analysis established, the next part of the analysis was to determine which correlation structure (across time) was the best fitting for these data. The scatterplots, correlation matrices, and principle component analyses were used to inform the choice of covariance structure used in the repeated measure analysis. Some summary points from the above exploratory analyses are that temporal correlations within a response variable tended to be stronger than correlations among response variables within a time point; variance for a given response variable was not homogenous among the CCAs; and that the pairwise correlations did not consistently decrease as the distance between the temporal pairs increased.

Before conducting any comparisons among treatments or CCAs, several different correlation structures to best fit the temporal correlation were evaluated. The structures that were fitted included:

- **Compound symmetry (CS)**: assumes equal correlation for all pairwise correlations (regardless of distance of time point).
- **Compound symmetry with heterogeneous variance (CSH):** Estimates a unique variance at each time point, but assumes equal correlation for all pairwise correlations (regardless of distance of time point).
- **Autoregressive correlation (AR(1))**. Assumes equal correlation between adjacent time points. Time points further apart have a lesser correlation.
- **Heterogeneous Toeplitz (ToepH):** models a unique variance for each time point and separate correlations for equidistant time points (*e.g.*, correlation between CCA3 and CCA5 is the same as the correlation between CCA4 and CCA6).

More information about each of the covariance structures available in the REPEATED statement in SAS can be found here: https://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#statug_mixed_sect019.htm . The full SAS output is provided in **Attachment 2**.

To compare covariance structure fits, Bayesian Information Criterion (BIC) was utilized[10]. The BIC is a function of the log likelihood with a penalty for an increase in the number of covariance parameters to be fitted. The BIC value for each fitted model for all response variables is reported in **Table A.2; s**maller values of the BIC indicate a better fit (bolded). For many of the endpoints, heterogeneity of variance at different time points was indicated as compound symmetry with heterogeneous variance (CSH) and heterogeneous Toeplitz (ToepH) were the covariance structures providing the best fits. This is not surprising as unequal variances were observed in the exploratory multivariate/principle component analysis.

Table A.2. BIC values for fitted models. CCA3 – CCA8 -thiamethoxam

Variable → Model ↓	Adults	Eggs	Larvae (open)	Pupae (capped)	Pollen	Honey	Live	Brood	Food
CS	2281	1910	1959	2598	1849	2982	3152	2980	3044
CSH	2262	1784@	**1791**	2429	1836	2953	3027	2814	3023
AR(1)	2202@	1891	1949	2593	1821	2913	3093	2959	2964
ToepH	**2172**	**1767@**	DNC	**2414@**	**1790**	**2877**	**2954**	**2772@**	**2932**

*Within a response variable, smaller BIC values (bolded) indicate better covariance model fit. Kass and Raferty (1995) suggested that differences of greater than 10 in BIC values provides very strong evidence that model fits are not equivalent.
@Convergence was attained, but estimated G matrix was not positive definite and not all covariance parameters could be estimated.
DNC – Model algorithm did not converge.

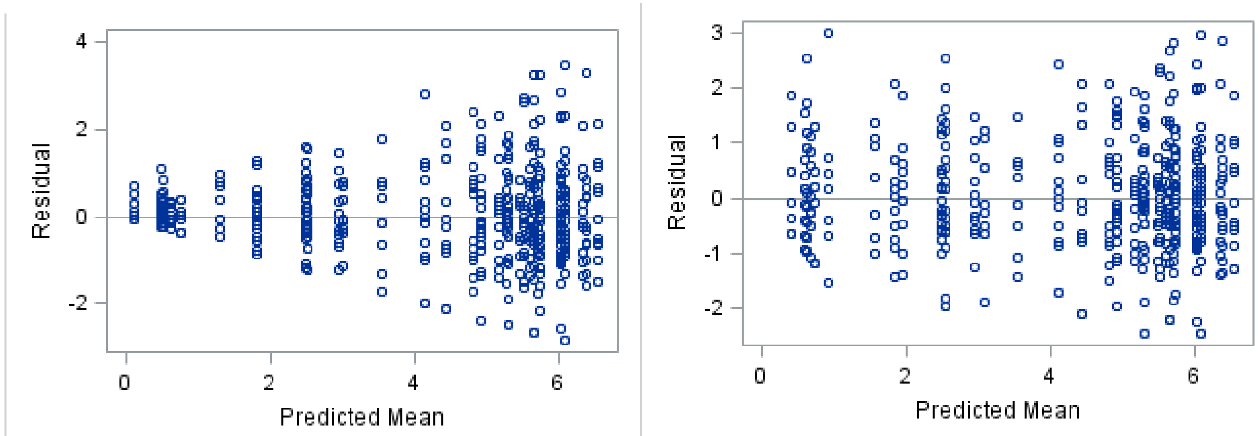
For all the evaluated response variables except larvae, ToepH was identified as the 'best fitting' covariance structure; however, all covariance parameters could not be estimated for three of the endpoints. In addition, convergence was not attained for larvae when fitting the ToepH covariance structure. For larvae (for which ToepH did not converge), CSH was the best fitting covariance structure. If CS, CSH, and AR(1) covariance structures are evaluated without considering ToepH, it is noted that CS is never the 'best fitting' based on BIC comparisons. CSH fits better than AR(1) for five response variables and AR(1) fits better than CSH for four response variables based on BIC alone. Both CSH and AR(1) have one instance when not all covariance parameters could be estimated.

Residual plots were also evaluated for each of the response variables and covariance structures. Patterns indicative of heterogeneous variance of the residuals were evident for many of the response variables and models where an assumption of equal variance at each time point was made. For many of the residual plots when CS or AR(1) covariance structure was modeled, the vertical spread of the residuals around increased as the predicted mean increased (indicating larger variances as the mean increased) (see **Figure A.1** for example). These response variables are counts, hence the distribution of the response variable and the residuals may not meet assumptions of normality and/or equal variance. More specifically, review of the residual plots indicates that estimating utilizing a covariance structure that

[10] Schwarz, Gideon. Estimating the Dimension of a Model. Ann. Statist. 6 (1978), no. 2, 461--464. doi:10.1214/aos/1176344136. http://projecteuclid.org/euclid.aos/1176344136.

estimated unique variances for each CCA (*e.g.*, CSH, ToepH covariance structures) appears to improve overall model fit.

Figure A.1. Studentized residual plots for eggs with covariance structures of (left) compound symmetry (CS) and (right) compound symmetry with heterogeneous variance (CSH). Distribution of the residuals indicates a better fitting model for the CSH covariance structure.



For this suite of response variables, both the results of the BIC analysis and review of residual plots were informative in selecting a covariance structure for the mixed model. Comparison of covariance structures indicated that while ToepH fit better than others for some endpoints, there were also issues of non-estimation of covariance parameters and non-convergence. Comparison of BICs for CS, CSH, and AR(1) did not identify a best covariance structure for a majority of the endpoints. However, a review of the residual plots did indicate that CSH was providing an advantage as clear evidence of heterogeneity of variance was evident in the CS an AR(1) residual plots for a majority of the endpoints. Therefore, the review team elected to move forward with the heterogeneous compound symmetry (CSH) covariance structure for the final analyses.

Treatment by Time Interaction and Follow-up Contrasts

The text box below provides the SAS code for the mixed model that was used for follow-up statistical contrasts to address the following questions:

- At each CCA, was there a reduction in the response relative to the control?
- At each treatment level, was there a difference in the response relative to the baseline time point (CCA3)?

The contrasts that were utilized for this analysis were Dunnett's test. Dunnett's test is a set of pairwise contrasts in which each treatment mean is compared to the control mean; the tests can be one- or two-sided. For a given set of contrasts, the experiment-wise error-rate is controlled as the specified alpha-level. In this case, a 'set of contrasts' is either (1) comparisons of treatment means to the control for a specific endpoint at a specific CCA or (2) comparison of time-points CCA4, CCA5, CCA6, CCA7, and CCA8 to the baseline CCA3 for a given endpoint. For all analyses, the CSH covariance matrix was used for each of the variables.

Text Box: SAS Code for the mixed model used to run the statistical analysis

```
title 'Thiamethoxam - ColonyFeedingStudy(2015) data analysis';
proc mixed data=cca3_8  ;
      title2 "Dunnett's tests - adult_scale";
      class apiary cca conc hive;
               model adult_scale = conc|cca /DDFM=SATTERTHWAITE;
               random apiary  ;
               repeated cca/ subject=hive*conc(apiary) type=csh ;
               lsmeans  conc*cca/cl;
               slice conc*cca /sliceby=cca diff=controll adjust=dunnett;
               slice conc*cca /sliceby=conc diff=control adjust=dunnett;
               run;
```

Williams' test was also considered for use for one set of the follow-up contrasts - comparisons of treatment means to the control for a specific endpoint at a specific CCA. Williams' test has been shown to be more powerful than Dunnett's test when the assumption of monotonicity is met. Williams' requires the assumption that if there is an effect of the chemical, it follows the classic dose-response relationship (*i.e.*, assuming the test material has a negative effect on the response variable, then as the test concentration increases, mean response is equal to or less than the mean response of the next lower dose concentration). The test procedure then determines the lowest dose level for which the mean is significantly less than the control mean. This concentration is identified as the LOAEC and the next lower concentration is identified as the NOAEC. Williams' test was not utilized for this analysis for several reasons:

- Review of the treatment means identified several instances when the underlying assumption of monotonicity does not appear to be met. Given the large variation in the measured responses in general, it could not be determined if the observed deviations from monotonicity were due to large background variation or to a non-monotone treatment response.
- For any one response variable, the data are combined across CCAs into one mixed model analysis. Incorporating data from all CCAs improves the variance/covariance estimates and increases the degrees of freedom for hypothesis testing. As the degrees of freedom for hypothesis testing increases, any differences in power between Dunnett's test and Williams' test would become very small.
- It has not been codified in the PROC MIXED procedure in SAS, and the level of effort to code and QA the test would be significant.

An analysis approach where data from each CCA was analyzed separately as a randomized complete block design was also considered as SAS has options for use of Williams' test for simpler experimental designs. This approach was not selected for several reasons:

- Equality of variance across treatment groups would still need to be evaluated. If the assumption of homogenous variances was not met for some CCAs, then transforming the response or non-parametric analyses would need to be considered. Incorporating the heterogeneous variances into the error matrices of the general linear model (GLM) would increase the complexity of the model such that the Williams' options in SAS could no longer be utilized.
- A statistical analysis approach that does not utilize the strength of the correlations among time points to improve estimates of error variance would not be as powerful as one that does incorporate that additional information about the nature of the responses.

Treatment effects within a CCA

The table of p-values resulting from the Dunnett's tests (for evaluating whether within a CCA, the treatment means are significantly less than control means) are summarized in **Table A.3. Figures 7-15 in section 3** show the results for each response variable across all CCAs analyzed (CCA3-CCA8) and all treatment levels. For all figures, significant reductions from the negative control with p-values below the 0.05 alpha level are denoted by a red dot at a given treatment level and CCA and those reductions with p-values between 0.05 and 0.1 are denoted by a black dot. Statistical NOAECs and LOAECs will be determined using an alpha-level of 0.05. Additional comparisons using and alpha-level of 0.10 are included for additional characterization. These will be integrated into the final biological interpretation. Error bars represent one standard error from the mean calculated from the model residual mean squares estimate. The tables of p-values resulting from the Dunnett's test are summarized in **Table A.3**. The associated SAS output containing the full results of the Dunnett's comparisons can be found in **Attachment 3.**

	Adults	**Eggs**	**Larvae (Open)**	**Pupae (Capped)**	**Pollen**	**Honey**	**Live**	**Brood**	**Food**
CCA3	NS	*25*	**25**	**25**	NS	NS	**25**	**25**	NS
CCA4	NS	NS	NS	**100**	*25* **100**	NS	**100**	**100**	NS
CCA5	*50* **100**	**100**	**50** **100**	**37.5** **50** **100**	**25** *50* **100**	*12.5*	**50** **100**	**50** **100**	NS
CCA6	**100**	*12.5* **100**	**100**	**100**	**100**	NS	**100**	**100**	NS
CCA7	**100**	**NS**	NS	**100**	**25** **100**	NS	**100**	**100**	NS
CCA8	**100**	**NS**	*50*	NS	NS	NS	NS	NS	NS

* NS indicates that there were no test concentrations with means significantly less than the control (p>0.10).
Bolded concentration = significantly less than control (p< 0.05)
Italicized concentration = less than control (0.05< P < 0.10)

Temporal trends within a treatment level

A second component to evaluating the "treatment x CCA" interaction is to look at the temporal changes within a treatment group. This was accomplished by comparing each CCA (CCA4 through CCA8) to

CCA3 by use of a two-sided Dunnett's test (**Table A.4** and **Table A.5**). This suite of comparisons is not as informative as the contrasts of control against the treatment group within a CCA for establishing a NOAEC and LOAEC. However, it may aid in interpretations and further biological understanding of temporal shifts in the life stages and food components present in the hive. Differences in patterns of temporal shifts between the control and various treatment groups can provide further understanding of the potential impacts of clothianidin on beehive population dynamics.

Table A.4 Results of two-sided Dunnett's test (comparing CCA3 to each following CCA), correlations modeled using CSH.*

Trt Group	Response Variable					
	Adults	**Eggs**	**Open**	**Capped**	**Live**	**Brood**
Control	CCA4** and CCA5**, CCA7 and CCA8 <CCA3	CCA7 and CCA8 < CCA3	CCA7 and CCA8<CCA3	CCA5, CCA6**, CCA7 and CCA8 < CCA3	CCA5, CCA7-CCA8<CCA3	CCA7 and CCA8<CCA3
12.5	CCA8<CCA3	CCA7 and CCA8 < CCA3	CCA7 and CCA8<CCA3	CCA7 and CCA8 < CCA3	CCA5, CCA7-CCA8<CCA3	CCA5**, CCA7-CCA8<CCA3
25	CCA5, CCA6 and CCA8 < CCA3	CCA4** CCA7 and CCA8 < CCA3	CCA6** CCA5, CCA7, and-CCA8<CCA3	CCA5, CCA7 and CCA8 < CCA3	CCA4-CCA5, CCA7-CCA8<CCA3	CCA5, CCA7-CCA8<CCA3
37.5	CCA8<CCA3	CCA7** and CCA8 < CCA3	CCA7 and CCA8<CCA3	CCA7** and CCA8 < CCA3	CCA7 and CCA8<CCA3	CCA7 and CCA8<CCA3
50	CCA8<CCA3	CCA7 and CCA8 < CCA3	CCA7 and CCA8<CCA3	CCA7 and CCA8 < CCA3	CCA7 and CCA8<CCA3	CCA7 and CCA8<CCA3
100	CCA5-CCA8<CCA3	CCA5 – CCA8 <CCA3	CCA5-CCA8<CCA3	CCA5 – CCA8 <CCA3	CCA4-CCA8<CCA3	CCA4-CCA8<CCA3

*Unless otherwise stated significance from CCA3 is ($p<0.05$)
** Significant at $0.05<p<0.1$
NS – No significant differences from CCA3 ($p>0.05$)

Table A.5. Results of two-sided Dunnett's test (comparing CCA3 to each following CCA), correlations modeled using CSH.*

Trt Group	Response Variable		
	Pollen	**Honey**	**Food**
Control	CCA8<CCA3	CCA6<CCA3	CCA5** and CCA6**<CCA3
12.5	NS	NS	NS
25	CCA4**<CCA3	NS	NS
37.5	NS	NS	NS
50	NS	NS	NS
100	CCA4-CCA8<CCA3	CCA4-CCA5 <CCA3	CCA5<CCA3

Unless otherwise stated significance from CCA3 is (p>0.05)
** Significant at 0.05<p<0.1
NS – No significant differences from CCA3 (p>0.05)

Tables A.6 – A.14 tabulate the summary statistics (including mean and standard deviation) of each response variable for all treatment levels across CCAs 3-8.

Table A.6. Summary statistics for adults

CCA	Treatment Group µg/L	Mean	SE	DF	Lower	Upper
3	0	11.0402	1.0201	42.3	8.982	13.0985
4	0	12.7383	0.9493	40	10.8198	14.6568
5	0	13.0853	1.0846	56.5	10.913	15.2575
6	0	11.5786	0.9479	40.5	9.6636	13.4937
7	0	9.0371	0.9244	39.1	7.1675	10.9067
8	0	4.749	0.7519	19.6	3.1783	6.3197
3	12.5	8.9627	1.3154	56.6	6.3283	11.597
4	12.5	10.3778	1.2046	63	7.9706	12.7851
5	12.5	11.111	1.4148	76.6	8.2936	13.9284
6	12.5	9.7727	1.2417	68.9	7.2955	12.2498
7	12.5	8.9278	1.2026	67.4	6.5276	11.3279
8	12.5	4.9827	0.8988	34.8	3.1576	6.8079
3	25	8.2639	1.3154	56.6	5.6296	10.8982
4	25	10.3928	1.2046	63	7.9856	12.8001
5	25	12.0837	1.4148	76.6	9.2663	14.9011
6	25	11.1413	1.2145	65.5	8.7161	13.5665
7	25	9.3521	1.1767	64.3	7.0016	11.7027
8	25	5.5522	0.8837	33	3.7543	7.3501
3	37.5	10.5697	1.3154	56.6	7.9354	13.2041
4	37.5	12.0279	1.2046	63	9.6206	14.4352
5	37.5	11.3155	1.4426	79.7	8.4445	14.1865
6	37.5	9.9277	1.2145	65.5	7.5025	12.353
7	37.5	9.2447	1.1767	64.3	6.8941	11.5953
8	37.5	5.7487	0.8837	33	3.9508	7.5466
3	50	10.0062	1.3154	56.6	7.3718	12.6405
4	50	11.7762	1.2046	63	9.369	14.1835
5	50	9.8803	1.4148	76.6	7.0629	12.6977

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

CCA	Treatment Group µg/L	Mean	SE	DF	Lower	Upper
6	50	9.8116	1.2417	68.9	7.3344	12.2888
7	50	8.0158	1.2026	67.4	5.6156	10.416
8	50	5.5436	0.9157	36.8	3.6878	7.3994
3	100	11.6786	1.3154	56.6	9.0443	14.3129
4	100	11.3337	1.2046	63	8.9265	13.741
5	100	7.211	1.4148	76.6	4.3936	10.0284
6	100	4.9539	1.2417	68.9	2.4767	7.4311
7	100	3.9155	1.3127	79	1.3027	6.5284
8	100	2.1947	0.9643	42.6	0.2495	4.1399

Table A.7. Summary statistics for eggs

CCA	Treatment Group µg/L	Mean	SE	DF	Lower	Upper
3	**0**	6.063	0.5155	81.1	5.0373	7.0887
4	**0**	6.02	0.512	82.9	5.0015	7.0385
5	**0**	5.289	0.4491	83.4	4.3958	6.1822
6	**0**	5.6499	0.5428	75	4.5686	6.7313
7	**0**	2.5399	0.2768	68.8	1.9877	3.0921
8	**0**	0.6236	0.1382	58.8	0.3471	0.9001
3	12.5	5.375	0.729	81.1	3.9245	6.8255
4	12.5	5.3607	0.7241	82.9	3.9204	6.801
5	12.5	6.1633	0.6351	83.4	4.9001	7.4265
6	12.5	3.4677	0.8122	76.7	1.8503	5.085
7	12.5	2.4052	0.4141	70.4	1.5794	3.2311
8	12.5	0.7035	0.2033	59.8	0.2969	1.1101
3	25	4.1137	0.729	81.1	2.6631	5.5642
4	25	6.0773	0.7241	82.9	4.637	7.5176
5	25	4.9593	0.6351	83.4	3.6961	6.2225
6	25	5.3716	0.7814	75.6	3.8151	6.9281
7	25	1.864	0.3984	69.3	1.0692	2.6588
8	25	0.6979	0.1956	58.8	0.3065	1.0892
3	37.5	4.8017	0.729	81.1	3.3511	6.2522
4	37.5	6.0343	0.7241	82.9	4.594	7.4746
5	37.5	5.715	0.6565	84.8	4.4097	7.0202
6	37.5	5.2596	0.7814	75.6	3.7031	6.8161
7	37.5	3.0741	0.3984	69.3	2.2793	3.8689
8	37.5	0.5917	0.1956	58.8	0.2003	0.983
3	50	5.59	0.729	81.1	4.1395	7.0405
4	50	6.364	0.7241	82.9	4.9237	7.8043
5	50	4.4433	0.6351	83.4	3.1801	5.7065
6	50	4.9032	0.8122	76.7	3.2858	6.5205
7	50	1.9499	0.4141	70.4	1.124	2.7758
8	50	0.3983	0.2119	60.9	-0.02539	0.8219
3	100	6.536	0.729	81.1	5.0855	7.9865
4	100	5.504	0.7241	82.9	4.0637	6.9443
5	100	2.9383	0.6351	83.4	1.6751	4.2015
6	100	2.484	0.8122	76.7	0.8666	4.1013
7	100	1.5797	0.4794	73.5	0.6242	2.5351
8	100	0.9278	0.2353	62.9	0.4576	1.3981

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table A.8. Summary statistics for larval (open) cells

CCA	Treatment Group µg/L	Mean	SE	DF	Upper	Lower
3	**0**	5.9197	0.494	80.7	4.9367	6.9026
4	**0**	6.0343	0.6069	82.3	4.827	7.2417
5	**0**	6.7223	0.5277	79.3	5.6721	7.7726
6	**0**	5.6075	0.6523	70.2	4.3066	6.9084
7	**0**	1.6513	0.3264	60.7	0.9985	2.3041
8	**0**	0.4781	0.1191	27.3	0.2337	0.7224
3	12.5	4.902	0.695	80.7	3.5192	6.2848
4	12.5	6.2207	0.8554	82	4.519	7.9223
5	12.5	6.1347	0.7428	79.1	4.6561	7.6132
6	12.5	5.4005	0.9666	72.9	3.474	7.327
7	12.5	2.1823	0.479	63.5	1.2252	3.1394
8	12.5	0.5024	0.1574	48.3	0.1859	0.8189
3	25	3.44	0.695	80.7	2.0572	4.8228
4	25	5.8337	0.8554	82	4.132	7.5353
5	25	6.0487	0.7428	79.1	4.5701	7.5272
6	25	5.6949	0.9342	70.9	3.8321	7.5577
7	25	0.6867	0.4632	61.6	-0.2393	1.6127
8	25	0.2741	0.1529	45.6	-0.03376	0.5819
3	37.5	4.945	0.695	80.7	3.5622	6.3278
4	37.5	6.3067	0.8554	82	4.605	8.0083
5	37.5	6.2395	0.7643	81.5	4.7189	7.7601
6	37.5	5.759	0.9342	70.9	3.8961	7.6218
7	37.5	1.7959	0.4632	61.6	0.8699	2.722
8	37.5	0.5985	0.1529	45.6	0.2907	0.9063
3	50	4.988	0.695	80.7	3.6052	6.3708
4	50	5.8767	0.8554	82	4.175	7.5783
5	50	4.3573	0.7428	79.1	2.8788	5.8359
6	50	4.6815	0.9666	72.9	2.755	6.608
7	50	1.4615	0.479	63.5	0.5043	2.4186
8	50	0.1012	0.1626	51.1	-0.2253	0.4277
3	100	4.945	0.695	80.7	3.5622	6.3278
4	100	4.085	0.8554	82	2.3834	5.7866
5	100	2.666	0.7428	79.1	1.1875	4.1445
6	100	2.2644	0.9666	72.9	0.3379	4.1909
7	100	0.8091	0.5453	69.5	-0.2786	1.8967
8	100	0.3163	0.1771	57.5	-0.03823	0.6709

Table A.9. Summary statistics for pupal (capped) cells

CCA	Treatment Group µg/L	Mean	SE	DF	Min	Max
3	**0**	14.0682	0.8515	74.9	12.3719	15.7645
4	**0**	13.33	1.2346	78.5	10.8724	15.7876
5	**0**	17.3863	1.0887	80	15.2197	19.5529
6	**0**	10.9905	1.3821	69.4	8.2337	13.7473
7	**0**	3.1487	0.5533	57.4	2.0409	4.2564
8	**0**	1.1669	0.2525	40.5	0.6568	1.677
3	12.5	12.1833	1.2023	76.1	9.7887	14.578

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

CCA	Treatment Group µg/L	Mean	SE	DF	Min	Max
4	12.5	11.7963	1.7447	79.2	8.3237	15.2689
5	12.5	15.7667	1.5382	80.9	12.706	18.8273
6	12.5	11.056	2.0648	72.2	6.9401	15.1719
7	12.5	4.3783	0.8239	61.2	2.7308	6.0258
8	12.5	1.8241	0.3646	60.2	1.0948	2.5535
3	25	10.5063	1.2023	76.1	8.1117	12.901
4	25	11.739	1.7447	79.2	8.2664	15.2116
5	25	14.448	1.5382	80.9	11.3873	17.5087
6	25	10.9297	1.9878	70.6	6.9658	14.8937
7	25	1.4547	0.7933	59.7	-0.1323	3.0416
8	25	0.7581	0.3511	58.3	0.05528	1.4609
3	37.5	12.3697	1.2023	76.1	9.975	14.7643
4	37.5	12.6133	1.7447	79.2	9.1407	16.0859
5	37.5	12.7454	1.5888	82.9	9.5852	15.9055
6	37.5	10.765	1.9878	70.6	6.801	14.7291
7	37.5	3.2046	0.7933	59.7	1.6176	4.7916
8	37.5	1.5497	0.3511	58.3	0.8469	2.2525
3	50	12.7997	1.2023	76.1	10.405	15.1943
4	50	10.5637	1.7447	79.2	7.0911	14.0363
5	50	10.0763	1.5382	80.9	7.0157	13.137
6	50	9.2467	2.0648	72.2	5.1307	13.3626
7	50	1.7136	0.8239	61.2	0.06612	3.3611
8	50	1.1756	0.3797	62	0.4167	1.9346
3	100	13.1437	1.2023	76.1	10.749	15.5383
4	100	7.0233	1.7447	79.2	3.5507	10.4959
5	100	4.257	1.5382	80.9	1.1963	7.3177
6	100	4.6079	2.0648	72.2	0.4919	8.7238
7	100	0.2375	0.9516	65.5	-1.6628	2.1377
8	100	1.0002	0.421	65.4	0.1596	1.8407

Table A.10. Summary statistics for total individuals

CCA	Treatment Group µg/L	Mean	SE	DF	Min	Max
3	**0**	37.091	2.174	79.6	32.7644	41.4177
4	**0**	38.1226	2.5169	82.3	33.116	43.1293
5	**0**	42.483	2.5186	84.7	37.4751	47.4908
6	**0**	33.6219	2.8208	75.1	28.0028	39.241
7	**0**	16.3324	1.4849	64.8	13.3666	19.2981
8	**0**	7.0926	0.8641	42.1	5.3491	8.8362
3	12.5	31.423	3.0414	78.1	25.3682	37.4778
4	12.5	33.7555	3.5309	80.9	26.7299	40.7811
5	12.5	39.1757	3.5333	83.2	32.1483	46.203
6	12.5	29.4253	4.1085	77.7	21.2454	37.6052
7	12.5	17.8406	2.1243	68.8	13.6026	22.0786
8	12.5	7.9604	1.1635	60.9	5.6338	10.287
3	25	26.3239	3.0414	78.1	20.2691	32.3787
4	25	34.0428	3.5309	80.9	27.0172	41.0684
5	25	37.5397	3.5333	83.2	30.5123	44.567
6	25	32.8367	4.0081	74.9	24.8519	40.8214
7	25	13.2651	2.0737	66.5	9.1255	17.4047

8	25	7.2446	1.1373	58.8	4.9686	9.5205
3	37.5	32.6861	3.0414	78.1	26.6313	38.7409
4	37.5	36.9823	3.5309	80.9	29.9566	44.0079
5	37.5	35.9161	3.6046	86.1	28.7504	43.0818
6	37.5	31.588	4.0082	74.9	23.6032	39.5728
7	37.5	17.2478	2.0737	66.5	13.1081	21.3874
8	37.5	8.4623	1.1373	58.8	6.1863	10.7383
3	50	33.3838	3.0414	78.1	27.329	39.4386
4	50	34.5806	3.5309	80.9	27.555	41.6062
5	50	28.7573	3.5333	83.2	21.73	35.7847
6	50	28.1544	4.1085	77.7	19.9745	36.3343
7	50	13.0838	2.1243	68.8	8.8457	17.322
8	50	7.2735	1.1927	63.2	4.8901	9.6569
3	100	36.3033	3.0414	78.1	30.2485	42.358
4	100	27.9461	3.5309	80.9	20.9205	34.9717
5	100	17.0723	3.5333	83.2	10.045	24.0997
6	100	14.0192	4.1085	77.7	5.8393	22.1991
7	100	6.3152	2.3386	77	1.6584	10.972
8	100	4.5	1.2764	68.7	1.9535	7.0465

Table A.11. Summary statistics for nectar (honey) cells

CCA	Treatment Group µg/L	Mean	SE	DF	Min	Max
3	**0**	15.3582	1.3232	67.1	12.7172	17.9991
4	**0**	16.7557	1.3322	66.2	14.096	19.4153
5	**0**	18.6118	1.7232	73.3	15.1778	22.0459
6	**0**	20.1786	2.1601	76.7	15.8769	24.4803
7	**0**	13.4115	1.5727	69.6	10.2746	16.5485
8	**0**	18.7394	2.0549	61.1	14.6305	22.8483
3	12.5	12.3983	1.8336	80.1	8.7495	16.0472
4	12.5	12.6277	1.8466	80.6	8.9532	16.3021
5	12.5	12.728	2.4081	78.3	7.9341	17.5219
6	12.5	17.3371	3.1698	80	11.029	23.6452
7	12.5	12.0618	2.2909	79.1	7.5019	16.6216
8	12.5	17.6326	2.9739	65.4	11.6939	23.5713
3	25	14.1757	1.8336	80.1	10.5268	17.8245
4	25	15.3223	1.8466	80.6	11.6479	18.9968
5	25	15.351	2.4081	78.3	10.5571	20.1449
6	25	17.3619	3.0744	77.7	11.2409	23.4828
7	25	13.8175	2.2228	76.8	9.3912	18.2439
8	25	19.7936	2.8846	63.7	14.0304	25.5568
3	37.5	17.5297	1.8336	80.1	13.8808	21.1785
4	37.5	17.9597	1.8466	80.6	14.2852	21.6341
5	37.5	18.9453	2.4691	80.7	14.0322	23.8583
6	37.5	22.5023	3.0744	77.7	16.3812	28.6233
7	37.5	15.8999	2.2228	76.8	11.4736	20.3263
8	37.5	20.9586	2.8847	63.7	15.1954	26.7219
3	50	14.3907	1.8336	80.1	10.7418	18.0395
4	50	16.1823	1.8466	80.6	12.5079	19.8568
5	50	17.8307	2.4081	78.3	13.0368	22.6246
6	50	20.0261	3.1698	80	13.718	26.3342

7	50	15.473	2.2909	79.1	10.9132	20.0329
8	50	19.8588	3.0726	67.1	13.726	25.9915
3	100	15.824	1.8336	80.1	12.1751	19.4729
4	100	22.3457	1.8466	80.6	18.6712	26.0201
5	100	27.4483	2.4081	78.3	22.6544	32.2422
6	100	19.945	3.1698	80	13.6369	26.2532
7	100	15.7232	2.5766	86.3	10.6013	20.845
8	100	14.1472	3.3483	70.7	7.4704	20.824

Table A.12. Summary statistics for pollen cells

CCA	Treatment Group µg/L	Mean	SE	DF	Min	Max
3	**0**	3.7553	0.5481	75.1	2.6635	4.8472
4	**0**	3.2178	0.4432	76	2.3351	4.1005
5	**0**	4.6082	0.4878	72.6	3.6359	5.5805
6	**0**	3.7682	0.4174	60.6	2.9334	4.603
7	**0**	3.7741	0.4444	60	2.8852	4.6629
8	**0**	2.4393	0.3614	43.3	1.7107	3.1679
3	12.5	3.096	0.7582	78.4	1.5867	4.6053
4	12.5	3.1533	0.6057	78.5	1.9476	4.359
5	12.5	3.6407	0.6708	77.2	2.305	4.9763
6	12.5	3.5344	0.5948	80.9	2.351	4.7179
7	12.5	3.5934	0.6364	75	2.3257	4.8611
8	12.5	2.2668	0.5008	67.3	1.2674	3.2663
3	25	3.139	0.7582	78.4	1.6297	4.6483
4	25	1.5767	0.6057	78.5	0.371	2.7824
5	25	2.5943	0.6708	77.2	1.2587	3.93
6	25	2.703	0.5762	78.6	1.556	3.8499
7	25	2.0256	0.6163	73.3	0.7973	3.2539
8	25	1.8856	0.4854	65.1	0.9162	2.8551
3	37.5	4.128	0.7582	78.4	2.6187	5.6373
4	37.5	2.8953	0.6057	78.5	1.6896	4.101
5	37.5	3.5771	0.6891	79.1	2.2056	4.9486
6	37.5	3.3326	0.5762	78.6	2.1856	4.4795
7	37.5	3.2485	0.6163	73.3	2.0203	4.4768
8	37.5	2.783	0.4854	65.1	1.8136	3.7525
3	50	2.967	0.7582	78.4	1.4577	4.4763
4	50	1.8633	0.6057	78.5	0.6576	3.069
5	50	2.8667	0.6708	77.2	1.531	4.2023
6	50	2.7015	0.5948	80.9	1.518	3.8849
7	50	2.7404	0.6364	75.1	1.4727	4.0082
8	50	2.2081	0.5177	69.5	1.1755	3.2407
3	100	4.171	0.7582	78.4	2.6617	5.6803
4	100	1.3903	0.6057	78.5	0.1846	2.596
5	100	0.8887	0.6708	77.2	-0.447	2.2243
6	100	0.9494	0.5948	80.9	-0.2341	2.1328
7	100	1.2598	0.7203	80	-0.1736	2.6932
8	100	1.4989	0.5649	74.5	0.3734	2.6244

Table A.13. Summary statistics for Brood

CCA	Treatment Group µg/L	Mean	SE	DF	Min	Max
3	**0**	26.0508	1.6271	76	22.8101	29.2916
4	**0**	25.3843	1.9894	78.3	21.4239	29.3447
5	**0**	29.3977	1.7983	79.7	25.8187	32.9766
6	**0**	22.1232	2.2647	70.7	17.6073	26.6391
7	**0**	7.3222	0.9489	56.7	5.4219	9.2224
8	**0**	2.2802	0.4507	30.2	1.36	3.2003
3	12.5	22.4603	2.2902	76.5	17.8994	27.0212
4	12.5	23.3777	2.8046	78.6	17.7948	28.9605
5	12.5	28.0647	2.5333	80	23.0233	33.106
6	12.5	19.8457	3.3441	74.4	13.1831	26.5083
7	12.5	8.9353	1.3839	61.1	6.1681	11.7025
8	12.5	3.0071	0.6152	50	1.7714	4.2427
3	25	18.06	2.2902	76.5	13.4991	22.6209
4	25	23.65	2.8046	78.6	18.0671	29.2329
5	25	25.456	2.5333	80	20.4146	30.4974
6	25	21.8342	3.2408	71.9	15.3735	28.2948
7	25	3.9728	1.3419	59.1	1.2878	6.6578
8	25	1.7155	0.5975	47.4	0.5137	2.9173
3	37.5	22.1163	2.2902	76.5	17.5554	26.6772
4	37.5	24.9543	2.8046	78.6	19.3715	30.5372
5	37.5	24.6831	2.5999	82.9	19.512	29.8542
6	37.5	21.6707	3.2409	71.9	15.21	28.1314
7	37.5	8.0574	1.3419	59.1	5.3724	10.7425
8	37.5	2.7414	0.5975	47.4	1.5396	3.9432
3	50	23.3777	2.2902	76.5	18.8168	27.9386
4	50	22.8043	2.8046	78.6	17.2215	28.3872
5	50	18.877	2.5333	80	13.8356	23.9184
6	50	18.5525	3.3441	74.4	11.8899	25.2151
7	50	5.0989	1.384	61.1	2.3317	7.8662
8	50	1.6631	0.635	52.6	0.3891	2.937
3	100	24.6247	2.2902	76.5	20.0638	29.1856
4	100	16.6123	2.8046	78.6	11.0295	22.1952
5	100	9.8613	2.5333	80	4.82	14.9027
6	100	9.1763	3.3441	74.4	2.5137	15.8389
7	100	2.6162	1.5604	68	-0.4976	5.73
8	100	2.3012	0.6907	58.6	0.919	3.6833

Table A.14. Summary statistics for Food

CCA	Treatment Group µg/L	Mean	SE	DF	Min	Max
3	**0**	19.1135	1.5637	69.2	15.9942	22.2328
4	**0**	19.9735	1.4199	65.5	17.1382	22.8088
5	**0**	23.22	1.853	76.4	19.5297	26.9103
6	**0**	23.9329	2.2477	76.2	19.4564	28.4093
7	**0**	17.1938	1.7423	67.6	13.7168	20.6708
8	**0**	21.1434	2.244	61.9	16.6576	25.6291
3	12.5	15.4943	2.1798	79.7	11.1562	19.8324

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

CCA	Treatment Group µg/L	Mean	SE	DF	Min	Max
4	12.5	15.781	1.9731	80.8	11.8549	19.7071
5	12.5	16.3687	2.5939	78.9	11.2055	21.5318
6	12.5	20.8523	3.296	80.6	14.2938	27.4108
7	12.5	15.5833	2.5421	78.4	10.5227	20.6438
8	12.5	19.7647	3.2494	66.5	13.278	26.2514
3	25	17.3147	2.1798	79.7	12.9766	21.6528
4	25	16.899	1.9731	80.8	12.9729	20.8251
5	25	17.9453	2.5939	78.9	12.7822	23.1085
6	25	20.037	3.1996	78.3	13.6675	26.4065
7	25	15.8295	2.4684	76.1	10.9134	20.7456
8	25	21.6347	3.1544	64.8	15.3346	27.9348
3	37.5	21.6577	2.1798	79.7	17.3196	25.9958
4	37.5	20.855	1.9731	80.8	16.9289	24.7811
5	37.5	22.5205	2.6577	81.3	17.2328	27.8082
6	37.5	25.7557	3.1996	78.3	19.3861	32.1252
7	37.5	19.1143	2.4684	76.1	14.1981	24.0305
8	37.5	23.6597	3.1544	64.8	17.3595	29.9599
3	50	17.3577	2.1798	79.7	13.0196	21.6958
4	50	18.0457	1.9731	80.8	14.1196	21.9717
5	50	20.6973	2.5939	78.9	15.5342	25.8605
6	50	22.6254	3.296	80.6	16.067	29.1839
7	50	18.1754	2.5421	78.4	13.1148	23.236
8	50	21.8537	3.3543	68.3	15.1609	28.5465
3	100	19.995	2.1798	79.7	15.6569	24.3331
4	100	23.736	1.9731	80.8	19.8099	27.6621
5	100	28.337	2.5939	78.9	23.1738	33.5002
6	100	20.7528	3.296	80.6	14.1943	27.3113
7	100	16.8394	2.8518	85.9	11.1701	22.5087
8	100	15.1786	3.6481	72.1	7.9065	22.4507

Appendix B – PMRA Data Statistical Analysis Report

Analysis Strategy

Hive condition data:

To analyze colony condition data which contains many components over many assessments at different times, a primary analysis was set out to effectively prevent multiplicities from interfering with the interpretation of p_values and confidence intervals. These multiplicities arise from having multiple dose levels, multiple outcomes and multiple time points, and are dealt with as follows:

The multiplicities from having multiple dose levels was dealt with by using step down testing, the highest dose group's data was compared directly to the control group's data, if statistically significant at a chosen alpha level the next lowest dose group's data was compared to the control group's data and this was continued down to the dose where statistical significance was no longer achieved. A technical reference for this step down testing would be Multiple Comparison Procedures in Dose Response Studies. Tamhane, Ajit C. and Logan, Brent R., in Dose Finding in Drug Development edited by Ting, Naitee. Springer New York 2006. This step down procedure (referred to as the SD2PC procedure in the technical reference) was chosen as it provides good power for detecting the minimum effective dose (lowest does where effect is present) when monotonic dose effects are expected while providing stringent control of type one error, regardless of the true pattern of dose effects. That is, with minimal assumptions, the procedure strongly controls family wise type one error rate while maintaining good power for effect patterns that are expected.

This step down procedure is implemented by PMRA using only data from the control group and the dose group being tested in that step which alleviates any concern about heterogeneity of variance across dose groups. Especially with outcome data that involves estimates of underlying counts, it is expected that effects at a given dose necessarily involves both the mean and variance. When this is the case - the use of data from a higher does with a putative effect in the comparison of a lower dose would thus be inappropriate and would invalidate the control of type one error.

The applicant's choice of multiplicity adjustment procedure, which was William's trend test (Williams 1972), presumably chosen to be in accord with OECD. 2003. Draft guidance document for the statistical analysis of ecotoxicity data. They are both step down procedures but ours differs from William's in that it uses only within dose group data based estimates of means rather than maximum likelihood estimates of dose group means using all group's data simultaneously - under monotonicity assumption (i.e. order restricted or isotonic means) additionally assuming homogeneous variances . Although these additional assumptions may not be problematic and are within the OECD guidelines, we simply chose not to rely on them (and by doing so, exceed the OECD guidelines.)

The multiplicities from having multiple outcomes, was dealt with by choosing to focus on the assessment of total life in the hive – simply the number of viable life forms at any stage in the hive. It is considered that the total number of individuals includes all live individuals in hives and is expected to be a better indicator of the hive status at the colony level than any single stage of bees alone. This outcome would provide good power when background knowledge is lacking on the stage most likely to be affected (i.e. it cannot be well anticipated) and it is not expected that there will be simultaneous trade-offs effects between the stages. That is, when it is not expected that a toxic effect on one stage would have a beneficial effect for another stage at the same point in time.

The multiplicities from having multiple time points was dealt with by choosing to focus on the time when the effects were believed most pronounced both in terms of having an impact on total life and having a high powered assessment of that. In this case CCA5 was selected for the following reasons.

1. CCA5 – assessed effects to the colony after 7 weeks of exposure (which was expected to result in whole hive exposure), and occurs before the start of hive decline prior to overwintering. In the case of this study, the initiation of feeding occurred later in the season (in comparison to the clothianidin and imidacloprid studies), which most likely led to preparation for overwintering at CCA 6.
2. CCA6 - was not selected as the start of the natural decline of hive size in the fall was clearly apparent and the width of the confidence intervals started to expand (the precision of estimates declined.).
3. CCA7 and CCA8 was not selected simply due to the natural decline of hive size in the late fall that may mask the effect of treatment.
4. CCA9 and CCA10 were not selected because of the high hive mortality observed in the controls.

While the total individuals at CCA5 is considered as a primary parameter to control multiplicity for statistical analysis, all parameters including eggs, open brood and capped brood, adults, hive weight, pollen and nectar store, that were observed during the entire study including CCA4, CCA5, CCA6,CCA7 and CCA8 were also considered in the review. Given that the primary analysis has prevented multiplicities from interfering with the interpretation of p_values and confidence intervals, if statistical significance has been achieved (at given dose levels), further analysis with all other outcomes is undertaken “with prejudice” for the assessment of similar effects as being significant. More formally, re-allowance for multiplicities is not required and less stringent alpha levels are allowed. Essentially the price has been paid for searching for the pattern in the primary analysis (measures taken to prevent multiplicities) and it need not be re-paid evaluating the same pattern elsewhere. On the other hand, if statistical significance has not been achieved (at given dose levels), further analysis with all other outcomes is undertaken “with prejudice” for assessment of other effects as likely being just noise. Here though dramatic effects should not be ignored but carefully considered and noted.

Analysis methods for hive conditions

For all hive conditions total life, eggs, open brood and capped brood, adults, hive weight, pollen and nectar store at CCA4, CCA5, CCA6, CCA7 and CCA8 a conventional analysis of block randomised experiments with a baseline measurements was undertaken. In line with the statistical strategy discussed above, the focus was on total life at CCA5(with step down adjustment for multiplicities applied) but identical analysis was carried out (less the step down adjustment) on all other hive conditions assessed at the given assessment points. This analysis comprised of linear modeling (or ANOVA) stratified on Apiary (block) and adjusted for baseline measurements at CCA3 with one-side testing for harm using only the control group data and the data from a single dose group at a time, starting with the highest and then through lowest dose groups. It is a series of robust “t.test like” analyses that conservatively implement the step down testing procedure. Under the assumption of no effect in the single dose group being tested (relevant to type one error control), the means and variances and covariate effects should be identical in both the control group and the single dose group being tested. (In an analysis that includes all dose group data together e.g. William’s procedure, an impact of a treatment effect on the variance and covariate effects at a higher dose, in addition to an effect on the mean, would invalidate the assumptions needed to control type one error rate in the lower doses.) The results of all analyses are presented in tables of unadjusted p_values (adjusted p_values can be simply read off as the maximum of all p_values in any higher dose), effect estimates and upper and lower confidence intervals (in file Thia_summariesF) as well as plots of the confidence intervals (pdf file Bees8.pdf).

The code snippet to implement these analyses in R was:

```
glm(outcome~Apiary + baseline + exposed, data= x[x$exposed == " control " | x$exposed == dose,])
```

Sensitivity analysis was undertaken by extensive graphical analyses sometimes using the square root transformation as well as calculating non-parametric randomisation (permutation) tests on the differences between high dose group and control group average within Apiary.These are in given in the column named PermP_value in Thia_summariesF.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table B.1. Summary of the differences between treatment and controls on the basis of observations and model estimations, and P values.

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Adults	3	12.5	2078	983	12	0	0	0	0	0	0	11040	-1.717
Adults	3	25	2776	968	12	0	0	0	0	0	0	11040	-1.717
Adults	3	37.5	470	866	12	0	0	0	0	0	0	11040	-1.717
Adults	3	50	1034	791	12	0	0	0	0	0	0	11040	-1.717
Adults	3	100	-638	992	12	0	0	0	0	0	0	11040	-1.717
Adults	4	12.5	2360	1388	12	465.854	1011.542	0.325	0.037	0.173	-0.1	12738	-1.717
Adults	4	25	2345	1626	12	-626.911	1162.225	0.702	-0.049	0.107	-0.206	12738	-1.717
Adults	4	37.5	710	1194	12	273.457	807.929	0.369	0.021	0.13	-0.087	12738	-1.717
Adults	4	50	962	1033	12	151.83	855.342	0.43	0.012	0.127	-0.103	12738	-1.717
Adults	4	100	1405	1320	12	1916.704	950.49	0.028	0.15	0.279	0.022	12738	-1.717
Adults	5	12.5	1974	774	12	1332.61	1014.834	0.101	0.102	0.235	-0.031	13085	-1.717
Adults	5	25	1002	2035	12	-2250.84	1498.899	0.926	-0.172	0.025	-0.369	13085	-1.717

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Adults	5	37.5	1481	1535	11	1028.226	1183.261	0.197	0.079	0.234	-0.077	13085	-1.721
Adults	5	50	3205	1582	12	2400.807	1254.876	0.034	0.183	0.348	0.019	13085	-1.717
Adults	5	100	5874	1782	12	6292.225	1371.698	0	0.481	0.661	0.301	13085	-1.717
Adults	6	12.5	1747	931	10	1652.605	1400.572	0.126	0.141	0.349	-0.066	11686	-1.729
Adults	6	25	183	1727	11	-1443.1	1789.536	0.785	-0.123	0.141	-0.388	11686	-1.725
Adults	6	37.5	1682	1258	11	1490.868	1353.398	0.142	0.128	0.327	-0.072	11686	-1.725
Adults	6	50	2528	1638	11	1954.227	1649.23	0.125	0.167	0.411	-0.076	11686	-1.725
Adults	6	100	6563	1401	10	6890.493	1468.408	0	0.59	0.807	0.372	11686	-1.729
Adults	7	12.5	-196	517	10	-526.859	1566.501	0.63	-0.058	0.24	-0.356	9079	-1.729
Adults	7	25	-409	1003	11	-1907.23	1665.135	0.867	-0.21	0.106	-0.526	9079	-1.725
Adults	7	37.5	-28	1053	11	-227.895	1477.933	0.561	-0.025	0.256	-0.306	9079	-1.725
Adults	7	50	1020	1376	10	593.505	1790.472	0.372	0.065	0.406	-0.276	9079	-1.729
Adults	7	100	5594	1491	7	6898.915	2174.979	0.003	0.76	1.178	0.342	9079	-1.746
Adults	8	12.5	-324	690	10	-585.345	958.941	0.725	-0.119	0.219	-0.458	4912	-1.734
Adults	8	25	-823	964	11	-1746.76	1110.662	0.934	-0.356	0.035	-0.747	4912	-1.729

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Adults	8	37.5	-844	683	11	-813.68	871.911	0.819	-0.166	0.141	-0.473	4912	-1.729
Adults	8	50	-668	1000	9	-968.17	1073.057	0.81	-0.197	0.183	-0.577	4912	-1.74
Adults	8	100	2703	992	7	3470.592	1214.753	0.006	0.707	1.14	0.273	4912	-1.753
Adults	9	12.5	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
Adults	9	25	-3968	336	2	-4005.85	NA	NA	-0.966	NA	NA	4148	NA
Adults	9	37.5	-1062	NA	1	NA	NA	NA	NA	NA	NA	NA	NA
Adults	9	50	-3745	2855	4	-2553.83	3178.295	0.747	-0.616	1.622	-2.853	4148	-2.92
Adults	9	100	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
Honey	3	12.5	2960	3129	12	0	0	0	0	0	0	15358	-1.717
Honey	3	25	1182	2275	12	0	0	0	0	0	0	15358	-1.717
Honey	3	37.5	-2172	2115	12	0	0	0	0	0	0	15358	-1.717
Honey	3	50	968	2062	12	0	0	0	0	0	0	15358	-1.717
Honey	3	100	-466	2225	12	0	0	0	0	0	0	15358	-1.717
Honey	4	12.5	4128	3118	12	1413.722	1143.924	0.115	0.084	0.202	-0.033	16756	-1.717
Honey	4	25	1433	2723	12	389.484	1536.613	0.401	0.023	0.181	-0.134	16756	-1.717

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Honey	4	37.5	-1204	1886	12	496.245	1225.842	0.345	0.03	0.155	-0.096	16756	-1.717
Honey	4	50	573	2014	12	-192.6	1306.776	0.558	-0.011	0.122	-0.145	16756	-1.717
Honey	4	100	-5590	2040	12	-5284.74	1573.762	0.999	-0.315	-0.154	-0.477	16756	-1.717
Honey	5	12.5	5884	3549	12	3372.694	2650.901	0.108	0.181	0.426	-0.063	18612	-1.717
Honey	5	25	3261	3410	12	2251.552	2810.035	0.216	0.121	0.38	-0.138	18612	-1.717
Honey	5	37.5	-399	2505	11	1023.878	2769.842	0.358	0.055	0.311	-0.201	18612	-1.721
Honey	5	50	781	2703	12	35.836	2575.857	0.495	0.002	0.24	-0.236	18612	-1.717
Honey	5	100	-8836	2497	12	-8492.71	2464.922	0.999	-0.456	-0.229	-0.684	18612	-1.717
Honey	6	12.5	-1367	2710	10	-1292.68	2532.99	0.692	-0.065	0.156	-0.286	19809	-1.729
Honey	6	25	1454	4380	11	2013.298	3078.98	0.26	0.102	0.37	-0.166	19809	-1.725
Honey	6	37.5	-3377	4057	11	-500.9	3096.905	0.563	-0.025	0.244	-0.295	19809	-1.725
Honey	6	50	2603	3845	11	1985.883	3009.392	0.258	0.1	0.362	-0.162	19809	-1.725
Honey	6	100	-585	4332	10	349.337	2861.585	0.452	0.018	0.267	-0.232	19809	-1.729
Honey	7	12.5	-1428	2175	10	-1464.58	2194.118	0.744	-0.111	0.177	-0.4	13158	-1.729
Honey	7	25	-1298	2720	11	-954.117	2224.217	0.664	-0.073	0.219	-0.364	13158	-1.725

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4.5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Honey	7	37.5	-3159	3573	11	-491.248	2722.557	0.571	-0.037	0.32	-0.394	13158	-1.725
Honey	7	50	-2571	2574	10	-2671.61	2224.197	0.878	-0.203	0.089	-0.495	13158	-1.729
Honey	7	100	-3243	4024	7	-1602.15	3195.269	0.689	-0.122	0.302	-0.546	13158	-1.746
Honey	8	12.5	-2150	3339	10	-1971.07	4067.71	0.683	-0.109	0.282	-0.501	18017	-1.734
Honey	8	25	-2377	2212	11	-2087	3421.135	0.725	-0.116	0.212	-0.444	18017	-1.729
Honey	8	37.5	-3252	3597	11	-555.28	3891.151	0.556	-0.031	0.343	-0.404	18017	-1.729
Honey	8	50	-96	2712	9	-350.486	4035.232	0.534	-0.019	0.37	-0.409	18017	-1.74
Honey	8	100	6155	4291	7	7209.036	5059.696	0.087	0.4	0.892	-0.092	18017	-1.753
Honey	9	12.5	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
Honey	9	25	-5160	7052	2	34801.33	NA	NA	2.555	NA	NA	13622	NA
Honey	9	37.5	2408	NA	1	NA	NA	NA	NA	NA	NA	NA	NA
Honey	9	50	2881	2159	4	2539.491	2986.889	0.242	0.186	0.827	-0.454	13622	-2.92
Honey	9	100	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
Pollen	3	12.5	659	688	12	0	0	0	0	0	0	3755	-1.717
Pollen	3	25	616	705	12	0	0	0	0	0	0	3755	-1.717

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Pollen	3	37.5	-373	782	12	0	0	0	0	0	0	3755	-1.717
Pollen	3	50	788	514	12	0	0	0	0	0	0	3755	-1.717
Pollen	3	100	-416	1010	12	0	0	0	0	0	0	3755	-1.717
Pollen	4	12.5	64	575	12	-274.182	529.486	0.695	-0.085	0.197	-0.368	3218	-1.717
Pollen	4	25	1641	472	12	1338.308	489.69	0.006	0.416	0.677	0.155	3218	-1.717
Pollen	4	37.5	322	747	12	524.949	586.77	0.19	0.163	0.476	-0.15	3218	-1.717
Pollen	4	50	1354	440	12	995.281	531.877	0.037	0.309	0.593	0.025	3218	-1.717
Pollen	4	100	1828	451	12	1976.704	533.579	0.001	0.614	0.899	0.33	3218	-1.717
Pollen	5	12.5	968	831	12	626.829	845.907	0.233	0.136	0.451	-0.179	4608	-1.717
Pollen	5	25	2014	698	12	1773.621	837.4	0.023	0.385	0.697	0.073	4608	-1.717
Pollen	5	37.5	954	772	11	1039.421	943.221	0.141	0.226	0.578	-0.127	4608	-1.721
Pollen	5	50	1742	621	12	1388.486	803.124	0.049	0.301	0.601	0.002	4608	-1.717
Pollen	5	100	3720	624	12	3805.417	848.688	0	0.826	1.142	0.51	4608	-1.717
Pollen	6	12.5	146	804	10	72.159	802.08	0.465	0.019	0.39	-0.351	3741	-1.729
Pollen	6	25	751	622	11	526.567	707.645	0.233	0.141	0.467	-0.185	3741	-1.725

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Pollen	6	37.5	109	558	11	326.383	796.257	0.343	0.087	0.454	-0.28	3741	-1.725
Pollen	6	50	1157	701	11	669.109	711.586	0.179	0.179	0.507	-0.149	3741	-1.725
Pollen	6	100	2778	851	10	3005.044	922.899	0.002	0.803	1.23	0.377	3741	-1.729
Pollen	7	12.5	112	917	10	41.086	966.295	0.483	0.011	0.46	-0.438	3720	-1.729
Pollen	7	25	1345	475	11	1121.167	758.894	0.078	0.301	0.653	-0.05	3720	-1.725
Pollen	7	37.5	109	468	11	385.35	834.496	0.325	0.104	0.491	-0.283	3720	-1.725
Pollen	7	50	731	601	10	236.918	833.164	0.39	0.064	0.451	-0.324	3720	-1.729
Pollen	7	100	1831	884	7	2298.613	1247.892	0.042	0.618	1.204	0.032	3720	-1.746
Pollen	8	12.5	9	666	10	-36.682	660.37	0.522	-0.015	0.462	-0.492	2401	-1.734
Pollen	8	25	281	456	11	123.306	514.202	0.407	0.051	0.422	-0.319	2401	-1.729
Pollen	8	37.5	-625	433	11	-282.961	527.598	0.701	-0.118	0.262	-0.498	2401	-1.729
Pollen	8	50	134	542	9	-91.816	585.371	0.561	-0.038	0.386	-0.462	2401	-1.74
Pollen	8	100	614	914	7	1000.501	906.927	0.144	0.417	1.079	-0.245	2401	-1.753
Pollen	9	12.5	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
Pollen	9	25	-1290	1462	2	-2752	NA	NA	-1.404	NA	NA	1961	NA

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4.5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Pollen	9	37.5	172	NA	1	NA	NA	NA	NA	NA	NA	NA	NA
Pollen	9	50	-1935	1004	4	-1239.68	873.448	0.854	-0.632	0.668	-1.933	1961	-2.92
Pollen	9	100	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
Capped	3	12.5	1885	1302	12	0	0	0	0	0	0	14068	-1.717
Capped	3	25	3562	1981	12	0	0	0	0	0	0	14068	-1.717
Capped	3	37.5	1698	1597	12	0	0	0	0	0	0	14068	-1.717
Capped	3	50	1268	1033	12	0	0	0	0	0	0	14068	-1.717
Capped	3	100	924	952	12	0	0	0	0	0	0	14068	-1.717
Capped	4	12.5	1534	1940	12	868.417	2287.868	0.354	0.065	0.36	-0.23	13330	-1.717
Capped	4	25	1591	1688	12	-203.405	2154.713	0.537	-0.015	0.262	-0.293	13330	-1.717
Capped	4	37.5	717	1915	12	-485.599	2050.363	0.593	-0.036	0.228	-0.301	13330	-1.717
Capped	4	50	2766	1328	12	2134.483	1958.734	0.144	0.16	0.412	-0.092	13330	-1.717
Capped	4	100	6307	1777	12	5894.047	2125.093	0.006	0.442	0.716	0.168	13330	-1.717
Capped	5	12.5	1620	909	12	835.091	1238.42	0.254	0.048	0.17	-0.074	17386	-1.717
Capped	5	25	2938	1835	12	492.217	1465.202	0.37	0.028	0.173	-0.116	17386	-1.717

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Capped	5	37.5	4605	2016	11	3446.378	1658.596	0.025	0.198	0.362	0.034	17386	-1.721
Capped	5	50	7310	1885	12	7062.109	1769.461	0	0.406	0.581	0.231	17386	-1.717
Capped	5	100	13129	1567	12	12668.53	1508.311	0	0.729	0.878	0.58	17386	-1.717
Capped	6	12.5	-103	1516	10	280.291	1953.853	0.444	0.025	0.33	-0.279	11087	-1.729
Capped	6	25	203	1803	11	-693.87	2024.965	0.632	-0.063	0.252	-0.378	11087	-1.725
Capped	6	37.5	876	2190	11	-20.494	2101.09	0.504	-0.002	0.325	-0.329	11087	-1.725
Capped	6	50	2807	2447	11	2439.957	2383.828	0.159	0.22	0.591	-0.151	11087	-1.725
Capped	6	100	6312	1736	10	6579.909	2035.95	0.002	0.593	0.911	0.276	11087	-1.729
Capped	7	12.5	-1041	942	10	-629.7	938.611	0.745	-0.189	0.298	-0.676	3332	-1.729
Capped	7	25	1384	977	11	1568.674	1009.999	0.068	0.471	0.993	-0.052	3332	-1.725
Capped	7	37.5	-164	1206	11	-62.613	1133.697	0.522	-0.019	0.568	-0.606	3332	-1.725
Capped	7	50	1462	600	10	1638.468	845.043	0.034	0.492	0.93	0.053	3332	-1.729
Capped	7	100	1867	980	7	1867.429	1045.354	0.046	0.56	1.108	0.013	3332	-1.746
Capped	8	12.5	-654	563	10	-519.544	542.462	0.825	-0.407	0.33	-1.145	1276	-1.734
Capped	8	25	336	209	11	211.226	378.348	0.292	0.166	0.678	-0.347	1276	-1.729

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Capped	8	37.5	-367	451	11	-456.96	484.771	0.821	-0.358	0.299	-1.015	1276	-1.729
Capped	8	50	-143	297	9	-214.838	464.816	0.675	-0.168	0.465	-0.802	1276	-1.74
Capped	8	100	-467	438	7	-466.857	538.258	0.8	-0.366	0.374	-1.106	1276	-1.753
Capped	9	12.5	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
Capped	9	25	-5504	2408	2	-5426.32	NA	NA	-0.73	NA	NA	7430	NA
Capped	9	37.5	-1892	NA	1	NA	NA	NA	NA	NA	NA	NA	NA
Capped	9	50	-5031	5148	4	-7475.75	2913.255	0.938	-1.006	0.139	-2.151	7430	-2.92
Capped	9	100	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
Open	3	12.5	1018	831	12	0	0	0	0	0	0	5920	-1.717
Open	3	25	2480	942	12	0	0	0	0	0	0	5920	-1.717
Open	3	37.5	975	824	12	0	0	0	0	0	0	5920	-1.717
Open	3	50	932	653	12	0	0	0	0	0	0	5920	-1.717
Open	3	100	975	690	12	0	0	0	0	0	0	5920	-1.717
Open	4	12.5	-186	1032	12	-858.507	1033.015	0.793	-0.142	0.152	-0.436	6034	-1.717
Open	4	25	201	876	12	-916.841	1159.785	0.781	-0.152	0.178	-0.482	6034	-1.717

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Open	4	37.5	-272	611	12	-931.856	824.644	0.865	-0.154	0.08	-0.389	6034	-1.717
Open	4	50	158	817	12	-678.321	854.933	0.782	-0.112	0.131	-0.356	6034	-1.717
Open	4	100	1949	903	12	1149.816	929.037	0.114	0.191	0.455	-0.074	6034	-1.717
Open	5	12.5	588	798	12	65.307	804.633	0.468	0.01	0.215	-0.196	6722	-1.717
Open	5	25	674	750	12	-116.926	953.828	0.548	-0.017	0.226	-0.261	6722	-1.717
Open	5	37.5	555	899	11	293.801	945.175	0.379	0.044	0.286	-0.198	6722	-1.721
Open	5	50	2365	873	12	2011.076	904.088	0.018	0.299	0.53	0.068	6722	-1.717
Open	5	100	4056	663	12	3523.042	746.233	0	0.524	0.715	0.333	6722	-1.717
Open	6	12.5	335	570	10	39.984	1013.403	0.484	0.007	0.302	-0.289	5934	-1.729
Open	6	25	235	1166	11	-1328.15	1267.95	0.846	-0.224	0.145	-0.592	5934	-1.725
Open	6	37.5	360	948	11	3.834	1078.98	0.499	0.001	0.314	-0.313	5934	-1.725
Open	6	50	1564	1184	11	1240.532	1229.488	0.163	0.209	0.566	-0.148	5934	-1.725
Open	6	100	3870	940	10	2997.233	1065.815	0.006	0.505	0.816	0.195	5934	-1.729
Open	7	12.5	-206	773	10	-390.251	701.588	0.708	-0.212	0.447	-0.871	1842	-1.729
Open	7	25	1048	445	11	1042.041	583.558	0.045	0.566	1.112	0.019	1842	-1.725

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4.5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Open	7	37.5	31	493	11	-117.567	530.054	0.587	-0.064	0.433	-0.56	1842	-1.725
Open	7	50	318	707	10	280.351	661.722	0.338	0.152	0.773	-0.469	1842	-1.729
Open	7	100	356	725	7	331.341	693.158	0.32	0.18	0.837	-0.477	1842	-1.746
Open	8	12.5	0	186	10	-33.062	198.726	0.565	-0.065	0.612	-0.742	509	-1.734
Open	8	25	164	122	11	38.619	180.761	0.417	0.076	0.69	-0.538	509	-1.729
Open	8	37.5	-133	110	11	-133.331	154.527	0.801	-0.262	0.263	-0.787	509	-1.729
Open	8	50	363	182	9	269.007	193.836	0.092	0.529	1.191	-0.134	509	-1.74
Open	8	100	-12	264	7	-40.823	241.085	0.566	-0.08	0.75	-0.911	509	-1.753
Open	9	12.5	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
Open	9	25	-4300	1032	2	-2335.87	NA	NA	-0.606	NA	NA	3853	NA
Open	9	37.5	-2408	NA	1	NA	NA	NA	NA	NA	NA	NA	NA
Open	9	50	-2451	2421	4	-2700.72	3149.925	0.759	-0.701	1.686	-3.088	3853	-2.92
Open	9	100	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
Eggs	3	12.5	688	781	12	0	0	0	0	0	0	6063	-1.717
Eggs	3	25	1949	1063	12	0	0	0	0	0	0	6063	-1.717

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4.5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Eggs	3	37.5	1261	1056	12	0	0	0	0	0	0	6063	-1.717
Eggs	3	50	473	680	12	0	0	0	0	0	0	6063	-1.717
Eggs	3	100	-473	596	12	0	0	0	0	0	0	6063	-1.717
Eggs	4	12.5	659	671	12	512.178	679.245	0.229	0.085	0.279	-0.109	6020	-1.717
Eggs	4	25	-57	917	12	-539.733	840.224	0.736	-0.09	0.15	-0.329	6020	-1.717
Eggs	4	37.5	-14	684	12	-383.721	652.181	0.719	-0.064	0.122	-0.25	6020	-1.717
Eggs	4	50	-344	873	12	-494.63	754.614	0.741	-0.082	0.133	-0.297	6020	-1.717
Eggs	4	100	516	775	12	620.721	732.1	0.203	0.103	0.312	-0.106	6020	-1.717
Eggs	5	12.5	-874	681	12	-1052.4	847.229	0.886	-0.199	0.076	-0.474	5289	-1.717
Eggs	5	25	330	492	12	-146.206	816.494	0.57	-0.028	0.237	-0.293	5289	-1.717
Eggs	5	37.5	-461	779	11	-1021.58	840.179	0.881	-0.193	0.08	-0.466	5289	-1.721
Eggs	5	50	874	1117	12	681.408	1012.774	0.254	0.129	0.458	-0.2	5289	-1.717
Eggs	5	100	2351	481	12	2517.267	745.13	0.001	0.476	0.718	0.234	5289	-1.717
Eggs	6	12.5	2348	733	10	1840.078	1025.765	0.044	0.323	0.633	0.012	5705	-1.729
Eggs	6	25	305	922	11	-1022.46	1026.986	0.834	-0.179	0.131	-0.49	5705	-1.725

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Eggs	6	37.5	367	973	11	-441.412	1000.926	0.668	-0.077	0.225	-0.38	5705	-1.725
Eggs	6	50	1235	1311	11	776.626	1180.261	0.259	0.136	0.493	-0.221	5705	-1.725
Eggs	6	100	3466	676	10	3525.367	1042.032	0.002	0.618	0.934	0.302	5705	-1.729
Eggs	7	12.5	112	357	10	76.195	674.86	0.456	0.03	0.486	-0.426	2558	-1.729
Eggs	7	25	610	335	11	396.183	654.163	0.276	0.155	0.596	-0.286	2558	-1.725
Eggs	7	37.5	-625	468	11	-746.558	674.229	0.859	-0.292	0.163	-0.746	2558	-1.725
Eggs	7	50	559	403	10	485.487	679.011	0.242	0.19	0.649	-0.269	2558	-1.729
Eggs	7	100	725	369	7	788.178	823.767	0.176	0.308	0.87	-0.254	2558	-1.746
Eggs	8	12.5	-86	257	10	-74.885	240.469	0.62	-0.116	0.53	-0.763	645	-1.734
Eggs	8	25	-78	231	11	-167.544	222.122	0.77	-0.26	0.336	-0.855	645	-1.729
Eggs	8	37.5	16	196	11	34.87	209.763	0.435	0.054	0.616	-0.508	645	-1.729
Eggs	8	50	220	158	9	191.919	198.451	0.174	0.298	0.833	-0.238	645	-1.74
Eggs	8	100	-393	516	7	-379.17	388.547	0.828	-0.588	0.468	-1.644	645	-1.753
Eggs	9	12.5	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
Eggs	9	25	-1118	2666	2	3947.4	NA	NA	0.948	NA	NA	4162	NA

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
Eggs	9	37.5	-688	NA	1	NA	NA	NA	NA	NA	NA	NA	NA
Eggs	9	50	-1290	2689	4	347.554	894.384	0.368	0.083	0.711	-0.544	4162	-2.92
Eggs	9	100	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
TotalLife	3	12.5	5668	3513	12	0	0	0	0	0	0	37091	-1.717
TotalLife	3	25	10767	4383	12	0	0	0	0	0	0	37091	-1.717
TotalLife	3	37.5	4405	3798	12	0	0	0	0	0	0	37091	-1.717
TotalLife	3	50	3707	2515	12	0	0	0	0	0	0	37091	-1.717
TotalLife	3	100	788	2489	12	0	0	0	0	0	0	37091	-1.717
TotalLife	4	12.5	4367	3999	12	942.431	3798.456	0.403	0.025	0.196	-0.146	38123	-1.717
TotalLife	4	25	4080	4212	12	-3289.24	3853.76	0.799	-0.086	0.087	-0.26	38123	-1.717
TotalLife	4	37.5	1140	3831	12	-2022.78	3258.133	0.729	-0.053	0.094	-0.2	38123	-1.717
TotalLife	4	50	3542	3482	12	733.75	3323.912	0.414	0.019	0.169	-0.13	38123	-1.717
TotalLife	4	100	10177	3555	12	9634.56	3370.598	0.005	0.253	0.405	0.101	38123	-1.717
TotalLife	5	12.5	3307	2444	12	694.456	2864.883	0.405	0.016	0.132	-0.099	42483	-1.717
TotalLife	5	25	4943	4192	12	-2840.58	3277.988	0.802	-0.067	0.066	-0.199	42483	-1.717

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
TotalLife	5	37.5	6180	4311	11	3162.085	3439.368	0.184	0.074	0.214	-0.065	42483	-1.721
TotalLife	5	50	13754	4869	12	11624.68	4131.08	0.005	0.274	0.441	0.107	42483	-1.717
TotalLife	5	100	25411	3685	12	24887.04	3151.779	0	0.586	0.713	0.458	42483	-1.717
TotalLife	6	12.5	4327	2285	10	2865.989	4443.573	0.263	0.083	0.307	-0.14	34411	-1.729
TotalLife	6	25	926	4828	11	-6504.93	4896.464	0.901	-0.189	0.056	-0.434	34411	-1.725
TotalLife	6	37.5	3285	4448	11	135.529	4447.651	0.488	0.004	0.227	-0.219	34411	-1.725
TotalLife	6	50	8134	5407	11	5352.874	5452.809	0.169	0.156	0.429	-0.118	34411	-1.725
TotalLife	6	100	20211	3425	10	19401.15	4514.48	0	0.564	0.791	0.337	34411	-1.729
TotalLife	7	12.5	-1331	1564	10	-1491.87	3041.223	0.685	-0.089	0.224	-0.402	16812	-1.729
TotalLife	7	25	2632	2114	11	1071.462	3265.271	0.373	0.064	0.399	-0.271	16812	-1.725
TotalLife	7	37.5	-786	2446	11	-1184.94	3148.394	0.645	-0.07	0.253	-0.393	16812	-1.725
TotalLife	7	50	3359	2432	10	2877.337	3316.56	0.198	0.171	0.512	-0.17	16812	-1.729
TotalLife	7	100	8542	2793	7	8845.245	3844.574	0.018	0.526	0.925	0.127	16812	-1.746
TotalLife	8	12.5	-1064	1242	10	-1157.8	1526.336	0.771	-0.158	0.203	-0.518	7342	-1.734
TotalLife	8	25	-401	1308	11	-1899.2	1466.454	0.895	-0.259	0.087	-0.604	7342	-1.729

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Parameter	Time (CCA)[1]	Test conc. (µg/l)	mean	SE (standard error observed mean)	n	Estimate (model estimate mean difference from control)[3,4]	Std.Error (of estimated mean)	p_value for comparison with the control	Estimated reduction from control (%) [4,5]	Estimate (90% confidence upper limit) [4])	Estimate (90% confidence lower limit)[4,5]	Control Mean (observed means in control)	t-test confidence limit
TotalLife	8	37.5	-1328	946	11	-1163.46	1322.765	0.805	-0.158	0.153	-0.47	7342	-1.729
TotalLife	8	50	-228	1263	9	-949.986	1512.152	0.731	-0.129	0.229	-0.488	7342	-1.74
TotalLife	8	100	1831	1703	7	2103.263	1790.473	0.129	0.286	0.714	-0.141	7342	-1.753
TotalLife	9	12.5	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
TotalLife	9	25	-14890	6441	2	8870.319	NA	NA	0.453	NA	NA	19594	NA
TotalLife	9	37.5	-6050	NA	1	NA	NA	NA	NA	NA	NA	NA	NA
TotalLife	9	50	-12517	12936	4	-2269.79	1581.988	0.856	-0.116	0.12	-0.352	19594	-2.92
TotalLife	9	100	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA

Analysis and discussion of results

Control trends

As shown in **Figure B.1**, the number of adults increased until CCA 5 (August) and then started to decline. This trend was also observed in open cells and pollen, and was very evident with capped cells, whereby a dramatic decline was observed following CCA 5. The number of eggs appeared to drop slightly at CCA 5, but then increased by CCA 6 before a drop in numbers at CCA 7. **Figure B.2** shows a comparison between the numbers of live bees in the three different neonicotinoid colony feeding studies for the control. Live bee counts in the imidacloprid and clothianidin studies both appear to be in decline beginning after CCA 6 (September) in the control colonies, whereas the decline in live bees started to decline in the thiamethoxam study after CCA 5 (August). This is likely the result of a later start date for the thiamethoxam study, which resulted in the 6th colony condition assessment being taken in late September, which is closer to the period of time in which the colony is preparing for overwintering. As a result, any effects observed in the thiamethoxam study at CCA 5 were difficult to follow to CCA 6 (or thereafter) for potential recovery of effects, since the control colony was declining in numbers at this time, resulting in less sensitivity in the analysis. **Figure B.3** shows the variability in the mean of the controls for total life and the beginning of the "die off" of bees at CCA 5.

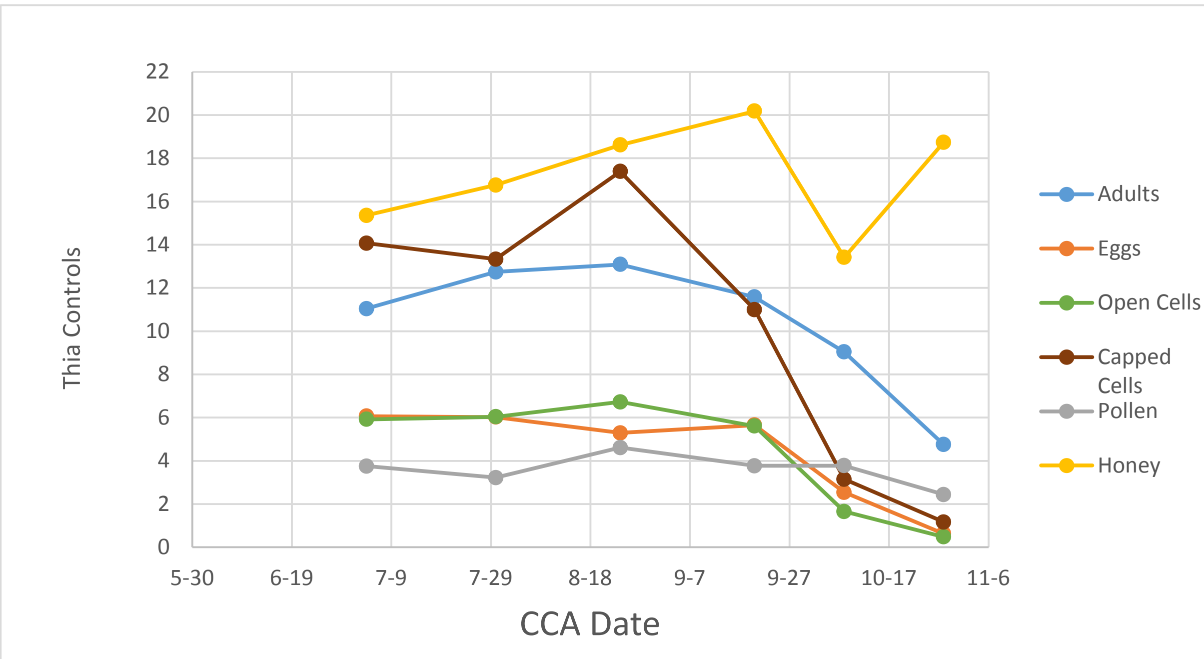


Figure B.1. Control data for all endpoints for CCA 3 to CCA 8.

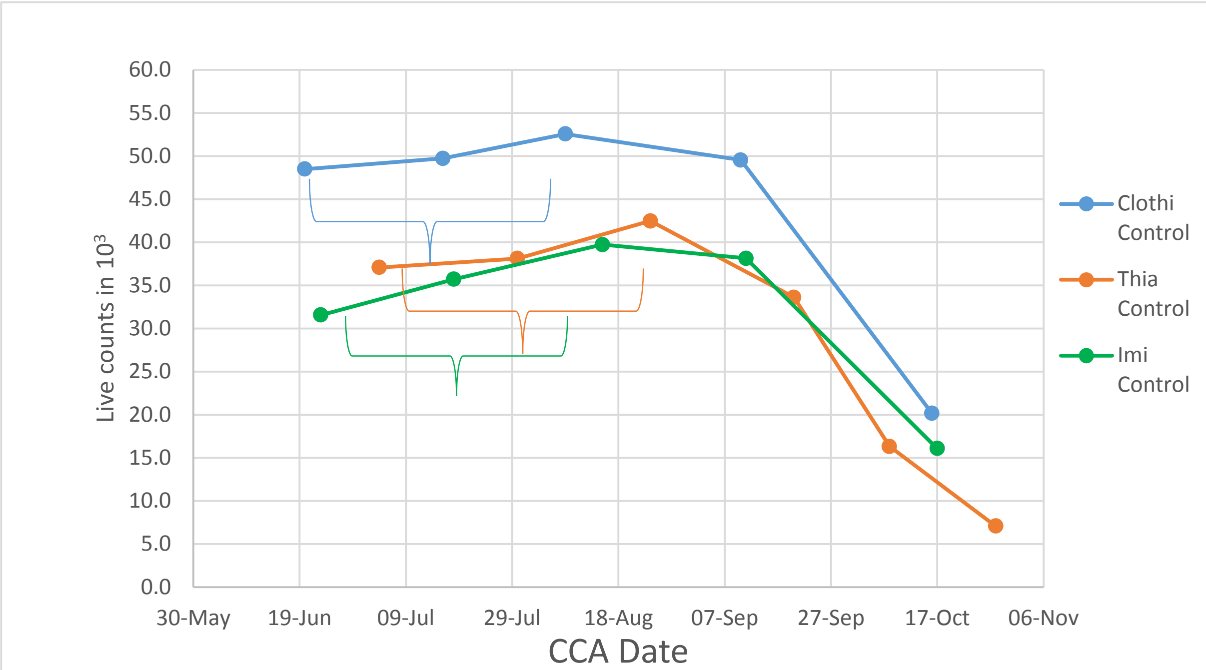


Figure B.2. Comparison between the numbers of live bees in the three different neonicotinoid colony feeding studies for the controls.

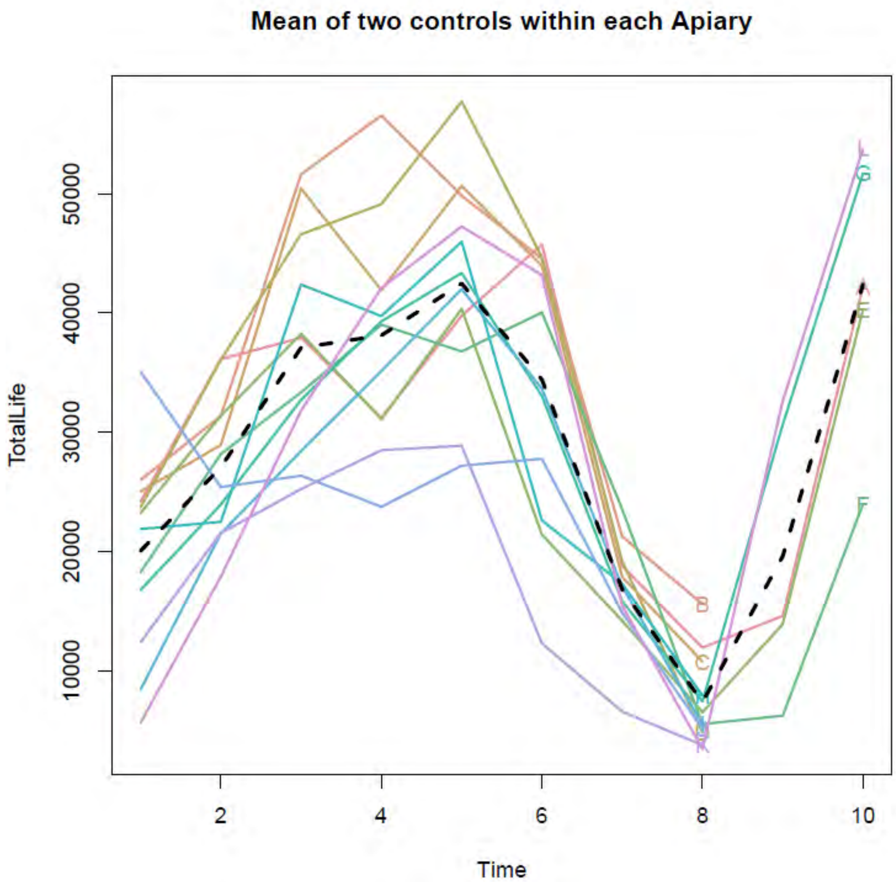


Figure B.3. Mean of two controls within each apiary for CCAs for total life.

Colony Condition Assessments: Life stages in the hive

Total number of individuals (total life)

Figures B.4 and **Table B.2** show the effects on total life (total number of individual bees) across CCAs and treatment groups. CCA 5 is of particular interest in this study since it provides an observation period which has allowed for the whole colony to be exposed to thiamethoxam for up to 7 weeks following exposure. Subsequent CCAs (for example CCA 6 and onward) are difficult to use for comparison to the control since all the hives are beginning to prepare for overwintering. This was the likely consequence of a latter study initiation which resulted in CCA 6 occurring at the end of September, and CCA 7/8 in October. Compared with the control, no differences in the number of TOTAL LIFE adults in hives (P>0.1) was observed for any CCA in the 12.5, 25 or 37.5 µg a.i/L dose groups. In the 50 µg a.i/L group, there was a significant reduction from the control at CCA 5 (27% reduction, p=0.005). There was no other significant reduction in the following CCAs (CCA 6, and 7), although percent reduction from the control were 15 and 17%. Variability in the data and the preparation of hives for overwintering resulted in difficulty comparing the treatment and control groups following CCA 5. In the 100 µg a.i/L group, there was a significant reduction from the control at all CCAs (except CCA 8). Total number of individuals was reduced by 25% at CCA 4 (p=0.005), 58% at CCA 5 (p<<0.05p<.001), 56% at CCA 6 (p<<0.05 p<.001) and 52% at CCA 7 (p=0.018). It is noted that the largest decrease in total life was at CCA 5. **Figure B.4 and B.5a** shows a general increase in the reduction from the control as the dose increases. In the 37.5 µg a.i/L dose group 6/11 apiaries are reduced compared to the control, in the 50 µg a.i/L dose group 9/12 apiaries are reduced compared to the control, and in the 100 µg a.i/L all apiaries are reduced. In general, the standard error bars were observed mostly to increase with increasing CCAs and dose. Figure X5b shows the number of hives which were performing more poorly (for total number of individuals) than the control at CCA 3 which is prior to the beginning of exposure. In particular, it is noted that 10/12 hives in the 25 µg a.i/L had reductions from the control for total life.

Table B.2. Estimated percent reduction from control for total number of individuals (total life).

Test concentration (µg/L)	Estimated reduction from control (%) (P value)					
	CCA4 (3 WAE) July 28-31	CCA5 (6-7 WAE) 20-28 Aug	CCA6 (10-11 WAE) 17-23 Sep	CCA7 (13 WAE) 6-10 Oct	CCA8 (16 WAE) 27-29 Oct	CCA9 (After over winter) 31 Mar
12.5	2.5 (0.403)	1.6 (0.405)	8.3 (0.263)	-8.9 (0.685)	-15.8 (0.771)	NA
25	-8.6 (0.799)	-6.7 (0.802)	-18.9 (0.901)	6.4 (0.373)	-25.9 (0.895)	45.3 (NA)
37.5	-5.3 (0.729)	7.4 (0.184)	0.4 (0.488)	-7 (0.645)	-15.8 (0.805)	NA
50	1.9 (0.414)	27 (0.005**)	15.6 (0.169)	17 (0.198)	-12.9 (0.731)	-11.6 (0.856)
100	25.3 (0.005**)	58.6 (0**)	56.4 (0**)	52.6 (0.018**)	28.6 (0.129)	NA

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<P<0.1
**P<0.05
NA – not applicable because the sample sizes were too low.
NOTE: WAE = weeks after exposure. CCA 1 was 7 weeks before exposure, CCA 2 was 4 weeks before exposure, and CCA 3 was 1 week before exposure. Tables present post exposure CCA data only.

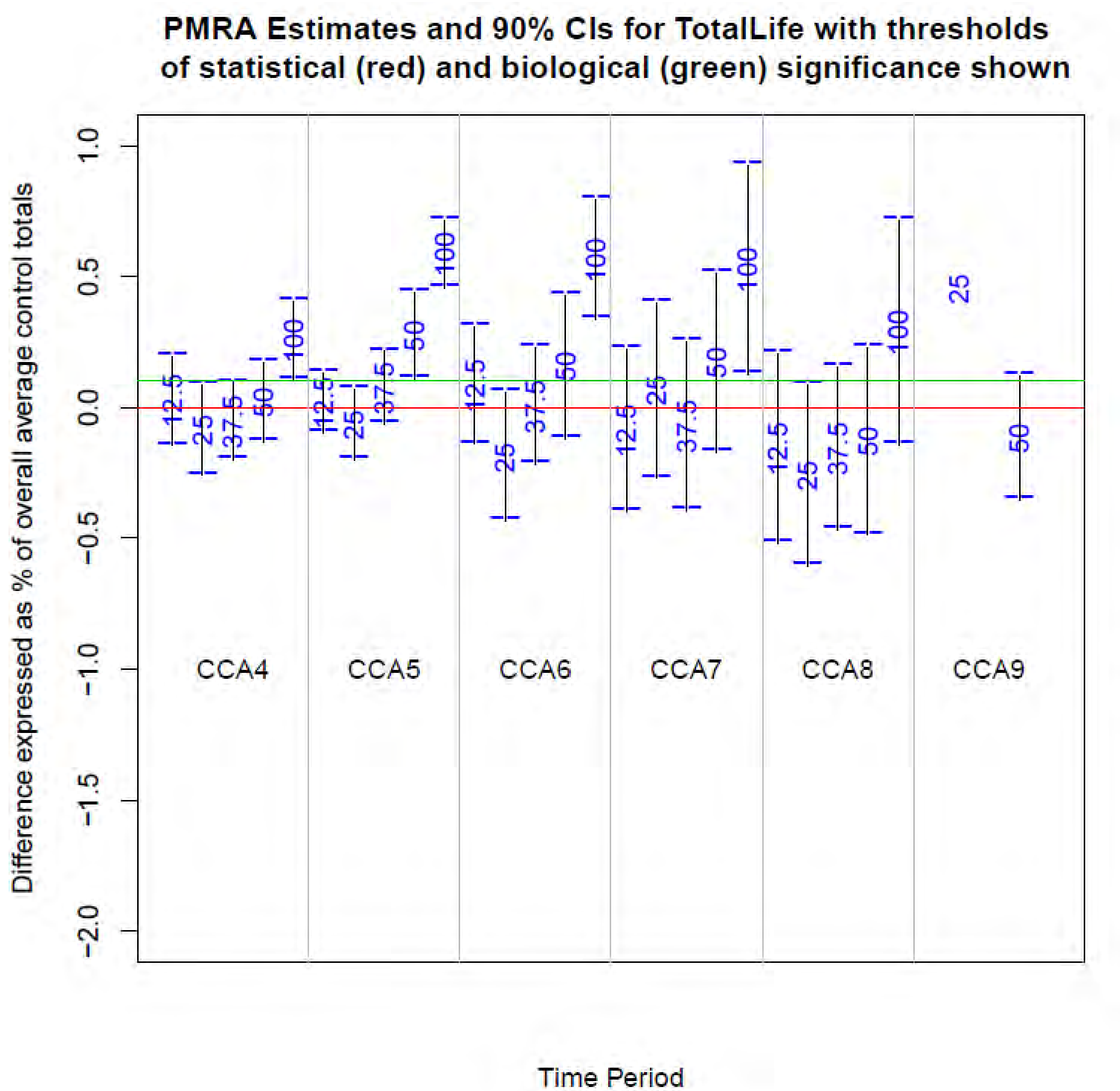


Figure B.4. Difference from control for the means for total life for CCA 4 to 9.

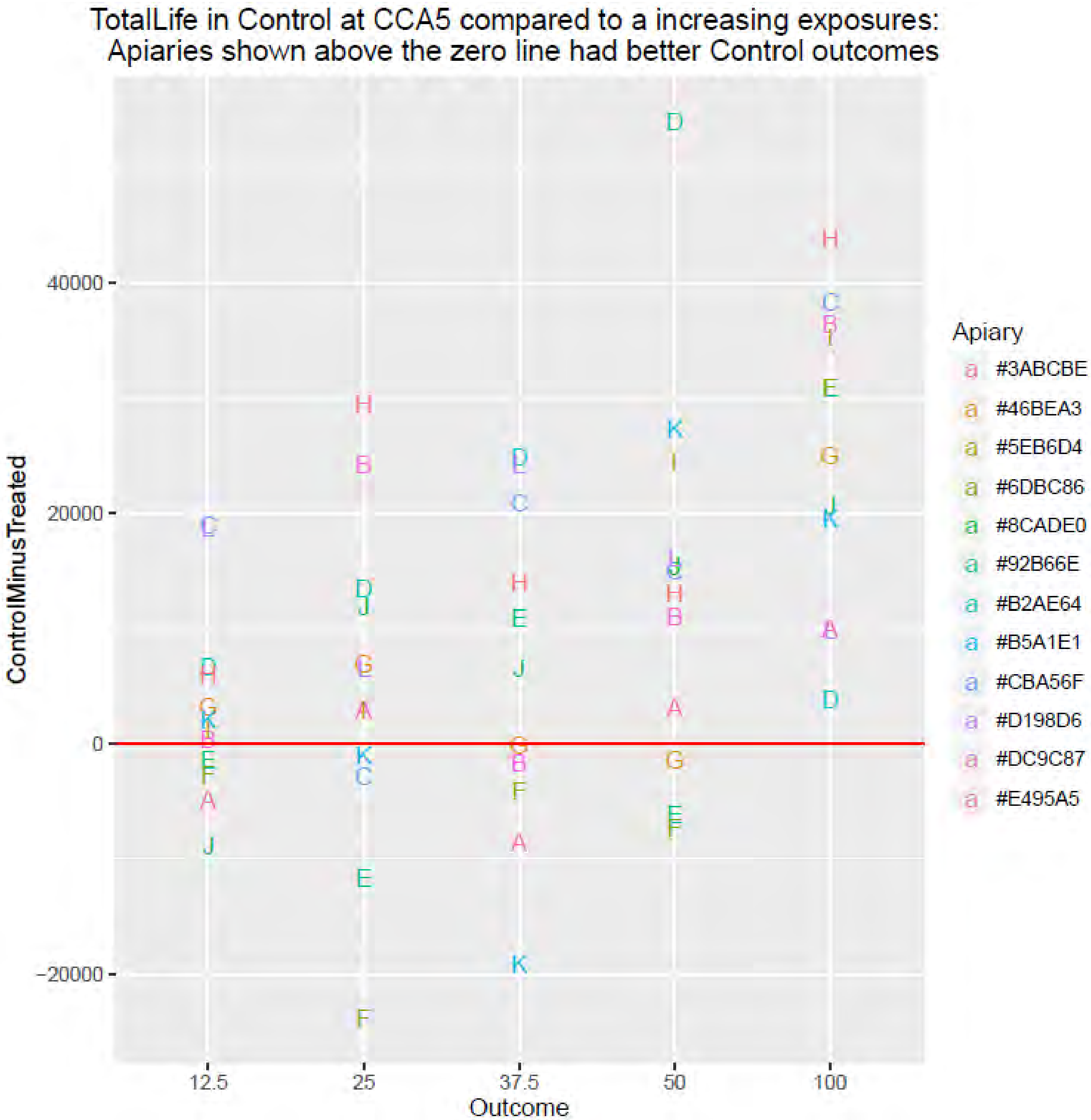


Figure B.5a. Difference from control for all treatments and apiaries at CCA3 (before exposure) for total life.

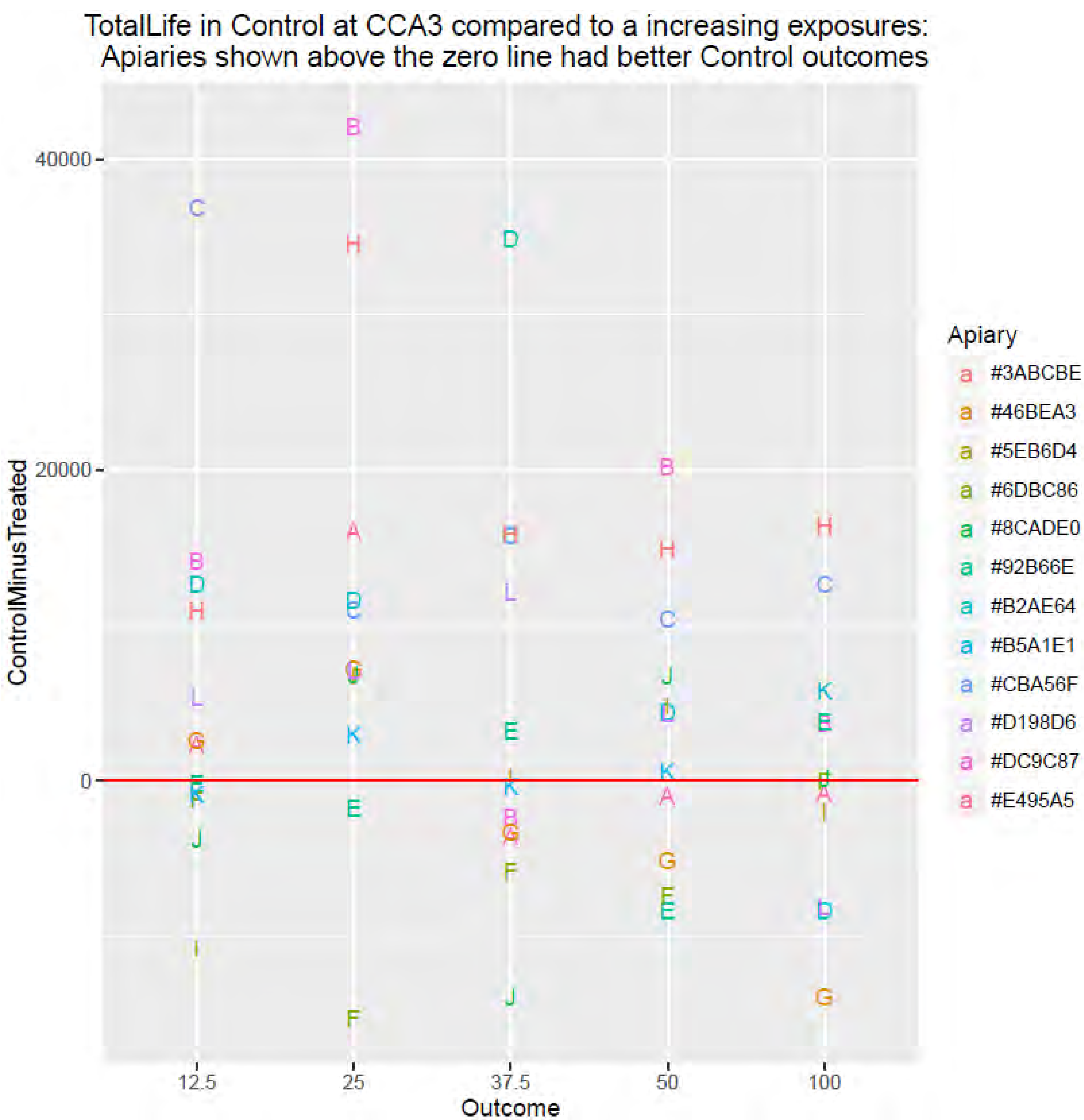


Figure B.5b. Difference from control for all treatments and apiaries at CCA5 for total life.

Adults

Figure B.6 and **Table B.3** shows the effects on adult honey bees across CCAs and treatment groups. Compared with the control, no differences in the number of adults in hives (P>0.1) was observed for any CCA in the 12.5, 25 or 37.5 µg a.i/L dose groups. In the 50 µg a.i/L group, there was an 18% reduction from the control at CCA 5 (p=0.034). By the next CCA (6), there was no statistical significance from the control, however, the reduction was very similar at 16.7%. Since the thiamethoxam study started later in the season, by CCA 6 and onward, the colonies appeared to be starting to prepare for overwintering, and therefore, the consistent decline in most colonies made a comparison difficult. In the 100 µg a.i/L group, there was a consistent trend of significant reduction from the control at all CCAs with the reduction of the number of adults generally increasing with each subsequent CCA (CCA 4, 15% reduction from control; CCA 5, 48% reduction; CCA6, 59% reduction; CCA 7, 76% reduction; and CCA 8, 70% reduction). In the 50 µg a.i/L the decline in adults may have been a large contributing factor to the decline in total life. **Figure B.6** and **B.7** shows a general increase in the reduction from the control as the dose increases. In the 37.5 µg a.i/L dose group 6/10 apiaries are reduced compared to the control, in the 50 µg a.i/L dose group 8/12 apiaries are reduced compared to the control, and in the 100 µg a.i/L 11/12 apiaries are reduced. In general, the observed standard error bars mostly increase with increasing CCAs and dose.

Table B.3. Estimated percent reduction from control for adults.

Test concentration (µg/L)	Estimated reduction from control (%) (P value)					
	CCA4 (3 WAE) July 28-31	CCA5 (6-7 WAE) 20-28 Aug	CCA6 (10-11 WAE) 17-23 Sep	CCA7 (13 WAE) 6-10 Oct	CCA8 (16 WAE) 27-29 Oct	CCA9 (After over winter) 31 Mar
12.5	3.7 (0.325)	10 (0.101)	14 (0.126)	-5.8 (0.63)	-11.9 (0.725)	NA
25	-4.9 (0.702)	-17 (0.926)	-12 (0.785)	-21 (0.867)	-35.6 (0.934)	-96.6 (NA)
37.5	2.1 (0.369)	7.9 (0.197)	12.8 (0.142)	-2.5 (0.561)	-16.6 (0.819)	NA
50	1.2 (0.43)	18 (0.034**)	16.7 (0.125)	6.5 (0.372)	-19.7 (0.81)	-61.6 (0.747)
100	15 (0.028**)	48 (0**)	59 (0**)	76 (0.003**)	70.7 (0.006**)	NA

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<P<0.1
**P<0.05
NA – not applicable because the sample sizes were too low.
NOTE: WAE = weeks after exposure. CCA 1 was 7 weeks before exposure, CCA 2 was 4 weeks before exposure, and CCA 3 was 1 week before exposure. Tables present post exposure CCA data only.

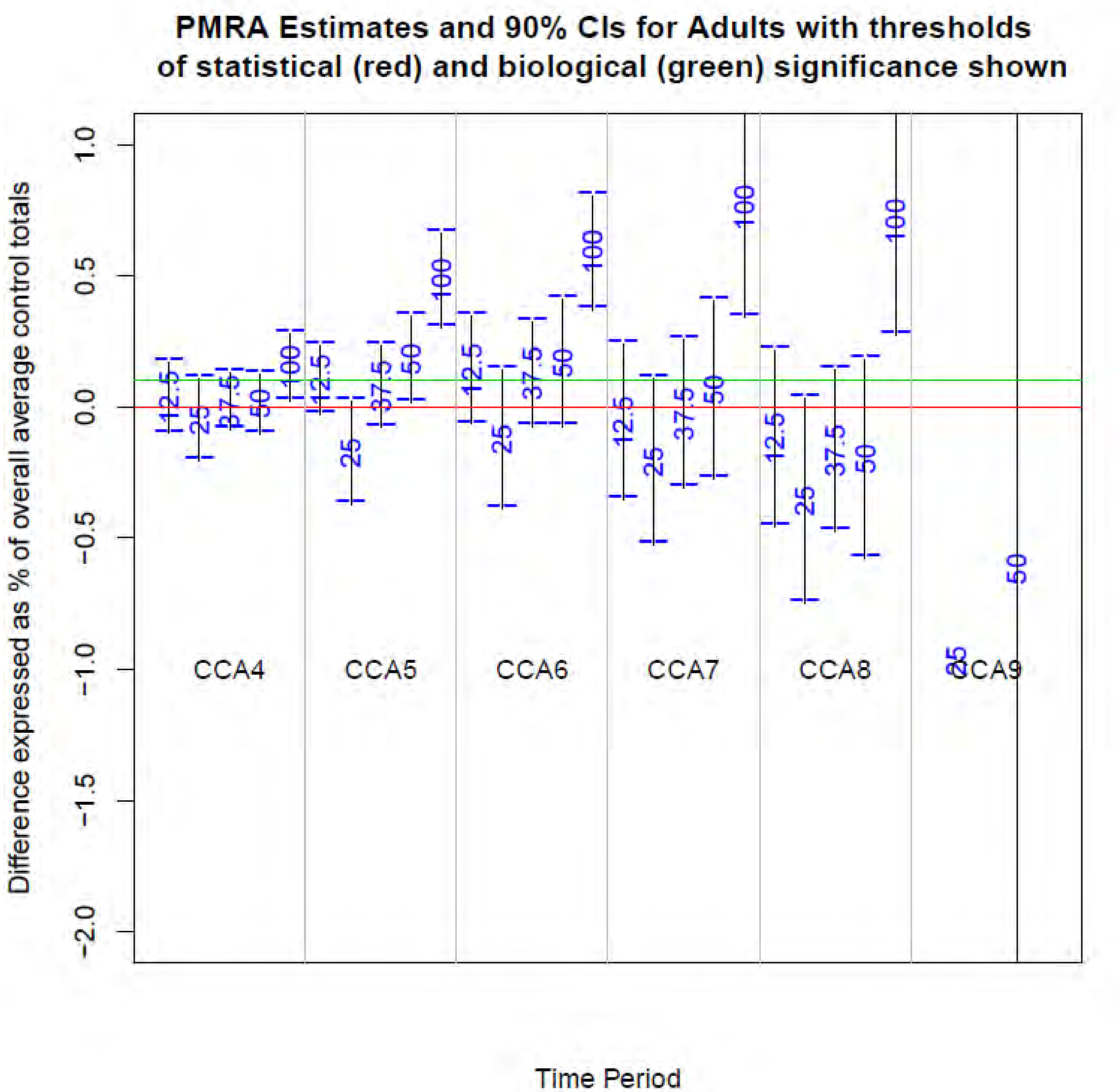


Figure B.6. Difference from control for the means for number of adults for CCA 4 to 9.

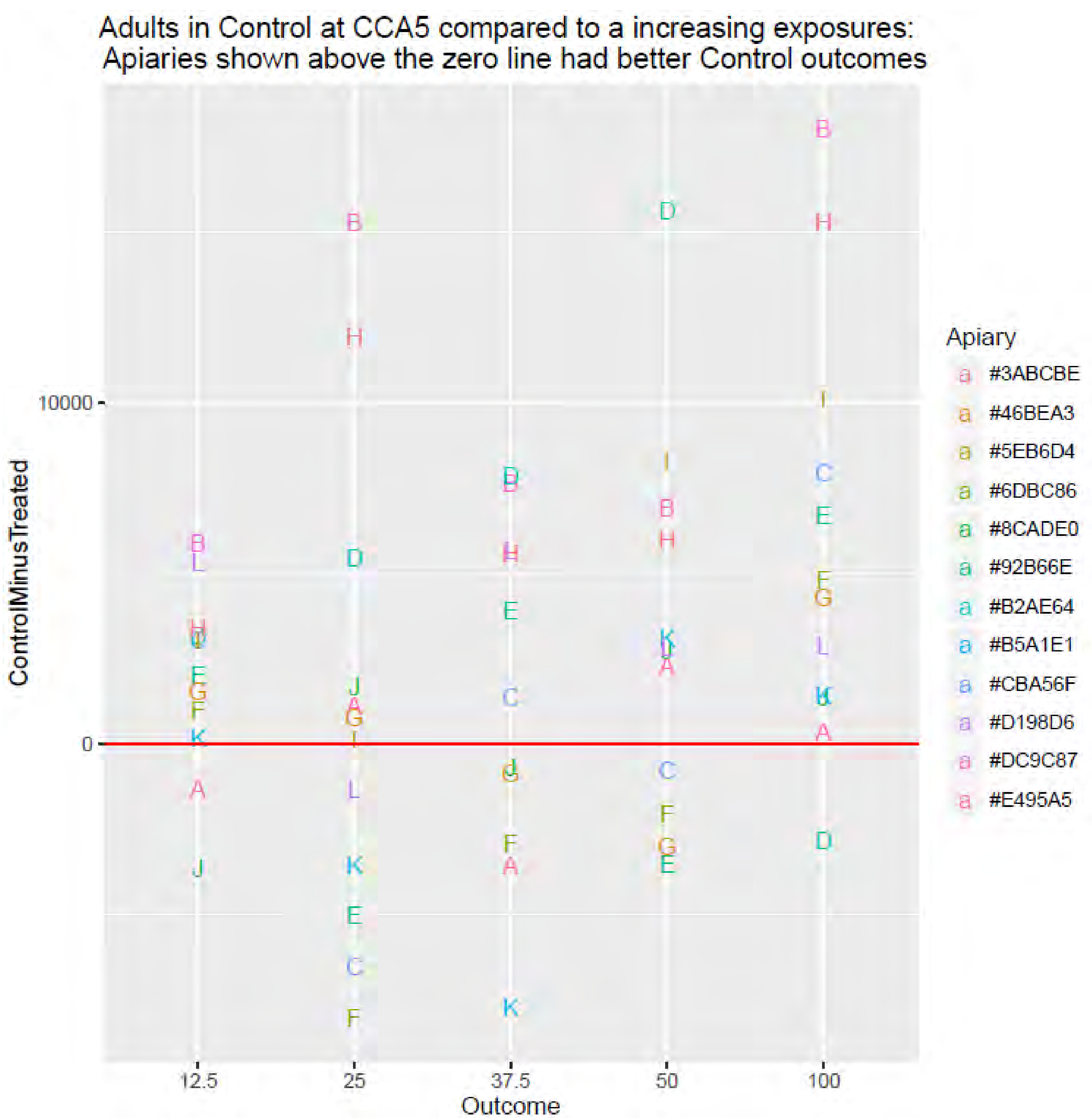


Figure B.7. Difference from control for all treatments and apiaries at CCA5 for adults.

Eggs

Figure B.8 and **Table B.4** shows the effects on eggs across CCAs and treatment groups. Compared with the control, no differences in the number of eggs in hives (P>0.1) was observed for any CCA in the 25, 37.5 or 50 µg a.i/L dose groups. In the 12.5 µg a.i/L group, there was a significant reduction from control at CCA 6 only (32% reduction, p=0.04). However, in the previous CCAs at 12.5 µg a.i/L, and the higher dose groups (25, 37.5 and 50 µg a.i/L) at CCA 6 (and all other CCAs) there were no significant reductions from the control, and many of the treatment groups actually had more eggs compared to the control over the course of the study. However, in the 100 µg a.i/L group there was a significant reduction from the control at CCA 5 (- you to be consistent in the number of decimal places you give and how you round or truncate 47.6% reduction, p=0.001) and CCA6 (62% reduction, p=0.002). As previously discussed, despite a lack of statistically observed effects at CCA 7 (30% reduction), it is likely that the colonies preparation for overwintering resulted in a less ability to distinguish effects compared to the control, since all colonies were in decline. **Figure B8** and **B.9** shows a general increase in the reduction from the control as the dose increases. In the 37.5 µg a.i/L dose group 5/11 apiaries are reduced compared to the control, in the 50 µg a.i/L dose group 7/12 apiaries are reduced compared to the control, and in the 100 µg a.i/L 10/12 apiaries are reduced. In general, the observed standard error bars mostly increase with increasing CCAs and dose.

Table B.4. Estimated percent reduction from control for eggs.

Test concentration (µg/L)	Estimated reduction from control (%) (P value)					
	CCA4 (3 WAE) July 28-31	CCA5 (6-7 WAE) 20-28 Aug	CCA6 (10-11 WAE) 17-23 Sep	CCA7 (13 WAE) 6-10 Oct	CCA8 (16 WAE) 27-29 Oct	CCA9 (After over winter) 31 Mar
12.5	8.5 (0.229)	-20 (0.886)	32 (0.04**/0.834)	3 (0.456)	-11.6 (0.62)	NA
25	-9 (0.736)	-2.8 (0.57)	-17.9 (0.834)	15 (0.276)	-26 (0.77)	94.8 (NA)
37.5	-6.4 (0.719)	-19.3 (0.881)	-7.7 (0.668)	-29 (0.859)	5.4 (0.435)	NA
50	-8.2 (0.741)	12.9 (0.254)	13.6 (0.259)	19 (0.242)	29.8 (0.174)	8.3 (0.368)
100	10 (0.203)	47.6 (0.001**)	62.8 (0.002**)	30.8 (0.176)	-58.8 (0.828)	NA

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<P<0.1
**P<0.05
NOTE: When two p values are presented, it is the result of a step down approach in the statistical analysis. We gave this rationale for not having to do that other than total life at CCA5 – but you can if you wish)
NA – not applicable because the sample sizes were too low.
NOTE: WAE = weeks after exposure. CCA 1 was 7 weeks before exposure, CCA 2 was 4 weeks before exposure, and CCA 3 was 1 week before exposure. Tables present post exposure CCA data only.

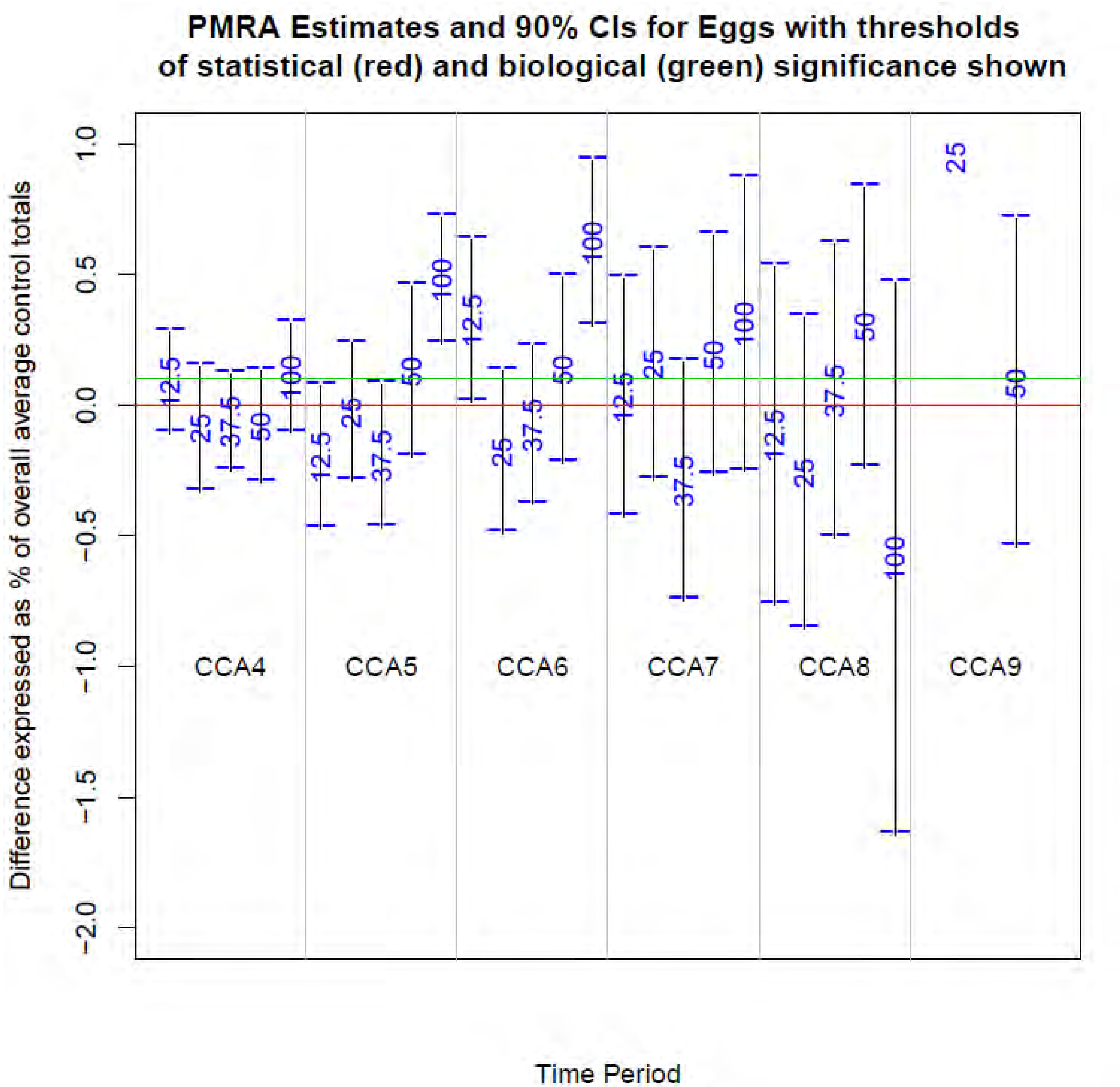


Figure B.8. Difference from control for the means for number of eggs for CCA 4 to 9.

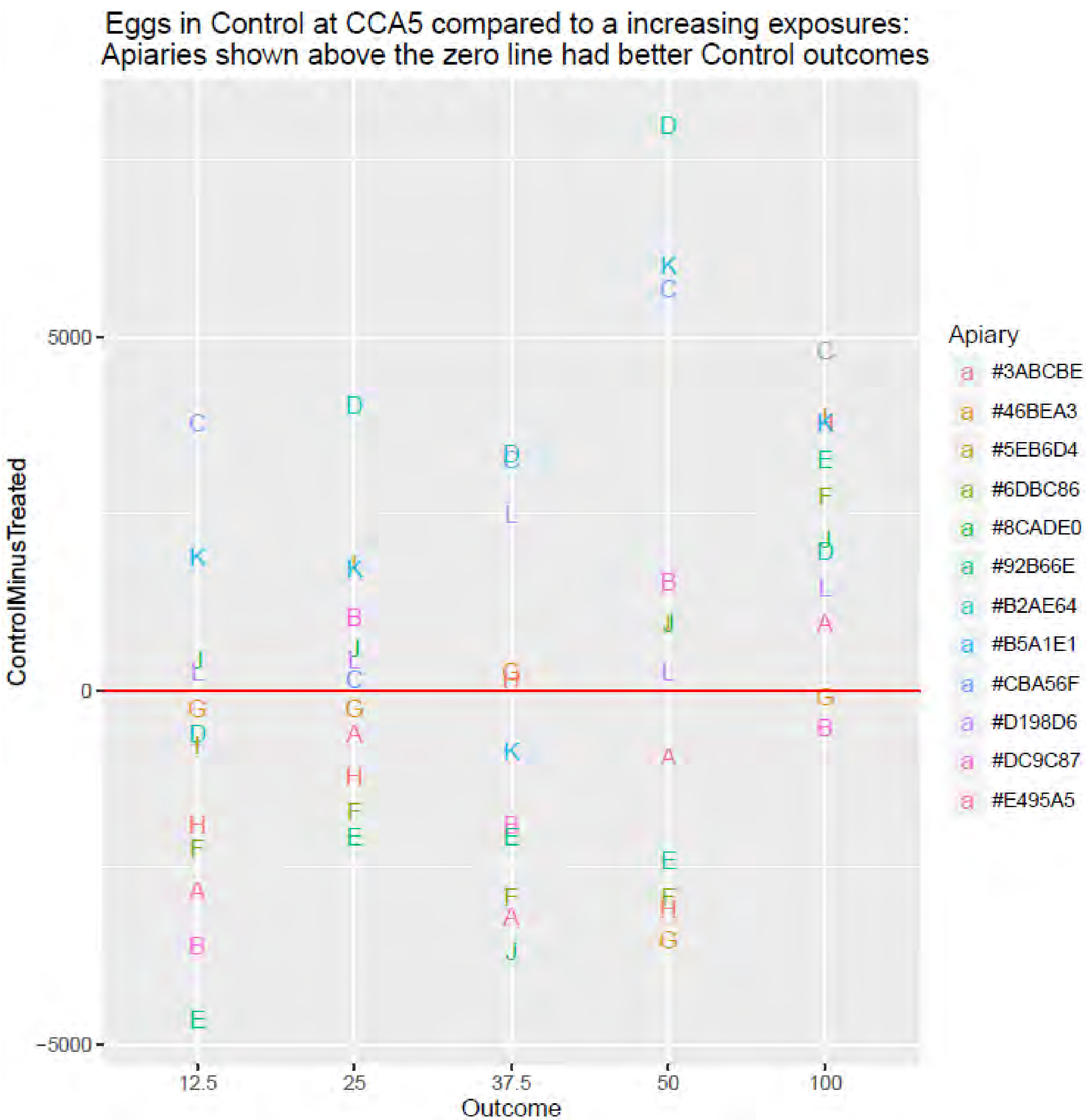


Figure B.9. Difference from control for all treatments and apiaries at CCA5 for eggs.

<u>**Larvae (Open/uncapped brood)**</u>

Figure B.10 and **Table B.5** shows the effects on open brood (larvae) across CCAs and treatment groups. Compared with the control, no differences in the open brood (larvae) in hives (P>0.1) was observed for any CCA in the 12.5, or 37.5 µg a.i/L dose groups. In the 25 µg a.i/L group, there was a significant reduction from control at CCA 7 only (56.6% reduction, p=0.045). However, in the previous CCAs at 25 µg a.i/L, and the higher dose groups (37.5, 50 and 100 µg a.i/L) at CCA 7 there were no significant reductions from the control. However, in the 50 and 100 µg a.i/L group there was a significant reduction from the control at CCA 5 (29% reduction, p=0.018 for 50 µg a.i/L, and 52% reduction, p<<0.05 for 100 µg a.i/L). At 50 µg a.i/L, there was no significant reduction from the control at CCA 6 and 7 (with reductions of 20 and 15%, respectively), however, by CCA 8 there was a significant reduction (52%, p=0.092). In the 100 µg a.i/L group, there was also a reduction at CCA 6 (50%, p=0.006), however, there were no further reductions from the control at any subsequent CCAs. Once again, this could be the result of all hives (including the control) preparing for overwintering. The reduction in larvae may have contributed to the overall decline in total individuals. **Figure B.10 and B.11** shows a general increase in the reduction from the control as the dose increases. In the 37.5 µg a.i/L dose group 6/10 apiaries are reduced compared to the control, in the 50 µg a.i/L dose group 8/12 apiaries are reduced compared to the control, and in the 100 µg a.i/L 11/12 apiaries are reduced. In general, the observed standard error bars mostly increase with increasing CCAs and dose.

Table B.5 Estimated percent reduction from control for larvae (open).

Test concentration (µg/L)	Estimated reduction from control (%) (P value)					
	CCA4 (3 WAE) July 28-31	CCA5 (6-7 WAE) 20-28 Aug	CCA6 (10-11 WAE) 17-23 Sep	CCA7 (13 WAE) 6-10 Oct	CCA8 (16 WAE) 27-29 Oct	CCA9 (After over winter) 31 Mar
12.5	-14 (0.793)	1 (0.468)	7 (0.484)	-21 (0.708)	-6.5 (0.565)	NA
25	-15 (0.781)	-1.7 (0.548)	-22.4 (0.846))	56.6 (0.045**/ 0.587)	7.6 (0.417)	-60.6 (NA)
37.5	-15 (0.865)	4.4 (0.379)	1 (0.499)	-6.4 (0.587)	-26 (0.801)	NA
50	-12 (0.78)	29.9 (0.018**)	20.9 (0.163)	15 (0.338)	52.9 (0.092*)	-70 (0.759)
100	19 (0.114)	52.4 (0**)	50.5 (0.006**)	18 (0.32)	-8 (0.566)	NA

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<P<0.1
**P<0.05
NA – not applicable because the sample sizes were too low.
NOTE: When two p values are presented, it is the result of a step down approach in the statistical analysis.
NOTE: WAE = weeks after exposure. CCA 1 was 7 weeks before exposure, CCA 2 was 4 weeks before exposure, and CCA 3 was 1 week before exposure. Tables present post exposure CCA data only.

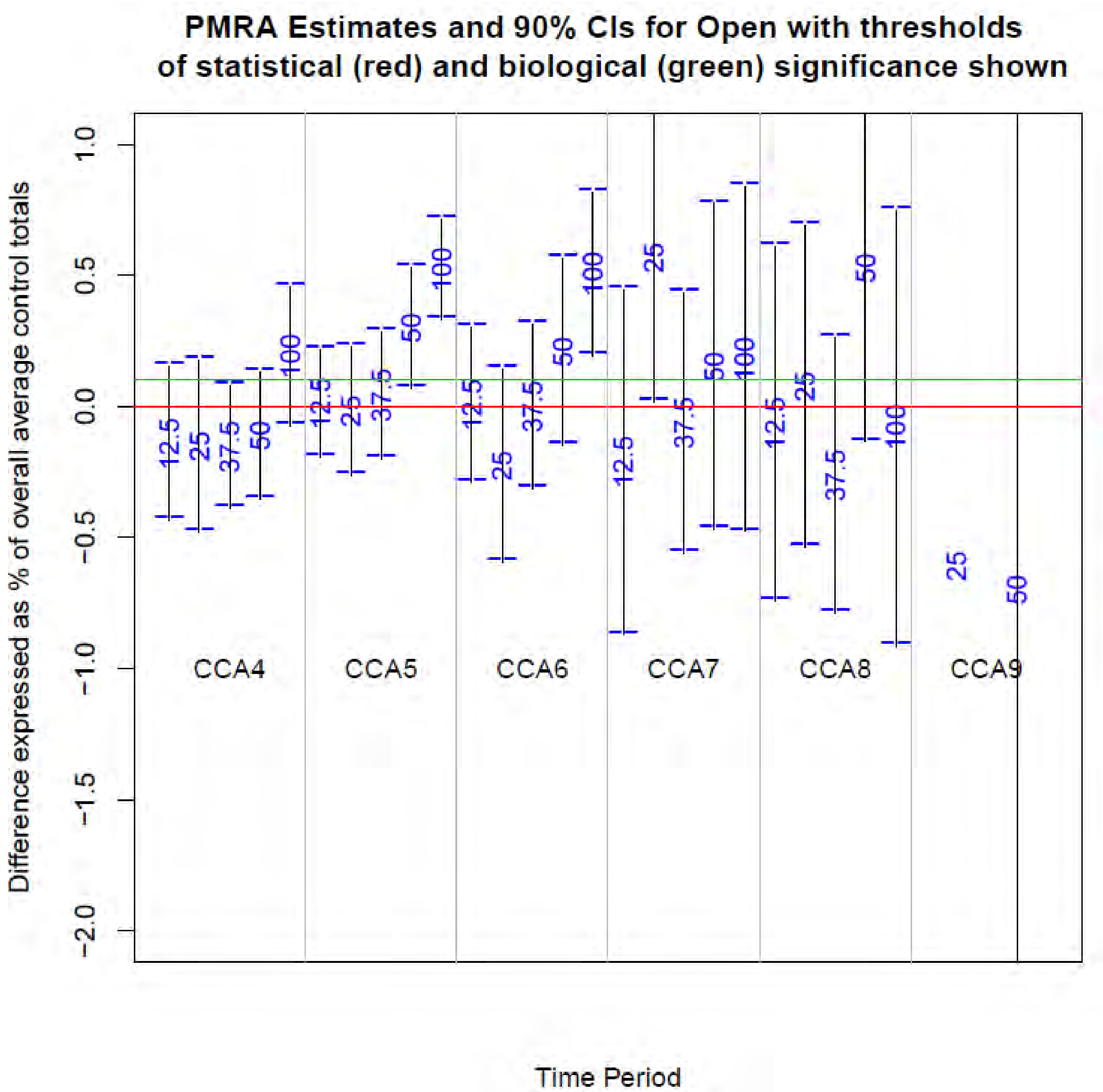


Figure B.10. Difference from control for the means for open cells (larvae) for CCA 4 to 9.

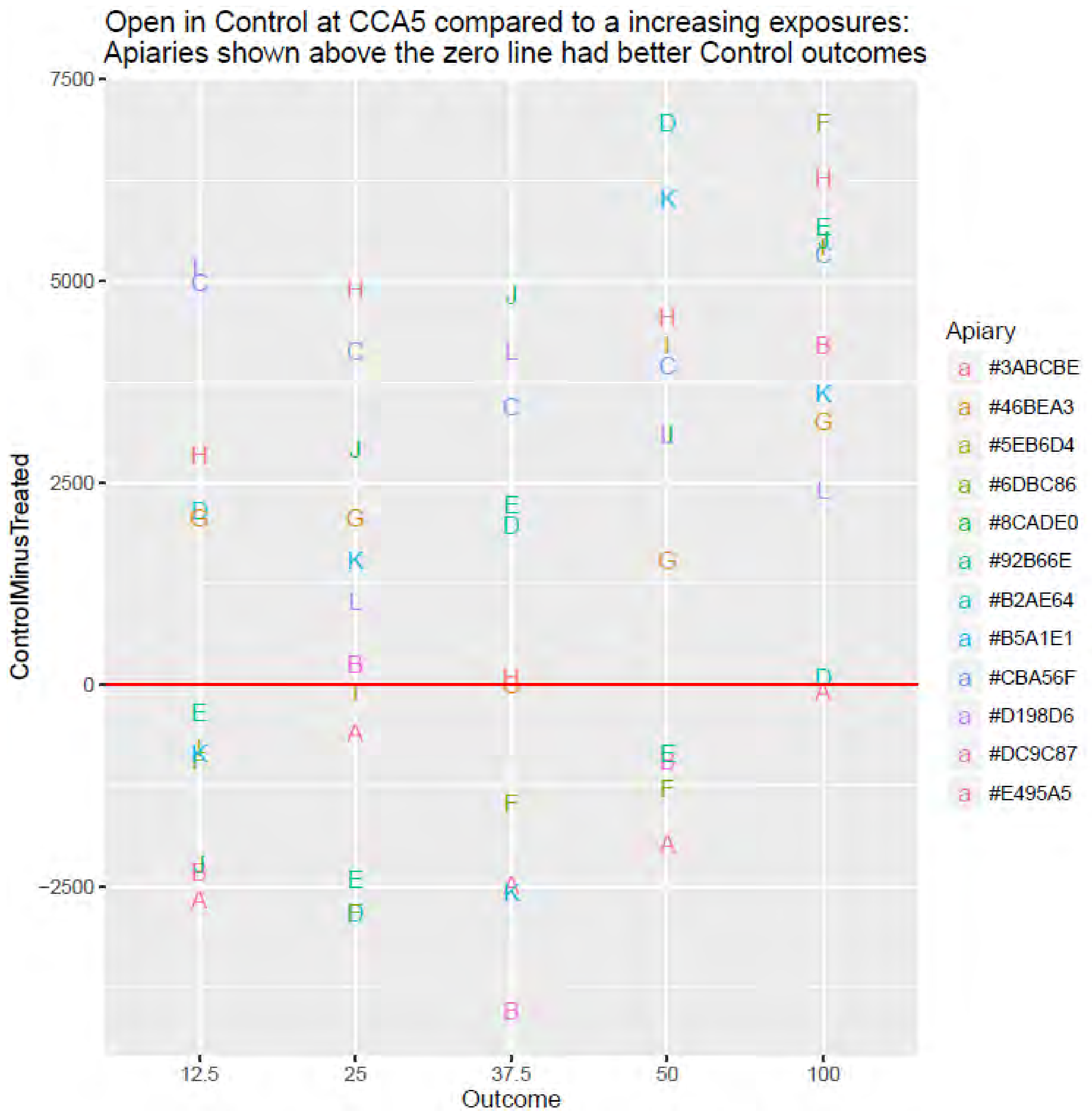


Figure B.11. Difference from control for all treatments and apiaries at CCA5 for open cells (larvae).

__Pupae (Capped brood)__

Figure B.12 and **Table B.6** shows the effects on capped brood (pupae) across CCAs and treatment groups. Compared with the control, no differences in the capped brood (pupae) in hives (P>0.1) was observed for any CCA in the 12.5 µg a.i/L dose group. In the 25 µg a.i/L group, there was a significant reduction from the control at CCA 7 only (47% reduction, p=0.068) which was not observed at any other CCA. In previous CCAs (4, 5 and 6), the number of pupae were either close to the control, or higher. In the 37.5 µg a.i/L group, there was a significant reduction from the control at CCA 5 only (19.8%, p=0.025). In the 50 µg a.i/L group, there was a significant reduction from the control at CCA 5 (40%, p<<0.05p<0.001) and CCA 7 (49%, p=0.034). At CCA 6 there was 22 % reduction, which was not statistically significant. It is interesting to note that the reduction in pupae appeared dose related at CCA 5, with percent reduction increasing from 19.8% at 37.5 µg a.i/L, to 40% at 50 µg a.i/L, up to 72.9% at 100 µg a.i/L. The reduction in pupae may have contributed to the overall reduced live individuals. **Figure B.12** and **B.13** shows a general increase in the reduction from the control as the dose increases. In the 37.5 µg a.i/L dose group 9/11 apiaries are reduced compared to the control, in the 50 µg a.i/L dose group 10/12 apiaries are reduced compared to the control, and in the 100 µg a.i/L 12/12 apiaries are reduced. In general, the standard error bars increase with increasing CCAs and dose.

Table B.6. Estimated percent reduction from control for pupae (capped).

Test concentration (µg/L)	Estimated reduction from control (%) (P value)					
	CCA4 (3 WAE) July 28-31	**CCA5 (6-7 WAE) 20-28 Aug**	**CCA6 (10-11 WAE) 17-23 Sep**	**CCA7 (13 WAE) 6-10 Oct**	**CCA8 (16 WAE) 27-29 Oct**	**CCA9 (After over winter) 31 Mar**
12.5	6.5 (0.354)	4.8 (0.254)	2.5 (0.444)	-18.9 (0.745)	-40.7 (0.825)	NA
25	-1.5 (0.537)	2.8 (0.37)	-6.3 (0.632)	47 (0.068*/ 0.522)	16.6 (0.292)	-73 (NA)
37.5	-3.6 (0.593)	19.8 (0.025**)	-2 (0.504)	-1.9 (0.522)	-35.8 (0.821)	NA
50	16 (0.144)	40.6 (0**)	22 (0.159)	49.2 (0.034**)	-16.8 (0.675)	-100 (0.938)
100	44 (0.006**)	72.9 (0**)	59.3 (0.002**)	56 (0.046**)	-36.6 (0.8)	NA

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<P<0.1
**P<0.05
NA – not applicable because the sample sizes were too low.
NOTE: When two p values are presented, it is the result of a step down approach in the statistical analysis.
NOTE: WAE = weeks after exposure. CCA 1 was 7 weeks before exposure, CCA 2 was 4 weeks before exposure, and CCA 3 was 1 week before exposure. Tables present post exposure CCA data only.

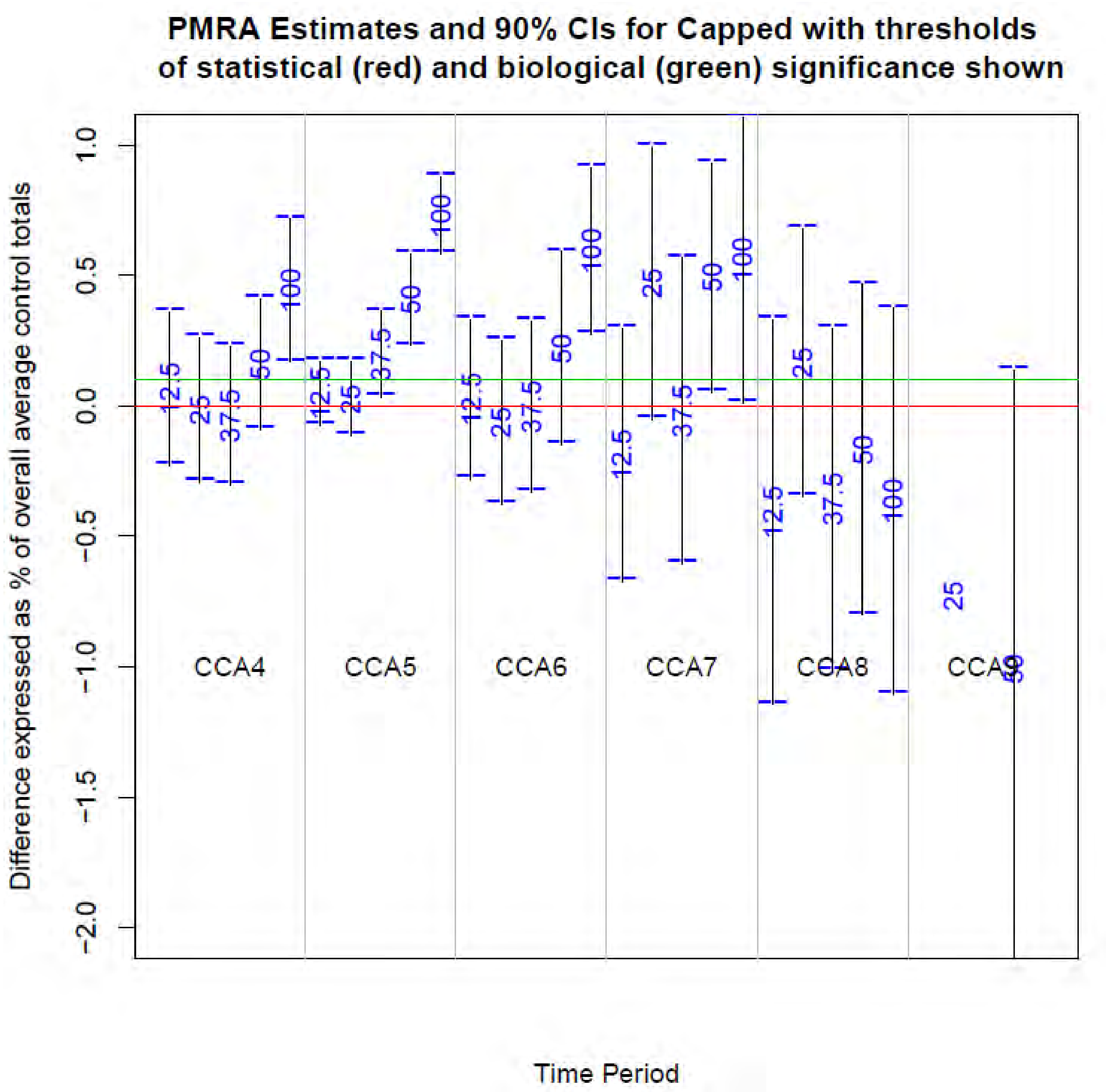


Figure B.12. Difference from control for the means for capped cells (pupae) for CCA 4 to 9.

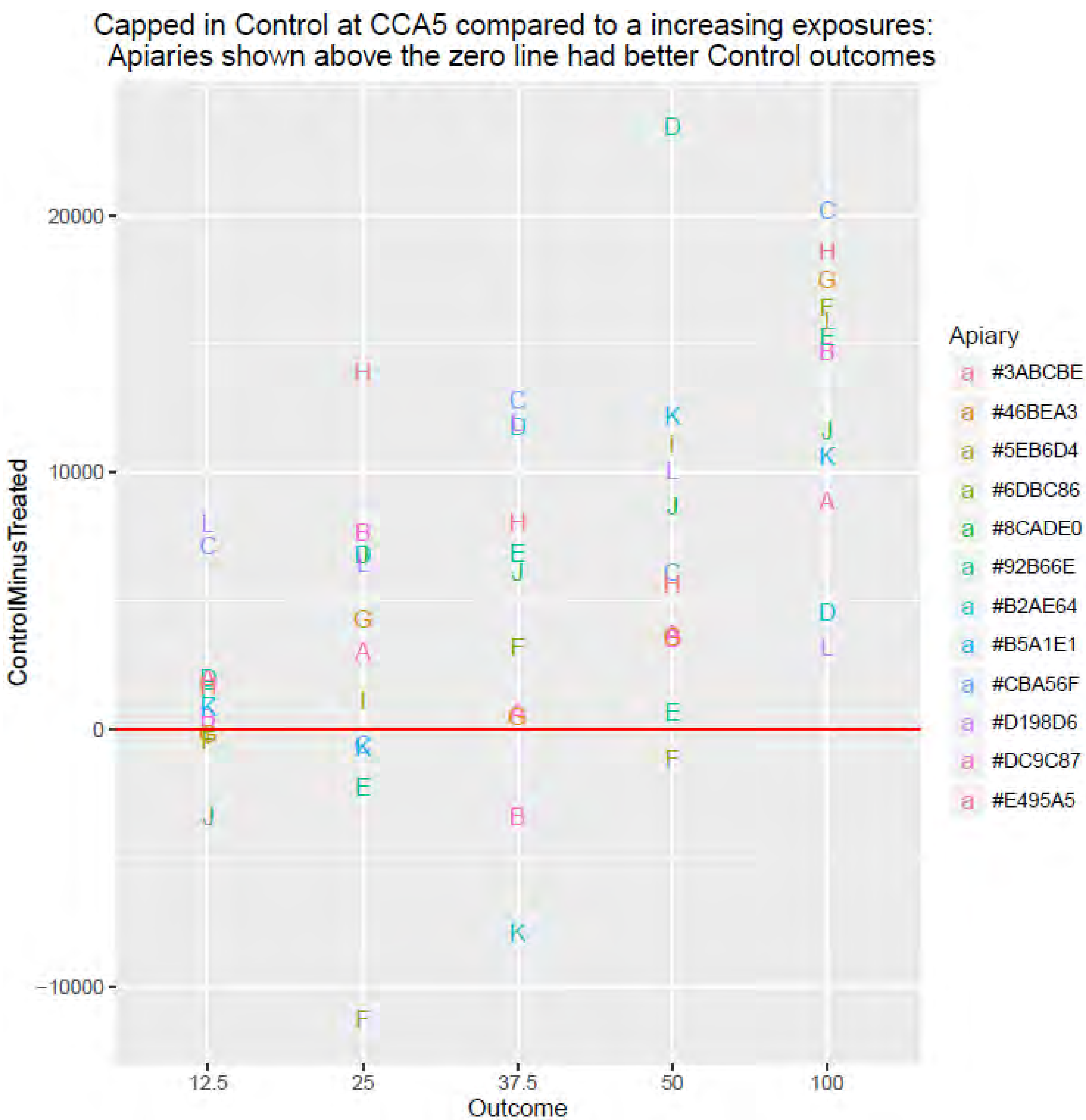


Figure B.13. Difference from control for all treatments and apiaries at CCA for capped cells (pupae).

Colony Condition Assessments – Food Stores

Pollen

Figure B.14 and **Table B.7** shows the effects on pollen across CCAs and treatment groups. Compared with the control, no differences in the pollen stores (P>0.1) was observed for any CCA in the 12.5 and 37.5 µg a.i/L dose groups. This trend was similar to the larval effects. In the 25 and 50 µg a.i/L groups, there were significant reductions from the control at CCA 4 and 5 (with lower pollen stores in the lower test concentration). The percent reduction in the 25 µg a.i/L group was 41.6 % (p=0.006) and 38.5% (p=0.023) at CCA 4 and 5, respectively. In the 50 µg a.i/L group, reduction was 30% at both CCA 4 and 5 (p=0.037 and 0.049, respectively). In the 100 µg a.i/L group, the effects were observed across all CCAs at relatively consistent reductions. Percent reduction from control ranged from 61 to 82.6% from CCA 4 to 7. By CCA 8, the reduction was not statistically significant, but was still at 41.7%. It is interesting to note that this food store response variable, compared to life forms in the hive, appeared to reflect earlier onset of effects. Figure **B.14** and **B.15** shows a general increase in the reduction from the control as the dose increases. In the 25 µg a.i/L dose group 10/12 apiaries are reduced compared to the control, in the 37.5 µg a.i/L dose group 7/10 apiaries are reduced compared to the control, in the 50 µg a.i/L dose group 10/12 apiaries are reduced compared to the control, and in the 100 µg a.i/L 11/12 apiaries are reduced. In general, the standard error bars increase with increasing CCAs.

Table B.7. Estimated percent reduction from control for pollen stores

Test concentration (µg/L)	Estimated reduction from control (%) (P value)					
	CCA4 (3 WAE) July 28-31	**CCA5 (6-7 WAE) 20-28 Aug**	**CCA6 (10-11 WAE) 17-23 Sep**	**CCA7 (13 WAE) 6-10 Oct**	**CCA8 (16 WAE) 27-29 Oct**	**CCA9 (After over winter) 31 Mar**
12.5	-8.5 (0.695)	13.6 (0.233)	1.9 (0.465)	1.1 (0.483)	-1.5 (0.522)	NA
25	41.6 (0.006**/ 0.19)	38.5 (0.023**/ 0.141)	14 (0.233)	30 (0.078*)	5.1 (0.407)	-140 (NA)
37.5	16.3 (0.19)	22.6 (0.141)	8.7 (0.343)	10 (0.325)	-11.8 (0.701)	NA
50	30.9 (0.037**)	30 (0.049**)	17.9 (0.179)	6.4 (0.39)	-3.8 (0.561)	-63 (0.854)
100	61.4 (0.001**)	82.6 (0**)	80.3 (0.002**)	61.8 (0.042**)	41.7 (0.144)	NA

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<P<0.1
**P<0.05
NA – not applicable because the sample sizes were too low.
NOTE: When two p values are presented, it is the result of a step down approach in the statistical analysis.
NOTE: WAE = weeks after exposure. CCA 1 was 7 weeks before exposure, CCA 2 was 4 weeks before exposure, and CCA 3 was 1 week before exposure. Tables present post exposure CCA data only.

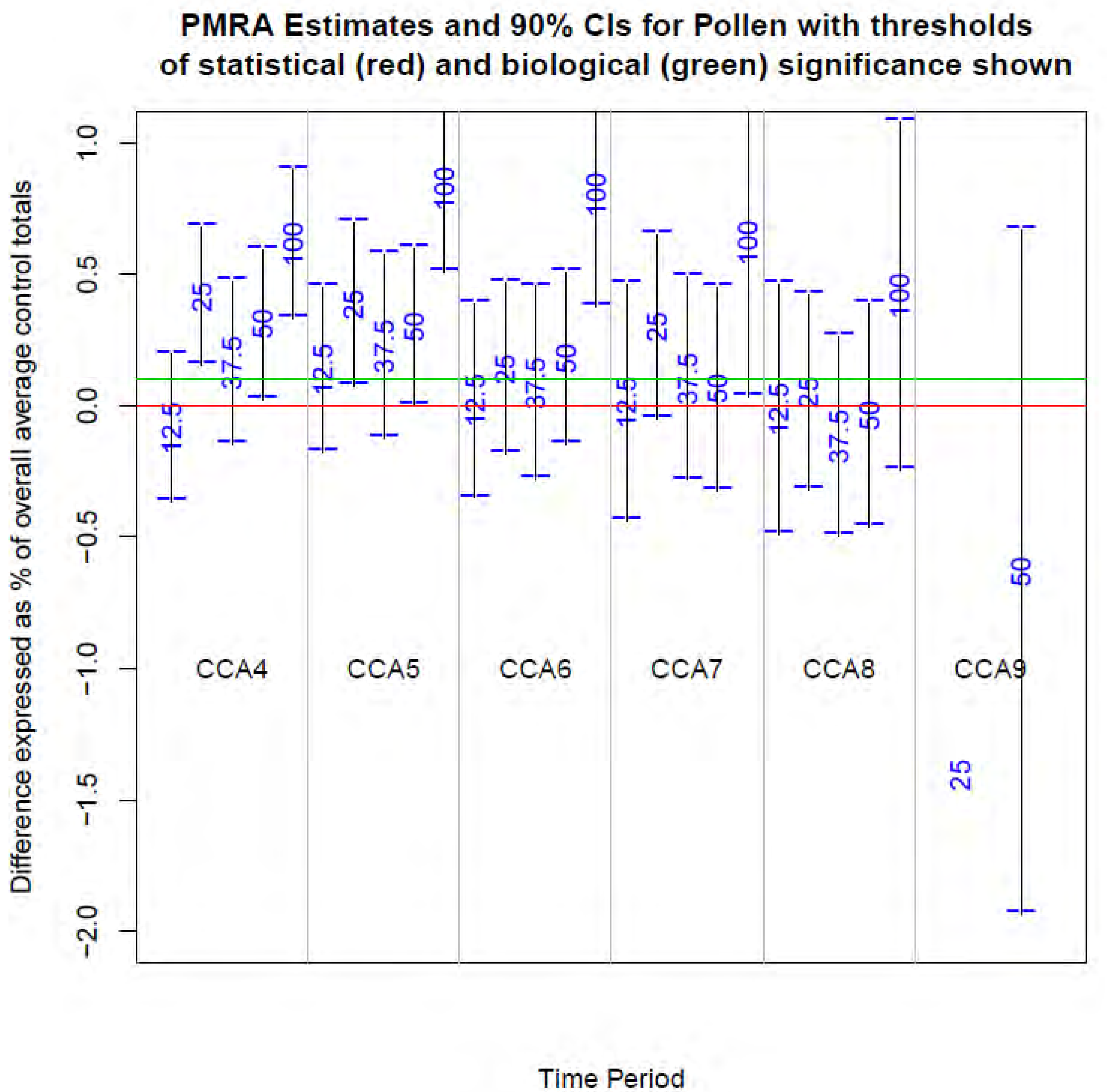


Figure B.14. Difference from control for the means for pollen for CCA 4 to 9.

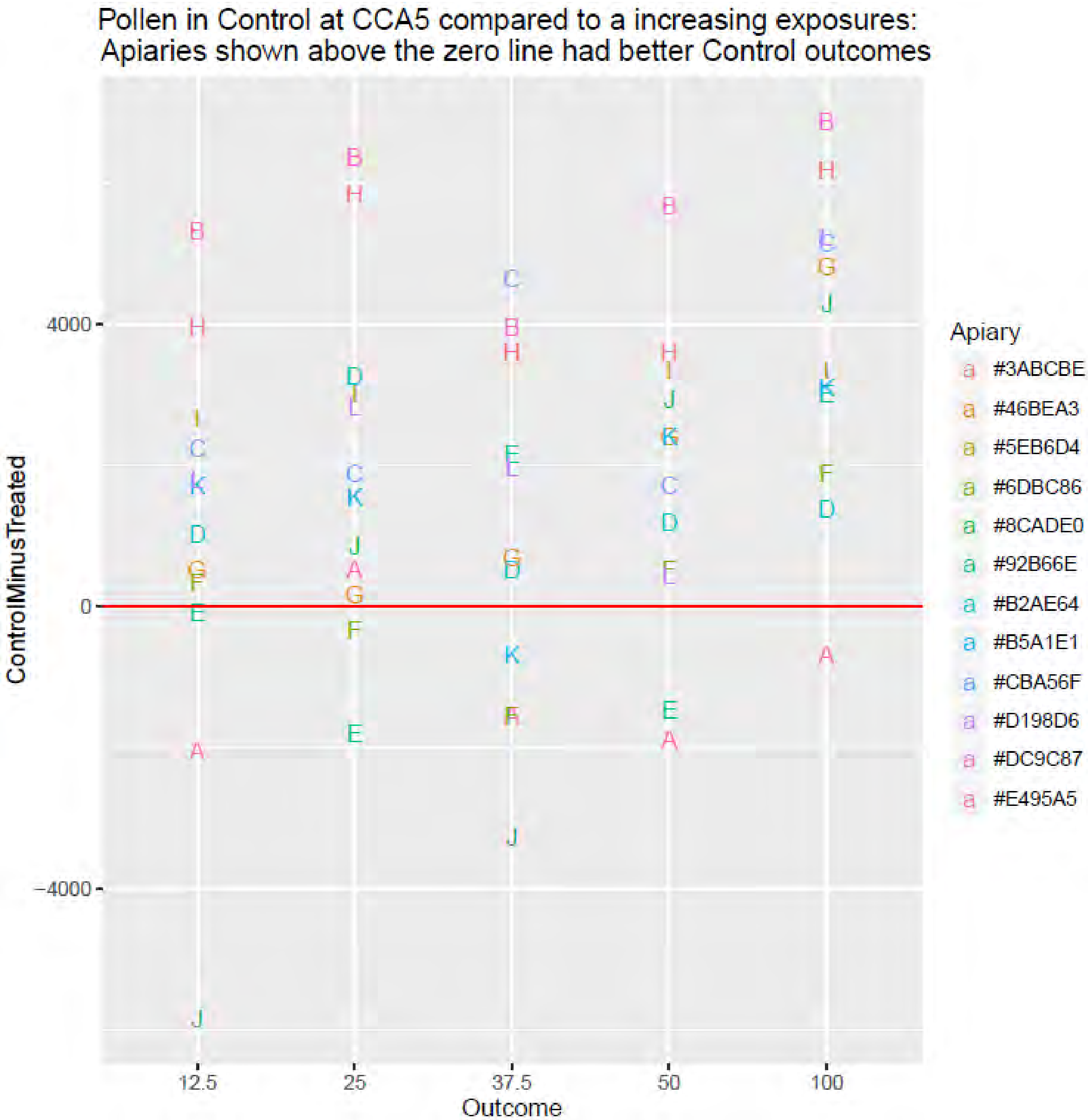


Figure B.15. Difference from control for all treatments and apiaries at CCA5 for pollen.

Honey

Figure B.16 and **Table B.8** shows the effects on honey stores across CCAs and treatment groups. Compared with the control, no differences in the honey stores (P>0.1) was observed for any CCA in the 12.5, 25, 37.5, and 50 µg a.i/L dose groups. Note, in CCA4 and CCA5 at the 100 µg a.i/L dose the confidence interval is below zero but as testing is one sided for harm it is not statistically significant. There was only one CCA (8) in the 100 µg a.i/L group which was marginally reduced from the control (40% reduction, p=0.087). It is noted that the feeding solutions (sugar solutions) provided during the exposure period might have affected natural honey storage patterns; however, effects on honey storage are still able to be considered as all treatments were compared to control hives (which also received feeding solutions). **Figure B.16** and **B.17** shows a general decrease in the reduction from the control as the dose increases. This may also be the effect of a lower number of individuals available to consume the honey.

Table B.8. Estimated percent reduction from control for honey stores.

Test concentration (µg/L)	Estimated reduction from control (%) (P value)					
	CCA4 (3 WAE) July 28-31	CCA5 (6-7 WAE) 20-28 Aug	CCA6 (10-11 WAE) 17-23 Sep	CCA7 (13 WAE) 6-10 Oct	CCA8 (16 WAE) 27-29 Oct	CCA9 (After over winter) 31 Mar
12.5	8.4 (0.115)	18.1 (0.108)	-6.5 (0.692)	-11 (0.744)	-10.9 (0.683)	NA
25	2.3 (0.401)	12 (0.216)	10 (0.26)	-7.3 (0.664)	-11.6 (0.725)	255 (NA)
37.5	3 (0.345)	5.5 (0.358)	-2.5 (0.563)	-3.7 (0.571)	-3.1 (0.556)	NA
50	-1.1 (0.558)	0.2 (0.495)	10 (0.258)	-20 (0.878)	-1.9 (0.534)	18.6 (0.242)
100	-31.5 (0.999)	-45.6 (0.999)	1.8 (0.452)	-12 (0.689)	40 (0.087*)	NA

Note: Negative value indicates increased number of individuals in comparison to control.
*0.05<P<0.1
**P<0.05
NA – not applicable because the sample sizes were too low.
NOTE: WAE = weeks after exposure. CCA 1 was 7 weeks before exposure, CCA 2 was 4 weeks before exposure, and CCA 3 was 1 week before exposure. Tables present post exposure CCA data only.

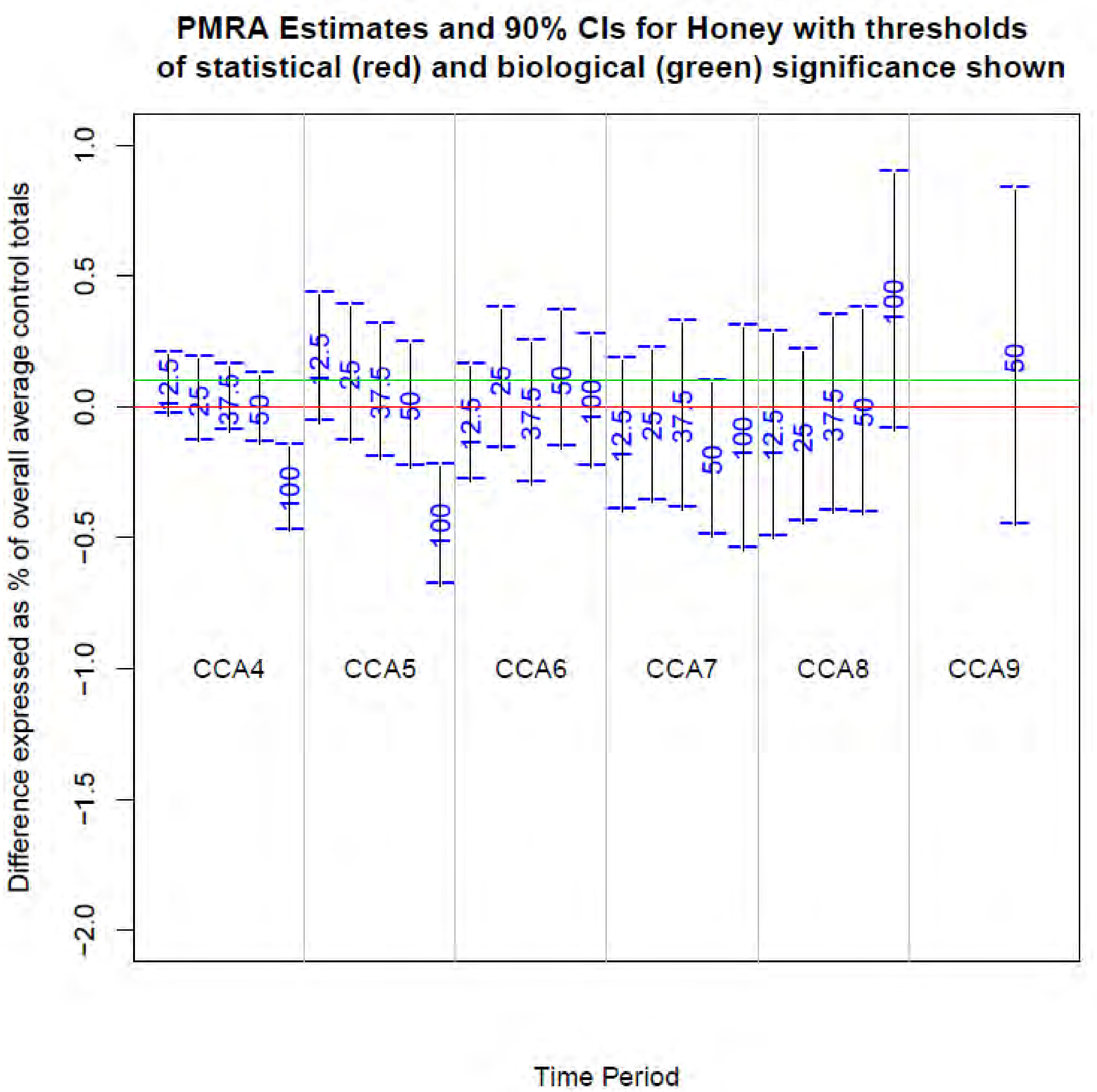


Figure B.16. Difference from control for the means for honey for CCA 4 to 9.

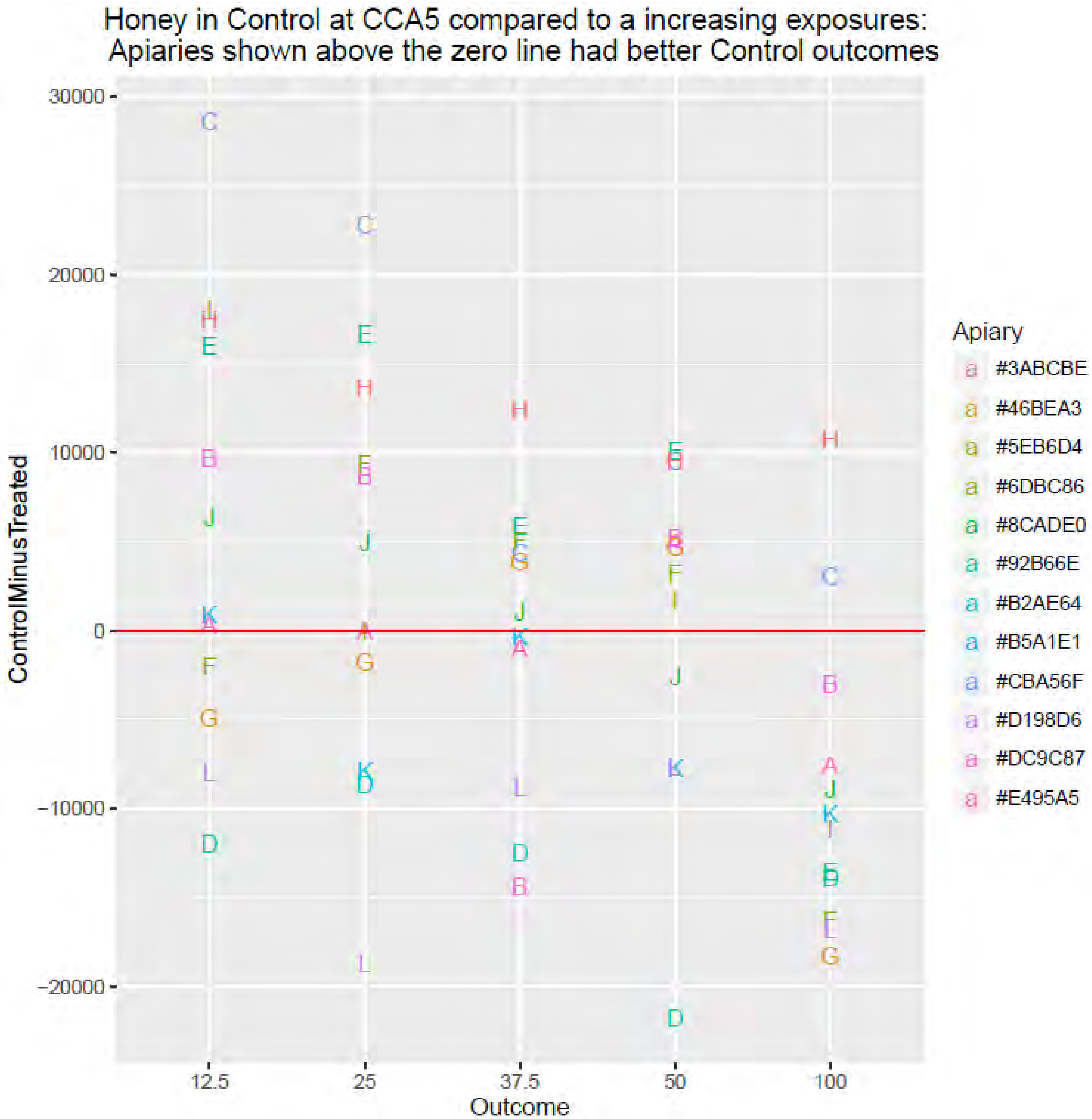


Figure B.17. Difference from control for all treatments and apiaries at CCA5 for honey.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table B.9. Summary of observed effects at each treatment level (Note: Values reported in the table are the % reduction compared to control, based on model estimated raw numbers corrected for baseline measurements).

Treatment (µg/l)	Observations (excluding overwintering data)	
	No significant reduction from control for the following endpoints	**Significant reduction from control for the following endpoints**
12.5	Total number of individuals (total life), Larvae (open), Pupae (capped), Adults, Pollen stores, Honey stores.	**Eggs** at CCA 6 only (32% reduction, p=0.04).
25	Total number of individuals (total life), Eggs, Honey stores, Adults. **NOTE:** In the 25 µg a.i/L dose, at CCA 3 (before exposure began), 10/12 hives were performing more poorly than control hives (for example, total number of individuals).	**Larvae** (open) at CCA 7 only (56% reduction, p=0.045), **Pupae** (capped) at CCA 7 only (47% reduction, p=0.068), **Pollen** stores at CCA 4 (41% reduction, p=0.006), CCA 5 (38% reduction, p=0.023) and CCA 7 (30% reduction, p=0.078).
37.5	Total number of individuals (total life), Eggs, Larvae (open), Pollen stores, Honey stores, Adults.	**Pupae** (capped) at CCA 5 only (19.8% reduction, p=0.025).
50	Eggs, Honey	**Total number of individuals** at CCA 5 only (27% reduction, p=0.005), **Larvae** at CCA 5 (29.9% reduction, p=0.018) and CCA 8 (52.9% reduction, p=0.092), **Pupae** at CCA 5 (40% reduction, p<<0.05) and CCA 7 (49% reduction, p=0.034), **Pollen** at CCA 4 (30.9% reduction, p=0.037) and CCA 5 (30% reduction, p=0.049), **Adults** at CCA 5 (18% reduction, p=0.034).
100		**Total number of individuals** at CCA 4 (25% reduction, p=0.005), CCA 5 (58.6% reduction, p<<0.05), CCA 6 (56% reduction, p<<0.05) and CCA 7 (52% reduction, p=0.018). **Eggs** at CCA 5 (47% reduction, p=0.001) and CCA 6 (62.8% reduction, p=0.002), **Larvae** at CCA 5 (52% reduction, p<<0.05), CCA 6 (50% reduction, p=0.006), **Pupae** at CCA 4 (44% reduction, p=0.006), CCA 5 (72.9% reduction, p<<0.05), CCA 6 (59% reduction, p=0.002), CCA 7 (56% reduction, p=0.046), **Pollen** at CCA 4 (61% reduction, p=0.001), CCA 5 (82.6% reduction, p<<0.05), CCA 6 (80% reduction, p=0.002) and CCA 7 (61.8% reduction, p=0.042),

		Honey stores at CCA 8 only (40% reduction, p=0.087) **Adults** at CCA 4 (15% reduction, p=0.028), CCA 5 (48% reduction, p<<0.05), CCA 6 (59% reduction, p<<0.05), CCA 7 (76% reduction, p=0.003) and CCA 8 (70.7% reduction, p=0.006).
OVERALL ENDPOINT	The thiamethoxam study began later in the season, and thus by CCA 6 the colonies were likely starting to prepare for overwinter, making a comparison between control and treatment groups difficult since all colonies were declining in strength. High overwinter mortality in the control, resulted in an inability to assess overwinter success. Data was highly variable which also led to amibiguity in some of the data interpretation. Overall, there appears to be effects at the 50 µg/L dose group. Another study is being conducted, and will be taken into consideration when completed and reviewed. **In the interim, the quantitative NOEC will be 37.5 µg/L. The quantitative LOEC will be 50 µg/L.**	

Consideration of combined doses response modelling

Figure B.18 shows a dose modeling of toxicity (the reduction of control life), with vertical lines showing the best estimate of the dose which causes 10% reduction from control for total life at CCA 5 for thiamethoxam (T), imidacloprid (I) and clothianidin (C), and all data combined (A). Here, the analysis is being specifically focussed on a 10% reduction from control as well as contrasting and utilising data from all three neonicotinoids (which are believed to have the same biological mode of action). This may assist in the biological interpretation of the data for thiamethoxam which had large standard errors for total life at CCA5 (and others) that resulted in difficulties of interpretation. It should be noted that the 25 µg a.i/L dose group was excluded in this analysis due the large statistically significant imbalance at baseline (pre-treatment condition of the hives). In the analysis that accompanies this graph, in addition to the BMD (benchmark dose) (which is the dose expected to result in 10% reduction of total life from control) the lower confidence for the BMD (the BMDL (benchmark dose lower confidence limit) was also calculated, which is the dose that with 95% confidence (one sided) can be expected to result in less than 10% effect (from the control). This provides a "safe" estimate of the dose that would result in less than a 10% reduction.

For thiamethoxam alone (considering only the data in that submission), the BMD was 29.19 µg a.i/L and the BMDL was 14.36 µg a.i/L. Considering all data together under the assumption that all three neonicotinoids have the exactly same biological mode of action, the BMD was 15.91 µg a.i/L and the BMDL was 9.69 µg a.i/L. To allow biological mode of action to differ in some aspects according to the data, testing methods suggested in Hydrogen Sulfide: Integrative Analysis of Acute Toxicity Data for Estimating Human Health Risk. J Stanek, J Gift, G Woodall, and G Foureman, US EPA/ORD/NCEA, Research Triangle Park, NC, USA were followed. This compromise method resulted in a BMD of 31.69 µg a.i/L and BMDL 18.07 µg a.i/L (higher in part due to increased sample size). As a comparison, the compromise BMDs were 20.02 µg a.i/L for imidacloprid, and 13.14 µg a.i/L for clothianidin with BMDLs 12.53 µg a.i/L and 7.2 µg a.i/L, respectively. On the other hand, if all three neonicotinoids truly have exactly the same biological mode of action, the BMD and BMDL arguable should be taken as 15.91 and 9.69. [The statement "BMD and BMDL arguable should be taken as 15.91 µg a.i/L and 9.69 µg a.i/L" represents some real uncertainty given the limitation of the studies even if the data suggested otherwise – it is not definitive.] Therefore, the observed deviation of thiamethoxam from the other two neonicotinoids introduces some additional uncertainty in the assessment, and provides further support for considering effects at the 50 µg a.i/L for the LOEC.

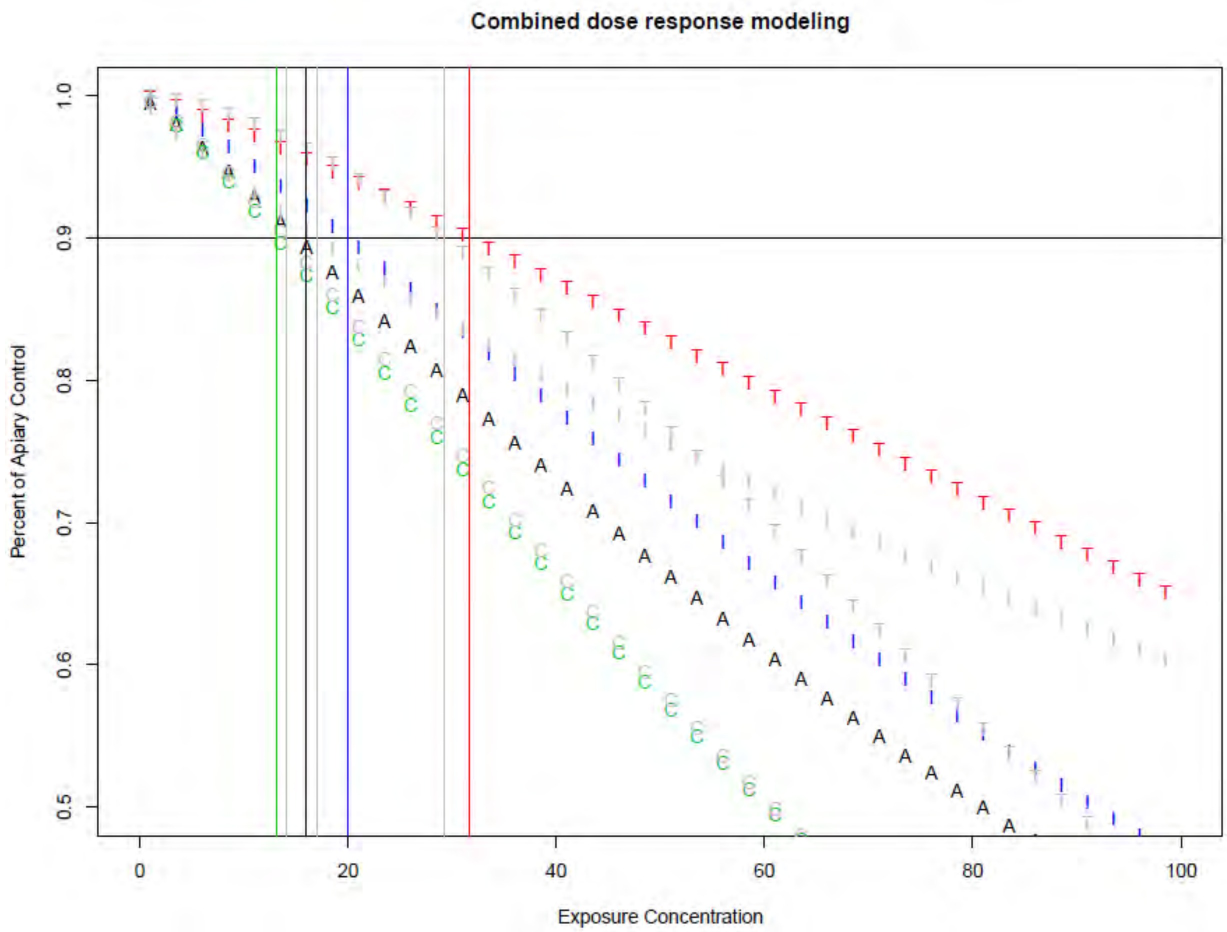
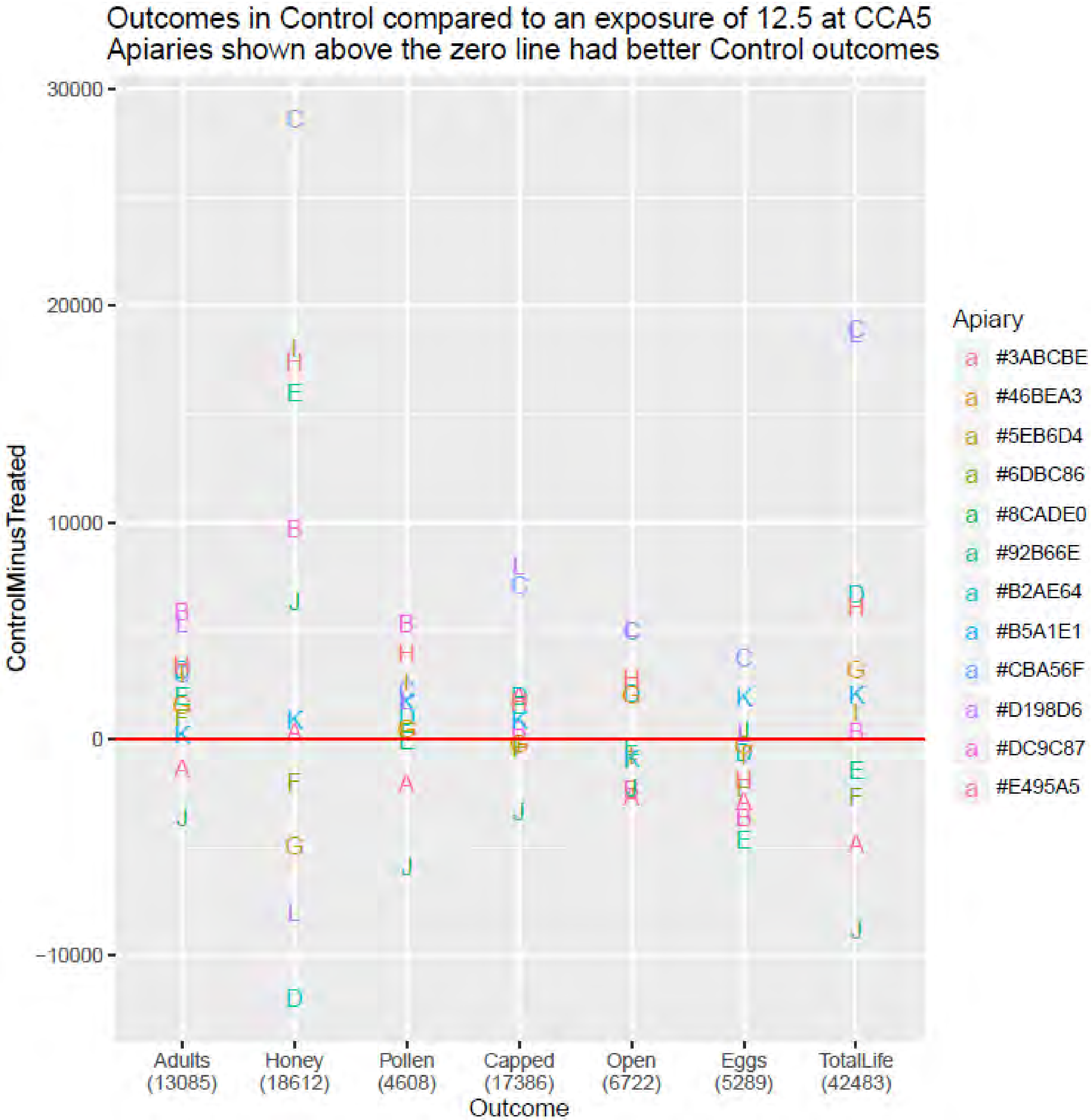


Figure B.18. Best estimate of the dose which causes 10% reduction from control for total life at CCA 5 (for T=thiamethoxam, I=imidacloprid, C=clothianidin and A = "all" or combined). The grey lines represent the individual analysis and the coloured lines represent the BMD of the combined analysis.

Graphical representation of all parameters at CCA 5

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document



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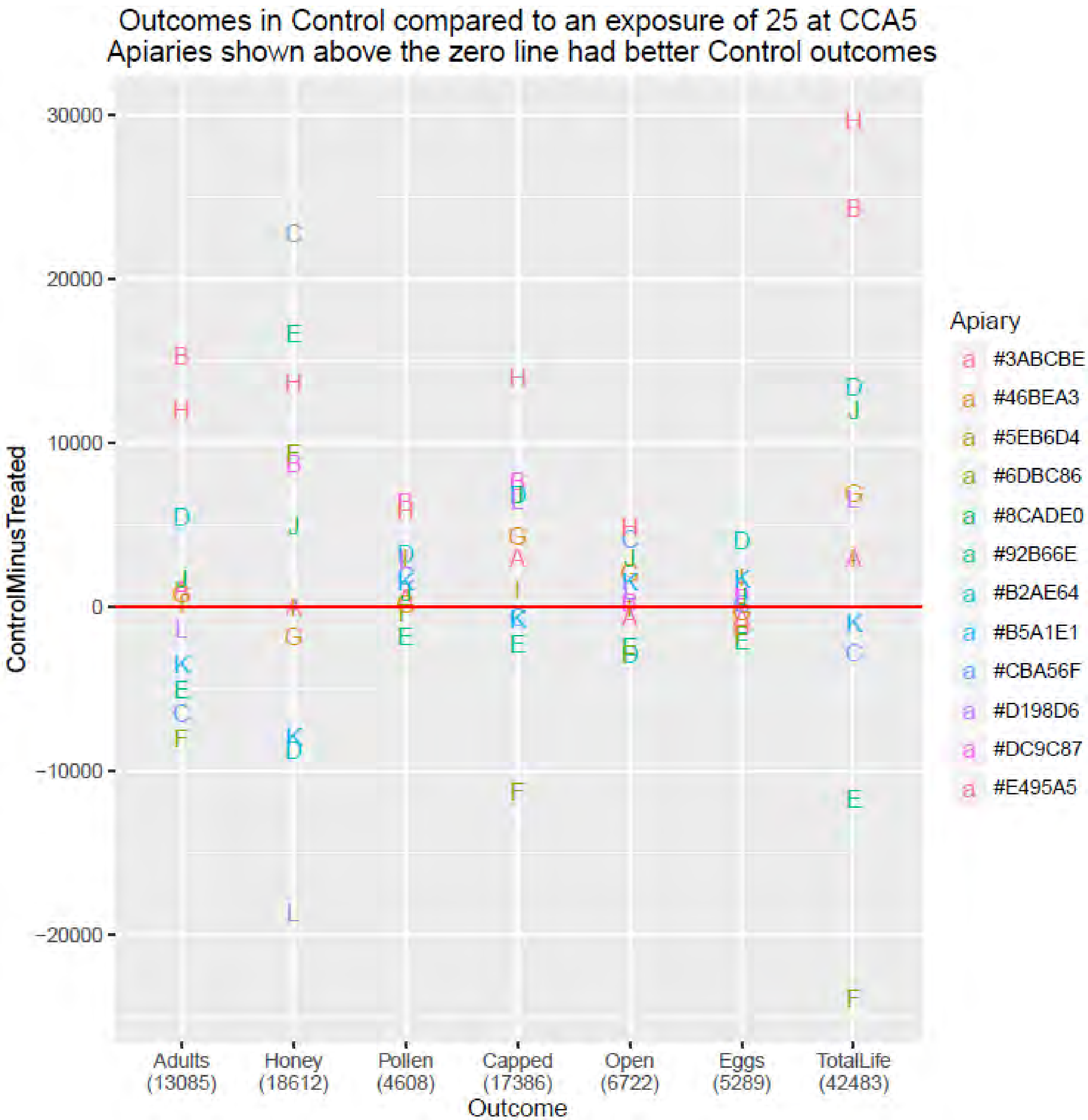
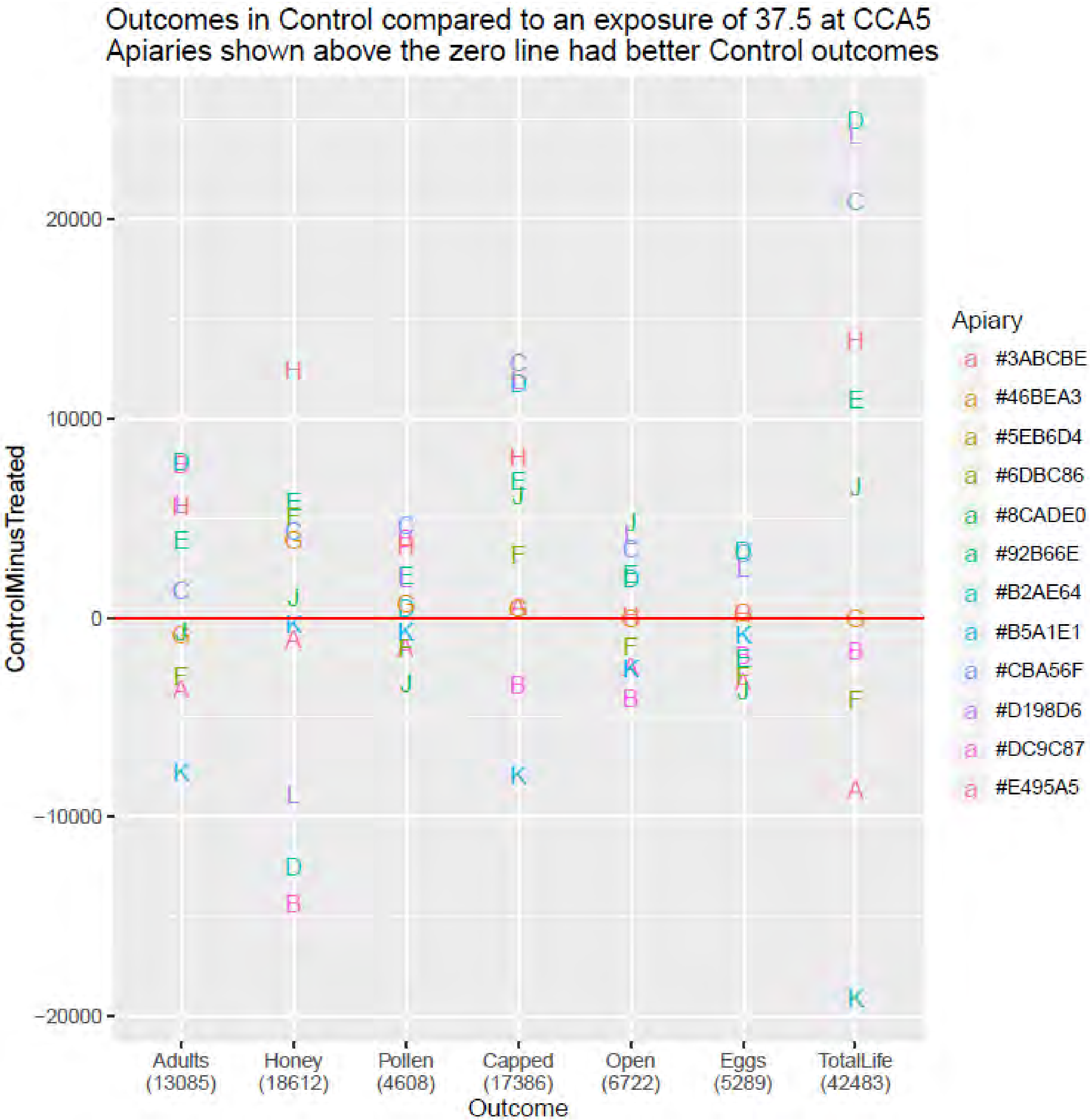
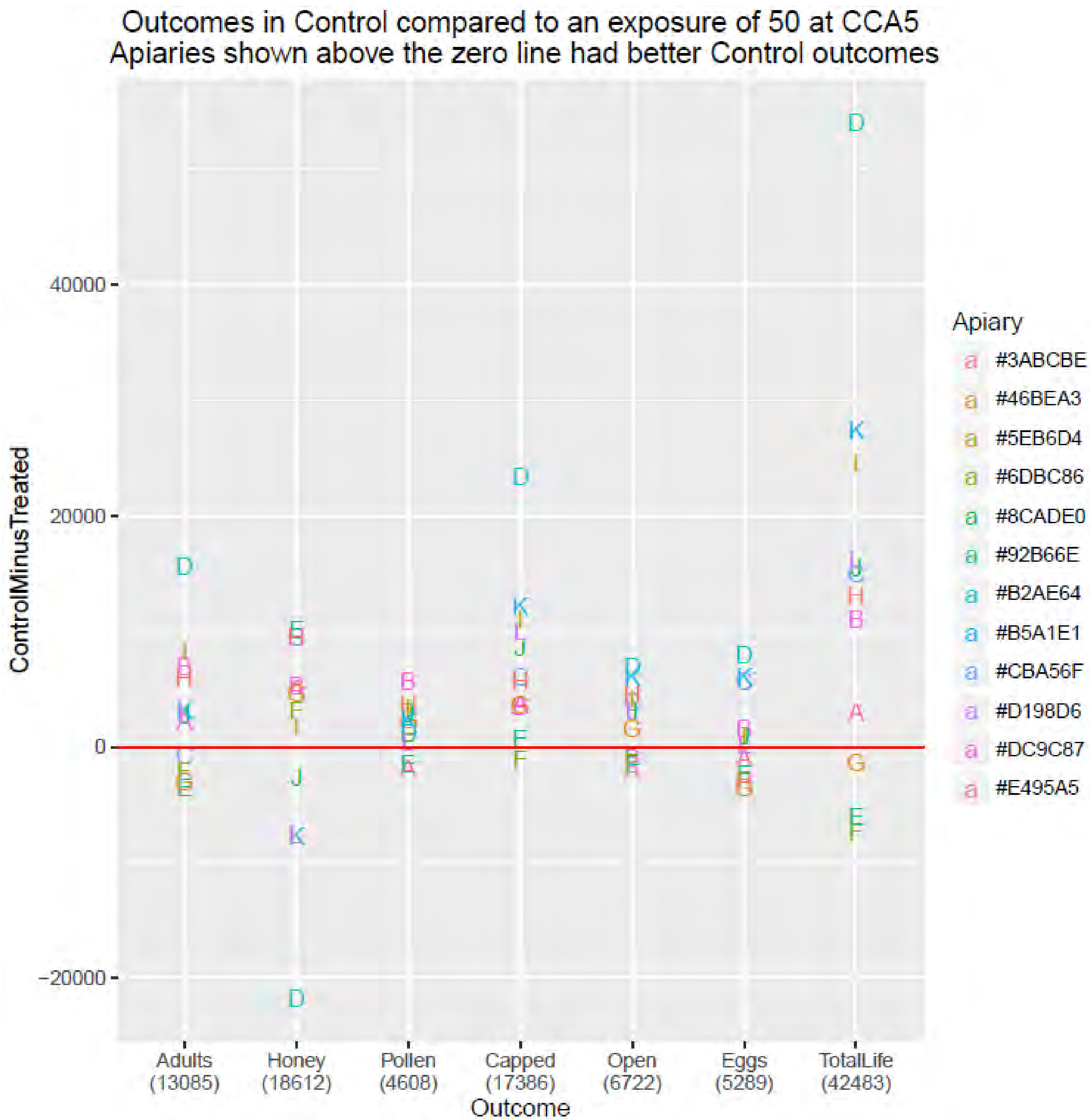
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

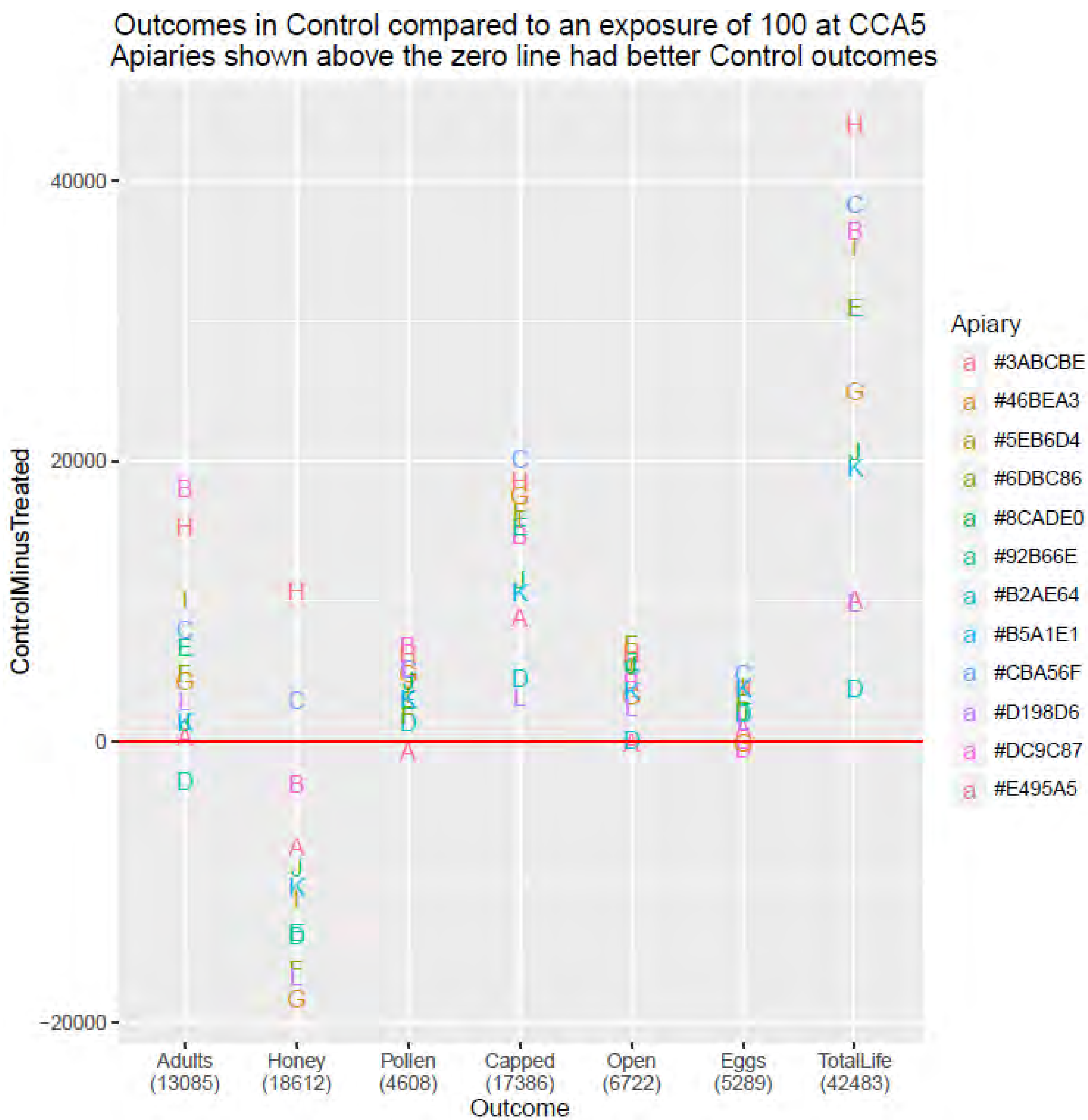



Outcomes in Control compared to an exposure of 37.5 at CCA5
Apiaries shown above the zero line had better Control outcomes
ControlMinusTreated
20000
10000
0
−10000
−20000
Adults (13085)
Honey (18612)
Pollen (4608)
Capped (17386)
Open (6722)
Eggs (5289)
TotalLife (42483)
Outcome
Apiary
#3ABCBE
#46BEA3
#5EB6D4
#6DBC86
#8CADE0
#92B66E
#B2AE64
#B5A1E1
#CBA56F
#D198D6
#DC9C87
#E495A5

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document



Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document



Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

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EVALUATION REPORT - Thiamethoxam Nectar Colony Feeding Study: Repeat Study Conducted in 2016-2017

John Troiano, Research Scientist III

June, 2018

A review of: - Bocksch, S. (2017). Thiamethoxam Technical – Honey Bee Brood and Colony Level Effects Following Thiamethoxam Intake via Treated Sucrose Solution in a Field Study in North Carolina – USA 2016: Final Report. Unpublished study prepared by Eurofins Agroscience Service EcoChem Gmbh, & Eurofins Agroscience Services Ecotox GmbH. 481p., Laboratory Report Number S16-02808. MRID 50432101. CDPR Study ID 304522.

Introduction

A colony feeding study was conducted to determine the effects of graded levels of thiamethoxam on the health of honey bee hives where doses mimicked exposure from foraging on nectar. Thiamethoxam was dosed directly to hives, supplied in a sugar solution that mimicked a nectar source for food supply. Hive health was determined by Colony Condition Assessments (CCAs) where measurements were made over time on the number of individuals in each bee life stage in the hive, the storage of honey and pollen food supplies in the hives, and the weight of hives. This study, conducted in 2016-2017, was a repeat of a study conducted in 2014-2015 (Bocksch, S., 2015). The second study was conducted in the same area as the first study where locations of apiary sites were distributed throughout a forested area of North Carolina. Not all sites were in the exact location as in the previous study. The distance between each apiary site was approximately 3 miles apart. The majority of land near the apiaries was non-intensively managed pasture and forest with low potential exposure of bees to pesticides applied for agricultural purposes.

Measurements made over time were indicated by sequential numbering of the colony condition assessments (CCAs), which were conducted at approximately monthly intervals. Timing of assessments were made at similar time intervals for the two studies. The exposure period for both studies was initiated in early July with the treatment period lasting 6 weeks. The CCAs included in this analysis are:

- Just prior to initiation of treatments, denoted CCA2 in this analysis
- 3 weeks into the exposure period, denoted CCA3 in this analysis
- 6 weeks after initiation of exposure, denoted CCA4 in this analysis
- 10 weeks after initiation of exposure, denoted CCA5 in this analysis
- 13 weeks after initiation of exposure, denoted CCA6 in this analysis
- 16 weeks after initiation of exposure, denoted CCA7 in this analysis

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

These CCAs were chosen because this was the time period used to determine No Observed Effects Concentrations (NOECs) and Lowest Observed Effects Concentrations (LOECs) in the previous neonicotinoid nectar colony feeding studies.

The similarity of the study design between the two studies facilitated an analysis of the data combined between years. Addition of variance between years in the statistical analysis provides confidence that detection of significant effects are biologically significant and that they are not limited to the year in which the study was conducted. Data from the previous study was analyzed jointly by DPR, U.S. EPA, and Canada's PMRA staff scientists (U.S. EPA, PMRA, & DPR, 2017).

Statistical Analysis

Evaluation of the data followed the statistical approach used by DPR and EPA scientists to analyze previously reviewed neonicotinoid colony feeding studies. Since measurements for each variable were made in each hive over time, the statistical analysis was conducted as a repeated measures over time (McIntosh, 1982). Additionally, a mixed model was used where apiary location was identified as a random variable and thiamethoxam levels of dose as a fixed effect. The mixed model was chosen because the results of the analysis were to be applied to the larger population of bee hives. The analysis was conducted on the data combined from both years. In the first year, data collected for CCAs number 3-8 corresponded to CCAs in the second year numbered 2-7. As indicated previously, data collected from the timing of these assessments conducted from July through September were the basis for development of NOEC and LOEC values on previous neonicotinoid colony feeding studies. Normality tests were conducted for each CCA within each year as indicated by Shapiro-Wilk and Kolmogorov-Smirnov test statistics produced by the PROC CAPABILITY procedure in Statistical Analysis System (SAS, version 9.4). For comparison, data were also transformed to natural logarithms to determine if transformation provided better results. The majority of results indicated that the distributions of the raw data were normal with many of the logarithm transformed data indicating many instances of non-normality. Based on these results the raw data were used in the analyses. The mixed model approach used to analyze the data included tests to determine the appropriate covariance model that describes the covariance structure reflected by the data. Inclusion of a covariance model in the analysis accounts for heterogeneity of variances that often are measured between treatment levels.

The PROC MIXED procedure in the Statistical Analysis System (SAS, version 9.4) was used to run the repeated measures effects mixed model. Measurements of colony health and hive weight were conducted approximately 1 month apart so CCAs were treated as equally spaced intervals. The effects side of the model statement included testing differences in the response between years, between CCAs indicating changes in response over the monthly measurements, between the levels of thiamethoxam dose, and the potential interaction for effects of dose over time with CCA and year factors. SAS Program 1 below reflects the structure of the program used to analyze the combined data from both years. Statistical options were included in the 'Slice' statement to protect against falsely discovering significant multiple comparisons for paired mean values between the value at the control and each level of dose. The 'Simulated' option is a Monte Carlo approach that computes adjusted p-values from simulated distributions based on distributional statistics generated during the analysis (Edwards, D., and Berry, 1987). In addition, the 'Stepdown' option was invoked because it tends to increase the power of the multi-comparison tests (SAS, version 9.4). SAS Program 2 indicates the structure of the program used to conduct the analyses for each year.

There were two statements in the mixed model used to analyze the data, where a covariance model could be specified. One was in the 'Random' statement with apiary indicated as a random variable. The second was in the 'Repeated' measures statement where each hive was indicated as the subject for the repeated measure. For the random statement only the Variance Component (VC) model successfully paired with the covariance model specified in the repeated statement: Specifying more complex covariance models in the random statement resulted in indications of converge problems for that model. As observed in the previous colony feeding studies the correlation structure indicated greater correlation between samples taken at close time intervals and, conversely, decreased correlation the further apart the samples were taken in time. Since this structure is normally represented by autoregressive covariance models, the covariance structure for the repeated statement was tested using variance component (VC), compound symmetry (CS), compound symmetry with heterogeneity (CSH), autoregressive first order (AR(1)), autoregressive first order with heterogeneity (ARH(1)), and unstructured (UN) models. Covariance model selection was based on the statistic generated for the Bayesian Information Criteria (BIC) where a lower value of the criterion indicated a better fit of the covariance model. A statistical basis for choosing the appropriate model was determined from Chi-square tests conducted on the difference of the value of the BIC criteria between the two models tested with the number of degrees of freedom determined as the difference between the number of parameters in the model and where the significance level of probability was at 0.01 (Hammer, 2000; Littell et al., 2006). With the VC covariance model specified in the random statement, the best fit covariance model in the repeated statement for the combined years analysis was AR(1) for adult bees and ARH(1) for pupae, larvae, eggs, nectar, and pollen cells (Table 1). Values for numbers of cells measured for each bee life stage and food supply were divided by 1000 prior to statistical analysis to minimize potential convergence problems due to magnitude of values.

SAS Program 1

```
proc mixed data=a3 order=data;
class apiary dose cca hive rep year;
model transvalue =year cca dose dose*cca year*cca year*dose year*dose*cca/ddfm=sat
htype=1;
random apiary(year)/type=vc;
repeated cca/ subject=hive*rep(dose) type=arh(1);
slice dose*cca /sliceby=cca diff=controll stepdown(report) adjust=simulate adjdfe=row;
run;
```

Results

Data Combined for Years: Means and standard deviation for each response variable measured at each dose and each CCA are the same as presented in this and the previous report of study results so they are not reprinted in this analysis (Bocksch, S., 2015). Results from the combined years repeated measures model indicated numerous effects due to dose of thiamethoxam and its interactions with primarily CCA assessment for all bee life stages and food storage variables (Table 2). There was no interactive effect between the three factors for analysis of number of adult bees, or pupal, larval, and nectar cells indicating similar responses between for the effects of treatments where significance was indicated. The interactive effect of dose with CCA reflects the varying magnitude in the level of differences for significant effects over the sampling interval. For example, there were no differences between the levels of dose for the first CCA2 samples as these were taken prior to the study and indicate no bias in treatments at the start of the study (Figures 1 to 6). In later CCAs, the number of adult bees at the 100 ng/g treatment indicates a downward trend where the magnitude in difference compared to the controls becomes progressively greater at CCA4 and 5, but then lessens as hive activity normally decreases toward the end of the season at the last CCA (Figure 1). Results from the pairwise comparisons between values for control and each dose level indicate a specific pattern (Table 3). Except for nectar cells, effects were first indicated on the number of pupal, larval, and pollen cells at CCA3 at the highest dose of 100 ng/g when measurements were made midway through the exposure period (Figures 2, 3, and 6). These effects were sustained until CCA7 where, as previously indicated, all measurement of hive health decreased due to the normal yearly pattern of growth. At the next CCA (CCA4) the number of adult bees and egg cells were then affected at the 100 ng/g dose and these effects were also sustained throughout the entire season. Significant ($P<0.05$) effects were also measured at the next lowest dose at 50 ng/g for number of adult bees, pupal cells, and pollen cells that also appear to be sustained for a number of consecutive sampling intervals (Figures 1, 2, and 6). A trend was indicted ($P<0.1$) at CCA4 for number of larval cells. Sporadic indications of effects were noted at the next lowest dose at 37.5 ng/g for number of pupal and pollen cells but they were not sustained for consecutive sampling intervals, most likely indicating spurious effects. Effects on egg and nectar cells were minimal (Figures 4 and 5). This pattern of effects indicates that the 50 ng/g dose is the LOEC value and the 37.5 g/g dose is the NOEC value. Actual values measured for these concentrations in the dosing solutions as reported in Table 58 of the report were 50 ng/g for the LOEC value and 34 ng/g for the NOEC value.

For completeness, graphs for each year are presented in Figures 7-12. Overlap for treatments below 50 ng/g is obvious, clouding the consistency for effects between years at the 50 ng/g level of dose. Figures 13 through 18 compare the effects on each variable for only the 0, 50 and 100 ng/g. Graphs for number of adult bees and pupal, larval and pollen cells clearly show the consistent effect between year for decreased numbers at the 50 ng/g treatment.

Conclusion

In both replicate studies, the authors of the reports concluded that the 100 ng/g treatment was a nominal LOEC value and that the 50 ng/g treatment was the NOEC value due to inconsistent effects at that concentration. Statistical analyses conducted independently by the three agencies on data generated from the first study disagreed with that conclusion (U.S. EPA, PMRA, & DPR, 2017). This analysis of the combined data between years strongly supports the previous conclusion that the 50 ng/g level of dose was the LOEC value and the 37.5 ng/g treatment was the appropriate NOEC value: Effects at the 50 ng/g level were evident and sustained across CCAs for number of adult bees and pupal, larval, and pollen cells (Tables 2 and 3). The measured value of thiamethoxam in the sucrose patties in the nominal 37.5 ng/g treatment group was 34 ng/g, which is the actual NOEC value based on mean measured concentrations of thiamethoxam. This study has been determined to be scientifically sound and can be used quantitatively to assess risks to honey bee colonies.

References

Bocksch, S. (2015). Thiamethoxam Technical - Honey Bee Brood and Colony Level Effects Following Thiamethoxam Intake via Treated Sucrose Solution in a Field Study in North Carolina: Final Report. Unpublished study prepared by Eurofins Agroscience Services EcoChem Gmbh. 468p., Laboratory Report Number S14-02633. MRID 49757201. CDPR Study ID 288917.

Edwards, D., and Berry, J. J. (1987). "The Efficiency of Simulation-Based Multiple Comparisons." Biometrics 43:913–928

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Mcintosh, M. (1983). Analysis of combined experiments. Agronomy Journal (75) 153-155.

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U.S. EPA, PMRA, & DPR. (2017). Data evaluation report: Bocksch, S. (2015) -Thiamethoxam Technical - Honey Bee Brood and Colony Level Effects Following Thiamethoxam Intake via Treated Sucrose Solution in a Field Study in North Carolina. Washington, D.C.: U.S. EPA.

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 1. Mixed Model Analysis of Variance: BIC goodness-of-fit values generated for each covariance model structure tested in the repeated measures analysis of variance program. Shaded cells indicate the covariance structure used for the analysis. DNC indicates that the model failed to converge to solution.

CV Model Tested	Number of Parameters	Combined Years Model BIC Value for:					
		Adults	Pupae	Larvae	Eggs	Nectar	Pollen
VC	2	DNC	DNC	4280.6	DNC	7262.7	4829.9
CS	3	5296.5	5228.7	4158.3	3836.4	6930.8	4670.9
AR(1)	3	5080.3	5213.7	4155.2	3826.1	DNC	4627.8
CSH	8	5235.7	5049.7	4037.1	3722.9	6899.3	4609.3
ARH(1)	8	DNC	5026.2	4030.3	3713.6	6784.9	4536.1

Table 2. Combined Years: Results of the repeated measures mixed model testing the response of each variable to clothianidin dosed surrogate honey.

Mixed Model Results for Repeated Measures Analysis of Variance: Combined Data from Both Years					
Variable	Effect	DF	Den DF	F Value	Pr > F
Adult Bees	Year	1	22.1	3.49	0.0752
	cca	5	628	64.99	<.0001
	dose	5	164	2.91	0.0152
	dose*cca	25	632	1.79	0.0110
	cca*Year	5	628	26.94	<.0001
	dose*Year	5	165	0.48	0.7925
	dose*cca*Year	25	631	0.76	0.7973
Pupal Cells	Year	1	214	31.79	<.0001
	cca	5	419	465.24	<.0001
	dose	5	214	9.52	<.0001
	dose*cca	25	438	3.43	<.0001
	cca*Year	5	417	44.4	<.0001
	dose*Year	5	215	0.72	0.6106
	dose*cca*Year	25	449	1.02	0.4420
Larval Cells	Year	1	59.4	65.34	<.0001
	cca	5	403	261.04	<.0001
	dose	5	196	5.83	<.0001
	dose*cca	25	404	2.1	0.0017
	cca*Year	5	402	15.47	<.0001
	dose*Year	5	197	0.16	0.9778
	dose*cca*Year	25	402	1.17	0.2596
Egg Cells	Year	1	38.9	1.38	0.2464
	cca	5	368	220.65	<.0001
	dose	5	220	2.15	0.0611
	dose*cca	25	378	1.2	0.2365
	cca*Year	5	369	5.66	<.0001
	dose*Year	5	221	0.32	0.9001
	dose*cca*Year	25	388	1.67	0.0236
Nectar Cells	Year	1	24.7	667.71	<.0001
	cca	5	396	68.29	<.0001
	dose	5	141	0.85	0.5176
	dose*cca	25	398	2.41	0.0002
	cca*Year	5	395	63.06	<.0001
	dose*Year	5	142	0.56	0.7274
	dose*cca*Year	25	396	1.26	0.1817
Pollen Cells	Year	1	31.8	106.36	<.0001
	cca	5	365	65.62	<.0001
	dose	5	156	9.13	<.0001
	dose*cca	25	370	3.78	<.0001
	cca*Year	5	366	54.13	<.0001
	dose*Year	5	156	3.95	0.0021
	dose*cca*Year	25	371	1.85	0.0084

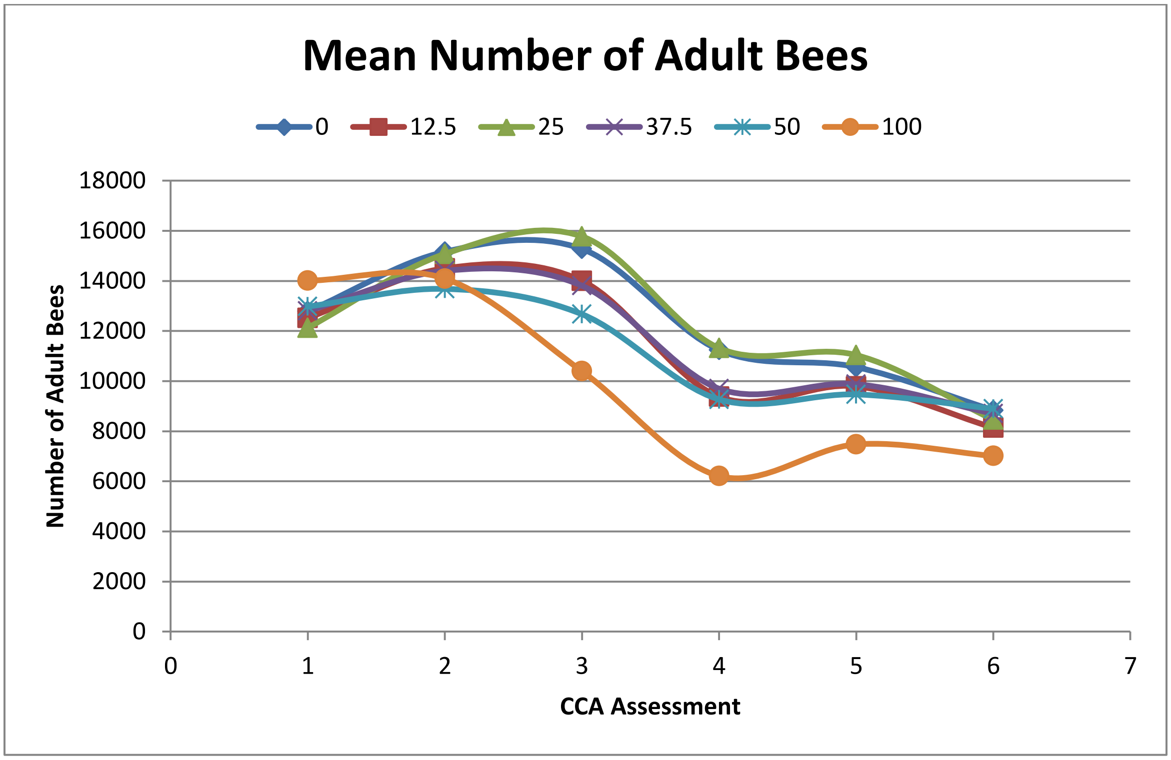
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 3. Combined Years: Probability value for the contrast of the control to each clothianidin dose at each CCA and for each variable measured. Dark shaded cells indicate significance at P<0.01 and lighter shaded cells at 0.1>P>0.05.

Response and (ng/g)	Dose	Probability Value for Contrast of the Control to Each Dinotefuran Dose at Each CCA					
		CCA2	CCA3	CCA4	CCA5	CCA6	CCA7
Bees	12.5	0.774	0.493	0.212	0.155	0.548	0.691
	25	0.671	0.493	0.677	0.502	0.649	0.691
	37.5	0.801	0.493	0.170	0.176	0.548	0.691
	50	0.801	0.297	0.031	0.095	0.270	0.691
	100	0.870	0.422	<.0001	<.0001	0.001	0.040
Pupae	12.5	0.274	0.467	0.090	0.477	0.477	0.888
	25	0.187	0.467	0.090	0.477	0.111	0.642
	37.5	0.274	0.467	0.016	0.454	0.302	0.690
	50	0.274	0.053	0.000	0.092	0.184	0.642
	100	0.309	<.0001	<.0001	0.001	0.001	0.532
Larvae	12.5	0.430	0.626	0.166	0.296	0.711	0.819
	25	0.190	0.592	0.166	0.296	0.225	0.240
	37.5	0.430	0.626	0.166	0.435	0.564	0.240
	50	0.430	0.488	0.078	0.117	0.564	0.240
	100	0.663	0.005	<.0001	<.0001	0.049	0.240
Eggs	12.5	0.742	0.352	0.795	0.281	0.654	0.435
	25	0.172	0.762	0.329	0.639	0.654	0.494
	37.5	0.430	0.803	0.795	0.639	0.654	0.494
	50	0.778	0.803	0.337	0.435	0.465	0.494
	100	0.778	0.339	0.014	0.002	0.034	0.411
Nectar	12.5	0.976	0.976	0.464	0.203	0.187	0.155
	25	0.982	0.982	0.717	0.520	0.482	0.439
	37.5	0.982	0.982	0.744	0.520	0.482	0.439
	50	0.958	0.958	0.615	0.137	0.080	0.142
	100	0.961	0.961	0.958	0.203	0.122	0.010
	Pollen 12.5	0.942	0.913	0.792	0.787	0.364	0.296
	25	0.942	0.827	0.748	0.550	0.071	0.296
	37.5	0.942	0.312	0.046	0.365	0.071	0.296
	50	0.931	0.030	0.002	0.026	0.017	0.170
	100	0.942	<.0001	<.0001	0.000	<.0001	0.002

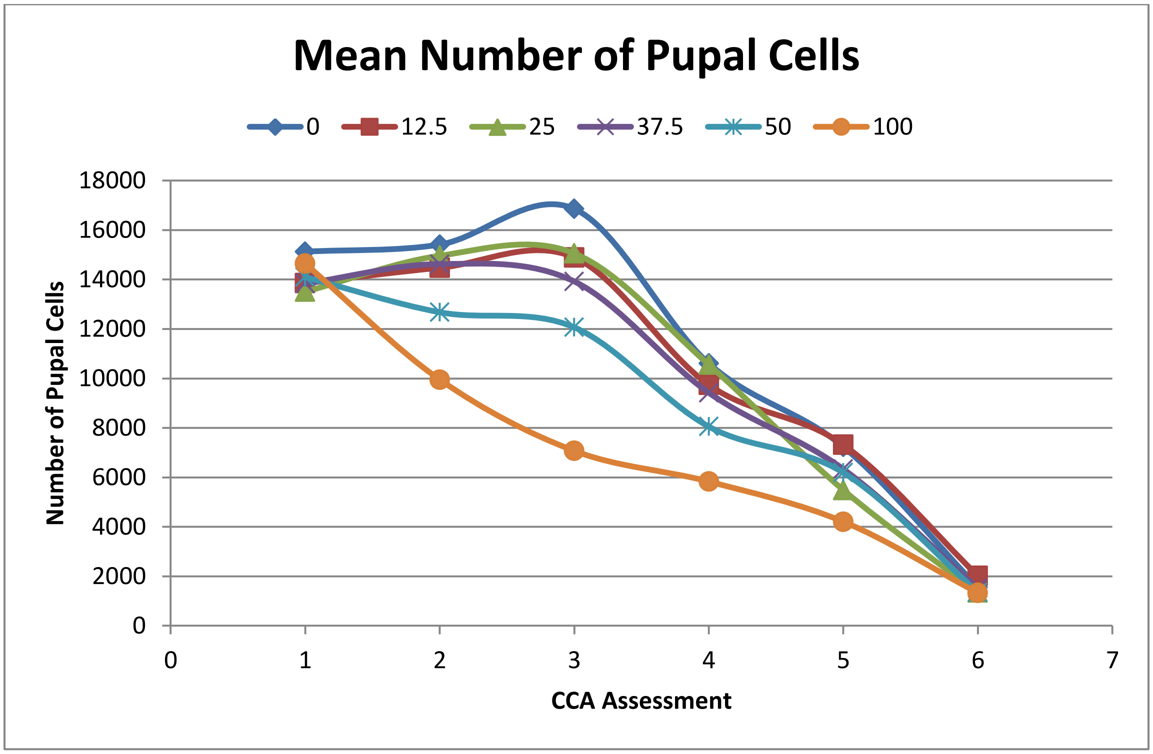
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 1. Combined Years: Mean number of adult bee in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.



Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 2. Combined Years: Mean number of pupal cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.



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Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 3. Combined Years: Mean number of larval cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

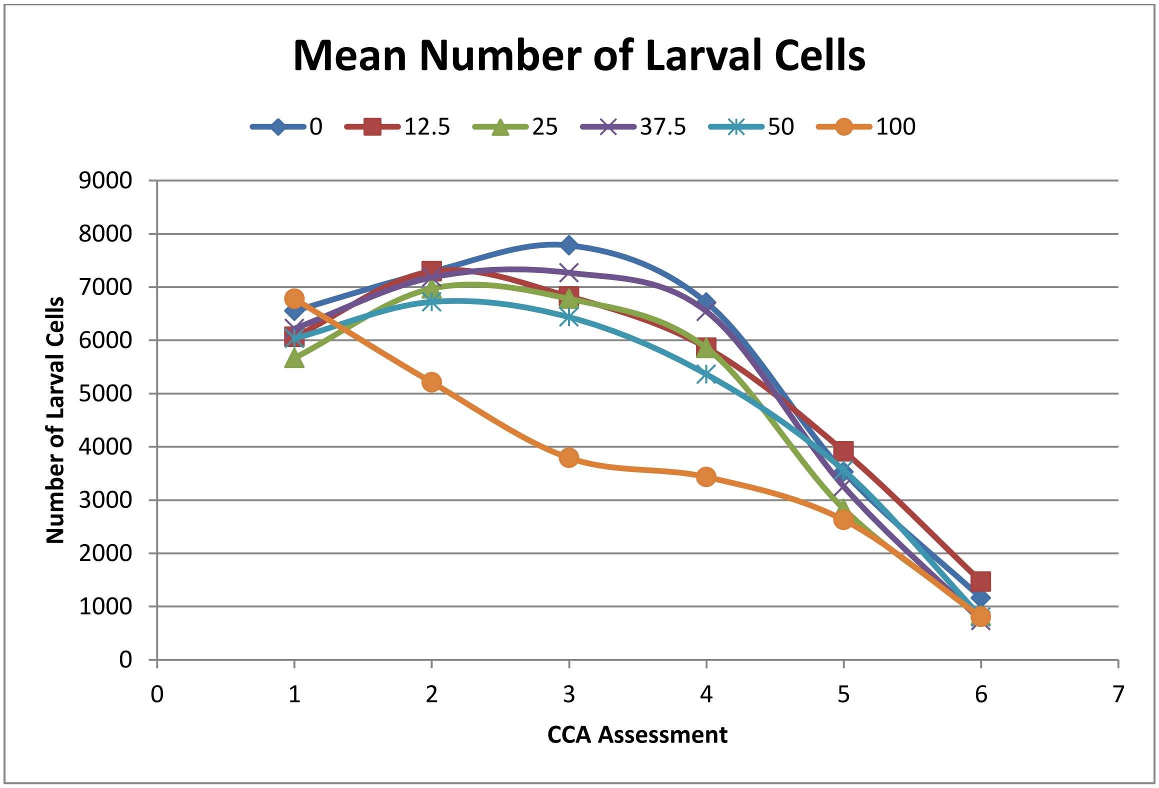

Figure 4. Combined Years: Mean number of larval cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

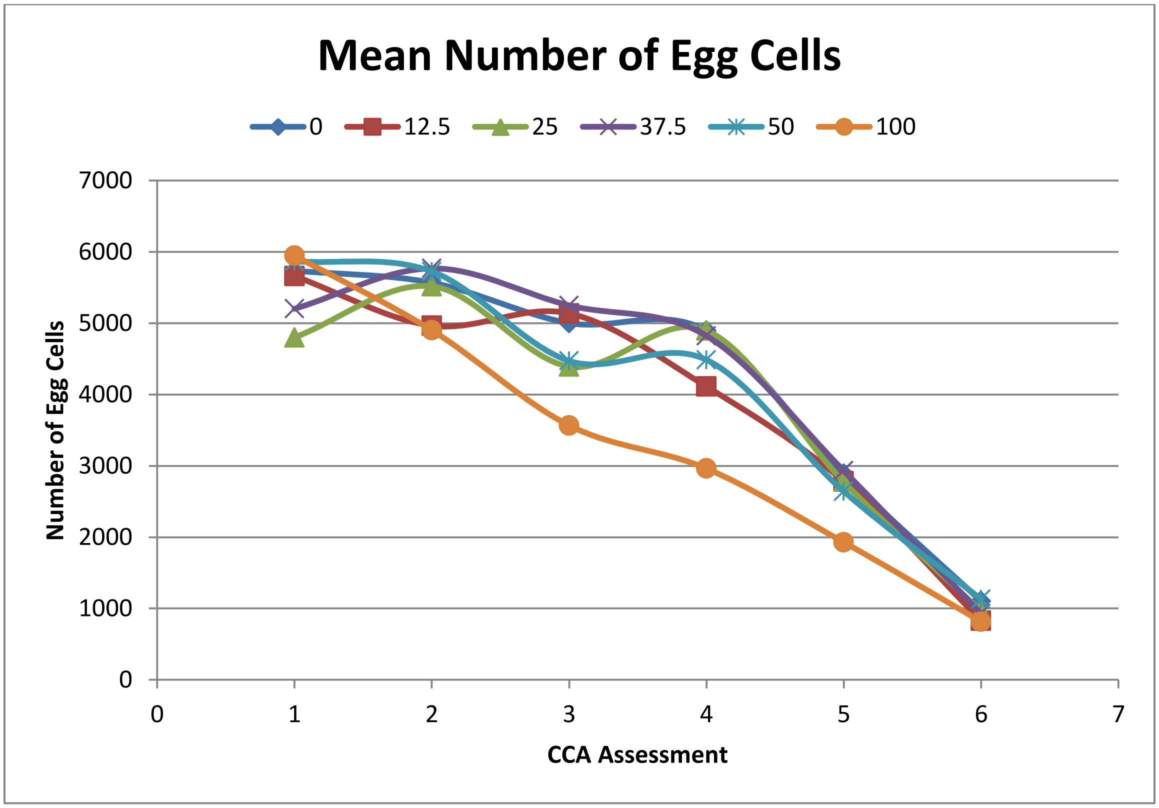

Figure 5. Combined Years: Mean number of nectar cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

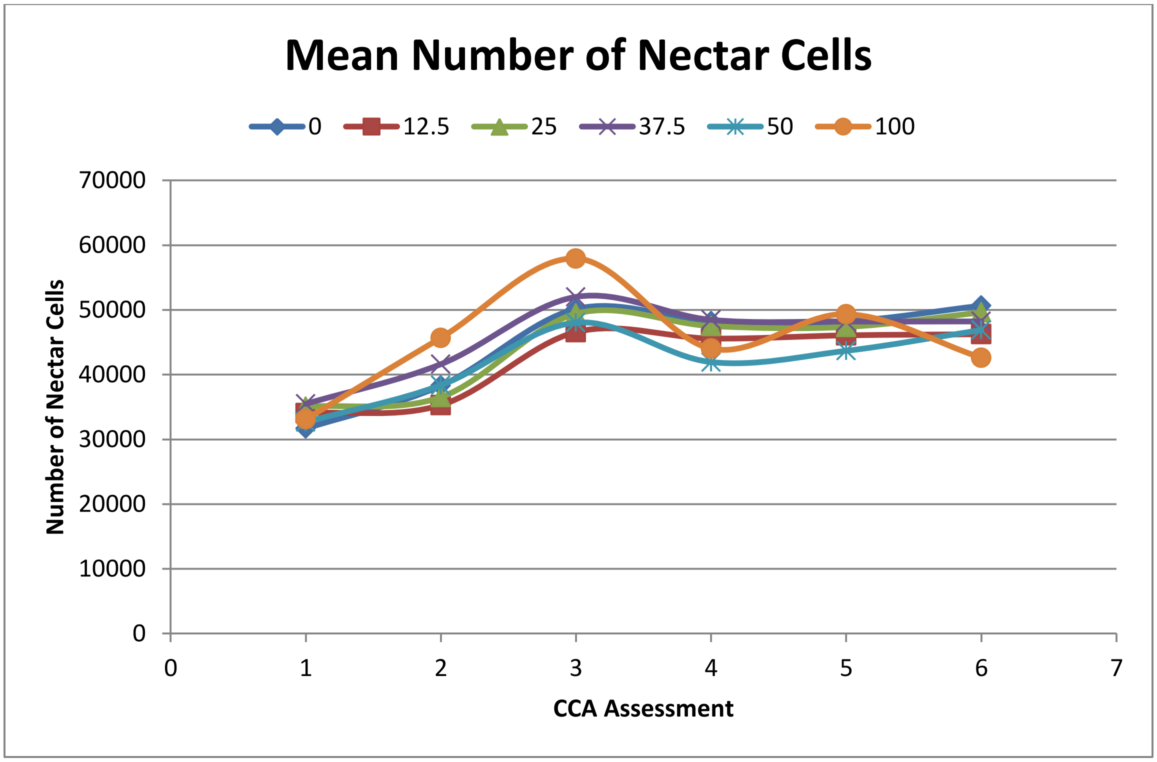

Figure 6. Combined Years: Mean number of pollen cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

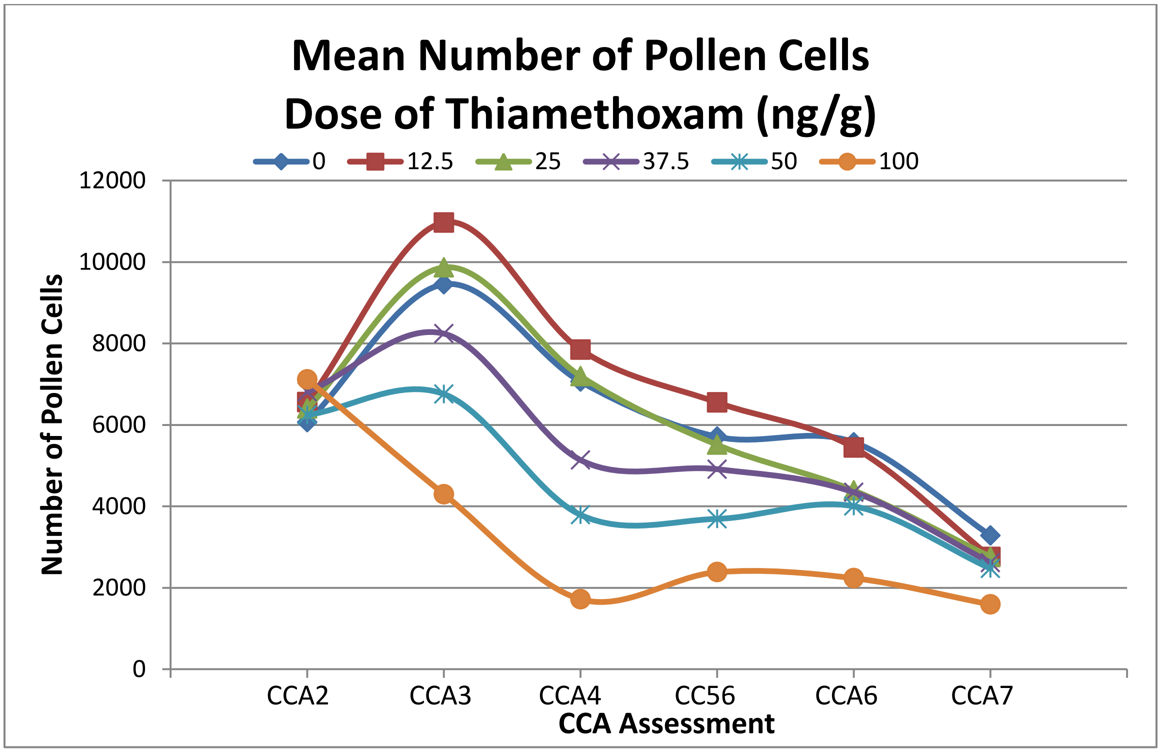

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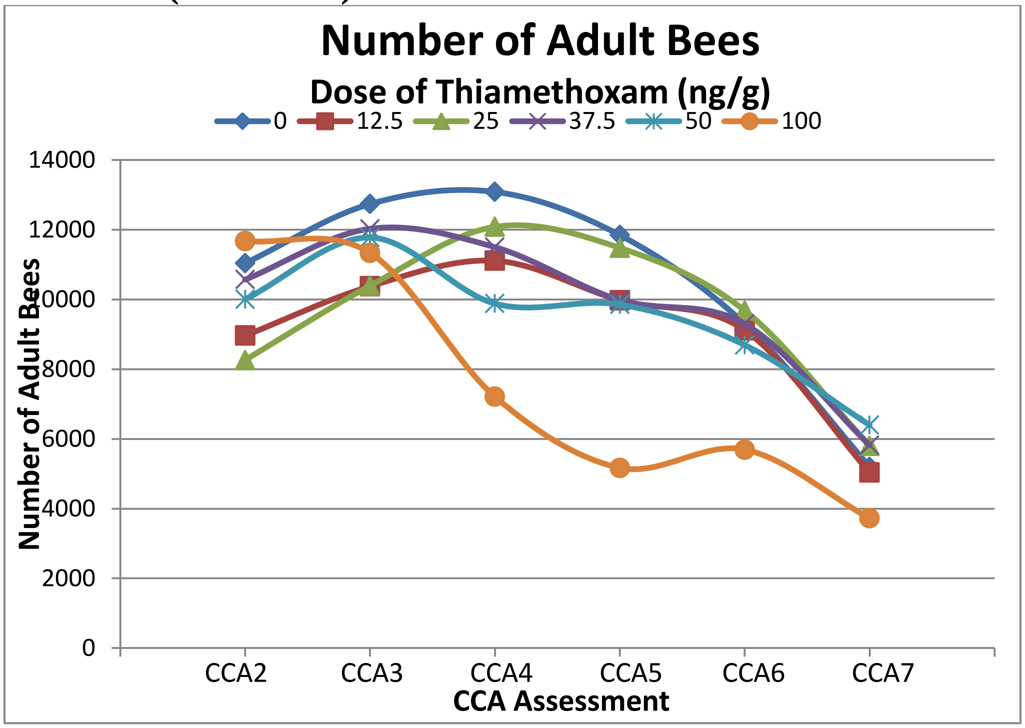
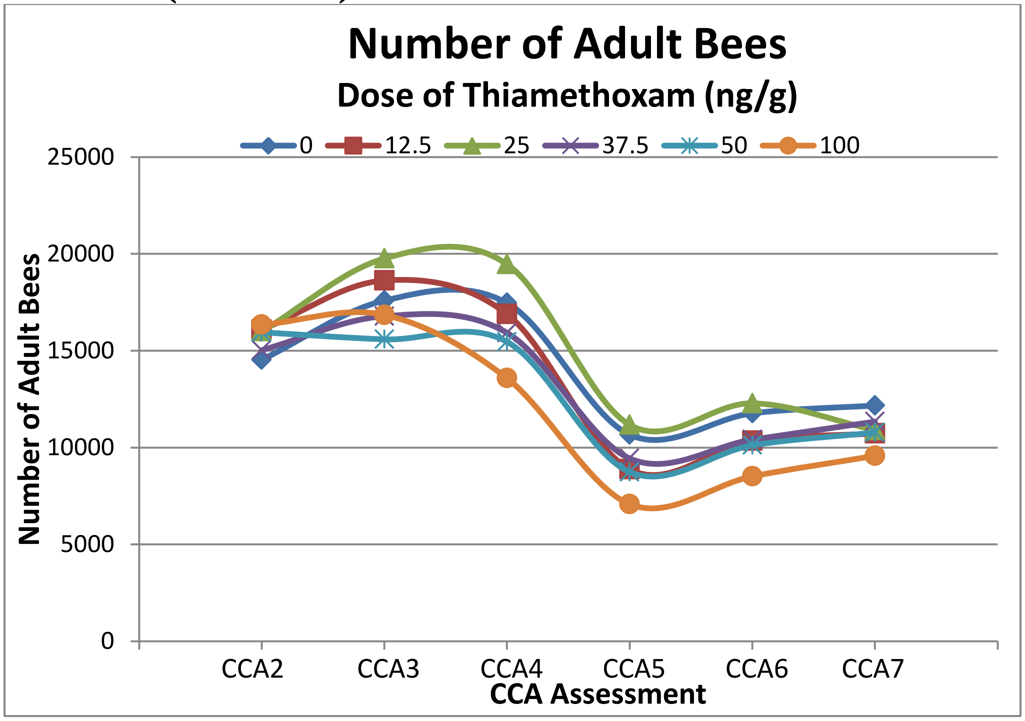
Figure 7. Separate Years: Mean number of adult bees in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

A. Year 1 (2014-2015)



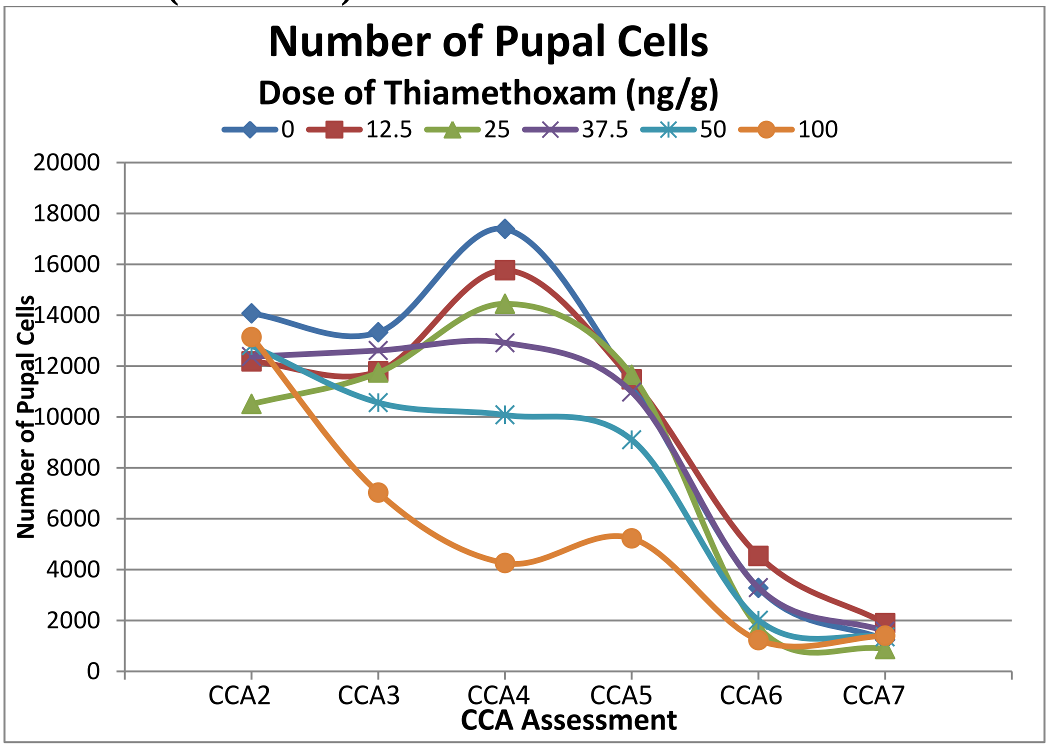
B. Year 2 (2016-2017)



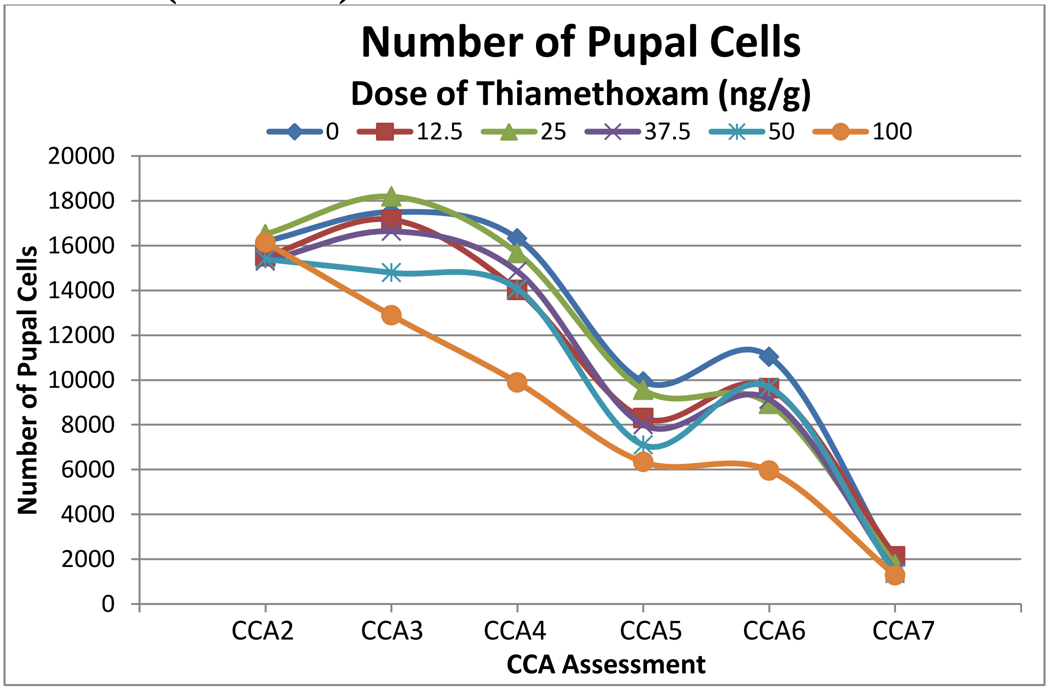
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 8. Separate Years: Mean number of pupal cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

A. Year 1 (2014-2015)



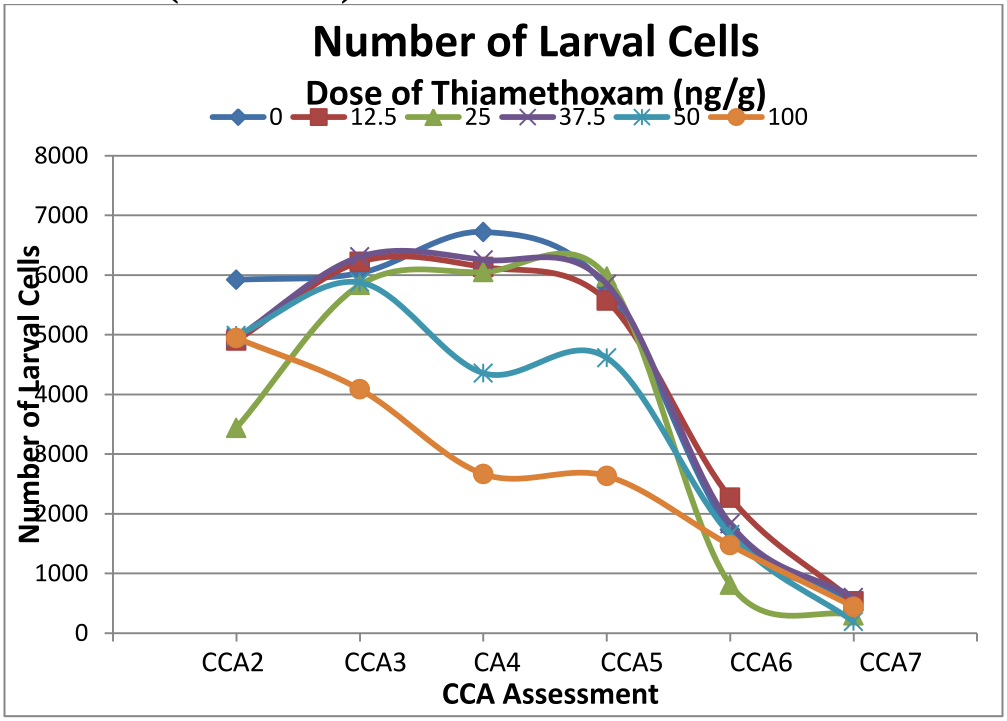
B. Year 2 (2016-2017)



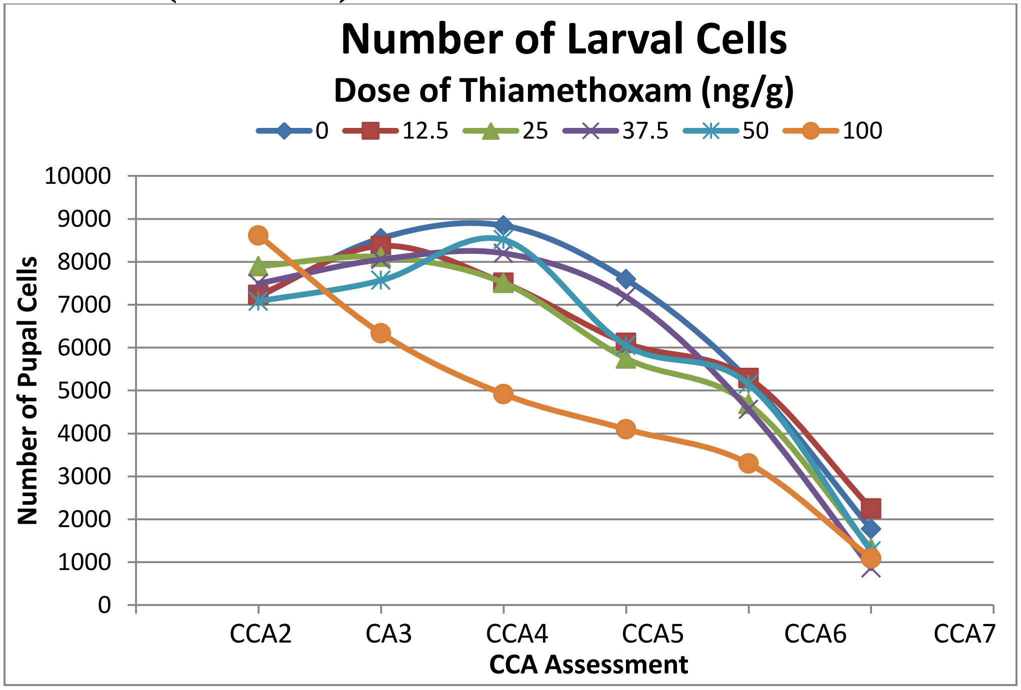
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 9. Separate Years: Mean number of larval cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

A. Year 1 (2014-2015)



B. Year 2 (2016-2017)



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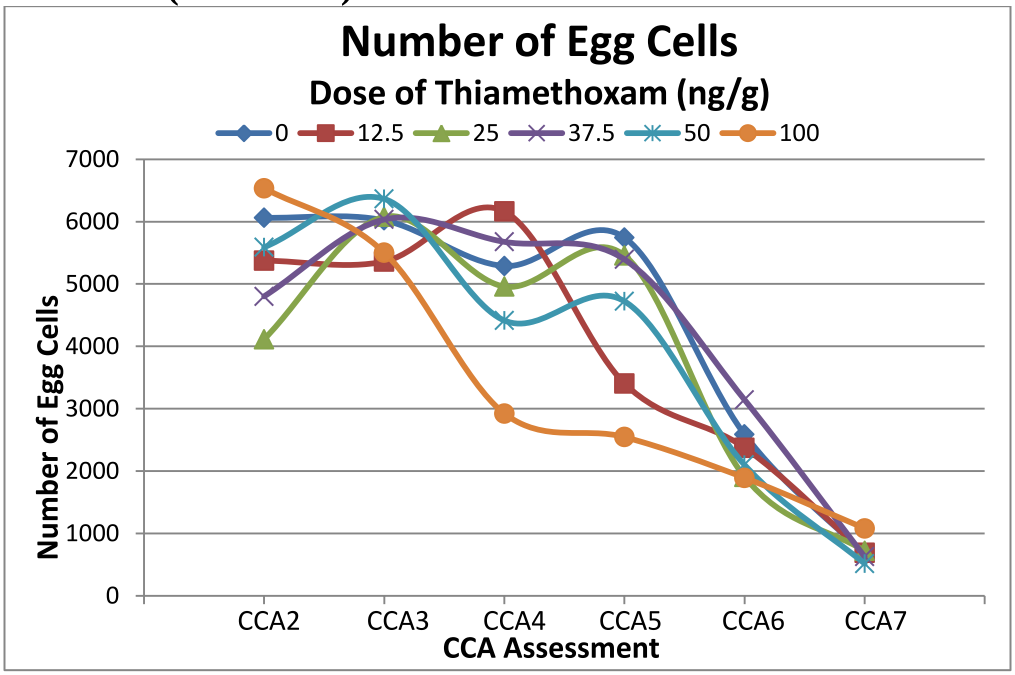
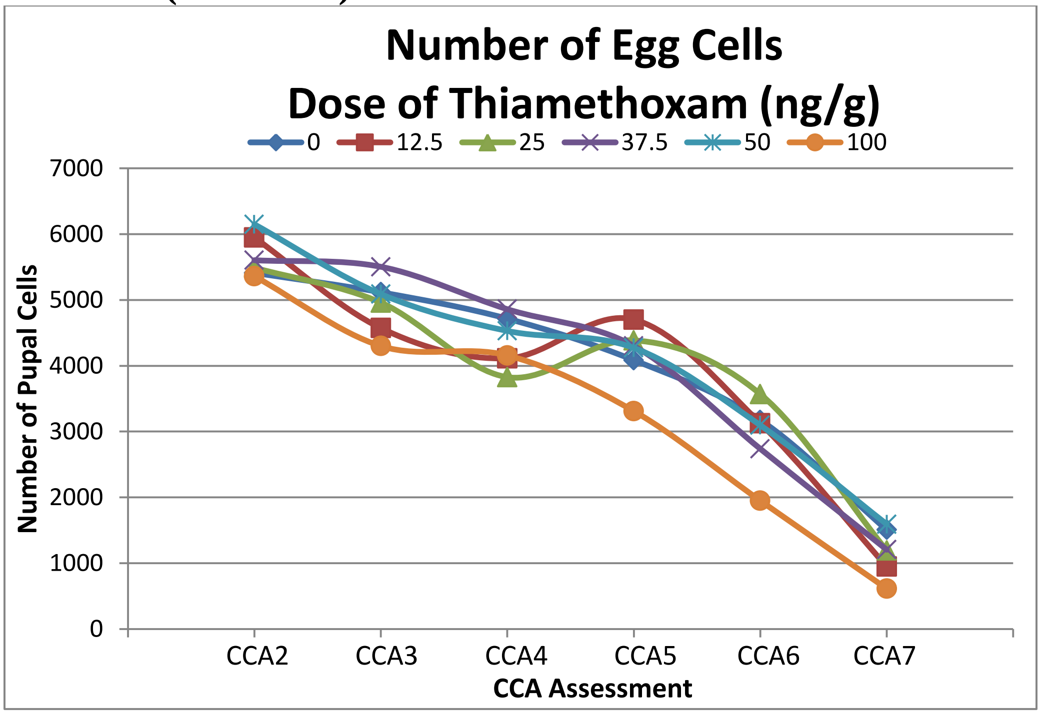
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 10. Separate Years: Mean number of egg cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

A. Year 1 (2014-2015)



B. Year 2 (2016-2017)



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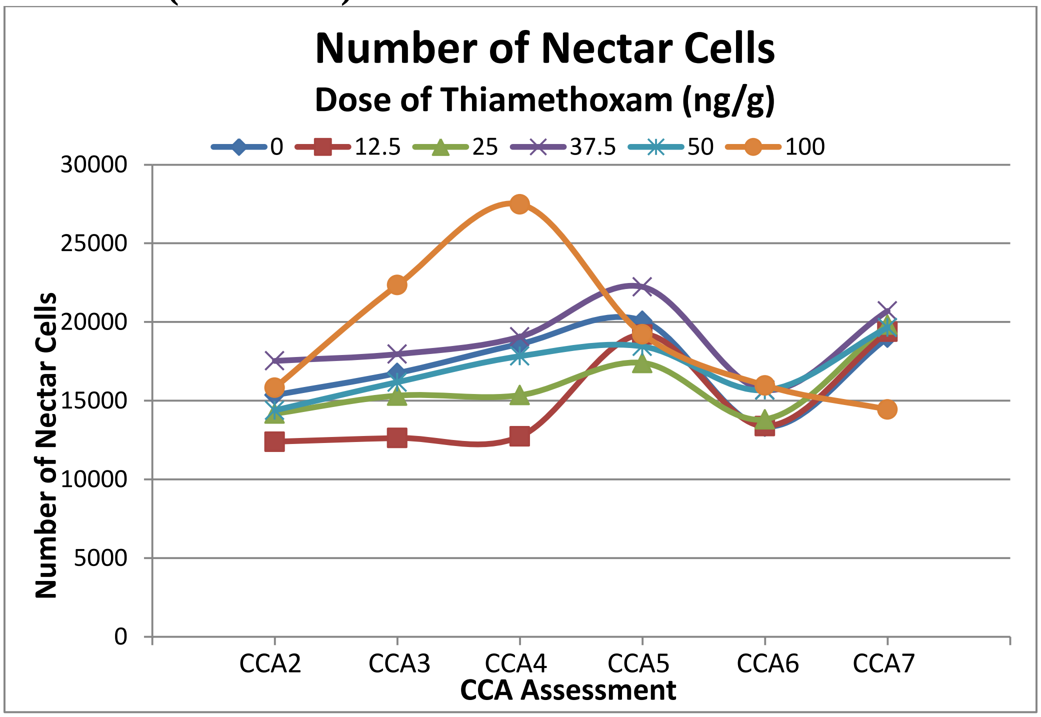
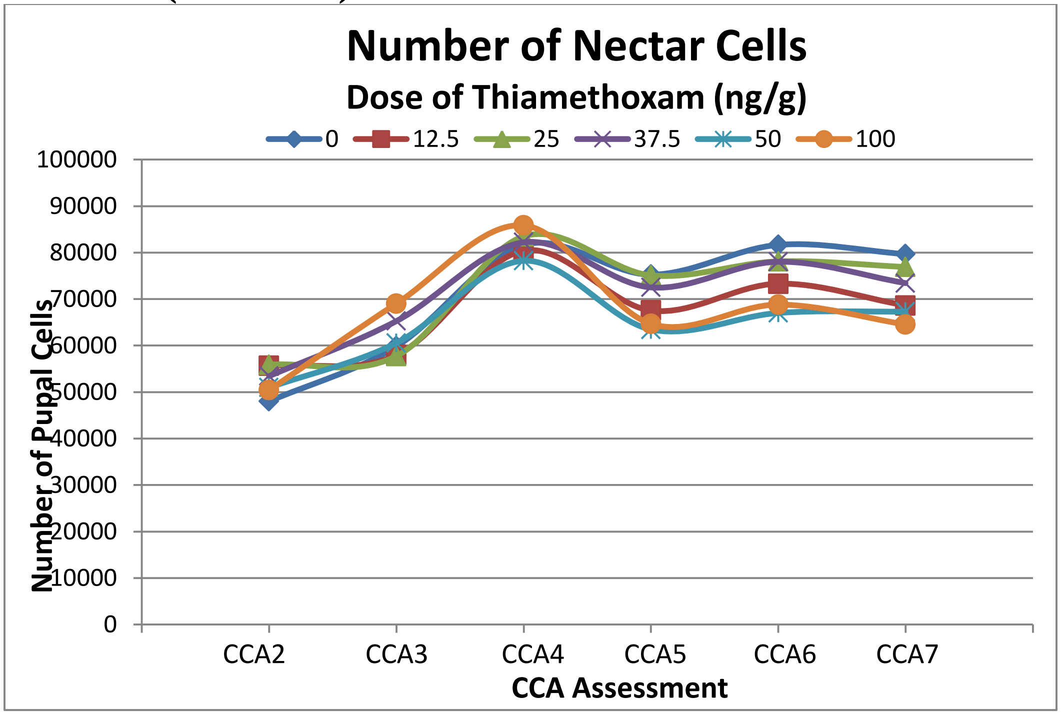
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 11. Separate Years: Mean number of nectar cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

A. Year 1 (2014-2015)



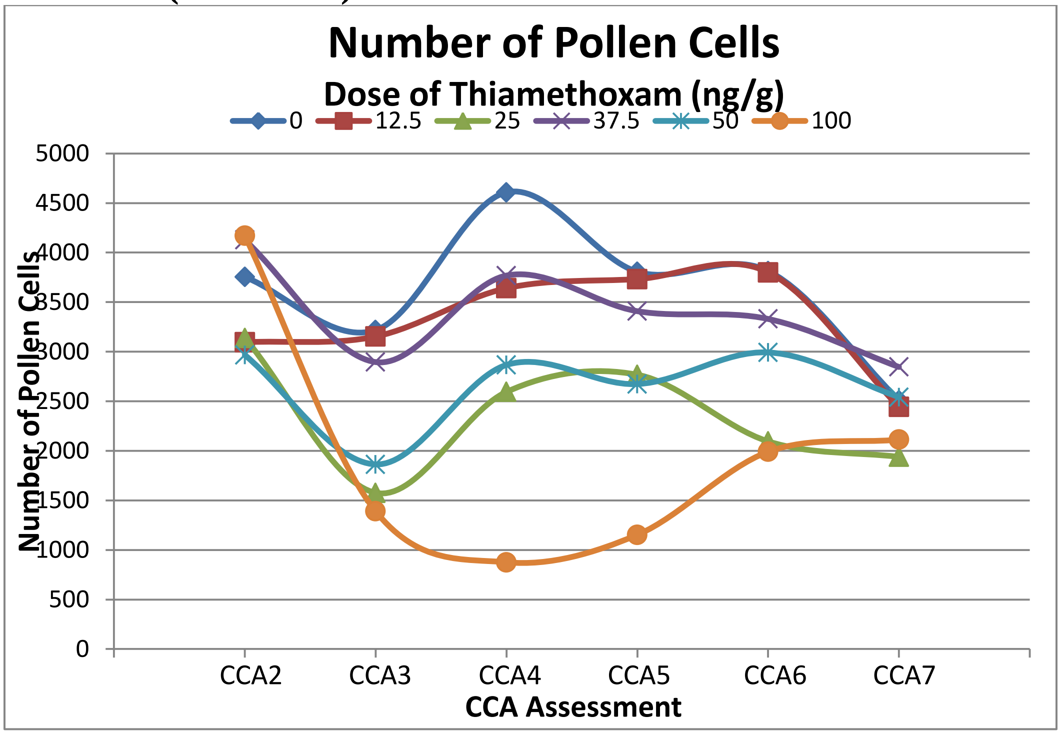
B. Year 2 (2016-2017)



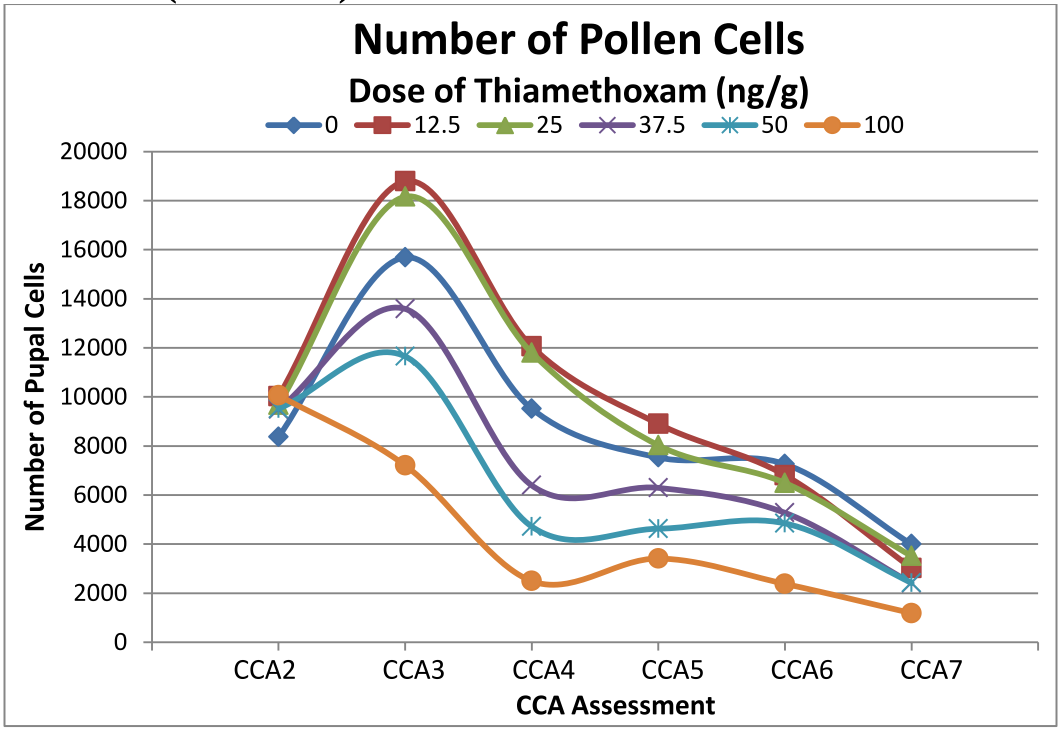
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 12. Separate Years: Mean number of pollen cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

A. Year 1 (2014-2015)



B. Year 2 (2016-2017)



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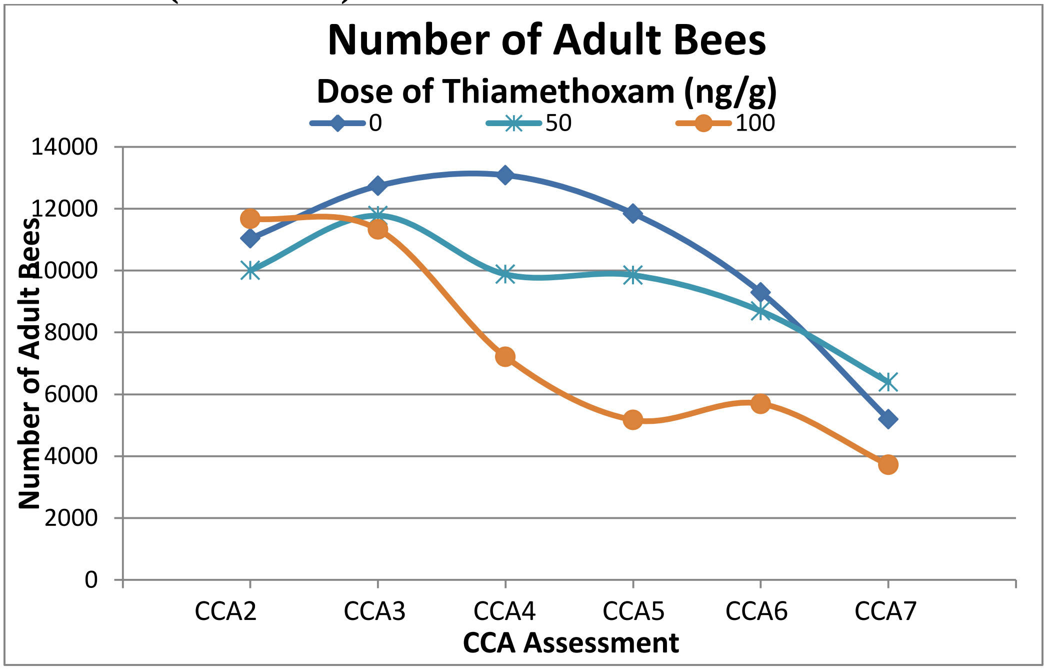
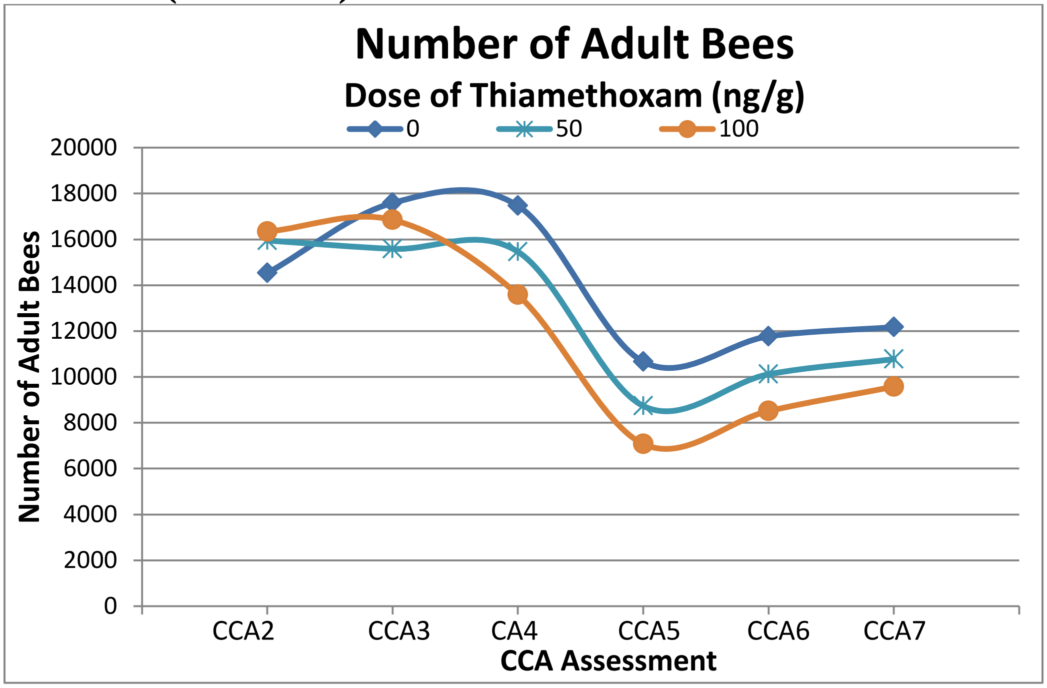
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 13. Separate Years-0, 50 100 ng/g Comparison: Mean number of adult bees in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

A. Year 1 (2014-2015)



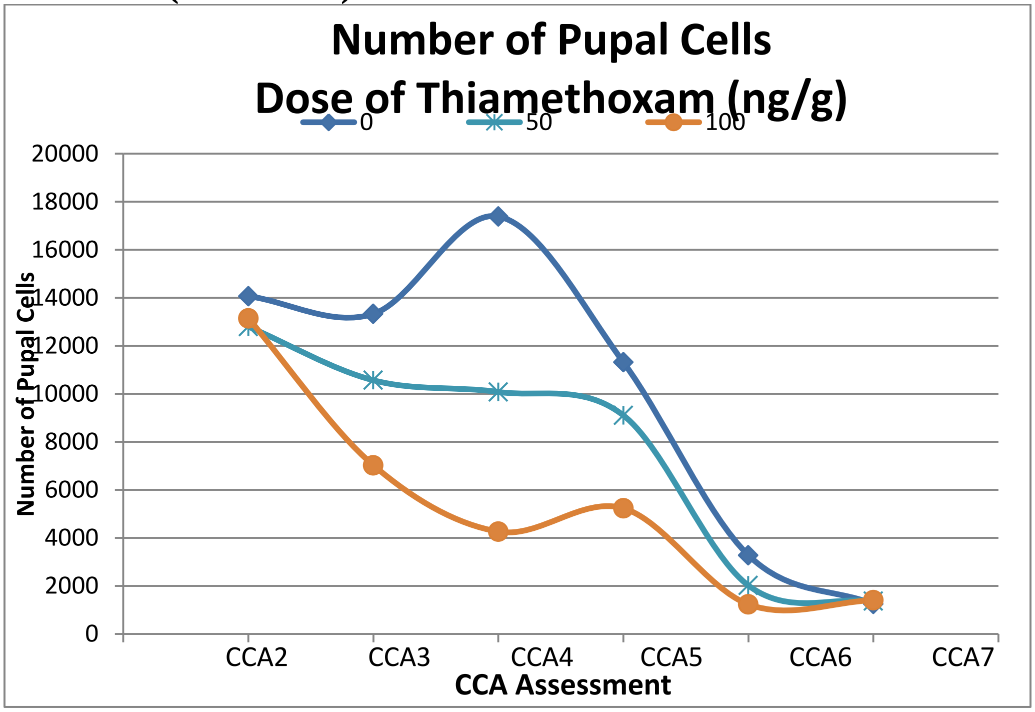
B. Year 2 (2016-2017)



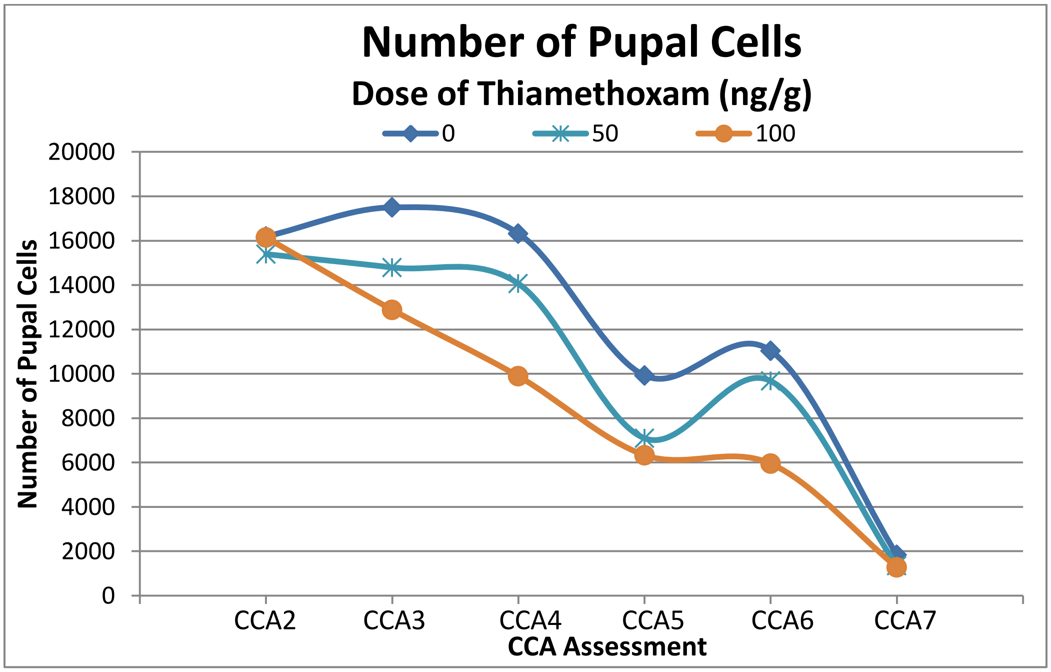
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 14. Separate Years-0, 50 100 ng/g Comparison: Mean number of pupal cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

A. Year 1 (2014-2015)



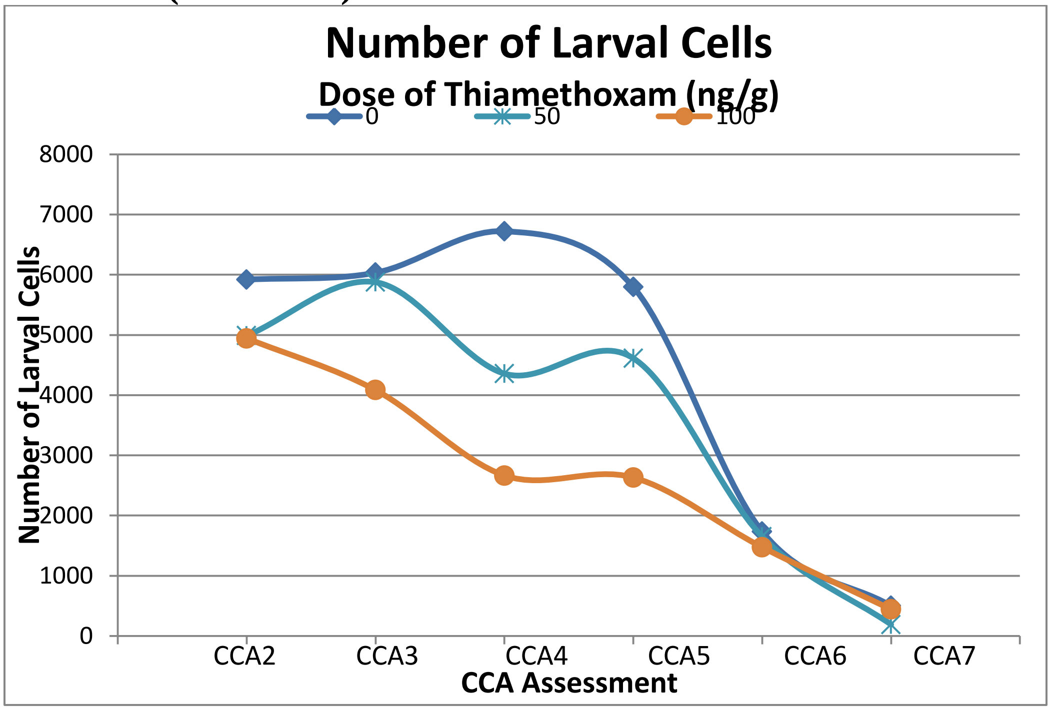
B. Year 2 (2016-2017)



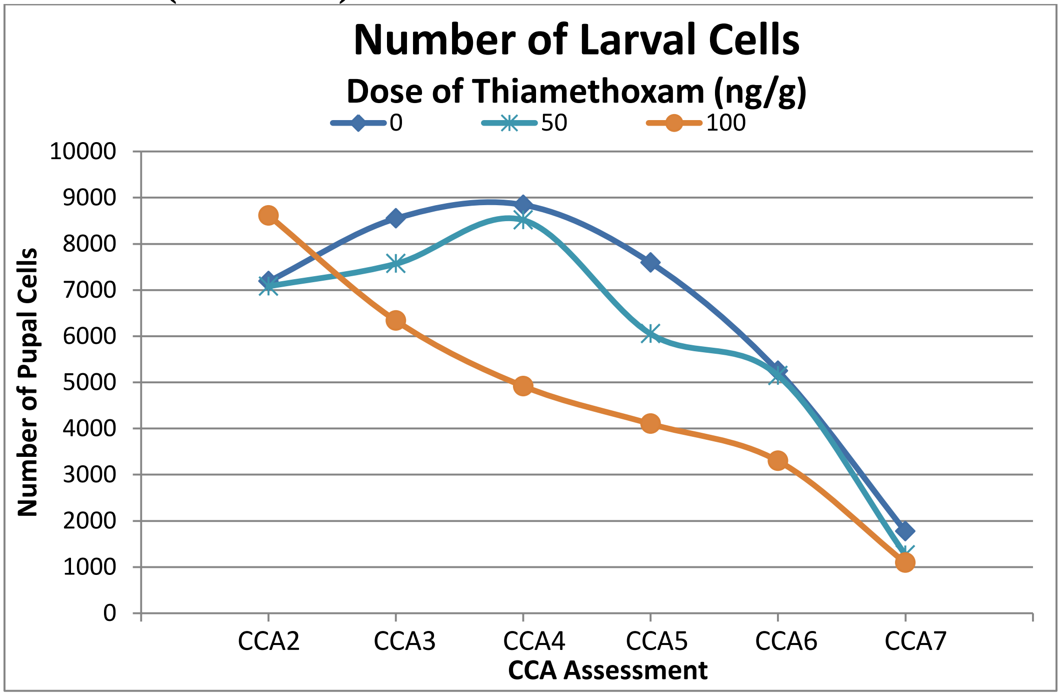
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 15. Separate Years-0, 50 100 ng/g Comparison: Mean number of larval cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

A. Year 1 (2014-2015)



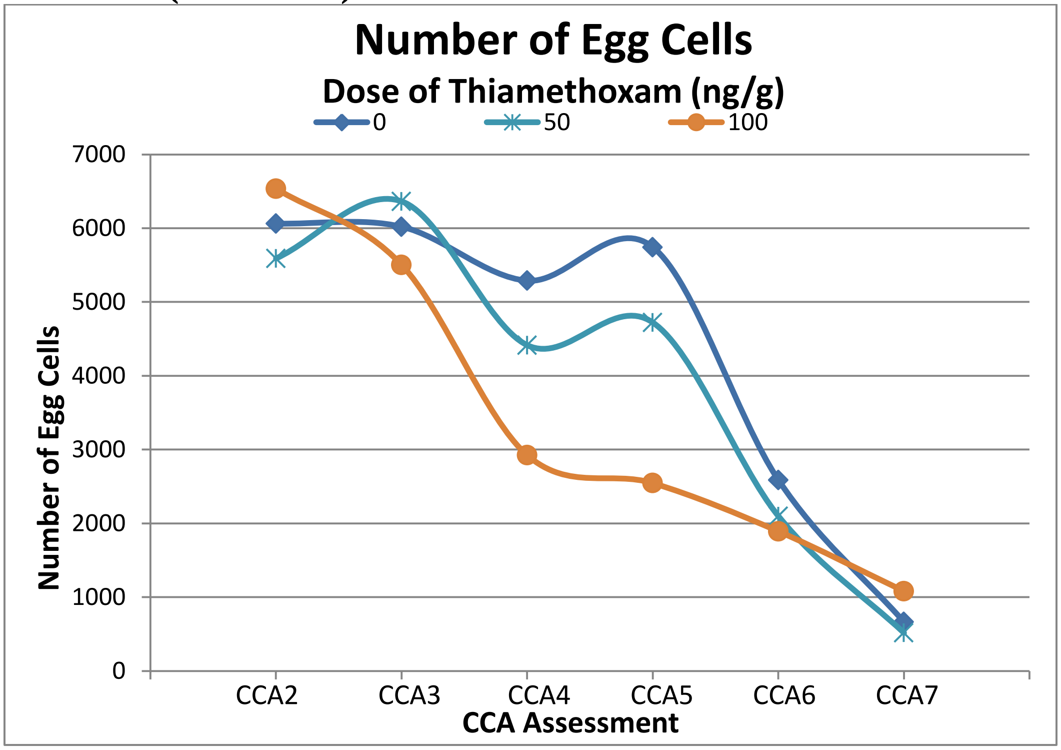
B. Year 2 (2016-2017)



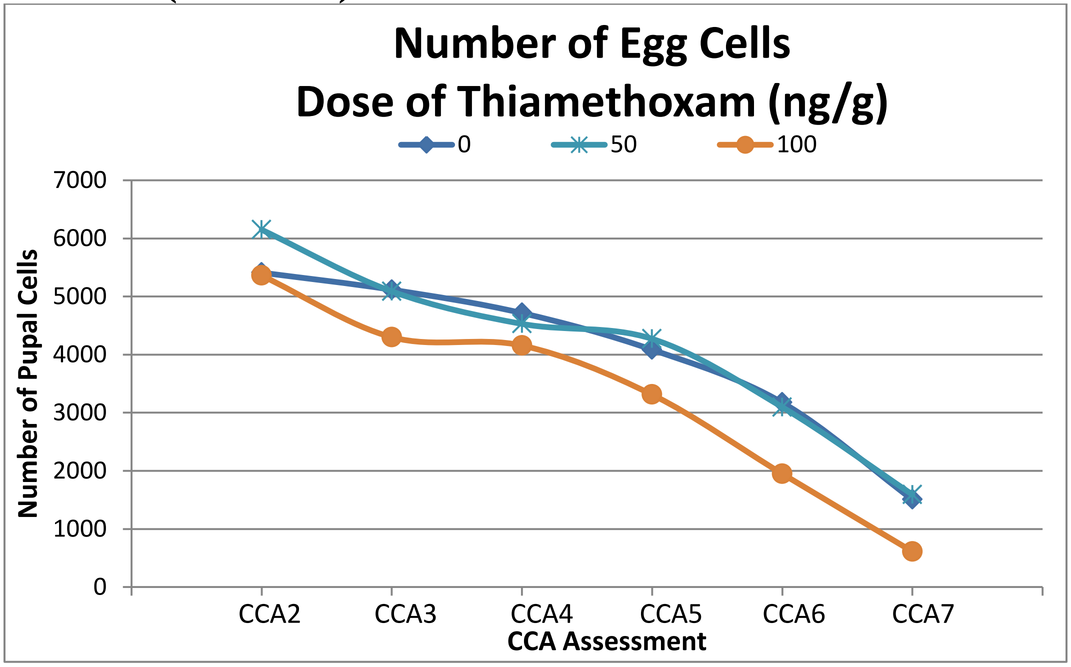
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 16. Separate Years-0, 50 100 ng/g Comparison: Mean number of egg cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

A. Year 1 (2014-2015)



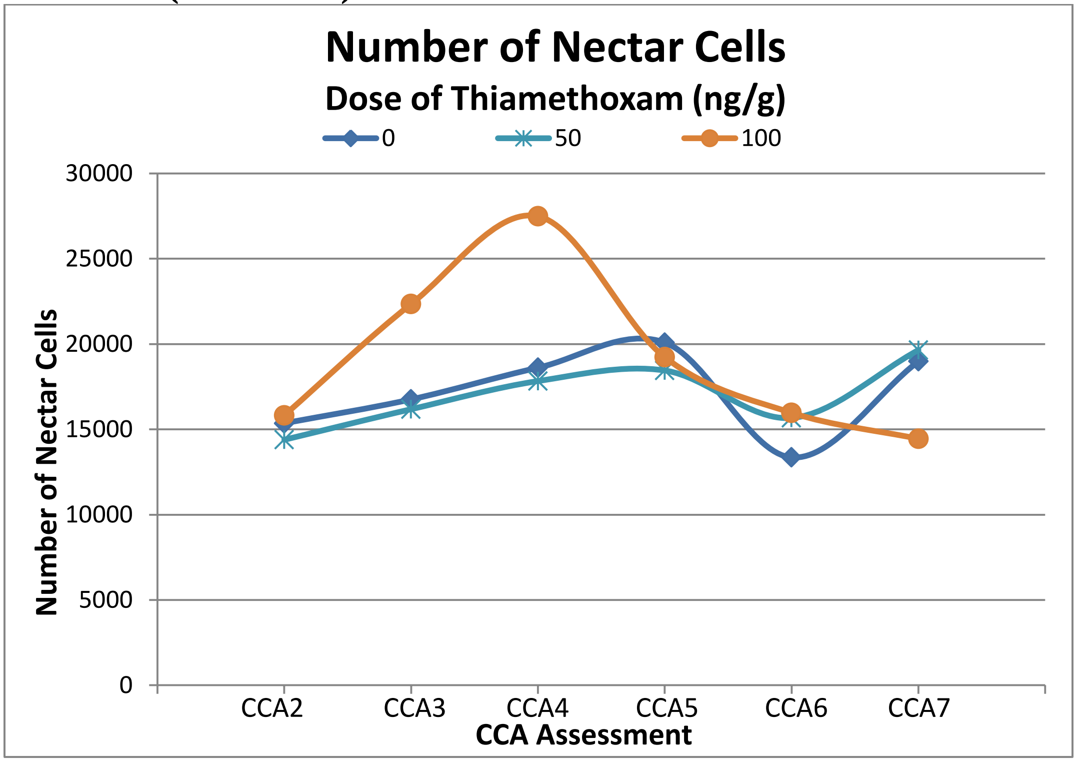
B. Year 2 (2016-2017)



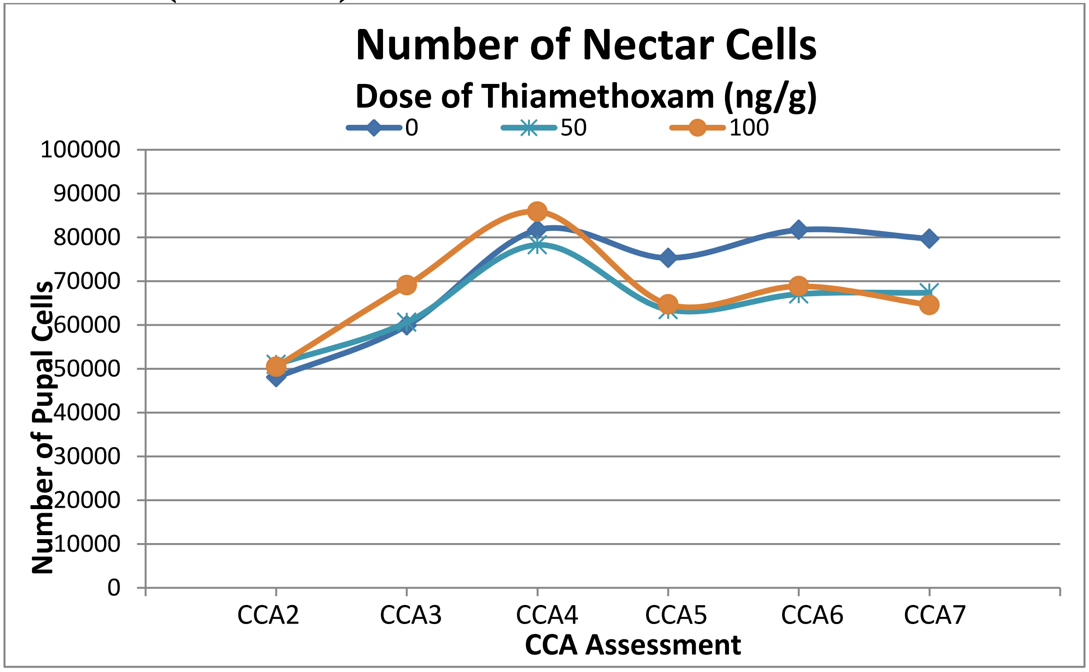
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 17. Separate Years-0, 50 100 ng/g Comparison: Mean number of nectar cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

A. Year 1 (2014-2015)



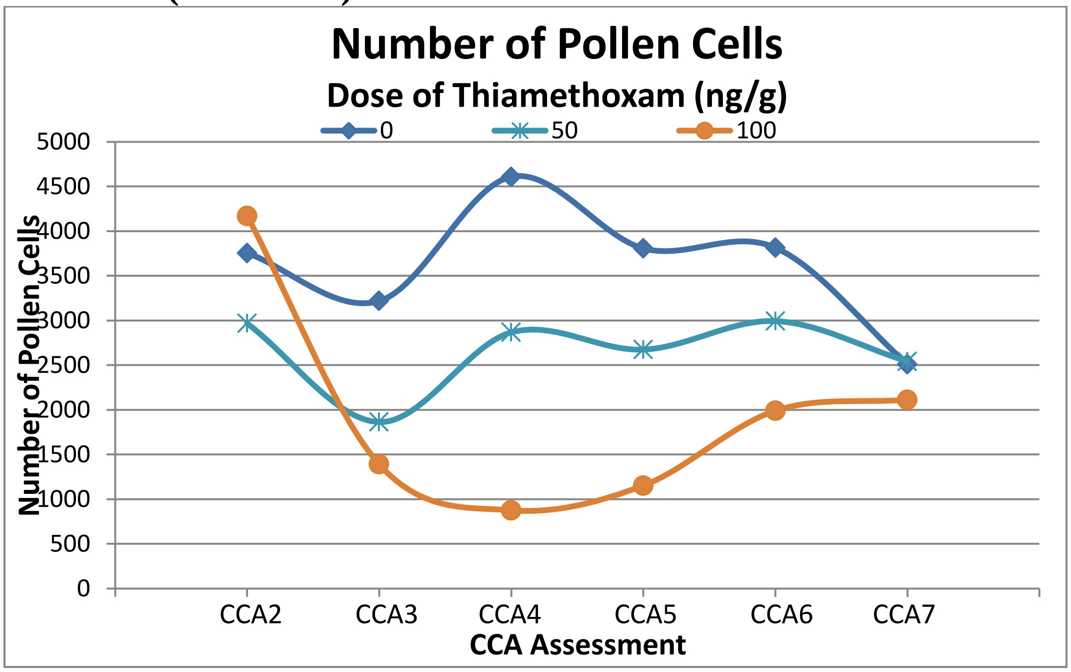
B. Year 2 (2016-2017)



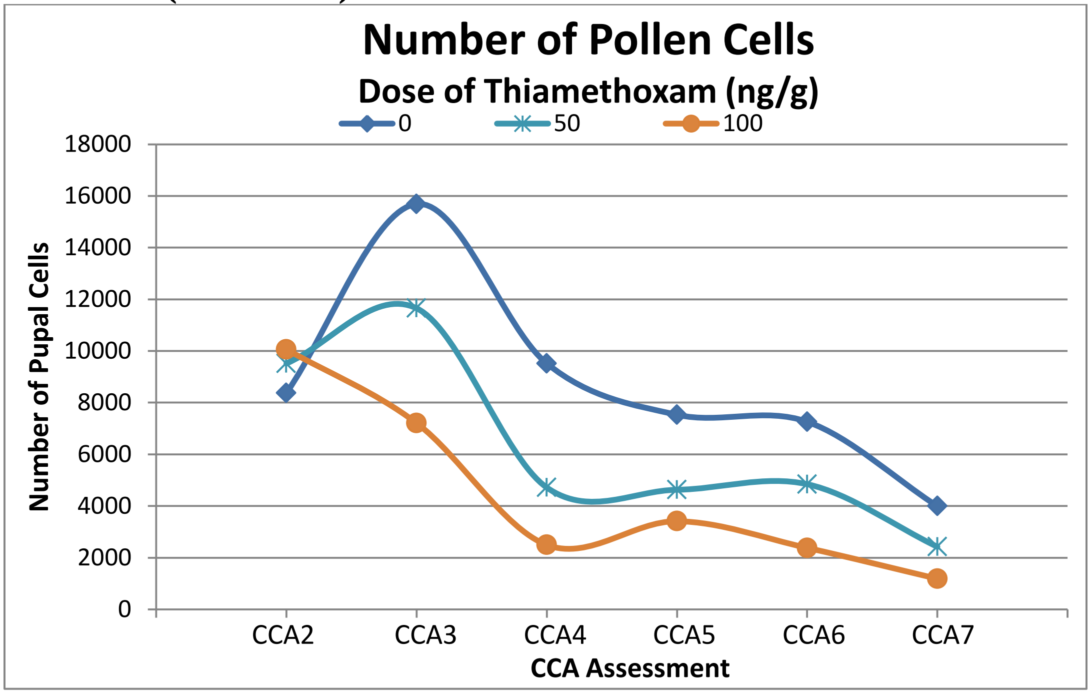
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 18. Separate Years-0, 50 100 ng/g Comparison: Mean number of pollen cells in each treatment group measured at every CCA resulting from nectar feeding at the indicated dose of thiamethoxam.

A. Year 1 (2014-2015)



B. Year 2 (2016-2017)



EVALUATION REPORT - Dinotefuran Nectar Colony Feeding Study

John Troiano, Research Scientist III

May, 2018

A review of: - Bocksch, S. (2016). Honey Bee Brood and Colony Level Effects Following Dinotefuran Intake via Treated Sucrose Solution in a Field Study in North Carolina: Final Report. Unpublished study prepared by Eurofins Agroscience Services EcoChem GmbH, & Eurofins Agroscience Services Ecotox GmbH. 523p., Laboratory Report Number S15-00102. MRID 50147001. CDPR Study ID 296826.

Introduction

A colony feeding study was conducted to determine the effects of dinotefuran on the health of honey bees and their hives. Dinotefuran was dosed directly to hives, supplied in a sugar solution that mimicked a nectar source for food supply. Health of the bee colonies was determined by measuring the number of individuals in each bee life stage in the hive over time, the storage of honey and pollen food supplies in the hives, and the weight of hives over time. The statistical design of the study was the same as that used in previous colony feeding studies on potential effects of imidacloprid, thiamethoxam, and clothianidin on honey bee colony health. Dinotefuran was mixed into sugar feeding solutions (i.e. nectar surrogate) at nominal concentrations of 10, 20, 40, 80, and 160 ng/g (ppb; ng of active ingredient per g of sugar solution). Colonies were exposed to the sugar feeding solutions for six weeks. Each treatment was replicated at 12 different apiary sites that were located throughout forested areas of North Carolina. Assessments of land use conducted in a three mile radius around each apiary site indicated that cultivated crops comprised a maximum of 3% of the surrounding land, so the potential for exposure to other agricultural pesticides was low. The untreated control group was replicated twice at each apiary, resulting in 24 replicates at the 0 (control) dose and 12 replicates at each of the treatment group levels of dinotefuran. The distance between each apiary site was sufficient to minimize potential for bees to cross-contaminate the apiary sites. Measurements made over time were indicated by numbering of the colony condition assessments (CCA). For this analysis CCAs 3 through 8 were included. Observations at CCA3 were taken prior to initiation of dinotefuran feeding treatments. Observations at CCA 4 were taken during the middle of the six week feeding (exposure) period with CCA 5 through 8 assessments taken after the exposure. Observations were taken at approximately 1 month intervals. Additional observations taken after overwintering were indicated as CCA9 and CCA10. Since determination of No Observed Effects Concentrations (NOECs) and Lowest Observed Effects Concentrations (LOECS) in previous studies had been based on effects noted during the growing season, data were analyzed for CCA3 to CCA8 in this study, which corresponded to the same time interval in previous studies. Observations made at CCA9 and CCA10 were not included in this statistical analysis of the data because of complications which arose in previously evaluated colony feeding studies.

Statistical Analysis

Evaluation of the data followed the statistical approach used by DPR and EPA scientists to analyze data from previously reviewed neonicotinoid colony feeding studies. Since measurements for each parameter were made in each hive over time, the statistical analysis was conducted as a repeated measures over time. Additionally, a mixed model was used where apiary location was identified as a random variable and dinotefuran levels of dose a fixed effect. The mixed model was chosen because the results of the analysis were to be applied to the larger population of bee hives. Normality tests were conducted for each CCA as indicated by Shapiro-Wilk and Kolmogorov-Smirnov test statistics produced by the PROC CAPABILITY procedure in Statistical Analysis System (SAS, version 9.4). For comparison, data were also transformed to natural logarithms to determine if transformation provided better results. Results were mixed where in some cases the probability levels did not agree between the two test statistics or the transformation gave more instances of non-normality, such as for pupal and honey cell counts. Based on these results the raw data were used in the analyses. The mixed model approach included tests to determine the appropriate model that describes the covariance structure reflected by the data. Inclusion of a covariance model in the analysis accounts for heterogeneity of variances that often are measured between treatment levels.

The repeated measures analysis of variance was conducted to determine potential effects of dinotefuran dose on each measurement of hive health over time. Data collected from colony condition assessments (CCAs) numbered CCA3 to CCA8 were included because these are the time intervals where effects were observed in the previous neonicotinoid feeding studies. The PROC MIXED procedure in the Statistical Analysis System (SAS, version 9.4) was used to run the repeated measures effects mixed model. Since measurements of colony health were conducted and hive weight reported approximately 1 month apart, they were treated as equally spaced intervals in the analysis. A regression model was used to determine the effects of dose on each response variable (SAS Program 1 below). The regression model was run twice. First, all dose levels were included where the levels of dose were expressed as orthogonal polynomial contrasts that reflected the uneven spacing between the levels – 0, 10, 20, 40, 80, and 160 ng/g. Based on these results, a reduced regression model was run that included the concentrations that appeared to define LOEC and the NOEC concentrations. The second run was intended to remove extraneous variance produced from treatments that were not contributing information to the model. The linear and quadratic coefficients for the effect of dose concentration and their interaction with CCA assessments were tested in the models. The interaction term provided information on changes in the relative effect of dinotefuran concentration over the duration of the study. For each regression model, an additional analysis was run that compared the probability values between the control and each level of dinotefuran treatment at each CCA (SAS Program 2 below). This analysis provided guidance on the potential concentration of dinotefuran that defined the NOEC and LOEC values. In order to protect against falsely discovering a significant comparison between mean values, the 'Simulated' option was used to generate comparisons between the control and each dose level (Edwards, D., and Berry, 1987). The 'Simulated' option computes adjusted p-values from simulated distributions that are based on distributional statistics generated during the analysis.

In the regression mixed model used to analyze the data, there are two places where a covariance model could be specified. One is in the 'Random' statement where apiary was identified as the random variable. The second is in the 'Repeated' measures statement where each hive was identified as the subject for the repeated measure. For the random statement only the Variance Component (VC) model successfully paired with the covariance model specified in the repeated statement: Specifying more complex covariance models in the random statement resulted in indications of converge problems for that model. For the repeated measures statement the correlation structure generally indicated greater correlation between samples taken at close time intervals and, conversely, decreased correlation the further apart the samples were taken in time (Appendix I). Since this structure is normally represented by autoregressive covariance models, the covariance structure for the repeated statement was tested using variance component (VC), compound symmetry (CS), compound symmetry with heterogeneity (CSH), autoregressive first order (AR(1)), autoregressive first order with heterogeneity (ARH(1)), and unstructured (UN) models. Covariance model selection was based on the statistic generated for the Bayesian Information Criteria (BIC) where a lower value of the criterion indicated a better fit of the covariance model. A statistical basis for choosing the appropriate model was determined from Chi-square tests conducted on the difference of the value of the BIC criteria between the two models tested with the number of degrees of freedom determined as the difference between the number of parameters in the model and where the significance level was at 0.01 (Hammer, 2000; Littell et al., 2006). With the VC covariance model specified in the random statement, the best fits covariance models in the repeated statement were AR(1) for adult bees and honey, ARH(1) for larvae, eggs, pollen and hive weight, and UN for pupae (Table 1).

SAS Program 1

```
proc mixed data=b6 order=data;
class apiary dose cca hive;
model transvalue =cca lin quad lin*cca quad*cca /ddfm=sat htype=1 solution;
random apiary/type=vc;
repeated cca/ subject=hive*dose type=ar(1);
run;
```

SAS Program 2

```
proc mixed data=b6 order=data;
class apiary dose cca hive;
model transvalue =cca dose dose*cca /ddfm=sat htype=1;
random apiary/type=vc;
repeated cca/ subject=hive*dose type=ar(1);
slice dose*cca /sliceby=cca diff=controll adjust=simulate adjdfe=row;
run;
```

Results

Means and standard deviation for each response variable measured at each dose and each CCA are presented in Appendix B. Data for CCA3 through CCA8 were included in the repeated measures analysis. Hive C2 was excluded from the analysis because data were lacking for CCA6, CCA7, and CCA8. For the repeated measures regression model that included all levels of dose (i.e. exposure concentrations) there were a few indications of trends in the data (Probability level between 0.05 and 0.1): Linear effect of dose for number of adult bee cells, a linear interaction over time (linear*CCA) for pupal cells, and a quadratic interaction over time for larval cells (Table 2). There was an indication of a significant linear interaction term for honey cells (Probability level <0.05). For the pairwise comparisons of each dose level to the control value, only trends, indicating decreases in numbers, were observed at the highest dose level (160 ng/g) for adult bee and pupal cells (Table 3). These results indicated that 160 ng/g is the LOEC value, which makes the 80 ng/g concentration the NOEC value.

Results from reduced analyses, using data for 0, 80, and 160 ng/g dose levels, confirmed the NOEC and LOEC values. The linear effect for dose for adult bees was significant (Probability level < 0.05). Comparison to the control value indicated significant decreases at CCA6 and trends indicated at CCA5 and CCA7 (Tables 4 and 5). The graphical comparison of the effects measured at each CCA indicates that upon exposure after CCA3 the number of adult bee cells were consistently lower at the 160 ng/g treatment over the rest of the CCAs (Figure 1). The graph comparing only the 3 treatment levels (0, 80, and 160 ng/g) clearly indicates that the decrease was maintained over successive colony assessments. Although there was no indication of significance for pupal cells in the regression table, the individual comparisons reflected the trend observed in the overall analysis where the effect at CCA6 was significant for the comparison of the effect at 160 ng/g to the control value (Table 5). The graph comparing the number of pupal cells reflects this effect with a clear reduction at the 160 ng/g treatment shown in the graph containing the 3 treatment levels (Figure 2). Although there was an indication of a significant linear interaction for honeys cells in the reduced analysis, there were no indication of dose-related effects in the individual comparisons (Figures 3). Potential effects noted in the full analysis for larval cells were also non-significant in the reduced analysis.

Potential dose-related effects were indicated for honey and pollen food stores where a significant linear interaction over time was indicated in the full and reduced analyses (Tables 2 and 4). For honey, none of the specific dose comparisons were significant, indicating no sustained effects at any level of dose (Tables 3 and 5 and Figure 5). There were no dose-related effects for pollen indicated in pairwise comparisons made for the full analyses, but results in the reduced analysis indicated a trend for a decreases in the number of cells at the 160 ng/g dose at CCA4 and CCA5 (Tables 2 and4). The graph comparing responses between the three treatment levels reflects a reduction in the mean number of pollen cells at the 160 ng/g dose at those CCAs (Figure 6).

One additional objective of colony feeding studies is to determine potential effects of treatments on overwintering of hives. The number of surviving hives measured at the last CCA after overwintering (CCA10 on April 27, 2016) indicated a high survival rate for all treatments with no dose-related effects: Rates were 88%, 83%, 67% , 92%, 83%, and 83% for 0, 10, 20, 40, 80, and 160 ng/g treatments, respectively. Analyses for hive weights also indicated no dose-related effects (Tables 2 through 5 and Figure 8).

Conclusion

Results of the statistical analyses indicated effects of the highest dinotefuran dose on various bee life stages and food stores. The most sustained effect was a decrease in the number of adult bees at 160 ng/g indicated from CCA5 through CCA7. The graph comparing the projected NOEC and LOEC values to the control values (0 80, and 160 ng/g) show that the number of adult bee cells between the treatments were clearly similar at CCA 3 prior to initiation of the treatments, followed by a steady decline at the highest 160 ng/g treatment after imposition of the dinotefuran treatments (Figure 1). Other effects measured at the 160 ng/g treatment were decreased number of pupal cells and pollen food stores (Figures 2 and 6). The number of adult bees, often referred to as colony strength, is one of the primary indicators of colony health. Therefore, the Lowest Observed Effect Concentration (LOEC) was determined to be 160 ng/g and the No Observed Effect Concentration (NOEC) was established at a nominal concentration of 80 ng/g. The actual measured value of clothianidin in the nectar feeding solution was 71 ng/g, which is the actual NOEC value. The study authors reached the same conclusion, stating that the NOEC was established at 80 ng/g as based on a significant reduction in bee bread storage at CCA4 and on a significant reduction in the number of cells containing pupae at CCA6. Overall, this study has been determined to be scientifically sound and can be used quantitatively to assess risks to honey bee colonies.

References

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Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 1. Repeated Measures Analysis of Variance: BIC goodness-of-fit values generated for each covariance model structure tested in the repeated measures analysis of variance program. Shaded cells indicate the covariance structure used for the analysis. Number of parameters in parenthesis refers to hive weight analysis

CV Model Tested	Model BIC Value for: Number of Parameters	Adults	Pupae	Larvae	Eggs	Honey	Pollen	Number of Parameters	Hive Weight
VC/VC	2	2735.7	2766.3	2256.0	2156.3	3960.1	2753.1	2	2491.3
VC/CS	3	2690.6	2676.6	2207.3	2139.5	3635.6	2618.8	3	2397.8
VC/AR(1)	3	2601.1	2636.8	2204.4	2118.3	3542.9	2582.3	3	2354.2
VC/CSH	8	2686.6	2591.8	2092.7	1980.6	3646.6	2609.4	7	2320.3
VC/ARH(1)	8	2601.8	2560.7	2089.2	1963.7	3548.9	2566.5	7	2250.8
VC/UN	22	2587.4	2545.8	2094.8	1977.7	3543.6	2566.4	16	2192.4

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Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 2. All Dose Levels: Results of the repeated measures mixed model testing the response of each variable to dinotefuran dosed surrogate honey.

Mixed Model Results for Repeated Measures Analysis of Variance All Doses					
Variable	Effect	DF	Den DF	F Value	Pr > F
Adult Bees	CCA	5	325	50.29	<.0001
	Lnear	1	86.9	3.45	0.07
	Quadratic	1	86.9	0.13	0.72
	Linear*CCA	5	325	0.21	0.96
	Quadratic*CCA	5	325	0.52	0.76
Pupae	CCA	5	80	174.52	<.0001
	Linear	1	79	0.96	0.33
	Quadratic	1	79	0.63	0.43
	Linear*CCA	5	80	2.07	0.08
	Quadratic*CCA	5	80	1.14	0.35
Larvae	CCA	5	172	183.56	<.0001
	Linear	1	113	0.02	0.88
	Quadratic	1	113	2.19	0.14
	Linear*CCA	5	172	0.4	0.85
	Quadratic*CCA	5	172	2.06	0.07
Eggs	CCA	5	161	166.89	<.0001
	Linear	1	119	1	0.32
	Quadratic	1	119	0.01	0.93
	Linear*CCA	5	161	0.69	0.63
	Quadratic*CCA	5	161	0.5	0.77
Honey	CCA	5	375	40.04	<.0001
	Linear	1	80.5	0.29	0.59
	Quadratic	1	80.5	0.06	0.81
	Linear*CCA	5	375	2.46	0.03
	Quadratic*CCA	5	375	1.29	0.27
Pollen	CCA	5	234	24.03	<.0001
	Linear	1	75.6	1.46	0.23
	Quadratic	1	75.7	0.48	0.49
	Linear*CCA	5	234	3.22	0.01
	Quadratic*CCA	5	234	0.36	0.88
Hive Weight	Month	4	73	29.32	<.0001
	Linear	1	62.3	0.01	0.91
	Quadratic	1	62.7	1.46	0.23
	Linear*Month	4	73	1.06	0.38
	Quadratic*Month	4	73	0.7	0.59

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 3. All Dose Levels: Probability value for the contrast of the control to each dinotefuran dose at each CCA and for each variable measured.

Response and Dose (ng/g)		Probability Value for Contrast of the Control to Each Dinotefuran Dose at Each CCA					
		CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
Bees	10	0.391	0.137	0.200	0.273	0.805	0.532
	20	0.613	0.826	0.328	0.450	0.493	0.520
	40	0.691	0.882	0.677	0.901	0.932	0.903
	80	0.336	0.257	0.508	0.795	0.848	0.782
	160	0.588	0.231	0.131	0.058	0.167	0.279
Pupae	10	0.678	0.668	0.826	0.595	0.940	0.516
	20	0.519	0.930	0.663	0.892	0.961	0.487
	40	0.799	0.988	0.960	0.524	0.973	0.817
	80	0.841	0.985	0.916	0.630	0.887	0.825
	160	0.854	0.621	0.550	0.088	0.258	0.781
Larvae	10	0.573	0.760	0.925	0.905	0.840	0.681
	20	0.416	0.541	0.674	0.996	0.911	0.973
	40	0.935	1.000	0.884	0.986	0.998	0.391
	80	0.531	0.997	0.950	0.999	0.995	0.617
	160	0.682	0.766	0.773	0.977	0.921	0.478
Eggs	10	0.090	0.797	0.892	0.583	0.700	0.631
	20	0.662	0.916	0.954	0.815	0.968	0.631
	40	0.931	0.964	0.970	0.882	0.863	0.172
	80	0.871	0.846	0.542	0.998	0.596	0.972
	160	0.641	0.979	0.954	0.973	0.941	1.000
Honey	10	0.940	0.997	0.994	0.961	0.998	0.991
	20	0.163	0.557	0.556	0.349	0.929	0.761
	40	0.613	0.580	0.906	0.915	0.992	0.930
	80	0.817	0.901	0.942	0.937	0.995	0.960
	160	0.739	0.999	0.999	0.934	0.980	0.649
Pollen	10	0.802	0.690	0.719	0.671	0.846	0.594
	20	0.921	0.982	0.850	0.811	0.770	0.578
	40	0.948	0.628	0.816	0.954	0.977	0.809
	80	0.985	0.937	0.767	0.881	0.785	0.520
	160	0.978	0.113	0.110	0.434	0.929	0.349
Hive Weight		**June**	**July**	**August**	**September**	**October**	
	10	1.000	1.000	1.000	1.000	0.903	
	20	0.826	0.931	0.511	0.098	0.832	
	40	0.825	1.000	0.997	0.939	0.644	
	80	0.740	1.000	1.000	0.991	0.768	
	160	0.866	0.651	0.840	0.879	0.991	

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 4 Reduced Dose Levels: Results of the repeated measures mixed model testing the response of each variable to dinotefuran dosed surrogate honey. Dose levels tested were 0, 80, and 160 ng/g because the full analysis indicated 80 ng/g as a LOEC value and 160 ng/g as a NOEC value.

Mixed Model Results for Repeated Measures Analysis of Variance Data for 0, 80, and 160 ug/g Analyzed					
Variable	Effect	DF	Den DF	F Value	Pr > F
Adult Bees	CCA	5	184	29.96	<.0001
	Lnear	1	39.6	4.6	0.04
	Quadratic	1	39.5	0.01	0.94
	Linear*CCA	5	184	0.26	0.93
	Quadratic*CC	5	184	0.53	0.76
Pupae	CCA	5	44	106.58	<.0001
	Linear	1	40.7	1.44	0.24
	Quadratic	1	40.6	0.57	0.45
	Linear*CCA	5	44	1.57	0.19
	Quadratic*CC	5	44	0.66	0.65
Larvae	CCA	5	99.8	95.46	<.0001
	Linear	1	60.4	0.01	0.94
	Quadratic	1	60.3	1.19	0.28
	Linear*CCA	5	99.8	0.38	0.86
	Quadratic*CC	5	99.8	1.09	0.37
Eggs	CCA	5	67.5	88.46	<.0001
	Linear	1	38.9	0.17	0.68
	Quadratic	1	38.8	0.02	0.88
	Linear*CCA	5	67.5	0.33	0.89
	Quadratic*CC	5	67.5	0.66	0.65
Honey	CCA	5	202	18.94	<.0001
	Linear	1	37.7	0.36	0.55
	Quadratic	1	37.6	0.02	0.90
	Linear*CCA	5	202	3.18	0.01
	Quadratic*CC	5	202	0.65	0.66
Pollen	CCA	5	139	10.66	<.0001
	Linear	1	44.4	1.55	0.22
	Quadratic	1	44.3	0.28	0.60
	Linear*CCA	5	139	2.81	0.02
	Quadratic*CC	5	139	0.57	0.73
Hive Weight	Month	4	73	29.24	<.0001
	Linear	1	62.5	0.11	0.74
	Quadratic	1	62.1	1.24	0.27
	Linear*Month	4	73	1	0.42
	Quadratic*Mo	4	73	0.44	0.78

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

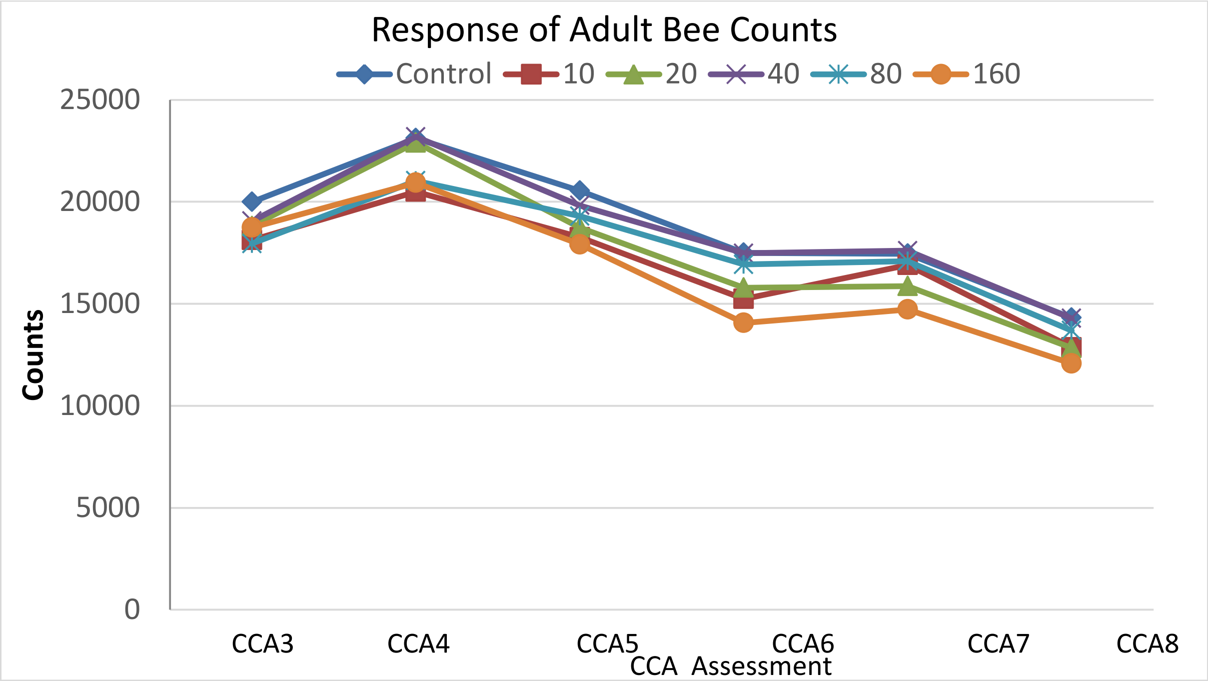
Table 5. Reduced Dose Levels: Probability value for the contrast of the control to each dinotefuran dose at each CCA and for each variable measured.

Response and Dose (ng/g)	Probability Value for Contrast of the Control to Each Dinotefuran Dose at Each CCA					
	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
Bees 80	0.179	0.143	0.304	0.578	0.638	0.558
160	0.368	0.126	0.067	0.034	0.087	0.153
Pupae 80	0.640	0.915	0.775	0.397	0.704	0.626
160	0.661	0.398	0.309	0.030	0.144	0.574
Larvae 80	0.327	0.952	0.822	0.990	0.909	0.388
160	0.457	0.566	0.525	0.899	0.736	0.276
Eggs 80	0.685	0.646	0.330	0.956	0.367	0.841
160	0.446	0.888	0.826	0.854	0.801	0.970
Honey 80	0.623	0.743	0.801	0.797	0.956	0.844
160	0.529	0.984	0.981	0.790	0.901	0.435
Pollen 80	0.908	0.769	0.551	0.673	0.555	0.316
160	0.883	0.067	0.063	0.239	0.774	0.203
Hive Weight	**June**	**July**	**August**	**September**	**October**	
80	0.475	0.997	0.972	0.844	0.524	
160	0.615	0.448	0.574	0.626	0.894	

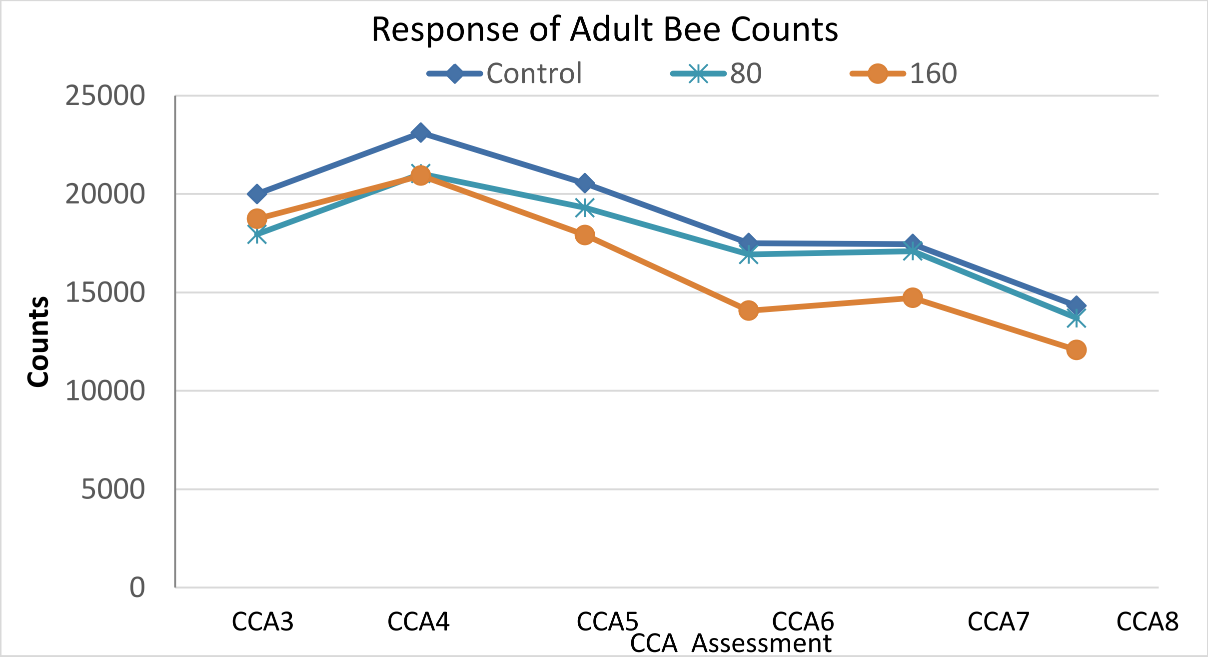
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 1. Mean number of adult bee cells in each treatment group measured at every CCA.

A) All Levels of Dose (ng/g)



B) 0, 80 , and 160 ng/g Dose Levels



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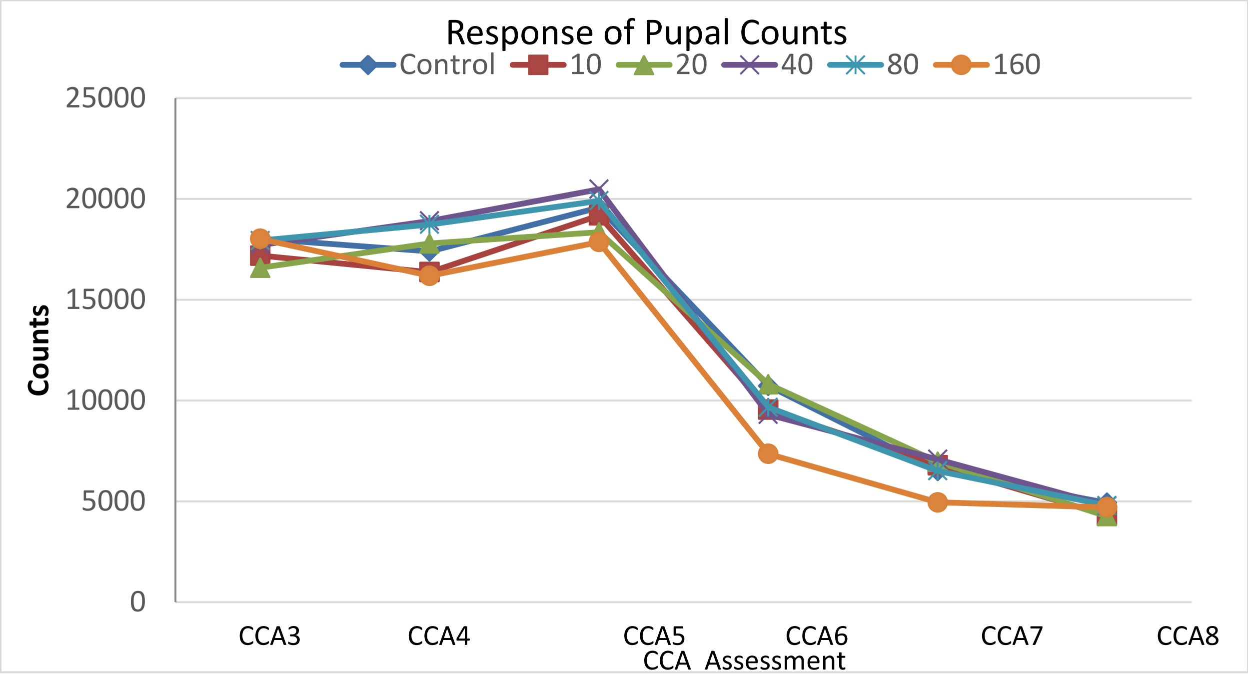
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

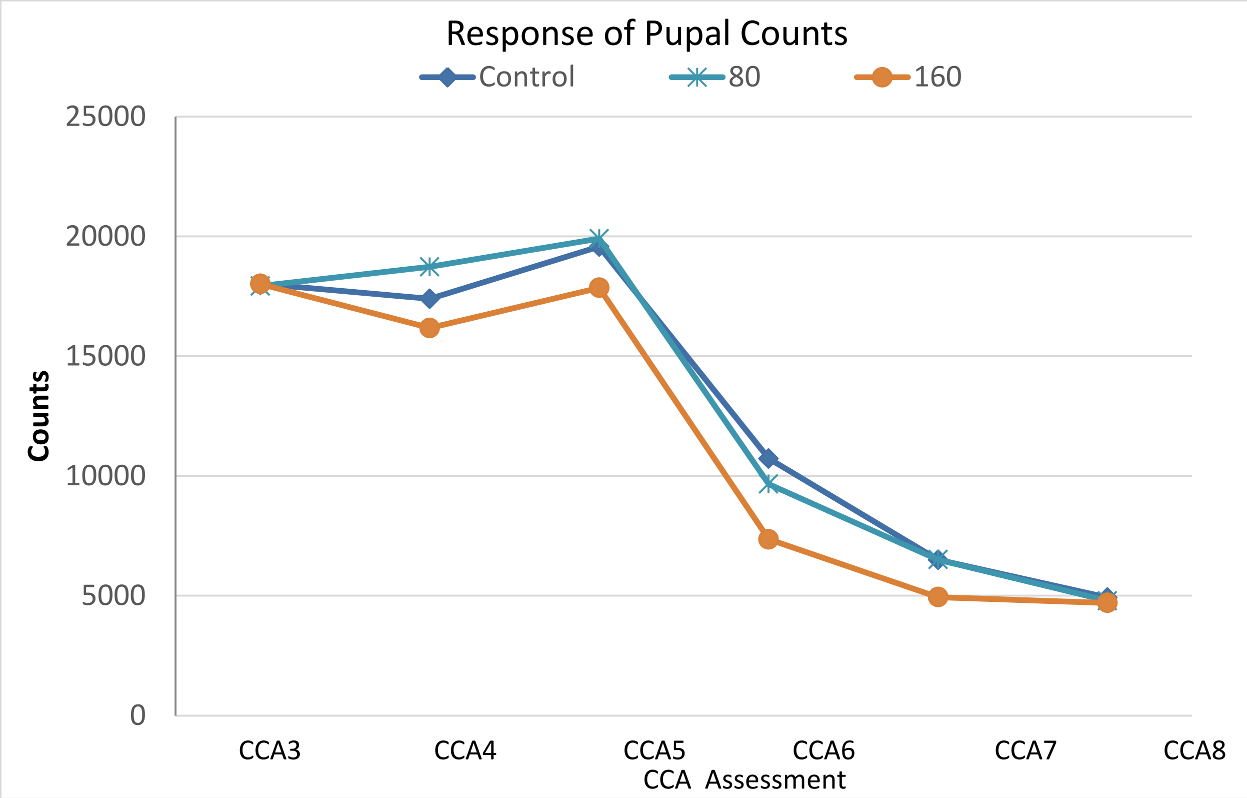
Figure 2. Mean number of pupal cells in each treatment group measured at every CCA.

A) All Levels of Dose (ng/g)



B) 0, 80 , and 160 ng/g Dose Levels

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document



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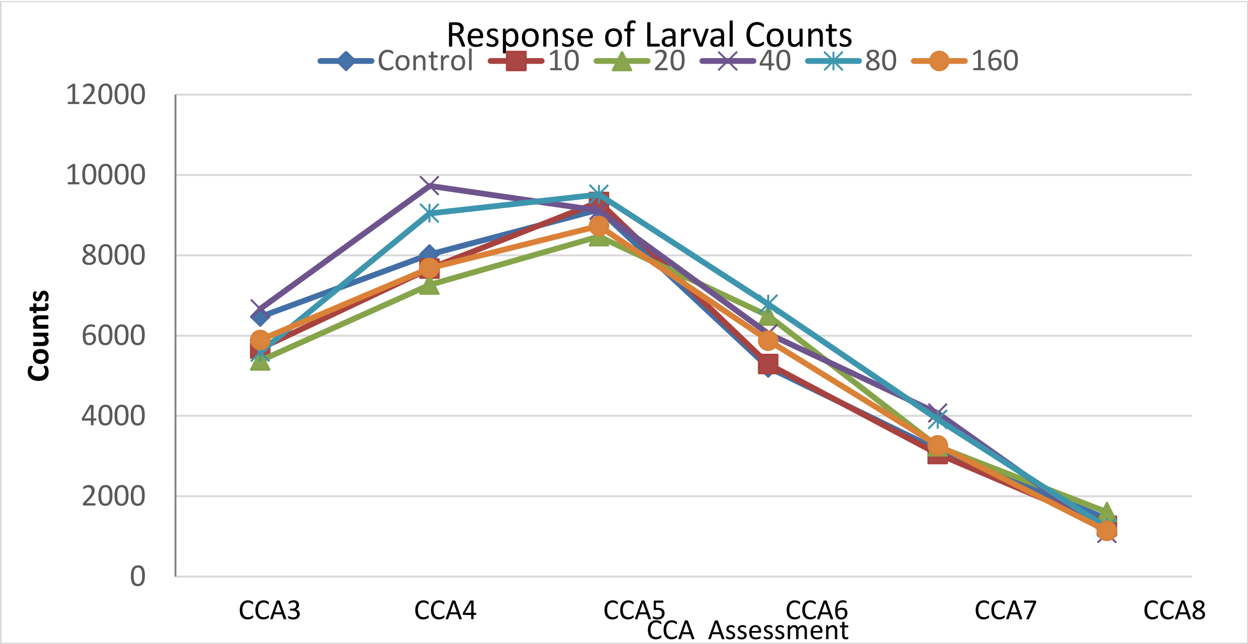
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 3. Mean number of larval cells in each treatment group measured at every CCA.

A) All Levels of Dose (ng/g)



B) 0, 80 , and 160 ng/g Dose Levels

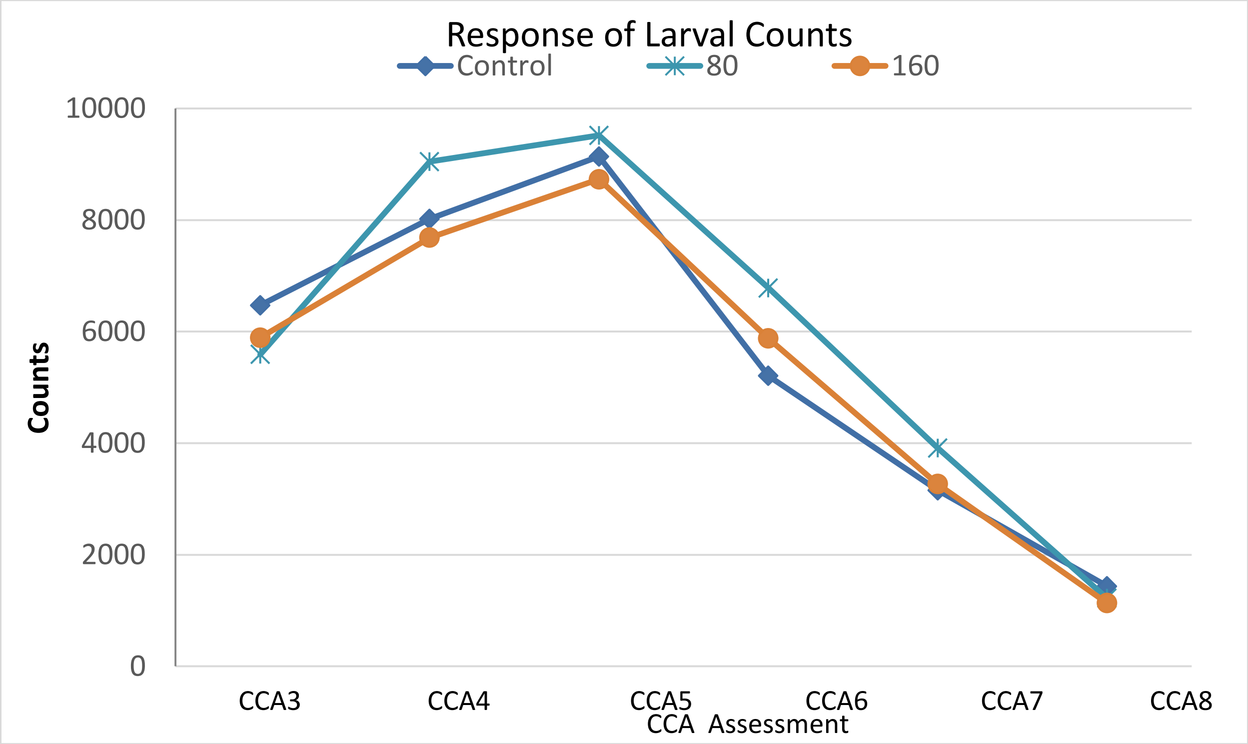
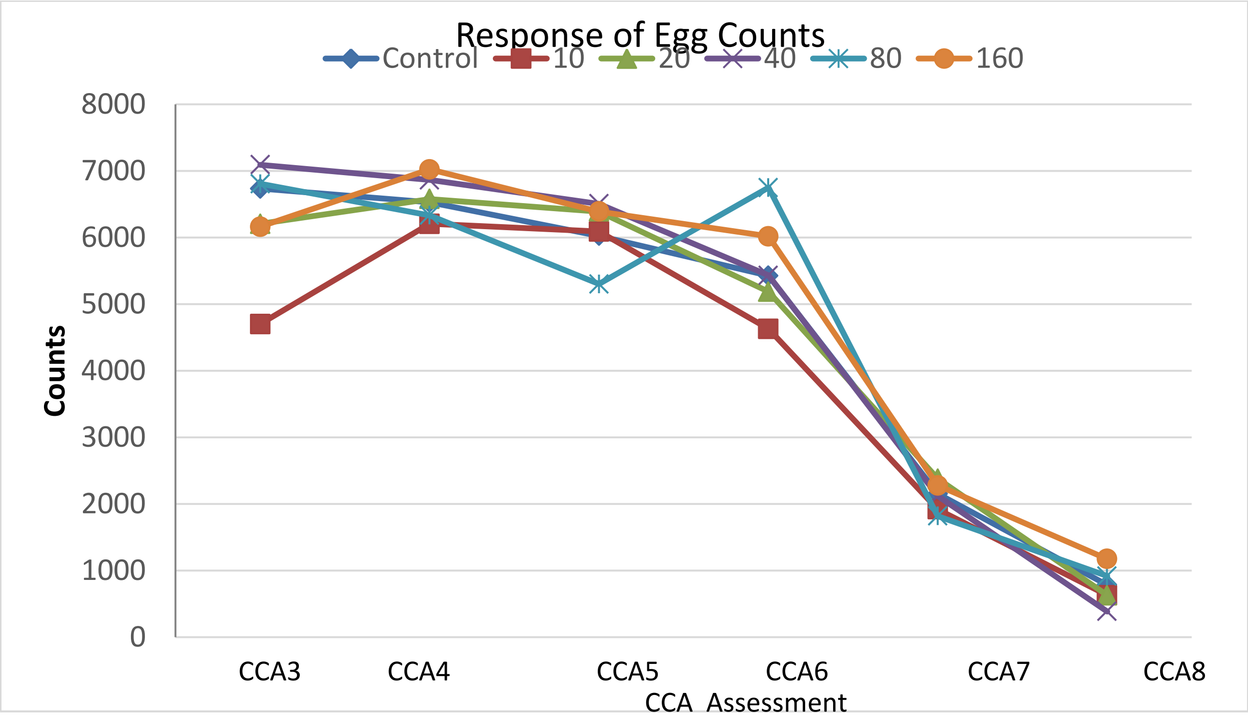
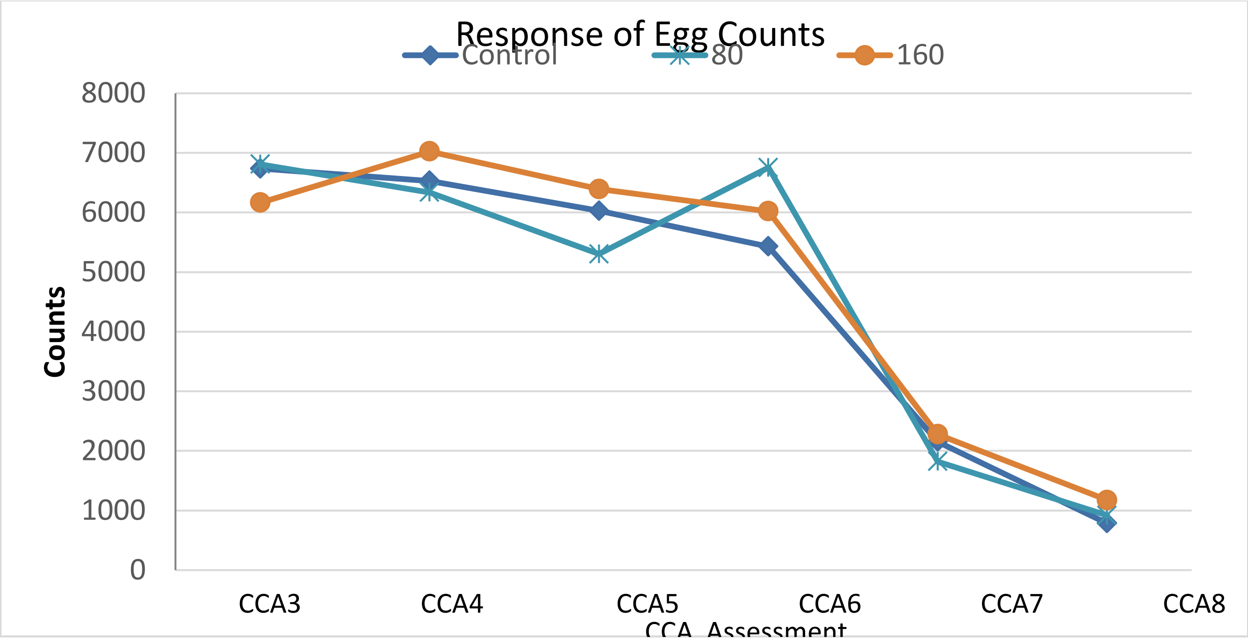


Figure 4. Mean number of egg cells in each treatment group measured at every CCA.

A) All Levels of Dose (ng/g)



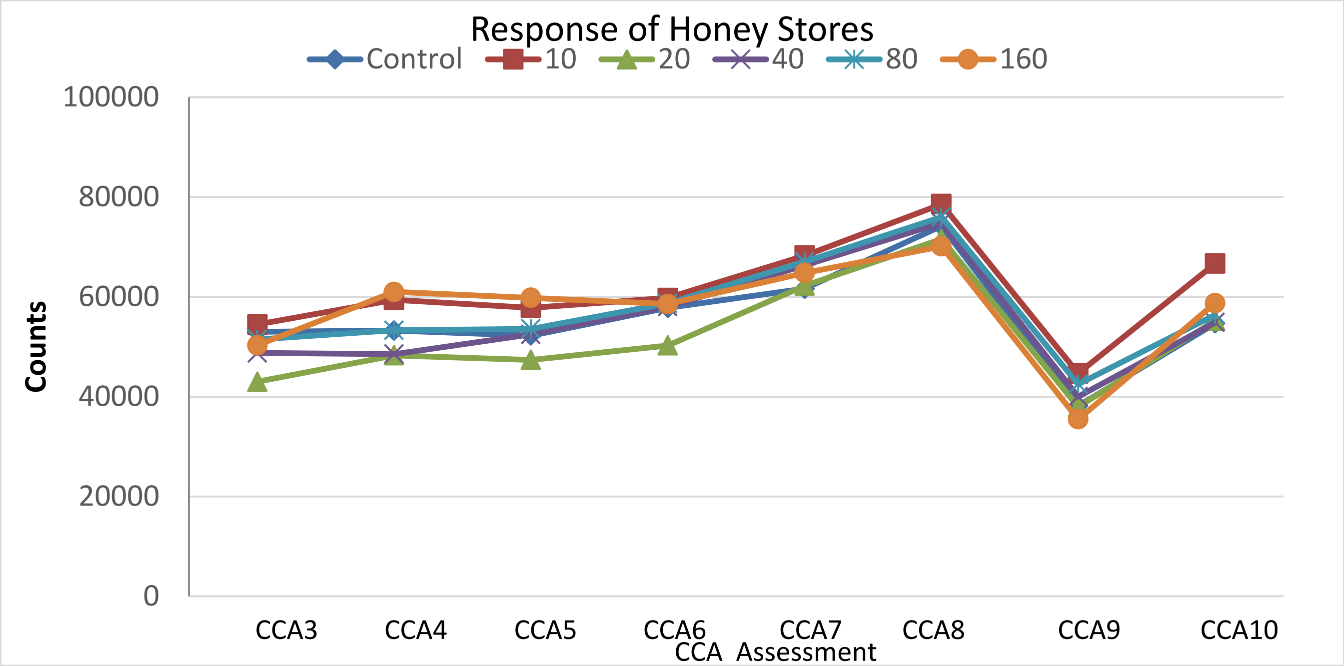
B) 0, 80 , and 160 ng/g Dose Levels



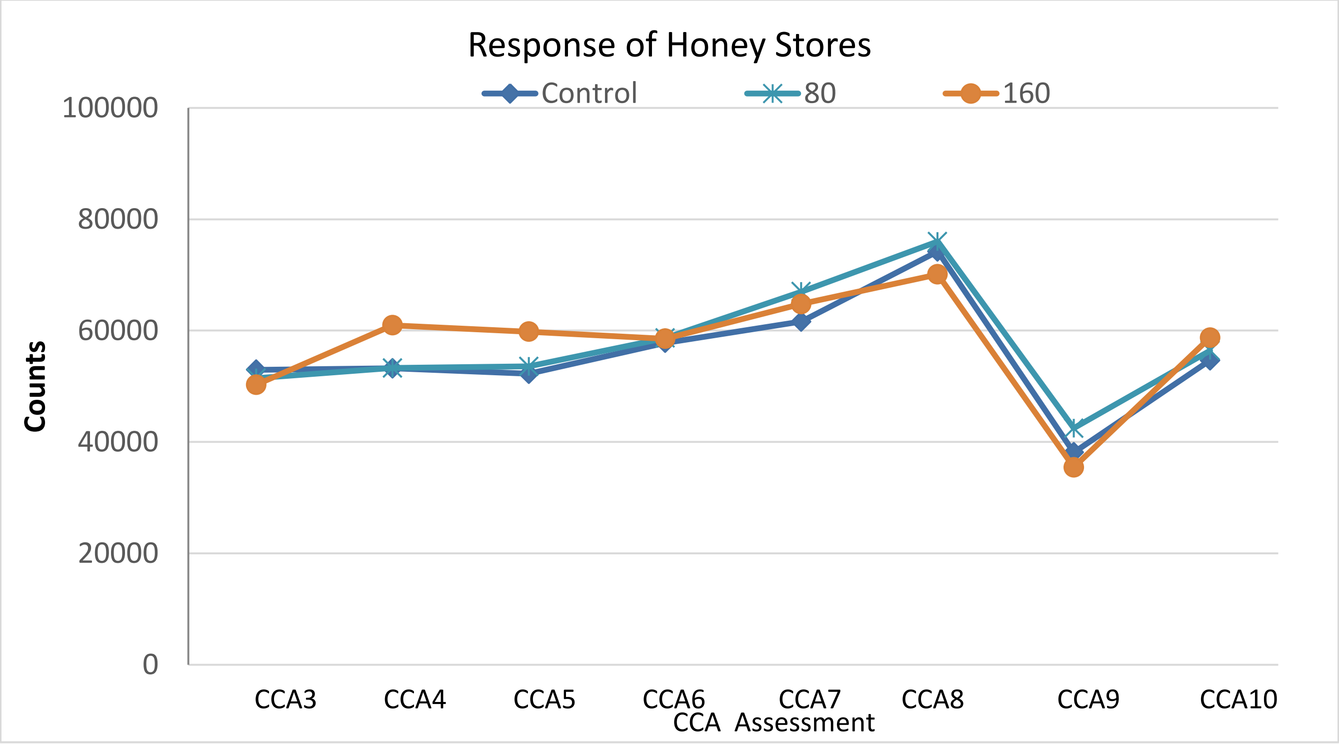
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 5. Mean number of honey cells in each treatment group measured at every CCA.

A) All Levels of Dose (ng/g)



B) 0, 80 , and 160 ug/g Dose Levels



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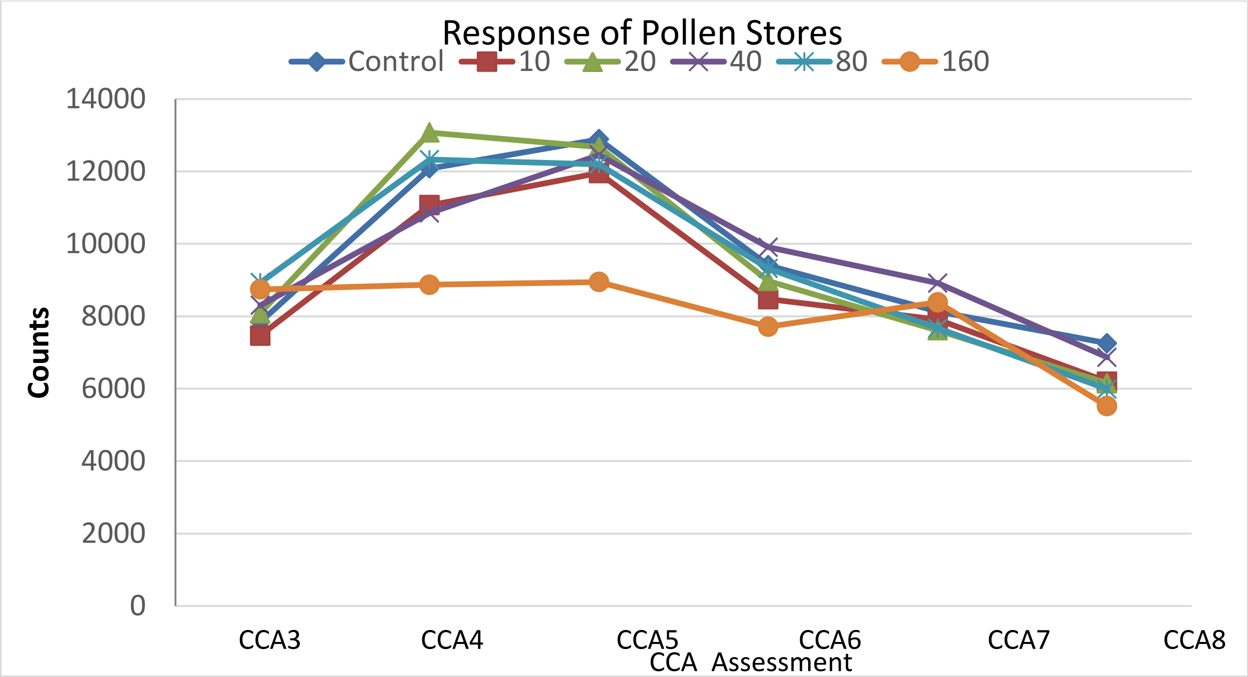
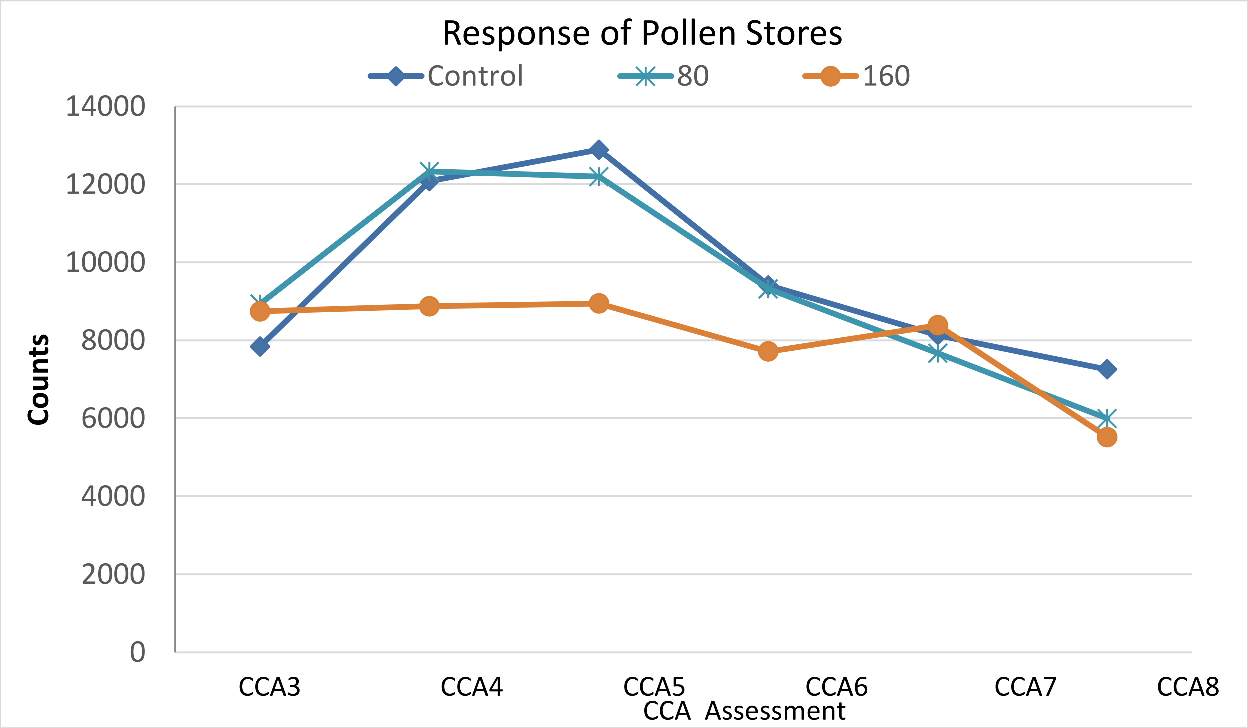
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 6. Mean number of pollen cells in each treatment group measured at every CCA.

A) All Levels of Dose (ng/g)



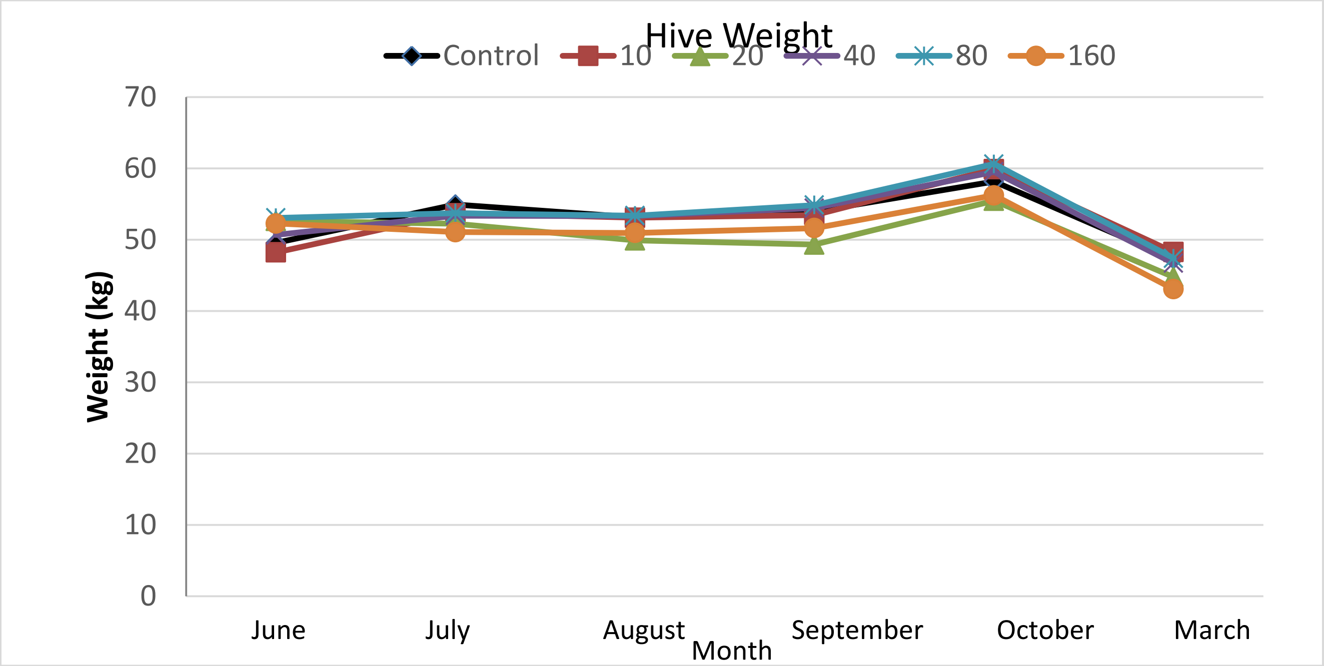
B) 0, 80 , and 160 ng/g Dose Levels



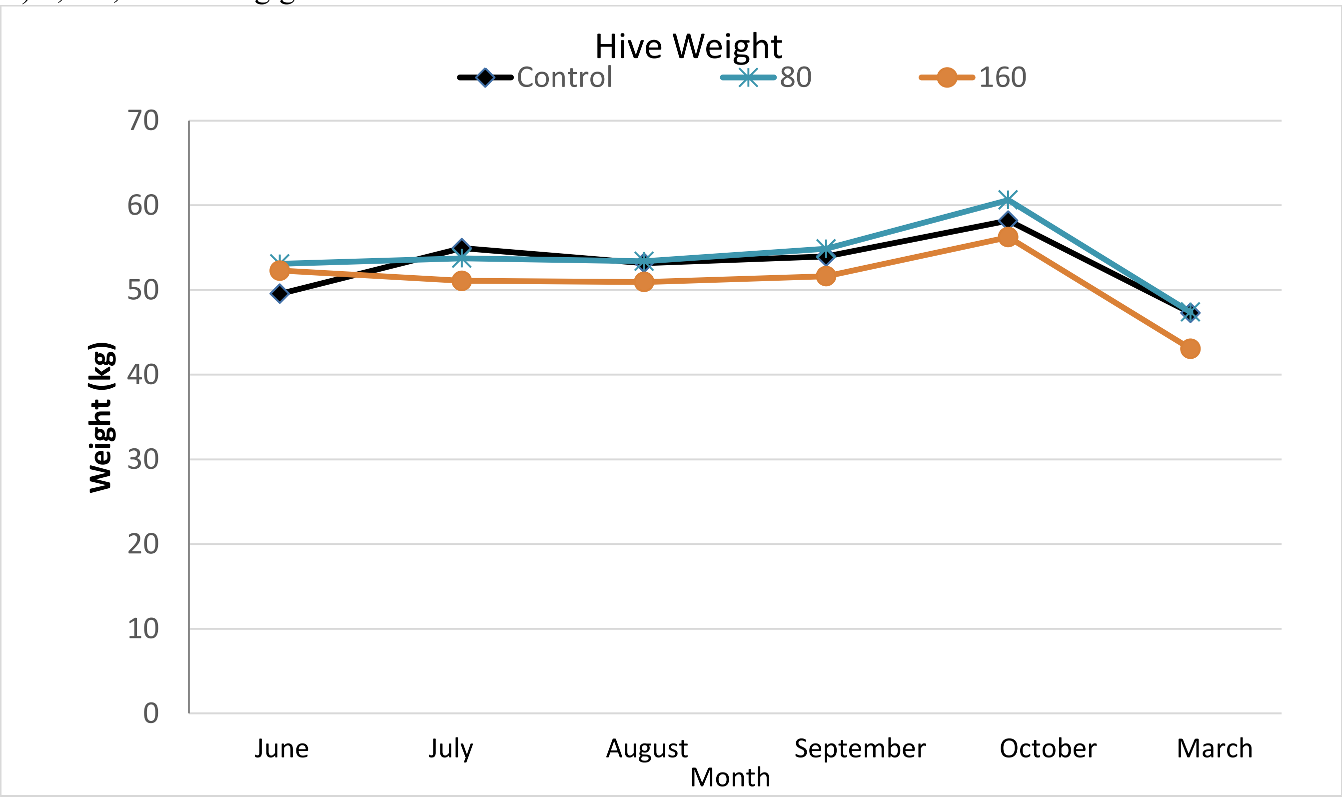
Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Figure 7. Weight of hives in each treatment group measured at every month.

A) All Levels of Dose (ng/g)



B) 0, 80 , and 160 ug/g Dose Levels



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APPENDIX A

Dinotefuran Feed Study

Correlation Statistics for Measurements Taken at each CCA for Each Variable

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table I-1. Pearson Correlation Coefficients for correlation of cell counts or hive weight measured between the CCAs or months.

A) Adult Bee Cells

Adult Bee Cells: Pearson Correlation Coefficients for Number Measured at Each CCA (N = 83)						
CCA	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
CCA3	1.000	0.689	0.555	0.410	0.384	0.125
		<.0001	<.0001	0.000	0.000	0.262
CCA4		1.000	0.801	0.691	0.656	0.361
			<.0001	<.0001	<.0001	0.001
CCA5			1.000	0.755	0.656	0.426
				<.0001	<.0001	<.0001
CCA6				1.000	0.901	0.746
					<.0001	<.0001
CCA7					1.000	0.770
						<.0001
CCA8						1.000

B) Pupal Cells

Pupal Cells: Pearson Correlation Coefficients for Number Measured at Each CCA (N = 83)						
CCA	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
CCA3	1.000	0.600	0.475	0.446	0.161	0.055
		<.0001	<.0001	<.0001	0.147	0.622
CCA4		1.000	0.734	0.628	0.373	0.104
			<.0001	<.0001	0.001	0.352
CCA5			1.000	0.583	0.299	0.279
				<.0001	0.006	0.011
CCA6				1.000	0.262	0.100
					0.017	0.369
CCA7					1.000	0.462
						<.0001
CCA8						1.000

Table 2. Continued.

C) Larval Cells

Larval Cells: Pearson Correlation Coefficients for Number Measured at Each CCA (N = 83)						
CCA	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
CCA3	1.000	0.429	0.375	0.380	0.257	0.006
		<.0001	0.001	0.000	0.019	0.960
CCA4		1.000	0.548	0.397	0.202	0.108
			<.0001	0.000	0.067	0.331
CCA5			1.000	0.389	0.254	0.045
				0.000	0.021	0.683
CCA6				1.000	0.271	0.012
					0.013	0.914
CCA7					1.000	0.121
						0.275
CCA8						1.000

D) Egg Cells

Egg Cells: Pearson Correlation Coefficients for Number Measured at Each CCA (N = 83)						
CCA	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
CCA3	1.000	0.542	0.384	0.320	0.072	0.136
		<.0001	0.000	0.003	0.518	0.222
CCA4		1.000	0.513	0.339	0.187	0.252
			<.0001	0.002	0.090	0.021
CCA5			1.000	0.365	0.308	0.148
				0.001	0.005	0.182
CCA6				1.000	0.306	0.119
					0.005	0.285
CCA7					1.000	0.063
						0.571
CCA8						1.000

Table 2 Continued.

E) Honey Cells

Honey Cells: Pearson Correlation Coefficients for Number Measured at Each CCA (N = 83)						
CCA	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
CCA3	1.000	0.829	0.736	0.701	0.597	0.568
		<.0001	<.0001	<.0001	<.0001	<.0001
CCA4		1.000	0.806	0.744	0.709	0.681
			<.0001	<.0001	<.0001	<.0001
CCA5			1.000	0.837	0.757	0.677
				<.0001	<.0001	<.0001
CCA6				1.000	0.857	0.817
					<.0001	<.0001
CCA7					1.000	0.934
						<.0001
CCA8						1.000

F) Pollen Cells

Pollen Cells: Pearson Correlation Coefficients for Number Measured at Each CCA (N = 83)						
CCA	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
CCA3	1.000	0.554	0.337	0.436	0.371	0.253
		<.0001	0.002	<.0001	0.001	0.021
CCA4		1.000	0.718	0.661	0.593	0.366
			<.0001	<.0001	<.0001	0.001
CCA5			1.000	0.631	0.485	0.357
				<.0001	<.0001	0.001
CCA6				1.000	0.685	0.575
					<.0001	<.0001
CCA7					1.000	0.747
						<.0001
CCA8						1.000

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table 2 Continued.

G) Hive Weight

Hive Weight: Pearson Correlation Coefficients for Number Measured at Each CCA (N = 84)						
Month	June	July	August	September	October	March
June	1.000	0.305	0.210	0.109	0.125	0.192
		0.005	0.055	0.323	0.257	0.081
July		1.000	0.535	0.460	0.364	0.136
			<.0001	<.0001	0.001	0.216
August			1.000	0.846	0.764	0.483
				<.0001	<.0001	<.0001
September				1.000	0.931	0.578
					<.0001	<.0001
October					1.000	0.635
						<.0001
March						1.000

APPENDIX B

Dinotefuran Feed Study

Mean Statistics for Response Variables Measured at Each CCA

Appendix 8. Data Evaluations for the Colony Feeding Studies that were Included in this Risk Determination Document

Table B-1. Adult Bees: Number of replicate hives (N), mean number of cells in each hive with adult bees (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the dinotefuran feeding study.

Dinotefuran Dose	Statistic	Number of Adult Bee Cells Measured at Each CCA									
		CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9	CCA10
0 ng/g	N	24	24	24	24	24	23	23	23	21	21
	Mean	5128.83	12377.6	19996	23117	20541	17504	17456.5	14312.9	12487.1	15931.5
	SD	1293.4	1947.21	3140.25	4198.16	4790.43	5598.87	6519.65	5458.08	5629.43	7560.55
10 ng/g	N	12	12	12	12	12	12	12	12	10	10
	Mean	5566.83	12475.2	18130.3	20496.6	18260.8	15242	16900.5	12866.3	13561.3	19291.3
	SD	1227.37	2009.03	3178.42	4431.82	4603.56	3767.35	6360.42	4582.8	6937.39	9216.13
20 ng/g	N	11	12	12	12	12	12	12	12	9	8
	Mean	5153.18	12489.1	18810.5	22905.2	18740.7	15792.7	15871	12830	11509.1	18768.4
	SD	1657.19	2010.08	5629.12	8118.46	7129.21	7102.8	7524.05	5384.53	6413.38	9396.52
40 ng/g	N	12	12	12	12	12	12	12	12	12	11
	Mean	5347.83	12465.8	19052.8	23189.3	19821.3	17473.5	17604	14282.4	11734.4	14457.7
	SD	1000.95	2070.98	4912.33	6847.72	5280.9	5880.49	7535.97	4819.93	7323.72	8483.29
80 ng/g	N	12	12	12	12	12	12	12	12	12	10
	Mean	5235.92	12530.8	17948.7	21032.3	19299.5	16928.4	17096.1	13690.9	12265.4	12834.7
	SD	1218.93	2157.97	3225.53	3932.31	4684.47	5413.23	6969.97	4974.02	7523.05	5611.61
160 ng/g	N	12	12	12	12	12	12	12	12	11	10
	Mean	5804.42	12600.8	18735.9	20934.5	17915.9	14068.3	14724.9	12069.8	10778.6	14539.6
	SD	1783.31	2388.2	4479.31	6477.22	5413.95	5428.6	7225.7	5646.75	6090.96	6676.96

Table B-2. Pupae: Number of replicate hives (N), mean number of cells in each hive with pupae (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the dinotefuran feeding study.

Dinotefuran Dose	Statistic	Number of Pupal Cells Measured at Each CCA									
		CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9	CCA10
0 ng/g	N	24	24	24	24	24	23	23	23	21	21
	Mean	9675	19543.5	18024.2	17393.5	19572.2	10723.8	6498.61	4920.7	15611	10721.3
	SD	2158.58	2494.48	4921.93	5030.83	5333.08	3276.88	2910.15	1805.25	5931.61	6854.22
10 ng/g	N	12	12	12	12	12	12	12	12	10	10
	Mean	9316.67	19034.7	17185.7	16368.7	19178	9546	6779.67	4300	15136	12968.8
	SD	1856.61	3933.39	4748.77	4783.97	4295.62	3192.23	3066.72	1557.53	7067.6	6384.47
20 ng/g	N	11	12	12	12	12	12	12	12	9	8
	Mean	8264.73	19235.3	16583.7	17787.7	18346.7	10807.3	6951.67	4257	14027.6	11266
	SD	2363.36	2967.22	5842.23	5805.75	9527.66	5809.16	2755.38	1974.94	6719.63	5688.27
40 ng/g	N	12	12	12	12	12	12	12	12	12	11
	Mean	10205.3	18203.3	17716	18905.7	20482.3	9302.33	7080.67	4773	13186.7	9647.64
	SD	1866	5529.76	6297.52	4698.1	4875.09	5770.43	3040.63	1685.45	8836.4	7308.87
80 ng/g	N	12	12	12	12	12	12	12	12	12	10
	Mean	9832.67	19894.7	17931	18733.7	19909	9660.67	6507.33	4787.33	15107.3	11885.2
	SD	2284	3592.46	4079.59	5077.22	5164.23	6106.2	3691.78	2310.92	8521.41	8692.18
160 ng/g	N	12	12	12	12	12	12	12	12	11	10
	Mean	9187.67	18834	18017	16182.3	17859.3	7353	4945	4701.33	13072	10578
	SD	2261.07	3701	3829.31	3990.49	4237.2	3396.83	2470.98	2362.14	8138.34	8507.48

Table B-3. Lavae: Number of replicate hives (N), mean number of cells in each hive with lavae (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the dinotefuran feeding study

Dinotefuran Dose	Statistic	Number of Larval Cells Measured at Each CCA									
		CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9	CCA10
0 ng/g	N	24	24	24	24	24	23	23	23	21	21
	Mean	4765.83	7324.33	6471.5	8019.5	9138.5	5204.87	3155.83	1435.83	8968.57	7838.29
	SD	1253.18	1782.37	2640.27	2929.85	3703.3	2760.4	1723.6	873.357	3322.89	5952.19
10 ng/g	N	12	12	12	12	12	12	12	12	10	10
	Mean	4773	7582.33	5676	7668.33	9331	5289	3053	1261.33	8101.2	10130.8
	SD	1207.62	2128.73	2524.68	2258.69	2577.52	2804.4	1645.07	794.434	3554.16	4039.01
20 ng/g	N	11	12	12	12	12	12	12	12	9	8
	Mean	4581.45	6794	5375	7267	8471	6493	3239.33	1605.33	8332.44	9804
	SD	1445.77	2179.97	2500.19	2501.27	4257.02	3390.49	1840.6	606.266	3839.41	4701.43
40 ng/g	N	12	12	12	12	12	12	12	12	12	11
	Mean	5805	6980.33	6665	9732.33	9116	6048.67	4070.67	1075	7123.67	4941.09
	SD	1405.25	3092.92	2588.98	1786.66	2354.92	3909.04	2113.36	668.672	3897.76	5395.48
80 ng/g	N	12	12	12	12	12	12	12	12	12	10
	Mean	5733.33	6822.67	5590	9044.33	9517.33	6779.67	3913	1218.33	7252.67	5228.8
	SD	2153.7	2226.16	2783.58	2181.15	3395.41	3716.92	2405.9	899.612	4116.15	5282.89
160 ng/g	N	12	12	12	12	12	12	12	12	11	10
	Mean	4672.67	6478.67	5891	7682.67	8729	5876.67	3268	1132.33	7912	7671.2
	SD	1601.51	1880.36	3093.5	2257.35	1935.04	2500.95	2071.8	929.027	4601.76	6486.12

Table A-4. Eggs: Number of replicate hives (N), mean number of cells in each hive with eggs (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the dinotefuran feeding study

Dinotefuran Dose	Statistic	Number of Egg Cells Measured at Each CCA									
		CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9	CCA10
0 ng/g	N	24	24	24	24	24	23	23	23	21	21
	Mean	4722.83	7009	6736.67	6528.83	6027.17	5429.22	2153.74	785.217	4824.19	4938.86
	SD	1346.69	2448.39	2727.43	2507.68	2680.25	2555.82	958.876	870.608	2237.64	3707.84
10 ng/g	N	12	12	12	12	12	12	12	12	10	10
	Mean	4027.67	6292.33	4701.33	6206.33	6091.67	4629.67	1920.67	630.667	5366.4	5504
	SD	1181.36	2039.7	2182.23	2296.47	1774.58	2071.1	1280.5	504.581	2732.58	2193.7
20 ng/g	N	11	12	12	12	12	12	12	12	9	8
	Mean	4597.09	7338.67	6206.33	6579	6392.67	5188.67	2379.33	630.667	5140.89	5547
	SD	982.332	2235.8	3274.54	3433.84	3206.8	2324.85	1664.1	410.535	2740.48	3139.04
40 ng/g	N	12	12	12	12	12	12	12	12	12	11
	Mean	4902	7023.33	7095	6865.67	6507.33	5432.33	2121.33	387	5246	3502.55
	SD	1697.18	2046.77	1973.58	2296.47	2818.47	2113.52	794.434	452.848	3540.56	4262.18
80 ng/g	N	12	12	12	12	12	12	12	12	12	10
	Mean	4013.33	7467.67	6808.33	6335.33	5303.33	6751	1820.33	917.333	4945	2855.2
	SD	1203.63	3807.71	3856.13	3001.46	2719.39	5354.96	1103.68	871.905	2995.45	3751.02
160 ng/g	N	12	12	12	12	12	12	12	12	11	10
	Mean	4515	7396	6163.33	7023.33	6392.67	6020	2279	1175.33	4237.45	4506.4
	SD	2027.35	1710.59	3890.42	2752.81	2140.55	2516.14	1426.15	842.092	2960.47	4454.18

Table B-5. Honey: Number of replicate hives (N), mean number of cells in each hive with honey (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the dinotefuran feeding study

Dinotefuran Dose	Statistic	Number of Cells Containing Honey Measured in Hives at Each CCA									
		CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9	CCA10
0 ng/g	N	24	24	24	24	24	23	23	23	21	21
	Mean	11538.3	32386.2	52961.7	53234	52237.8	57784.5	61628.3	74206.8	38143	54671.4
	SD	4080.34	14475.1	19613.8	21175.6	18004.7	19435.1	21043.2	20641.8	15393.8	20204.7
10 ng/g	N	12	12	12	12	12	12	12	12	10	10
	Mean	12719.7	32995.3	54452.3	59368.7	57820.7	59755.7	68241	78561	44651.2	66650
	SD	3993.52	12054.7	17139.9	18484.7	17495.8	18457	14130.5	16307.6	16195.3	32580.6
20 ng/g	N	11	12	12	12	12	12	12	12	9	8
	Mean	10929.8	31863	42985.7	48260.3	47371.7	50238.3	62221	71566.3	37916.4	55212
	SD	4881.51	11043.9	11430.5	13659.5	18287.6	18983.8	17228.3	15359.4	12115.1	26407.5
40 ng/g	N	12	12	12	12	12	12	12	12	12	11
	Mean	11051	31605	48762	48532.7	52460	57992.7	66334.7	74848.7	39990	54852.4
	SD	3590.81	5936.15	17575.5	13165.7	12092.2	15014.7	16914.3	14820.7	14498.3	27499.8
80 ng/g	N	12	12	12	12	12	12	12	12	12	10
	Mean	8571.33	31605	51485.3	53291.3	53578	58666.3	66994	75995.3	42484	56416
	SD	2124.15	12436.5	16061.2	16622.7	12317.7	19425.6	19161.7	22149	15720.5	30542.6
160 ng/g	N	12	12	12	12	12	12	12	12	11	10
	Mean	12154.7	32766	50295.7	60974	59798.7	58537.3	64786.7	70104.3	35463.3	58738
	SD	3943.63	11300.8	15397.9	17658.8	15406.7	16563.6	18071.1	18756.6	10548.5	24326.8

Table B-6. Pollen: Number of replicate hives (N), mean number of cells in each hive with pollen (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the dinotefuran feeding study

Dinotefuran Dose	Statistic	Number of Cells Containing Pollen Measured in Hives at Each CCA									
		CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9	CCA10
0 ng/g	N	24	24	24	24	24	23	23	23	21	21
	Mean	2142.83	4973.67	7840.33	12083	12885.7	9407.65	8131.65	7253.91	17724.2	20541.7
	SD	784.079	3012.15	3641.94	4280.14	5302.59	4412.19	3690.99	3925.51	11117.3	9024.44
10 ng/g	N	12	12	12	12	12	12	12	12	10	10
	Mean	2594.33	6765.33	7467.67	11065.3	11954	8472.33	7912	6192	15462.8	22618
	SD	1712.88	3270.33	3898.45	4088.94	4962.05	4152.93	3572.7	2978.22	7421.71	9283.49
20 ng/g	N	11	12	12	12	12	12	12	12	9	8
	Mean	2517.45	5618.67	8084	13072	12670.7	8972.67	7611	6149	12938.2	20769
	SD	997.816	2821.8	4004.31	5108.67	6830.31	5015.6	5296.38	3853.81	6983.38	7532.03
40 ng/g	N	12	12	12	12	12	12	12	12	12	11
	Mean	3526	5575.67	8299	10850.3	12455.7	9904.33	8915.33	6865.67	14390.7	17309.5
	SD	1304.77	1812.07	3407.89	3263.85	4586.28	4351.58	4667.88	4708.96	8735.39	9007.7
80 ng/g	N	12	12	12	12	12	12	12	12	12	10
	Mean	2623	6134.67	8929.67	12326.7	12197.7	9316.67	7668.33	5991.33	15580.3	20622.8
	SD	981.579	4537.87	4681.47	5406.55	6680.26	5144.78	3746.47	4514.99	11788.6	10407.1
160 ng/g	N	12	12	12	12	12	12	12	12	11	10
	Mean	2752	4687	8743.33	8872.33	8944	7711.33	8385	5518.33	14776.4	19797.2
	SD	1348.36	2690.85	3421.84	4906.43	5731.4	3844.52	5285.71	4257.86	9885.27	8598.26

Table B-7. Hive Weight: Number of replicate hives (N), mean number of cells in each hive with pollen (Mean), and standard deviation for the number of cells measured at each CCA (SD) at each treatment level in the dinotefuran feeding study

Dinotefuran Dose	Statistic	Weight of Hives Measured at Each Month					
		June	July	August	September	October	March
0 ng/g	N	22	23	24	23	23	23
	Mean (kg)	49.5464	54.9398	53.1717	53.95815	58.1913	47.2969
	SD (kg)	9.29501	7.9595	7.12342	8.656816	10.3452	8.98094
10 ng/g	N	12	12	12	12	12	10
	Mean (kg)	48.2321	53.732	53.0841	53.48075	59.9183	48.2816
	SD (kg)	10.4867	7.62297	7.48246	7.493645	6.75777	7.70813
20 ng/g	N	11	12	12	12	12	11
	Mean (kg)	52.6477	52.2401	49.9195	49.32529	55.4768	44.7787
	SD (kg)	8.19024	4.37797	4.9698	8.233907	7.7581	8.72248
40 ng/g	N	9	12	11	11	11	10
	Mean (kg)	50.6675	53.3843	53.3459	54.43734	59.5305	46.7136
	SD (kg)	12.8747	5.09707	4.55789	6.000668	8.33407	7.42551
80 ng/g	N	12	12	12	12	12	12
	Mean (kg)	53.0802	53.7356	53.3899	54.86597	60.6274	47.3962
	SD (kg)	11.7014	6.19684	6.45758	7.542952	9.111	9.20402
160 ng/g	N	12	12	12	12	12	11
	Mean (kg)	52.2784	51.0845	50.954	51.62743	56.2386	43.0622
	SD (kg)	13.5021	8.55456	7.1154	6.600616	7.18878	4.59698

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

The following tables present the foliar and soil residue studies that were considered for use in this Risk Determination Document. For reviews of the studies that were found to be acceptable, refer to Appendix 10.

Imidacloprid Soil or Foliar Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
8 – Fruiting Vegetables	Tomato (EBNTN012)	1 soil application at 0.376 lb ai/A and 2 foliar applications at 0.06 lb ai/A/each. Total app. rate = 0.5 lbs ai/A.	Acceptable
8 – Fruiting Vegetables	Tomato (EBNTL056-05)	2 soil applications at 0.13 lb ai/A each OR 1 soil application at 0.18 lb ai/A/each.	Not acceptable for use in this risk determination document. Study was not conducted at the maximum annual application rate.
9 – Cucurbit Vegetables	Melon (EBNTL056-02)	Soil application at 0.29-0.36 lb ai/A in 2011 (year prior to sampling) and in previous years.	Not acceptable for use in this risk determination document. Bee-collected matrices (tented) including pollen from pollen traps and hive deposited nectar. In addition, applications could have been made a lot closer to bloom according to label.
10 – Citrus	Citrus (EBNTL056-7)	5 different soil treatment trials testing a variety of citrus crops. Soil applications were made at 1x and 2x the maximum annual application rate. Trials with applications made at the maximum annual application rate were used in this risk determination document. Data was not separated by crop due to poor replication for statistical analysis.	Acceptable Only data from applications made at the maximum annual application rate were included in this risk determination document. Data was not separated by crop due to poor replication for statistical analysis.

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

Imidacloprid Soil or Foliar Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
10 – Citrus	Orange (EBNTY007)	2 foliar applications at 0.25 lb ai/A each. Total app. rate = 0.5 lbs ai/A.	Acceptable for 2/3 sites which were conducted at the max annual foliar application rate.
11 – Pome Fruits	Apple (EBNTN014)	1 soil at 0.38 lb ai/A + 2 foliar applications at 0.06 lb ai/A/each. Total app. rate = 0.5 lbs ai/A.	Acceptable
12 – Stone Fruits	Cherry (EBNTY008)	5 x 0.1 lbs ai/A foliar applications. Total app. rate = 0.5 lbs ai/A.	Acceptable
12 – Stone Fruits	Stone fruit (EBNTN013)	One soil (0.38 lb ai/A) and two foliar applications (0.06 lb ai/A/each) Total app. rate = 0.5 lbs ai/A. Multiple stone fruit crops tested. However, data was not be separated by crop due to poor replication for statistical analysis.	Acceptable However, data was not be separated by crop due to poor replication for statistical analysis.
13 – Berries	Blueberry (EBNTY006)	1 soil application at 0.5 lb ai/A.	Acceptable
13 – Berries	Strawberry (EBNTL056-04)	1 soil application at 0.5 lb ai/A.	Acceptable

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

Imidacloprid Soil or Foliar Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
20 – Oilseed	Cotton (EBNTY010)	Seed treatment (0.375 mg ai/seed or 0.047 lb ai/A) and 5 x 0.06 lb ai/A foliar applications.	Acceptable
20 – Oilseed	Cotton (EBNTN011)	1 soil application (0.34 lb ai/A) and 3 foliar applications (0.058 lb ai/A/each). The total seasonal application rate was 0.5 lbs ai/A.	Acceptable
20 – Oilseed	Cotton (EBNTL056-01)	1 foliar app at 0.063 lb ai/A made during flowering.	Not acceptable for use in this risk determination document. Study was not conducted at the maximum annual application rate.

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

Clothianidin Foliar and Soil Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
1 – Root and Tuber Vegetables	Potato (VP-38985)	Trial 1: 1 soil application at 0.2 lb ai/A. Trial 2: foliar application at 0.05 lb ai/A.	Acceptable Trial 2 is excluded from analysis as it was not conducted at the maximum annual application rate.
9 – Cucurbit Vegetables	Cucurbit (VP-38938)	1 soil application at 0.20 lb ai/A.	Acceptable Data was broken up by crop (pumpkin, cucumber, squash and melon) for crop-specific analysis.
9 – Cucurbit Vegetables	Pumpkin (VP-38263)	1 soil application at 0.2 lb/ai/A. Second soil application was made at 3/9 sites a month later (this data is not included because 2nd application is not permitted by the label).	Acceptable Data for 2nd soil application not included as 2nd application is not permitted by the label.
9 – Cucurbit Vegetables	Pumpkin (VP-38313)	2 pre-bloom foliar applications at 0.0935 lb ai/A/application (0.18 lb ai/A/season).	Acceptable
9 – Cucurbit Vegetables	Pumpkin (VP-38971)	TRT-2: 1 pre-emergent soil application at 0.2 lb ai/A. TRT-3: 1 post-emergent soil application at 0.2 lb ai/A. TRT-4: 1 foliar application at 0.1 lb ai/A.	Acceptable Only TRT-3 included for analysis in the risk determination document as this treatment represented a worst-case application.
9 – Cucurbit Vegetables	Cantaloupe (VP-39242)	1 soil application at 0.2 lb ai/A. Bee-collected matrices (pollen traps and bee stomachs) in 3 plots, flower collected matrices in 1 plot.	Not acceptable for use in this risk determination.

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

Clothianidin Foliar and Soil Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
10 – Citrus Fruit	Citrus (VP-38685)	1 soil application at 0.6 g ai/tree. Applications were made at different times in relation to bloom for each plot (anywhere from 21 days – 6 months prior to sample collection.	Not acceptable for use in this risk determination. Clothianidin is not registered on Citrus in California.
10 – Citrus Fruit	Citrus (VP-38980)	TRT-2, TRT-3, and TRT-4 had soil applications at 0.59 g ai/tree/application at 6, 3, and 1 month before bloom, respectively. TRT-5 had soil applications at both 6 and 1 month before bloom.	Not acceptable for use in this risk determination. Clothianidin is not registered on Citrus in California.
10 – Citrus Fruit	Citrus (VP-39259)	Clothianidin applied in 2 soil applications at 0.6 g ai/tree/application. Multiple neonicotinoids were applied in the 9 months prior to test initiation.	Not acceptable for use in risk determination. Clothianidin is not registered on Citrus in California.
10 – Pome Fruits	Apple (VP-38552)	1 post-bloom foliar application at 0.1874 lb ai/A.	Acceptable
12 – Stone Fruits	Peach (VP-38563)	2 post-bloom foliar applications at 0.1 lb ai/A/application.	Acceptable

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

Clothianidin Foliar and Soil Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
13 – Berries	Grape (VP-38992)	3 trials. TRT-2: post-bloom foliar application at 0.1 lb ai/A. TRT-3: Pre-bloom soil application at 0.2 lb ai/A. TRT-4: pre-bloom foliar application at 0.1 lb ai/A	Acceptable TRT-2 and TRT-4 are excluded for analysis as they were not conducted at the maximum annual application rate.
14 – Tree Nuts	Almond (VP-38473)	2 post-bloom foliar applications at 0.1 lb. ai/A/application.	Acceptable
15 – Cereal Grains	Corn (VP-39240)	1 Soil Application at 0.2 lb ai/A.	Not acceptable for use in risk determination. Clothianidin is not registered for soil applications to corn in California.
15 – Cereal Grains	Corn (VP-39071)	1 Soil Application at 0.2 lb ai/A.	Not acceptable for use in risk determination. Clothianidin is not registered for soil applications to corn in California.
15 – Cereal Grains	Corn (VP-39234)	1 soil application at planting (0.16 lb ai/A).	Not acceptable for use in risk determination. Clothianidin is not registered for soil applications to corn in California.
15 – Cereal Grains	Corn (VP-39422)	1 soil application at planting (0.20 lb ai/A).	Not acceptable for use in risk determination. Clothianidin is not registered for soil applications to corn in California.

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

Clothianidin Foliar and Soil Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
20 – Oilseed	Cotton (VP-38259)	2 foliar applications at 0.1 lb. ai/A/application.	Acceptable
20 – Oilseed	Cotton (EBNIN115)	1 pre-bloom foliar application at 0.085 lb. ai/A.	Not acceptable for use in risk determination. Study was not conducted as maximum annual application rate.

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

Thiamethoxam Foliar and Soil Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
6 – Legume Vegetables (Succulent or Dried)	Soybean (TK0250070)	Two foliar applications at 0.063 lbs ai/A for a total annual rate of 0.126 lbs ai/A.	Acceptable
8 – Fruiting Vegetables	Pepper (TK0236306)	One soil application at a rate of 0.172 lbs ai/A.	Acceptable
8 – Fruiting Vegetables	Tomato (TK0025811)	One soil application at a rate of 0.078 lbs ai/A or 0.172 lbs ai/A.	Not acceptable for use in this risk determination. Samples of whole flowers were taken but not samples of pollen.
8 – Fruiting Vegetables	Tomato (TK0222531)	Two foliar applications at 0.086 lbs ai/A for a total annual rate of 0.172 lbs ai/A.	Acceptable
8 – Fruiting Vegetables	Tomato (TK0242072)	One soil application at a rate of 0.125 lbs ai/A or 0.172 lbs ai/A.	Acceptable. Only data from the maximum rate allowed by the label were included in statistical analysis.
9 – Cucurbit Vegetables	Cucumber (TK0024668)	One soil application at 0.172 lbs ai/A.	Acceptable
9 – Cucurbit Vegetables	Cucumber (TK0222532)	Two foliar applications at 0.086 lbs ai/A for total annual rate of 0.172 lbs ai/A.	Acceptable

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

Thiamethoxam Foliar and Soil Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
9 – Cucurbit Vegetables	Cucurbits (Pumpkin) (TK0222530)	One soil treatment at a rate of 0.125 lbs ai/A or 0.172 lbs ai/A.	Acceptable From a study with multiple cucurbit crops. Only data from the maximum rate allowed by the label were included in statistical analysis.
9 – Cucurbit Vegetables	Pumpkin (TK0242074)	Two foliar applications at a rate of 0.023 lbs ai/A or 0.086 lbs ai/A. Total annual rates were 0.046 lbs ai/A or 0.172 lbs ai/A.	Acceptable Only data from the maximum rate allowed by the label were included in statistical analysis.
9 – Cucurbit Vegetables	Cucurbits (Muskmelon) (TK0222530)	One soil treatment at a rate of 0.0858 lbs ai/A or 0.172 lbs ai/A.	Acceptable From a study with multiple cucurbit crops. Only data from the maximum rate allowed by the label were included in statistical analysis.
9 – Cucurbit Vegetables	Cucurbits (Summer Squash) (TK0222530)	One soil treatment at a rate of 0.172 lbs ai/A.	Acceptable From a study with multiple cucurbit crops.
10 – Citrus Fruit	Orange (TK0124743)	One soil application at a rate of 0.086, 0.172, or 0.558 lbs ai/A for the first year. For the second year, one soil application was made at a rate of 0.256 lbs ai/A.	Not acceptable for use in this risk determination. The maximum label rate was only investigated after the first yearin navel oranges, which do not produce pollen. After the first year, only one rate was used, which was significantly higher than the maximum application rate allowed.
10 – Citrus Fruit	Citrus (TK0124745)	One soil application at a rate of 0.086, 0.129, 0.172, 0.257, or 0.556 lbs ai/A.	Not acceptable for use in this risk determination. Classified as supplemental per study DER due to only one geographical location used. Not included in the risk determination as other acceptable studies were available that also assessed soil applications to citrus.

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

Thiamethoxam Foliar and Soil Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
10 – Citrus Fruit	Citrus (TK0177221)	One soil application at 0.172 lbs ai/A.	Acceptable
10 – Citrus Fruit	Sweet Orange (TK0250069)	One or two foliar applications at 0.086 lbs ai/A. Total annual rates were 0.086 lbs ai/A or 0.172 lbs ai/A.	Acceptable Only data from the maximum rate allowed by the label were included in statistical analysis.
11 – Pome Fruits	Apple (TK0250071)	One foliar application at 0.086 lbs ai/A.	Acceptable with limitations. The application rate is lower than the maximum rate permitted by the label, but residue concentrations still exceed the NOEC by a wide margin. In addition, lower than acceptable recoveries in nectar indicate that actual values may be even higher than reported.
12 – Stone Fruits	Stone Fruit (peach, plum, and sweet cherry) (TK0177222)	Two foliar applications at 0.086 lb ai/A for a total annual rate of 0.172 lbs ai/A.	Acceptable
13 – Berries	Strawberry (TK0177224)	Three foliar applications at 0.063 lbs ai/A for a total annual rate of 0.189 lbs ai/A.	Acceptable
13 – Berries	Strawberry (TK0250068)	One soil application at either 0.129 lbs ai/A or at 0.188 lbs ai/A.	Acceptable Only data from the maximum rate allowed by the label were included in statistical analysis.

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

Thiamethoxam Foliar and Soil Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
13 – Berries	Cranberry (TK0236307)	Three foliar applications at a rate of 0.0626 lbs ai/A for a total annual rate of 0.188 lbs ai/A.	Acceptable
13 – Berries	Blueberry (TK0250072)	One or three foliar applications at a rate of 0.063 lbs ai/A. Total annual rates were either 0.063 lbs ai/A or 0.188 lbs ai/A.	Acceptable Only data from the maximum rate allowed by the label were included in statistical analysis.
15 – Cereal Grains	Corn (TK0258214)	Seed treatment (1.25 mg ai/seed) and two foliar applications at a rate of 0.043 lbs ai/A or 0.063 lbs ai/A. Total annual foliar rates were either 0.086 lbs ai/A or 0.126 lbs ai/A.	Acceptable Foliar applications were made to corn grown from treated seeds. Only data from the maximum rate allowed by the label were included in statistical analysis.
20 – Oilseed	Cotton (TK0177223)	Two foliar applications at a rate of 0.063 lbs ai/A for two years.	Acceptable

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

Dinotefuran Soil or Foliar Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
1 – Root and Tuber Vegetables	Potato (10934.4100)	One soil application at 0.38 lbs ai/A.	Acceptable
8 – Fruiting Vegetables	Tomato (10934.4103)	Two soil applications at 0.206 & 0.330 lbs ai/A or two foliar applications at 0.089 & 0.179 lbs ai/A.	Acceptable
8 – Fruiting Vegetables	Bell Pepper (S16-01167)	Two soil applications at 0.206 & 0.330 lbs ai/A.	Acceptable
9 – Cucurbit Vegetables	Butternut Squash, Yellow Crookneck Squash, Cucumber, Pumpkin (S16-02009)	Two soil applications at 0.206 & 0.330 lbs ai/A.	A four cucurbit study. Not acceptable for use in this risk determination document. Nectar samples collected from within the hives.
9 – Cucurbit Vegetables	Cantaloupe (S16-01165)	Two soil applications at 0.206 & 0.330 lbs ai/A.	Not acceptable for use in this risk determination document. Pollen and nectar samples collected from within the hives.
9 – Cucurbit Vegetables	Cucumber (10934.4102)	Two pre-bloom soil or foliar applications.	Not acceptable for use in this risk determination document. Pollen and nectar collected by bees and samples collected from within the hives.
9 – Cucurbit Vegetables	Pumpkin (10934.4104)	Two soil applications at 0.206 & 0.330 lbs ai/A.	Acceptable
9 – Cucurbit Vegetables	Pumpkin (S16-02008)	Two foliar applications at a rate of 0.089 & 0.179 lb ai/A.	Not acceptable for use in this risk determination document. Nectar collected by bees and sampled from within the hives.

Appendix 9. Foliar and Soil Residue Studies Considered for Use in this Risk Determination Document

Dinotefuran Soil or Foliar Application Residue Studies			
Crop Group	**Crop (Lab Study ID #)**	**Application Type and Rate**	**Notes/Acceptability**
9 – Stone Fruits	Cherry (10934.4105)	Two foliar applications at 0.232 & 0.304 lbs ai/A or one trunk injection application of 2 g product per inch of diameter at breast height.	Acceptable
13 – Berries	Cranberry (10934.4101)	Two foliar applications at a rate of 0.18 lbs ai/A.	Acceptable
13 – Berries	Blueberry (10934.4107)	Two foliar applications at a rate of 0.18 lbs ai/A.	Acceptable
20 – Oilseed	Cotton (43411B104)	Two foliar applications at a rate 0.129 to 0.136 lbs ai/A.	Acceptable

Data evaluations for the residue studies that were found to be acceptable and included in this risk determination document are presented below. These data evaluations are a collection of DPR Data Evaluation Reports (DERs), Study Summary Tables (for those residue studies that did not have full DERs available at the time this document was finalized), and citations for DERs conducted by and available through U.S. EPA. Measured residue concentrations reported in the following data evaluations are those reported by the study author and thus may not match the residue concentrations used to make risk determinations. DPR independently calculated descriptive statistics for each of the acceptable studies. These descriptive statistics are presented in Appendix 11.

Imidacloprid Data Evaluations (begin on next page)

Reference
Gould, T., and Jerkins, E. (2015) Determination of the Residues of Imidacloprid, 5-Hydroxy Imidacloprid, and Imidacloprid Olefin in Bee Relevant Matrices Collected from Tomatoes Following Application of Imidacloprid Over Two Successive Years: Final Report. Project Number: EBNTN012. Unpublished study prepared by Bayer CropScience 466pg. MRID 49665201, CDPR study ID 285680, Data Volume 51950-0899, Tracking ID#270950

1. STUDY INFORMATION

Chemical:	Imidacloprid	**PC Code**	129099
Test Material:	Admire Pro Systemic Protectant (SC)	**Purity:**	43.50% a.i. w.w.
Study Type:	Non-Guideline field residue study on tomatoes to establish imidacloprid and metabolite levels in pollen, nectar, and leaves after at plant soil drip/drench application followed by two foliar spray applications in each of two successive years and three different soil types (fine, medium, and coarse).		
Sponsor:	Bayer CropScience 2T.W. Alexander Drive Research Triangle Park, NC USA 27709	**Experiment Start and End Date:**	July 2, 2013 - April 9, 2015
Sponsor Study ID:	EBNTN012	**Study Locations:**	9 Field Trials in the cities of: **Fresno, Guadalupe, Kerman, Porterville, Sanger, San Joaquin, San Luis Obispo, California**
Study Completion Date:	June 29, 2015		
Date of Amendment:	April 15, 2016		
GLP Status:	GLP-compliant; protocol reviewed by EPA, PMRA, CDPR. [CDPR study ID 254887, Data Volume 51950-0757, Tracking ID# 241047.]		

2. REVIEWER INFORMATION

Primary Reviewers: California Department of Pesticide Regulation	**John Troiano, Ph.D.**, Research Scientist III, Environmental Monitoring Branch **Richard Bireley**, Senior Environmental Scientist (Specialist), Ecotoxicology Group, Pesticide Registration Branch **Denise Alder**, Senior Environmental Scientist (Specialist), Lead Reevaluation Coordinator, Pesticide Registration Branch **Russell Darling**, Environmental Scientist, Reevaluation Coordinator, Pesticide Registration Branch
Secondary Reviewer:	TBD

3. EXECUTIVE SUMMARY

A total of nine field trials were conducted to measure the magnitude of imidacloprid residues in transplanted tomato pollen and in/on transplanted tomato leaves following three applications of Admire Pro Systemic Protectant, EPA Reg. No. 264-827 in each of two successive years. Admire Pro Systemic Protectant is a suspension concentrate formulation containing 550 g/L imidacloprid.

Across all reported trials and years, treated plots received one soil (in-furrow) drip/drench application of Admire Pro five to seven days after tomato transplantation followed by two equivalent Admire Pro foliar spray applications per planting season. Individual soil application rates ranged from 0.37 to 0.38 lb imidacloprid/acre per application (0.42 to 0.43 kg/ha). The interval between the soil and first foliar applications was 48 to 78 days. Individual foliar application rates ranged from 0.058 to 0.062 lb imidacloprid/A/application (0.065 to 0.070 kg/ha). All foliar applications were made to flowering tomato plants, after the first two sampling events were complete. The interval between foliar applications was four to five days. The foliar spray volumes ranged from 50 to 101 gal/A, with the exception of the second foliar spray in 2013 to trial NT018-13ZA (48 gal/A). Total seasonal application rates were 0.49 to 0.50 lb imidacloprid/A (0.55 to 0.56 kg/ha).

All applications were made using ground-based equipment. The adjuvant Dyne-Amic (0.25 or 0.5 % v/v) was used in all foliar applications, with the exception of the first foliar application in trial NT017-13ZB and both foliar applications in trial NT039-13ZA.

Each trial year, one bee tunnel was erected on an untreated plot (UTC), and two bee tunnels were erected on a treated plot (TRTD), except in trials NT013-13ZA, NT040-13ZA, and NT041-13ZA, when only one TRTD tunnel was erected. Bumble bee *(Bombus impatiens)* colonies (1 to 3 per tunnel) were placed in each tunnel for the collection of pollen. One sample was collected per bee tunnel, yielding two TRTD samples and one UTC sample at each sampling interval, except in trials NT013-13ZA, NT040-13ZA, and NT041-13ZA, when two replicate samples were collected from the single erected TRTD tunnel. Additionally, in trial NT042-13ZA, the first pollen sampling of 2015 was made by hand-collecting pollen directly from the flowers in the field due to a bee shortage.

Tomato leaf and pollen samples were collected at four sampling intervals each year: two samples were collected after the soil application, approximately 14 days apart (31 to 68 and 45 to 77 days after the soil application, respectively), and two samples were collected after the last foliar application, approximately 14 days apart (2 to 8 and 16 to 22 days after the last foliar application, respectively). At each interval, fresh bumble bee colonies were placed in each bee tunnel, and the bumble bees were allowed to forage from the tomato flowers for several days. Then, bumble bees carrying pollen were collected from the tunnels and the pollen was removed from them. To ensure a large enough pollen sample for analysis was collected, some trials collected bees over multiple days (up to seven) per sampling event. Multi-day pollen samples from the same sampling interval and bee tent were composited together into one sample vial.

During the described sampling intervals, composite samples of tomato leaves were collected from within the tunnels of the treated plots. Composite samples of tomato pollen and leaves were collected from the control plot tunnel of each trial during the same sampling intervals and using the methods as samples collected from the treated plots.

The residues of Admire Pro Systemic Protectant (imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

4. STUDY VALIDITY

Guideline Followed:	Non-guideline study (protocol was reviewed by U.S. EPA/PMRA/CDPR)
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	**Acceptable For Quantitative Use**
Rationale:	The data from the study will provide a basis for developing a quantitative assessment of exposure levels to bees that can be used in a risk assessment scenario.
Reparability:	N/A

5. MATERIALS AND METHODS

Test Material Characterization			
Test item:	Admire Pro Systemic Protectant (Imidacloprid) 550 g a.i./L SC	**CAS #:**	138261-41-3
Description:	Suspension concentrate (SC)	**Purity:**	43.50% w/w
Lot No./Batch No.	Batch No. NK41CX0578	**Density:**	1.41 – 1.54 g/mL
Material Source:	Bayer CropScience	**Cert. #**	213CJ2446
Material Receipt Date:	Not Reported	**Analysis Date:**	12/03/2012
Expiration Date:	12/03/2014	**Solubility:**	0.51 to 0.61 g/L
Storage of Test Material:	Ambient (35-86ºF) except trials NT010-13ZA and NT016-13ZA when the temperature briefly reached 95ºF; trial NT017-13ZB when the temperature briefly reached 90ºF; and, trial NT018-13ZA when the temperature reached as low as 32ºF and as high as 90ºF.	**Sample Storage:**	-27ºC to -7ºC -16ºF to 19ºF

5A. Study Design

This study requirement was part of the imidacloprid special review at the California Department of Pesticide Regulation (CDPR). The study design and protocol were approved by CDPR prior to study initiation. This study was conducted using GLP standards and following an approved protocol. The study initiation date was June 21, 2013. The experimental start date was July 02, 2013 (first application), and the experimental end date was December 5, 2016 (last analysis).

Nine trials in California were conducted for this study, representing all three soil texture categories (fine, medium, and coarse). Each trial includes one treated plot to be planted and treated for two consecutive years and one untreated plot.

Indeterminate (continuously flowering and fruiting) tomato varieties representing those commonly grown in the area of the trials and agronomic practices typical for commercial production of tomato were used at all trial locations. Once bloom began, the tomato plots were sampled at four intervals. The plots were sampled twice at early bloom approximately 14 days apart, prior to any foliar sprays, to assess the residues in bee-relevant matrices resulting from the at-plant soil application. The flowering tomatoes were then given two foliar applications of imidacloprid. The tomatoes were sampled twice more after the last foliar application, approximately 14 days apart, to assess the residues resulting from the soil application at planting plus two additional at-bloom foliar applications.

Homogenization and analysis of the samples from this study were performed by Bayer CropScience in Research Triangle Park (RTP), NC. Final report preparation was performed by Critical Path Services, LLC, located in RTP, NC.

All raw data associated with this study are retained along with the protocol, protocol amendments, and final report under Notebook Number EBNTN012 at Bayer CropScience, 2 T. W. Alexander Drive, RTP, NC 27709.

5B. Application Timing and Rates

The full study report provides (1) Chronological listing of significant study dates (**Appendix 1**); (2) Field report summaries for each trial detailing the actual amount of test substance applied, plot sizes, dates of treatment, dates of sample collection, maintenance chemicals, climatic data, and irrigation data (**Appendix 2**); and (3) Quality assurance statements for each trial (**Appendix 3**). Information on application timing is provided in **Table 1.** Soil and meteorological characteristics of the study sites are provided in **Table 2** and **Table 3**. **Table 4** provides the sampling dates and tomato developmental stages.

BBCH or Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie, identifies the specific phenological development stages of tomato. Plot TRTD received one soil (in-furrow) drip/drench application of Admire Pro five to seven days after tomato transplantation followed by two equivalent Admire Pro foliar applications per planting season. Individual soil application rates ranged from 0.37 to 0.38 lb imidacloprid/A per application (0.42 to 0.43 kg/ha). The interval between the soil and first foliar applications was 48 to 78 days. Individual foliar application rates ranged from 0.058 to 0.062 lb imidacloprid/A/application (0.065 to 0.070 kg/ha). All foliar applications were made to flowering tomato plants, after the first two sampling events were complete. The interval between foliar applications was four to five days. The foliar spray volumes ranged from 50 to 101 gal/A, with the exception of the second foliar spray in 2013 to trial NT018-13ZA (48 gal/A). Total seasonal application rates were 0.49 to 0.50 lb imidacloprid/A (0.55 to 0.56 kg/ha).

Temperature and precipitation data were recorded for each trial and are summarized in **Appendix 2** of the study report EBNTN012. Temperatures recorded during the field phase of the study were similar to average historical records except in trial NT013-13ZA, which had a slightly warmer spring 2014. Recorded rainfall was slightly lower than historical records in trials NT010-13ZA, NT016-13ZA, and NT018-13ZA. However, there were no significantly unusual weather conditions that would affect the conclusions of the study.

CDPR requested that the trial sites be distributed as three coarse, three medium, and three fine textured soils [per USDA's Soil Survey Geographic database (SSURGO) mapping units]. There are nine trial sites in this tomato study design: three in fine texture soils, one in medium, and five in coarse; three sandy loams (as determined by SSURGO and shown in the general texture description of were considered coarse textured. Two years of data for each site are presented in this report.

Table 1. Summary of soil and foliar application rates and timing*.

Trial Identification	Location (City, State, NAFTA Region)	Formulation	Plot Name	Year	Method	Timing/Growth Stage (BBCH)	Actual Spray Volume, GPA (L/ha)[a]	Rate, lb a.i./A (kg a.i./ha)	Retreatment Interval (Days)	Total Rate	Adjuvant
NT010-13ZA	Porterville, CA Region 10	Admire Pro Systemic Protectant	TRTD	2013	Soil Drench/Drip	13	218 (2037)	0.376 (0.422)	NA[b]	0.50 (0.56)	NA
					Foliar Spray	71	57 (530)	0.061 (0.068)	57		Dyne-Amic 0.5% v/v
					Foliar Spray	71	57 (535)	0.061 (0.069)	4		Dyne-Amic 0.5% v/v
				2014	Soil Drench/Drip	17	218 (2037)	0.376 (0.422)	279	0.50 (0.56)	NA
					Foliar Spray	65	57 (533)	0.061 (0.069)	75		Dyne-Amic 0.5% v/v
					Foliar Spray	65	57 (532)	0.061 (0.069)	5		Dyne-Amic 0.5% v/v
NT013-13ZA	Fresno, CA Region 10	Admire Pro Systemic Protectant	TRTD	2013	Soil Drench/Drip	19	24 (220)	0.377 (0.422)	NA	0.50 (0.56)	NA
					Foliar Spray	68	60 (558)	0.060 (0.068)	78		Dyne-Amic 0.25% v/v
					Foliar Spray	69	62 (576)	0.062 (0.070)	5		Dyne-Amic 0.25% v/v
				2014	Soil Drench/Drip	19	26 (247)	0.374 (0.419)	262	0.50 (0.56)	NA
					Foliar Spray	71	61	0.061	48		Dyne-

Trial Identification	Location (City, State, NAFTA Region)	Formulation	Plot Name	Year	Method	Timing/Growth Stage (BBCH)	Actual Spray Volume, GPA (L/ha)[a]	Rate, lb a.i./A (kg a.i./ha)	Retreatment Interval (Days)	Total Rate	Adjuvant
							(568)	(0.069)			Amic 0.25% v/v
					Foliar Spray	71	61 (568)	0.062 (0.069)	4		Dyne-Amic 0.25% v/v
NT016-13ZA	Porterville, CA Region 10	Admire Pro Systemic Protectant	TRTD	2013	Soil Drench/Drip	12	218 (2037)	0.376 (0.422)	NA	0.50 (0.56)	NA
					Foliar Spray	71	57 (530)	0.061 (0.068)	61		Dyne-Amic 0.5% v/v
					Foliar Spray	68	57 (353)	0.061 (0.069)	4		Dyne-Amic 0.5% v/v
				2014	Soil Drench/Drip	13	218 (2037)	0.376 (0.422)	280	0.50 (0.56)	NA
					Foliar Spray	65	57 (532)	0.061 (0.069)	75		Dyne-Amic 0.5% v/v
					Foliar Spray	65	57 (532)	0.061 (0.069)	5		Dyne-Amic 0.5% v/v
NT017-13ZB	Guadalupe, CA Region 10	Admire Pro Systemic Protectant	TRTD	2014	Soil Drench/Drip	19	8530 (79781)	0.377 (0.422)	NA	0.50 (0.56)	NA
					Foliar Spray	75	56 (520)	0.061 (0.069)	61		NA
					Foliar Spray	75	55 (516)	0.061 (0.068)	5		Dyne-Amic 0.25%

Trial Identification	Location (City, State, NAFTA Region)	Formulation	Plot Name	Year	Method	Timing/Growth Stage (BBCH)	Actual Spray Volume, GPA (L/ha)[a]	Rate, lb a.i./A (kg a.i./ha)	Retreatment Interval (Days)	Total Rate	Adjuvant
											v/v
				2015	Soil Drench/Drip	16	10353 (96829)	0.377 (0.422)	296	0.49 (0.55)	NA
					Foliar Spray	79	53 (495)	0.058 (0.065)	75		Dyne-Amic 0.25% v/v
					Foliar Spray	81	54 (507)	0.060 (0.067)	4		Dyne-Amic 0.25% v/v
NT018-13ZA	Sanger, CA Region 10	Admire Pro Systemic Protectant	TRTD	2013	Soil Drench/Drip	19	6787 (63477)	0.380 (0.426)	NA	0.50 (0.56)	NA
					Foliar Spray	83	51 (477)	0.062 (0.069)	72		Dyne-Amic 0.5% v/v
					Foliar Spray	NR[c]	48 (451)	0.058 (0.066)	5		Dyne-Amic 0.5% v/v
				2014	Soil Drench/Drip	15	6787 (63477)	0.380 (0.426)	210	0.50 (0.56)	NA
					Foliar Spray	71	50 (467)	0.060 (0.068)	72		Dyne-Amic 0.5% v/v
					Foliar Spray	71	50 (466)	0.060 (0.068)	5		Dyne-Amic 0.5% v/v
NT039-13ZA	San Luis Obispo, CA Region 10	Admire Pro Systemic Protectant	TRTD	2014	Soil Drench/Drip	NR	7 (63)	0.378 (0.423)	NA	0.50 (0.56)	NA
					Foliar Spray	72	51	0.062	64		NA

Trial Identification	Location (City, State, NAFTA Region)	Formulation	Plot Name	Year	Method	Timing/Growth Stage (BBCH)	Actual Spray Volume, GPA (L/ha)[a]	Rate, lb a.i./A (kg a.i./ha)	Retreatment Interval (Days)	Total Rate	Adjuvant
							(473)	(0.069)			
					Foliar Spray	73	51 (474)	0.062 (0.069)	5		NA
				2015	Soil Drench/Drip	NR	7 (63)	0.378 (0.423)	256	0.50 (0.56)	NA
					Foliar Spray	75	50 (468)	0.061 (0.068)	77		Dyne-Amic 0.5% v/v
					Foliar Spray	75	50 (469)	0.061 (0.068)	5		Dyne-Amic 0.5% v/v
NT040-13ZA	San Joaquin, CA Region 10	Admire Pro Systemic Protectant	TRTD	2014	Soil Drench/Drip	19	26 (247)	0.374 (0.419)	NA	0.50 (0.56)	NA
					Foliar Spray	69	100 (933)	0.061 (0.068)	52		Dyne-Amic 0.25% v/v
					Foliar Spray	71	100 (935)	0.061 (0.069)	5		Dyne-Amic 0.25% v/v
				2015	Soil Drench/Drip	16	26 (247)	0.374 (0.419)	195	0.50 (0.56)	NA
					Foliar Spray	75	100 (933)	0.061 (0.069)	60		Dyne-Amic 0.25% v/v
					Foliar Spray	76	99 (930)	0.061 (0.068)	5		Dyne-Amic 0.25%

Trial Identification	Location (City, State, NAFTA Region)	Formulation	Plot Name	Year	Method	Timing/Growth Stage (BBCH)	Actual Spray Volume, GPA (L/ha)[a]	Rate, lb a.i./A (kg a.i./ha)	Retreatment Interval (Days)	Total Rate	Adjuvant
											v/v
NT041-13ZA	Kerman, CA Region 10	Admire Pro Systemic Protectant	TRTD	2014	Soil Drench/Drip	19	26 (247)	0.374 (0.419)	NA	0.50 (0.56)	NA
					Foliar Spray	65	101 (942)	0.062 (0.069)	50		Dyne-Amic 0.25% v/v
					Foliar Spray	81	100 (931)	0.061 (0.068)	5		Dyne-Amic 0.25% v/v
				2015	Soil Drench/Drip	19	26 (247)	0.374 (0.419)	245	0.50 (0.56)	NA
					Foliar Spray	69	100 (936)	0.061 (0.069)	51		Dyne-Amic 0.25% v/v
					Foliar Spray	71	100 (937)	0.061 (0.069)	5		Dyne-Amic 0.25% v/v
NT042-13ZA	Sanger, CA Region 10	Admire Pro Systemic Protectant	TRTD	2014	Soil Drench/Drip	11	9044 (84585)	0.380 (0.426)	NA	0.50 (0.56)	NA
					Foliar Spray	72	65 (612)	0.061 (0.068)	52		Dyne-Amic 0.25% v/v
					Foliar Spray	72	55 (514)	0.062 (0.069)	5		Dyne-Amic 0.25%

Trial Identification	Location (City, State, NAFTA Region)	Formulation	Plot Name	Year	Method	Timing/Growth Stage (BBCH)	Actual Spray Volume, GPA (L/ha)[a]	Rate, lb a.i./A (kg a.i./ha)	Retreatment Interval (Days)	Total Rate	Adjuvant
											v/v
				2015	Soil Drench/Drip	14	9973 (93277)	0.377 (0.422)	217	0.50 (0.56)	NA
					Foliar Spray	85	67 (929)	0.061 (0.068)	655		Dyne-Amic 0.25% v/v
					Foliar Spray	87	68 (632)	0.062 (0.069)	5		Dyne-Amic 0.25% v/v

[a] In trials NT010-13ZA, NT013-13ZA, NT039-13ZA, NT040-13ZA, NT041-13ZA and NT042-13ZA, additional irrigation (0.2 to 0.75 inches) occurred as part of the drench/drip applications that is not captured in the listed spray volumes. See Appendix 2 of the study report for details.

[b] NA= Not applicable.

[c] NR= Not Reported; the BBCH at this application was not reported by the PFI.

***Table 4** of the study report.

5C. STUDY SITE LOCATION AND CHARACTERISTICS

Table 2. Soil and meteorological characteristics of the study sites*.

Trial ID[a]	Trial Location (City, Country/State, Year, GPS Coordinates[b])	Soil Characteristics[c]							Meteorological Data[d]			Variety
		OM (%)	pH	CEC (meq/ 100 g soil)	% Sand	% Silt	% Clay	Type	Total Rainfall (in)	Temp. Range (°F)		
NT010-13ZA	Porterville, CA, 2013-2014 (36.005, -119.0721)	0.97	7.1	9.6	84	13	3	Loamy Sand	4.56	29	97	Garden Delight
NT013-13ZA	Fresno, CA, 2013-2014 (36.7362, -119.87476)	0.93	7.2	12.9	69	22	9	Sandy Loam	3.62	30	102	Big Beef
NT016-13ZA	Porterville, CA, 2013-2014 (36.0348, -118.9964)	2.2	7.7	31.6	40	27	33	Clay Loam	4.62	29	97	Garden Delight
NT017-13ZB	Guadalupe, CA, 2014 (N34.96917, W120.60196)	0.81	8.0	14.1	84	9	7	Loamy Sand	5.87	43	81	Sungold
NT018-13ZA	Sanger, CA, 2013-2014 (36.739659, -119.576766)	0.25	8.2	6.5	76	18	6	Loamy Sand	4.34	28	100	Cherry Tomato
NT039-13ZA	San Luis Obispo, CA, 2014 (35.306478, -120.677548)	2.7	7.6	27.77	50	19	31	Sandy Clay Loam	12.15	45	81	Naomi
NT040-13ZA	San Joaquin, CA, 2014 (36.59885, -120.20671)	1.8	7.9	46.8	15	20	65	Clay	5.58	37	97	Naomi
NT041-13ZA	Kerman, CA, 2014 (36.79380, -120.05320)	0.38	6.1	3.9	89	8	3	Sand	6.46	53	98	Naomi
NT042-13ZA	Sanger, CA, 2014 (36.70034, -119.461982)	0.25	6.6	5.1	75	20	5	Loamy Sand	6.27	35	97	Cherry Tomato (Golden Gem)

[a] Site conditions listed are for the TRTD plot. For UTC plot conditions, see **Appendix 2** of the study report.

[b] GPS coordinates are in the form (latitude, longitude).

[c] These soil characteristics are based on analyses of soil samples collected from within approximately 500 ft of the treated plot. Abbreviations used: %OM = percent organic matter; CEC = cation exchange capacity.

[d] Data is for the interval of the month of first application through the month of last sampling. Meteorological data were obtained from nearby government weather stations.

* Combined table from **Table 3A** and **Table 8** of the study report.

Table 3. SSURGO soil characteristics of the study sites*.

Trial ID	Trial Location	General Texture Description (SSURGO)	Component %[a]	CDPR Texture Category	Drainage Class	Hydrologic Group	Runoff	Particle Size
NT010-13ZA	Porterville, CA	Tujunga loamy sand, 0-2% slopes	85	Coarse	Somewhat excessively drained	A	Negligible	NA[b]
NT013-13ZA	Fresno, CA	Ramona loam	80	Medium	Well drained	C	Low	Fine Loamy
NT016-13ZA	Porterville, CA	Centerville clay, 2-9% slopes	80	Fine	Well drained	D	NA	Fine
NT017-13ZB	Guadalupe, CA	Mocho sandy loam, 0-3% slopes	85	Coarse	Well drained	A	Negligible	Fine Loamy
NT018-13ZA	Sanger, CA	Delhi loamy sand, 0-3% slopes	85	Coarse	Somewhat excessively drained	A	Very Low	NA
NT039-13ZA	San Luis Obispo, CA	Salinas silty clay loam, 0-2% slope	85	Fine	Well Drained	C	Medium	Fine loamy
NT040-13ZA	San Joaquin, CA	Merced clay, slightly saline	85	Fine	Very Poorly Drained	C	Medium	Fine
NT041-13ZA	Kerman, CA	Hanford coarse sandy loam	85	Coarse	Well drained	A	Very Low	Coarse-loamy
NT042-13ZA	Sanger, CA	Hanford fine sandy loam, gravelly substratum	85	Coarse	Well Drained	A	Very Low	Coarse-loamy

[a] Major component of the soil as a percentage of total soil.
[b] NA = Not applicable.

5D. SAMPLE COLLECTION, HANDLING, PROCESSING

Tomato Plant Matrices.

Composite samples (one per each bee tunnel) of tomato leaves and pollen were collected from plots UTC and TRTD in years one and two of the study, except in trials NT017-13ZB and NT039-13ZA through NT042-13ZA, which were started/restarted in 2014 and have only completed one year of the study. All tomato leaf and pollen samples were collected from within the erected bee tunnels on both plots at four sampling intervals. The first and second samples were collected at early bloom approximately 14 days apart, prior to any foliar sprays (31 to 68 and 45 to 77 days after the soil application, respectively). The third and fourth samples were collected after the last foliar application, approximately 14 days apart (2 to 8 and 16 to 22 days after the last foliar application, respectively). UTC samples were collected at the same sampling intervals and via the same methods as the TRTD samples.

All samples were protected from sunlight and placed in field coolers containing ice substitute. Upon arrival at the site facility, all composite leaf samples were placed directly into frozen storage. All composite pollen samples were placed directly into frozen storage after their removal from the bumble bees (used as a sampling device).

Composite samples were placed into labeled (study number and sample number) containers for shipment. All samples were frozen within four hours of their collection. Samples were shipped and remained frozen until receipt at Bayer CropScience (RTP, NC).
During the described sampling intervals, duplicate composite samples (one per bee tunnel) of tomato leaves were collected from the treated plots (one day of sampling only).

All composite leaf samples were collected by hand from different areas of the tomato plants (top and middle, left and right). Each composite sample of leaves contained a target of 150 g collected from any number of healthy-appearing plants located inside the bee tunnel.

Tomato Pollen Samples and Bee Handling.

Details on the pollinator portions of the study are located in **Appendix 5**. One bee tunnel was erected on untreated plot UTC, and two bee tunnels were erected on treated plot TRTD, except in trials NT013-13ZA, NT040-13ZA, and NT041-13ZA, when only one TRTD tunnel was erected. Bumble bee (*Bombus impatiens*) colonies (1 to 3 per tunnel) were placed in each tunnel for the collection of pollen. One sample was collected per bee tunnel, yielding two TRTD samples and one UTC sample at each sampling interval, except in trials NT013-13ZA, NT040-13ZA, and NT041-13ZA, when two replicate samples were collected from the single erected TRTD tunnel.

At each testing location, the control and treated plots were divided into sampling subplots for the construction of bee-tight, ventilated mesh-covered tents (tunnels). When the tomato bloom was sufficient to support pollen sampling after the first (soil) application, the tunnels were erected. The tunnels were 100 to 210 feet long and 20 to 40 feet wide, and each tunnel enclosed four to eight rows of tomato plants. In some trials, the tunnel was removed after sampling interval two to allow for the foliar applications and re-erected prior to sampling interval three.

Normally developed, apparently healthy bumble bees (*Bombus impatiens*) were used for pollen collection. One to three bumble bee colonies were placed in each bee tunnel. Bumble bee colonies were contained in Class A research hives, Class C garden hives, or boxed colonies. In all trials, the bumble bee colonies were allowed access to sugar water feeders or nectar/syrup bags.

Fresh bumble bee colonies were placed in each bee tunnel prior to each sampling event; the bumble bees were allowed to forage from the tomato flowers for several days. Bumble bees were observed for visible pollen collected in the pollen basket on their legs. Bees with pollen were collected using nets or vacuums and placed in containers with dry ice at the field. The bees were transported to the site facility for pollen removal. All available pollen was collected from the bees using an appropriate tool such as tweezers or forceps, and the pollen samples were placed in 40-mL amber glass containers.

To ensure a large enough pollen sample for analysis, some trials collected bees over multiple days (up to seven) per sampling event (see **Appendix 2**). Multi-day pollen samples from the same sampling interval and bee tent were composited together into one sample vial. Bumble bee colonies were removed from the tents after the end of the sampling event.

Bumble bee colonies were removed from the tents after the end of the sampling event

Table 4. Sampling dates and tomato developmental stages for plant residue analysis*

Pollen and Leaf Sampling							
Year 1		Year 2		Year 1		Year 2	
BBCH†	Dates‡	BBCH†	Dates‡	BBCH†	Dates‡	BBCH†	Dates‡
Fresno = NT013-13ZA				**Sanger 1 = NT018-13ZA**			
XX[2]	62-68 DASA, (-16 to -10 DA1FA)	XX	31-33 DASA, (-17 to -15 DA1FA)	71	55 DASA, (-17 DA1FA)	61	52-54 DASA, (-20 to -18 DA1FA)
XX	75-77 DASA, (-3 to -1 DA1FA)	XX	45-47 DASA, (-3 to -1 DA1FA)	83	70-71 DASA, (-2 to -1 DA1FA)	71	69 DASA, (-3 DA1FA)
XX	2-8 DA2FA	XX	4 DA2FA[1]	85	6 DA2FA	72	7 DA2FA
XX	17-20 DA2FA	XX	19-21 DA2FA	85	20 DA2FA	86	20 DA2FA
Guadalupe = NT017-13ZB				**Sanger 2 = NT042-13ZA**			
69	46 DASA, (-15 DA1FA)		58 DASA (-17 DA1FA)	XX	36-38 DASA, (-16 to -14 DA1FA)		58 DASA (-7 DA1FA)
72	59 DASA, (-2 DA1FA)		72 DASA (-3 DA1FA)	XX	49-51 DASA, (-3 to -1 DA1FA)		62-64 DASA (-3 to -1 DA1FA)
74	8 DA2FA		4-5 DA2FA	XX	5-6 DA2FA		5-7 DA2FA
79	19-20 DA2FA		18 DA2FA	XX	16-18 DA2FA		1-18 DA2FA
Kerman = NT041-13ZA				**San Joaquin = NT040-13ZA**			
XX	33-37 DASA, (-17 to -13 DA1FA)		34-36 DASA (-15 to -17 DA1FA)	XX	35-37 DASA, -17 to -15 DA1FA		43-44 DASA (-16 to -17 DA1FA)
XX	49 DASA, (-1 DA1FA[1])		48-50 DASA (-3 to -1 DA1FA)	XX	50 DASA, -2 DA1FA[1]		57-58 DASA (-3 to -2 DA1FA)
XX	4-7 DA2FA		6 DA2FA[1]	XX	4-6 DA2FA		4-6 DA2FA
XX	19-21 DA2FA		21 DA2FA[1]	XX	19-22 DA2FA		17-19 DA2FA
Porterville 1 = NT010-13ZA				**San Luis Obispo = NT039-13ZA**			
XX	44-45 DASA, (-13 to -12 DA1FA)	XX	61 DASA, (-14 DA1FA)	XX	50 DASA, (-14 DA1FA[1])		61-63 DASA (-14 to -16 DA1FA)
XX	54-56 DASA, (-3 to -1 DA1FA)	XX	72-73 DASA, (-3 to -2 DA1FA)	XX	61-63 DASA, (-3 to -1 DA1FA)		74-76 DASA (-3 to -1 DA1FA)
XX	4 DA2FA[1]	XX	4-6 DA2FA	XX	5-6 DA2FA		4-6 DA2FA
XX	20-21 DA2FA	XX	17-19 DA2FA	XX	17-18 DA2FA		14-16 DA2FA
Porterville 2 = NT016-13ZA							
XX	48-50 DASA, (-13 to -11 DA1FA)	XX	59-61 DASA, (-16 to -14 DA1FA)				
XX	59 DASA, (-2 DA1FA[1])	XX	72-73 DASA, (-3 to -2 DA1FA)				

Pollen and Leaf Sampling							
Year 1		Year 2		Year 1		Year 2	
BBCH†	Dates‡	BBCH†	Dates‡	BBCH†	Dates‡	BBCH†	Dates‡
XX	5 DA2FA[1]	XX	4-6 DA2FA				
XX	20 DA2FA	XX	17-19 DA2FA				

†BBCH = **B**iologische Bundesanstalt, **B**undessortenamt und **CH**emische Industrie growth stage scale for tomato.
‡DASA = Days after the soil (in-furrow) application; DA1FA = days after the first foliar application;
DA2FA = days after the second foliar (and last) application. A negative number designates days prior to the indicated application. Ranges indicate that samples were collected over several days and composited together to create a large enough volume for analysis.
[1]Leaf sampling ONLY. No pollen data, insufficient sample weight.
[2]BBCH XX indicates no growth stage reported in the field data summary.
*Combination of **Appendix 1** and **Appendix 5** of the study report.

Sample Storage.

Composite samples were placed into labeled (study number and sample number) containers for shipment. All samples were frozen within four hours of their collection. Samples were shipped and remained frozen until receipt at Bayer CropScience (RTP, NC).

Storage stability studies indicate that the imidacloprid residues would have been stable during frozen storage for at least 1,080 days (36 months) in tomato leaves prior to analysis. Transit stability samples showed that imidacloprid residues were stable in pollen for the duration of the study. The maximum storage period of frozen samples in this study for Admire Pro Systemic Protectant was 561 days for tomato leaves and 560 days for tomato pollen.

Stability studies have indicated that imidacloprid residues are stable (<30% decomposition) for 24 months (728 to 769 days) of freezer storage in the following representative crops: an oilseed (tomatoseed), a non-oily grain (wheat), a leafy vegetable (lettuce), a root crop (potato), a tree fruit (apple), and a fruiting vegetable (tomato).[4-10] An additional stability study has indicated that imidacloprid residues are stable (<30% decomposition) for 36 months of freezer storage in wheat (grain), orange (fruit), tomato (fruit), bean (seed), and rape (seed)16. Demonstrated freezer stability in all of the above crops is representative of the freezer stability of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin residues to be expected for tomato leaves from this study.

Based on the available storage stability data,[4-11] the imidacloprid residues in tomato leaves would be representative of the residues to be expected after the use of Admire Pro Systemic Protectant on tomato.

5E. ANALYTICAL METHODS

The analytical methods[1-2] used in this study measured the residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin in tomato leaves and pollen. These data are reported in **Appendix 4** of the full study report titled, "*Analytical Report for EBNTN012 Determination of the Residues of Imidacloprid, 5-Hydroxy Imidacloprid, and Imidacloprid Olefin in Bee Relevant Matrices Collected from Tomato Plants Following Soil and Foliar Applications of Admire Pro Over Two Successive Years.*"

All neat analytical reference standards were stored frozen prior to dilution. All reference standard solutions were prepared in parent equivalents and corrected for purity during initial preparation. The reference standard solutions were stored refrigerated or frozen and have been shown to be stable for the length of storage time required for this study.

For the tomato leaves[1], a 2.5 g sample was weighed into a 50-mL polypropylene conical centrifuge tube, and 10 mL HPLC-grade water was added. The tube was mixed manually for 1 minute, followed by adding 20 mL of acetonitrile and mechanical shaking (HS501 digital, IKA-Werke, Wilmington, NC) for an additional 1 minute. Then, 3 g of $MgSO_4$ and 1.5 g of NaCl were added. The sample was amended with a mixed internal standard solution, capped, and shaken for 1 minute. For leaf samples which were found to contain high residues of imidacloprid (>2 ppm), the sample was amended with a 10X mixed internal standard solution before the salts were added. The sample was centrifuged. For low imidacloprid residue samples, 20 mL of organic supernatant was transferred into a separate 50-mL polypropylene conical centrifuge tube containing 0.3 g of Bondesil-PSA and 1.8 g of $MgSO_4$. For high imidacloprid residue samples, 2.0 mL of organic supernatant and 18.0 mL of acetonitrile were transferred into a separate 50-mL polypropylene conical centrifuge tube containing 0.3 g of Bondesil-PSA and 1.8 g of $MgSO_4$, which was capped and shaken for 1 minute. The sample extract was centrifuged, and a 1.25 mL aliquot of supernatant was transferred into a clean culture tube. The sample aliquot was evaporated to near dryness on the Turbo-Vap (Biotage, Charlotte, NC). The solid was reconstituted with 1.25 mL of 9:1 H_2O:MeOH and transferred into a 2-mL sample vial for LC/MS/MS analysis.

For pollen[2], a 0.1 g sample was weighed into a 2-mL centrifuge tube containing 2.8 mm steel balls. If the available pollen amount was less than 0.1 g but greater than 0.025 g for a sample, the sample was considered sufficient for analysis. A 1 mL portion of methanol/water (3:1 v/v) was added, and the mixture was homogenized with a bead mixer at 5000 beats/minute for 1 minute on a Precellys homogenizer (Bertin Technologies, Rockville, MD). The isotopically labeled internal standards were added and mixed, and the mixture was centrifuged at 12,000 rpm for 2 minutes. The supernatant was transferred into a clean culture tube containing 2.5 mL of water and was evaporated to an aqueous remainder, then applied to a 3-mL ChemElut SPE cartridge. After 10 to 15 minutes, the cartridge was washed with 4 mL of hexane/ethyl acetate (1:1 v/v) three times into a clean culture tube. The combined eluates were evaporated to dryness. The analytes were dissolved from the tube with 0.5 mL of MeOH/H_2O (1:4 v/v). The solution was transferred into a 2 mL sample vial for analysis by LC/high resolution mass spectrometry (LC/HRMS).

Quantitation of each analyte was based on the daughter ion transitions of the analyte and the respective internal standard analog. The responses of the LC/MS/MS and LC/HRMS systems to each analyte and its internal standard were measured in samples and in standards, and a relative response was calculated (as the ratio of the analyte and the stable isotopically labeled internal standard responses). The relative response of the analyte in each sample was compared to the relative response of the analyte in the standards.

The relative responses of imidacloprid and its analytes were measured over the range of 0.00012 to 2.0 ppm. The analyte relative responses were fit to a linearity curve calculated using linear regression analysis with 1/x weighting (Thermo Finnigan XCalibur 2.7.0.20 or 2.2 SP1.48). Correlation coefficients were calculated with the same software.

All data are reported in parent equivalents, and the individual measured residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin are summed to give a total imidacloprid residue.

The methods for determining imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin residues in/on tomato leaves and pollen were validated by measuring the recovery of these analytes from control matrices fortified at their respective LOQs. Additional recoveries at higher fortification levels validated the method for the highest residues observed in individual matrices. Concurrent recoveries of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin from fortified samples were measured with each set of samples to verify method performance.

5F. Quality Assurance Results

The responses of the LC/HRMS and LC/MS/MS systems to imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin were linear in solvent over the range of 0.00012 to 2.0 ppm. The coefficients of determination were >0.99. The response data are located in **Appendix 4** in the study report.

Control interferences for tomato matrices are discussed in this paragraph; no total imidacloprid residue was calculated for the UTC samples, so the levels of imidacloprid as an individual analyte are described. Imidacloprid (parent) residues in UTC tomato pollen ranged from below the analyte LOD to 0.060 ppm (trial NT018-13ZA). Imidacloprid residues in UTC tomato leaves ranged from below the analyte LOD to 0.031 ppm (trial NT017-13ZB).

All recoveries were corrected for any interferences in corresponding controls. The overall means of the recoveries for each matrix at each fortification level were within the acceptable range of 70 to 120%, and the standard deviation values were below 20%. The analytical data summaries are located in **Appendix 4** in the study report.

The limit of quantitation (LOQ) is defined as the lowest fortification level of an analyte at which acceptable recovery has been achieved. The LOQ for a total residue is the highest of the LOQ values assigned to the individual analytes for a particular matrix.

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be determined to be statistically different from a blank. The LODs were determined from method validation data obtained from control samples fortified at the respective analyte LOQs. The LODs were calculated by multiplying the standard deviation of recovery measurements at the LOQ by $t_{0.99}$ [where $t_{0.99}$ is the one-tailed t-statistic at the 99% confidence level for the number of replicates (n)].[4] The LOD for the total imidacloprid residue in each matrix is the highest LOD value of any one individual analyte for that particular matrix.

Table 5. Limits of quantification and detection for imidacloprid and metabolites*.

Matrix	Analyte	LOQ (ppb)	LOD (ppb)
Tomato Leaves	Imidacloprid	5.0	2.2
	5-Hydroxy Imidacloprid	5.0	0.7
	Imidacloprid Olefin	5.0	1.0
	Total Imidacloprid	5.0	2.2
Pollen	Imidacloprid	1.0	0.4
	5-Hydroxy Imidacloprid	1.0	0.5
	Imidacloprid Olefin	1.0	0.3
	Total Imidacloprid	1.0	0.5

***From page 22 of the study report.**

6. RESULTS:

6.A. Comparison of Concentrations Measured for Parent and Degradates

Comparison of the distribution of concentrations measured for parent imidacloprid and degradation products in pollen and leaves are presented in **Table 6-1** and **Table 6-2**. The LOD of values for pollen were very low ranging from 0.3 ppb to 0.5 ppb. Reporting concentration in ppb in this report indicates a weight to weight basis. The LOD for leaves ranged from 0.7 to 2.2 ppb. Data reported as below the LOD were assigned one-half the LOD value. Comparison of the contribution of each chemical to the total residue indicates that parent imidacloprid comprised essentially 90% or greater of the total residue measured in each plant part. Since a small portion of the degradation products (considered to be as toxic as parent Imidacloprid) were present in the measured residue, the following discussions will focus on total imidacloprid residue measured in plant tissues. Statistical procedures used in the Statistical Analysis System (SAS) software to provide distribution statistics or statistical tests were PROC CAPABILITY, PROC T-TEST, PROC SHEWHART, and PROC NPAR1WAY.

6.B. Magnitude of Residues in Bee-Relevant Matrices

Figure 6-1 explains the statistical aspects relayed in the Box-and-Whisker plots used to compare the distribution of total imidacloprid concentrations calculated for each sampling interval. For each data set analyzed, the box graphic presents values for the mean, median, minimum, maximum, and 25th and 75th percentiles. Replicate samples were taken at each interval. All data have been used to determine the expected distributional properties for concentration in pollen and leaves because each analysis is representative of the potential distribution encountered in field sampling.

Pollen. Comparison of overall statistics for total imidacloprid residue indicated much greater concentrations at the third sampling interval than at the other three sampling intervals (**Figure 6-2**). The soil application occurred at planting and two samplings were conducted after the soil application but before the foliar applications. The first sampling, noted as "Interval 1", occurred at a mean of 49 days after the soil application with a range of values from 31 to 63 days. The second sampling interval, noted as "Interval 2", was also made prior to foliar applications at a mean of 62 days after the soil application with a range of 45 to 77 days. Median total imidacloprid residues in pollen, which is relevant to potential bee exposure, were 41 and 30 ppb with maximum values of 679 and 138 ppb for sampling intervals 1 and 2, respectively (**Table 6-1**).

After the second sampling interval, plants received two additional foliar applications at approximately 5 day intervals. The third sampling interval, noted as "Interval 3", occurred approximately 6 days after the second foliar spray. Median concentrations measured at the third interval increased nearly tenfold to 442 ppb with a maximum value of 1763 ppb. The fourth and final sampling interval, noted as “Interval 4”, occurred around 19 days after the second foliar application where the median total imidacloprid residue concentration dropped to 66 ppb and the maximum concentration was also lower at 354 ppb. With respect to timing after soil application, the means were 74 and 87 days after application for intervals 3 and 4, respectively.

6.C. MAGNITUDE OF RESIDUES IN LEAVES

For leaves, the same pattern was measured as for pollen where median total imidacloprid values were 125, 100, 726, and 96 ppb for sampling intervals 1, 2, 3, and 4, respectively (**Table 6-2**). The sharp decrease in concentration between intervals 3 and 4 does not appear to be due to wash-off from rainfall or irrigation because drench/drip irrigation was indicated at each site. Also, there was essentially no recorded rainfall during the interval between foliar applications and sampling of plant tissues.

6.D. SITE SPECIFIC TRENDS

Temporal patterns in residue concentration for each site are depicted for pollen in **Figures 6-3A and 6-3B** and for leaves in **Figures 6-4A and 6-4B**. For pollen data, five of the individual studies lacked data for sampling interval 3. Based on the pattern noted in section 6.B, interval 3 is obviously an important sampling event where concentrations were noted to spike upwards. Graphs are not presented for sites missing data for interval 3, which were Fresno in 2014, Kerman in 2015, Porterville sites 1 and 2 in 2013, and San Joaquin in 2015. In addition, data were not available for sampling interval 2 at the San Joaquin and Kerman site in 2014 and for interval 1 at the San Luis Obispo site in 2014, but the curves are presented for these sites because they conformed to the noted trend for sharp increases at interval 3. Except for the San Joaquin site **in Figure 6-3B**, the general pattern of mean concentrations rising nearly tenfold at the third sampling interval was observed at all other sites (**Figures 6-3A and 6-3B**). A sharp decline in concentration between intervals 3 and 4 was also predominant except for the San Luis Obispo site where there was only a slight decrease at interval 4. The concentrations measured in interval 4 are nearly equal to those measured before the foliar application. An additional treatment that measured longevity of residues due to the soil application would have provided data to determine if the residues measured at interval 4 were due to the soil application or simply the dissipation curve for residues deposited from the foliar application.

For leaves, data were available for all sites and all years. Except for a few sites, a steep rise at the third sampling interval was observed with many reflecting an order of magnitude increase (**Figures 6-4A and 6-4B**). Curves not reflective of the predominant pattern were indicated at the Sanger 1 site in 2014, the Fresno site in 2013, and the Kerman site for both years. In contrast, pollen data for these sites reflect a significant rise in concentration at the third sampling interval. The lack of a similar pattern between leaves and pollen for these two sites indicate a larger variability in distribution of concentration in leaf tissue, which might be due to variability in application or sampling methodology.

6.E. Potential Carry-over of Residues

Paired T-tests were conducted at each interval to determine potential carry-over of residues between years. Two replicates were taken at each interval within each year. These replicates, however, have no relationship with respect to sampling between years so means were calculated. Tests for normality indicated that that base 10 logarithm transformations produced distributions that approximated normality. Paired T-tests were conducted for each interval to reduce potential problems with variance caused by increased concentrations measured at interval 3. Within each site, the study was conducted for two years with some sites spanning years 2013 to 2014 and others spanning years 2014 to 2015. T-tests were conducted on the difference between year 1 and year 2 for the logarithmic values of the total imidacloprid residue concentration. Results generally indicated no difference in values between years for most of the tests (**Table 6-5**). There were two instances where significance was indicated but the values for year 1 were greater than year 2. These results indicate no potential carry-over between years for imidacloprid residues under the conditions of this study.

6.F. Leaf and Nectar Concentration in Relation to Soil Type

Originally, the proposed study design suggested sampling sites located in coarse, medium, and fine-textured soils with three replicates assigned to each soil type. There is an inherent difficulty in fulfilling the proposed design when the study is conducted after fields have been planted and then attempting to procure cooperating growers. The distribution of sites with respect to soil texture category was 5 sites in coarse-textured soil, 1 site in medium-textured soil, and 3 sites in fine-textured soil. Using all replicate data pooled over all years to represent potential distribution of residues within a soil type, the total number of samples within each soil type was 20, 4, and 12 for coarse, medium, and fine-textured soil, respectively. The replication was too low in medium-textured soil so comparisons were limited to coarse and fine-textured categories. Non-parametric statistical tests were used to test for differences in distributions between specified comparisons. Non-parametric tests do not require tests for normality as they are robust to differences in distribution and they are also robust for experimental designs with low replicates. The PROC NPAR1WAY procedure in the Statistical Analysis System (SAS) statistical package was used to conduct Wilcoxon-Mann –Whitney (Wilcoxon) and Median non-parametric tests. A significant result from the Wilcoxon test indicates differences in the shape of distributions and a significant result from the Median test indicates differences in the location of the medians between distributions. The Exact option for each statistic was implemented as it provides permutation testing, a statistical method that minimizes the effect of sample size and distributional differences. Under the Exact option, the Monte Carlo procedure was also implemented which provided 10,000 separate runs for each statistic to produce the permutation distributions.

Statistics for the distributions of total imidacloprid residue by each soil category for pollen and leaves are in **Tables 6-4 and 6-5,** respectively. Significant differences in distributions between soil category were indicated for pollen and leaves for sampling intervals 1 and 2, which potentially represent differences in uptake due to soil application (**Table 6-6**). No significant differences were measured at interval 3 when residues were greatly increased by the foliar applications. Foliar applications are not expected to be influenced by soil type because residues are deposited directly onto the plant. By the last sampling at interval 4, concentrations in leaves appear to retain differences noted prior to foliar sprays. No difference in pollen concentration was measured at the last sampling interval. Even though the uneven replication amongst the soil categories provides some uncertainty in the statistical analysis, the results appear to indicate that prior to the foliar sprays, concentrations in leaves and pollen were greater in the coarse soil category.

Table 6-1. Cumulative distributional statistics for concentration of imidacloprid and related metabolites in tomato pollen. Acronyms in the table are: IMI= imidacloprid; olefin = imidacloprid olefin; 5-OH = 5 hydroxy imidacloprid; Total = sum of IMI, olefin, and 5-OH (in bold); N=number of observations; SD=standard deviation; CV = coefficient of variation. Numbered interval denotes timing of sampling where intervals 1 and 2 were approximately at 49 and 62 days after the first soil application and intervals 3 and 4 were at 6 and 19 days after a second foliar spray coinciding with 74 and 87 days after the soil application.

Pollen Sampling Statistics	Interval 1				Interval 2				Interval 3				Interval 4			
	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**
N	32	32	32	**32**	27	27	27	**27**	22	22	22	**22**	32	32	32	**32.0**
Mean (ppb)	5.2	2.1	99.6	**106.9**	1.7	0.7	38.0	**40.4**	25.3	7.9	560.0	**593.2**	5.9	2.1	71.1	**79.0**
SD (ppb)	8.5	2.6	140.6	**151.1**	1.8	1.0	33.1	**35.4**	14.7	4.8	381.1	**397.2**	7.3	1.7	56.6	**64.1**
CV (%)	163.4	125.0	141.1	**141.4**	104.9	138.1	87.1	**87.7**	58.1	61.2	68.1	**66.9**	125.2	85.2	79.6	**81.2**
Min (ppb)	0.3	0.2	6.6	**7.0**	0.3	0.2	1.4	**1.8**	9.7	2.6	232.9	**249.2**	0.8	0.2	15.5	**16.5**
Median (ppb)	1.6	0.8	39.3	**41.0**	1.0	0.2	28.7	**29.9**	19.7	7.1	420.5	**442.4**	4.3	1.3	58.8	**66.3**
75th (ppb)	6.2	3.1	121.9	**130.8**	2.3	0.7	48.6	**51.9**	30.3	9.6	560.4	**602.9**	6.1	3.9	84.5	**90.7**
90th (ppb)	11.7	6.6	225.7	**242.3**	4.6	2.8	94.8	**102.6**	43.9	12.7	975.7	**1015.9**	9.6	4.4	141.0	**154.1**
95th (ppb)	30.3	8.1	490.0	**526.9**	5.3	3.2	103.3	**106.7**	58.7	15.9	1448.8	**1520.8**	21.8	4.6	148.7	**174.5**
Max (ppb)	37.2	9.2	632.8	**679.2**	6.4	3.3	128.6	**138.3**	63.5	24.1	1679.7	**1762.5**	39.4	6.0	312.2	**354.0**
% of Total	**4.9**	**2.0**	**93.2**		**4.2**	**1.7**	**94.1**		**4.3**	**1.3**	**94.4**		**7.4**	**2.6**	**90.0**	

Table 6-2. Cumulative distributional statistics for concentration of imidacloprid and related metabolites in tomato leaves. Acronyms in the table are: IMI= imidacloprid; olefin = imidacloprid olefin; 5-OH = 5 hydroxy imidacloprid; Total = sum of IMI, olefin, and 5-OH (in bold); N=number of observations; SD=standard deviation; CV = coefficient of variation. Numbered interval denotes timing of sampling where intervals 1 and 2 were approximately at 49 and 62 days after the first soil application and intervals 3 and 4 were at 6 and 19 days after a second foliar spray coinciding with 74 and 87 days after the soil application.

Leaf Sampling Statistics	Interval 1				Interval 2				Interval 3				Interval 4			
	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**
N	36.0	36.0	36.0	**36.0**	36.0	36.0	36.0	**36.0**	36.0	36.0	36.0	**36.0**	36.0	36.0	36.0	**36.0**
Mean (ppb)	8.9	7.7	151.0	**167.6**	5.7	6.2	134.0	**145.9**	57.5	31.8	1139.8	**1229.2**	8.7	7.4	130.5	**146.5**
SD (ppb)	11.8	7.5	148.2	**163.5**	6.1	5.6	128.3	**139.4**	51.6	22.5	1272.8	**1341.7**	6.6	5.0	107.6	**117.7**
CV (%)	132.2	97.4	98.2	**97.6**	107.7	91.6	95.7	**95.6**	89.7	70.6	111.7	**109.2**	76.7	68.5	82.5	**80.3**
Min (ppb)	0.4	0.5	1.1	**2.0**	0.4	0.5	4.6	**5.5**	1.7	1.5	41.3	**44.5**	0.1	1.5	12.7	**14.3**
Median (ppb)	4.3	4.9	103.7	**125.0**	3.3	5.0	89.5	**100.1**	41.8	26.3	660.3	**726.2**	7.8	6.4	84.8	**96.4**
75th (ppb)	9.8	11.6	182.9	**798.3**	9.4	11.0	231.7	**252.0**	93.3	39.9	1133.9	**1271.1**	12.0	9.7	143.8	**170.0**
90th (ppb)	27.2	18.0	362.6	**397.0**	15.2	14.0	330.5	**361.5**	111.8	70.9	3222.0	**3427.9**	18.5	15.8	320.6	**356.1**
95th (ppb)	43.5	24.2	492.9	**544.0**	18.4	16.8	381.2	**417.5**	136.6	79.9	3339.8	**3539.8**	21.1	19.1	370.8	**423.1**
Max (ppb)	43.5	25.7	541.4	**592.3**	23.2	19.0	459.0	**496.4**	261.3	81.1	5907.9	**6249.1**	31.2	21.1	402.8	**434.9**
% of Total	**5.3**	**4.6**	**90.1**		**3.9**	**4.2**	**91.9**		**4.7**	**2.6**	**92.7**		**5.9**	**5.0**	**89.1**	

Table 6-3. Probability levels for paired T-tests for the effects of year on concentration of total imidacloprid residues.

Paired T-test for Total Imidaclprid Residue				
Source	Interval	N	Pr>t-value	Comment
Pollen	1	16	0.88	
	2	12	0.64	
	3	7	0.47	
	4	16	0.004	Year 1 Greater
Leaves	1	18	0.42	
	2	18	0.03	Year 1 Greater
	3	18	0.11	
	4	18	0.55	

Table 6-4. Cumulative distributional statistics for total imidacloprid concentration measured in tomato pollen for categorized texture of soil at the site plants were grown. Acronyms in the table are; N=NUMBER OF OBSERVATIONS; SD=STANDARD DEVIATION; CV = COEFFICIENT OF VARIATION. NUMBERED INTERVAL DENOTES TIMING OF SAMPLING WHERE INTERVALS 1 AND 2 WERE APPROXIMATELY AT 49 AND 62 DAYS AFTER THE FIRST SOIL APPLICATION AND INTERVALS 3 AND 4 WERE AT 6 AND 19 DAYS AFTER A SECOND FOLIAR SPRAY COINCIDING WITH 74 AND 87 DAYS AFTER THE SOIL APPLICATION.

Pollen: Distribution of Total Imidacloprid Residues by Site Soil Type Classification												
	Interval 1			Interval 2			Interval 3			Interval 4		
Statistic	Coarse	Medium	Fine	Coarse	Medium	Fine	Coarse	Medium	Fine	Coarse	Medium	Fine
N	20	4	12	20	4	12	20	4	12	20	4	12
Mean (ppb)	242	93	69	187	150	75	1174	541	1550	205	58	81
SD (ppb)	182	24	70	137	180	109	1520	377	1185	131	17	30
CV (%)	75	25	102	73	120	145	130	70	76	64	29	37
Min (ppb)	13	71	2	13	29	5	45	68	226	14	41	30
Median (ppb)	176	89	49	154	77	35	529	594	1131	161	58	82
75th (ppb)	394	111	101	282	254	51	952	840	2894	309	72	96
90th (ppb)	517	124	197	373	418	291	3502	906	3120	390	74	124
95th (ppb)	568	124	199	441	418	322	4894	906	3428	429	74	135
Max (ppb)	592	124	199	496	418	322	6249	906	3428	435	74	135

Table 6-5. Cumulative distributional statistics for total imidacloprid concentration measured in tomato leaves for categorized texture of soil at the site plants were grown. Acronyms in the table are; N=NUMBER OF OBSERVATIONS; SD=STANDARD DEVIATION; CV = COEFFICIENT OF VARIATION. NUMBERED INTERVAL DENOTES TIMING OF SAMPLING WHERE INTERVALS 1 AND 2 WERE APPROXIMATELY AT 49 AND 62 DAYS AFTER THE FIRST SOIL APPLICATION AND INTERVALS 3 AND 4 WERE AT 6 AND 19 DAYS AFTER A SECOND FOLIAR SPRAY COINCIDING WITH 74 AND 87 DAYS AFTER THE SOIL APPLICATION.

Leaves: Distribution of Total Imidacloprid Residues by Site Soil Type Classification												
	Interval 1			Interval 2			Interval 3			Interval 4		
Statistic	Coarse	Medium	Fine	Coarse	Medium	Fine	Coarse	Medium	Fine	Coarse	Medium	Fine
N	19	4	9	16	3	8	14	2	6	18	4	10
Mean (ppb)	144	82	40	50	55	15	557	1268	453	77	66	88
SD (ppb)	185	34	53	37	45	12	387	357	195	35	51	118
CV (%)	129	42	131	73	82	76	70	28	43	46	78	16
Min (ppb)	18	47	7	10	25	2	265	1016	249	24	20	49
Median (ppb)	41	82	13	44	33	15	442	1268	375	70	60	80
75th (ppb)	232	111	41	69	107	25	561	1521	603	87	109	264
90th (ppb)	527	118	170	103	107	30	972	1521	769	154	124	354
95th (ppb)	679	118	170	138	107	30	1763	1521	769	155	124	354
Max (ppb)	679	118	170	138	107	30	1763	1521	769	155	124	354

Table 6-6. Results of non-parametric Wilcoxon and Median tests comparing distributions of total imidacloprid residue concentration in pollen and leaves with coarse and fine-textured soil categories.

Exact Probability Levels for Wilcoxon and Median Non-Parametric Tests Comparing Total Imidacloprid Residue Distributions in Coarse to Fine-Textured soil								
	Interval 1		Interval 2		Interval 3		Interval 4	
Source	Wilcoxon	Median	Wilcoxon	Median	Wilcoxon	Median	Wilcoxon	Median
Pollen	0.028	0.42	0.005	0.026	0.66	0.64	0.21	0.24
Leaves	0.005	0.01	0.024	0.009	0.17	0.28	0.003	0.01

7. STUDY STRENGTHS, LIMITATIONS AND CONCLUSIONS

The data from this study provide an expected distribution for the concentration of imidacloprid residues that bees are exposed to in pollen in tomato flowers grown under actual agronomic practices in California. Relating concentrations measured in flower parts to bee health is possible by comparing the concentrations measured in bee relevant plant parts to target values that define acute or chronic exposure scenarios. Although the detected levels of imidacloprid residues indicate substantial presence of residues in pollen samples, results do not reflect potential maximum exposure concentrations to foliar applications because samples were taken approximately 6 days after a second foliar application. Therefore, there is some uncertainty if the sampling schedule reflected a maximal exposure scenario.

In the context of documenting the magnitude of imidacloprid residues in bee-related matrices of tomato, the following strengths are observed in this study.

1. Data provide quantitative values of total imidacloprid residues expected in pollen and leaves of tomato plants grown under California conditions.

2. Measurements were taken at four time intervals in an attempt to quantify levels expected in plant tissues over time and from different methods of application: The first and second interval reflected concentrations following a period of time after a soil application at planting, and third and fourth interval reflected concentrations after two additional foliar applications were made to the crop.

3. Each site was replicated over two years so potential carry-over effects could be measured.

Limitations noted in this study include:

1. The values most likely do not reflect expected maximum concentrations in pollen because sampling did not occur directly after foliar application. Sampling occurred on average 6 days after a second foliar application. Substantial decreases at nearly an order of magnitude were noted in residues from plants sampled between the 3rd and 4th foliar application interval. The average sampling between the 3rd and the 4th foliar application was 13 days. Since there was no potential for redistribution of residues due to water movement from either irrigation or rainfall, the steep dissipation indicates that concentrations would most likely have been highest if samples were taken directly after the foliar applications.

2. Data were missing for pollen sampling at interval 3 at three sites. Highest concentrations were measured at this sampling interval so characterization of the distribution for the third sampling interval across all sites is incomplete.

3. Since data from coarse soils were disproportionately represented as compared to the medium and fine soil types, observed statistical effects provide only preliminary evidence for differences measured in residue uptake in tomato plants due to soil type.

Overall, considering the strengths and limitations of this study, the following conclusions can be drawn:

1. Imidacloprid residues were measured in pollen from soil application: The distribution of total imidacloprid residues resulting from the soil application (the mean sampling time occurred at approximately 48 days after application of 0.38 lb ai/A) produced a median concentration of 41 ppb, a maximum value measured at 679 ppb and a 90th percentile value at 242 ppb. These values represented 32 samples taken over two years from 9 sites sampled.

2. Imidacloprid residues in pollen increased from additional foliar sprays: The distribution of total imidacloprid residues from 2 additional foliar applications (approximately 0.068 lb ai/A), which were sampled approximately six days after the second application, resulted in a median of 442 ppb, a maximum value of 1763 ppb and a 90th percentile value of 1016 ppb. These values represented 22 samples taken over two years from 9 sites.

3. Patterns noted in concentration over time were similar between leaf and pollen samples: The pattern in leaves was similar to that observed for bee-relevant pollen samples. Leaf concentrations that were measured approximately 48 days after the soil application resulted in a median value of 125 ppb, a maximum of 592 ppb and a 90th percentile value of 347 ppb. These values were increased for samples taken after the second foliar application where the median value was increased to 726 ppb with a maximum value at 6249 ppb and a 90th percentile value of 3428 ppb. These values represented 36 samples taken over 2 years from 9 sites.

4. Imidacloprid concentration indicated dissipation between the third and fourth sampling intervals: Residues were observed to drop at a second sampling date taken approximately 19 days after the second foliar application. Median and maximum values for pollen were measured at 66 and 354 ppb, respectively, and 46 and 435 ppb for leaves, respectively. For the median values, this was an 86% decrease for pollen and a 94% decrease for leaves over the approximately 11 day period between the two sampling intervals taken after the second foliar application of imidacloprid.

5. No carry-over effect between years: There was no increase in concentrations over years measured in either pollen or leaf samples.

6. Concentrations in leaf and pollen tissue was greater in coarse-textured soil due to soil application: Although the replication was uneven between the soil texture categories, the distribution of total imidacloprid residues was greater in the coarse-texture soil compared to the fine-textured soil in the sampling intervals conducted after the soil application but prior to foliar application. Greater uptake in coarse-textured soil has been measured in previous studies.

8. STUDY VALIDITY/CLASSIFICATION

The data from this study provide an expected distribution of the concentration of imidacloprid residues that bees are exposed to in pollen of tomato plants grown under actual agronomic practices in California. These data, however, do not represent the maximum expected concentrations in pollen due to samples not being taken directly after foliar applications. They were taken on average 6 days after a second foliar application. Therefore, there is uncertainty if these values reflect maximum exposure scenarios. The study is considered scientifically sound and useful for risk assessment purposes. The concentrations reported for pollen are high enough to require comparison to target values that define acute or chronic exposure scenarios when they are calculated. The study is classified as ACCEPTABLE for quantitative use in risk assessment

9. REFERENCES

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Figure 6-1. Explanation of statistical meaning of the Box-and-Whisker plots.

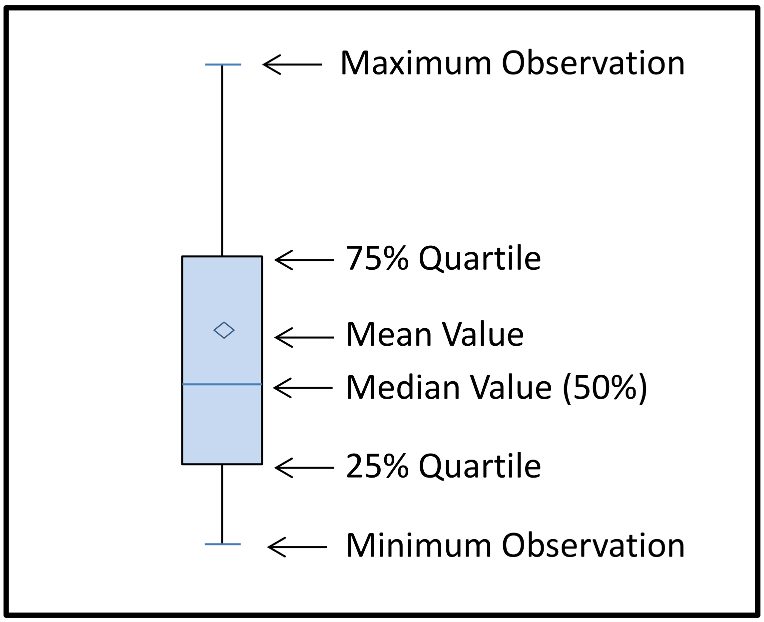


Figure 6-2. Relative concentration of total imidacloprid residues measured in tomato (A) pollen and (B) leaves compared between sampling intervals. Data were averaged over all sites. NUMBERED INTERVAL DENOTES TIMING OF SAMPLING WHERE INTERVALS 1 AND 2 WERE APPROXIMATELY AT 49 AND 62 DAYS AFTER THE FIRST SOIL APPLICATION AND INTERVALS 3 AND 4 WERE AT 6 AND 19 DAYS AFTER A SECOND FOLIAR SPRAY COINCIDING WITH 74 AND 87 DAYS AFTER THE SOIL APPLICATION.

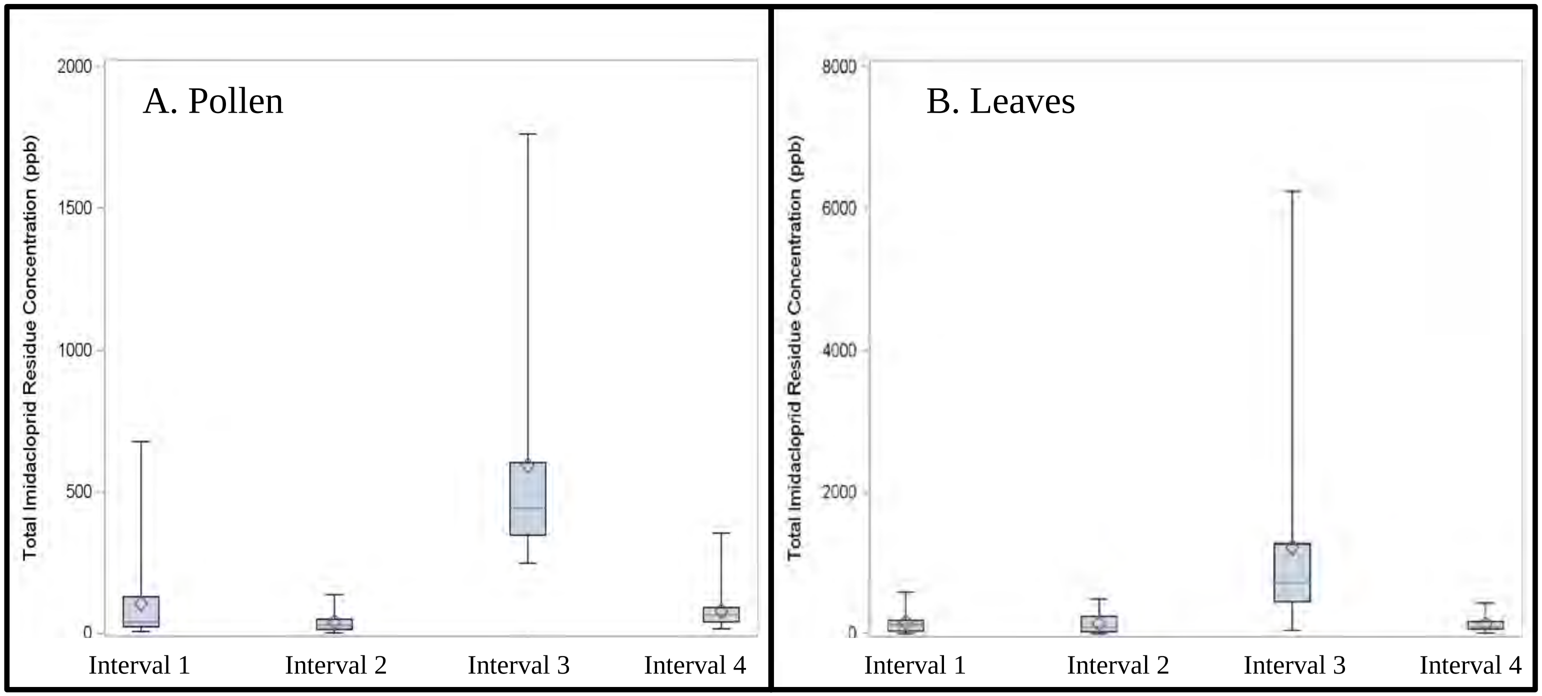
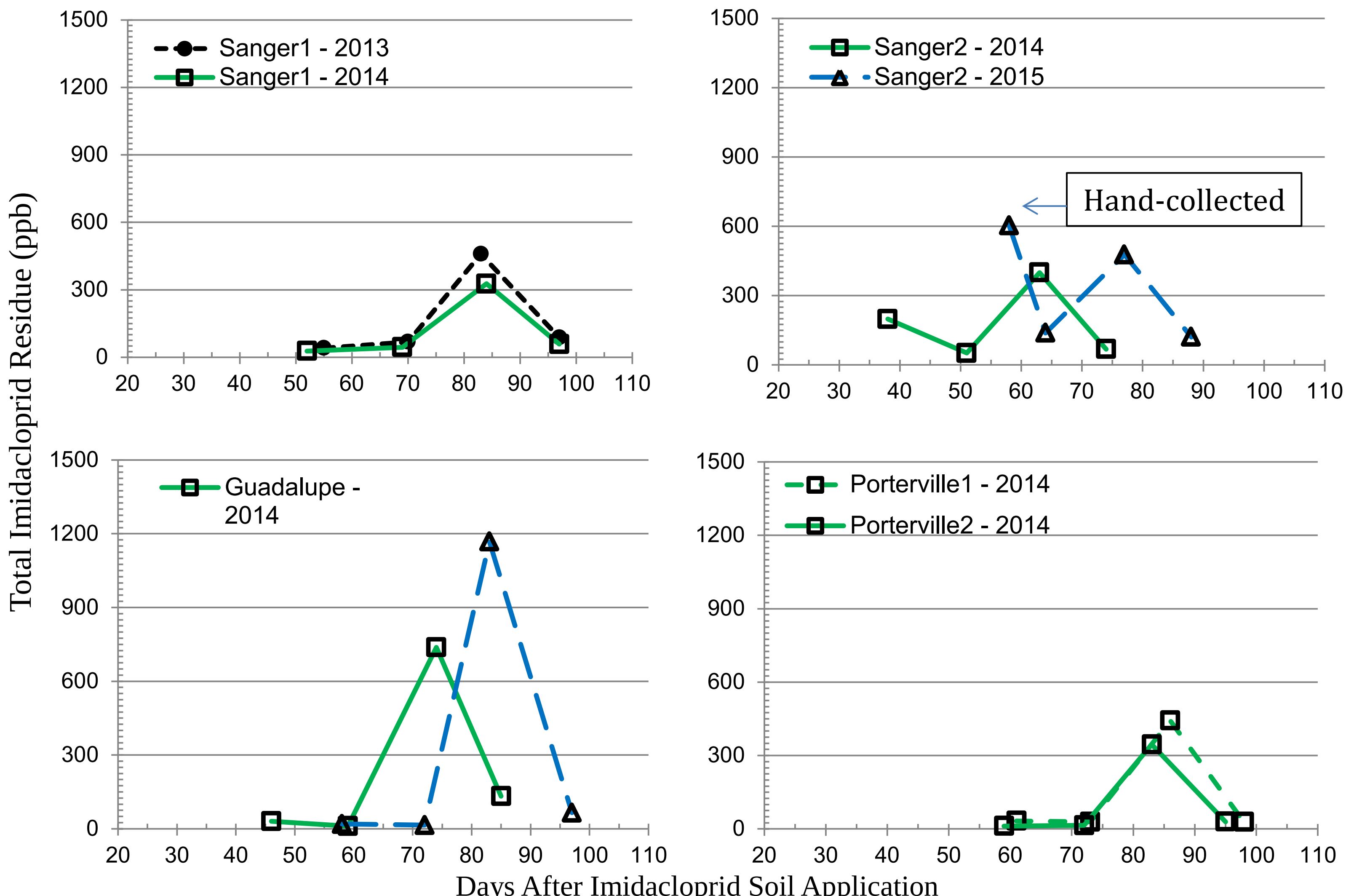


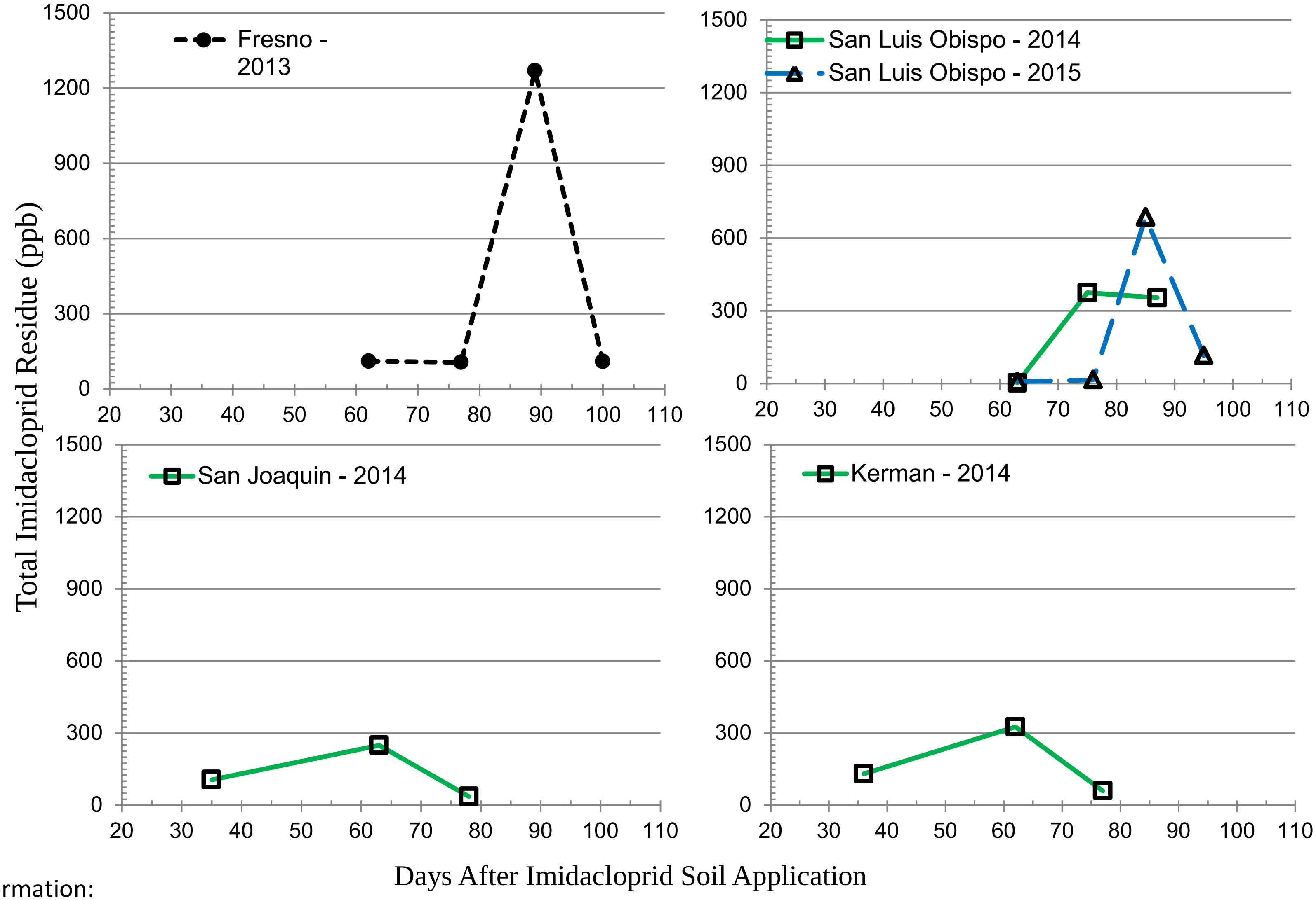
Figure 6-3A. Trend in total imidacloprid residue measured in pollen at each site. Within each panel markers, marker color and line style denote the year, i.e. year 2013 is a solid black circle and a small-dashed black line; year 2014 is a hollow square and a green solid line; year 2015 isa hollow triangle and a large-dashed blue line.



<u>Specific Site Information:</u>

Sanger1=NT018-13ZA; Sanger2=NT042-13ZA; Guadalupe=NT017-13ZB; Porterville1=NT010-13ZA; Porterville2=NT016-13ZA

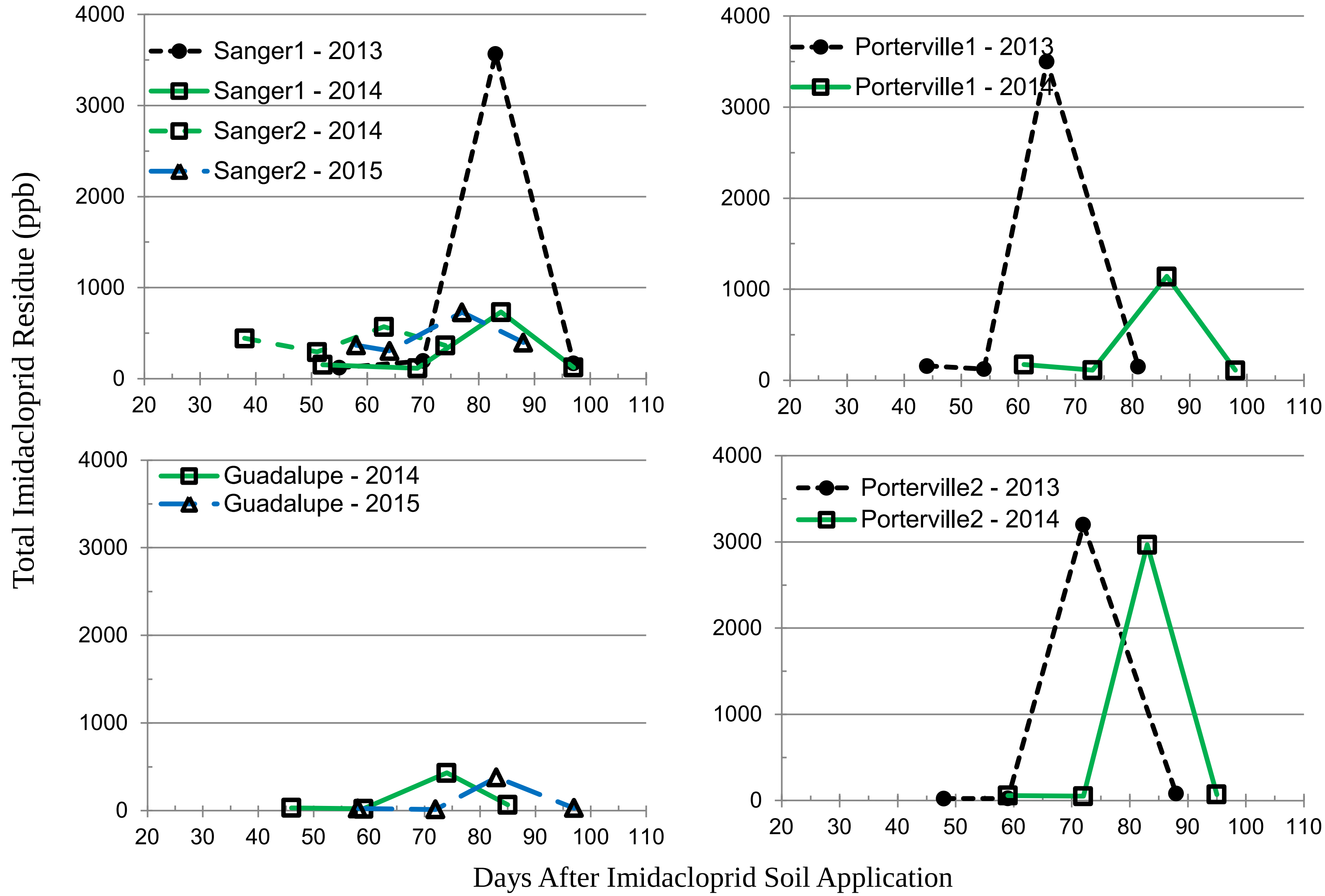
Figure 6-3B. Trend in total imidacloprid residue measured in pollen at each site. Within each panel markers, marker color and line style denote the year, i.e. year 2013 is a solid black circle and a small-dashed black line; year 2014 is a hollow square and a green solid line; year 2015 is a hollow triangle and a large-dashed blue line.



<u>Specific Site Information:</u>

Fresno=NT013-13ZA; ; San Luis Obispo=NT039-13ZA; San Joaquin=NT040-13ZA; Kerman=NT041-13ZA

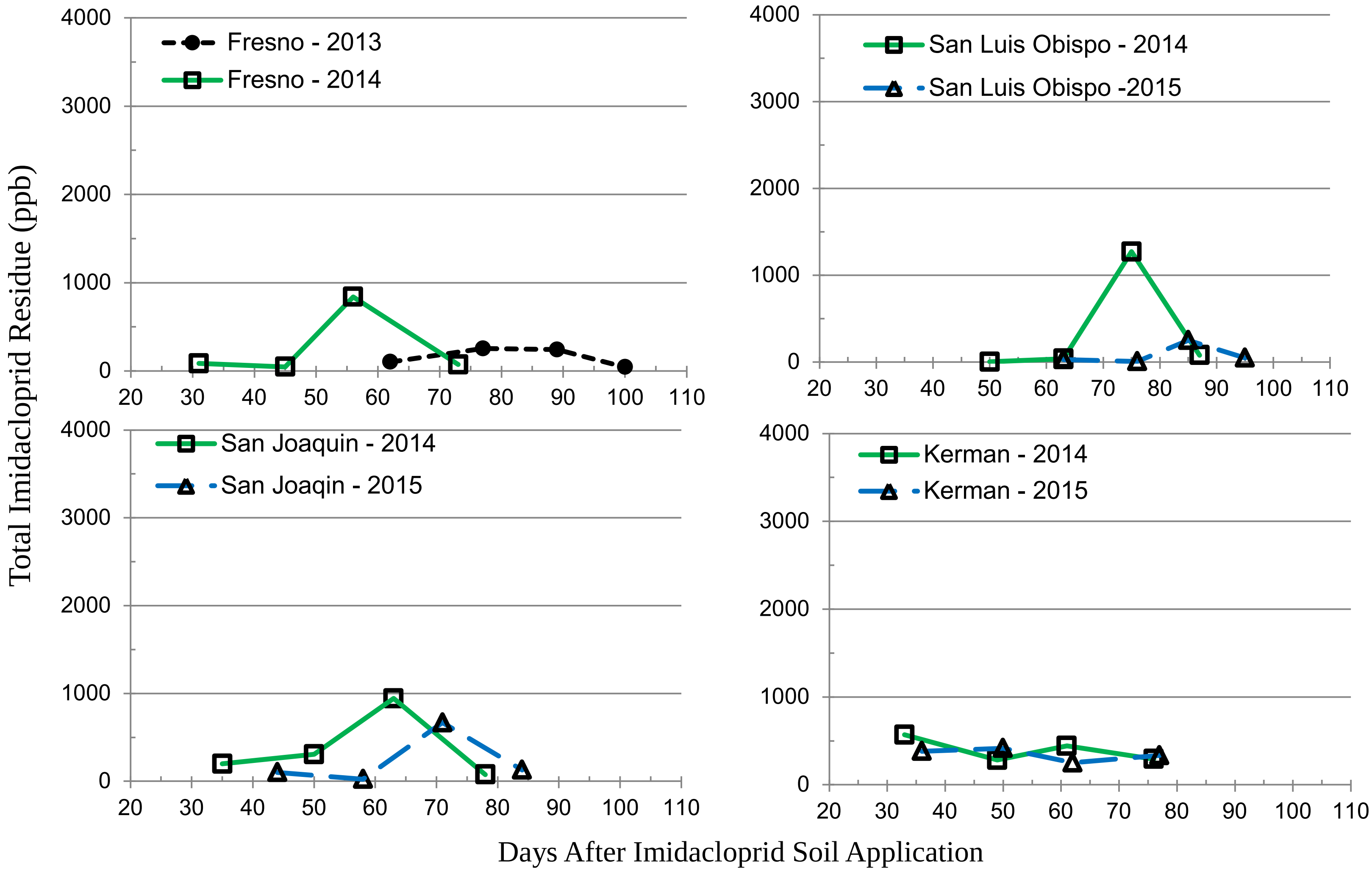
Figure6-4 A. Trend in total imidacloprid residue measured in leaf tissue at each site. Within each panel markers, marker color and line style denote the year, i.e. year 2013 is a solid black circle and a small-dashed black line; year 2014 is a hollow square and a green solid line; year 2015 is a hollow triangle and a large-dashed blue line. Note both Sanger both sites are plotted on the same graph with site 2 year 2014 a green dashed line.



<u>Specific Site Information:</u>

Sanger1=NT018-13ZA; Sanger2=NT042-13ZA; Guadalupe=NT017-13ZB; Porterville1=NT010-13ZA; Porterville2=NT016-13ZA

Figure 6-4B. Trend in total imidacloprid residue measured in leaf tissue at each site. Within each panel markers, marker color and line style denote the year, i.e. year 2013 is a solid black circle and a small-dashed black line; year 2014 is a hollow square anda green solid line; year 2015 is a hollow triangle and a large-dashed blue line



<u>Specific Site Information:</u>

Fresno=NT013-13ZA; ; San Luis Obispo=NT039-13ZA; San Joaquin=NT040-13ZA; Kerman=NT041-13ZA

MRID 49090504 CDPR IMI Citrus (2011)

Reference
Byrne, F.; Morse, J.; Visscher, P.; Grafton-Cardwell, E.; Leimkuehler, W. (2011) Determination of exposure levels of honey bees foraging on flowers of citrus trees previously treated with imidacloprid. Project Number: EBNTL056/7, M/408424/012. Unpublished study prepared by University of California, Riverside. 70p. MRID 49090504, CDPR Study ID 259131, Data Volume 51950-0787, Tracking ID# 246252

1. STUDY INFORMATION

Study Reviewed By:	CDPR, U.S. EPA and PMRA	**Study Completion Date:**	March 30, 2011
Sponsor:	Bayer CropScience 2T.W. Alexander Drive Research Triangle Park, NC USA 27709	**Study Location:** 1) Hemet, California 2) Lindcove Research and Extension Center 3) Ventura County, California 4) Temecula, California 5) Tulare County, California 6) University of California, Riverside	
Sponsor Study ID:	EBNTL056-7	**PC Code:**	129099
GLP Status:	Non-GLP; Final protocol was submitted to CDPR for review. [CDPR study ID 253951, Data Volume 51950-0756, Tracking ID# 240317]		
Study Type:	Non-Guideline field residue study on Southern California Citrus Groves that had been previously treated with Imidacloprid to evaluate bee exposure.		

2. REVIEWER INFORMATION

Primary Reviewers: California Department of Pesticide Regulation	**John Troiano, Ph.D.**, Research Scientist III, Environmental Monitoring Branch **Richard Bireley**, Senior Environmental Scientist (Specialist), Ecotoxicology Group, Pesticide Registration Branch **Denise Alder**, Senior Environmental Scientist (Specialist), Lead Reevaluation Coordinator, Pesticide Registration Branch **Russell Darling**, Environmental Scientist, Reevaluation Coordinator, Pesticide Registration Branch
Secondary Reviewer:	TBD

3. TEST MATERIAL CHARACTERIZATION

Test Material:	1) Admire Pro 2) Unknown Formulations	**Percent Active Ingredient:**	1) 42.8% A.I. 2) Unknown
Description:	1) Suspension concentrate (SC) 2) Unknown	**Density:**	1.41-1.54 g/mL
Material Source:	Bayer Corporation	**Solubility:**	0.51 to 0.61 g/L
CAS #:	138261-41-3	**pH (24°C):**	7.8

4. EXECUTIVE SUMMARY

A series of field investigations were conducted between 2008 and 2011 to determine to what extent honey bees foraging on citrus blossoms may be exposed to imidacloprid when citrus trees are treated with systemic applications (soil treatments) of this insecticide.

The approach that was taken was to compare the imidacloprid residues in floral nectar with nectar extracted from the crops, foraging honey bees and from stored nectar within their hives. Two experimental systems were established. First, honey bee colonies were confined within large tunnel cage enclosures where foraging access to treated trees was limited. Second, honey bee colonies were situated in an open-field system, in which hives were placed in a large acreage of commercial citrus that had been treated with imidacloprid. In this second system, the foraging activity of the honey bees was not limited to any specific trees or to citrus.

In addition to the honey bee studies, the study quantifies the levels of imidacloprid in nectar from trees that had been treated under a number of different scenarios. The intent was to determine residues in trees treated in successive years to test for potential imidacloprid accumulation and persistence. For this, groves that were growing in different soil types were included because of the impact that soil has on the availability of imidacloprid for uptake. The effect of differing application rates were also evaluated on imidacloprid residues in nectar.

The subject study report consists of five different trials that will make up the composition of this Data Evaluation Report. Detailed findings, study limitations, study validity and results from each trial are found below.

5. STUDY VALIDITY

Guideline Followed:	Non-guideline study (final protocol submitted by CDPR)
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	**Supplemental**
Rationale:	N/A
Reparability:	N/A

6. SAMPLE COLLECTION, HANDLING, PROCESSING

Nectar Collections by Hand

Nectar was sampled from individual flowers using a micro-capillary tube inserted into a bulb dispenser. After insertion of the tip of the tube into the nectar at the base of the flower, the nectar was drawn into the tube by capillary action. The nectar was then transferred to an autosampler vial. The target volume for each sample was 150 µl. During field trips to collect the nectar, the samples were kept in an ice chest containing dry ice until they were stored in a -20°C freezer.

Nectar collections by Honey Bees

Nectar was collected from the crops of honey bees returning to their hives from foraging trips to determine the concentrations of imidacloprid and metabolites. Bees were intercepted at the entrance to the hives using a small net and were immediately transferred to a cooler containing dry ice to anesthetize them. After 30 seconds in the cooler, individual bees were forced to regurgitate the contents of their crops by pressing gently on the lateral sides of their abdomen region (the gaster) with paddle forceps. The nectar formed a droplet at the top of their mandibles where it was collected using a micro-capillary tube. The nectar was transferred to an autosampler vial and then placed in a cooler with dry ice. For each sample it was necessary to composite nectar from several bees in order to meet the minimum target volume of 75 µl. At least 5 bees were used to prepare 1 sample and a maximum of 20 samples from each hive were collected.

Stored Nectar

In addition to the stomach nectar, nectar was also collected from deposits made to new comb within each hive. A sample consisted of pooled nectar from comb cells near each other. Sufficient nectar was collected for residue analysis and to measure sugar concentration using a refractometer to provide information on possible changes in imidacloprid concentration during conversion from nectar to honey. The target sample number was 10 samples from each hive.

7. ANALYTICAL METHODS

Nectar

Nectar collected from citrus trees (orange, mandarins and grapefruit) was analyzed by dilution with mobile phase containing the stable isotope internal standards. 50 µL of nectar was pipetted into an autosampler vial containing a 300 µL conical insert. 50 µL of mobile phase (9:1 water/methanol containing 10 mM NH_4HCO_3) containing 10 ng/mL of the stable isotope internal standards was added and the sample mixed a couple times by pulling up and down with the Gilson Microman pipette. The final concentration in the diluted nectar of the internal standard was 5 ng/mL. The samples were analyzed by LC/MS/MS. Response ratios (NA/IS) were directly compared to a calibration curve having concentrations of 5 ng/mL for the internal standards and native analyte concentrations ranging from 0.1 to 100 ng/mL and adjusted for dilution, i.e., 50 µL of nectar diluted to 100 µL (2X dilution). Recoveries (QC) samples were prepared by diluting control nectar with the QC dilution solution as described.

Pollen

A 0.05 to 0.5 g sample of pollen was weighed into a 13 mL polypropylene conical screw cap centrifuge tube. 1.5 mL of HPLC grade water (pesticide residue grade) was added and the pollen dissolved. 20 ng of the stable isotope internal standard mixture of imidacloprid, imidacloprid olefin and 5-hydroxy imidacloprid was added followed by 4 mL of ACN. The tube was then shaken vigorously for one minute. Then 1 g of $MgSO_4$ (anhydrous) and 0.5 g NaCl was added and the tube again shaken and vortexed vigorously for 1 minute. The tubes were then centrifuged at 4000 RPM for 5 minutes using an Eppendorf 5810 R centrifuge.

The supernatant, nominally 4 mL, was decanted into another 13 mL centrifuge tube containing, 0.040 g BONDESIL-PSA and 170 g Mg SO_4 (anhydrous). The tube was shaken and vortexed vigorously for 1 minute. The tubes were centrifuged as before. A 1.0 mL portion of the supernatant (ACN) was transferred to a LC autosampler vial and the ACN was avaporated to dryness using a N-Evap or Tubovap. The residue was re-dissolved in 1.0 mL of HPLC mobile phase (9:1 water/MeOH containing 10 mM NH_4HCO_3) in preparation for analysis by LC/MS/MS.

Recoveries (QC) samples (5 ppb) were prepared by fortifying control pollen (500 mg) with mixed native stds (25 µL of the 0.1 µg/mL mixed standard).

Imidacloprid, imidacloprid olefin and 5-hydroxy imidacloprid were measured in nectar and pollen. Nectar was measured directly by dilution with HPLC mobile phase and the pollen was extracted using QuEChERS methodology. The final extracts were analyzed by LC/MS/MS employing stable isotope internal standards. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated based on 3X and 10X, respectively, of the standard deviation of 4 QC recovery samples from stomach nectar at 1 ppb. From this evaluation, the LOD and LOQ were calculated to be 0.018, 0.36 and 0.30 ppb (LOD) and 0.6, 1.2 and 1 ppb (LOQ) for imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin, respectively.

8. TRIALS

7A. TRIAL INFORMATION

Title:	Tunnel Cage Trial		
Application Date:	September 3, 2009	**Sampling Date:**	April 24, 2010
Section of Study:	Section 2	**Trial Location:**	Lindcove Research and Extension Center (LREC)

TRIAL SUMMARY

The objective of this component of the study was to examine citrus groves that were treated with a soil application of imidacloprid systemic insecticide, to understand the levels of imidacloprid that occurred in (a) nectar extracted by hand from citrus flowers, (b) nectar collected by forager honey bees and transported back to the hive, and (c) nectar or "uncapped honey" deposited by bees in cells of the Brood comb.

Honey bee colonies were confined within tunnel cage enclosures, each containing 3 flowering citrus trees that had been treated with soil applications of systemic imidacloprid (Admire Pro®). Nectar was collected by hand from each tree within each tunnel cage. In addition, nectar was extracted from the stomachs of honey bees foraging within the tunnels so that a comparison could be made between hand-collected and honey bee-collected nectar. At the end of the confinement period within the tunnels, stored nectar was sampled from new comb that had been placed within each hive at the start of the exposure period. Four tunnels were constructed, providing three imidacloprid-treated and one untreated control data set.

METHOD

APPLICATION TIMING AND RATES

Admire Pro® was applied at the maximum label rate of 14 fl oz per acre (0.104 fl oz per tree) on Sept. 3, 2009. Trees were pre-irrigated for 2 hours prior to applications. The insecticide was applied to each tree using a watering can, taking care to apply the solution evenly within the irrigated area defined by the sprinklers. Watering cans were used to administer the insecticide to the trees in order to ensure that the correct amount of active ingredient, as defined by the insecticide label, was delivered to each tree. Trees were irrigated for another 4 hours following completion of the applications, and then subjected to the standard 8 hour, once-per-week irrigation regime during the first 4 weeks post-treatment. Thereafter, trees were irrigated according to tensiometer measurements.

STUDY SITE LOCATION AND CHARACTERISTICS

This trial was conducted at the Lindcove Research and Extension Center (LREC) in Exeter, California. The trees were Washington Parent Navels on C35 rootstock, which were planted in June 1992 (17 years old at the time of the study). The soil type at LREC is a loam soil consisting of 15-25% clay and 00.5-1% organic matter. There were 134 trees per acre with a tree spacing of 18’ x 18’ in the 3.9 acre block. Each tree was irrigated by 2 sprinklers located on opposite sides of the trunk with 10.3 gph output on a weekly schedule.

The enclosures were constructed from transparent netting with a maximum light exclusion of 13%. The netting was supported on a frame constructed from 1" PVC tubing to provide a semi cylindrical tunnel that fully enclosed 3 citrus trees. There was sufficient clearance to allow movement of personnel between the citrus trees and the netting so that all sections of the trees could be used for nectar sampling. Tunnel dimensions were 26' x 96' x 16'.

The Italian honey bee, *Apis mellifera ligustica,* was used in the subject trial. When the trees were in full bloom, one small colony of bees (a nuclear hive consisting of 5 frames of comb, a queen, developing brood, and workers to cover about 4 frames) was introduced into each tunnel. Hives were initially placed in the tunnels at 8.00 a.m. on April 20, 2010. Due to heavy rainfall, bees were confined within the hives until the morning of April 22, 2010 when the bees were released to begin foraging.

SAMPLING METHOD

Nectar was sampled directly from the flower by hand using a micro-capillary tube. The target volume for each sample was 150 µl, with nine composite nectar samples collected, three from each tree. Samples were also collected using honey bees that were contained over the three trees by a tunnel constructed from transparent netting (Combined Clear Net 13%) with 1” PVC tube frames. The bees were intercepted at the hive entrance and chilled with dry ice for 30 seconds. A composite sample from at least 5 bees was collected. In addition, stored nectar was collected from new combs within each hive. A pooled sample was collected from cells near each other with a target of ten samples per hive. Nectar sugar analysis was measured by refractometry.

RESULTS:

The mean residues of total imidacloprid in hand or bee collected citrus nectar ranged from 13.97-21.19 ppb, while mean residues in hive stored nectar ranged from 44.65-72.81 ppb. The highest residues were found in hive stored nectar, corresponding with the highest sugar concentration.

Table 1. Summary of imidacloprid residues in nectar collected from orange trees within four tunnel enclosures that had been treated the previous two years as a soil drench at the maximum labelled rate. Maximum application rate applied to individual trees on 9/3/2009.

Lindcove Research and Extension Center (LREC) Spring 2010 Loam Soil [15-25% clay, 0.5 – 1% organic matter] Mean Residue Concentration (ppb) (minimum – maximum)						
Tunnel	Hand Collected Nectar N[1] = 9 [3 samples from each tree, 3 trees per tunnel] Sampling occurred 4/22/2010		Bee Collected Nectar N[1] = 20 [one hive per tunnel, mean of 20 samples] Sampling occurred 4/22/2010		Uncapped Stored Nectar N[1] = varies [one hive per tunnel, mean of up to 10 samples] Sampling occurred 4/24/2010	
	IMI	Total IMI[2]	IMI	Total IMI[2]	IMI	Total IMI[2]
Control	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1	8.3 (2.86 – 13.64)	13.96 (5.30 – 22.73)	10.61 (5.6 – 21.22)	17.55 (9.17 – 37.12)	25.49 (19.71 – 36.06)	44.65 (35.40 – 62.75)
2	7.64 (4.38 – 12.38)	14.01 (8.06 – 22.84)	8.97 (3.02 – 16.17)	15.08 (4.92 – 29.75)	27.33 (24.81 – 30.83)	49.65 (42.52 – 57.58)
3	12.81 (8.72 – 21.91)	21.19 (9.18 – 34.64)	8.00 (1.66 – 18.99)	13.38 (2.81 – 34.15)	40.12 (27.14 – 54.14)	72.81 (48.75 – 95.18)
Mean for Tunnels 1-3	**9.58 (7.64 – 12.81)**	**16.39 (13.96 – 21.19)**	**9.23 (8.00 – 10.61)**	**15.40 (13.38 – 17.55)**	**30.98 (25.49 – 40.12)**	**55.70 (44.65 – 72.81)**

[1] "N" is the total number of samples collected

2 "Total IMI" combines magnitude of residues of imidacloprid plus degradants IMI-olefin and IMI-5-OH

CONCLUSION TO TUNNEL CAGE

In this first trial, oranges (Washington Parent Navels on C35 rootstock) in a 3.9 acre block were treated in the fall of 2009 with 14 fl oz per acre (the maximum current U.S. label rate). The trial was located at the Lindcove Research and Extension Center (LREC) in Exeter, CA. The soil type at LREC is a loam soil consisting of 15-25% clay and 0.5-1% organic matter. Honey bee colonies were confined within tunnel cage enclosures, each containing 3 flowering citrus trees.

Nectar was collected by hand from each tree within each tunnel cage. In addition, nectar was extracted from the stomachs of honey bees foraging within the tunnels so that a comparison could be made between hand-collected and honey bee-collected nectar. At the end of the confinement period within the tunnels, stored nectar was sampled from new comb that had been placed within each hive at the start of the exposure period.

The mean residues of total imidacloprid in hand or bee collected citrus nectar ranged from 13.97-21.19 ppb, while mean residues in hive stored nectar ranged from 44.65-72.81 ppb. The highest residues were found in hive stored nectar, corresponding with the highest sugar concentration.

7B. TRIAL INFORMATION

Title:	Open Field Trial		
Application Date:	Fall 2009	**Sampling Date:**	April 25, 2010
Section of Study:	Section 3	**Trial Location:**	Lindcove Research and Extension Center (LREC)

TRIAL SUMMARY

Honey bee colonies were situated within a large area of treated commercial citrus. Within the area, there were multiple citrus varieties, some weeds, but no other commercial crops. The bees were allowed to forage freely. Nectar was collected by hand from trees within several different blocks within the perceived foraging area. In addition, nectar was extracted from the stomachs of honey bees foraging within the citrus region so that a comparison could be made between hand-collected and honey bee-collected nectar. Whereas in the tunnel study, hand-collected and honey bee-collected sources were controlled, the foraging range of the honey bees was not controlled. At the end of the 3-day foraging period, nectar was sampled from new comb that had been placed within each hive at the start of the trial. Pollen traps were also included in the hives and these were operated for a 24-hour period prior to the completion of the experiment.

The Italian honey bee *Apis mellifera ligustica* was also used in this study. Five large colonies consisting of 10 frames were situated in the citrus area on the evening of April 22. The bees were confined within the hives until the morning of April 23 when the bees were released to begin foraging. The larger hives used for the open field study were equipped with pollen traps that were set into operation for 24 hours beginning on April 24, 2010 and concluding on April 25, 2010.

Hand-collect nectar, bee-collected nectar, and stored nectar were collected in the same way as in the tunnel trial. Pollen samples were also collected from pollen traps within a 24-hour collection period prior to the completion of the experiment. Pollen samples from only two hives were available for residue analysis. It was found that the pollen load in each trap was very low given the 24-hour collection period.

Pollen sources were not distinguished for residue analysis. Upon visual examination, there were clearly several pollen sources within the traps indicating that the bees were not foraging exclusively on citrus flowers.

METHOD

APPLICATION TIMING AND RATES

All citrus groves in a radius of 1-2 miles on and around the LREC were treated for aphids with imidacloprid in the fall of 2009. A very large contiguous area of citrus had been treated in 2009 with various imidacloprid commercial formulations at half the maximum field rate (0.25 lb. a.i. per acre regardless of the commercial formulations used). A large block of citrus was selected in the center of such treatments for this honey bee study with the knowledge that bees can forage very long distances from their hives. Although there were other blooming plants in the vicinity of this block that honey bees could have foraged from, there were no nearby agricultural crops other than citrus. All nearby citrus within 1 mile had been treated with the half rate of imidacloprid the previous fall.

STUDY SITE LOCATION AND CHARACTERISTICS

The Lindcove Research and Extension Center (LREC) contains much of the collection of important rootstock and scions for the Citrus Clonal Protection Program (CCPP) and research projects for the State of California. In 2009, a higher than normal number of trees in the region became infected with Citrus tristeza virus (CTV). CTV is a pathogen vectored by several species of aphids. The LREC has soils characterized as loam soils (15-25% clay, 0.5-1% organic matter).

RESULTS:

In this trial, the mean residues of total imidacloprid in hand collected citrus nectar ranged from 1.81-9.42 ppb while the mean residues in nectar extracted from foraging honey bees ranged from 1.11-7.59 ppb. Also, the mean residues of total imidacloprid in hive stored nectar ranged from 6.95-11.63 ppb. Moreover, the maximum mean residues for hive pollen were found to be 10.2 ppb while the average residue measurement was 9.39 ppb.

There appears to be large variations in the level of residues among citrus varieties. Due to the limited samples and information provided, it is unknown if this variation was due to citrus varieties or other factors, including age of the trees or level or irrigation.

Table 2. Summary of imidacloprid residues in hand collected nectar randomly selected within six blocks of citrus surrounding the location of five honey bee hives. Commercial citrus trees were treated in the Fall of 2009 at half (½X) the labelled rate.

Lindcove Research and Extension Center (LREC) Spring 2010 Loam Soil [15-25% clay, 0.5 – 1% organic matter] Mean Residue Concentration (ppb) (minimum – maximum)					
Citrus Variety	Hand Collected Nectar				
	N[1]	IMI	5-OH	Olefin	Total IMI[2]
Valencia Oranges – South East	10	<1.0 (<1.0 – 1.18)	<1.0	<1.0	1.81 (1.15 – 2.48)
Valencia Oranges – South West	5	2.73 (1.53 – 3.55)	<1.0	<1.0 (<1.0 – 2.22)	5.18 (3.17 – 6.21)
Navel Oranges – North East	4	1.79 (<1.0 – 2.48)	<1.0	<1.0 (1.02 – 1.95)	3.51 (2.11 – 4.42)
Navel Oranges – South East	4	3.51 (2.03 – 6.15)	<1.0	<1.0 (<1.0 – 1.73)	4.87 (2.64 – 8.42)
Tangerines	10	6.82 (2.84 – 13.26)	<1.0	1.99 (<1.0 – 3.67)	9.42 (4.03 – 18.28)
Young Oranges	5	2.76 (1.34 – 4.21)	<1.0	<1.0 (<1.0 – 1.11)	3.73 (1.76 – 5.88)
Means		**3.29 (<1.0 – 6.82)**	**<1.0**	**1.19**	**4.75 (1.81 – 9.42)**

[1] "N" is the total number of samples collected
[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH

Table 3. Summary of imidacloprid residues in nectar collected from free-ranging honey bees in commercial citrus trees treated in the fall of 2009 at half the labelled rate.

Lindcove Research and Extension Center (LREC) Spring 2010 Loam Soil [15-25% clay, 0.5 – 1% organic matter] Mean Residue Concentration (ppb) (minimum – maximum)						
Hive	Bee Collected Nectar N[1] = varies [mean of up to 20 samples] Sampling occurred 4/23/2010		Uncapped Stored Nectar N[1] = varies [mean of up to 10 samples] Sampling occurred 4/25/2010		Hive Pollen [Each value represents a single measurement for the analysis of the entire pollen retrieved from traps within individual hives] Sampling occurred 4/25/2010	
	IMI	Total IMI[2]	IMI	Total IMI[2]	IMI	Total IMI[2]
1	1.05 (<1.0 – 2.43)	2.11 (<1.0 – 5.07)	6.25 (4.73 – 8.67)	11.63 (9.31 – 15.53)	N/A	
2	3.77 (<1.0 – 9.31)	7.59 (1.16 – 16.02)	N/A		N/A	
3	1.94 (<1.0 – 7.56)	3.59 (<1.0 – 12.16)	N/A		N/A	
4	<1.0 (<1.0 – 2.69)	1.11 (<1.0 – 3.71)	3.23 (2.47 – 4.98)	6.96 (4.47 – 9.25)	6.58	8.57
5	1.94 (<1.0 – 4.2)	3.23 (<1.0 – 7.29)	5.98 (3.81 – 8.18)	10.60 (8.13 – 13.98)	5.84	10.2
Mean for Hive 1-5	**1.88 (<1.0 – 3.77)**	**3.53 (1.11 – 7.59)**	**5.15 (3.23 – 6.25)**	**9.72 (6.96 – 11.63)**	**6.21**	**9.39**

[1] "N" is the total number of samples collected
[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH

CONCLUSION TO OPEN FIELD TRIAL

The second trial, also at the LREC, consisted of an open field area with multiple citrus varieties. A very large contiguous area of citrus had been treated in 2009 with various imidacloprid commercial formulations at 0.25 lbs. a.i./acre (half the maximum label rate).

Five large 10-frame colonies were situated in the citrus orchard area. Pollen was successfully collected from only two of five hives via pollen traps that were operated for 24 hours. Nectar was either hand collected from randomly selected citrus trees, extracted from honey stomachs of honey bees allowed to forage in the treated orchard and intercepted at the hive, and from the hive as uncapped honey. Nectar sugar analysis was measured by refractometry.

In this trial, the mean residues of total imidacloprid in hand collected citrus nectar ranged from 1.81-9.42 ppb while the mean residues in nectar extracted from foraging honey bees ranged from 1.11-7.59 ppb. Also, the mean residues of total imidacloprid in hive stored nectar ranged from 6.95-11.63 ppb. Moreover, the maximum mean residues for hive pollen were found to be 10.2 ppb while the average residue measurement was 9.39 ppb.

7C. TRIAL INFORMATION

Title:	One Year Nectar Collections		
Application Date:	September 3 and 9, 2009	**Sampling Date:**	Spring 2010
Section of Study:	Section 4	**Trial Location:**	Lindcove Research and Extension Center (LREC) and Bakersfield, CA

TRIAL SUMMARY

As research into the potential uses of imidacloprid for the management of Asian Citrus Psylliid (*Diaphorina citri*), different imidacloprid rates at several locations within the citrus producing areas of southern and central California were evaluated. Nectar from trees treated with either the maximum label rate (1X) or double the maximum label rate (2X) of imidacloprid (Admire Pro) were evaluated to determine whether there was a relationship between application rates and residue levels. For this component of the trial, all data were derived from analysis of hand-collected nectar.

METHOD

APPLICATION TIMING AND RATES

The treatments were applied by water can on September 3, 2009 and September 8, 2009 at the LREC and Bakersfield sites, respectively. Citrus trees were treated with Admire Pro at the maximum label rate (1X=14 fl oz/ acre) and double the maximum label rate (2X= 28 fl oz/acre) in the Fall of 2009. Residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin were then analyzed from hand-collected nectar samples.

STUDY SITE LOCATION AND CHARACTERISTICS

For this trial, Lindcove Research and Extension Center (LREC) and a commercial citrus farm in Bakersfield were used to collect data from. The LREC trees were in the same block of navel oranges that were used for the honey bee trial. At the commercial citrus farm in Bakersfield, a 3.9 acre block of Valencia oranges that were planted in 1985 were provided. There were 100 trees per acre compared to the 134 trees were acre at the LREC site. The LREC has soils classified as loam soil (15-25% clay, 0.5-1% organic matter). Soil characteristics were not provided in the study for Bakersfield.

RESULTS:

The 1X label rate treatments resulted in an average residue measurement of 19.30 ppb in nectar at the Bakersfield grove and 16.30 ppb at the LREC. While the 2X label rate treatments resulted in an average residue measurement of 47.36 ppb at the Bakersfield grove and 35.83 ppb at the LREC.

Residues of imidacloprid, 5-hydroxy Imidacloprid and imidacloprid olefin were higher in nectar sampled from the trees treated with the 2X label rate compared with the 1X label rate, with the *ca.* twofold difference in residue levels reflecting the twofold difference in application rates. The imidacloprid and total residues were lower at the LREC site. The most likely reason for this difference is the higher tree density at the LREC site. Since the insecticide was applied on a per acre basis (1X=14 fl oz./acrea and 2X=28 fl oz./acre), the amount of active ingredient per tree would have been higher at the Bakersfield site and this could, therefore, account for the higher residues in the nectar.

Table 4. Summary of imidacloprid residues in hand collected nectar resulting from a soil treatment at maximum label rate (1X) and twice maximum label rate (2X). Application made by watering can to individual trees in Bakersfield on 9/8/2009 and LREC on 9/3/09.

Spring 2010 Mean Residue Concentration (ppb) (minimum – maximum)					
Location Citrus Variety	Rate	Hand Collected Nectar (N[1] = 10)			
		IMI	5-OH	Olefin	Total IMI[2]
Bakersfield Valencia Oranges (soil type unknown)	1X	12.13 (3.62 – 18.82)	2.53 (1.10 – 3.61)	4.64 (1.58 – 6.93)	19.30 (6.30 – 29.10)
	2X	31.10 (9.70 – 92.05)	5.67 (1.88 – 15.95)	10.60 (3.12 – 31.15)	47.36 (14.70 – 139.15)
LREC Navel Oranges [Loam Soil]	1X	9.51 (3.31 – 15.49)	3.09 (1.18 – 5.39)	3.70 (1.56 – 6.04)	16.30 (6.81 – 26.45)
	2X	22.23 (5.98 – 43.94)	6.70 (2.25 – 11.74)	6.89 (1.70 – 13.06)	35.82 (9.93 – 68.30)

[1] "N" is the total number of samples collected
[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH.

CONCLUSION TO ONE YEAR NECTAR COLLECTIONS

The third trial was conducted at the LREC and a commercial citrus farm in Bakersfield, CA. Navel oranges (LREC) and Valencia oranges (Bakersfield) were treated with either 1X (the maximum label rate) or 2X the label rate in Fall 2009. Hand collected nectar was quantified in Spring 2010.

Residues of imidacloprid, 5-hydroxy Imidacloprid and imidacloprid olefin were higher in nectar sampled from the trees treated with the 2X label rate compared with the 1X label rate, with the *ca.* twofold difference in residue levels reflecting the twofold difference in application rates

7D. TRIAL INFORMATION

Title:	Citrus Nectar Collected from Field Sites Treated in Successive Years		
Application Years:	2008 & 2009	**Sampling Date:**	Spring 2010
Section of Study:	Section 5	**Trial Location:**	1) Hemet, California 2) Temecula, California 3) Lindcove Research and Extension Center (LREC)

TRIAL SUMMARY

The objective of this trial was to determine if imidacloprid residues in nectar could persist and/or accumulate in situations where the insecticide was used on the same trees in successive years. Residues in nectar sampled from citrus trees that had been treated in 2 successive years were quantified. Sites were chosen with different soil types for the sampling program. Imidacloprid uptake into trees can be affected by soil type and this could potentially affect the residues in nectar. All analyses were conducted on nectar that was extracted by hand.

METHOD

APPLICATION TIMING AND RATES

Hemet. The first treatments with Admire Pro were applied in Fall 2008 and the second treatments were applied in Fall 2009. The intent was to evaluate both the 1X label rate and the 2X label rate in all possible application sequences. In order to do this, the block was subdivided into four 2.5 acre plots so that the following sequences of treatments could be established: 1X (Fall 2008) followed by 1X (Fall 2009), 1X (Fall 20.08) followed by 2X (Fall 2009), 2X (Fall 2008) followed by 1X (Fall 2009), and 2X (Fall 2008) followed by 2X (Fall 2009). Nectar Samples were taken from trees chosen at random from within each plot.

Temecula. Six commercial orchards in Temecula, California that had been treated both years of 2008 and 2009 in an area-wide control program were used in this study. The sites consisted of five Grapefruit and one Valencia orange orchard, which were first treated between June 7 and June 16, 2008 and then treated a second time between April 7 and May 27, 2009. The maximum label rate (1X) for Admire Pro was used in both treatment years.

Lindcove Research and Extension Center. Five citrus blocks at the LREC had been treated with imidacloprid for two successive years (2008 and 2009). The sites consisted of four navel oranges and one Valencia orange orchard, which were first treated on September 17 and September 18, 2008 and then treated a second time between September 10 and September 16, 2009. The maximum label rate (1X) for Admire Pro was used in both treatment years.

STUDY SITE LOCATION AND CHARACTERISTICS

Hemet. The trial was conducted in a 10 acre block of 50 year old Ruby Red Grapefruit trees on Troyer Rootstock. Soil type was sandy loam and irrigation was scheduled weekly according to tensiometer measurements. Nectar samples were collected from treated trees at random within the commercial grapefruit orchard.

Temecula. A total of 68.25 acres of Star Ruby Grapefruit and 1.65 acres of Valencia oranges were treated with imidacloprid for two successive years. The soil type at all six site locations is considered to be Sandy Loam. At each site, two trees were randomly selected for hand-collected nectar sampling.

Lindcove Research and Extension Center. Five citrus blocks with 2 successive years of imidacloprid applications were chosen from the LREC. These blocks were independent from the block used for the tunnel study. The soil type at all five site locations is considered to be Loam Soil. A total of 15.44 acres of navel oranges and 2.5 acres of Valencia oranges were treated with imidacloprid. Within each block, 2 trees were randomly selected for hand-collected nectar sampling.

RESULTS:

Imidacloprid nectar residues in Hemet were lowest in the 1X – 1X label rate treatment with an average residue measurement of 23.84 ppb and highest in the 2X – 2X label rate treatment with an average residue measurement of 58.66 ppb. Following the 1X – 1X label rate treatments in two successive years at Temecula and LREC, an average residue measurement of the nectar residues were 5.15 ppb and 11.16 ppb, respectively. The maximum mean residues of 21.65 ppb were measured at LREC.

The imidacloprid and total residues measured in the nectar sampled from trees treated with the 1X-2X label rate sequence were approximately 2-fold higher than those measured for the 1X- 1X label rate treatments, suggesting that the residue levels reflected the rate of imidacloprid used in the most recent application. Thus, residues for the 1X- 1X and 2X-1X rate sequences were not significantly different, and data for the 1X-2X and 2X-2X rate sequences were not significantly different. While the means for the 2X treatments exceeded the 1X measurements by more than twofold, the statistical analysis shows that imidacloprid and its metabolites did not accumulate significantly from one year to the next.

Data from six commercial groves in Temecula that were treated in two successive years (2008 and 2009) with the maximum label rate of imidacloprid (1X-1X) were completed by June each year. The highest total residues (9.56 ppb and 15.53 ppb for the two trees sampled) were measured at Site 6, where the youngest trees (seven years of age) used in the sampling program was located. The overall means for the Temecula samples were lower than those measured for the Hemet site where the 1X rate was applied for two successive years.

Data from five blocks at the LREC that were treated in two successive years (2008 and 2009) with the label rate of imidacloprid (1X-1X) had higher residues than those measured at the Temecula site, possibly reflecting the later timing of treatments. Despite the higher values at the LREC site, the residues were still lower than those measured for trees at Hemet. The major difference between the LREC and Hemet sites is the soil type and it seems that the lighter and sandier soil at Hemet allowed better uptake of imidacloprid into the trees resulting in higher residues in nectar the following spring.

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Table 5. Summary of imidacloprid residues in hand collected nectar of Ruby Red grapefruit trees resulting from two successive year soil treatments at maximum label rate (1X) and twice maximum label rate (2X) in Fall 2008/Fall 2009.

Hemet Site Spring 2010 [Sandy Loam] Mean Residue Concentration (ppb) (minimum – maximum)					
Rate	Hand Collected Nectar				
	N	IMI	5-OH	Olefin	Total IMI[2]
1X – 1X	10	16.07 (9.88 – 25.67)	5.05 (3.62 – 6.43)	2.72 (1.57 – 4.00)	23.84 (15.57 – 35.47)
1X – 2X	9	35.13 (13.28 – 63.60)	9.70 (6.70 – 12.33)	6.04 (3.77 – 9.31)	50.87 (27.54 – 85.03)
2X – 1X	7	14.50 (11.77 – 22.66)	4.78 (3.63 – 7.49)	2.44 (1.73 – 3.66)	21.73 (17.62 – 33.81)
2X – 2X	7	41.41 (28.62 – 62.67)	10.78 (7.14 – 13.56)	6.47 (4.38 – 10.48)	58.67 (40.14 – 86.71)

[1] "N" is the total number of samples collected
[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH.

Table 6. Summary of imidacloprid residues in hand collected nectar sampled in Spring 2010 from citrus trees receiving a soil treatment at maximum label rate (1X) in successive years 2008 and 2009.

Spring 2010 Mean Residue Concentration (ppb) (minimum – maximum) Sampling occurred 4/2010						
Location	Rate	N[1]	Hand Collected Nectar			
			IMI	5-OH	Olefin	Total IMI[2]
Temecula	1X – 1X	11	3.17 (<1.0 – 10.60)	<1.0 (<1.0 – 1.18)	1.48 (<1.0 – 3.76)	5.15 (<1.0 – 15.54)
LREC	1X – 1X	9	6.51 (<1.0 – 13.65)	1.80 (<1.0 – 5.40)	2.86 (<1.0 – 8.42)	11.17 (1.53 – 21.64)

[1] "N" is the total number of samples collected
[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-5-OH and IMI-olefin

CONCLUSION FOR FIELD SITES TREATED IN SUCCESSIVE YEARS

The fourth trial was conducted to determine if imidacloprid residues in nectar could accumulate from year to year following successive year applications at three different locations (Hemet, Temecula, and LREC). Hand collected nectar samples were obtained with either 1X or 2X soil applications were made in two successive years (2008, 2009) prior to sampling during bloom 2010.

Imidacloprid residues at the Hemet site appear to be a function of the rate applied at the most recent application only, with no evidence of carryover from previous years. The overall means for the Temecula samples were lower than those measured for the Hemet site where the 1X rate was applied for two successive years. Data from five blocks at the LREC that were treated in two successive years (2008 and 2009) with the label rate of imidacloprid (1X-1X) had higher residues than those measured at the Temecula site, possibly reflecting the later timing of treatments. Despite the higher values at the LREC site, the residues were still lower than those measured for trees at Hemet. The major difference between the LREC and Hemet sites is the soil type and it seems that the lighter and sandier soil at Hemet allowed better uptake of imidacloprid into the trees resulting in higher residues in nectar the following spring.

7E. TRIAL INFORMATION

Title:	Citrus Nectar Collection from Field Sites Treated in Successive Years		
Application Years:	2008, 2009 and 2010	**Sampling Date:**	Spring 2011
Section of Study:	Appendix B (Supplemental)	**Trial Location:**	1) Tulare County, California 2) Temecula, California 3) Lindcove Research and Extension Center (LREC) 4) Ventura County, California 5) University of California, Riverside 6) Hemet, California

TRIAL SUMMARY

The objective of this trial was to determine if residues of imidacloprid and its important metabolites could persist and/or accumulate in nectar in situations where the insecticide was used on the same trees in successive years. Also, because imidacloprid uptake into trees can be affected by soil type, sites were chosen to reflect the variety of soil types where citrus is gown in California. In section 3 of this Data Evaluation Report, data and information was provided for several sites where citrus was grown in soils that ranged from sandy loam to loam. To expand on the previous trial, this study will include data and information on heavier clay soils and lighter soils. Nectar was extracted from flowers by hand at all sites during bloom and imidacloprid, imidacloprid olefin and 5-hydroxy imidacloprid were quantified by LC/MS/MS.

METHOD

APPLICATION TIMING AND RATES

Tulare County. Five of the citrus sites had been treated with the full label rate of imidacloprid for at least 3 previous years. The sixth citrus site was treated with the full label rate of imidacloprid for 2 successive years. Two composite nectar samples were collected from each grove and all nectar samples were hand-collected.

Temecula Valley. Nectar samples were hand-collected from six groves where the trees have been treated for three successive years with the full label rate of imidacloprid.

Lindcove Research and Extension Center. Nectar samples were hand-collected from five citrus blocks at the LREC that had been treated with the full label rate of imidacloprid for three successive years (September 2008, 2009, and 2010).

Ventura County. Nectar samples were hand-collected from a lemon grove where the trees had been treated with the full label rate of imidacloprid at different timings during the season. The treatment timings were in May, July and September of 2010. The subject grove had not been treated in 2009. Two composite nectar samples were collected from trees treated at application timing 1 and 3, while one composite nectar sample was collected from trees treated at application 2.

University of California, Riverside. In October of 2010, nectar samples were collected from a citrus block where the trees had been treated with the full label rate of imidacloprid. Sixteen composite samples were collected from the trees.

Hemet. In the previous trial titled "Citrus Nectar Collected from Field Sites Treated in Successive Years" (Section 5 of study report), data was provided for a grapefruit grove that had been treated in successive years (Fall 2008 nad Fall 2009) with different combinations of application rates. The initial trial began with either a 1X label rate of imidacloprid (Admire Pro at 14 fl oz/ acre) or a 2X label rate (Admire Pro at 28 fl oz/ acrea). In 2009, the same trees were treateed to give different treatment rate scenarios. To expand on the subject trial, an additional year of treatment was provided to the trees in 2010.

STUDY SITE LOCATION AND CHARACTERISTICS

Table 7. Site location with information regarding soil type and treated commodity.

SITE AND COMMODITY INFORMATION		
TEMELCULA	**SOIL TYPE**	**COMMODITY**
1	FALLBROCK ROCKY SANDY LOAM	STAR RUBY GRAPEFRUIT
2	FALLBROCK ROCKY SANDY LOAM	STAR RUBY GRAPEFRUIT
3	FALLBROCK ROCKY SANDY LOAM	VALENCIA ORANGE
4	FALLBROCK ROCKY SANDY LOAM	STAR RUBY GRAPEFRUIT
5	FALLBROCK ROCKY SANDY LOAM	STAR RUBY GRAPEFRUIT
6	GREENFIELD SANDY LOAM	STAR RUBY GRAPEFRUIT
TULARE COUNTY		
1	PORTERVILLE CLAY	TANGELOS
2	CENTERVILLE CLAY	NAVEL ORANGES
3	PORTERVILLE CLAY	NAVEL ORANGES
4	PORTERVILLE CLAY	NAVEL ORANGES
5	PORTERVILLE CLAY	NAVEL ORANGES
6	PORTERVILLE CLAY	VALENCIA ORANGES
OTHER SITES		
LINDCOVE RESEARCH AND EXTENSION CENTER	SAN JOAQUIN LOAM	NAVEL AND VALENCIA ORANGES
UNIVERSITY OF CALIFORNIA, RIVERSIDE	ARLINGTON LOAM	VALENCIA ORANGES
VENTURA COUNTY	MOCHO LOAM	LEMON

RESULTS:

Total mean residues in nectar of imidacloprid from trees treated three years in a row at the Temecula site ranged from 1.02 to 5.91 ppb with an average residue measurement of 2.57 ppb. Mean nectar residues from Tulare County ranged from 0.29 to 4.21 ppb with an average residue measurement of 1.54 ppb. Total mean nectar residues from trees at the LREC with a loam soil ranged from 0.52 to 23.95 ppb with an average residue measurement of 4.55 ppb. Total mean nectar residues from the UCR site

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with a loam soil ranged from 0.83 to 13.88 ppb with an average residue measurement of 3.68 ppb. Imidacloprid nectar residues from Ventura County sites treated at various times during 2010 were all less than 1 ppb regardless of the application timing. Following three years of treatments at the Hemet site at various application rates, the average residue measurement in nectar for the 1X-1X-1X treatment regimen was 24.40 ppb. The average residue measurement following the third year of treatment for sites treated once per year with 2X the label rate (2X-2X-2X) was 44.81 ppb. For the 1X-2X-0X and 2X-1X-0X treatments the average residue measurement was 31.67 and 32.97 ppb. The residues following three years of applications at 2X the maximum label rate were slightly lower than residues after just two years.

Table 8. Summary of imidacloprid residues in hand collected nectar sampled in Spring 2011 from citrus trees receiving a soil treatment at maximum label rate (1X) in successive years 2008, 2009, and 2010.

Spring 2011 Mean Residue Concentration (ppb) (minimum – maximum)						
Location	Rate	N[1]	Hand Collected Nectar			
			IMI	5-OH	Olefin	Total IMI[2]
Temecula	1X – 1X – 1X	12	1.68 (<1.0 – 3.48)	<1.0	<1.0 (<1.0 – 1.62)	2.58 (1.01 – 5.91)
LREC	1X – 1X – 1X	10	3.50 (<1.0 – 16.87)	<1.0 (<1.0 – 3.88)	<1.0 (<1.0 – 3.20)	4.95 (<1.0 – 23.95)

[1] "N" is the total number of samples collected
[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH

Table 9. Supplement to **Table E-10.** Summary of imidacloprid residues in hand collected nectar resulting from successive year soil treatments at maximum label rate (1X), twice maximum label rate (2X), and no application (0X) in Fall 2008/Fall 2009/Fall 2010.

Hemet Site Spring 2011 [Sandy Loam] Mean Residue Concentration (ppb) (minimum – maximum)				
Rate	Hand Collected Nectar (N[1] = 10)			
	IMI	5-OH	Olefin	Total IMI[2]
1X – 1X – 1X	19.68 (12.26 – 33.82)	3.13 (2.02 – 4.31)	1.59 (<1.0 – 4.96)	24.40 (17.56 – 39.86)
1X – 2X – 0X	26.01 (23.05 – 30.00)	3.93 (3.35 – 4.37)	1.73 (1.44 – 1.92)	31.67 (28.97 – 36.27)
2X – 1X – 0X	27.02 (20.01 – 33.45)	3.87 (2.28 – 5.43)	2.08 (1.51 – 3.11)	32.97 (25.12 – 41.99)
2X – 2X – 2X	36.86 (26.67 – 41.92)	5.18 (3.86 – 5.76)	2.77 (2.27 – 3.16)	44.81 (32.80 – 50.35)

[1] "N" is the total number of samples collected
[2] "Total IMI" combines magnitude of residues of imidacloprid plus degradates IMI-olefin and IMI-5-OH

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Table 10. Information on trees used for hand collected nectar collected from six commercial orchards in Tulare County during bloom in Spring 2011 after three successive years of imidacloprid application for control of glassy-winged sharpshooter 2008, 2009, 2010.

Tulare County [Porterville Clay – 40% clay] Spring 2011				
Site	Citrus Variety	2008 Treatment Date	2009 Treatment Date	2010 Treatment Date
1	Tangelos	Not treated	6/16/2009	5/21/2010
2	Navel Oranges	5/22/2008	6/29/2009	6/18/2010
3	Navel Oranges	7/8/2008	6/18/2009	6/9/2010
4	Navel Oranges	5/17/2008	6/18/2009	6/6/2010
5	Navel Oranges	5/21/2008	6/29/2009	6/15/2010
6	Valencia Oranges	7/3/2008	6/26/2009	6/16/2010

Table 11. Information on trees used for hand collected nectar collections during bloom in Spring 2011 after three successive years of imidacloprid application at the label rate 2008, 2009, 2010.

Temecula [Sandy Loam] Spring 2011				
Site	Citrus Variety	2008 Treatment Date	2009 Treatment Date	2010 Treatment Date
1	Star Ruby Grapefruit	6/7/2008	5/27/2009	5/22/10
2	Star Ruby Grapefruit	6/9/2008	4/13/2009	6/11/10
3	Valencia Orange	6/6/2008	4/7/2009	6/12/10
4	Star Ruby Grapefruit	6/9/2008	4/21/2009	5/17/10
5	Star Ruby Grapefruit	6/7/2008	4/10/2009	6/2/10
6	Star Ruby Grapefruit	6/16/2008	4/17/2009	5/14/10
LREC [Loam Soil] Spring 2011				
Site	Citrus Variety	2008 Treatment Date	2009 Treatment Date	2010 Treatment Date
1	Atwood Navel	9/18/2008	9/10/2009	9/13/2010
2	Atwood Navel	9/17/2008	9/10/2009	9/10/2010
3	Caracara Navel	9/18/2008	9/16/2009	9/09/2010
4	Parent Navel	9/18/2008	9/16/2009	9/09/2010
5	Red Valencia	9/17/2008	9/14/2009	9/13/2010

Table 12. Summary of imidacloprid residues in hand collected nectar sampled in Spring 2011 from citrus trees receiving a soil treatment at maximum labelled rate as part of the area side control program for glassy-winged sharpshooter in successive years 2008, 2009 and 2010.

Spring 2011 Mean Residue Concentration (ppb) (minimum – maximum)						
Location	Rate	N[1]	Hand Collected Nectar			
			IMI	4/5-OH	Olefin	Total IMI[2]
Tulare County	1X[3] – 1X – 1X	12	1.29 (<1.0 – 3.31)	<1.0	<1.0	1.70 (<1.0 – 4.21)

[1] "N" is the total number of samples collected
[2] "Total IMI" combines magnitude of residues of imidacloprid plus IMI-olefin and IMI-5-OH
[3] One site did not receive an application in 2008.

CONCLUSION

In 2011, the researchers followed up with a third year of treatments at the Temecula, LREC, and Hemet sampling sites. Nectar was again collected from six groves previously treated in Temecula where the soil type is sandy loam and five citrus blocks at the LREC with a loam soil (20% clay).

In addition, sites were added at University of California, Riverside (UCR), Ventura County and Tulare County to address different soil types not previously represented. Nectar was collected from six citrus groves in Tulare County grown in Porterville clay (clay content 40%). Of these six sites, five had been treated with 1X imidacloprid for the past three years and the remaining site was treated similarly the past two years. A new site in Ventura County with a soil consisting of 23% clay/35% sand was sampled following applications at the full label rate of imidacloprid at different times during the season. The treatment timings for this site were May, July and September 2010 and untreated in 2009. Also, a citrus block from the farm at UCR, where the soil type is loam, was treated in October 2010 and sampled in 2011.

In Temecula and Tulare County, the 2010 treatments at the 1X label rate were made in mid-May to mid-June. The LREC and UCR 2010 treatments at 1X label rate were made in September and October. Treatments at the four Hemet sites were 1X-1X-1X, 1X-2X-0X, 2X-1X-0X, or 2X-2X-2X representing years 2008, 2009 and 2010, where 1X-1X-1X represents a single application at the maximum label rate per year for three consecutive years.

9. STUDY VALIDITY/CLASSIFICATION AND STUDY LIMITATIONS

Classification/Utility for Bee Risk Assessment. This study is classified as supplemental for use in risk assessment due to no pollen data available and a potential underestimation of a worst case scenario due to current labels not restricting pre bloom and during bloom applications whereas this study done post bloom. The study results characterize expected imidacloprid residues in citrus nectar from applications in various soils ranging from fine to coarse and following different application rates and application timing in California. These results may not extrapolate directly to expected results in other regions of the U.S.

Concentrations in nectar extracted from the stomachs of free-ranging bees (open field study) were somewhat lower than samples collected directly from flowers of nearby trees. This may reflect a "dilution effect" from bees foraging on other (untreated) flower types. The few pollen samples obtained during the open field study had imidacloprid concentrations roughly equal to the nectar sampled from the same hives. Concentrations in flower nectar samples appear to be linearly related to the application rate, based on *ca.* twofold increases in residue levels with doubling the application rate in the Hemet trials.

Temporal Variability in Residues. Nectar samples were obtained from two locations (citrus blocks in the Temecula region and at LREC) where the 1X soil application rate of imidacloprid had been made in two successive years (2008, 2009) prior to sampling in April 2010. Residue levels at these 11 sites averaged 8 ppb and ranged from 1 to 18 ppb. The application timing (May, July, September, October) appears to be an important factor in determining residue levels in flower nectar the following year particularly for sites planted to coarse soils which consistently yielded the higher imidacloprid residues. Fall (Sept) applications resulted in about twofold higher residue concentrations than spring (April-June) applications.

Spatial Variability in Residues. The six locations for the citrus trials were in relatively close proximity. Soil types reflect sandy loam, loam or clay compositions (20-40% clay) and low organic carbon content (0.35-1.9%). Weather conditions (temperature and precipitation) were similar across the three trials. As a result of the close proximity of trial sites, this study provides very limited information on how differences in environmental conditions across different areas of the U.S. may affect accumulation of total imidacloprid in pollen and nectar.

Pesticide Carryover. The authors speculated that imidacloprid residues at the Hemet site appear to be a function of the rate applied at the most recent application only, with no evidence of carryover from previous years. However, following the third year of application at the Hemet site, residues were higher at the two sites receiving no treatment in 2010 than at the site treated all three years with 1X. This indicates some degree of carryover from previous application years, at least for sites treated with the 2X rate during one of the two years prior to the no treatment year. This was the only site where samples were collected following a year without treatment.

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Reference
Miller, A., and Jerkins, E. (2016) Determination of the Residues of Imidacloprid and its Metabolites 5-Hydroxy Imidacloprid and Imidacloprid Olefin in Bee Relevant Matrices Collected from Apple Trees following Soil and Foliar Applications of Imidacloprid over Two Successive Years: Final Report. Project Number: EBNTN014. Unpublished study prepared by Bayer Cropscience LP. 406. MRID 49662101, CDPR Study ID 289057, Data Volume 51950-0901, Tracking ID# 273842

1. STUDY INFORMATION

Chemical:	Imidacloprid	**PC Code**	129099
Test Material:	Admire Pro Systemic Protectant	**Percent Active Ingredient:**	42.8%
Study Type:	Field residue study on apple orchards to measure the residues of imidacloprid and metabolite levels in nectar, pollen and on leaves following one soil and two foliar applications.		
Sponsor:	Bayer CropScience 2T.W. Alexander Drive Research Triangle Park, NC USA 27709	**Experiment Start and End Date:**	August 1, 2013 – July 22, 2015
Sponsor Study ID:	EBNTN014	**Study Locations:**	Nine Apple Orchard field trials located in California.
Study Completion Date:	January 13, 2016		
GLP Status:	GLP; protocol reviewed by CDPR. [CDPR Study ID 289057, Data Volume 51950-0901, Tracking ID# 273842]		

2. REVIEWER INFORMATION

Study Reviewed by: California Department of Pesticide Regulation	Richard Bireley, Sr. Environmental Scientist (Specialist) John Troiano, Ph.D., Research Scientist III Alexander Kolosovich, Environmental Scientist Brigitte Tafarella, Environmental Scientist Denise Alder, Sr. Environmental Scientist (Specialist) Russell Darling, Environmental Scientist

3. EXECUTIVE SUMMARY

A total of nine field trials were conducted to measure the magnitude of imidacloprid residues in apple nectar, pollen and leaves following one soil and two foliar applications of Admire Pro® Systemic Protectant in each of two successive years. Admire Pro Systemic Protectant is a suspension concentrate formulation containing 550 g/L imidacloprid. Applications were made in the fall of 2013 and 2014, post-bloom.

Across both years, individual soil application rates ranged from 0.38 to 0.39 lb. imidacloprid/acre. The interval between the soil application and first foliar application was 3 to 5 days. For all foliar applications, individual rates ranged from 0.059 to 0.064 lb. imidacloprid/acre. The interval between first and second foliar application was 8 to 10 days. Application volumes ranged from 13,000 to 15,200 gal/acre (GPA) for the soil applications and from 55 to 75 GPA for the foliar applications. Total seasonal application rates ranged from 0.50 to 0.52 lb. imidacloprid/acre.

In 2013, trials NT031-13ZA and NT036-13ZA made applications prior to apple harvest, while the other trials made all applications post-harvest. Soil applications were made at BBCH growth stages 79 to 99, while the two foliar applications were made at BBCH growth stages 81 to 99 and 85 to 99, respectively. In 2014, all applications were made prior to apple harvest. Soil applications were targeted for 21 days prior to apple harvest and made at BBCH growth stages 75 to 89. The two foliar applications were targeted such that the last would occur 7 days prior to harvest, with sprays made at BBCH growth stages 65 to 85 and 67 to 89, respectively.

All applications were made using ground-based equipment. The adjuvant Dyne-Amic (0.25 % v/v) was used in all foliar applications.

Apple flower (also called blossom) and leaf samples were collected once in the spring of 2014, following the fall 2013 applications, and once in the spring of 2015, following the fall 2014 applications. At each sampling interval, two composite samples of apple flowers (to be hand-processed to obtain apple nectar and pollen) and apple leaves were collected by hand when the apple trees were at bloom.

Single composite samples of apple flowers and leaves were collected from the control plot of each trial on the same days that samples were collected from the treated plots.

After their collection, apple flowers were hand-processed at the field site to obtain the bee relevant matrices of apple nectar and pollen. The processed flowers were discarded.

The residues of Admire Pro Systemic Protectant (imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

4. STUDY VALIDITY

Guideline Followed:	See Section 7; Protocol was reviewed and accepted by CDPR
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	**Acceptable**
Rationale:	N/A
Reparability:	N/A

5. MATERIALS

Test Material Characterization			
Test item:	Admire Pro Systemic Protectant	**Percent A.I.:**	42.8% A.I.
pH (20°):	7.8	**Density (20°C):**	1.41 to 1.54 g/mL
CAS #:	138261-41-3	**Solubility:**	0.51 to 0.61 g/L

5A. Study Design

This study requirement was part of the Neonicotinoid Reevaluation at the California Department of Pesticide Regulation (CDPR). The study design and protocol were approved by the CDPR prior to study initiation. This study was conducted using GLP standards and following an approved protocol. The study initiation date was August 01, 2013. The experimental start date was August 30, 2013 (first application), and the experimental end date was July 22, 2015 (last analysis).

Nine trials were conducted for this study, representing each of the three soil texture categories (fine, medium, and coarse) throughout multiple locations in California. Each trial includes one untreated control plot and one TRTD plot to be treated in two consecutive years. Apple varieties in this study represented those commonly grown in the area and agronomic practices typical for commercial production of apples were used at all trial locations.

Homogenization and analysis of the leaf, nectar, and pollen samples from this study were performed by Bayer CropScience in Research Triangle Park (RTP), NC. Final report preparation was performed by Critical Path Services, LLC, located in RTP, NC.

5B. Application Timing and Rates

Applications were made in the fall of 2013 and 2014, post-bloom. Across both years, individual soil application rates ranged from 0.38 to 0.39 lb. imidacloprid/acre. The interval between the soil application and first foliar application was 3 to 5 days. For all foliar applications, individual rates ranged from 0.059 to 0.064 lb. imidacloprid/acre. The interval between first and second foliar applications was 8 to 10 days. Application volumes ranged from 13,000 to 15,200 gal/acre (GPA) for the soil applications and from 55 to 75 GPA for the foliar applications. Total seasonal application rates ranged from 0.50 to 0.52 lb. imidacloprid/acre.

In 2013, trials NT031-13ZA and NT036-13ZA made applications prior to apple harvest, while the other trials made all applications post-harvest. Soil applications were made at BBCH growth stages 79 to 99 (BBCH 79: fruit about 90% final size; BBCH 99: harvested product), while the two foliar applications were made at BBCH growth stages 81 to 99 (BBCH 81: beginning of ripening, first appearance of cultivar-specific color) and 85 to 99 (BBCH 85: advanced ripening, increase in intensity of cultivar-specific color), respectively. In 2014, all applications were made prior to apple harvest. Soil applications were targeted for 21 days prior to apple harvest and made at BBCH growth stages 75 to 89 (BBCH 75: fruit about half final size; BBCH 89: fruit ripe for consumption, fruit have typical taste and firmness). The two foliar applications were targeted such that the last would occur 7 days prior to harvest, with sprays made at BBCH growth stages 65 to 85 (BBCH 65: full flowering, at least 50% of flowers open, first petals falling) and 67 to 89 (BBCH 67: flowers fading, majority of petals fallen), respectively.

All applications were made using ground-based equipment. The adjuvant Dyne-Amic (0.25 % v/v) was used in all foliar applications.

Table 1. Application and Location Information

Trial Identification	Location (City, State, NAFTA Region)	Application Year	Method	Timing BBCH[a]	Rate, lb. a.i./Acre	Total Rate, lb. a.i./Acre
NT028-13ZA	Clarksburg, California Region 10	2013	Drip Application	99	0.381	0.50
			Airblast Application	99	0.059	
			Airblast Application	99	0.060	
		2014	Drip Application	76	0.381	0.50
			Airblast Application	81	0.060	
			Airblast Application	81	0.060	
NT029-13ZA	Stockton, California Region 10	2013	Drip Application	99	0.381	0.50
			Airblast Application	99	0.060	
			Airblast Application	99	0.060	
		2014	Drip Application	NR[b]	0.379	0.50
			Airblast Application	81	0.061	
			Airblast Application	81	0.060	
NT030-13ZA	Linden, California Region 10	2013	Drip Application	99	0.380	0.50
			Airblast Application	99	0.060	
			Airblast Application	99	0.060	
		2014	Drip Application	77	0.379	0.50
			Airblast Application	81	0.061	

Trial Identification	Location (City, State, NAFTA Region)	Application Year	Method	Timing BBCH[a]	Rate, lb. a.i./Acre	Total Rate, lb. a.i./Acre
			Airblast Application	81	0.060	
NT031-13ZA	Clarksburg, California Region 10	2013	Drip Application	79	0.381	0.50
			Airblast Application	81	0.060	
			Airblast Application	85	0.060	
		2014	Drip Application	78	0.381	0.50
			Airblast Application	81	0.060	
			Airblast Application	81	0.060	
NT032-13ZA	Linden, California Region 10	2013	Drip Application	99	0.381	0.50
			Airblast Application	99	0.060	
			Airblast Application	99	0.060	
		2014	Drip Application	75	0.381	0.50
			Airblast Application	81	0.060	
			Airblast Application	81	0.060	
NT033-13ZA	Linden, California Region 10	2013	Drip Application	99	0.380	0.50
			Airblast Application	99	0.060	
			Airblast Application	99	0.060	
		2014	Drip Application	76	0.381	0.50
			Airblast Application	81	0.061	
			Airblast Application	81	0.060	

Trial Identification	Location (City, State, NAFTA Region)	Application Year	Method	Timing BBCH[a]	Rate, lb. a.i./Acre	Total Rate, lb. a.i./Acre
NT034-13ZA	Madera, California Region 10	2013	Drip Application	99	0.391	0.52
			Airblast Application	99	0.063	
			Airblast Application	99	0.063	
		2014	Drip Application	NR	0.391	0.52
			Airblast Application	65	0.062	
			Airblast Application	67	0.063	
NT035-13ZA[c]	Madera, California Region 10	2013	Drip Application	99	0.377	0.50
			Airblast Application	99	0.063	
			Airblast Application	99	0.063	
NT036-13ZA	Sanger, California Region 10	2013	Drip Application	87	0.380	0.50
			Airblast Application	91	0.061	
			Airblast Application	91	0.059	
		2014	Drip Application	89	0.380	0.50
			Airblast Application	85	0.059	
			Airblast Application	89	0.059	

[a] Typical commercial apple harvest generally occurs between BBCH 87 and 89. In 2013, trials NT031-13ZA and NT036-13ZA made applications prior to apple harvest, and all other trials applied post-harvest (BBCH 99). In 2014, all applications were made prior to apple harvest.
[b] NR = Not reported; the BBCH growth stage at this application was not reported by the PFI.
[c] Trial NT035-13ZA was not able to complete the second year of sampling, so only one year of test substance application data are reported.

5C. STUDY SITE LOCATION AND CHARACTERISTICS

A variety of soil types were included in the study design. Bayer CropScience conducted the study in three soil texture types, fine, medium, and coarse, based on Soil Survey Geographic (SSURGO) Database mapping units. There are nine trial sites in this apple study design: three in fine texture soils, one in medium, and five in coarse. Two years of data for each site are presented in this report with the exception of trial NT035-13ZA (coarse soil), which could not complete year 2 because the apple trees were removed from the plot, so only first year data are reported from this trial.

Table 2. Trial Site Conditions for Apple Orchard

Trial (Field) Identification	Trial Location (County, State)	OM (%)	pH	CEC (meq/100g soil)	% Sand	% Silt	% Clay	Soil Types	Rainfall (in)	Temperature Range (°F)
NT028-13ZA	Clarksburg, California	6.1	6.5	26.9	16	30	54	Clay (Fine)	23.0	28 - 89
NT029-13ZA	Stockton, California	2.8	7.4	28.2	22	38	40	Clay Loam (Fine)	17.6	29 - 92
NT030-13ZA	Linden, California	2.5	7.1	22.8	48	24	28	Sandy Clay Loam (Coarse)	17.6	29 - 92
NT031-13ZA	Clarksburg, California	4.7	6.7	26.2	12	30	58	Clay (Fine)	23.0	28 - 89
NT032-13ZA	Linden, California	5.0	7.0	21.6	26	40	34	Clay Loam (Medium)	17.6	29 - 92
NT033-13ZA	Linden, California	1.6	6.9	11.6	68	20	12	Sandy Loam (Coarse)	17.6	29 - 92
NT034-13ZA	Madera, California	1.3	7.0	8.0	68	22	10	Sandy Loam (Coarse)	10.3	31 - 97
NT035-13ZA	Madera, California	0.88	7.1	8.4	68	20	12	Sandy Loam (Coarse)	3.6	28 - 77
NT036-13ZA	Sanger, California	0.83	6.1	6.1	67	26	7	Sandy Loam (Coarse)	10.3	31 - 97

5D. Sample Collection, Handling, Processing

Apple flower (also called blossom) and leaf samples were collected once in the spring of 2014, following the fall 2013 applications, and once in the spring of 2015, following the fall 2014 applications. The exception is trial NT035-13ZA, in which the year 2 (2015) sample collection was cancelled because the apple trees were removed from the trial field. Each TRTD plot was divided into two subplots. At each sampling interval, two composite samples (one from each subplot) of apple flowers (to be hand-processed to obtain apple nectar and pollen) and apple leaves were collected by hand when the apple trees were at bloom, BBCH 65 to 69 (BBCH 69: end of flowering, all petals fallen). Exceptions are the leaf samples collected in 2014 from trials NT034-13ZA and NT035-13ZA and in 2015 from trial NT036-13ZA, which were collected at BBCH 71 (BBCH 71: fruit size up to 10 mm, fruit fall after flowering). In 2014, apple flower samples were collected at 138 to 193 days after the last application (DAA), and apple leaf samples were collected at 151 to 214 DAA. In 2015, apple flower samples were collected at 131 to 287 DAA, and apple leaf samples were collected at 147 to 293 DAA.

Single composite samples of apple flowers and leaves were collected from the control plot of each trial on the same days that samples were collected from the treated plots.

Apple flowers and leaves were collected by hand into Ziplock bags from all four quadrants (high, low, inside, and outside) of the trees in the subplot (UTC plot: 6 to 24 trees; TRTD subplots: 8 to 15 trees). Each composite flower sample contained a minimum of 125 g, and each composite leaf sample contained a minimum of 100 g.

After their collection, apple flowers were hand-processed at the field site to obtain the bee relevant matrices of apple nectar and pollen. Nectar processing began the same day as flower collection. Nectar from the floral nectary was removed by a micropipette and placed into a pre-weighed glass collection vial. The blossoms were then allowed to dry overnight at room temperature to desiccate the pollen. The next day, pollen was removed from the apple blossoms either by vacuum aspiration with collection in filter tips or by tapping the pollen from the blossoms onto wax paper and collection of the accumulated pollen into a vial. All resulting nectar and pollen samples were labeled and placed in the freezer immediately after they were generated. After processing was completed, the flowers were discarded.

Composite samples of apple nectar, pollen, and leaves were placed into labeled (study number and sample number) containers for shipment. All leaf, nectar, and pollen samples were placed in frozen storage within 6 hours and 10 minutes of collection.

Sample Storage.

Upon arrival at Bayer CropScience, all leaf, nectar, and pollen samples were immediately transferred to frozen storage. The leaf samples were homogenized with dry ice using a Robot Coupe chopper and were returned to frozen storage immediately following homogenization. Pollen and nectar were used without further processing. All samples remained frozen at all times except during subsampling for analysis.

Stability studies have indicated that imidacloprid residues are stable (<30% decomposition) for 24 months (728 to 769 days) of freezer storage in the following representative crops: an oilseed (tomatoseed), a non-oily grain (wheat), a leafy vegetable (lettuce), a root crop (potato), a tree fruit (apple), and a fruiting vegetable (tomato)[4-10]. An additional stability study has indicated that imidacloprid residues are stable (<30% decomposition) for 36 months of freezer storage in wheat (grain), orange (fruit), tomato (fruit), bean (seed), and rape (seed)[11]. Demonstrated freezer stability in all of the above crops is representative of the freezer stability of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin residues to be expected for apple leaves from this study. The apple leaves analyzed in this study were held in frozen storage for a maximum of 561 days (18 months) prior to extraction.

To demonstrate that imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin were stable in the apple nectar and pollen, samples of nectar surrogate and commercial pollen were fortified with a mixture of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin, each at a level of 100 or 200 ppb. These transit stability samples were shipped to the field site at the start of each study year and were subsequently stored with the study samples. The transit stability samples were analyzed after all sample analyses were complete. The transit stability analyses indicate that the imidacloprid residues are stable (<30% decomposition) during concurrent freezer storage with the nectar and pollen samples from this study report.

5E. ANALYTICAL METHODS

The analytical methods[1-2] used in this study measured the residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin in apple nectar, pollen, and leaves. All neat analytical reference standards were stored frozen prior to dilution. All reference standard solutions were prepared in parent equivalents and corrected for purity during initial preparation.

For the apple leaves[1], a 2.5 g sample was weighed into a 50-mL polypropylene conical centrifuge tube, and 10 mL of HPLC-grade water was added. The tube was mixed manually for 1 minute, followed by adding 20 mL of acetonitrile and manual shaking for an additional 1 min. Then, 3 g of MgSO4 and 1.5 g of NaCl were added. The sample was amended with a mixed internal standard solution and manually mixed for 1 minute. For leaf samples found to contain high imidacloprid residues (>2 ppm), as determined by an initial run in which the response exceeded the calibration curve, the sample was amended with a 10X mixed internal standard solution before the salts were added. The sample was centrifuged, and 20 mL of the organic supernatant was transferred into a separate 50-mL polypropylene conical centrifuge tube containing 0.3 g of Bondesil-PSA and 1.8 g of $MgSO_4$. For the high imidacloprid residue samples, 2.0 mL of the organic supernatant and 18.0 mL of acetonitrile were instead transferred. The sample was again manually mixed for 1 minute. The sample extract was centrifuged and a 1.25 mL aliquot of supernatant was transferred into a clean culture tube. The sample aliquot was evaporated to near dryness on a Turbo-Vap. The extract was reconstituted with 1.25 mL of 9:1 water/methanol and transferred into a 2-mL sample vial for high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) analysis.

For nectar[2], a 0.1-mL sample was weighed into a 20 x 150 mm culture tube and dissolved in 4 mL of water. If the total sample volume was less than 0.1 mL, the entire sample was weighed and recorded. The mixture was amended with isotopically labeled internal standards, and the resultant solution was mixed well and applied to an Agilent BondElut Solid Phase Extraction (SPE) cartridge (50 mg resin; previously conditioned with methanol then water). The cartridge was washed with 1 mL of water/methanol (19:1 v/v), and the combined eluates were discarded. The analytes were extracted from the cartridge with 0.5 mL of water/methanol (4:1 v/v). The eluate was collected into a 2 mL sample vial for analysis by LC/MS/MS.

For pollen[2], a 0.1-g sample was weighed into a small Precellys vial containing 2.8 mm steel balls. If the available pollen sample amount was not sufficient for an analysis, samples of the same interval were composited and analyzed. The composite sample was weighed and recorded. A 1-mL portion of water/methanol (3:1 v/v) was added, and the mixture was homogenized with a bead mixer at 5000 beats/minute for 1 minute on a Precellys homogenizer. The isotopically labeled internal standards were added and mixed, and the mixture was centrifuged at 12,000 rpm for 2 minutes. The supernatant was transferred into a clean culture tube containing 2.5 mL of water and was evaporated to an aqueous remainder, then applied to a 3-mL ChemElut SPE cartridge. After 10 to 15 minutes, the cartridge was washed with 4 mL of hexane/ethyl acetate (1:1 v/v) three times into a clean culture tube. The combined eluates were evaporated to dryness. The analytes were dissolved in 0.5 mL of water/methanol (4:1 v/v). The solution was transferred into a 2 mL sample vial for analysis by LC/high resolution mass spectrometry (LC/HRMS).

Quantitation of each analyte was based on the daughter ion transitions of the analyte and the respective internal standard analog. The responses of the LC/MS/MS and LC/HRMS systems to each analyte and its internal standard were measured in samples and in standards, and a relative response was calculated (as the ratio of the analyte and the stable isotopically labeled internal standard responses). The relative response of the analyte in each sample was compared to the relative response of the analyte in the standards.

The relative responses of imidacloprid and its analytes were measured over the range of 0.12 to 2000 ppb. The analyte relative responses were fit to a linearity curve calculated using linear regression analysis with 1/x weighting (AB Sciex Analyst 1.6.1, 1.6.2 or Thermo Finnigan XCalibur 2.7.0.20, 2.2 SP1.48; Appendix 4 of the study report). Correlation coefficients were calculated with the same software.

All data are reported in parent equivalents, and the individual measured residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin are summed to give a total imidacloprid residue.

5F. QUALITY ASSURANCE RESULTS

The responses of the LC/HRMS and LC/MS/MS systems to imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin were linear in solvent over the range of .12 to 2000 ppb. The coefficients of determination were >0.99.

No total imidacloprid residue was calculated for the UTC samples, so the levels of imidacloprid as an individual analyte are described. Imidacloprid (parent) residues in UTC apple nectar ranged from below the analyte LOD to below the analyte LOQ. Imidacloprid (parent) residues in UTC apple pollen ranged from below the analyte LOD to 23 ppb (trial NT034-13ZA). Imidacloprid residues in UTC apple leaves were all below the analyte LOD with the exception of the year 2 samples from trial NT029-13ZA, which was likely, contaminated and had an imidacloprid residue of 2200 ppb.

The limit of quantitation (LOQ) is defined as the lowest fortification level of an analyte at which acceptable recovery has been achieved. The LOQ for a total residue is the highest of the LOQ values assigned to the individual analytes for a particular matrix.

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be determined to be statistically different from a blank. The LODs were determined from method validation data obtained from control samples fortified at the respective analyte LOQs. The LODs were calculated by multiplying the standard deviation of recovery measurements at the LOQ by $_{t0.99}$ [where $t_{0.99}$ is the one-tailed t-statistic at the 99% confidence level for the number of replicates (n)][3]. The LOD for the total imidacloprid residue in each matrix is the highest LOD value of any one individual analyte for that particular matrix.

The LOQs and LODs are summarized in the table below.

Summary of LOQs and LODs

Matrix	Analyte	LOQ (ppb, parent equivalents)	LOD (ppb, parent equivalents)
Apple Nectar	Imidacloprid	1.0	0.3
	5-hydroxy Imidacloprid	1.0	0.7
	Imidacloprid olefin	1.0	0.6

Matrix	Analyte	LOQ (ppb, parent equivalents)	LOD (ppb, parent equivalents)
	Total Imidacloprid	1.0	0.7
Apple Pollen	Imidacloprid	1.0	0.4
	5-hydroxy Imidacloprid	1.0	0.5
	Imidacloprid olefin	1.0	0.3
	Total Imidacloprid	1.0	0.5
Apple Leaves	Imidacloprid	5.0	0.9
	5-hydroxy Imidacloprid	5.0	0.5
	Imidacloprid olefin	5.0	0.8
	Total Imidacloprid	5.0	0.9

6. RESULTS:

The imidacloprid residue data for apple nectar, pollen, and leaves are provided in Table 3. Only residue values above the respective analyte LODs are reported. Any residue value that was below the LOD is reported as less than the LOD (<LOD). The total imidacloprid residue is a sum of the analyte residue values that are greater than the respective analyte LODs. If the analyte value was less than the LOD, a default value equal to half of the analyte LOD (half-LOD) was added into the sum. For samples with a reported total residue of less than LOD, the sum of the analyte half-LOD values for the respective matrix was used to calculate the average residue values.

Table 3. Results of Imidacloprid and Imidacloprid Metabolite Residue from Apple

Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	Imidacloprid Olefin Residues (ppb)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
Apple Nectar					**LODs (ppb):**	**0.6**	**0.7**	**0.3**	**0.7**
NT028-13ZA	Clarksburg, California Region 10 2014	Granny Smith	Fine	0.50	193	1.4	26.0	8.9	36.3 **Avg.: 36.3**
NT028-13ZA	Clarksburg, California Region 10 2015	Granny Smith	Fine	0.50	279	0.7 <LOD	<LOD <LOD	<LOD <LOD	1.1 0.8 **Avg.: 1.0**
NT029-13ZA	Stockton, California Region 10	York Imperial	Fine	0.50	177	<LOD <LOD	0.9 <LOD	1.1 0.4	2.3 1.0 **Avg.: 2.0**

Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	Imidacloprid Olefin Residues (ppb)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
	2014								
NT029-13ZA	Stockton, California Region 10 2015	York Imperial	Fine	0.50	278	0.7 <LOD	<LOD <LOD	<LOD <LOD	1.2 0.8 **Avg.: 1.0**
NT030-13ZA	Linden, California Region 10 2014	Granny Smith	Coarse	0.50	186	<LOD <LOD	<LOD <LOD	<LOD 1.2	0.8 1.8 **Avg.: 2.0**
NT030-13ZA	Linden, California Region 10 2015	Granny Smith	Coarse	0.50	287	<LOD 1.0	<LOD <LOD	1.0 0.7	1.6 2.0 **Avg.: 2.0**
NT031-13ZA	Clarksburg, California Region 10 2014	Fuji	Fine	0.50	193	<LOD <LOD	<LOD <LOD	1.2 2.8	1.9 3.4 **Avg.: 3.0**
NT031-13ZA	Clarksburg, California Region 10 2015	Fuji	Fine	0.50	273	<LOD <LOD	<LOD <LOD	<LOD <LOD	0.8 0.8 **Avg.: 1.0**
NT032-13ZA	Linden, California Region 10 2014	Shirely Ranch	Medium	0.50	177	<LOD <LOD	0.8 <LOD	0.9 0.4	2.0 1.0 **Avg. 2.0**
NT032-13ZA	Linden, California Region 10 2015	Shirley Ranch	Medium	0.50	278	<LOD <LOD	<LOD <LOD	<LOD <LOD	0.8 0.8 **Avg.: 1.0**
NT033-13ZA	Linden, California Region 10 2014	Granny Smith	Coarse	0.50	186	0.7 <LOD	<LOD <LOD	0.4 <LOD	1.4 0.8 **Avg.: 1.0**
NT033-13ZA	Linden, California	Granny Smith	Coarse	0.50	287	1.3 0.9	<LOD <LOD	0.7 0.7	2.3 2.0

Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	Imidacloprid Olefin Residues (ppb)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
	Region 10 2015								**Avg.: 2.0**
NT034-13ZA	Madera, California Region 10 2014	Fuji	Coarse	0.52	145	<LOD <LOD	<LOD <LOD	<LOD <LOD	0.8 0.8 **Avg.: 1.0**
NT034-13ZA	Madera, California Region 10 2015	Fuji	Coarse	0.52	265	<LOD 0.8	<LOD <LOD	<LOD <LOD	0.8 1.3 **Avg.: 1.0**
NT035-13ZA	Madera, California Region 10 2014	Gala	Coarse	0.50	138	<LOD <LOD	<LOD <LOD	0.6 0.5	1.2 1.2 **Avg.: 1.0**
NT036-13ZA	Sanger, California Region 10 2014	Pink Lady Apple	Coarse	0.50	151	1.6 1.8	<LOD <LOD	0.9 0.7	2.8 2.8 **Avg.: 3.0**
NT036-13ZA	Sanger, California Region 10 2015	Pink Lady Apple	Coarse	0.50	131	2.0 2.5	0.7 <LOD	1.3 1.2	4.0 4.0 **Avg.: 4.0**
Apple Pollen					**LODs (ppb):**	**0.3**	**0.5**	**0.4**	**0.7**
NT028-13ZA	Clarksburg, California Region 10 2014	Granny Smith	Fine	0.50	193	<LOD <LOD	0.8 <LOD	6.6 8.0	7.7 8.4 **Avg.:8.0**
NT028-13ZA	Clarksburg, California Region 10 2015	Granny Smith	Fine	0.50	279	0.5 3.4	<LOD 0.8	4.7 2.8	5.4 7.1 **Avg.: 6.0**
NT029-13ZA	Stockton, California Region 10 2014	York Imperial	Fine	0.50	177	0.9 <LOD	1.1 0.5	28.4 18.8	30.4 18.8 **Avg.: 25.0**

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Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	Imidacloprid Olefin Residues (ppb)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
NT029-13ZA	Stockton, California Region 10 2015	York Imperial	Fine	0.50	278	7.9 1.3	2.0 <LOD	4.8 5.6	14.6 7.1 **Avg.: 11.0**
NT030-13ZA	Linden, California Region 10 2014	Granny Smith	Coarse	0.50	186	<LOD <LOD	<LOD 0.5	20.5 20.2	20.9 20.8 **Avg.: 21.0**
NT030-13ZA	Linden, California Region 10 2015	Granny Smith	Coarse	0.50	287	14.7 9.0	3.6 2.4	34.2 91.3	52.4 102.7 **Avg.: 78**
NT031-13ZA	Clarksburg, California Region 10 2014	Fuji	Fine	0.50	193	<LOD <LOD	0.7 1.1	19.2 45.9	20.1 47.2 **Avg.: 34.0**
NT031-13ZA	Clarksburg, California Region 10 2015	Fuji	Fine	0.50	273	52.1 14.2	13.9 3.0	10.2 6.6	76.2 23.8 **Avg.: 50.0**
NT032-13ZA	Linden, California Region 10 2014	Shirely Ranch	Medium	0.50	177	0.3 0.4	<LOD 0.5	29.5 20.5	30.1 21.4 **Avg.: 26.0**
NT032-13ZA	Linden, California Region 10 2015	Shirley Ranch	Medium	0.50	278	<LOD 4.2	<LOD 1.0	5.0 2.0	5.4 7.2 **Avg.: 6.0**
NT033-13ZA	Linden, California Region 10 2014	Granny Smith	Coarse	0.50	186	<LOD <LOD	<LOD <LOD	14.2 0.7	14.6 1.1 **Avg.: 8.0**
NT033-13ZA	Linden, California Region 10	Granny Smith	Coarse	0.50	287	1.8 8.0	<LOD 1.8	13.6 28.7	15.6 38.5

Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	Imidacloprid Olefin Residues (ppb)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
	2015								
NT034-13ZA	Madera, California Region 10 2014	Fuji	Coarse	0.52	145	<LOD <LOD	<LOD <LOD	13.7 8.7	14.1 9.1 **Avg.: 12.0**
NT034-13ZA	Madera, California Region 10 2015	Fuji	Coarse	0.52	265	<LOD 0.8	<LOD <LOD	7.5 1.3	7.9 2.3 **Avg.: 5.0**
NT035-13ZA	Madera, California Region 10 2014	Gala	Coarse	0.50	138	<LOD <LOD	<LOD <LOD	3.8 1.4	4.2 1.8 **Avg.: 3.0**
NT036-13ZA	Sanger, California Region 10 2014	Pink Lady Apple	Coarse	0.50	151	1.0 1.9	<LOD 0.6	5.1 13.2	6.3 15.8 **Avg.: 11.0**
NT036-13ZA	Sanger, California Region 10 2015	Pink Lady Apple	Coarse	0.50	131	9.4 9.5	1.2 1.2	92.8 47.8	103.4 58.5 **Avg.: 81.0**
Apple Leaves					**LODs (ppb):**	**0.8**	**0.5**	**0.9**	**0.9**
NT028-13ZA	Clarksburg, California Region 10 2014	Granny Smith	Fine	0.50	214	<LOD <LOD	<LOD <LOD	<LOD <LOD	1.1 1.1 **Avg.: 1.0**
NT028-13ZA	Clarksburg, California Region 10 2015	Granny Smith	Fine	0.50	293	1.9 1.4	<LOD <LOD	1.1 <LOD	3.2 2.1 **Avg.: 3.0**
NT029-13ZA	Stockton, California Region 10 2014	York Imperial	Fine	0.50	198	2.1 4.0	<LOD <LOD	2.4 3.6	4.7 7.8 **Avg.: 6.0**
NT029-	Stockton,	York	Fine	0.50	293	11.7	30.0	3533.2	3575.0

Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	Imidacloprid Olefin Residues (ppb)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
13ZA	California Region 10 2015	Imperial				13.2	25.3	3164.0	3202.5 **Avg.: 3400.0**
NT030-13ZA	Linden, California Region 10 2014	Granny Smith	Coarse	0.50	204	6.0 6.2	1.2 1.5	8.1 8.9	15.3 16.9 **Avg.: 16.0**
NT030-13ZA	Linden, California Region 10 2015	Granny Smith	Coarse	0.50	293	4.5 4.9	0.8 0.6	8.0 5.9	13.3 11.4 **Avg.: 12.0**
NT031-13ZA	Clarksburg, California Region 10 2014	Fuji	Fine	0.50	214	0.8 1.0	<LOD <LOD	<LOD <LOD	1.5 1.7 **Avg.: 2.0**
NT031-13ZA	Clarksburg, California Region 10 2015	Fuji	Fine	0.50	293	1.9 3.1	<LOD <LOD	<LOD <LOD	2.6 3.8 **Avg.: 3.0**
NT032-13ZA	Linden, California Region 10 2014	Shirely Ranch	Medium	0.50	198	1.5 1.8	<LOD <LOD	1.0 3.4	2.8 5.4 **Avg.: 4.0**
NT032-13ZA	Linden, California Region 10 2015	Shirley Ranch	Medium	0.50	293	3.9 4.9	<LOD 0.6	3.4 4.9	7.6 10.4 **Avg.: 9.0**
NT033-13ZA	Linden, California Region 10 2014	Granny Smith	Coarse	0.50	204	9.4 5.5	1.5 1.4	8.5 7.5	19.3 14.4 **Avg.: 17.0**
NT033-13ZA	Linden, California Region 10 2015	Granny Smith	Coarse	0.50	293	8.6 10.0	1.1 1.5	7.8 10.3	17.5 21.8 **Avg.: 20.0**

Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	Imidacloprid Olefin Residues (ppb)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
NT034-13ZA	Madera, California Region 10 2014	Fuji	Coarse	0.52	175	6.4 3.7	1.4 0.9	2.9 1.8	10.7 6.3 **Avg.: 9.0**
NT034-13ZA	Madera, California Region 10 2015	Fuji	Coarse	0.52	265	12.9 14.0	1.3 1.1	6.7 5.8	20.9 20.9 **Avg.: 9.0**
NT035-13ZA	Madera, California Region 10 2014	Gala	Coarse	0.50	175	8.6 7.5	1.7 1.9	7.1 8.0	17.4 17.4 **Avg.: 17.0**
NT036-13ZA	Sanger, California Region 10 2014	Pink Lady Apple	Coarse	0.50	151	28.6 30.6	4.7 4.3	27.0 21.7	60.3 56.7 **Avg.: 59.0**
NT036-13ZA	Sanger, California Region 10 2015	Pink Lady Apple	Coarse	0.50	147	68.7 81.1	14.2 17.2	54.1 70.4	137.0 168.6 **Avg.: 150.0**

7. STUDY VALIDITY/CLASSIFICATION AND STUDY LIMITATIONS

Classification/Utility for Bee Risk Assessment. This study is classified as **ACCEPTABLE** with limitations. This study provides a snapshot of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin residues in apple leaves, pollen, and nectar during bloom. The residue values presented should be considered to be reliable. However, it is important to note that it is unclear if concentrations were increasing or decreasing at the time the samples were collected. It is also important to note that there are many concerns regarding the timing of applications in this study. In 2013, applications to 7 of the 9 trial sites were made at BBCH 99. At this growth stage, apples have been harvested and all of the trees' leaves have fallen. This may be an inappropriate application time for a residue study as leaves play an important part in uptake of residues by the plant, especially for foliar applications. In addition, the label for Admire Pro® Systemic Protectant states that applications may not be made during pre-bloom or bloom periods. However, in 2014, applications at 1 of the 9 sites were made during BBCH 65-67, which is when the plant is in full flowering.

Temporal Variability in Residues. This study was not designed for temporal analysis of declining concentrations, but rather, to provide a snapshot of residue concentrations during flowering.

Spatial Variability in Residues. All nine sites in this study were located in central California and experienced similar climatic conditions throughout the study duration. Three of the sites had fine soil, one had medium soil, and five sites had course soil. Statistical comparison for potential differences in soil type was conducted in Section 8 comparing the coarse soil to fine-texture soil data. The medium-textured soil did not contain an adequate number of replicates for comparison. The findings indicated that there were significantly higher concentrations in leaves of plants grown in coarse soils that in those grown in fine soils. This pattern was not reflected in nectar or pollen samples.

Pesticide Carryover. The extent to which prior year applications of imidacloprid contributed to year-to-year carryover was not analyzed by the study authors. In Section 8, analyses were conducted to determine if there were significant differences in distribution of imidacloprid residues between years. The findings indicated that, generally, there were no significant differences between the two years. Thus, it is not expected that residues that resulted from the first year of applications contributed to the residue levels that resulted from the second year of applications.

Magnitude of Residues. A summary of imidacloprid residue data for apple nectar, pollen, and leaves are provided in Table 4 of the study (copied below). Only residue values above the respective analyte's LOD are reported. Any residue value that was below the LOD is reported as less than the LOD (<LOD). The total imidacloprid residue is a sum of the analyte residue values that are greater than the respective analyte LODs. If the analyte value was less than the LOD, a default value equal to half of the analyte LOD (half-LOD) was added into the sum. For samples with a reported total residue of less than LOD, the sum of the analyte's half-LOD value for the respective matrix was used to calculate the average residue values.

The maximum total imidicloprid residues were 36, 100, and 3600 ppb in nectar, pollen, and leaves, respectively. The mean total imidicloprid residues were 3, 24, and 220 ppb in nectar, pollen, and leaves, respectively.

Table 4. Summary of Residue Data for Imidacloprid in/on Apple, All Trials.*

Matrix	Plot	Years	DAA[a]	Seasonal Application Rates (lb a.i./A)	Total Imidacloprid Residue Levels (ppb)[b]						
					n[c]	Min	Max	90^{th} percentile	Median	Mean	Standard Deviation
Nectar	TRTD	2014	138-193	0.50-0.52	17	1.0	36	3.0	1.0	4.0	8.0
		2015	131-287	0.50-0.52	16	1.0	4.0	3.0	1.0	2.0	1.0
		2014, 2015	131-287	0.50-0.52	33	<LOD	36	3.0	1.0	3.0	6.0
Pollen	TRTD	2014	138-193	0.50-0.52	18	1.0	47	30	15	16	12
		2015	131-287	0.50-0.52	16	2.0	100	89	15	33	35
		2014, 2015	131-287	0.50-0.52	34	1.0	100	57	15	24	27

Leaves	TRTD	2014	151-214	0.50-0.52	18	1.0	60	31	9.0	14	17
		2015	147-293	0.50-0.52	16	2.0	3600[d]	1700	15	450	1100
		2014, 2015	147-293	0.50-0.52	34	1.0	3600	110	12	220	810

a DAA = Days after the last application.
b For the purpose of calculating the total imidacloprid residues, any individual analyte value reported as <LOD was summed into the total at a default value equal to ½ the LOD.
c n = Number of individual treated samples analyzed.
d The next highest residue from 2015 leaf samples in trial NT029-13ZA was 4.0 ppb.
* Values rounded to the nearest whole number

8. STATISTICAL ANALYSIS

1. There were a number of values below the limit of detection (LOD). These values were substituted with ½ LOD values for the represented year and plant tissue. The total was a simple addition of all analytes.

2. Analyses were conducted to determine if there were significant differences in distribution of imidacloprid residue concentrations between years in the plant samples. Tables S-1 through S-3 contain a comparison of the distributions for each analyte between years for leaves, nectar, and pollen, respectively. Figures S-1 through S-3 provide a graphical view of the distributional differences between years for parent imidacloprid and total residues in leaves, nectar, and pollen, respectively. The data are presented as distributions of the natural logarithms because they provide a clearer separation of the data. As shown in the graph for leaves, there were 2 rather large values of 3,575 and 3,202.5 ppb measured from samples taken at the Stockton site in 2015 (Figure S-1). Generally, there were no significant differences between the two years as indicated by non-parametric tests for Wilcoxon rank sum, which tests for a general difference in distributions, and Median tests, which tests for differences in the location of the median value between the distributions (Table S-4).

3. Tables S-5 through S-7 contain the distributions combined from both years for data in each soil type that was measured for leaves, nectar and pollen, respectively. The highest nectar value at 36.3 ppb was measured in the fine-textured soil with the next highest value measured at 4 ppb in the coarse-textured soil (Table S-6). For pollen, the highest values were measured in coarse soil at 103.4 and 102.7 ppb, respectively (Table s-7). Comparison of the median values between nectar and pollen indicate that the pollen distribution tended to contain higher values. The medians for the 3 soils typed for nectar ranged from 0.9 to 1.5 ppb compared to 14.3 to 16.7 ppb for pollen.

4. Statistical comparison for potential differences in soil type were conducted comparing the coarse soil to fine-texture soil data: the medium-textured soil did not contain an adequate number of values for comparison to the other two categories. Although there were a few rather large values measured for the fine-textured soils for imidacloprid in leaves, the non-parametric tests indicated a significant difference in the distribution. The findings indicated that leaves in coarse-textured soils tended to have a greater number of higher values than in the fine-textured soils (Table S-8). The patterns in the graphic for leaves in Figure S-4 illustrate that although there were a few large values in the fine-texture soil, the bulk of the concentration were at the lower tail compared to the coarse-texture soils distribution. No differences were noted for nectar and pollen (Figures S-5 and S-6).

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Conclusion: The median pollen concentration was generally 10x greater than nectar thus requiring a comparison between these values and benchmark values to determine biological significance. Difference in concentration due to soil type became evident as higher concentrations were measured in leaves of plants grown in coarse soils. This pattern was not reflected in nectar or pollen samples.

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Table S-1. Leaves: Comparison of statistics for the distribution between years 2014 and 2015 for concentrations of imidacloprid, its degradation products and their total in leaves of apples. Residues are reported in parts per billion (ppb).

	Olefin		Hydroxy		Imidacloprid		Total Residue	
Statistic	2014	2015	2014	2015	2014	2015	2014	2015
N	18	16	18	16	18	16	18	16
Mean	6.9	15.4	1.3	5.9	6.3	429.8	14.5	451.2
SD	8.7	23.7	1.3	9.9	7.3	1141.6	17.3	1149.8
CV (%)	126.4	153.9	106.5	167.5	116.0	265.6	119.1	254.8
Min	0.4	1.4	0.3	0.3	0.5	0.5	1.1	2.1
Median	4.8	6.8	1.1	1.0	3.5	6.3	9.3	15.4
75th	7.5	13.1	1.5	7.9	8.1	32.2	17.4	79.4
90th	28.6	68.7	4.3	25.3	21.7	3164.0	56.7	3202.5
Max	30.6	81.1	4.7	30.0	27.0	3533.0	60.3	3575.0
% of Total	47.6	3.4	8.6	1.3	43.6	95.3		

Table S-2. Nectar: Comparison of statistics for the distribution between years 2014 and 2015 for concentration of imidacloprid, its degradation products and their total in nectar of apples. Residues are reported in parts per billion (ppb).

	Olefin		Hydroxy		Imidacloprid		Total Residue	
Statistic	2014	2015	2014	2015	2014	2015	2014	2015
N	17	16	17	16	17	16	17	16
Mean	0.6	0.8	1.9	0.4	1.2	0.4	3.7	1.6
SD	0.5	0.7	6.2	0.1	2.1	0.4	8.5	1.1
CV (%)	92.9	86.5	323.7	23.5	172.0	95.2	230.6	68.5
Min	0.3	0.3	0.4	0.4	0.2	0.2	0.8	0.8
Median	0.3	0.5	0.4	0.4	0.6	0.15	1.4	1.2
75th	0.3	1.0	0.4	0.4	1.1	1.2	2.3	2.0
90th	1.6	2.0	0.9	0.4	2.8	1.3	3.4	4.0
Max	1.8	2.5	26.0	0.7	8.9	1.3	36.3	4.0
% of Total	15.0	49.0	52.5	23.6	33.1	28.0		

Table S-3. Pollen: Comparison of statistics for the distribution between years 2014 and 2015 for concentration of imidacloprid, its degradation products and their total in pollen of apples. Residues are reported in parts per billion (ppb).

	Olefin		Hydroxy		Imidacloprid		Total Residue	
Statistic	2014	2015	2014	2015	2014	2015	2014	2015
N	18	16	18	16	18	16	18	16
Mean	0.4	8.6	0.5	2.0	15.5	22.4	16.3	33.0
SD	0.5	12.6	0.3	3.3	11.5	30.2	11.7	35.2
CV (%)	129.3	147.0	63.5	165.0	74.2	134.6	71.8	105.6
Min	0.2	0.2	0.3	0.3	0.7	1.3	1.1	2.3
Median	0.2	6.1	0.3	1.1	14.0	7.1	15.2	15.1
75th	0.3	9.5	0.6	2.2	20.5	31.5	20.9	55.5
90th	1.0	14.7	1.1	3.6	29.5	91.3	30.4	102.7
Max	1.9	52.1	1.1	13.9	45.9	92.8	47.2	103.4
% of Total	2.2	26.0	2.8	6.2	95.1	68.0		

Table S-4. Exact probability levels for non-parametric tests between years 2014 and 2015 for distribution of concentrations of imidacloprid and its degradation products in leaves, nectar, and pollen of apples.

Plant Tissue	Probability Level for Non-Parametric Test Between Years							
	Olefin		Hydroxy		Imdacloprid		Total Residue	
	Wilcoxon	Median	Wilcoxon	Median	Wilcoxon	Median	Wilcoxon	Median
Leaves	0.13	1	0.66	1	0.34	0.72	0.12	0.73
Nectar	0.2	0.2	0.3	0.6	0.1	0.3	0.4	0.7
Pollen	<0.0001	0.0003	0.02	0.19	0.63	0.3	0.49	1

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Table S-5. Leaves: Distribution statistics for imidacloprid and degradates for applications made in three soil texture categories and with data combined over 2014 and 2015. Residues are reported in parts per billion (ppb).

	Coarse Textured Soil				Medium Textured Soil				Fine Textured Soil			
Statistic	**5-OH**	**Olefin**	**IMI**	**Total**	**5-OH**	**Olefin**	**IMI**	**Total**	**5-OH**	**Olefin**	**IMI**	**Total**
N	18	18	18	18	4	4	4	4	12	12	12	12
Mean	3.2	17.6	15.0	35.9	0.3	3.0	3.2	6.6	4.8	3.5	559.0	567.3
SD	4.7	22.3	18.4	45.2	0.2	1.6	1.6	3.2	10.7	4.3	1305.4	1320.3
CV (%)	144.8	126.3	122.5	125.8	51.9	54.3	50.8	49.3	222.4	123.9	233.6	232.8
Min	0.6	3.7	1.8	6.3	0.3	1.5	1.0	2.8	0.3	0.4	0.5	1.1
Median	1.5	8.6	8.0	17.5	0.3	2.9	3.4	6.5	0.3	1.9	0.5	2.9
75th	1.9	14.0	10.3	21.8	0.4	4.4	4.2	9.0	0.3	3.6	3.0	6.3
90th	14.2	68.7	54.1	137.0	0.6	4.9	4.9	10.4	25.3	11.7	3.6	3202.5
Max	17.2	81.1	70.4	168.6	0.6	4.9	4.9	10.4	30.0	13.2	3533.2	3575.0
% of Total	9.0	49.1	41.9		5.2	46.3	48.5		0.8	0.6	98.5	

Table S-6. Nectar: Distribution statistics for imidacloprid and degradates for applications made in three soil texture categories and with data combined over 2014 and 2015. Residues are reported in parts per billion (ppb).

	Coarse Textured Soil				Medium Textured Soil				Fine Textured Soil			
Statistic	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total
N	18	18	18	18	4	4	4	4	11	11	11	11
Mean	0.4	0.9	0.6	1.8	0.5	0.3	0.4	1.2	2.7	0.5	1.4	4.6
SD	0.1	0.7	0.4	1.0	0.2	0.0	0.4	0.6	7.7	0.4	2.6	10.6
CV (%)	22.3	83.1	67.2	57.5	48.7	0.0	88.4	50.0	282.6	73.3	188.5	230.3
Min	0.4	0.3	0.2	0.8	0.4	0.3	0.2	0.8	0.4	0.3	0.2	0.8
Median	0.4	0.5	0.7	1.5	0.4	0.3	0.3	0.9	0.4	0.3	0.2	1.1
75th	0.4	1.3	0.9	2.3	0.6	0.3	0.7	1.5	0.4	0.7	1.2	2.3
90th	0.4	2.0	1.2	4.0	0.8	0.3	0.9	2.0	0.9	0.7	2.8	3.4
Max	0.7	2.5	1.3	4.0	0.8	0.3	0.9	2.0	26.0	1.4	8.9	36.3
% of Total	20.6	47.2	33.3		40.0	26.1	34.8		59.6	10.3	30.3	

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Table S-7. Pollen: Distribution statistics for imidacloprid and degradates for applications made in three soil texture categories and with data combined over 2014 and 2015. Residues are reported in parts per billion (ppb).

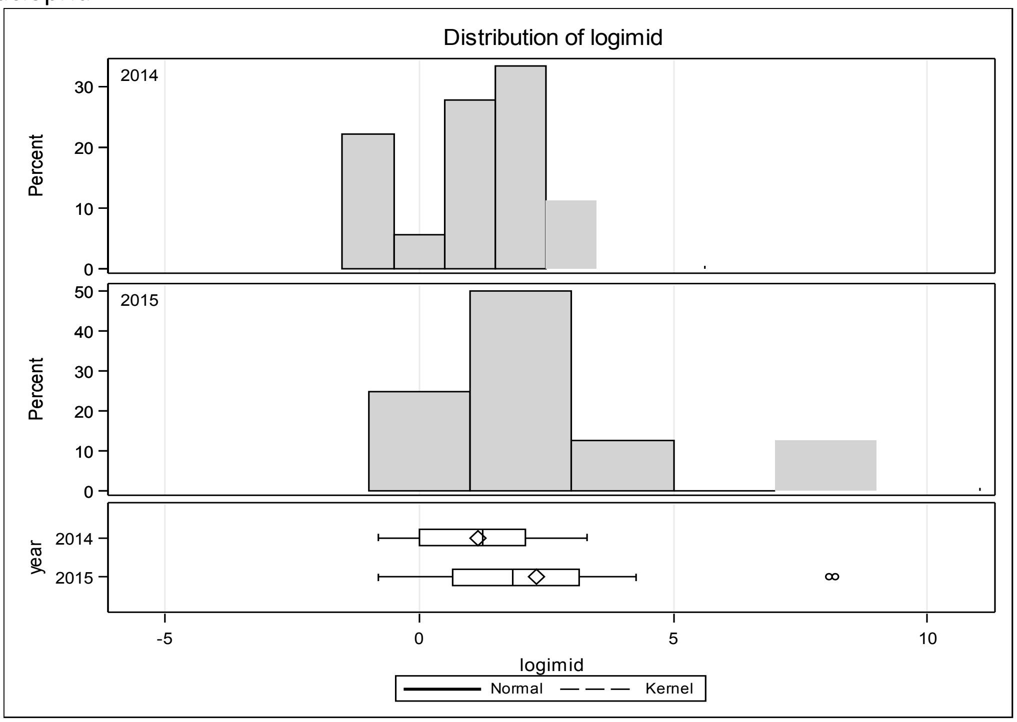
Statistic	Coarse Textured Soil				Medium Textured Soil				Fine Textured Soil			
	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total
N	18	18	18	18	4	4	4	4	12.0	12.0	12.0	12.0
Mean	0.8	3.2	23.7	27.2	0.5	1.3	14.3	16.0	2.1	6.8	13.5	22.2
SD	0.9	4.6	27.9	32.1	0.4	2.0	13.0	11.8	3.8	14.9	12.8	20.9
CV (%)	121.0	145.2	20.0	118.0	70.7	155.3	91.3	74.6	185.8	220.8	95.0	94.2
Min	0.3	0.2	0.7	1.1	0.3	0.2	2.0	5.4	0.3	0.2	2.8	5.4
Median	0.3	0.5	13.7	15.1	0.4	0.4	12.8	14.3	0.8	0.7	7.3	16.7
75th	1.2	8.0	28.7	38.5	0.8	2.3	25.0	25.8	1.6	5.7	19.0	27.1
90th	2.4	9.5	91.3	102.7	1.0	4.2	29.5	30.1	3.0	14.2	28.4	47.2
Max	3.6	14.7	92.8	103.4	1.0	4.2	29.5	30.1	13.9	52.1	45.9	76.2
% of Total	2.9	11.7	86.9		3.1	7.9	88.9		9.2	30.4	60.6	

Table S-8. Exact probability levels for the Wilcoxon rank sum and Median tests for the difference in imidacloprid and total imidacloprid residue distribution between coarse and fine-textured soils.

Plant Sample	Probability Level for Non-Parametric Test Between Soils			
	Imidacloprid		Total Residue	
	Wilcoxon	Median	Wilcoxon	Median
Leaves	0.0034	0.0068	0.0012	0.008
Nectar	0.7	0.4	0.5	0.5
Pollen	0.43	0.27	0.9	1

Figure S-1. Leaf Concentration: Comparison of imidacloprid and total imidacloprid residue between data collected in 2014 and 2015.

A. Imidacloprid



B. Total imidacloprid residue.

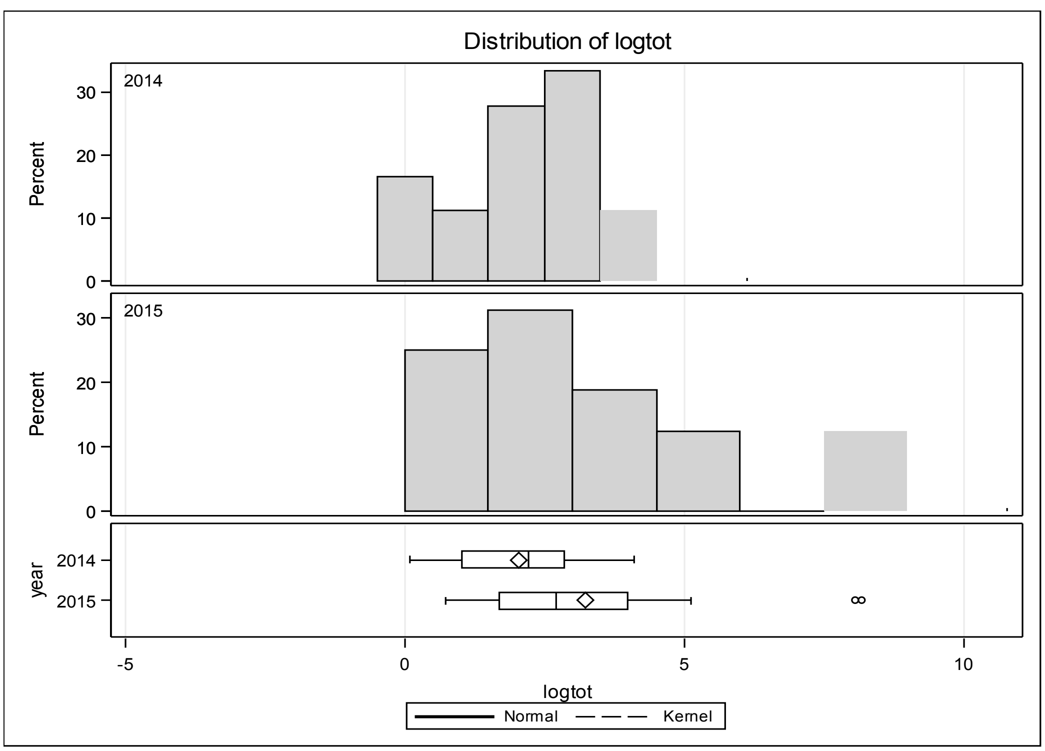
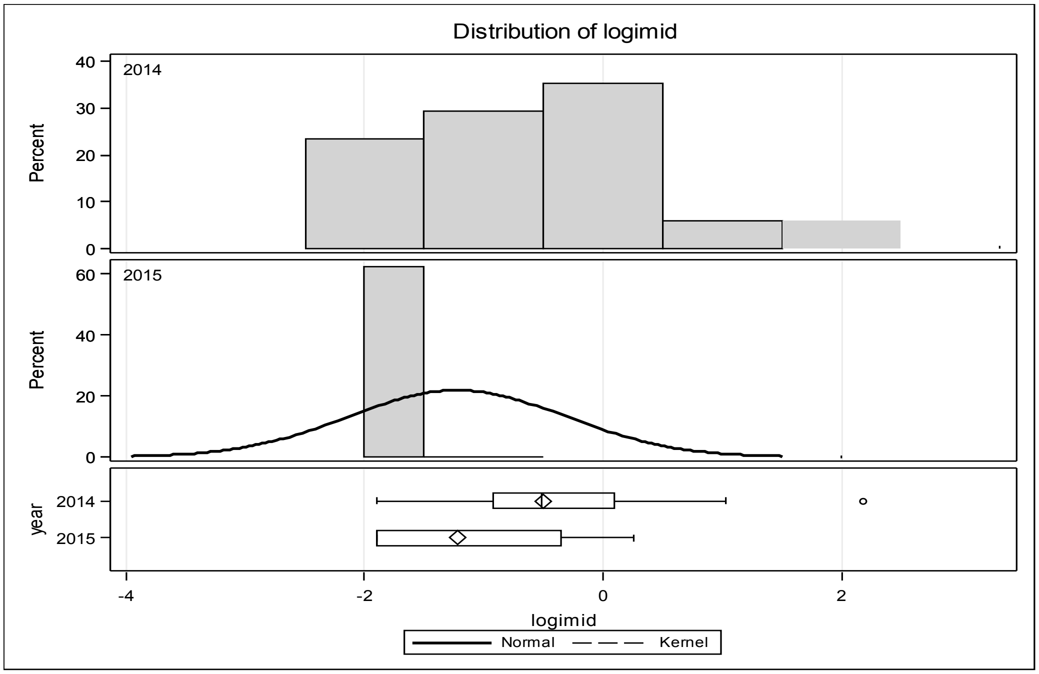


Figure S-2. Nectar Concentration: Comparison of imidacloprid and total imidacloprid residue between data collected in 2014 and 2015.

A. Imidacloprid



B. Total imidacloprid residue.

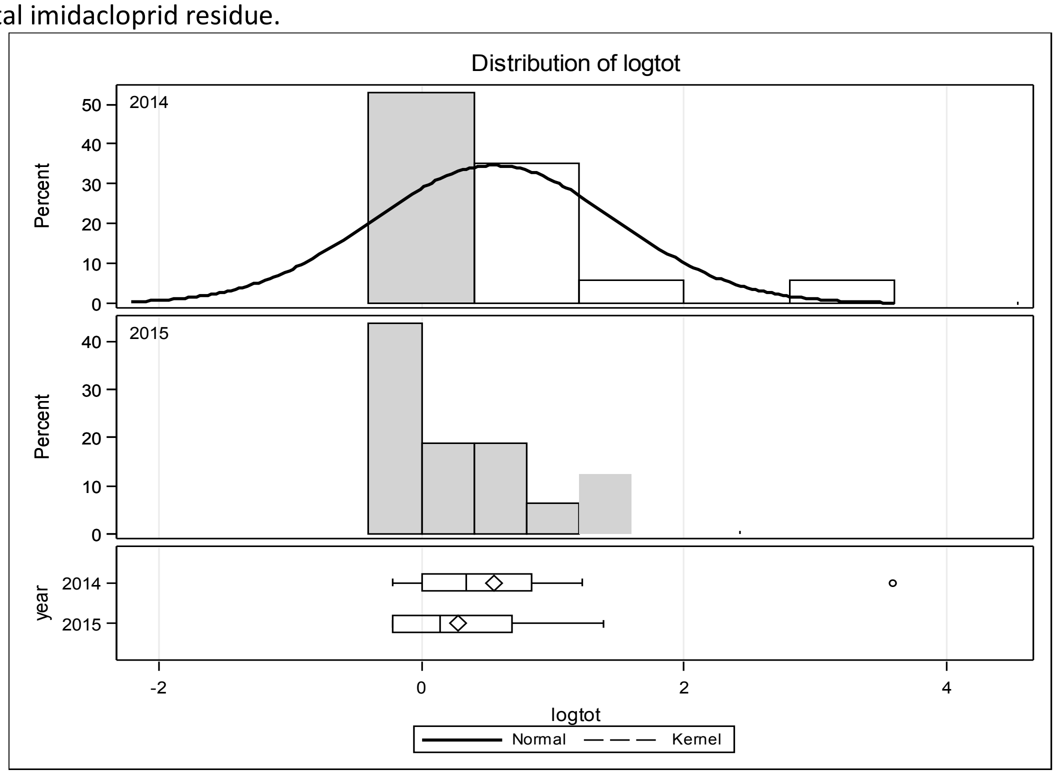
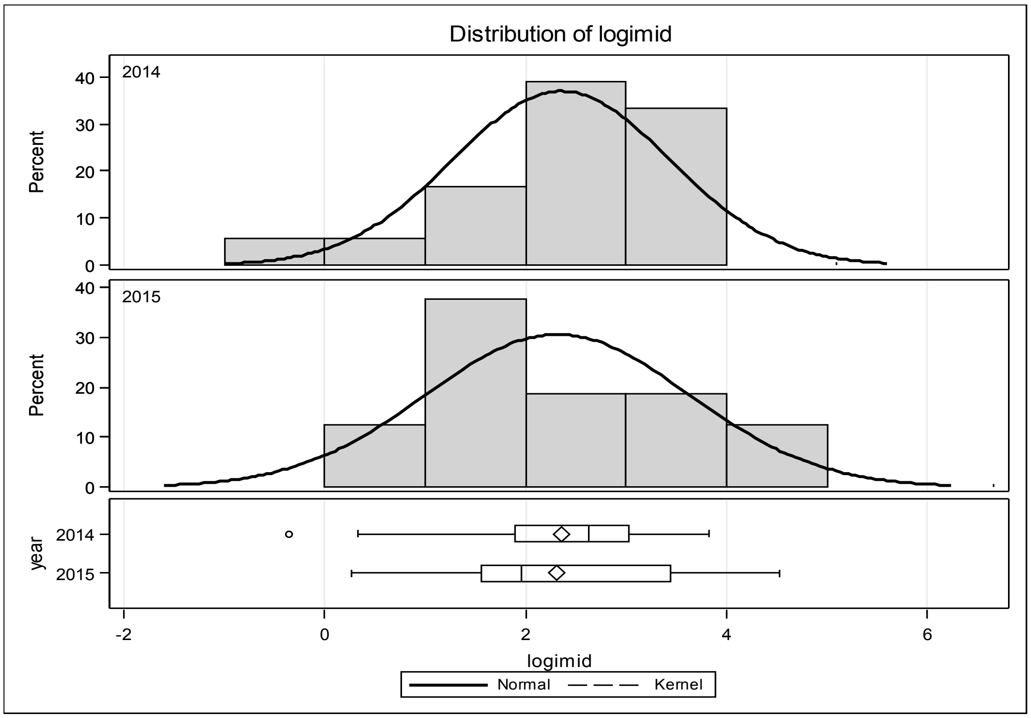


Figure S-3. Pollen Concentration: Comparison of imidacloprid and total imidacloprid residue between data collected in 2014 and 2015.

A. Imidacloprid



B. Total imidacloprid residue.

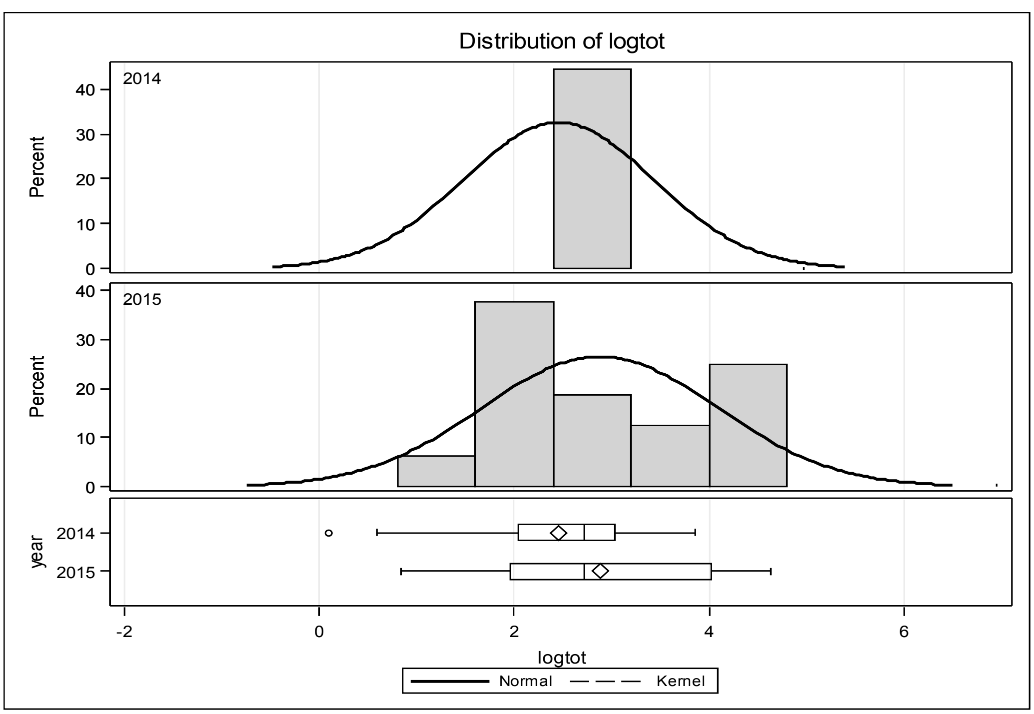
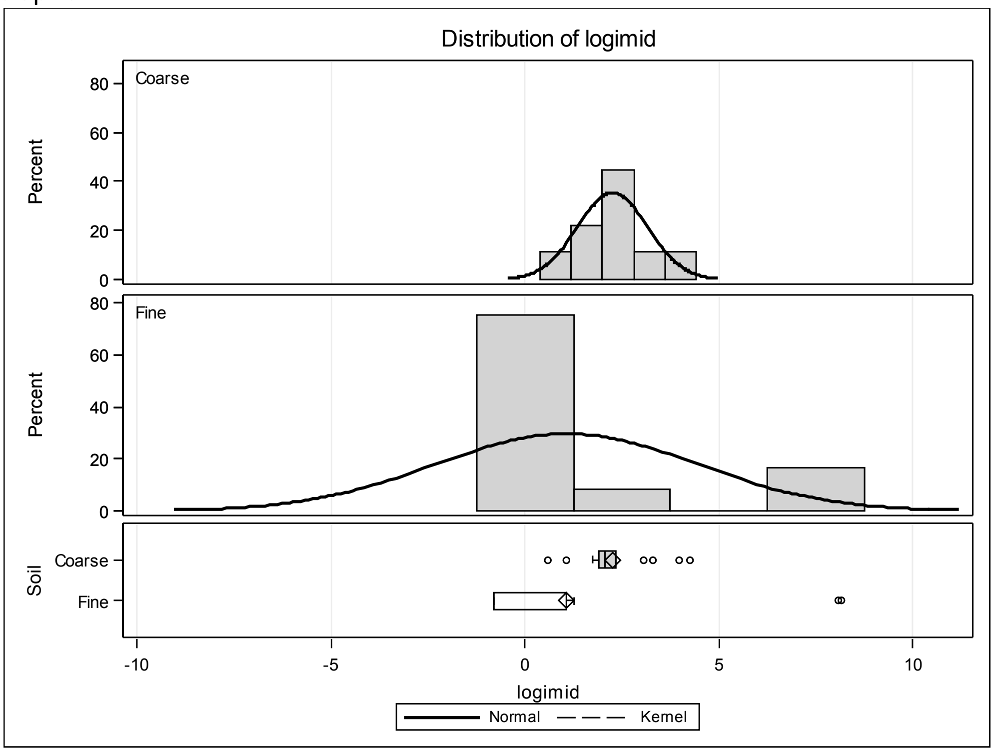


Figure S-4. Leaves: comparison of imidacloprid and total imidacloprid residue between coarse and fine textured soil derived data.

A. Imidacloprid



B. Total imidacloprid residue.

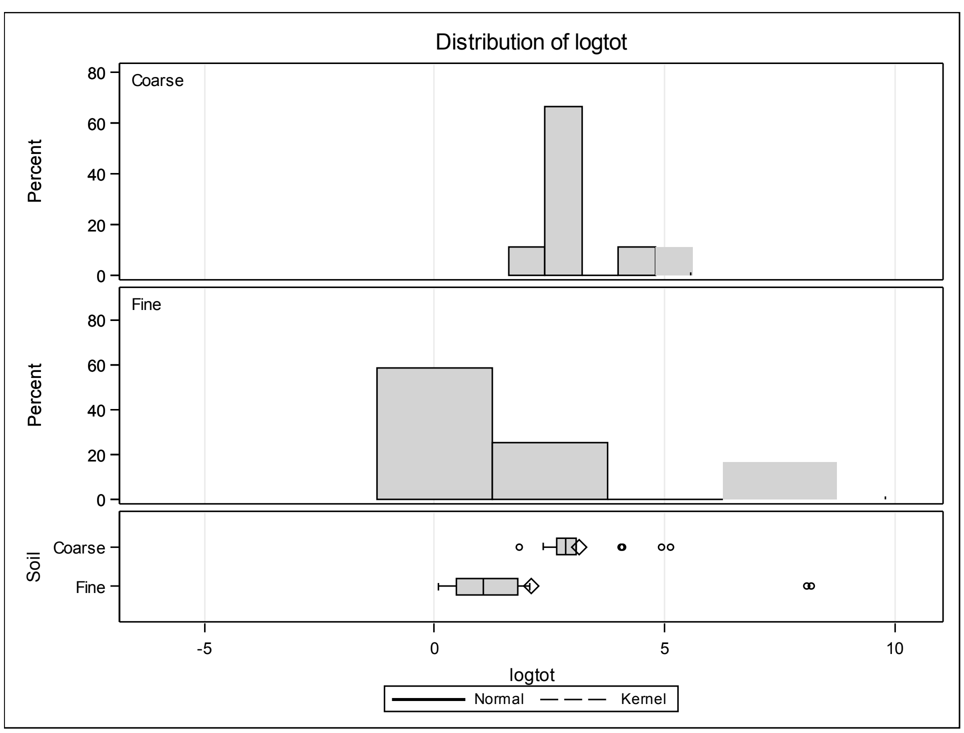
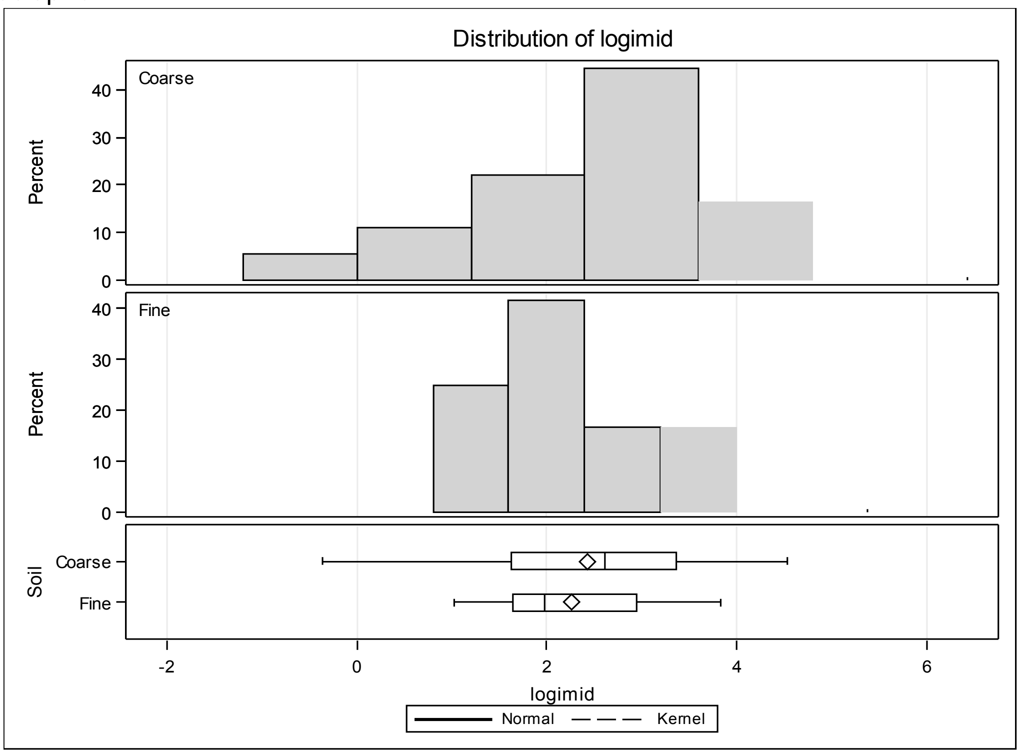


Figure S-5. Nectar: comparison of imidacloprid and total imidacloprid residue between coarse and fine textured soil derived data.

A. Imidacloprid



B. Total imidacloprid residue.

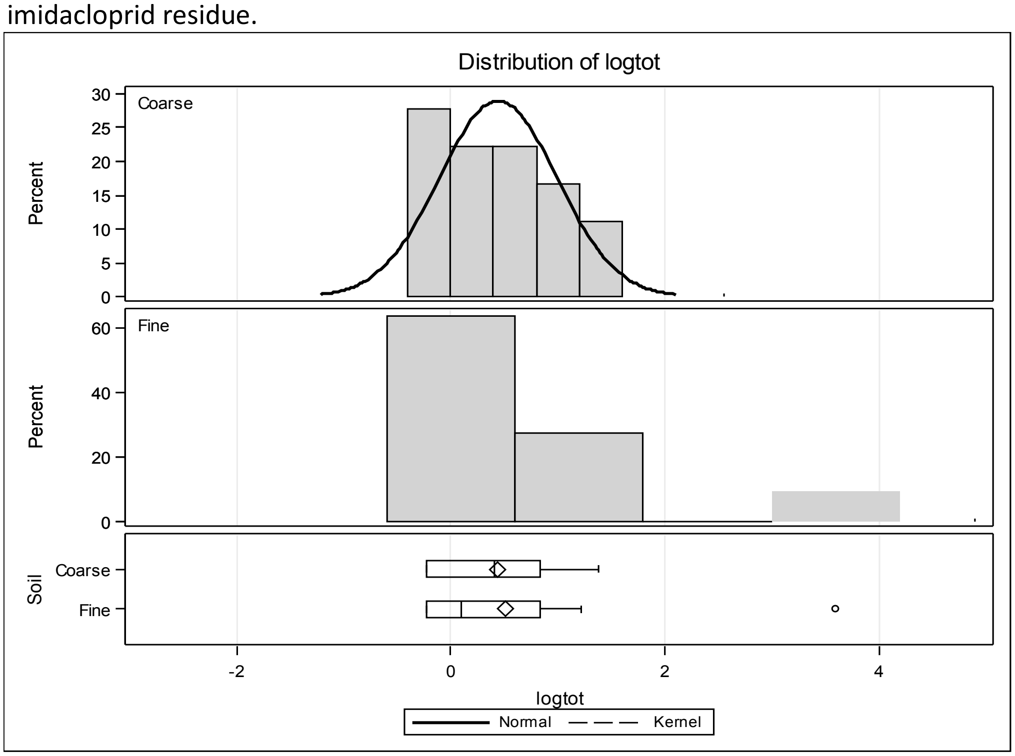
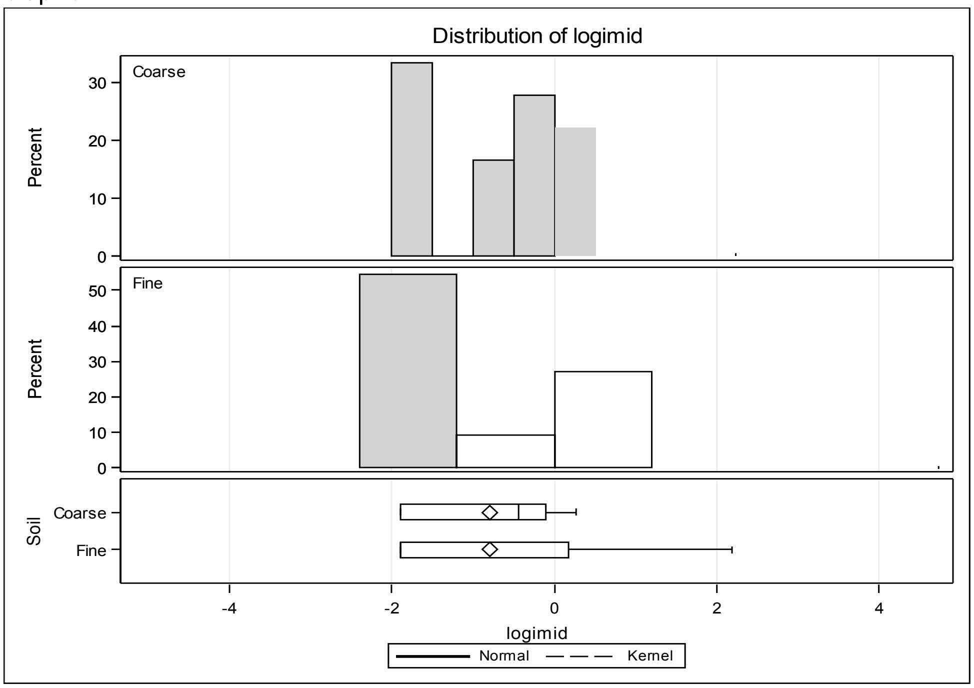
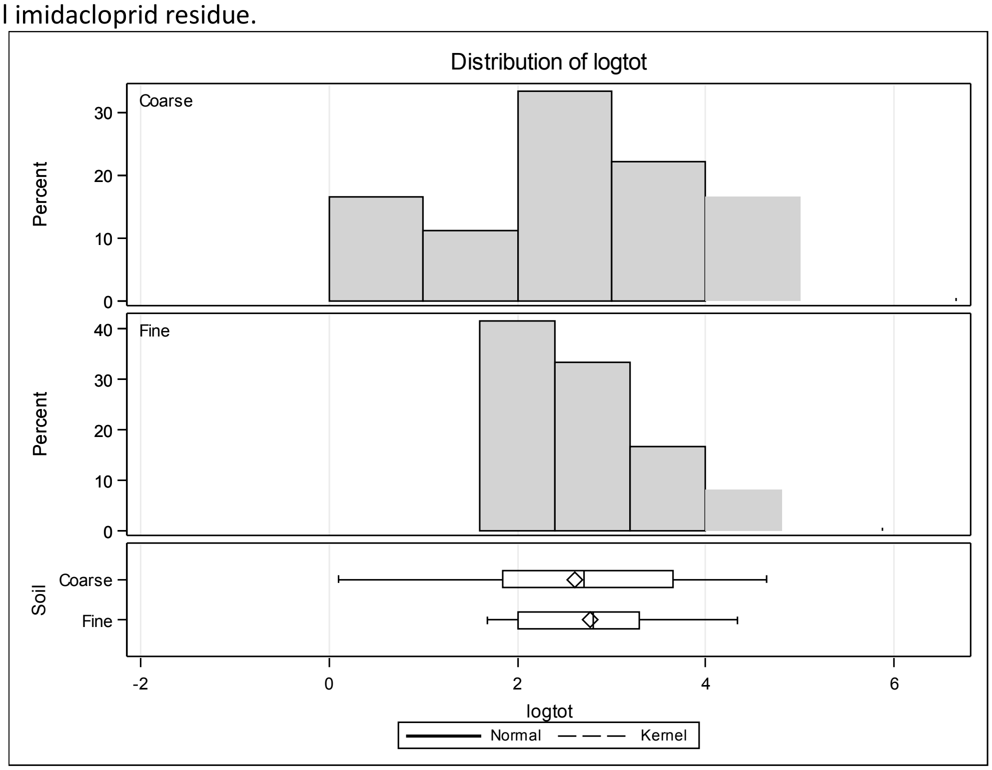


Figure S-6. Pollen: comparison of imidacloprid and total imidacloprid residue between coarse and fine textured soil derived data.

A. Imidacloprid



B. Total imidacloprid residue.



9. REFERENCES

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Reference
Gould, T., and Jerkins, E. (2016) Determination of the Residues of Imidacloprid, 5-Hydroxy Imidacloprid, and Imidacloprid Olefin in Bee Relevant Matrices Collected from Stone Fruit Trees following Application of Imidacloprid over Two Successive Years: Final Report. Project Number: EBNTN013. Unpublished study prepared by Bayer Cropscience. 466p. MRID 49819401, CDPR Study ID 289102, Data Volume 51950-0904, Tracking ID# 273965

1. STUDY INFORMATION

Chemical:	Imidacloprid	**PC Code**	129099
Test Material:	Admire Pro Systemic Protectant	**Percent Active Ingredient:**	42.8%
Study Type:	Field residue study on Stone Fruit to establish imidacloprid and metabolite levels in nectar, pollen and leaves in 9 trial site locations following one soil and two foliar applications of Admire Pro® Systemic Protectant in two successive years.		
Sponsor:	Bayer CropScience 2T.W. Alexander Drive Research Triangle Park, NC USA 27709	**Experiment Start and End Date:**	September 13, 2013 – July 16, 2015
Sponsor Study ID:	EBNTN013	**Study Locations:**	Nine trial sites that included cherry, plum, apricot and peach located in California.
Study Completion Date:	January 13, 2016		
GLP Status:	GLP; protocol reviewed by CDPR. [CDPR Study ID 289102, Data Volume 51950-0904, Tracking ID# 273965]		

2. REVIEWER INFORMATION

Study Reviewed by: California Department of Pesticide Regulation	Richard Bireley, Sr. Environmental Scientist (Specialist) John Troiano, Ph.D., Research Scientist III Alexander Kolosovich, Sr. Environmental Scientist (Specialist) Brigitte Tafarella, Environmental Scientist Denise Alder, Sr. Environmental Scientist (Specialist) Russell Darling, Sr. Environmental Scientist (Specialist)

3. EXECUTIVE SUMMARY

A total of nine field trials were conducted to measure the magnitude of imidacloprid residues in stone fruit (cherry, plum, apricot and peach) nectar, pollen and leaves following one soil and two foliar applications of Admire Pro® Systemic Protectant in two successive years. Admire Pro Systemic Protectant is a suspension concentrate formulation containing 550 g/L of imidacloprid.

One soil and two foliar applications were made in 2013 and 2014, post-bloom. Across both years, individual soil application (drip irrigation) rates were 0.38 lb imidacloprid/acre. The interval between the soil application and the first foliar application was 3 to 7 days. For all foliar applications (airblasts), individual rates ranged from 0.058 to 0.064 lb imidacloprid/acre. The interval between the first and second foliar application was 7 to 11 days. Application volumes ranged from 13,000 to 16,600 gal/acre (GPA) for the soil applications and 53 to 90 GPA for the foliar applications. Total annual application rates ranged from 0.50 to 0.51 lb imidacloprid/acre, which is the maximum labeled amount allowed. In 2013, all applications were made after stone fruit harvest at BBCH growth stages 91 to 99. In 2014, soil applications were targeted for 21 days prior to stone fruit harvest and made at BBCH growth stages 77 to 81. The two foliar applications were targeted such that the last would occur 7 days prior to fruit harvest, with sprays made at BBCH growth stages 76 to 89.

All applications were made using ground-based equipment. The adjuvant Dyne-Amic was used in all foliar applications at a rate of 0.25% v/v, except in trial NT027-13ZA, when a rate of 0.025% v/v was used.

Stone fruit flowers (blossoms) and leaf samples were collected once in the spring of 2014, following the post-harvest fall 2013 applications, and once in the spring of 2015, following the pre-harvest fall 2014 applications. In 2014, flower samples were collected at 133 to 160 days after the last application (DALA), and leaf samples were collected at 155 to 188 DALA. In 2015, flower samples were collected at 211 to 309 DALA, and leaf samples were collected at 230 to 323 DALA.

Single composite samples of cherry, plum, apricot, or peach flowers and leaves were collected from the control plot of each trial on the same days that samples were collected from the treated plot.

The residues of Admire Pro Systemic Protectant (imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

4. STUDY VALIDITY

Guideline Followed:	Protocol was reviewed by CDPR
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	**ACCEPTABLE**
Rationale:	N/A
Reparability:	N/A

5. MATERIALS AND METHODS

Test Material Characterization			
Test item:	Admire Pro Systemic Protectant	**Percent Active Ingredient:**	42.8% A.I.
		EPA Reg. No.	264-827
Description:	Suspension Concentrate	**Density (20°C):**	1.41 - 1.54 g/mL
CAS #:	138261-41-3	**Solubility:**	0.51 to 0.61 g/L

5A. STUDY DESIGN

This study requirement was part of the imidacloprid special review at the California Department of Pesticide Regulation (CDPR). The study design and protocol were approved by the CDPR prior to study initiation. This study was conducted using GLP standards and following an approved protocol. The study initiation date was August 02, 2013. The experimental start date was September 13, 2013 (first field application) and the experimental end date was July 16, 2015 (last sample analysis). Nine trials were conducted for this study in multiple locations in California, representing each of the three soil texture categories (fine, medium, and coarse). Each trial includes one untreated control plot (UTC) and one treated (TRTD) plot to be treated in each of two consecutive years.

Stone fruit varieties representing those commonly grown in the area of the trials and agronomic practices typical for commercial production of cherry, plum, apricot, and peach were used at all trial locations.

Homogenization and analysis of the leaf, nectar, and pollen samples from this study were performed by Bayer CropScience in Research Triangle Park (RTP), NC. Final report preparation was performed by Critical Path Services, LLC, located in RTP, NC.

5B. APPLICATION TIMING AND RATES

In 2013, applications were made post-harvest. In 2014, the soil application was made 21 days before harvest and the last foliar application was made 7 days before harvest, which is in compliance with the pre-harvest intervals on the label. Across both years, individual soil application rates were 0.38 lb imidacloprid/acre, which is the maximum allowed for soil applications. The interval between the soil application and the first foliar application was 3 to 7 days. For all foliar applications, individual rates ranged from 0.058 to 0.064 lb imidacloprid/acre. The interval between the first and second foliar application was 7 to 11 days. Application volumes ranged from 13,000 to 16,600 gallons per acre (GPA) for the soil applications and 53 to 90 GPA for the foliar applications; 16,600 gallons per acre is equivalent in volume to approximately one-half inch of water covering one acre. Total annual application rates ranged from 0.50 to 0.51 lb imidacloprid/acre, which the label states is the maximum annual rate permitted regardless of formulation or method of application.

In 2013, all applications were made after the stone fruit was harvested; at BBCH growth stages 91 to 99 (BBCH 91: shoot growth completed, foliage still fully green; BBCH 99: harvested product). In 2014, soil applications were targeted for 21 days prior to stone fruit harvest and made at BBCH growth stages 77 to 81 (BBCH 77: fruit about 70% of final size; BBCH 81: beginning of fruit coloring); the two foliar applications were targeted such that the last would occur 7 days prior to fruit harvest, with sprays made at BBCH growth stages 76 to 89 (BBCH 76: fruit about 70% of final size; BBCH 89: fruit ripe for consumption, fruit have typical taste and firmness).

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5C. STUDY SITE LOCATION AND CHARACTERISTICS

The CDPR required a variety of soil types to be included in the study design. Bayer CropScience conducted the study in three soil texture types (fine, medium, and coarse) based on Soil Survey Geographic (SSURGO) Database mapping units. There are nine trial sites in this study design: three in fine texture soils, three in medium, and three in coarse (Table 1).

Table 1. Study Site Location and Characteristics

Trial (Field) Identification	Trial Location (City, State)	OM (%)	pH	CEC (meq/100g soil)	% Sand	% Silt	% Clay	Soil Types	Rainfall (in)	Temperature Range (°F)
NT019-13ZA	Stockton, California	2.3	7.5	16.8	46	30	24	Loam	16.4	29 – 92
NT020-13ZA	Merced, California	1.6	6.7	21.2	36	28	36	Clay loam	11.5	25 – 96
NT021-13ZA	Yuba City, California	1.5	6.5	17.2	44	28	28	Clay loam	20.7	26 – 92
NT022-13ZA	Yuba City, California	1.6	6.6	19.0	26	36	38	Clay loam	20.7	26 – 92
NT023-13ZA	Yuba City, California	1.7	5.7	11.5	52	26	22	Sandy clay loam	20.7	26 – 92
NT024-13ZA	Kerman, California	0.50	6.4	5.2	84	14	2	Loamy sand	7.5	30 – 98
NT025-13ZA	Kerman, California	0.21	8.3	5.8	84	14	2	Loamy sand	7.2	29 – 98
NT026-13ZA	Sanger, California	1.3	6.3	6.2	60	34	6	Sandy loam	10.3	31 – 97
NT026-13ZA	Kingsburg, California	1.7	7.2	11.6	56	32	12	Sandy loam	10.3	31 - 97

5D. SAMPLE COLLECTION, HANDLING, PROCESSING

Stone fruit flower (also called blossom) and leaf samples were collected once in the spring of 2014, following the post-harvest fall 2013 applications, and once in the spring of 2015, following the pre-harvest fall 2014 applications. Each TRTD plot was divided into two subplots. At each sampling interval, two composite flower samples (one from each subplot) of cherry, plum, apricot, or peach flowers (to be hand-processed to obtain nectar and pollen) were collected by hand when the stone fruit trees were at bloom, BBCH 65 (BBCH 65: full flowering, at least 50% of flowers open, first petals falling). Two composite leaf samples were also collected for (one from each subplot) cherry, plum, apricot, or peach leaves after bloom, once the leaves had expanded at BBCH 69 to 75 (BBCH 69: end of flowering, all petals fallen; BBCH 75: fruit about half final size) or at BBCH 19 (first leaves fully expanded). In 2014, flower samples were collected at 133 to 160 days after the last application (DALA), and leaf samples were collected at 155 to 188 DALA. In 2015, flower samples were collected at 211 to 309 DALA, and leaf samples were collected at 230 to 323 DALA.

Single composite samples of stone fruit flowers and leaves were collected from the control plot of each trial on the same days that samples were collected from the treated plot. Stone fruit flowers and leaves were collected by hand into Ziplock bags. Each composite flower sample contained a minimum of 125 g (minimum 250 flowers), which was collected from at least 12 different areas of the plot, avoiding the edges (except in trial NT026-13ZA, when unfavourable weather caused the 2015 samples to be underweight). Each composite leaf sample contained a minimum of 100 g.

After collection, flowers were hand-processed at a facility near the field site to obtain the bee-relevant matrices of nectar and pollen. Nectar processing began the same day as flower collection. Nectar from the floral nectary was removed by micropipette and placed into a pre-weighed glass collection vial. The flowers were then allowed to dry overnight at room temperature to desiccate the pollen. The next day, pollen was removed from the flowers either by vacuum aspiration with collection in filter tips or by tapping the pollen from the flowers onto wax paper with collection of the accumulated pollen into a vial. All resulting nectar and pollen samples were labelled and placed in the freezer immediately after they were generated. After processing was completed, the flowers were discarded.

The samples of stone fruit leaves, nectar, and pollen were placed into labelled (study number and sample number) containers for shipment. All leaf, nectar, and pollen samples were placed in frozen storage within 4 hours of collection with the exception of the 2014 leaf samples from trial NT020-13ZA, which were frozen in 5 hours and 15 minutes, and the 2015 pollen samples from trial NT027-13ZA, which were frozen in 6 hours and 10 minutes. Samples remained frozen until receipt at Bayer CropScience in RTP, NC.

Sample Storage.

Upon arrival at Bayer CropScience, all leaf, nectar, and pollen samples were immediately transferred to frozen storage. The leaf samples were homogenized with dry ice using a Robot Coupe chopper and then were returned to frozen storage immediately following homogenization. Pollen and nectar were used without further preparation. All samples remained frozen at all times except during subsampling for analysis.

Stability studies have indicated that imidacloprid residues are stable (<30% decomposition) for 24 months (728 to 769 days) of freezer storage in the following representative crops: an oilseed (tomato seed), a non-oily grain (wheat), a leafy vegetable (lettuce), a root crop (potato), a tree fruit (apple), and a fruiting vegetable (tomato)[9-15]. An additional stability study has indicated that imidacloprid residues are stable (<30% decomposition) for 36 months of freezer storage in wheat (grain), orange (fruit), tomato (fruit), bean (seed), and rape (seed)[16]. Demonstrated freezer stability in all of the above crops is representative of the freezer stability of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin residues to be expected for cherry, plum, apricot, and peach leaves from this study. The leaves analyzed in this study were held in frozen storage for a maximum of 561 days (18 months) prior to extraction.

To demonstrate that imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin were stable in stone fruit nectar and pollen, samples of a nectar surrogate and commercial pollen were fortified with a mixture of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin at a level of 100 or 200 ppb. These transit stability samples were shipped to the field site at the start of each study year and were subsequently stored with the study samples; the exception is trial NT025-13ZA, when the field site did not receive 2014 stability samples. The transit stability samples were analyzed after all sample analyses were complete. The transit stability analyses indicate that the imidacloprid residues are stable (<30%

decomposition) during concurrent transit and freezer storage with the nectar and pollen samples from this study.

5E. ANALYTICAL METHODS

The analytical methods[6-7] used in this study measured the residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin in stone fruit leaves, nectar, and pollen. All neat analytical reference standards were stored frozen prior to dilution. All reference standard solutions were prepared in parent equivalents and corrected for purity during initial preparation.

For stone fruit leaves[6], a 2.5 g sample was weighed into a 50-mL polypropylene conical centrifuge tube, and 10 mL of HPLC-grade water was added. The tube was mixed manually for 1 minute, followed by adding 20 mL of acetonitrile and mechanical shaking (HS501 digital) for an additional 1 minute. Then, 3 g of $MgSO_4$ and 1.5 g of NaCl were added. The sample was amended with a mixed internal standard solution and manually mixed for 1 minute. The sample was centrifuged and 20 mL of organic supernatant was transferred into a separate 50-mL polypropylene conical centrifuge tube containing 0.3 g of Bondesil-PSA and 1.8 g of $MgSO_4$. The sample was again manually mixed for 1 minute. The sample extract was centrifuged, and a 1.25 mL aliquot of supernatant was transferred into a clean culture tube. The sample aliquot was evaporated to near dryness on a Turbo-Vap. The extract was reconstituted with 1.25 mL of 9:1 water/methanol containing 10 mM NH_3HCO_3 by vortexing, then transferred into a 2-mL sample vial for high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) analysis.

For stone fruit nectar[7], a 0.1-mL sample was weighed into a 20 x 150 culture tube and dissolved in 4 mL of water. If the total nectar sample was less than 0.1 mL, the entire sample was weighed and extracted. The mixture was amended with isotopically labeled internal standards, and the resultant solution was mixed well and applied to an Agilent BondElut Solid Phase Extraction (SPE) cartridge (50 mg resin; previously conditioned with methanol then water). The cartridge was washed with 1 mL of water/methanol (19:1 v/v), and the combined eluates were discarded. The analytes were extracted from the cartridge with 0.5 mL of water/methanol (4:1 v/v). The eluate was collected into a 2 mL sample vial for analysis by LC/MS/MS.

For stone fruit pollen[7], a 0.1-g sample was weighed into a small Precellys vial containing 2.8 mm steel balls. If the available pollen sample amount was not sufficient for an analysis, samples of the same interval and trial site were composited and analyzed. The composite sample was weighed and extracted. A 1-mL portion of water/methanol (3:1 v/v) was added, and the mixture was homogenized at 5000 beats/minute for 1 minute on a Precellys homogenizer. The isotopically labeled internal standards were added and mixed, and the mixture was centrifuged at 12,000 rpm for 2 minutes. The supernatant was transferred into a clean culture tube containing 2.5 mL of water and was evaporated to an aqueous remainder, then applied to a 3-mL ChemElut SPE cartridge. After 10 to 15 minutes, the cartridge was washed with 4 mL of hexane/ethyl acetate (1:1 v/v) three times into a clean culture tube. The combined eluates were evaporated to dryness. The analytes were dissolved in 0.5 mL of water/methanol (4:1 v/v). The solution was transferred into a 2 mL sample vial for analysis by LC/high resolution mass spectrometry (LC/HRMS).

Quantitation of each analyte was based on the daughter ion transitions of the analyte and the respective internal standard analog. The responses of the LC/MS/MS and LC/HRMS systems to each analyte and its internal standard were measured in samples and in standards, and a relative response was calculated (as the ratio of the analyte and the stable isotopically labeled internal standard responses). The relative response of the analyte in each sample was compared to the relative response of the analyte in the standards.

The relative responses of imidacloprid and its analytes were measured over the range of 0.12 to 2000 ppb. The analyte relative responses were fit to a linearity curve calculated using linear regression analysis with 1/x weighting. Correlation coefficients were calculated with the same software. All data are reported in parent equivalents, and the individual measured residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin are summed to give a total imidacloprid residue.

5F. Quality Assurance Results

No total imidacloprid residue was calculated for the UTC samples, so individual analyte levels are described. Imidacloprid (parent) residues in UTC stone fruit pollen ranged from below the analyte LOD to 29 ppb (trial NT023-13ZA, peach). Imidacloprid residues in UTC stone fruit nectar ranged from below the analyte LOD to 1.0 ppb (trial NT024-13ZA, cherry). Imidacloprid residues in UTC stone fruit leaves ranged from below the analyte LOD to 1.0 ppb (trial NT023-13ZA, peach). 5-Hydroxy imidacloprid residues in UTC stone fruit pollen ranged from below the analyte LOD to 32 ppb (trial NT023-13ZA, peach). 5-Hydroxy imidacloprid residues in UTC stone fruit nectar ranged from below the analyte LOD to 31 ppb (trial NT026-13ZA, apricot). 5-Hydroxy imidacloprid residues in UTC stone fruit leaves were all below the analyte LOD. Imidacloprid olefin residues in UTC stone fruit pollen ranged from below the analyte LOD to 190 ppb (trial NT022-13ZA, plum). Imidacloprid olefin residues in UTC nectar and leaves were all below the analyte LOD.

All recoveries were corrected for any interferences in corresponding controls. The overall means of the recoveries for each matrix at each fortification level were within the range of 70 to 120%, and the standard deviation values were below 20%.

The limit of quantitation (LOQ) is defined as the lowest fortification level of an analyte at which acceptable recovery has been achieved. The LOQ for a total residue is the highest of the LOQ values assigned to the individual analytes for a particular matrix.

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be determined to be statistically different from a blank. The LODs were determined from method validation data obtained from control samples fortified at the respective analyte LOQs. The LODs were calculated by multiplying the standard deviation of recovery measurements at the LOQ by $t_{0.99}$ [where $t_{0.99}$ is the one-tailed t-statistic at the 99% confidence level for the number of replicates (n)].[8] The LOD for the total imidacloprid residue in each matrix is the highest LOD value of any one individual analyte for that particular matrix.

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Table 2. Summary of LOQs and LODs

Matrix	Analyte	LOQ (ppb, parent equivalents)	LOD (ppb, parent equivalents)
Cherry, plum, apricot, and peach leaves	Imidacloprid	5.0	0.5
	5-hydroxy Imidacloprid	5.0	0.4
	Imidacloprid olefin	5.0	1.6
	Total Imidacloprid	5.0	1.6
Cherry, plum, apricot, and peach nectar	Imidacloprid	1.0	0.3
	5-hydroxy Imidacloprid	1.0	0.7
	Imidacloprid olefin	1.0	0.6
	Total Imidacloprid	1.0	0.7
Cherry, plum, apricot, and peach pollen	Imidacloprid	1.0	0.4
	5-hydroxy Imidacloprid	1.0	0.5
	Imidacloprid olefin	1.0	0.3
	Total Imidacloprid	1.0	0.5

6. RESULTS:

The imidacloprid residue data for cherry, plum, apricot, and peach (stone fruit) leaves, nectar, and pollen are provided in Table 3. Only residue values above the respective analyte LODs are reported. Any residue value that was below the LOD is reported as less than the LOD (<LOD). The total imidacloprid residue is a sum of the analyte residue values that are greater than the respective analyte LODs. If the analyte value was less than the LOD, a default value equal to half of the analyte LOD (half-LOD) was added into the sum. For samples with a reported total residue of less than LOD, the sum of the analyte half-LOD values for the respective matrix was used to calculate the average residue values.

Table 3. Imidacloprid Residue Data from Cherry, Plum, Apricot and Peach

Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop and Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Olefin Residues (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
Stone Fruit Leaves					**LODs (ppb):**	**0.4**	**1.6**	**0.5**	**1.6**
NT024-13ZA	Kerman, California Region 10, 2014	Cherry Brooks	Coarse	0.50	174	26.4 6.8	8.6 2.8	164.1 40.3	199.1 49.9 Avg.: 124.5
NT024-13ZA	Kerman, California Region 10, 2015	Cherry Brooks	Coarse	0.50	323	25.6 25.9	12.8 13.4	148.8 148.3	187.1 187.6 Avg.: 187.35

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Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop and Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Olefin Residues (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
NT025-13ZA	Kerman, California Region 10, 2014	Cherry Brooks	Coarse	0.50	168	17.7 22.0	9.4 9.9	204.4 245.5	231.4 277.5 Avg.: 254.45
NT025-13ZA	Kerman, California Region 10, 2015	Cherry Brooks	Coarse	0.50	292	21.0 22.6	14.1 18.2	146.2 160.0	181.3 200.8 Avg.: 191.05
NT020-13ZA	Merced, California Region 10, 2014	Cherry Brooks	Fine	0.50	182	<LOD <LOD	<LOD <LOD	2.6 4.2	3.6 5.2 Avg.: 4.4
NT020-13ZA	Merced, California Region 10, 2015	Plum French	Fine	0.50	249	1.6 2.3	5.3 5.6	13.8 19.7	20.7 27.6 Avg.: 24.15
NT021-13ZA	Yuba City, California Region 10, 2014	Plum French	Fine	0.50	180	1.0 1.9	2.1 3.6	2.3 6.4	5.4 11.9 Avg.: 8.65
NT021-13ZA	Yuba City, California Region 10, 2015	Plum French	Fine	0.50	243	1.9 3.4	6.7 8.2	9.2 18.8	17.7 30.4 Avg.: 24.05
NT022-13ZA	Yuba City, California Region 10, 2014	Plum French	Medium	0.50	180	0.5 <LOD	<LOD <LOD	5.6 0.6	6.9 1.6 Avg.: 4.25
NT022-13ZA	Yuba City, California Region 10, 2015	Plum French	Medium	0.50	243	0.8 0.4	4.7 <LOD	4.8 4.5	10.4 5.7 Avg.: 8.05
NT026-13ZA	Sanger, California Region 10 2014	Apricot Castlebrite	Coarse	0.50	174	3.0 2.1	8.4 7.7	28.7 20.0	40.1 29.8 Avg.: 34.95

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Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop and Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Olefin Residues (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
NT026-13ZA	Sanger, California Region 10 2015	Apricot Castlebrite	Coarse	0.50	309	0.8 1.1	3.6 5.4	12.5 15.5	16.8 21.9 Avg.: 19.35
NT019-13ZA	Stockton, California Region 10, 2014	Peach Flavor Crest	Fine	0.50	155	0.5 0.5	4.6 5.2	18.6 23.0	23.6 28.6 Avg.: 26.1
NT019-13ZA	Stockton, California Region 10, 2015	Peach Flavor Crest	Fine	0.50	230	1.2 0.7	7.7 6.2	4.0 4.1	12.9 11.1 Avg.: 12
NT023-13ZA	Yuba City, California Region 10, 2014	Peach Bounty	Medium	0.50	159	<LOD <LOD	<LOD <LOD	6.4 11.2	7.4 12.2 Avg.: 9.8
NT023-13ZA	Yuba City, California Region 10, 2015	Peach Bounty	Medium	0.50	230	0.6 0.5	3.3 2.5	6.2 4.8	10.0 7.8 Avg.: 8.9
NT027-13ZA	Kingsburg, California Region 10 2014	Peach Late Ross	Medium	0.51	188	16.4 8.9	21.8 13.2	56.0 27.5	94.2 49.6 Avg.: 71.9
NT027-13ZA	Kingsburg, California Region 10 2015	Peach Late Ross	Medium	0.51	264	5.6 9.5	10.7 18.7	17.8 28.5	34.1 56.7 Avg.: 45.4
Stone Fruit Nectar					**LODs (ppb):**	**0.7**	**0.6**	**0.3**	**0.7**
NT024-13ZA	Kerman, California Region 10, 2014	Cherry Brooks	Coarse	0.50	160	1.6 0.9	<LOD <LOD	5.5 3.9	7.4 5.1 Avg.: 6.25
NT025-13ZA	Kerman, California Region 10,	Cherry Brooks	Coarse	0.50	152	1.1 0.9	<LOD 0.8	5.9 7.1	7.3 8.8 Avg.: 8.05

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Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop and Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Olefin Residues (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
	2014								
NT025-13ZA	Kerman, California Region 10, 2015	Cherry Brooks	Coarse	0.50	279	1.2 1.5	<LOD <LOD	5.9 8.9	7.4 10.7 Avg.: 9.05
NT020-13ZA	Merced, California Region 10, 2014	Plum French	Fine	0.50	156	<LOD <LOD	<LOD <LOD	<LOD 0.3	0.8 1.0 Avg.: 0.9
NT020-13ZA	Merced, California Region 10, 2015	Plum French	Fine	0.50	231	<LOD <LOD	<LOD <LOD	<LOD <LOD	0.8 0.8 Avg.: 0.8
NT021-13ZA	Yuba City, California Region 10, 2014	Plum French	Fine	0.50	155	<LOD <LOD	<LOD <LOD	0.4 <LOD	1.1 0.8 Avg.: 0.95
NT021-13ZA	Yuba City, California Region 10, 2015	Plum French	Fine	0.50	223	<LOD <LOD	<LOD <LOD	<LOD <LOD	0.8 0.8 Avg.: 0.8
NT022-13ZA	Yuba City, California Region 10, 2014	Plum French	Medium	0.50	155	<LOD <LOD	<LOD <LOD	0.4 0.3	1.0 1.0 Avg.: 1.0
NT022-13ZA	Yuba City, California Region 10, 2015	Plum French	Medium	0.50	223	<LOD <LOD	<LOD <LOD	<LOD <LOD	0.8 0.8 Avg.: 0.8
NT026-13ZA	Sanger, California Region 10 2014	Apricot Castlebrite	Coarse	0.50	152	28.7 28.8	0.8 1.4	2.5 3.4	31.9 33.6 Avg.: 32.75
NT026-13ZA	Sanger, California Region 10	Apricot Castlebrite	Coarse	0.50	291	<LOD <LOD	<LOD 0.7	1.0 0.8	1.7 1.8 Avg.: 1.75

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Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop and Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Olefin Residues (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
	2015								
NT019-13ZA	Stockton, California Region 10, 2014	Peach Flavor Crest	Fine	0.50	133	7.5 5.5	0.8 0.7	1.2 0.6	9.6 6.7 Avg.: 8.15
NT019-13ZA	Stockton, California Region 10, 2015	Peach Flavor Crest	Fine	0.50	214	<LOD <LOD	3.5 <LOD	<LOD <LOD	4.0 0.8 Avg.: 2.4
NT023-13ZA	Yuba City, California Region 10, 2014	Peach Bounty	Medium	0.50	141	<LOD <LOD	0.7 0.8	0.6 0.8	1.6 1.9 Avg.: 1.75
NT023-13ZA	Yuba City, California Region 10, 2015	Peach Bounty	Medium	0.50	211	<LOD <LOD	<LOD <LOD	<LOD <LOD	0.8 0.8 Avg.: 0.8
NT027-13ZA	Kingsburg, California Region 10 2014	Peach Late Ross	Medium	0.51	145	2.3 1.9	0.9 0.7	0.6 0.5	3.8 3.0 Avg.: 3.4
NT027-13ZA	Kingsburg, California Region 10 2015	Peach Late Ross	Medium	0.51	246	1.0 0.7	0.8 <LOD	<LOD <LOD	1.9 1.2 Avg.: 1.55
Stone Fruit Pollen					**LODs (ppb):**	**0.5**	**0.3**	**0.4**	**0.5**
NT024-13ZA	Kerman, California Region 10, 2014	Cherry Brooks	Coarse	0.50	160	1.2 3.0	0.4 1.3	11.8 12.0	13.4 16.6 Avg.: 15
NT024-13ZA	Kerman, California Region 10, 2015	Cherry Brooks	Coarse	0.50	309	<LOD <LOD	0.6 <LOD	5.1 5.4	5.9 5.8 Avg.: 5.85
NT025-13ZA	Kerman, California	Cherry Brooks	Coarse	0.50	152	3.9 1.3	2.9 0.7	18.5 18.6	25.3 20.5

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Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop and Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Olefin Residues (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
	Region 10, 2014								Avg.: 22.9
NT025-13ZA	Kerman, California Region 10, 2015	Cherry Brooks	Coarse	0.50	279	0.8 1.4	0.9 1.9	8.3 12.4	10.0 15.7 Avg.: 12.85
NT020-13ZA	Merced, California Region 10, 2014	Plum French	Fine	0.50	156	2.1 4.0	0.6 1.7	53.6 108.7	56.3 114.4 Avg.: 85.35
NT020-13ZA	Merced, California Region 10, 2015	Plum French	Fine	0.50	231	<LOD <LOD	<LOD <LOD	1.9 28.0	2.3 28.4 Avg.: 15.35
NT021-13ZA	Yuba City, California Region 10, 2014	Plum French	Fine	0.50	155	1.3	0.5	33.5	35.3 Avg.: 35.3
NT021-13ZA	Yuba City, California Region 10, 2015	Plum French	Fine	0.50	223	0.5 1.1	0.7 4.0	3.2 7.5	4.4 12.6 Avg.: 8.5
NT022-13ZA	Yuba City, California Region 10, 2014	Plum French	Medium	0.50	155	1.1	0.3	29.6	31.0 Avg.: 31.0
NT022-13ZA	Yuba City, California Region 10, 2014	Plum French	Medium	0.50	223	<LOD	<LOD	3.1	3.5 Avg.: 3.5
NT026-13ZA	Sanger, California Region 10 2014	Apricot Castlebrite	Coarse	0.50	152	1.7 1.4	1.3 1.0	49.4 27.3	52.5 29.8 Avg.: 41.15
NT026-13ZA	Sanger, California	Apricot Castlebrite	Coarse	0.50	291	<LOD	<LOD	2.7	3.1 Avg.: 3.1

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Trial Identification	Location (City, State, NAFTA Region, Sampling Year)	Crop and Variety	Soil Type	Total Rate, lb. a.i./acre	DAA (Days After the Last Application)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Olefin Residues (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
	Region 10 2015								
NT019-13ZA	Stockton, California Region 10, 2014	Peach Flavor Crest	Fine	0.50	133	9.4 3.7	3.9 2.0	328.0 127.7	341.3 133.4 Avg.: 237.35
NT019-13ZA	Stockton, California Region 10, 2015	Peach Flavor Crest	Fine	0.50	214	2.4 0.8	3.7 1.8	38.4 52.3	44.5 54.9 Avg.: 49.7
NT023-13ZA	Yuba City, California Region 10, 2014	Peach Bounty	Medium	0.50	141	1.0	1.3	54.7	57.0 Avg.: 57.0
NT023-13ZA	Yuba City, California Region 10, 2015	Peach Bounty	Medium	0.50	211	2.6 2.8	14.3 40.3	122.1 144.7	139.0 187.8 Avg.: 163.4
NT027-13ZA	Kingsburg, California Region 10 2014	Peach Late Ross	Medium	0.51	145	3.7	2.1	27.8	33.6 Avg.: 33.6
NT027-13ZA	Kingsburg, California Region 10 2015	Peach Late Ross	Medium	0.51	246	1.9 <LOD	3.3 2.7	3.2 3.8	8.4 6.7 Avg.: 7.55

7. STATISTICAL ANALYSIS

Study Objectives and Design

The objective of the study was to determine the concentration of imidacloprid and its degradation products, 5-Hydroxy imidacloprid and imidacloprid olefin, in leaves, nectar, and pollen of stone fruit trees in response to one soil and two foliar applications of a imidacloprid pesticide product applied in the previous year. The two-year study had applications made in 2013 and 2014 and plant matrices harvested in the respective following years of 2014 and 2015. Trees received one soil application applied at 0.38 lbs imidacloprid per acre and two foliar applications ranging between 0.058 and 0.064 lbs imidacloprid per acre. The first foliar application was made 3 to 7 days after the soil application and the second foliar spray was applied 7 to 11 days after the first foliar application. In 2014, flower samples were collected 133 to 160 days after the last foliar application and in 2015, flower samples were collected 211 to 309 days after the last foliar application. In 2014, leaf samples were collected 155 to 188 days after the last foliar application and in 2015, sampling ranged from 230 to 323 days after the last foliar application. As specified in the data call-in, the study was conducted at 9 separate sites with the study being replicated at each site in the next year. Two composite samples were collected from each treated plot for each plant matrix. Untreated control plots were also included at each site with only one composite sample taken at the same time sampling was occurring for treated plots.

Non-parametric statistical tests were used to test for differences in distribution of concentrations between years, untreated control to treated plants, extra floral nectar concentration between sampling intervals, and between soil type. Non-parametric tests do not require tests for normality as they are robust to differences in distribution and they are also robust for experimental designs with low replicates (Helsel and Hirsch, 2002). The PROC NPAR1WAY procedure in the Statistical Analysis System (SAS) statistical package was used to conduct Wilcoxon-Mann –Whitney (Wilcoxon), Median non-parametric, and Kuiper tests. A significant result from the Wilcoxon test indicates differences in the shape of distributions; A significant result from the Median test indicates differences in the location of the medians between distributions; and A significant result from the Kuiper test indicates differences in the empirical distributions between two groups. The Exact option for each statistic was implemented as it provides permutation testing, a statistical method that minimizes the effect of sample size and distributional differences. Using the Exact option, the Monte Carlo procedure was also implemented which provided 10,000 separate runs for each statistic to produce the permutation distributions. The test for potential differences in soil type had 3 levels so the DSCF option in PROC NPAR1WAY, which invokes the Dwass, Steel, Critchlow-Fligner multiple comparison test, was used to provide pairwise tests for two-sample rankings.

Additional procedures used for descriptive statistics were PROC MEANS to calculate mean values from the replicates at each site, PROC CAPACITY to produce cumulative statistics, and PROC BOXPLOT to produce comparative graphics. Data from treated sites were averaged to test for the effects of years, soil type, and to compare to untreated control plots where only 1 replicate sample was available. Graphical comparisons are presented with data transformed to a natural logarithm scale, providing clearer contrasts between the distributions. Although both limits of detection (LOD) and limits of quantification (LOQ) were indicated, only data less than the LOD were indicated as <LOD in the data set. Values were provided when samples were between the LOD and LOQ. For statistical analyses, values noted as below the limit of detection (LOD) were assigned half the value of the respective detection limit (Table 2). Values between the LOD and LOQ were used as reported. Results were reported in ppm on a wet weight/weight basis. The distribution of concentrations in bee relevant plant matrices was

calculated using all the raw data because these values represent the actual range of exposure to bees and other organisms that feed off the nectar and pollen of plants.

Detection rate noted for each plant matrix: Counts for the number of samples reported below the respective detection limit for each matrix are presented in Table 4 for treated plants and untreated control plants. The LOQ for the leaf matrix was at 5 ng/g (ppb) and at 1 ng/g (ppb) for nectar and pollen matrices. For treated plants, the majority of concentrations for parent imidacloprid were above the LOQ for leaves and pollen, but not for nectar. The percent of values above the LOQ for imidacloprid were 75% for leaves, 100% for pollen, and 26% for nectar. None of the olefin imidacloprid values were above the LOQ, whereas, 5-Hydroxy imidacloprid values were measured above the LOQ but at lower frequency than for the parent: 33%, 60%, and 29% for leaves, pollen and nectar, respectively. Figure 1 provides a graphical comparison for the relative range in concentrations measured between the residues in treated plants. A few imidacloprid residues were reported above the LOQ in untreated control plants in leaf (11%) and nectar (12%) matrices but nearly all samples for pollen were above the LOQ at 89% of the total number of samples taken. Except for pollen, results for the imidacloprid olefin and 5-Hydroxy imidacloprid metabolites were below the LOD. For pollen, no residues above the LOQ were reported for imidacloprid olefin but 33% of the values were above the LOQ for the 5-Hydroxy imidacloprid metabolite. Figure 2 provides a graphical comparison for the relative range in concentrations measured between the residues in untreated control plants.

Comparison between years: Potential difference between years was measured to indicate the presence of carry-over effects of residues between years for treated plants. Except for imidacloprid residue measured in pollen, there was no statistical difference between years (Table 5, Difference Between Years for Treated Plants heading; Figure 3). The significant result for imidacloprid residues in pollen show a greater range in concentrations measured in the first year of the study. This pattern indicates no potential for carry-over of residues due to the pattern of application used in this study.

Comparison between treated and untreated control plants: The distribution statistics for all treatments are presented for leaves, nectar, and pollen in Table 6. Non-parametric tests conducted on the mean of replicate samples for foliar treated plots indicated a significantly greater range in imidacloprid residues for treated plants compared to untreated control plants for leaf and pollen matrices but not for nectar samples (Table 5, Treated vs UTC heading; Figure 4). The results for the metabolites did not indicate a consistent difference between treated and untreated plants (Figures 5 and 6). The inconsistency was caused by the lower range in residues reported for the metabolites coupled with a few detections in untreated plants that were above the LOQ. For example, a value of 190 ng/g was reported for imidacloprid olefin in pollen sampled from an untreated plant. The highest value in treated plants was 40.3 ng/g. This disparity resulted in a wider range in values noted for untreated plants.

Comparison of distribution between soil types: As reported in Table 3B of the report, the sites were distributed among the three requested coarse, medium, and fine soil texture categories. There were 3 sites in each category. Results of non-parametric tests indicated that the range in concentrations for parent imidacloprid in leaves and nectar was greater in plants grown in coarse-textured soils than in the other two soil categories (Table 7; Figure 7). No other significant effects were observed for parent imidacloprid in pollen samples or for the two metabolites in the plant matrices (Figures 7 and 8).

Magnitude of residues in bee-relevant matrices: The observed distributions derived from the individual analyses ostensibly determines the expected range in concentrations of imidacloprid residues in bee relevant plant samples for the combination of plant species and application scenario tested in this study (Table 6 Treated Plants heading). The median and maximum values for total residue in nectar were 1.7 and 33.6 ng/g, respectively, on a wet weight basis. For pollen, median and maximum values were higher at 26.9 and 341.3 ng/g, respectively.

8. STUDY STRENGTHS, LIMITATIONS AND CONCLUSIONS

In the context of documenting the magnitude of imidacloprid residues in bee-related matrices of stone fruit, the following strengths are observed with this study.

1. Data provide quantitative values of total imidacloprid residues expected in pollen, nectar, and leaves of various varieties of stone fruit.

2. The study was replicated over two years with measurements in plant samples taken at a mean of 158 days after the last application in 2014 and at a mean of 251 days after the last application in 2015.

Limitations noted in this study include:

1. Samples were taken from a variety of stone fruits (cherry, peach, plum, or apricot). Since the effect of different varieties on distribution of residues is unknown, the results will reflect general observations made to all stone fruits.

2. Leaf and flower samples were collected at different times, so establishing correlations between concentrations in leaves and flowers (nectar and pollen) is not possible.

Overall, considering the strengths and limitations of this study, the following conclusions can be drawn:

1. **Bee-relevant matrices:** Significant concentrations of imidacloprid residues were measured in pollen samples taken in the year following one soil and two foliar spray applications. For example, the average concentration of total imidacloprid residues in pollen at one of the sites was 160 ng/g for samples taken 211 days after the last application made in the previous year. Maximum concentrations in pollen was measured at 341 ng/g with a median value of 27 ng/g for 30 samples. Concentrations in nectar were lower with many samples reported at the LOD for each residue. The maximum nectar concentration was 34 ng/g with a median value of 2 ng/g for 34 samples.

2. **Soil type:** An effect of soil type was measured but it was inconsistent between the plant matrices: Higher concentrations were indicated for leaves and nectar of plants sampled from coarse-textured soil sites but no effect was observed for pollen. The range in concentrations for the residues was similar between plants grown in medium and fine-textured soil sites.

3. **The untreated control plots were contaminated with imidacloprid residues.** Elevated concentrations of imidacloprid and metabolite residues were measured in untreated control plants. For example, two pollen samples collected in 2015 from untreated plots had higher total imidacloprid residue values than from their corresponding treated plot. In site NT020-13ZA-H004, which was a plum orchard, the total imidacloprid residue in the untreated plot in 2015 was 246.4 ng/g (32.1 IMI-5-OH, 187.3 IMI Olefin, and 27.0 ng/g parent imidacloprid), whereas total residue from the replicate samples from the treated plot was 31.0 ng/g (0.3 IMI-5-OH, 1.1 IMI Olefin, and 29.6 ng/g parent imidacloprid) and 3.5 ng/g (0.15 IMI-5-OH, 0.25 IMI Olefin, and 3.1 ng/g parent imidacloprid). It is unclear how this contamination occurred.

9. STUDY VALIDITY/CLASSIFICATION

Classification/Utility for Bee Risk Assessment. This study is classified as acceptable. It provides an accurate assessment of Imidacloprid (and its metabolites) residues in leaves, pollen, and nectar during bloom for stone fruit trees under the exposure and cultural conditions used in this study. The study was conducted using the maximum annual application rate (0.5 lbs ai/A) and residue values presented should be considered reliable. The label for Admire Pro Systemic Protectant prohibits soil applications to stone fruit pre-bloom, during bloom, or when bees are foraging, but this restriction does not specify the amount of time before bloom that applications are prohibited.

Temporal Variability in Residues. This study was not designed for temporal analysis of declining concentrations, but rather, to provide an annual snapshot of residue concentrations during flowering. Only one sample per year of each matrix was collected and analyzed so there is no way to know the rate at which concentrations were decreasing.

Spatial Variability in Residues. All nine sites were located in the Central Valley of California. The southernmost sites were located just north of Visalia (Kingsburg) and the northernmost sites were in Yuba City. Climatic conditions were similar across all nine sites. Peaches, of the varieties Flavor Crest, Bounty, and Late Ross were grown in Stockton (NT019-13ZA), Yuba City (NT023-13ZA), and Kingsburg (NT027-13ZA), respectively. Brooks variety cherries were grown in two sites in Kerman (NT025-13ZA and NT024-13ZA). French variety plums were grown in fine soil in Merced (NT020-13ZA) and in fine (NT021-13ZA) and medium soil (NT022-13ZA) in Yuba City. Castlebright variety apricots were grown in Sanger (NT026-13ZA). Concentrations of total Imidacloprid in nectar were highest in Apricots grown in Sanger in 2014 (33 µg/g), but in 2015 the same trees had concentrations of only 2 ng/g. Concentrations of total Imidacloprid in pollen in the same trees (Apricots in Sanger) followed a similar pattern, with an average of 41 µg/g in 2014 and an average of 3 µg/g in 2015. The study authors did not offer any explanations as to why total imidacloprid concentrations would vary so widely in the same trees in two consecutive years. The interval from the time of the last application to the time that samples were collected was longer in 2015, but it the difference in these intervals was similar for the other sites and none of the other sites had such a drastic change in residue levels from year to year. This introduces significant uncertainty to the results. Total Imidacloprid residues in pollen, nectar, and leaves in other sites were similar to each other, and all of the other nectar samples in all other stone fruits and locations contained 10.7 µg/g or less of total imidacloprid. The highest concentration in pollen was 341 ng/g from peaches grown in Stockton (NT019-13ZA), but that was only one individual sample, and all the other samples contained less than 200 µg/g, with most of those below 100 ng/g. Leaf samples were collected at different times than flower (i.e., pollen and nectar) samples, so the concentrations in leaves cannot be correlated with concentrations in pollen and nectar.

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Table 4. Counts of chemical analytical results for parent imidacloprid and 5-Hydroxy imidacloprid and imidacloprid olefin metabolites that were indicated as above the LOQ, between the LOQ and LOD, and below the LOD (Table 2) for treated and untreated control plants.

Treatment and Plant Sample	Comparison of Total Number of Samples Reported Above the LOQ, Between the LOQ and LOD, and Below the LOD											
	Imidacloprid				5-Hydroxy Imidacloprid				Olefin Imidacloprid			
	Total Number	Number Above LOQ	Number Between LOD and LOQ	Number Below LOD	Total Number	Number Above LOQ	Number Between LOD and LOQ	Number Below LOD	Total Number	Number Above LOQ	Number Between LOD and LOQ	Number Below <LOD
Treated Plants: Foliar Application												
Leaf	36	27	9	0	36	12	19	5	36	0	29	7
Nectar	34	9	11	14	34	10	4	20	34	0	12	22
Pollen	30	30	0	0	30	18	4	8	30	0	25	5
Untreated Control Plants												
Leaf	18	2	2	14	18	0	0	18	18	0	0	18
Nectar	17	2	4	11	17	1	0	16	17	0	0	17
Pollen	18	16	2	0	18	6	4	8	18	0	7	11

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Table 5. Statistical results for test of differences in concentration of parent imidacloprid and 5-Hydroxy imidacloprid and imidacloprid olefin metabolites measured between replicate years for treated plants and between treated and untreated control plants.

Comparison and Plant Matrix	Exact Probability Levels for Wilcoxon, Median, and Kuiper Tests Comparing Concentrations Between Years								
	Imidacloprid			5-Hydroxy Metabolite			Olefin Metabolite		
	Wilcoxon	Median	Kuiper	Wilcoxon	Median	Kuiper	Wilcoxon	Median	Kuiper
Difference Between Years for Treated Plants									
Leaves	1	0.98	0.89	0.37	1	0.57	0.34	1	0.89
Nectar	0.4	0.16	0.04	0.14	0.33	0.93	0.4	0.63	0.91
Pollen	0.03	0.06	0.26	0.02	0.09	0.66	0.98	1	0.26
Treated vs Untreated Plants									
Leaves	0.001	0.01	0.003	0.001	0.001	0.003	0.001	0.001	0.008
Nectar	0.16	0.18	0.95	0.04	0.04	0.53	0.003	0.003	0.3
Pollen	0.001	0.02	0.08	0.02	0.09	0.08	0.14	0.09	0.03

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Table 6. Distributional statistics for concentration of parent imidacloprid (IMI) and 5-Hydroxy imidacloprid (5-OH) and imidacloprid olefin (Olefin) metabolites and total residue (Total) in leaves, nectar, and pollen of stone fruit trees treated with imidacloprid in the previous year and in untreated control plants. Acronyms in the table are; N=NUMBER OF OBSERVATIONS; SD=STANDARD DEVIATION; CV= COEFFICIENT OF VARIATION.

	Leaves				Nectar				Pollen			
Statistic	**IMI**	**5-OH**	**Olefin**	**Total**	**IMI**	**5-OH**	**Olefin**	**Total**	**IMI**	**5-OH**	**Olefin**	**Total**
Treated Plants												
N	36.0	36.0	36.0	**36.0**	34	34	34	**34**	30.0	30.0	30.0	**30.0**
Mean (ng/g)	45.4	6.5	6.9	**58.9**	1.6	2.7	0.6	**4.8**	44.8	1.8	3.2	**49.7**
SD (ng/g)	66.8	9.0	5.5	**78.4**	2.4	6.8	0.6	**7.7**	66.8	1.9	7.5	**71.7**
CV (%)	148.0	138.0	80.0	**133.0**	153.0	255.0	104.0	**161.0**	149.0	105.0	236.0	**144.0**
Min (ng/g)	0.6	0.2	0.8	**1.6**	0.2	0.4	0.3	**0.8**	1.9	0.3	0.2	**2.3**
Median (ng/g)	16.7	1.8	5.5	**22.9**	0.4	0.4	0.3	**1.7**	23.0	1.3	1.3	**26.9**
75th (ng/g)	34.5	9.2	9.7	**53.3**	1.2	1.2	0.7	**6.8**	52.3	2.6	2.7	**54.9**
90th (ng/g)	160.0	22.6	14.1	**199.1**	5.9	5.5	0.8	**9.5**	124.9	3.8	4.0	**136.2**
95th (ng/g)	204.4	25.9	18.7	**231.5**	7.1	28.7	1.4	**32.0**	144.7	4.0	14.3	**187.8**
Max (ng/g)	245.5	26.4	21.8	**277.4**	8.9	28.8	3.5	**33.6**	328.0	9.4	40.3	**341.3**
% of Total	**77.1**	**11.0**	**11.7**		**32.3**	**55.4**	**11.7**		**90.1**	**3.6**	**6.4**	
Untreated Control Plants												
N	18	18	18	**18**	17	17	17	**17**	18	18	18	**18**
Mean (ng/g)	1.3	0.2	0.8	**2.3**	0.9	2.1	0.3	**3.4**	6.5	4.2	22.5	**33.2**
SD (ng/g)	2.9	0.0	0.0	**2.9**	1.8	7.4	0.0	**7.6**	8.5	9.8	60.6	**73.8**
CV (%)	220.0	0.0	0.0	**125.0**	196.0	345.0	0.0	**227.0**	131.0	234.0	269.0	**222.0**
Min (ng/g)	0.3	0.2	0.8	**1.3**	0.2	0.4	0.3	**0.8**	0.6	0.3	0.2	**1.0**
Median (ng/g)	0.3	0.2	0.8	**1.3**	0.2	0.4	0.3	**0.8**	3.1	0.6	0.2	**5.7**
75th (ng/g)	0.3	0.2	0.8	**1.3**	0.5	0.4	0.3	**1.2**	6.5	1.7	2.7	**14.8**
90th (ng/g)	6.4	0.2	0.8	**7.4**	4.8	0.4	0.3	**6.9**	27.0	30.1	187.3	**220.9**
95th (ng/g)	11.2	0.2	0.8	**12.2**	6.2	30.9	0.3	**32.1**	28.9	32.1	190.0	**246.4**
Max (ng/g)	11.2	0.2	0.8	**12.2**	6.2	30.9	0.3	**32.1**	28.9	32.1	190.0	**246.4**
% of Total	**56.5**	**8.7**	**34.8**		**26.5**	**61.8**	**8.8**		**19.6**	**12.7**	**67.8**	

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Table 7. Statistical results for test of differences in concentration of parent imidacloprid and 5-Hydroxy imidacloprid (5-Hydroxy) and imidacloprid olefin (Olefin) metabolites measured between plants grown in coarse, medium, and fine-textured soils.

Treatment, Plant Matrix, and Specific Soil Contrasts	Exact Probability Levels for Non-parametric Tests of Differences Between Soil Type		
	Imidacloprid	5-Hydroxy	Olefin
	Wilcoxon	Wilcoxon	Wilcoxon
Treated Plants			
Leaves - Overall	0.001	0.02	0.22
Fine vs. Medium	0.88	1.00	0.94
Fine vs. Coarse	0.0345	0.064	0.13
Medium vs. Coarse	0.0431	0.064	0.50
Nectar - Overall	0.002	0.15	0.93
Fine vs. Medium	0.96	0.91	0.96
Fine vs. Coarse	0.016	0.21	0.92
Medium vs. Coarse	0.016	0.28	0.99
Pollen - Overall	0.22	0.66	0.57
Fine vs. Medium	0.70	0.92	0.97
Fine vs. Coarse	0.18	0.70	0.60
Medium vs. Coarse	0.60	0.75	0.65
Untreated Plants			
Leaves - Overall	0.25	1.00	1.00
Fine vs. Medium	0.27	1.00	1.00
Fine vs. Coarse	0.58	1.00	1.00
Medium vs. Coarse	0.48	1.00	1.00
Nectar - Overall	0.32	0.29	1.00
Fine vs. Medium	0.33	1.00	1.00
Fine vs. Coarse	0.55	0.52	1.00
Medium vs. Coarse	0.87	0.52	1.00
Pollen - Overall	0.43	0.75	0.47
Fine vs. Medium	0.50	1.00	1.00
Fine vs. Coarse	0.92	0.82	0.55
Medium vs. Coarse	0.50	0.73	0.43

Figure 1. Treated Plants: Comprison of the relative range in concentrations for parent imidacloprid and 5-Hydroxy imidacloprid and imidacloprid olefin metabolite residues in leaves, nectar, and pollen samples from stone fruit trees exposed to imidacloprid treatments in the previous year. Values were transformed to natural logarithms.

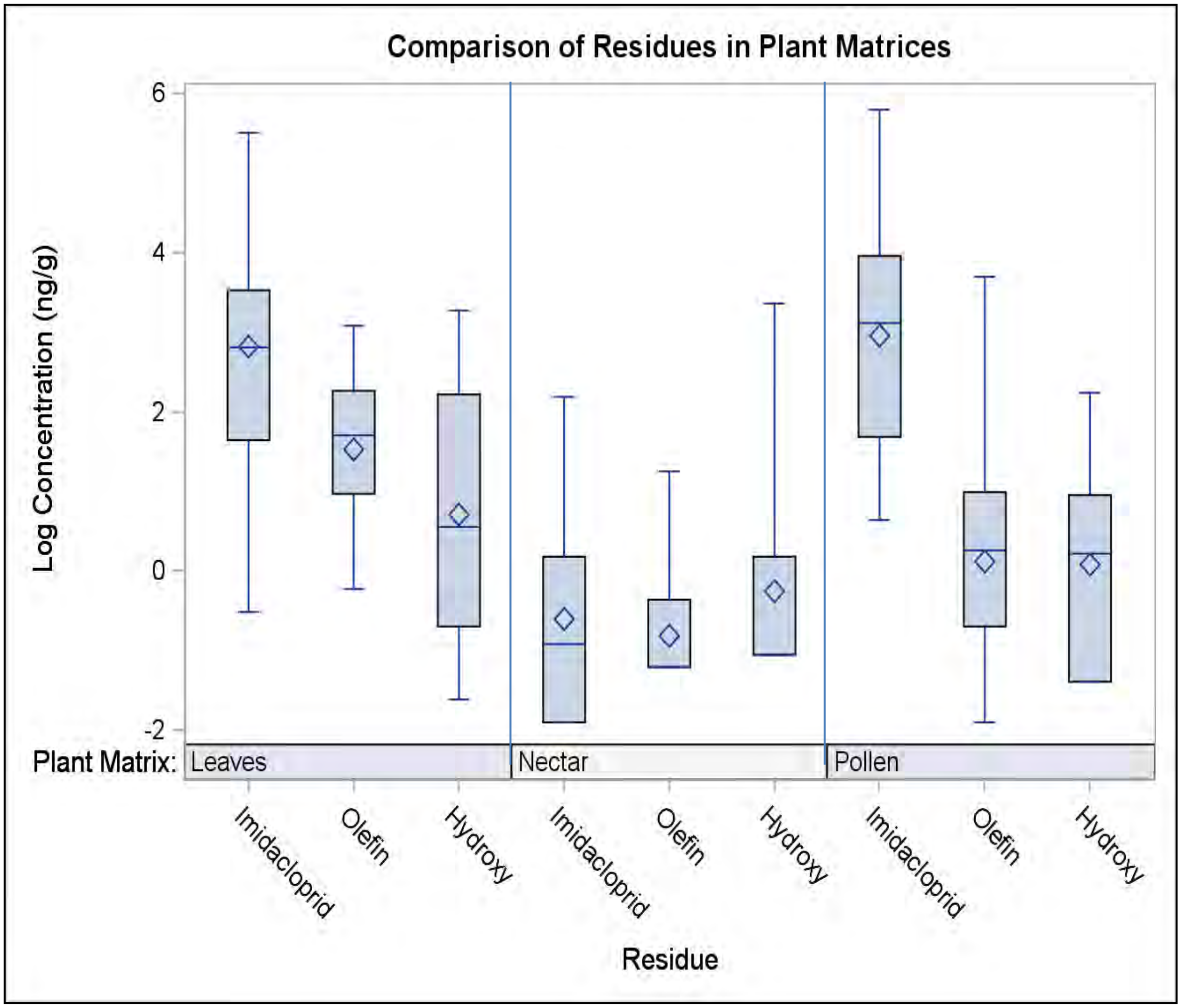


Figure 2. Untreated Control Plants: Comparison of the relative range in concentrations for parent imidacloprid and 5-Hydroxy imidacloprid and imidacloprid olefin metabolite residues in leaves, nectar, and pollen samples from stone fruit trees grown in untreated control plots. Values were transformed to natural logarithms.

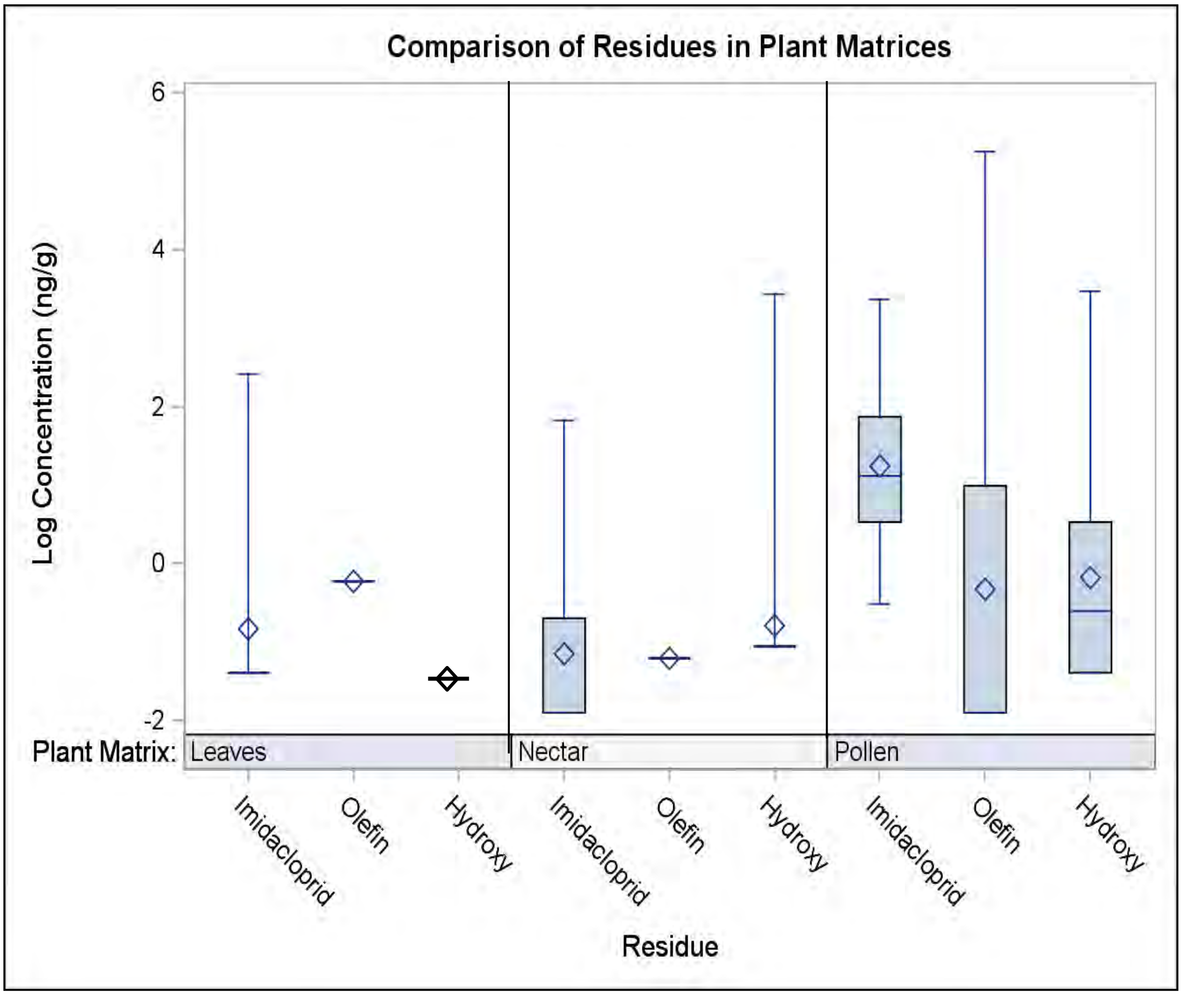


Figure 3. Treated Plants: Comparison of the relative range in concentrations between replicate years for midacloprid residues in leaves, nectar, and pollen samples obtained from stone fruit trees treated with imidacloprid in the previous year. Values were transformed to natural logarithms.

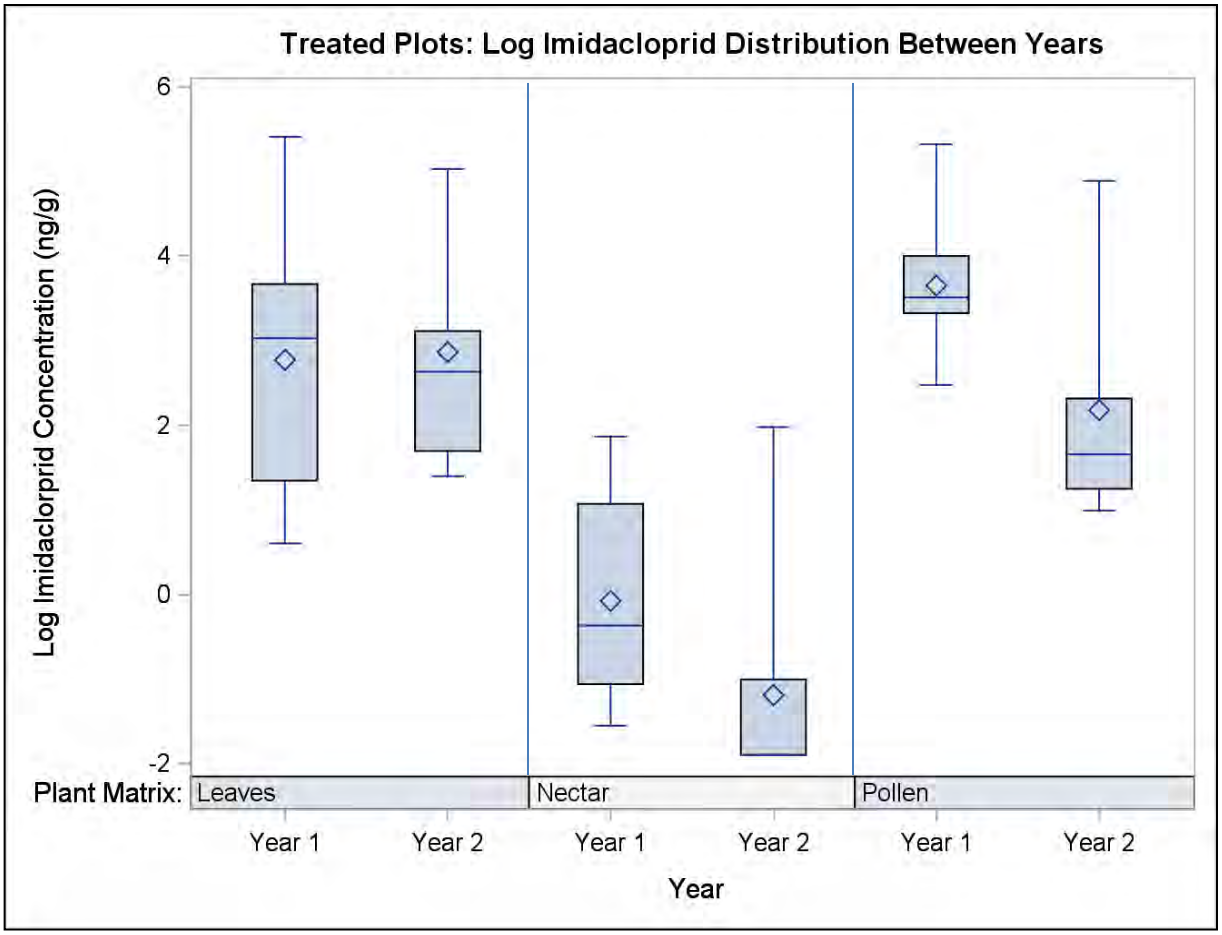


Figure 4. Treated vs Untreated Control Plants: Comparison of the relative range in imidacloprid concentrations between treated and untreated control plants for residues measured in leaves, nectar, and pollen samples obtained from stone fruit trees treated with imidacloprid in the previous year. Values were transformed to natural logarithms.

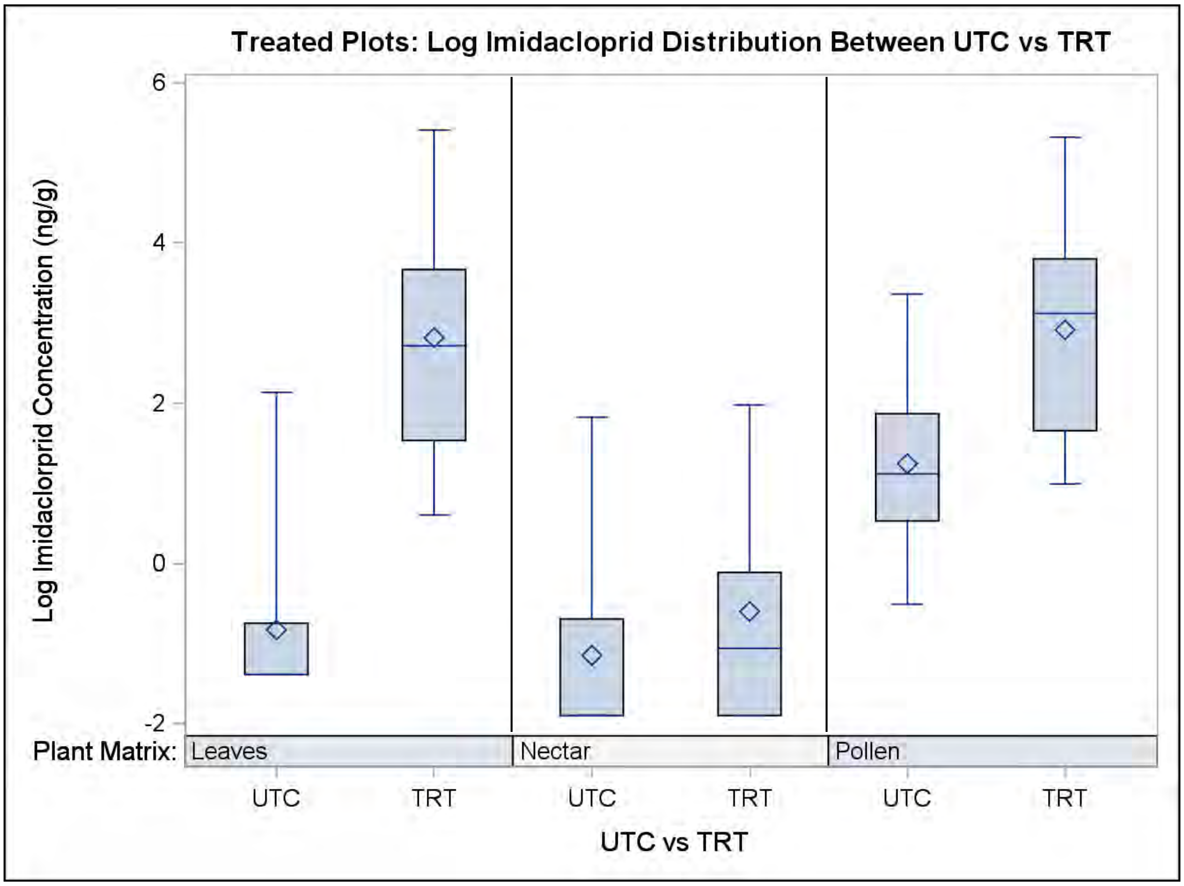


Figure 5. Treated vs Untreated Control Plants: Comparison of the relative range in 5-Hydroxy imidacloprid metabolite concentrations between treated and untreated control plants for residues measured in leaves, nectar, and pollen samples obtained from stone fruit trees treated with imidacloprid in the previous year. Values were transformed to natural logarithms.

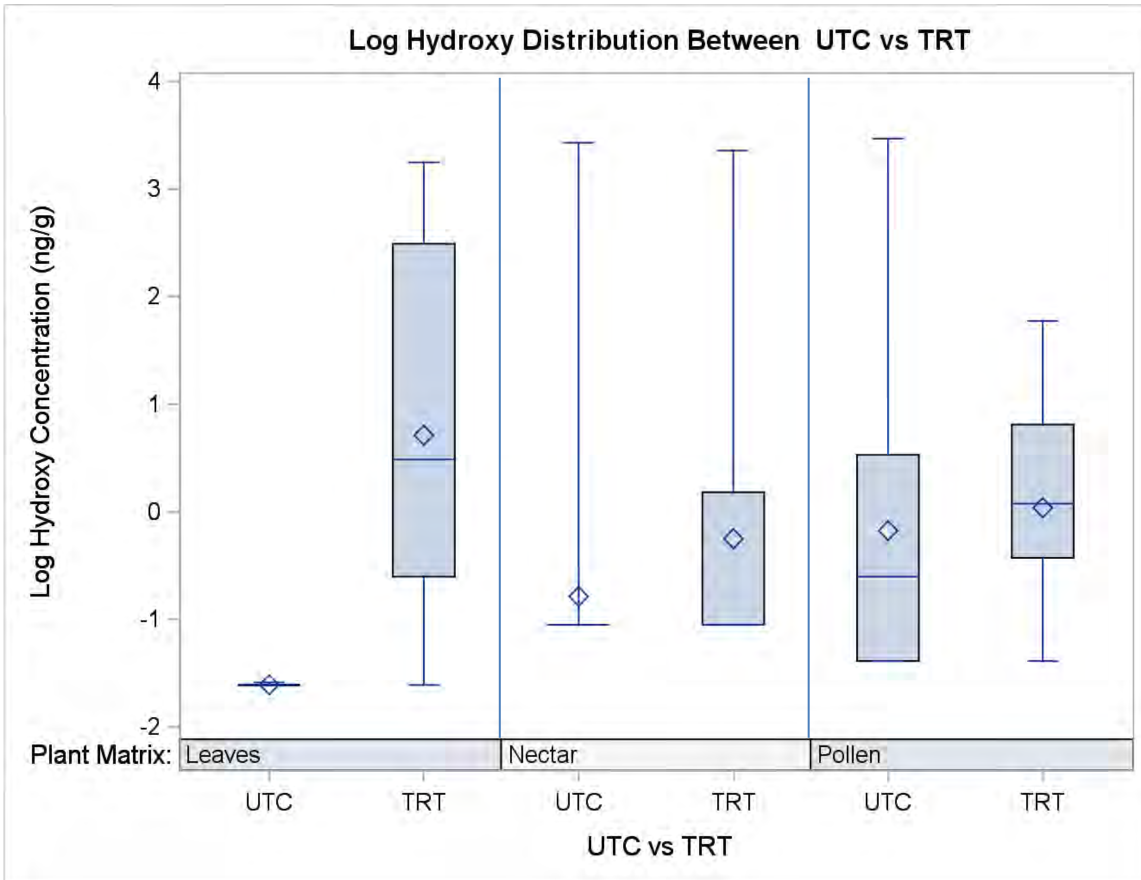


Figure 6. Treated vs Untreated Control Plants: Comparison of the relative range in imidacloprid olefin metabolite concentrations between treated and untreated control plants for residues measured in leaves, nectar, and pollen samples obtained from stone fruit trees treated with imidacloprid in the previous year. Values were transformed to natural logarithms.

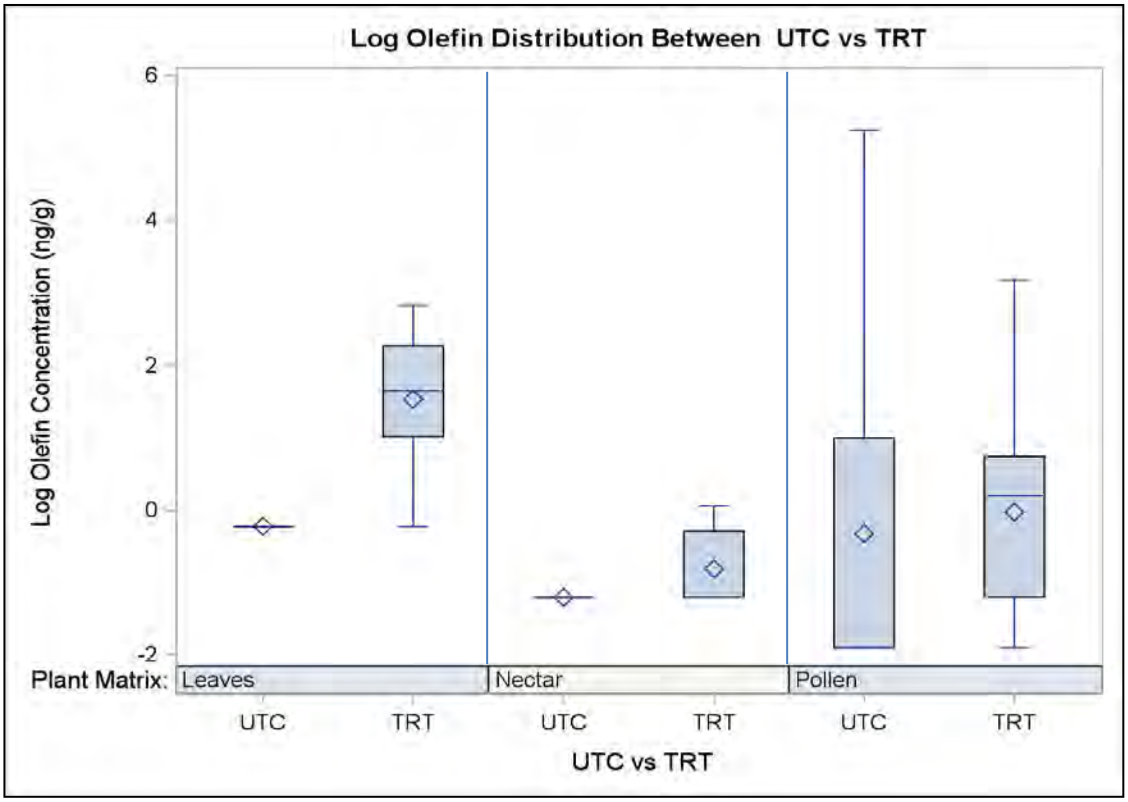


Figure 7. Soil Type: Comparison of the relative range in imidacloprid concentrations between plants grown in coarse, medium, and fine-textured soil types. Residues were measured in leaves, nectar, and pollen samples obtained from stone fruit trees treated with imidacloprid in the previous year. Values were transformed to natural logarithms.

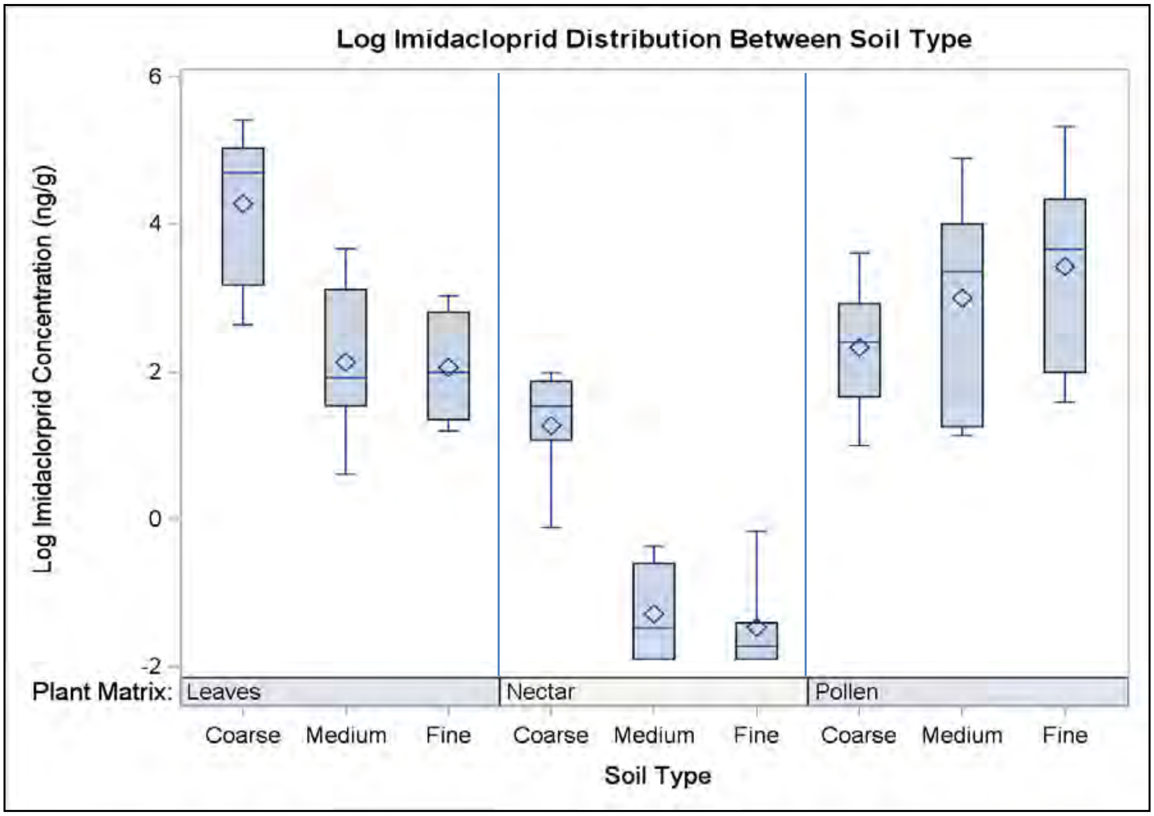


Figure 8. Soil Type: Comparison of the relative range in 5-Hydroxy imidacloprid metabolite concentrations between plants grown in coarse, medium, and fine-textured soil types. Residues were measured in leaves, nectar, and pollen samples obtained from stone fruit trees treated with imidacloprid in the previous year. Values were transformed to natural logarithms.

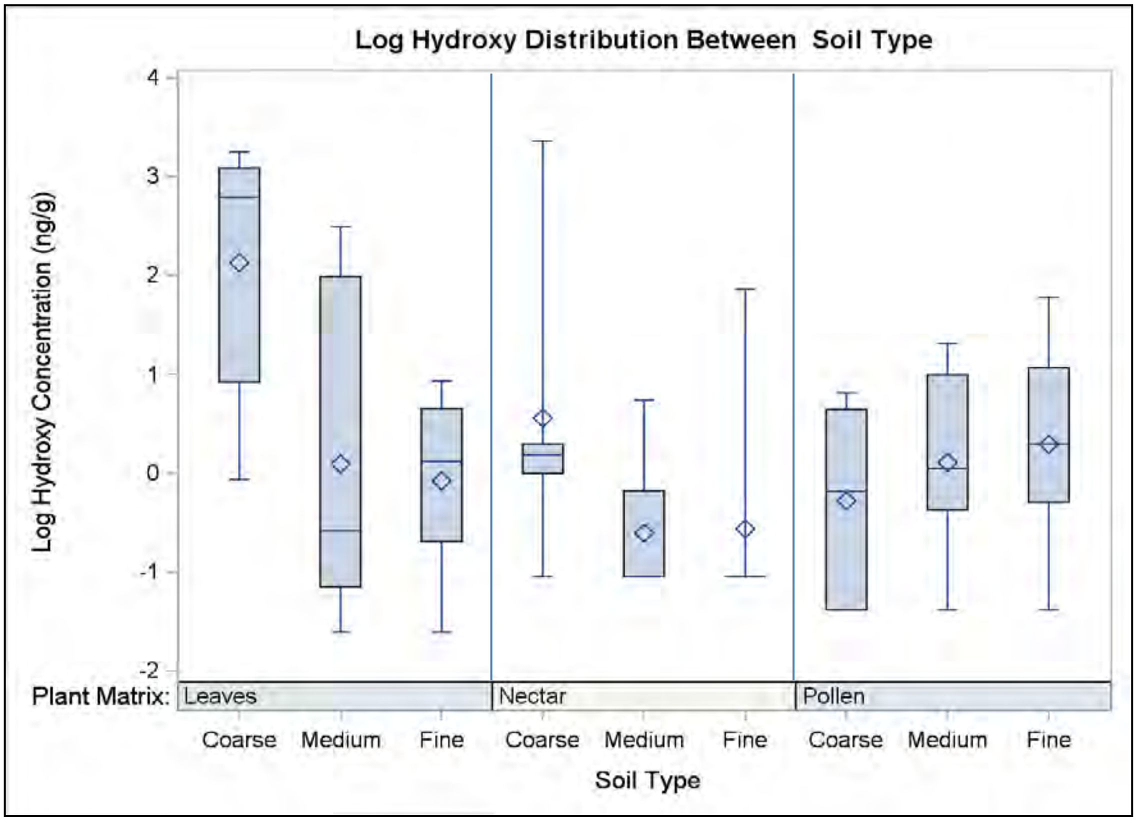
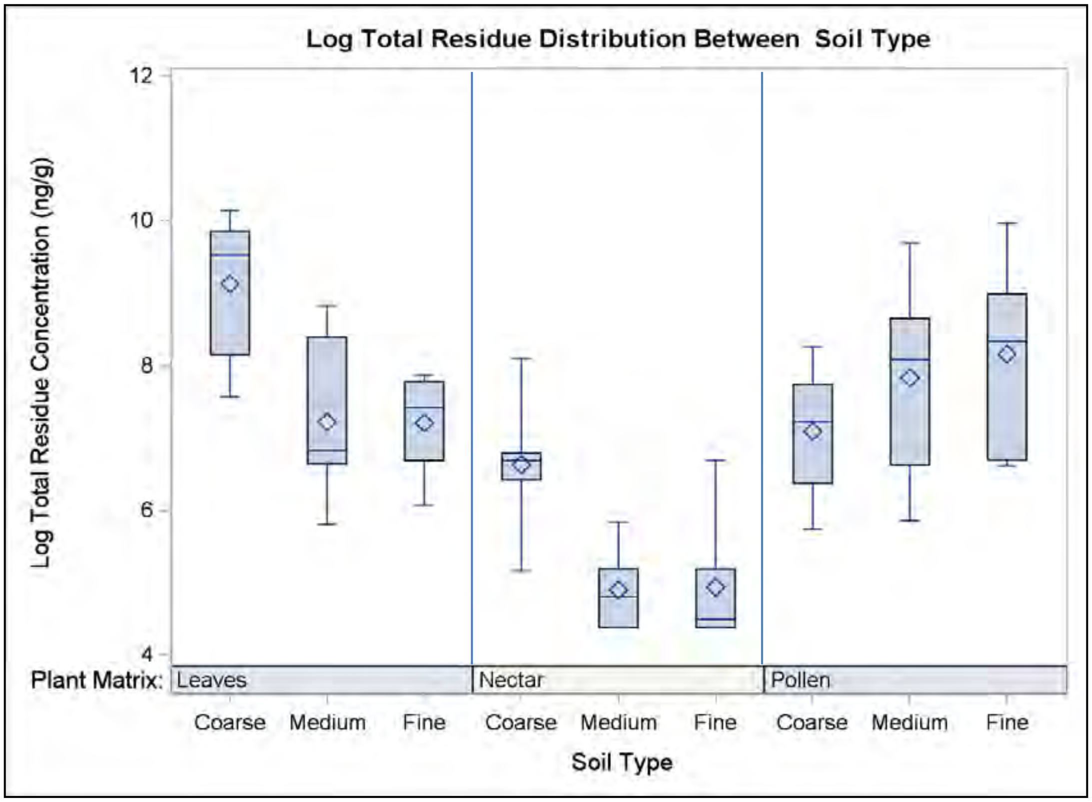


Figure 9. Soil Type: Comparison of the relative range in imidacloprid olefin metabolite concentrations between plants grown in coarse, medium, and fine-textured soil types. Residues were measured in leaves, nectar, and pollen samples obtained from stone fruit trees treated with imidacloprid in the previous year. Values were transformed to natural logarithms.



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MRID 49090502 CDPR IMI Strawberry

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1. STUDY INFORMATION

Chemical:	Imidacloprid	**PC Code**	129099
Test Material:	1) Admire Pro 2) Alias 4F	**Percent Active Ingredient:**	1) 42.8% 2) 40%
Study Type:	Non-Guideline field residue study to establish imidacloprid and metabolite levels in blossoms, anthers, pollen and leaves from strawberry in site locations that have been previously treated with imidacloprid at least once for two successive years.		
Sponsor:	Bayer CropScience 2T.W. Alexander Drive Research Triangle Park, NC USA 27709	**Experiment Start and End Date:**	September 2011 – December 13, 2012
Sponsor Study ID:	EBNTL056-04	**Study Locations:**	Seven treated fields sites within California that consist of either sand or loam soil.
Study Completion Date:	December 27, 2012		
GLP Status:	Non-GLP; protocol reviewed by CDPR. [CDPR study ID 260012, Data Volume 51950-0791, Tracking ID# 247269]		

2. REVIEWER INFORMATION

Primary Reviewers: California Department of Pesticide Regulation	**John Troiano, Ph.D.**, Research Scientist III, Environmental Monitoring Branch **Richard Bireley**, Senior Environmental Scientist (Specialist), Ecotoxicology Group, Pesticide Registration Branch **Denise Alder**, Senior Environmental Scientist (Specialist), Lead Reevaluation Coordinator, Pesticide Registration Branch **Russell Darling**, Environmental Scientist, Reevaluation Coordinator, Pesticide Registration Branch

3. EXECUTIVE SUMMARY

Blossom and leaf samples were collected from seven treated field sites in Santa Barbara County, California to determine the residues of imidacloprid and its metabolites (5-hydroxy imidacloprid and imidacloprid olefin) in blossoms, anthers, pollen and leaves collected from strawberry plants grown at locations treated with imidacloprid at least once per year for two years. The site locations consisted of either a sand soil (3 sites, “light”) or a loam soil (4 sites, “medium”), which had all previous received applications of either Alias 4F or Admire Pro at a rate of 0.5 lb. a.i./acre in the prior year as well as an application of imidacloprid in 2010.

Duplicate composite samples of strawberry blossoms for direct analysis, strawberry blossoms for anther samples, strawberry blossoms for pollen samples and strawberry leaves were collected at a BBCH ranging from 61 to 69 (flowering) at each field site.

The residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

4. STUDY VALIDITY

Guideline Followed:	Non-guideline study (protocol was reviewed by U.S. EPA/PMRA/CDPR)
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	**Acceptable**
Rationale:	N/A
Reparability:	N/A

5. MATERIALS AND METHODS

Test Material Characterization			
Test item:	Admire Pro, Alias and Unknown Formulations	**pH (24°C)**	7.8
Description:	Unknown	**Density (20°C):**	1.54 g/mL
CAS #:	138261-41-3	**Solubility:**	0.51 to 0.61 g/L

5A. STUDY DESIGN

Seven treated field sites in California representing two soil categories classified as either 'light,' generally sand soil, or 'medium,' generally loam soil, were selected based on previous application(s) of imidacloprid according to grower interviews. Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service. All test sites had received applications of imidacloprid in the preceding years (2011, 2010).

The field sampling phase of this study occurred in September, 2011, and was not conducted under GLP. The GLP experimental start date was August 22, 2012 (leaf sample homogenization), and the experimental end date was December 13, 2012 (last analysis). The field sampling phase of the study was conducted by California Agricultural Research, Inc. (CAR).

The sampling of blossoms and leaves, as well as the separation of the pollen and anthers, were performed at the test site. Homogenization of the leaves and blossoms for direct analysis from this study were performed at the Bayer Research Park (BRP) located in Stilwell, KS. Sample analysis and report preparation was performed at Bayer CropScience located in Research Triangle Park, NC. All raw data associated with this study are retained along with the protocol, protocol amendments and the final report under Notebook Number EBNTL056-04 at locations specified by Bayer CropScience.

5B. APPLICATION TIMING AND RATES

In 2011, one application of either Alias 4F or Admire Pro was made to the soil at a rate of 0.5 lb. a.i./acre in each of the seven field sites. In 2010, one soil application of an imidacloprid product (the subject products were not identified for four out of seven of the sites) was made at a rate of 0.5 lb. a.i./acre in each of the seven field sites. Method, timing or growth stage, and spray volume of these applications were not reported by the study authors. Thus, it is uncertain when the sampling was conducted in relation to the imidacloprid applications.

The test substance use pattern for imidacloprid in 2011 and 2010 for each site is described in Table 1. All site locations were in Santa Barbara county in California, NAFTA Region 10. For the applications in 2011, either Alias 4F or Admire Pro were applied at a rate of 0.5 lb ai/A.

Table 1. Study Use Pattern for 2011 Application of Imidacloprid on Strawberry.

Trial Identification	Location	Year	End Use Product [a]	Timing/Growth Stage	Spray Volume [b]	Rate (lb. a.i./A) [a]
NT031-11ZA	Santa Maria, CA Region 10	2011 2010	Alias 4F Alias 4F	Unknown Unknown	Unknown Unknown	0.5 0.5
NT032-11ZA	Santa Maria, CA Region 10	2011 2010	Alias 4F Alias 4F	Unknown Unknown	Unknown Unknown	0.5 0.5
NT033-11ZA	Santa Maria, CA Region 10	2011 2010	Alias 4F Alias 4F	Unknown Unknown	Unknown Unknown	0.5 0.5
NT035-11ZA	Santa Maria, CA Region 10	2011 2010	Alias 4F Not Available [c]	Unknown Unknown	Unknown Unknown	0.5 0.5
NT037-11ZA	Santa Maria, CA Region 10	2011 2010	Admire Pro Not Available [c]	Unknown Unknown	Unknown Unknown	0.5 0.5
NT038-11ZA	Santa Maria, CA Region 10	2011 2010	Admire Pro Not Available [c]	Unknown Unknown	Unknown Unknown	0.5 0.5
NT039-11ZA	Santa Maria, CA Region 10	2011 2010	Admire Pro Not Available [c]	Unknown Unknown	Unknown Unknown	0.5 0.5

[a] imidacloprid use was obtained from verbal communications with the growers or pest control advisors.
[b] Rates and spray volumes that were provided in the application order/recommendation. See Appendix 2 of the study report for field trial summary data.
[c] imidacloprid use was confirmed by the grower, but no documentation was available .

5C. STUDY SITE LOCATION AND CHARACTERISTICS

The trial site conditions including soil characteristics are listed in Table 2. All sites used agronomic practices typical for commercial production of strawberries. A chronological listing of significant study dates is given in Appendix 1 of the study report.

Temperatures and rainfall recorded during the field phase of the study were similar to average historical records, with no significantly unusual weather conditions that would affect the conclusions of the study. Temperature and precipitation data for each trial were obtained from the nearest National Climatic Data Center (NCDC) weather station. Application, sampling and climatic data are located in Appendix 2 of the study report.

Table 2. Trial Site Conditions for Imidacloprid on Strawberry.

Trial Identification	Trial Location	Soil Type [a]	Rainfall (in) [b]	Temperature Range (°F) [b]
NT031-11ZA	Santa Maria, CA Region 10	Batteravia Loamy Sand, Light	0.1	58-78
NT032-11ZA	Santa Maria, CA Region 10	Oceano Sand, Light	0.1	58-78
NT033-11ZA	Santa Maria, CA Region 10	Batteravia Loamy Sand, Light	0.1	58-78
NT035-11ZA	Santa Maria, CA Region 10	Sorento Sandy Loam, Medium	0.1	58-78
NT037-11ZA	Santa Maria, CA Region 10	Sorrento Loam, Medium	0.1	58-78
NT038-11ZA	Santa Maria, CA Region 10	Sorrento Loam, Medium	0.1	58-78
NT039-11ZA	Santa Maria, CA Region 10	Sorento Sandy Loam, Medium	0.1	58-78

[a] Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service.
[b] Data is for the interval of the month of sampling. Meteorological data were obtained from nearby government weather stations.

5D. Sample Collection, Handling, Processing

At each sampling interval, duplicate samples of strawberry blossoms, strawberry blossoms for anther samples, strawberry blossoms for pollen samples, and strawberry leaves were collected from the treated sites during flowering.

Strawberry blossoms and leaves were placed into coolers with dry ice directly after sampling. Strawberry blossoms for anther samples were processed by removing the anthers from the blossom with tweezers/small scissors and transferred to a sampling jar and frozen. Strawberry blossoms for pollen samples were allowed to dry overnight, and the pollen was removed by vacuum and frozen.

Untreated (control) blossom and leaf samples used for validation of the analytical method and for transit stability samples were obtained from a nearby farm that was believed to have no recent imidacloprid application.

All samples were placed in frozen storage at CAR Samples were shipped to BRP frozen via Agricultural Chemicals Development Services (ACDS) and were placed in frozen storage upon receipt and remained frozen throughout the storage period at BRP.

The anther and pollen samples, along with the transit stability samples, were shipped frozen to Bayer CropScience, Research Triangle Park (RTP), NC, via ACDS. Homogenization of the leaf and blossom samples was conducted at BRP and shipped frozen via FedEx to RTP. During storage at RTP, the anther and pollen samples, along with the transit stability samples, reached room temperature for approximately three days due to freezer failure. Transit stability samples accompanied the field samples during all sample handling and preparation steps to ensure the validity of the sampling practices.

Sample Storage.

Upon arrival at BRP, all samples were immediately transferred to frozen storage. The leaf samples were homogenized with dry ice using a Robot Coupe chopper (Jackson, MS). The leaf samples were returned to frozen storage immediately following homogenization. Samples were transferred to Bayer CropScience, Research Triangle Park, NC, for analysis. Samples remained frozen at all times except during preparation for analysis A summary of collection, shipment, and homogenization dates for the trials is given in Appendix 1 of the study report.

Additionally, freezer storage stability studies have indicated the imidacloprid residues are stable (<30% decomposition) for 24 months (728 to 769 days) of freezer storage in the following representative crops: an oilseed (cottonseed), a non-oily grain (wheat), a leafy vegetable (lettuce), a root crop (potato), a tree fruit (apple), and a fruiting vegetable (tomato). [7-11]

Demonstrated freezer stability in the transit stability samples from this study, as well as stability demonstrated in the above crops is representative of the freezer stability of imidacloprid residues to be expected in the blossoms, anthers, pollen, and leaves collected in this study.

5E. Analytical Methods

The analytical method used in this study measured the residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin in strawberry blossoms, anthers, pollen and leaves.[4]

All neat analytical reference standards were stored frozen when not in use and all solutions were stored refrigerated or frozen. Analytical standard solutions used in this study were not stored for longer than approximately 2 months and have been shown to be stable for this period of time. [5] All reference standard solutions were prepared in parent imidacloprid equivalents and corrected for purity during initial preparation.

Samples of anthers, blossoms, leaves, and pollen were analyzed for residues of imidacloprid, imidacloprid olefin, and imidacloprid 5-hydroxy. For anthers, blossoms, and leaves, a sample was extracted by blending samples with 2:1 acetonitrile/water (ACN/H_20). The mixture was amended with $MgS0_4$, NaCl, and isotopically labeled internal standards, mixed well, and centrifuged. An aliquot of the supernatant was transferred to a 15-mL centrifuge tube containing $MgS0_4$ and Bondesil-PSA. The contents of the tube were mixed well and centrifuged. About 1.2 ml of the supernatant was percolated through a C18 SPE cartridge (50 mg Bond Elut) into a 20x150mm culture tube. The eluate was evaporated to dryness and the residue was reconstituted with 1:9 methanol/water ($MeOH/H_20$) for analysis by high performance liquid chromatography/tandem mass spectrometry (LC/MS/MS).

For pollen a sample was extracted by shaking with water for 10 minutes, adding ACN and shaking for an additional 55 minutes. The mixture was amended with isotopically labeled internals standards, mixed well and centrifuged. The supernatant was drawn off, evaporated to an aqueous remainder, amended with water and applied to a Chern Elute cartridge (3-ml). The analytes were extracted from the cartridge with 3 x 5 ml of hexane/ethyl acetate (1:1 v/v). The eluate was evaporated to dryness and the residue was reconstituted with 1:9 methanol/water ($MeOH/H_20$) for analysis by LC/MS/MS.

Quantitation of each analyte was based on the daughter ion transitions of the analytes and the respective internal standard analogs. The responses of LC/MS/MS system to each analyte and its internal standard were measured in samples and in standards, and a relative response was calculated as the ratio of the analyte and the stable isotopically labeled internal standard responses. The relative response of the analyte in each sample was compared to the relative response of the analyte in the standards (Appendix 3 of the study report).

The relative responses of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin in solvent were measured over the range of 3 ppb to 3000 ppb for each analyte. The coefficients of determination (r^2) were calculated using linear regression analysis (Microsoft Excel 2010; Appendix 3 of the study report).

The total imidacloprid residue is the sum of the individual measured residue values of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin in parent equivalents. For the purpose of calculating total imidacloprid residues where individual analyte residue values were less than the limit of detection (LOD), the residues were assigned a finite value of ½ the value of the respective LOD. This value is only an estimate of potential residue in a sample, not a measured value.

Blossoms and leaves were collected from the control test site and were used for validation, LOD determination, and concurrent recoveries. Commercially obtained bee pollen was also used for validation and recovery. Because only small, untreated control samples of anthers and pollen were generated in the field phase of this study, surrogate anther and pollen controls were used for validation, LOD determination, and concurrent recoveries. Blossom validation LOD data was used for anthers while the control strawberry blossoms were used for anther concurrent recoveries. Commercial bee pollen was obtained from a local health food store and stored at room temperature. The bee pollen was used without any preparation. The commercial pollen was used for pollen validation, LOD determination, and for concurrent recoveries.

The method for determining imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin residues in/on strawberry blossoms, anthers, pollen and leaves was validated by measuring the recovery of these analytes from control matrices fortified at their respective LOQs.

Additional recoveries at higher fortification levels validated the method for the highest residues observed in individual matrices. Concurrent recoveries of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin were measured with each set of samples to verify method performance.

5F. Quality Assurance Results

The responses of the LC/MS/MS system to imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin were linear in solvent over the range of 3 ppb to 3000 ppb for each analyte. The coefficients of determination were >0.99.

All recoveries were corrected for any interferences in corresponding controls. The overall mean values of the recoveries for each matrix were within the acceptable range of 70% to 120%, and the standard deviation values were below 20%, except for 5-hydroxy imidacloprid recovery from blossoms which had a percent recovery standard deviation of 24%.

The limit of quantitation (LOQ) is defined as the lowest fortification level of an analyte at which acceptable recovery has been achieved. The LOQ for total residue is the highest of the LOQ values assigned to the individual analytes for a particular matrix.

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be determined to be statistically different from a blank. The LODs were determined from method validation data obtained from control samples fortified at the respective analyte LOQs. The LODs were calculated by multiplying the standard deviation of recovery measurements at the LOQ by $t_{0.99}$ [where $t_{0.99}$ is the one-tailed t-statistic at the 99% confidence level for the number of replicates (n)] [6]. The LOD for the total imidacloprid residue in each matrix is the highest LOD value of any individual analyte for that particular matrix.

The LOQs and LODs are summarized in the table below.

Summary of LOQs and LODs

Matrix	Analyte	LOQ (ppb, parent equivalents)	LOD (ppb, parent equivalents)
Strawberry Blossom	Imidacloprid	5.0	0.87
	5-hydroxy Imidacloprid	5.0	3.9
	Imidacloprid olefin	5.0	1.3
	Total Imidacloprid	5.0	3.9
Strawberry Anthers	Imidacloprid	5.0	0.87
	5-hydroxy Imidacloprid	5.0	3.9
	Imidacloprid olefin	5.0	1.3
	Total Imidacloprid	5.0	3.9
Strawberry Pollen	Imidacloprid	10	1.1
	5-hydroxy Imidacloprid	10	2.6
	Imidacloprid olefin	10	2.2
	Total Imidacloprid	10	2.6
Strawberry Leaves	Imidacloprid	10	2.2
	5-hydroxy Imidacloprid	10	3.0
	Imidacloprid olefin	10	4.6
	Total Imidacloprid	10	4.6

Any individual analyte or total residue measured to be less than the Limit of Quantitation (LOQ) was reported as <5 ppb for blossoms and anthers and <10 ppb for leaves and pollen. In Appendix 3 of the study report, any residue value that was below the LOD was reported as less than the LOD and measured residue values between the LOQ and LOD are also provided.

6. RESULTS:

Summary statistics for concentration of total imidacloprid in strawberry blossoms, anthers, pollen and leaves are contained in Table 3. The individual analyte residues were summed to give a total imidacloprid residue. The study authors stated that when individual analyte residue values were less than the LOD, the residues were assigned a finite value of half the value of the respective LOD.

Table 3. Magnitude of total imidacloprid residues in strawberry blossoms, anthers, pollen, and leaves.

Commodity	Soil Texture Category[a]	Total Imidacloprid Residue Levels (ppb)[b]						
		N	Min	Max	Highest Avg. Site Residue	Median	Mean	Standard Deviation
Strawberry Blossoms	Coarse	6	210	530	500	380	360	130
Strawberry Anthers	Coarse	6	81	300	250	200	180	82
Strawberry Pollen	Coarse	6	78	320	280	210	190	95
Strawberry Leaves	Coarse	6	1700	2800	2400	2100	2200	410
Strawberry Blossoms	Medium	8	<5.0	31	18	6.4	9.4	9.1
Strawberry Anthers	Medium	8	11	33	23	13	18	7.9
Strawberry Pollen	Medium	8	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0
Strawberry Leaves	Medium	8	<10.0	18	17	11	11	<10.0

[a] Classification of the soils was obtained from the Soil Survey Geographic (SSURGO) Database provided by the Natural Resources Conservation Service and converted to soil texture categories.
[b] Abbreviations used are as follows: Min is the lowest treated residue value; Max is the highest treated residue value; Median is the geometric median of the treated residue values; Mean is the mathematical average of the treated residue values; Standard Deviation is the standard deviation for a small population of "N" samples.

The total imidacloprid residue data for strawberry blossoms, anthers, pollen and leaves are provided in Table 4.

Table 4. Imidacloprid Residue Data from Strawberry.

MRID 49090502 CDPR IMI Strawberry

Trial Identification	Location (City, State, Region)	Plot Name	Commodity	Rate, lb a.i./A	Residue Sample Collection DAT (Days after 2010	Imidacloprid Olefin Residue (ppb)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
Blossoms						**Residue (ppb) [a]**			
NT031-1ZA	Santa Maria, CA, Region 10	Sand 1	Blossom	0.5	NA	10 12	46 33	470 420	530 470 Average 500
NT032-1ZA	Santa Maria, CA, Region 10	Sand 2	Blossom	0.5	NA	8.7 8.2	31 27	340 350	380 380 Average 380
NT033-1ZA	Santa Maria, CA, Region 10	Sand 3	Blossom	0.5	NA	8.5 10	38 40	160 170	210 220 Average 220
NT035-1ZA	Santa Maria, CA, Region 10	Loam 1	Blossom	0.5	NA	<5.0 <5.0	7.0 <5.0	22 <5.0	31 <5.0 Average 18
NT037-1ZA	Santa Maria, CA, Region 10	Loam 3	Blossom	0.5	NA	<5.0 <5.0	7.4 <5.0	<5.0 <5.0	11 6.0 Average 8.6
NT038-1ZA	Santa Maria, CA, Region 10	Loam 4	Blossom	0.5	NA	<5.0 <5.0	5.1 6.8	<5.0 <5.0	6.7 8.8 Average 7.7
NT039-1ZA	Santa Maria, CA, Region 10	Loam 5	Blossom	0.5	NA	<5.0 <5.0	<5.0 <5.0	<5.0 <5.0	<5.0 <5.0 Average <5.0
Anthers						**Residue (ppb) [a]**			
NT031-1ZA	Santa Maria, CA, Region 10	Sand 1	Anthers	0.5	NA	19 9.5	43 26	240 170	300 210 Average 250
NT032-1ZA	Santa Maria, CA, Region 10	Sand 2	Anthers	0.5	NA	7.5 9.5	24 35	160 180	190 220 Average 210
NT033-1ZA	Santa Maria, CA, Region 10	Sand 3	Anthers	0.5	NA	5.5 7.6	26 20	64 54	96 81 Average 89

MRID 49090502 CDPR IMI Strawberry

Trial Identification	Location (City, State, Region)	Plot Name	Commodity	Rate, lb a.i./A	Residue Sample Collection DAT (Days after 2010	Imidacloprid Olefin Residue (ppb)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
NT035-1ZA	Santa Maria, CA, Region 10	Loam 1	Anthers	0.5	NA	<5.0 <5.0	13 7.6	38 <5.0	19 11 Average 15
NT037-1ZA	Santa Maria, CA, Region 10	Loam 3	Anthers	0.5	NA	<5.0 <5.0	13 22	<5.0 <5.0	14 25 Average 19
NT038-1ZA	Santa Maria, CA, Region 10	Loam 4	Anthers	0.5	NA	<5.0 8.8	8.7 23	<5.0 <5.0	13 33 Average 23
NT039-1ZA	Santa Maria, CA, Region 10	Loam 5	Anthers	0.5	NA	<5.0 <5.0	11 11	<5.0 <5.0	12 13 Average 13
Pollen						**Residue (ppb) [a]**			
NT031-1ZA	Santa Maria, CA, Region 10	Sand 1	Pollen	0.5	NA	17 14	42 33	260 200	320 250 Average 280
NT032-1ZA	Santa Maria, CA, Region 10	Sand 2	Pollen	0.5	NA	14 10	32 25	190 150	240 190 Average 210
NT033-1ZA	Santa Maria, CA, Region 10	Sand 3	Pollen	0.5	NA	<10 <10	21 18	57 54	87 78 Average 83
NT035-1ZA	Santa Maria, CA, Region 10	Loam 1	Pollen	0.5	NA	<10 <10	<10 <10	<10 <10	<10 <10 Average <10
NT037-1ZA	Santa Maria, CA, Region 10	Loam 3	Pollen	0.5	NA	<10 <10	<10 <10	<10 <10	<10 <10 Average <10
NT038-1ZA	Santa Maria, CA, Region 10	Loam 4	Pollen	0.5	NA	<10 <10	<10 <10	<10 <10	<10 <10 Average <10
Leaves						**Residue (ppb) [a]**			
NT031-1ZA	Santa Maria, CA, Region 10	Sand 1	Leaves	0.5	NA	14 17	50 64	1600 2200	1700 2300 Average 2000

Trial Identification	Location (City, State, Region)	Plot Name	Commodity	Rate, lb a.i./A	Residue Sample Collection DAT (Days after 2010	Imidacloprid Olefin Residue (ppb)	5-Hydroxy Imidacloprid Residue (ppb)	Imidacloprid Residue (ppb)	Total Imidacloprid Residue (ppb)
NT032-1ZA	Santa Maria, CA, Region 10	Sand 2	Leaves	0.5	NA	27 18	53 48	2300 1900	2400 1900 Average 2200
NT033-1ZA	Santa Maria, CA, Region 10	Sand 3	Leaves	0.5	NA	42 25	100 70	2700 1800	2800 1900 Average 2400
NT035-1ZA	Santa Maria, CA, Region 10	Loam 1	Leaves	0.5	NA	<10 <10	<10 <10	14 <10	18 16 Average 17
NT037-1ZA	Santa Maria, CA, Region 10	Loam 3	Leaves	0.5	NA	<10 <10	<10 <10	<10 <10	<10 10 Average <10
NT038-1ZA	Santa Maria, CA, Region 10	Loam 4	Leaves	0.5	NA	<10 <10	<10 <10	<10 <10	12 13 Average 13
NT039-1ZA	Santa Maria, CA, Region 10	Loam 5	Leaves	0.5	NA	<10 <10	<10 <10	<10 <10	<10 <10 Average <10

[a] See Appendix 3 of the study report for analytical data summaries and refined data used to prepare this summary. Total
imidacloprid is the sum of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin .

In coarse-textured soils, mean residues of total imidacloprid were 360, 180, 190, and 2200 ppb in blossoms, anthers, pollen, and leaves, respectively and, maximum residues were 530, 300, 320, and 2800 ppb in blossoms, anthers, pollen, and leaves respectively. In medium-textured soils, mean residues of total imidacloprid were 9.4, 18, <0.010 (LOQ), and 11 ppb in blossoms, anthers, pollen, and leaves, respectively, and maximum residues were 31, 33, <0.010 (LOQ), and 18 ppb in blossoms, anthers, pollen, and leaves respectively. Thus, in all matrices (blossoms, anthers, pollen, and leaves) mean concentrations of total imidacloprid were approximately ten times higher in strawberries grown in coarse-textured soil than in medium-textured soil.

7. STUDY VALIDITY/CLASSIFICATION AND STUDY LIMITATIONS

Classification/Utility for Bee Risk Assessment. The study is **ACCEPTABLE** for considering the level of residues of imidacloprid in pollen after soil application in strawberry. The level of imidacloprid residues appeared to be much higher in strawberries grown in coarse-textured sandy soil than in medium-textured loamy soil. In sandy soil, soil application at 0.5 lb ai/A of imidacloprid for two consecutive years resulted in maximum total imidacloprid residues of 320 ppb (mean ± SEM, 190 ± 95), 300 ppb (180±82), 530 ppb (360±130) and 2800 ppb (2200±410) respectively in strawberry pollen, anthers, blossoms and leaves. Residues in strawberry nectar, another bee-relevant matrix, were not measured. Another concern is that the date of the last soil application was not provided, thus the duration from the soil application to the collection of the samples could not be estimated.

Temporal Variability in Residues. This study was not designed to measure temporal variability in residues. All samples were taken in 2011, within five days of each other. Thus, this study was designed to analyze imidacloprid residues at a single time point. In addition, time of sampling in relation to the imidacloprid applications, is unknown.

Spatial Variability in Residues. All seven sites for this strawberry study were located in Santa Maria, CA. As expected, reported weather conditions (temperature and precipitation) were the same across all seven sites. As a result of the close proximity of trial sites, this study provides very limited information on how differences in environmental conditions across different areas of the US may affect accumulation of total imidacloprid in bee-relevant matrices. However, because there are different soil types represented (3 sand soil sites and 4 loam soil sites), this study may offer insight to how soil type may affect accumulation of total imidacloprid in bee-relevant matrices. In all matrices (blossoms, anthers, pollen, and leaves) mean concentrations of total imidacloprid were higher in strawberries grown in sandy soil than in loam soil.

Pesticide Carryover. This study was not designed to measure pesticide carryover. All samples were taken in 2011, within five days of each other. This this study is designed to analyze imidacloprid residues at a single time point.

8. CONCLUSION

Samples were obtained from 7 field sites where soil in 3 of the sites was classified as coarse-textured and the other 4 classified as medium-textured. The cultivar of strawberry was not reported that was grown in each field. Parent imidacloprid and 2 breakdown products, the 5-Hdroxy and Olefin residues, were measured. Concentrations were measured for whole blossoms, anthers and pollen separated from the blossom, and leaves. Chemical analysis was conducted on samples stored for more than 1 year. Samples were purported to store well over this period but storage data were only referenced and not provided.

In coarse-textured soils, mean residues of total imidacloprid were 360, 180, 190, and 2200 ppb in blossoms, anthers, pollen, and leaves, respectively and, maximum residues were 530, 300, 320, and 2800 ppb in blossoms, anthers, pollen, and leaves respectively. In medium-textured soils, mean residues of total imidacloprid were 9.4, 18, <0.010 (LOQ), and 11 ppb in blossoms, anthers, pollen, and leaves, respectively, and maximum residues were 31, 33, <0.010 (LOQ), and 18 ppb in blossoms, anthers, pollen, and leaves respectively. Thus, in all matrices (blossoms, anthers, pollen, and leaves) mean concentrations of total imidacloprid were approximately ten times higher in strawberries grown in coarse-textured soil than in medium-textured soil.

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MRID 49665202 CDPR IMI Soil and Foliar Cotton Study

Reference
Fischer, D.R., and Jerkins, E. (2015) Determination of the Residues of Imidacloprid, 5-Hydroxy Imidacloprid, and Imidacloprid Olefin in Bee Relevant Matrices Collected from Cotton During Two Successive Years: Final Report. Project Number: EBNTN011. Unpublished study prepared by Bayer CropScience 632pg. MRID 49665202, CDPR study ID 285681, Data Volume 51950-0900, Tracking ID# 270950

1. STUDY INFORMATION

Chemical:	Imidacloprid	**PC Code**	129099
Test Material:	Admire Pro Systemic Protectant (SC)	**Purity:**	43.50% a.i. w.w.
Study Type:	Non-Guideline field residue study on cotton to establish imidacloprid and metabolite levels in pollen, nectar (floral and extrafloral), and leaves following four applications in each of two successive years and three different soil types (fine, medium, and coarse).		
Sponsor:	Bayer CropScience 2T.W. Alexander Drive Research Triangle Park, NC USA 27709	**Experiment Start and End Date:**	April 12, 2013 - April 9, 2015
Sponsor Study ID:	EBNTN011	**Study Locations:**	9 Field Trials in the cities of: **Davis, Fresno, Kerman, Sanger, Wheatland, and Yuba City, California**
Study Completion Date:	June 19, 2015		
Date of Amendment:	April 13, 2016		
GLP Status:	GLP-compliant; protocol reviewed by EPA, PMRA, CDPR, CDPR study ID 266879, Data Volume 51950-0808, Tracking ID# 254696.]		

2. REVIEWER INFORMATION

Primary Reviewers: California Department of Pesticide Regulation	**John Troiano, Ph.D.**, Research Scientist III, Environmental Monitoring Branch **Richard Bireley**, Senior Environmental Scientist (Specialist), Ecotoxicology Group, Pesticide Registration Branch **Denise Alder**, Senior Environmental Scientist (Specialist), Lead Reevaluation Coordinator, Pesticide Registration Branch **Russell Darling**, Environmental Scientist, Reevaluation Coordinator, Pesticide Registration Branch
Secondary Reviewer:	TBD

3. EXECUTIVE SUMMARY

A total of nine (9) field trials were conducted to measure the magnitude of imidacloprid residues in bee-relevant cotton pollen, nectar, and in/on leaves following four applications of Admire Pro Systemic Protectant, EPA Reg. No. 264-827 in each of two successive years. Admire Pro Systemic Protectant is a suspension concentrate formulation containing 550 g/L imidacloprid. Three (3) trials have one year of data and six (6) trials have two years of data.

Treated plots received one soil (in-furrow) spray application of Admire Pro at planting (BBCH 00: dry seed) followed by 3 equivalent Admire Pro foliar spray applications per planting season. Individual soil application rates ranged from 0.37 to 0.38 kg imidacloprid/ha per application (0.33 to 0.34 lb/A), and spray volumes were 13 to 15 gal/A. The interval between the soil and first foliar application was 75 to 99 days. Individual foliar application rates ranged from 0.063 to 0.067 kg imidacloprid/ha per application (0.056 to 0.060 lb/A). Foliar and soil applications were made using ground-based equipment. Also, the adjuvant Dyne-Amic (0.25% v/v) was used in all foliar applications. Moreover, all foliar applications were made between BBCH growth stages 61 and 72 (BBCH 61: beginning of flowering; BBCH 72: about 20% of bolls have attained their final size). The interval between foliar applications was 6 to 7 days. The foliar spray volumes ranged from 14 to 20 gal/A. Total seasonal application rates ranged from 0.56 to 0.57 kg imidacloprid/ha (0.50 to 0.51 lb/A).

Cotton leaf and flower samples were collected at three sampling intervals: 4 to 5 days prior to the first foliar application (70 to 95 days after the soil application), 4 to 5 days after the last foliar application, and 12 to 14 days after the last foliar application. At each sampling interval, duplicate composite samples (two separate runs through the plot) of cotton flowers and cotton leaves were collected from the treated plots when the plants were at bloom, BBCH 61 (begin flowering, early bloom) to BBCH 73 (about 30% of bolls have attained their final size). Single composite samples of cotton leaves and flowers were collected from the control plot of each trial on the same days that samples were collected from the treated plots.

After their collection, cotton flowers were hand-processed at the field site to obtain the bee-relevant samples of cotton pollen, floral nectar, and extra floral nectar. The processed flowers were discarded. The residues of Admire Pro Systemic Protectant (imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin) were quantitated by high performance liquid chromatography/triple stage quadrupole mass spectrometry (LC/MS/MS) and LC/high resolution mass spectrometry (LC/HRMS) using stable isotopically labeled internal standards. The individual analyte residues were summed to give a total imidacloprid residue.

Storage stability studies indicate that the imidacloprid residues would have been stable during frozen storage for at least 1,080 days (36 months) in cotton leaves prior to analysis. Transit spikes showed that imidacloprid residues were stable in pollen and nectar for the duration of the study. The maximum storage period of frozen samples in this study for Admire Pro Systemic Protectant was 569 days for cotton leaves, 226 days for cotton pollen, and 211 days for cotton floral and extrafloral nectar.

4. STUDY VALIDITY

Guideline Followed:	Non-guideline study (protocol was reviewed by EPA/PMRA/CDPR
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	**Quantitative**
Rationale:	The data from the study will provide a basis for developing a quantitative assessment of exposure levels to bees that can be used in a risk assessment scenario.
Reparability:	N/A

5. MATERIALS AND METHODS

Test Material Characterization			
Test item:	Admire Pro Systemic Protectant (Imidacloprid) 550 g a.i./L SC	**CAS #:**	138261-41-3
Description:	Suspension concentrate (SC)	**Purity:**	43.50% w/w
Lot No./Batch No.	Batch No. NK41CX0578	**Density:**	1.41 – 1.54 g/mL
Material Source:	Bayer CropScience	**Cert. #**	213CJ2446
Material Receipt Date:	Not Reported	**Analysis Date:**	12/03/2012
Expiration Date:	12/03/2014	**Solubility:**	0.51 to 0.61 g/L
Storage of Test Material:	Ambient (35-86ºF) except trials NT001-13ZB, NT002-13ZB, and NT005-13ZB when the temperature briefly reached 94ºF.	**Sample Storage:**	-47ºC to -11ºC -116ºF to -52ºF

5A. Study Design

This study requirement was part of the imidacloprid special review at the California Department of Pesticide Regulation (CDPR). The study design and protocol were approved by the CDPR prior to study initiation. This study was conducted using GLP standards and following an approved protocol. The study initiation date was April 12, 2013. The experimental start date was May 10, 2013 (first application), and the experimental end date was January 26, 2016 (last analysis).

Two plots were included in each trial, to be planted and treated in each of two consecutive years. Only the first year of trial NT002-13ZB could be completed and reported because the plot location was no longer available. Trials NT001-13ZB and NT005-13ZB were restarted in 2014 and ran for a two year time period.

5B. Application Timing and Rates

The full study report provides (1) Chronological listing of significant study dates (**Appendix 1**); (2) Field report summaries for each trial detailing the actual amount of test substance applied, plot sizes, dates of treatment, dates of sample collection, maintenance chemicals, climatic data, and irrigation data (**Appendix 2**); and (3) Quality assurance statements for each trial (**Appendix 3**). Information on application timing is provided in **Table 1.** Soil and meteorological characteristics of the study sites are provided in **Table 2** and **Table 3**. **Table 4** provides the sampling dates and cotton developmental stages.

BBCH or Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie, identifies the specific phenological development stages of cotton. Plot TRTD received one soil (in-furrow) spray application of Admire Pro at planting (BBCH 00: dry seed) followed by 3 equivalent Admire Pro foliar spray applications per planting season. Individual soil application rates ranged from 0.35 to 0.38 kg imidacloprid/ha per application (0.32 to 0.34 lb/A), and spray volumes were 13 to 15 gal/A. The interval between the soil and first foliar application was 75 to 99 days. Individual foliar application rates ranged from 0.063 to 0.067 kg imidacloprid/ha/application (0.056 to 0.060 lb/A). All foliar applications were made between BBCH growth stages 61 and 72 (BBCH 61: beginning of flowering; BBCH 72: about 20% of bolls have attained their final size). The interval between foliar applications was 6 to 8 days. The foliar spray volumes ranged from 14 to 20 gal/A. Total seasonal application rates ranged from 0.55 to 0.57 kg imidacloprid/ha (0.49 to 0.51 lb/A).

Temperature and precipitation data were recorded for each trial and are summarized in **Appendix 2** of the study report EBNTN011. Temperatures recorded during the field phase of the study were similar to average historical records. Recorded rainfall was lower than historical records across all trials, but this would not affect the conclusions of the study. Irrigation supplemented normal rainfall as needed in some trials. CDPR requested that the trial sites be distributed as three coarse, three medium, and three fine textured soils [per USDA's Soil Survey Geographic database (SSURGO) mapping units]; however, due to various issues, the final trials contained four coarse, three medium and two fine textured sites (in some cases, the plots were shifted a short distance within the designated field, but this placed the plot in a coarse texture mapping unit instead of the intended medium texture area). The two-year study includes one year of residue data from each of two fine texture fields, one to two years of data from the three medium texture fields, and one to two years of data from four coarse texture fields. See **Table 3** for more site specific soil information.

Table 1. Summary of foliar and soil application rates and timing*.

Trial Identification	Location (City, State, NAFTA Region)	Formulation	Plot Name	Year	Application[a]						
					Method	Timing/Growth Stage (BBCH)	Actual Spray Volume, GPA (L/ha)	Rate, lb a.i./A (kg a.i./ha)	Retreatment Interval (days)	Total Rate, lb a.i./A (kg a.i./ha)	Adjuvant
NT001-13ZB	Yuba City, CA Region 10	Admire Pro	TRTD	2014	Soil Spray	00	14 (133)	0.328 (0.368)	NA[b]	0.50 (0.56)	NA
					Foliar Spray	61	16 (150)	0.058 (0.065)	93		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	16 (147)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	16 (148)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
				2015	Soil Spray	00	14 (134)	0.316 (0.354)	308	0.49 (0.55)	NA
					Foliar Spray	61	20 (188)	0.057 (0.064)	88		Dyne-Amic 0.25 % v/v
					Foliar Spray	61	20 (188)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	20 (187)	0.057 (0.064)	8		Dyne-Amic 0.25 % v/v
NT002-13ZB[c]	Wheatland, CA Region 10	Admire Pro	TRTD	2014	Soil Spray	00	15 (142)	0.331 (0.371)	NA	0.50 (0.56)	NA
					Foliar Spray	65	15 (142)	0.057 (0.064)	92		Dyne-Amic 0.25 % v/v
					Foliar Spray	69	15 (144)	0.058 (0.065)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	72	15 (140)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v

Trial Identification	Location (City, State, NAFTA Region)	Formulation	Plot Name	Year	Application[a]						
					Method	Timing/Growth Stage (BBCH)	Actual Spray Volume, GPA (L/ha)	Rate, lb a.i./A (kg a.i./ha)	Retreatment Interval (days)	Total Rate, lb a.i./A (kg a.i./ha)	Adjuvant
NT003-13ZA	Fresno, CA Region 10	Admire Pro	TRTD	2013	Soil Spray	00	14 (132)	0.330 (0.370)	NA	0.51 (0.57)	NA
					Foliar Spray	65	15 (142)	0.058 (0.066)	81		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (140)	0.058 (0.065)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (142)	0.058 (0.066)	7		Dyne-Amic 0.25 % v/v
				2014	Soil Spray	00	14 (132)	0.330 (0.370)	254	0.51 (0.57)	NA
					Foliar Spray	61	15 (141)	0.058 (0.065)	77		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (142)	0.059 (0.066)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	16 (145)	0.060 (0.067)	7		Dyne-Amic 0.25 % v/v
NT004-13ZA	Davis, CA Region 10	Admire Pro	TRTD	2013	Soil Spray	00	14 (127)	0.332 (0.372)	NA	0.51 (0.57)	NA
					Foliar Spray	61	15 (142)	0.058 (0.065)	91		Dyne-Amic 0.25 % v/v
					Foliar Spray	61	15 (140)	0.058 (0.065)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	61	14 (136)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
				2014	Soil Spray	00	15 (139)	0.327 (0.367)	253	0.50 (0.56)	NA
					Foliar Spray	61	14 (135)	0.057 (0.064)	99		Dyne-Amic 0.25 % v/v
					Foliar Spray	61	17 (156)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	61	16 (148)	0.057 (0.063)	7		Dyne-Amic 0.25 % v/v
NT005-13ZB	Yuba City, CA Region 10	Admire Pro	TRTD	2014	Soil Spray	00	15 (143)	0.337 (0.377)	NA	0.51 (0.57)	NA
					Foliar Spray	61	15 (142)	0.056 (0.063)	91		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (144)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (142)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
				2015	Soil Spray	00	15 (141)	0.333 (0.374	307	0.50 (0.56)	NA
					Foliar Spray	61	20 (187)	0.057 (0.064)	91		Dyne-Amic 0.25 % v/v

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Trial Identification	Location (City, State, NAFTA Region)	Formulation	Plot Name	Year	Application[a]						
					Method	Timing/Growth Stage (BBCH)	Actual Spray Volume, GPA (L/ha)	Rate, lb a.i./A (kg a.i./ha)	Retreatment Interval (days)	Total Rate, lb a.i./A (kg a.i./ha)	Adjuvant
					Foliar Spray	61	20 (187)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	20 (187)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
NT006-13ZA	Sanger, CA Region 10	Admire Pro	TRTD	2013	Soil Spray	00	13 (118)	0.328 (0.368)	NA	0.50 (0.56)	NA
					Foliar Spray	61	16 (146)	0.058 (0.065)	95		Dyne-Amic 0.25 % v/v
					Foliar Spray	61	15 (142)	0.058 (0.065)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (144)	0.058 (0.065)	6		Dyne-Amic 0.25 % v/v
				2014	Soil Spray	00	13 (125)	0.332 (0.372)	249	0.51 (0.57)	NA
					Foliar Spray	61	19 (179)	0.058 (0.065)	77		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	19 (181)	0.058 (0.065)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	20 (185)	0.057 (0.064)	6		Dyne-Amic 0.25 % v/v
NT007-13ZA	Fresno, CA Region 10 2013	Admire Pro	TRTD	2013	Soil Spray	00	14 (132)	0.331 (0.372)	NA	0.50 (0.56)	NA
					Foliar Spray	61	15 (141)	0.057 (0.064)	81		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (141)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (140)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
				2014	Soil Spray	00	14 (132)	0.330 (0.369)	262	0.50 (0.56)	NA
					Foliar Spray	61	15 (140)	0.057 (0.064)	75		Dyne-Amic 0.25 % v/v
					Foliar Spray	61	15 (140)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (141)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v

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Trial Identification	Location (City, State, NAFTA Region)	Formulation	Plot Name	Year	Application[a]						
					Method	Timing/Growth Stage (BBCH)	Actual Spray Volume, GPA (L/ha)	Rate, lb a.i./A (kg a.i./ha)	Retreatment Interval (days)	Total Rate, lb a.i./A (kg a.i./ha)	Adjuvant
NT008-13ZA	Kerman, CA Region 10	Admire Pro	TRTD	2013	Soil Spray	00	14 (130)	0.328 (0.368)	NA	0.50 (0.56)	NA
					Foliar Spray	65	15 (141)	0.057 (0.064)	83		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (141)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (142)	0.058 (0.065)	7		Dyne-Amic 0.25 % v/v
				2014	Soil Spray	00	14 (130)	0.326 (0.365)	226	0.50 (0.56)	NA
					Foliar Spray	61	15 (138)	0.056 (0.063)	83		Dyne-Amic 0.25 % v/v
					Foliar Spray	61	15 (141)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (140)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
NT009-13ZA	Kerman, CA Region 10	Admire Pro	TRTD	2013	Soil Spray	00	14 (132)	0.331 (0.371)	NA	0.50 (0.56)	NA
					Foliar Spray	61	15 (139)	0.056 (0.063)	83		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (141)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (141)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
				2014	Soil Spray	00	14 (131)	0.330 (0.369)	225	0.50 (0.56)	NA
					Foliar Spray	61	15 (141)	0.057 (0.064)	83		Dyne-Amic 0.25 % v/v
					Foliar Spray	61	15 (141)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v
					Foliar Spray	65	15 (141)	0.057 (0.064)	7		Dyne-Amic 0.25 % v/v

[a] Values for spray volume, rate, and total rate have been rounded using values provided in Appendix 2 of the study report.
[b] NA = Not applicable.
[c] Only the first year of trial NT002-13ZB will be reported because the plot location was no longer available.
*Table 4 of the study report

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Table 2. Soil and meteorological characteristics of the study sites*.

Trial ID[a]	Trial Location (City, Country/State, Year, GPS Coordinates[b])	Soil Characteristics[c] OM (%)	pH	CEC (meq/100 g soil)	% Sand	% Silt	% Clay	Type	Meteorological Data[d] Total Rainfall (in)	Temp. Range (°F)		Variety
NT001-13ZB	Yuba City, CA, 2014 (38°59.055' N, 121°36.115' W)	2.0	6.1	19	19	46	35	Silty clay loam	18.92	41	97	Pima
NT002-13ZB	Wheatland, CA, 2014 (39°00.22' N, 121°27.59 W)	1.5	6.5	14	39	36	25	Loam	0.92	47	97	Pima
NT003-13ZA	Fresno, CA, 2013-2014 (36.73628, -119.87515)	1.0	7.2	14	67	18	15	Sandy Loam	3.92	30	102	DP 358 RF PHY 804 RF
NT004-13ZA	Davis, CA, 2013-2014 (38.5337, -121.7793)	1.6	7.0	19	39	36	25	Loam	14.93	32	93	Pima
NT005-13ZB	Yuba City, CA, 2014 (39°02.24' N, 121°37.63' W)	2.7	6.5	19	25	44	31	Clay loam	18.92	41	97	Pima
NT006-13ZA	Sanger, CA, 2013-2014 (36.69982, -119.46196)	0.38	5.9	5.7	69	24	7	Sandy Loam	5.69	31	99	Pima
NT007-13ZA	Fresno, CA, 2013-2014 (36.73916, -119.87599)	0.81	7.4	12	63	28	9	Sandy Loam	3.92	30	102	PHY 802 RF
NT008-13ZA	Kerman, CA, 2013-2014 (36.79552, -120.05406)	0.51	5.7	4.5	73	26	1	Loamy sand	2.74	29	99	Delta Pine 358 RF
NT009-13ZA	Kerman, CA, 2013-2014(36.79516, -120.05676)	0.34	5.6	4.5	85	14	1	Loamy sand	2.74	29	99	Delta Pine 358 RF PHY 802 RF

a Site conditions listed are for the TRTD plot. For UTC plot conditions, see Appendix 2 of the study report.

b GPS coordinates are in the form (latitude, longitude).

c Soil characteristics are based on analyses of composite soil samples colle3cted from within the treated plot. The central area of the treated plot was identified (e.g., the central 100 by 200 ft. section from within a 200 by 400 ft. plot), and soil subsamples were collected from the four corners of that central area. The four subsamples were composited into one sample for analysis. Abbreviations used: %OM = percent organic matter; CEC = cation exchange capacity.

d Data is for the interval of the month of first application through the month of last sampling. Meteorological data were obtained from nearby government weather stations or on-site weather stations.

* Combined table from Table 3A and Table 8 of the study report.

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Table 3. SSURGO soil characteristics of the study sites*.

Trial ID	Trial Location	General Texture Description (SSURGO)	CDPR Texture Category	Component %[a]	Drainage Class	Hydrologic Group	Runoff	Particle Size
NT001-13ZB	Yuba City, CA	Marcum-Gridley Clay Loam, 0-1% slopes	Fine	45 / 40	Moderately Well Drained	C / D	Low / Medium	Fine
NT002-13ZB	Wheatland, CA	Columbia Fine Sandy Loam, 0-1% slopes (~10% Kimball Loam)	Coarse	85	Somewhat Poorly Drained	A	Very Low	Coarse-loamy
NT003-13ZA	Fresno, CA	Ramona Loam	Medium	80	Well Drained	C	Low	Fine-loamy
NT004-13ZA	Davis, CA	Reiff Very Fine Sandy Loam	Coarse	85	Well Drained	A	Very Low	Coarse-loamy
NT005-13ZB	Yuba City, CA	Marcum-Gridley Clay Loam, 0-1% slopes	Fine	45 / 40	Moderately Well Drained	C / D	Low / Medium	Fine
NT006-13ZA	Sanger, CA	Hanford fine sandy loam, gravelly substratum	Coarse	85	Well Drained	A	Very Low	Coarse-loamy
NT007-13ZA	Fresno, CA	Greenfield Sandy Loam, 0-3% slopes	Coarse	85	Well Drained	A	Very Low	Coarse-loamy
NT008-13ZA	Kerman, CA	Hanford Coarse Sandy Loam	Coarse	85	Well Drained	A	Very Low	Coarse-loamy
NT009-13ZA	Kerman, CA	Hanford Sandy Loam, silty substratum	Coarse	85	Well Drained	A	Very Low	Coarse-loamy

[a] Major component(s) of the soil as a percentage of total soil; for Marcum-Gridley: Marcum is 45%, hydrologic group C, and Low runoff; Gridley is 40%, hydrologic group B, and Medium runoff.
* From Table 3B of the study report.

5D. Sample Collection, Handling, Processing

Cotton Plant Matrices.

Cotton leaf and flower samples were collected at three sampling intervals. The first samples were collected at early bloom, prior to any foliar sprays, to measure residues in bee-relevant matrices as a result of the at-plant soil application. The second and third sampling intervals measured residues that were a result of the soil application at planting plus three additional at-bloom sprays. The first, second, and third sampling intervals corresponded to 4 to 5 days prior to the first foliar application (70 to 95 days after the soil application), 4 to 5 days after the last foliar application, and 10 to 14 days after the last foliar application, respectively (**Table 4**). At each sampling interval, duplicate composite samples (two separate runs through the plot) of cotton flowers and cotton leaves were collected from the treated plots when the plants were at bloom, BBCH 61 (begin flowering, early bloom) to BBCH 73 (about 30% of bolls have attained their final size). Single composite samples of cotton leaves and flowers were collected from the control plot of each trial on the same days that samples were collected from the treated plots.

Cotton flowers and leaves were collected by hand into Ziplock bags. Each composite flower sample contained a minimum of 125 g (minimum 250 blossoms) collected from at least 12 different areas of the plot, avoiding the edges. Exceptions occurred in trials NT001-13ZB and NT005-13ZB, when flower sample weights were below the minimum or were not recorded, however sufficient flowers were collected from processing. Each composite leaf sample contained a minimum of 100 g. All samples were protected from sunlight and placed in field coolers containing ice or ice substitute or in portable freezers.

After their collection, cotton flowers were hand-processed at the field site to obtain the bee-relevant samples of cotton pollen, floral nectar, and extrafloral nectar; all available matrices were collected. Processing occurred the same day as flower collection, except during the last 2015 sampling interval in trial NT001-14ZB, when nectar samples were collected same day and then the flowers were allowed to air dry overnight, with pollen samples being collected the following day. Extrafloral nectar from the sub bracteal and inner bracteal nectaries was removed using a micropipette and placed into a pre-weighed amber glass collection vial. Nectar from the floral nectary was removed by micropipette and placed in a separate vial. Pollen was removed from the cotton blossoms either by vacuum aspiration with collection in filter tips or by tapping the pollen from the blossoms onto wax paper and collection of the accumulated pollen into a vial. All resulting nectar and pollen samples were labeled and placed in the frozen storage (via freezer or dry ice) immediately after they were generated. After processing was completed, the flowers were discarded.

Figures 4 through 6 provide trends in total imidacloprid residue measured in extrafloral nectar, pollen, and leaf tissue at each site. **Figure 7** depicts the relationship between concentration of total imidacloprid residues measured in leaves and floral nectar parsed out by soil type.

Table 4. Sampling dates and cotton developmental stages for plant residue analysis*

Pollen, Nectar, Extrafloral Nectar, and Leaf Sampling							
Year 1		Year 2		Year 1		Year 2	
BBCH†	Dates‡	BBCH†	Dates‡	BBCH†	Dates‡	BBCH†	Dates‡
Davis 1 = NT004-13ZA				**Sanger 1 = NT006-13ZA**			
61	86 DASA, -5 DA1FA	61	95 DASA, -4 DA1FA	61	90 DASA, -5 DA1FA	61	73 DASA, -4 DA1FA
61	5 DA3FA	65	4 DA3FA	65	4 DA3FA	65	4 DA3FA
61	13 DA3FA	67	13 DA3FA	65	14 DA3FA	67	14 DA3FA
Fresno 1 = NT003-13ZA				**Wheatland = NT002-13ZB**			
65	76 DASA, -5 DA1FA	61	72 DASA, -5 DA1FA	65	88 DASA, -4 DA1FA	NC[1]	NC
67	5 DA3FA	65	5 DA3FA	69	5 DA3FA	NC	NC
67	14 DA3FA	67	14 DA3FA	73	14 DA3FA	NC	NC
Fresno 2 = NT007-13ZA				**Yuba City 1 = NT001-13ZB**			
65	76 DASA, -5 DA1FA	61	70 DASA, -5 DA1FA	61	88 DASA, -5 DA1FA	TBC[2]	TBC
65	5 DA3FA	65	5 DA3FA	61	5 DA3FA	TBC	TBC
67	14 DA3FA	67	14 DA3FA	61	14 DA3FA	TBC	TBC
Kerman 1 = NT008-13ZA				**Yuba City 2 = NT005-13ZB**			
65	78 DASA, -5 DA1FA	61	78 DASA, -5 DA1FA	61	86 DASA, -5 DA1FA	TBC	TBC
65	4 DA3FA	65	4 DA3FA	XX[3]	5 DA3FA	TBC	TBC
67	14 DA3FA	67	12 DA3FA	67	14 DA3FA	TBC	TBC
Kerman 2 = NT009-13ZA							
65	78 DASA, -5 DA1FA	61	78 DASA, -5 DA1FA				
65	4 DA3FA	65	5 DA3FA				
67	14 DA3FA	67	14 DA3FA				

†BBCH = **B**iologische Bundesanstalt, **B**undessortenamt und **CH**emische Industrie growth stage scale for cotton.
‡DASA = Days after the soil (in-furrow) application; DA1FA = days after the first foliar application; DA3FA = days after the third foliar (and last) application. A negative number designates days prior to the indicated application.
[1]NC = Not collected; no year 2 samples from trial NT002-13ZB can be collected.
[2]TBC = To Be Collected; the samples will be collected when the second year of the trial is performed.
[3]BBCH XX indicates no growth stage reported in the field data summary.
*Combination of Appendix 1 and Appendix 4 (Section 6) of the study report.

Sample Storage.

Composite samples of cotton leaves, pollen, floral nectar, and extrafloral nectar were placed into labeled (study number and sample number) containers for shipment. All samples were frozen within 4 hours of collection with the exception of certain samples in trial NT004-13ZA, which were frozen within 9 hours and 1 minute, and 2015 samples from trials NT001-13ZB and NT005-13ZB took up to 7 hours and 20 minutes to be stored frozen (in a freezer or on dry ice). Samples remained frozen until receipt at Bayer CropScience in RTP, NC.

Stability studies have indicated that imidacloprid residues are stable {<30% decomposition) for 24 months (728 to 769 days) of freezer storage in the following representative crops: an oilseed (cottonseed), a non-oily grain (wheat), a leafy vegetable {lettuce), a root crop {potato), a tree fruit {apple), and a fruiting vegetable {tomato)[4-10]. An additional stability study has indicated that imidacloprid residues are stable (<30% decomposition) for 36 months of freezer storage in wheat (grain), orange {fruit), tomato (fruit), bean {seed), and rape (seed)[11]. Demonstrated freezer stability in all of the above crops is representative of the freezer stability of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin residues to be expected for cotton leaves from this study. The cotton leaves analyzed in this study were held in frozen storage for a maximum of 569 days (19 months) prior to extraction.

Based on the available storage stability data,[4-11] the imidacloprid residues would be representative of the residues to be expected after the use of Admire Pro Systemic Protectant on the tested crops.

5E. ANALYTICAL METHODS

The analytical methods[1-2] used in this study measured the residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin in cotton leaves, floral nectar, extra floral nectar, and pollen. These data are reported in Appendix 4 of the full study report titled, "*Analytical Report for EBNTN011 Determination of the Residues of Imidacloprid, 5-Hydroxy Imidacloprid, and Imidacloprid Olefin in Bee Relevant Matrices Collected from Cotton During Two Successive Years.*"

For the cotton leaves, a 2.5-g sample was weighed into a 50-mL polypropylene conical centrifuge tube, and 10 mL HPLC-grade water was added. The tube was mixed manually for 1 minute, followed by adding 20 mL of acetonitrile and shaking for an additional 1 minute. Then, 3 g of MgSO4 and 1.5 g of NaCl were added. The sample was amended with a mixed internal standard solution and mixed manually for 1 minute. For leaf samples which were found to contain high residues of imidacloprid (>2 ppm), as determined by an initial run in which the response exceeded the calibration curve, the sample was amended with a 10X mixed internal standard solution before the salts were added. The sample was centrifuged. For samples containing low levels of imidacloprid residue, 20 mL of organic supernatant was transferred into a separate 50-mL polypropylene conical centrifuge tube containing 0.3 g of Bondesil-PSA and 1.8 g of MgSO4. For samples containing high levels of imidacloprid residue, 2.0 mL of organic supernatant and 18.0 mL of acetonitrile were transferred into a separate 50-mL polypropylene conical centrifuge tube containing 0.3 g of Bondesil-PSA and 1.8 g of MgSO4, which was manually mixed for 1 minute. The sample extract was centrifuged, and a 1.25 mL aliquot of supernatant was transferred into a clean culture tube. The sample aliquot was evaporated to near dryness using a Turbo-Vap (Biotage, Charlotte, NC). The solid was reconstituted with 1.25 mL of 9:1 water/MeOH containing 10 mM NH4HCO3 by vortexing, and the resulting solution was transferred into a 2-mL sample vial for LC/MS/MS analysis.

For nectar, a 0.1-mL sample was weighed into a 20 x 150 mm culture tube and dissolved in 4 mL of water. If the total sample volume was less than 0.1 mL, the entire sample was weighed and recorded. The mixture was amended with isotopically labeled internal standards, mixed well, and applied to an Agilent BondElut SPE cartridge (50 mg resin; previously conditioned with methanol then water). The cartridge was washed with 1 mL of MeOH/H2O (1:19 v/v), and the combined eluates were discarded. The analytes were eluted from the cartridge with 0.5 mL of MeOH/H2O (1:4 v/v). The eluate was collected in a 2 mL sample vial for analysis by LC/MS/MS.

For pollen, a 0.1-g sample was weighed into a 2-mL centrifuge tube containing 2.8 mm steel balls. If an individual sample volume was not sufficient for analysis, the two samples collected at the same interval were composited and analyzed. The composite sample was weighed and recorded. A 1-mL portion of methanol/water (3:1 v/v) was added, and the mixture was homogenized with a bead mixer at 5000 beats/min for 1 minute on a Precellys homogenizer (Bertin Technologies, Rockville, MD). The isotopically labeled internal standards were added and mixed, and the mixture was centrifuged at 12,000 rpm for 2 minute. The supernatant was transferred into a clean culture tube containing 2.5 mL of water. The sample was evaporated to an aqueous remainder and applied to a 3-mL ChemElut SPE cartridge. After 10 to 15 minutes, the cartridge was washed three times with 4 mL of hexane/ethyl acetate (1:1 v/v), and the eluates were collected in a clean culture tube. The combined eluates were evaporated to dryness. The analytes were dissolved in 0.5 mL of MeOH/H2O (1:4 v/v), and the resulting solution was transferred into a 2 mL sample vial for analysis by LC/high resolution mass spectrometry (LC/HRMS).

Quantitation of each analyte was based on the daughter ion transitions of the analyte and the respective internal standard analog. The responses of the LC/MS/MS and LC/HRMS systems to each analyte and its internal standard were measured in samples and in standards, and a relative response was calculated (as the ratio of the analyte and the stable isotopically labeled internal standard responses). The relative response of the analyte in each sample was compared to the relative response of the analyte in the standards provided in **Appendix 4** of the study report.

The relative responses of imidacloprid were measured over the range of 0.00012 to 4 ppm. The correlation coefficients (R) were calculated using linear regression analysis with 1/x weighting.

All data are reported in parent equivalents, and the individual measured residues of imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin are summed to give a total imidacloprid residue.

5F. Quality Assurance Results

The responses of the LC/MS/MS and LC/HRMS systems to imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin were linear in solvent over the range of 0.00012 to 4 ppm. The correlation coefficients were >0.99. The response data and analytical data summaries are located in Appendix 4.

Control interferences for cotton matrices are discussed in this paragraph; no total imidacloprid residue was calculated for the UTC samples, so the levels of imidacloprid as an individual analyte are described. Imidacloprid (parent) residues in UTC cotton floral nectar ranged from below the analyte LOD to 0.067 ppm. Imidacloprid residues in UTC cotton extrafloral nectar ranged from below the analyte LOD to 0.053 ppm. Imidacloprid residues in UTC cotton pollen ranged from below the analyte LOD to 0.017 ppm. Imidacloprid residues in UTC cotton leaves ranged from below the analyte LOD to 0.052 ppm.

All recoveries were corrected for any interferences in corresponding controls. The overall means of the recoveries for each matrix at each fortification level were within the acceptable range of 70 to 120%, and standard deviation values were below 20%.

The limit of quantitation (LOQ) is defined as the lowest fortification level of an analyte at which acceptable recovery has been achieved. The LOQ for a total residue is the highest of the LOQ values assigned to the individual analytes for a particular matrix. The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be determined to be statistically different from a blank. The LODs were determined from method validation data obtained from control samples fortified at the respective analyte LOQs. The LODs were calculated by multiplying the standard deviation of recovery measurements at the LOQ by $t_{0.99}$ [where $t_{0.99}$ is the one-tailed t-statistic at the 99% confidence level for the number of replicates (n)].[3] The LOD for the total imidacloprid residue in each matrix is the highest LOD value of any one individual analyte for that particular matrix.

Limits of quantification and detection for imidacloprid and metabolites*.

Matrix	Analyte	LOQ (ppb)	LOD (ppb)
Cotton Leaves	Imidacloprid	5.0	1.2
	5-Hydroxy Imidacloprid	5.0	0.7
	Imidacloprid Olefin	5.0	0.8
	Total Imidacloprid	5.0	1.2
Cotton Extrafloral Nectar	Imidacloprid	1.0	0.3
	5-Hydroxy Imidacloprid	1.0	0.7
	Imidacloprid Olefin	1.0	0.6
	Total Imidacloprid	1.0	0.7
Cotton Floral Nectar	Imidacloprid	1.0	0.3
	5-Hydroxy Imidacloprid	1.0	0.7
	Imidacloprid Olefin	1.0	0.6
	Total Imidacloprid	1.0	0.7
Cotton Pollen	Imidacloprid	1.0	0.4
	5-Hydroxy Imidacloprid	1.0	0.5
	Imidacloprid Olefin	1.0	0.3
	Total Imidacloprid	1.0	0.5

* From page 20 of the study report.

6. RESULTS:

6.A. COMPARISON OF CONCENTRATIONS MEASURED FOR PARENT AND DEGRADATES

Comparison of the relative concentrations measured for parent imidacloprid and degradation products in floral nectar, extrafloral nectar, pollen, and leaves are presented in **Table 6-3** through **Table 6-6**. Concentrations were reported as ppm on a weight/weight basis. The LOD of values were low, ranging from 0.3 ug/g to 1.2 ug/g (ppb), so data reported as below the LOD were assigned one-half the LOD value. Comparison of the contribution of each chemical to the total residue indicates that parent imidacloprid represented the majority of the total residue measured in each plant part. For floral nectar, extrafloral nectar, and pollen (bee relevant tissue), parent imidacloprid comprised over 90% or greater of the total for most comparisons. When the levels of residues are low the ratios could be affected by the insertion of ½ the LOD where the tendency would be for overestimation of the contribution of a degradation product. Since the degradation products (considered as toxic as parent imidacloprid) are comprised of a small portion of the measured residue, the following discussions will focus on comparing total imidacloprid residue measured in plant tissues. Statistical procedures used in the Statistical Analysis System (SAS) software to provide distribution statistics and statistical tests were PROC CAPABILITY, PROC SHEWHART, PROC TTEST, PROC UNIVARIATE, and PROC NPAR1WAY. **Figure 6-1** illustrates the statistical aspects relayed in the Box-and-Whisker plots used to compare the distribution of concentrations calculated for total imidacloprid residue at each sampling interval. For each represented data set, the box graphic presents values for the mean, median, minimum, maximum, and 25th and 75th percentiles of the distribution.

6.B. Potential for Yearly Carry-over of Residues

Site specific curves provide comparison between years for mean concentrations of total imidacloprid residue measured at each sampling interval for 8 of the 9 sites **(Figure 6-2 through Figure 6-5)**. The study was conducted only one year at the Wheatland site (NT002-13ZB). Some curves are very similar between the years, such as floral nectar at the Sanger site and leaves at the Kerman and Davis sites. But some indicate a greater concentration measured at sampling interval 2 in 2013 than in 2014, such as floral nectar at the Davis site and Kerman site 1 and leaves at Fresno site 1. In addition, mean values at the first sampling interval were very similar between years at each site for floral nectar, extrafloral nectar, and leaf samples (**Table 6-1**). The similarity in starting values for each year and the variation in patterns observed between years indicated no consistent effect for a carry-over of residues. These observations were confirmed by lack of a significant difference between years using either Wilcoxon's Signed Rank Test for zero as the location for the difference in value between the 2 years or Student's paired t-Test (**Table 6-2**). Most sites reported 2 replicate samples so tests were conducted on mean values calculated for each site at each sampling interval within each year.

For pollen samples, there was a noticeable difference in the magnitude of concentrations and trend measured between 2013 and 2014 data **(Figure 6-4).** Concentrations in pollen samples at all sites and **at all sampling intervals** were lower in the first year of the study compared to when measured in the second year of the study. This pattern was not consistent with all other patterns measured for that year in the other plant tissues or for the pattern in pollen residues measured in the second year. Since the abnormal pattern noted for pollen was limited to the first year of the study, this potentially indicates problems caused by sampling procedures and/or chemical analyses for pollen in 2013. One might argue that the effect could be caused by climatic or site differences, but if true, then the patterns would also be expected in the other plant samples as they were obtained at the same sites and at the same sampling interval. Therefore, yearly comparisons for pollen appear to be compromised due to experimental anomalies encountered in the analyses for the first year.

6.C. MAGNITUDE OF RESIDUES IN BEE-RELEVANT MATRICES

Based on the lack of differences measured between years, data were pooled from both years to determine the expected distributional properties for concentrations in plant samples. General patterns for total imidacloprid residues in plant samples are illustrated in **Figure 6.6**.

Floral Nectar. Comparison of overall statistics for total imidacloprid residue indicated much greater concentrations in all plant parts at the second sampling interval than at the other two sampling intervals (**Figure 6-6**). The first soil application occurred at planting. Sampling for the first interval occurred at a mean of 81 days after the soil application with a range of values from 70 to 95 days (**Table 4 above**). The median total imidacloprid residue at the first sampling interval was 9 ppb in floral nectar with a maximum value of 127 ppb and 90th percentile value of 50 ppb (**Table 6-3**). The second sampling interval was conducted after 3 foliar applications of imidacloprid and was approximately 23 days after the first interval. Concentrations in floral nectar at the second sampling interval increased to a median of 70 ppb with a maximum value of 171 ppb and a 90th percentile value of 144 ppb. The third sampling interval occurred approximately 9 days after the second interval with the total imidacloprid residue concentration dropping to a median of 35 ppb with a maximum value of 117 ppb and a 90th percentile value of 113 ppb.

Extrafloral Nectar. Although a similar increase in concentration was observed at sampling interval 2 for extrafloral nectar, the increase in concentration measured was relatively much larger than observed for floral nectar. The median value at the second sampling interval was 276 ppb, which was approximately 4 times greater than the value measured for floral nectar (**Table 6-4**). At the second interval the maximum value was 2775 ppb and the 90th percentile value was 1882 ppb.

Pollen. Even though the pattern in the first year indicated potential analytical problems, the trend for pooled pollen data was similar to floral and extrafloral nectar where increased concentrations were observed at sampling interval 2. The median value at the first sampling interval was 1 ppb which increased to 46 ppb at the second sampling interval (**Table 6-5**). At the second interval the maximum value was 2906 ppb and the 90th percentile value was 409 ppb.

6.D. Magnitude of Residues in Leaves

As expected, direct foliar applications of imidacloprid to plants between the first and second sampling interval greatly increased the magnitude of residues of total imidacloprid in leaves measured at the second sampling interval **(Table 6-6).** At the first sampling interval that occurred around 81 days after the soil application, the median total residue in leaves was 24 ppb. The median concentration at sampling interval 2 was 80 times greater at 1956 ppb. At interval 3, the levels in leaves were decreased with the median value down to 441 ppb, a value that was still greater than residues measured at the first interval.

6.E. Site Specific Trends

Temporal patterns in residue concentrations for the 9 separate sites are depicted in **Figure 6-2** through **Figure 6-5** for floral nectar, extrafloral nectar, pollen, and leaves, respectively. The general pattern noted above where concentrations rise steeply at the second sampling interval is reflected in floral nectar samples with 13 of the 15 curves **(Figure 6-2).** Curves at three sites (Fresno site 1 in 2013, and both Yuba City sites in 2015) reflect a different pattern where a rise in concentration over time is noted. Concentrations at a fourth site, Kerman site 2 in 2013 were stable over time. The cause for these differences is unknown but uncertainties in sampling methodology may cause this variation. All other sites, though, indicated that there should be sharp increases in concentrations caused by foliar applications. Decreases in concentration from interval 2 to 3 were predominant but there were subtle differences in slopes where some indicated deep decreases in concentrations between the intervals while others indicated only a shallow, slight decrease in concentration over time. Data in 2014 for the two Kerman sites (green lines) exemplify the potential differences in slopes measured between intervals 2 and 3 where a large decrease is indicated at Kerman1 (solid dots) but a small decrease is indicated at Kerman2 (open squares).

Trends in total imidacloprid residue measured in extrafloral nectar also reflected a large increase in concentration at sampling interval 2 followed by reductions at sampling interval 3 **(Figure 6-3).** The exception was data for Fresno site 1 in 2013. Data in 2014 reflected the pattern measured at the rest of the sites. The increase at some sites was rather large where, for example at Davis site 1 in 2013 the average concentration was 1629 ppb.

For pollen samples, there were noticeable differences in the trend and magnitude of concentrations measured between 2013 and 2014 data as previously indicated **(Figure 6-4).** Concentrations in pollen at all sites in the first year of the study were lower than when measured in the second year of the study. The pattern for the second year of the study is consistent with the general trend observed for all other plant samples in both years where foliar application greater increased the measured concentrations.

Lastly, all curves for leaf concentrations exhibited an increase at interval 2 and a sharp decline at interval 3 **(Figure 6-5).** The sharp decrease in concentrations between intervals 2 and 3 does not appear to be due to washoff from rainfall or irrigation. For example, at the Fresno sites and the Sanger sites, there was no recorded rainfall during this time interval. Also, the irrigation systems used had a low potential for wetting the leaves because drip irrigation was used at the Fresno sites and a combination of drip and furrow methods at the Sanger site.

6.F. Leaf and Nectar Concentration in Relation to Soil Type

Originally, the proposed study design suggested sampling sites located in coarse, medium, and fine textured soils with 3 replicates assigned to each soil type. There is an inherent difficulty in fulfilling the proposed design when the study is conducted after fields have been planted and then growers are contacted in an effort to procure their cooperation. The reported soils in this study were biased towards coarse soils where in 2013 five of six sites were located in coarse soil, one in medium textured soils and none in the fine textured category. In 2014, two sites were located in the fine textured category, one site in medium, and six sites in the coarse textured category. Then in 2015, there were only 2 sites in fine-textured soil. Analysis for the effect of soil is not possible in light of the noted differences measured in concentration between years and the uneven replication in soil type between years.

7. STUDY STRENGTHS, LIMITATIONS AND CONCLUSIONS

In the context of documenting the magnitude of imidacloprid residues in bee-related matrices of cotton, the following strengths are observed with this study.

1. Data provide quantitative values of total imidacloprid residues expected in floral nectar, extrafloral nectar, pollen, and leaves of cotton.

2. Measurements were taken at 3 time intervals in an attempt to quantify levels expected in plant tissues: The first interval reflected concentrations following a period of time after a soil application at planting, and the second and third sampling intervals expected concentrations expected in plants after three additional foliar applications.

Limitations noted in this study include:

1. The values most likely do not reflect a maximal exposure to bee relevant matrices because sampling did not occur directly after foliar application. Sampling after the third foliar application averaged 5 days. Substantial decreases at nearly an order of magnitude were noted in residues from plants sampled between the 2rd and 3th foliar application where the sampling interval averaged 9 days. Since there was no potential for redistribution of residues due to water movement from either irrigation or rainfall, the steep dissipation indicates that concentrations would most likely have been highest if samples were taken directly after foliar applications.

2. The study did not follow the residue data protocol calling for three replicates each in fine, medium and coarse soils resulting in uncertainty in regards to the effects of soil type on imidacloprid concentration in cotton flower parts and leaves.

3. An inadequate sample of extrafloral nectar to analyse was collected following the soil application and prior to the foliar application in replicate NT003-13ZA.

4. Pollen analysis appeared problematic in the first year of the study, which was initiated in 2013. Patterns for all other plant tissues and for analyses of pollen in 2014 indicated a very large increase in total imidacloprid concentration for interval 2 due to foliar application, an effect that was extended to the third sampling interval. This apparent anomalous pattern was evident at all experimental locations. Since the other plant samples were obtained simultaneously, effects of climate or site should be minimal. This condition indicates that an experimental condition such as an analytical problem was most likely the cause for lower concentrations measured in the first year.

5. Data are inadequate to compare concentrations in cotton matrices between soil types because data from medium and fine soil types were not adequately represented.

Overall, considering the strengths and limitations of this study, the following conclusions can be drawn:

1. Imidacloprid residues were measured in bee-relevant matrices from soil application: Maximum concentrations of total imidacloprid residues at approximately 81 days after soil application of 0.34 lb ai/A were 127, 36, and 43 ppb in floral nectar, extrafloral nectar, and pollen, respectively. Median concentrations were 9, 3, and 1 ppb in floral nectar, extrafloral nectar, and pollen, respectively.

2. Imidacloprid residues that were measured in bee-relevant matrices increased from additional foliar sprays: The distribution of total imidacloprid residues resulting from 3 additional foliar sprays each at 0.056 – 0.067 lb ai/A increased the maximum values to 170, 2775, and 2906 ppb, in floral nectar, extrafloral nectar, and pollen, respectively. Median concentrations were 70, 276, and 46 ppb in floral nectar, extrafloral nectar, and pollen, respectively.

3. Concentrations in bee-relevant matrices generally decreased overtime following the foliar applications: Subsequent sampling 14 days after the 3rd foliar application resulted in maximum total imidacloprid residues of 117, 136, and 182 ppb in nectar, extrafloral nectar, and pollen, respectively. Median concentrations were 35, 27, and 15 ppb in floral nectar, extrafloral nectar, and pollen, respectively.

4. No evidence for carry-over effects between years: As indicated in the discussion, comparison of the starting values between years and the patterns over time compared between years for each site for leaf, floral and extra-floral nectar indicated no consistent evidence for carry-over effects. Comparison between years for pollen samples appeared compromised so comparisons between years were not worthwhile.

8. STUDY VALIDITY/CLASSIFICATION

The data from this study provide an expected distribution of the concentrations of imidacloprid residues that bees are exposed to in nectar, pollen and extrafloral nectaries of cotton plants grown under actual agronomic practices in California. Relating concentrations measured in flower parts to bee health is possible by comparing the concentrations measured in bee relevant plant parts to target values that define acute or chronic exposure scenarios. These data, however, would represent a minimal exposure assessment to foliar applications because samples were not taken during the period of foliar application. Therefore, there is uncertainty as to how reflective the values obtained at the 3 sampling intervals represent maximum exposure scenarios. The study is considered scientifically sound and useful for risk assessment purposes. The study is classified as ACCEPTABLE for quantitative use in risk assessment.

9. REFERENCES

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Table 6-1. Comparison of cumulative distributional statistics for concentration of total imidacloprid residues between the first year of the study (Year 1) and the second year of the study (Year 2) in floral nectar (ppb). Acronyms in the table are: Total Imidacloprid Residue = Sum of parent and degrades; N=Number of paired observations; SD=Standard Deviation; CV=Coefficient of Variation. Numbered Interval denotes timing of sampling where interval 1 was approximately 81 days after the first soil application and intervals 2 and 3 were at 5 and 14 days after a third foliar spray coinciding with 91 and 100 days after the soil application.

Total Imidacloprid Residue												
	Floral Nectar						Extrafloral Nectar					
	Interval 1		Interval 2		Interval 3		Interval 1		Interval 2		Interval 3	
Statistic	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
N	8	8	8	8	8	8	6	6	8	8	8	8
Mean (ug/L)	27.3	19.6	91.4	65.6	37.7	44.0	5.1	13	528.0	651.5	38.5	42.3
SD (ug/L)	34.6	16.1	49.0	36.5	24.6	28.5	3.4	13.7	650.0	580.6	21.1	33.3
CV (%)	127.0	82.1	53.6	55.7	65.2	64.8	67.0	105.5	123.0	89.1	54.7	78.8
Min (ug/L)	1.7	1	20.9	17.9	10.5	17.6	1.2	0.9	54.3	225.2	7.6	16.2
Median (ug/L)	6.2	18.5	83.1	72.3	31.2	37.6	4.9	10.2	194.8	373.1	34.8	26.3
Max (ug/L)	83.1	53.4	153.3	113.4	78.8	103.9	9.7	35.9	1951.5	1629.6	63.1	111.2
Statistic	Leaves						Pollen					
N	8	8	8	8	8	8	8	8	8	8	8	8
Mean (ug/L)	41.5	53.3	1532.4	1882.5	318.5	434.4	0.9	10.8	21.0	524.0	8.1	97.1
SD (ug/L)	63.6	48.9	715.6	500.7	176.1	122.0	0.5	13.4	16.1	757.6	6.6	45.7
CV (%)	153.2	91.7	46.7	26.6	55.3	28.1	50.7	124.3	76.5	144.6	82.0	47.1
Min (ug/L)	1.4	5.6	800.9	1196.8	103.8	293.7	0.6	0.6	4.2	45.6	3.2	17.2
Median (ug/L)	19.7	44.6	1417.9	1762.6	261.1	420.7	0.8	6.9	19.5	231.1	5.8	105.5
Max (ug/L)	192.9	140.1	3098.2	2860.3	698.6	616.5	2.0	41.1	45.4	2316.8	22.8	153.3

Table 6-2. Statistical results for test of differences in concentration measured between years 1 and 2 for concentration of total imidacloprid residue.

Total Imidacloprid Residue			
Plant Sample	Interval Sampled	Probability Level for	
		T-Test	Sign Rank
Extrafloral Nectar	1	0.15	0.16
	2	0.75	0.84
	3	0.79	0.95
Floral Nectar	1	0.44	0.55
	2	0.29	0.38
	3	0.56	0.64
Leaves	1	0.64	0.25
	2	0.16	0.25
	3	0.21	0.25
Pollen	1	0.08	0.03
	2	0.10	0.01
	3	0.00	0.01

Table 6-3. Floral Nectar: Cumulative distributional statistics for concentration of imidacloprid and related metabolites in cotton floral nectar (ppb). Acronyms in the table are: IMI=Imidacloprid; Olefin=Imidacloprid Olefin; 5-OH=5 Hydroxy Imdacloprid; Total Imidacloprid Residue (in bold) = Sum of parent and degrades; N=Number of observations; SD=Standard Deviation; CV=Coefficient of Variation. Numbered Interval denotes timing of sampling where interval 1 was approximately 81 days after the first soil application and intervals 2 and 3 were at 5 and 14 days after a third foliar spray coinciding with 91 and 100 days after the soil application.

Floral Nectar: Distribution of Imidacloprid Residues by Interval Sampled												
	Interval 1				Interval 2				Interval 3			
Statistic	**5-OH**	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**
N	32	32	32	**32**	33	33	33	**33**	33	33	33	**33**
Mean (ppb)	0.6	0.6	20.0	**21.2**	2.1	1.3	71.2	**74.6**	1.2	1.1	38.1	**40.4**
SD (ppb)	0.4	0.5	26.6	**27.4**	1.2	0.9	41.1	**42.8**	0.7	0.8	26.2	**27.2**
CV (%)	71.4	87.2	133.2	**129.4**	54.9	67.4	57.8	**57.4**	57.2	69.6	68.8	**67.2**
Min (ppb)	0.4	0.3	0.3	**1.0**	0.4	0.3	11.5	**12.2**	0.4	0.3	9.0	**10.2**
Median (ppb)	0.4	0.3	8.6	**9.2**	1.9	1.2	66.3	**69.6**	1.0	1.0	32.4	**35.1**
75th (ppb)	0.8	0.8	34.1	**35.2**	2.7	1.4	93.8	**97.7**	1.5	1.4	45.9	**50.1**
90th (ppb)	1.4	1.4	47.4	**50.2**	3.5	2.8	128.8	**134.9**	2.0	1.9	66.0	**70.1**
95th (ppb)	1.5	1.8	71.0	**74.0**	4.6	3.1	139.0	**144.0**	3.0	2.1	109.2	**113.1**
Max (ppb)	1.8	2.0	123.4	**127.0**	4.7	3.7	164.0	**170.6**	3.2	4.5	112.5	**117.3**
% of Mean Total	**2.8**	**2.8**	**94.3**		**2.8**	**1.7**	**95.4**		**3.1**	**2.7**	**94.3**	

Table 6-4. Extrafloral Nectar: Cumulative distributional statistics for concentration of imidacloprid and related metabolites in cotton extrafloral nectar (ppb). Acronyms in the table are: IMI=Imidacloprid; Olefin=Imidacloprid Olefin; 5-OH=5 Hydroxy Imdacloprid; Total Imidacloprid Residue (in bold) = Sum of parent and degrades; N=Number of observations; SD=Standard Deviation; CV=Coefficient of Variation. Numbered Interval denotes timing of sampling where interval 1 was approximately 81 days after the first soil application and intervals 2 and 3 were at 5 and 14 days after a third foliar spray coinciding with 91 and 100 days after the soil application.

ExtraFloral Nectar: Distribution of Imidacloprid Residues by Interval Sampled												
	Interval 1				Interval 2				Interval 3			
Statistic	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**
N	28.0	28.0	28.0	**28.0**	33.0	33.0	33.0	**33.0**	33.0	33.0	33.0	**33.0**
Mean (ppb)	0.5	0.5	5.6	**6.6**	15.2	2.9	543.0	**561.5**	1.6	0.6	37.4	**39.7**
SD (ppb)	0.3	0.4	7.9	**8.1**	16.5	3.3	603.2	**622.2**	1.1	0.7	31.6	**32.7**
CV (%)	69.0	79.7	140.0	**122.2**	108.8	117.4	111.0	**110.1**	70.5	115.5	84.5	**82.5**
Min (ppb)	0.4	0.3	0.2	**1.1**	3.2	0.8	43.8	**47.3**	0.4	0.3	5.2	**7.6**
Median (ppb)	0.4	0.3	1.9	**3.3**	8.3	1.5	266.9	**276.4**	1.3	0.3	25.1	**26.7**
75th (ppb)	0.4	0.7	6.8	**7.9**	20.8	2.7	584.0	**607.9**	1.9	0.7	43.2	**45.6**
90th (ppb)	1.0	1.2	17.9	**19.3**	36.9	6.0	1426.5	**1470.4**	3.5	1.1	85.6	**90.8**
95th (ppb)	1.4	1.6	18.3	**19.3**	53.9	8.5	1819.7	**1881.5**	4.1	2.0	110.5	**113.2**
Max (ppb)	1.6	1.9	34.1	**35.9**	77.0	17.5	2680.0	**2774.5**	5.0	4.1	130.4	**136.1**
% of Mean Total	**7.1**	**7.9**	**84.8**		**2.7**	**0.5**	**96.7**		**4.0**	**1.5**	**94.2**	

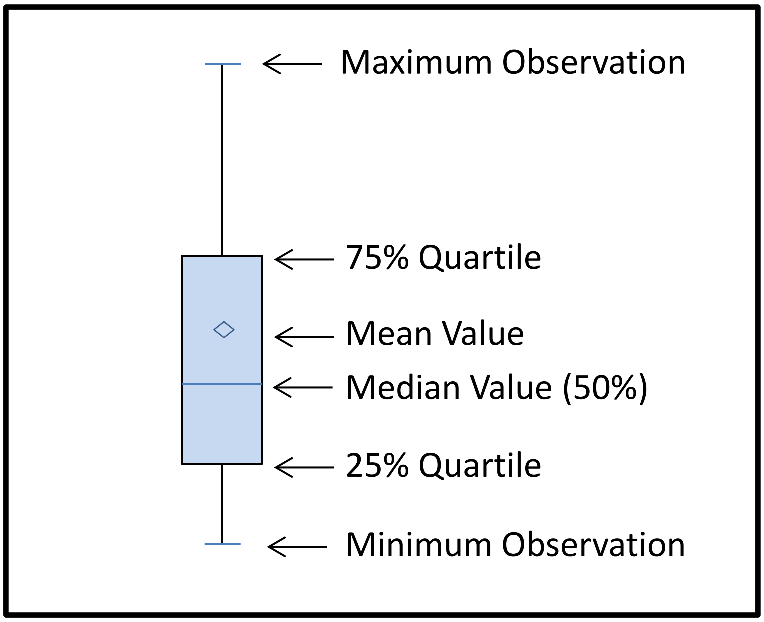
Table 6-5. Pollen: Cumulative distributional statistics for concentration of imidacloprid and related metabolites in cotton pollen (ppb). Acronyms in the table are: IMI=Imidacloprid; Olefin=Imidacloprid Olefin; 5-OH=5 Hydroxy Imdacloprid; Total Imidacloprid Residue (in bold) = Sum of parent and degrades; N=Number of observations; SD=Standard Deviation; CV=Coefficient of Variation. Numbered Interval denotes timing of sampling where interval 1 was approximately 81 days after the first soil application and intervals 2 and 3 were at 5 and 14 days after a third foliar spray coinciding with 91 and 100 days after the soil application.

Pollen: Distribution of Imidacloprid Residues by Interval Sampled												
	Interval 1				Interval 2				Interval 3			
Statistic	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**
N	34.0	34.0	34.0	**34.0**	34.0	34.0	34.0	**34.0**	34.0	34.0	34.0	**34.0**
Mean (ppb)	0.3	0.3	5.0	**5.6**	4.0	1.6	252.4	**258.0**	0.9	0.6	48.8	**50.2**
SD (ppb)	0.2	0.6	10.1	**10.2**	9.5	3.4	568.2	**580.9**	1.0	0.7	57.0	**58.4**
CV (%)	76.3	209.5	203.6	**184.1**	237.6	206.6	225.1	**225.1**	120.5	115.5	116.9	**116.3**
Min (ppb)	0.3	0.2	0.2	**0.6**	0.3	0.2	3.4	**3.8**	0.3	0.2	2.5	**2.9**
Median (ppb)	0.3	0.2	0.5	**1.0**	0.9	0.2	41.9	**45.5**	0.3	0.2	14.0	**14.6**
75th (ppb)	0.3	0.2	3.9	**5.3**	2.7	1.5	197.5	**200.9**	1.2	0.8	92.9	**94.4**
90th (ppb)	0.3	0.2	14.7	**15.8**	5.5	3.9	401.4	**409.0**	2.2	1.6	146.9	**150.0**
95th (ppb)	0.8	8.0	37.9	**38.9**	34.5	10.6	1682.2	**1727.3**	3.7	2.3	160.0	**165.5**
Max (ppb)	1.5	3.3	42.5	**43.4**	44.4	15.5	2846.3	**2906.2**	4.0	2.3	175.9	**182.2**
% of Mean Total	**5.7**	**4.6**	**89.3**		**1.6**	**0.6**	**97.8**		**1.8**	**1.2**	**97.2**	

Table 6-6. Leaves: Cumulative distributional statistics for concentration of imidacloprid and related metabolites in cotton leaves (ppb). Acronyms in the table are: IMI=Imidacloprid; Olefin=Imidacloprid Olefin; 5-OH=5 Hydroxy Imdacloprid; Total Imidacloprid Residue (in bold) = Sum of parent and degrades; N=Number of observations; SD=Standard Deviation; CV=Coefficient of Variation. Numbered Interval denotes timing of sampling where interval 1 was approximately 81 days after the first soil application and intervals 2 and 3 were at 5 and 14 days after a third foliar spray coinciding with 91 and 100 days after the soil application.

Leaves: Distribution of Imidacloprid Residues by Interval Sampled												
	Interval 1				Interval 2				Interval 3			
Statistic	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**	5-OH	Olefin	IMI	**Total**
N	34.0	34.0	34.0	**34.0**	34.0	34.0	34.0	**34.0**	34.0	34.0	34.0	**34.0**
Mean (ppb)	3.4	2.5	39.1	**45.0**	103.1	41.3	1493.4	**1637.9**	43.4	26.9	287.0	**357.9**
SD (ppb)	4.8	3.7	59.5	**67.6**	35.1	16.7	649.7	**41.5**	16.7	10.5	167.0	**51.5**
CV (%)	142.8	146.2	151.9	**150.1**	34.0	40.3	43.5	**481.5**	37.9	38.9	58.2	**56.8**
Min (ppb)	0.4	0.4	0.6	**1.4**	53.9	16.0	401.4	**1497.0**	14.7	5.4	38.0	**321.4**
Median (ppb)	1.9	0.7	20.7	**23.9**	91.6	22.0	1352.9	**1956.1**	42.5	28.7	233.1	**440.4**
75th (ppb)	4.2	3.5	39.2	**46.5**	121.1	42.3	1776.0	**2745.7**	56.6	32.6	378.3	**3651.9**
90th (ppb)	9.3	4.7	103.5	**119.3**	172.3	47.6	2554.8	**2992.7**	64.6	37.0	561.9	**708.3**
95th (ppb)	12.0	10.7	132.5	**154.3**	173.0	77.1	2869.4	**3203.7**	70.5	45.2	620.9	**719.3**
Max (ppb)	24.3	17.1	316.5	**357.9**	196.6	79.7	3043.9		88.7	50.8	625.6	
% of Mean Total	**7.6**	**5.6**	**86.9**		**6.3**	**2.5**	**91.2**		**12.1**	**7.5**	**80.2**	

Figure 6-1. Explanation of statistical meaning of the Box-and-Whisker plots.



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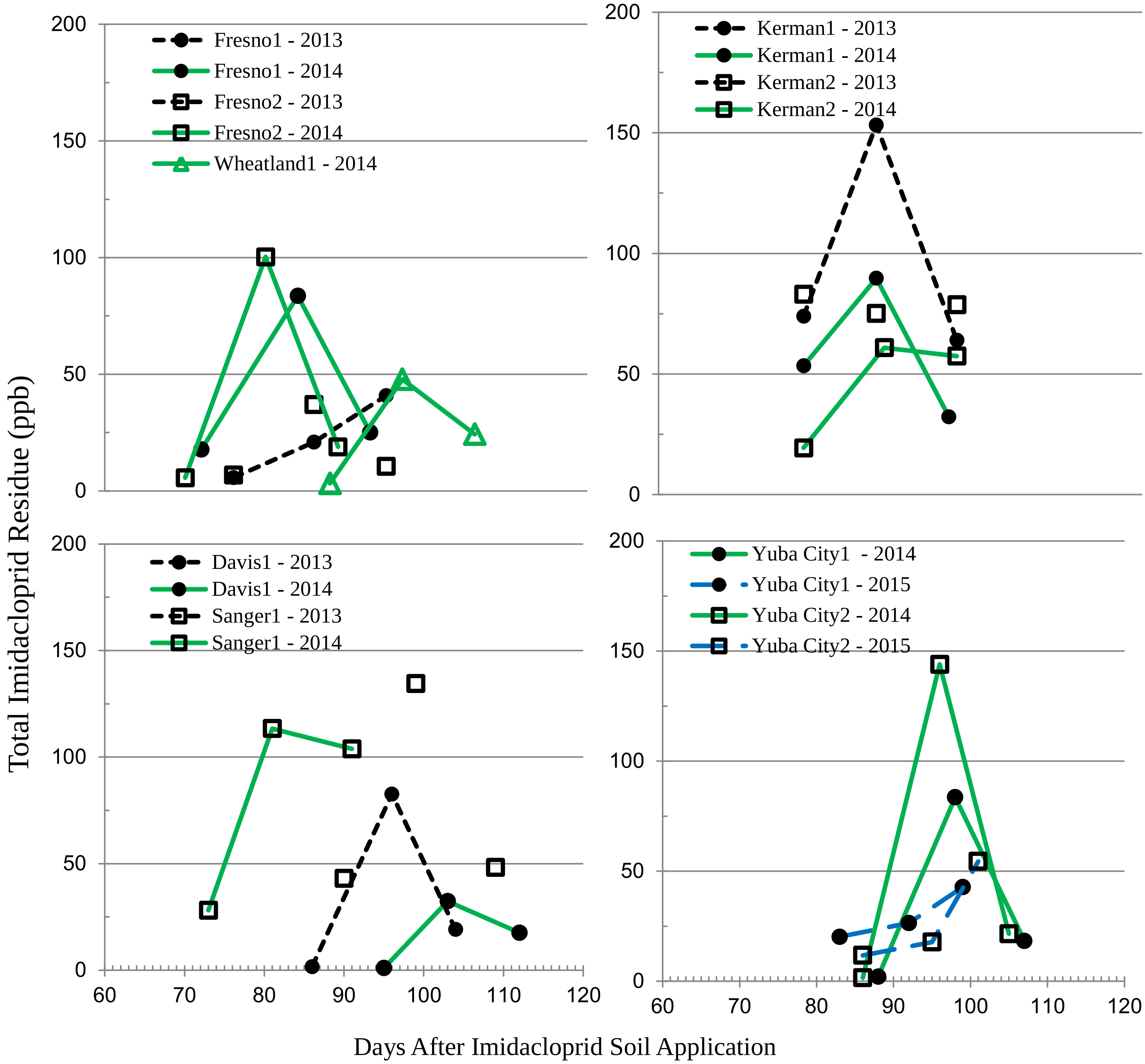
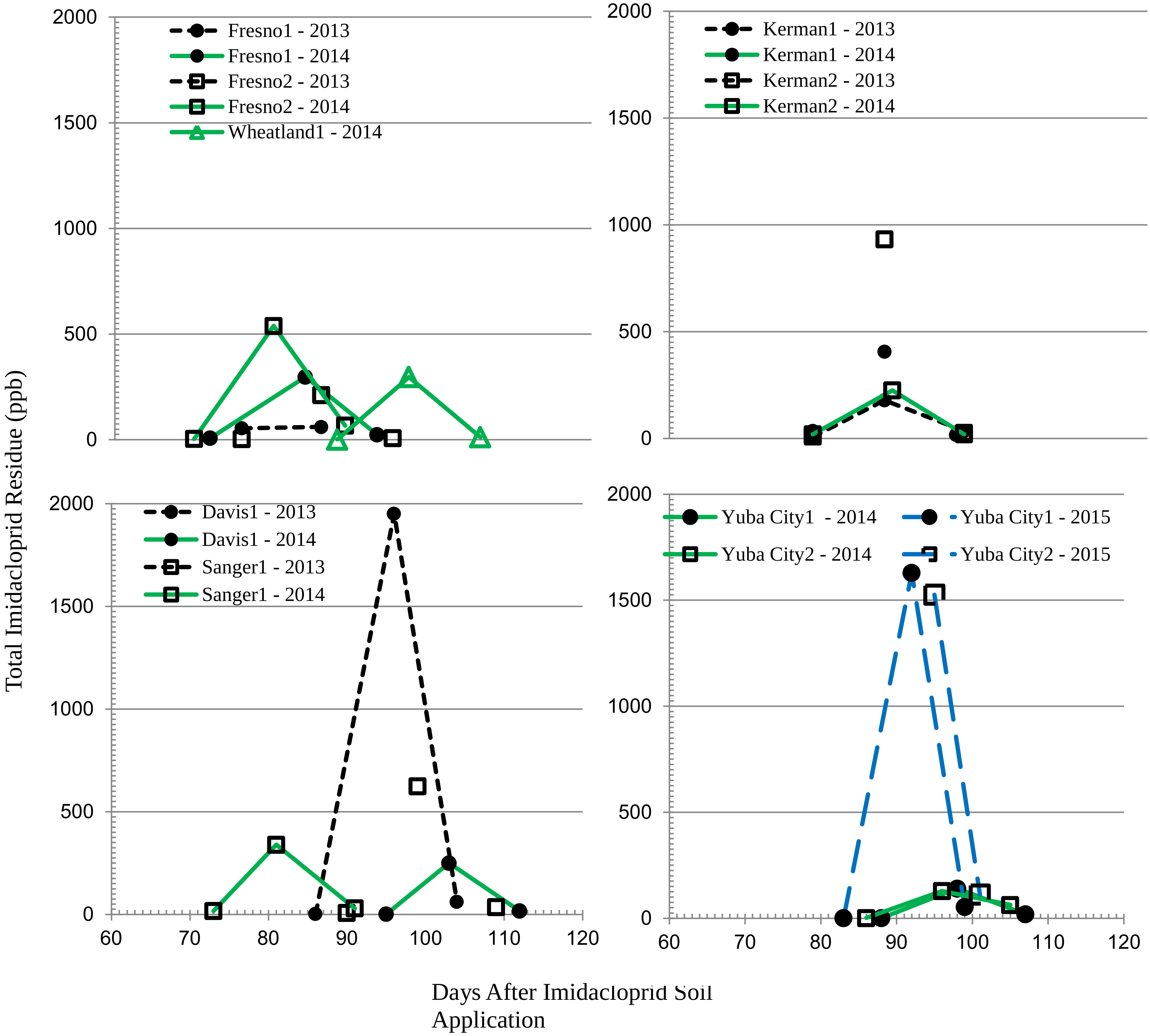
igure 6-2. Floral Nectar: Trend in total imidacloprid residue measured in floral nectar at each site. Within each panel, markers denote the site. For example, Fresno site 1 are solid circles and Fresno site 2 are open squares and Wheatland site 1 are open triangles. Years are reflected by the color and style of lines where black small-dashed lines are 2012, green solid lines are 2014 data, and blue large-dashed lines are 2015 data.



Specific Site Information:
Fresno1=NT003-13ZA; Fresno2=NT007-13ZA
Wheatland=NT002-13ZB;
Davis1=NT004-13ZA; Sanger1=NT006-13ZA

Specific Site Information:
Kerman1=NT008-13ZA; Kerman2=NT009-13ZA
Yuba City1=NT001-13ZB; Yuba City2=NT005-13ZB

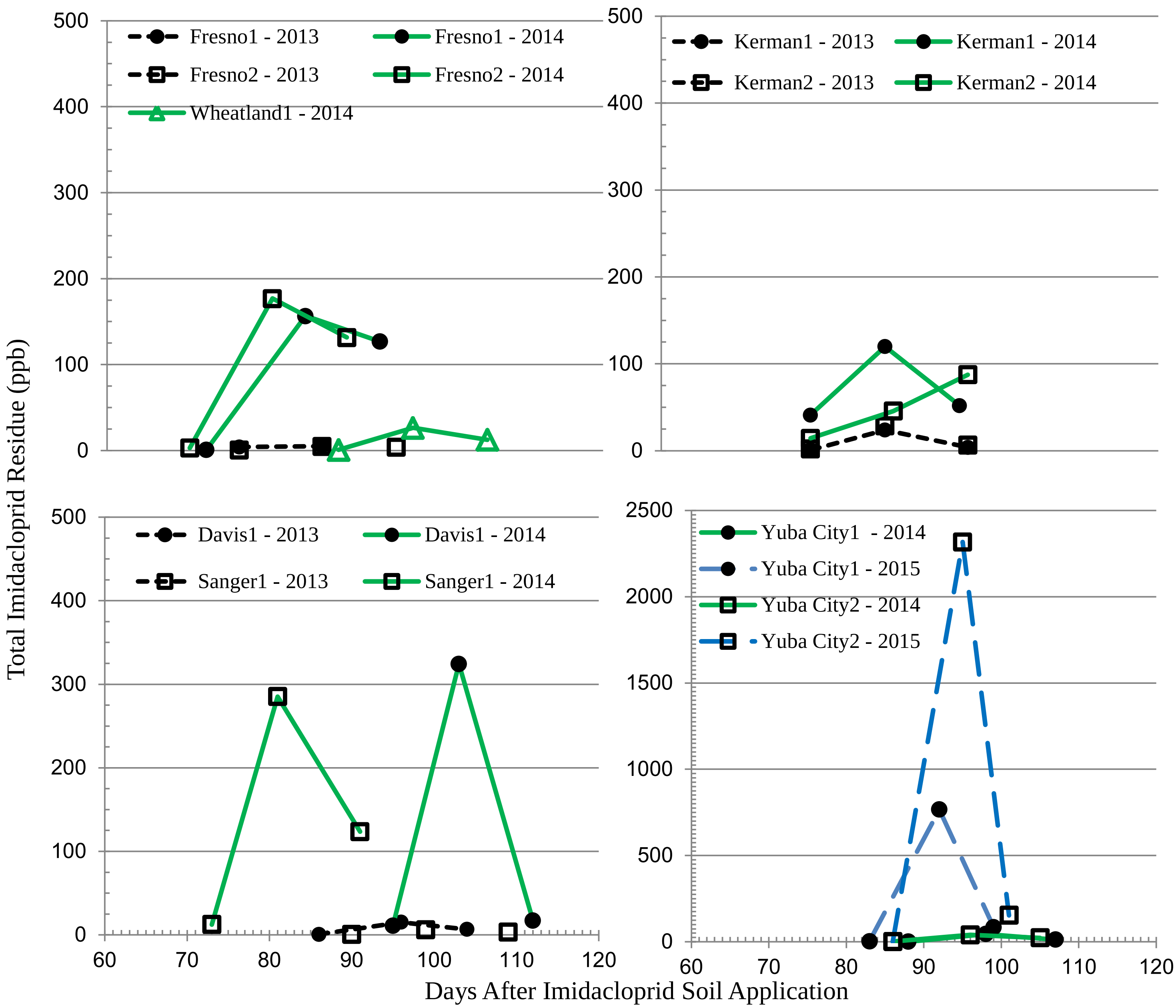
Figure 6-3. Extrafloral Nectar: Trend in total imidacloprid residue measured in extrafloral nectar at each site. Within each panel, markers denote the site. For example, Fresno site 1 are solid circles and Fresno site 2 are open squares and Wheatland site 1 are open triangles. Years are reflected by the color and style of lines where black small-dashed lines are 2012, green solid lines are 2014 data, and blue large-dashed lines are 2015 data.



<u>Specific Site Information:</u>
Fresno1=NT003-13ZA; Fresno2=NT007-13ZA
Wheatland=NT002-13ZB;
Davis1=NT004-13ZA; Sanger1=NT006-13ZA

<u>Specific Site Information:</u>
Kerman1=NT008-13ZA; Kerman2=NT009-13ZA
Yuba City1=NT001-13ZB; Yuba City2=NT005-13ZB

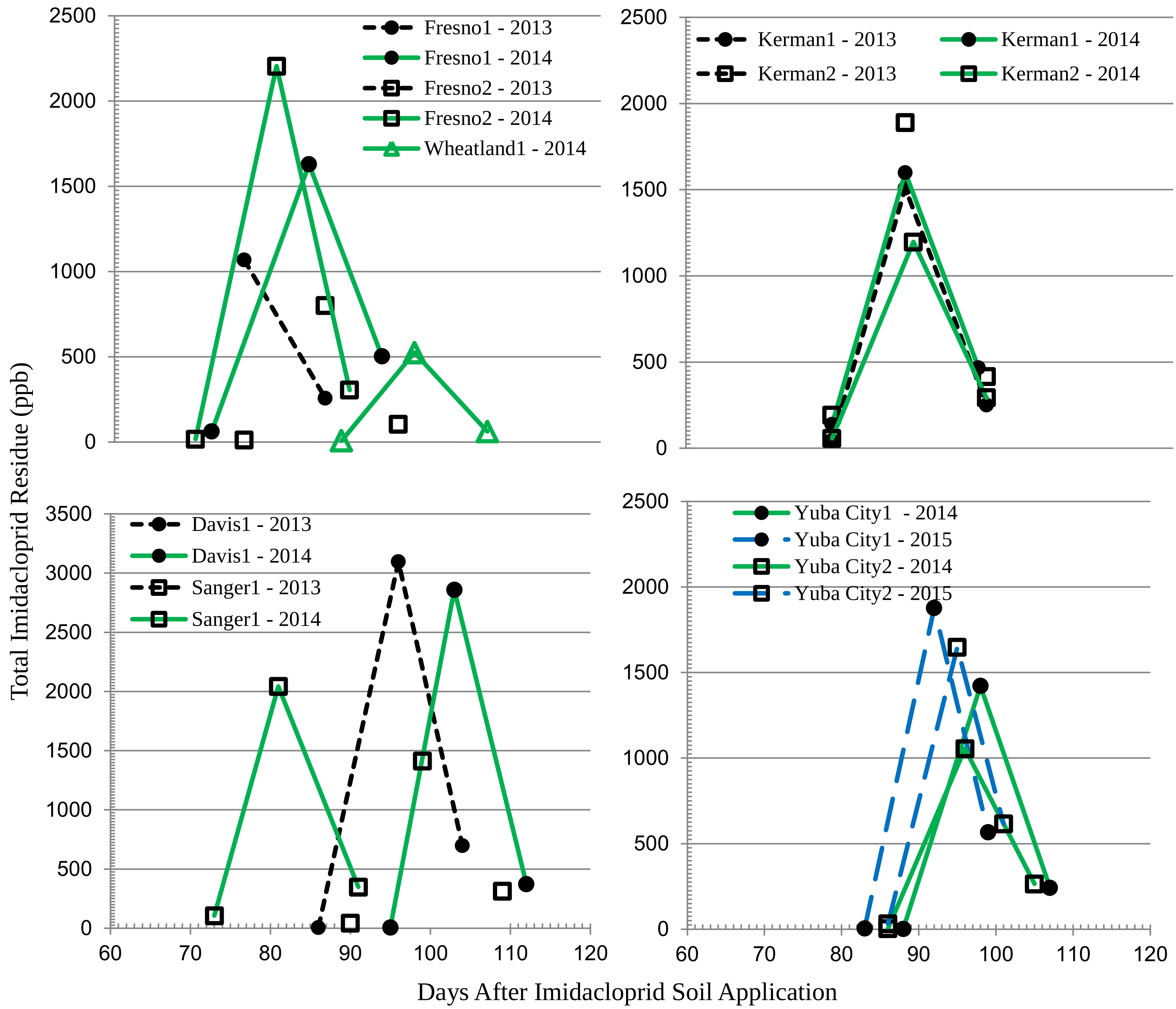
Figure 6-4. Pollen: Trend in total imidacloprid residue measured in pollen at each site. Within each panel markers denote the site, for example Fresno site 1 are solid circles and Fresno site 2 are open squares and Wheatland site 1 are open triangles. Years are reflected by the color and style of lines where black small-dashed lines are 2012, green solid lines are 2014 data, and blue large-dashed lines are 2015 data. Note the larger scale in the plots for the Yuba City sites.



<u>Specific Site Information:</u>
Fresno1=NT003-13ZA; Fresno2=NT007-13ZA
Wheatland=NT002-13ZB;
Davis1=NT004-13ZA; Sanger1=NT006-13ZA

<u>Specific Site Information:</u>
Kerman1=NT008-13ZA; Kerman2=NT009-13ZA
Yuba City1=NT001-13ZB; Yuba City2=NT005-13ZB

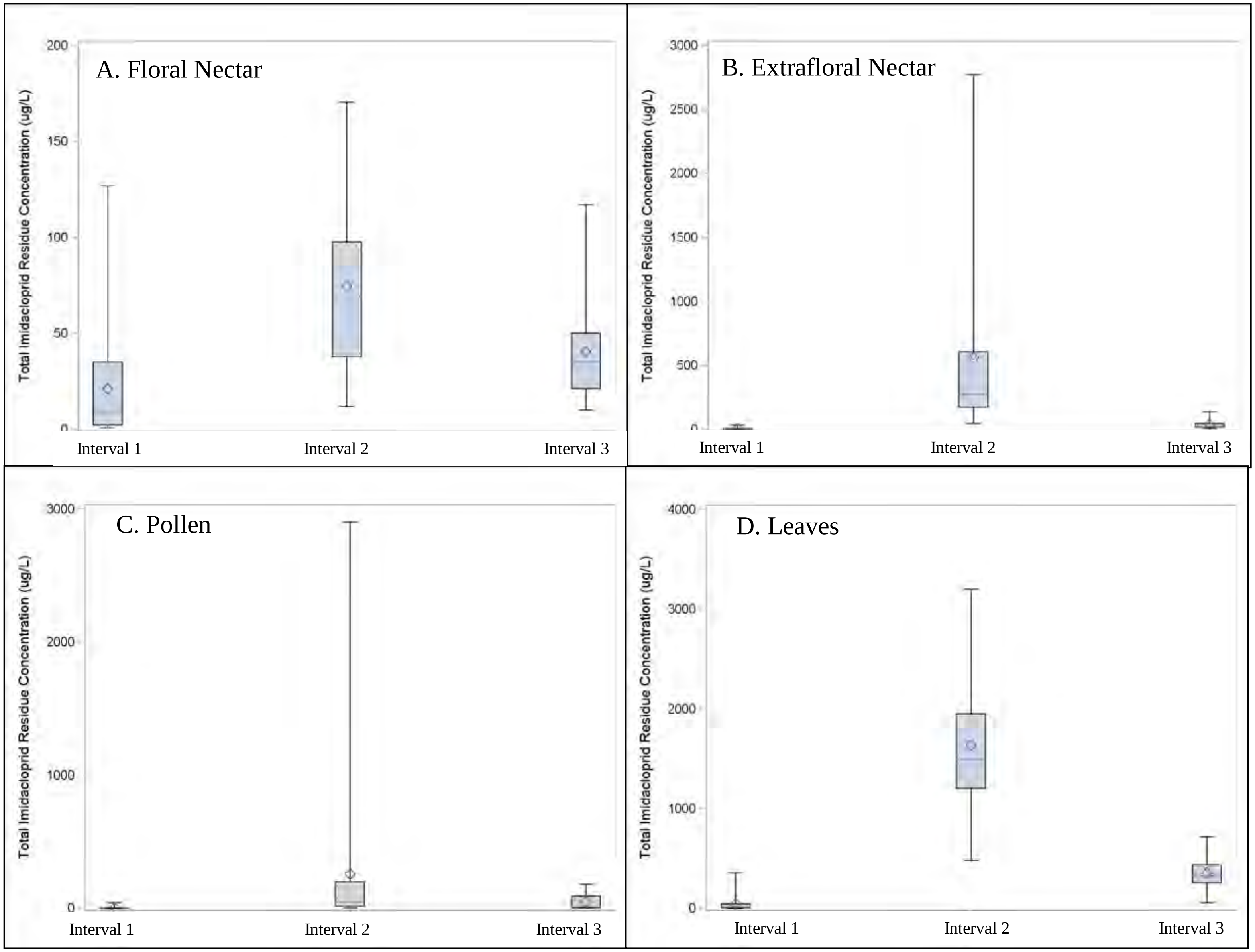
Figure 6-5. Leaves: Trend in total imidacloprid residue measured in leaves at each site. Within each panel, markers denote the site. For example, Fresno site 1 are solid circles and Fresno site 2 are open squares and Wheatland site 1 are open triangles. Years are reflected by the color and style of lines where black small-dashed lines are 2012, green solid lines are 2014 data, and blue large-dashed lines are 2015 data. Note the larger scale in the plots for the Davis site.



<u>Specific Site Information:</u>
Fresno1=NT003-13ZA; Fresno2=NT007-13ZA
Wheatland=NT002-13ZB;
Davis1=NT004-13ZA; Sanger1=NT006-13ZA

<u>Specific Site Information:</u>
Kerman1=NT008-13ZA; Kerman2=NT009-13ZA
Yuba City1=NT001-13ZB; Yuba City2=NT005-13ZB

Figure -6-6. Relative distribution of concentration of total imidacloprid residues compared between intervals as measured in (A) Floral Nectar; (B) Extrafloral Nectar; (C) Pollen; and (D) Leaves. Data were averaged over all sites.



U.S. EPA Data Evaluation Reports (Imidacloprid):

U.S. EPA. (2016). Data evaluation report: determination of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from blueberries following soil application of imidacloprid over two successive years. Washington, D.C.: Author. Laboratory Report Number EBNTY006.

U.S. EPA. (2016). Data evaluation report: determination of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from cherry trees following foliar application of imidacloprid over two successive years. Washington, D.C.: Author. Laboratory Report Number EBNTY008.

U.S. EPA. (2016). Data evaluation report: admire pro - magnitude of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from citrus trees following foliar applications of imidacloprid over two successive years. Washington, D.C.: Author. Laboratory Report Number EBNTY007.

U.S. EPA. (2016). Data evaluation report: determination of the residues of imidacloprid and its metabolites 5-hydroxy imidacloprid and imidacloprid olefin in bee relevant matrices collected from treated cotton during two successive years and in white clover planted after treated cotton. Washington, D.C.: Author. Laboratory Report Number EBNTY010.

Clothianidin Data Evaluations (begin on next page)

Reference
Rose, A. (2015) Clothianidin: Quantitation of Residues of Clothianidin, TZNG, and TZMU in Leaves, Nectar, Pollen and Soil Following Soil Application of Belay Insecticide to Cucurbits: Final Report. Project Number: VP-38263. Unpublished study prepared by Valent U.S.A. Corporation and Rose Consulting. 1103. MRID 49602801, CDPR Study ID 283866, Data Volume 52884-0245, Tracking ID# 269547

1. STUDY INFORMATION

Chemical:	Clothianidin	**PC Code**	44309
Test Material:	Belay Insecticide	**Percent Active Ingredient:**	23.0%
Study Type:	Field residue study on pumpkin crops to meansure clothianidin and its metabolite levels in soil, leaves, nectar and pollen after a single soil application per year for three years.		
Sponsor:	Valent U.S.A. Corporation 1600 Riviera Ave., Suite 200 Walnut Creek, U.S.A. 94596	**Experiment Start and End Date:**	June 12, 2012 – December 12, 2014
Sponsor Study ID:	VP-38263	**Study Locations:**	A total of 12 pumpkin trial sites in multiple locations throughout California.
Study Completion Date:	March 16, 2015		
GLP Status:	Non Good Laboratory Practice; protocols reviewed by CDPR. [CDPR Study ID 265308, Data Volume 52884-0174, Tracking ID#253142 *REVISED* CDPR Study ID 266052, Data Volume 52884-0175, Tracking ID# 254177]		

2. REVIEWER INFORMATION

Study Reviewed by: California Department of Pesticide Regulation	Richard Bireley, Sr. Environmental Scientist (Specialist) John Troiano, Ph.D., Research Scientist III Alexander Kolosovich, Environmental Scientist Brigitte Tafarella, Environmental Scientist Russell Darling, Environmental Scientist Denise Alder, Sr. Environmental Scientist (Specialist)

Study Reviewed by: United States Environmental Protection Agency	Michael Wagman, Biologist, EPA/EFED/ERB6 Amy Blankinship, Senior Scientist, EPA/EFED/ERB6 **EPA Reviewer Comments:** EPA considers the study to be scientifically sound and it is classified as **Acceptable**

3. EXECUTIVE SUMMARY

The objective of this study was to quantify the extent to which insect pollinators may be exposed to clothianidin and its degradates thiazolylnitroguanidine (TZNG) and thiazolylmethylurea (TZMU) following applications of Belay® Insecticide to cucurbits. This was accomplished by measuring residue concentrations of these chemicals in leaves, nectar and pollen from pumpkin (a cucurbit) flowers. The study continued for a total of 3 years to examine possible year-over-year accumulation of clothianidin and clothianidin-related residues in soil and their possible uptake by subsequent (second and third year) cucurbit crops.

Belay® Insecticide (active ingredient, clothianidin) was applied with pumpkin (*Cucurbita pepo L.*) seeding to 9 field sites in California. At 3 of the 9 sites a second use pattern (application to pumpkin plants at BBCH growth stage 201-229) was tested. Single applications were applied to the soil (chemigation or in-furrow) of all trial sites at the maximum product label-allowed rate of 0.2 lb. a.i./acre. Plants were grown following local agronomic practice. When the plants were in full bloom, around BBCH growth stage 605, leaf punches and male flowers were collected. Floral nectar and pollen were collected and processed from the flowers. In study years 2 and 3, soil cores (0-12 inch soil horizon) were collected. All samples were stored and remained frozen pending residue analysis.

Leaf punches, nectar, pollen and soil were analyzed by LC-MS/MS using validated analytical methods.

4. STUDY VALIDITY

Guideline Followed:	Non-guideline study (protocol was reviewed by CDPR)
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	**ACCEPTABLE**
Rationale:	N/A
Reparability:	N/A

5. MATERIALS AND METHODS

Test Material Characterization			
Test item:	Belay Insecticide	**Percent Active Ingredient:**	23.0% A.I.
Description:	Soluble Concentrate (SC)	**Molecular Formula:**	$C_6H_8ClN_5O_2S$
Material Source:	Valent U.S.A. Corporation	**Molecular Weight:**	205.68 g/mol
CAS #:	210880-92-5	**Valent Lot Number:**	AS 2351a

5A. STUDY DESIGN

Belay® Insecticide is currently registered for foliar and soil use on cucurbits in California. Residue data for clothianidin in nectar and pollen collected from cucurbit plants and clothianidin in soil was requested by California Department of Pesticide Regulation (CDPR). Interim reports (report dates May 17, 2013 and February 27, 2014) were submitted to CDPR. In addition, portions of this study were published[1].

Pumpkin (Cucurbita pepo L.) seeds were purchased by the field Principal Investigators and planted at the time of the Belay® Insecticide application or planted for a later season application (plants at BBCH growth stage 201 to 229; side shoots visible on main stem). Although at the same field site, separate plots were used for the at-planting and late season applications of Belay® Insecticide. Seeds were either hand planted (Sites 1, 2, 5, 6, 7, and 9) or planted by a single row planter (sites 3, 4, and 8).

Pumpkin plants were thinned as necessary so that the distance between plants was 2 to 3 feet. Plants were irrigated as necessary to maintain a healthy crop. Irrigation was applied by subsurface drip tape or by furrow flood irrigation. Occasionally some insect pests were observed and were controlled by application of non-neonicotinoid insecticides such as Sevin, Pristine, or Asana.

Mature pumpkins were removed from the plot and irrigation stopped. The vines were allowed to desiccate in the plot, and then the plot was cultivated to a depth of about 6 inches. Cultivation mixed the soil and likely distributed clothianidin residues more evenly across the plot. Plots remained fallow pending planting of the same pumpkin variety in 2013 and 2014.

5B. APPLICATION TIMING AND RATES

Belay® Insecticide was applied by chemigation (subsurface drip tape) at sites 1, 2, 5, 6, 7, and 9 on the same day that the pumpkin seeds were planted. At sites 3, 4, and 8 seeds were planted in a furrow, Belay® Insecticide sprayed into the furrow (using a C02 canister powered single, hand-held flat fan nozzle), then the furrow covered with soil. At 3 of the 9 sites a second use pattern application to pumpkin plants at BBCH growth stage 201-229 was tested.

Belay® Insecticide was applied in a single application per year at an application rate of 0.2 lb. a. i./acre at all sites.

Table 1a. Application Method and Application Dates for At-Planting Belay Insecticide on Pumpkins.

Site Number	Application Method	Application Date		
		Year 1	Year 2	Year 3
1	Chemigation - subsurface drip	7/12/2012	8/9/2013	6/20/2014
2	Chemigation - subsurface drip	7/13/2012	7/31/2013	6/23/2014
3	In-furrow spray	6/26/2012	6/20/2013	5/15/2014
4	In-furrow spray	6/26/2012	6/20/2013	5/15/2014
5	Chemigation-subsurface drip	7/11/2012	7/12/2013	6/2/2014
6	Chemigation - subsurface drip	7/6/2012	7/12/2013	6/24/2014
7	Chemigation - subsurface drip	7/5/2012	7/12/2013	6/23/2014
8	In-furrow spray	7/6/2012	6/26/2013	5/14/2014
9	Chemigation - subsurface drip	7/6/2012	7/9/2013	6/23/2014

Table 1b. Application Method and Application Date for Late Season Belay Insecticide Applications.

Site Number	Application Method	Application Date		
		Year 1	Year 2	Year 3
1a	Chemigation - subsurface drip	8/15/2012	9/12/2013	7/20/2014
4a	In-furrow subsurface shank	7/27/2012	8/9/2013	7/11/2014
7a	Chemigation - subsurface drip	7/25/2012	8/23/2013	7/17/2014

5C. Study Site Location and Characteristics

Belay® Insecticide (active ingredient, clothianidin) was applied concomitant with pumpkin (Cucurbita pepo L.) seeding to 9 field sites in California. The soil characterization data and field site location are summarized in Table 2.

Table 2. Field Site Locations and Soil Series, Characterization and Organic Matter.

Site Number	Field Site County	Soil Texture	Soil Series & Texture	Soil Characterization (%Sand/Silt/Clay)	Percent Organic Matter
1	Fresno	Medium	Ramona Loam	65/24/11	0.43
2	Fresno	Coarse	Hanford Coarse Sandy Loam	84/13/3	0.34
3	Madera	Coarse	Awater Loamy Sand	85/10/5	0.81
4	Madera	Coarse	Atwater Sandy Loam	73/16/11	1.08
5	San Luis Obispo	Medium	Nacimieno-Los Osos complex, Loam	45/31/24	1.6
6	Tulare	Coarse	Nord Sandy Loam	69/24/7	1.2
7	Tulare	Fine	Centerville Clay	31/25/44	2.1
8	Madera	Coarse	Grangeville Loamy Sand	77/16/7	1.5
9	Tulare	Medium	Centerville Sandy Clay Loam	50/17/33	3.7

5D. Sample Collection, Handling, Processing

Collection of Soil Coarse

Soil cores were collected prior to Belay® Insecticide applications in study years 2 and 3. For soil coring, each treatment plot was divided into 3 approximately equal size subplots (Subplots 1, 2, and 3) then each of the subplots divided into 16 approximately equal size sectors (numbered 1 through 16). Seven sectors were randomly selected for coring. Soil cores (0-12 inch horizon) in plastic sleeves were taken with a slide hammer or tractor-mounted Giddings hydraulic soil probe. The seven soil cores from each sector were combined in the field to give a total of three replicates per plot (field site). Soil cores were stored in temperature-monitored freezers at the field site until they were shipped frozen to the Analytical Laboratory.

Collection of Leaf Punches and Flowers

Leaf punches and male flowers were collected when the field was in full bloom, generally around BBCH growth stage 605 (also referred to as stage 61; 51h flower open on main stem). Approximately 100 male flower buds were indiscriminately selected the afternoon before anthesis occured and covered with paper bags in order to prevent visits from insects. Male flowers can be easily distinguished from female flowers by their much longer and thinner styles. The following day, on the day of sampling, duplicate leaf punch samples were collected. Two punches were collected from leaves near 25 of the 100 covered male flowers (total 50 leaf punches; except 55 leaf punches Site 2 in year 1). Leaf punches were placed into tared, labeled Ziploc plastic bags and the gross weight recorded. Leaf punch samples were transported to the laboratory in coolers containing Blue Ice (or equivalent).

Duplicate samples of covered flowers were collected and transported to the laboratory in coolers containing Blue Ice (or equivalent). The number of flowers collected and their weights were recorded. In general, each field site indiscriminately collected 35 to 75 flowers per replicate.

Nectar and Pollen Samples

Flowers were processed on the day they were harvested from the field plot. Each flower was cut around the sepals, then the sepals and petals were removed (discarded) to expose the receptacle and nectary and the single pollen-laden stamen. Nectar was collected from the cavity within the receptacle with a glass pipette. The nectar was transferred into a label and tared glass vial. Nectar extraction from multiple flowers continued until at least 0.5 g of nectar had been collected. The weight of the vial, plus the nectar, was recorded. A scalpel or razor blade was used to scrape pollen from the filament. Pollen was collected from the same flowers from which nectar was collected. Pollen was transferred to a tared, label vial and the weight of the vial, plus the pollen, was recorded. The number of flowers processed was recorded. The flower dissection, nectar extraction and pollen collection was repeated with the second replicate of flowers. After processing, the flowers were discarded.

Sample Storage

Soil cores were placed into coolers in the field and stored in freezers at the field sites pending shipment to the Analytical Laboratory. Leaf punch samples were hand carried to the laboratory in coolers. Once in the laboratory, the samples were placed into freezers (temperature <0°F) at each field site.

Immediately after final weights were recorded, the labeled sample vials containing nectar and pollen were placed into a freezer (temperature <0°F) at each field site where they remained until they were shipped to the analytical laboratory.

Samples were packaged for shipment by the field Principal Investigators. Chain of Custody forms were prepared and accompanied the samples. Samples were shipped by freezer truck (Agricultural Chemical Delivery Services, Inc. (ACDS, Inc.)) or by overnight delivery (United Parcel Service or FedEx). Samples for overnight delivery were packaged in a cooler containing Blue Ice to keep the samples cold.

Once at the Valent Technical Center, samples were placed into a temperature monitored, walk-in freezer until analyzed.

5E. Analytical Methods

Methods used in this analytical study were Valent U.S.A. Corporation's methods RM-39N-l, RM-39Pl, RM-39S-l, RM-39S-2, RM-39L-l, RM-39L-2.

Samples of pumpkin nectar were dissolved in methanol/water (40:60, v/v) acidified with 0.05% formic acid and analyzed by LC/MS-MS after spiking with isotopically labeled internal standards (d_3 clothianidin, $^{13}C/^{15}N$-TZNG, and d_3TZMU) to compensate for matrix effect (method RM-39N-l). The limit of detection (LOD) was 0.2 ppb, and the limit of quantitation (LOQ) was 1 ppb for clothianidin and its metabolites, TZNG and TZMU and reported in Table 3 below.

Samples of pumpkin pollen were extracted with water and acetonitrile followed by adding sodium chloride and anhydrous magnesium sulfate salts. The acetonitrile extract was partitioned with n-hexane, and the acetonitrile phase was collected and concentrated to dryness. Residues were re-dissolved in methanol/water (40:60, v/v) acidified with 0.05% formic acid and analyzed by LC/MS-MS after spiking with isotopically labeled internal standards (d_3 clothianidin, $^{13}C,^{15}N$-TZNG, and d_3 TZMU) to compensate for matrix effect (method RM-39P-l). Using this method, the LOD was 0.25 ppb, and the LOQ was 1 ppb for clothianidin and its metabolites, TZNG and TZMU.

Residues in leaves were measured using two methods. The first method measured clothianidin concentration in leaves. Leaf samples were extracted with methanol and water (40:60, v/v) acidified with 0.05% formic acid and analyzed using an accurate mass UPLC/Q-TOF MS-MS after spiking with isotopically labeled internal standard (d_3 clothianidin) to compensate for matrix effect (method RM39L-l). The LOD in this method was 2.5 ppb, and the LOQ was 5 ppb for clothianidin. This method was superseded following discussions with regulatory authorities to include determination of clothianidin and its major metabolites, TZNG and TZMU in leaves. Leaf samples were extracted with water acidified with 0.05% formic acid and acetonitrile followed by adding sodium chloride and anhydrous magnesium sulfate salts. An aliquot of the acetonitrile extract was cleaned through a Strata Cl8-E column and concentrated to dryness. Residues were re-dissolved in water/methanol (75:25, v/v) acidified with 0.05% formic acid and analyzed by accurate mass UPLC/Q-TOF MS-MS after spiking with isotopically labeled internal standard (d_3 clothianidin, $^{13}C,^{15}N$-TZNG, and d_3 TZMU) to compensate for matrix effect (method RM-39L-2). Using this method, the LOD was 1.3 ppb, and the LOQ was 5 ppb for clothianidin and its metabolites, TZNG and TZMU.

To measure “total” clothianidin and its major metabolites, TZNG and TZMU in soil, soil samples were extracted with water and methanol (75:25, v/v) acidified with 0.05% formic acid and analyzed using an accurate mass UPLC/Q-TOF MS-MS after spiking with isotopically labeled internal standards (d_3 clothianidin, $^{13}C,^{15}N$-TZNG, and d_3 TZMU) to compensate for matrix effect. The LOD was 1.3 ppb, and the LOQ was 5 ppb for clothianidin, TZNG and TZMU in this method.

To estimate the "bioavailable" concentration of clothianidin, soil samples with total clothianidin concentration greater than the LOQ were shaken with 0.01 M calcium chloride solution for 24 hours, and an aliquot of the sample was analyzed using an accurate mass UPLC/Q-TOF MS-MS after spiking with isotopically labeled internal standard (d_3 clothianidin) to compensate for matrix effect. The LOD is 0.3 ppb, and the LOQ is 5 ppb for clothianidin in this method.

A total of 72 pumpkin floral nectar samples, 72 pumpkin pollen samples, 58 leaf samples and 72 soil samples were analyzed for clothianidin and its metabolite residues; 48 leaf samples were analyzed only for clothianidin residues and 54 soil samples were analyzed for “bioavailable” clothianidin residues. A total of 7 soil transit stability samples were analyzed for clothianidin, 16 nectar and 16 pollen transit stability samples were analyzed for clothianidin, TZNG and TZMU.

Table 3. Summary of Limit of Quantitation and Limit of Detection.

Matrix	Analyte	LOQ (ppb, parent equivalents)	LOD (ppb, parent equivalents)
Pumpkin Soil	Clothianidin, TZNG and TZMU	5.0	1.3
Pumpkin Pollen	Clothianidin, TZNG and TZMU	1.0	0.25
Pumpkin Nectar	Clothianidin, TZNG and TZMU	1.0	0.2
Pumpkin Leaves	Clothianidin, TZNG and TZMU	5.0	1.3

5F. QUALITY ASSURANCE RESULTS

Quality assurance measures taken during the analytical phase of this study included, but were not limited to the following:

All analytical standards used in this study were kept at reduced temperature in a refrigerator or in a freezer at all times when not in use.

At least five different standard concentrations were injected within each analytical set. The concentration (ng/mL) of clothianidin and its metabolites detected in sample extracts was interpolated from the standard calibration curve. The LC/MS-MS and accurate mass UPLC/Q-TOF MS-MS systems were calibrated for each set of samples by analyzing these calibrating standard concentrations, with these standards interspersed within the analytical sequence. A second-order polynomial fit (weighted relative to 1/concentration) was then calculated from the concentrations and the detector response of the calibration standards. To verify performance, the percent difference between the actual concentration and the calculated concentration for each of the calibration standards (based on the curve) was also calculated. Each of the standards were required to be within 15% of the theoretical concentration and the coefficient of determination (r^2) of the weighted polynomial calibration curve was required be greater than or equal to 0.99. Minor exceedance of these criteria for the calibration standards were accepted for the lowest standards in some cases, however the coefficient of determination (r^2) of the weighted polynomial calibration curve was always greater than or equal to 0.99.

The reproducibility of the LC/MS-MS and accurate mass UPLC/Q-TOF MS-MS systems was verified by comparison of instrument responses obtained from the repeated analysis of a continuing standard (a mid-level calibration standard) analyzed with the study samples. The continuing calibration standards were interspersed within the samples in the analytical sequence, and the analytical sequence began and ended with a continuing calibration standard. For an analytical set (injection sequence) to be acceptable, the coefficient of variation (CV) of these responses was required to be 10% or less. Minor exceedance of these criteria for the continuing calibration standards were accepted in some cases.

Laboratory fortification samples were analyzed concurrently with each analytical set to demonstrate method performance. Laboratory fortification samples were prepared using artificial nectar and commercially available pollen when not enough untreated control pumpkin nectar or pollen were available, while untreated control soil from pumpkin fields and pumpkin leaf samples were used for soil and leaf laboratory fortification samples. Each sample set included at least one untreated control (UTC) and two laboratory fortification samples. Fortifications ranged from 1 to 10 ppb for nectar and pollen samples and 5 to 50 ppb for leaf and soil samples. Generally, concurrent fortification recoveries for clothianidin, TZNG and TZMU in the laboratory fortified samples were in the range of 70 to 120. In some cases, minor exceedance of these criteria was accepted.

6. RESULTS:

6.1 Soil Results

Table 4. Mean Concentrations of Total Clothianidin, TZNG and TZMU Residues in Pumpkin Soil following Soil Applications of Belay Insecticide At-Planting.

Site Number	Mean Concentration (ppb)[1]					
	Year 2			Year 3		
	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU
1	6.5	[0.7][2]	[0.7][2]	15.8	[0.7][2]	[0.7][2]
2	(2.5)[3]	[0.7][2]	[0.7][2]	(3.7)[3]	[0.7][2]	[0.7][2]
3	[0.7][2]	[0.7][2]	[0.7][2]	(4.6)[3]	[0.7][2]	[0.7][2]
4	(2.7)[3]	[0.7][2]	[0.7][2]	6.7	[0.7][2]	[0.7][2]
5	13.4	[0.7][2]	[0.7][2]	9.3	[0.7][2]	[0.7][2]
6	[0.7][2]	[0.7][2]	[0.7][2]	6.6	[0.7][2]	[0.7][2]
7	11.2	[0.7][2]	[0.7][2]	[0.7][2]	[0.7][2]	[0.7][2]
8	13.3	[0.7][2]	[0.7][2]	11.1	[0.7][2]	[0.7][2]
9	5.9	[0.7][2]	[0.7][2]	17.5	[0.7][2]	[0.7][2]

[1] LOQ= 5 ppb; LOD= 1.3 ppb
[2] Values in square brackets are <LOD and entered as ½ LOD
[3] Values in parenthesis are between the LOQ and the LOD

Table 5. Mean Concentrations of "Bioavailable" Clothianidin Residues in Pumpkin Soil following Soil Applications of Belay Insecticide At-Planting.

Site #	Mean Concentrations of Clothianidin (ppb)[1]	
	Year 2	Year 3
1	(4.0)[2]	10.8
2	NE[3]	NE[3]
3	NE[3]	(3.0)[2]
4	NE[3]	(2.3)[2]
5	6.7	6.3
6	NE[3]	(2.9)[2]
7	6.1	NE[3]
8	5.0	5.2
9	(3.2)[2]	9.9

[1] LOQ= 5 ppb; LOD= 1.3 ppb
[2] Values in parenthesis are between the LOQ and the LOD

[3] NE, not extracted because total clothianidin residues were <LOQ

Table 6. Mean Total Concentrations of Clothianidin, TZNG and TZMU in Pumpkin Soil following Soil Applications of Belay Insecticide at Growth Stage BBCH 201-299.

Site Number	Mean Concentrations (ppb)[1]					
	Year 2			Year 3		
	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU
1a	7.1	[0.7][2]	[0.7][2]	10.6	[0.7][2]	[0.7][2]
4a	8.0	[0.7][2]	[0.7][2]	10.5	[0.7][2]	[0.7][2]
7a	8.7	[0.7][2]	[0.7][2]	39.9	[0.7][2]	[0.7][2]

[1] LOQ= 5 ppb; LOD= 1.3 ppb
[2]Values in square brackets are <LOD and entered as ½ LOD

Table 7. Mean Bioavailable Concentrations of Clothianidin in Pumpkin Soil following Soil Applications of Belay Insecticide at Growth Stage BBCH 201-299.

Site Number	Mean Concentrations of Clothianidin (ppb)[1]	
	Year 1	Year 2
1a	(4.4)[2]	6.6
4a	(3.6)[2]	6.7
7a	(3.4)`[2]	19.4

[1] LOQ= 5 ppb; LOD= 1.3 ppb
[2]Values in parenthesis are between the LOQ and the LOD

6.2 Leaf Results

Table 8. Mean Concentration of Clothianidin, TZNG and TZMU in Pumpkin Leaves following Soil Applications of Belay Insecticide At-Planting.

Site Number	Mean Concentration (ppb)[1]								
	Year 1			Year 2			Year 3		
	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU
1	16.3	3.6	4.5	63.2	10.1	25.6	111	15.3	20.5
2	80.0	14.2	19.4	38.1	5.8	12.5	26.6	3.5	8.4
3	15.3	3.2	7.6	16.2	1.6	6.6	45.6	6.6	9.0
4	52.0	6.3	28.0	13.0	3.2	4.7	44.1	7.3	11.9
5	26.1	4.2	7.3	28.8	4.5	7.4	56.3	6.3	9.1
6	13.1	3.6	2.0	26.9	NS[2]	NS[2]	71.3	7.2	7.0
7	150	NS[2]	NS[2]	12.4	NS[2]	NS[2]	28.8	2.1	3.9
8	50.5	5.5	12.5	32.5	6.0	8.0	31.0	4.9	3.2
9	18.1	NS[2]	NS[2]	19.5	NS[2]	NS[2]	20.4	3.2	5.2

[1] LOQ= 5 ppb; LOD=1.3 ppb
[2] NS, No sample remaining for analysis using method RM-39L-2

Table 9. Mean Concentrations of Clothianidin, TZNG and TZMU in Pumpkin Leaves Following Soil Applications of Belay Insecticide at Growth Stage BBCH 201-299.

Site Number	Mean Concentration (ppb)[1]								
	Year 1			Year 2			Year 3		
	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU
1a	137	20.6	27.1	431	27.1	77.4	80.5	10.0	11.1
4a	103	19.8	27.6	72.9	10.8	22.8	111	16.5	40.7
7a	26.5	NS[2]	NS[2]	7.9	NS[2]	NS[2]	14.5	1.5	2.9

[1]LOQ= 5 ppb; LOD=1.3 ppb
[2]NS, No sample remaining for analysis using method RM-39L-2

6.3 Nectar Residues

Table 10. Mean Concentrations of Clothianidin, TZNG and TZMU in Pumpkin Floral Nectar following Soil Applications of Belay Insecticide At-Planting.

Site Number	Mean Concentration (ppb)[1]								
	Year 1			Year 2			Year 3		
	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU
1	2.06	[0.1][2]	[0.1][2]	4.1	(0.3)[3]	(0.6)[3]	4.9	(0.4)[3]	(0.4)[3]
2	5.44	(0.43)[3]	[0.1][2]	2.0	(0.2)[3]	(0.6)[3]	1.3	[0.1][2]	[0.1][2]
3	(0.7)[3]	[0.1][2]	[0.1][2]	(0.7)[3]	[0.1][2]	(0.3)[3]	2.0	[0.1][2]	[0.1][2]
4	1.7	[0.1][2]	(0.2)[3]	(0.7)[3]	[0.1][2]	[0.1][2]	2.1	[0.1][2]	[0.1][2]
5	1.0	(0.4)[3]	[0.1][2]	1.5	1.4	(0.3)[3]	2.2	1.1	(0.2)[3]
6	1.9	[0.1][2]	[0.1][2]	2.0	[0.1][2]	(0.2)[3]	4.0	[0.1][2]	(0.5)[3]
7	5.8	(0.3)[3]	(0.3)	1.1	[0.1][2]	[0.1][2]	4.6	[0.1][2]	(0.2)[3]
8	2.9	(0.2)[3]	[0.1][2]	3.2	(0.3)[3]	(0.3)[3]	1.1	[0.1][2]	[0.1][2]
9	2.2	(0.3)[3]	[0.1][2]	1.4	[0.1][2]	[0.1][2]	1.6	[0.1][2]	[0.1][2]

[1] LOQ= 1 ppb; LOD= 0.2 ppb
[2] Values in square brackets are <LOD and entered as ½ LOD
[3] Values in parenthesis are between the LOQ and the LOD

Table 11. Mean Concentrations of Clothianidin, TZNG and TZMU in Pumpkin Floral Nectar following Soil Applications of Belay Insecticide at Growth Stage BBCH 201-299.

Site Number	Mean Concentration (ppb)[1]								
	Year 1			Year 2			Year 3		
	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU
1a	12.8	(0.7)[3]	(0.6)[3]	17.0	(0.9)[3]	2.0	11.1	(0.9)[3]	(0.9)[3]
4a	5.4	(0.5)[3]	(0.5)[3]	3.1	(0.2)[3]	(0.4)[3]	4.94	(0.4)[3]	(0.7)[3]
7a	4.2	(0.4)[3]	(0.2)[3]	2.1	[0.1][2]	(0.4)[3]	(0.9)[3]	[0.1][2]	[0.1][2]

[1] LOQ= 1 ppb; LOD= 0.2 ppb
[2] Values in square brackets are <LOD and entered as ½ LOD
[3] Values in parenthesis are between the LOQ and the LOD

6.4 Pollen Residues

Table 12. Mean Concentrations of Clothianidin, TZNG and TZMU in Pumpkin Pollen following Soil Applications of Belay Insecticide At-Planting.

Site Number	Mean Concentration (ppb)[1]								
	Year 1			Year 2			Year 3		
	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU
1	3.9	(0.5)[2]	(0.5)[2]	9.4	(0.9)[2]	1.1	14.2	1.9	1.2
2	7.5	(0.7)[2]	(0.6)[2]	6.2	(0.6)[2]	(0.7)[2]	3.8	(0.4)[2]	(0.3)[2]
3	2.8	(0.3)[2]	(0.3)[2]	1.3	[0.1][3]	[0.1][3]	4.6	(0.5)[2]	(0.4)[2]
4	4.3	(0.6)[2]	(0.8)[2]	1.9	[0.1][3]	[0.1][3]	11.6	1.0	1.7
5	2.1	(0.8)[2]	[0.1][3]	4.2	2.6	(0.9)[2]	4.7	2.8	(0.8)[2]
6	4.0	(0.5)[2]	(0.5)[2]	3.9	(0.3)[2]	(0.3)[2]	7.7	(0.7)[2]	(0.9)[2]
7	15.5	1.3	1.0	2.2	[0.1][3]	(0.4)[2]	10.0	(0.6)[2]	(0.5)[2]
8	6.4	(0.5)[2]	(0.9)[2]	7.3	(0.6)[2]	1.1	2.0	(0.2)[2]	[0.1][3]
9	7.3	1.1	(0.8)[2]	5.4	(0.7)[2]	(0.9)[2]	5.0	(0.8)[2]	(0.5)[2]

[1] LOQ= 1 ppb; LOD= 0.2 ppb
[2] Values in parenthesis are between the LOQ and the LOD
[3] Values in square brackets are <LOD and entered as ½ LOD

Table 13. Mean Concentrations of Clothianidin, TZNG and TZMU in Pumpkin Pollen following Soil Application of Belay Insecticide at Growth Stage BBCH 201-299.

Site Number	Mean Concentration (ppb)[1]								
	Year 1			Year 2			Year 3		
	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU	Clothianidin	TZNG	TZMU
1a	33.3	2.2	2.5	27.4	1.5	1.3	37.9	3.9	2.7
4a	18.3	1.3	1.6	9.8	(0.8)[2]	(0.8)[2]	18.2	1.6	2.0
7a	9.8	1.1	1.1	1.8	[0.1][3]	(0.3)[2]	1.5	[0.1][3]	[0.1][3]

[1] LOQ= 1 ppb; LOD= 0.2 ppb
[2] Values in parenthesis are between the LOQ and the LOD
[3] Values in square brackets are <LOD and entered as ½ LOD

7. STUDY VALIDITY/CLASSIFICATION AND STUDY LIMITATIONS

Classification/Utility for Bee Risk Assessment. This study is classified as acceptable. It provides a comprehensive overview of clothianidin concentrations in soil, as well as pumpkin leaves, pollen, and nectar during bloom. The residue values presented should be considered to be fully reliable. However, a decline curve cannot be constructed because samples were only collected from each matrix at one time point in each year.

Temporal Variability in Residues. This study was conducted over a three year period. Soil applications of 0.2 lb ai/acre were made annually for three years at planting, or, approximately one month later, at BBCH 201 (first shoots visible) to 229 (ninth leaf unfolded on main stem). Samples were collected during bloom, approximately two months after planting. Concentrations in soil, leaves, pollen, and nectar were all significantly higher following applications at BBCH 201-229, compared to applications at planting.

Coarse soils had significantly higher concentrations of clothianidin in nectar and pollen than fine soils following applications at BBCH 201-229 (Tables 18 and 20).

Spatial Variability in Residues. Eight of the nine sites were located in the San Joaquin Valley of California (Fresno, Madera, and Tulare Counties), and one site was located in California's Central Coast Region (San Luis Obispo County). Temperatures were similar across all sites. There were no apparent differences in residue concentrations based on location.

Pesticide Carryover. The study authors stated that a two-way ANOVA showed that there was a significant effect on concentrations in soil based on year of sampling ($p = 0.04$), but that the year of sampling did not have significant effects on concentrations in leaves, pollen, or nectar. The average number of days between the last application and sample collection was also slightly shorter during Year 3 (331 days for the At-Planting applications, and 314 days for the BBCH 201-229 applications), than during year 2 (368 days for the At-Planting applications, and 359 days for the BBCH 201-229 applications).

Table 14. Summary Statistics for Soil Grouped by Year and Time of Application.

Matrix	Application Time	Year	N	Mean ± SD[a] (µg a.i./kg ww)	Median (µg a.i./kg ww)	90th Percentile (µg a.i./kg ww)
Soil	At Planting	2	27	6.4 ± 5.9	4.9	14
		3	27	8.4 ± 5.8	7.4	16
	BBCH 201-299	2	9	7.9 ± 3.2	6.9	11
		3	9	20 ± 16	18	39

[a] Standard Deviation

Table 15. Results of a Two-Way Analysis of Variance Testing the Effects of Application Timing and Year on Clothianidin Residues in Soil.

Source	Type III Sum of Squared	Degrees of Freedom	Mean Squares	F-Ratio	P
Application Timing (Main Effects)	1.59	1	1.59	8.26	0.01
Year of Sampling (Main Effect)	0.82	1	0.82	4.25	0.04
Application Timing x Year (Interaction Effect)	0.04	1	0.04	0.18	0.67
Error	13.12	68	0.19		

Note: Data were log-transformed prior to analysis to improve data normality

Relationships between Leaf and Nectar/Pollen Residues. The study authors determined that there were significant relationships between clothianidin concentrations in pumpkin leaves compared to nectar ($n = 36$, $r^2 = 0.66$, $p<0.001$) and pollen ($n = 36$, $r^2 = 0.67$, $p<0.001$) collected from plants grown under the same conditions. The relationship between clothianidin concentrations in leaves and pollen is represented by the following equation: $C_N = 0.17(C_L)^{0.75}$, where C_N is the concentration in nectar and C_L is the concentration in leaves. The relationship between clothianidin concentrations in leaves and pollen is represented by the following equation: $C_P = 0.31(C_L)^{0.81}$, where C_P represents the concentration in pollen and C_L represents the concentration in leaves. The data used in these equations is not transformed. The study authors stated that there is a large degree of uncertainty in these relationships, because it is unclear how these relationships would apply to pumpkins grown in other locations, or to other use patterns. Therefore these equations should not be considered reliable until more data is acquired, so they can be tested and refined. Future studies should attempt to generate similar equations so that these relationships can be better understood.

Magnitude of Residues. Descriptive statistics for concentrations of Clothianidin, TZNG, and TZMU residues in nectar, pollen, soil, and leaves are presented in Table 16. The study authors conducted statistical analyses and found that, except for soil (Table 14), there were no significant differences between years, but that there were significant differences associated with the timing of applications for all matrices (i.e., at planting vs. at BBCH 201-229).

Table 16. Summary Statistics for Pumpkin Nectar, Pollen, Leaves and Pollen Grouped by Year and Time of Application.

Matrix	Application Time	Year	n	Mean ± SD[a] (µg ai/kg ww)	Median (µg ai/kg ww)	90th Percentile (µg ai/kg ww)
Nectar	At Planting	1	18	2.6 ± 2.3	1.9	5.1
		2	18	1.9 ± 1.2	1.6	3.1
		3	18	2.7 ± 1.5	2.2	4.5
	BBCH 201- 229	1	6	7.5 ± 4.5	6.8	13
		2	6	7.4 ± 7.5	3.5	17
		3	6	5.7 ± 4.6	4.9	11
Pollen	At Planting	1	18	6 ± 5.3	4.8	7.8
		2	18	4.7 ± 2.7	4.2	7.9
		3	18	7.1 ± 4.2	6	12
	BBCH 201- 229	1	6	20 ± 12	20	33
		2	6	13 ± 12	9.8	27
		3	6	19 ± 17	18	38
Leaves	At Planting	1	18	47 ± 44	26	100
		2	18	28 ± 18	23	38
		3	18	48 ± 32	42	70
	BBCH 201 - 229	1	6	89 ± 57	90	150
		2	6	170 ± 200	73	430
		3	6	69 ± 54	68	120

[a] Standard Deviation

The registrant collected samples from pumpkins grown in different soil types, but did not analyze the effects that different soil types have on clothianidin concentrations in bee-relevant matrices, so DPR conducted independent analyses (Tables 17-20). Site 7 was classified as fine soil, Sites 5 and 9 were classified as medium soils, and Sites 1, 2, 3, 4, 6, and 8 were classified as coarse soils. The study authors also calculated consumption and exposure (ng/bee) based on mean concentrations and 90th percentile residue values.

Table 17. Clothianidin Concentrations in Nectar in Different Soil Types Resulting from At-Planting Applications (all units in ng/g).

Soil Type	Mean (± SD)	Median	Maximum
Fine	3.85 (± 3.30)	2.89	9.58
Medium	1.68 (± 0.54)	1.52	2.91
Coarse	2.39 (± 1.55)	1.97	6.36

Table 18. Clothianidin Concentrations in Nectar in Fine and Coarse Soils Resulting from the BBCH 201-229 Applications (all units in ng/g).

Soil Type	Mean (± SD)	Median	Maximum
Fine	2.43 (± 2.00)	1.87	5.98
Coarse	9.06 (± 5.26)*	9.04	18.01

* Significantly higher concentrations resulted from application at BBCH 201-229 in coarse soil compared to fine soil (Welch's t-test, p = 0.002)

Table 19. Clothianidin Concentrations in Pollen in Different Soil Types Resulting from At-Planting Applications (all units in ng/g).

Soil Type	Mean (± SD)	Median	Maximum
Fine	9.24 (± 8.86)	7.07	25.81
Medium	4.76 (± 1.76)	4.81	8.27
Coarse	5.72 (± 3.59)	4.85	17.03

Table 20. Clothianidin Concentrations in Pollen in Fine and Coarse Soils Resulting from the BBCH 201-229 Applications (all units in ng/g).

Soil Type	Mean (± SD)	Median	Maximum
Fine	4.37 (± 4.79)	1.86	13.41
Coarse	24.14 (± 11.20)*	25.29	44.47

* Significantly higher concentrations resulted from application at BBCH 201-229 in coarse soil compared to fine soil (Mann-Whitney-Wilcoxson test, p = 0.0023)

8. STATISTICAL ANALYSIS

1. Table S-1 summarizes the number of plant samples that were obtained from each trial site for each year with an indication of the soil texture at each site, the method of chemical application used at each site, and the timing of applications. Note that there is confounding in the experimental design with respect to method of application and soil type because not all sites received the same method of application. Also, three of the sites were split across method of application to test timing of application. Site 4, which received a furrow application, had a second plot where the application occurred 1 month after planting. Then for chemigation applications, Site 1 and 7 were similarly treated. The analysis of the data was conducted to provide guidance for these questions:

1. Was there bias in analyses of clothianidin residues over years?

2. Was there a difference in chemical concentration in plant samples due to timing of application?

3. Was there a difference in chemical concentration in plant samples due to method of application?

4. Was there a difference in chemical concentration due to soil type?

When no bias was measured, this indicated that the data could potentially be pooled over the factor tested to proceed on to the next test. For example, bias between years was tested. If no difference in distribution between years was measured then data for years at a treatment level were pooled. Two methods of chemical analysis were indicated. One denoted L-1, which only reported on concentration of parent and the other denoted L-2, which reported on parent and degradation products. Only data from L-2 are presented in this analysis. Values were reported that were below the limit of detection (LOD). These values were substituted with ½ LOD stated for the year and plant sample. Total clothianidin residue was determined as a simple addition of all analytes.

2. Bias in concentration between years. Analyses were conducted to determine if there were significant differences in distribution of clothianidin residue concentration between years in the plant samples. Tables S-2 through S-4 contain a comparison of the distributions for each analyte between years for leaves, nectar, and pollen, respectively. Non-parametric tests were conducted to measure potential differences in the concentration between the 3 years. A significant Wilcoxon test provides an indication of general differences in the distribution, whereas, the Median test provides an indication if differences in the median values that are present between the distributions. The tests were run using the exact option in Proc Npar1way in the SAS program with the Monte Carlo option where 10,000 iterations of the tests were run. No significant differences in distributions between years were indicated for chemical analysis in any plant sample. Figures S-1 contains an example for the comparison of the distribution of clothianidin concentration in leaves between years.

3. Bias in concentration due to timing of applications. Based on the results of the analysis comparing distribution between years, the data were pooled over years to test the potential effect of timing of application. Tables S-6 through S-8 compare the distributions for application at planting to applications made 30 days after planting for leaves, nectar, and pollen, respectively. The analyses were conducted on data from trial sites 1, 4 and 7 because these were sites with the split application treatments. Statistical results indicated that the distribution of values measured at the later application was shifted toward higher values (Table S-9). The shift in distribution was highly significant for leaf samples (Figure S-2). For nectar, the Wilcoxon test indicated a significant difference for all chemicals but only the Median test indicated a trend. Graphical comparison indicates there is a trend for higher concentrations for the later applications (Figure S-3). Results for pollen were similar with graphical comparison indicating a shift toward higher values at the later application data (Figure S-4). Median total clothianidin values for all plant samples were 2 to 3 times greater for the later applications and maximum values were 2.5, 2, and 1.8 times greater in leaves, nectar, and pollen samples.

4. Bias in concentration between methods of application. This aspect of the study was confounded whereby effects of method of application were unevenly distributed amongst the soil types (Table S-1). For example, only coarse soils were located in furrow application treatments so potential effects due to the other soil types were not represented. Owing to the presence of confounding effects, these comparisons are presented for informational purposes only. Table S-10 compares the distribution for total clothianidin residues between furrow and chemigation methods of application. Based on differences measured for timing of application, separate statistical analyses were conducted for at planting and 1 month later application comparisons. The majority of results indicated no difference in the overall distribution and in the median values for both methods of application (Table S-11 and Figures S-5 through S-7). The only significant effects were indicated at planting for Wilcoxon test for distributions of TZNG and total residue in nectar: Tests for location of the median value were not significant.

5. Bias in concentration due to soil type. As for comparisons between methods of application, the design of the study was confounded with respect to effects of soil type with method of application (Table S-1). Again, these comparisons are presented for informational purposes only. Since there appeared to be no consistent differences in distribution of residue concentrations between furrow or chemigation methods of application, these data were combined and the distributions compared between soil types. Tables S-12 through S-14 contain the distributions by each soil type as measured for leaves, nectar, and pollen, respectively. The highest total clothianidin residue nectar value was 20.8 ppb, which was measured in a medium-textured soil plot for an application made 1 month after planting. The second highest value was 10.3 ppb, which was measured in a fine-textured soil for an application made at planting. For pollen, the pattern was similar with 51.6 ppb being the highest value measured at the fine-textured site for the 1 month later application.

6. Concentration distribution of residue in bee relevant matrices. Tables S-7 and S-8 contain the distribution of residues measured in nectar and pollen, respectively, for the trial sites combined within the two timings of application treatments. For nectar, the maximum and median values were 10.3 and 2.3 ppb for at planting treatments, and 29.4 and 6.7 ppb for treatments made 1 month after planting. Pollen values tended to be higher with maximum and median values of 20.8 and 6.1 ppb for at planting treatments, and 51.6 and 18.7 ppb for treatments made 1 month after planting.

7. Relationship in clothianidin concentrations between plant samples. Figure S-9 shows the relationship measured in all replicate samples for concentrations of clothianidin between leaves and nectar (A), leaves and pollen (B), and nectar and pollen (C) samples. The R-square for leaves and nectar is 0.68 and the relationship indicates a good relationship where nectar values increase with increase in leaf concentration. The R-square for leaves and pollen is lower for all data at 0.39, but removal of the one potential outlier increases the R-square value to 0.56. The general response is similar to that observed for nectar where concentration in pollen tends to increase with increased concentration in leaves. The relationship is not as clear cut between nectar and pollen concentrations as there is obviously more scatter associated with that graph.

8. Soil concentration. Based on the analyses for plant samples, the most relevant comparison is the distribution of soil concentrations between applications made at planting or 1 month after planting. Table S-15 contains the statistics for the comparison of these two distributions where soil data was combined over 2013 and 2014 for each treatment. Non-parametric statistical tests indicated that the Wilcoxon test was significant at P=0.021 but the test for location of the Median values was not significant with P=0.41. In Figure S-8, concentrations for applications made 1 month after planting indicate a skew towards higher concentrations where maximum and median values for the 1 month later applications are 1.9 and 1.5 times greater than at planting values, respectively.

Conclusion:

1. For bee relevant matrices, the response of pumpkin to the pattern of application used in this study indicated higher concentrations for total clothianidin residues measured in pollen than in nectar samples. The highest pollen value was 51.6 ppb compared to 29.4 ppb for nectar. Potential for biological significance relies upon comparison to chronic feeding benchmark values, but they have not yet been established.

2. The study design was confounded by too many instances of adding a treatment factor, but at the expense of producing an incomplete treatment design matrix. For example, applications at some trial sites were made to furrows rather than by chemigation. A trial site represented a soil type so adding the furrow application method without also applying chemigation at that site confounded comparisons of soil type. Effects could have been caused by method of application and not due to soil type. Thus it was not possible to conclude potential effects of soil type.

3. A comparison was made for effect and timing of application on concentrations measured in plant tissues. Delaying application from at planting to 1 month after planting resulted in higher concentrations, especially significant for leaf samples. Analysis of soil sampled between these two dates of application indicated potentially higher concentrations for applications made 1 month after planting. The measurement of increased concentration in plant samples may in part be due to higher soil concentrations as well as a decreased time interval between application and plant sampling.

4. Increasing concentrations of clothianidin leaf tissue resulted in concomitant increases in concentration measured in nectar and pollen samples.

5. Lack of difference in concentration between years indicated no carry-over effect from the application rates and methods used in the studies: i.e. rate at 0.2 lbs/acre applied either as a chemigation treatment or furrow injection, and applied either at planting or 30 days after planting.

Table S-1. Summary of the number of samples taken at each year and for each sample type at each trial site. The method used was indicated as L-2 that measured parent and degradation products. Note that there were different methods of application and different timing of applications distributed across the trial sites and across the soil categories.

Application Timing, Trial Site Number, and Soil Texture	Furrow/Soil Application										
	Leaves			Nectar			Pollen			Soil	
At Planting	2012	2013	2014	2012	2013	2014	2012	2013	2014	2013	2014
1-Medium											
2-Coarse											
3-Coarse	2	2	2	2	2	2	2	2	2	3	3
4-Coarse	2	2	2	2	2	2	2	2	2	3	3
5-Medium											
6-Coarse											
7-Fine											
8-Coarse	2	2	2	2	2	2	2	2	2	3	3
9-Medium											
Total	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**9**	**9**
Month Delay	2012	2013	2014	2012	2013	2014	2012	2013	2014	2013	2014
4A-Coarse	2	2	2	2	2	2	2	2	2	3	3
Total	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**3**	**3**

	Chemigation										
	Leaves			Nectar			Pollen			Soil	
At Planting	2012	2013	2014	2012	2013	2014	2012	2013	2014	2013	2014
1-Medium	2	2	2	2	2	2	2	2	2	3	3
2-Coarse	2	2	2	2	2	2	2	2	2	3	3
3-Coarse											
4-Coarse											
5-Medium	2	2	2	2	2	2	2	2	2	3	3
6-Coarse	2	0	2	2	2	2	2	2	2	3	3
7-Fine	0	0	2	2	2	2	2	2	2	3	3
8-Coarse											
9-Medium	0	0	2	2	2	2	2	2	2	3	3
Total	**8**	**6**	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**18**	**18**
Month Delay	2012	2013	2014	2012	2013	2014	2012	2013	2014	2013	2014
1A-Medium	2	2	2	2	2	2	2	2	2	3	3
7A-Fine	0	0	2	2	2	2	2	2	2	3	3
Total	**2**	**2**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**6**	**6**

Table S-2. Leaves: Comparison of statistics for the distribution for years 2012, 2013, and 2014 for concentration of clothianidin, its degradation products and total residue in leaves of pumpkin.

Statistic	Clothianidin			TZNG			TZMU			Total Residue		
	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014
N	18	16	24	18	16	24	18	16	24	18	16	24
Mean	65.2	91.1	53.4	9.0	8.6	7.1	15.1	20.6	11.1	89.3	120.4	71.5
SD	51.7	130.3	38.4	7.5	8.0	5.2	10.7	24.4	11.3	68.5	161.6	52.9
CV (%)	79.3	142.9	71.8	83.2	92.1	74.1	71.1	118.2	102.4	76.7	134.2	73.9
Min	15.2	11.8	13.1	2.6	0.7	1.3	0.7	4.4	2.8	20.7	17.0	17.4
Median	51.8	39.9	42.5	4.6	5.8	6.2	12.5	9.3	8.1	70.2	54.3	58.4
75th	95.7	90.3	66.4	13.5	10.4	7.8	22.8	22.8	11.4	133.6	128.8	82.7
90th	171.3	409.8	118.1	25.0	24.1	16.2	33.2	73.3	31.5	231.2	515.4	166.5
95th	184.4	423.6	129.4	26.0	30.1	16.8	33.9	81.5	32.2	239.3	527.0	169.7
Max	184.4	423.6	156.4	26.0	30.1	21.4	33.9	81.5	49.2	239.3	527.0	209.3
% of Total	73.0	75.7	74.7	10.1	7.2	9.9	16.9	17.1	15.4			

Table S-3. Nectar: Comparison of statistics for the distribution for years 2012, 2013, and 2014 for concentration of clothianidin, its degradation products and total residue in nectar of pumpkin flowers.

Statistic	Clothianidin			TZNG			TZMU			Total Residue		
	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014
N	24	24	24	24	24	24	24	24	24	24	24	24
Mean	3.9	3.3	3.4	0.3	0.3	0.3	0.2	0.4	0.3	4.4	4.0	4.0
SD	3.6	4.4	2.9	0.2	0.4	0.4	0.2	0.5	0.3	3.9	5.1	3.3
CV (%)	92.6	135.1	83.6	63.7	118.1	110.1	82.7	114.5	86.8	88.5	125.7	81.1
Min	0.6	0.7	0.6	0.1	0.1	0.1	0.1	0.1	0.1	0.8	0.9	0.8
Median	2.4	1.9	2.4	0.3	0.2	0.1	0.1	0.3	0.2	2.8	2.8	3.3
75th	5.3	3.0	4.3	0.5	0.3	0.3	0.3	0.5	0.5	6.0	3.7	5.0
90th	9.6	5.4	6.3	0.6	0.9	0.8	0.6	1.7	0.8	10.3	6.5	7.2
95th	12.3	15.9	10.6	0.7	1.4	0.9	0.6	7.9	0.9	13.5	18.9	12.3
Max	13.3	18.0	11.6	0.7	1.5	1.5	0.8	2.0	1.0	14.7	20.8	13.4
% of Total	87.5	80.7	84.6	7.0	8.4	7.9	5.2	10.9	7.7			

Table S-4. Pollen: Comparison of statistics for the distribution for years 2012, 2013, and 2014 for concentration of clothianidin, its degradation products and total residue in pollen of pumpkin flowers.

Statistic	Clothianidin			TZNG			TZMU			Total Residue		
	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014
N	24	24	24	24	24	24	24	24	24	24	24	24
Mean	9.6	6.7	10.1	0.9	0.7	1.2	0.9	0.7	1.0	11.4	8.1	12.3
SD	9.7	7.1	10.2	0.7	0.7	1.2	0.7	0.4	0.8	11.0	7.7	11.9
CV (%)	101.3	104.5	100.8	75.0	99.8	95.7	77.0	63.4	88.9	96.0	95.0	96.9
Min	1.7	1.2	1.4	0.1	0.1	0.1	0.1	0.1	0.1	2.6	1.5	1.6
Median	5.7	4.8	6.8	0.7	0.6	0.7	0.6	0.7	0.6	6.6	6.9	9.2
75th	9.5	8.0	12.6	1.2	0.9	1.6	1.1	1.0	1.2	11.7	9.8	15.8
90th	26.0	10.6	19.9	2.0	1.6	3.1	2.1	1.2	2.5	29.9	12.9	23.6
95th	28.9	24.6	31.2	2.1	2.4	3.7	2.3	1.3	2.6	33.5	27.2	37.5
Max	37.6	30.3	44.5	2.4	2.9	4.2	2.6	1.4	2.9	42.3	33.3	51.6
% of Total	84.1	83.0	82.4	8.0	8.9	9.8	8.0	8.1	7.8			

Table S-5. Results of non-parametric statistical tests for measuring differences in distributions for years 2012, 2013, and 2014 clothianidin, its degradation products and total residue in leaves nectar and pollens samples of pumpkin.

Plant Sample	Non-Parametric Test Significance Levels For Differences Between Years							
	Clothianidin		TZNG		TZMU		Total Residue	
	Wilcoxon	Median	Wilcoxon	Median	Wilcoxon	Median	Wilcoxon	Median
Leaves	0.95	0.76	0.86	0.94	0.19	0.40	0.86	0.76
Nectar	0.39	0.73	0.50	0.41	0.17	0.25	0.62	0.93
Pollen	0.25	0.56	0.32	0.95	0.67	0.91	0.33	0.42

Table S-6. Leaves: Comparison of statistics between timing of application for the distribution of clothianidin, its degradation products and total residue in leaves of pumpkin.

Statistic	Clothianidin		TZNG		TZMU		Total Residue	
	At Planting	30 Days After Planting	At Planting	30 Days After Planting	At Planting	30 Days After Planting	At Planting	30 Days After Planting
N	14	14	14	14	14	14	14	14
Mean	50.1	143.1	6.8	15.3	14.2	29.9	71.1	188.3
SD	39.3	126.5	5.1	8.9	13.1	23.9	55.3	156.4
CV (%)	78.5	88.4	74.8	58.6	92.7	79.8	77.8	83.1
Min	14.0	13.1	1.9	1.3	3.1	2.8	21.4	17.4
Median	38.6	111.2	5.9	15.4	8.7	25.1	55.3	157.8
75th	65.7	171.3	8.7	24.1	22.7	33.9	84.3	231.2
90th	98.5	409.3	11.4	26.0	33.2	73.3	153.1	515.4
95th	156.4	423.6	21.4	30.1	43.2	81.5	209.3	527.0
Max	156.4	423.6	21.4	30.1	43.2	81.5	209.3	527.0
% of Total	70.5	76.0	9.6	8.1	19.9	15.9		

Table S-7. Nectar: Comparison of statistics between timing of application for the distribution of clothianidin, its degradation products and total residue in nectar of pumpkin flowers.

Statistic	Clothianidin		TZNG		TZMU		Total Residue	
	At Planting	30 Days After Planting	At Planting	30 Days After Planting	At Planting	30 Days After Planting	At Planting	30 Days After Planting
N	18	18	18	18	18	18	18	18
Mean	3.0	6.8	0.2	0.5	0.2	0.7	3.5	8.0
SD	2.4	5.4	0.1	0.3	0.2	0.6	2.6	6.2
CV (%)	78.3	79.3	56.5	66.8	75.2	85.4	74.4	77.8
Min	0.7	0.6	0.1	0.1	0.1	0.1	0.9	0.8
Median	2.4	4.9	0.2	0.4	0.2	0.5	2.8	6.1
75th	3.8	11.6	0.3	0.7	0.3	0.8	4.2	13.4
90th	6.3	15.9	0.3	0.9	0.5	1.9	7.2	18.9
95th	9.6	18.0	0.5	0.9	0.7	2.0	10.3	20.8
Max	9.6	18.0	0.5	0.9	0.7	2.0	10.3	20.8
% of Total	87.0	85.5	6.1	5.8	6.9	8.1		

Table S-8. Pollen: Comparison of statistics between timing of application for the distribution of clothianidin, its degradation products and total residue in pollen of pumpkin flowers.

Statistic	Clothianidin		TZNG		TZMU		Total Residue	
	At Planting	30 Days After Planting	At Planting	30 Days After Planting	At Planting	30 Days After Planting	At Planting	30 Days After Planting
N	18	18	18	18	18	18	18	18
Mean	8.1	17.6	0.8	1.4	0.8	1.4	9.7	20.3
SD	6.3	13.4	0.7	1.2	0.6	1.0	7.3	15.4
CV (%)	77.4	76.4	86.1	83.5	69.5	70.2	75.2	75.5
Min	1.6	1.4	0.1	0.1	0.1	0.1	1.9	1.6
Median	6.8	15.0	0.6	1.4	0.6	1.3	8.0	18.7
75th	11.1	28.9	0.9	2.0	1.2	2.1	12.9	33.3
90th	17.0	37.6	2.1	3.7	1.4	2.6	20.6	42.3
95th	25.8	44.5	2.4	4.2	2.5	2.9	29.4	51.6
Max	25.8	44.5	2.4	4.2	2.5	2.9	29.4	51.6
% of Total	83.7	86.3	7.9	6.9	8.4	6.7		

Table S-9. Results of non-parametric statistical tests for measuring differences in distributions for years 2012, 2013, and 2014 clothianidin, its degradation products and total residue in leaves nectar and pollens samples of pumpkin.

Clothianidin Residue	Non-Parametric Test Significance Levels For Differences Between Applications at Planting or Made One Month Later					
	Leaves		Nectar		Pollen	
	Wilcoxon	Median	Wilcoxon	Median	Wilcoxon	Median
TZNG	0.0067	0.1130	0.0100	0.0550	0.1200	0.3100
TZMU	0.0960	0.0560	0.0031	0.0158	0.1400	0.1300
Clothianidin	0.0084	0.0074	0.0190	0.1000	0.1300	0.3200
Total Residue	0.0200	0.0064	0.0190	0.0930	0.0700	0.0900

Table S-10. Total Clothianidin Residue: Comparison of statistics between applications applied either to the furrow or through chemigation for the distribution of total clothianidin residues leaves, nectar, and pollen of pumpkin plants.

Application at Planting						
	Leaves		Nectar		Pollen	
Statistic	Chemigation	Furrow	Chemigation	Furrow	Chemigation	Furrow
N	26	18	36	18	36	18
Mean	64.7	51.8	3.3	2.0	8.1	5.8
SD	45.4	25.4	2.0	1.2	5.2	4.3
CV (%)	70.2	49.0	61.4	59.1	63.5	73.5
Min	20.7	17.0	1.3	0.8	2.6	1.5
Median	52.9	54.7	2.8	1.5	7.4	4.6
75th	81.0	64.7	4.1	3.0	9.3	8.7
90th	133.9	82.0	6.5	4.3	12.9	11.2
95th	153.1	115.0	7.2	4.4	20.6	17.6
Max	209.3	115.0	10.3	4.4	29.4	17.6
Application One Month After Planting						
	Leaves		Nectar		Pollen	
Statistic	Chemigation	Furrow	Chemigation	Furrow	Chemigation	Furrow
N	8	6	12	6	12	6
Mean	209.7	159.8	9.2	5.4	21.4	18.1
SD	207.5	40.7	7.2	2.1	18.3	7.7
CV (%)	99.0	25.5	78.2	38.7	85.3	42.4
Min	17.2	114.0	0.8	2.9	1.6	10.5
Median	154.8	155.0	9.6	5.1	22.3	16.3
75th	377.4	169.7	14.1	6.7	35.5	23.6
90th	527.0	231.2	18.9	8.8	42.3	29.9
95th	527.0	231.2	20.8	8.8	51.6	29.9
Max	527.0	231.2	20.8	8.8	51.6	29.9

Table S-11. Results of non-parametric statistical tests conducted to compare the distribution of clothianidin, its degradation products, and total residue between chemigation and furrow application methods that were made either at planting or 1 month after planting. Concentrations are compared for leaf, nectar, and pollens samples of pumpkin plants.

	Application at Planting					
	Leaves		Nectar		Pollen	
Chemical	Wilcoxon	Median	Wilcoxon	Median	Wilcoxon	Median
TZNG	0.6300	1.0000	0.0300	0.1400	0.0030	0.0060
TZMU	0.6500	0.3700	0.3300	0.7700	0.3100	0.6800
Clothianidin	0.4000	0.7600	0.0170	0.3900	0.0750	0.3900
Total Residue	0.5700	1.0000	0.0058	0.1400	0.0390	0.1540
	Application One Month After Planting					
	Leaves		Nectar		Pollen	
Chemical	Wilcoxon	Median	Wilcoxon	Median	Wilcoxon	Median
TZNG	0.8800	1.0000	0.5300	0.6100	0.9500	1.0000
TZMU	0.4100	0.5900	0.9400	0.6200	0.8900	1.0000
Clothianidin	0.7500	0.5900	0.5500	0.6300	0.8900	1.0000
Total Residue	1.0000	1.0000	0.6100	0.6200	0.9000	1.0000

Table S-12. Leaves: Comparison of distribution statistics for concentration of clothianidin and total residue in leaves of pumpkin grown in each soil type. Note that data were pooled for furrow and chemigation applications within a soil type when indicated (see Table S-1). Separate analyses are presented for applications made at planting or 30 days after planting.

	Application At Planting						Application 1 Month After Planting					
	Clothianidin			Total Residue			Clothianidin			Total Residue		
Statistic	Coarse	Medium	Fine	Coarse	Medium	Fine	Coarse	Medium	Fine	Coarse	Medium	Fine
N	28	14	2	28	14	2	6	6	2	6	6	2
Mean	41.5	49.4	28.9	57.1	67.5	34.9	113.7	215.5	14.5	159.8	273.2	19.0
SD	23.0	37.9	6.3	31.1	52.6	7.7	30.6	163.4	2.0	40.0	202.1	2.2
CV (%)	55.4	76.8	21.8	54.4	77.9	22.1	27.0	75.8	13.6	72.0	74.0	11.6
Min	11.8	15.7	24.4	17.0	23.2	29.4	82.0	31.6	13.1	25.5	42.1	17.4
Median	39.6	36.7	28.9	55.1	50.7	34.9	106.0	156.9	14.5	114.0	200.2	19.0
75th	51.8	58.6		73.0	74.2		118.1	409.8		155.0	515.4	
90th	75.5	98.5		115.0	153.1		171.3	423.6		169.7	527.0	
95th	88.7	156.4		147.6	209.3		171.3	423.6		231.2	527.0	
Max	95.7	156.4	33.3	133.9	209.3	40.3	171.3	423.6	15.9	231.2	527.0	20.5
% of Total	72.5	73.2	82.8				71.2	78.9	76.5			

Table S-13. Nectar: Comparison of distribution statistics for concentration of clothianidin and total residue in nectar of pumpkin plants grown in each soil type. Note that data were pooled for furrow and chemigation applications within a soil type when indicated (see Table S-1). Separate analyses are presented for applications made at planting or 30 days after planting.

	Application At Planting						Application 1 Month After Planting					
	Clothianidin			Total Residue			Clothianidin			Total Residue		
Statistic	Coarse	Medium	Fine	Coarse	Medium	Fine	Coarse	Medium	Fine	Coarse	Medium	Fine
N	30	18	6	30	18	6	6	6	6	6	6	6
Mean	2.2	2.4	0.4	2.5	3.1	4.2	4.5	13.6	2.4	5.4	15.6	2.9
SD	1.4	1.5	3.3	1.5	1.6	3.5	1.8	2.8	2.0	2.1	3.4	2.3
CV (%)	65.2	61.5	85.8	61.0	53.3	82.4	39.5	20.7	82.3	38.7	22.1	81.0
Min	0.6	0.9	1.1	0.8	1.3	1.3	2.5	10.6	0.6	2.9	12.3	0.8
Median	1.8	1.7	2.9	2.1	3.0	3.2	4.2	12.8	1.9	5.1	14.1	2.1
75th	2.6	2.9	5.6	3.1	3.6	6.1	5.3	15.9	3.2	6.7	18.9	3.9
90th	4.1	5.4	9.6	4.6	6.5	10.3	7.5	18.0	6.0	8.8	20.8	7.0
95th	4.5	6.3	9.6	5.0	7.2	10.3	7.5	18.0	6.0	8.8	20.8	7.0
Max	6.4	6.3	9.6	7.2	7.2	10.3	7.5	18.0	6.0	8.8	20.8	7.0
% of Total	86.1	76.3	9.0				82.9	87.4	85.0			

Table S-14. Pollen: Comparison of distribution statistics for concentration of clothianidin and total residue in pollen of pumpkin plants grown in each soil type. Note that data were pooled for furrow and chemigation applications within a soil type when indicated (see Table S-1). Separate analyses are presented for applications made at planting or 30 days after planting.

	Application At Planting						Application 1 Month After Planting					
	Clothianidin			Total Residue			Clothianidin			Total Residue		
Statistic	Coarse	Medium	Fine	Coarse	Medium	Fine	Coarse	Medium	Fine	Coarse	Medium	Fine
N	30	18	6	30	18	6	6	6	6	6	6	6
Mean	5.0	6.3	9.2	6.1	8.4	10.5	15.4	32.9	4.4	18.1	37.6	5.3
SD	2.9	3.8	8.9	3.6	4.3	10.0	6.6	7.1	4.8	7.7	8.5	6.2
CV (%)	56.9	60.0	95.9	58.3	51.3	95.1	42.9	21.6	109.7	42.4	22.6	117.2
Min	1.2	1.7	2.2	1.5	2.6	2.6	9.1	24.6	1.4	10.5	27.2	1.6
Median	4.3	5.3	7.1	5.7	7.5	8.0	13.6	30.7	1.9	16.3	35.5	2.1
75th	7.4	8.3	11.1	8.8	10.1	12.3	19.9	37.6	6.2	23.6	42.3	6.7
90th	7.7	11.5	25.8	9.4	14.1	29.4	26.0	44.5	13.4	29.9	51.6	17.4
95th	9.5	17.0	25.8	11.2	20.6	29.4	26.0	44.5	13.4	29.9	51.6	17.4
Max	13.8	17.0	25.8	17.6	20.6	29.4	26.0	44.5	13.4	29.9	51.6	17.4
% of Total	82.3	74.8	87.8				85.1	87.5	82.3			

Table S-15. Soil: Comparison of statistics for the distribution concentration of clothianidin in soil for applications made at planting compared to those made 1 month after planting.

Statistic	Clothianidin	
	At Planting	1 Month After Planting
N	54	18
Mean	7.4	14
SD	5.9	12.7
CV (%)	79.6	89.6
Min	0.7	3.6
Median	5.6	8.5
75th	11.0	18.3
90th	15.8	38.4
95th	18.8	43.1
Max	22.2	43.1

Figure S-1. Leaf Samples: Comparison of box plots for the distribution of clothianidin residues between data collected in 2012, 2013, and 2014.

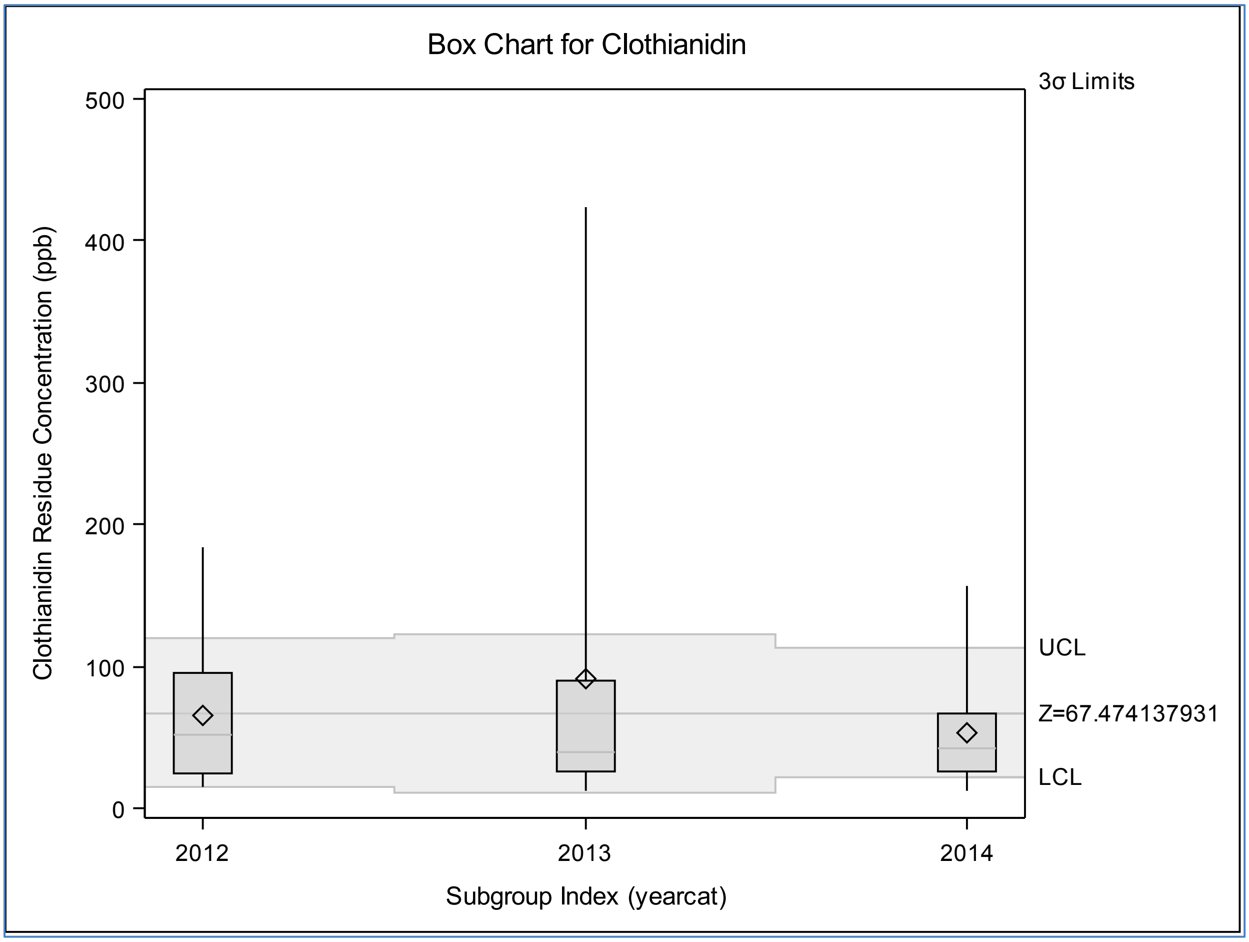


Figure S-2. Leaf Samples: Comparison of the distribution of clothianidin concentration measured for applications made at planting or one month after application. Data has been pooled for all years.

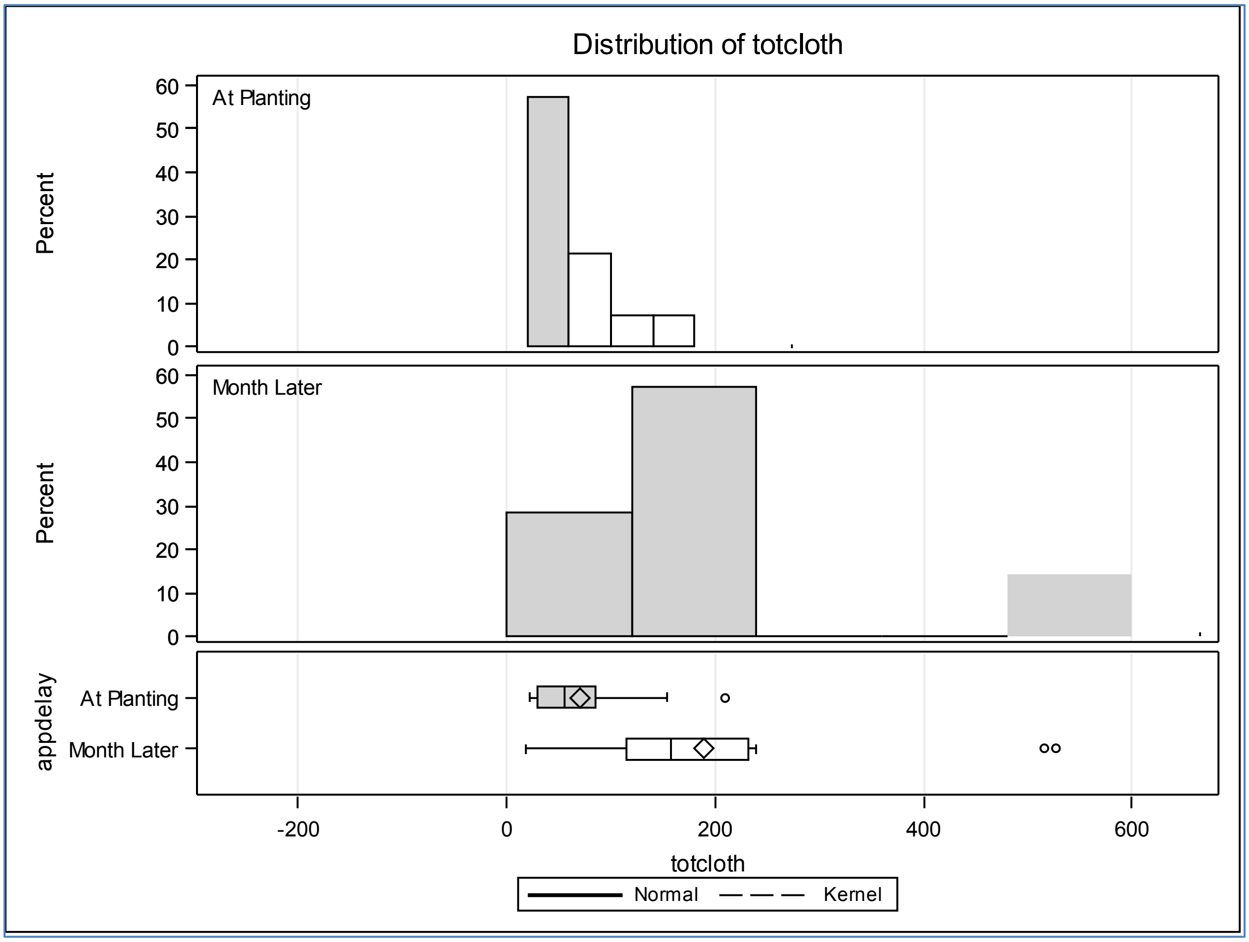


Figure S-3. Nectar Samples: Comparison of the distribution of total clothianidin residue concentration measured for applications made at planting or one month after application. Data has been pooled for all years.

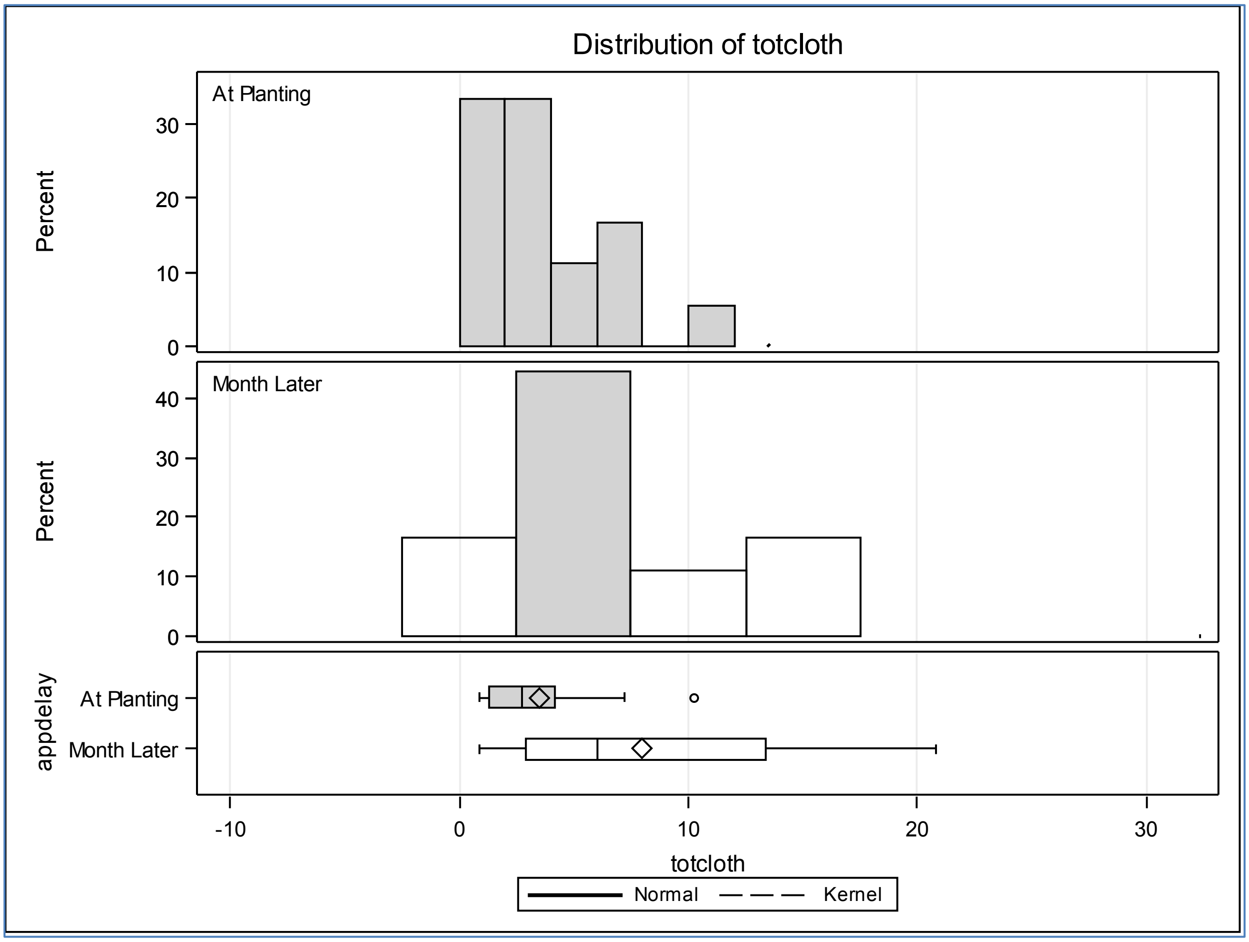


Figure S-4. Pollen Samples: Comparison of the distribution of total clothianidin residue concentration measured for applications made at planting or one month after application. Data has been pooled for all years.

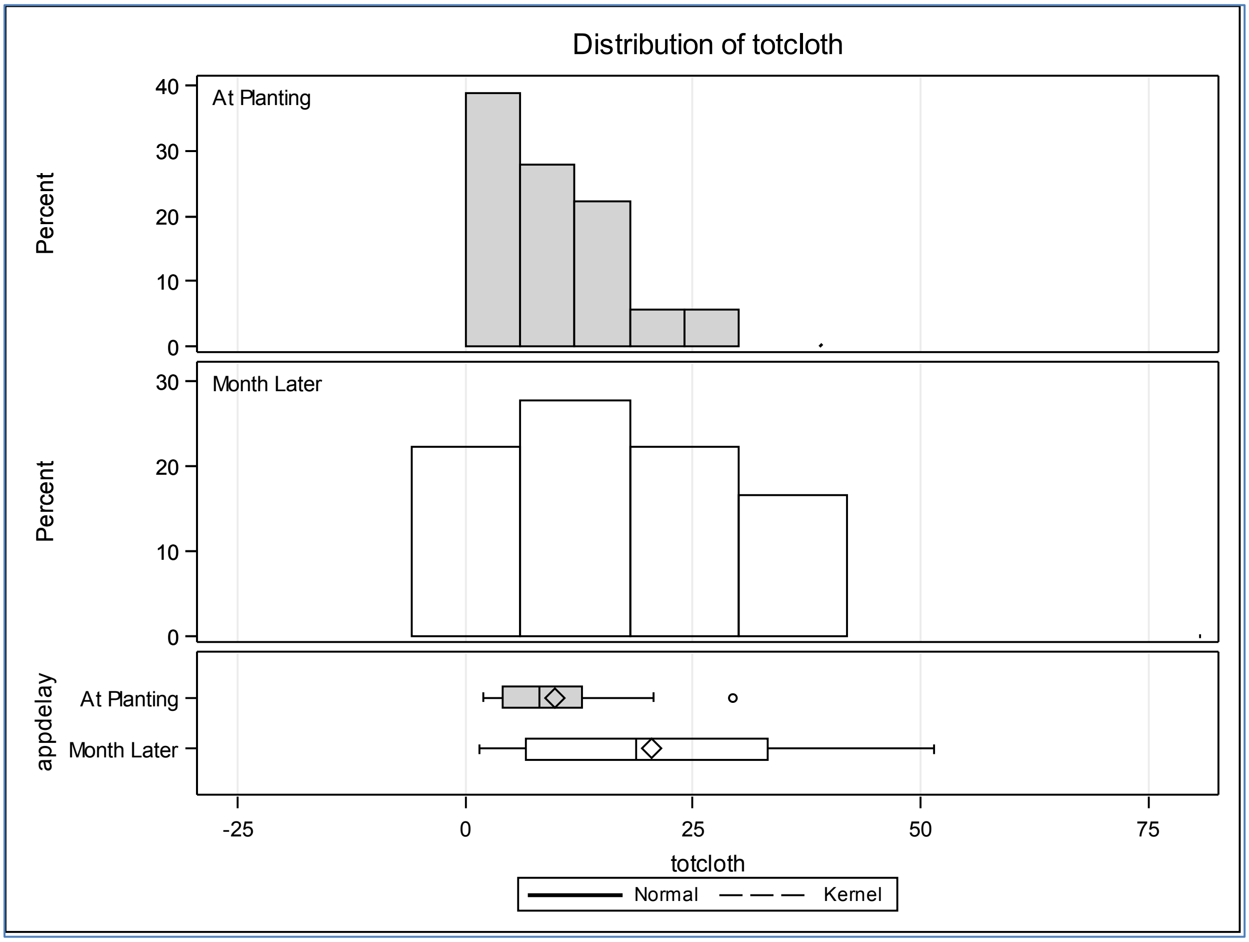


Figure S-5. Leaf Samples: Comparison of the distribution of total clothianidin residue concentration measured for applications made either directly to the furrow or through the chemigation system for application made at planting. Data has been pooled for all years.

Figure S-5 (A). Application At Planting

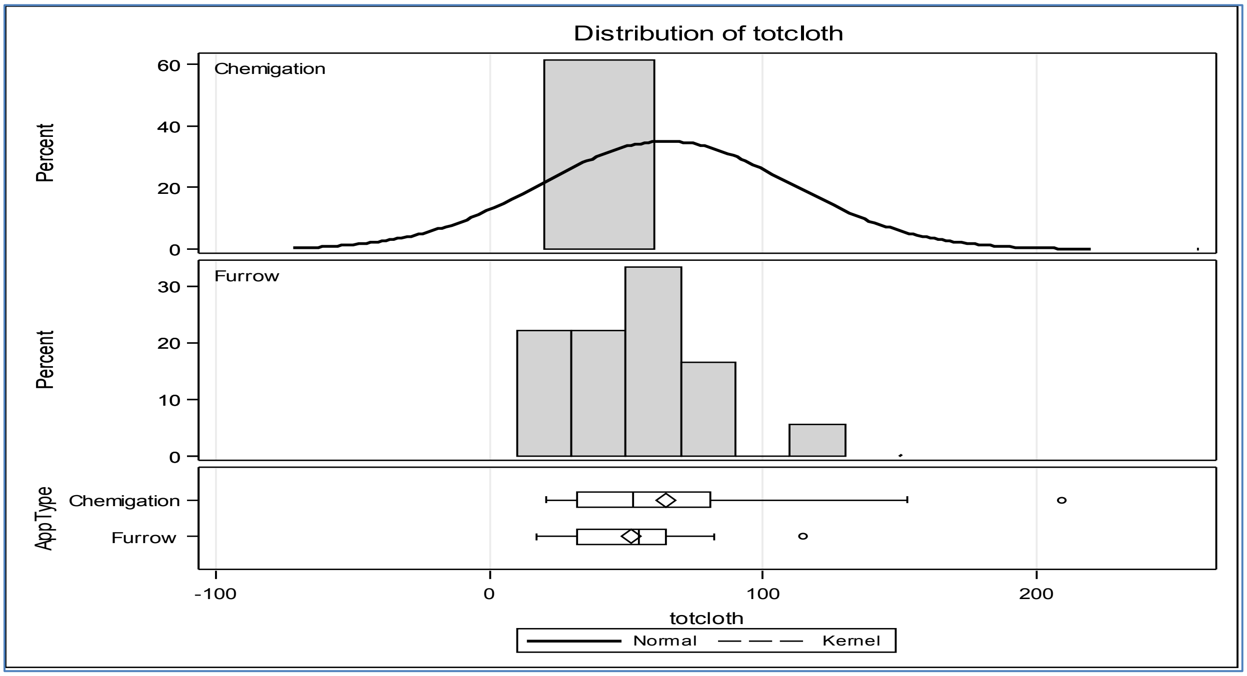


Figure S-5 (B). Application 1 Month After Planting

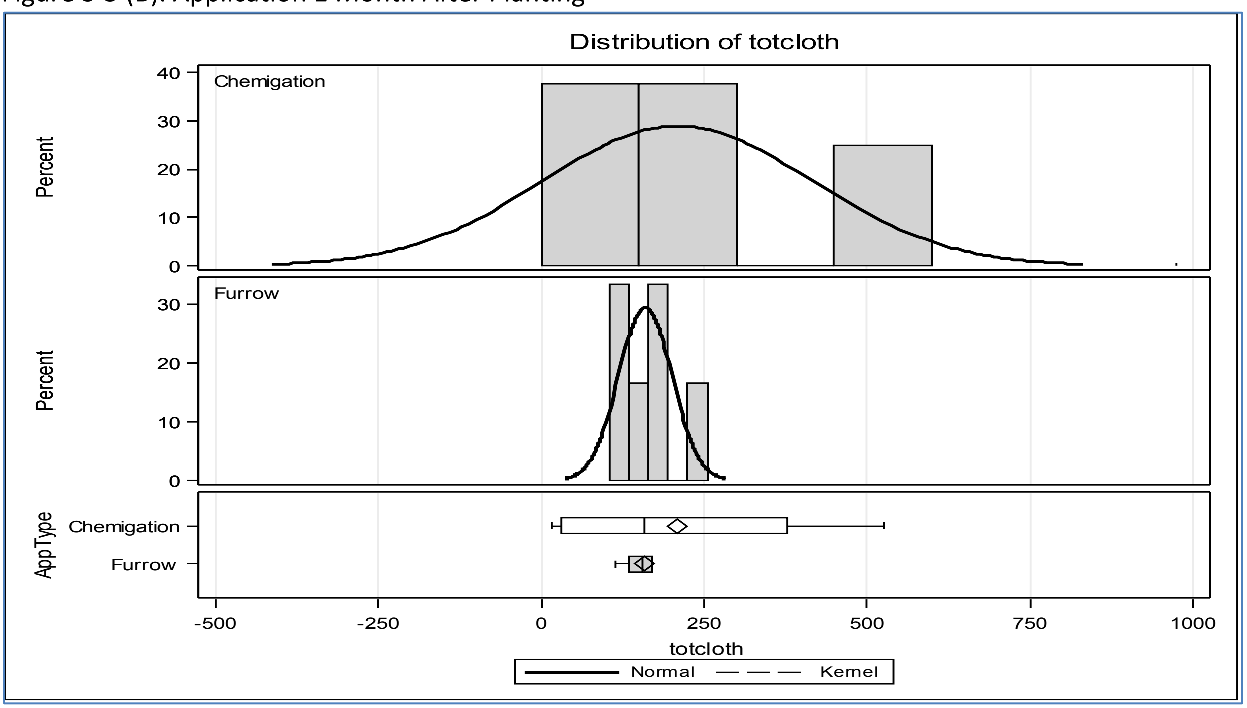


Figure S-6. Nectar Samples: Comparison of the distribution of total clothianidin residue concentration measured for applications made either directly to the furrow or through the chemigation system for application made at planting. Data has been pooled for all years.

Figure S-6 (A). Application At Planting

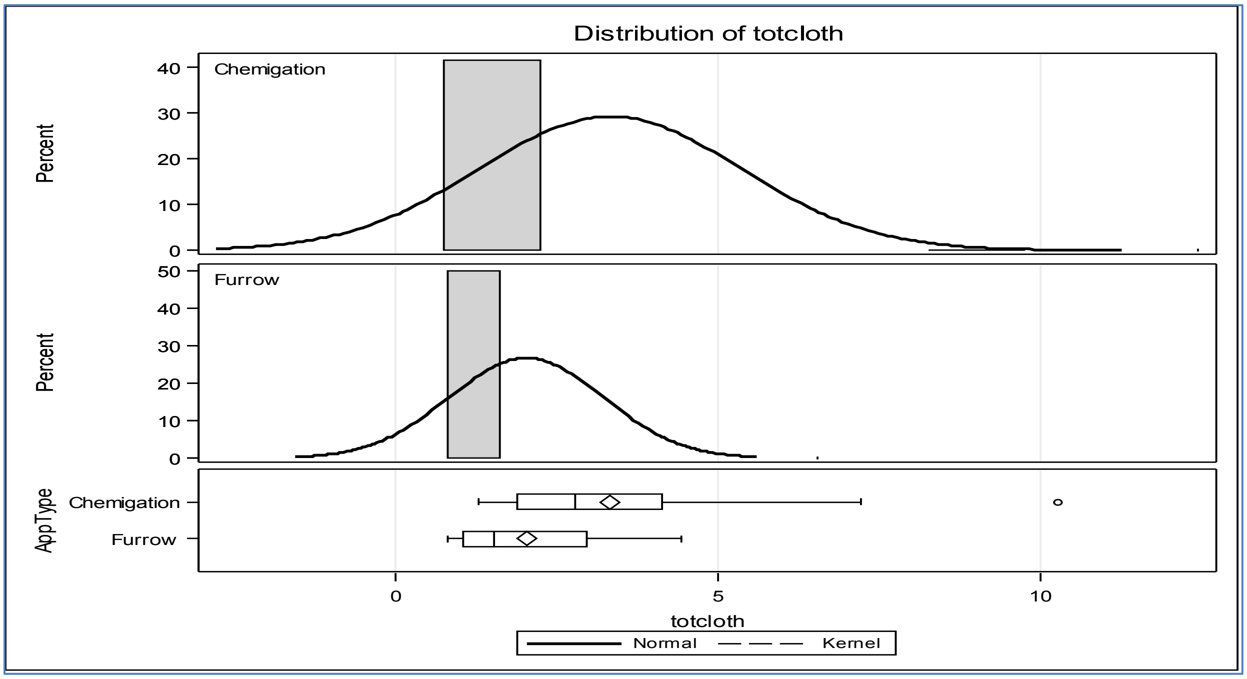


Figure S-6 (B). Application 1 Month After Planting

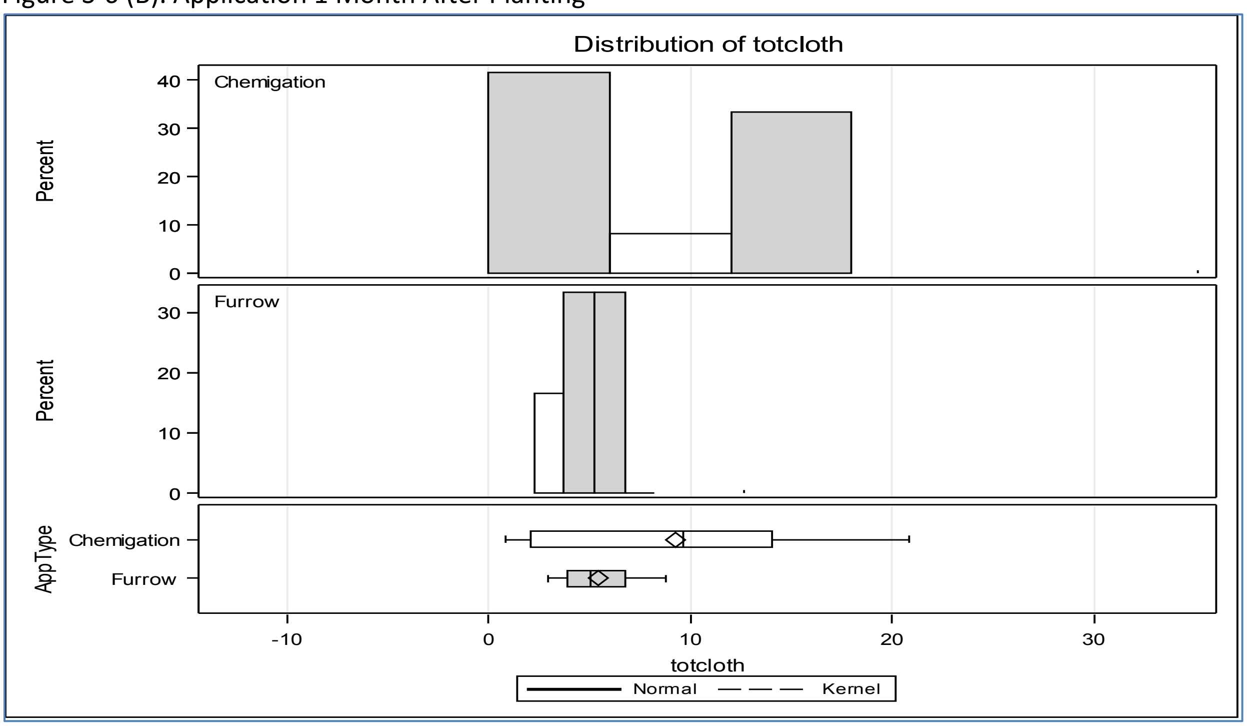


Figure S-7. Pollen Samples: Comparison of the distribution of total clothianidin residue concentration measured for applications made either directly to the furrow or through the chemigation system for application made at planting. Data has been pooled for all years.

Figure S-7 (A). Application At Planting

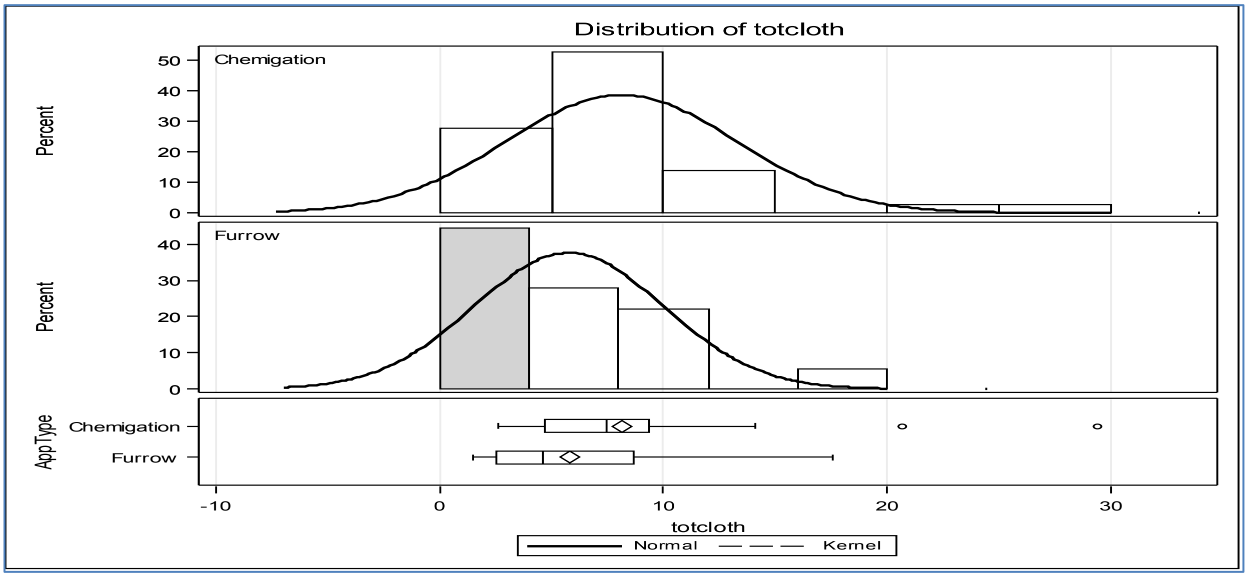


Figure S-7 (B). Application 1 Month After Planting

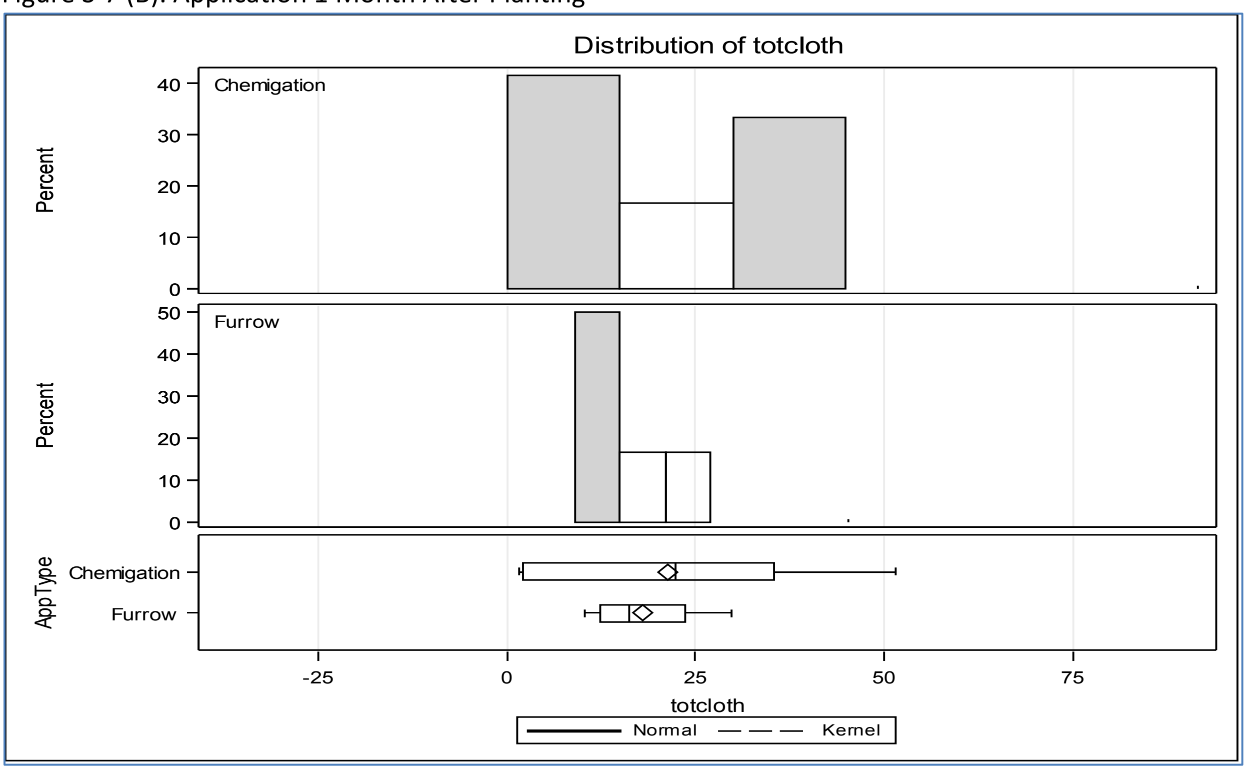


Figure S-8. Soil Samples: Comparison of the distribution of clothianidin residue concentration measured for applications made either at planting or 1 month after planting. Data has been pooled over 2013 and 2014 years.

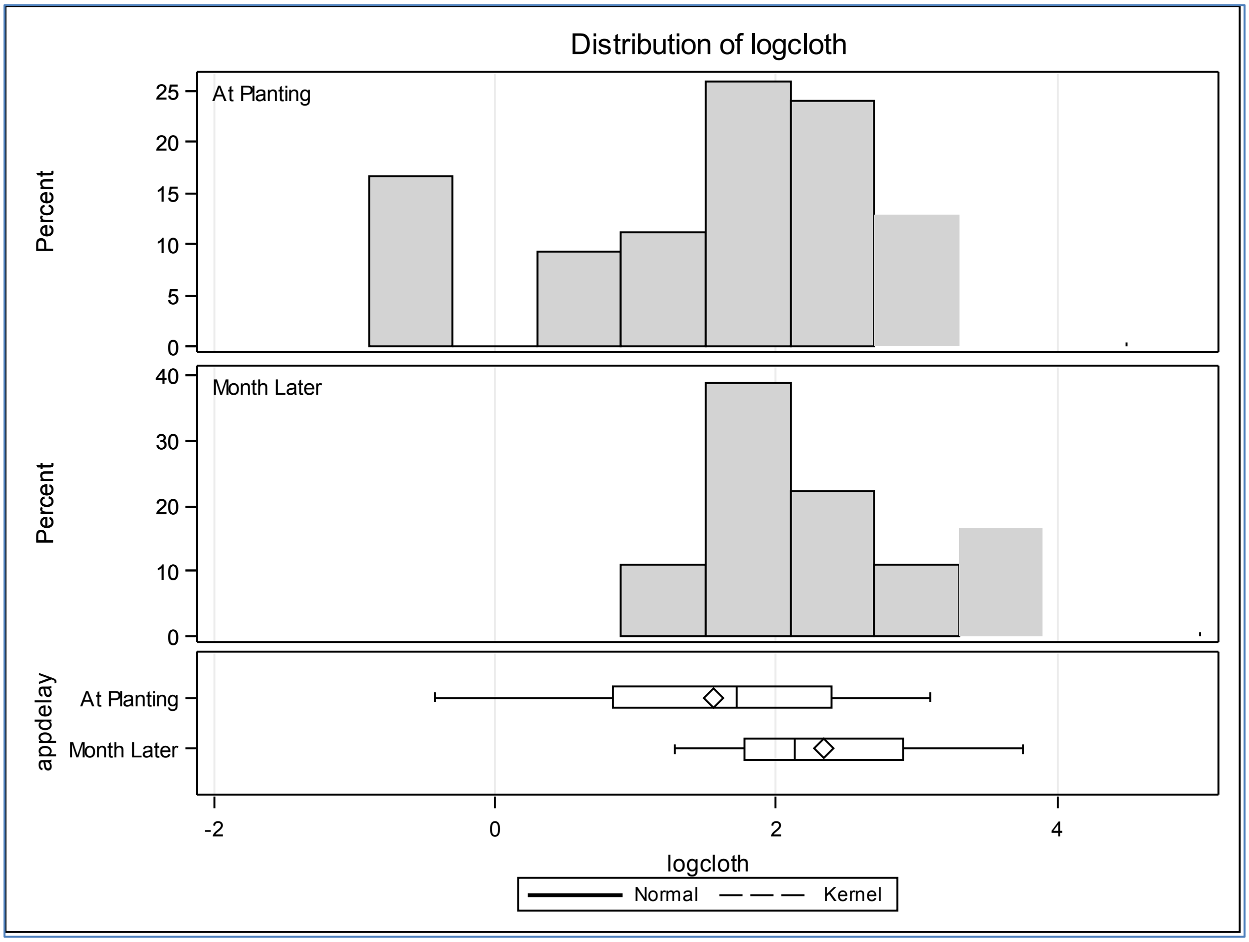


Figure S-9. Relationship of concentration of clothianidin measured between A) Leaves and Nectar; B) Leaves and Pollen; C) Nectar and Pollen.

Figure S-9 (A). Leaves and Nectar

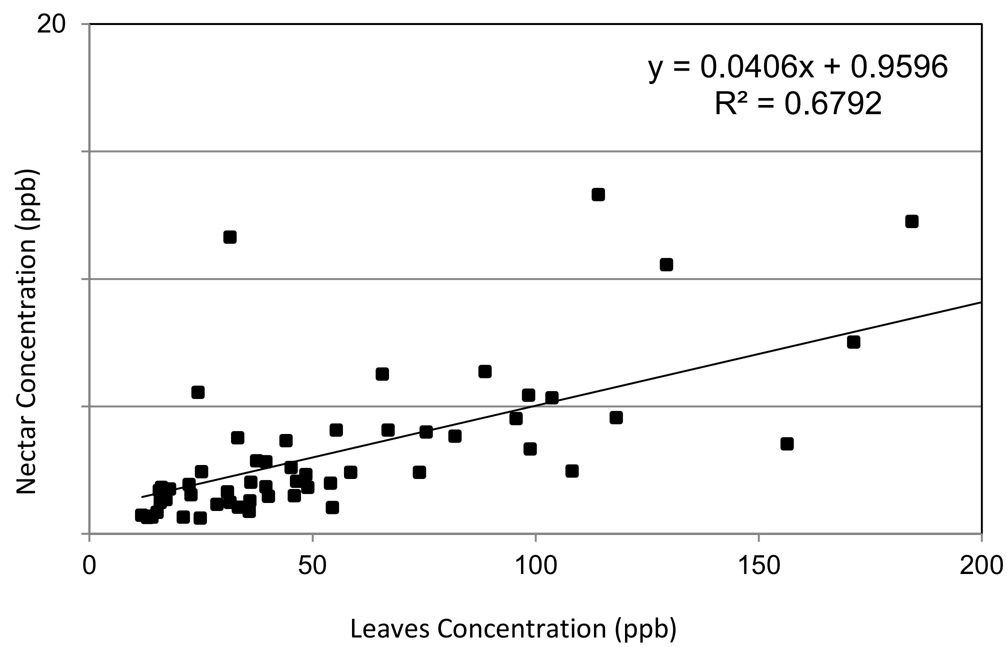


Figure S-9 (B). Leaves and Pollen

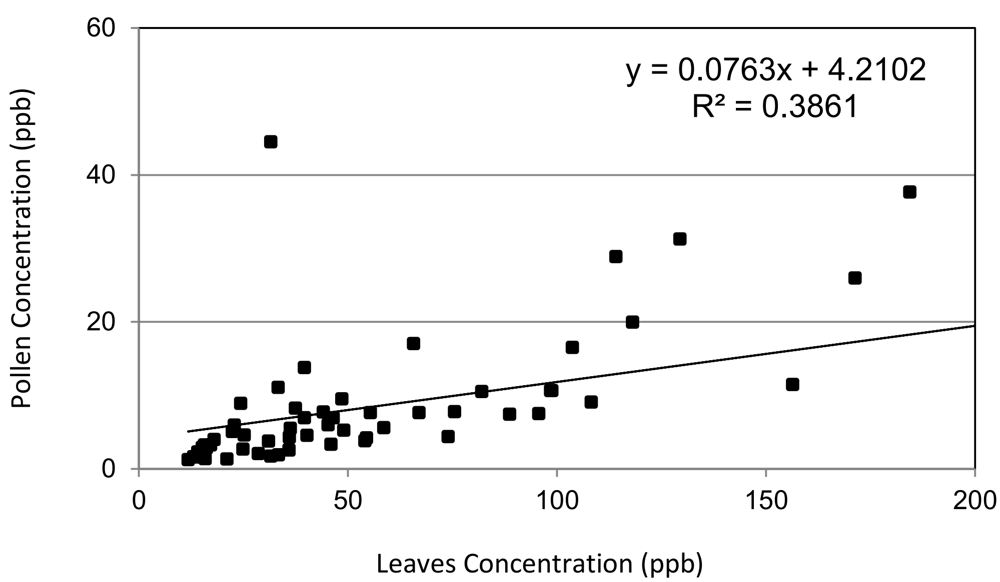
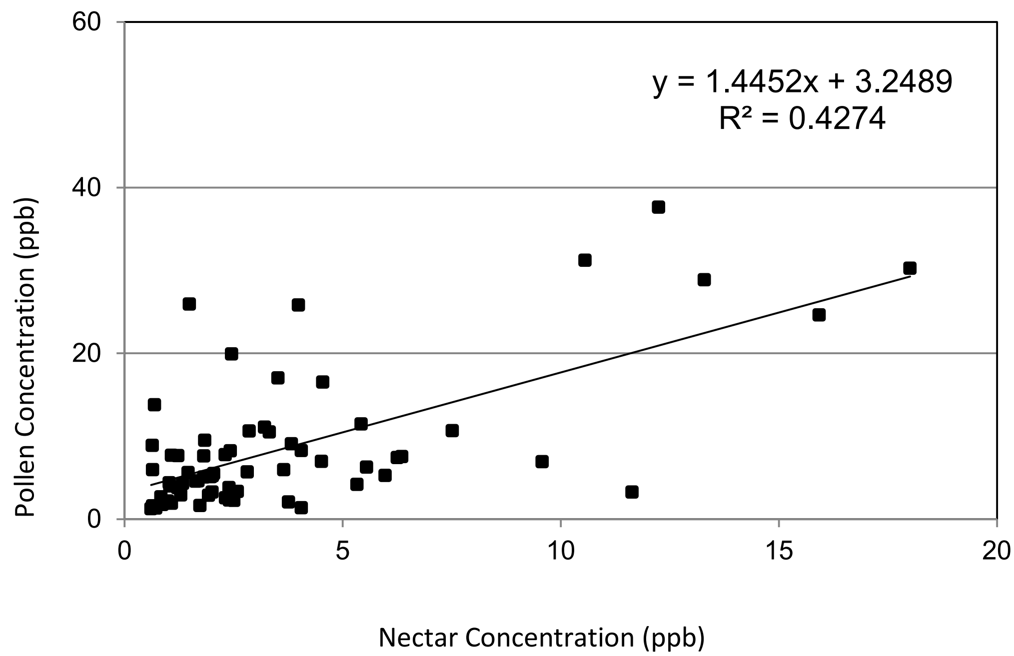


Figure S-9 (C). Nectar and Pollen



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1. STUDY INFORMATION

Chemical:	Clothianidin	**PC Code**	044309
Test Material:	Belay Insecticide	**Percent Active Ingredient:**	20%
Study Type:	Residue study to measure the magnitude of Clothianidin and its major metabolites, TZNG and TZMU, in almond leaves, pollen, and nectar following foliar applications.		
Sponsor:	Valent U.S.A. Corporation 6560 Trinity Court Dublin, California 94568	**Experiment Start and End Date:**	March 24, 2014 – November 14, 2016
Sponsor Study ID:	43411B104	**Study Locations:**	Nine (9) trial sites including
Study Completion Date:	February 23, 2017		
GLP Status:	TBD; protocol reviewed by CDPR. [CDPR Study ID 298000, Data Volume 52884-0279, Tracking ID# 280318]		

2. REVIEWER INFORMATION

Study Reviewed by: California Department of Pesticide Regulation	Richard Bireley, Sr. Environmental Scientist (Specialist) John Troiano, Ph.D., Research Scientist III Alexander Kolosovich, Sr. Environmental Scientist (Specialist) Brigitte Tafarella, Environmental Scientist Denise Alder, Sr. Environmental Scientist (Specialist) Russell Darling, Sr. Environmental Scientist (Specialist)

3. EXECUTIVE SUMMARY

The study was conducted to measure residues of clothianidin and its metabolites, TZNG and TZMU, in nectar, pollen, and leaves collected from almond trees treated with Belay Insecticide (active ingredient clothianidin) over two years. The treatment regimen (two post-bloom foliar applications of Belay Insecticide at BBCH growth stage 7.5 and 21 days before harvest in 2014 and 2015) was evaluated at nine trials located in California in 2014-2016. An NIS (nonionic surfactant) adjuvant (0.20 % v/v) was used in all foliar applications. The total amount of clothianidin applied to almond trees each year was at the maximum annual application rate of 0.2 lb a.i./Acre.

Each trial consisted of a single treated plot with a minimum of 9 almond trees. Each treated plot consisted of three subplots. In the 2014 blooming season, control samples of nectar, pollen, leaves, and soil were collected at the field sites during evaluation of the sampling method and the same field plots were then used as treated plots in the study. Samples of almond flowers were collected by hand from each subplot during the blooming period in 2015 and 2016. The flower collection was done after 139 days (shortest) and 252 days (longest) after the last Belay Insecticide application. The collected flowers were then processed to nectar and pollen. The leaves were collected at BBCH growth stage ca. 6.7 in 2015 and 2016. Soil cores were collected from each trial to characterize clothianidin and its metabolites background in soil before treatment if no pesticide history was available. Soil cores were also used to obtain an estimate of the residues in the root zone of almond trees on the day of/day after the second and forth applications of Belay Insecticide in 2014 and 2015 and during the 2015 and 2016 bloom samplings. Nectar, pollen, anthers, leaves, and soil were analyzed for clothianidin, TZNG, and TZMU residues using liquid chromatography mass spectrometry (LC-MS/MS).

4. STUDY VALIDITY

Guideline Followed:	Protocol was reviewed and approved by CDPR
Guideline Deviations:	N/A
Other Deviations:	See Section 6
Classification:	**ACCEPTABLE**
Rationale:	N/A
Reparability:	N/A

5. MATERIALS

Test Material Characterization for Foliar Application End Use Product			
Test item:	Belay Insecticide	**Percent A.I.:**	23% A.I.
Formulation Type:	Soluble Concentrate (SC)	**Lot/Batch Number:**	V13C-1742-2,V15C-1742-1
CAS #:	210880-92-5	**Expiration Date:**	1/25/2015, 3/3/2016

5A. STUDY DESIGN

The objective of this study is to determine residues of clothianidin and its metabolites, TZNG and TZMU, in almond nectar, pollen, and leaves collected following post-bloom foliar applications of Belay Insecticide to almond trees over two years. One treated plot received two post-bloom foliar applications of Belay Insecticide to almond trees in 2014 and two post-bloom foliar applications of Belay Insecticide to almond trees in 2015. The first foliar application of Belay Insecticide was made at the application rate of 0.1 lb a.i./Acre at BBCH growth stage 7.5 (fruit at half size), and the second foliar application of Belay

Insecticide was made at the application rate of 0.1 lb a.i./Acre at 21 days before harvest. An NIS (nonionic surfactant) adjuvant (0.20 % v/v) was used in all foliar applications. The total amount of clothianidin applied in each year is equivalent to the maximum annual use rate of 0.2 lb a.i./A (224 g a.i./ha).

Each field trial consisted of one treated plot of a minimum of 9 almond trees and each treated plot comprised of three individual subplots. Standard agronomic practices for growing almonds were used on the treated plots.

5B. STUDY SITE LOCATION AND CHARACTERISTICS

The nine field trials were located in the United States of America in California, a commercial area for growing almonds, on either coarse-textured or medium-textured soils. The trial location and soil characterization information is presented in appendix 3 through appendix 12 of the study report, and is summarized in the table below:

Table 1. Site Locations and Cotton Varieties

Trial Site	Site Identification	Trial Location	Height of Trees at Bloom(ft)	Almond Variety	Plot Area (Acres)
1	V-38473-A	Dos Palos, Merced	16	Butte	0.2727
2	V-38473-B	Kerman, Fresno	12	Monterey	0.2500
3	V-38473-C	Madera, Madera	13	Nonpareil	0.1697
4	V-38473-D	Strathmore, Tulare	14-16	Fritz	0.1212
5	V-38473-E	Dinuba, Tulare	18-20	Sonora	0.1212
6	V-38473-F	Lost Hills, Kern	18	Nonpareil/Monterey	0.7651
7	V-38473-G	Shafter, Kern	15-20	Nonpareil/Monterey/Fritz	0.4309
8	V-38473-H	Arbuckle, Colusa	16	Winters	0.2962
9	V-38473-I	Winters, Yolo	18	Mission	0.3182

Table 2. Trial Site Conditions

Site Identification	Sand %	Silt %	Clay %	USDA Textural Class	CEC Meq/100g	Organic Matter %	Soil pH
V-38473-A	53	23	24	Sandy Clay Loam	20.9	1.4	7.6
V-38473-B	77	19	4	Loamy Sand	19.3	1.5	7.1
V-38473-C	86	9	5	Loamy Sand	6.4	0.58	8.0
V-38473-D	53	31	16	Sandy Loam	12.7	2.5	7.3
V-38473-E	71	25	4	Sandy Loam	7.3	1.5	7.9
V-38473-F	48	21	31	Sandy Clay Loam	22.9	1.2	8.1
V-38473-G	56	25	19	Sandy Loam	14.6	0.80	6.1
V-38473-H	39	29	32	Clay Loam	22.1	1.9	7.1
V-38473-I	47	29	24	Loam	17.0	1.4	5.7

MRID 50154302 CDPR Cloth Almond

5C. Application Timing and Rates

One treated plot received two post-bloom foliar applications of Belay Insecticide to almond trees in 2014 and two post-bloom foliar applications of Belay Insecticide to almond trees in 2015. The first foliar application of Belay Insecticide was made at the application rate of 0.1 lb a.i./Acre at BBCH growth stage 7.5 (fruits at half size), and the second foliar application of Belay Insecticide was made at the application rate of 0.1 lb a.i./Acre at 21 days before harvest with exceptions for trials V-38473-F and V38473-G. For V-38473-F trial, the second and fourth applications of Belay Insecticide were made at 32 and 44 days before harvest, respectively. For V-38473-G trial, the second application of Belay Insecticide was done after almond harvest. The total clothianidin amount applied in each year is equivalent to the maximum annual use rate of 0.2 lb a.i./A (224 g a.i./ha). Belay Insecticide was sprayed using an orchard air blast to both sides of the tree rows. Non-ionic surfactant at 0.20% (v/v) was applied with each application

Table 3. Study Use Pattern for Clothianidin

Trial ID	Application Number	Application Date	Application Type	BBCH Growth Stage	Spray Volume	Application rate (lbs ai./Acre)		% of Target Applied
						Spray	Total	
Year: 2014								
V-38473-A	1	3/28/14	Foliar	7.5	150.5	0.101	0.200	101
V-38473-A	2	10/2/14	Foliar	8.9	147.7	0.099		99
V-38473-B	1	3/27/14	Foliar	7.5	150.7	0.101	0.202	101
V-38473-B	2	9/29/14	Foliar	8.9	150.7	0.101		101
V-38473-C	1	4/2/14	Foliar	7.5	149.8	0.099	0.198	98
V-38473-C	2	7/21/14	Foliar	21 days PHI[a]	150.2	0.099		99
V-38473-D	1	4/8/14	Foliar	7.5	125.7	0.101	0.201	101
V-38473-D	2	6/12/14	Foliar	8.1	122.8	0.100		100
V-38473-E	1	4/9/14	Foliar	7.5	125.3	0.101	0.201	101
V-38473-E	2	6/11/14	Foliar	8.1	119.1	0.100		100
V-38473-F	1	4/4/14	Foliar	7.5	124.9	0.118	0.213	118[b]
V-38473-F	2	8/5/14	Foliar	8.5[c]	118.1	0.096		96
V-38473-G	1	3/24/14	Foliar	7.5	104.1	0.103	0.203	103
V-38473-G	2	9/23/14	Foliar	9.1[d]	114.6	0.100		100
V-38473-H	1	4/30/14	Foliar	7.5	127.1	0.101	0.202	101
V-38473-H	2	7/16/14	Foliar	8.5	101.0	0.101		101
V-38473-I	1	5/9/14	Foliar	7.5	138.2	0.101	0.200	101
V-38473-I	2	7/16/14	Foliar	8.5	110.0	0.100		100

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Trial ID	Application Number	Application Date	Application Type	BBCH Growth Stage	Spray Volume	Application rate (lbs ai./Acre)		% of Target Applied
						Spray	Total	
Year: 2015								
V-38473-A	3	4/9/15	Foliar	7.5	151.6	0.102	0.201	101
V-38473-A	4	8/14/15	Foliar	8.9	148.5	0.100		100
V-38473-B	3	4/8/15	Foliar	7.5	148.8	0.100	0.201	100
V-38473-B	4	8/25/15	Foliar	8.9	150.4	0.101		101
V-38473-C	3	4/6/15	Foliar	7.5	152.1	0.100	0.199	100
V-38473-C	4	7/14/15	Foliar	21 days PHI[a]	150.9	0.099		100
V-38473-D	3	5/6/15	Foliar	7.5	122.7	0.100	0.200	100
V-38473-D	4	6/12/15	Foliar	8.1	126.4	0.101		101
V-38473-E	3	5/7/15	Foliar	7.5	122.1	0.100	0.200	100
V-38473-E	4	6/12/15	Foliar	8.1	125.9	0.100		101
V-38473-F	3	3/30/15	Foliar	7.5	110.8	0.103	0.200	103
V-38473-F	4	8/9/15	Foliar	8.5[c]	108.3	0.097		97
V-38473-G	3	3/31/15	Foliar	7.5	112.3	0.101	0.200	101
V-38473-G	4	7/24/15	Foliar	8.5	112.7	0.099		99
V-38473-H	3	4/16/15	Foliar	7.5	121.6	0.100	0.203	101
V-38473-H	4	6/12/15	Foliar	8.5	124.8	0.103		103
V-38473-I	3	4/16/15	Foliar	7.5	133.0	0.100	0.202	100
V-38473-I	4	6/12/15	Foliar	8.5	137.3	0.103		103

[a] Re-treatment interval (Number of days between applications). Not applicable= n/a.
b Application rate outside the acceptable range, see deviation;
c For trial V-38473-F, applications 2 and 4 were made at 32 and 44 days before harvest, respectively, instead of at 21 days before harvest as specified in the protocol;
d Application occurred after the harvest.

5D. SAMPLE COLLECTION, HANDLING, PROCESSING

Soil

The soil core samples were collected at each trial to characterize soil properties and to measure clothianidin residue background from previous agricultural activities when no data were provided about clothianidin use. Typically, three soil cores of 2 inches (5.1 cm) were sampled using a soil auger or a probe to a depth of 12 inches (30 cm) from each plot area. The three soil cores (one from each subplot) were composited together to generate one sample per plot.

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The soil core samples were also collected at each site from three subplots on the day of/day after the second application of Belay Insecticide in 2014 and 2015 and during the 2015 and 2016 bloom samplings, except at trials V-38473-D and V-38473-E. These sites soil samples were not collected after the second application in 2014. To collect a soil sample, two random adjacent almond trees were selected from each subplot. Five (5) soil cores were then collected between the two trees to a depth of 12 inches (30 cm) and 2 inches (5.1 cm) in diameter using a hand held device. The only exception was for trial V-38473-F where soil cores from the 2015 bloom sampling were collected to a depth of 18–23 cm. This sampling technique was used to obtain an estimate of the residues in the root zone of the almond tree.

Once collected, soil samples were placed into coolers with blue ice or wet ice, transported to the field facility, and stored in a temperature-monitored freezer pending shipment to the analytical laboratory. Aliquots of composited soil samples from each plot were shipped to AGVISE Laboratories for soil characterization analysis when no characterization data were provided.

Nectar, Pollen, and Leaf

A single composite flower sample and leaf sample was taken from each replicate subplot resulting in collection of three samples of each matrix for each treated plot. The flower samples were collected during the blooming period in 2015 and 2016 and the leaf samples were collected at BBCH growth stage 6.7 in 2015 and 2016. The flower sampling was done when there were enough flowers in the field to obtain sufficient amounts of pollen and nectar for residue analysis. The leaf sampling was done when first leaves were unfolded. Single composite samples of almond flowers and leaves were collected from each site in the 2014 blooming season and used as control samples. Sample collection dates are summarized in the table below.

Table 4. Sampling Events and Timing.

Sampling Event	Flower			Leaves		
	Timing	Collection Date	DALA[a] (Days)	Timing (BBCH)	Collection Date	DALA[a] (Days)
Trial V-38473-A						
2015	Bloom	2/18/2015	139	7.2	3/17/15	166
2016	Bloom	2/18/2016	188	7.2	3/16/16	215
Trial V-38473-B						
2015	Bloom	2/20/15	144	7.2	3/20/15	172
2016	Bloom	2/22/16	181	7.2	3/17/16	205
Trial V-38473-C						
2015	Bloom	2/18/15	212	6.7	3/2/15	224
2016	Bloom	2/17/16	218	6.7	3/4/16	234
Trial V-38473-D						
2015	Bloom	2/17/15	250	6.7	3/18/15	279
2016	Bloom	2/17/16	250	6.7	3/3/16	265
Trial V-38473-E251						
2015	Bloom	2/18/15	252	6.7	3/19/15	281
2016	Bloom	2/18/16	251	6.7	3/3/16	265

Sampling Event	Flower			Leaves		
	Timing	Collection Date	DALA[a] (Days)	Timing (BBCH)	Collection Date	DALA[a] (Days)
Trial V-38473-F						
2015	Bloom	2/18/15	197	6.7	3/4/15	211
2016	Bloom	2/20/16	195	6.7	2/29/16	204
Trial V-38473-G						
2015	Bloom	2/16/15	146	6.7	2/26/15	156
2016	Bloom	2/16/16	207	6.7	2/29/16	220
Trial V-38473-H						
2015	Bloom	2/10/15	209	6.7	3/13/15	240
2016	Bloom	2/17/16	250	6.7	3/21/16	283
Trial V-38473-I						
2015	Bloom	2/19/15	218	6.7	3/13/15	240
2016	Bloom	2/17/16	250	6.7	3/16/16	278

Flowers from the same subplot were randomly collected from each quadrant of the tree at the middle of the current season's terminal shoots of the lower, middle, and upper lateral branches of the tree and composited in a 1-gallon bag. The collected flowers were placed in a cooler containing blue ice or wet ice and transported to the field facility for processing into pollen and nectar samples. The bag(s) with the flowers were left in a cooler for 1–3 hours (for conditioning), except at trial V-38473-C. The conditioning allowed a pool of nectar to form in the flower nectaries. Nectar and pollen processed from individual flowers collected from the same subplot were composited to generate one sample of nectar and one sample of pollen. Nectar was collected at the base of the flower filament using a glass microcapillary and transferred through pre-split septa into a 1.8-mL pre-labeled vial at the field facility with exceptions for trials V-38473-A and V-38473-B where nectar was collected in the field from flowers. After extraction of nectar, the blossoms were placed on a dry, clean surface and allowed to dry for several hours. The drying allowed the release of pollen from the anthers. The released pollen was "vacuumed" into a pipette tip containing a barrier, using a vacuum system. After pollen processing was complete, the pipette tips were transferred into a vial.

Immediately after final weights were recorded, the labeled sample vials containing nectar and pollen were placed into a freezer where they remained until they were shipped to the analytical laboratory. After processing was completed, the flowers were discarded.

New emerged leaves were sampled from the middle of the current season's terminal shoots of the lower, middle, and upper lateral branches of the tree and transferred into a pre-labeled container. Leaf samples were collected and placed in a cooler containing blue or wet ice and transported to the field facility where they were stored in a temperature-monitored freezer pending shipment to the analytical laboratory.

Sample Storage.

In the field facility, all collected samples were stored in a temperature-monitored freezer pending shipment to the analytical laboratory. Samples were shipped by a freezer truck (Agricultural Chemical Delivery Services, Inc.) or by FedEx☐ on dry ice accompanied by the chain of custody documents. Once samples were received at the analytical laboratory, they were placed into a temperature monitored, walk in freezer pending analysis.

The almond nectar and pollen samples generated during the study were stored up to 176 and 196 days before analysis, respectively. Valent U.S.A. Corporation is conducting freezer storage stability studies of clothianidin, TZNG, and TZMU in surrogate nectar[1] and corn pollen[2]. Interim analyses after 1 year of frozen storage indicate that these chemicals are stable in both matrices.

The almond leaf samples generated during the study were stored up to 152 days before analysis. Clothianidin residues have been shown to be stable on a variety of leafy vegetable crops when stored frozen for up to 242 days[3 and 4]; therefore, a storage stability study on almond leaves was not conducted with this study. Lettuce leaf samples from the head lettuce residue study were stored for up to 242 days, and turnip top leaves and mustard greens from the rotational crop study were stored for 309 days.

The soil samples generated during the study were stored up to 334 days before analysis except only three soil samples from V-38473-C trial were stored up to 489 days. Clothianidin, TZNG, and TZMU residues have been shown to be stable in soil when stored frozen for up to 356 days[5]; therefore, a storage stability study on soil was not conducted with this study.

5E. ANALYTICAL METHODS

Method Summary for Analyzing Almond Nectar Samples

The method used to analyze samples of nectar in this study was Valent Method RM-39N-1, and it allowed the quantitative determination of residues of clothianidin and its metabolites, TZNG and TZMU. A copy of the method is included in appendix 13 of the study report. Generally, 0.100 g of nectar sample was weighed into an autosampler vial and dissolved in 1.0 mL of methanol/water solution (40:60, v/v) acidified with 0.05% formic acid. The sample was spiked with isotopically labeled internal standards (clothianidin-d_3, TZNG-^{13}C-^{15}N, and TZMU-d_3), filtered through a Whatman 0.2 µm nylon membrane syringe filter, if particles were present, and then analyzed by LC-MS/MS.

Due to the small sample size of some nectar samples, the entire sample was used for the analysis.

Method Summary for Analyzing Almond Pollen and Anther Samples

The method used to analyze pollen and anthers in this study was Valent Method RM-39P-1, and it allowed the quantitative determination of residues of clothianidin, TZNG, and TZMU. A copy of the method is included in appendix 14 of the study report. Generally, 0.100 g of pollen/anther sample was weighed into a 50-mL polypropylene centrifuge tube and extracted with water (10.0 mL), and acetonitrile (10.0 mL) followed by adding sodium chloride (1.0 g) and anhydrous magnesium sulfate (2.0 g) salts. Further, the upper acetonitrile phase (9.0 mL) was removed, partitioned with n-hexane (5 mL) containing magnesium sulfate (0.5 g), then collected (8.0 mL) and concentrated to dryness. Residues were re-dissolved in 1.0 mL of methanol/water (40:60, v/v) acidified with 0.05% formic acid, filtered through a Whatman 0.2 µm nylon membrane syringe filter, and analyzed by LC-MS/MS after spiking with isotopically labeled internal standards (clothianidin-d_3, TZNG-^{13}C-^{15}N, and TZMU-d_3) to compensate for matrix effect.

Due to the small sample size of some pollen or anther samples, the entire sample was used for the analysis and the final volume of the sample was adjusted to either 0.2 or 0.5 mL, depending on the sample size. For these samples, instead of filtering, the sample extract was centrifuged to remove any particles before analysis.

Method Summary for Analyzing Almond Leaf Samples

The method used to analyze almond leaf samples in this study was Valent Method RM-39L-2 and it allowed quantitative determination of clothianidin, TZNG, and TZMU. A copy of the method is included in appendix 15 of the study report. Generally, 2.0 g of homogenized leaf sample was weighed into a 50-mL polypropylene centrifuge tube and extracted with 10 mL of water acidified with 0.05% formic acid and acetonitrile (10 mL), followed by adding sodium chloride (2.0 g) and anhydrous magnesium (4.0 g) sulfate salts. Further, an aliquot (1.0 mL) of the acetonitrile extract was cleaned through a Strata C18-E column and concentrated to dryness. Residues were re-dissolved in 1.0 mL of water/methanol (75:25, v/v) acidified with 0.05% formic acid, filtered through a Whatman 0.2 µm nylon membrane syringe filter into a vial, and analyzed by accurate mass UPLC/Q-TOF MS-MS after spiking with isotopically labeled internal standards (clothianidin-d_3, TZNG-^{13}C-^{15}N, and TZMU-d_3) to compensate for matrix effect.

Method Summary for Analyzing Soil Samples

The method used to analyze soil samples in this study was Valent Method RM-39S-2, and it allowed quantitative determination of clothianidin, TZNG, and TZMU. A copy of the method is included in appendix 16 of the study report. Typically, a 20.0-g soil sample was weighed into a 50-mL polypropylene centrifuge tube and extracted with 25.0 mL of methanol/water (25:75, v/v) acidified with 0.05% formic acid. The sample was shaken on a shaker for 1 hour and then centrifuged. Extraction was repeated again with a fresh portion of solvent (25.0 mL). The supernatants were combined and then spiked with isotopically labeled internal standards (clothianidin-d_3, TZNG-^{13}C-^{15}N, and TZMU-d_3) to compensate for matrix effect. Then an aliquot was filtered through a Whatman 0.2 µm nylon membrane syringe filter directly into an autosampler vial and then analyzed by accurate mass UPLC/Q-TOF MS-MS.

For the site selection when no appropriate documentation was provided that clothianidin and thiamethoxam had not been used in the last year at the trial site, collected soil samples were analyzed for clothianidin residues using Valent Method RM-39S-1. This method allowed quantitative determination of clothianidin. A copy of the method is included in appendix 16 of the study report. Typically, a 20.0-g soil sample was weighed into a 50-mL polypropylene centrifuge tube and extracted with 25.0 mL of methanol/water (40:60, v/v) acidified with 0.05% formic acid. The sample was shaken on a shaker for 1 hour and then centrifuged. Extraction was repeated again with a fresh portion of solvent (25.0 mL). The supernatants were combined and then spiked with the isotopically labeled internal standard (clothianidin-d_3) to compensate for matrix effect. Then an aliquot was filtered through a Whatman 0.2 µm nylon membrane syringe filter directly into an autosampler vial and analyzed by accurate mass UPLC/Q-TOF MS-MS.

5F. QUALITY ASSURANCE RESULTS

Quality control measures taken during the analytical phase of this study included, but were not limited to, the following:

All analytical standards used in this study were kept at reduced temperature in a refrigerator or in a freezer at all times when not in use. The temperatures of all refrigerators and freezers used to store samples and standards at Valent Technical Center for this study were continuously monitored using a datalogger. Data from the datalogger are printed on a regular schedule and archived at the Valent Technical Center. Valent certified the analytical reference standards used in this study prior to use. Certification documents are included in this report in appendix 2 of the study report. Certification data and retain samples of these materials are archived at the Valent Technical Center. All raw data generated from this study will be archived at the Valent Technical Center.

At least five different standard concentrations were injected within each analytical set. The concentration (ng/mL) of clothianidin and its metabolites detected in sample extracts was interpolated from the standard calibration curve. The LC/MS-MS and accurate mass UPLC/Q-TOF MS-MS systems were calibrated for each set of samples by analyzing these calibrating standard concentrations, with these standards interspersed within the analytical sequence. A second-order polynomial fit (weighted relative to 1/concentration) was then calculated from the concentrations and the detector response of the calibration standards. To verify performance, the percent difference between the actual concentration and the calculated concentration for each of the calibration standards (based on the curve) was also calculated. Each of the standards was required to be within 15% (20% for method RM-39S-2) of the theoretical concentration and the coefficient of determination (r^2) of the weighted polynomial calibration curve was required to be greater than or equal to 0.99. No exceedance of these criteria for the calibration standards was observed in the study.

The reproducibility of the LC/MS-MS and accurate mass UPLC/Q-TOF MS-MS systems was verified by comparison of instrument responses obtained from the repeated analysis of a continuing standard (a midlevel calibration standard) analyzed with the study samples. The continuing calibration standards were interspersed within the samples in the analytical sequence, and the analytical sequence began and ended with a continuing calibration standard. For an analytical set (injection sequence) to be acceptable, the coefficient of variation (CV) of the back calculated concentration of the continuing standards was required to be 10% (method RM-39N-1 and RM-39P-1) and 15% (method RM-39L-2 and RM-39S-2). No exceedance of these criteria for the continuing calibration standards was observed in the study.

At least one control sample and two fortified samples were analyzed with each set of the study samples to verify method performance. Fortifications were made at 1.00 and 10.0 ng/g for nectar and pollen samples, and at 5.0 and 50.0 ng/g for leaves and soil. For an analytical run to be acceptable, method recoveries were required to be between 70 and 120%. Recoveries of the concurrent laboratory fortified samples were within this range. Generally, recoveries of the concurrent laboratory fortified samples were within this range. In some cases, minor exceedance of these criteria was accepted.

Method verification for each matrix was conducted for clothianidin, TZNG, and TZMU. Method RM 39N-1 was verified at 1.00 ng/g (LOQ) and 10.0 ng/g (10× LOQ) using almond nectar or artificial nectar. Method RM-39P-1 was verified at 1.00 ng/g (LOQ) and 10.0 ng/g (10× LOQ) using commercially available pollen. Methods RM-39L-2 and RM-39S-2 were verified at 5.0 ng/g (LOQ) and 50.0 ng/g (10× LOQ) levels using untreated control almond leaves and untreated control soil.

Table 5. Summary of LOQs and LODs

Method	Matrix	Analyte	LOD (ppb, parent equivalents)	LOQ (ppb, parent equivalents)
RM-39N-1	Nectar	Clothianidin, TZNG and TZMU	0.20	1.0
RM-39P-1	Pollen	Clothianidin, TZNG and TZMU	0.25	1.0
RM-39P-1	Anthers	Clothianidin, TZNG and TZMU	0.25	1.0
RM-39L-2	Leaves	Clothianidin, TZNG and TZMU	1.3	5.0
RM-39S-1	Soil	Clothianidin	1.3	5.0
RM-39S-2	Soil	Clothianidin, TZNG and TZMU	1.3	5.0

6. DEVIATIONS DURING FIELD PHASE

During the field portion of this study the following deviations occurred:

Trial V-38473-C:

Several weather data points were not collected for the trial period because Weather Station CIMIS #188 had some gaps in recording daily temperatures.

The 2015 pollen sample size did not meet protocol requirements because a significant amount of pollen was diluted/washed off by heavy fog and could not be collected. Also, flowers were not placed in a cooler with blue ice for the 1- to 3-hour conditioning period during sampling events.

Trial V-38473-F:

During the first application of Belay Insecticide to the almond plot, a spray error was made resulting in the actual application rate to the center (sample) row was likely 90.8% of the target rate. This deviation was due to a technical error by the field trial personnel and the sprayer malfunction during application.

Due to excessively moist soil, soil samples were collected 2 days after flower collection in 2015 instead of on the day of/day after flower collection. Also, the soil cores were collected to a depth of 18–23 cm (7– 9 inches) instead 30 cm (12 inches.).

The timing of the second application in 2014 was 32 days before harvest and the timing of the fourth application in 2015 was 44 days before harvest, instead of 21 days before harvest as specified in the protocol. Almond harvest timing is variable from season to season, so despite efforts to meet the 21-day requirement through communication with the grower, the actual timings were longer than desired.

Transport temperatures for the test substance were not monitored between the field test facility storage and the field site, as required by Valent SOP VP-203.

Trial V-38473-G:

The second application of Belay Insecticide to almond trees was conducted after the final almond harvest instead of 21 days pre-harvest as required by the protocol. The orchard had three varieties of almonds with different harvest dates, so the application was delayed.

Transport temperatures of the test substance were not monitored from the field test facility storage to the field site, as required by Valent SOP VP-203.

Flower, pollen, and nectar samples were not collected in 2014 because the almond bloom was too far along to collect these samples. This sampling was only to be used to evaluate the sampling method during the 2014 bloom.

No weight was recorded for the leaf sample V-38473-G-5 because the Field Residue Data Book forms had not yet been received and field personnel neglected to record the weight. This deviation had no negative impact on the study. Additionally, this sample was collected at BBCH 7.2, instead of at BBCH 6.7 as required by the protocol. This sampling was only to be used to evaluate the sampling method and use the collected control sample for method verification and QC samples.

No soil core sample was collected from subplot 3 (2014, second application) as required by the protocol because the soil core sampler handle broke during sampling.

7. RESULTS:

Raw data as reported for concentrations of Clothianidin, TZNG, and TZMU are reproduced in Tables 6 through 11 where Table 6 contains data for almond nectar, Table 7 for almond pollen, Table 8 for almond anthers, Table 9 for almond leaves, Table 10 for soil samples taken at bloom, and Table 11 for soil samples taken after applications 2 and 4. Summary statistics for total clothianidin residues are reproduced in Table 12.

Table 6. Clothianidin, TZNG and TZMU Residues in Almond Nectar

Trial	Sample ID	DALA[a]	Clothianidin		TZNG		TZMU	
			ppb[b]	Average	ppb[b]	Average	ppb[b]	Average
2015								
	V-38473-A-15		<0.20		<0.20		<0.20	
A	V-38473-A-16	139	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
	V-38473-A-17		<0.20		<0.20		<0.20	
	V-38473-B-15		(0.34)		<0.20		<0.20	
B	V-38473-B-16	144	1.28	0.67	<0.20	<0.20	(0.53)	0.28
	V-38473-B-17		(0.40)		<0.20		(0.20)	
	V-38473-C-15		<0.20		<0.20		<0.20	
C	V-38473-C-16	212	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
	V-38473-C-17		(0.21)		<0.20		(0.22)	
	V-38473-D-15		(0.70)		<0.20		<0.20	
D	V-38473-D-16	250	(0.56)	0.70	<0.20	<0.20	<0.20	<0.20
	V-38473-D-17		(0.84)		<0.20		<0.20	
	V-38473-E-15		(0.73)		<0.20		<0.20	
E	V-38473-E-16	252	(0.47)	0.50	<0.20	<0.20	<0.20	<0.20
	V-38473-E-17		(0.30)		<0.20		<0.20	
	V-38473-F-15		<0.20		<0.20		<0.20	
F	V-38473-F-16	197	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
	V-38473-F-17		<0.20		<0.20		<0.20	
	V-38473-G-15		<0.20		<0.20		<0.20	
G	V-38473-G-16	146	(0.40)	0.24	<0.20	<0.20	<0.20	<0.20
	V-38473-G-17		(0.23)		<0.20		<0.20	
	V-38473-H-15		<0.20		<0.20		<0.20	
H	V-38473-H-16	209	<0.20	0.20	<0.20	<0.20	<0.20	<0.20
	V-38473-H-17		<0.20		<0.20		<0.20	
	V-38473-I-15		<0.20		<0.20		<0.20	
I	V-38473-I-16	218	<0.20	0.20	<0.20	<0.20	<0.20	<0.20
	V-38473-I-17		<0.20		<0.20		<0.20	
		Minimum:	<0.20		<0.20		<0.20	
		Maximum:	1.28		<0.20		(0.53)	
		Average:	(0.28)		[c]		[c]	
		Median:	<0.20		[c]		[c]	
		90^{th} Percentile:	(0.71)		[c]		[c]	

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2016								
A	V-38473-A-33	188	(0.87)	1.35	<0.20	<0.20	<0.20	<0.20
	V-38473-A-34		1.15		<0.20		<0.20	
	V-38473-A-35		2.04		<0.20		<0.20	
B	V-38473-B-33	181	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
	V-38473-B-34		<0.20		<0.20		<0.20	
	V-38473-B-35		<0.20		<0.20		<0.20	
C	V-38473-C-33	218	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
	V-38473-C-34		<0.20		<0.20		<0.20	
	V-38473-C-35		(0.37)		<0.20		<0.20	
D	V-38473-D-33	250	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
	V-38473-D-34		<0.20		<0.20		<0.20	
	V-38473-D-35		<0.20		<0.20		<0.20	
E	V-38473-E-33	251	(0.33)	0.57	<0.20	<0.20	<0.20	<0.20
	V-38473-E-34		1.09		<0.20		<0.20	
	V-38473-E-35		(0.29)		<0.20		<0.20	
F	V-38473-F-33	195	(0.29)	<0.20	<0.20	<0.20	<0.20	<0.20
	V-38473-F-34		<0.20		<0.20		<0.20	
	V-38473-F-35		<0.20		<0.20		<0.20	
G	V-38473-G-33	207	<0.20	0.26	<0.20	<0.20	<0.20	<0.20
	V-38473-G-34		(0.41)		<0.20		<0.20	
	V-38473-G-35		(0.26)		<0.20		<0.20	
H	V-38473-H-33	250	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
	V-38473-H-34		<0.20		<0.20		<0.20	
	V-38473-H-35		<0.20		<0.20		<0.20	
I	V-38473-I-33	250	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
	V-38473-I-34		<0.20		<0.20		<0.20	
	V-38473-I-35		<0.20		<0.20		<0.20	
		Minimum:	**<0.20**		**<0.20**		**<0.20**	
		Maximum:	**2.04**		**<0.20**		**<0.20**	
		Average:	**0.33**		[c]		[c]	
		Median:	**<0.20**		[c]		[c]	
		90th Percentile:	**0.96**		[c]		[c]	

[a] DALA= days after last application
[b] Values in parenthesis are between the LOQ and LOD
[c] Descriptive statistics were not calculated because >50% of the results are below the LOD
In calculating the average concentration and descriptive statistics, values below the LOD are substituted with half of the LOD value. LOD= 0.20 ppb and LOQ= 1.00 ppb for Clothianidin, TZNG and TZMU.

Table 7. Clothianidin, TZNG and TZMU Residues in Almond Pollen

Trial	Sample ID	DALA[a]	Clothianidin		TZNG		TZMU	
			ppb[b]	Average	ppb[b]	Average	ppb[b]	Average
2015								
A	V-38473-A-18	140	3.06	2.73	8.19	2.86	22.8	8.86
	V-38473-A-19		1.90		<0.25		(0.98)	
	V-38473-A-20		3.22		(0.25)		2.78	
B	V-38473-B-18	145	4.58	5.30	(0.27)	(0.41)	1.70	2.79
	V-38473-B-19		7.08		(0.64)		3.59	
	V-38473-B-20		4.26		(0.32)		3.09	
C	V-38473-C-18	214	12.7	13.4	(0.83)	(0.64)	3.63	2.68
	V-38473-C-20		14.0		(0.44)		1.73	
F	V-38473-F-18	198	(0.77)	1.16	<0.25	(0.72)	(0.56)	1.60
	V-38473-F-19		1.60		1.90		3.74	
	V-38473-F-20		1.10		<0.25		(0.49)	
G	V-38473-G-18	147	2.21	1.91	<0.25	<0.25	(0.30)	<0.25
	V-38473-G-19		1.29		<0.25		(0.25)	
	V-38473-G-20		2.23		<0.25		<0.25	
H	V-38473-H-18	210	10.4	11.5	2.48	1.66	5.85	5.08
	V-38473-H-19		13.3		<0.25		2.03	
	V-38473-H-20		11.0		2.39		7.37	
I	V-38473-I-18	219	7.45	11.9	(0.90)	(0.56)	2.23	1.59
	V-38473-I-19		8.26		(0.26)		(0.95)	
	V-38473-I-20		20.0		(0.51)		1.58	
		Minimum:	**(0.77)**		**<0.25**		**<0.25**	
		Maximum:	**20.0**		**8.19**		**22.8**	
		Average:	**6.52**		**1.01**		**3.29**	
		Median:	**4.42**		**(0.30)**		**1.88**	
		90th Percentile:	**13.4**		**2.40**		**6.00**	
2016								
A	V-38473-A-36	189	5.42	4.82	<0.25	<0.25	(0.45)	(0.43)
	V-38473-A-37		3.83		<0.25		(0.38)	
	V-38473-A-38		5.23		<0.25		(0.47)	
B	V-38473-B-36	182	3.04	3.21	<0.25	<0.25	(0.44)	(0.61)
	V-38473-B-37		2.76		<0.25		(0.80)	
	V-38473-B-38		3.82		<0.25		(0.59)	
C	V-38473-C-36	219	11.7	7.80	(0.54)	(0.26)	1.25	(0.77)
	V-38473-C-37	220	4.54		<0.25		(0.63)	
	V-38473-C-38	221	7.20		<0.25		(0.45)	
F	V-38473-F-36	196	1.04	(0.75)	<0.25	<0.25	<0.25	<0.25
	V-38473-F-37		(0.62)		<0.25		<0.25	
	V-38473-F-38		(0.60)		<0.25		<0.25	

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Trial	Sample ID	DALA[a]	Clothianidin		TZNG		TZMU	
			ppb[b]	Average	ppb[b]	Average	ppb[b]	Average
G	V-38473-G-36	208	1.15	(0.90)	<0.25	<0.25	<0.25	<0.25
	V-38473-G-37		1.00		<0.25		<0.25	
	V-38473-G-38		(0.55)		<0.25		<0.25	
H	V-38473-H-36	251	8.81	11	(0.32)	(0.41)	(0.98)	(0.99)
	V-38473-H-37		13.8		(0.51)		1.19	
	V-38473-H-38		10.4		(0.40)		(0.81)	
I	V-38473-I-36	251	5.98	4.92	<0.25	<0.25	(0.56)	(0.50)
	V-38473-I-37		4.32		<0.25		(0.45)	
	V-38473-I-38		4.46		<0.25		(0.49)	
		Minimum:	**(0.55)**		**<0.25**		**<0.25**	
		Maximum:	**13.8**		**(0.54)**		**1.25**	
		Average:	**4.77**		**<0.25**		**(0.51)**	
		Median:	**4.32**		**<0.25**		**(0.45)**	
		90th Percentile:	**10.4**		**(0.40)**		**(0.98)**	

[a] DALA= days after last application
[b] Values in parenthesis are between the LOQ and the LOD
In calculating the average concentration and descriptive statistics, values below the LOD are substituted with half of the LOD value
LOD= 0.25 ppb and LOQ= 1.00 ppb for clothianidin, TZNG and TZMU

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Table 8. Clothianidin, TZNG and TZMU Residues in Almond Anthers

Trial	Sample ID	DALA[a]	Clothianidin		TZNG		TZMU	
			ppb[b]	Average	ppb[b]	Average	ppb[b]	Average
2015								
D	V-38473-D-18	250	23.1	43.4	11.0	4.25	43.3	16.5
	V-38473-D-19		88.1		1.42		4.57	
	V-38473-D-20		19.2		(0.35)		1.70	
E	V-38473-E-18	252	15.2	18.7	1.04	(0.83)	(0.84)	(0.69)
	V-38473-E-19		27.0		(0.75)		(0.72)	
	V-38473-E-20		13.9		(0.70)		(0.50)	
		Minimum:	**13.9**		**(0.35)**		**(0.50)**	
		Maximum:	**88.1**		**11.0**		**43.3**	
		Average:	**31.1**		**2.54**		**8.61**	
		Median:	**21.1**		**(0.89)**		**1.27**	
		90th Percentile:	**57.5**		**6.21**		**23.9**	
2016								
D	V-38473-D-36	250	1.38	1.06	<0.25	<0.25	<0.25	<0.25
	V-38473-D-37		(0.75)		<0.25		<0.25	
	V-38473-D-38		1.04		<0.25		<0.25	
E	V-38473-E-36	251	9.34	3.96	(0.45)	(0.36)	(0.28)	<0.25
	V-38473-E-37		2.19		(0.32)		<0.25	
	V-38473-E-38		(0.35)		(0.31)		<0.25	
		Minimum:	**(0.35)**		**<0.25**		**<0.25**	
		Maximum:	**9.34**		**(0.45)**		**(0.28)**	
		Average:	**2.51**		**<0.25**		**<0.25**	
		Median:	**1.21**		**<0.25**		**<0.25**	
		90th Percentile:	**5.77**		**(0.38)**		**<0.25**	

[a] DALA= days after last application
[b] Values in parenthesis are between the LOQ and the LOD
In calculating the average concentration and descriptive statistics, values below the LOD are substituted with half of the LOD value
LOD= 0.25 ppb and LOQ= 1.00 ppb for clothianidin, TZNG and TZMU

Table 9. Clothianidin, TZNG and TZMU Residues in Almond Leaves

Trial	Sample ID	DALA[a]	Clothianidin		TZNG		TZMU	
			ppb[b]	Average	ppb[b]	Average	ppb[b]	Average
2015								
A	V-38473-A-21	166	(3.01)	(3.09)	<1.3	<1.3	(1.94)	(1.66)
	V-38473-A-22		(3.08)		<1.3		(1.56)	
	V-38473-A-23		(3.18)		<1.3		(1.48)	
B	V-38473-B-21	172	<1.3	5.62	<1.3	(1.55)	<1.3	<1.3
	V-38473-B-22		(3.64)		<1.3		<1.3	
	V-38473-B-23		12.6		(3.35)		<1.3	
C	V-38473-C-21	224	5.57	10.1	<1.3	<1.3	(1.38)	(1.53)
	V-38473-C-22		9.39		<1.3		(1.54)	
	V-38473-C-23		15.4		<1.3		(1.65)	
D	V-38473-D-21	279	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
	V-38473-D-22		<1.3		<1.3		<1.3	
	V-38473-D-23		<1.3		<1.3		<1.3	
E	V-38473-E-21	281	10.0	8.43	5.64	(4.36)	<1.3	<1.3
	V-38473-E-22		8.29		(3.72)		<1.3	
	V-38473-E-23		6.97		(3.73)		<1.3	
F	V-38473-F-21	211	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
	V-38473-F-22		<1.3		<1.3		<1.3	
	V-38473-F-23		<1.3		<1.3		<1.3	
G	V-38473-G-21	156	(2.41)	(2.61)	<2.61)	(2.30)	<1.3	<1.3
	V-38473-G-22		(2.01)		(2.01)		<1.3	
	V-38473-G-23		(3.39)		(2.28)		<1.3	
H	V-38473-H-21	240	<1.3	(1.15)	<1.3	<1.3	<1.3	<1.3
	V-38473-H-22		(2.15)		<1.3		<1.3	
	V-38473-H-23		<1.3		<1.3		<1.3	
I	V-38473-I-21	240	(2.16)	(1.43)	<1.3	<1.3	<1.3	<1.3
	V-38473-I-22		(1.47)		<1.3		<1.3	
	V-38473-I-23		<1.3		<1.3		<1.3	
		Minimum:	**<1.3**		**<1.3**		**<1.3**	
		Maximum:	**15.4**		**5.64**		**(1.94)**	
		Average:	**(3.75)**		**(1.35)**		**<1.3**	
		Median:	**(2.16)**		**<1.3**		**<1.3**	
		90th Percentile:	**9.64**		**(3.50)**		**(1.55)**	
2016								
A	V-38473-A-39	215	(3.99)	(4.32)	<1.3	<1.3	(1.45)	(1.20)
	V-38473-A-40		(3.94)		<1.3		(1.49)	
	V-38473-A-41		5.02		<1.3		<1.3	
B	V-38473-B-39	205	<1.3	(2.38)	<1.3	(1.08)	<1.3	<1.3
	V-38473-B-40		(1.38)		<1.3		<1.3	
	V-38473-B-41		5.12		(1.95)		<1.3	

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Trial	Sample ID	DALA[a]	Clothianidin		TZNG		TZMU	
			ppb[b]	Average	ppb[b]	Average	ppb[b]	Average
C	V-38473-C-39	234	(1.58)	(1.81)	<1.3	<1.3	<1.3	<1.3
	V-38473-C-40		(2.04)		<1.3		<1.3	
	V-38473-C-41		(1.82)		<1.3		<1.3	
D	V-38473-D-39	265	<1.3	(1.26)	<1.3	<1.3	<1.3	<1.3
	V-38473-D-40		(1.56)		<1.3		<1.3	
	V-38473-D-41		(1.58)		<1.3		<1.3	
E	V-38473-E-39	265	(3.72)	(4.71)	(2.83)	(3.34)	<1.3	<1.3
	V-38473-E-40		5.94		(3.52)		<1.3	
	V-38473-E-41		(4.45)		(3.67)		<1.3	
F	V-38473-F-39	204	(3.43)	(2.68)	(1.79)	(1.80)	<1.3	<1.3
	V-38473-F-40		(2.82)		(2.08)		<1.3	
	V-38473-F-41		(1.79)		(1.54)		<1.3	
G	V-38473-G-39	220	(4.30)	(4.49)	(2.77)	(2.95)	<1.3	<1.3
	V-38473-G-40		(3.87)		(2.89)		<1.3	
	V-38473-G-41		5.31		(3.20)		<1.3	
H	V-38473-H-39	283	(2.62)	(3.55)	<1.3	<1.3	<1.3	<1.3
	V-38473-H-40		(2.67)		<1.3		<1.3	
	V-38473-H-41		5.35		<1.3		<1.3	
I	V-38473-I-39	278	(3.72)	5.10	<1.3	<1.3	<1.3	<1.3
	V-38473-I-40		(4.62)		<1.3		<1.3	
	V-38473-I-41		6.96		<1.3		<1.3	
		Minimum:	**<1.3**		**<1.3**		**<1.3**	
		Maximum:	**6.96**		**(3.67)**		**(1.49)**	
		Average:	**(3.37)**		**(1.38)**		**<1.3**	
		Median:	**(3.72)**		**<1.3**		**<1.3**	
		90th Percentile:	**5.33**		**(3.01)**		**<1.3**	

[a] DALA= days after last application
[b] Values in parenthesis are between the LOQ and the LOD
In calculating the average concentration and descriptive statistics, values below the LOD are substituted with half of the LOD value
LOD= 1.3 ppb and LOQ= 5.0 ppb for clothianidin, TZNG and TZMU

Table 10. Clothianidin, TZNG and TZMU Residues in Soil Collected during Bloom

Trial	Sample ID	DALA[a]	Clothianidin		TZNG		TZMU	
			ppb[b]	Average	ppb[b]	Average	ppb[b]	Average
2015								
A	V-38473-A-9	139	32.8	45.0	<1.3	<1.3	<1.3	(0.93)
	V-38473-A-10		38.3		<1.3		<1.3	
	V-38473-A-11		64.0		<1.3		(1.48)	
B	V-38473-B-9	144	20.1	19.0	<1.3	<1.3	<1.3	<1.3
	V-38473-B-10		17.0		<1.3		<1.3	
	V-38473-B-11		19.8		<1.3		<1.3	
C	V-38473-C-9	212	6.15	7.04	<1.3	<1.3	<1.3	<1.3
	V-38473-C-10		10.4		<1.3		<1.3	
	V-38473-C-11		(4.59)		<1.3		<1.3	
D	V-38473-D-9	250	6.88	11.5	<1.3	<1.3	<1.3	<1.3
	V-38473-D-10		16.3		<1.3		<1.3	
	V-38473-D-11		11.3		<1.3		<1.3	
E	V-38473-E-9	252	(3.90)	11.7	<1.3	(1.09)	<1.3	<1.3
	V-38473-E-10		25.8		(1.97)		<1.3	
	V-38473-E-11		5.49		<1.3		<1.3	
F	V-38473-F-9	199	6.74	(4.75)	<1.3	<1.3	<1.3	<1.3
	V-38473-F-10		(2.60)		<1.3		<1.3	
	V-38473-F-11		(4.93)		<1.3		<1.3	
G	V-38473-G-9	146	10.6	8.40	<1.3	<1.3	<1.3	<1.3
	V-38473-G-10		6.96		<1.3		<1.3	
	V-38473-G-11		7.61		<1.3		<1.3	
H	V-38473-H-9	209	6.78	6.81	<1.3	<1.3	<1.3	<1.3
	V-38473-H-10		8.27		<1.3		<1.3	
	V-38473-H-11		5.37		<1.3		<1.3	
I	V-38473-I-9	218	5.02	10.3	<1.3	<1.3	<1.3	<1.3
	V-38473-I-10		11.8		<1.3		<1.3	
	V-38473-I-11		14.0		<1.3		<1.3	
		Minimum:	**(2.60)**		**<1.3**		**<1.3**	
		Maximum:	**64.0**		**(1.97)**		**(1.48)**	
		Average:	**13.8**		[c]		[c]	
		Median:	**8.27**		[c]		[c]	
		90th Percentile:	**28.6**		[c]		[c]	
2016								
A	V-38473-A-27	188	44.0	45.5	<1.3	<1.3	(1.78)	(1.31)
	V-38473-A-28		42.7		<1.3		<1.3	
	V-38473-A-29		49.9		<1.3		(1.50)	
B	V-38473-B-27	181	9.65	11.0	<1.3	<1.3	<1.3	<1.3
	V-38473-B-28		10.8		<1.3		<1.3	
	V-38473-B-29		12.5		<1.3		<1.3	

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Trial	Sample ID	DALA[a]	Clothianidin		TZNG		TZMU	
			ppb[b]	Average	ppb[b]	Average	ppb[b]	Average
C	V-38473-C-27A	218	7.86	(4.87)	<1.3	<1.3	<1.3	<1.3
	V-38473-C-28B		(3.55)		<1.3		<1.3	
	V-38473-C-29C		(3.21)		<1.3		<1.3	
D	V-38473-D-27	250	(4.76)	6.48	<1.3	<1.3	<1.3	<1.3
	V-38473-D-28		9.53		<1.3		<1.3	
	V-38473-D-29		5.16		<1.3		<1.3	
E	V-38473-E-27	251	(3.43)	5.45	<1.3	<1.3	<1.3	<1.3
	V-38473-E-28		(4.16)		<1.3		<1.3	
	V-38473-E-29		8.74		<1.3		<1.3	
F	V-38473-F-27	195	(4.43)	5.90	<1.3	<1.3	<1.3	<1.3
	V-38473-F-28		8.47		<1.3		<1.3	
	V-38473-F-29		(4.78)		<1.3		<1.3	
G	V-38473-G-27	207	9.13	11.6	<1.3	<1.3	<1.3	<1.3
	V-38473-G-28		10.0		<1.3		<1.3	
	V-38473-G-29		15.6		<1.3		<1.3	
H	V-38473-H-27	250	22.2	27.1	<1.3	<1.3	<1.3	<1.3
	V-38473-H-28		28.3		<1.3		<1.3	
	V-38473-H-29		30.9		<1.3		<1.3	
I	V-38473-I-27	250	16.7	25.9	<1.3	<1.3	<1.3	<1.3
	V-38473-I-28		23.3		<1.3		<1.3	
	V-38473-I-29		37.8		<1.3		<1.3	
		Minimum:	**(3.21)**		**<1.3**		**<1.3**	
		Maximum:	**49.9**		**<1.3**		**(1.78)**	
		Average:	**16.0**		c		c	
		Median:	**9.65**		c		c	
		90th Percentile:	**39.8**		c		c	

[a] DALA= days after last application
[b] Values in parenthesis are between the LOQ and the LOD
[c] Descriptive statistics were not calculated because >50% of the results are below the LOD
Reported concentration is based on dry weight
In calculating average concentration and descriptive statistics, values below the LOD are substituted with half of the LOD value
LOD= 1.3 ppb and LOQ= 5.0 ppb for clothianidin, TZNG and TZMU

Table 11. Clothianidin, TZNG and TZMU Residues in Soil Collected after Applications 2 and 4

Trial	Sample ID	DALA[a]	Clothianidin		TZNG		TZMU	
			ppb[b]	Average	ppb[b]	Average	ppb[b]	Average
2014								
A	V-38473-A-6	0	39.5	34.0	<1.3	<1.3	<1.3	<1.3
	V-38473-A-7		42.2		<1.3		<1.3	
	V-38473-A-8		20.3		<1.3		<1.3	
B	V-38473-B-6	0	32.1	29.6	<1.3	<1.3	<1.3	<1.3
	V-38473-B-7		26.9		<1.3		<1.3	
	V-38473-B-8		29.7		<1.3		<1.3	
C	V-38473-C-6	0	20.5	19.5	<1.3	<1.3	<1.3	<1.3
	V-38473-C-7		20.2		<1.3		<1.3	
	V-38473-C-8		17.7		<1.3		<1.3	
F	V-38473-F-6	0	11.0	10.4	<1.3	<1.3	<1.3	<1.3
	V-38473-F-7		14.5		<1.3		<1.3	
	V-38473-F-8		5.79		<1.3		<1.3	
G	V-38473-G-6	0	20.7	15.0	<1.3	<1.3	<1.3	<1.3
	V-38473-G-7		9.23		<1.3		<1.3	
H	V-38473-H-6	0	41.5	28.0	<1.3	<1.3	<1.3	<1.3
	V-38473-H-7		21.6		<1.3		<1.3	
	V-38473-H-8		21.0		<1.3		<1.3	
I	V-38473-I-6	0	21.1	19.7	<1.3	<1.3	<1.3	<1.3
	V-38473-I-7		17.0		<1.3		<1.3	
	V-38473-I-8		21.1		<1.3		<1.3	
		Minimum:	**5.79**		**<1.3**		**<1.3**	
		Maximum:	**42.2**		**<1.3**		**<1.3**	
		Average:	**22.7**		[c]		[c]	
		Median:	**20.8**		[c]		[c]	
		90th Percentile:	**39.7**		[c]		[c]	
2015								
A	V-38473-A-24	0	91.0		(1.50)	(0.93)	(3.40)	(2.52)
	V-38473-A-25		86.3		<1.3		(2.04)	
	V-38473-A-26		99.8		<1.3		(2.13)	
B	V-38473-B-24	0	58.8		<1.3	<1.3	<1.3	<1.3
	V-38473-B-25		59.7		<1.3		<1.3	
	V-38473-B-26		57.2		<1.3		<1.3	
C	V-38473-C-24	0	58.2		<1.3	<1.3	<1.3	<1.3
	V-38473-C-25		51.3		<1.3		<1.3	
	V-38473-C-26		96.5		<1.3		<1.3	
D	V-38473-D-24	0	14.7		<1.3	<1.3	<1.3	<1.3
	V-38473-D-25		20.4		<1.3		<1.3	
	V-38473-D-26		8.09		<1.3		<1.3	

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Trial	Sample ID	DALA[a]	Clothianidin		TZNG		TZMU	
			ppb[b]	Average	ppb[b]	Average	ppb[b]	Average
E	V-38473-E-24	0	(4.36)		<1.3	<1.3	<1.3	<1.3
	V-38473-E-25		11.3		<1.3		<1.3	
	V-38473-E-26		(2.69)		<1.3		<1.3	
F	V-38473-F-24	0	15.8		<1.3	<1.3	<1.3	<1.3
	V-38473-F-25		16.8		<1.3		<1.3	
	V-38473-F-26		16.1		<1.3		<1.3	
G	V-38473-G-24	0	24.3		<1.3	<1.3	<1.3	<1.3
	V-38473-G-25		36.1		<1.3		<1.3	
	V-38473-G-26		20.1		<1.3		<1.3	
H	V-38473-H-24	0	23.2		<1.3	<1.3	<1.3	<1.3
	V-38473-H-25		19.6		<1.3		<1.3	
	V-38473-H-26		19.8		<1.3		<1.3	
I	V-38473-I-24	0	54.6		<1.3	<1.3	<1.3	<1.3
	V-38473-I-25		**65.2**		**<1.3**		**<1.3**	
	V-38473-I-26		**70.5**		**<1.3**		**<1.3**	
		Minimum:	**(2.69)**		**<1.3**		**<1.3**	
		Maximum:	**99.8**		**(1.50)**		**(3.40)**	
		Average:	**40.8**		c		c	
		Median:	**24.3**		c		c	
		90th Percentile:	**88.2**		c		c	

[a] DALA= days after last application
[b] Values in parenthesis are between the LOQ and the LOD
[c] Descriptive statistics were not calculated because >50% of the results are below the LOD
Reported concentration is based on dry weight
In calculating average concentration and descriptive statistics, values below the LOD are substituted with half of the LOD value
LOD= 1.3 ppb and LOQ= 5.0 ppb for clothianidin, TZNG and TZMU

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Table 12. Summary Statistics for Clothianidin Residues in Almond Nectar, Pollen, Anthers, Leaves, and Soil Collected at Bloom

Matrix	Year	N	Minimum (ppb)	Maximum (ppb)	Mean±SD (ppb)	Median (ppb)	90th Percentile (ppb)
Nectar	2015	27	<0.20	1.28	[a]	[a]	[a]
	2016	27	<0.20	2.04	[a]	[a]	[a]
	Combined	54	<0.20	2.04	(0.31)±0.38	<0.20	(0.81)
Pollen	2015	20	(0.77)	20.0	6.52±5.45	4.42	13.4
	2016	21	(0.55)	13.8	4.77±3.79	4.32	10.4
	Combined	41	(0.55)	20.0	5.62±4.70	4.32	12.7
Anthers	2015	6	13.9	88.1	31.1±28.3	21.1	57.5
	2016	6	(0.35)	9.34	2.51±3.41	1.21	5.77
	Combined	12	(0.35)	88.1	16.8±24.3	11.6	26.6
Leaves[b]	2015	27	<1.30	15.4	(3.75)±4.07	(2.16)	9.64
	2016	27	<1.30	6.96	(3.37)±1.69	(3.72)	5.33
	Combined	54	<1.30	15.4	(3.56)±3.09	(2.91)	6.97
Soil	2015	27	(2.60)	64.0	[a]	[a]	[a]
	2016	27	(3.21)	49.9	[a]	[a]	[a]
	Combined	54	(2.60)	64.0	[a]	[a]	[a]

[a] Descriptive statistics were not calculated because >50% of the results are below the LOD
[b] Almond leaves were not present at bloom; they were collected later ca. 1 month after bloom
Values in parenthesis are between the LOQ and the LOD
In calculating the average concentration and descriptive statistics, values below the LOD are substituted with half of the LOD value.
For nectar, LOD= 0.20 ppb and LOQ= 1.00 ppb for clothianidin, TZNG, and TZMU
For pollen and anthers, LOD= 0.25 ppb and LOQ= 1.00 ppb for clothianidin, TZNG, and TZMU
For leaves and soil, LOD= 1.3 ppb and LOQ= 5.0 ppb for clothianidin, TZNG, and TZMU

8. STATISTICAL ANALYSIS

Study Objectives and Design

The study was conducted to determine the concentration of clothianidin and its metabolites TZNG, and TZMU in anthers, nectar, leaves, and pollen of almond trees in response to a previous year's foliar applications of a clothianidin pesticide product. Two applications of Belay were made post bloom where the first foliar application was made at an application rate of 0.1 lb a.i./Acre (BBCH growth stage 7.5, fruits at half size), and the second foliar application of Belay Insecticide was made at an application rate of 0.1 lb a.i./Acre at 21 days before harvest. In the second year, flower parts and leaves were harvested and analyzed for clothianidin and its degradation products. The crops received a second set of foliar treatments after bloom in the second year and the same sampling scheme was then followed at bloom in the third year. Soil samples were taken after the second application in each year and again when samples were taken the next year at bloom.

Non-parametric statistical tests were used to test for differences in distribution of concentrations between years and between soil type. Non-parametric tests do not require tests for normality as they are robust to differences in distribution and experimental designs with low replicates (Helsel and Hirsch, 2002). The PROC NPAR1WAY procedure in the Statistical Analysis System (SAS) statistical package was used to conduct Wilcoxon-Mann –Whitney (Wilcoxon), Median non-parametric, and Kuiper tests. A significant result from the Wilcoxon test indicates differences in the shape of distributions. A significant result from the Median test indicates differences in the location of the medians between distributions; and a significant result from the Kuiper test indicates differences in the empirical distributions between two groups. The Exact option for each statistic was implemented as it provides permutation testing, a statistical method that minimizes the effect of sample size and distributional differences. Using the Exact option, the Monte Carlo procedure was also implemented that provided 10,000 separate runs for each statistic to produce the permutation distributions. The test for potential differences due to soil type had 3 levels so the DSCF option in PROC NPAR1WAY, which invokes the Dwass, Steel, Critchlow-Fligner multiple comparison test was used to provide pairwise tests for two-sample rankings. Additional procedures used for descriptive statistics were PROC MEANS to calculate mean values from the replicates at each site, PROC CAPACITY to produce cumulative statistics, and PROC BOX plot to produce comparative graphics. Statistical analysis for effect of years and soil type were conducted on the mean of the replicate samples taken from each site. Graphical comparisons are presented on data transformed to a natural logarithm scale, providing clearer contrasts between the distributions. Values indicated as less than the limit of detection (LOD) were assigned ½ their respective LOD value (Table 5). Values were reported between the limit of detection and limit of quantification (LOQ) in parentheses so these were used as reported. For determination of the potential distribution of concentrations in bee relevant plant matrices, the distribution of the raw data is presented as these values represent the actual range of exposure to bees and other organisms that feed off the nectar and pollen of plants.

Detection rate noted for each plant matrix: Counts for the number of samples reported below respective detection limits for each matrix are presented in Table 13. Parent clothianidin residues were above the LOD for all samples except for nectar where 55% of the samples were below the LOD. Except for pollen samples, the majority of the other analyses for degradation products were reported below the LOD. Statistical analyses were not conducted for plant and soil matrices where the majority of results were reported below the LOD. In addition, since summation of the residues would be highly biased due to the large amount of substitution, statistical analyses were not conducted on total residues.

Comparison of distribution between years: Potential difference between years was measured for two reasons. First, greater concentrations measured in year 2 would indicate potential for carry-over effects between years. Second, if there was no effect of years then the data could be pooled for subsequent tests between soil types. The result for analyses conducted on plant matrices with sufficient detections indicated no significant difference in the distribution of concentration of residues between years, as based on exact probability levels for a two-sided test (Table 14). Graphical comparisons between years indicated significant overlap in the distribution of concentration for clothianidin residues between years in the plant samples taken at bloom (Figure 1) and for soil samples (Figure 2). The data for anthers indicated a higher distribution for the first year of the study, but samples were only taken from a subset of two sites in each year so this difference could be due to the low number of replicates. The result for clothianidin residues indicated that the data for both years can be combined in further tests for comparison of effect of soil type.

Comparison of distribution between soil types: Based on the soil characteristics provided in the Table embedded in section '3.2 Test Sites' in the report, the sites were classified as: coarse-textured sites are B, C, and E; medium-textured sites are D, G, and I; and moderately fine-textured sites are A, F, and H. Although the soil description at sites A,F, and H were not strictly indicated as fine-textured, their classification into a moderately fine-textured classification provided for potential distinction within the medium-textured classification. These categories are based on the USDA classification of soils (Soil Science Division Staff, 2017, see Table 3.1). Results of the non-parametric test indicated no difference in the distribution between the three soil types for parent clothianidin (Table 15). Graphic comparisons indicated significant overlap in the distributions for all sampled matrices (Figures 3 and 4).

Data for bee relevant matrices: The distribution derived from the individual analyses ostensibly determines the expected range in concentrations of clothianidin and TZNG, and TZMU degradation product residues in bee relevant plant samples for this combination of plant species and application scenario (Table 16). Also, although the number of samples noted below the LOD was problematic for conducting meaningful statistical tests, the presence of parent clothianidin indicated a potential for degradation products to be present. Therefore, total residues were calculated as the addition of all residues with results indicated as <LOD set at one-half the respective LOD for each matrix. For nectar, most concentrations were below the LOD so the total residue concentration was low with a maximum value estimated at 2.2 ng/g and the median value at 0.3 ng/g. Concentrations in pollen were higher where the maximum total residue value was estimated at 34.1 ng/g and the median value at 6.0 ng/g. The number of samples taken for anthers was small since only two of the plots were monitored in each year. The distribution for total residue concentration appeared to be higher than measured for pollen with the maximum value at 94.1 ng/g and the median value at 12.6 ng/g.

Longevity of residues in soil: The distribution of soil concentration measured after the second foliar application in each year and then at bloom the next year is presented in Table 17. The mean soil concentration at bloom was 14.9 ng/g compared to 32.7 ng/g after application. The length of time between the last foliar application and sampling at bloom in the next year varied at each site, ranging from 139 to 252 days. Estimates of the dissipation half-life can be calculated from the initial concentration measured after application and at bloom according to Equation 1:

Equation 1 Half-life (days) = ln(2) / ((ln(Co)-ln(Cn)/DALA)

In Equation 1, Co is the concentration at application, Cn is the concentration at bloom, and DALA is the days after the last application. Basing the calculation on the mean values in Table 17, Co is 32.7 and Cn is 14.9 ng/g. At an average DALA of 208 days, the terrestrial field dissipation half-life estimate is 162 days. This is a comparatively large value with respect to dissipation of pesticide residues in soil and indicates that the residues are long-lived in the soil.

Conclusions

1. Utility of the data: The study followed the design as indicted in the data call-in where the study was replicated in two years at 9 sites. The 9 sites appeared to be evenly distributed amongst the soil types with 3 replicates in coarse, medium, and fine- textured soils.

2. Concentrations in Bee Relevant Matrices: By default, the distributions reported in Table 16 represent the expected concentrations in bee relevant matrices that result from foliar clothianidin treatments applied to almond trees in the previous growing season. Median and maximum values for total clothianidin residues in pollen are 6.0 and 34.1 ng/g on wet weight basis and 0.3 and 2.2 ng/g for nectar, respectively.

3. No carry-over effect between years: Concentrations measured in plant matrices were similar between the two years of the study, indicating low potential for carry-over effects due to sequential foliar applications at the concentrations and timing used in this study.

4. No effect of Soil Type: Concentrations in plant matrices at bloom were similar between plants grown in the 3 soil types, indicating that foliar sprays produce similar results regardless of the soil condition.

5. **Residues are long-lived in soil:** The estimated dissipation half-life for clothianidin in soil was 162 days, a value that indicates slow dissipation in the soil environment.

9. STUDY STRENGTHS, LIMITATIONS AND CONCLUSIONS

In the context of documenting the magnitude of clothianidin residues in bee-related matrices of almond trees, the following strengths are observed with this study.

1. Data provide quantitative values of total clothianidin residues expected in pollen, nectar, and leaves of almond trees when measured at bloom in response to foliar applications made in the previous growing season.

2. The study was replicated over two years with measurements in plant samples taken at a mean of 197 days after the last application in the first year of the study and at a mean of 221 days after the last application in the second year of the study.

3. The 9 sites were evenly replicated over the requested 3 soil texture categories that reflected stratification between coarse, medium, and moderately fine-textured classifications.

Limitations noted in this study include:

1. In this study, the treatment applications were not conducted according to the "worst case" scenario permitted by the product label. The product label allows foliar applications to be made after bloom and up to 21 days before harvest, with a minimum reapplication interval of 10 days. Thus, the "worst case" application schedule permitted by the label would have been one application at 31 days before harvest and a second application at 21 days before harvest. However, in this study, plots received two applications anywhere from one to six months apart, and some plots received the second application at 32 or 44 days before harvest. In addition, some applications were conducted at harvest or after harvest which is not permitted by the product label. It is unclear how these deviations from the "worst case" application schedule permitted by the label may have affected the residues measured in bee-relevant matrices the next season.

Classification/Utility for Bee Risk Assessment. This study is classified as acceptable. It provides a snapshot of Clothianidin, TZNG and TZMU residues in nectar, pollen, and leaves collected from almond trees treated with Belay Insecticide (active ingredient clothianidin) over two years. The residue values presented should be considered to be fully reliable.

Temporal Variability in Residues. This study was not designed for temporal analysis of declining concentrations, but rather, to provide a snapshot of residue concentrations during flowering. Samples were collected at only one time point per year and so there is no way to know if concentrations were increasing or decreasing.

Overall, considering the strengths and limitations of this study, the following conclusions can be drawn:

Bee-relevant matrices: Clothianidin residues were measured in pollen sampled in the year following applications to plants of two foliar sprays made in the previous year. Most of the analyses for nectar indicated concentrations below the LOD of 0.1 ng/g on a wet weight basis. Therefore, exposure to bees from nectar is minimal; whereas, exposure to pollen requires comparison of the measured distribution to target values that define acute or chronic exposure scenarios.

Spatial Variability in Residues. Concentrations in plant matrices at bloom were similar between plants grown in the 3 soil types, indicating that foliar sprays produce similar results regardless of the soil condition.

No carry-over effects of years: Concentrations measured in plant matrices were similar between the two years of the study indicating low potential for carry-over effects due to sequential foliar applications at the concentrations and timing of applications used in this study.

10. STUDY VALIDITY/CLASSIFICATION

The data from this study provide an expected distribution of the concentrations of clothianidin residues that bees are exposed to in nectar and pollen of almond trees under actual agronomic practices in California. Relating concentrations measured in flower parts to bee health is possible by comparing the concentrations measured in bee relevant plant parts to target values that define acute or chronic exposure scenarios. The study is considered scientifically sound and useful for risk assessment purposes. The study is classified as ACCEPTABLE for quantitative use in risk assessment.

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Table 13. Counts of chemical analytical results for clothianidin and TZNG, and TZMU degradation products comparing the total number of samples collected for each matrix and the number of analyses where the concentration was indicated below respective detection limits.

Plant Sample	Comparison of Total Number of Samples Reported Above the LOQ, Between the LOQ and LOD, and Below the LOD											
	Clothianidin				TZNG				TZMU			
	Total Number	Number >LOQ	Number <LOQ	Number <LOD	Total Number	Number >LOQ	Number <LOQ	Number <LOD	Total Number	Number >LOQ	Number <LOQ	Number <LOD
Soil: After Application	**47**	**45**	**2**	**0**	**47**	**0**	**2**	**45**	**47**	**0**	**3**	**44**
Soil: At Bloom	**54**	**43**	**11**	**0**	**54**	**0**	**1**	**53**	**54**	**0**	**3**	**51**
Leaves	**54**	**13**	**29**	**12**	**54**	**1**	**16**	**37**	**54**	**0**	**8**	**46**
Nectar	**54**	**4**	**18**	**32**	**54**	**0**	**0**	**54**	**54**	**1**	**2**	**51**
Pollen	**42**	**38**	**4**	**0**	**42**	**6**	**12**	**24**	**42**	**17**	**18**	**7**
Anthers	**12**	**10**	**2**	**0**	**12**	**3**	**6**	**3**	**12**	**3**	**4**	**5**

Table 14. Effect of Year: Exact probability levels for Wilcoxon, Median, and Kuiper non-parametric tests for differences in the distribution of clothianidin concentrations between years. NA=Not Analyzed.

	Nonparametric Test Exact Probability Levels: Effect of Year		
	Clothianidin		
Source	Wilcoxon	Median	Kuiper
Pollen	0.38	1	0.73
Nectar	NA	NA	NA
Leaves	0.65	1	0.26
Soil at Application	0.47	1	0.68
Soil at Bloom	1	1	0.57

Table 15. Effect of Soil Type: Exact probability levels for Wilcoxon and Median non-parametric tests for differences in the distribution of clothianidin clothianidin and TZNG, and TZMU degradation products between soil types. NA=Not Analyzed.

Source	Wilcoxon Nonparametric Test for Effect of Soil: Exact Probability Level		
	Clothianidin	TZNG	TZMU
Leaves	0.15	NA	NA
Nectar	0.42	NA	NA
Pollen	0.48	0.33	0.27
Soil at Application	0.84	NA	NA
Soil at Bloom	0.78	NA	NA

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Table 16. Distribution of clothianidin and TZNG and TZMU degradation products measured in nectar, pollen, and anthers sampled from almond trees that were exposed to two applications of clothianidin in the year previous to bloom. Samples were combined from two consecutive years of study.

	Plant Sample											
	Nectar				Pollen				Anthers			
Statistic	Clothianidin	TZNG	TZMU	**Total**	Clothianidin	TZNG	TZMU	**Total**	Clothianidin	TZNG	TZMU	**Total**
N	54	54	54	54	41	41	41	41	12	12	12	12
Mean (ng/g)	0.3	0.1	0.1	**0.52**	5.62	0.59	1.87	**8.08**	16.8	1.4	4.4	**22.6**
SD (ng/g)	0.4	0	0.06	**0.41**	4.7	1.34	37.1	**7.22**	24.4	3.1	12.3	**31.1**
CV (%)	123	0	55	**78**	83.6	228	199	**89**	145	219	281	**138**
Min (ng/g)	0.1	0.1	0.1	**0.3**	0.6	0.1	0.1	**0.8**	0.4	0.1	0.125	**0.8**
Median (ng/g)	0.1	0.1	0.1	**0.3**	4.3	0.1	0.6	**6.0**	11.6	0.4	0.39	**12.6**
75th (ng/g)	0.4	0.1	0.1	**0.6**	8.3	0.4	1.7	**11.3**	21.2	0.9	1.27	**24.9**
90th (ng/g)	0.8	0.1	0.1	**1.0**	12.7	0.9	3.6	**17.2**	27.0	1.4	4.57	**77.4**
95th (ng/g)	1.2	0.1	0.2	**1.4**	13.8	2.4	5.9	**20.8**	88.1	11.0	43.3	**94.1**
Max (ng/g)	2.0	0.1	0.5	**2.2**	20.0	8.2	22.8	**34.1**	88.1	11.0	43.3	**94.1**
% of Total	**57.7**	**19.2**	**19.2**		**69.6**	**7.3**	**23.1**		**74.3**	**6.2**	**19.5**	

Table 17. Soil concentrations: Distribution of soil concentration of clothianidin for samples measured directly after the second foliar application in each year and then at bloom in the following year.

Statistic	Clothianidin Soil Concentration	
	At Application	At Bloom
N	16	18
Mean (ng/g)	32.7	14.9
SD (ng/g)	24.7	12.9
CV (%)	75.4	86.4
Min (ng/g)	6.1	4.8
Median (ng/g)	23.9	10.6
Max (ng/g)	92.4	45.5

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Figure 1. Year Comparison for plant samples: Comparison of the distribution of clothianidin residues expressed as natural logarithms between a sequential study replicated over two years. Yearly comparisons are made for leaf, nectar, pollen, and anther samples taken at bloom when sampled in the year following respective foliar applications.

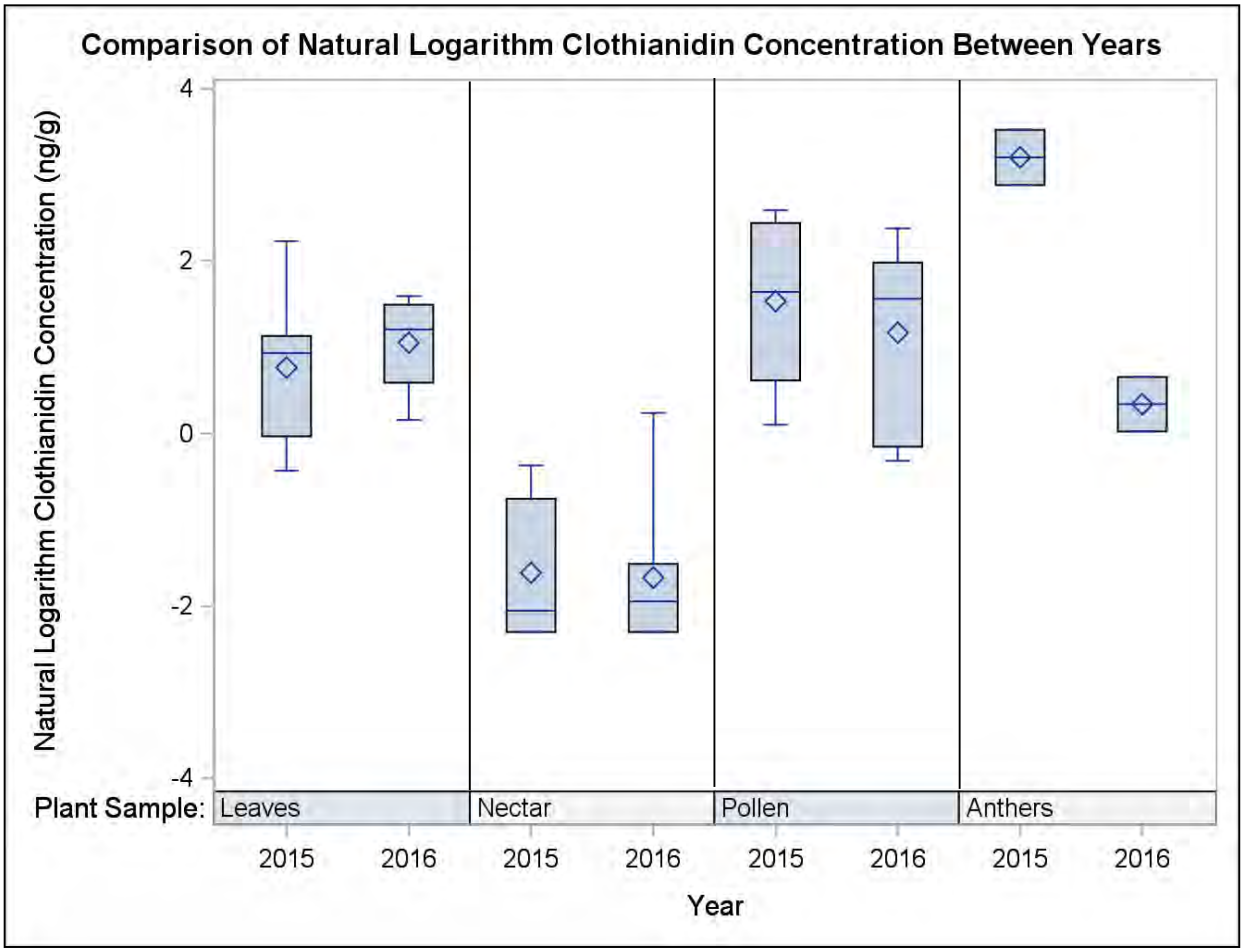


Figure 2. Year comparison for soil samples: Comparison of the distribution of clothianidin residues in soil samples expressed as natural logarithms between a sequential study replicated over two years. In the graphic 'At App' indicates samples taken directly after the second foliar application and 'At Bloom' indicates samples taken in the next year when trees were blooming.

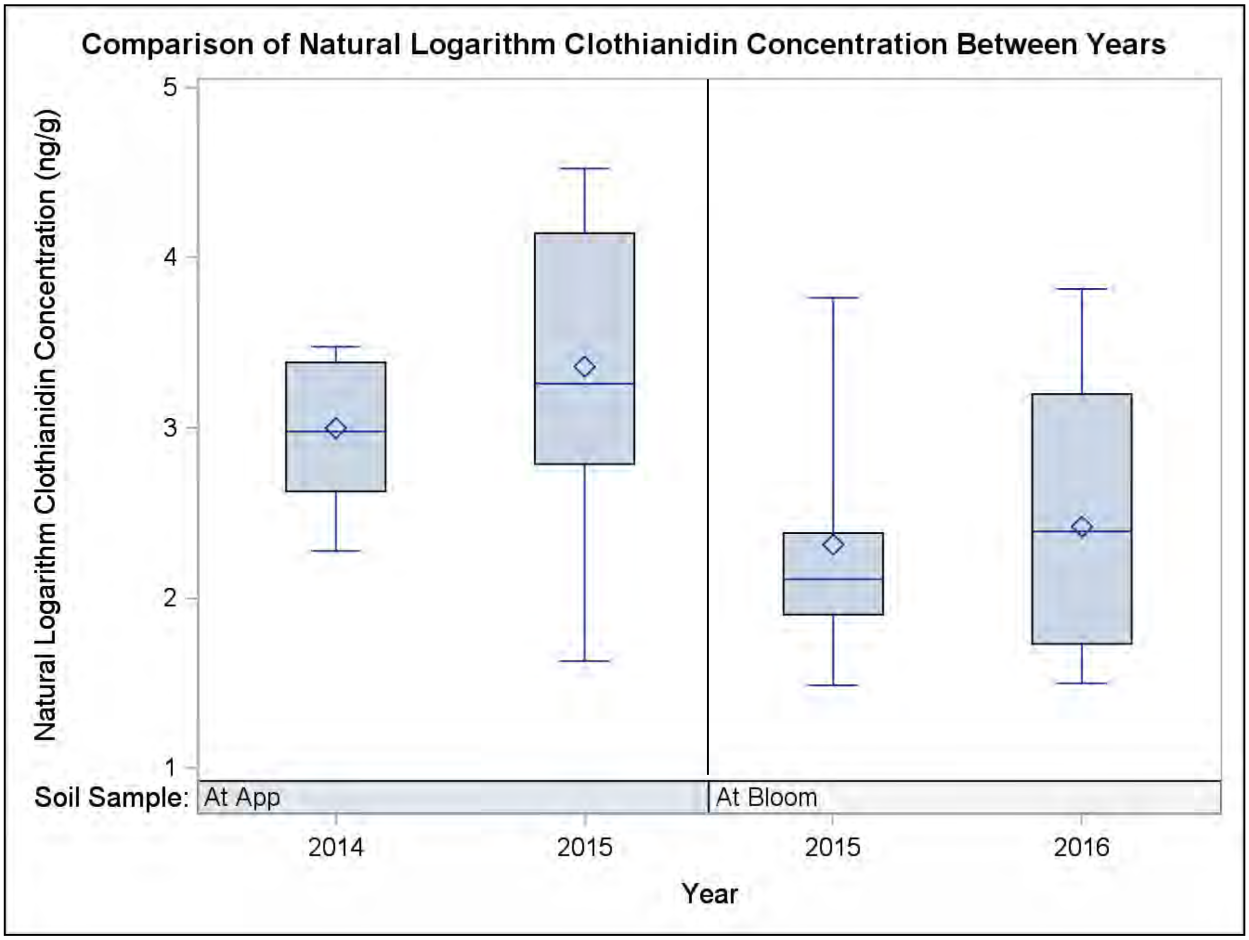


Figure 3. Comparison between soil type: Comparison of the distribution of clothianidin residues expressed as natural logarithms between a sequential study replicated over two years. Yearly comparisons are made for leaf, nectar, pollen, and anther samples taken at bloom when sampled in the year following respective foliar applications.

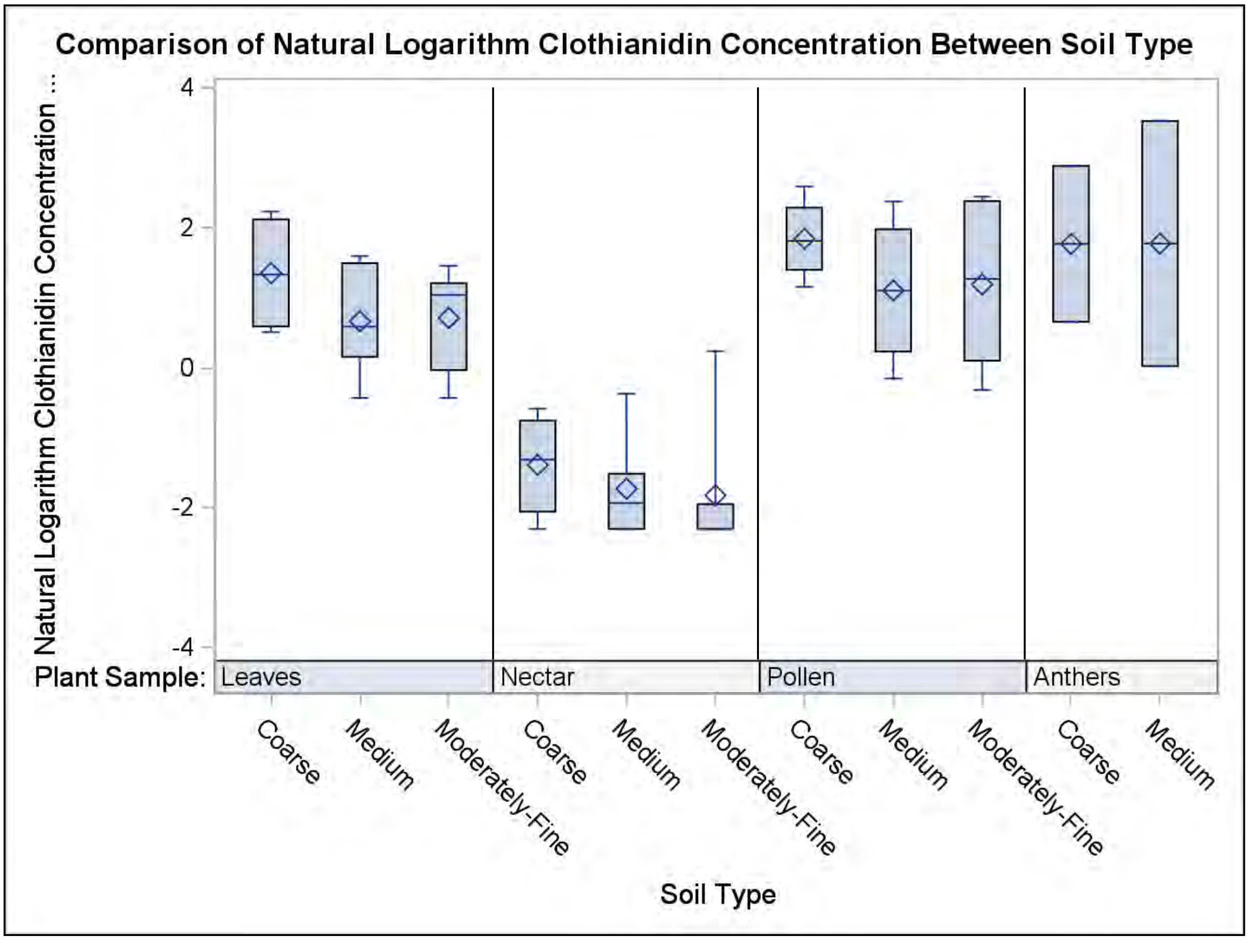
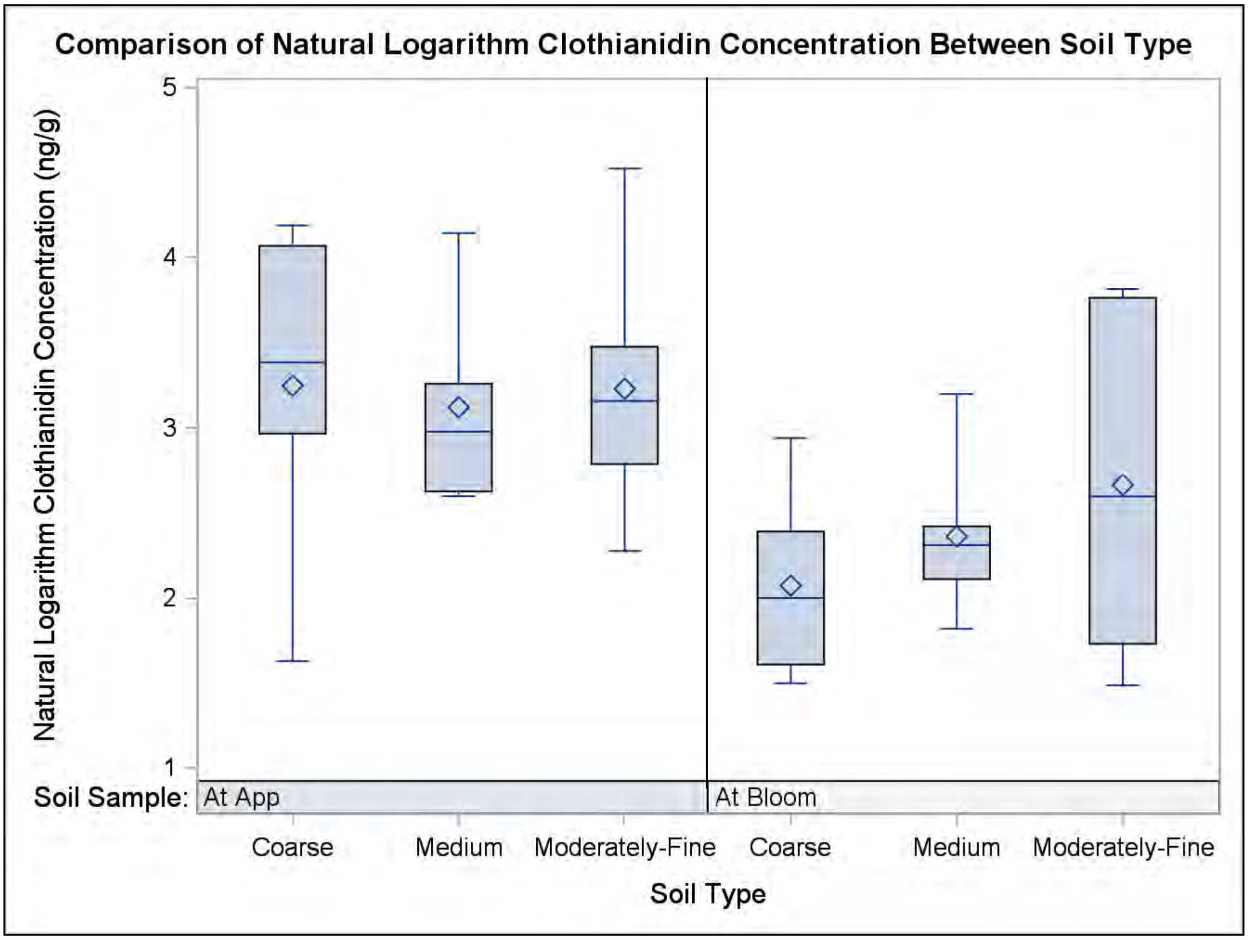


Table 4. Comparison between sequential replicate studies of the distribution of clothianidin residues measured between plants grown in 3 different soil types for soil samples taken either after full treatment application or at bloom in the year following respective foliar applications. Soil-App indicates samples taken directly after the second foliar application and Soil-Bloom indicates samples taken in the next year when trees were blooming.



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Reference
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1. STUDY INFORMATION

Chemical:	Clothianidin	**PC Code**	44309
Test Material:	Belay Insecticide	**Percent Active Ingredient:**	23.0%
Study Type:	Non-Guideline field residue study on cotton plants to establish clothianidin and metabolite levels in extrafloral nectars, floral nectar, pollen and leaves in site locations that were treated with two foliar applications.		
Sponsor:	Valent U.S.A. Corporation 1600 Riviera Ave., Suite 200 Walnut Creek, U.S.A. 94596	**Experiment Start and End Date:**	May 29, 2012 – May 9, 2013
Sponsor Study ID:	VP-38259	**Study Locations:**	Two trial sites that included Prima and Acala Cotton located in Fresno and Kerman, California.
Study Completion Date:	September 29, 2015		
GLP Status:	All phases of study were conducted under Good Laboratory Practice; protocol reviewed by CDPR. [CDPR study ID 264408, Data Volume 52884-0173, Tracking ID# 252080]		

2. REVIEWER INFORMATION

Study Reviewed by: California Department of Pesticide Regulation	Richard Bireley, Sr. Environmental Scientist (Specialist) John Troiano, Ph.D., Research Scientist III Alexander Kolosovich, Environmental Scientist Brigitte Tafarella, Environmental Scientist Russell Darling, Environmental Scientist Denise Alder, Sr. Environmental Scientist (Specialist)

Study Reviewed by: United States Environmental Protection Agency	Michael Wagman, Biologist, EPA/EFED/ERB6 **Date:** Amy Blankinship, Senior Scientist, EPA/EFED/ERB6 **Date:** **EPA Reviewer Comments:** EPA considers the study to be scientifically sound and it is classified as **Acceptable**

3. EXECUTIVE SUMMARY

Two field trials were conducted to quantify the extent to which insect pollinators may be exposed to clothianidin and its degradates thiazolylnitroguanidine (TZNG) and thiazolylmethylurea (TZMU) following two foliar applications of Belay® Insecticide to cotton. When applying Belay® Insecticide to field-grown cotton, leaf nectar, nectar and pollen from cotton flowers and concentrations of clothianidin in or on leaves were measured.

Cotton (*Gossypium hirsutum* variety Acala - Site 1 or *Gossypium barbadense* variety Pima – Site 2) was grown from seed at two field trial sites in California. The first foliar application of Belay® Insecticide (active ingredient, clothianidin) was applied approximately 7 days prior to floral bloom. The second Belay® Insecticide application occurred at floral bloom, 7 days after the first application. Each spray application of clothianidin was applied "over-the-top" at a rate of 0.1 lb. a.i./acre per application. No products containing clothianidin or thiamethoxam had been applied to either trial site in the three years prior to this study. Starting 21 days after planting and continuing until the second Belay® Insecticide application, treatment plots were surveyed for the presence of honey bees and, if present, their numbers quantified. In addition, plants were surveyed for the presence of main stem leaf nectar.

Leaves for residue analysis were collected from day 1 to day 28 after the last Belay® Insecticide application. Main stem leaves were collected from the upper portion of the plant where flowers were collected and received Belay® Insecticide applications. Leaf nectar was collected from 3 days after the first Belay® Insecticide application until 28 days after the second application.

First day open flowers were harvested and transported into the laboratory where they were immediately processed into subbracteal nectar, floral nectar and pollen. Subbracteal nectar was collected from Acala cotton beginning 5 days after the last Belay® Insecticide application but could not collect any subbracteal nectar from Pima cotton. Floral nectar and pollen was collected from the same flowers used to collect subbracteal nectar.

4. STUDY VALIDITY

Guideline Followed:	Non-guideline study (protocol was reviewed by CDPR)
Guideline Deviations:	N/A
Other Deviations:	No deviations were made during the analytical portion. Protocol was amended.
Classification:	**ACCEPTABLE**
Rationale:	N/A
Reparability:	N/A

5. MATERIALS AND METHODS

Test Material Characterization			
Test item:	Belay Insecticide	**Percent Active Ingredient:**	23.0% A.I.
Description:	Soluble Concentrate (SC)	**Molecular Formula:**	$C_6H_8ClN_5O_2S$
Material Source:	Valent U.S.A. Corporation	**Molecular Weight:**	205.68 g/mol
CAS #:	210880-92-5	**Valent Lot Number:**	AS 2351a

5A. Study Design

The experimental start date was May 29, 2012 and experimental end date was May 9, 2013 (last data collection). The field sampling phase of the study was conducted by California Agricultural Research, Inc.

Data was collected from two cotton trials conducted in outdoor plots owned by California Agricultural Research, Inc. and located in Fresno and Kerman, California. The soil types for these two locations are described as Sandy Loam and Loamy Sand. Treatment plot dimensions varied but, in general, were about 0.3 acres in area. Control plots were up to 0.3 acres in area. The site 1 control plot was about 200 feet from the treatment plot. This control plot was converted to a treatment plot (protocol amendment 2) and then subsequently abandoned (protocol amendment 3). The site 2 control plot was >200 feet from the treatment plot. Drip irrigation was installed in all plots. Bee hives were located about ¼ mile from site 2 and adjacent (within about 100 feet) to the treatment plot at site 1.

5B. Application Timing and Rates

Belay® Insecticide was applied to the foliage of the mature cotton plants using a tractor mounted boom containing flat fan Teejets. Belay® Insecticide was applied twice at application rates of 0.1 lb. a.i./acre and a spray interval of 7 days. The first foliar application of Belay® Insecticide (active ingredient, clothianidin) was applied approximately 7 days prior to floral bloom (about BBCH plant growth stage 51). The second Belay® Insecticide application occurred at floral bloom (defined in this study as when the site contained approximately 500 blossoms), 7 days after the first application.

Induce (0.25%, v/v), a non-ionic surfactant, was added to each tank mix prior to application. Key application information is collated in Table 1:

Table 1. Application Method and Application Dates for Belay Insecticide on Cotton

Site Number	Application Method	Application Date	Application Rate (lb. a.i./acre)
1	Foliar- Over the Top	July 16, 2012	0.1
1	Foliar- Over the Top	July 23, 2012	0.1
2	Foliar- Over the Top	July, 13, 2012	0.1
2	Foliar- Over the Top	July 20, 2012	0.1

5C. Study Site Location and Characteristics

Both field sites were in Fresno County located in California, where surface soil samples were collected from each treatment plot prior to planting. Agvise Laboratories (Northwood, ND) analysed soils for textures and percent organic matter. The soil characterization data and field site location are summarized in Table 2:

Table 2. Field Site Locations and Soil Series, Characterization and Organic Matter

Site Number	Field Site County	GPS Coordinates [1]	Soil Series & Texture	Soil Characterization (%Sand/Silt/Clay)	Percent Organic Matter
1	Fresno	N36.73709 W-119.87587	Ramona Sandy Loam	56/29/15	1.2
2	Fresno	N36.79409 W-120.05673	Hanford Loamy Sand	84/13/3	0.34

[1] Northwest Corner of field treatment plot

5D. Sample Collection, Handling, Processing

Leaf nectar was collected from 3 days after the first Belay® Insecticide application until 28 days after the second application. Leaf nectar was collected in the field on the same days that flowers were collected. Leaf nectar was collected using hand-held glass microcapillary tubes. Main stem leaves near the top of the plant were turned over to expose the single gland on the mid-rib of the leaf. If nectar was observed, the microcapillary was brought into contact with the gland and the nectar extracted by capillary action. Many leaves contained no nectar and those that did contained much less than 1 uL. Hundreds of leaves were examined in order to collect the amount (about 0.1 g) required for chemical analysis. The microcapillary was inserted into a tared, labeled glass vial and the leaf nectar expelled by closing the hole in the end of the bulb holder and gently squeezing the bulb. The vials were placed into a cooler containing Blue Ice in the field and then the cooler was hand carried to the field laboratory. The vials were placed into freezer storage on the day of collection. Due to extensive plant manipulation and handling during field collection, it is likely that leaf nectar was contaminated. In addition, evaporation of water during sample collection could artificially increase leaf nectar residue concentrations.

Flowers were hand carried in coolers to the field laboratory where they were processed on the day they were harvested from the field plot. Subbracteal nectar was collected by inserting a glass microcapillary on or into the subbracteal nectary located on the outside of the flower at the base of the bract. Most often the nectary was dry and no nectar could be collected. Subbracteal nectar was not present at any sampling time in Pima cotton (site 2). Subbracteal nectar extraction continued until the microcapillary tube contained a reasonable amount of visible nectar. The microcapillary was then inserted into a tared, labeled vial and the nectar expelled by closing the hole in the end of the bulb holder and gently squeezing the bulb. This process was continued with additional flowers until at least 0.1 g of subbracteal nectar was collected. For at least two samplings (days 14 and 21), this amount was not feasible.

A microcapillary tube was used to collect floral and/or inner bracteal nectar from the same flowers from which subbracteal nectar was collected. Occasionally, nonsubbracteal nectar extracted flowers were processed. The microcapillary was inserted under the lip of the calyx and into the floral nectary. Floral nectar was present in most of the flowers. The microcapillary was then inserted into a tared, labeled glass vial and the nectar expelled by closing the hole in the end of the bulb holder and gently squeezing the bulb.

Pollen was collected from the same flowers from which subbracteal and floral nectars were collected. When less than the minimum amount was collected, pollen was collected from untouched flowers. The petals were folded back to expose the pollen laden anthers. A plastic barrier pipette was connected to a small diaphragm vacuum pump by flexible tygon tubing. The small end of the barrier pipette was cut to enlarge the opening. The barrier pipette was then brushed over the tips of the anthers and the pollen, and likely dislodged parts of the anther, was retained within the pipette by the barrier. Pollen present at the base of the stigma was, if possible, also vacuum collected. The barrier pipette was replaced with a clean one as needed. The pipettes were placed into a tared, labeled glass vial. Flowers were discarded after pollen collection.

A total of 72 cotton floral nectar and extrafloral nectar samples, 29 cotton pollen samples, 30 cotton leaf samples and 28 stability samples were analyzed for clothianidin and its metabolites residues.

Sample Storage.

Leaves for residue analysis and flowers for processing were hand carried to the field laboratory in coolers with Blue Ice. Once in the laboratory, the leaf samples were placed into a freezer (temperature <0°F).

Pollen, nectar and leaf samples were stored in monitored freezers at the field sites pending shipment to the Valent Technical Center. Samples were shipped frozen and upon arrival at the Valent Technical Center samples were placed into a temperature monitored, walk-in freezer until analyzed. Pollen samples were stored (sampling to extraction) for a maximum of 122 days, nectar samples for up to 56 days, and leaves for up to 193 days. Transit stability samples for pollen were stored up to 283 days and for nectars were stored for up to 91 days prior to extraction for analysis.

The nectar stability samples were stored for up to 91 days, and pollen stability samples were stored up to 283 days. Clothianidin residues have been shown to be stable on a variety of leafy vegetable crops when stored frozen for up to 242 days[1], therefore a storage stability study on cotton leaf tissues was not conducted with this study.

5E. Analytical Methods

Analyses were conducted in a total of 16 sets. Each set included at least 6 standards for the calibration curve, at least one untreated sample, two laboratory fortification samples, and 7-15 samples. Instrument software was used to integrate the analyte and internal standard peak response (area integration) for each injection. Excel was used to calculate analyte concentrations in sample extracts based on peak area ratios (area analyte/area internal standard) and the standard curve (second order polynomial). Excel was also used to calculate sample residue concentrations based on the extract concentration, the extract volume, the dilution factor (if any) and the sample weight. Some samples were collected before the second application of Belay® Insecticide. Although these samples were analyzed, only samples analyzed after the second pesticide application are presented in this report.

Leaf samples were extracted with methanol and water (40:60, v/v) acidified with 0.05% formic acid and analyzed using an accurate mass UPLC/Q-TOF MS-MS after spiking with isotopically labeled internal standard (d3-clothianidin) to compensate for matrix effect (method RM-39L-1). The method was validated at 5 ppb and 50 ppb. Subsequently, the method was re-validated at 5,000 ppb and 25,000 ppb. Frozen leaf samples were homogenized in the presence of Dry Ice. After sublimation of the Dry Ice, an accurately weighed (about 2 g) leaf subsample was extracted in a mixture of acidified methanol and water. The solids were allowed to settle and an aliquot of the mother liquor was filtered through a nylon syringe filter. An aliquot of the filtered liquid was fortified with isotopically labeled clothianidin (internal standard) and analyzed by accurate mass UPLC/ Q-TOF MS-MS. The LOD in this method was 2.5 ppb, and the LOQ was 5.0 ppb for clothianidin.

Samples of cotton floral nectar or extrafloral nectar were dissolved in methanol/water (40:60, v/v) acidified with 0.05% formic acid and analyzed by LC/MS-MS after spiking with isotopically labeled internal standards (d3-clothianidin, 13C, 15N-TZNG, and d3-TZMU) to compensate for matrix effect (method RM-39N-1). The method was validated at 1 ppb and 10 ppb for each analyte before using it to analyze study samples. Subsequently, the method was validated at higher levels (250 ppb and 5,000 ppb) using artificial nectar. Nectar validation was conducted using artificial nectar that contained 11% sucrose, 40% fructose, and 49% glucose in water. Sugar content of the artificial nectar was 36% (36 ºBrix). Nectar samples were removed from freezer storage on the day of chemical analysis and allowed to warm to room temperature. An accurately weighed nectar subsample (about 0.1 g) was dissolved in a mixture of methanol and water, each containing 0.05% formic acid, fortified with isotopically labeled internal standards then an aliquot of each sample was filtered through a nylon syringe filter, if particles were present, into an autosampler vial prior to analysis by HPLC/MS-MS. The limit of detection (LOD) was 0.2 ppb, and the limit of quantitation (LOQ) was 1.0 ppb for clothianidin, TZNG and TZMU in this method.

Samples of cotton pollen were extracted with acetonitrile and water followed by adding sodium chloride and anhydrous magnesium sulfate salts. The acetonitrile extract was partitioned with n-hexane, and acetonitrile phase was collected and concentrated to dryness. Residues were re-dissolved in methanol/water (40:60, v/v) acidified with 0.05% formic acid and analyzed by LC/MS-MS after spiking with isotopically labeled internal standards (d3-clothianidin, 13C, 15N-TZNG, and d3-TZMU) to compensate for matrix effect (method RM-39P-1). The method was validated at 1 ppb and 10 ppb for each analyte before using it to analyze study samples. Using this method, the LOD was 0.25 ppb, and the LOQ was 1.0 ppb for clothianidin, TZNG and TZMU.

Subsequently, the method was validated at higher levels (100 ppb and 500 ppb) using commercially available bee pollen. The barrier pipettes were removed from the storage vial and the cotton pollen dislodged. The pollen was collected into a vial and homogenized by stirring. An accurately weighed pollen subsample (about 0.1 g) was removed and extracted in a mixture of acetonitrile and water. Sodium chloride and magnesium sulfate was added to increase the ionic strength and effect a clean phase separation during centrifugation. An aliquot of the organic phase (upper acetonitrile) was removed then partitioned against hexane (discarded). An aliquot of the acetonitrile solution was concentrated to dryness under a stream of nitrogen. Residues were dissolved in a mixture of acidified methanol and water, filtered through a nylon syringe filter into an autosampler vial then fortified with isotopically labeled internal standards. Extracts were analyzed by HPLC/MS-MS.

5F. QUALITY ASSURANCE RESULTS

Six to eight different standard concentrations were injected within each analytical set. The concentration (ng/mL) of clothianidin and its metabolites detected in sample extracts was interpolated from the standard calibration curve. The LC/MS-MS system was calibrated for each set of samples by analyzing these calibrating standard concentrations, with these standards interspersed within the analytical sequence. A second-order polynomial fit (weighted relative to 1/concentration) was then calculated from the concentrations and the instrument response of the calibration standards. To verify performance, the percent difference between the actual concentration and the calculated concentration for each of the calibration standards (based on the curve) was also calculated. Each of the standards was required to be within 15% of the theoretical concentration and the coefficient of determination (r^2) of the weighted polynomial calibration curve was required to be greater than or equal to 0.99. Minor exceedance of these criteria for the calibration standards were accepted for the lowest standards in some cases, however the coefficient of determination (r^2) of the weighted polynomial calibration curve was always greater than or equal to 0.99.

The reproducibility of the LC/MS-MS system was verified by comparison of instrument responses obtained from the repeated analysis of a reference standard (a continuing calibration standard) analyzed with the study samples. The continuing calibration standards were interspersed within the samples in the analytical sequence, and the analytical sequence began and ended with a continuing calibration standard. For an analytical data set (injection sequence) to be acceptable, the coefficient of variation (CV) of these responses was required to be 10% or less. Minor exceedance of these criteria for the continuing calibration standards were accepted in some cases.

One control sample and at least one fortified sample were analyzed with each set of study samples to verify method performance. Fortifications were made at 1.0 and 10.0 ppb for nectar and pollen samples, and 5.0 ppb and 50.0 ppb for leaves. For an analytical run to be acceptable, method recoveries were required to be between 70 and 120%. Generally, recoveries of the concurrent laboratory fortified samples were within this range. In some cases, minor exceedance of these criteria was accepted.

6. RESULTS:

6.1 LEAF RESIDUES

No clothianidin was detected (LOD, 2.5 ppb) in cotton leaves collected prior to the first Belay® Insecticide application or in control leaf samples. Although the same application equipment was used at both field sites, mean clothianidin leaf concentrations were higher in Pima cotton (site 2) than in Acala cotton (site 1). In both varieties, residues were highest immediately after the last application and declined thereafter. One day after the last application of Belay® Insecticide, clothianidin concentrations were 11,100 ppb in Pima cotton and 9,820 ppb in Acala cotton. Twenty-eight days after the application, leaf residue concentrations declined to 57.5 ppb in Pima cotton and 25.7 ppb in Acala cotton.

6.2 NECTAR ANALYSES

Mean concentrations of clothianidin in Acala (site 1) leaf nectar were highest (3,390 ppb) 1 day after the last application of Belay® Insecticide, declining to 14.6 ppb on day 28. Leaf nectar residue concentrations of clothianidin were highest in Pima cotton 1 day after the last Belay® Insecticide application (210 ppb), declining to 24.4 ppb on day 21. Only one replicate sample of Pima leaf nectar was collected on day 28 and the concentration of clothianidin in it were higher (57.0 ppb) than in the day 21 sample. Residue concentrations of metabolites were lower than parent concentrations (except TZMU in Pima at day 21) in both cotton varieties with TZMU concentrations higher than TZNG concentrations.

Clothianidin residues in subbracteal nectar were highest and approximately equal (about 620 ppb) in Acala on days 5 and 7 after the last application of Belay® Insecticide. Clothianidin concentrations declined thereafter and were 3.4 ppb at 28 days post application. Except for the last interval, TZMU concentrations were higher than TZNG concentrations in subbracteal nectar but always much lower than parent concentrations. Metabolite concentrations declined over time and were below the LOQ at day 28.

Floral nectar concentrations were initially higher in Acala cotton (site 1) than in Pima (site 2) cotton. Mean residue concentrations were highest 1 day after the last Belay® Insecticide application in Acala cotton (142 ppb) but in Prima cotton, the highest residue came 7 days after the last application (95.8 ppb). The unexpected high residue value in one replicate suggests that it was inadvertently contaminated, either during sample collection, sample processing, or during work-up for analysis. Clothianidin residues in floral nectar dissipated to concentrations below the LOQ by day 28 in both cotton varieties.

6.3 POLLEN ANALYSES

In general, clothianidin residues were highest 1 to 5 days after the last Belay® Insecticide application and then declined over the subsequent 21 days. In Acala cotton (site 1), mean clothianidin residues were highest (300 ppb) 1 day after the last pesticide application declining to <LOQ at day 28. Mean clothianidin residues were highest 3 days (130 ppb) and 5 days (123 ppb) after the last Belay® Insecticide application in Pima cotton (site 2). Mean residue concentrations then declined and were below the LOQ by day 28.

For the first 14 days after the last pesticide application, mean residue concentrations of TZNG and TZMU were about 10 to 100 times lower than clothianidin concentrations in Acala cotton pollen. TZNG and TZMU concentrations were usually higher in Pima (site 2) cotton pollen than in Acala (site 2) cotton pollen and sometimes (TZMU only) concentrations were higher than that of clothianidin. In Pima cotton, mean residues of TZNG increased following the last Belay® Insecticide application and were highest (45.9 ppb) on day 7; TZNG residue concentrations declined thereafter and were below the LOQ on day 28. Mean TZMU concentrations in Pima pollen also increased after the last pesticide application and were highest (109 ppb) 7 days post-application. TZMU mean concentrations declined thereafter and were 1.2 ppb at day 28.

6.4 SITE SUMMARIES

Table 3. *Site 1 (Acala cotton variety):* Average residues of clothianidin, TZNG and TZMU detected in floral nectar, extrafloral nectar, pollen and leaf tissues samples collected after the second Belay® Insecticide application are presented below:

Matrix	Days After Last Application (DALA)	Clothianidin Concentration (ppb)	TZNG Concentration (PPB)	TZMU Concentration (ppb)
Floral Nectar	1	142	20.9	4.6
	3	51.4	22.0	5.0
	5	22.5	16.6	2.5
	7	27.5	15.6	3.5
	14	11.4	2.8	1.9
	21	(0.48)[b]	2.8	1.5
	28	(0.40)[b]	3.4	(0.34)[b]
	Half-Life:	*3.2 Days*		
Subbracteal Nectar	1	-	-	-
	3	-	-	-
	5	621	8.2	41.9
	7	623	6.6	37.8
	14	171	2.5	17.0
	21	15.9	(0.89)[b]	2.0
	28	(3.4)[b]	(0.63)[b]	(0.51)[b]
	Half-Life	*2.9 Days*		
Leaf Nectar	3[a]	2288	32.9	208
	1	3393	28.3	67.6
	3	1386	30.5	215
	5	340	23.2	60.5
	7	126	19.2	20.2
	14	64.6	15.0	23.7

Matrix	Days After Last Application (DALA)	Clothianidin Concentration (ppb)	TZNG Concentration (PPB)	TZMU Concentration (ppb)
	21	16.2	2.8	5.2
	28	14.6	2.3	4.1
	Half-Life	*3.6 Days*		
Pollen	1	300	3.4	4.6
	3	125	2.0	8.6
	5	419	1.5	7.5
	7	79.9	1.9	5.4
	14	15.4	1.1	2.1
	21	1.5	(0.92)[b]	(0.22)[b]
	28	(0.45)[b]	(0.43)[b]	<0.25
	Half-Life	*2.9 Days*		
Leaf Tissue	1	9823	-	-
	3	5747	-	-
	5	2818	-	-
	7	1546	-	-
	14	202	-	-
	21	41.1	-	-
	28	25.7	-	-
	Half-Life	*3 Days*		

[a] Three days after the first Belay Insecticide application and 4 days before the second Belay Insecticide application.
[b] Values in parenthesis are between the LOQ and the LOD.

Residues of clothianidin, TZNG and TZMU were detected in floral nectar, extrafloral nectar, pollen and leaf tissues of Acala cotton plants. Average concentrations of clothianidin, TZNG and TZMU in floral nectar ranged from 0.40 to 142 ppb; 2.8 to 22.0 ppb; and 0.34 to 5.0 ppb, respectively. Average residues of clothianidin, TZNG, and TZMU in subbracteal nectar varied from 3.4 to 623 ppb; 0.63 to 8.2 ppb; and 0.51 to 41.9 ppb, respectively. In leaf nectar, average residues ranged from 14.6 to 3,393 ppb; 2.3 to 32.9 ppb; and 4.1 to 208 ppb for clothianidin, TZNG and TZMU, respectively. Average concentrations of clothianidin, TZNG and TZMU in pollen ranged from 0.45 to 419 ppb; 0.43 to 3.4 ppb; and below <0.25 to 8.6 ppb, respectively. High clothianidin concentration detected in pollen samples at 5 days after the last application may be a result of pollen sample contamination during field sample collection. Residues of clothianidin in leaf tissues ranged from 25.7 to 9,823 ppb.

Table 4. *Site 2 (Pima cotton variety):* Average residues of clothianidin, TZNG and TZMU detected in floral nectar, leaf nectar, pollen and leaf tissue samples collected after the second Belay® Insecticide application are presented below:

Matrix	Days After Last Application (DALA)	Clothianidin Concentration (ppb)	TZNG Concentration (PPB)	TZMU Concentration (ppb)
Floral Nectar	1	32.6	28.8	5.3
	3	18.2	22.3	4.7

Matrix	Days After Last Application (DALA)	Clothianidin Concentration (ppb)	TZNG Concentration (PPB)	TZMU Concentration (ppb)
	5	53.4	51.8	8.1
	7	95.8	36.4	8.2
	14	11.8	(0.99)[b]	3.7
	21	1.2	2.5	1.2
	28	(0.46)[b]	2.8	(0.82)[b]
	Half-Life	*4.2 Days*		
Leaf Nectar	3[a]	138	24.6	46.8
	1	210	29.6	45.8
	3	162	42.5	78.9
	5	147	31.5	61.2
	7	92.9	35.8	68.1
	14	49.6	11.5	23.3
	21	24.4	17.9	32.5
	28	57.0	13.4	29.7
	Half-Life	*6.2 Days*		
Pollen	1	19.2	1.3	2.0
	3	130	9.9	20.0
	5	123	27.4	65.5
	7	94.6	45.9	109
	14	28.6	17.7	38.0
	21	1.2	1.0	1.7
	28	(0.8)[b]	(0.54)[b]	1.2
	Half-Life	*2.8 Days*		
Leaf Tissue	1	11,076	-	-
	3	7,300	-	-
	5	5,200	-	-
	7	3,888	-	-
	14	709	-	-
	21	162	-	-
	28	57.5	-	-
	Half-Life	*3 Days*		

[a] Three days after the first Belay Insecticide application and 4 days before the second Belay Insecticide application.
[b] Values in parenthesis are between the LOQ and the LOD.

Residues of clothianidin, TZNG and TZMU were detected in floral nectar, extrafloral nectar, pollen and leaf tissues of Pima cotton plants. Average concentrations of clothianidin, TZNG and TZMU in floral nectar ranged from 0.46 to 95.8 ppb; 0.99 to 51.8 ppb; and 0.82 to 8.2 ppb, respectively. In leaf nectar, average residues ranged from 24.4 to 210 ppb; 11.5 to 42.5 ppb; and 23.3 to 78.9 ppb for clothianidin, TZNG and TZMU, respectively. Average concentrations of clothianidin, TZNG and TZMU in pollen ranged from 0.80 to 130 ppb; 0.54 to 45.9 ppb; and 1.2 to 109 ppb, respectively. Residues of clothianidin in leaf tissues ranged from 57.5 to 11,076 ppb.

7. STUDY VALIDITY/CLASSIFICATION AND STUDY LIMITATIONS

Classification/Utility for Bee Risk Assessment. This study is classified as acceptable. It provides quantitative information regarding the magnitude of clothianidin, TZNG and TZMU residues in floral nectar, extrafloral nectar, pollen and leaf tissues in cotton after two foliar applications of Belay® Insecticide (active ingredient, clothianidin). Applications were made at the maximum labeled rate, and thus the results are relevant to the current bee risk assessment. Residues were sampled over a period of 1 to 28 days after the last application and thus provide information regarding the temporal variability of residues after application. The study protocol was reviewed by CDPR before study initiation. It is important to note that the study authors stated that it is likely that leaf nectar was contaminated and, in addition, evaporation of water during sample collection could have artificially increased leaf nectar residue concentrations. Thus the results for residue concentrations in leaf nectar may not be reliable.

Temporal Variability in Residues. Sampling was conducted from 1 to 28 days after the last Belay® Insecticide application. In general, average residues of clothianidin, TZNG and TZMU detected in floral nectar, leaf nectar, pollen and leaf tissue samples were highest between 1 to 7 days after the second application and then declined thereafter.

Spatial Variability in Residues. Both field sites were in Fresno County, located in California. The soil was described as Ramona Sandy Loam at site 1 (Acala cotton) and Hanford Loamy Sand at site 2 (Pima cotton). Small variations in average residue concentrations were noted between the two study sites. Mean clothianidin leaf concentrations were higher in Pima cotton (site 2) than in Acala cotton (site 1). TZNG and TZMU concentrations were usually higher in Pima cotton (site 2) pollen than in Acala cotton (site 2) pollen as well.

Pesticide Carryover. This study was not designed to measure the extent to which prior year applications of clothianidin contributed to year-to-year carryover. Therefore, the effects of pesticide carryover in cotton are unknown.

Magnitude of Residues. Average residues of clothianidin, TZNG and TZMU detected in floral nectar, extrafloral nectar, pollen and leaf tissues samples collected after the second Belay® Insecticide application are presented for each study site (**Tables 3 and 4**).

Although the same application equipment was used at both field sites, mean clothianidin leaf concentrations were higher in Pima cotton (site 2) than in Acala cotton (site 1). In both varieties, residues were highest immediately after the last application and declined thereafter. One day after the last application of Belay® Insecticide, clothianidin leaf concentrations were 11,100 ppb in Pima cotton and 9,820 ppb in Acala cotton. Twenty-eight days after the application, leaf residue concentrations declined to 57.5 ppb in Pima cotton and 25.7 ppb in Acala cotton. The decline of clothianidin on leaves was first-order with a half-life of about 3 days at both sites.

Mean concentrations of clothianidin in Acala leaf nectar were highest (3,390 ppb) 1 day after the last application of Belay® Insecticide, declining to 14.6 ppb on day 28. Leaf nectar residue concentrations of clothianidin were highest in Pima cotton 1 day after the last Belay® Insecticide application (210 ppb), declining to 24.4 ppb on day 21. Residue concentrations of metabolites were lower than parent concentrations (except TZMU in Pima at day 21) in both cotton varieties with TZMU concentrations higher than TZNG concentrations. The decline in clothianidin in leaf nectar was first-order with a half-life of 3.6 and 6.2 days in Acala and Pima cotton, respectively.

Clothianidin residues in subbracteal nectar were highest and approximately equal (about 620 ppb) in Acala on days 5 and 7 after the last application of Belay® Insecticide. Clothianidin concentrations declined thereafter and were 3.4 ppb at 28 days post application. Except for the last interval, TZMU concentrations were higher than TZNG concentrations in subbracteal nectar but always much lower than parent concentrations. Metabolite concentrations declined over time and were below the LOQ at day 28. The decline in clothianidin in subbracteal nectar was first-order with a half-life of 2.9 days.

Floral nectar concentrations were initially higher in Acala cotton than in Pima cotton. Mean residue concentrations were highest 1 day after the last Belay® Insecticide application in Acala cotton (142 ppb) but in Prima cotton, the highest residue came 7 days after the last application (95.8 ppb). Clothianidin residues in floral nectar dissipated to concentrations below the LOQ by day 28 in both cotton varieties. The first-order half-life of clothianidin in floral nectar was 3.2 and 4.2 days in Acala and Pima cotton, respectively.

In Acala cotton, mean clothianidin residues in pollen were highest (300 ppb) 1 day after the last pesticide application declining to <LOQ at day 28. In Pima cotton, mean clothianidin residues in pollen were highest 3 days (130 ppb) and 5 days (123 ppb) after the last Belay® Insecticide application. Mean residue concentrations then declined and were below the LOQ by day 28. The first-order half-life of clothianidin in pollen was 2.9 and 2.8 days in Acala and Pima cotton, respectively.

For the first 14 days after the last pesticide application, mean residue concentrations of TZNG and TZMU were about 10 to 100 times lower than clothianidin concentrations in Acala cotton pollen. TZNG and TZMU concentrations were usually higher in Pima cotton pollen than in Acala cotton pollen. In Pima cotton, mean residues of TZNG and TZMU increased following the last Belay® Insecticide application and were highest on day 7; residue concentrations declined thereafter.

8. STATISTICAL ANALYSIS

Clothianidin was applied in two foliar sprays to cotton plants at two sites in Fresno County, CA in 2012. The first application was made 7 days prior to floral bloom and the second application was made at floral bloom. Soil at site 1 was a Ramona sandy loam, which is a medium-textured soil with 56% sand content, whereas, the soil at site 2 was a Hanford sandy loam, which is a coarse-textured soil with 84% sand content. Potential comparisons of the effect of soil were compromised because different cultivars were planted at each site where 'Acala' was planted at site 1 and 'Pima' was planted at site 2. Thus, effects from soil type could be confounded by differences due to cultivar used. Since the sprays were applied to foliage at bloom, effects of soil were not expected and not analyzed. The objective of the study design was to describe the degradation rate of residues of clothianidin and its transformation products after foliar application to cotton plants. An analysis of the potential distribution of clothianidin residues measured at a specific sampling time interval was restricted because only two replicates were obtained for each plant sample from only two experimental sites. The distribution of data pooled over all sampling dates at each site provided guidance on the maximum values that were measured.

The analysis of the data was conducted to provide guidance for the following questions:

A. What was the overall distribution of clothianidin, TZNG and TZMU concentrations for data pooled over the sampling intervals?
   i. Sampling intervals analyzed were at 1, 7, 14(15), 21 and 28 days after the second foliar application.
B. Was there a temporal pattern in residue concentrations over time?
C. Was there a relationship between residues in the plant tissues?

A. Distribution of concentration data pooled over sampling intervals. Tables S-1 through S-5 contain the distributional statistics for leaf, foliar nectar, leaf nectar, subbracteal nectar and pollen, respectively, for each site. For leaves, only parent residue was measured where the median concentration was 1,812.0 ppb and the maximum value at 11,127.0 ppb. For floral nectar, the median concentration of total residue was 40.9 ppb with a maximum at 239.9 ppb. Median and maximum values were higher in leaf nectar at 185.5 and 4,267.9 ppb, respectively. Subbracteal nectar samples were not measured at site 2 but samples at site 1 had median and maximum concentrations at 190.9 and 694.2 ppb, respectively. Pollen concentrations for median and maximum values were 38.5 and 771.4 ppb. The magnitude and range in these values potentially are biologically significant and should be compared to biological benchmarks, when established.

B. Temporal Pattern in Concentration of Residues. Figures S-1 through S-5 present residue concentrations measured over time for leaves, floral nectar, leaf nectar, subbracteal nectar, and pollen, respectively. For leaves, the raw data indicated decreased concentration over time with concave curvature in the response. A graph of data transformed to natural, base E, logarithms resulted in a highly significant straight line: R-square values were very high at 0.96 and 0.99 for sites 1 and 2, respectively. The transformation was also successful in linearizing the regressions for the other plant samples. Testing for significance of regression using the PROC REG procedure in the SAS analysis software similarly indicated highly significant regressions over time in the other plant samples, except for TZNG and TZMU residues in pollen. Equations based on natural log transformed data are used to provide estimates of dissipation half-lives with examples for determination of half-lives for data generated in aerobic and terrestrial field dissipation studies.

The logarithmic regression is expressed as in Equation 1:

Equation 1

$$\mathrm{Ln(Concentration) = Ln(Initial\ Concentration)\ +\ b(Time)}$$

The half-life is determined as Equation 2

Equation 2

$$\mathrm{T1/2 = Ln(.5)/b}$$

In Equation 2, Ln(.5) is the determination made at 50% of dissipated residues and b is the coefficient determined from fit of Equation 1 to the observed dissipation data. Table S-6 (A) compares the coefficients from the linear regression of the transformed data between the plant samples and between the two sites. Table S-6 (B) compares the half-live values calculated according to Equation 2. Addition of degradation products tended to increase the estimate of the half-life where, for example, at site 1 the half-life in floral nectar increased from 3.2 for parent clothianidin to 5.5 days when total residue was analyzed.

These regression equations also could be used to estimate the amount of time a residue would be above a benchmark value. For floral nectar, the mean total clothianidin value for the 3 samples taken from both sites 1 day after the second application was 100.3 ppb. If a chronic value was set at 25 ppb, then according to Equation 1:

Ln(Concentration) = 25, the target value
Ln(Initial Concentration) = 100.3, the mean of the 3 samples
b = 0.1285, the mean of the coefficients for total clothianidin for floral nectar in Table S-6 (A)
x = time

Equation 3

$$\ln(25) = \ln(100.3) + (-0.1285)(x)$$

$$3.219 = 4.608 - .1285(x)$$

$$x = 10.8 \text{ days}$$

Substituting and then solving Equation 3 results in an estimate of around 11 days above the 25 ppb benchmark value. Table S-7 contains the estimated number of days above the assumed benchmark values of 15, 20, 25 and 30 for floral nectar, leaf nectar, subbracteal nectar and pollen samples. The initial values were an average from the two sites as were the coefficient values for b. Individual analyses at site 2 would have resulted in longer estimated time intervals because concentrations were generally larger at that site. The averaged initial values in leaf nectar were large, which resulted in estimates greater than 30 days for the amount of time above the benchmark values.

C. Relationship between Residue in Plant Tissue. Figure S-6 contains the relationship for measured clothianidin concentration between leaf and floral nectar samples (A) and between leaf and pollen samples (B). Both graphs indicate a general relationship for increasing concentrations in floral nectar and pollen in response to increases in measured concentration leaves. However, the observed variance amongst the points only result in a low measure of R-square and thus a low accuracy in equations derived to describe the relationship.

Conclusion:

1. Foliar applications to cotton plants during bloom resulted in high concentration of clothianidin and its degradation products in bee relevant samples of nectar and pollen. Maximum and median values for total clothianidin residues are as followed: 239.9 and 40.9 ppb in floral nectar, respectively; 694.2 and 190.9 ppb in subbracteal nectar, respectively; 4,267.9 and 185.5 ppb in leaf nectar, respectively; and 771.4 and 38.5 ppb in pollen, respectively. These values will require additional analysis to determine their significance when related to derived benchmark values.

2. Significant regressions were measured for each of the residues in each of the plant samples where residues were observed to decrease within the 28 day sampling interval, as measured after the second clothianidin application. The derived equations can be used to calculate the days above which

concentrations would be expected to be higher than relevant benchmark values. An example is given for floral nectar where based on the mean of the initial values measured at the 2 sites in this study, concentrations would be expected to be greater than an assumed benchmark value of 25 ppb for approximately 11 days. Table S-7 contains a summary of estimated days above benchmark values of 15, 20, 25, and 30 ppb for floral nectar, subbracteal nectar, leaf nectar, and pollen. Values were calculated according to Equation 3.

3. There was a general relationship noted in graphs for increasing concentrations of clothianidin measured in leaf tissue to result in concomitant increases in concentration measured in nectar and pollen samples. The graphs, however, had a large amount of variability so resultant equations describing the relationships would have had low accuracy.

4. The applications were made to the foliage so effects due to soil were expected to be minimal. Potential differences due to soil type were not investigated because only one test site was investigated for each soil category and different cultivars were used at each site.

Table S-1. Leaves: Comparison of statistics for the distribution of year 2012 concentrations of clothianidin in cotton leaves where data have been pooled over all sampling dates (ranging from 1 to 28 days after the last foliar application to cotton plants). Note that the degradation products were not measured in leaf tissue.

Statistic	Distribution in Leaves (ng/g) Clothianidin
N	28
Mean	3,471.1
SD	3,759.2
CV (%)	108.3
Min	23.1
Median	1,812.0
75th	5,746.5
90th	9,917.0
95th	11,026.0

Table S-2. Floral Nectar: Comparison of statistics for the distribution of year 2012 concentrations of clothianidin in cotton floral nectar where data have been pooled over all sampling dates (ranging from 1 to 28 days after the last foliar application to cotton plants).

Statistic	Distribution in Floral Nectar (ng/g)			
	Clothianidin	TZNG	TZMU	Total
N	27	27	27	27
Mean	29.5	16.5	3.6	49.6
SD	43.2	15.8	2.6	56.3
CV (%)	146.4	96.2	72.5	113.4
Min	0.2	0.9	0.2	2.7
Median	17.4	14.2	3.1	40.9
75th	32.0	23.5	5.3	75.2
90th	79.4	40.9	7.5	127.8
95th	142.0	47.0	8.8	167.5
Max	182.0	62.7	10.9	239.9
% of Total	182.0	33.2	7.3	

Table S-3. Leaf Nectar: Comparison of statistics for the distribution of year 2012 concentrations of clothianidin in cotton leaf nectar where data have been pooled over all sampling dates (ranging from 1 to 28 days after the last foliar application to cotton plants).

Statistic	Distribution in Floral Nectar (ng/g)			
	Clothianidin	TZNG	TZMU	Total
N	27	27	27	27
Mean	448.5	22.0	53.4	523.9
SD	946.9	12.4	60.4	977.1
CV (%)	211.2	56.6	113.0	186.5
Min	9.9	2.3	3.6	15.9
Median	104.0	20.0	39.1	185.5
75th	213.0	32.9	67.7	338.9
90th	1,692.0	38.9	90.0	2,035.8
95th	2,624.0	39.5	110.0	2,710.9
Max	4,163.0	45.6	320.0	4,267.9
% of Total	85.6	4.2	10.2	

Table S-4. Subbracteal Nectar: Comparison of statistics for the distribution of year 2012 concentrations of clothianidin in cotton subbracteal nectar where data have been pooled over all sampling dates (ranging from 1 to 28 days after the last foliar application to cotton plants). Note that no subbracteal nectar samples were obtained from Site 2.

Statistic	Distribution in Floral Nectar (ng/g)			
	Clothianidin	TZNG	TZMU	Total
N	10	10	10	10
Mean	286.9	3.8	19.8	310.4
SD	295.6	3.3	18.4	316.9
CV (%)	103.1	87.2	92.9	102.1
Min	2.0	0.3	0.5	2.8
Median	171.5	2.5	17.0	190.9
75th	598.0	7.0	39.4	649.8
90th	647.0	8.3	41.9	692.9
95th	651.0	8.4	43.7	694.2
Max	651.0	8.4	43.7	694.2
% of Total	92.4	1.2	6.4	

Table S-5. Pollen: Comparison of statistics for the distribution of year 2012 concentrations of clothianidin in cotton pollen where data have been pooled over all sampling dates (ranging from 1 to 28 days after the last foliar application to cotton plants).

Statistic	Distribution in Floral Nectar (ng/g)			
	Clothianidin	TZNG	TZMU	Total
N	27	27	27	27
Mean	88.1	8.4	19.9	116.4
SD	156.4	18.2	41.0	175.4
CV (%)	177.6	217.2	205.8	150.7
Min	0.2	0.2	0.1	1.0
Median	19.7	1.5	4.6	38.5
75th	123.0	3.8	17.3	771.4
90th	246.0	28.8	58.6	308.0
95th	300.0	30.7	79.1	456.9
Max	760.0	87.9	199.0	771.4
% of Total	75.7	7.2	17.1	

Table S-6. Regression results measuring the change in observed concentrations in each plant sample over time. Table S-6 (A) contains the coefficient of the linear regression of the data transformed to natural logarithms, denoted as b, and the R-square value. Table S-6 (B) contains the estimated half-lives from each regression as calculated according to Equation 2. Except for TZNG and TZMU residue measures in pollen, regressions were highly significant.

Table S-6 (A) Regression Results

Plant Sample	Regression Results for Linear Coefficient (b) and R-square Values (R^2)															
	Clothianidin				TZNG				TZMU				Total Residue			
	Site 1		Site 2		Site 1		Site 2		Site 1		Site 2		Site 1		Site 2	
	b	R^2	b	R^2	b	R^2	b	R^2	b	R^2	b	R^2	b	R^2	b	R^2
Leaves	-0.233	0.96	-0.202	0.99	-	-	-	-	-	-	-	-	-	-	-	-
Floral Nectar	-0.216	0.90	-0.173	0.78	-0.086	0.88	-0.121	0.59	-0.088	0.84	0.081	0.81	-0.130	0.92	-0.127	0.78
Leaf Nectar	-0.193	0.85	-0.080	0.67	-0.102	0.90	-0.044	0.60	-0.126	0.81	-0.037	0.47	-0.180	0.86	-0.060	0.70
Subbracteal Nectar	-0.243	0.97	-	-	-0.127	0.91	-	-	-0.200	0.96	-	-	-0.233	0.97	-	-
Pollen	-0.253	0.93	-0.182	0.66	-0.062	0.80	-0.087	0.21	-0.173	0.92	-0.044	0.06	-0.223	0.92	-0.105	0.37

Table S-6 (B) Estimated Half-lives

Plant Sample	Estimated Half-lives for Each Plant Sample at Each Site (Days)							
	Clothianidin		TZNG		TZMU		Total Residue	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Leaves	2.97	3.43	-	-	-	-	-	-
Floral Nectar	3.21	4.01	8.06	5.73	7.88	8.56	5.33	5.46
Leaf Nectar	3.59	8.66	6.79	15.75	5.50	18.73	3.85	11.55
Subbracteal Nectar	2.85	-	5.46	-	3.47	-	2.97	-
Pollen	2.74	3.81	11.18	NS	4.01	NS	3.11	6.60

Table S-7. Estimated time interval that concentrations measured in bee relevant cotton plant samples would be above assumed benchmark values. Data were pooled from the 2 sites and initial values were determined as the average from both sites for those measured 1 day after the second foliar application. Values for days above assumed benchmark calculated according to Equation 3.

Plant Sample	Initial Value (ng/g)	Averaged Coefficient (b)	Estimated Days Over Assumed Benchmark Value Value (ng/g)			
			15.0	20.0	25.0	30.0
Floral Nectar	100.30	-0.129	14.8	12.5	10.8	9.4
Leaf Nectar	1885.90	-0.120	40.3	37.9	36.0	34.5
Subbracteal Nectar	670.65	-0.233	16.3	15.1	14.1	13.3
Pollen	117.30	-0.164	12.5	10.8	9.4	8.3

Figure S-1. Leaves: Concentration of clothianidin residues measured in cotton leaves over a 28 day period. Initial samples are indicated as those taken 1 day after the second foliar application to plants in bloom. Figure S-1 (A) contains raw data and Figure S-1 (B) displays the transformed data to natural logarithms.

Figure S-1 (A) Raw Data

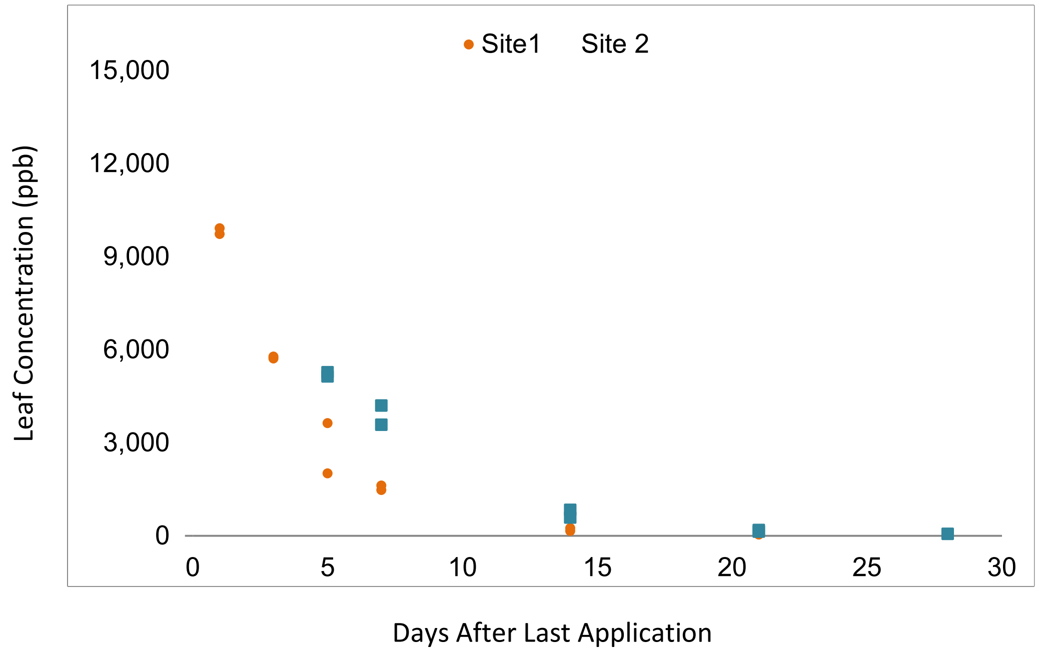


Figure S-1 (B) Transformed to Natural Logarithms

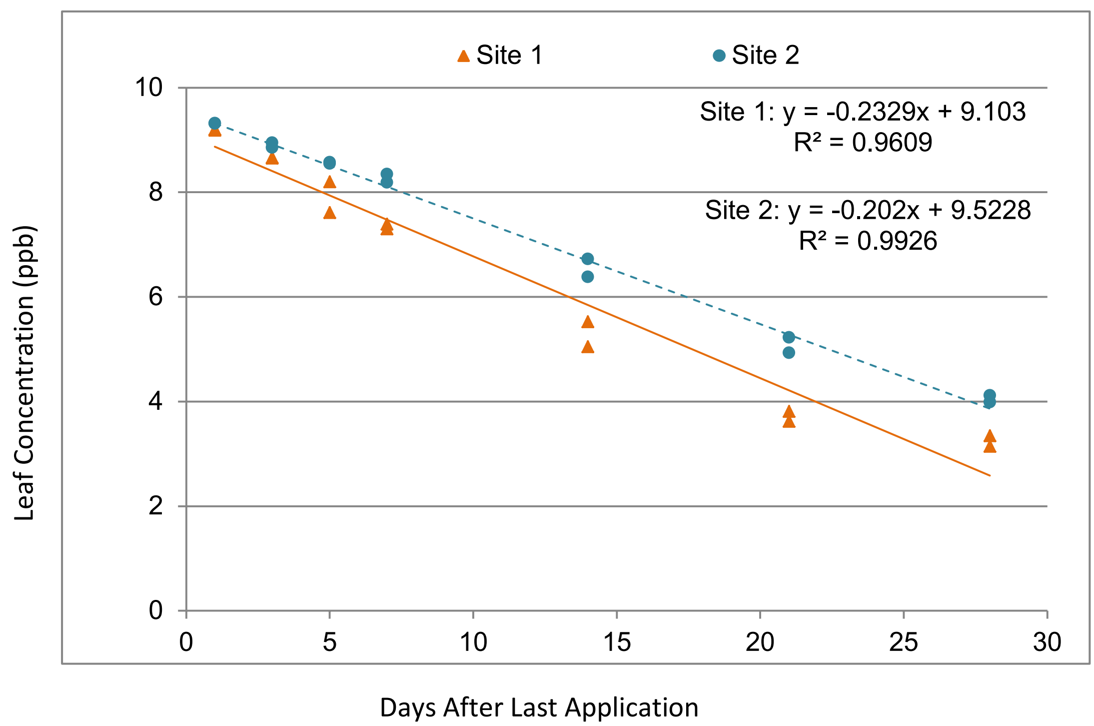
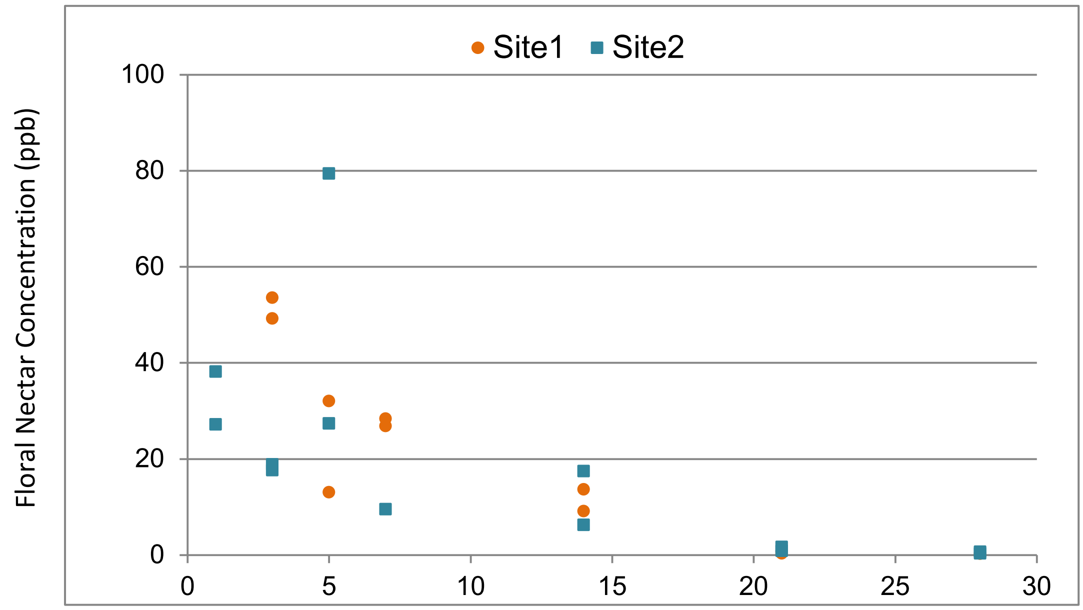


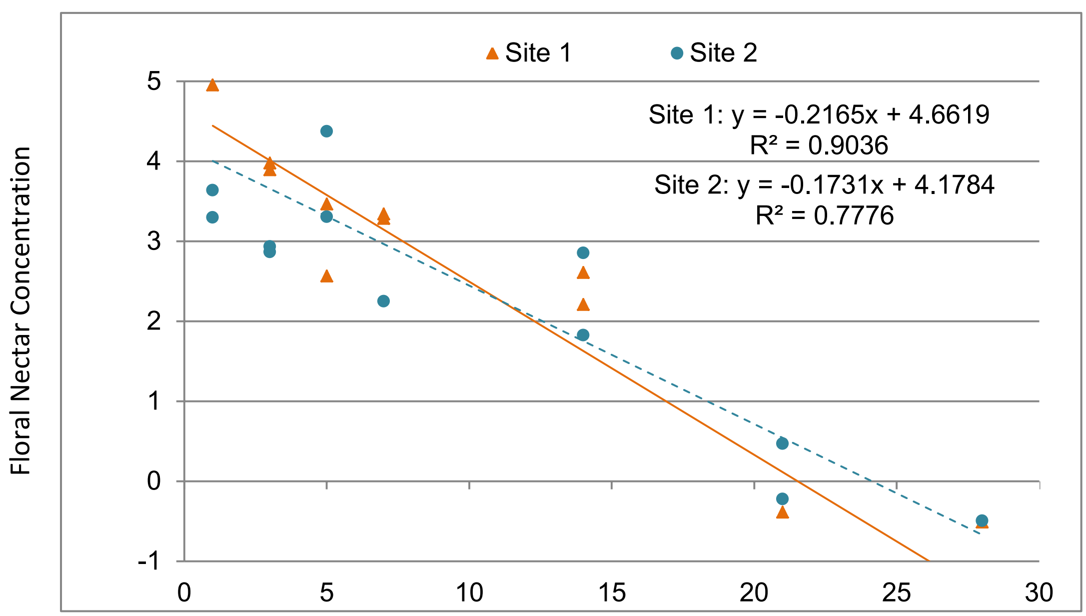
Figure S-2. Floral Nectar: Concentration of clothianidin residues measured in floral nectar of cotton plants over a 28 day period. Initial samples are indicated as those taken 1 day after the second foliar application to plants in bloom. Figure S-2 (A) contains raw data and Figure S-2 (B) displays the transformed data to natural logarithms.

Figure S-2 (A) Raw Data



Days After Last Application

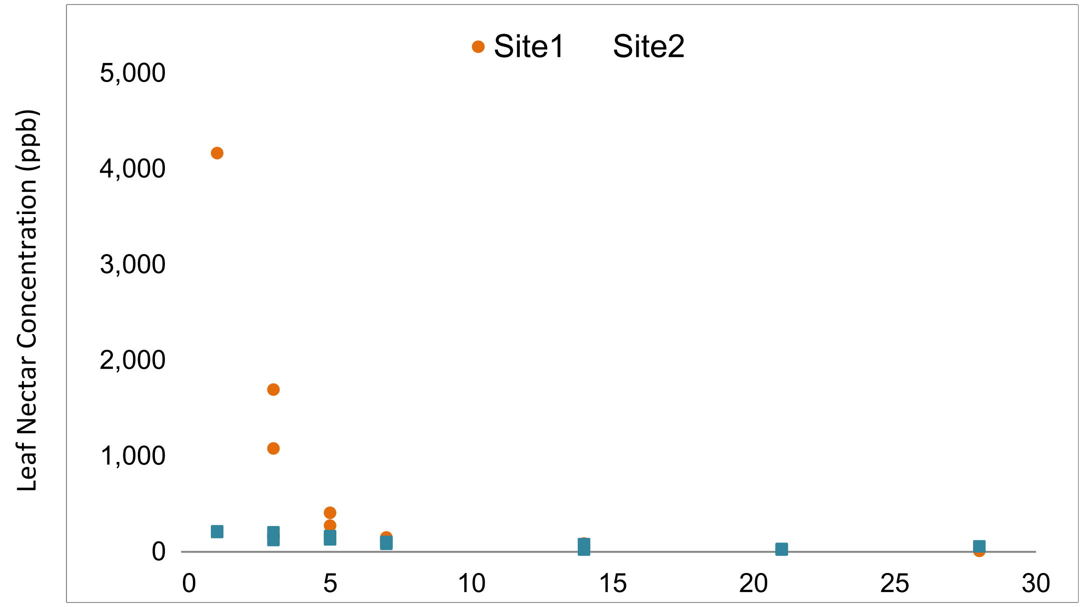
Figure S-2 (B) Transformed to Natural Logarithms



Days After Last Application

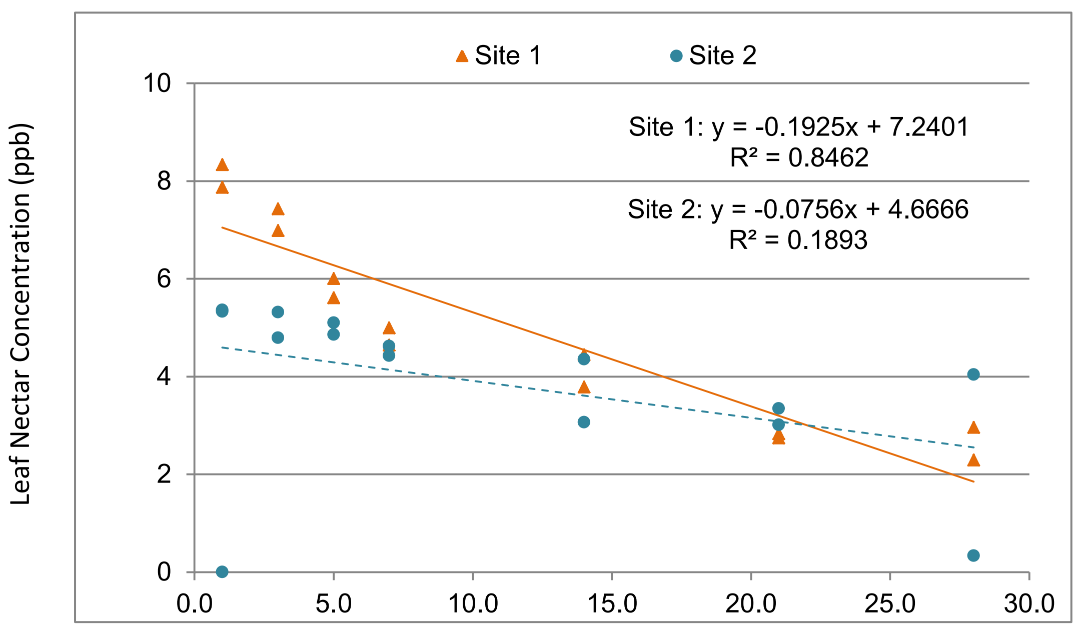
Figure S-3. Leaf Nectar: Concentration of clothianidin residues measured in leaf nectar of cotton plants over a 28 day period. Initial samples are indicated as those taken 1 day after the second foliar application to plants in bloom. Figure S-3 (A) contains raw data and Figure S-3 (B) displays the transformed data to natural logarithms.

Figure S-3 (A) Raw Data



Days After Last Application

Figure S-3 (B) Transformed to Natural Logarithms



Days After Last Application

Figure S-4. Subbracteal Nectar: Concentration of clothianidin residues measured in subbracteal nectar of cotton plants over a 28 day period. Initial samples are indicated as those taken 1 day after the second foliar application to plants in bloom. Figure S-4 (A) contains raw data and Figure S-4 (B) displays the transformed data to natural logarithms.

Figure S-4 (A) Raw Data

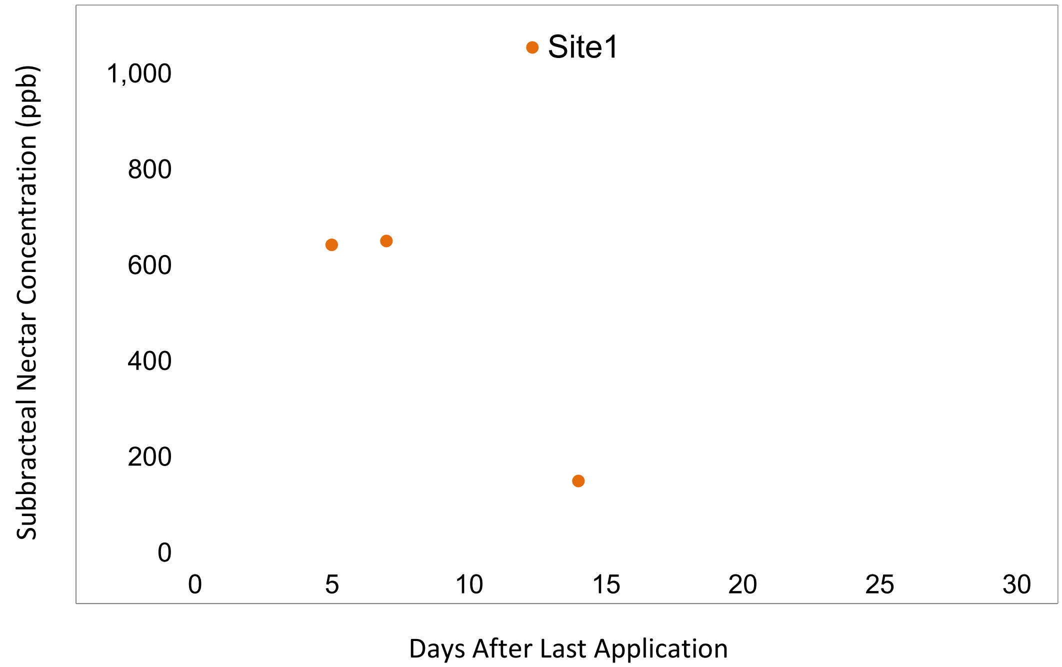


Figure S-4 (B) Transformed to Natural Logarithms

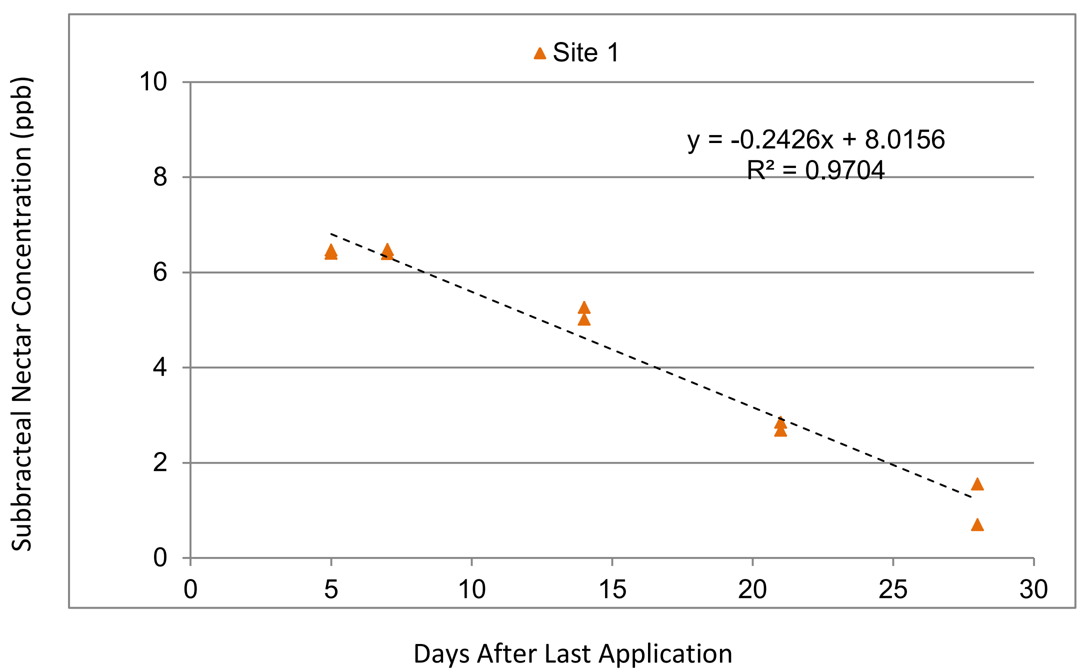
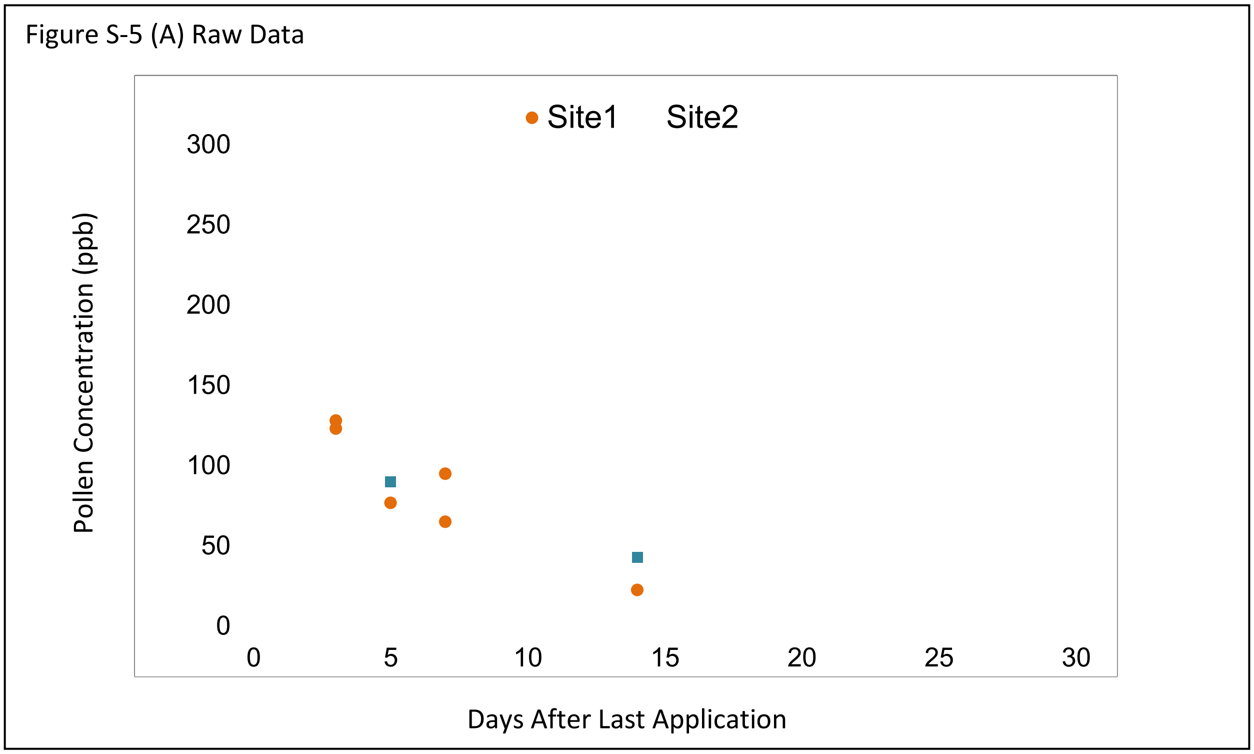


Figure S-5. Pollen: Concentration of clothianidin residues measured in pollen of cotton plants over a 28 day period. Initial samples are indicated as those taken 1 day after the second foliar application to plants in bloom. Figure S-5 (A) contains raw data and Figure S-5 (B) displays the transformed data to natural logarithms.



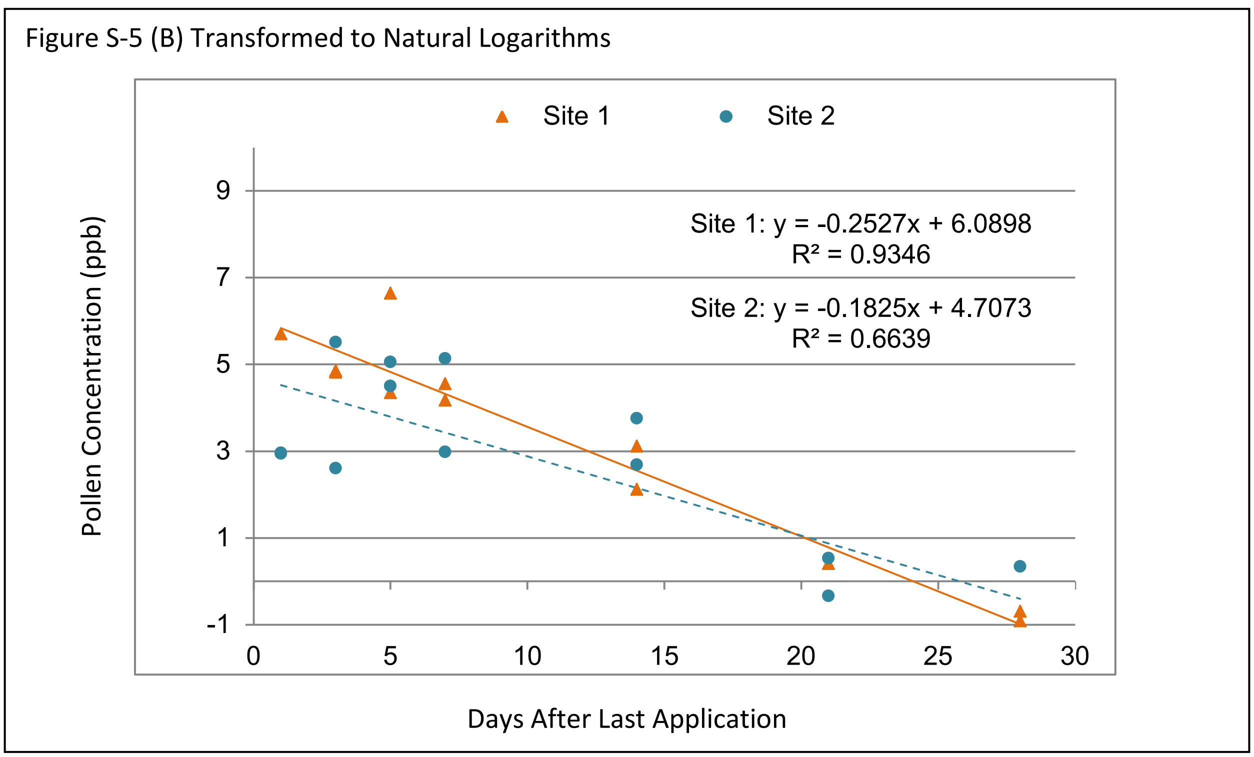


Figure S-6. Relationship measured for concentration of clothianidin residues. Figure S-6 (A) displays the relationship of clothianidin between leaf and floral nectars and Figure S-5 (B) displays the relationship of clothianidin between leaf and pollen.

Figure S-6 (A) Leaf and Floral Nectar

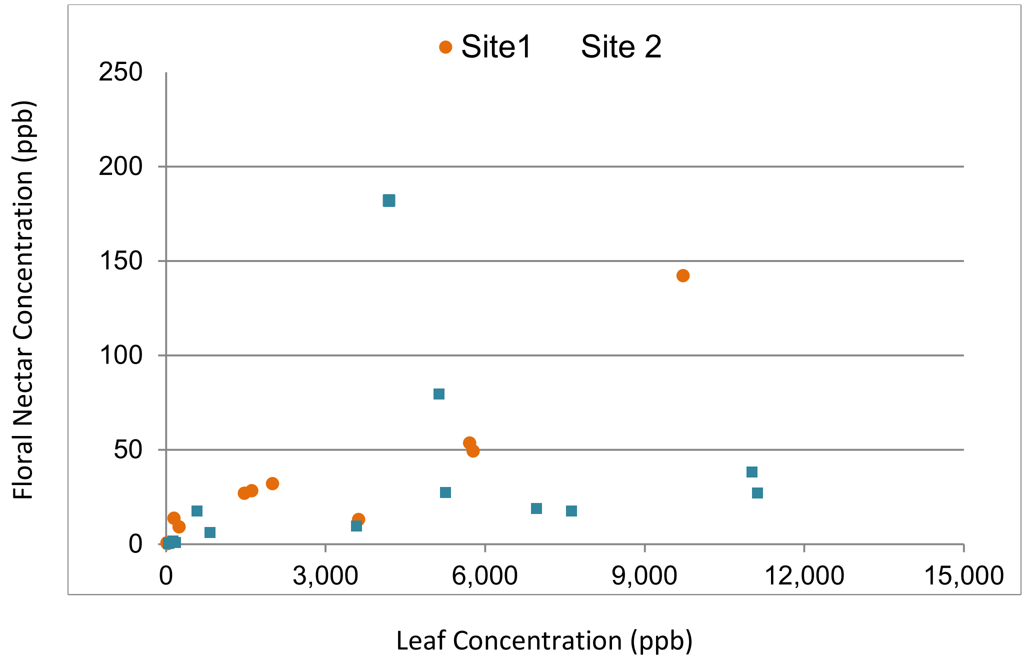
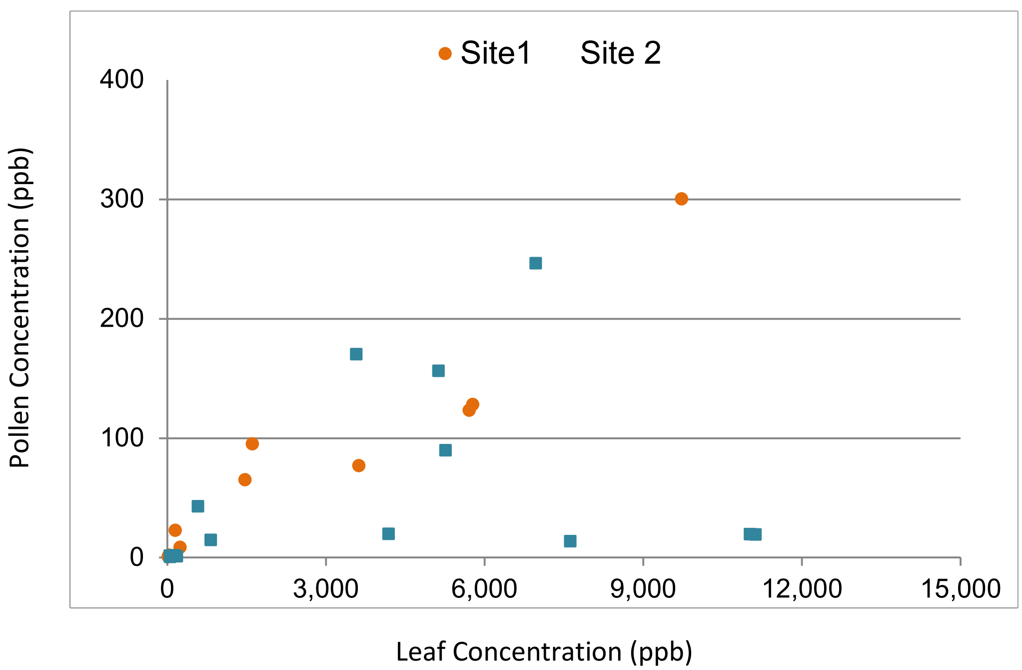


Figure S-6 (B) Leaf and Pollen



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Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Bondarenko, S. 2016. Clothianidin: Quantitation of Residues of Clothianidin, TZNG and TZMU in Nectar, Pollen and Leaves Collected from Pumpkins Following Soil Application of Belay Insecticide and Foliar Application of Belay 50 WDG Insecticide Valent Technical Cener Study Number: VP-38971	Non-Guideline field residue study on pumpkin to establish clothianidin and metabolite concentrations in leaves and manually-collected nectar and pollen following soil or foliar applications	This study quantified clothianidin, TZNG, and TZMU residues in pumpkin (*Cucurbita peto* L. var *pepo*) grown in three locations: North Dakota (ND; loam or sandy loam), California (CA; loamy sand or sand), and Oregon (OR; silt loam). Three replicate plots were used in each location for each treatment. One set of plots received a soil application at planting at a nominal rate of 0.2 lbs. ai/A, and another set of plots at each location received a single soil application at BBCH stage ca. 14 at a nominal rate 0.2 lbs. ai/A. The final set of plots received a single foliar application BBCH stage ca. 14 at a nominal rate 0.1 lbs. ai/A. The soil application rate equals the maximum label rate of 0.2 lbs. ai/A for Belay® 50 WDG, but the foliar application rate exceeded the maximum rate of 0.067 lbs. ai/A. Nectar and pollen were sampled 5 times during the blooming period. In the plots that received an at-plant soil application, samples of pollen and nectar were collected 47-75, 42-69, and 52-79 days after the application in ND, CA, and OR, respectively. In the plots that received a soil or foliar application at BBCH stage 14, samples of pollen and nectar were collected 25-53, 21-48, and 22-49 days after the application in ND, CA, and OR, respectively. Analyses of fortified samples of pollen (79-102% clothianidin, 88-96 TZNG, and 84-95 TZMU) and nectar (93-109% clothianidin, 88-101 TZNG, and 99-109 TZMU) were all within acceptable limits. Nectar and pollen samples were manually collected from flowers. Clothianidin residues in nectar from plots receiving at-plant soil applications across all locations were 5.84 ppb or less and decreased over the course of the sampling, and concentrations for TZNG or TZMU were low, always less than the LOQ of 1.0 ppb and often less than the LOD of 0.20 ppb. Clothianidin residues in pollen from plots receiving at-plant soil applications across all locations were 38.3 ppb or less and decreased over the course of the sampling in CA and OR but remain fairly constant in ND, and concentrations for TZNG or TZMU were low, always less than the LOQ of 1.0 ppb except in the initial samples in CA and often less than the LOD of 0.25 ppb. Clothianidin residues in nectar from plots receiving soil applications at BBCH stage 14 across all locations were 11.3 ppb or less and decreased over the course of the sampling, and concentrations for TZNG or TZMU were low, frequently less than the LOQ of 1.0 ppb or the LOD of 0.20 ppb. Clothianidin residues in pollen from plots receiving soil applications at BBCH stage 14 across all locations were 31.9 ppb or less and decreased over the course of the sampling in CA and OR but increased over time in ND, and concentrations for TZNG or TZMU were low, always less than the LOQ of 1.0 ppb except in CA and often less than the LOD of 0.25 ppb. Clothianidin residues in nectar from plots	Nothing that would affect the validity of the study.

		receiving foliar applications at BBCH stage 14 across all locations were consistently greater than other application scenarios at 43.5 ppb or less and decreased less dramatically over the course of the sampling, and concentrations for TZNG or TZMU were low, always less than the LOD of 0.20 ppb. Clothianidin residues in pollen from plots receiving foliar applications at BBCH stage 14 across all locations were always low at 3.03 ppb or less but did not show a consistent pattern as sampling progressed, and concentrations for TZNG or TZMU were low, always less than the LOD of 0.25 ppb. Mean residues for clothianidin in nectar and pollen were generally greater across all sample periods in CA than in ND or OR.	

Appendix 10. Evaluations of Residue Studies Included in this Risk Determination Document

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Bondarenko, S. 2017. Clothianidin: Quantitation of Residues of Clothianidin, TZNG, and TZMU in Nectar, Pollen, and Leaves Following Foliar Post Bloom Application of Belay Insecticide/Clutch 50 WDG Insecticide to Apple Trees. MRID: 50154304 Valent Study Number: VP-38552	Non-Guideline field residue study on apples to establish Clothianidin and metabolite levels in nectar, pollen, and leaves following foliar applications	This study quantified Clothianidin residues in apple (*Malus pumila*) grown in three locations: Ontario, Canada (CAN; loam), Hood River, Oregon (OR; loam), and Parkdale, OR (OR; sandy loam). One replicate plot was used in each location, and each plot received a post-bloom foliar application of Belay Insecticide/Clutch 50 WDG Insecticide to apple trees in 2014 and again in 2015. The post-bloom foliar application was made at the nominal application rate of 0.1874 lb ai/A at ca. 7 days before harvest in September 2014. The post-bloom foliar application was made at the nominal application rate of 0.1874 lb ai/A at ca. 7 days before harvest in August-September 2015. The maximum annual use rate of Clothianidin approved in Canada is 0.1874 lb ai/A. For foliar applications, the substance was applied using an orchard air blast to both sides of the tree rows. Soil samples were collected using a soil auger or a probe. Pollen and nectar samples were collected from single composite flower samples during the blooming period in spring 2015 and 2016. Leaf samples were collected from new emerged whole leaves in the terminal shoots. In 2015, flowers and whole leaves were collected 231, 218, and 229 days after the last application in Ontario, Canada, Hood River, OR, and Parkdale, OR, respectively. In 2016, flowers and whole leaves were collected 247, 231, and 245 days after the last application in Ontario, Canada, Hood River, OR, and Parkdale, OR, respectively. Average recoveries for Clothianidin (82-96%), TZNG (71-95%), and TZMU (83-100%) in nectar, pollen, leaf, and soil samples were all within the 70-120% acceptable range. The maximum measured Clothianidin residues resulting from post-bloom foliar application in 2015 were 0.71 ng/g in nectar, 57.4 ng/g in pollen, and 2.55 ng/g in leaves. The maximum measured Clothianidin residues resulting from post-bloom foliar application in 2016 were <0.20 ng/g in nectar, 31.1 ng/g in pollen, and 2.58 ng/g in leaves. This study is acceptable.	Minimum pollen and nectar sample amounts were not obtained in 2015 or 2016 for all subplot samples, as the flowers did not yield the necessary amounts. This deviation may lead to a gap in data for pollen and nectar results. In 2015 and 2016, poor quality pollen samples were collected, as the samples were contaminated with anthers, flower filaments, and other flower materials. In both years, scarce amounts of apple pollen were collected in all trial locations. This may affect the recoveries for Clothianidin in pollen.

Appendix 10. Evaluations of Residue Studies Included in this Risk Determination Document

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Bondarenko, S. 2017. Clothianidin: Quantitation of Residues of Clothianidin, TZNG, and TZMU in Nectar, Pollen, and Leaves Following Foliar Post Bloom Application of Belay Insecticide to Peach Trees. MRID: 50154303 Valent Study Number: VP-38563	Non-Guideline field residue study on peaches to establish Clothianidin and metabolite levels in nectar, pollen, and leaves following foliar applications	This study quantified Clothianidin residues in peach (*Prunus persica*) grown in three locations: Athens, Georgia (GA; sandy clay loam), Monetta, South Carolina (SC; sand), and Selma, California (CA; loamy sand). One replicate plot was used in each location, and each plot received two post-bloom foliar applications of Belay Insecticide to peach trees in 2014 and again in 2015. The first post-bloom foliar application in June-July 2014 was made at the nominal application rate of 0.1 lb ai/A at 35-40 days before harvest (BBCH 73-81). The second application in 2014 was made at least 10 days after the previous application, and at least 21 days before harvest (at the same nominal application rate). The second post-bloom foliar application in June-July 2015 was made at the nominal application rate of 0.1 lb ai/A at 35-40 days before harvest (BBCH 72-81). The second application in 2015 was made at least 10 days after the previous application, and at least 21 days before harvest (at the same nominal application rate). The maximum annual use rate of Clothianidin is 0.2 lb ai/A. For foliar applications, the substance was applied using an orchard air blast to both sides of the tree rows. Soil samples were collected using a soil auger or a probe. Pollen and nectar samples were collected from single composite flower samples during the blooming period in February – March 2015 and 2016. Leaf samples were collected from new emerged leaves in the terminal shoots at the BBCH growth stage ca. 72 in March – April 2015 and 2016. In 2015, flowers were collected 276, 248, and 234 days after the last application in Georgia, South Carolina, and California, respectively. In 2015, leaves were collected 301, 269, and 251 days after the last application in Georgia, South Carolina, and California, respectively. In 2016, flowers were collected 280, 245, and 233 days after the last application in Georgia, South Carolina, and California, respectively. In 2016, leaves were collected 314, 286, and 254 days after the last application in Georgia, South Carolina, and California, respectively. Average recoveries for Clothianidin (90-101%), TZNG (85-102%), and TZMU (84-99%) in nectar, pollen, leaf, and soil samples were all within the 70-120% acceptable range. The maximum measured Clothianidin residues resulting from post-bloom foliar application in 2015 were 0.21 ng/g in nectar, 6.19 ng/g in pollen, and 12.2 ng/g in leaves. The maximum measured Clothianidin residues resulting from post-bloom foliar application in 2016 were 0.30 ng/g in nectar, 130 ng/g in pollen, and 13.3 ng/g in leaves. This study is acceptable, but the abnormally high Clothianidin residue in pollen in 2016 suggests possible contamination.	The collected pollen sample size in 2015 and 2016 did not meet the protocol requirements. This led to a data gap in the pollen results. In 2015 and 2016, poor quality pollen samples were collected at all three sites. Pollen samples had contaminants such as anthers, flower filaments, and other flower materials. Additionally, scarce pollen was collected at the California site. This could have a potential impact on the integrity of the data collected. The maximum Clothianidin residue in peach pollen in 2016 is abnormally high, suggesting either contamination during the field sample or in analytical sample processing.

Appendix 10. Evaluations of Residue Studies Included in this Risk Determination Document

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Bondarenko, S. 2017. Clothianidin: Quantitation of Residues of Clothianidin, TZNG, and TZMU in Pollen and Leaves Collected from Grapevines Following Soil and Foliar Applications of Belay 50 WDG Insecticide (Clutch 50 WDG). MRID: 50154305 Valent Study Number: VP-38992	Non-Guideline field residue study on grapes to establish Clothianidin and metabolite levels in pollen and leaves following soil and foliar applications	This study quantified Clothianidin residues in grape (*Vitis vinifera*) grown in three locations: California (CA; sandy loam), Oregon (OR; loam), and Ontario, Canada (CAN; sandy loam and loam). Three replicate plots were used in each location, and each plot received a post-bloom foliar application, a pre-bloom soil application, or a pre-bloom foliar application of Belay 50 WDG/Clutch 50 WDG Insecticide. The post-bloom foliar application was made at the nominal application rate of 0.1 lb ai/A at BBCH growth stage ca. 71 (berry swelling) in 2015. The pre-bloom soil application was made at the nominal application rate of 0.2 lb ai/A at BBCH growth stage ca. 08 (bud break on grapevines) in 2016. The pre-bloom foliar application was made at the nominal application rate of 0.1 lb ai/A at BBCH growth stage ca.14 (approximately 4 leaves unfolded) in 2016. For foliar applications, the substance was applied using an air blast sprayer. For soil applications, the substance was applied using either a boom sprayer or drip irrigation. Soil samples were collected using a small shovel, core sampler, or soil tube. Pollen samples were collected from grape flower clusters, and leaf samples were collected using 1 inch leaf-punches. Average recoveries for Clothianidin (93-116%), TZNG (84-88%), and TZMU (79-80%) in pollen, leaf, and soil samples were all within the 70-120% acceptable range. The maximum measured Clothianidin residues resulting from pre-bloom foliar application at BBCH ca. 14 were 1564 ng/g in pollen and 12781 ng/g in leaves. Maximum measured Clothianidin residues resulting from pre-bloom soil application at BBCH ca. 08 were 206 ng/g in pollen and 417 ng/g in leaves. Maximum measured Clothianidin residues resulting from post-bloom foliar application at BBCH ca. 71 were 31.9 ng/g in pollen and 15932 ng/g in leaves. This study is acceptable.	Pollen sample weights required by the protocol for all samples and all treatments were not met, as flowers did not produce sufficient amounts of pollen. No pollen was collected at late bloom sampling, due to advanced stage of blooms. This may lead to a data gap in pollen results. Abnormally high Clothianidin residues in soil were observed in one of the trials after foliar application to the grapevines, with low residues in pollen and leaf samples collected during the bloom. Soil samples were collected on the same day that nearby plots received irrigation treatment. Soil samples from these plots may have inadvertently been miscollected, leading to the discrepancy in results.

. . E A Data Evaluation eports (Clothianidin)

U.S. EPA. (2017). Data evaluation report: clothianidin: quantitation of residues of clothianidin, tzng, and tzmu in nectar, pollen, and leaves following soil application of belay insecticide to four different species of cucurbit. Washington, D.C.: Author. Laboratory Report Number VP-38938.

U.S. EPA. (2017). Data evaluation report: clothianidin: quantitation of residues of clothianidin, tzng, and tzmu in nectar and pollen following foliar application of Clutch 50 WDG insecticide to cucurbits. Washington, D.C.: Author. Laboratory Report Number: VP-38313.

U.S. EPA. (2017). Data evaluation report: clothianidin: quantitation of residues of clothainidin, tzng, and tzmu in pollen and leaves collected from potatoes follwing soil and foliar applications of Belay insecticide. Washington, D.C.: Author. Laboratory Report Number VP-38985.

Thiamethoxam Data Evaluations (begin on next page)

MRID 49550801 CDPR THX Cucumber

Reference
Hampton. R. (2013) Thiamethoxam 75 SG (A9549C) - Magnitude of the Residues in Leaves, Flowers, Pollen, and Nectar of Cucumbers, Representative Commodity of Cucurbit Vegetables, EPA Crop Group 9, in California: Final Report. Project Number: TK0024668. Unpublished study prepared by Syngenta Crop Protection, LLC. 67. MRID 49550801, CDPR Study ID 269320, Data Volume 52691-0466, Tracking ID# 269320

1. STUDY INFORMATION

Chemical:	Thiamethoxam	**PC Code**	60109
Test Material:	Platinum 75SG	**Percent Active Ingredient:**	75%
Study Type:	Fild residue study on cucumber crops to measure the magnitude of Thiamethoxam and CGA322704 on the leaves, pollen and nectar of the plant following an in-furrow treatment at cucumber seeding.		
Sponsor:	Syngenta Crop Protection, LLC 410 Swing Road Greensboro, North Carolina 27409	**Experiment Start and End Date:**	May 6, 2011 – November 30, 2012
Sponsor Study ID:	TK0024668	**Study Locations:**	3 trial sites of cucumber which were located in Fresno, California and San Luis Obispo, California.
Study Completion Date:	January 18, 2013		
GLP Status:	Non-GLP; protocol reviewed by CDPR. [CDPR Study ID 269320, Data Volume 52691-0466, Tracking ID# 269320]		

2. REVIEWER INFORMATION

Study Reviewed by: California Department of Pesticide Regulation	Richard Bireley, Sr. Environmental Scientist (Specialist) John Troiano, Ph.D., Research Scientist III Alexander Kolosovich, Environmental Scientist Brigitte Tafarella, Environmental Scientist Denise Alder, Sr. Environmental Scientist (Specialist) Russell Darling, Environmental Scientist

MRID 49550801 CDPR THX Cucumber

3. EXECUTIVE SUMMARY

A two-year study was conducted in 2011 and 2012 to determine the magnitude of thiamethoxam and CGA322704 residues in cucumber leaves, flowers, pollen, and nectar. The study consisted of three trials that were located in California and each consisted of an untreated control plot and three replicated treated plots. The trials were conducted on coarse-, medium- and fine-textured soils, which were characterized as two sandy loam sites (9% clay and 14% clay) and one clay loam site (38% clay), respectively. Platinum® 75SG (active ingredient, thiamethoxam) was applied as an in-furrow treatment at cucumber seeding at a target rate of 0.172 lb ai/acre in Years 1 and 2. Composite samples of leaves, female flowers, male flowers, pollen, and nectar were collected for residue analysis from the untreated plot and treated plots at 43 to 57 days after planting in Year 2.

4. STUDY VALIDITY

Guideline Followed:	TBD; (protocol was reviewed by CDPR)
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	**ACCEPTABLE**
Rationale:	N/A
Reparability:	N/A

5. MATERIALS AND METHODS

Test Material Characterization			
Test item:	Platinum 75SG	**Percent A.I.:**	75.0% A.I.
Formulation Type:	Soluble Granule, SG	**Batch Number:**	592641
CAS #:	153719-23-4	**Expiration Date:**	April 30, 2013

5A. STUDY DESIGN

Residue data for thiamethoxam (CGA293343) and its major plant metabolite, CGA322704, in the pollen and nectar of cucurbit vegetables were requested by the California Department of Pesticide Regulations (CDPR) as part of the re-evaluation of the nitroguanidine class of neo-nicotinoid insecticides (Article 8, Subchapter 1, Chapter 2, Division 6 of Title 3 of the California Code of Regulations).

The purpose of this two-year study was to determine the amount of thiamethoxam and CGA322704 in cucurbit pollen and nectar from plants grown in fields after an at-plant soil application of Platinum® 75SG in two successive years. The effect of soil type on thiamethoxam uptake and resulting residues in pollen and nectar also was investigated by conducting trials on coarse-, medium-, and fine-textured soils. The choice of soils represented a range of soil types on which cucurbits may be grown commercially. The goal was for this study to provide realistic measurements of thiamethoxam and CGA322704 residues to which bees may be exposed to under typical growing conditions in California.

5B. Application Timing and Rates

The target application rate for cucurbit vegetables is 0.172 lb ai/acre (3.67 oz of product/acre) as per the Platinum® 75SG label. For the applications in 2011 and 2012 at each trial site, the maximum amount of test substance was added to the volume of water needed to cover the plot area (plus overage).

A CO2-pressurized, single-nozzle sprayer was calibrated with water prior to each application of test substance. To calibrate, the sprayer system was charged, and the volume of water discharged during 30 seconds was collected and recorded. The procedure was repeated 2 additional times, and the mean output was calculated. The acceptance criterion required that the three collections fell in the targeted range of 15 gallons per (GPA) ± 5% (or 140 L/ha) for Trial Sites 1 and 2 and 20 gallons/A ± 5% (or 187 L/ha) for Trial Site 3.

The single-nozzle sprayer was attached to the planter, and the spray nozzle directed the test substance application into the furrow prior to furrow closing to cover the seed.

Table 1. Applications of Platinum 75SG per year.

Site #	Year	Test Substance Mass (g of product)	Water Volume (gallons)
1	2011	27.7	4
	2012	27.7	4
2	2011	27.7	4
	2012	27.7	4
3	2011	20.8	4
	2012	20.8	4

5C. Study Site Location and Characteristics

The three trial sites were selected based on the USDA soil survey as the soils at the sites were identified as a loamy sand, a sandy loam, and a silty clay loam (coarse-, medium-, and fine textured soil, respectively). Trial Sites 1 and 2 were located in Fresno County on land leased and managed by Eurofins Agroscience Services, Inc. (EASI, Sanger, CA), and Trial Site 3 was located on the research farm of California Polytechnical University at San Luis Obispo in San Luis Obispo County.

No maintenance pesticides were applied to the treated plots, with the exception of a single application of Baythroid (2.8 fl. oz./A) to Trial Site 3 on July 5, 2012. Irrigation was required at each of the three trial sites from planting and test-substance application to harvest. Approximately 0.33 inches of water was applied at Trial Sites 1 and 2 on a ca. 3-day schedule; whereas, approximately 3 inches of water was applied at Trial Site 3 on ca. 14-day schedule.

After harvest of cucumbers in 2011 (Year 1), vines were mowed and the crop residue was left on the soil surface. Plots remained fallow until the plots were prepared (i.e., tilled and rebidded) the following spring prior to planting in Year 2 (2012).

The results of the soil-characterization analyses are summarized below. Although the soil survey maps indicated that the textural classes of the soils at Trials Sites 1, 2 and 3 were a loamy sand (‘coarse-’), sandy loam ('medium-') and silty clay loam ('fine-'), the soils within the plot areas at the Trial Sites 1 and 2 were determined by soil-characterization analyses to be sandy loams, whereas, the soil from Trial Site 3 was classified as a clay loam. The three soils differed in the percentages of clay and sand and in soil-solution pH.

Table 2. Trial Site Conditions for Melon Grown at Sites Previously Treated with Imidacloprid

Site #	Trial Location (County, State)	Percent Sand	Percent Silt	Percent Clay	Organic Matter (%)	pH	Soil Type	Soil Characteristics
1	Fresno, California	65	26	9	0.5	5.6	Medium	Sandy Loam
2	Fresno, California	56	30	14	0.7	6.9	Medium	Sandy Loam
3	San Luis Obispo, California	37	25	38	2.8	7.5	Fine	Clay Loam

5D. Sample Collection, Handling, Processing

Samples of leaves, female and male flowers, pollen and nectar were collected from all trial sites in Year 2 of the study. In preparation for sampling, the untreated and treated plots were enclosed in tunnels constructed of PVC® pipe covered by netting of a mesh size suitable for excluding foraging bees. In addition, plots were irrigated within 24 hours of sample collection to ensure that the plants were adequately hydrated thereby promoting nectar flow.

At each trial site, plant samples were collected from the untreated and treated plots by separate teams to minimize the potential for cross contamination. Approximately 400 male and 250 female flowers were collected, which pollen and nectar samples were extracted from (female and male flowers were collected on successive days to allow adequate time for extraction of nectar and pollen). The male and female flower samples were transported on blue ice in separate, labeled plastic bags to the field laboratory for pollen and nectar extraction. Leaves were subsequently sampled to confirm uptake of the test substance.

Female Flowers and Nectar

The nectary of the female flower was exposed by removal of the corolla and calyx using a dissection scalpel. Once exposed, the nectar was collected by capillary action using a 10-µL microcapillary pipette (preliminary method development indicated that nectar could be extracted from approximately 50% of the flowers in a given sample; if nectar was present, approximately 2 - 4 µL typically was collected from each flower). Approximately 250 female flowers were sampled in order to extract the minimum sample size of nectar required for analysis (ca. 100 µL). The total number of microcapillary pipettes needed to extract the sample of ca. 250 flowers was recorded, and the entire set of pipettes was then placed into a labeled, extraction-ready 15-mL centrifuge tube. The total sample weight was recorded, and the weights of the storage container and the pipettes were subtracted to determine the actual nectar sample mass. After nectar extraction, a ca. 500-1000 g flower sample was placed into a labeled, sealable plastic bag, and the sample mass was recorded. The nectar and flower samples were placed into the freezer until shipment.

Male Flowers and Pollen

Pollen was extracted from the male flowers using a laboratory vacuum pump; a rubber tube was attached to the pump and fitted with a filtered 1000-µL Eppendorf pipette tip. The pipette tips containing the trapped pollen were cut in two using nursery pruners (to facilitate solvent extraction), and the two pieces were placed into a 250-mL extraction bottle. The total sample mass was recorded, and the weights of the extraction bottle and the pipette tips were subtracted to determine the actual pollen sample mass. After pollen collection, ca. 500 - 1000 g flower samples were placed into labeled, sealable plastic bags, weighed and stored frozen until shipment.

Leaves

Representative samples of at least 500 g of leaves were collected from the untreated and treated plots. Leaves were removed by hand from the proximal (i.e., closest to the root), middle and distal portions of the cucumber vines.

Sample Storage.

Samples were transported from the field sites to freezers at EASI (Sanger, CA) where they were stored frozen until shipment to the analytical laboratory. Samples were shipped overnight on dry ice via FedEx to the analytical laboratory.

All samples were received frozen and in good condition at ABC Laboratories from the field trial sites. The samples were maintained in frozen condition, excluding periods during which the samples were removed from the freezer for sample preparation, weighing or residue analysis.

The leaf and flower samples were weighed and ground with dry ice using a Robot Coup; the homogenized samples were placed into labeled, plastic containers and stored in a freezer (allowing the dry ice to sublime). Extraction solution was added directly to pollen and nectar samples, which were then shaken on a mechanical shaker for approximately one hour. After sample preparation, the homogenized leaf and flower samples were stored in plastic containers and placed in a freezer until they were sub-sampled for analysis; the pollen and nectar extracts were stored directly in a freezer in the extraction containers. Freezer-storage temperatures were monitored and typically were maintained at -20 ± 5 °C.

5E. Analytical Methods

The analytical phase was conducted at ABC Laboratories, Inc. (Columbia, MO). The Principal Analytical Investigator was Richard Schierhoff. Validated analytical methods were provided by the Sponsor to ABC Laboratories, Inc. Prior to analysis of field samples, the analytical methods were verified by ABC Laboratories, Inc. as part of this study.

Leaf and Flower Analysis

Leaf and flower samples were analyzed for thiamethoxam and CGA 322704 based on the analytical method described in Syngenta Method REM 179.06, entitled “Residue Method for the Determination of Residues of Thiamethoxam (CGA 293343) and CGA 322704 in Lettuce, Tomato, Grape and Tobacco

Samples. Final Determination by LC-MS/MS". In summary, residues of thiamethoxam and CGA322704 were extracted with 50:50 methanol/water from 10-g leaf and flower samples using a VirtiShear homogenizer. Extracts were centrifuged and concentrated via SPE cleanup in preparation for LC-MS/MS analysis. The Limit of Quantitation (LOQ) for both thiamethoxam and CGA322704, in leaf and flower matrices, was 10.0 ppb. The Limit of Detection (LOD) was targeted to be 5 ppb in thiamethoxam and CGA322704.

Pollen and Nectar Analysis

Pollen and nectar samples were analyzed for thiamethoxam and CGA322704 based on the analytical method described in Syngenta Method REM 179.07, entitled "Thiamethoxam: Analytical Method for the Determination of Residues of Thiamethoxam (CGA 293343) and CGA 322704 in Bee and Hive Products. Final Determination by LC-MS/MS". The method is presented in APPENDIX 3. In summary, residues of thiamethoxam and CGA322704 were extracted with water from 0.05 g pollen and nectar samples. The extraction was conducted with a VirtiShear homogenizer, and extracts were subsequently centrifuged and passed through a solid-phase extraction cleanup in preparation for LC-MS/MS analysis. The Limit of Quantitation (LOQ) for both thiamethoxam and CGA322704, in pollen and nectar matrices, was 1.00 ppb. The Limit of Detection (LOD) was targeted to be 0.5 ppb in thiamethoxam and CGA322704.

The LOQs and LODs are summarized in the table below.

Summary of LOQs and LODs

Matrix	Analyte	LOQ (ppb, parent equivalents)	LOD (ppb, parent equivalents)
Cucumber Leaves and Flowers	Thiamethoxam and CGA322704	10	5.0
Cucumber Pollen and Nectar	Thiamethoxam and CGA322704	1.0	0.5

5F. Quality Assurance Results

For each matrix, at least one method-recovery (QC) sample per analytical set was prepared by fortifying an untreated control sample with thiamethoxam and CGA322704 at concentrations equal to the method LOQ or 10xLOQ . These samples were then analyzed concurrently with the treated field samples to demonstrate adequate method performance throughout the study, i.e. recoveries of 70-120%.

Syngenta Methods REM179.06 and REM179.07 were verified successfully at ABC Laboratories prior to the analysis of field samples. Mean percent recoveries fell within the acceptable range of 70 - 120% with relative standard deviations <20% between the three replicate analyses (n = 3).

6. RESULTS:

No residues >LOD were found in any untreated plant matrix, excluding a nectar sample from Trial Site 3. Residues greater than the respective LOQs were found in all plant matrices sampled from the treated cucumber plots. The residues in nectar ranged from 1.26 ppb to 11.48 ppb and from 1.29 ppb to 8.22 ppb in pollen.

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Table 3. Residues of Thiamethoxam and CGA322704 for Nectar, Pollen, Flowers and Leaves of Cucumber

Site #	Treatment	Matrix	Residue Concentraion	
			Thiamethoxam	CGA322704
1	TRT1	Nectar	7.77	1.18
		Pollen	2.81	0.81
		Female Flower	11.24	2.12
		Male Flower	13.25	1.50
		Leaf	60.27	10.52
	TRT2	Nectar	7.77	1.61
		Pollen	7.98	2.09
		Female Flower	16.97	1.72
		Male Flower	24.20	4.46
		Leaf	136.97	24.24
	TRT3	Nectar	11.48	1.75
		Pollen	2.45	0.94
		Female Flower	26.54	7.07
		Male Flower	9.84	2.24
		Leaf	110.25	22.65
2	TRT1	Nectar	6.41	1.14
		Pollen	4.67	1.22
		Female Flower	14.86	0.00
		Male Flower	13.33	2.22
		Leaf	59.83	10.41
	TRT2	Nectar	9.25	1.65
		Pollen	4.10	1.71
		Female Flower	21.57	6.27
		Male Flower	14.03	1.78
		Leaf	61.43	12.24
	TRT3	Nectar	7.50	1.04
		Pollen	8.22	2.58
		Female Flower	23.65	2.55
		Male Flower	17.73	2.68
		Leaf	87.68	16.69
3	TRT1	Nectar	1.26	0.00
		Pollen	1.29	0.23
		Female Flower	3.29	0.00
		Male Flower	3.77	0.00
		Leaf	20.59	0.00
	TRT2	Nectar	1.31	0.00
		Pollen	3.86	0.52
		Female Flower	3.56	0.00
		Male Flower	4.06	0.00
		Leaf	18.06	0.00

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Site #	Treatment	Matrix	Residue Concentraion	
			Thiamethoxam	CGA322704
	TRT3	Nectar	1.51	0.00
		Pollen	5.18	0.54
		Female Flower	4.80	0.00
		Male Flower	5.21	0.50
		Leaf	25.53	0.00

7. STUDY VALIDITY/CLASSIFICATION AND STUDY LIMITATIONS

Classification/Utility for Bee Risk Assessment. This study is classified as acceptable. It provides a snapshot of Thiamethoxam and CGA322704 residues in leaves, flowers, pollen, and nectar during bloom. The residue values presented should be considered to be fully reliable. However, it is important to note that it is unclear if concentrations were increasing or decreasing at the time the samples were collected.

Temporal Variability in Residues. This study was not designed for temporal analysis of declining concentrations, but rather, to provide a snapshot of residue concentrations during flowering. Only one sample of each matrix was collected and analyzed from each plot so there is no way to know if concentrations were increasing or decreasing.

Spatial Variability in Residues. Two sites were in Fresno County, in the San Joaquin Valley, and one site was in San Luis Obispo County, in the Central Coast region. Climatic conditions were similar, except that in Fresno County locations summer air temperatures were approximately 20°F warmer than the San Luis Obispo County location. The locations in Fresno County (Trial Sites 1 and 2) both had sandy loam soil but one of the sites had slightly coarser soil with more sand and less clay than the other site. The San Luis Obispo County had fine soil that was classified as clay loam. Residue concentrations were higher in the Fresno County sites than the San Luis Obispo County sites.

Pesticide Carryover. The extent to which prior year applications of imidacloprid contributed to year-to-year carryover was not a part of the study design. Therefore, the effects of pesticide carryover in cucumber are unknown.

Table 4. Magnitude of Thiamethoxam and CGA322704 residues in leaves, flowers, pollen, and nectar (Trial Sites 1 and 2 = Fresno County; Trial Site 3 = San Luis Obispo County).

Trial Site	Plant Matrix	Thiamethoxam Concentration (ppb)			CGA322704 Concentration (ppb)		
		Mean Residue	Standard Deviation	Maximum Residue	Mean Residue	Standard Deviation	Maximum Residue
1	Leaf	102.5	38.9	137.0	22.4	8.8	28.3
	Female Flower	18.2	7.7	26.5	4.2	3.5	8.3
	Male Flower	15.8	7.5	24.2	3.2	1.8	5.2
	Nectar	9.0	2.1	11.5	1.8	0.3	2.0
	Pollen	4.4	3.1	8.0	1.5	0.8	2.4

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Trial Site	Plant Matrix	Thiamethoxam Concentration (ppb)			CGA322704 Concentration (ppb)		
		Mean Residue	Standard Deviation	Maximum Residue	Mean Residue	Standard Deviation	Maximum Residue
2	Leaf	69.6	15.6	87.7	15.3	3.8	19.5
	Female Flower	20.0	4.6	23.7	3.4	3.7	7.3
	Male Flower	15.0	2.4	17.7	2.6	0.5	3.1
	Nectar	7.7	1.4	9.3	1.4	0.2	1.6
	Pollen	5.7	2.2	8.2	2.1	0.8	3.0
3	Leaf	21.4	3.8	25.5	0.0	0.0	0.0
	Female Flower	3.9	0.8	4.8	0.0	0.0	0.0
	Male Flower	4.3	0.8	5.2	0.2	0.3	0.6
	Nectar	1.4	0.1	1.5	0.0	0.0	0.0
	Pollen	3.4	2.0	5.2	0.5	0.2	0.6

8. STATISTICAL ANALYSIS

1. Sampling intervals between application and harvest of plant samples was nearly equal between the trial sites.

2. There were 3 sites where initially they were designated as coarse, medium, and fine-textured soils. Although the soil mapping unit indicated a loamy sand texture, the analysis of texture from soil sampled at the site indicated a lower sand content that was more aligned with a determination of medium texture (Table 2). Data were pooled from the medium-textured sites for comparison to data generated from the fine-textured site. Three trials were conducted within each site, providing 3 replicate values for each soil texture category. There was no true replication for effect of site. Results of statistical analysis for soil texture provides an indication of potential soil effects so conclusions are only tentative and require further testing to determine veracity.

3. Thiamethoxam parent residues were approximately 85% of the total residue measured for each plant sample (Table S-1).

4. The maximum concentrations for total thiamethoxam residues measured were 161 ppb in leaves, 13.2 ppb in nectar, and 11 ppb in pollen (Table S-1).

5. The box plots in Figure S-1 compare the range in total thiamethoxam residue concentration for the plant tissues. Concentrations in leaves were approximately 10x greater than in the other plant sample.

6. Comparison of the concentration of total thiamethoxam residue indicated a high correlation between concentrations in leaves and nectar and in leaves and female flowers where concentrations increased in direct response to increases in leaves (Figure S-2). The correlation was not as great between leaves and pollen, though there appeared to be a general positive relationship between concentrations measured in the male flowers and pollen (Figure S-3).

7. Tables S-2 through S-6 compare the distribution of total thiamethoxam residues measured between the samples from plants grown in medium and fine-textured soils. Figures S-4 though S-6 compare the distribution of concentrations for total thiamethoxam residue. A test of potential differences between the soil-texture categories was conducted using the Wilcoxon non-parametric rank sum test using the Exact option with the Monte Carlo procedure (Table S-5). A significant difference in the distribution was measured for all plant samples except for pollen. The pattern indicates that concentrations were higher in plants grown in medium-textured soils: Concentrations in leaves were approximated 5 times greater, approximately 7 times greater in nectar, approximately 6 times greater in female flowers, and approximately 4 times greater in male flowers from plants sampled in medium-textured soils compared to plants grown in the fine-texture soil site.

Conclusion: The pattern of application used in this study resulted in low values for the maximum concentration of total thiamethoxam residues in nectar and pollen of cucumber: Maximum concentration measured for total thiamethoxam residue was 13.2 ug/L in nectar and 10.8 ug/L in pollen. Differences in concentration due to soil texture measured where, except for pollen, the magnitude of concentrations measured in plant samples was at least 5 times greater in plants grown in medium-textured soils as compared to those at the fine-textured plot site.

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Table S-1. Statistical summary and distribution for concentration of thiamethoxam, CGA322704 degradant, and total residues measured in leaves, female flowers, nectar, male flowers, and pollen of cucumber plants.

Statistic	Leaves			Female Flowers			Nectar		
	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N	9	9	9	9	9	9	9	9	9
Mean	64.5	10.8	75.3	14.1	2.2	16.3	6.0	0.9	7.0
SD	41.1	9.4	50.4	8.9	2.7	11.2	3.8	0.7	5.0
CV (%)	63.8	87.5	70.0	63.3	123.9	69.2	62.6	79.4	64.6
Min	18.1	0.0	18.1	3.2	0.0	0.3	1.3	0.0	1.3
Median	60.3	10.5	70.8	14.9	1.7	14.9	7.5	1.1	8.5
75th	87.7	16.7	104.4	21.6	2.6	26.2	7.8	1.6	9.4
90th	137.0	24.2	161.2	26.5	7.1	33.6	11.5	1.8	13.2
95th	137.0	24.2	161.2	26.5	7.1	33.6	11.5	1.8	13.2
Max	137.0	24.2	161.2	26.5	7.1	33.6	11.5	1.8	13.2
% of Total	85.7	14.3		86.5	13.5		86.6	13.4	

Statistic	Male Flowers			Pollen		
	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N	9	9	9	9	9	9
Mean	11.7	1.7	13.4	4.5	1.2	5.7
SD	6.8	1.4	8.2	2.4	0.8	3.0
CV (%)	58.1	83.8	60.9	52.3	67.0	53.5
Min	3.8	0.0	3.8	1.3	0.2	1.5
Median	13.3	1.8	14.8	4.1	0.9	5.7
75th	14.0	2.2	15.8	5.2	1.7	5.9
90th	24.2	4.5	28.7	8.2	2.6	10.8
95th	24.2	4.5	28.7	8.2	2.6	10.8
Max	24.2	4.5	28.7	8.2	2.6	10.8
% of Total	87.3	12.7		79.3	20.7	

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Table S-2 Leaves: Statistical summary and distribution for concentration of thiamethoxam, CGA322704 degradant and total residues measured in leaves of cucumber plants grown in fine and medium-textured soil.

	Leaves					
	Medium Textured Soil			Fine Textured Soil		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N	6	6	6	3	3	3
Mean	86.1	16.1	102.2	21.4	0.0	21.4
SD	32.1	6.1	38.1	3.8	0.0	3.8
CV (%)	37.3	38.0	37.3	17.8	0.0	17.8
Min	59.8	10.4	70.2	18.1	0.0	18.1
Median	74.6	14.5	89.0	20.6	0.0	20.6
Max	137.0	24.2	161.2	25.5	0.0	25.5
% of Total	84.2	15.8		100.0	0.0	

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Table S-3 Nectar and female flowers: Statistical summary and distribution for concentration of thiamethoxam, CGA322704 degradant, and total residues measured in nectar and female flowers of cucumber plants grown in fine and medium-textured soil.

	Nectar					
	Medium Textured Soil			Fine Textured Soil		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N	6	6	6	3	3	3
Mean	8.4	1.4	9.8	1.4	0.0	1.4
SD	1.8	0.3	2.0	0.1	0.0	0.1
CV (%)	21.2	22.1	20.8	9.7	0.0	9.7
Min	6.4	1.0	7.6	1.3	0.0	1.3
Median	7.8	1.4	9.2	1.3	0.0	1.3
Max	11.5	1.8	13.2	1.5	0.0	1.5
% of Total	85.7	14.2		100.0	0.0	
	Female Flowers					
	Medium Textured Soil			Fine Textured Soil		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N	6	6	6	3	3	3
Mean	19.1	3.3	22.4	3.9	0.0	3.9
SD	5.8	2.8	8.0	0.8	0.0	0.8
CV (%)	30.2	84.2	35.8	20.7	0.0	20.7
Min	11.2	0.0	13.4	3.3	0.0	3.3
Median	19.3	2.3	22.5	3.6	0.0	3.6
Max	26.5	7.1	33.6	4.8	0.0	4.8
% of Total	85.2	14.7		100.0	0.0	

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Table S-4 Pollen and male flowers: Statistical summary and distribution for concentration of thiamethoxam, CGA322704 degradant, and total residues measured in nectar and female flowers of cucumber plants grown in fine and medium-textured soil

.

Statistic	Pollen					
	Medium Textured Soil			Fine Textured Soil		
	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N	6	6	6	3	3	3
Mean	5.0	1.6	6.6	3.4	0.4	3.9
SD	2.5	0.7	3.2	2.0	0.2	2.2
CV (%)	49.8	44.5	47.9	57.5	40.4	55.4
Min	2.5	0.8	3.4	1.3	0.2	1.5
Median	4.4	1.5	5.9	3.9	0.5	4.4
Max	8.2	2.6	10.8	5.2	0.5	5.7
% of Total	76.4	23.6		88.9	11.1	
Statistic	Male Flowers					
	Medium Textured Soil			Fine Textured Soil		
	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N	6	6	6	3	3	3
Mean	15.4	2.5	17.9	4.4	0.2	4.5
SD	5.0	1.1	5.93	0.8	0.3	1.0
CV (%)	32.4	42.4	33.2	17.5	173.0	23.2
Min	9.8	1.5	12.1	3.8	0.0	3.8
Median	13.7	2.2	15.7	4.1	0.0	4.1
Max	24.2	4.5	28.7	5.2	0.5	5.7
% of Total	86.1	13.9		96.5	3.8	

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Table S-5. Statistical comparison between concentration of total thiamethoxam residue between soil types measured in leaves, female flowers, nectar, male flowers, and pollen where C=coarse-textured, M=medium-textured, and F=fine-textured soil. Wilcoxon rank sum test is a nonparametric test for differences amongst the 3 categories with the test run using the Exact option. The T-test is a test for differences when there are two categories and it is run to provide guidance when the Wilcoxon test indicates a significant difference amongst the three soil categories.

Soil Comparison	Probability Level Wilcoxon Test				
	Leaves	Female Flowers	Nectar	Male Flowers	Pollen
Medium vs Fine Textured	0.022	0.024	0.024	0.021	0.26

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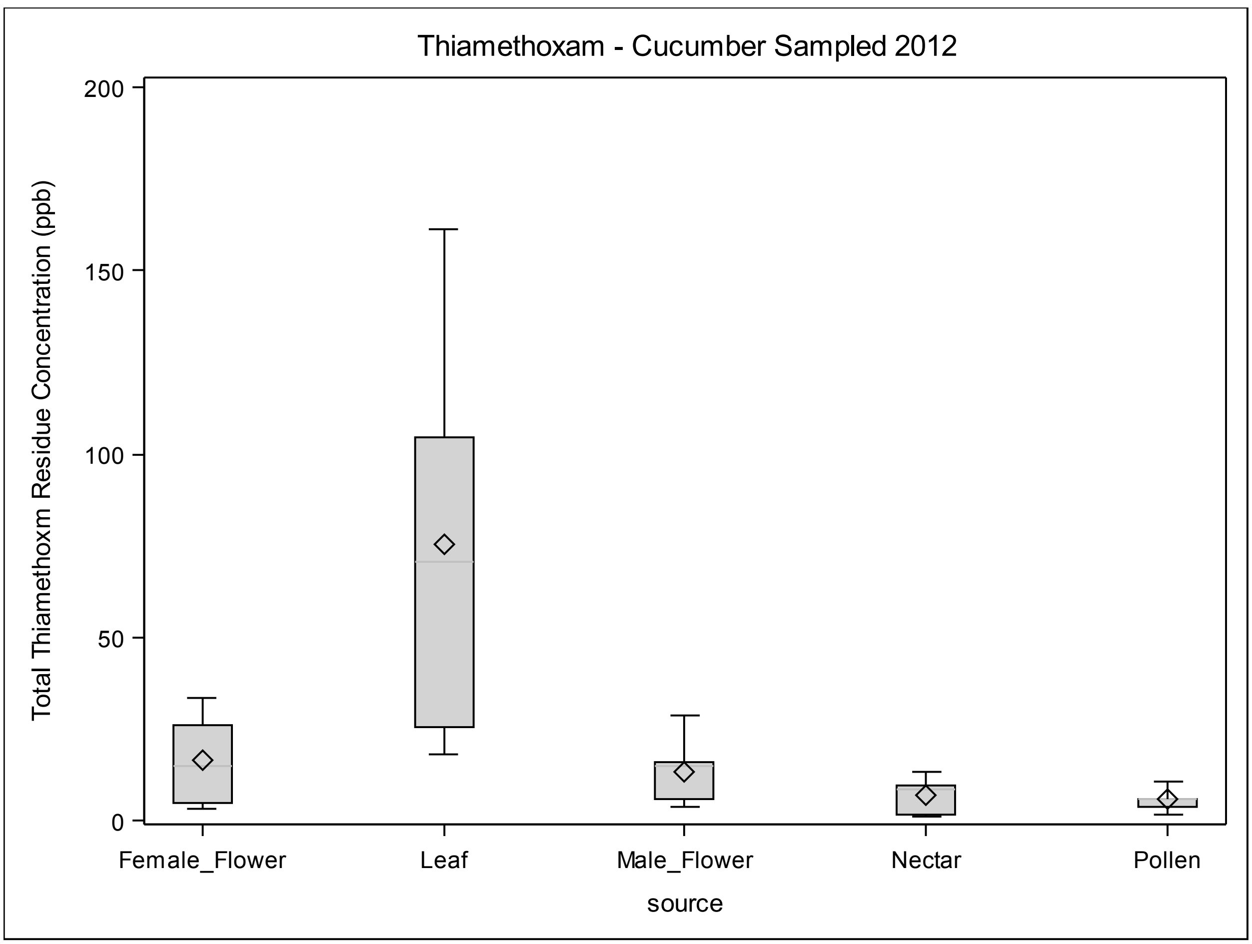
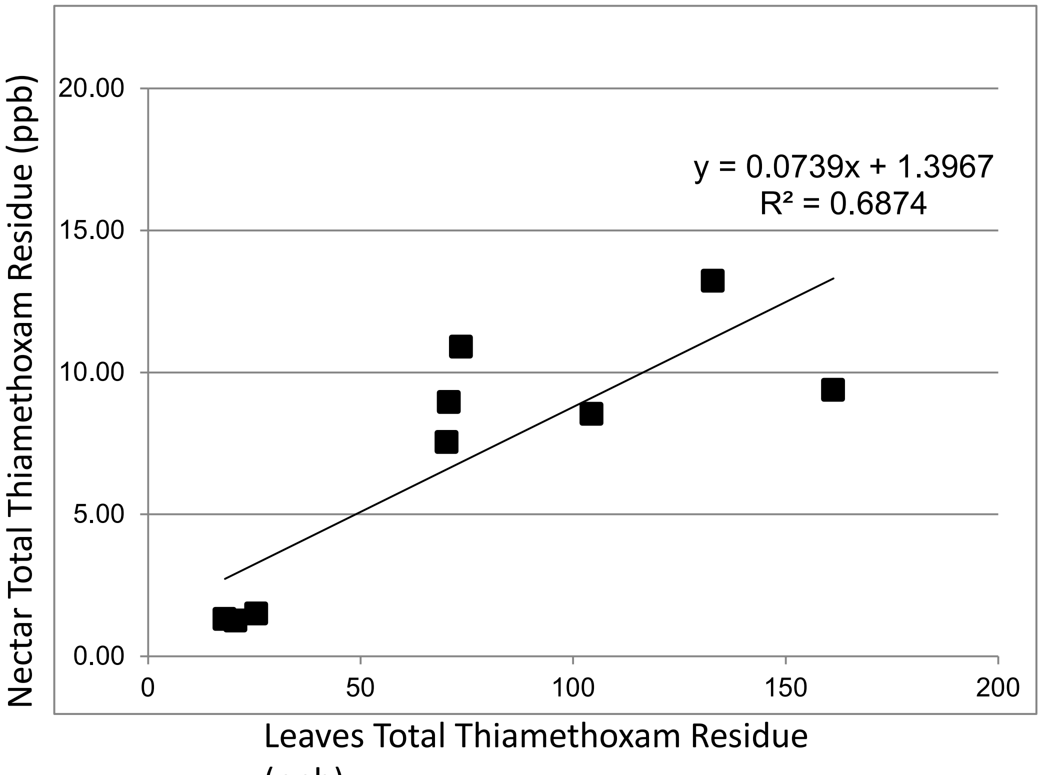
Figure S-1. Box plots comparing the distribution of concentration of total thiamethoxam residues in samples of plant tissue of cucumber.



MRID 49550801 CDPR THX Cucumber

Figure S-2. Relationship of total thiamethoxam residue measured A) in the leaves and nectar and B) in the female flowers and nectar.

A. Relationship between leaves and nectar



B. Relationship between female flowers and nectar.

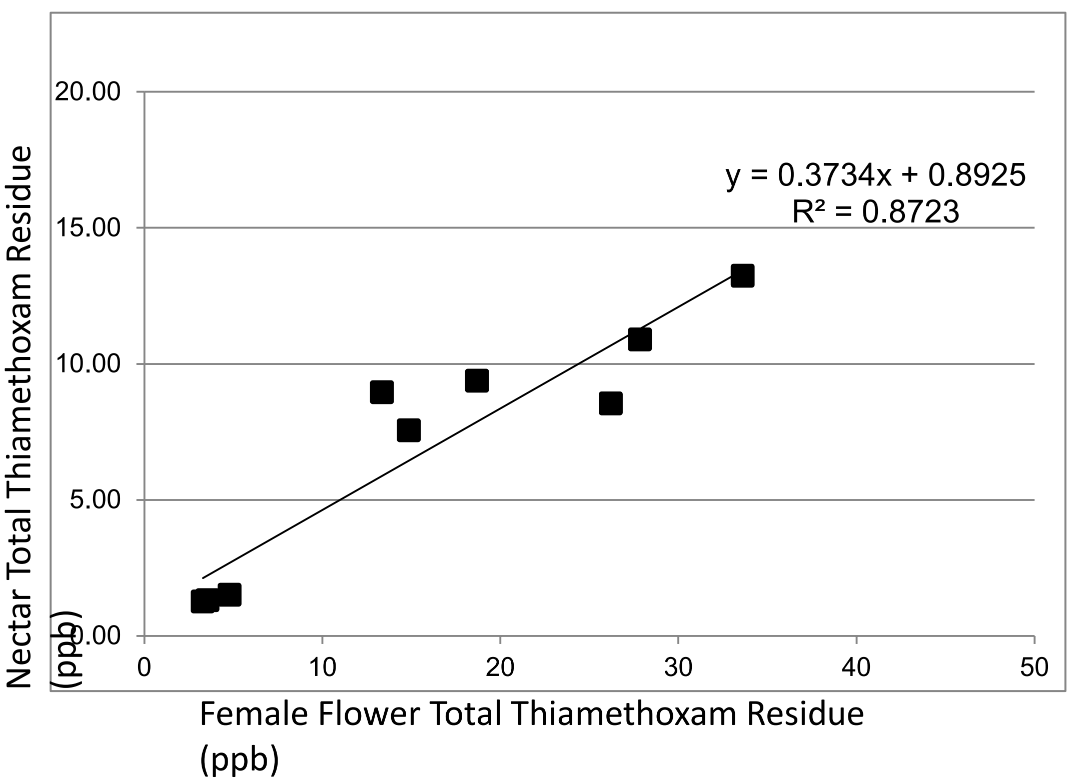
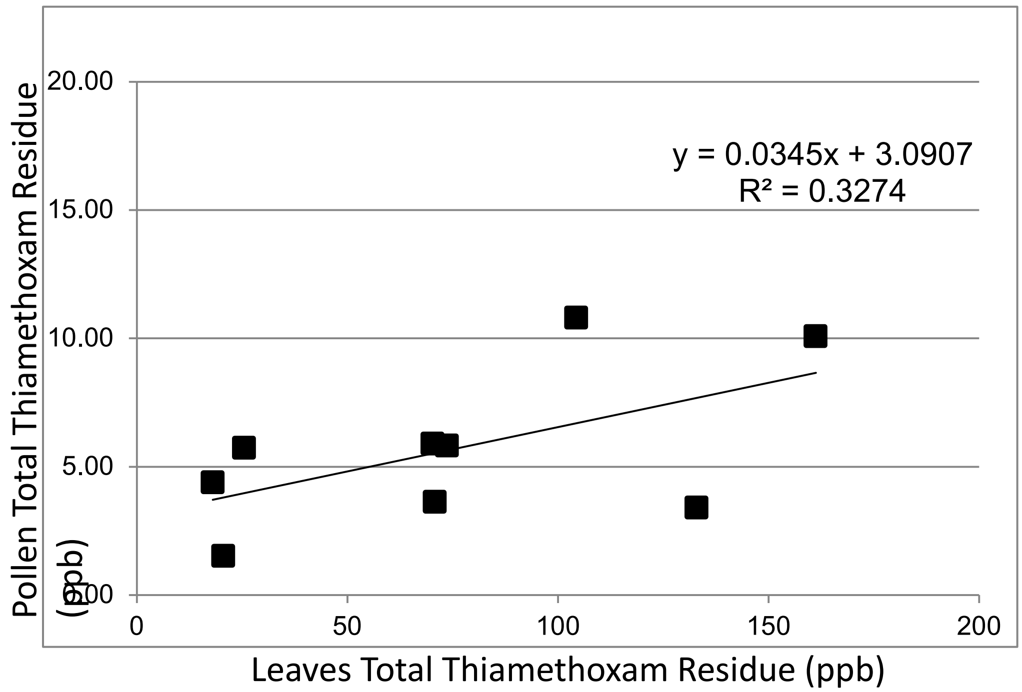
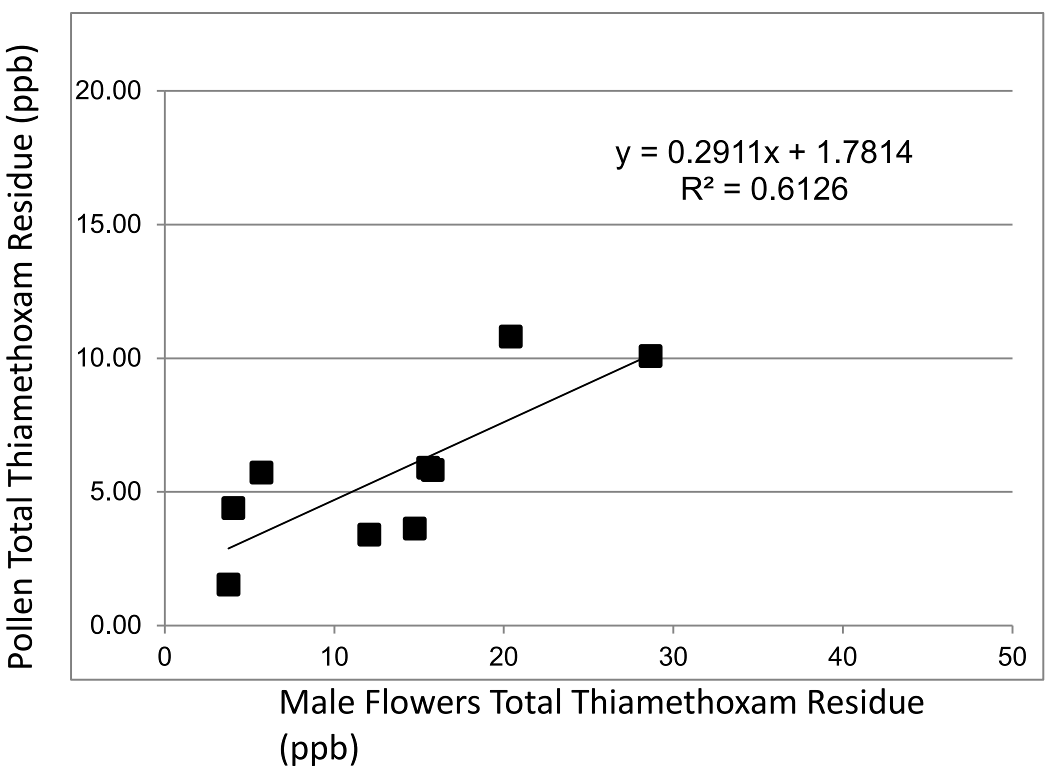


Figure S-3. Relationship of total thiamethoxam residue measured A) in the leaves and pollen and B) in the male flowers and pollen.

A. Relationship between leaves and pollen



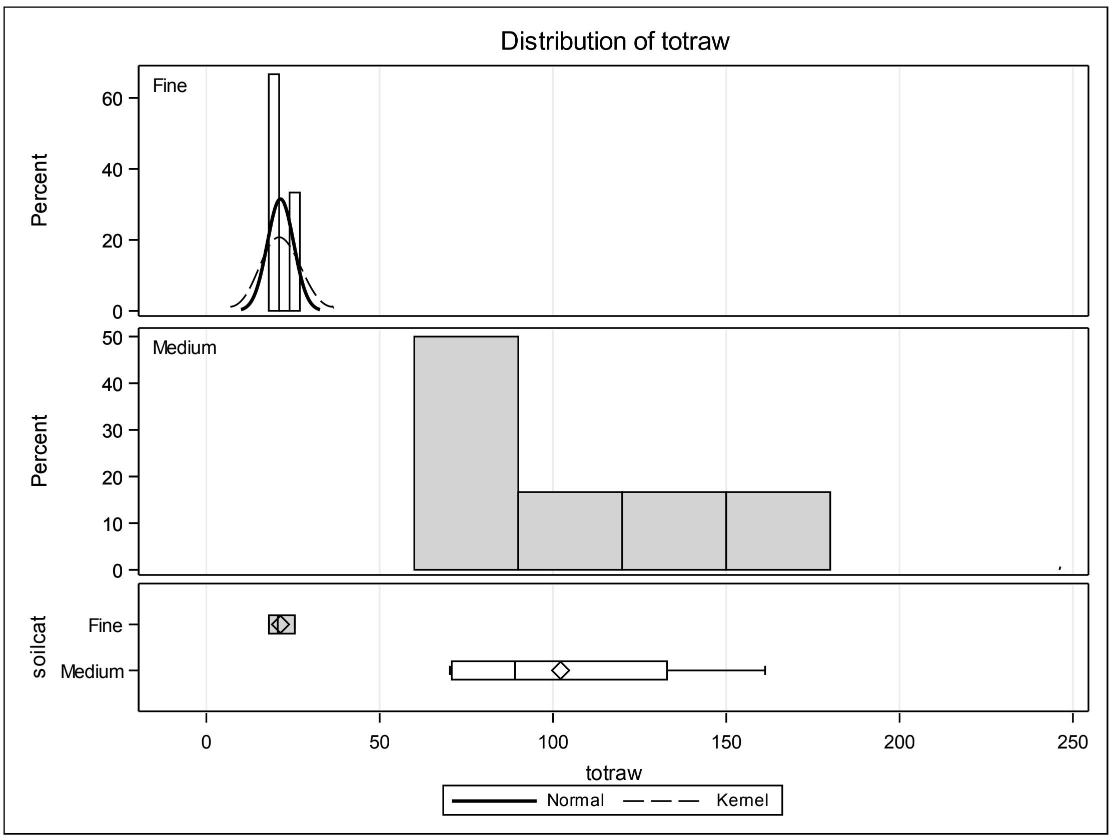
B. Relationship between male flowers and pollen.



MRID 49550801 CDPR THX Cucumber

Figure S-4 Leaves. Comparison of distribution of total thiamethoxam residue for medium, and fine-texture soils in leaves of cucumber.

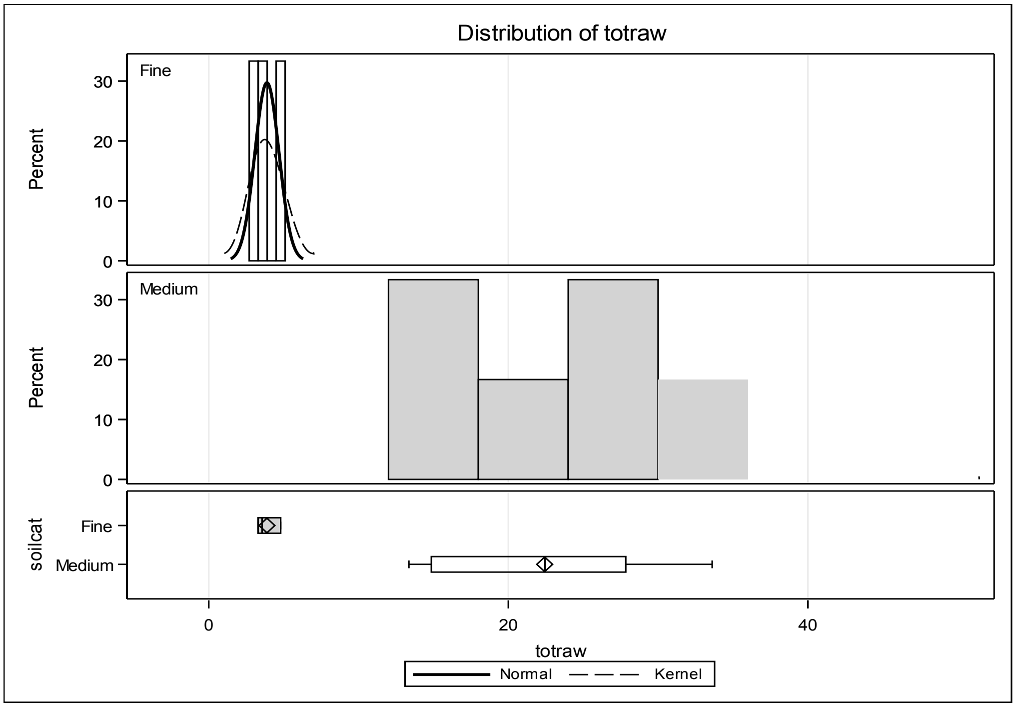
Cucumber Leaves



MRID 49550801 CDPR THX Cucumber

Figure S-5. Comparison of distribution of total thiamethoxam residue for medium, and fine-texture soils in female flowers and nectar of cucumber.

A. Female Flowers of Cucumber



B. Nectar

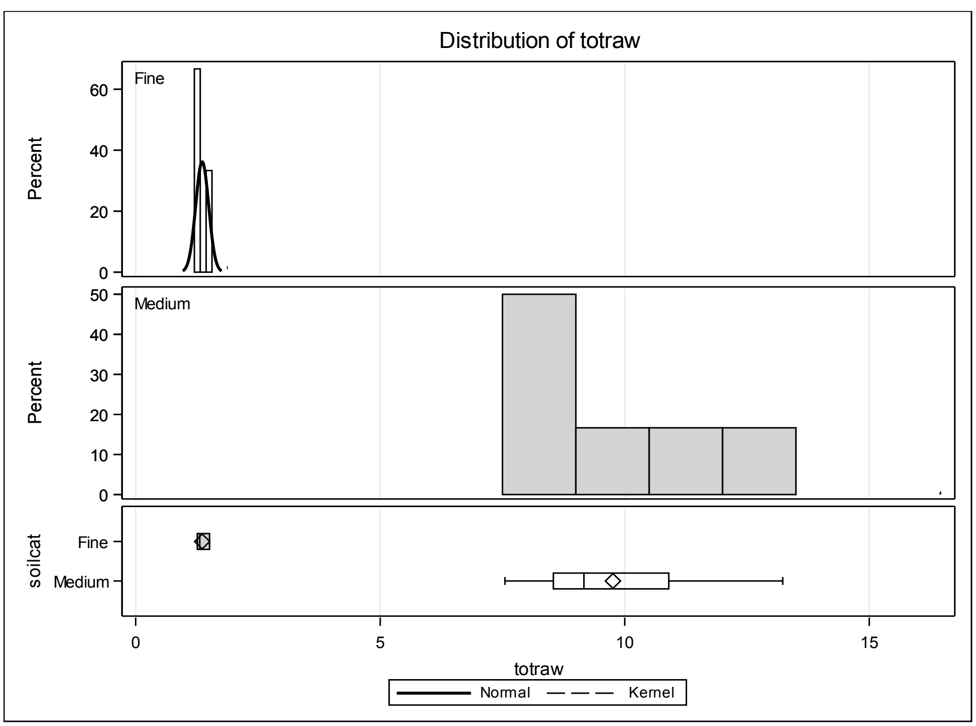
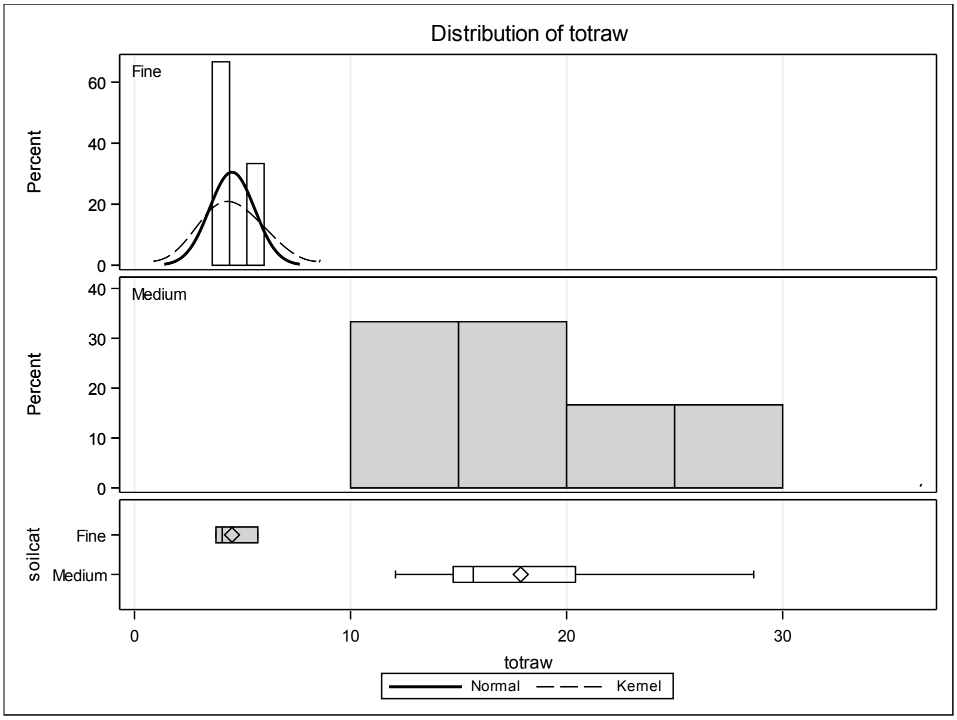
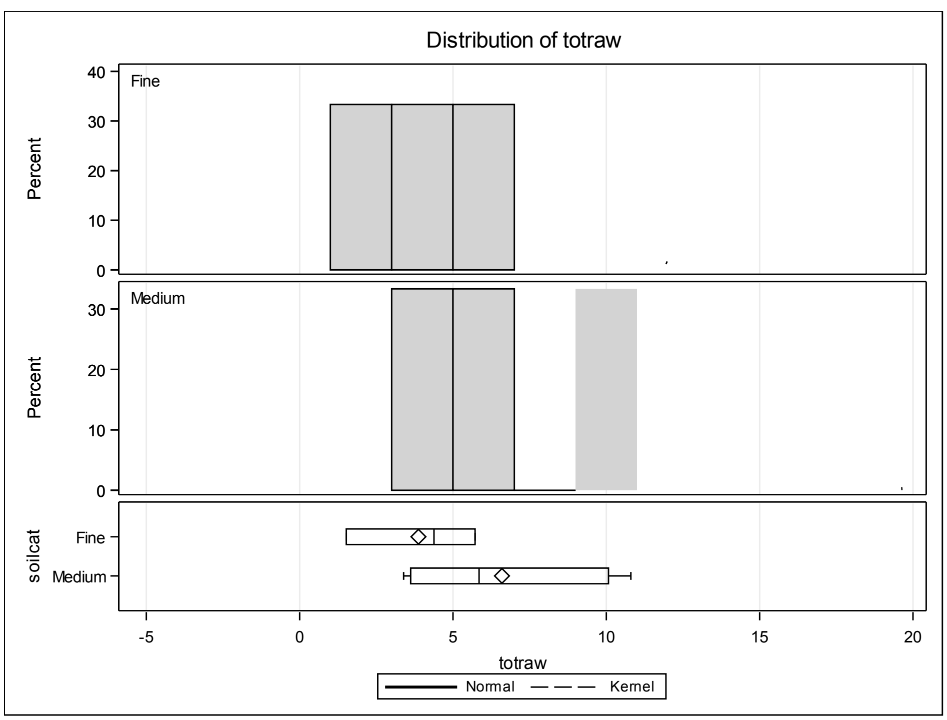


Figure S-6. Comparison of distribution of total thiamethoxam residue for medium, and fine-texture soils in male flowers and pollen of cucumber.

A. Male Flowers of Cucumber



B. Pollen



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5. Crook, S (2004) Residue Method for the Determination of Residues of Thiamethoxam (CGA293343) and CGA322704 in Lettuce, Tomato, Grape and Tobacco Samples. Final Determination by LC/MS/MS" (Syngenta Method REM179.06)

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MRID 50131102 CDPR THX Citrus

Reference
Lange,B., and Rice, F. (2016) Thiamethoxam 75SG (A9549C) – Magnitude of Residues in Leaves, Flowers, Pollen, and Nectar of Citrus After Soil Application with Platinum 75 SG in California: Final Report. Project Number: TK0177221. Unpublished study prepared by Syngenta Crop Protection, LLC. 337p. MRID 50131102, CDPR Study ID 297891, Data Volume 52691-0571, Tracking ID# 280248

1. STUDY INFORMATION

Chemical:	Thiamethoxam	**PC Code**	060109
Test Material:	Platinum 75 SG	**Percent Active Ingredient:**	42.8%
Study Type:	Field residue study on Citrus to establish thiamethoxam and metabolite levels in nectar, pollen, whole flowers and leaves in 9 trial site locations following two soil applications Platinum 75 SG in two successive years.		
Sponsor:	Syngenta Crop Protection, LLC 410 Swing Road Post Office Box 18300 Greensboro, North Carolina 27419-8300 USA	**Experiment Start and End Date:**	September 13, 2013 – July 16, 2015
Sponsor Study ID:	TK0177221	**Study Locations:**	Nine trial sites that included orange and lemon located in California.
Study Completion Date:	January 13, 2016		
GLP Status:	GLP; protocol reviewed by CDPR. [CDPR Study ID 297891, Data Volume 52691-0571, Tracking ID# 280248]		

2. REVIEWER INFORMATION

Study Reviewed by: California Department of Pesticide Regulation	Richard Bireley, Sr. Environmental Scientist (Specialist) John Troiano, Ph.D., Research Scientist III Alexander Kolosovich, Sr. Environmental Scientist Brigitte Tafarella, Environmental Scientist Denise Alder, Sr. Environmental Scientist (Specialist) Russell Darling, Sr. Environmental Scientist (Specialist)

3. EXECUTIVE SUMMARY

Nine (9) field trials were conducted in the United States for the purpose of quantifying residues of thiamethoxam and its major metabolite, CGA322704, in leaves, flowers, pollen, and nectar from citrus after soil treatment applications with Platinum® 75SG. This study was conducted and reported to satisfy data requested by the California Department of Pesticide Regulations (CDPR) as part of the re-evaluation of the nitro-guanidine class of neo-nicotinoid insecticides (Article 8, Subchapter 1, Chapter 2, Division 6 of Title 3 of the California Code of Regulations).

For this study, thiamethoxam (CGA293343), a 75% soluble granule formulation (75% w/w), was applied to commercial varieties/cultivars of citrus via soil directed spray application. At all field sites leaves, whole flower, pollen and nectar were collected 45 (± 10) days after the last application to fulfill early bloom sampling events, with the following exceptions. Year 2 samples for CA-3 were collected 59 days after the application. Year 1 samples for CA-7 were collected 30 days after the application. Year 2 samples were not collected from CA-7 and CA-8, so these trials were continued a third season, resulting in three annual applications for the "year 2" samples collected for these trials. Representative soil samples were collected to confirm soil-textural class.

Leaf, whole flower, pollen, and nectar samples were analyzed by validated methods using HPLC-MS/MS for thiamethoxam and CGA322704 (see Sections 2.4.4.1 and 2.4.4.2). Performance of analytical methods were verified using control (UTC) samples of leaf, whole flower, pollen, and nectar prior to any analysis of field samples by analyzing one UTC and three UTC samples fortified with thiamethoxam and CGA322704 at the respective LOQs and at 10xLOQs. The Limit of Quantitation (LOQ) was 1 ppb for both analytes in leaves, flowers, and pollen. The LOQ was 0.5 ppb for both analytes in nectar.

All matrices were analyzed for thiamethoxam and CGA322704 within 435 days (14 months) after sample collection. Residues have been shown to be stable for at least 24 months in a range of crops.

Individual recoveries from analyte-fortified samples that were analyzed concurrently with field samples ranged between 69.5 to 112% for thiamethoxam and 76.4 to 115% for CGA322704. The mean recovery of each matrix was within 70 to 120%, and the relative standard deviations (RSD) were < 20%, indicating acceptable performance of the analytical method during the conduct of this study.

4. STUDY VALIDITY

Guideline Followed:	Protocol was reviewed by CDPR
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	**ACCEPTABLE**
Rationale:	N/A
Reparability:	N/A

5. MATERIALS AND METHODS

Test Material Characterization			
Test item:	Platinum 75 SG	**Percent Active Ingredient:**	75% w.w A.I.
Description:	Soluble granule	**Design Code:**	A9549C
CAS #:	153719-23-4	**Molecular Weight:**	291.71

5A. Study Design

The study included nine geographically separated replicated trials that each consisted of a non-treated and a treated plot large enough to fulfil sample collection requirements. The treated plots were divided into 3 replicate sub-plots (A, B, and C). The size of each sub-plot varied at each location, measuring 100 ft x 21 ft (CA-1), 90 ft x 21 ft (CA-2), 60 ft x 28 ft (CA-3), 55 ft x 22 ft (CA-4), 110 ft x 20 ft (CA-5), 100 ft x 20 ft (CA-6), 105 ft x 20 ft (CA-7 and CA-8), and 50 ft x 20 ft (CA-9). At each location, the control plot was located up-slope and up-wind with regard to the prevailing wind direction and separated by a minimum of 200 ft. (exception CA-2, 125 ft) from the treated plot to minimize potential cross-contamination by runoff or pollen transfer.

5B. APPLICATION TIMING AND RATES

All trial locations established plots for a non-treated control plot (01) and a treated plot (02). The treatment list is presented in table 1 below.

Table 1. Application Rates and Timing

Treatment List								
Treatment ID (Plot)	**Application Number**	**Year**	**End-Use Product**	**Application Method**	**Volume (GPA)**	**Nominal Rate (lb ai/Acre) Per Application**	**Timing**	**Total Rate (lb ai/Acre) Per Year**
01	--	--	--	Control	--	--	--	--
02	1	1	Platinum 75 SG A9549C	Soil Directed Spray	>50	0.172 lb ai/Acre	45 (±10 days) Before Bloom	0.172
02	2	2	Platinum 75 SG A9549C	Soil Directed Spray	>50	0.172 lb ai/Acre	45 (±10 days) Before Bloom	0.172
***02**	3	3	Platinum 75 SG A9549C	Soil Directed Spray	>50	0.172 lb ai/Acre	45 (±10 days) Before Bloom	0.172

Application Notes:
GPA=gallons per acre
*Trials CA-7 and CA-8 only, per amendment 6.

5C. Study Site Location and Characteristics

Table 2. Study Site Location and Characteristics

Trial (Field) Identification and Crop	Trial Location (City, State)	OM (%)	pH	CEC (meq/100 g soil)	% Sand	% Silt	% Clay	Soil Types	Rainfall (in)	Temperature Range (°F)
CA-1 (Valencia Orange)	Sanger, California	0.22	7.5	5.3	84	9	7	Loamy Sand	0 to 2.4	26 to 108
		0.13	7.5	3.3	88	7	5	Sand		
		Not Collected								
CA-2 (Valencia Orange)	Orange Cove, California	0.53	5.5	7.7	78	15	7	Loamy Sand	0 to 2.4	26 to 108
		0.31	6.6	9.5	78	13	9	Sandy Loam		
		Not Collected								
CA-3 (Lisbon Lemon)	San Luis Obispo, California	3.1	7.4	24.8	46	26	28	Sandy Clay Loam	0 to 5.29	29 to 100
		1.4	7.4	24.3	48	26	26	Sandy Clay Loam		
		0.76	7.5	18.5	66	16	18	Sandy Loam		
CA-4 (Valencia Orange)	San Luis Obispo, California	1.8	7.4	28.4	42	26	32	Clay Loam	0 to 5.29	29 to 100
		1.3	7.6	32.7	34	30	36	Clay Loam		
		1.3	7.7	33.1	42	24	34	Clay Loam		
CA-5 (Valencia Orange)	Navelencia, California	1.10	7.8	9.1	77	13	10	Sandy Loam	0 to 2.15	27.3 to 105
		0.40	7.6	7.7	81	9	10	Loamy Sand		
		0.48	7.5	16.8	51	23	26	Sandy Clay Loam		
CA-6 (Valencia Orange)	Orange Cove, California	1.01	6.7	14.8	59	25	16	Sandy Loam	0 to 2.15	27.3 to 105
		0.57	6.5	16.7	53	27	20	Sandy Loam		
		0.62	6.6	17.4	55	27	18	Sandy Loam		
CA-7 (Pryor Lemon)	Porterville, California	2.3	7.5	31.2	46	19	35	Sandy Clay Loam	0 to 3.12	21.5 to 108
		0.94	7.6	32.7	44	19	37	Clay Loam		
		0.59	7.9	31.4	44	19	37	Clay Loam		
CA-8 (Lisbon Lemon)	Porterville, California	1.8	7.3	34.8	34	23	43	Clay	0 to 3.12	21.5 to 108
		1.02	7.7	35.9	30	23	47	Clay		
		0.54	7.7	37.3	32	23	45	Clay		
CA-9 (Valencia Orange)	Fresno, California	1.2	8.1	13.3	68	19	13	Sandy Loam	0 to 2.51	25.8 to 110
		1.04	8.0	15.9	66	19	15	Sandy Loam		
		Not Collected								

Precipitation and air temperature data summarized above are representative of the time period (whole months) from first application through final sample collection for each trial. Weather conditions did not negatively impact the crop growth or development. Irrigation was used to supplement rainfall at all trial locations and the data are provided in the Field Trial Summaries (Appendix 1 of the study report).

5D. SAMPLE COLLECTION, HANDLING, PROCESSING

For all trials, the non-treated control plots were sampled first or by different personnel to prevent contamination. For each matrix, one sample was collected from the control plot and each treated replicate plot A, B, and C.

At all field sites, leaves, whole flower, pollen and nectar were collected 45 (± 10) days after the last application to fulfill early bloom sampling, with the following exceptions: Year 2 samples for CA-3 were collected 59 days after the application; Year 1 samples for CA-7 were collected 30 days after the application; Year 2 samples were not collected from CA-7 and CA-8 due to low flowering so these trials were continued a third season.

Leaf and Whole Flower

Target weights of 250 g for leaves and flowers were collected, except for CA-3 where the Year 2 flower samples weighed between 45 to 70 g. Additionally, bulk, non-treated leaves and flowers with target weights of 500 g each were collected for laboratory verification and concurrent fortifications. Leaves and flowers were collected directly into labelled, sealable plastic bags and held in separate control and treatment ice chests on substitute ice until placed into frozen storage. Samples were collected from the lower-, middle- and upper-plant canopy for a representative, composite sample.

Pollen and Nectar

Flowers were collected from the untreated control and the treated sub-plots, bagged and placed in ice chests with substitute ice then transported to the field laboratory for pollen and nectar extraction. Pollen samples were extracted manually from flowers using a plastic filtered collection tip which was attached to a vacuum pump. The tips were weighed before and after pollen extraction and the net weight between the two represented the sample size. Once the target weight of 30 mg was obtained, or all flowers available for pollen sampling were used, the plastic tips containing pollen were wrapped in parafilm and placed in labeled plastic bottles. The bottles were sealed, placed in resealable plastic bags, and transferred immediately into separate freezers for treatment and control samples.

Nectar samples were collected manually. A glass microcapillary pipette was used to extract nectar from the inside base of the flower and then to transfer the nectar into a pre-weighed glass vial. Each vial was weighed before and after nectar extraction, with the net weight between the two representing the sample size. Once the target weight of 100 mg was obtained, or all flowers available for nectar sampling were used, the vials containing nectar were sealed in individual labeled plastic centrifuge tubes, then placed in resealable plastic bags and transferred immediately into separate freezers for treatment and control samples.

Target weights of 30 mg for pollen and 100 mg for nectar were collected, except for CA-3 where the Year 2 nectar and pollen samples did not meet size requirements.

Transit Stability

At trial CA-1 and CA-2, plant matrix samples from the field sites were pre-weighed at the laboratory. Homogenized control leaves and flower samples were used to prepare single control and triplicate treated samples of leaves and flowers for fortification at the field site. The pre-weighed leaves and flower samples and pre-measured vials of fortification solution were shipped to the field site. The contents of a vial were emptied into a bottle containing the homogenized sample, and then rinsed into the bottles with samples. The vials were then paced into the sample container. The target fortification level for all samples was 50X LOQ. The sample container was secured with electrical tape, and placed into an ice chest on substitute ice until placed into frozen storage.

Sample Storage.

All residue samples (leaf, whole flower, pollen, and nectar) were shipped from the test sites to EPL Bio Analytical Services via ACDS freezer truck.

Three separate storage-stability studies, MRID 44703525 (Reference 4), MRID 45108001 (Reference 5) and MRID 45659205 (Reference 6), were conducted to determine the stability of thiamethoxam and its metabolite, CGA322704, in various crop matrices stored under deep-freezer conditions. Storage stability for pollen and nectar stored under deep-freezer conditions was conducted in Syngenta Study No. 05-S508 (Reference 7). These studies showed that thiamethoxam and metabolite CGA322704 are stable in leaves, whole flower, pollen, and nectar for up to 24 months when stored frozen. Therefore, residues of thiamethoxam and CGA322704 in citrus leaf, whole flower, pollen, and nectar samples should not have been adversely affected by frozen storage during this study.

5E. ANALYTICAL METHODS

Leaves, whole flowers, pollen and nectar were analyzed for thiamethoxam and its metabolite CGA322704. Leaf samples were analyzed using EPL method 110G747D, entitled "Analytical Method for the Determination of Residues of Thiamethoxam and CGA322704 in Leaves by LC-MS/MS", which is an adaptation of Syngenta methods REM179.06 (Reference 1) and REM179.07 (Reference 2). Whole flower samples were analyzed using EPL method 110G747C, entitled "Analytical Method for the Determination of Residues of Thiamethoxam and CGA322704 in Flowers by LC-MS/MS", which is also an adaptation of Syngenta methods REM179.06 (Reference 1) and REM179.07 (Reference 2). Pollen samples were analyzed using EPL method 110G747B (and Revision 1), entitled "Analytical Method for the Determination of Residues of Thiamethoxam and CGA322704 in Pollen by LC-MS/MS," which is a revision of Syngenta method REM179.07 (Reference 2). Nectar samples were analyzed using EPL method 110G747A (and Revision 1), entitled "Analytical Method for the Determination of Residues of Thiamethoxam and CGA322704 in Nectar by LC-MS/MS," which is based on Syngenta method REM179.07 (Reference 2). The LOQ was 1 ppb, and the LOD was 0.5 ppb for both analytes in leaves, whole flowers and pollen. The LOQ was 0.5 ppb, and the LOD was 0.25 ppb for both analytes in nectar. The detailed analytical methods appear in appendix 2 of the study report.

Means, medians, standard deviations, and relative standard deviations were calculated using Microsoft Excel 2010 where appropriate.

Analysis of Leaf Samples

In summary, residues of thiamethoxam and CGA322704 were extracted with 50:50 methanol:0.2% formic acid from 0.1 g leaf samples. Extracts were centrifuged and aliquots were diluted with deionized (DI) water and stable isotope labelled internal standards were added. Sample extracts were then purified by solid-phase extraction (Oasis HLB) and analyzed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The LOQ for both analytes in leaves was 1.0 ppb and the LOD was 0.5 ppb.

Analysis of Whole Flower Samples

In summary, residues of thiamethoxam and CGA322704 were extracted with 50:50 methanol:0.2% formic acid from 0.1 g flower sub-samples. Extracts were centrifuged and aliquots were diluted with DI water, and stable isotope labelled internal standards were added. Sample extracts were then purified by solid-phase extraction (Oasis HLB) and analyzed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The LOQ for both analytes in whole flowers was 1.0 ppb and the LOD was 0.5 ppb.

Analysis of Pollen Samples

In summary, residues of thiamethoxam and CGA322704 were extracted with 50:50 methanol:0.2% formic acid from pollen samples. Pollen samples collected in the field were received in plastic pipette tips. To prepare these samples for extraction, the pipette tips containing pollen were cut into 3 pieces, and as much of the pollen as possible was transferred to a 50 mL plastic centrifuge tube. The filters used to contain the pollen in the pipette tips were also placed in the centrifuge tube, followed by the pipette tips. The extraction solvent was added to the tube which was then mixed with a vortexing apparatus to achieve extraction of the analytes. Weights of each pollen sample were recorded in the field and provided to EPL. The weights were needed to determine the residue concentrations and to determine the final volume of solvent needed following the solid phase extraction cleanup. Extracts were centrifuged and aliquots were diluted with DI water, and stable isotope labelled internal standards were added. Sample extracts were then purified by solid-phase extraction (Oasis HLB) and analyzed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The LOQ for both analytes in pollen was 1.0 ppb and the LOD was 0.5 ppb.

Analysis of Nectar Samples

In summary, residues of thiamethoxam and CGA322704 were extracted with 50:50 methanol:0.2% formic acid from 0.1 g nectar samples. Extracts were centrifuged and aliquots were diluted with DI water. Sample extracts were then purified by solid-phase extraction (Oasis HLB) and analyzed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The LOQ for both analytes in nectar was 0.5 ppb and the LOD was 0.25 ppb.

Table 3. Summary of LOQs and LODs

Matrix	Analyte	LOQ (ppb, parent equivalents)	LOD (ppb, parent equivalents)
Lemon and Orange Leaves	Thiamethoxam	1.0	0.5
	CGA322704	1.0	0.5
Lemon and Orange Pollen	Thiamethoxam	1.0	0.5
	CGA322704	1.0	0.5
Lemon and Orange Nectar	Thiamethoxam	0.5	0.25
	CGA322704	0.5	0.25
Lemon and Orange Whole Flower	Thiamethoxam	1.0	0.5
	CGA322704	1.0	0.5

5F. QUALITY ASSURANCE RESULTS

Method verification was performed on fortified leaf, whole flower, pollen, and nectar samples prior to the analysis of field collected samples. Triplicate fortifications at the LOQ and 10x LOQ concentrations were made for each analyte in each plant matrix. With only one exception, individual recoveries fell within the range of 70-120%. A single whole flower LOQ fortification yielded a CGA322704 recovery of 64.6%. Mean recoveries at each fortification level fell within the range of 70-120% for both analytes in all four plant matrices. Relative standard deviations (RSD) at each level were less than 20% for both analytes in all four plant matrices.

For each matrix, two concurrent recovery samples per analytical set were prepared by fortifying an untreated control sample with thiamethoxam and CGA322704 at concentrations samples to demonstrate acceptable method performance throughout the study. Mean concurrent method recoveries in the range of 70-120% were used to confirm analytical method performance. With a single exception, individual recoveries from analyte-fortified samples that were analyzed concurrently with field collected samples fell within the range of 70-120% for all matrices. One pollen LOQ fortification yielded a recovery of 69.5% for thiamethoxam. All mean recoveries fell within the range of 70-120%, and all RSD values were less than 20% for both analytes in all plant matrices.

An Agilent 1290 HPLC system with an AB Sciex Triple Quad 6500 mass spectrometer detector was used for the separation and quantitation of thiamethoxam and CGA322704. For the quantitation of the analytes of interest, standard curves were prepared by injecting constant volumes of standard solutions ranging in concentration from 0.004 - 1.0 ng/mL. Constant volume injections were used for sample extracts as well. A calibration standard typically was injected every 2-5 sample injections. Linear regression with 1/x weighting was used.

6. RESULTS:

Table 4. Thiamethoxam Residue Data from Orange and Lemon Trees

Trial Identification	Location (City, State)	Crop and Variety	Year	Treatment Code	Thiamethoxam Residue (ppb)	CGA322704 Residues (ppb)
Citrus Leaves						
CA-1	Sanger, California	Orange	1	UTC	<LOD	<LOD
				TRT A	58.8	42.2
				TRT B	84.9	75.6
				TRT C	17.0	9.70
CA-2	Orange Cove, California	Orange	1	UTC	<LOD	<LOD
				TRT A	14.0	5.83
				TRT B	33.9	16.1
				TRT C	15.6	10.3
CA-3	San Luis Obispo, California	Lemon	1	UTC	<LOD	<LOQ
				TRT A	3.69	1.07
				TRT B	2.36	<LOQ
				TRT C	2.72	<LOQ
CA-4	San Luis Obispo, California	Orange	1	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOQ	<LOD
				TRT C	<LOQ	<LOD
CA-5	Navelencia, California	Orange	1	UTC	<LOD	<LOD
				TRT A	2.43	2.16
				TRT B	3.02	2.23
				TRT C	1.29	1.44
CA-6	Orange Cove, California	Orange	1	UTC	<LOD	<LOD
				TRT A	2.95	2.28
				TRT B	3.10	2.72
				TRT C	3.14	2.63
CA-7	Porterville, California	Lemon	1	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOD	<LOD
CA-8	Porterville, California	Lemon	1	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOD	<LOD
				TRT C	1.10	<LOD

Trial Identification	Location (City, State)	Crop and Variety	Year	Treatment Code	Thiamethoxam Residue (ppb)	CGA322704 Residues (ppb)
CA-9	Fresno, California	Orange	1	UTC	<LOD	<LOD
				TRT A	<LOQ	<LOD
				TRT B	<LOD	<LOD
				TRT C	1.07	<LOQ
CA-1	Sanger, California	Orange	2	UTC	<LOD	<LOD
				TRT A	67.3	47.1
				TRT B	69.4	47.8
				TRT C	40.5	29.1
CA-2	Orange Cove, California	Orange	2	UTC	<LOD	<LOD
				TRT A	31.2	33.2
				TRT B	30.8	21.0
				TRT C	15.3	20.6
CA-3	San Luis Obispo, California	Lemon	2	UTC	<LOD	<LOD
				TRT A	14.5	20.2
				TRT B	5.11	2.11
				TRT C	6.49	3.38
CA-4	San Luis Obispo, California	Orange	2	UTC	<LOD	<LOD
				TRT A	<LOQ	<LOQ
				TRT B	1.56	1.07
				TRT C	1.07	<LOQ
CA-5	Navelencia, California	Orange	2	UTC	<LOD	<LOD
				TRT A	1.37	2.33
				TRT B	2.21	3.15
				TRT C	<LOQ	<LOQ
CA-6	Orange Cove, California	Orange	2	UTC	<LOD	<LOD
				TRT A	3.46	2.82
				TRT B	2.48	2.23
				TRT C	3.46	2.95
CA-7	Porterville, California	Lemon	2	UTC	<LOD	<LOD
				TRT A	<LOD	1.07
				TRT B	<LOD	<LOQ
				TRT C	<LOQ	<LOQ
CA-8	Porterville, California	Lemon	2	UTC	<LOQ	1.06
				TRT A	2.43	1.71
				TRT B	2.58	1.37

Trial Identification	Location (City, State)	Crop and Variety	Year	Treatment Code	Thiamethoxam Residue (ppb)	CGA322704 Residues (ppb)
				TRT C	2.32	1.93
CA-9	Fresno, California	Orange	2	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOD	<LOD
Citrus Whole Flower						
CA-1	Sanger, California	Orange	1	UTC	<LOD	<LOD
				TRT A	49.0	<LOD
				TRT B	16.7	10.6
				TRT C	31.4	4.02
CA-2	Orange Cove, California	Orange	1	UTC	<LOD	5.84
				TRT A	10.3	<LOD
				TRT B	14.9	1.74
				TRT C	12.9	3.09
CA-3	San Luis Obispo, California	Lemon	1	UTC	<LOD	2.58
				TRT A	<LOQ	<LOD
				TRT B	<LOQ	<LOD
				TRT C	<LOD	<LOD
CA-4	San Luis Obispo, California	Orange	1	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOD	<LOD
CA-5	Navelencia, California	Orange	1	UTC	<LOD	<LOD
				TRT A	2.02	<LOQ
				TRT B	2.06	<LOQ
				TRT C	<LOQ	<LOD
CA-6	Orange Cove, California	Orange	1	UTC	<LOD	<LOD
				TRT A	2.10	<LOQ
				TRT B	1.86	<LOQ
				TRT C	2.23	<LOQ
CA-7	Porterville, California	Lemon	1	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOQ	<LOQ
				TRT C	<LOD	<LOQ
				UTC	<LOD	<LOD

Trial Identification	Location (City, State)	Crop and Variety	Year	Treatment Code	Thiamethoxam Residue (ppb)	CGA322704 Residues (ppb)
CA-8	Porterville, California	Lemon	1	TRT A	<LOD	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOD	<LOD
CA-9	Fresno, California	Orange	1	UTC	<LOD	<LOD
				TRT A	<LOQ	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOQ	<LOD
CA-1	Sanger, California	Orange	2	UTC	<LOD	<LOD
				TRT A	46.9	13.6
				TRT B	46.4	12.4
				TRT C	59.4	17.3
CA-2	Orange Cove, California	Orange	2	UTC	<LOD	<LOD
				TRT A	37.7	7.57
				TRT B	30.5	6.58
				TRT C	28.4	7.20
CA-3	San Luis Obispo, California	Lemon	2	UTC	<LOD	<LOD
				TRT A	2.04	<LOQ
				TRT B	1.65	<LOQ
				TRT C	2.16	<LOQ
CA-4	San Luis Obispo, California	Orange	2	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOQ	<LOQ
				TRT C	<LOQ	<LOD
CA-5	Navelencia, California	Orange	2	UTC	<LOD	<LOQ
				TRT A	2.08	<LOQ
				TRT B	1.91	<LOQ
				TRT C	1.03	<LOD
CA-6	Orange Cove, California	Orange	2	UTC	<LOD	<LOD
				TRT A	3.09	1.32
				TRT B	2.50	<LOQ
				TRT C	1.92	<LOQ
CA-7	Porterville, California	Lemon	2	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOQ	<LOD
				TRT C	<LOQ	<LOD

Trial Identification	Location (City, State)	Crop and Variety	Year	Treatment Code	Thiamethoxam Residue (ppb)	CGA322704 Residues (ppb)
CA-8	Porterville, California	Lemon	2	UTC	<LOD	<LOD
				TRT A	<LOQ	<LOD
				TRT B	1.57	<LOD
				TRT C	1.38	<LOD
CA-9	Fresno, California	Orange	2	UTC	<LOD	<LOD
				TRT A	1.64	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOQ	<LOD
Citrus Pollen						
CA-1	Sanger, California	Orange	1	UTC	2.34	<LOD
				TRT A	51.1	36.0
				TRT B	32.9	29.4
				TRT C	27.9	21.2
CA-2	Orange Cove, California	Orange	1	UTC	16.6	<LOD
				TRT A	36.0	17.9
				TRT B	56.9	16.4
				TRT C	3.95	<LOD
CA-3	San Luis Obispo, California	Lemon	1	UTC	3.12	1.05
				TRT A	11.2	<LOQ
				TRT B	1.48	<LOD
				TRT C	1.57	<LOD
CA-4	San Luis Obispo, California	Orange	1	UTC	153	<LOQ
				TRT A	51.8	<LOQ
				TRT B	7.80	<LOQ
				TRT C	13.3	<LOQ
CA-5	Navelencia, California	Orange	1	UTC	1.28	<LOD
				TRT A	3.79	2.68
				TRT B	3.02	2.49
				TRT C	1.69	1.88
CA-6	Orange Cove, California	Orange	1	UTC	<LOQ	<LOD
				TRT A	3.47	2.88
				TRT B	4.04	3.27
				TRT C	2.66	2.14
CA-7	Porterville,	Lemon	1	UTC	3.68	<LOD
				TRT A	1.55	<LOD

Trial Identification	Location (City, State)	Crop and Variety	Year	Treatment Code	Thiamethoxam Residue (ppb)	CGA322704 Residues (ppb)
	California			TRT B	1.59	<LOD
				TRT C	1.75	<LOD
CA-8	Porterville, California	Lemon	1	UTC	3.44	<LOD
				TRT A	5.34	<LOD
				TRT B	3.41	<LOD
				TRT C	4.18	<LOD
CA-9	Fresno, California	Orange	1	UTC	2.66	<LOD
				TRT A	6.66	1.3
				TRT B	2.96	<LOQ
				TRT C	<LOQ	1.36
CA-1	Sanger, California	Orange	2	UTC	49.7	<LOD
				TRT A	67.2	40.6
				TRT B	104	30.0
				TRT C	73.6	61.5
CA-2	Orange Cove, California	Orange	2	UTC	1.08	<LOD
				TRT A	2.20	<LOQ
				TRT B	1.24	<LOD
				TRT C	1.18	<LOD
CA-3	San Luis Obispo, California	Lemon	2	UTC	37.2	101
				TRT A	12.0	13.6
				TRT B	10.8	12.3
				TRT C	36.1	13.3
CA-4	San Luis Obispo, California	Orange	2	UTC	<LOQ	<LOD
				TRT A	1.83	<LOQ
				TRT B	2.07	1.62
				TRT C	2.64	1.14
CA-5	Navelencia, California	Orange	2	UTC	1.75	<LOD
				TRT A	21.7	3.20
				TRT B	5.18	3.59
				TRT C	21.5	1.39
CA-6	Orange Cove, California	Orange	2	UTC	10.1	<LOD
				TRT A	2.27	3.17
				TRT B	4.73	<LOQ
				TRT C	6.94	3.33
				UTC	1.35	<LOD

Trial Identification	Location (City, State)	Crop and Variety	Year	Treatment Code	Thiamethoxam Residue (ppb)	CGA322704 Residues (ppb)
CA-7	Porterville, California	Lemon	2	TRT A	1.66	<LOD
				TRT B	1.15	<LOD
				TRT C	1.25	6.53
CA-8	Porterville, California	Lemon	2	UTC	2.48	<LOD
				TRT A	44.5	10.3
				TRT B	3.14	1.15
				TRT C	2.22	2.28
CA-9	Fresno, California	Orange	2	UTC	1.07	<LOD
				TRT A	1.71	<LOQ
				TRT B	1.95	<LOQ
				TRT C	2.86	<LOQ
Citrus Nectar						
CA-1	Sanger, California	Orange	1	UTC	<LOD	<LOD
				TRT A	22.5	3.19
				TRT B	10.4	1.84
				TRT C	9.34	1.41
CA-2	Orange Cove, California	Orange	1	UTC	<LOD	<LOD
				TRT A	5.30	0.714
				TRT B	5.03	0.535
				TRT C	5.62	0.774
CA-3	San Luis Obispo, California	Lemon	1	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOD	<LOD
CA-4	San Luis Obispo, California	Orange	1	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOD	<LOD
CA-5	Navelencia, California	Orange	1	UTC	<LOD	<LOD
				TRT A	0.691	<LOQ
				TRT B	0.760	<LOQ
				TRT C	<LOQ	<LOQ
CA-6	Orange Cove,	Orange	1	UTC	<LOD	<LOD
				TRT A	1.87	0.776
				TRT B	0.952	<LOQ

Trial Identification	Location (City, State)	Crop and Variety	Year	Treatment Code	Thiamethoxam Residue (ppb)	CGA322704 Residues (ppb)
	California			TRT C	1.56	0.691
CA-7	Porterville, California	Lemon	1	UTC	0.504	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOD	<LOD
CA-8	Porterville, California	Lemon	1	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOD	<LOD
CA-9	Fresno, California	Orange	1	UTC	<LOD	<LOD
				TRT A	0.520	<LOQ
				TRT B	<LOD	<LOD
				TRT C	<LOQ	<LOD
CA-1	Sanger, California	Orange	2	UTC	0.549	<LOD
				TRT A	20.8	6.96
				TRT B	16.9	4.69
				TRT C	NA	NA
CA-2	Orange Cove, California	Orange	2	UTC	<LOD	<LOD
				TRT A	8.69	1.49
				TRT B	7.22	1.14
				TRT C	5.85	1.38
CA-3	San Luis Obispo, California	Lemon	2	UTC	<LOD	<LOD
				TRT A	NA	NA
				TRT B	<LOQ	0.893
				TRT C	<LOQ	<LOD
CA-4	San Luis Obispo, California	Orange	2	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOQ	<LOD
				TRT C	<LOD	<LOD
CA-5	Navelencia, California	Orange	2	UTC	<LOD	<LOD
				TRT A	0.923	<LOQ
				TRT B	0.584	<LOQ
				TRT C	<LOD	<LOD
CA-6	Orange	Orange	2	UTC	<LOD	<LOD
				TRT A	1.12	0.672

Trial Identification	Location (City, State)	Crop and Variety	Year	Treatment Code	Thiamethoxam Residue (ppb)	CGA322704 Residues (ppb)
	Cove, California			TRT B	0.574	<LOQ
				TRT C	0.679	<LOQ
CA-7	Porterville, California	Lemon	2	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOD	<LOD
CA-8	Porterville, California	Lemon	2	UTC	<LOD	<LOD
				TRT A	<LOD	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOD	<LOD
CA-9	Fresno, California	Orange	2	UTC	<LOD	<LOD
				TRT A	<LOQ	<LOD
				TRT B	<LOD	<LOD
				TRT C	<LOD	<LOD

Table 5. Statistics of Residues of Thiamethoxam and CGA322704 in Citrus Plant Leaves, Whole Flowers, Pollen and Nectar.

Total Rate (lb ai/Acre)	Matrix and Timing	Analyte	N	Minimum (ppb)	Maximum (ppb)	Median (ppb)	Mean (ppb)	Standard Deviation (ppb)
0.172	Year 1 Leaves	Thiamethoxam	27	<LOD	84.9	2.36	9.67	19.7
		CGA322704	27	<LOD	75.6	1.00	6.97	16.1
0.172	Year 2 Leaves	Thiamethoxam	27	<LOD	69.4	2.43	11.5	19.5
		CGA322704	27	<LOD	47.8	2.11	9.38	14.4
0.172	Year 1 Whole Flowers	Thiamethoxam	27	<LOD	49.0	1.00	5.95	11.1
		CGA322704	27	<LOD	10.6	1.00	1.81	2.10
0.172	Year 2 Whole Flowers	Thiamethoxam	27	<LOD	59.4	1.65	10.4	17.7
		CGA322704	27	<LOD	17.3	1.00	3.19	4.55
0.172	Year 1 Pollen	Thiamethoxam	27	<LOQ	56.9	3.95	12.7	17.4
		CGA322704	27	<LOD	36.0	1.00	5.66	9.59
0.172	Year 2 Pollen	Thiamethoxam	27	1.15	104	2.86	16.2	26.5
		CGA322704	27	<LOD	61.5	1.62	8.11	14.3

Total Rate (lb ai/Acre)	Matrix and Timing	Analyte	N	Minimum (ppb)	Maximum (ppb)	Median (ppb)	Mean (ppb)	Standard Deviation (ppb)
0.172	Year 1 Nectar	Thiamethoxam	27	<LOD	22.5	0.500	2.67	4.83
		CGA322704	27	<LOD	3.19	0.500	0.720	0.582
0.172	Year 2 Nectar	Thiamethoxam	25	<LOD	20.8	0.500	2.83	5.35
		CGA322704	25	<LOD	6.96	0.500	1.05	1.50

LOQ: Limit of Quntitation. 1 ppb for leaves, whole flowers and pollen, 0.5 ppb for nectar.
LOD: Limit of Detection. 0.5 ppb for leaves, whole flowers and pollen, 0.25 ppb for nectar.

7. Statistical Analysis

Study Objectives and Design

The study was conducted to determine the concentration of thiamethoxam and its degradation product CGA322704 in whole flowers, nectar, pollen, and leaves of citrus trees in response to soil application of a thiamethoxam pesticide product applied 45 days prior to bloom. The rate of application of Platinum 75CA9549C was 0.172 lbs a.i./Acre. At approximately 45 days after application flower parts and leaves of orange or lemon trees were harvested and analyzed for thiamethoxam and its degradation product. The crops received a similar second soil application in the next year and the plants were sampled again at bloom.

Non-parametric statistical tests were used to test for differences in distribution of concentrations between years and between soil type. Non-parametric tests do not require tests for normality as they are robust to differences in distribution and they are also robust for experimental designs with low replicates (Helsel and Hirsch, 2002). The PROC NPAR1WAY procedure in the Statistical Analysis System (SAS) statistical package was used to conduct Wilcoxon-Mann –Whitney (Wilcoxon), Median non-parametric, and Kuiper tests. A significant result from the Wilcoxon test indicates differences in the shape of distributions; A significant result from the Median test indicates differences in the location of the medians between distributions; and a significant result from the Kuiper test indicated differences in the empirical distributions between two groups. The Exact option for each statistic was implemented as it provides permutation testing, a statistical method that minimizes the effect of sample size and distributional differences. Using the Exact option the Monte Carlo procedure was also implemented which provided 10,000 separate runs for each statistic to produce the permutation distributions. The test for potential differences due to soil type had 3 levels so the DSCF option in PROC NPAR1WAY, which invokes the Dwass, Steel, Critchlow-Fligner multiple comparison test was used to provide pairwise tests for two-sample rankings. Additional procedures used for descriptive statistics were PROC MEANS to calculate mean values from the replicates at each site, PROC CAPACITY to produce cumulative statistics, and PROC BOX plot to produce comparative graphics. Statistical analysis for effect of years and soil type were conducted on the mean of the replicate samples taken from each site. Graphical comparisons are presented on data transformed to a natural logarithm scale, providing clearer contrasts between the distribution. Also, for statistical analyses, values noted as below the limit of quantification (LOQ) or limit of detection (LOD) were assigned half the value of the respective detection limit (Table 3). Distribution of concentrations in bee relevant plant matrices was calculated using all the raw data because these values represent the actual range of exposure to bees and other organisms that feed off the nectar and pollen of plants.

Detection rate noted for each plant matrix: Counts for the number of samples reported below the respective detection limit for each matrix are presented in Table 6. For untreated control plots, most analytical results were below the LOD except for measurements of thiamethoxam in pollen samples where approximately 90% of pollen samples were above the LOQ. For treated plants, the rate of detection was again highest for pollen samples where thiamethoxam was measured in 98% of samples. The rate of detection above the LOQ for thiamethoxam was 42, 54, and 66% of samples for nectar, whole flowers, and leaves, respectively. Rate of detection above the LOQ for the CGA322704 degradation product was lower than for parent thiamethoxam in treated plants at 56, 29, 24, and 59% for pollen, nectar, whole flowers, and leaves, respectively.

Comparison of distribution between years: Potential difference between years was measured for two reasons. First, greater concentrations measured in year 2 would indicate potential for carry-over effects between years. Second, if there was no effect of years then the data could be pooled for subsequent tests between soil types. The result for analyses conducted on plant matrices with sufficient detections indicated no significant difference in the distribution of concentration of residues between years, based on exact probability levels for a two-sided test (Table 7). Graphical comparisons between years also indicated significant overlap in the distribution of concentration for thiamethoxam residues between years in plant samples taken at bloom (Figure 1). The result for thiamethoxam and CGA322704 residues indicated that the data for both years could be combined in further tests for comparison between untreated and treated plants, and for effect of soil type.

Comparison of distribution between untreated and treated plants: Non-parametric tests indicated significantly greater range in the distributions for treated plants for all matrices, except for parent thiamethoxam residue in pollen samples (Table 8 and Figure 2). There was one extreme value measured in pollen of untreated plants for parent thiamethoxam at 153 ng/g. Normally, this result could be determined as an outlier but most of the other samples had detections above the LOQ with two other detections noted at 49.7 and 37.2 ng/g. The authors of the report indicate that the source of the pollen samples could have been from the treated plants. A similar effect for similarity in distribution between pollen concentration of untreated and treated plants has been noted for other combinations of neonicotinoid treated trees.

Comparison of distribution between soil types: Mean data from the two years were combined from the sites to test for potential differences in distributions due to soil texture. Soil textural information was supplied in supplemental data submitted after the final report. Based on the supplemental soil characteristics the sites were classified as follows: coarse-textured soil sites were CA-1, CA-2, and Ca-5; medium-textured soil sites were CA-6 and CA-9; and fine-textured soil sites were CA-3, CA-4, CA-7, and CA-8. Results of the non-parametric test indicated a significant difference in the distribution of leaves, nectar, and whole flowers between citrus trees grown in the 3 soil types for parent thiamethoxam and CGA322704 degradation product (Table 9 and Figure 3). Many of the median concentration values for trees grown in medium and fine textured soils were at or near the values that were the substituted values for one-half the LOQ or LOD detection limit for thiamethoxam and CGA322704 analyses. This indicatates that many values in these soils were reported at the detection limits. In contrast, the median values in coarse-textured soils were well above the respective plant matrix LOQ and LOD limits. Differences in distribution between soil types for pollen were not significant but there was a greater range indicated in the graphs for trees grown in coarse-textured soils. For comparison, the contrast between soil types for untreated control trees is presented and no significant differences were measured for these comparisons, indicating no initial bias due to soil type (Figure 4).

A potential confounding factor was the location of three lemon trees sites used in the study in 3 fine-textured soil sites at CA-3, CA-7, and CA-8. The rest of the sites were planted with Valencia oranges. Potential differences in uptake due to plant species could have confounded the contrasts between soil type. The soil analyses were rerun with the soil categories assigned according to the distribution of the type of citrus. The result was 3 coarse-textured sites with Valencia oranges, 3 fine-textured sites with lemon, and the 3 remaining sites of Valencia oranges with 1 fine-textured and 2 medium-textured soil categories. The comparison for distribution between the sites was essentially the same as for the previous analyses where the concentration range in coarse-textured soils was greater than for the other two designated categories, as reflected in the comparison of thiamethoxam concentration (Figures 5 vs 3). Results of statistical analyses were also similar where specific comparisons for leaves, nectar, and whole flowers indicated differences between the coarse-textured soil sites and the other two categories (Table 10). Pollen as observed before lacked indication of a significant difference but the range in concentration was again greater in coarse-textured soil. These results support the overall observation that for these experimental conditions, plants grown in coarse-textured soils exhibit a greater range in concentration of thiamethoxam residues in leaves, nectar, and whole flowers. Plants grown in medium or fine-textured soils apparently do not take up as much residue from the soil. As indicated by the authors the lack of effect on pollen may be due to its higher potential for aerial movement between plots so the analyses are prone to confounding from applications made to adjacent treated plots.

Data for bee relevant matrices: The observed distributions derived from the individual analyses ostensibly determines the expected range in concentrations of thiamethoxam and CGA322704 degradation product residues in bee relevant plant samples for this combination of plant species and application scenario (Table 11). Although many samples were below detection limits, the presence of parent thiamethoxam in the various plant matrices indicates a potential for degradation products to be present. Therefore, the complete data set with values set at one-half the respective LOD for each matrix was included in the determination of distribution of concentrations. For nectar, although some concentrations were below the LOD, the maximum total residue concentration was 27.8 ng/g and the median value at 0.4 ng/g. Concentrations of total residue in pollen were higher where the maximum total residue value was 107.8 ng/g and the median value at 5.5 ng/g.

Conclusions

1. Utility of the data: The study followed the design as indicted in the data call-in where the study was replicated in two years at 9 sites. Given the limitations of finding experimental sites in existing fields, the 9 sites were reasonable representative of the 3 soil types requested in the data call-in with 3 sites in coarse-textured soils, 2 sites in medium-textured soil, and 4 sites in fine-textured soils.

2. Concentrations in Bee Relevant Matrices: By default, the distributions reported in Table 11 represent the expected concentrations in bee relevant matrices that result from soil application of thiamethoxam to citrus trees applied 45 days prior to bloom. Median and maximum values for total thiamethoxam residues in pollen are 5.5 and 107.8 ng/g on wet weight basis and for nectar are 0.5 and 27.8 ng/g, respectively.

3. No carry-over effect between years: Concentrations measured in plant matrices between the two years of the study was similar, indicating low potential for carry-over effects due to sequential soil applications at the concentrations and timing used in this study.

4. Effect of Soil Type: Concentrations in plant matrices at bloom were higher in plants grown in coarse-textured soil. The very low concentrations measured in medium and fine-textured soils indicate much less uptake from the soil.

8. STUDY STRENGTHS, LIMITATIONS AND CONCLUSIONS

In the context of documenting the magnitude of thiamethoxam residues in bee-related matrices of citrus trees, the following strengths are observed with this study.

1. Data provide quantitative values of thiamethoxam residues expected in pollen, nectar, and leaves of citrus trees when measured at bloom in response to one soil application of thiamethoxam applied approximately 45 days prior to bloom.

2. The study was replicated over two years with measurements in plant samples taken at bloom where citrus trees had received a soil application of thiamethoxam approximately 45 days before bloom.

3. The 9 sites were reasonably replicated over the requested 3 soil texture categories.

Limitations noted in this study include:

1. Approximately 90% of pollen samples from the untreated control were above the LOQ. This is similar to the rate of detection in pollen samples of treated plots where thiamethoxam was measured in 98% of samples. There was one extreme value measured in pollen of untreated plants for parent thiamethoxam at 153 ng/g. Normally, this result could be determined as an outlier but most of the other samples had detections above the LOQ with two other detections noted at 49.7 and 37.2 ng/g. It is unclear if the source of this control contamination may have an effect on the magnitude of residues in the treatment plots.

Overall, considering the strengths and limitations of this study, the following conclusions can be drawn:

1. **Bee-relevant matrices:** Thiamethoxam residues were measured in nectar and pollen sampled 45 days after a soil application. Median and maximum values for total thiamethoxam residues in pollen are 5.5 and 107.8 ng/g on wet weight basis and for nectar are 0.5 and 27.8 ng/g, respectively. Values in Table 11 indicate the potential range in concentrations that bees are exposed to in the field.

2. **No carry-over effect of years:** Concentrations measured in plant matrices were similar between the two years of the study indicating low potential for carry-over effects due to a single soil application at the concentration and timing of application used in this study.

3. **Effect of soil type:** Concentrations in plant matrices were higher in plants grown in coarse-textured soil. Many analyses were indicated as below detection limits in plants grown in medium or fine-textured soils indicating much less extraction of residues from these soil types.

4. **Temporal Variability in Residues**. This study was not designed for temporal analysis of declining concentrations, but rather, to provide a snapshot of residue concentrations during flowering. Samples were collected at only one time point during bloom.

9. STUDY VALIDITY/CLASSIFICATION

The study is classified as ACCEPTABLE for quantitative use in risk assessment. The data from this study provide an expected distribution of the concentrations thiamethoxam residues that bees are exposed to in nectar and pollen of citrus trees under actual agronomic practices in California. Relating concentrations measured in flower parts to bee health is possible by comparing the concentrations measured in bee relevant plant parts to target values that define acute or chronic exposure scenarios. However, approximately 90% of pollen samples from the untreated control were above the LOQ. This is similar to the rate of detection in pollen samples of treated plots where thiamethoxam was measured in 98% of samples. The similarity in distributions places a serve limit on comparing differences in distribution caused by years or varieties because results would be confused with the large amount +of background variance, i.e. what part of the measured effect is due to actual treatment difference in comparison to background variation. The study is considered scientifically sound and useful for risk assessment purposes.

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Table 6. Counts of chemical analytical results for thiamethoxam and CGA322704 degradation product comparing the total number of samples collected for each matrix to the number of analyses below the LOQ or LOD for untreated control and treated citrus trees. Trees had been treated with a soil application of Platinum 75CA9549C at 0.172 lbs a.i./Acre approximately 45 days prior to sampling at bloom.

Treatment and Plant Matrix	Comparison of Total Number of Samples Reported Above and Below Detection Limits							
	Thiamethoxam				CGA322704			
	Total Number	Number > LOQ	Number <LOQ>LOD	Number < LOD	Total Number	Number > LOQ	Number <LOQ>LOD	Number < LOD
Untreated Plants								
Leaves	18	0	1	17	18	1	1	16
Whole Flowers	18	0	0	18	18	0	1	17
Nectar	18	2	0	16	18	0	0	18
Pollen	18	16	2	0	18	2	1	15
Treated Plants								
Leaves	54	36	6	12	54	32	8	14
Whole Flowers	54	29	12	13	54	13	14	27
Nectar	52	22	6	24	52	15	9	28
Pollen	54	53	1	0	54	30	11	13

Table 7. Effect of Year: Exact probability levels for Wilcoxon, Median, and Kuiper non-parametric tests for differences in the distribution of thiamethoxam and its degradation product CGA322704 between replicate years for leaves, whole flowers, nectar, and pollen of citrus trees. Trees had been treated with a soil application of Platinum 75CA9549C at 0.172 lbs a.i./Acre approximately 45 days prior to sampling at bloom.

Treatment Plant Matrix	Exact Probability Levels for Non-parametric Tests of Differences Between Years					
	Thiamethoxam			CGA322704		
	Wilcoxon	Median	Kuiper	Wilcoxon	Median	Kuiper
Untreated Control Plants						
Leaves	1	1	1	1	1	1
Whole Flowers	1	1	1	1	1	1
Nectar	1	1	1	1	1	1
Pollen	0.51	0.34	0.89	1	1	1
Thiamethoxam Treated Plants						
Leaves	0.69	1	1	0.34	1	0.89
Whole Flowers	0.24	1	1	0.74	1	1
Nectar	0.78	1	1	0.92	1	1
Pollen	0.61	1	0.88	0.37	1	1

Table 8. Untreated vs Treated Plants: Exact probability levels for Wilcoxon, Median and Kuiper non-parametric tests for differences in the distribution of thiamethoxam and its degradation product CGA322704 between untreated control and treated citrus tree leaf, whole flower, nectar, and pollen samples. Trees had been treated with a soil application of Platinum 75CA9549C at 0.172 lbs a.i./Acre approximately 45 days prior to sampling at bloom.

Plant Matrix	Non-paramentric Test Exact Probability Levels for Comparing Concentration Distribution Between Untreated Control and Treated Plants					
	Thiamethoxam			CGA322704		
	Wilcoxon	Median	Kuiper	Wilcoxon	Median	Kuiper
Leaves	0.001	0.001	0.001	0.001	0.001	0.01
Whole Flowers	0.001	0.001	0.001	0.002	0.003	0.18
Nectar	0.001	0.002	0.078	0.001	0.001	0.078
Pollen	0.13	0.31	0.59	0.001	0.001	0.001

Table 9. Effect of Soil Type: Exact probability levels for Wilcoxon non-parametric tests for differences in the distribution of thiamethoxam, CGA322704 degradation product, and total residue between soil types in leaf, whole flower, nectar, and pollen samples of citrus trees. Trees had been treated with a soil application of Platinum 75CA9549C at 0.172 lbs a.i./Acre approximately 45 days prior to sampling at bloom. The Wilcoxon probability level is for the combined analysis of all three soil types for each plant matrix. The DSCF probability level is for the specific 1-degree of freedom contrasts between soil types, such as Coarse vs Fine-textured soil sites.

Treatment, Plant Matrix, and Specific Soil Contrasts	Exact Probability Levels for Non-parametric Tests of Differences Between Soil Type	
	Thiamethoxam	CGA322704
	Wilcoxon	Wilcoxon
Treated Plants		
Leaves	0.037	0.010
Coarse vs. Fine	0.053	0.012
Coarse vs. Medium	0.203	0.203
Fine vs. Medium	0.905	0.901
Whole Flowers	0.002	0.006
Coarse vs. Fine	0.012	0.013
Coarse vs. Medium	0.203	0.199
Fine vs. Medium	0.163	0.505
Nectar	0.001	0.002
Coarse vs. Fine	0.004	0.007
Coarse vs. Medium	0.203	0.203
Fine vs. Medium	0.023	0.133
Pollen	0.390	0.180
Coarse vs. Fine	0.476	0.188
Coarse vs. Medium	0.535	0.407
Fine vs. Medium	0.776	0.905
Untreated Plants		
Leaves	1	0.32
Whole Flowers	1	0.55
Nectar	0.84	1
Pollen	0.54	0.17

Table 10. Potential Species Confounding for Effect of Soil Type: Exact probability levels for Wilcoxon non-parametric tests for differences in the distribution of thiamethoxam, CGA322704 degradation product, and total residue between soil types in leaf, whole flower, nectar, and pollen samples of citrus trees. Trees had been treated with a soil application of Platinum 75CA9549C at 0.172 lbs a.i./Acre approximately 45 days prior to sampling at bloom. The Wilcoxon probability level is for the combined analysis of all three soil types for each plant matrix. The DSCF probability level is for the specific 1-degree of freedom contrasts where the acronyms are: C-Orange=orange trees in coarse-textured soil; F-Lemon=lemon trees in fine-textured soil; and M-F-Orange=orange trees grown in medium or fine-textured soils.

	Exact Probability Levels for Non-parametric Tests of Differences Between Soil Type		
	Thiamethoxam	CGA322704	Total Residue
Plant Sample	Wilcoxon	Wilcoxon	Wilcoxon
Treated Plants			
Leaves	0.044	0.013	0.025
C-Orange vs. F-Lemon	0.133	0.027	0.064
C-Orange vs. M-F-Orange	0.064	0.064	0.064
F-Lemon vs. M- F-Orange	0.997	0.985	0.969
Whole Flowers	0.009	0.011	0.011
C-Orange vs. F-Lemon	0.028	0.037	0.028
C-Orange vs. M-F-Orange	0.064	0.058	0.064
F-Lemon vs. M- F-Orange	0.743	0.956	0.912
Nectar	0.001	0.006	0.001
C-Orange vs. F-Lemon	0.008	0.022	0.013
C-Orange vs. M-F-Orange	0.064	0.062	0.064
F-Lemon vs. M- F-Orange	0.082	0.547	0.120
Pollen	0.440	0.180	0.340
C-Orange vs. F-Lemon	0.501	0.278	0.406
C-Orange vs. M-F-Orange	0.501	0.244	0.406
F-Lemon vs. M- F-Orange	0.986	0.986	0.986

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Table 11. Distribution of thiamethoxam, CGA322704 degradate, and total residue concentrations measured in nectar, pollen, whole flowers, and leaves of citrus trees that were exposed to a soil application of Platinum 75CA9549C at 0.172 lbs a.i./acre approximately 45 days prior to sampling at bloom. Data are the combined results of individual plant samples obtained from two consecutive years of study.

	Nectar			Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	52	52	52	54	54	54
Mean (ng/g)	2.6	0.6	3.2	14.4	6.6	21.1
SD (ng/G)	5.1	1.2	6.3	22.3	12.2	33.0
CV (%)	201.5	192.0	196.9	154.4	185.3	156.8
Min (ng/g)	0.1	0.1	0.3	0.5	0.3	1.4
Median (ng/g)	0.3	0.1	0.4	3.6	1.3	5.5
75th (ng/g)	1.3	0.7	2.0	13.3	3.6	23.1
90th (ng/g)	8.7	1.4	10.2	51.1	21.2	62.3
95th (ng/g)	16.9	3.2	21.6	67.2	36.0	107.8
Max (ng/g)	22.5	7.0	27.8	104.0	61.5	135.1
% of Total	80.2	19.8		68.2	31.3	
	Whole Flowers			Leaves		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	54	54	54	54	54	54
Mean (ng/g)	7.9	2.0	9.9	10.4	7.9	18.3
SD (ng/g)	15.0	3.8	18.7	19.6	15.3	34.7
CV (%)	189.5	190.7	189.2	188.4	193.8	189.6
Min (ng/g)	0.3	0.3	0.5	0.3	0.3	0.5
Median (ng/g)	1.5	0.4	1.7	2.4	1.6	1.1
75th (ng/g)	2.5	0.5	3.0	6.5	3.4	9.9
90th (ng/g)	31.4	7.2	37.2	33.9	29.1	64.4
95th (ng/g)	46.9	12.4	59.6	67.3	47.1	114.4
Max (ng/g)	59.4	17.3	76.7	84.9	75.6	160.5
% of Total	79.8	20.2		56.8	43.2	

Figure 1. Comparison between Years: Distribution of natural logarithm of thiamethoxam residues in leaves, nectar, pollen and whole flowers of citrus trees compared between sequential replicate studies years of the study. Trees were treated with a soil application of Platinum 75CA9549C at 0.172 lbs a.i./Acre approximately 45 days prior to sampling at bloom.

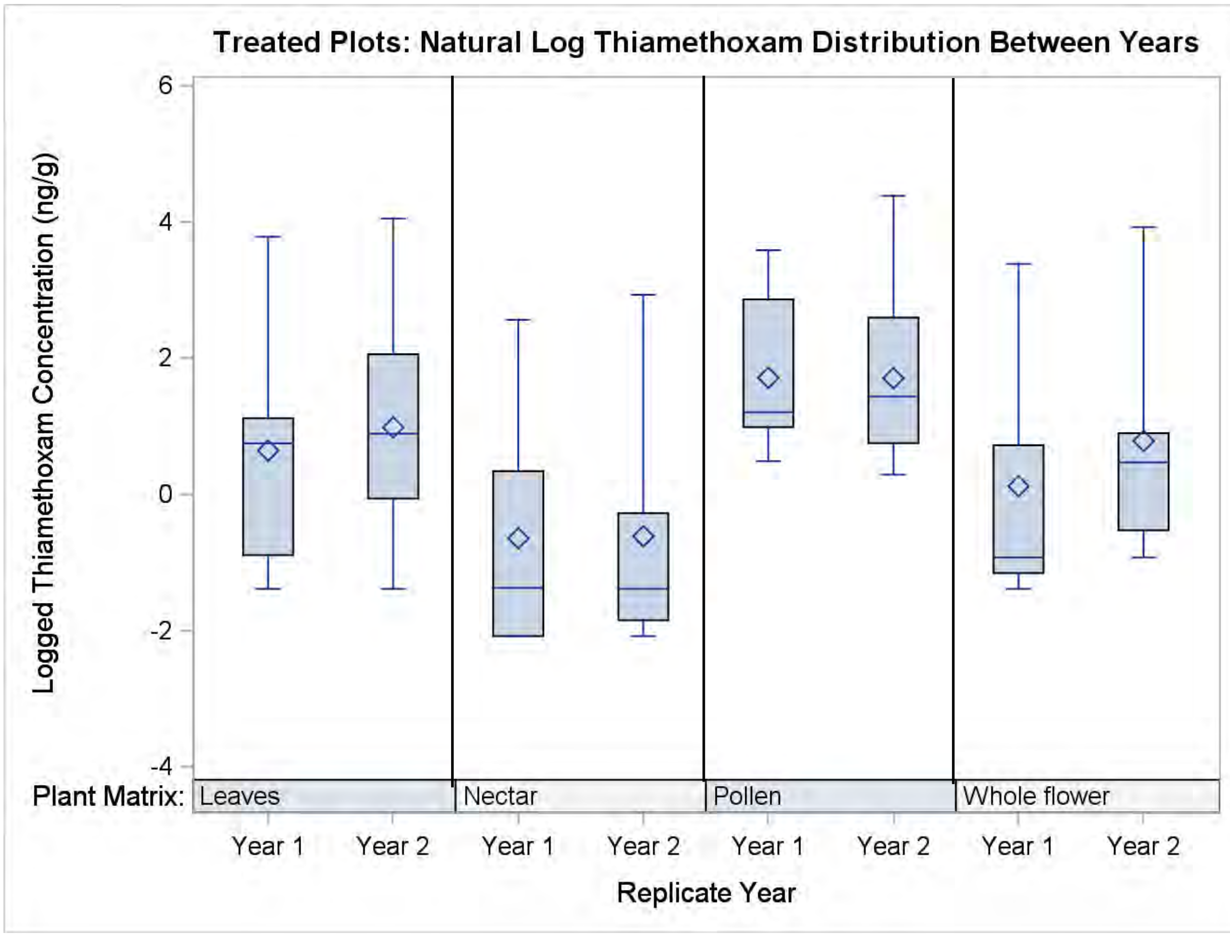


Figure 2. Untreated vs Treated Plots: Comparison of distribution of natural logarithm of thiamethoxam concentration between untreated control (UTC) and treated (TRT) plants for leaves, nectar, pollen, and whole flowers of citrus trees. Treated trees received a soil application of Platinum 75CA9549C at 0.172 lbs a.i./Acre approximately 45 days prior to sampling at bloom.

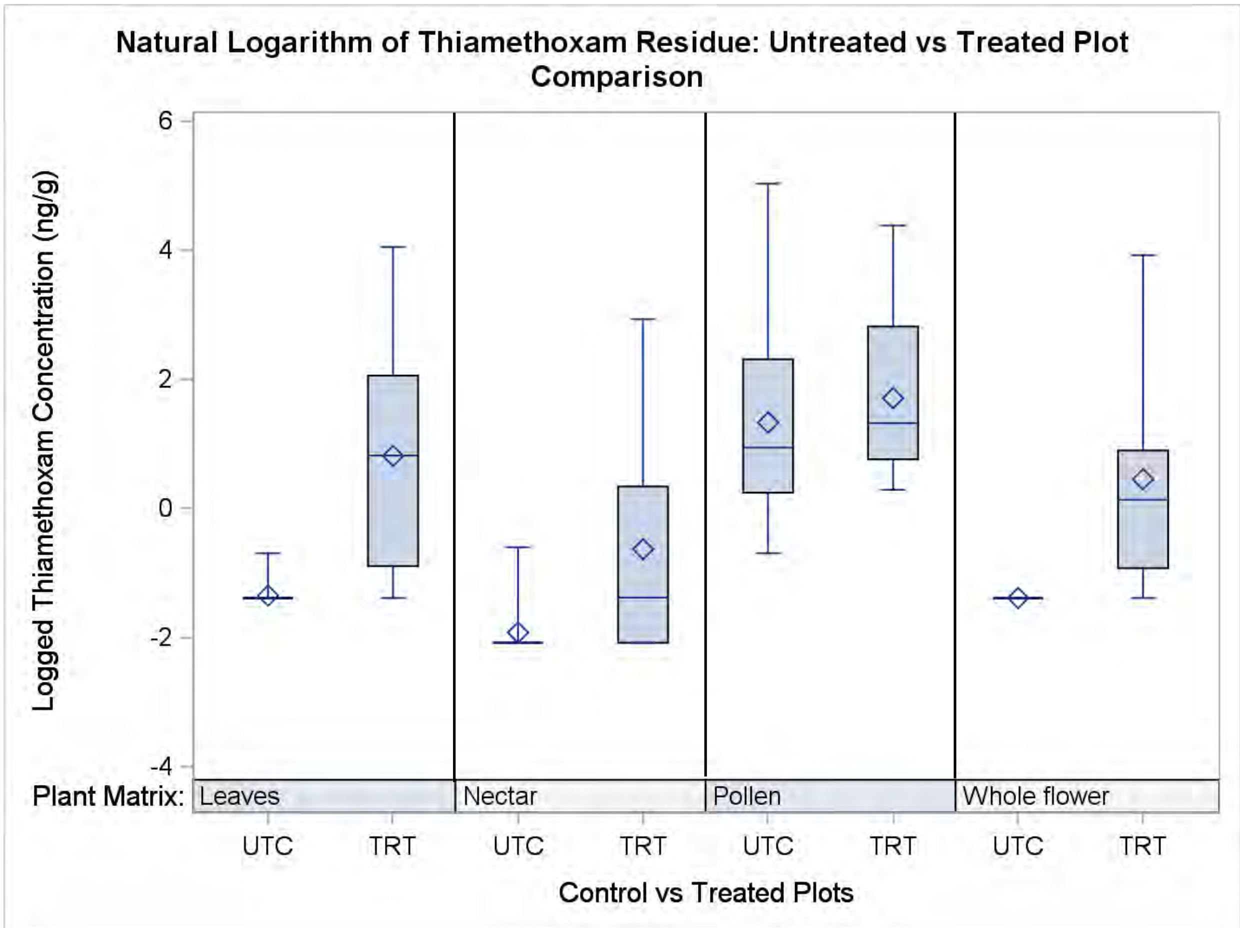


Figure 3. Comparison of Soil Type for Treated Plants: Comparison of distribution of natural logarithm of thiamethoxam concentration measured between Coarse, Medium, and Fine-textured soil types for leaves, nectar, pollen, and whole flowers of treated citrus trees. Treated trees received a soil application of Platinum 75CA9549C at 0.172 lbs a.i./Acre approximately 45 days prior to sampling at bloom.

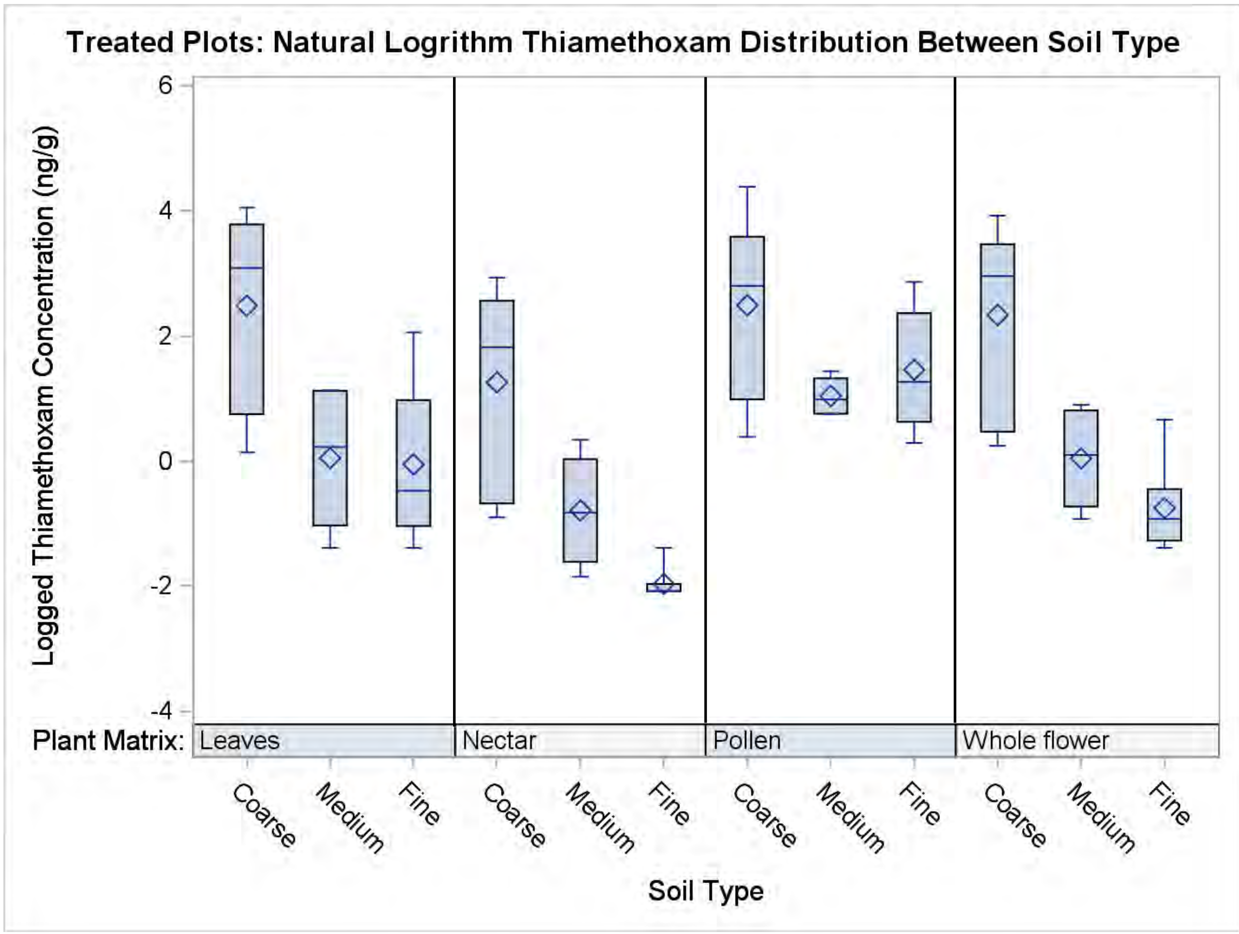


Figure 4. Comparison of Soil Type for Untreated Plants: Comparison of distribution of natural logarithm of thiamethoxam concentration measured between Coarse, Medium, and Fine-textured soil type for leaves, nectar, pollen, and whole flowers of untreated control citrus trees.

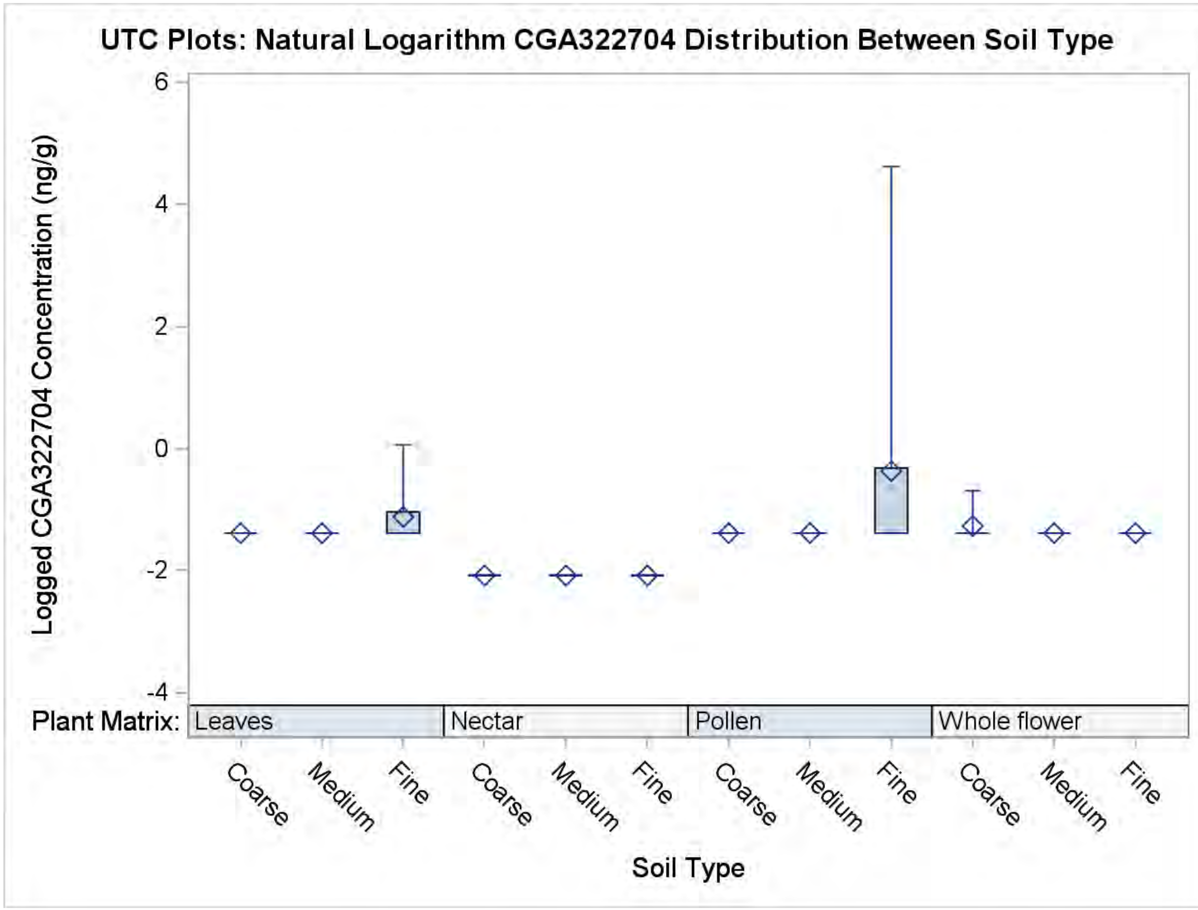
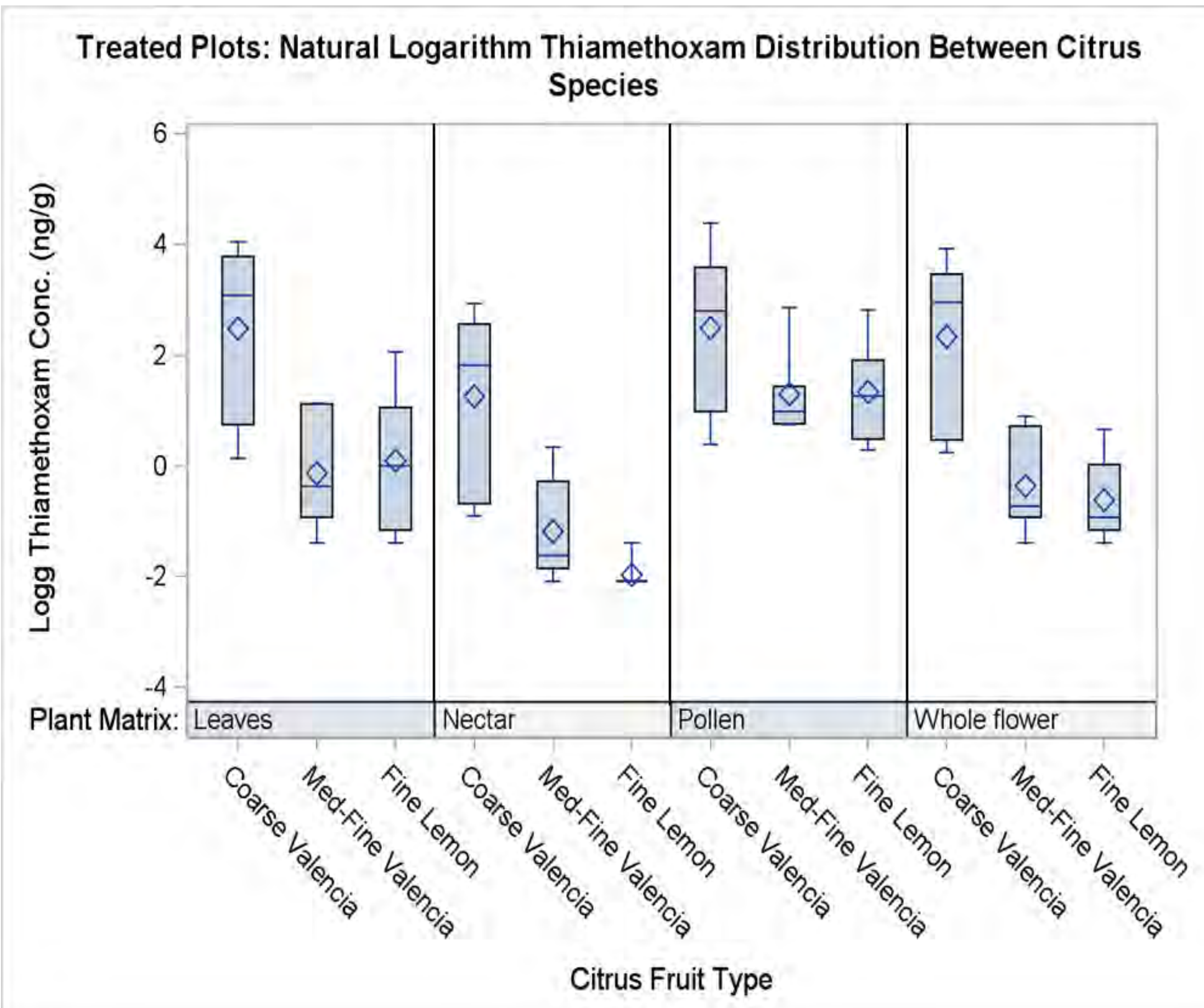


Figure 5. Comparison between Plant Species and Soil Type: Comparison of thiamethoxam concentration distribution in leaves, nectar, pollen, and whole flowers of treated citrus trees measured between Valencia and Lemon citrus plant species. The three categories indicate distribution of 3 Lemon tree sites located in 3 sites with fine-textured (Fine) soils, 3 sites where Valencia orange trees were grown in 1 fine-textured and 2 medium-textured (Med) soils, and 3 sites with Valencia orange trees grown in coarse-textured (Coarse) soils.



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MRID 50096606 CDPR THX Stone Fruit

Reference
Rice, F., Lange, B. (2015) Thiamethoxam 25 WG (A9584C) - Magnitude of Residues in Pollen, Nectar, Flowers, and Leaves of Stone Fruit After Foliar Application with Actara® 25WG in California: Final Report. Project Number: TK0177222. Unpublished study prepared by Syngenta Crop Protection, LLC. 644. MRID 50096606, CDPR Study ID 288446, Data Volume 52691-0531, Tracking ID# 273604

1. STUDY INFORMATION

Chemical:	Thiamethoxam	**PC Code**	60109
Test Material:	Actara 25WG	**Percent Active Ingredient:**	25.0%
Study Type:	Non-Guideline field residue study on Stone Fruit to measure Thiamethoxam and CGA322704 residue levels in nectar, pollen, anthers, flowers and leaves in site locations that have been treated with Thiamethoxam for two successive years.		
Sponsor:	Syngenta Crop Protection, LLC 410 Swing Road Greensboro, North Carolina 27409	**Experiment Start and End Date:**	April 24, 2013 – September 25, 2015
Sponsor Study ID:	TK0177222	**Study Locations:**	10 stone fruit trial sites including peach, plum and sweet cherry located throughout California.
Study Completion Date:	December 17, 2015		
Report Amendment Date:	October 21, 2016		
GLP Status:	Non-GLP; protocol reviewed by CDPR. [CDPR Study ID 288446, Data Volume 52691-0531, Tracking ID# 273604]		

2. REVIEWER INFORMATION

Study Reviewed by: California Department of Pesticide Regulation	Richard Bireley, Sr. Environmental Scientist (Specialist) John Troiano, Ph.D., Research Scientist III Alexander Kolosovich, Sr. Environmental Scientist (Specialist) Brigitte Tafarella, Environmental Scientist Denise Alder, Sr. Environmental Scientist (Specialist) Russell Darling, Sr. Environmental Scientist (Specialist)

3. EXECUTIVE SUMMARY

A two-year study was initiated in 2013 to determine the magnitude of the residue of thiamethoxam (CGA293343) and its major metabolite, CGA322704, in stone fruit (peach, plum, and sweet cherry) leaves, flowers, anthers, pollen, and nectar following foliar applications with Actara® 25WG (EPA Reg. No. 100-938). The study consisted of 10 trials located in California, each with an untreated control plot and a treated plot large enough to ensure adequate plants for collection. Over two consecutive growing seasons, thiamethoxam, formulated as Actara® 25WG, was applied to treated plots as a broadcast foliar spray twice (7-day interval) during each growing season at the maximum labeled-use rate of 5.5 oz formulated product per acre (0.086 lb ai/acre) for each application. Applications were targeted 21- and 14-days before normal harvest of mature fruit. Composite samples of leaves, flowers, anthers, pollen and nectar were collected for residue analysis from untreated control (UTC) and treated (TRT) plots the following bloom period (the following spring).

This report is amended to include information on collection and analysis of second year samples collected at site CA 10 and to add further explanation regarding residues in control pollen samples.

4. STUDY VALIDITY

Guideline Followed:	Deviations Exist; (protocol was reviewed by CDPR)
Guideline Deviations:	Site CA2 was terminated, Site CA10 was added
Other Deviations:	Additional year of samples are being collected
Classification:	**ACCEPTABLE**
Rationale:	N/A
Reparability:	N/A

5. MATERIALS AND METHODS

Test Material Characterization			
Test item:	Actara 25WG	**Percent A.I.:**	25.0% A.I.
Formulation Type:	Water Dispersible Granule, WG	**pH:**	9.4 (1% aqueous solution)
CAS #:	153719-23-4	**Solubility:**	4.1 g/L @ 25°C

5A. STUDY DESIGN

The purpose of this two-year study was to determine the amount of thiamethoxam and CGA322704 in stone fruit leaves, flowers, anthers, pollen, and nectar after foliar applications of Actara® 25WG (EPA Reg. No. 100-938) in two successive years. The study was initiated on April 24, 2013 and the experimental termination date was on September 25, 2015.

The two-year study initially included nine trial sites (CA-1 through CA-9) each with an untreated control (UTC) plot and a treated (TRT) plot large enough to ensure adequate plants for collection of sufficient quantities of leaves, flowers, anthers, pollen, and nectar for residue analysis. Per Amendment 6 (September 02, 2014), a tenth trial site (CA-10) was added to replace CA-2 which had a plum variety that did not produce pollen. The test substance was applied to treated plots as a foliar broadcast treatment twice each year. Representative composite samples of leaves, flowers, anthers, pollen, and nectar were collected from the UTC plot and each TRT replicate plot for residue analysis.

5B. APPLICATION TIMING AND RATES

Airblast sprayers were calibrated prior to test-substance application. Sprayer-pass times were recorded to confirm application rate accuracy. All trials were within the acceptance criteria range of 95 to 105% of the target 5.5 oz formulated product per acre application for both years.
Application rates and dates are summarized below in table 1.

Table 1. Summary of Applications on Stone Fruit

Trial Site	Year	Application Date	Target Rate (oz/acre)	Actual Rate (oz/acre)
CA-1	1	9/4/2013[1]	5.5	5.599
		9/11/2013[1]	5.5	5.470
	2	6/20/2014	5.5	5.480
		6/27/2014	5.5	5.582
CA-2	1	5/8/2013	5.5	5.532
		5/15/2013	5.5	5.556
	2	4/28/2014	5.5	5.606
		5/5/2014	5.5	5.506
CA-3	1	5/8/2013	5.5	5.524
		5/15/2013	5.5	5.515
	2	4/28/2014	5.5	5.342
		5/5/2014	5.5	5.559
CA-4	1	4/30/2013	5.5	5.531
		5/7/2013	5.5	5.632
	2	5/15/2014	5.5	5.586
		5/22/2014	5.5	5.628
CA-5	1	6/18/2013	5.5	5.591
		6/25/2013	5.5	5.594
	2	7/21/2014	5.5	5.624
		7/28/2014	5.5	5.562
CA-6	1	4/30/2013	5.5	5.515
		5/7/2013	5.5	5.480
	2	4/24/2014	5.5	5.548
		5/1/2014	5.5	5.531
CA-7	1	4/29/2013	5.5	5.433
		5/7/2013	5.5	5.467
	2	5/28/2014	5.5	5.543
		6/4/2014	5.5	5.546
CA-8	1	5/28/2013	5.5	5.516
		6/4/2013	5.5	5.497
	2	7/15/2014	5.5	5.512
		7/22/2014	5.5	5.570
CA-9	1	4/29/2013	5.5	5.489
		5/7/2013	5.5	5.517
	2	4/28/2014	5.5	5.665
		5/6/2014	5.5	5.533
CA-10	1	8/20/2014	5.5	5.749
		8/27/2014	5.5	5.493
	2	8/28/2015	5.5	5.476
		9/4/2015	5.5	5.487

[1] Applications made after harvest of fruit, to avoid making applications too close to harvest.

5C. Study Site Location and Characteristics

The 10 trial sites were distributed within typical peach-, plum-, and sweet cherry-growing areas of the Central Valley of California (northern, middle, and southern sections) and represented management practices and weather conditions under which stone fruit is commercially produced. Stone fruit varieties and site conditions of the test sites are provided below in Table 2 and Table 3.

At each sampling event, 0-12-inch soil cores were collected from control and treated plots. A minimum of 5 cores were collected randomly from each control plot and a minimum of 10 cores were collected randomly from all replicates of treated plots. Soil cores were then sectioned into 0-6 inch and 6-12 inch segments; soil was removed from segmented plastic tubes and placed into labeled plastic bags, and double bagged. For each trial, soil was combined into a single control and a single treated sample by depth.

Table 2. Trial Site Conditions for Stone Fruit

Trial ID	Trial Location (County, State)	Crop	Variety	OM (%)	pH	Cation Exchange Capacity (meq/100g soil)	Soil Types	Temperature Range (°F) [b]
CA-1	King, California	Peach	Late Ross Cling	1.6 – 1.8	7.6 – 7.7	11.7 -12.1	Sandy Loam	30.9 – 98.5
CA-2	Madera, California	Plum	Apple Dandy	1.3 – 1.6	6.7 – 6.8	7.9 – 9.4	Sandy Loam	27.8 – 97.2
CA-3	Fresno, California	Cherry	Washington	0.52	6.5 – 6.6	8.5 – 9.1	Loamy Sand/Sandy	29.6 – 97.0
CA-4	San Joaquin, California	Peach	Flavorcrest	1.8 – 2.4	7.3 – 7.5	18.1 – 18.3	Loam	28.9 – 91.6
CA-5	Merced, California	Prune	French	0.70 – 1.5	5.0 – 5.7	20.0 – 23.4	Clay Loam/Loam	28.9 – 91.6
CA-6	San Joaquin, California	Cherry	Sweet Tart	1.9 – 2.6	7.2 – 7.5	18.8 – 19.9	Loam	28.9 – 91.6
CA-7	Sutter, California	Peach	Elegant Lady	2.2 – 2.4	7.0 – 7.5	17.9 – 18.3	Clay Loam	26.2 – 92.1
CA-8	Sutter, California	Prune	French	1.3 – 1.5	6.4 – 6.7	15.2 – 15.7	Loam	26.2 – 92.1
CA-9	Sutter, California	Cherry	Bing	0.82 – 1.6	6.9	6.4 – 7.7	Sandy Loam	36.6 – 94.7
CA-10	Tulare, California	Plum	Angeleno	1.3 – 2.4	7.2	16.3 – 17.0	Sandy Loam	35.3 – 95.6

Table 3. Soil Characterization Results.

Trail Site	Soil Type	% Sand	% Silt	% Clay
CA-1	Sandy Loam	67	24	9
		65	26	9
CA-2	Sandy Loam	67	23	10
		65	23	12
CA-3	Loamy Sand/Sand	85	10	5
		87	10	3
CA-4	Loam	40	35	25
		38	35	27
CA-5	Clay Loam/Loam	32	39	29
		36	37	27
CA-6	Loam	38	37	25
		40	35	25
CA-7	Clay Loam	28	39	33
		22	43	35
CA-8	Loam	48	28	24
		46	28	26
CA-9	Sandy Loam	64	25	11
		68	23	9
CA-10	Sandy Loam	60	24	16
		58	26	16

5D. Sample Collection, Handling, Processing

Leaf samples were collected from UTC and replicated TRT (A, B, C) plots at each site after the last application of each season. Whole flower, anther, pollen, and nectar samples were collected at an early bloom stage (50–75% bloom) in the spring following test substance applications in the fall. Leaf samples were collected after flowering, when leaves had emerged and were near normal size. Also, at appropriate timings, bulk samples were collected from one site for analytical method development and method verification as follows: approximately 500 g of leaf and flower samples, 1g of anther samples, and 500 mg of nectar samples. Bulk pollen for method development/verification was purchased from a commercial source. In all cases, samples were collected from the UTC plot first, then from TRT plots or by different personnel to minimize the potential for cross contamination.

Soil samples were collected during the 2014 growing season and again when flowers were collected in spring 2015. For trial CA-10, soil samples were also collected when flowers were collected in spring of 2016.

Leaves

Representative samples of at least 500 g of leaves were collected from UTC and TRT plots after the second application of the test substance during both growing seasons. Samples were collected when leaves were near normal size after the bloom period was complete. Leaves were removed by hand from the upper, middle, and lower portions of the trees. All samples met the protocol minimum sample size requirement.

Flowers

Flowers were removed by hand from the high, low, and middle portions of the trees. Representative samples of at least 1000 flowers (500 g target sample size) were collected from UTC and TRT plots. From the 1000 flowers, approximately 400 flowers (200 g target sample size) were used for whole flower sample analysis and approximately 600 flowers (300 g target sample size) were used for subsequent collection of nectar and pollen samples. It should be noted that in some cases whole flowers and flowers for collection of nectar and pollen were not counted, nor did they meet the minimum weights. Flowers that had not yet opened (popcorn stage) were collected and processed for anther samples. Flowers for direct analysis were placed into plastic bags then into the freezer until shipment to the analytical laboratory.

Anthers

Anthers were collected from flowers that were in the popcorn stage and had not yet opened. Target weight for anther samples was approximately 50 mg from the UTC plots and 100 mg from the TRT plots. All samples, except sample numbers 369, 377, and 393 from CA-6, met the protocol for the minimum sample size requirement.

Pollen

Pollen was collected using a vacuum pump that was connected by tubing to a 1000-µL pre-weighed filtered pipette tip. Pollen was vacuumed directly from the anthers into the pipette tips. After weighing the samples, the pipette tips were placed into plastic bottles and sealed for additional containment. Pollen samples were then placed into the freezer until shipment. The minimum pollen sample size required for analysis was approximately 30 mg for both the UTC and TRT samples. All pollen samples, except sample numbers 119, 127, and 131 from CA-6, and samples 447, 451, 459, and 467 from CA-10, met the protocol's minimum sample size requirement. No pollen samples were collected from CA-2 due to male-sterile plum variety.

Nectar

Nectar was collected using 10 and 20-µL microcapillary pipettes. Approximately 300 flowers were collected to extract the minimum sample size of nectar required for analysis (≥30 mg for TRT samples, ≥30 mg for UTC samples). Nectar samples were placed into sealed and labelled containers where sample weights were then recorded. Nectar samples were then placed into the freezer until shipment. All samples met the protocol's minimum sample size requirement.

Soil

Soil samples were collected during the 2014 growing season and again when flowers were collected in spring 2015. For trial CA-10, soil samples were also collected when flowers were collected in spring 2016.

At each sampling event, 0-12 inch soil cores were collected from control and treated plots. A minimum of 5 cores were collected randomly from each control plot and a minimum of 10 cores were collected randomly from all replicates of treated plots. Soil cores were then sectioned into 0-6 and 6-12 inch segments; soil was removed from segmented plastic tubes and placed into labelled plastic bags, and double bagged. For each trial, soil was combined into a single control and a single treated sample by depth.

Sample Storage.

Samples were transported from field sites to field facility freezers where they were stored frozen until shipment to the analytical laboratory. For trials CA-1 through CA-9, anther, pollen, and nectar samples were shipped to Eurofins Agrosciences, Inc. (EASI) in East Brunswick, NJ. Leaf, whole flower, and soil samples were shipped to the EASI Sample Processing Laboratory in Forsyth, Georgia. Then the leaf, whole flower, and soil samples were shipped to the EASI laboratory in East Brunswick, NJ after processing was completed. In March 2015, first year anther and all year-2 samples were sent from the EASI laboratory to SynTech Research Laboratory Services (SRLS) in Stilwell, KS for analysis, per Amendment 7 (Appendix 5). For CA-10, leaves collected post application 1 were shipped to the EASI Sample Processing Laboratory in Forsyth, Georgia, and after processing, they were shipped to the EASI Laboratory in East Brunswick, New Jersey. All other CA-10 samples were shipped to SynTech Research Laboratory Services (SRLS) in Stilwell, Kansas for analysis.

Previous storage stability studies[2,3,4] show thiamethoxam and CGA322704 are stable in a variety of matrices for up to 12 months. Therefore, residues of thiamethoxam and CGA322704 in stone fruit leaf and flower samples should not have been adversely affected by freezer storage during this study. The maximum freezer storage period for samples was 577 days. A 2 year freezer stability study[1] (Amendment 7 listed in the study report) shows that thiamethoxam and CGA322704 were stable in plant material for 2 years.

All samples were received frozen and in good condition at EASI or SRLS. Samples were maintained frozen (-20 ± 5 °C), except during periods when samples were removed from the freezer for sample preparation, weighing, or residue analysis. Leaf and flower samples were weighed and homogenized with dry ice using a Robot Coupe; the homogenized samples were placed into labelled plastic containers and stored in a freezer (allowing the dry ice to sublime) until sub-sampled for analysis. Pollen, anther and nectar samples were stored directly in a freezer until analysis.

5E. Analytical Methods

The analytical phase for year 1 samples (except for anthers) and year 2 post-second application leaves was conducted at EASI. The Principal Analytical Investigator was Chelsea Bonetti. From March 2015 to trial completion, per Amendment 7 listed in the study report, the analytical phase was conducted at SRLS in Stilwell, KS. The Principal Analytical Investigator was Ying Li. Samples analyzed by SynTech included first year anthers and all year-2 samples, as well as the CA10 samples.

Leaf and Whole Flower Sample Analysis

At EASI Laboratory, residues of thiamethoxam and CGA322704 were extracted with 50:50 methanol/water from 10-g of leaf and flower samples using a TomTec automatic homogenizer unit. Extracts were cleaned up with PSA and diluted with water in preparation for LC-MS/MS analysis. The

Limit of Quantitation (LOQ) for both analytes was 10 ppb in the leaf and 1 ppb in the flower matrices. The Limit of Detection (LOD) was targeted to be 5.0 ppb and 0.5 ppb in leaf and flowers, respectively.

At SRLS Laboratory, residues of thiamethoxam and CGA322704 were extracted with 50:50 (v:v) methanol:water containing 0.2% formic acid from approximately 10-g of leaf and flower samples using a blender. Extracts were centrifuged and diluted with water in preparation for LC-MS/MS analysis. The LOQ for both analytes was 1 ppb in the leaf and flower matrices. The Limit of Detection (LOD) was targeted to be 0.5 ppb in both leaf and flower matrices.

Anther, Pollen and Nectar Sample Analysis

At EASI Laboratory, residues of thiamethoxam and CGA322704 were extracted with 50:50 methanol/water containing 0.2% acetic acid from approximately 50 mg of pollen and 100 mg nectar samples. Extraction was conducted with a MP FastPrep-24 homogenizer; extracts were subsequently centrifuged and prepared for online SPE-LC-MS/MS analysis. The LOQ for both analytes was 1.0 ppb in pollen matrices and 0.5 ppb in the nectar matrix. The LOD was targeted to be 0.35 ppb and 0.186 ppb in pollen and nectar matrices, respectively. Anther samples were not analyzed at EASI.

At SRLS Laboratory, residues of thiamethoxam and CGA322704 were extracted with 50:50 (v:v) methanol:water containing 0.2% formic acid from approximately 0.1-g of pollen, nectar, and anther samples. Extraction solution and matrices were mixed well, centrifuged, diluted with water and cleaned by solid phase extraction in preparation for LC-MS/MS analysis. The LOQ for both analytes was 1 ppb in the pollen and anther matrices and 0.5 ppb for nectar matrix. The LOD was targeted to be 0.5 ppb in pollen and anther matrices and 0.25 ppb in a nectar matrix, respectively.

The LOQs and LODs are summarized in table 4 below.

Table 4. Summary of LOQs and LODs

Site Laboratory	Matrix	LOQ (Total Thiamethoxam PPB)	LOD (Total Thiamethoxam PPB)
EASI Laboratory	Leaf	10	5.0
	Flower	1.0	0.5
	Nectar	0.5	0.186
	Pollen	1.0	0.35
	Anthers	-	-
SRLS Laboratory	Leaf	1.0	0.5
	Flower	1.0	0.5
	Nectar	0.5	0.25
	Pollen	1.0	0.5
	Anthers	1.0	0.5

5F. Quality Assurance Results

At EASI Analytical Laboratory: A Shimadzu Nexera X2 HPLC system coupled to an API 4000 mass-spectrometric detector was used for separation and quantitation of thiamethoxam and CGA322704 for leaf matrices. A Shimadzu Nexera X2 HPLC system coupled to an API QTRAPP 6500 mass-spectrometric detector was used for separation and quantitation of thiamethoxam and CGA322704 for flower matrices. An API QTRAPP 6500 mass spectrometric detector equipped with a Spark Holland Symbiosis online HPLC-SPE system was used for separation and quantitation of thiamethoxam and CGA322704 for pollen and nectar matrices.

At SRLS Analytical Laboratory: A Waters Acquity UPLC system coupled to an API 5500 AB-Sciex mass spectrometric detector was used for the separation and quantitation of thiamethoxam and CGA322704 for leaf, flower, pollen, anther and nectar matrices. To quantify analytes of interest, standard curves were prepared by injecting constant volumes of solvent-based standard solutions at appropriate concentrations. Constant volume injections were used for sample extracts as well. Linear regression with 1/X weighting was used.

EASI successfully verified both methods prior to analysis of samples. Control samples of each matrix were fortified with thiamethoxam and CGA322704 at concentrations equal to the method 1xLOQ and 10xLOQ and analyzed according to the appropriate methods. No additional method verification was performed at SRLS prior to sample analysis as the methods were in-house for other studies. Concurrent recoveries were used to demonstrate method performance.

For each matrix, at least one method-recovery (QC) sample per analytical set was prepared by fortifying an untreated control sample with thiamethoxam and CGA322704 at concentrations equal to the method LOQ or higher and analyzing concurrently with treated samples to demonstrate adequate method performance throughout the study. Percent recoveries for QCs analyzed at EASI fell within the acceptable range of 70 to 120% with the exception of one flower and two leaf QCs with recoveries ranging from 128 to 133%. Percent recoveries for QCs analyzed at SRLS fell within the acceptable range of 70 to 120%.

6. RESULTS:

During sample analysis at EASI, no thiamethoxam or CGA322704 residues >LOQ were found in any UTC leaf matrices. Only thiamethoxam residues >LOQ were found in UTC flower (3 samples, 1.97 ppb max), pollen (6 samples, 39.1 ppb max) and nectar (4 samples, 2.75 ppb max) samples. Residues greater than respective LOQs were found in TRT flowers, pollen, and nectar samples. No residues were found in TRT leaf samples collected near bloom. Anther samples collected in Year 1 were analyzed by SLRS. Residues greater than LOQ were found in all leaf samples collected post application 2. The following table provides a summary of the average residues for samples analyzed at EASI.

Table 5. Residue Analysis on Leaves and Flowers at EASI

Year	Timing	Matrix	Crop	Average Residue Concentration	
				Thiamethoxam (ppb)	CGA322704 (ppb)
Year 1	Post Application 2	Leaf	Peach	4956	343
			Plum	6164	287
			Sweet Cherry	3941	279
	Bloom	Leaf	Peach	<LOD	<LOD
			Plum	<LOD	<LOD
			Sweet Cherry	<LOD	<LOD
		Flower	Peach	1.84	ND
			Plum	2.65	<LOD
			Sweet Cherry	<LOQ	<LOQ

LOQ= 10 ppb for leaf samples, 1 ppb for whole flower and pollen samples and 0.5 ppb for nectar samples.
LOD= 5 ppb for leaf samples, 0.5 ppb for whole flower, 0.35 ppb for pollen and 0.186 ppb for nectar samples.

Table 6. Residue Analysis on Pollen, Nectar and Leaves at EASI

Year	Timing	Matrix	Crop	Average Residue Concentration	
				Thiamethoxam (ppb)	CGA322704 (ppb)
Year 1	Bloom	Pollen	Peach	16.2	<LOQ
			Plum	18.9	<LOQ
			Sweet Cherry	30.3	1.08
		Nectar	Peach	<LOQ	<LOQ
			Plum	1.11	<LOD
			Sweet Cherry	<LOQ	<LOQ
Year 2	Post Application 2	Leaf	Peach	5589	276
			Plum	8316	356
			Sweet Cherry	4684	344

LOQ= 10 ppb for leaf samples, 1 ppb for whole flower and pollen samples and 0.5 ppb for nectar samples.
LOD= 5 ppb for leaf samples, 0.5 ppb for whole flower, 0.35 ppb for pollen and 0.186 ppb for nectar samples.

During sample analysis at SRLS, thiamethoxam and CGA322704 residues >LOQ were found in some UTC samples of all plant matrices at very low levels with the exception of whole flower samples in which no residues were found >LOQ. Both thiamethoxam and CGA322704 residues >LOQ were found in UTC leaves (5.07 and 1.15 ppb max, respectively), pollen (382.04 and 2.70 ppb max), nectar (0.74 and 5.10 ppb max), and anthers (5.75 and 1.83 ppb max) samples. Residues, greater than the respective LOQs, were found in some TRT samples of all plant matrices. The following table provides a summary of the average residues for treated plots post-application 2 analyzed at SRLS. Anther samples collected Year 1 and 2 were analyzed at SLRS.

Table 7. Residue Analysis on Leaves, Flowers, Pollen and Anthers at SRLS

Year	Timing	Matrix	Crop	Average Residue Concentration	
				Thiamethoxam (ppb)	CGA322704 (ppb)
Year 1	≤ 1.0 Day	Leaf	Plum[a]	11765	299
	Bloom	Leaf	Plum[a]	4.00	1.79
		Flower	Plum[a]	ND	ND
		Pollen	Plum[a]	110	1.55
		Anther	Peach	2.34	2.15
			Plum[a]	4.39	1.95
			Sweet Cherry	<LOQ (0.83)	ND
		Nectar	Plum[a]	<LOQ (0.42)	ND
Year 2	≤ 1.0 Day	Leaf	Plum	16132	951
	Bloom	Leaf	Peach	ND	1.14
			Plum	1.54	1.21
			Sweet Cherry	1.81	3.07
		Flower	Peach	ND	ND
			Plum	1.88	ND
			Sweet Cherry	ND	ND
		Pollen	Peach	75.4	1.75
			Plum	25.0	1.91
			Sweet Cherry	117	1.04
		Anther	Peach	2.18	1.58
			Plum	ND	2.01
			Sweet Cherry	10.4	2.92
		Nectar	Peach	<LOQ (0.44)	ND
			Plum	0.58	<LOQ (0.45)
			Sweet Cherry	<LOQ (0.35)	<LOQ (0.32)

[a] Year 1 data is for CA-10 trial that was started to replace CA-2
<LOQ= <1 ppb for leaf, whole flower, pollen and anther samples; and <0.5 ppb for nectar samples.
ND= <LOD= ≤0.5 ppb for leaf, whole flower, pollen and anther samples; and ≤0.25 ppb for nectar samples

7. STATISTICAL ANALYSIS

Study Objectives and Design

The study was conducted to determine the concentration of thiamethoxam and its metabolite CGA322704 in flowers, anthers, nectar, and pollen of stone fruit trees in response to previous year's foliar application of a thiamethoxam pesticide product. In year 1 of the study, two foliar sprays at an application rate of 0.086 lbs/acre were applied at 21 to 14 days before harvest of the fruit for a total application of 0.172 lbs/ai. Leaves were sampled after the second foliar spray and analyzed for concentrations of thiamethoxam and CGA322404 metabolite. Flower parts and leaves were harvested in the second year of the study following the foliar applications and also analyzed. The crops at the same study sites received a second set of foliar treatments in the second year and the same sampling scheme was then followed with the study due for completion upon sampling at bloom in the third year.

Initially there were 9 sites, denoted as CA-1 through CA-9, chosen for the study with 3 sites planted with plums, 3 sites planted with sweet cherries, and 3 sites planted with peach. The plum variety at site C2 named 'Apple Dandy' did not produce sufficient pollen for sampling so in year 2 another site, denoted CA-10, was substituted that was planted to a plum variety that produced sufficient pollen. An amended final report was submitted that contained data for the first replicate year of site CA-2 and for two years of data at site CA-10. Site CA-2 was omitted from further analysis due to incomplete data.

The data call-in specified that the test sites were to be distributed across general soil texture categories with 3 sites each in coarse, medium, and fine-textured soils. The amended report contains information on particle size distribution, organic matter content, cation exchange capacity, pH and percent moisture held at 1/3 bar. Based on this data, sites CA-3 and CA-9 are classified as coarse-textured soil; Sites CA-1, CA-4, CA-6, CA-8, and CA-10 are classified as medium-textured soil; and Sites CA-5 and CA-7 as fine-texture soil.

Chemical analytical results that were below detection limits were reported as less than the limit of quantification (LOQ), less than the limit of detection (LOD), and ND, an apparent acronym for not-detected. In the footnotes on pages 181 and 448 of the report, the ND designation appears to be the same as LOD. For example on page 181 the footnote is: 'ND=<LOD=<5 ng/g for leaf samples'. In the tables, data are indicated as <LOD but are also denoted ND. Both of these acronyms appear to indicate values below the LOD so they were treated as the same. A different laboratory analyzed the data obtained from year 2 where results specified as <LOQ also had a value noted in parentheses. For consistency between years, one-half the value of the LOQ rather than the noted value in parentheses was assigned to the data. There were a few differences noted in the detection levels between the laboratories. The LOQ for leaf samples in the first year was at 10ng/g whereas for the second year it was at 1 ng/g. Leaf samples taken directly after thiamethoxam application were all greater than 10 ng/g so the difference in LOQ did not affect these analyses. For samples taken at bloom, most of the concentrations were below either the LOQ or the LOD so substituting different values for each year would bias comparisons made between years. Subsequently statistical analyses were not conducted on data for leaf samples taken at bloom. The very low leaf concentration concentrations measured at bloom in the second set of analyses logically indicates lack of thiamethoxam parent or degradate residues in leaves at this sampling interval. Differences were also noted for the LOD for pollen and nectar samples between years where in the first year the values were slightly lower: For pollen LOD was at 0.35 ng/g in the first year compared to 0.5 ng/g in the second year; For nectar the LOD was at 0.186 ng/g in the first year compared to 0.25 ng/g in the second year. For consistency, one-half the LOD of the value noted in the second year was substituted for data indicated as <LOD or as ND for thiamethoxam parent and degradate. Detection limits are given in Table 8.

Non-parametric statistical tests were used to test for differences in distribution of concentrations between years, between soil type and between untreated and treated sites. Non-parametric tests do not require tests for normality as they are robust to differences in distribution and they are also robust for experimental designs with low replicates (Helsel and Hersch, 2002). The PROC NPAR1WAY procedure in the Statistical Analysis System (SAS) statistical package was used to conduct Wilcoxon-Mann – Whitney (Wilcoxon), Median non-parametric, and Kuiper tests. A significant result from the Wilcoxon test indicates differences in the shape of distributions; A significant result from the Median test indicates differences in the location of the medians between distributions; and A significant result from the Kuiper test indicates differences in the empirical distributions between two groups. The Exact option for each statistic was implemented as it provides permutation testing, a statistical method that minimizes the effect of sample size and distributional differences. Using the Exact option the Monte Carlo procedure was also implemented which provided 10,000 separate runs for each statistic to produce the permutation distributions. The test for potential differences due to soil type had 3 levels so the DSCF option in PROC NPAR1WAY, which invokes the Dwass, Steel, Critchlow-Fligner multiple comparison test , was used to provide pairwise tests for two-sample rankings. Additional procedures used for descriptive statistics were PROC MEANS to calculate mean values from the replicates at each site, PROC CAPACITY to produce cumulative statistics, and PROC BOX plot to produce comparative graphics. Statistical analysis for effect of years and soil type were conducted on the mean of the replicate samples taken from each site. Since many of the data at bloom were indicated below detection limits, graphical comparisons are presented on data transformed to a natural logarithm scale, providing clearer contrasts between the distributions. Final presentation of the potential distribution of concentrations in bee relevant plant matrices is based on all raw data because these values represent the actual range of exposure to bees and other organisms that feed off the nectar and pollen of plants.

Detection rate noted for each plant matrix: Counts for the number of samples reported above each of the noted detection limits are presented in Table 9 where Table 9A contains data for treated plants and Table 9B contains data for untreated plants. For leaf analyses, the data appear as expected where sampling post application resulted in all concentrations for leaf samples above the LOQ in treated plants and below the LOQ in untreated plants. For samples taken the next year at bloom, the range in concentration for leaves between treated and untreated plants was similar where counts of most leaf concentrations were below the LOQ but above the LOD. Except for pollen samples, the distribution of detections for the other plant matrices between treated and untreated plants was similarly below detection limits. For pollen, numerous samples above the LOQ were reported for both treated and untreated plants where, for example, the overall frequency of detection for thiamethoxam was 100% for treated plants and 94% for untreated plants (Table 10).

Comparison of distribution between years: Potential difference between years was measured for two reasons. First, greater concentrations measured in year 2 would indicate potential for carry-over effects between years. Second, if there was no effect of years then the data could be pooled for subsequent tests between soil type and to untreated control data. Comparison of the distribution between years was conducted for treated plants because of the probability for detection of thiamethoxam residues. No significant difference in the distribution of concentration of residues between years was measured as indicated by the lack of significance in the majority of the non-parametric tests (Table 11). Tests for some degradation products indicated significance but most of these analyses were below detection limits so those results are most likely an artifact of the substitution of ½ of the respective detection limits. Graphical comparisons between years indicate significant overlap in the distribution of concentration for total residue between years: Figure 1 illustrates the comparison for leaf samples between years taken after application and at bloom and Figure 2 illustrates the comparison for samples taken at bloom from the flowers.

Comparison of distribution between untreated and treated plants: As indicated in the previous comparison for counts of data above the detection limits, the frequency of detection in pollen, nectar, anthers, and whole flowers was similar between treated and untreated plants. Non-parametric tests indicated no significant difference in distribution between untreated and treated plants (Table 12). Figure 3 illustrates the comparison for between treated and untreated plants for leaves and Figure 4 illustrates the comparison for bloom plant samples. For all matrices at bloom, except pollen, concentrations of thiamethoxam were below detection limits in untreated plants. The result for pollen was not typical because the expectation is for no detection of residues in plants in untreated plots. Maximum concentrations measured for pollen were similar between treated and untreated plants at 383 ng/g and 382 ng/g, respectively. The effect appears to be localized to the blooms because residues in leaves from untreated plants were essentially non-detected

Comparison of distribution between soil type: The distributions for parent thiamethoxam and degradation product were similar between the plant matrices (Figures 5 and 6). The only indication of a potential effect was for pollen samples where a comparison between the coarse and fine textured soil types indicated a trend where concentrations in trees grown in coarse textured soils tended to be greater (Table 13 and Figure 6).

Data for bee relevant matrices: Except for pollen samples, the similarity in distributions for plant matrices taken at bloom between untreated and treated plants indicate that the data reported for treated most samples indicate concentrations were below detections limits. Owing to the uncertainty as to the source for residues measured in untreated plants for pollen, the observed distributions from treated plots ostensibly determines the expected range in concentrations of thiamethoxam and CGS322704 residues in bee relevant plant samples for this combination of plant species and application scenario (Tables 14A and 14B). For pollen, the median total residue value was 30.5 ng/g with a maximum value measured at 383 ng/g. For nectar, the median total residue value was 0.4 ng/g with a maximum value at 2.6 ng/g. Although many of the values reported for anthers were below the detection limits, resulting in a low median value at 0.8 ng/g, a maximum total residue value was reported at 91.7 ng/g. In contrast, most of the whole flower concentrations were also low but the maximum total residue value was low at 5.8 ng/g in flowers. Note that many of these total values were a summation of the substituted values inserted for data reported below the detection limits.

2. Concentrations in Bee Relevant Matrices: By default, the distributions reported for treated plants in pollen and nectar in table 14A represent the expected distributions from foliar thiamethoxam treatments applied to stone fruit trees in the previous growing season. Median and maximum values for total thiamethoxam residues in pollen are 30.5 and 383 ng/g on wet weight basis and for nectar at 0.4 and 2.6 ng/g, respectively. Of note is that many of the values represent the summation of values substituted for ½ respective detections limits.

8. STUDY STRENGTHS, LIMITATIONS AND CONCLUSIONS

In the context of documenting the magnitude of thiamethoxam residues in bee-related matrices of stone fruit (peach, plum, and sweet cherry), the following strengths are observed with this study.

1. Data provide quantitative values for thiamethoxam and the major degradation product, CGA-322704, expected in leaves, flowers, anthers, pollen, and nectar of various stone fruit when measured at bloom in response to foliar applications made in the previous growing season.

2. The study was replicated over two years. Each year, whole flower, anther, pollen, and nectar samples were collected at early bloom stage (50–75% bloom) in the spring following test substance applications in the fall.

3. Sites CA-3 and CA-9 are classified as coarse-textured soil; Sites CA-1, CA-4, CA-6, CA-8, and CA-10 are classified as medium-textured soil; and Sites CA-5 and CA-7 as fine-texture soil, allowing for a comparison of the effect of soil type on concentrations measured in plant samples.

Limitations noted in this study include:

1. Samples were taken from three types of stone fruit (peach, plum, and sweet cherry). Since the effect of different varieties on distribution of residues is unknown, the results reflect general observations made to all planted stone fruit.

2. The frequency of detection in pollen, nectar, anthers, and whole flowers was similar between treated and untreated plants with no significant difference in the distributions between them. The similarity in distributions places a serve limit on comparing differences in distribution caused by years or varieties because results would be confused with the large amount +of background variance, i.e. what part of the measured effect is due to actual treatment difference in comparison to background variation.

Overall, considering the strengths and limitations of this study, the following conclusions can be drawn:

Classification/Utility for Bee Risk Assessment. This study is classified as acceptable. Although the distribution of thiamethoxam residues in leaves indicated large differences between untreated and treated plants when sampled soon after the second applications, differences were not measured between untreated and treated plants when samples were taken at bloom for all matrices, except for pollen. The similarity in distributions places a serve limit on comparing differences in distribution caused by years or varieties because results would be confused with the large amount of background variance, i.e. what part of the measured effect is due to actual treatment difference in comparison to background variation.

Magnitude of Residues in Bee-relevant Matrices. By default, the distributions reported for treated plants in pollen and nectar in table 14A represent the expected distributions from foliar thiamethoxam treatments applied to stone fruit trees in the previous growing season. For data from treated plot, the median and maximum values for total thiamethoxam residues in pollen are 30.5 and 383 ng/g on wet weight basis and for nectar at 0.4 and 2.6 ng/g, respectively. Of note is that many of the values represent the summation of values substituted for ½ respective detections limits.

Temporal Variability in Residues. This study was not designed for temporal analysis of declining concentrations, but rather, to provide a snapshot of residue concentrations during flowering. Samples were collected at only one-time point during bloom.

Effect of Soil Type. There was generally no difference in the magnitude and distribution of concentrations of thiamethoxam and degradate between soils. One trend was indicated for pollen samples where concentrations tended to be greater in coarse textured soils when compared to fine textured soils. This effect requires more study for confirmation.

Pesticide Carryover. In general, there was no significant difference in the distribution of concentration of residues between years. Graphical comparisons between years indicate significant overlap in the distribution of concentration for total residue between years and, subsequently, no carryover effect.

9. STUDY VALIDITY/CLASSIFICATION

This study is classified as acceptable. The data from this study provide an expected distribution of the concentrations of thiamethoxam residues that bees are exposed to in extra-floral nectar, nectar, and pollen of stone fruits grown under actual agronomic practices in California. Relating concentrations measured in flower parts to bee health is possible by comparing the concentrations measured in bee relevant plant parts to target values that define acute or chronic exposure scenarios. However, differences were not measured between untreated and treated plants when samples were taken at bloom for all matrices. The similarity in distributions places a serve limit on comparing differences in distribution caused by years or varieties because results would be confused with the large amount +of background variance, i.e. what part of the measured effect is due to actual treatment difference in comparison to background variation.

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Table 8. Detection limits reported for thiamethoxam and CGA322704 for each year of the study and for each plant sample. Note that the for nectar and pollen samples the slightly higher values for one-half the ND/LOD from the second year were used for substitution.

Year and Plant Sample	Detection Limit Noted for Thiamethoxam and CGA322704			
	LOQ (ng/g Wet Weight)	1/2 LOQ (ng/g Wet Weight)	ND/LOD (ng/g Wet Weight)	1/2 ND/LOD (ng/g Wet Weight)
Year 1				
Leaf	10.00	5.00	5.00	2.50
Flower	1.00	0.50	0.50	0.25
Nectar	0.50	0.25	0.186	0.093
Pollen	1.00	0.50	0.35	0.18
Year 2				
Leaf	1.00	0.50	0.50	0.25
Flower	1.00	0.50	0.50	0.25
Nectar	0.50	0.25	0.25	0.125
Pollen	1.00	0.50	0.50	0.25
Year 1 and 2				
Anthers	1.00	0.50	0.50	0.25

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Table 9A. Treated Plants: Counts of chemical analytical results for thiamethoxam and CGA322704 that were indicated as above the LOQ, between the LOQ and LOD, and below the LOD.

Year and Plant Sample	Treated Plants: Comparison of total Number of Samples to Results Reported Above the LOQ, Between the LOQ and LOD, or Below the ND/LOD							
	Thiamethoxam				CGA322704			
	Total Number	Number >LOQ	Number LOQ>LOD	Number <ND/LOD	Total Number	Number >LOQ	Number LOQ>LOD	Number <ND/LOD
Year 1								
Leaves: After Application	27	27	0	0	27	27	0	0
Leaves: At Bloom	27	1	24	2	27	3	24	0
Anthers	27	3	6	18	27	4	1	22
Pollen	23	23	0	0	23	9	8	6
Whole Flowers	27	4	7	16	27	2	4	21
Nectar	27	3	11	13	27	0	8	19
Year 2								
Leaves: After Application	27	27	0	0	27	27	0	0
Leaves: At Bloom	27	6	3	18	27	17	6	4
Anthers	27	9	5	13	27	13	7	7
Pollen	27	27	0	0	27	18	3	6
Whole Flowers	27	8	2	17	27	1	3	23
Nectar	27	7	6	14	27	6	4	17

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Table 9B. Untreated Plants: Counts of chemical analytical results for thiamethoxam and CGA322704 that were indicated as above the LOQ, between the LOQ and LOD, and below the LOD.

Year and Plant Sample	Untreated Plants: Comparison of total Number of Samples to Results Reported Above the LOQ, Between the LOQ and LOD, or Below the ND/LOD							
	Thiamethoxam				CGA322704			
	Total Number	Number >LOQ	Number LOQ>LOD	Number <ND/LOD	Total Number	Number >LOQ	Number LOQ>LOD	Number <ND/LOD
Year 1								
Leaves: After Application	9	0	8	1	9	1	8	0
Leaves: At Bloom	9	0	8	1	9	0	8	1
Anthers	9	5	0	4	9	1	0	8
Pollen	8	7	1	0	8	1	2	5
Whole Flowers	9	2	0	9	9	0	1	8
Nectar	9	4	3	2	9	0	3	6
Year 2								
Leaves: After Application	9	0	0	9	9	0	0	9
Leaves: At Bloom	9	0	0	9	9	0	1	8
Anthers	9	2	1	6	9	5	2	2
Pollen	9	9	0	0	9	5	4	0
Whole Flowers	9	0	0	9	9	0	0	9
Nectar	9	0	1	8	9	2	1	6

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Table 10. Comparison of the frequency of detection of thiamethoxam and CGA322704 in leaves, anthers, Counts of chemical analytical results for thiamethoxam and CGA322704 that were indicated as above the LOQ, between the LOQ and LOD, and below the LOD.

Chemical and Plant Sample	Overall Frequency (%) of Samples Reported Above the LOQ, Between the LOQ and LOD, or Below the LOD					
	Treatment Treated			Untreated Trees		
	Proportion Above LOQ	Proportion Below LOQ	Proportion Below LOD	Proportion Above LOQ	Proportion Below LOQ	Proportion Below LOD
Thiamethoxam						
Leaves: After Application	100	0	0	0	44	56
Leaves: At Bloom	13	50	37	0	44	56
Anthers: At Bloom	22	20	57	39	6	56
Pollen: At Bloom	100	0	0	94	6	0
Whole Flowers: At Bloom	22	17	61	11	0	89
Nectar: At Bloom	19	31	50	22	22	56
CGA322704						
Leaves: After Application	100	0	0	6	44	50
Leaves: At Bloom	37	56	7	0	50	50
Anthers: At Bloom	31	15	54	33	11	56
Pollen: At Bloom	54	22	24	35	35	29
Whole Flowers: At Bloom	6	13	81	0	6	94
Nectar: At Bloom	11	22	67	11	22	67

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Table 11. Effect of Years: Exact probability levels for Wilcoxon and Median non-parametric tests for differences in the distribution of thiamethoxam and CGA322704 degradate between years.

Plant Sample and Interval	Exact Probability Levels for Non-parametric Tests of Differences Between Years								
	Thiamethoxam Treated Plants								
	Thiamethoxam			CGA322704			Total Residue		
	Wilcoxon	Median	Kuiper	Wilcoxon	Median	Kuiper	Wilcoxon	Median	Kuiper
Leaves: After Application	0.6	1	0.89	0.6	1	0.37	0.54	0.63	0.97
Anthers	0.4	0.35	1	0.02	0.06	0.09	0.039	0.35	0.89
Pollen	0.09	0.35	0.89	0.05	0.35	0.57	0.1	0.34	0.89
Whole Flowers	0.73	1	0.89	0.98	1	1	0.82	1	1
Nectar	0.6	1	0.89	0.48	1	0.89	0.51	0.69	0.57

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Table 12. Untreated vs Treated Plants: Exact probability levels for Wilcoxon and Median non-parametric tests for differences in the distribution in chemical analyses conducted on untreated control plants and plants treated with a foliar spray of thiamethoxam.

	Exact Probability Levels for Non-parametric Tests of Differences in Concentration Distribution Between Untreated Control and Treated Plants								
	Thiamethoxam			CGA322704			Total Residue		
Plant Sample	Wilcoxon	Median	Kuiper	Wilcoxon	Median	Kuiper	Wilcoxon	Median	Kuiper
Anthers	0.89	1	0.89	0.42	1	0.89	0.53	1	0.89
Pollen	0.34	0.34	0.89	0.15	0.34	1	0.35	0.33	0.89
Whole Flowers	0.07	0.06	0.57	0.21	0.56	1	0.03	0.06	0.27
Nectar	0.5	1	0.89	0.86	1	1	0.93	1	0.89

Table 13. Effect of Soil: Exact probability levels for Wilcoxon and Median non-parametric tests for differences in the distribution of thiamethoxam and CGA322704 degradate between trees grown in different soil types.

A) Non-Parametric Test Results

Plant Sample and Interval	Exact Probability Levels for Non-parametric Tests of Differences Between Soil Type		
	Thiamethoxam	CGA322704	Total Residue
	Wilcoxon	Wilcoxon	Wilcoxon
Leaves: After Application	0.039	0.11	0.045
Anthers	0.48	0.89	0.7
Pollen	0.08	0.78	0.08
Whole Flowers	0.64	0.22	0.19
Nectar	0.32	0.62	0.41

B**) Pollen Pairwise Tests Between Soil Types**

Pairwise Two-Sided Multiple Comparison Analysis			
Dwass, Steel, Critchlow-Fligner Method			
Variable: mthia			
soil	Wilcoxon Z	DSCF Value	Pr > DSCF
Medium vs. Coarse	1.6971	2.4	0.2063
Medium vs. Fine	-1.2728	1.8	0.4106
Coarse vs. Fine	-2.3094	3.266	0.0545

MRID 50096606 CDPR THX Stone Fruit

Table 14A. Distribution of thiamethoxam and CGA322704 degradate measured in pollen, nectar, and anthers sampled from stone fruit that were exposed to two applications of thiamethoxam in the year previous to bloom. Samples were combined from two consecutive years of study.

	Pollen			Nectar			Anthers		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	50	50	50	54	54	54	54	54	54
Mean (ng/g)	54.2	1.1	55.3	0.4	0.2	0.6	2.9	1.6	4.5
SD (ng/g)	75.5	1.2	75.5	0.5	0.3	0.6	12.0	2.9	13.1
CV (%)	139.3	104.7	136.5	135.7	117.0	98.8	420.4	175.2	290.9
Min (ng/g)	0.4	0.2	0.5	0.1	0.1	0.3	0.3	0.3	0.5
Median (ng/g)	29.6	0.9	30.5	0.2	0.1	0.4	0.3	0.3	0.8
75th (ng/g)	63.3	1.6	63.6	0.3	0.3	0.5	0.5	2.2	3.5
90th (ng/g)	132.0	2.4	133.2	1.0	0.5	1.6	4.4	4.4	7.9
95th (ng/g)	181.6	2.6	182.1	1.8	0.7	2.0	7.2	5.8	11.7
Max (ng/g)	382.0	5.9	383.0	2.4	1.7	2.6	87.5	15.8	91.7
% of Total	98.0	2.0		60.7	39.3		63.7	36.3	

MRID 50096606 CDPR THX Stone Fruit

Table 14B. Distribution of thiamethoxam and CGA322704 degradate measured in whole flowers and leaves sampled either after application or in the next year after application at bloom. Samples are from stone fruit that were exposed to two applications of thiamethoxam in the year previous to bloom. Samples were combined from two consecutive years of study.

Statistic	Whole Flowers			Leaves: Post Application			Leaves: At Bloom		
	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	54	54	54	54	51	51	54	54	54
Mean (ng/g)	0.9	0.3	1.2	6741.6	351.0	7197.7	1.9	2.1	4.0
SD (ng/G)	1.4	0.2	1.4	3648.8	188.7	388.5	1.9	1.3	2.7
CV (%)	154.9	67.7	114.3	54.1	53.8	53.4	95.6	63.0	68.2
Min (ng/g)	0.3	0.3	0.5	2097.0	110.0	2273.0	0.3	0.3	0.5
Median (ng/g)	0.3	0.3	0.8	5257.5	308.0	5630.0	2.5	2.5	5.0
75th (ng/g)	0.5	0.3	1.3	9508.0	399.0	9828.0	2.5	2.5	5.0
90th (ng/g)	3.5	0.5	4.2	11723.0	478.0	12006.4	4.4	3.4	5.7
95th (ng/g)	4.9	1.1	5.2	13116.2	766.7	14004.4	4.5	5.3	10.9
Max (ng/g)	5.5	1.2	5.8	20996.5	1232.4	22229.0	10.4	6.3	12.5
% of Total	71.9	27.3		93.7	4.9		47.5	52.5	

MRID 50096606 CDPR THX Stone Fruit

Figure. 1. Year Comparison for Leaves: Comparison of the distribution between years for leaf thiamethoxam residues transformed to natural logarithms where sampled were obtained either directly after foliar application of thiamethoxam denoted 'PostApp' or at bloom in the year following application denoted 'Bloom'.

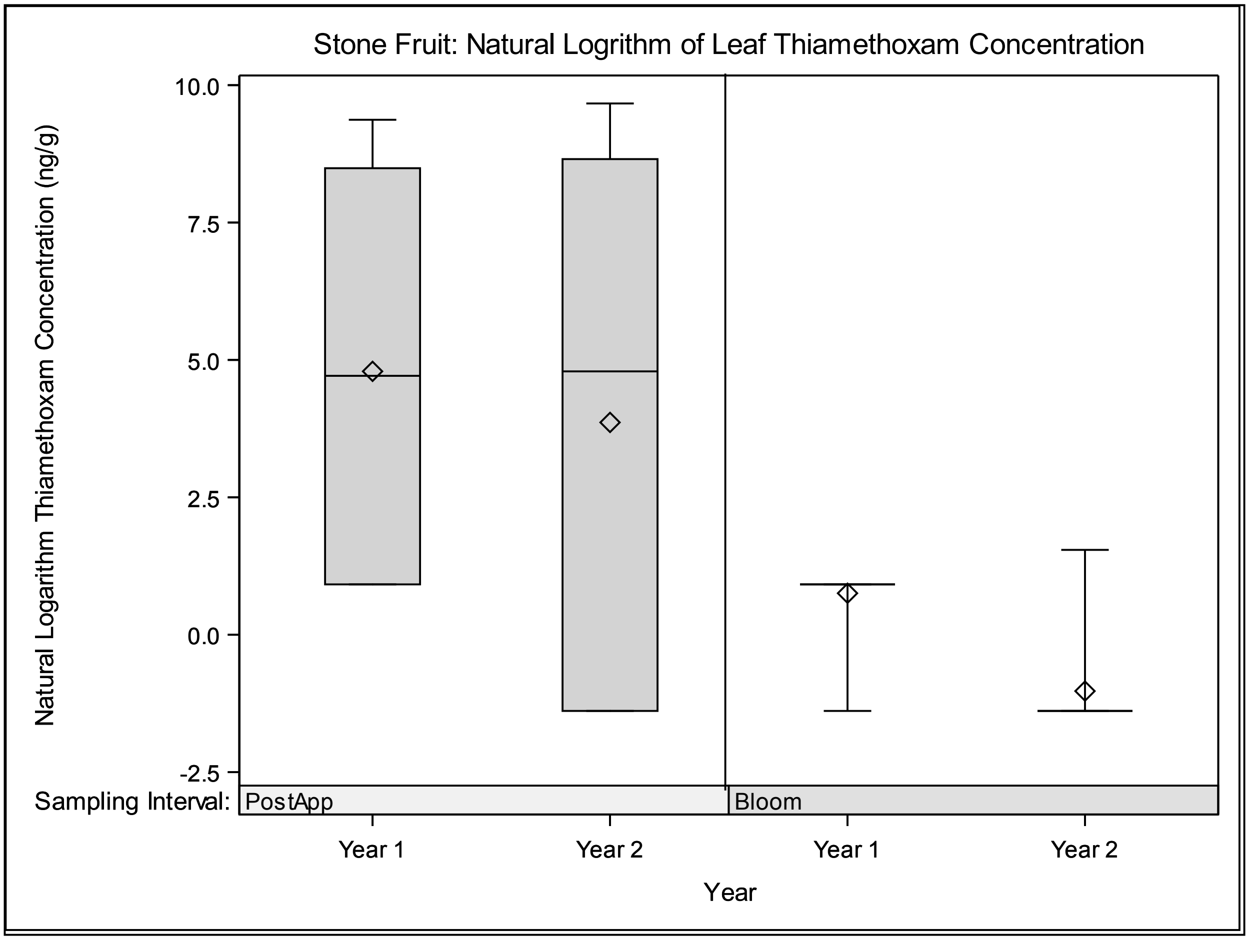


Figure 2. Year Comparison for Bloom Plant Samples: Comparison of the distribution between years for nectar, pollen, anthers, and whole flowers thiamethoxam residues transformed to natural logarithms. Samples were taken in the year that followed a previous foliar application.

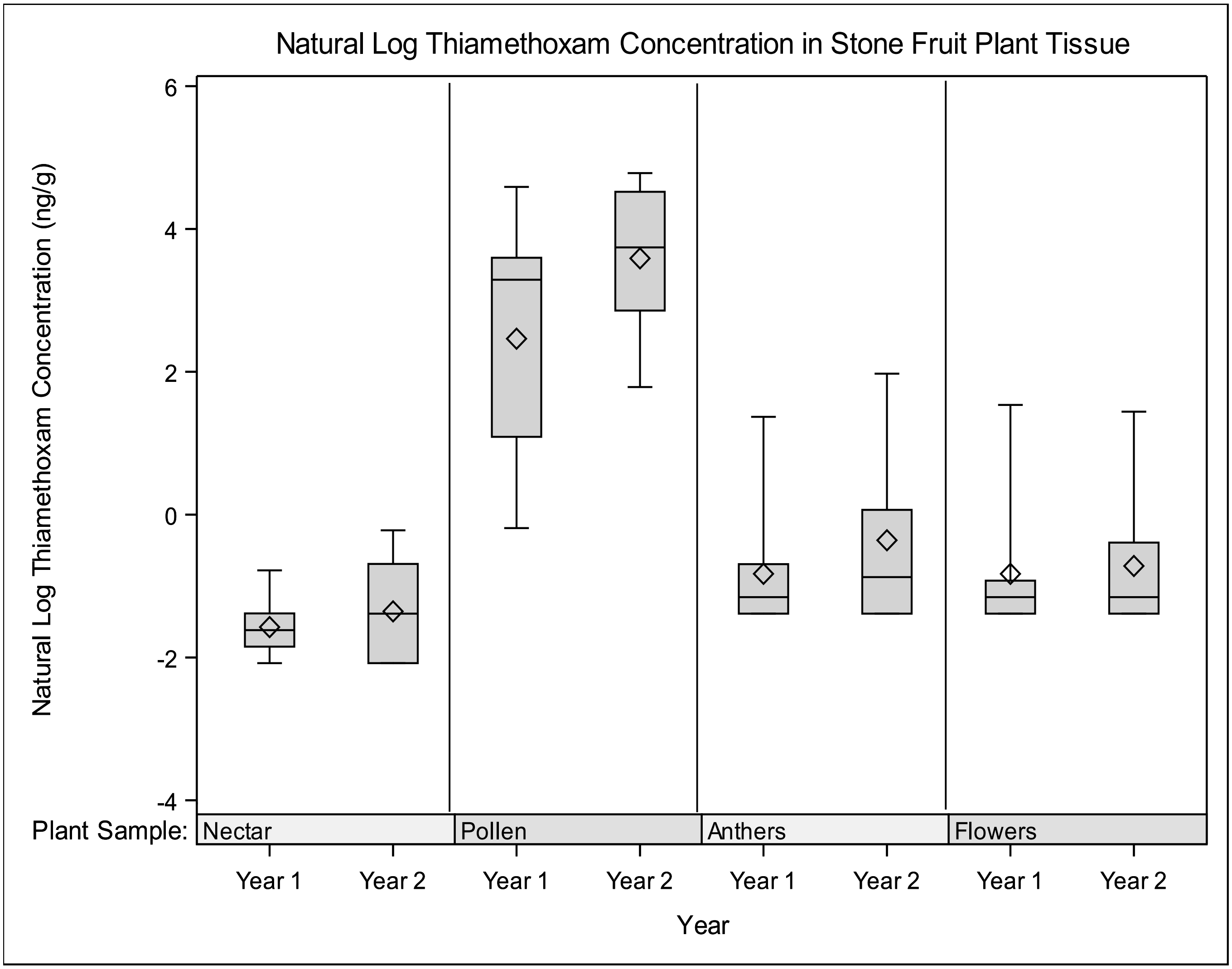


Figure 3. Untreated vs Treated Leaf Samples: Comparison of the distribution of total thiamethoxam residues measured between leaves of untreated control (UTC) and treated (TRT) stone fruit trees where samples were obtained after the second foliar spray application denoted 'PostApp' and then at bloom in the next year denoted 'Bloom' .

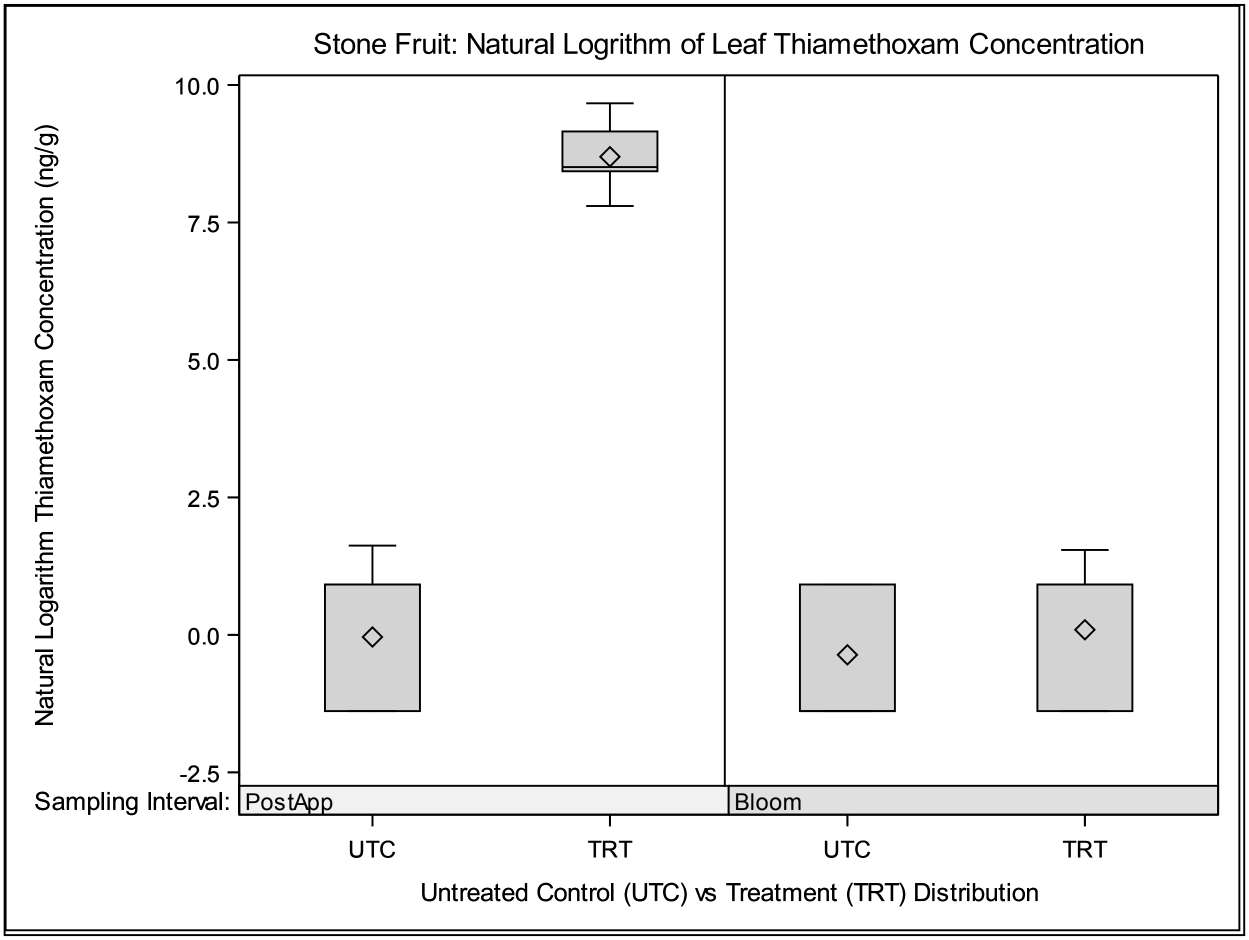
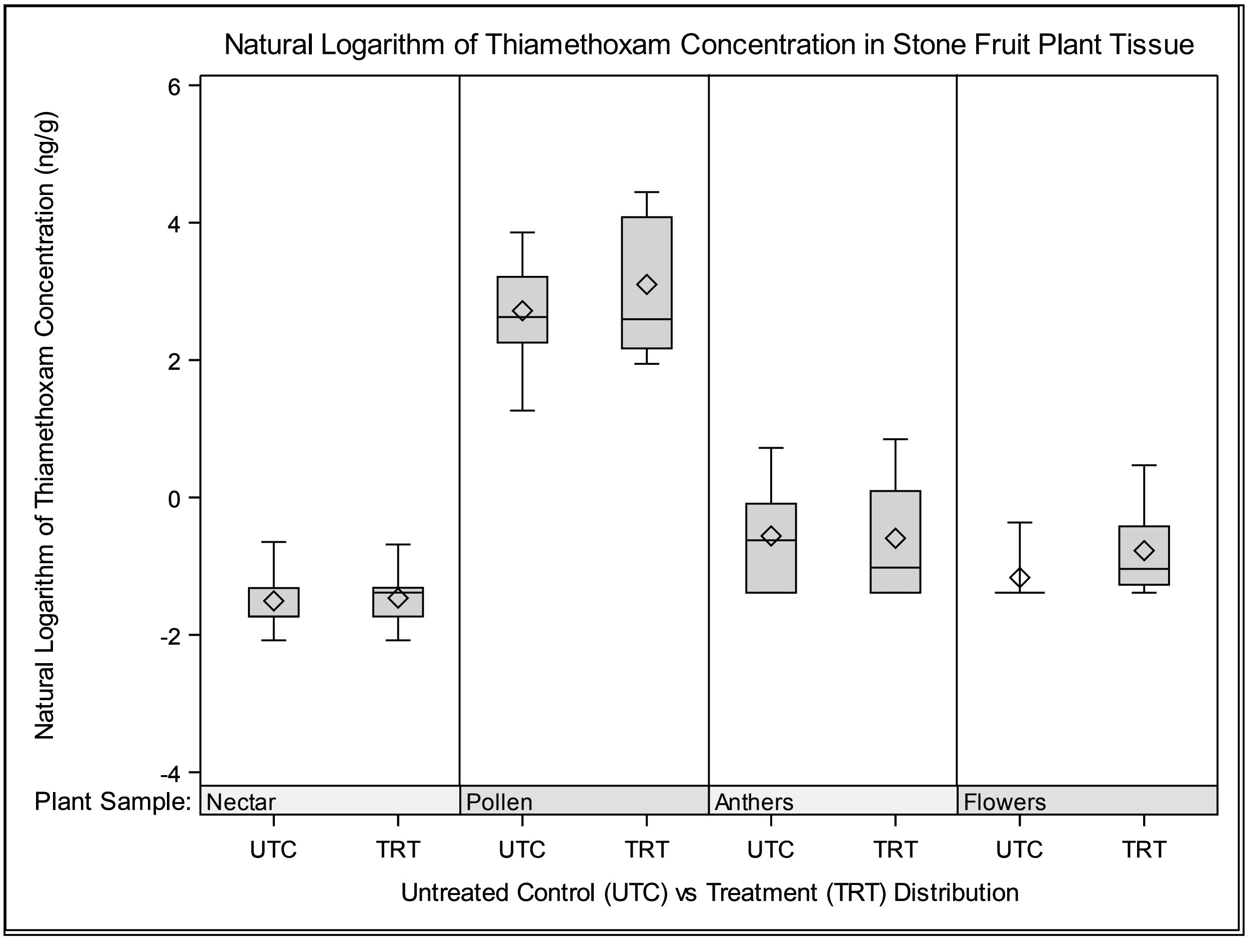


Figure 4. Untreated vs Treated Bloom Plant Samples: Comparison of the distribution of thiamethoxam residues measured between untreated control (UTC) and treated (TRT) plants A) Pollen and B) nectar, anthers, and whole flowers. Samples were taken in the year that followed a previous foliar application.



MRID 50096606 CDPR THX Stone Fruit

Figure 5. Soil Texture Comparison for Leaf Analyses: Comparison of the distribution of thiamethoxam residues measured between stone fruit trees grown in coarse, medium, and fine-textured soils. Samples were taken in the year that followed a previous foliar application.

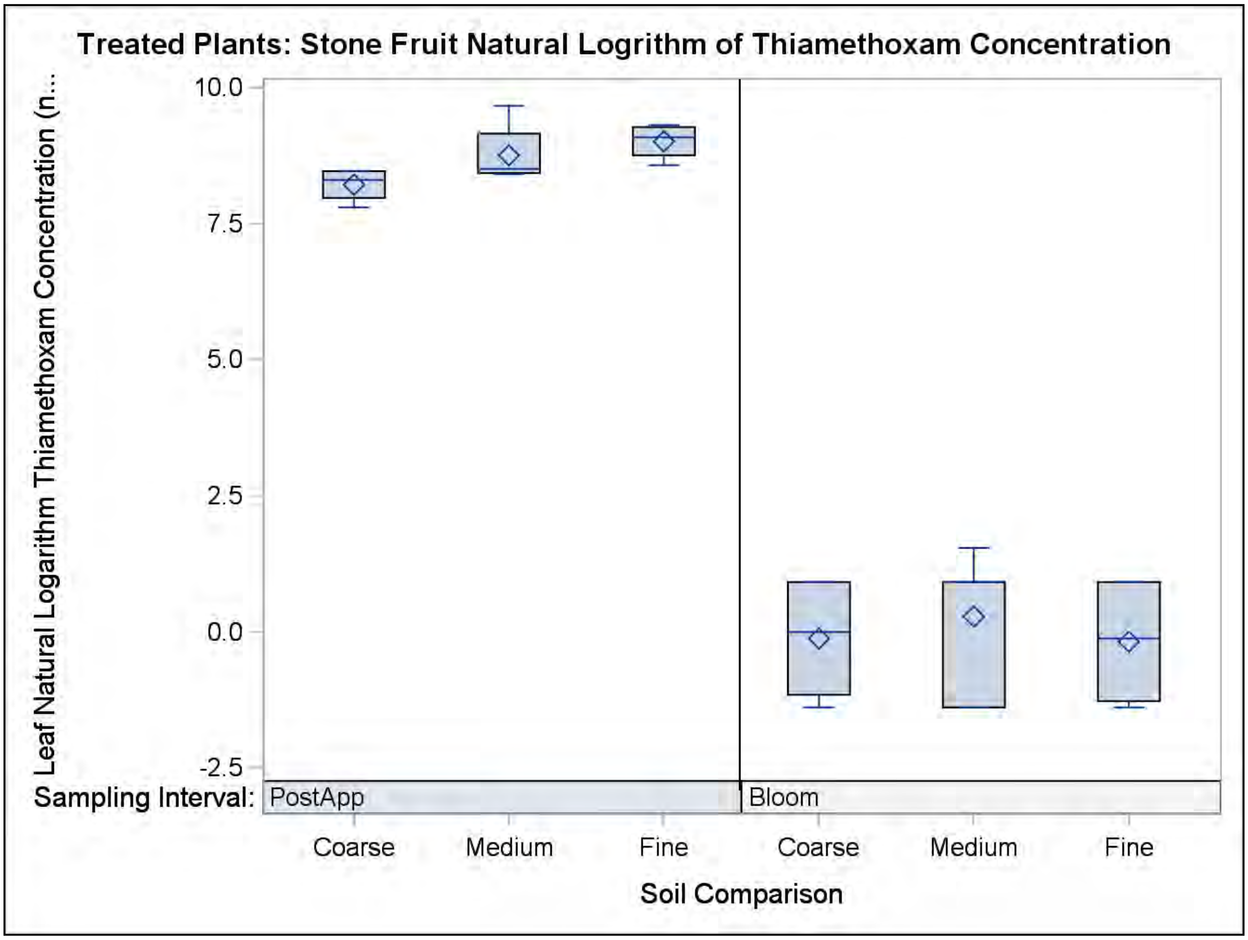
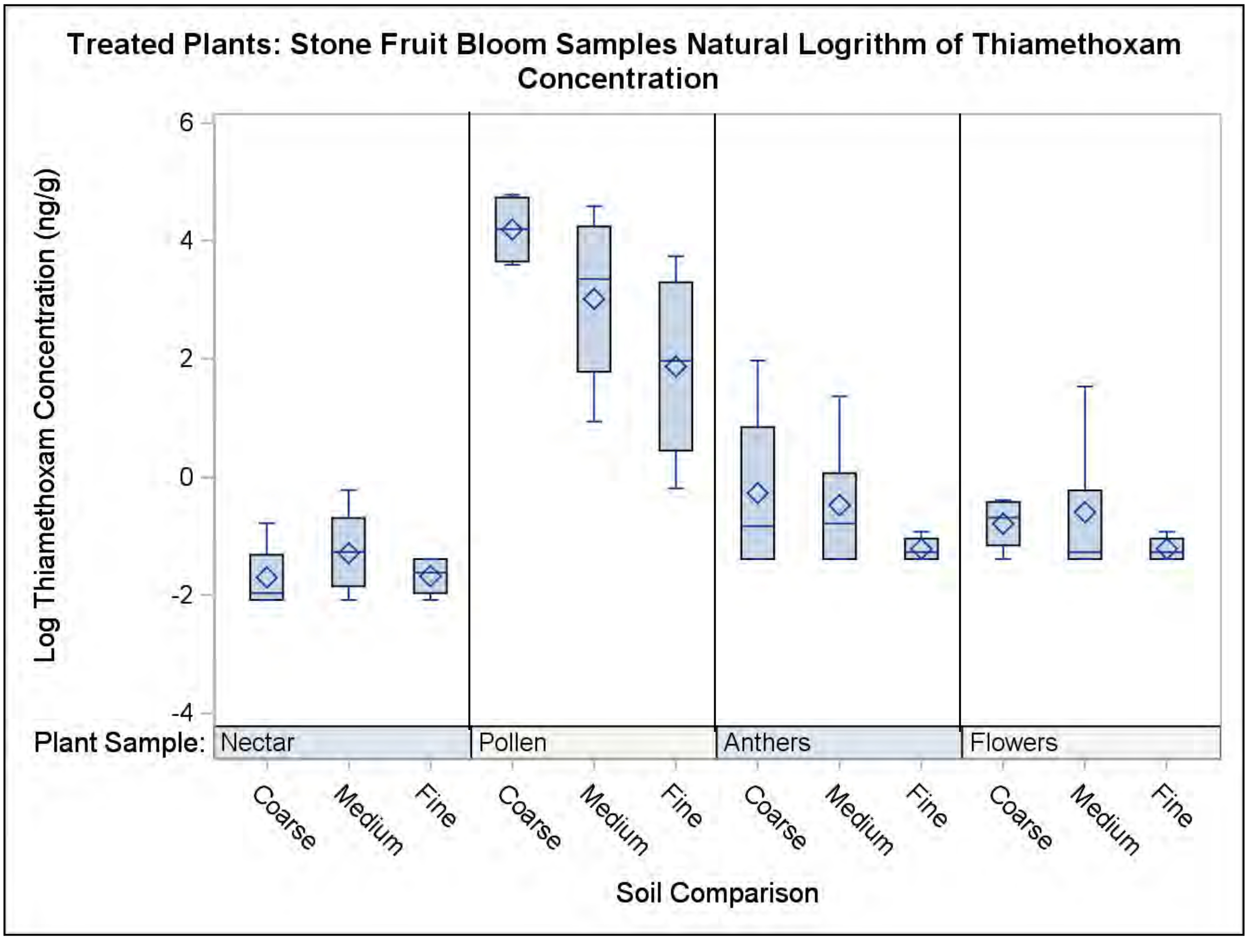


Figure 6. Soil Texture Comparison for Flower Analyses: Comparison of the distribution of thiamethoxam residues measured between stone fruit trees grown in coarse, medium, and fine-textured soils. Samples were taken in the year that followed a previous foliar application.



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MRID 50265502 CDPR THX Strawberry

Reference
Trask, J. (2017) Thiamethoxam 25 WG (A9584C) - Magnitude of Residues in Pollen, Nectar, Flowers, and Leaves of Strawberry After Foliar Application with Actara 25WG in California: Final Report. Report Number: TK0177224. Unpublished study prepared by Syngenta Crop Protection, LLC. 346. MRID 50265502, CDPR Study ID 301205, Data Volume 52691-0574, Tracking ID# 282051

1. STUDY INFORMATION

Chemical:	Thiamethoxam	**PC Code**	60109
Test Material:	Actara 25WG	**Percent Active Ingredient:**	25%
Study Type:	Study to measure Thiamethoxam and CGA322704 residues in strawberry pollen, nectar, flowers and leaves from nine field trials that received foliar applications of Actara 25 WG for two growing seasons.		
Sponsor:	Syngenta Crop Protection, LLC 410 Swing Road Greensboro, North Carolina 27409	**Experiment Start and End Date:**	April 28, 2014 – June 1, 2017
Sponsor Study ID:	TK0177224	**Study Locations:**	Nine strawberry field trials located in in the Central Valley and Coastal Region of California.
Study Completion Date:	June 1, 2017		
GLP Status:	Non-GLP; protocol reviewed by CDPR. [CDPR Study ID 301205, Data Volume 52691-0574, Tracking ID# 282051]		

2. REVIEWER INFORMATION

Study Reviewed by: California Department of Pesticide Regulation	Richard Bireley, Sr. Environmental Scientist (Specialist) John Troiano, Ph.D., Research Scientist III Alexander Kolosovich, Sr. Environmental Scientist (Specialist) Brigitte Tafarella, Environmental Scientist Denise Alder, Sr. Environmental Scientist (Specialist) Russell Darling, Environmental Scientist

3. EXECUTIVE SUMMARY

A nine-site field trial study on strawberries was conducted in the primary strawberry producing areas of California. This study was conducted to measure the levels of thiamethoxam and its major metabolite, CGA322704, in leaves, flowers, soil, pollen, and nectar of strawberries after foliar treatment applications of Actara® 25WG were made at the maximum labeled use rate. Residue data for thiamethoxam and its major metabolite, CGA322704, in the pollen and nectar of strawberry were requested by the California Department of Pesticide Regulations (CDPR) as part of the reevaluation of the nitroguanidine class of neonicotinoid insecticides (Article 8, Subchapter 1, Chapter 2, Division 6 of Title 3 of the California Code of Regulations), according to the “Data Requirements Regarding Re-evaluation of Certain Neonicotinoid Products” (Prichard, September 15, 2009) and “Recommendations for Additional Thiamethoxam

Residue Studies in Almond, Cotton, and Strawberry" (Bireley and Troiano, October 4, amended December 7, 2012). This study was designed to satisfy this request.

4. STUDY VALIDATION

Guideline Followed:	TBD; (protocol was reviewed by CDPR)
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	**TBD**
Rationale:	N/A
Reparability:	N/A

5. MATERIALS AND METHODS

Test Material Characterization			
Test item:	Actara 25WG	**Percent A.I.:**	25%
Formulation Type:	Water Dispersible Granule	**Batch Number:**	697333/731279
CAS #:	153719-23-4	**EPA Reg. No.**	100-938

5A. STUDY DESIGN

The study included nine geographically separated replicated trials each consisting of a non-treated and a treated plot large enough to fulfill sample collection requirements. The treated plots were divided into 3 replicate sub-plots (A, B, and C). The size of each sub-plot varied at each location, measuring 130 ft x 36.7 ft (CA-1 season 1), 110 ft x 43 ft (CA-1 season 2), 150 ft x 30 ft (CA-2 season 1), 125 ft x 30 ft (CA-2 season 2), 100 ft x 60 ft (CA-3) , 100 ft x 60 ft (CA-4), 100 ft x 50 ft (CA-5), 150 ft x 32 ft (CA-6), 150 ft x 32 ft (CA-7), 230 ft x 35 ft (CA-8) and 100 ft x 75 ft (CA-9). At each location, the control plot was located up-slope and up-wind with regard to the prevailing wind direction and separated by a minimum of 200 ft. for all sites from the treated plot to minimize potential cross-contamination by runoff or pollen transfer except CA-4, which was 75 feet due to site constraints.

The original two-year study design included test substance applications made during two growing seasons with sample collection during bloom in each season. However, after the first season application at the three central valley locations (CA-1, CA-2 and CA-3), weather conditions resulted in poor flower production and residues could not be analyzed. Therefore, the study design was modified in the following ways: (1) New plants were re-established at the sites (CA-1, CA-2 and CA-3) in the fall of 2014. Season 2 applications were made in the spring of 2015, matrices collected, and the resulting residue data is reported. (2) Two additional Central Valley trial locations were added (CA-4 and CA-5) with matrices collected after single season applications (spring of 2015). (3) A one-season study design was also used for the four coastal region trial locations (CA-6 through CA-9) with matrices collected after applications in the spring of 2015.

5B. Application Timing and Rates

Treatment specifications, including target application rate, spray timing and volume, is presented in the following table.

Table 1. Thiamethoxam Applications with Actara 25WD.

Treatment List								
TRT ID (plot)	Application Number	Application Method	Volume (GPA)	Nominal Rate (+10%) (lb ai/acre)	Tank Mix Additives	Timing	RTI (days)	Total Rate (lb ai/acre)
CA-1*								
UTC	--	Control	--	--	--	--	--	--
TRT	1	Foliar Broadcast Spray	52.9	0.063	None	25	10	0.189
	2		54.8	0.063		15	10	
	3		55.9	0.063		5	--	
	4		51.0	0.063		25	10	0.189
	5		50.7	0.063		15	11	
	6		50.4	0.063		4	--	
CA-2*								
UTC	--	Control	--	--	--	--	--	--
TRT	1	Foliar Broadcast Spray	56.0	0.063	None	25	10	0.189
	2		55.3	0.063		15	10	
	3		55.5	0.063		5	--	
	4		51.0	0.063		25	9	0.189
	5		50.9	0.063		16	11	
	6		50.4	0.063		5	--	
CA-3*								
UTC	--	Control	--	--	--	--	--	--
TRT	1	Foliar Broadcast Spray	65.3	0.063	None	25	10	0.189
	2		65.4	0.063		15	10	
	3		65.3	0.063		5	--	
	4		65.7	0.063		25	10	0.189
	5		65.8	0.063		15	10	
	6		65.7	0.063		5	--	
CA-4								
UTC	--	Control	--	--	--	--	--	--
TRT	1	Foliar Broadcast Spray	65.2	0.063	None	25	10	0.189
	2		65.7	0.063		15	10	
	3		65.8	0.063		5	--	
CA-5								
UTC	--	Control	--	--	--	--	--	--
TRT	1	Foliar Broadcast Spray	59.8	0.063	None	25	10	0.189
	2		60.2	0.063		15	10	
	3		59.6	0.063		5	--	

Treatment List								
TRT ID (plot)	Application Number	Application Method	Volume (GPA)	Nominal Rate (+10%) (lb ai/acre)	Tank Mix Additives	Timing	RTI (days)	Total Rate (lb ai/acre)
CA-6								
UTC	--	Control	--	--	--	--	--	--
TRT	1	Foliar Broadcast Spray	50.0	0.063	Adjuvant (Dnye-Amic)	25	10	0.189
	2		49.8	0.063		15	10	
	3		49.9	0.063		5	--	
CA-7								
UTC	--	Control	--	--	--	--	--	--
TRT	1	Foliar Broadcast Spray	74.9	0.063	Adjuvant (Spreader 90)	25	9	0.189
	2		74.8	0.063		16	11	
	3		74.4	0.063		5	--	
CA-8								
UTC	--	Control	--	--	--	--	--	--
TRT	1	Foliar Broadcast Spray	69.7	0.063	Adjuvant (Kinetic)	25	10	0.189
	2		69.8	0.063		15	10	
	3		70.2	0.063		5	--	
CA-9								
UTC	--	Control	--	--	--	--	--	--
TRT	1	Foliar Broadcast Spray	49.8	0.063	Adjuvant (Spreader 90)	28	12	0.189
	2		49.8	0.063		16	9	
	3		49.7	0.063		5	--	
Application Notes: GPA = gallons per acre; RTI = Retreatment Interval Adjuvants applied at labeled use rates *Season 1 (2014) applications are application #1-3; season 2 (2015) applications are application #4-6								

5C. STUDY SITE LOCATION AND CHARACTERISTICS

Table 2. Trial Site Conditions for Strawberry Trials

Trial Identification (City, State/Year)	Soil Characteristics							Meteorological Data[1]	
	Texture Class	%Sand	%Silt	%Clay	%OM	pH	CEC (meq/ 100g)	Study Monthly Rainfall Ranch (inches)	Overall Temperature Range (°F)
CA-1 Porterville, California 2014-2015	Clay Loam	37	23	40	1.8	6.9	28.9	0.00 to 2.27	22 to 107

Trial Identification (City, State/Year)	Soil Characteristics							Meteorological Data[1]	
	Texture Class	%Sand	%Silt	%Clay	%OM	pH	CEC (meq/ 100g)	Study Monthly Rainfall Ranch (inches)	Overall Temperature Range (°F)
CA-2 Porterville, California 2014-2015	Loamy Sand	81	13	6	0.72	5.7	14.5	0.00 to 2.27	22 to 107
CA-3 Yuba City, California 2014-2015	Sandy Clay Loam	53	25	22	1.9	6.3	13.4	0.00 to 7.92	23 to 106
CA-4 Woodland, California 2015	Loam	47	31	22	1.6	7.9	18.0	0.09 to 7.10	27 to 107
CA-5 Fresno, California 2015	Loamy Sand	82	14	4	0.53	7.7	4.5	0.00 to 1.28	37 to 107
CA-6 Nipomo, California 2015	Sandy Loam	71	19	10	1.2	7.8	11.1	0.00 to 4.02	32 to 98
CA-7 Guadalupe, California 2015	Loamy Sand	77	17	6	0.85	7.9	9.6	0.00 to 4.03	32 to 98
CA-8 Salinas, California 2015	Clay Loam	33	33	34	2.3	7.6	24.5	0.00 to 3.06	30 to 98
CA-9 Salinas, California 2015	Clay Loam	37	33	30	2.1	7.3	21.7	0.00 to 3.06	30 to 98

OM= Organic Matter, CEC= Cation Exchange Capacity

[1] Precipitation and air temperature data summarized are representative of the time period (whole months) from planting through sample collection for each trial.

The weather conditions (high air temperatures) at CA-1, CA-2 and CA-3 in the spring and summer of 2014 resulted in poor flower production for the first season of this study. Therefore, new plants were re-established in November 2014 to the same plots. Irrigation was used to supplement rainfall at all trial locations and is presented in the Field Trial Summaries (Appendix 1 of the study report).

5D. SAMPLE COLLECTION, HANDLING, PROCESSING

For all trials, the non-treated control plots were sampled first or by separate personnel, to prevent contamination. For each matrix, one sample was collected from each treated replicate plot A, B, and C, as well as from the control plot.

Leaf and Whole Flower

At all field sites, leaf and whole flower samples were collected at 5 days after the third application (5 DA3A), also referred to as days after last application (DALA) during bloom. In addition, leaf samples were collected before (0 DA3A) and after bloom (4 and 8 weeks after the last application) to establish a decline curve. Target weights of 250g for leaves and 100g for flowers were collected. Additionally, bulk, non-treated leaves and flowers with target weights of 500g for leaves and 100g for flowers were collected for laboratory verification and concurrent fortifications. Leaves and flowers were collected directly into labelled sealable plastic bags and held in separate control and treated ice chests on wet or blue ice until placed into frozen storage. Samples were collected from the lower, middle and upper plant canopy for a representative, composite sample; sample weights are presented in the field trial summaries located in Appendix 1 of the study report.

Pollen and Nectar

At all field sites, pollen and nectar samples were collected at 5 DA3A during bloom. Male and female flowers were collected from the untreated control and the treated sub-plots, bagged and placed in ice chests with wet or blue ice and then transported to the field laboratory for pollen and nectar extraction. Pollen samples were extracted manually from male flowers using a plastic filtered collection tip attached to a vacuum pump. The tips were weighed before and after pollen extraction and the net weight represented the sample size. Once the target weight of 100mg was obtained, (or all flowers available for pollen sampling were used), the plastic tips containing pollen were wrapped in parafilm and placed in labeled plastic bottles. The bottles were sealed, placed in resealable plastic bags, and transferred immediately into separate freezers for the treated and untreated samples.

Female flowers were left in ice chests with blue ice for several hours after collection to maximize nectar collection. Nectar samples were collected manually by cutting off the flower petals and sepals with scissors. The remaining flower was placed into a centrifuge tube with a filter insert and mesh screen. The nectar was then harvested using a micro centrifuge by spinning for approximately five seconds. The filter insert and mesh screen was removed and the centrifuge tube was weighed. Samples were then wrapped in aluminum foil and placed into a 60-mL amber HDPE bottle. Once the target weight of 100mg was obtained, (or all flowers available for nectar sampling were used), the vials were transferred into separate freezers for the treated and untreated samples. Additionally, 3g of nectar and pollen samples were collected for analytical method development and verification.

Soil

At all field sites, soil samples were collected prior to planting and after the last leaf sampling, targeting 120 DA3A. At each sampling event, five 0-6-inch soil cores were collected from each plot (treated and untreated) and composited by plot. The target weight for soil samples was 200g.

Soil samples were placed into labelled bags and placed into frozen storage. Freezer-storage temperatures were monitored and typically were maintained at -20°C.

Sample Storage.

All residue samples (leaf, whole flower, pollen, nectar and soil) were shipped from the test sites in separate ice chests for treated and untreated with dry ice to SynTech analytical laboratory via ACDS overnight service.

Three separate storage-stability studies; MRID 44703525 (Reference 5), MRID 45108001 (Reference 6) and MRID 45659205 (Reference 7) were conducted to determine the stability of thiamethoxam (CGA293343) and its metabolite, CGA322704, in various crop matrices and soil stored under deep-freezer conditions. Storage stability for pollen and nectar stored under deep-freezer conditions was conducted in Syngenta Study No. 05-S508 (Reference 8). These studies showed that thiamethoxam and metabolite CGA322704 are stable in leaves, whole flower, pollen, and nectar for up to 12 months when stored frozen.

Additionally a storage stability study, MRID 47751401 (Reference 9), was conducted on soil stored under deep-freezer conditions for thiamethoxam and metabolite CGA322704. This study showed that thiamethoxam and CGA322704 are stable in soil for up to 12 months when stored frozen.

Therefore, residues of thiamethoxam and CGA322704 in strawberry leaf, whole flower, pollen, and soil samples should not have been adversely affected by frozen storage during this study.

5E. ANALYTICAL METHODS

Analysis of Leaf and Whole Flower

Leaf and whole flower samples were analyzed for thiamethoxam and CGA322704 based on the analytical method described in Syngenta Method REM 179.06, entitled "Residue Method for the Determination of Residues of Thiamethoxam (CGA 293343) and CGA 322704 in Lettuce, Tomato, Grape and Tobacco Samples. Final Determination by LC-MS/MS" (Reference 1). The method is presented in the Analytical Phase Report located in Appendix 2 of the study report. In summary, residues of thiamethoxam and CGA322704 were extracted with 50:50 methanol/water from 10g leaf and whole flower samples using a high-speed homogenizer. Extracts were centrifuged and concentrated via SPE cleanup in preparation for LC-MS/MS analysis. The Limit of Quantitation (LOQ) for both analytes in leaves and flowers was 1.0 ppb. The Limit of Detection (LOD) was targeted to be ≤0.5 ppb for both analytes and matrices. Field samples were diluted for analysis as appropriate, to keep the analyte response within the initial calibration range.

Analysis of Pollen and Nectar

Pollen and nectar samples were analyzed for thiamethoxam and CGA322704 based on the analytical method described in Syngenta Method REM 179.07, entitled "Thiamethoxam: Analytical Method for the Determination of Residues of Thiamethoxam (CGA293343) and CGA322704 in Bee and Hive Products. Final Determination by LC-MS/MS", with modifications found in EPL_BAS Method No. 110G747B, "Analytical Method for the Determination of Residues of Thiamethoxam and CGA3222704 in Pollen by

LC-MS/MS" (References 3 and 4). The method is presented in the Analytical Phase Report located in Appendix 2 of the study report.

In summary, residues of thiamethoxam and CGA322704 in pollen samples were extracted with methanol:0.2% formic acid in deionized (DI) water (50:50 v/v). Aliquots were diluted with DI water and stable isotope labeled internal standards were added. Sample extracts were then purified by solid-phase extraction (Oasis HLB) and analyzed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The Limit of Quantitation (LOQ) for both analytes in pollen was 1.0 ppb. Residues of thiamethoxam and CGA322704 in nectar were extracted with 50:50 methanol/water:0.2% formic acid (aq). Extracts were centrifuged and passed through a solid-phase extraction cleanup in preparation for LC-MS/MS analysis. The Limit of Quantitation (LOQ) for both analytes in nectar was 0.5 ppb. The Limit of Detection (LOD) was targeted to be ≤0.5 ppb and ≤0.25 ppb, respectively. Field samples were diluted for analysis as appropriate, to keep the analyte response within the initial calibration range.

Analysis of Soil

Soil samples were analyzed for thiamethoxam and CGA322704 based on the analytical method described in Syngenta Method T009171-04, entitled "Analytical Method for the Determination of CGA-293343 and its Degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406 and NOA-459602 in Soil by Direct Injection High Performance Liquid Chromatography with Mass Spectrometric Detection" (Reference 4). The method is presented in the Analytical Phase Report located in Appendix 2 of the study report.

In summary, soil was extracted twice with 100 mL of 20% 10mM ammonium acetate/acetonitrile for thirty minutes at room temperature using mechanical shaking. The sample was centrifuged and filtered. Solvent was evaporated via turbovap to approximately 20-25 mL. After evaporation, the aqueous sample is transferred to a centrifuge tube and diluted with 10% methanol adjusted to 0.1% acetic acid to a final volume of 50 mL. The sample was then transferred into an HPLC autosampler vial for analysis by LC/MS/MS. The Limit of Quantitation (LOQ) for both analytes, in soil, was 1.0 ppb. The Limit of Detection (LOD) was targeted to be ≤0.5 ppb. Soil sample residues were corrected for moisture in the results.

5F. QUALITY ASSURANCE RESULTS

The analytical method used to quantify residues of thiamethoxam and CGA322704 was verified on control samples prior to the analysis of treated samples in this study. The performance of the method for determination of thiamethoxam and CGA322704 residues in strawberry matrices was demonstrated by fortifying at least one non-treated control sample of each matrix with thiamethoxam and CGA322704 prior to each extraction. These fortified samples were analyzed concurrently with each analytical sample set. The concurrent fortification levels bracketed the actual residue levels found in treated samples over the course of the study. Individual recoveries are presented in the Analytical Phase Report located in Appendix 2 of the study report.

Syngenta analytical methods REM179.06 for leaves and whole flowers, REM179.07 for pollen and nectar, and T009171-04 for soil were used with modifications approved by the study director (Appendix 2 of the study report).

The methods were successfully proven by analysis of blank untreated control (UTC) samples fortified with 1.0 ppb each of thiamethoxam and CGA for leaves, whole flower, pollen, and soil, and 0.5 ppb for nectar. These data support a method limit of quantitation (LOQ) of 1.0 ppb for leaves, whole flowers, pollen, and soil, and 0.5 ppb for nectar. The LOQ is the lowest fortification level at which acceptable recovery was achieved.

For the quantitation of the analytes of interest, standard curves were prepared by injecting constant volumes of solvent-based standard solutions at appropriate concentrations (see Section 3.2). Constant volume injections were used for sample extracts as well.

Calibration curves and residue values were calculated using Analyst 1.5.1 data handling software using linear regression with 1/x weighting. Representative calibration curves are presented in Appendix 4.

For each matrix, at least one method-recovery (QC) sample per analytical set was prepared by fortifying an untreated control sample with thiamethoxam and CGA322704 at concentrations equal to the method LOQ and 10xLOQ and analyzing concurrently with the treated field samples to demonstrate adequate method performance throughout the study, i.e. recoveries of 70-120%.

Site Laboratory	Matrix	Analyte	LOQ (ppb)	LOD (ppb)
SynTech Research Laboratory Services, LLC	Leaves	Thiamethoxam	1.0	≤0.5
		CGA322704	1.0	≤0.5
	Whole Flowers	Thiamethoxam	1.0	≤0.5
		CGA322704	1.0	≤0.5
	Pollen	Thiamethoxam	1.0	≤0.5
		CGA322704	1.0	≤0.5
	Nectar	Thiamethoxam	0.5	≤0.25
		CGA322704	0.5	≤0.25
	Soil	Thiamethoxam	1.0	≤0.5
		CGA322704	1.0	≤0.5

6. RESULTS:

Table 3. Summary of Residues (ppb) for Thiamethoxam and CGA322704 in Control Samples

Sample Description/Index Number	Matrix	Thiamethoxam Residue Concentration (ppb)	CGA322704 Residue Concentration (ppb)
24.2015.CA-1.01.SB.LV.4WK-218	Leaves	<LOQ	<LOQ
24.2015.CA-1.01.SB.NC.BL.5	Nectar	61.91	<LOQ
24.2015.CA-1.01.SB.PO.BL-4	Pollen	8.12	ND
24.2015.CA-2.01.SB.NC.BL.29	Nectar	6.52	0.9

Sample Description/Index Number	Matrix	Thiamethoxam Residue Concentration (ppb)	CGA322704 Residue Concentration (ppb)
24.2015.CA-2.01.SB.PO.BL-28	Pollen	44.39	1.8
24.2015.CA-3.01.SB.LV.8WK-243	Leaves	1.06	ND
24.2015.CA-3.01.SB.NC.BL-53	Nectar	104.91[1]	ND
24.2015.CA-3.01.SB.PO.BL-52	Pollen	669.04[1]	1.73
24.2015.CA-4.01.SB.LV.BL-75	Leaves	0.51	<LOQ
24.2015.CA-4.01.SB.LV.8WK-255	Leaves	ND	1.53
24.2015.CA-4.01.SB.LV.0DA3A.253	Leaves	1.15	ND
24.2015.CA-4.01.SB.NC.BL-77	Nectar	22.4[2]	<LOQ
24.2015.CA-4.01.SB.PO.BL-76	Pollen	249.74[2]	ND
24.2015.CA-4.01.SB.SOIL.POST.78	Soil	ND	<LOQ
24.2015.CA-4.01.SB.SOIL.PRE.73	Soil	ND	<LOQ
24.2015.CA-4.01.SB.WF.BL-74	Whole Flower	0.53	ND
24.2015.CA-5.01.SB.NC.BL-101	Nectar	8.95	ND
24.2015.CA-5.01.SB.PO.BL-100	Pollen	47.33[3]	<LOQ
24.2015.CA-6.01.SB.LV.0DA3A.277	Leaves	<LOQ	ND
24.2015.CA-6.01.SB.LV.BL-123	Leaves	3.44	ND
24.2015.CA-6.01.SB.LV.8WK-279	Leaves	<LOQ	ND
24.2015.CA-6.01.SB.NC.BL.125	Nectar	1.89	ND
24.2015.CA-6.01.SB.PO.BL-124	Pollen	20.04	<LOQ
24.2015.CA-6.01.SB.SOIL.POST-126	Soil	<LOQ	ND

Sample Description/Index Number	Matrix	Thiamethoxam Residue Concentration (ppb)	CGA322704 Residue Concentration (ppb)
24.2015.CA-6.01.SB.SOIL.PRE.121	Soil	1.20	<LOQ
24.2015.CA-6.01.SB.WF.BL-122	Whole Flower	5.93	ND
24.2015.CA-7.01.SB.LV.0DA3A.289	Leaves	2.01	<LOQ
24.2015.CA-7.01.SB.LV.8WK-291	Leaves	1.5	<LOQ
24.2015.CA-7.01.SB.LV.BL-147	Leaves	1.7	<LOQ
24.2015.CA-7.01.SB.NC.BL.149	Nectar	1.16	ND
24.2015.CA-7.01.SB.PO.BL-148	Pollen	42.99[4]	3.82
24.2015.CA-7.01.SB.SOIL.POST-150	Soil	5.15	<LOQ
24.2015.CA-7.01.SB.SOIL.PRE-145	Soil	1.29	<LOQ
24.2015.CA-7.01.SB.WF.BL-146	Whole Flower	1	ND
24.2015.CA-8.01.SB.LV.0DA3A-301	Leaves	41.48	<LOQ
24.2015.CA-8.01.SB.LV.4WK-302	Leaves	2254.41[5]	75.46
24.2015.CA-8.01.SB.LV.8WK-303	Leaves	1714.64[5]	8.15
24.2015.CA-8.01.SB.LV.BL-171	Leaves	18.05	1.05
24.2015.CA-8.01.SB.NC.BL-173	Nectar	4.17	0.64
24.2015.CA-8.01.SB.PO.BL-172	Pollen	9.78	ND
24.2015.CA-8.01.SB.SOIL.POST-174	Soil	20.62	<LOQ
24.2015.CA-8.01.SB.SOIL.PRE-169	Soil	10.92	<LOQ
24.2015.CA-8.01.SB.WF.BL-170	Whole Flower	2.24	ND
24.2015.CA-9.01.SB.LV.0DA3A-313	Leaves	31.44	2.09

Sample Description/Index Number	Matrix	Thiamethoxam Residue Concentration (ppb)	CGA322704 Residue Concentration (ppb)
24.2015.CA-9.01.SB.LV.4WK-314	Leaves	28.85	1.86
24.2015.CA-9.01.SB.LV.8WK-315	Leaves	638.33[5]	48.03
24.2015.CA-9.01.SB.LV.BL.195	Leaves	30.15	1.35
24.2015.CA-9.01.SB.NC.BL-197	Nectar	17.35	ND
24.2015.CA-9.01.SB.PO.BL-196	Pollen	12.00	<LOQ
24.2015.CA-9.01.SB.SOIL.POST-198	Soil	16.41	1.35
24.2015.CA-9.01.SB.SOIL.PRE-193	Soil	19.03	1.15
24.2015.CA-9.01.SB.WF.BL-194	Whole Flower	3.39	ND

[1] For CA-3 applications, the wind direction was consistently in the direction of the control plot and were less than 5 miles per hour. Sample weights were also lower than the target weight specified in the protocol.
[2] For CA-4 applications, the wind direction was consistently in the direction of the control plot and were less than 5 miles per hour. Sample weights were also lower than the target weight specified in the protocol. The control plot was also located within 75 feet of the test plots, which is less than the target distance specified in the protocol.
[3] For CA-5 applications, the wind direction was consistently in the direction of the control plot.
[4] CA-7 sample weight for pollen was lower than the target weight specified in the protocol.
[5] At CA-8 and CA-9 test sites, three additional inadvertent applications of the test substance (Actara) were made within the study area throughout the 4 week and 8 week sample activities.

Table 4. Thiamethoxam and CGA322704 Residues in Leaves, Nectar, Pollen, Whole Flowers and Soil

Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxa m Residues (ppb)	CGA322704 Residues (ppb)
24.2015.CA-1.01.SB.LV.BL-3	Leaves	UTC	Bloom	NA	ND	<LOQ
24.2015.CA-1.02.SB.LV.BL.A-9	Leaves	TRTD	Bloom	0.189	1736.79	38.54
24.2015.CA-1.02.SB.LV.BL.B-15	Leaves	TRTD	Bloom	0.189	1984.57	50.72
24.2015.CA-1.02.SB.LV.BL.C-21	Leaves	TRTD	Bloom	0.189	2145.93	46.92
24.2015.CA-1.01.SB.LV.4WK-218	Leaves	UTC	4 Week	NA	<LOQ	<LOQ

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
24.2015.CA-1.02.SB.LV.4WK.A-221	Leaves	TRTD	4 Week	0.189	192.04	13.03
24.2015.CA-1.02.SB.LV.4WK.B-224	Leaves	TRTD	4 Week	0.189	218.2	14.36
24.2015.CA-1.02.SB.LV.4WK.C-227	Leaves	TRTD	4 Week	0.189	240.36	15.43
24.2015.CA-1.01.SB.LV.8WK-219	Leaves	UTC	8 Week	NA	ND	<LOQ
24.2015.CA-1.02.SB.LV.8WK.A-222	Leaves	TRTD	8 Week	0.189	71.54	10.68
24.2015.CA-1.02.SB.LV.8WK.B-225	Leaves	TRTD	8 Week	0.189	71.34	15.26
24.2015.CA-1.02.SB.LV.8WK.C-228	Leaves	TRTD	8 Week	0.189	61.27	10.48
24.2015.CA-1.01.SB.NC.BL.5	Nectar	UTC	Bloom	NA	61.91	<LOQ
24.2015.CA-1.02.SB.NC.BL.A.11	Nectar	TRT A	Bloom	0.189	296.17	4.33
24.2015.CA-1.02.SB.NC.BL.B.17	Nectar	TRT B	Bloom	0.189	647.25	12.76
24.2015.CA-1.02.SB.NC.BL.C.23	Nectar	TRT C	Bloom	0.189	199.37	5.38
24.2015.CA-1.01.SB.PO.BL-4	Pollen	UTC	Bloom	NA	8.12	ND
24.2015.CA-1.02.SB.PO.BL.A-10-A	Pollen	TRT A	Bloom	0.189	287.87	7.12
24.2015.CA-1.02.SB.PO.BL.B-16-A	Pollen	TRT B	Bloom	0.189	169.57	4.10
24.2015.CA-1.02.SB.PO.BL.C-22-A	Pollen	TRT C	Bloom	0.189	173.32	6.76

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
24.2015.CA-1.01.SB.SOIL.POST.6	Soil	UTC	Post	NA	ND	ND
24.2015.CA-1.01.SB.SOIL.POST.A.12	Soil	TRT A	Post	0.189	3.29	2.79
24.2015.CA-1.02.SB.SOIL.POST.B.18	Soil	TRT B	Post	0.189	1.17	5.62
24.2015.CA-1.02.SB.SOIL.POST.C.24	Soil	TRT C	Post	0.189	1.77	2.42
24.2015.CA-1.01.SB.SOIL.PRE.1	Soil	UTC	Pre	NA	ND	ND
24.2015.CA-1.02.SB.SOIL.PRE.A.7	Soil	TRT A	Pre	0.189	3.48	2.30
24.2015.CA-1.02.SB.SOIL.PRE.B.13	Soil	TRT B	Pre	0.189	3.25	3.72
24.2015.CA-1.02.SB.SOIL.PRE.C.19	Soil	TRT C	Pre	0.189	5.31	3.62
24.2015.CA-1.01.SB.WF.BL-2*	Whole Flower	UTC	Bloom	NA	ND	ND
24.2015.CA-1.02.SB.WF.BL.A-8	Whole Flower	TRT A	Bloom	0.189	373.47	41.15
24.2015.CA-1.02.SB.WF.BL.B-14	Whole Flower	TRT B	Bloom	0.189	359.66	10.77
24.2015.CA-1.02.SB.WF.BL.C-20	Whole Flower	TRT C	Bloom	0.189	325.72	9.94
24.2015.CA-2.01.SB.LV.BL-27	Leaves	UTC	Bloom	NA	ND	ND
24.2015.CA-2.02.SB.LV.BL.A-33	Leaves	TRTD	Bloom	0.189	1506.08	32.85
24.2015.CA-2.02.SB.LV.BL.B-39	Leaves	TRTD	Bloom	0.189	1864.73	41.40
24.2015.CA-	Leaves	TRTD	Bloom	0.189	1931.85	44.41

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
2.02.SB.LV.BL.C-45						
24.2015.CA-2.01.SB.LV.4WK-230	Leaves	UTC	4 Week	NA	ND	ND
24.2015.CA-2.02.SB.LV.4WK.A-233	Leaves	TRTD	4 Week	0.189	283.13	54.84
24.2015.CA-2.02.SB.LV.4WK.B-236	Leaves	TRTD	4 Week	0.189	164.94	21.05
24.2015.CA-2.02.SB.LV.4WK.C-239	Leaves	TRTD	4 Week	0.189	286.57	19.92
24.2015.CA-2.01.SB.LV.8WK-231	Leaves	UTC	8 Week	NA	ND	ND
24.2015.CA-2.02.SB.LV.8WK.A-234	Leaves	TRTD	8 Week	0.189	13.94	4.20
24.2015.CA-2.02.SB.LV.8WK.B-237	Leaves	TRTD	8 Week	0.189	13.00	5.27
24.2015.CA-2.02.SB.LV.8WK.C-240	Leaves	TRTD	8 Week	0.189	16.1	13.43
24.01.CA-2.01.SB.LV.BL.A*	Leaves	UTC	Bloom	NA	ND	ND
24.2015.CA-2.01.SB.NC.BL.29	Nectar	UTC	Bloom	NA	6.52	0.90
24.2015.CA-2.02.SB.NC.BL.A.35	Nectar	TRT A	Bloom	0.189	164.62	4.69
24.2015.CA-2.02.SB.NC.BL.B.41	Nectar	TRT B	Bloom	0.189	149.38	3.62
24.2015.CA-2.02.SB.NC.BL.C.47	Nectar	TRT C	Bloom	0.189	129.43	4.97
24.2015.CA-	Pollen	UTC	Bloom	NA	44.39	1.80

Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
2.01.SB.PO.BL-28						
24.2015.CA-2.02.SB.PO.BL.A-34	Pollen	TRT A	Bloom	0.189	101.69	6.23
24.2015.CA-2.02.SB.PO.BL.B-40	Pollen	TRT B	Bloom	0.189	171.79	11.43
24.2015.CA-2.02.SB.PO.BL.C-46	Pollen	TRT C	Bloom	0.189	249.75	13.62
24.2015.CA-2.01.SB.SOIL.POST.30	Soil	UTC	Post	NA	ND	ND
24.2015.CA-2.02.SB.SOIL.POST.A.36	Soil	TRT A	Post	0.189	ND	ND
24.2015.CA-2.02.SB.SOIL.POST.B.42	Soil	TRT B	Post	0.189	3.36	1.10
24.2015.CA-2.02.SB.SOIL.POST.C.48	Soil	TRT C	Post	0.189	4.74	1.45
24.2015.CA-2.01.SB.WF.BL-26	Whole Flower	UTC	Bloom	NA	ND	ND
24.2015.CA-2.02.SB.WF.BL.A-32	Whole Flower	TRT A	Bloom	0.189	482.99	12.46
24.2015.CA-2.02.SB.WF.BL.B-38	Whole Flower	TRT B	Bloom	0.189	466.22	13.02
24.2015.CA-2.02.SB.WF.BL.C-44	Whole Flower	TRT C	Bloom	0.189	424.28	11.82
24.2015.CA-3.02.SB.LV.0DA3A.A.244	Leaves	TRTD	0 Day	0.189	8433.93	45.80
24.2015.CA-3.02.SB.LV.0DA3A.B.247	Leaves	TRTD	0 Day	0.189	9057.67	41.66
24.2015.CA-3.02.SB.LV.0DA3A.C-	Leaves	TRTD	0 Day	0.189	7605.97	37.01

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
250						
24.2015.CA-3.01.SB.LV.BL-51	Leaves	UTC	Bloom	NA	ND	ND
24.2015.CA-3.02.SB.LV.BL.A-57	Leaves	TRTD	Bloom	0.189	2151.76	71.32
24.2015.CA-3.02.SB.LV.BL.B-63	Leaves	TRTD	Bloom	0.189	2061.65	68.89
24.2015.CA-3.02.SB.LV.BL.C-69	Leaves	TRTD	Bloom	0.189	1923.48	67.20
24.2015.CA-3.01.SB.LV.4WK-242	Leaves	UTC	4 Week	NA	ND	ND
24.2015.CA-3.02.SB.LV.4WK.A-245	Leaves	TRTD	4 Week	0.189	185.65	10.47
24.2015.CA-3.02.SB.LV.4WK.B-248	Leaves	TRTD	4 Week	0.189	266.47	14.96
24.2015.CA-3.02.SB.LV.4WK.C-251	Leaves	TRTD	4 Week	0.189	289.27	15.8
24.2015.CA-3.01.SB.LV.8WK-243	Leaves	UTC	8 Week	NA	1.06	ND
24.2015.CA-3.02.SB.LV.8WK.A-246	Leaves	TRTD	8 Week	0.189	267.15	14.43
24.2015.CA-3.02.SB.LV.8WK.B-249	Leaves	TRTD	8 Week	0.189	318.21	17.25
24.2015.CA-3.02.SB.LV.8WK.C-252	Leaves	TRTD	8 Week	0.189	342.12	18.75
24.2015.CA-3.01.SB.LV.0DA3A.241*	Leaves	UTC	Day 0	NA	ND	ND
24.2015.CA-3.01.SB.NC.BL-53	Nectar	UTC	Bloom	NA	104.91	ND
24.2015.CA-3.02.SB.NC.BL.A-59	Nectar	TRT A	Bloom	0.189	211.95	1.76
24.2015.CA-3.02.SB.NC.BL.B-65	Nectar	TRT B	Bloom	0.189	136.22	1.93
24.2015.CA-3.02.SB.NC.BL.C-71	Nectar	TRT C	Bloom	0.189	189.62	2.62

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
24.2015.CA-3.01.SB.PO.BL-52	Pollen	UTC	Bloom	NA	669.04	1.73
24.2015.CA-3.02.SB.PO.BL.A-58	Pollen	TRT A	Bloom	0.189	1202.23	18.20
24.2015.CA-3.02.SB.PO.BL.B-64	Pollen	TRT B	Bloom	0.189	860.91	14.21
24.2015.CA-3.02.SB.PO.BL.C-70	Pollen	TRT C	Bloom	0.189	773.85	20.14
24.2015.CA-3.01.SB.SOIL.POST.54	Soil	UTC	Post	NA	ND	ND
24.2015.CA-3.02.SB.SOIL.POST.A.60	Soil	TRT A	Post	0.189	15.61	<LOQ
24.2015.CA-3.02.SB.SOIL.POST.B.66	Soil	TRT B	Post	0.189	28.45	1.78
24.2015.CA-3.02.SB.SOIL.POST.C.72	Soil	TRT C	Post	0.189	23.12	2.48
24.2015.CA-3.01.SB.SOIL.PRE.49	Soil	UTC	Pre	NA	ND	ND
24.2015.CA-3.02.SB.SOIL.PRE.A.55	Soil	TRT A	Pre	0.189	20.09	<LOQ
24.2015.CA-3.02.SB.SOIL.PRE.B.61	Soil	TRT B	Pre	0.189	36.37	1.24
24.2015.CA-3.02.SB.SOIL.PRE.C.67	Soil	TRT C	Pre	0.189	30.38	1.39
24.2015.CA-3.01.SB.WF.BL-50	Whole Flower	UTC	Bloom	NA	ND	ND
24.2015.CA-3.02.SB.WF.BL.A-56	Whole Flower	TRT A	Bloom	0.189	352.87	8.14
24.2015.CA-3.02.SB.WF.BL.B-62	Whole Flower	TRT B	Bloom	0.189	492.36	10.18
24.2015.CA-3.02.SB.WF.BL.C-68	Whole Flower	TRT C	Bloom	0.189	479.24	10.19
24.2015.CA-4.02.SB.LV.0DA3A.A.256	Leaves	TRTD	0 Day	0.189	8703.72	24.73
24.2015.CA-	Leaves	TRTD	0 Day	0.189	9099.27	25.87

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
4.02.SB.LV.0DA3A.B.259						
24.2015.CA-4.02.SB.LV.0DA3A.C-262	Leaves	TRTD	0 Day	0.189	8577.80	28.73
24.2015.CA-4.01.SB.LV.BL-75	Leaves	UTC	Bloom	NA	0.51	<LOQ
24.2015.CA-4.02.SB.LV.BL.A-81	Leaves	TRTD	Bloom	0.189	1242.7	33.40
24.2015.CA-4.02.SB.LV.BL.B-87	Leaves	TRTD	Bloom	0.189	1644.59	40.46
24.2015.CA-4.02.SB.LV.BL.C-93	Leaves	TRTD	Bloom	0.189	1685.71	34.85
24.2015.CA-4.01.SB.LV.4WK-254	Leaves	UTC	4 Week	NA	ND	ND
24.2015.CA-4.02.SB.LV.4WK.A-257	Leaves	TRTD	4 Week	0.189	45.26	6.43
24.2015.CA-4.02.SB.LV.4WK.B-260	Leaves	TRTD	4 Week	0.189	59.29	4.80
24.2015.CA-4.02.SB.LV.4WK.C-263	Leaves	TRTD	4 Week	0.189	135.37	9.75
24.2015.CA-4.01.SB.LV.8WK-255	Leaves	UTC	8 Week	NA	ND	1.53
24.2015.CA-4.02.SB.LV.8WK.A-258	Leaves	TRTD	8 Week	0.189	29.47	4.80
24.2015.CA-4.02.SB.LV.8WK.B-261	Leaves	TRTD	8 Week	0.189	31.03	5.05
24.2015.CA-4.02.SB.LV.8WK.C-264	Leaves	TRTD	8 Week	0.189	58.84	8.15
24.2015.CA-4.01.SB.LV.0DA3A.253	Leaves	UTC	Day 0	NA	1.15	ND
24.2015.CA-4.01.SB.NC.BL-77	Nectar	UTC	Bloom	NA	22.40	<LOQ
24.2015.CA-4.02.SB.NC.BL.A-83	Nectar	TRT A	Bloom	0.189	118.42	2.35

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
24.2015.CA-4.02.SB.NC.BL.B-89	Nectar	TRT B	Bloom	0.189	214.74	9.86
24.2015.CA-4.02.SB.NC.BL.C-95	Nectar	TRT C	Bloom	0.189	51.17	0.96
24.2015.CA-4.01.SB.PO.BL-76	Pollen	UTC	Bloom	NA	249.74	ND
24.2015.CA-4.02.SB.PO.BL.A-82	Pollen	TRT A	Bloom	0.189	1477.68	32.23
24.2015.CA-4.02.SB.PO.BL.B-88	Pollen	TRT B	Bloom	0.189	633.51	19.38
24.2015.CA-4.02.SB.PO.BL.C-94	Pollen	TRT C	Bloom	0.189	1159.00	13.45
24.2015.CA-4.01.SB.SOIL.POST.78	Soil	UTC	Post	NA	ND	<LOQ
24.2015.CA-4.02.SB.SOIL.POST.A.84	Soil	TRT A	Post	0.189	11.52	1.46
24.2015.CA-4.02.SB.SOIL.POST.B.90	Soil	TRT B	Post	0.189	17.14	1.20
24.2015.CA-4.02.SB.SOIL.POST.C.96	Soil	TRT C	Post	0.189	8.81	1.27
24.2015.CA-4.01.SB.SOIL.PRE.73	Soil	UTC	Pre	NA	ND	<LOQ
24.2015.CA-4.02.SB.SOIL.PRE.A.79	Soil	TRT A	Pre	0.189	ND	<LOQ
24.2015.CA-4.02.SB.SOIL.PRE.B.85	Soil	TRT B	Pre	0.189	ND	<LOQ
24.2015.CA-4.02.SB.SOIL.PRE.C.91	Soil	TRT C	Pre	0.189	ND	<LOQ
24.2015.CA-4.01.SB.WF.BL-74	Whole Flower	UTC	Bloom	NA	0.53	ND
24.2015.CA-4.02.SB.WF.BL.A-80	Whole Flower	TRT A	Bloom	0.189	511.72	10.04
24.2015.CA-4.02.SB.WF.BL.B-86	Whole Flower	TRT B	Bloom	0.189	247.61	5.05
24.2015.CA-4.02.SB.WF.BL.C-92	Whole Flower	TRT C	Bloom	0.189	305.94	5.03

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
24.2015.CA-5.01.SB.LV.0DA3A.265	Leaves	UTC	0 Day	NA	ND	ND
24.2015.CA-5.02.SB.LV.0DA3A.A.268	Leaves	TRTD	0 Day	0.189	6709.22	13.32
24.2015.CA-5.02.SB.LV.0DA3A.B.271	Leaves	TRTD	0 Day	0.189	7718.34	16.54
24.2015.CA-5.02.SB.LV.0DA3A.C-274	Leaves	TRTD	0 Day	0.189	6851.73	19.04
24.2015.CA-5.01.SB.LV.BL-99	Leaves	UTC	Bloom	NA	ND	ND
24.2015.CA-5.02.SB.LV.BL.A-105	Leaves	TRTD	Bloom	0.189	516.33	12.15
24.2015.CA-5.02.SB.LV.BL.B-111	Leaves	TRTD	Bloom	0.189	428.12	10.78
24.2015.CA-5.02.SB.LV.BL.C-117	Leaves	TRTD	Bloom	0.189	475.79	10.85
24.2015.CA-5.01.SB.LV.4WK-266	Leaves	UTC	4 Week	NA	ND	ND
24.2015.CA-5.02.SB.LV.4WK.A-269	Leaves	TRTD	4 Week	0.189	204.73	82.53
24.2015.CA-5.02.SB.LV.4WK.B-272	Leaves	TRTD	4 Week	0.189	154.91	24.86
24.2015.CA-5.02.SB.LV.4WK.C-275	Leaves	TRTD	4 Week	0.189	248.96	22.28
24.2015.CA-5.01.SB.LV.8WK-267	Leaves	UTC	8 Week	NA	ND	ND
24.2015.CA-5.02.SB.LV.8WK.A-270	Leaves	TRTD	8 Week	0.189	58.36	9.20
24.2015.CA-5.02.SB.LV.8WK.C-273	Leaves	TRTD	8 Week	0.189	46.48	9.46
24.2015.CA-5.02.SB.LV.8WK.C-276	Leaves	TRTD	8 Week	0.189	56.08	11.22
24.2015.CA-	Nectar	UTC	Bloom	NA	8.95	ND

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
5.01.SB.NC.BL-101						
24.2015.CA-5.02.SB.NC.BL.A-107	Nectar	TRT A	Bloom	0.189	177.45	5.39
24.2015.CA-5.02.SB.NC.BL.B-113	Nectar	TRT B	Bloom	0.189	143.65	3.74
24.2015.CA-5.02.SB.NC.BL.C-119	Nectar	TRT C	Bloom	0.189	108.07	3.79
24.2015.CA-5.01.SB.PO.BL-100	Pollen	UTC	Bloom	NA	47.33	<LOQ
24.2015.CA-5.02.SB.PO.BL.A-106	Pollen	TRT A	Bloom	0.189	334.49	19.94
24.2015.CA-5.02.SB.PO.BL.B-112	Pollen	TRT B	Bloom	0.189	337.62	21.86
24.2015.CA-5.02.SB.PO.BL.C-118	Pollen	TRT C	Bloom	0.189	309.42	22.81
24.2015.CA-5.01.SB.SOIL.POST.102	Soil	UTC	Post	NA	ND	ND
24.2015.CA-5.02.SB.SOIL.POST.A.108	Soil	TRT A	Post	0.189	4.52	<LOQ
24.2015.CA-5.02.SB.SOIL.POST.B.114	Soil	TRT B	Post	0.189	7.05	1.68
24.2015.CA-5.02.SB.SOIL.POST.C.120	Soil	TRT C	Post	0.189	1.36	ND
24.2015.CA-5.01.SB.SOIL.PRE.97	Soil	UTC	Pre	NA	ND	ND
24.2015.CA-5.02.SB.SOIL.PRE.A.103	Soil	TRT A	Pre	0.189	ND	ND
24.2015.CA-5.02.SB.SOIL.PRE.B.109	Soil	TRT B	Pre	0.189	ND	ND
24.2015.CA-5.02.SB.SOIL.PRE.C.115	Soil	TRT C	Pre	0.189	ND	ND
24.2015.CA-5.01.SB.WF.BL-98	Whole Flower	UTC	Bloom	NA	ND	ND
24.2015.CA-5.02.SB.WF.BL.A-104	Whole Flower	TRT A	Bloom	0.189	241.31	6.59

Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
24.2015.CA-5.02.SB.WF.BL.B-110	Whole Flower	TRT B	Bloom	0.189	283.29	6.14
24.2015.CA-5.02.SB.WF.BL.C-116	Whole Flower	TRT C	Bloom	0.189	263.98	6.57
24.2015.CA-6.01.SB.LV.0DA3A.277	Leaves	UTC	0 Day	NA	<LOQ	ND
24.2015.CA-6.02.SB.LV.0DA3A.A.280	Leaves	TRTD	0 Day	0.189	5365.14	25.92
24.2015.CA-6.02.SB.LV.0DA3A.B.283	Leaves	TRTD	0 Day	0.189	4751.53	26.75
24.2015.CA-6.02.SB.LV.0DA3A.C-286	Leaves	TRTD	0 Day	0.189	3487.21	21.24
24.2015.CA-6.01.SB.LV.BL-123	Leaves	UTC	Bloom	NA	3.44	ND
24.2015.CA-6.02.SB.LV.BL.A-129	Leaves	TRTD	Bloom	0.189	1889.24	33.79
24.2015.CA-6.02.SB.LV.BL.B-135	Leaves	TRTD	Bloom	0.189	1826.40	34.69
24.2015.CA-6.02.SB.LV.BL.C-141	Leaves	TRTD	Bloom	0.189	1483.09	25.84
24.2015.CA-6.01.SB.LV.4WK-278*	Leaves	UTC	4 Week	NA	ND	ND
24.2015.CA-6.02.SB.LV.4WK.A-281	Leaves	TRTD	4 Week	0.189	144.00	38.54
24.2015.CA-6.02.SB.LV.4WK.B-284	Leaves	TRTD	4 Week	0.189	210.05	20.38
24.2015.CA-6.02.SB.LV.4WK.C-287	Leaves	TRTD	4 Week	0.189	67.45	9.54
24.2015.CA-6.01.SB.LV.8WK-279	Leaves	UTC	8 Week	NA	<LOQ	ND
24.2015.CA-6.02.SB.LV.8WK.A-282	Leaves	TRTD	8 Week	0.189	106.27	5.67
24.2015.CA-6.02.SB.LV.8WK.B-	Leaves	TRTD	8 Week	0.189	134.08	5.79

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
285						
24.2015.CA-6.02.SB.LV.8WK.C-288	Leaves	TRTD	8 Week	0.189	162.54	6.55
24.2015.CA-6.01.SB.NC.BL.125	Nectar	UTC	Bloom	NA	1.89	ND
24.2015.CA-6.02.SB.NC.BL.A.131-001	Nectar	TRT A	Bloom	0.189	183.33	4.04
24.2015.CA-6.02.SB.NC.BL.B.137-001	Nectar	TRT B	Bloom	0.189	228.42	5.14
24.2015.CA-6.02.SB.NC.BL.C.143-001	Nectar	TRT C	Bloom	0.189	175.03	4.14
24.2015.CA-6.01.SB.PO.BL-124	Pollen	UTC	Bloom	NA	20.04	<LOQ
24.2015.CA-6.02.SB.PO.BL.A-130	Pollen	TRT A	Bloom	0.189	2486.10	36.63
24.2015.CA-6.02.SB.PO.BL.B-136	Pollen	TRT B	Bloom	0.189	2156.36	31.93
24.2015.CA-6.02.SB.PO.BL.C-142	Pollen	TRT C	Bloom	0.189	1935.33	30.42
24.2015.CA-6.01.SB.SOIL.POST-126	Soil	UTC	Post	NA	<LOQ	ND
24.2015.CA-6.02.SB.SOIL.POST.A-132	Soil	TRT A	Post	0.189	1.01	<LOQ
24.2015.CA-6.02.SB.SOIL.POST.B-138	Soil	TRT B	Post	0.189	2.59	<LOQ
24.2015.CA-6.02.SB.SOIL.POST.C-144	Soil	TRT C	Post	0.189	4.40	<LOQ
24.2015.CA-6.01.SB.SOIL.PRE.121	Soil	UTC	Pre	NA	1.20	<LOQ
24.2015.CA-6.02.SB.SOIL.PRE.A.127	Soil	TRT A	Pre	0.189	<LOQ	<LOQ
24.2015.CA-6.02.SB.SOIL.PRE.B.133	Soil	TRT B	Pre	0.189	<LOQ	ND

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
24.2015.CA-6.02.SB.SOIL.PRE.C.139	Soil	TRT C	Pre	0.189	<LOQ	<LOQ
24.2015.CA-6.01.SB.WF.BL-122	Whole Flower	UTC	Bloom	NA	5.93	ND
24.2015.CA-6.02.SB.WF.BL.A-128	Whole Flower	TRT A	Bloom	0.189	829.3	81.79
24.2015.CA-6.02.SB.WF.BL.B-134	Whole Flower	TRT B	Bloom	0.189	895.27	25.87
24.2015.CA-6.02.SB.WF.BL.C-140	Whole Flower	TRT C	Bloom	0.189	973.27	22.12
24.2015.CA-7.01.SB.LV.0DA3A.289	Leaves	UTC	0 Day	NA	2.01	<LOQ
24.2015.CA-7.02.SB.LV.0DA3A.A.292	Leaves	TRTD	0 Day	0.189	5256.56	25.78
24.2015.CA-7.02.SB.LV.0DA3A.B.295	Leaves	TRTD	0 Day	0.189	4772.30	24.39
24.2015.CA-7.02.SB.LV.0DA3A.C-298	Leaves	TRTD	0 Day	0.189	4851.20	23.31
24.2015.CA-7.01.SB.LV.4WK-290	Leaves	UTC	4 Week	NA	ND	ND
24.2015.CA-7.02.SB.LV.4WK.A-293	Leaves	TRTD	4 Week	0.189	289.29	14.54
24.2015.CA-7.02.SB.LV.4WK.B-296	Leaves	TRTD	4 Week	0.189	151.03	6.26
24.2015.CA-7.02.SB.LV.4WK.C-299	Leaves	TRTD	4 Week	0.189	159.04	6.20
24.2015.CA-7.02.SB.LV.8WK.A-294	Leaves	TRTD	8 Week	0.189	72.17	4.16
24.2015.CA-7.01.SB.LV.8WK-291	Leaves	UTC	8 Week	NA	1.50	<LOQ
24.2015.CA-7.02.SB.LV.8WK.B-297	Leaves	TRTD	8 Week	0.189	60.08	3.37
24.2015.CA-	Leaves	TRTD	8 Week	0.189	74.05	4.06

Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
7.02.SB.LV.8WK.C-300						
24.2015.CA-7.01.SB.LV.BL-147	Leaves	UTC	Bloom	NA	1.70	<LOQ
24.2015.CA-7.02.SB.LV.BL.A-153	Leaves	TRTD	Bloom	0.189	1156.66	24.78
24.2015.CA-7.02.SB.LV.BL.B-159	Leaves	TRTD	Bloom	0.189	1132.48	24.49
24.2015.CA-7.02.SB.LV.BL.C-165	Leaves	TRTD	Bloom	0.189	1059.15	23.34
24.2015.CA-7.01.SB.NC.BL.149	Nectar	UTC	Bloom	NA	1.16	ND
24.2015.CA-7.02.SB.NC.BL.A.155	Nectar	TRT A	Bloom	0.189	215.15	<LOQ
24.2015.CA-7.02.SB.NC.BL.B.161	Nectar	TRT B	Bloom	0.189	206.77	4.15
24.2015.CA-7.02.SB.NC.BL.C.167	Nectar	TRT C	Bloom	0.189	200.6	3.28
24.2015.CA-7.01.SB.PO.BL-148	Pollen	UTC	Bloom	NA	42.99	3.82
24.2015.CA-7.02.SB.PO.BL.A-154	Pollen	TRT A	Bloom	0.189	4238.76	43.7
24.2015.CA-7.02.SB.PO.BL.B-160	Pollen	TRT B	Bloom	0.189	5251.74	62.34
24.2015.CA-7.02.SB.PO.BL.C-166	Pollen	TRT C	Bloom	0.189	7473.47	66.10
24.2015.CA-7.01.SB.SOIL.POST-150	Soil	UTC	Post	NA	5.15	<LOQ
24.2015.CA-7.02.SB.SOIL.POST.A-156	Soil	TRT A	Post	0.189	17.15	2.13
24.2015.CA-7.02.SB.SOIL.POST.B-162	Soil	TRT B	Post	0.189	24.88	2.32
24.2015.CA-7.02.SB.SOIL.POST.C-168	Soil	TRT C	Post	0.189	25.78	2.93
24.2015.CA-7.01.SB.SOIL.PRE-145	Soil	UTC	Pre	NA	1.29	<LOQ
24.2015.CA-7.02.SB.SOIL.PRE.A-	Soil	TRT A	Pre	0.189	<LOQ	ND

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
151						
24.2015.CA-7.02.SB.SOIL.PRE.B-157	Soil	TRT B	Pre	0.189	<LOQ	ND
24.2015.CA-7.02.SB.SOIL.PRE.C-163	Soil	TRT C	Pre	0.189	<LOQ	ND
24.2015.CA-7.01.SB.WF.BL-146	Whole Flower	UTC	Bloom	NA	1.00	ND
24.2015.CA-7.02.SB.WF.BL.A-152	Whole Flower	TRT A	Bloom	0.189	493.67	11.15
24.2015.CA-7.02.SB.WF.BL.B-158	Whole Flower	TRT B	Bloom	0.189	443.68	9.50
24.2015.CA-7.02.SB.WF.BL.C-164	Whole Flower	TRT C	Bloom	0.189	468.15	8.36
24.2015.CA-8.01.SB.LV.0DA3A-301	Leaves	UTC	0 Day	NA	41.48	<LOQ
24.2015.CA-8.02.SB.LV.0DA3A.A-304	Leaves	TRTD	0 Day	0.189	2653.53	32.97
24.2015.CA-8.02.SB.LV.0DA3A.B-307	Leaves	TRTD	0 Day	0.189	2290.42	32.96
24.2015.CA-8.02.SB.LV.0DA3A.C-310	Leaves	TRTD	0 Day	0.189	2266.73	34.49
24.2015.CA-8.01.SB.LV.4WK-302	Leaves	UTC	4 Week	NA	2254.41	75.46
24.2015.CA-8.02.SB.LV.4WK.A-305	Leaves	TRTD	4 Week	0.189	1035.89	83.59
24.2015.CA-8.02.SB.LV.4WK.B-308	Leaves	TRTD	4 Week	0.189	1333.77	89.31
24.2015.CA-8.02.SB.LV.4WK.C-311	Leaves	TRTD	4 Week	0.189	1187.42	83.04
24.2015.CA-8.01.SB.LV.8WK-303	Leaves	UTC	8 Week	NA	1714.64	8.15
24.2015.CA-8.02.SB.LV.8WK.A-306	Leaves	TRTD	8 Week	0.189	1351.70	77.69

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
24.2015.CA-8.02.SB.LV.8WK.B-309	Leaves	TRTD	8 Week	0.189	1912.47	83.24
24.2015.CA-8.02.SB.LV.8WK.C-312	Leaves	TRTD	8 Week	0.189	2405.98	65.44
24.2015.CA-8.01.SB.LV.BL-171	Leaves	UTC	Bloom	NA	18.05	1.05
24.2015.CA-8.02.SB.LV.BL.A-177	Leaves	TRTD	Bloom	0.189	972.95	26.29
24.2015.CA-8.02.SB.LV.BL.B-183	Leaves	TRTD	Bloom	0.189	893.71	22.86
24.2015.CA-8.02.SB.LV.BL.C-189	Leaves	TRTD	Bloom	0.189	851.27	20.27
24.2015.CA-8.01.SB.NC.BL-173	Nectar	UTC	Bloom	NA	4.17	0.64
24.2015.CA-8.02.SB.NC.BL.A-179	Nectar	TRT A	Bloom	0.189	98.09	1.04
24.2015.CA-8.02.SB.NC.BL.B-185	Nectar	TRT B	Bloom	0.189	86.87	2.41
24.2015.CA-8.02.SB.NC.BL.C-191	Nectar	TRT C	Bloom	0.189	79.18	1.83
24.2015.CA-8.01.SB.PO.BL-172	Pollen	UTC	Bloom	NA	9.78	ND
24.2015.CA-8.02.SB.PO.BL.A-178	Pollen	TRT A	Bloom	0.189	1489.59	7.89
24.2015.CA-8.02.SB.PO.BL.B-184	Pollen	TRT B	Bloom	0.189	656.39	7.73
24.2015.CA-8.02.SB.PO.BL.C-190	Pollen	TRT C	Bloom	0.189	540.42	5.86
24.2015.CA-8.01.SB.SOIL.POST-174	Soil	UTC	Post	NA	20.62	<LOQ
24.2015.CA-8.02.SB.SOIL.POST.A-180	Soil	TRT A	Post	0.189	84.92	2.35
24.2015.CA-8.02.SB.SOIL.POST.B-186	Soil	TRT B	Post	0.189	85.19	3.13
24.2015.CA-8.02.SB.SOIL.POST.C-192	Soil	TRT C	Post	0.189	81.02	3.01

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
24.2015.CA-8.01.SB.SOIL.PRE-169	Soil	UTC	Pre	NA	10.92	<LOQ
24.2015.CA-8.02.SB.SOIL.PRE.A-175	Soil	TRT A	Pre	0.189	5.52	<LOQ
24.2015.CA-8.02.SB.SOIL.PRE.B-181	Soil	TRT B	Pre	0.189	3.94	<LOQ
24.2015.CA-8.02.SB.SOIL.PRE.C-187	Soil	TRT C	Pre	0.189	3.88	ND
24.2015.CA-8.01.SB.WF.BL-170	Whole Flower	UTC	Bloom	NA	2.24	ND
24.2015.CA-8.02.SB.WF.BL.A-176	Whole Flower	TRT A	Bloom	0.189	274.95	5.42
24.2015.CA-8.02.SB.WF.BL.B-182	Whole Flower	TRT B	Bloom	0.189	264.55	5.12
24.2015.CA-8.02.SB.WF.BL.C-188	Whole Flower	TRT C	Bloom	0.189	286.81	5.63
24.2015.CA-9.01.SB.LV.0DA3A-313	Leaves	UTC	0 Day	NA	31.44	2.09
24.2015.CA-9.02.SB.LV.0DA3A.A-316	Leaves	TRTD	0 Day	0.189	3920.73	33.73
24.2015.CA-9.02.SB.LV.0DA3A.B-319	Leaves	TRTD	0 Day	0.189	4322.57	37.56
24.2015.CA-9.02.SB.LV.0DA3A.C-322	Leaves	TRTD	0 Day	0.189	4111.73	45.89
24.2015.CA-9.01.SB.LV.4WK-314*	Leaves	UTC	4 Week	NA	28.85	1.86
24.2015.CA-9.02.SB.LV.4WK.A-317*	Leaves	TRTD	4 Week	0.189	124.95	8.82
24.2015.CA-9.02.SB.LV.4WK.B-320*	Leaves	TRTD	4 Week	0.189	170.94	12.59
24.2015.CA-9.02.SB.LV.4WK.C-323*	Leaves	TRTD	4 Week	0.189	165.90	12.09

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Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
24.2015.CA-9.01.SB.LV.8WK-315	Leaves	UTC	8 Week	NA	638.33	48.03
24.2015.CA-9.02.SB.LV.8WK.A-318	Leaves	TRTD	8 Week	0.189	413.45	52.79
24.2015.CA-9.02.SB.LV.8WK.B-321	Leaves	TRTD	8 Week	0.189	531.94	51.25
24.2015.CA-9.02.SB.LV.8WK.C-324	Leaves	TRTD	8 Week	0.189	528.89	35.59
24.2015.CA-9.01.SB.LV.BL.195	Leaves	UTC	Bloom	NA	30.15	1.35
24.2015.CA-9.02.SB.LV.BL.A.201	Leaves	TRTD	Bloom	0.189	919.86	34.48
24.2015.CA-9.02.SB.LV.BL.B.207	Leaves	TRTD	Bloom	0.189	1177.57	38.45
24.2015.CA-9.02.SB.LV.BL.C.213	Leaves	TRTD	Bloom	0.189	1177.37	33.01
24.2015.CA-9.01.SB.NC.BL-197	Nectar	UTC	Bloom	NA	17.35	ND
24.2015.CA-9.02.SB.NC.BL.A-203	Nectar	TRT A	Bloom	0.189	375.96	5.22
24.2015.CA-9.02.SB.NC.BL.B-209	Nectar	TRT B	Bloom	0.189	232.96	4.16
24.2015.CA-9.02.SB.NC.BL.C-215	Nectar	TRT C	Bloom	0.189	152.5	4.19
24.2015.CA-9.01.SB.PO.BL-196	Pollen	UTC	Bloom	NA	12.00	<LOQ
24.2015.CA-9.02.SB.PO.BL.A-202	Pollen	TRT A	Bloom	0.189	7349.43	61.96
24.2015.CA-9.02.SB.PO.BL.B-208	Pollen	TRT B	Bloom	0.189	7444.74	47.89
24.2015.CA-9.02.SB.PO.BL.C-214	Pollen	TRT C	Bloom	0.189	5354.97	40.91
24.2015.CA-9.01.SB.SOIL.POST-198	Soil	UTC	Post	NA	16.41	1.35
24.2015.CA-9.02.SB.SOIL.POST.A-204	Soil	TRT A	Post	0.189	52.25	1.59
24.2015.CA-	Soil	TRT B	Post	0.189	31.27	2.13

Trail Number/Sample	Matrix	Treatment	Timing	Nominal Total Treatment Rate	Thiamethoxam Residues (ppb)	CGA322704 Residues (ppb)
9.02.SB.SOIL.POST.B-210						
24.2015.CA-9.02.SB.SOIL.POST.C-216	Soil	TRT C	Post	0.189	30.94	1.64
24.2015.CA-9.01.SB.SOIL.PRE-193	Soil	UTC	Pre	NA	19.03	1.15
24.2015.CA-9.02.SB.SOIL.PRE.A-199	Soil	TRT A	Pre	0.189	19.45	1.14
24.2015.CA-9.02.SB.SOIL.PRE.B-205	Soil	TRT B	Pre	0.189	17.46	1.08
24.2015.CA-9.02.SB.SOIL.PRE.C-211	Soil	TRT C	Pre	0.189	15.02	1.02
24.2015.CA-9.01.SB.WF.BL-194	Whole Flower	UTC	Bloom	NA	3.39	ND
24.2015.CA-9.02.SB.WF.BL.A-200	Whole Flower	TRT A	Bloom	0.189	368.60	9.28
24.2015.CA-9.02.SB.WF.BL.B-206	Whole Flower	TRT B	Bloom	0.189	383.65	6.42
24.2015.CA-9.02.SB.WF.BL.C-212	Whole Flower	TRT C	Bloom	0.189	296.31	7.90

ND = No Detect, NA = Not Applicable LOQ = Limit of Quantitation
* Average of duplicate analyses

7. STATISTICAL ANALYSIS

Study objectives and design

The study was conducted to determine the concentration of thiamethoxam and its metabolite, CGA322704, in leaves, flowers, nectar, and pollen of strawberry plants in response to foliar applications of a thiamethoxam pesticide product applied before bloom. Plants received three foliar sprays at an application rate of 0.063 lbs./acre, resulting in a total application of 0.189 lbs./ai. The first application was applied at approximately 28 days before bloom. The second application was made at approximately 18 days before bloom with the third spray applied approximately 7 days prior to harvesting of plant samples at bloom. Leaves were sampled after the third foliar application and extended over time with samples being taken at 4 and 8 weeks after bloom. Soil samples were also taken prior to the initiation of the study and then at the end of the study, after the last sampling of leaves. Contaminated samples were taken from untreated control plots. Three replicate samples from each matrix were targeted for treated plots and only one sample from each matrix was obtained from the untreated control plots.

Plant and soil samples were analyzed for concentrations of thiamethoxam and its metabolite, CGA322404. The study was conducted only for 1 year.

Data was submitted for 9 sites, denoted as CA-1 through CA-9. The data call-in specified that the test sites were to be distributed across soil texture categories with 3 sites in each coarse, medium, and fine-textured soil. The sites were classified as coarse-textured sites for CA-2, CA5, and CA7; medium-textured sites for CA-6 and CA-4; and moderately-fine textured sites for CA-1, CA-3, CA-8 and CA-9 based on the reported soil textures. Although not strictly denoted as fine-textured, the moderately-fine textured soils were clayey in nomenclature, representing a finer-textured contrast to plants grown in loamy textured soil. These categories are based on the USDA classification of soils (Soil Science Division Staff, 2017, see Table 3.1).

Chemical analytical results that were reported as less than the limit of detection (LOD) and less than the limit of quantification (LOQ) (Table 3). The LOD was 0.5 ng/g (ppb) for leaves, pollen, whole flowers, soil and 0.25 ng/g for nectar. The LOQ was twice the LOD at 1.0 ng/g for leaves, pollen, whole flowers, soil and 0.5 ng/g for nectar. Data reported as <LOD or <LOQ were assigned ½ their respective detection limits.

Non-parametric statistical tests were used to test for differences in distribution of concentrations between soil type and between untreated and treated sites. Non-parametric tests do not require tests for normality as they are robust to differences in distribution and they are also robust for experimental designs with low replicates (Helsel and Hersch, 2002). The PROC NPAR1WAY procedure in the Statistical Analysis System (SAS) statistical package was used to conduct Wilcoxon-Mann –Whitney (Wilcoxon), Median non-parametric, and Kuiper tests. A significant result from the Wilcoxon test indicates differences in the shape of distributions; a significant result from the Median test indicates differences in the location of the medians between distributions; and a significant result from the Kuiper test indicates differences in the empirical distributions between two groups. The Exact option for each statistic was implemented as it provides permutation testing, which is a statistical method that minimizes the effect of sample size and distributional differences. Using the Exact option, the Monte Carlo procedure was also implemented which provided 10,000 separate runs for each statistic to produce the permutation distributions. The test for potential differences due to soil type had 3 levels so the DSCF option in PROC NPAR1WAY, which invokes the Dwass, Steel, Critchlow-Fligner multiple comparison test, was used to provide pairwise tests for two-sample rankings.

Additional procedures used for descriptive statistics were PROC MEANS to calculate mean values from the replicates at each site, PROC CAPACITY to produce cumulative statistics, and PROC BOXPLOT to produce comparative graphics. Most of the previous studies conducted for the data call-in were replicated over years so the mean from each site were used in the statistical analysis. This study was not replicated over years so the replicate samples taken within each site were used to provide guidance on potential effects of soil type and comparison between untreated and treated plants. Some comparison data was transformed to a natural logarithm scale to provide clear contrasts between distributions presented in the graphics. Figure 1 provides an explanation of the statistics summarized in Box-and-Whisker plots used to compare distributions. Final presentation of the potential distribution of concentrations in bee relevant plant matrices is based on all raw data because these values represent the actual range of exposure to bees and other organisms that feed off the nectar and pollen of plants.

Detection rates in each plant matrix with comparison between treated and untreated control plants: Counts for the number of samples reported above each detection limit are presented in Table 6 where

Table 6A contains data for treated plants and Table 6B contains data for untreated plants. Concentrations of thiamethoxam and CGA322704 in treated plant samples were above the LOQ. Parent thiamethoxam residues comprised greater than 90% of the total residue with most percentages above 95% (Tables 7 and 8). Concentrations in leaves and pollen matrices were greater than those measured in nectar and whole flower matrices (Figure 2). In untreated control plants, residues in nectar and pollen were reported above the LOQ. Nectar and pollen samples had a higher range in values than leaves and whole flowers in untreated control plants (Figure 3).

As expected, non-parametric tests comparing the distributions between untreated control plants and treated plants were significant for all plant tissues (Table 9). Graphical comparisons illustrate the higher range in concentrations measured in samples taken at bloom in treated plants for parent thiamethoxam (Figure 4) and CGA322704 (Figure 5). Although some thiamethoxam residues were measured above the LOQ in untreated plants, the means were orders of magnitude lower than those in treated plots (Tables 7 and 8).

Comparison of distribution between soil types: There was no effect of soil type on the distribution for parent thiamethoxam or CGA322704 in the plant matrices (Table 9; Figure 6).

Concentration in leaves measured over time: Leaves were sampled over time to determine potential dissipation of residues (Table 10 and Table 7 for samples taken at bloom). Concentrations for both parent thiamethoxam and CGA322704 metabolite decreased over time (Figures 7 and 8). Comparison between treated and untreated control plants at 8 weeks after application indicate that thiamethoxam residues in leaves remained at elevated levels and had not completely dissipated.

Concentrations measured in soil samples: Soil samples were obtained from treated and untreated control plots prior to the start of the study and then after completion of the study (Table 11). Non-parametric test comparing the distributions between treated and untreated control plants indicated no significant difference in thiamethoxam concentrations in samples taken before the start of the study where Wilcoxon and Median exact probability values were 0.17 and 0.65, respectively. For samples taken after the study, the tests indicated elevated thiamethoxam concentration in soil sampled from treated plots where Wilcoxon and Median exact probability values were 0.002 and 0.06, respectively (Figure 9).

Concentrations in Bee Relevant Matrices: Additional statistics for the distribution of thiamethoxam and CGA322704 in pollen and nectar samples of treated plants are given in Table 12. Median and maximum values for total thiamethoxam residues in pollen were 875 and 7540 ng/g on a wet weight basis and for nectar were 182 and 660 ng/g, respectively.

8. STUDY STRENGTHS, LIMITATIONS AND CONCLUSIONS

In the context of documenting the magnitude of thiamethoxam residues in bee-related matrices of strawberry, the following strengths are observed with this study.

1. Data provide quantitative values for thiamethoxam and the major degradation product, CGA-322704, expected in leaves, flowers, nectar, and pollen of strawberry plants in response to foliar applications made prior to bloom.

2. The study was not replicated over two years but samples were obtained from 9 distinct sites. Leaf, whole flower, nectar, and pollen were collected at bloom.

3. Sites were distributed across requested soil types, allowing for a comparison of the effect of soil type on concentrations measured in plant samples.

Limitations noted in this study include:

1. Potential for carryover effects could not be determined because the study was conducted in only one of two requested years.

Overall, considering the strengths and limitations of this study, the following conclusions can be drawn:

Classification/Utility for Bee Risk Assessment. This study is classified as acceptable. Although thiamethoxam residue concentrations were reported above the LOQ in plant matrices sampled from untreated plants, the range in concentrations in treated plants were much greater. Values from treated plants reflect the range that would be expected from foliar applications.

Magnitude of Residues in Bee-relevant Matrices. The distributions reported for treated plants in pollen and nectar in Table 12 represent the expected distributions from pre-bloom foliar thiamethoxam treatments applied to strawberry plants. For data from treated plots, the median and maximum values for total thiamethoxam residues in pollen were 875 and 7540 ng/g on a wet weight basis and for nectar at 182 and 660 ng/g, respectively. Total residue values represent predominantly parent thiamethoxam, as it comprised 98% of the total.

Temporal Variability in Residues. Leaf samples taken over time indicated dissipation of residues over time. Concentrations in treated plants remained elevated when compared to untreated plants 8 week after bloom.

Effect of Soil Type. There were no differences in the magnitude and distribution of concentrations of thiamethoxam and CGA322704 between soils.

Pesticide Carryover. The study was not replicated over years so it was not possible to determine potential for carryover of residues.

9. STUDY VALIDITY/CLASSIFICATION

This study is classified as ACCEPTABLE. The data from this study provide an expected distribution of the concentrations of thiamethoxam residues that bees are exposed to in nectar and pollen of strawberry plants grown in California under the thiamethoxam exposures used in this study. Relating concentrations measured in flower parts to bee health is possible by comparing the concentration measured in bee relevant plant parts to target values that define acute or chronic exposure scenarios. Potential for carryover effects within a site could not be determined because the study was not replicated over years.

10. REFERENCES

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Table 6A. Treated Plants: Counts of chemical analytical results for thiamethoxam and CGA322704 that were indicated as above the LOQ, between the LOQ and LOD, and below the LOD.

Plant Sample	Treated Plants: Comparison of Total Number of Samples Reported Above the LOQ, Between the LOQ and LOD, and Below the LOD							
	Thiamethoxam				CGA322704			
	Total Number	Number >LOQ	Number <LOQ	Number <LOD	Total Number	Number >LOQ	Number <LOQ	Number <LOD
Nectar	27	27	0	0	27	26	0	1
Pollen	27	27	0	0	27	27	0	0
Whole Flowers	27	27	0	0	27	27	0	0
Leaves: Bloom	27	27	0	0	27	27	0	0
Leaves: After Third App	21	21	0	0	21	21	0	0
Leaves: 4 Wks After Bloom	27	27	0	0	27	27	0	0
Leaves: 8 Wks After Bloom	27	27	0	0	27	27	0	0
Soil: Pre Study	24	12	6	6	24	8	8	8
Soil: Post Study	27	26	0	1	27	20	2	5

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Table 6B. Untreated Control Plants: Counts of chemical analytical results for thiamethoxam and CGA322704 that were indicated as above the LOQ, between the LOQ and LOD, and below the LOD.

Plant Sample	Untreated Plants: Comparison of Total Number of Samples Reported Above the LOQ, Between the LOQ and LOD, and Below the LOD							
	Thiamethoxam				CGA322704			
	Total Number	Number >LOQ	Number <LOQ	Number <LOD	Total Number	Number >LOQ	Number <LOQ	Number <LOD
Nectar	9	9	0	0	9	2	5	2
Pollen	9	5	0	4	9	3	3	3
Whole Flowers	9	5	0	4	9	0	9	0
Leaves: Bloom	10	5	0	5	10	2	5	3
Leaves: After Third App	7	4	1	1	6	1	3	2
Leaves: 4 Weeks After Bloom	9	2	6	1	9	2	1	6
Leaves: 8 Weeks After Bloom	9	4	1	4	9	3	4	2
Soil: Pre Study	8	4	0	4	8	1	3	4
Soil: Post Study	9	3	1	5	9	1	5	3

Table 7. Untreated vs Treated Plants: Distribution of thiamethoxam (Parent) and CGA322704 (Metabolite) in strawberry plant samples taken at bloom.

Samples Taken at Bloom						
Statistic	Untreated Control Plants			Thiamethoxam Treated Plants		
	Parent	Metabolite	Total	Parent	Metabolite	Total
Leaves						
N (#)	10	10	10	27	27	27
Mean (ng/g)	5.5	0.5	6.0	1401	35	1437
SD	10.3	0.4	10.6	529	16	543
CV (%)	186.0	75.0	176.3	38	46	38
Min (ng/g)	0.3	0.3	0.5	428	11	439
Median (ng/g)	0.4	0.4	0.9	1483	34	1509
Max (ng/g)	30.0	1.4	31.5	2152	71	2223
% of Total	91.7	8.3		97.5	2.4	
Whole Flowers						
N (#)	9	9	9	27	27	27
Mean (ng/g)	1.6	0.3	1.8	429	14	443
SD	2.0	0.0	2.0	191	16	201
CV (%)	126.0	0.0	109.0	44	116	45
Min (ng/g)	0.3	0.3	0.5	241	5	248
Median (ng/g)	0.5	0.3	0.8	373	10	390
Max (ng/g)	5.9	0.3	6.2	973	82	995
% of Total	88.9	13.9		96.8	3.2	
Nectar						
N (#)	9	9	9	27	27	27
Mean (ng/g)	25.5	0.3	25.8	192	4	196
SD	35.3	0.3	35.2	114	3	116
CV (%)	138.0	95.0	136.5	59	65	59
Min (ng/g)	1.2	0.1	1.3	51	0	52
Median (ng/g)	9.0	0.1	9.1	177	4	182
Max (ng/g)	105.0	0.9	105.0	647	13	660
% of Total	98.8	1.2		98.0	2.0	
Pollen						
N (#)	9	9	9	27	27	27
Mean (ng/g)	122.6	1.1	123.7	2023	25	2048
SD	218.4	1.2	218.6	2432	19	2449
CV (%)	178.0	113.0	177.0	120	74	120
Min (ng/g)	8.1	0.3	8.4	102	4	108
Median (ng/g)	43.0	0.5	46.2	861	20	875
Max (ng/g)	669.0	3.8	670.8	7473	66	7540
% of Total	99.1	0.9		98.8	1.2	

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Table 8. Untreated vs Treated Plants: Exact probability levels for Wilcoxon, Median, and Kuiper non-parametric tests for differences in the distribution in chemical analyses conducted on untreated control plants and plants treated with a foliar spray of thiamethoxam.

Plant Matrix	Non-parametric Test Exact Probability Levels for Comparing Concentration Distribution Between Untreated Control and Treated Plants					
	Thiamethoxam			CGA322704		
	Wilcoxon	Median	Kuiper	Wilcoxon	Median	Kuiper
Whole Flowers	0.001	0.001	0.001	0.001	0.001	0.001
Nectar	0.001	0.001	0.002	0.001	0.001	0.001
Pollen	0.001	0.01	0.009	0.01	0.01	0.01
Leaves: Bloom	0.001	0.001	0.001	0.001	0.001	0.001
Leaves: After Third App	0.001	0.01	0.003	0.001	0.01	0.003
Leaves: 4 Wks After Bloom	0.001	0.009	0.001	0.001	0.008	0.001
Leaves: 8 Wks After Bloom	0.003	0.06	0.001	0.001	0.01	0.09
Soil: Pre Study	0.18	0.66	1	0.23	0.3	1
Soil: Post Study	0.02	0.06	0.09	0.001	0.01	0.09

Table 9. Comparison Between Soil Type: Exact probability levels for Wilcoxon rank sum test for differences amongst the 3 soil texture categories on the distribution of thiamethoxam and CGA322704 metabolite residues in strawberry plants exposed to 3 foliar sprays. Samples were taken at bloom.

Treatment, Plant Matrix, and Specific Soil Contrasts	Exact Probability Levels for Non-parametric Tests of Differences Between Soil Type	
	Thiamethoxam	CGA322704
	Wilcoxon	Wilcoxon
Treated Plants		
Leaves	0.19	0.05
Coarse vs. MedFine	0.295	0.071
Coarse vs. Medium	0.981	0.676
MedFine vs. Medium	0.225	0.225
Whole Flowers	0.20	0.62
Coarse vs. MedFine	0.714	0.580
Coarse vs. Medium	0.292	0.840
MedFine vs. Medium	0.276	0.826
Nectar	0.78	0.97
Coarse vs. MedFine	0.837	0.933
Coarse vs. Medium	0.789	1.000
MedFine vs. Medium	0.970	0.970
Pollen	0.44	0.25
Coarse vs. MedFine	0.670	0.330
Coarse vs. Medium	0.617	0.389
MedFine vs. Medium	0.539	0.970
Untreated Plants		
Leaves	0.51	0.32
Whole Flowers	0.46	1
Nectar	0.35	1
Pollen	0.53	0.17

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Table 10. Distribution of thiamethoxam (Parent) and CGA322704 (Metabolite) in strawberry plant leaf samples taken over time.

	Leaf Residue Concentration Sampled Over Time					
	Untreated Control Plants			Thiamethoxam Treated Plants		
Statistic	Parent	Metabolite	Total	Parent	Metabolite	Total
After Third Foliar Application						
N (#)	6	6	6	21	21	21
Mean (ng/g)	13.0	0.6	13.4	5753.0	29.0	5782.0
SD	19.0	0.7	19.0	2297.0	9.0	2297.0
CV (%)	145.0	113.0	142.0	40.0	31.0	40.0
Min (ng/g)	0.3	0.3	0.5	227.0	13.0	2301.0
Median (ng/g)	1.6	0.4	2.0	5257.0	27.0	5282.0
Max (ng/g)	41.5	2.1	42.0	9099.0	46.0	9125.0
% of Total	97.0	4.5		99.5	0.5	
4 Weeks After Bloom						
N (#)	9	9	9	27	27	27
Mean (ng/g)	254.0	8.8	262.7	297.0	26.0	323.0
SD	750.0	25.0	775.0	330.0	27.0	352.0
CV (%)	295.0	284.0	255.0	111.0	101.0	109.0
Min (ng/g)	0.3	0.3	0.5	45.0	5.0	52.0
Median (ng/g)	0.3	0.3	0.5	192.0	15.0	205.0
Max (ng/g)	2254.0	75.5	2330.0	1334.0	89.0	1423.0
% of Total	96.7	3.3		92.0	8.0	
8 Weeks After Bloom						
N (#)	9	9	9	27	27	27
Mean (ng/g)	262.0	7.0	269.0	341.0	29.0	362.0
SD	584.0	16.0	591.0	597.0	24.0	617.0
CV (%)	223.0	237.0	220.0	175.0	116.0	171.0
Min (ng/g)	0.3	0.3	0.5	13.0	3.0	18.0
Median (ng/g)	0.5	0.5	1.3	72.0	10.0	82.0
Max (ng/g)	1715.0	48.0	1723.0	2406.0	83.0	2471.0
% of Total	97.4	2.6		94.2	8.0	

Table 11. Distribution of thiamethoxam (Parent) and CGA322704 (Metabolite) in soil samples.

Statistic	Residue Concentration in Soil Samples					
	Untreated Control Plants			Thiamethoxam Treated Plants		
	Parent	Metabolite	Total	Parent	Metabolite	Total
Before Start of Study						
N (#)	8	8	8	24	24	24
Mean (ng/g)	4.2	0.5	4.7	7.0	0.9	7.9
SD	7.0	0.3	7.3	10.4	1.0	10.6
CV (%)	168.0	60.0	156.0	148.0	10.0	134.0
Min (ng/g)	0.3	0.3	0.5	0.3	0.3	0.5
Median (ng/g)	0.7	0.5	1.2	1.9	0.5	2.6
Max (ng/g)	19.0	1.2	20.2	36.4	3.7	37.6
% of Total	89.4	10.6		88.6	11.4	
After Completion of Study						
N (#)	9	9	9	27	27	27
Mean (ng/g)	4.9	0.5	5.4	21.2	1.8	23.0
SD	8.0	0.4	8.2	25.8	1.2	26.3
CV (%)	163.0	78.0	154.0	121.0	67.0	114.0
Min (ng/g)	0.3	0.3	0.5	0.3	0.3	0.5
Median (ng/g)	0.3	0.3	0.8	11.5	1.6	13.0
Max (ng/g)	20.6	1.4	21.1	85.2	5.6	88.0
% of Total	90.7	9.3		92.2	7.8	

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Table 12. Bee Relevant Plant Matrices: Distribution of thiamethoxam, CGA322704 metabolite, and total thiamethoxam residues in nectar and pollen samples of strawberry plants exposed to 3 foliar sprays of thiamethoxam prior to bloom.

Statistic	Distribution of Residues		
	Thiamethoxam	CGA322704	Total
Nectar			
N (#)	27	27	27
Mean (ng/g)	192	4	196
SD (ng/G)	114	3	116
CV (%)	59	65	59
Min (ng/g)	51	0	52
Median (ng/g)	177	4	182
75th (ng/g)	214	5	215
90th (ng/g)	296	5	301
95th (ng/g)	376	10	381
Max (ng/g)	647	13	660
% of Total	98.0	2.0	
Pollen			
N (#)	27	27	27
Mean (ng/g)	2023	25	2048
SD (ng/G)	2432	19	2449
CV (%)	120	74	120
Min (ng/g)	102	4	108
Median (ng/g)	861	20	875
75th (ng/g)	2486	37	2522
90th (ng/g)	7349	62	7411
95th (ng/g)	7445	62	7493
Max (ng/g)	7473	66	7540
% of Total	98.8	1.2	

Figure 1. Explanation of the meaning of graphics presented in a Box-and-Whisker plot.

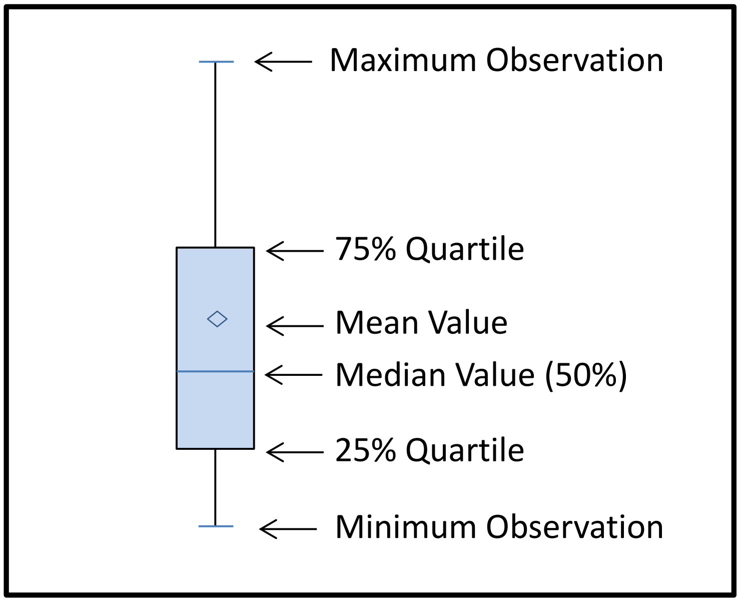


Figure 2. Treated Plants: Comparison of the distribution of thiamethoxam residues measured between leaf, nectar, pollen, and whole flower samples. Values transformed to natural logarithms.

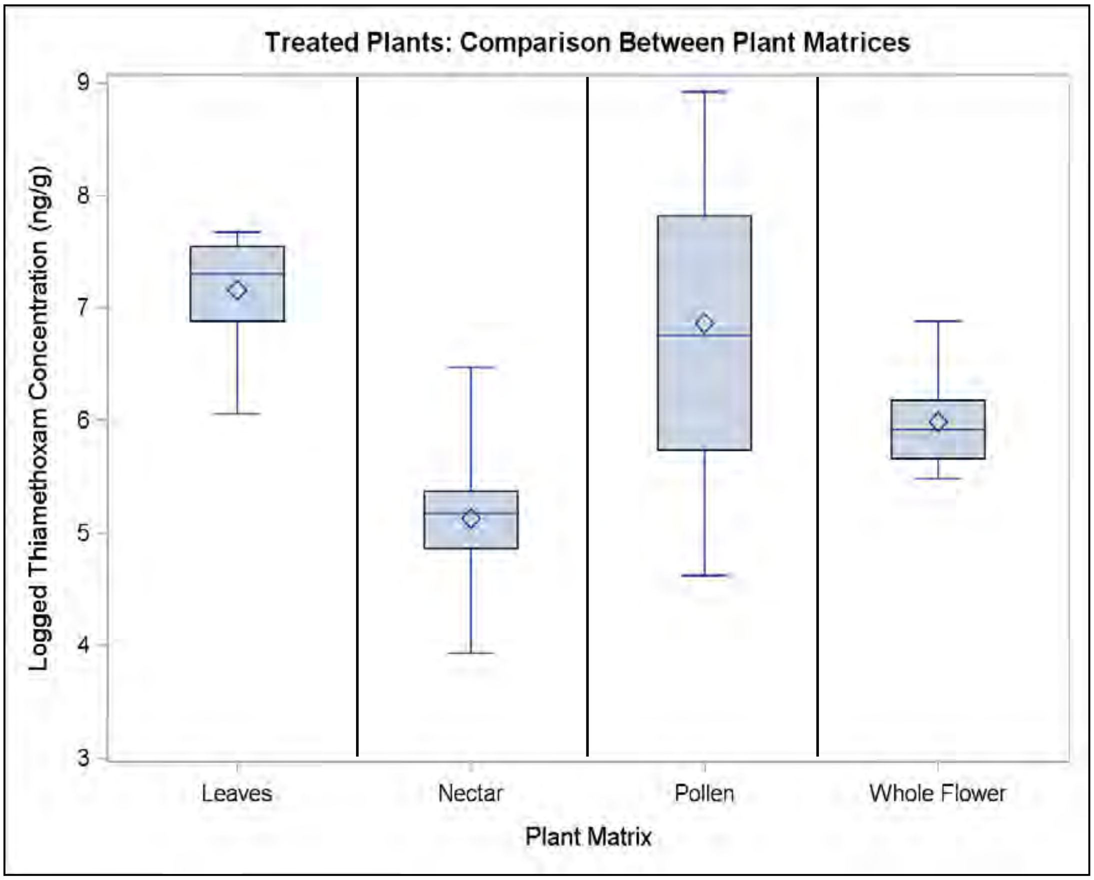


Figure 3. Untreated Control Plants: Comparison of the distribution of thiamethoxam residues measured between leaf, nectar, pollen, and whole flower samples. Values transformed to natural logarithms.

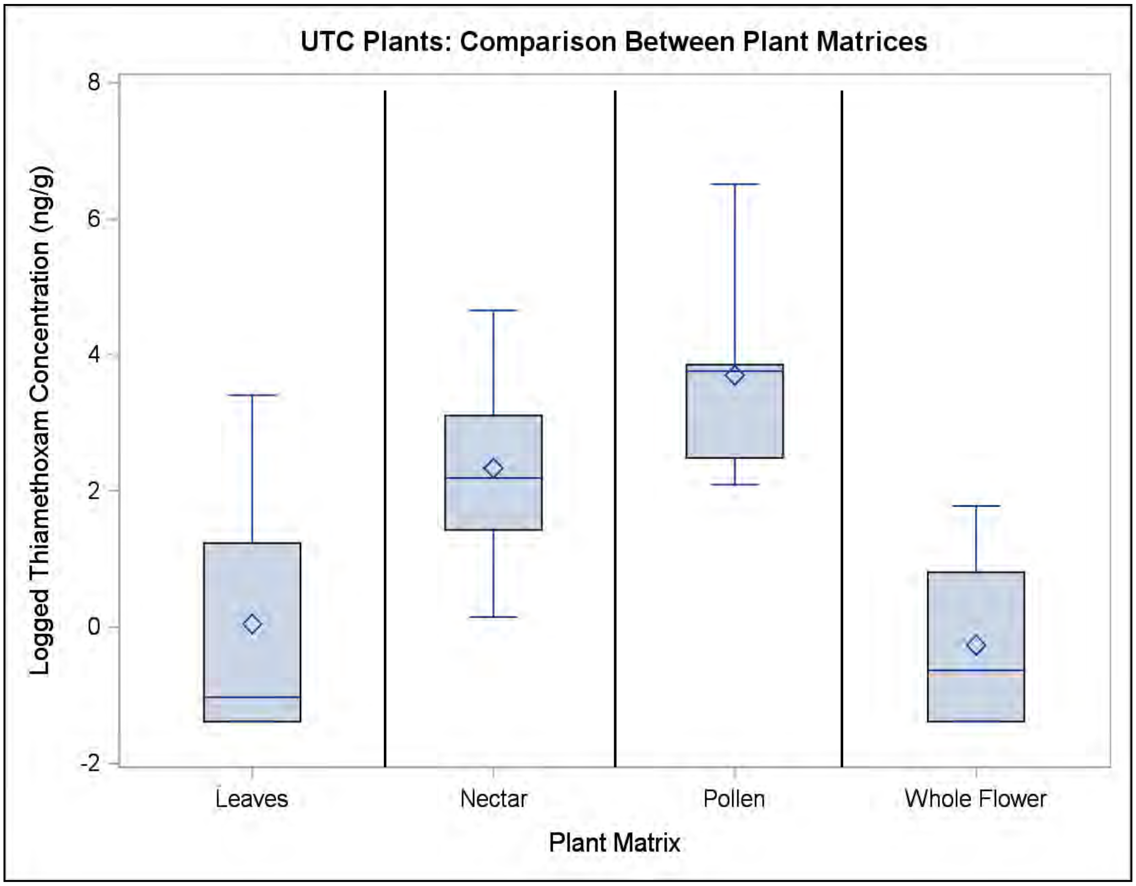


Figure 4. Untreated vs Treated Bloom Plant Samples: Comparison of the distribution of thiamethoxam residues measured between untreated control (UTC) and treated (TRT) plants for leaves, nectar, pollen, and whole flowers.

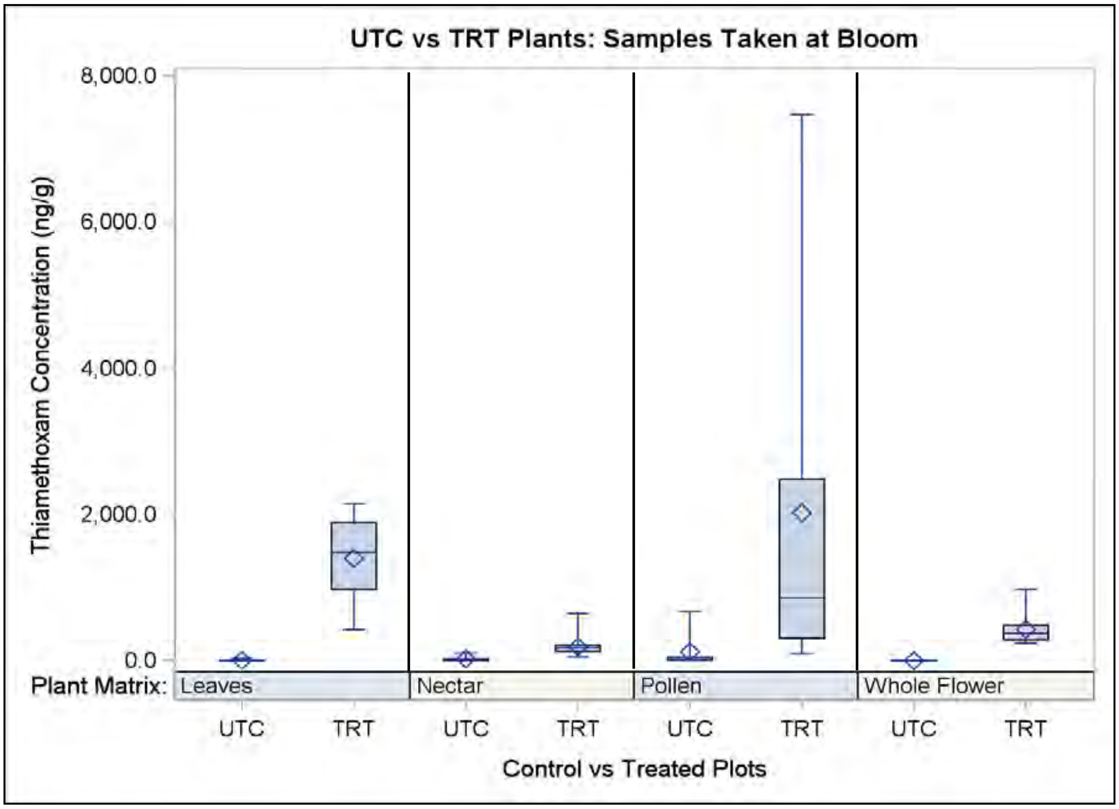
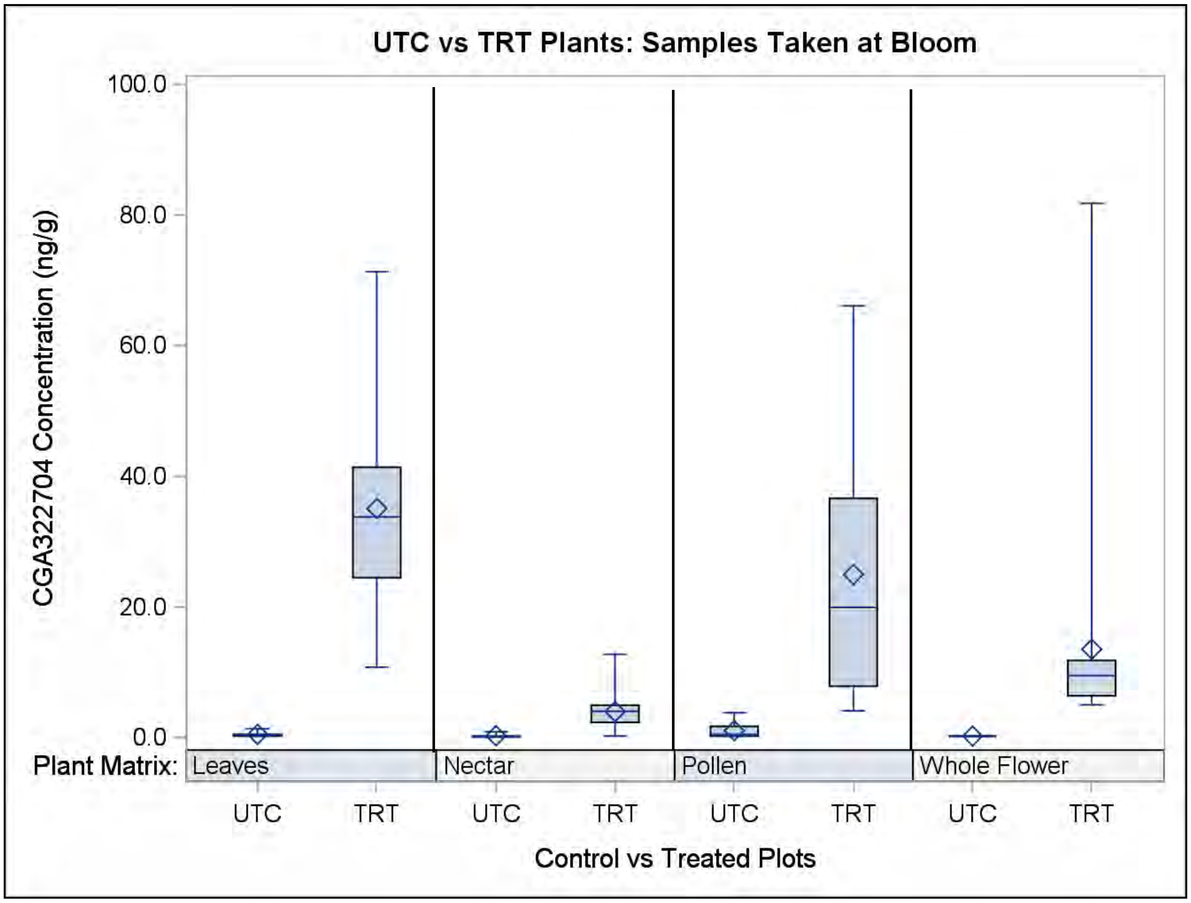


Figure 5. Untreated vs Treated Bloom Plant Samples: Comparison of the distribution of CGA322704 thiamethoxam metabolite residues measured between untreated control (UTC) and treated (TRT) plants for leaves, nectar, pollen, and whole flowers.



MRID 50265502 CDPR THX Strawberry

Figure 6. Comparison Between Soil Type: Distribution of thiamethoxam residues measured in leaves, nectar, pollen, and whole flowers between plants grown in soil at 3 different textures. Plants sampled at bloom and values were transformed to natural logarithms.

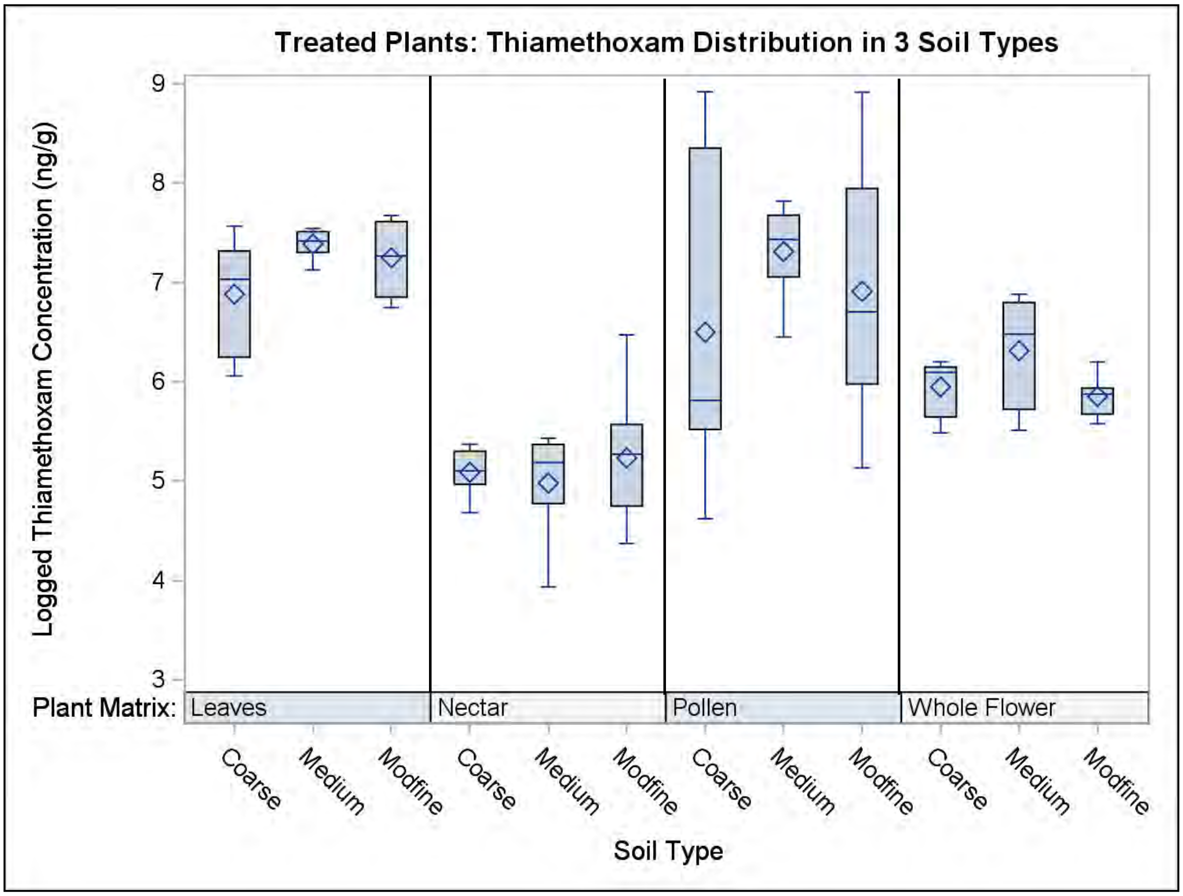


Figure 7. Thiamethoxam Concentration in Leaves Over Time: Comparison of the distribution of thiamethoxam residues measured in leaves sampled after the third foliar application, at bloom, and then at 4 and 8 weeks after bloom. Leaves from untreated control plants (UTC) were sampled at the same time as treated (TRT) plants. Values were transformed to natural logarithms.

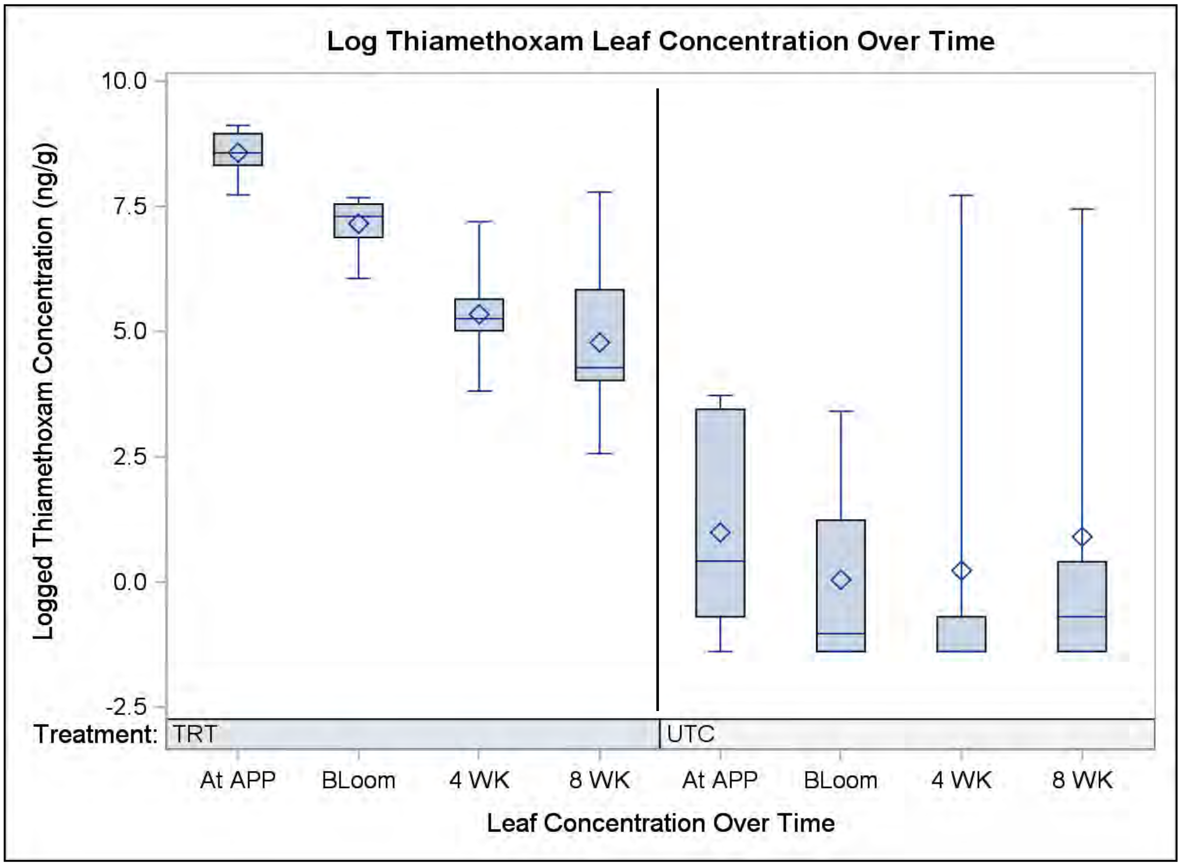


Figure 8. Thiamethoxam Concentration in Leaves Over Time: Comparison of the distribution of CGA322704 thiamethoxam metabolite residues measured in leaves sampled after the third foliar application, at bloom, and then at 4 and 8 weeks after bloom. Leaves from untreated control plants (UTC) were sampled at the same time as treated (TRT) plants. Values were transformed to natural logarithms.

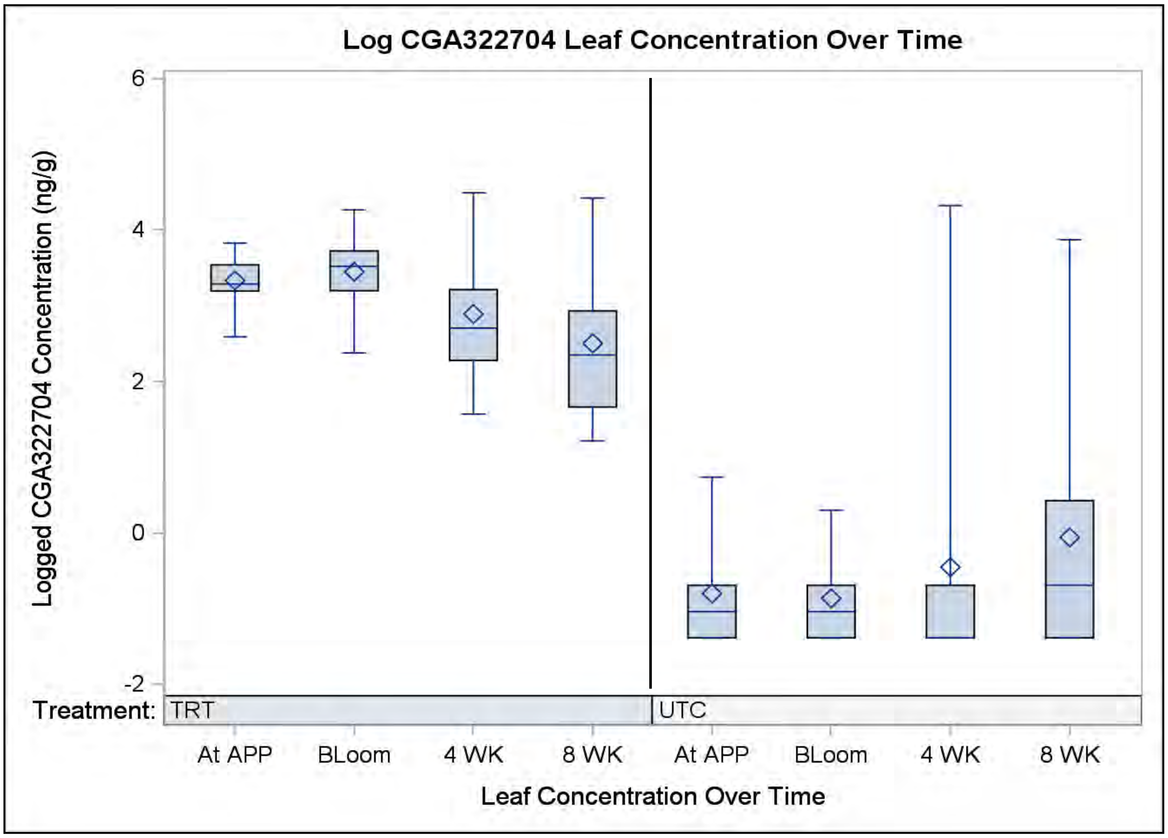
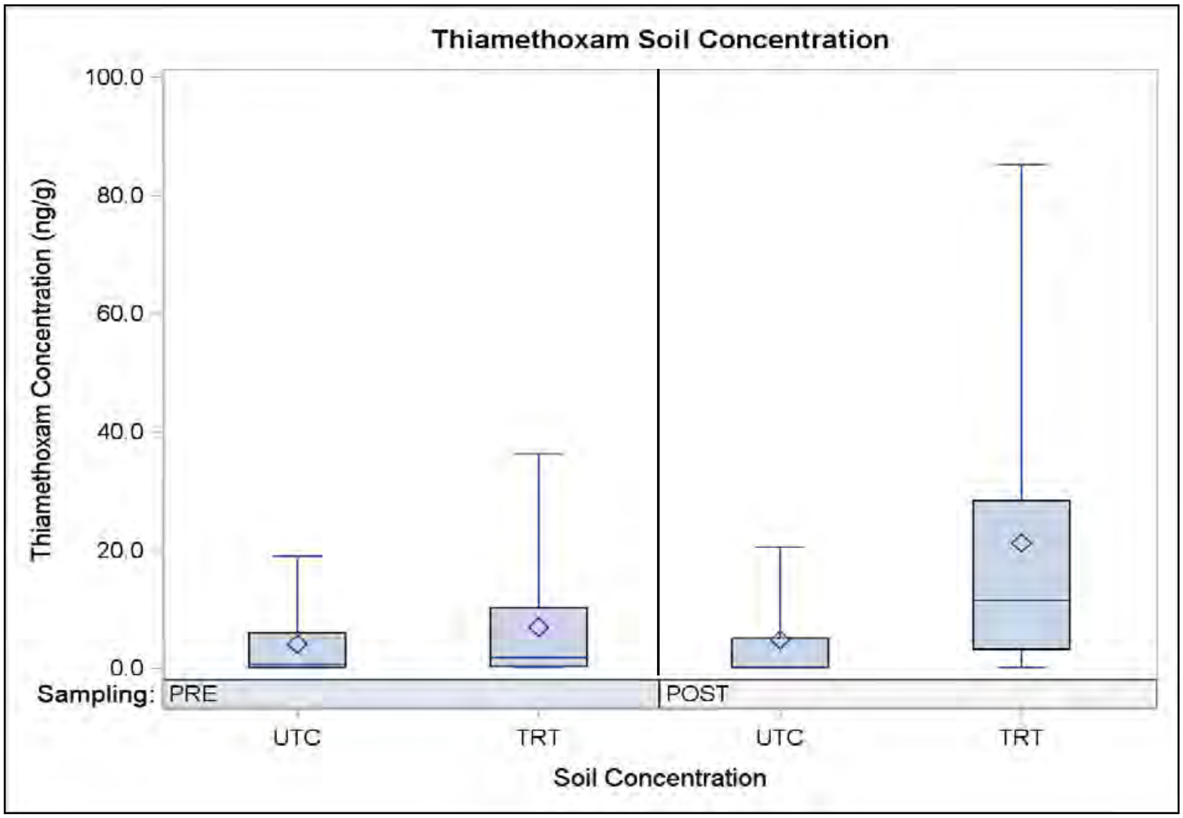


Figure 9. Thiamethoxam Concentration in Soil Samples: Comparison of the distribution of thiamethoxam residues measured in soil sampled prior to the start of the study and then after the completion of the study. Soil from untreated control plants (UTC) were sampled at the same time as from treated (TRT) plants.



Appendix 10. Evaluations of Residue Studies Included in this Risk Determination Document

Reference
Oakes, T., Mäyer, T., Rice, F., Jacobson, B. Grant, J. (2017) Thiamethoxam 40 WG (A11963C) and Thiamethoxam FS (A9765N) – Magnitude of Residues in Leaves, Flowers, Pollen, Nectar and Extra Floral Nectar of Cotton Plants After Foliar Application with Centric® 40WG in California or After Application as a Seed Treatment with Cruiser® 5FS: Amended Final Report. Project Number: TK0177223. Unpublished study prepared by Syngenta Crop Protection, LLC. 512. MRID 49686801, CDPR Study ID 304439, Data Volume 52691-0590, Tracking ID# 283477

1. STUDY INFORMATION

Chemical:	Thiamethoxam	**PC Code**	60109
Test Material:	Centric 40WG Cruiser 5FS	**Percent Active Ingredient:**	40% 47.6%
Study Type:	Non-Guideline field residue study on cotton to establish the magnitude of Thiamethoxam residues in leaves, flowers, pollen, nectar and extra floral nectar during a two year period.		
Sponsor:	Syngenta Crop Protection, LLC 410 Swing Road Post Office Box 18300 Greensboro, North Carolina 27419-8300 USA	**Experiment Start and End Date:**	May 8, 2013- January 4, 2017
Sponsor Study ID:	TK0177223	**Study Locations:**	Nine trial sites that were either foliar or seed applications located in California.
Study Completion Date:	July 17, 2015		
Amendment Date:	October 30, 2017		
GLP Status:	TBD; protocol reviewed by CDPR. [CDPR Study ID 304439, Data Volume 52691-0590, Tracking ID# 283477]		

2. REVIEWER INFORMATION

Study Reviewed by: California Department of Pesticide Regulation	Richard Bireley, Sr. Environmental Scientist (Specialist) John Troiano, Ph.D., Research Scientist III Alexander Kolosovich, Sr. Environmental Scientist (Specialist) Brigitte Tafarella, Environmental Scientist Denise Alder, Sr. Environmental Scientist (Specialist) Russell Darling, Environmental Scientist

MRID 49686801 THX Cotton DER

3. EXECUTIVE SUMMARY

The reason for the final report amendment is to correct the study title and the analytical phase report to incorporate transit stability data, freezer storage stability data, percent Brix data, reassignment of study responsibilities, and to clarify and correct sample residues for Year 1 nectar and extra floral samples from Site 6. The analytical report has also been amended for a more consistent reporting format and to add clarifications throughout. An extensive list of changes to the Analytical Phase Report can be found in Appendix 1 of the study report.

The study was designed to include nine trials each consisting of an untreated control plot and a three-replicate treated plots to be conducted on coarse-, medium- and fine-textured soils. Centric® 40WG (active ingredient, thiamethoxam) was applied as a foliar broadcast spray two times during the growing season at a target rate of 0.063 lb ai/acre/application for two consecutive years. The interval between applications was 5 days with the last application targeted 12 days before significant flowering. In the first year, three of the nine trials also included a three-replicate plot planted with Cruiser® 5FS (active ingredient, thiamethoxam) treat seed at a targeted rate of 0.375 mg a.i. per seed.

Samples of leaf, whole flower, pollen, nectar and extra floral nectar were collected from all trial sites in Year 1 (2013) and Year 2 (2014) of the study. The target sampling period at all trials (including seed treatment trials) was at early bloom stage (50–75% bloom). In the foliar-application trials, sampling was targeted to occur 12 days after the second (last) test substance application. Additionally for Year 2, at six trial sites extra floral nectar (EFN) was collected at 3 additional target intervals: 5 days after first application (5 DA1A), 5 days after second application (5 DA2A), and 24 days after the second application (24DA2A). These samples were collected to characterize residues of thiamethoxam and CGA322704 in EFN during bloom.

Method verification sets were performed at ABC Laboratories prior to the analysis of field samples. Methods were verified successfully for thiamethoxam and CGA322704 in leaves and whole flowers, and CGA322704 in nectar. Method verification for pollen resulted in mean percent recoveries below acceptance criteria for both analytes, and nectar mean percent recovery was below acceptance criteria for thiamethoxam. Concurrent procedural recovery samples were diluted in subsequent analyses to improve results and achieve acceptable method performance for these analytes and matrices.

4. STUDY VALIDITY

Guideline Followed:	TBD; (protocol was reviewed by CDPR)
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	**TBD**
Rationale:	N/A
Reparability:	N/A

5. MATERIALS

Test Material Characterization for Foliar Application End Use Product			
Test item:	Centric 40 WG	**Percent A.I.:**	40% A.I.
Formulation Type:	Wettable Granule, WG	**Design Code:**	A11963C
CAS #:	153719-23-4	**Specific Gravity:**	26 – 34 lb/ft^3

Test Material Characterization for Seed Treatment End Use Product			
Test item:	Cruiser 5FS	**Percent A.I.:**	47.6% A.I.
Formulation Type:	Flowable Suspension	**Concentration:**	5.18 lb a.i./gallon
CAS #:	153719-23-4	**Specific Gravity:**	1.295 g/mL

5A. STUDY DESIGN

The purpose of this two-year study was to determine the amount of thiamethoxam and CGA322704 that was present in cotton leaves, whole flowers, pollen, nectar, and extra floral nectar in fields after two successive years of foliar broadcast spray applications of Centric® 40WG. Also, the two analytes were measured in plants grown from seed treated with Cruiser® 5FS in the first year in three of the trials. The effect of soil type on thiamethoxam uptake and resulting residues in pollen and nectar were examined by conducting trials on coarse-, medium-, and fine textured soils, as available. The study initiation date was May 8, 2013 and the experimental termination date (analytical phase) was January 4, 2017.

Foliar-Application Trials (CA-1 – CA-9)

The study included nine geographically separated trials in the Central Valley of California. Each trial consisted of an untreated control (UTC) plot and a treated (TRT) plot (divided into three replicate areas) that were each large enough to collect sufficient quantities of flowers, leaves, pollen, nectar, and extra floral nectar for residue analysis. The plot size for the UTC and the TRT replicate plot at each location was a minimum of 0.115 A. The UTC plot was located up-slope and up-wind with regard to the prevailing wind direction and separated by a minimum of 50 ft. from the TRT plot to minimize potential cross-contamination by run-off or pollen transfer.

Representative composite samples of leaves, whole flowers, pollen, nectar, and extra floral nectar were collected from the UTC plot and each of the treated replicate plots for residue analysis in Year 1 and Year 2 of the study (n = 27 samples per matrix each year).

Seed-Treatment Application Trials (CA-2A, CA-6A, and CA-8A)

One additional treated plot (Year 1 only) was added at three of the foliar application locations. Each trial consisted of three replicated TRT plots that were approximately the same size (0.115 A) as indicated above for the foliar-application trials.

Representative composite samples of leaves, whole flowers, pollen, nectar and extra floral nectar were collected from each of the treated replicate plots for residue analysis in Year 1 of the study (n = 9 samples per matrix).

5B. Application Timing and Rates

Foliar Application Trials:

In Year 1 and Year 2, Centric® 40WG (thiamethoxam formulated) was applied to treated plots as a broadcast foliar spray two times at the maximum labeled-use rate of 2.5 oz formulated product per acre (0.063 lb ai/acre) for each application. The interval between applications was 5 days and the last application was scheduled to occur 12 days before significant bloom. The test substance was applied using a calibrated, boom sprayer in a volume of water that ensured uniform application (20 - 30 gallons/acre). The tables below present the application information for each trial.

All applications were made using ground-based equipment. The adjuvant Dyne-Amic (0.25 % v/v) was used in all foliar applications.

Table 1. Year 1 (2013)

Trial Number	Application 1			Application 2		
	Date	Calibrated GPA[a]	Tank Mix Volume	Date	Calibrated GPA[a]	Tank Mix Volume
1	8/30/2013	25.47	13.0	9/4/2013	25.93	13.0
2	8/2/2013	25.50	13.0	8/7/2013	25.66	13.0
3	9/6/2013	29.47	19.0	9/11/2013	20.04	13.46
4	8/9/2013	30.0	14.0	8/14/2013	30.0	14.0
5	7/22/2013	30.0	14.0	7/27/2013	30.0	14.0
6	8/2/2013	30.0	14.0	8/7/2013	30.0	14.0
7	8/2/2013	20.0	10.5	8/7/2013	20.0	10.5
8	8/5/2013	20.0	10.1	8/10/2013	20.0	10.1
9	8/10/2013	20.0	9.0	8/15/2013	20.0	9.0

[a] **Gallons Per Acre**

Table 2. Year 2 (2014)

Trial Number	Application 1			Application 2		
	Date	Calibrated GPA[a]	Tank Mix Volume	Date	Calibrated GPA[a]	Tank Mix Volume
1	8/8/2014	24.89	13.0	8/13/2014	24.96	13.0
2	7/18/2014	25.44	13.0	7/23/2014	25.79	14.0
3	7/27/2014	26.61	20.6	8/1/2014	25.71	19.9
4	7/18/2014	30.0	14.0	7/23/2014	30.0	14.0
5	7/7/2014	30.0	14.0	7/12/2014	30.0	14.0
6	7/21/2014	30.0	14.0	7/26/2014	30.0	14.0
7	7/18/2014	20.0	10.5	7/23/2014	20.0	10.5
8	7/1/2014	20.0	11.0	7/6/2014	20.0	11.0
9	7/11/2014	20.0	9.0	7/16/2014	20.0	9.0

[a] **Gallons Per Acre**

Seed-Treatment Application Trials:

These treated plots were planted with a commercial variety of cotton (Phytogen 499) that was treated with Cruiser® 5FS at a rate of 0.375 mg a.i. per seed. The seed was treated at Syngenta Seed Care Institute, Stanton, MN, using commercial seed treatment procedures. The seed treatment rate was verified by analysis of the treated seed at SGS NAM GLP Laboratory, Brookings, SD. The actual seed treatment rate based upon the verification analysis was 93% of target or 0.349 mg a.i. per seed. Treated cotton seed from a batch of certified seed (0.375 mg a.i. per seed) was provided to each selected facility. The table below presents the planting date and planting rate (seeds/A) for each of the three seed-treatment trials.

Table 3. Seed Treatment Applications

Trial Number	Application/Planting Date	Planting Rate[1] (Seeds/A)
2a	5/31/2013	59,739
6a	6/11/2013	144,500
8a	6/6/2013	36,000

[1] Thiamethoxam treated seed (0.375 mg ai/seed)

5C. Study Site Location and Characteristics

Trial site locations were selected based on soil-survey maps, soil characterization information, site availability, security, and cotton cultural significance to ensure representation of different soil textures (e.g., loamy sand, sandy loam, and loam, as availability allowed). The table below presents the field cooperator facility along with site information that includes county, soil series and textural class for each trial.

The test plots (UTC and TRT) were managed to mimic typical commercial cotton production in California. Plot areas were prepared, planted, and maintained according to local agricultural practice with regard to tillage, fertilizer inputs, irrigation, and weed and pest control. Irrigation was applied as needed according to commercial good agricultural practices for maintaining good crop health and yield.

After harvest of cotton in 2013 (Year 1), the cotton was shredded and the plant matter was disked/tilled into the ground in preparation for Year-2. Plots remained fallow until the plots were prepared, i.e., tilled and re-bedded, the following spring prior to planting in Year 2 (2014).

Table 4. Results from the Soil Characterization Analysis

Field Cooperator	Application Type	Trial Number	Trial Location (County, State)	Organic Matter (%)	Soil pH	Cation Exchange Capacity (meq/100g soil)	% Sand	% Silt	% Clay	Soil Types
Research For Hire	Foliar Application	1	Tulare, California	1.60	7.8	11.6	48	34	18	Exeter Loam (Fine/ Medium)
		2	Tulare, California	1.4	6.6	14.6	80	15	5	Tujunga Loamy Sand (Coarse)
				1.3	6.4	15.6	76	19	5	
	Seed Treatment	2a	Tulare, California	0.64	6.5	20.5	78	17	5	Tujunga Loamy Sand (Coarse)
				0.59	5.7	14.0	80	15	5	

Field Cooperator	Application Type	Trial Number	Trial Location (County, State)	Organic Matter (%)	Soil pH	Cation Exchange Capacity (meq/100g soil)	% Sand	% Silt	% Clay	Soil Types
Syntech Research	Foliar Application	3	Fresno, California	0.49	6.1	5.6	60	32	8	Hanford Fine Sandy Loam (Medium)
				0.40	6.1	5.5	58	32	10	
Excel Research	Foliar Application	4	Madera, California	0.77	7.2	6.6	78	15	7	Atwater Loamy Sand (Coarse)
				0.86	6.7	7.0	78	17	5	
		5	Madera, California	0.47	7.2	6.1	84	9	7	Chino Loamy Sand (Coarse)
				0.43	7.1	5.7	82	11	7	
		6	Madera, California	0.90	7.2	8.4	78	15	7	Atwater Loamy Sand (Coarse)
				0.43	6.7	7.8	76	11	13	
	Seed Treatment	6a	Madera, California	0.90	7.6	7.4	82	11	7	Atwater Loamy Sand (Coarse)
				0.77	7.6	6.7	82	11	7	
Cal Ag Research	Foliar Application	7	Fresno, California	0.87	7.6	13.0	50	32	18	Ramona Loam (Fine/Medium)
				0.61	7.6	14.1	48	36	16	
		8	Fresno, California	0.52	5.6	4.7	78	16	6	Hanford Sandy Loam (Medium)
				0.26	5.8	3.8	78	16	6	
	Seed Treatment	8a	Fresno, California	0.39	5.9	4.8	72	20	8	Hanford Sandy Loam (Medium)
				0.78	6.1	5.3	72	22	6	
Eurofins	Foliar Application	9	Sanger, California	1.10	7.6	9.7	66	24	10	Ramona Sandy Loam (Medium)
				1.05	7.5	11.0	70	22	8	

5D. SAMPLE COLLECTION, HANDLING, PROCESSING

Samples of leaf, whole flower, pollen, nectar, and extra floral nectar were collected from all trial sites in Year 1 of the study. In the foliar-application trials, sampling was targeted to occur 12 days after the second (last) test substance application. The target sampling period at all trials (including seed treatment trials) was at early bloom stage (50–75% bloom). All trials were sampled 12 days after the second application, except trials CA-3 and CA-9, which were sampled 9 and 14 days after the second application, respectively. These changes from the targeted 12 day schedule were made in order to obtain enough flowers to meet target sample size requirements.

In Year 2, samples of leaf, whole flower, pollen, nectar, and extra floral nectar were also collected from all trial sites. At all trials, sampling was targeted to occur 12 days after the second (last) test substance application. The target sampling period at all trials was at early bloom stage (50–75% bloom). All trials were sampled 12 days after the second application. In Year 2 the CA-1 nectar samples collected at 12 Days after second application (bloom) and the CA-2 extra floral nectar samples collected at 24 days after second application were lost in shipment to the laboratory.

Additionally for Year 2, at six trial sites (CA-1, CA-2, CA-3, CA-4, CA-6 and CA-7), Extra Floral Nectar (EFN) was collected at 3 additional target intervals: 5 days after first application (5 DA1A), 5 days after second application (5 DA2A), and 24 days after second application (24 DA2A). These samples were collected to be able to characterize residues of thiamethoxam and CGA322704 in EFN over time.

At each trial site, samples were collected from the UTC plot before the collection of samples from the TRT plot or by separate teams to minimize potential cross contamination. Gloves were worn and replaced between flower and leaf sampling and between plots.

Whole Flower and Leaf Samples

Each whole flower sample was a minimum of 500 intact flowers, if available. Flower samples were divided into 2 bags, one bag for the whole flower sample (200 flowers minimum) and another bag for the processing flowers (300 flowers minimum) from which pollen and nectar were collected. The only whole-flower samples that did not have the 200-flower minimum was at CA-9 in Year 2. Each leaf sample was a minimum of 500 g. Samples were collected from the UTC and each TRT replicate plot (A, B, and C). Samples were collected from the lower, middle and upper plant canopy for a representative, composite sample. The UTC and TRT leaf and flower samples were stored and transported in separate, labelled plastic sealable bags to the field laboratory on blue or wet ice for freezer storage or further processing.

Pollen, Nectar and Extra Floral Nectar Samples

The remaining 300 or more flowers were used for collection of pollen and nectar. An additional 300 flowers, when available, with the receptacle, peduncles, and the calyculus bracteoles were collected for extra floral nectar. At some trials, the 300 flowers for pollen and nectar collection were also used for extra floral nectar collection or the number of flowers processed for extra floral nectar were less than 300 due a limited availability of flowers at sampling.

Extraction approaches entail the use of:

(1) a vacuum pump fitted with a disposable 1000-µL filtered pipette tips to vacuum and trap the pollen; and,
(2) a 10-µL or larger capillary micro-pipettes to extract nectar from the exposed nectary.

The floral nectar was collected from the internal whorl of epicalyx bract nectaries, which occur on the inner side of the sepal base. The extra floral nectar was collected from the calycular nectaria.

The nectar collected in each micro-pipette was expelled into a pre-labelled 2 mL glass vial. Pipette tips and vials were weighed prior to and immediately after pollen and nectar collection to enable calculation of sample mass. The target pollen and nectar sample weight required for analysis – minus the pipette or vial weight – was >100 mg for TRT plots and >250 mg for UTC plots. Pipette tips (containing pollen) were placed directly into labelled vessels suitable for solvent extraction. Pollen and nectar samples were stored frozen until shipment. The target sample weight for all matrices collected in the treated replicate plots were met except for extra floral nectar (23 samples). The sample weight for some extra floral nectar samples collected from the treated replicate plots were less than the target (>100mg) at trials CA-1, CA-3, CA-5 and CA-9 in Year 1 and CA-1 and CA-2 in Year 2.

Transit Stability

For transit and freezer stability data, at least two concurrent recovery samples per analytical set were prepared by fortifying an untreated control sample with thiamethoxam and CGA322704 at the same level as the stability samples (50 ppb) and analyzed concurrently with the stability samples to demonstrate adequate method performance. The recoveries for both the fresh fortified samples as well as the stored samples were corrected for any control residues, prior to calculating percent recovery.

Sample Storage.

The leaf and whole flower samples were weighed and homogenized with dry ice using a Robot Coupe; the homogenized samples were placed into labeled, plastic containers and stored in a freezer (allowing the dry ice to sublime). After sample preparation, the homogenized leaf and whole flower samples were stored in plastic containers and placed in a freezer until they were sub-sampled for analysis. Pollen, nectar, and extra floral nectar extracts needed no homogenization and were stored directly in a freezer. Freezer-storage temperatures were monitored and typically were maintained at -10 to -25 °C.

For the samples in this study that were analyzed for thiamethoxam and CGA322704, the maximum frozen storage period experienced for any matrix was 303 days (10 months), from the sampling date of treated samples through the extraction of treated samples.

Previous storage stability studies show thiamethoxam and CGA322704 are stable in a variety of matrices for up to 12 months. Therefore, residues of thiamethoxam and CGA322704 in cotton leaf, whole flower, pollen, and nectar samples should not have been adversely affected by freezer storage during this study.

5E. ANALYTICAL METHODS

The reference standard information, as required by 40 CFR Part 160.185(a)(4), as well as representative LC-MS/MS chromatograms and typical calibration curves, can be found in the Analytical Phase Report located in Appendix 1 of the final study report.

Analysis of Leaves and Flower Samples

Leaf and whole flower samples were analyzed for thiamethoxam and CGA 322704 based on the analytical method described in Syngenta Method REM 179.06, entitled "Residue Method for the Determination of Residues of Thiamethoxam (CGA 293343) and CGA 322704 in Lettuce, Tomato, Grape and Tobacco Samples. Final Determination by LC-MS/MS" [6]. In the subject method, residues of thiamethoxam and CGA322704 were extracted with 50:50 methanol/water from 10-g leaf and whole flower samples using a high-speed homogenizer. Extracts were centrifuged and concentrated via SPE cleanup in preparation for LC-MS/MS analysis. The Limit of Quantitation (LOQ) for both analytes in leaves was 5.0 ppb and the Limit of Detection (LOD) was targeted to be 2.5 ppb. The Limit of Quantitation (LOQ) for both analytes in whole flowers was 1.0 ppb and the Limit of Detection (LOD) was targeted to be 0.50 ppb.

Analysis of Pollen, Nectar, and Extra Floral Nectar Samples

Pollen, nectar, and extra floral nectar samples were analyzed for thiamethoxam and CGA322704 based on the analytical method described in Syngenta Method REM 179.07, entitled "Thiamethoxam: Analytical Method for the Determination of Residues of Thiamethoxam (CGA 293343) and CGA 322704 in Bee and Hive Products. Final Determination by LC-MS/MS" [7]. In summary, residues of thiamethoxam and CGA322704 were extracted with 50:50 methanol/water: 0.2% formic acid (aq) from 0.05 g pollen and nectar samples. Pollen extracts were centrifuged and passed through a solid-phase extraction cleanup in preparation for LC-MS/MS analysis. The Limit of Quantitation (LOQ) for both analytes, in pollen, nectar, and extra floral nectar, was 1.0 ppb. The Limit of Detection (LOD) was targeted to be 0.50 ppb.

Summary of LOQs and LODs

Matrix	Analyte	LOQ (ppb, parent equivalents)	LOD (ppb, parent equivalents)
Leaves	Total Thiamethoxam	5.0	2.5
Whole Flowers	Total Thiamethoxam	1.0	0.50
Pollen, Nectar, and Extra Floral Nectar	Total Thiamethoxam	1.0	0.50

5F. QUALITY ASSURANCE RESULTS

Validated analytical methods were provided by the Sponsor to ABC Laboratories, Inc. Prior to analysis of field samples, the analytical methods were verified by ABC Laboratories, Inc. as part of this study. The standard (calibration) curve generated for each analytical set was used for the quantitation of thiamethoxam and CGA322704 in the samples. For this study, the correlation coefficient (r) for each calibration curve was equal to or greater than 0.990 (r^2 equal to or greater than 0.98).

To verify performance of the validated analytical methods at ABC Laboratories prior to analysis of field samples, UTC samples of each matrix were fortified with thiamethoxam and CGA322704 at concentrations equal to the method LOQ as well as from 5× to 50×LOQ, and analyzed according to the methods described in the "Analytical Methods" portion of this document.

For each matrix, at least one concurrent recovery sample per analytical set was prepared by fortifying an untreated control sample with thiamethoxam and CGA322704 at concentrations equal to the method LOQ and up to 200×LOQ (1000× LOQ for extra floral nectar), and analyzing concurrently with the treated field samples to demonstrate adequate method performance throughout the study, i.e. recoveries of 70 to 120%. In light of method verification recoveries that did not meet acceptance criteria, concurrent procedural recovery samples were diluted in subsequent analyses to effect improved results.

6. RESULTS:

Year 1

Thiamethoxam residues >LOD were found in 15 of 36 UTC plant matrices and CGA322704 residues >LOD were found in 3 of 36 UTC plant matrices. Residues greater than the LOQ were found in all plant matrices sampled from the treated cotton plots.

Table 5. Summary of Thiamethoxam and CGA322704 Residues in Samples from the Treated Plots of the Foliar Application Trials Year 1 (2013)

Trial Site	Soil Texture	Plant Matrix	Thiamethoxam Concentrations (ppb)			CGA322704 Concentration (ppb)		
			Mean Residue	Standard Deviation	Maximum Residue	Mean Residue	Standard Deviation	Maximum Residue
Year 1 (Foliar Broadcast Plot)								
1	Fine/Medium	Leaves	485	236	667	70.3	18	84.8
2	Coarse		337	99	448	70.4	26	100
3	Medium		420	108	505	50.1	19	70.0
4	Coarse		236	62	307	50.3	4.3	54.0
5	Coarse		57.3	5.2	61.6	27.4	2.5	29.2
6[a]	Coarse		98.7	33	126	31.6	18	49.2
7	Fine/Medium		92.1	33	130	24.7	16	42.8
8	Medium		92.7	17	108	41.0	3.4	44.8
9	Medium		162	101	278	52.3	12	65.2
1	Fine/Medium	Whole Flowers	147	13	156	28.4	4.8	33.8
2	Coarse		173	16	185	35.7	3.4	37.7
3	Medium		194	36	235	37.8	5.7	43.8
4	Coarse		93.3	6.9	101	27.2	2.4	28.8
5	Coarse		56.2	6.6	61.1	10.2	0.29	10.5
6[a]	Coarse		87.6	6.0	94.4	15.1	1.3	16.5
7	Fine/Medium		78.5	5.5	84.6	8.48	7.2	13.4
8	Medium		25.3	2.9	28.6	16.7	1.8	18.1
9	Medium		28.8	1.7	30.7	15.3	2.6	18.1
1	Fine/Medium	Pollen	3.23	0.73	3.79	0.507	0.44	1.02
2	Coarse		2.64	1.1	3.78	ND	N/A	ND
3	Medium		5.21	2.3	7.72	0.410	0.28	0.729
4	Coarse		1.31	0.49	1.78	ND	N/A	ND
5	Coarse		4.13	2.3	6.78	0.623	0.65	1.37
6[a]	Coarse		24.3	19	46.1	1.36	0.81	2.29
7	Fine/Medium		7.50	8.3	17.0	0.786	0.64	1.50
8	Medium		2.09	2.9	5.43	1.01	1.3	2.54
9	Medium		1.05	1.4	2.66	ND	N/A	ND
1	Fine/Medium	Nectar	0.983	0.07	1.06	ND	N/A	ND
2	Coarse		4.41	2.1	5.87	ND	N/A	ND
3	Medium		3.61	0.42	4.07	ND	N/A	ND
4	Coarse		1.84	1.1	3.02	0.37	0.20	0.595
5	Coarse		3.34	3.1	6.85	ND	N/A	ND
6[a]	Coarse		2.34	0.30	2.67	ND	N/A	ND
7	Fine/Medium		1.33	0.17	1.49	0.349	0.2	0.548
8	Medium		0.908	0.13	1.06	0.493	0.22	0.662

Trial Site	Soil Texture	Plant Matrix	Thiamethoxam Concentrations (ppb)			CGA322704 Concentration (ppb)		
			Mean Residue	Standard Deviation	Maximum Residue	Mean Residue	Standard Deviation	Maximum Residue
9	Medium		0.623	0.10	0.740	0.362	0.19	0.585
1	Fine/Medium	Extra Floral Nectar	127	89	228	7.26	6.2	14.3
2	Coarse		48.2	24	75.6	1.56	0.93	2.63
3	Medium		176	36	201	3.95	0.51	4.31
4	Coarse		26.7	7.7	32.2	1.37	0.20	1.60
5	Coarse		33.1	9.4	42.5	1.11	0.29	1.44
6[a]	Coarse		115	36	153	2.32	0.71	3.01
7	Fine/Medium		37.8	11	44.1	1.18	0.18	1.38
8	Medium		35.7	18	50.2	1.54	0.19	1.74
9	Medium		27.8	18	48.6	2.51	2.2	5.00

[a] Site 6 control samples EFN 105 and NC 104, as well as treated samples EFN 110 and NC 109 are suspected to have been mis-labeled but placed in the correct sample bags. The discrepancy between labels and bags was discovered at the lab, and the bottles were switched. Residues confirm the samples were probably mis-labeled and had originally been placed in the correct bags; therefore, results from the sample labeled as EFN Sample 105 are reported for NC Sample 104, and EFN Sample 110 are reported for NC Sample 109.

Note: For the purposes of calculations, ND samples were treated as ½ the LOD (0.25 ppb for whole flowers, pollen and nectar; 1.25 ppb for leaves).

Table 6. Summary of Thiamethoxam and CGA322704 Residues in Samples from the Treated Plots of the Seed Treatment Trials.

Trial Site	Soil Texture	Plant Matrix	Thiamethoxam Concentration (ppb)			CGA322704 Concentrations (ppb)		
			Mean Residue	Standard Deviation	Maximum Residue	Mean Residue	Standard Deviation	Maximum Residue
2a	Medium	Leaves	6.17	0.85	7.08	4.88	1.4	6.36
6a	Coarse		9.81	12	23.5	7.30	3.7	10.4
8a	Coarse		1.56	0.4	1.94	ND	N/A	ND
2a	Medium	Whole Flowers	6.38	1.1	7.30	2.72	0.52	3.06
6a	Coarse		2.07	1.5	3.78	0.854	0.45	1.36
8a	Coarse		ND	N/A	ND	ND	N/A	ND
2a	Medium	Pollen	ND	N/A	ND	ND	N/A	ND
6a	Coarse		ND	N/A	ND	ND	N/A	ND
8a	Coarse		ND	N/A	ND	ND	N/A	ND
2a	Medium	Nectar	0.559	0.27	0.759	0.849	0.17	1.04
6a	Coarse		0.664	0.46	1.16	ND	N/A	ND
8a	Coarse		0.195	0.0	0.220	ND	N/A	ND

Trial Site	Soil Texture	Plant Matrix	Thiamethoxam Concentration (ppb)			CGA322704 Concentrations (ppb)		
			Mean Residue	Standard Deviation	Maximum Residue	Mean Residue	Standard Deviation	Maximum Residue
2a	Medium	Extra Floral Nectar	0.650	0.69	1.45	ND	N/A	ND
6a	Coarse		0.493	0.21	0.624	ND	N/A	ND
8a	Coarse		ND	N/A	ND	ND	N/A	ND

Note: For the purpose of calculations, ND samples were treated as ½ the LOD (0.25 ppb for the whole flowers, pollen and nectar; 1.25 ppb for leaves).

Year 2

Thiamethoxam residues >LOD were found in 18 of 60 untreated control samples and CGA322704 residues >LOD were found in 5 of 60 untreated control samples. Residues greater than the LOQ were found in all plant matrices sampled from the treated cotton plots .

Table 6. Summary of Thiamethoxam and CGA322704 Residues in Samples from the Treated Plots of the Foliar Application Trials Year 2 (2014).

Trial Site	Soil Texture	Plant Matrix	Thiamethoxam Concentrations (ppb)			CGA322704 Concentration (ppb)		
			Mean Residue	Standard Deviation	Maximum Residue	Mean Residue	Standard Deviation	Maximum Residue
Year 2 (Foliar Broadcast Plot)								
1	Fine/Medium	Leaves	ND	N/S	ND	ND	N/A	ND
2	Coarse		173	31	195	21.7	2.4	24.1
3	Medium		22.1	2.1	23.7	9.76	2.6	11.8
4	Coarse		73.6	3.4	77.3	26.7	0.0	26.7
5	Coarse		29.1	16	40.8	11.0	0.82	11.9
6	Coarse		24.9	3.4	26.9	13.0	2.5	15.9
7	Fine/Medium		113	20	136	13.8	3.3	17.6
8	Medium		129	60	186	9.40	2.7	11.0
9	Medium		51.1	3.1	53.9	7.49	1.4	8.93
1	Fine/Medium	Whole Flowers	19.0	4.3	24.0	5.38	0.74	6.22
2	Coarse		89.1	27	119	18.2	7.6	26.0
3	Medium		32.3	4.8	36.8	9.63	1.5	10.9
4	Coarse		24.8	5.2	28.1	6.13	1.0	6.84
5	Coarse		46.7	4.3	50.0	11.6	0.55	12.0
6	Coarse		16.6	2.6	19.2	4.30	0.56	4.94
7	Fine/Medium		69.6	9.9	80.6	12.5	1.1	13.7
8	Medium		58.6	3.7	62.5	11.8	0.55	12.3
9	Medium		14.6	3.4	18.5	3.30	0.65	4.03

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Trial Site	Soil Texture	Plant Matrix	Thiamethoxam Concentrations (ppb)			CGA322704 Concentration (ppb)		
			Mean Residue	Standard Deviation	Maximum Residue	Mean Residue	Standard Deviation	Maximum Residue
1	Fine/Medium	Pollen	ND	N/A	ND	ND	N/A	ND
2	Coarse		5.61	2.7	8.64	ND	N/A	ND
3	Medium		55.0	21	79.2	3.74	1.5	5.24
4	Coarse		69.0	47	122	6.18	3.5	10.2
5	Coarse		1.19	0.55	1.76	ND	N/A	ND
6	Coarse		57.5	41	96.4	3.64	2.5	6.06
7	Fine/Medium		205	130	351	11.4	4.6	15.4
8	Medium		51.2	78	141	3.50	5.6	10.0
9	Medium		4.84	5.2	10.9	0.421	0.3	0.762
1[a]	Fine/Medium	Nectar	--	--	--	--	--	--
2	Coarse		20.9	22	46.2	0.560	0.27	0.774
3	Medium		1.38	0.16	1.49	1.35	0.29	1.53
4	Coarse		3.32	1.2	4.70	0.537	0.26	0.746
5	Coarse		2.22	1.5	3.94	0.460	0.36	0.880
6	Coarse		1.80	1.4	2.89	0.492	0.22	0.680
7	Fine/Medium		1.70	0.61	2.22	0.435	0.16	0.540
8	Medium	Nectar	3.99	2.1	6.47	0.642	0.16	0.832
9	Medium		1.05	0.36	1.28	ND	N/A	ND

[a] No sample available for analysis.

Note: For the purpose of calculations, ND samples were treated as ½ the LD (0.25 ppb for whole flowers, pollen and nectar/extra floral nectar; 1.25 ppb for leaves).

Table 7. Summary of Thiamethoxam and CGA322704 Residues in Extra Floral Nectar Samples from the Treated Plots of the Foliar Application Trials Year 2 (2014)

Trial Site	Soil Texture	Sample Timing	Thiamethoxam Concentration (ppb)			CGA322704 Concentration (ppb)		
			Mean Residue	Standard Deviation	Maximum Residue	Mean Residue	Standard Deviation	Maximum Residue
Extra Floral Nectar								
1	Fine/Medium	5DA1A	197	94	303	2.01	1.0	3.16
		5DA2A[a]	--	--	--	--	--	--
		Bloom	24.6	4.7	29.3	0.612	0.32	0.864
		24DA2A	0.593	0.59	1.28	ND	N/A	ND
2	Coarse	5DA1A	68.6	62	122	1.03	0.68	1.53
		5DA2A	35.3	40	81.5	0.824	0.68	1.58
		Bloom	49.4	64	123	1.73	1.0	2.90
		24DA2A[a]	--	--	--	--	--	--
3	Medium	5DA1A	239	71	288	3.59	0.96	4.48
		5DA2A	542	197	268	13.1	4.3	18.0
		Bloom	39.8	7.8	45.1	1.71	0.33	1.90
		24DA2A	4.24	0.96	5.06	0.337	0.15	0.512
4	Coarse	5DA1A	71.9	10	83.4	1.42	0.32	1.73
		5DA2A	47.2	4.6	52.5	1.17	0.25	1.42
		Bloom	104	65	178	5.04	2.9	8.40
		24DA2A	1.38	0.74	2.23	ND	N/A	ND
5	Coarse	Bloom	50.5	14	66.3	1.78	0.60	2.47
6	Coarse	5DA1A	29.4	9.8	40.7	0.735	0.48	1.21
		5DA2A	50.4	23	67.2	1.37	0.33	1.63
		Bloom	6.02	4.5	10.7	ND	N/A	ND
		24DA2A	0.588	0.02	0.612	ND	N/A	ND
7	Fine/Medium	5DA1A	112	37	154	1.21	0.20	1.43
		5DA2A	186	83	253	2.20	0.87	2.99
		Bloom	57.6	16	76.5	1.49	0.29	1.82
		24DA2A	0.626	0.34	0.922	ND	N/A	ND
8	Medium	Bloom	33.6	18	54.3	1.14	0.55	1.74
9	Medium	Bloom	9.75	1.6	10.7	0.360	0.19	0.581

[a] No sample available for analysis.

Note: For the purpose of calculations, ND samples were treated as ½ the LOD (0.25 ppb).

7. Statistical Analysis

Study Objectives and Design

The main objective of the study was to determine the concentration of thiamethoxam and its degradation product CGA322704 in whole flowers, nectar, extra floral nectar, pollen, and leaves of cotton plants in response to foliar applications of a thiamethoxam pesticide product. Additional test plots were included in year 1 to measure concentrations of thiamethoxam residues in plant matrices in response to a seed-treatment application. For the foliar spray study, the rate of application of Centric 40WG was 0.063 lbs a.i./Acre. Applications were made twice at 5 day intervals with the second application scheduled to occur 12 days before significant bloom. For the seed treatment study, treated seed at approximately 0.375 mg a.i./seed were planted at 3 of the trial sites in year 1 of the study. The foliar application portion of the study was replicated in the next year whereas the seed treatment portion was discontinued. Sampling of plant matrices were targeted for 12 days after the second foliar application, which was denoted as an early bloom period where blooms were at 50 to 70%. In order to provide enough sample for analysis, sampling commenced a few days later at two sites in year 1. In year 2, extra floral nectar was sampled at additional time intervals to characterize concentration of thiamethoxam residues over time. Additional extra floral nectar samples were taken at 5 days after the first foliar application, at 5 days after the second foliar application, and finally at 24 days after the second foliar application. An amended study report was submitted to update and correct various aspects of the previous study report. A complete list of changes is published on pages 59 through 65 of the amended study report. Data used for this analysis were obtained from pages 128 through 147 of the amended study report.

Non-parametric statistical tests were used to test for differences in distribution of concentrations between years, untreated control to treated plants, extra floral nectar concentration between sampling intervals, and between soil type. Non-parametric tests do not require tests for normality as they are robust to differences in distribution and they are also robust for experimental designs with low replicates (Helsel and Hirsch, 2002). The PROC NPAR1WAY procedure in the Statistical Analysis System (SAS) statistical package was used to conduct Wilcoxon-Mann –Whitney (Wilcoxon), Median non-parametric, and Kuiper tests. A significant result from the Wilcoxon test indicates differences in the shape of distributions; A significant result from the Median test indicates differences in the location of the medians between distributions; and A significant result from the Kuiper test indicates differences in the empirical distributions between two groups. The Exact option for each statistic was implemented as it provides permutation testing, a statistical method that minimizes the effect of sample size and distributional differences. Using the Exact option, the Monte Carlo procedure was also implemented, which provided 10,000 separate runs for each statistic to produce the permutation distributions. The test for potential differences in extra floral nectar concentrations over time had 4 levels so the DSCF option in PROC NPAR1WAY, which invokes the Dwass, Steel, Critchlow-Fligner multiple comparison test, was used to provide pairwise tests for two-sample rankings. Additional procedures used for descriptive statistics were PROC MEANS to calculate mean values from the replicates at each site, PROC CAPACITY to produce cumulative statistics, and PROC BOXPLOT to produce comparative graphics. Statistical analysis for effects and soil type were conducted on the replicate sample mean taken from each site. Due to limited site and year data, all replicate data was used to compare seed to foliar treatments (year 1 data) and to compare concentrations of extra floral nectar between sampling intervals (year 2 data).

Some graphical comparisons are presented with data transformed to a natural logarithm scale, providing clearer contrasts between the distributions. Although both limits of detection (LOD) and quantification (LOQ) were indicated, only data less than the LOD were indicated as ND in the data set. Values were provided between the LOD and LOQ. For statistical analyses, values noted as below the limit of detection (LOD) were assigned half the value of the respective detection limit (Table 5). Values between the LOD and LOQ were used as reported. The distribution of concentrations in bee relevant plant matrices were calculated using all the raw data because these values represent the actual range of exposure to bees and other organisms that feed off the nectar and pollen of plants.

Detection rate noted for each plant matrix: Counts for the number of samples reported below the respective detection limit for each matrix are presented in Table 8A for treated plants and Table 8B for untreated control plants. For plants treated with a foliar spray, the majority of concentrations for thiamethoxam and CGA322704 metabolite were above the LOQ in leaf and flowers sampled at bloom; Percent of values above the LOQ ranged from 94% to 100% of respective sample sizes. For nectar and pollen samples, the number of samples above the LOQ was greater for thiamethoxam than for the CGA322704 metabolite. For nectar, 80% of thiamethoxam values were above the LOQ, whereas only 6% of CGA322704 concentrations were above the LOQ: Values for pollen were 83% and 33%, respectively. The majority of concentrations in extra floral nectar samples were again above the LOQ for samples taken before and during bloom. The pattern observed for samples obtained at 24 days after the 2nd foliar application indicated declining concentrations as the percentage of thiamethoxam samples above the LOQ decreased from 100% to 40% of samples and none of the samples were above the LOQ for CGA322704.

The distribution of values in seed treated plants indicated much lower proportions of concentrations measured above the LOQ with similar distributions for both parent and metabolite. In leaf and flower samples, the percentage of samples above the LOQ ranged from 33% to 44%. For nectar, pollen, and extra floral nectar samples, the range was from 0% to 13% of concentrations above the LOQ (Table 8A).

Although the majority of concentrations measured in plant matrices in untreated control plants were reported below the LOD, concentrations were reported above the LOQ, especially for thiamethoxam concentration in pollen samples and in extra floral nectar samples taken at the first sampling interval, 5 days after the 1st foliar application (Table 8b).

Comparison of distribution between years: Potential difference between years was measured to indicate the presence of carry-over effects of residues. Results for analyses of thiamethoxam concentrations for foliar treated plants were mostly non-significant (Table 9). Two significant Wilcoxon tests were indicated for leaves and flowers but the graphic shows that the values were potentially greater in year 1 than in year 2 (Figure 2). This pattern implies no potential for carry-over of residues due to foliar sprays.

Comparison of distribution between untreated and treated plants: The distribution statistics for all treatments are presented for nectar, pollen, and extra floral nectar in Table 10 and for leaves and whole flowers in Table 11. Non-parametric tests conducted on the replicate sample mean for foliar treated plots indicated a significantly greater range in the distribution for foliar treated plants compared to untreated control plants for all matrices (Table 12; Figures 3 and 4). The exception was CGA322704 concentrations in pollen where detection of residues due to treatment were minimal.

Residue concentrations in seed treated plants were low and essentially similar to the range measured in untreated control plants with the exception of leaf and whole flower matrices where concentrations were slightly greater in seed treated plants (Table 12; Figures 5 and 6). Although statistical tests for thiamethoxam in flower samples were not significant, the graphical comparison indicated an elevated range in concentrations for seed treated plants.

Since concentrations were low in seed treated plants, comparison of concentrations to foliar treated plants indicated a higher range in residues in most matrices in plants treated with foliar sprays (Table 12; Figures 7 and 8). The results for CGA322704 residues in nectar and pollen indicated no differences due to minimal detection of residues in treated plants.

Comparison of distribution between soil types: Based on the soil characteristics provided in the study report, the sites were distributed between coarse and medium textured soil types: No sites were in a fine, clayey soil type. Sites C-1, C-3, C-7, and C-9 were classified as medium textured soil and sites C-2, C-4, C-5, C-6 and C-8 were classified as coarse-textured soil. Results of non-parametric tests show no significant differences in the distributions in the plant matrices between the two soil types (Table 13; Figures 9 and 10). There was an indication of a significant difference in thiamethoxam concentrations for nectar but the values in nectar were minimal and the effect is most likely circumspect.

Concentration in extra floral nectar sampled over time: Additional extra floral nectar samples were taken to determine concentration over time. This aspect of the study was only conducted in year 2 so analyses were based on the replicate samples obtained with each plot. Distributional statistics for the sampling intervals are presented in Table 14. Concentrations at the final sampling date were approaching background levels measured in untreated control plants so each contrast to concentrations at the 3 previous sampling dates were significant (Table 15; Figures 11 and 12). Additional differences were shown for thiamethoxam residues where concentrations at the first 2 sampling dates were similar but both higher in range in concentration than for those at bloom.

Data for bee relevant matrices: The observed distributions derived from the individual analyses ostensibly determines the expected range in concentrations of thiamethoxam and CGA322704 residues in bee relevant plant samples for the studies combination of plant species and application scenario (Table 10). The median and maximum values for total residue in nectar were 2.4 and 47 ng/g, respectively, on a wet weight basis. For pollen, median and maximum values were 4 and 366 ng/g, respectively. For extra floral nectar median and maximum values at bloom were 43 and 242 ng/g, respectively. Additional sampling of extra floral nectar after each application indicated that potential exposure occurs prior to bloom where median and maximum concentrations after the first application were 92 and 306 ng/g and after the second foliar treatment were 69 and 786 ng/g, respectively.

8. Conclusions

1. Utility of the data: The study followed the design as directed in the data call-in with the study being replicated in two years at 9 sites. Given the limitations of finding experimental sites in existing fields, the 9 sites were reasonable representatives of only 2 of the 3 soil types requested in the data call-in.

2. Concentrations in Bee Relevant Matrices: By default, the distributions reported in Table 10, under the Foliar Treated heading, represent the expected concentrations in bee relevant matrices that result from two foliar applications of thiamethoxam to cotton plants where the last application was 12 days prior to bloom. Median and maximum values for total thiamethoxam residues in plant matrices taken at bloom for nectar were 2.4 and 47 ng/g, for pollen were 4 and 366 ng/g and for extra floral nectar were 43 and 242 ng/g, respectively.

3. Extended Exposure from Extra Floral Nectar: Samples of extra floral nectar were obtained prior to bloom after the second application. Concentrations of residues were higher than when measured at bloom, indicating potential exposure prior to bloom.

4. No carry-over effect between years: Concentrations measured in plant matrices between the two years of the study were similar, indicating low potential for carry-over effects due to foliar applications at the concentrations and timing used in this study.

5. No Effect of Soil Type: Data for 2 of the 3 soil types were available, coarse and medium textured soils, for this study. There were no differences in the range of either thiamethoxam or CGA322704 metabolite residues between the two soil types.

6. Comparison to Seed Treated Plants: In the first year of the study, a few plots were added to compare concentrations in plant matrices that result from a seed treatment application. Values measured at bloom indicated low concentrations in bee relevant matrices in seed treatments.

9. STUDY STRENGTHS, LIMITATIONS AND CONCLUSIONS

In the context of documenting the magnitude of thiamethoxam residues in bee-related matrices of cotton plants, the following strengths are observed with this study.

> 1. The study provided quantitative values for cotton plant matrices exposed to foliar application of thiamethoxam.
>
> 2. The study was replicated over two years with measurements in plant samples taken at bloom after two foliar applications of thiamethoxam to cotton plants. Blooms were sampled approximately 12 days after the second application.
>
> 3. The 9 sites were reasonably replicated over the 2 of the requested 3 soil texture categories.

Limitations noted in this study include:

> 1. Additional aspects of the study such as seed treatment effects were lacking in replication with respect to the foliar application.

Overall, considering the strengths and limitations of this study, the following conclusions can be drawn:

1. **Bee-relevant matrices:** Thiamethoxam residues were measured in nectar, pollen, and extra floral nectar plant matrices sampled 12 days after a second foliar application to cotton plants. Values in Table 10, under the Foliar Treated heading, indicate the potential range in concentrations that bees are exposed to in the field.

2. **Potential for Extended Exposure to Extra Floral Nectar:** Additional samples of extra floral nectar taken after each application indicate potential for significant exposure prior to the blooming period through foraging on extra floral nectar. The range in concentrations after an application is greater than when sampled at bloom.

3. **No carry-over effect of years:** Concentrations measured in plant matrices were similar between the two years of the study indicating low potential for carry-over effects due to foliar treatments at the concentration and timing of application used in this study.

4. **Effect of soil type:** No differences were measured in the range of residue concentrations in plant matrices between plants grown in coarse or medium textured soils.

5. **Effect of Seed Treatment:** Although the number of replications were low for seed treatments, the data indicate lower concentrations than from foliar applications. Concentrations in plant matrices from seed treatment were similar to the range measured in untreated control plants.

10. STUDY VALIDITY/CLASSIFICATION

The data from this study provide an expected distribution of the thiamethoxam residue concentrations that bees are exposed to in nectar, pollen, and extra floral nectar in cotton plants under actual agronomic practices in California. Relating concentrations measured in flower parts to bee health is possible by comparing the concentrations measured in bee relevant plant parts to target values that define acute or chronic exposure scenarios. The study is considered scientifically sound and useful for risk assessment purposes. The study is classified as ACCEPTABLE for quantitative use in risk assessment.

11. REFERENCES

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2. Hohl, J. (1999) Stability of Residues of CGA-322704 in Plant Material and Soil Stored Under Deep Freezer Conditions (Study No. 779-00) MRID 45108001.

3. Oakes, T. (2002) Stability of CGA-293343 and CGA-322704 in Crops and Processed Fractions Under Freezer Storage Conditions (Study No. 269-98) MRID 45659205.

4. Hampton, R. (2012) "Thiamethoxam 75 SG (A9549C) - Magnitude of the Residues in Leaves, Flowers, Pollen, and Nectar of Cucumbers, Representative Commodity of Cucurbit Vegetables, EPA Crop Group 9, in California" (Report Number: TK0024668).

5. Anderson, L. (2007) Thiamethoxam (CGA293343) and CGA322704. Validation of Residue Analytical Method REM 179.07 for the determination of Residues in Bee and Hive Products and Storage Stability in Hive Pollen, Wax and Nectar, stored Deep Frozen for 12 months. (Study No. 05-S508).

6. Crook, S (2004) Residue Method for the Determination of Residues of Thiamethoxam (CGA293343) and CGA322704 in Lettuce, Tomato, Grape and Tobacco Samples. Final Determination by LC/MS/MS" (Syngenta Method REM179.06).

7. Crook, S (2007) Analytical Method for the Determination of Residues of Thiamethoxam (CGA293343) and CGA322704 in Bee and Hive Products. Final Determination by LC-MS/MS (Syngenta Method REM179.07).

8. Mohan, P. and Khadi, B.M. (2007) Nectar Glands in Gossypium. CICR Technical Bulletin No: 37. Central Institute of Cotton Research Nagpur.

Table 8A. Treated Plants: Counts of chemical analytical results for thiamethoxam and CGA322704 that were indicated as above the LOQ, between the LOQ and LOD, and below the LOD.

Plant Sample	Treated Plants: Comparison of Total Number of Samples Reported Above the LOQ, Between the LOQ and LOD, and Below the LOD							
	Thiamethoxam				CGA322704			
	Total Number	Number >LOQ	Number <LOQ	Number <LOD	Total Number	Number >LOQ	Number <LOQ	Number <LOD
Foliar Application								
Leaf	54	51	0	3	54	51	0	3
Flower	54	54	0	0	54	53	0	1
Nectar	50	40	9	1	50	3	17	30
Pollen	54	45	3	6	54	18	5	31
Extra Floral Nectar								
5 DA1App	18	18	0	0	18	15	1	2
5 DA2App	15	15	0	0	15	11	3	1
Bloom	53	53	0	0	53	41	6	6
24 DA2App	15	6	6	3	15	0	1	14
Seed Application								
Leaf	9	4	1	4	9	3	3	3
Flower	9	3	0	6	9	4	2	3
Nectar	8	1	3	4	8	1	2	5
Pollen	9	0	0	9	9	0	0	9
Extra Floral Nectar	9	1	2	6	9	0	0	9

Table 8B. Untreated Control Plants: Counts of chemical analytical results for thiamethoxam and CGA322704 that were indicated as above the LOQ, between the LOQ and LOD, and below the LOD.

Plant Sample	Untreated Control Plants: Comparison of Total Number of Samples Reported Above the LOQ, Between the LOQ and LOD, and Below the LOD							
	Thiamethoxam				CGA322704			
	Total Number	Number >LOQ	Number <LOQ	Number <LOD	Total Number	Number >LOQ	Number <LOQ	Number <LOD
Leaf	18	1	0	17	18	0	1	17
Flower	18	3	1	14	18	0	1	17
Nectar	16	3	1	12	16	0	0	16
Pollen	17	4	5	8	17	2	3	12
Extra Floral Nectar								
5 DA1App	6	4	1	1	6	0	0	6
5 DA2App	5	1	1	3	5	0	0	5
Bloom	16	3	2	11	16	2	1	13
24 DA2App	5	1	0	4	5	0	0	5

Table 9. Statistical results for test of differences in concentrations of thiamethoxam or CGS322704 metabolite residues measured between years 1 and 2.

Source	Nonparametric Test Exact Probability Levels: Effect of Year		
	Thiamethoxam		
	Wilcoxon	Median	Kuiper
Leaf	0.02	0.34	0.57
Flower	0.02	0.35	0.57
Nectar	0.54	1	0.95
Pollen	0.11	0.34	0.26
Extra Floral Nectar	0.49	1	0.89

Table 10. Distributional statistics for concentrations of thiamethoxam (Parent) and CGA322704 (Metabolite) measured in nectar, pollen, and extra floral nectar sampled from untreated control, foliar treated or seed treated cotton plants.

Statistic	Distribution of Thiamethoxam Residue Concentration at Bloom								
	Nectar			Pollen			Extra Floral Nectar		
	Parent	Metab	Total	Parent	Metab	Total	Parent	Metab	Total
Untreated Control Plants									
N (#)	16	16	16	17	17	17	16	16	16
Mean (ng/g)	1.2	0.3	1.4	1.1	0.5	1.6	1.7	1.0	2.7
SD (ng/g)	2.7	0.0	2.7	1.5	0.5	1.7	3.5	2.4	4.5
CV (%)	231	0	190	137	95	107	208	237	168
Min (ng/g)	0.3	0.3	0.5	0.3	0.3	0.5	0.3	0.3	0.5
Median (ng/g)	0.3	0.3	0.5	0.7	0.3	0.9	0.3	0.3	0.5
75th (ng/g)	0.4	0.3	0.7	0.8	0.6	1.5	0.7	0.3	1.5
90th (ng/g)	2.0	0.3	2.3	4.6	1.3	4.8	8.8	2.6	10.4
95th (ng/g)	10.9	0.3	11.2	5.2	1.9	5.9	12.3	9.6	14.9
Max (ng/g)	10.9	0.3	11.2	5.2	1.9	5.9	12.3	9.6	14.9
% of Total	**85.7**	**17.9**		**68.8**	**31.3**		**63.0**	**37.0**	
Foliar Treated									
N (#)	50	50	50	54	54	54	53	53	53
Mean (ng/g)	3.3	0.4	3.7	27.8	1.9	29.8	53.4	2.0	55.4
SD (ng/g)	6.5	0.3	6.6	58.7	3.3	61.9	50.0	2.3	51.8
CV (%)	201	69	178	211	170	208	94	112	94
Min (ng/g)	0.3	0.3	0.8	0.3	0.3	0.5	1.8	0.3	2.1
Median (ng/g)	1.7	0.3	2.4	3.8	0.3	4.3	41.1	1.4	42.8
75th (ng/g)	2.9	0.6	3.4	17.0	2.2	18.5	59.3	2.4	61.9
90th (ng/g)	5.3	0.8	5.8	96.4	6.1	102.5	123.0	3.8	125.9
95th (ng/g)	6.9	1.0	7.3	141.0	10.2	151.0	178.0	5.0	186.4
Max (ng/g)	46.2	1.5	47.0	351.0	15.4	366.4	228.0	14.3	242.3
% of Total	**89.2**	**10.8**		**93.3**	**6.4**		**96.4**	**3.6**	
Seed Treated									
N (#)	8	8	8	9	9	9	9	9	9
Mean (ng/g)	0.5	0.5	1.0	0.3	0.3	0.5	0.5	0.3	0.7
SD (ng/g)	0.3	0.3	0.5	0.0	0.0	0.0	0.4	0.0	0.4
CV (%)	64	68	51	0	0	0	87	0	56
Min (ng/g)	0.3	0.3	0.5	0.3	0.3	0.5	0.3	0.3	0.5
Median (ng/g)	0.4	0.3	0.9	0.3	0.3	0.5	0.3	0.3	0.5
75th (ng/g)	0.7	0.8	1.4	0.3	0.3	0.5	0.6	0.3	0.9
90th (ng/g)	1.2	1.0	1.8	0.3	0.3	0.5	1.5	0.3	1.7
95th (ng/g)	1.2	1.0	1.8	0.3	0.3	0.5	1.5	0.3	1.7
Max (ng/g)	1.2	1.0	1.8	0.3	0.3	0.5	1.5	0.3	1.7
% of Total	**50.0**	**50.0**		**50.0**	**50.0**		**71.4**	**35.7**	

Table 11. Distributional statistics for concentrations of thiamethoxam (Parent) and CGA322704 (Metabolite) measured in leaves and flowers sampled from untreated control, foliar treated or seed treated cotton plants.

Statistic	Distribution of Thiamethoxam Residue Concentration at Bloom					
	Leaves			Whole Flower		
	Parent	Metab	Total	Parent	Metab	Total
Untreated Control Plants						
N (#)	18	18	18	18	18	18
Mean (ng/g)	1.5	1.3	2.8	0.6	0.3	0.9
SD (ng/g)	1.1	0.4	1.4	0.7	0.1	0.7
CV (%)	73	26	51	120	40	81
Min (ng/g)	1.3	1.3	2.5	0.3	0.3	0.5
Median (ng/g)	1.3	1.3	2.5	0.3	0.3	0.5
75th (ng/g)	1.3	1.3	2.5	0.3	0.3	1.0
90th (ng/g)	1.3	1.3	2.5	1.8	0.3	2.0
95th (ng/g)	5.9	2.7	8.7	2.7	0.7	2.9
Max (ng/g)	5.9	2.7	8.7	2.7	0.7	2.9
% of Total	**53.6**	**46.4**		**66.7**	**33.3**	
Foliar Treated						
N (#)	54	54	54	54	54	54
Mean (ng/g)	144.4	29.6	173.9	69.7	15.4	85.1
SD (ng/g)	150.0	23.0	169.0	54.0	10.5	63.7
CV (%)	104	78	97	78	68	75
Min (ng/g)	1.3	1.3	2.5	12.3	0.3	15.1
Median (ng/g)	94.3	24.4	116.3	58.4	12.2	69.8
75th (ng/g)	190.0	45.6	219.1	87.7	18.1	110.9
90th (ng/g)	307.0	56.4	361.8	156.0	32.5	189.8
95th (ng/g)	505.0	76.4	553.0	180.0	37.6	217.1
Max (ng/g)	667.0	100.0	743.4	235.0	43.8	278.8
% of Total	**83.0**	**17.0**		**81.9**	**18.1**	
Seed Treated						
N (#)	9	9	9	9	9	9
Mean (ng/g)	5.7	4.5	10.2	2.9	1.3	4.2
SD (ng/g)	7.1	3.3	9.7	2.9	1.2	4.0
CV (%)	123	73	95	99	91	97
Min (ng/g)	1.3	1.3	2.5	0.3	0.3	0.5
Median (ng/g)	4.7	3.7	9.1	1.3	0.7	1.9
75th (ng/g)	6.0	6.4	11.6	5.1	2.1	7.3
90th (ng/g)	23.5	10.4	33.9	7.3	3.1	10.3
95th (ng/g)	23.5	10.4	33.9	7.3	3.1	10.3
Max (ng/g)	23.5	10.4	33.9	7.3	3.1	10.3
% of Total	**55.9**	**44.1**		**69.0**	**31.0**	

Table 12. Statistical results for test of differences in concentrations of thiamethoxam or CGA322704 metabolite measured between untreated control plants and plants treated with foliar sprays; between untreated control plants and seed treated plants; and between foliar treated and seed treated plants.

Source	Nonparametric Test Exact Probability Levels					
	Thiamethoxam			CGA322704		
	Wilcoxon	Median	Kuiper	Wilcoxon	Median	Kuiper
Foliar Treated vs UTC						
Leaf	0.001	0.001	0.001	0.001	0.001	0.001
Flower	0.001	0.001	0.001	0.001	0.001	0.001
Nectar	0.001	0.001	0.003	0.001	0.001	0.03
Pollen	0.001	0.001	0.001	0.03	0.04	0.68
Extra Floral Nectar	0.001	0.001	0.001	0.001	0.001	0.001
Seed Treated vs UTC						
Leaf	0.03	0.03	0.57	0.01	0.008	0.26
Flower	0.18	0.36	0.89	0.02	0.05	0.57
Nectar	0.48	0.61	0.99	0.2	0.2	0.99
Pollen	0.08	0.08	0.99	0.21	0.21	1
Extra Floral Nectar	0.15	0.61	1	0.17	0.17	1
Foliar vs Seed Treatments						
Leaf	0.001	0.001	0.001	0.001	0.001	0.001
Flower	0.001	0.001	0.001	0.001	0.001	0.001
Nectar	0.001	0.04	0.02	0.16	0.35	0.9
Pollen	0.001	0.001	0.001	0.07	0.08	0.95
Extra Floral Nectar	0.001	0.001	0.001	0.001	0.001	0.001

Table 13. Statistical results for test of differences in concentrations of thiamethoxam or CGS322704 metabolite residues measured between plants grown in coarse or medium textured soils.

Source	Nonparametric Test Exact Probability Levels: Effect of Soil Type					
	Thiamethoxam			CGA322704		
	Wilcoxon	Median	Kuiper	Wilcoxon	Median	Kuiper
Leaf	0.96	1	0.75	0.63	0.63	0.88
Flower	0.9	1	0.99	0.68	1	0.88
Nectar	0.03	0.05	0.15	0.31	0.34	0.51
Pollen	0.9	1	0.96	1	0.65	0.96
Extra Floral Nectar	0.9	1	0.93	0.83	1	0.93

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Table 14. Distributional statistics for concentrations of thiamethoxam (Parent) and CGA322704 (Metabolite) measured in extra floral nectar sampled over time from untreated control or foliar treated cotton plants.

Statistic	Distribution of Thiamethoxam Concentration in Extra Floral Nectar Over Time											
	5 Days After 1st App			5 Days After 2nd App			Bloom			24 Days After 2nd App		
	Parent	Metab	Total	Parent	Metab	Total	Parent	Metab	Total	Parent	Metab	Total
Untreated Control Plants												
N (#)	6	6	6	5	5	5	16	16	16	5	5	5
Mean (ng/g)	3.0	0.3	3.3	0.5	0.3	0.8	1.7	1.0	2.7	1.0	0.3	1.3
SD (ng/g)	2.9	0.0	2.9	0.4	0.0	0.4	3.5	2.4	4.5	1.7	0.0	1.7
CV (%)	96	0	88	81	0	55	208	237	168	169	0	136
Min (ng/g)	0.3	0.3	0.5	0.3	0.3	0.5	0.3	0.3	0.5	0.3	0.3	0.5
Median (ng/g)	1.9	0.3	2.1	0.3	0.3	0.5	0.3	0.3	0.5	0.3	0.3	0.5
75th (ng/g)	6.1	0.3	6.3	0.6	0.3	0.9	0.7	0.3	1.5	0.3	0.3	0.5
90th (ng/g)	7.1	0.3	7.4	1.2	0.3	1.5	8.8	2.6	10.4	4.1	0.3	4.4
95th (ng/g)	7.1	0.3	7.4	1.2	0.3	1.5	12.3	9.6	14.9	4.1	0.3	4.4
Max (ng/g)	7.1	0.3	7.4	1.2	0.3	1.5	12.3	9.6	14.9	4.1	0.3	4.4
% of Total	**90.9**	**7.6**		**62.5**	**31.3**		**63.0**	**37.0**		**76.9**	**19.2**	
Foliar Treated												
N (#)	18	18	18	15	15	15	53	53	53	15	15	15
Mean (ng/g)	119.7	1.7	121.3	172.2	3.7	175.9	53.4	2.0	55.4	1.5	0.3	1.8
SD (ng/g)	90.3	1.1	91.3	216.0	5.1	221.0	50.0	2.3	51.8	1.6	0.1	1.6
CV (%)	75	68	75	125	138	126	94	112	94	104	25	91
Min (ng/g)	1.1	0.3	1.4	9.0	0.3	9.7	1.8	0.3	2.1	0.3	0.3	0.5
Median (ng/g)	90.7	1.4	91.8	67.2	1.5	68.7	41.1	1.4	42.8	0.9	0.3	1.1
75th (ng/g)	158.0	1.7	160.6	253.0	3.0	256.0	59.3	2.4	61.9	2.2	0.3	2.5
90th (ng/g)	288.0	3.7	292.5	444.0	11.1	454.1	123.0	3.8	125.9	4.5	0.3	5.0
95th (ng/g)	303.0	4.5	306.2	768.0	18.0	786.0	178.0	5.0	186.4	5.1	0.5	5.3
Max (ng/g)	303.0	4.5	306.2	768.0	18.0	786.0	228.0	14.3	242.3	5.1	0.5	5.3
% of Total	**98.7**	**1.4**		**97.9**	**2.1**		**96.4**	**3.6**		**83.3**	**16.7**	

Table 15. Extra floral nectar concentration over time: Exact probability levels for non-parametric test for changes in concentration over time.

Treatment, Plant Matrix, and Specific Soil Contrasts	Exact Probability Levels for Non-parametric Tests of Differences Over Time	
	Thiamethoxam	CGA322704
	Wilcoxon	Wilcoxon
Treated Plants		
Overall Effect	0.001	0.001
App 1 vs. App 2	0.99	0.93
App 1 vs. Bloom	0.004	0.88
App 2 vs. Bloom	0.004	0.54
Final vs. App 1	0.001	0.001
Final vs. App 2	0.001	0.001
Final vs. Bloom	0.001	0.001
Untreated Plants		
Overall Effect	0.042	1.000
App 1 vs. App 2	0.20	1.00
App 1 vs. Bloom	0.11	0.85
App 2 vs. Bloom	0.74	0.88
Final vs. App 1	0.31	1.00
Final vs. App 2	0.98	1.00
Final vs. Bloom	0.99	0.88

Figure 1. Explanation of statistical meaning of the Box-and-Whisker plots.

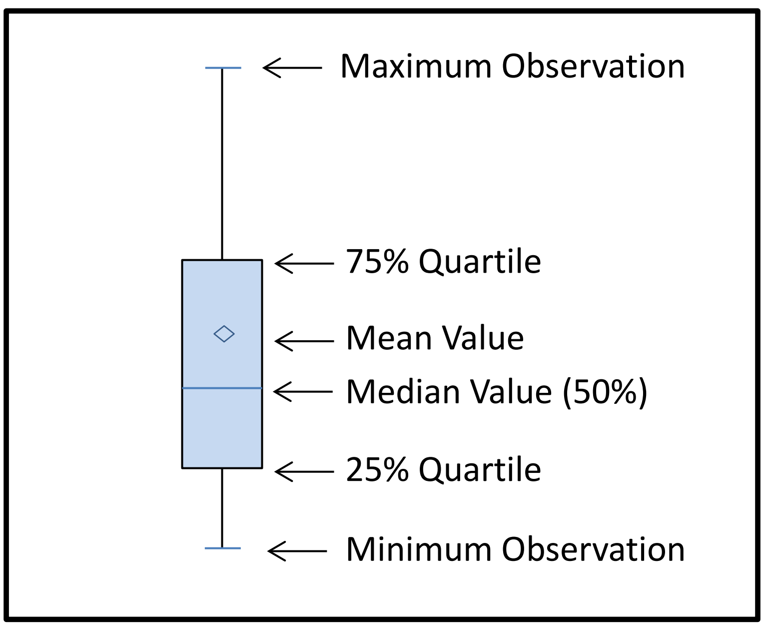

Figure 2. Comparison between years: Distribution of concentrations of thiamethoxam residues measured in plant matrices sampled at bloom compared between Year 1 and Year 2 of the study. Values were transposed to natural logarithms.

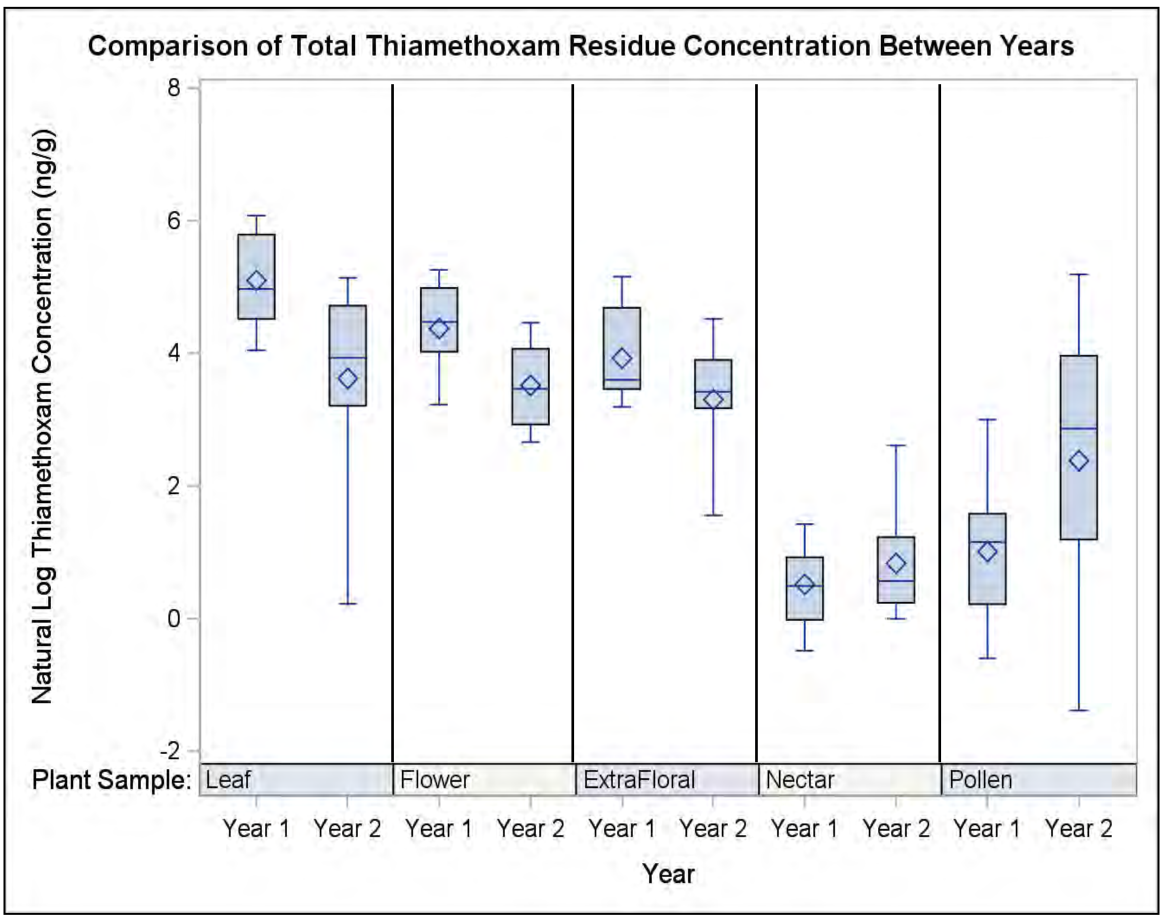

Figure 3. Foliar treated plants compared to untreated controls: Distribution of concentrations of thiamethoxam residues measured in plant matrices sampled at bloom. Concentrations in foliar treated plants (FTRT) are compared to untreated control plants (UTC). Values were transposed to natural logarithms.

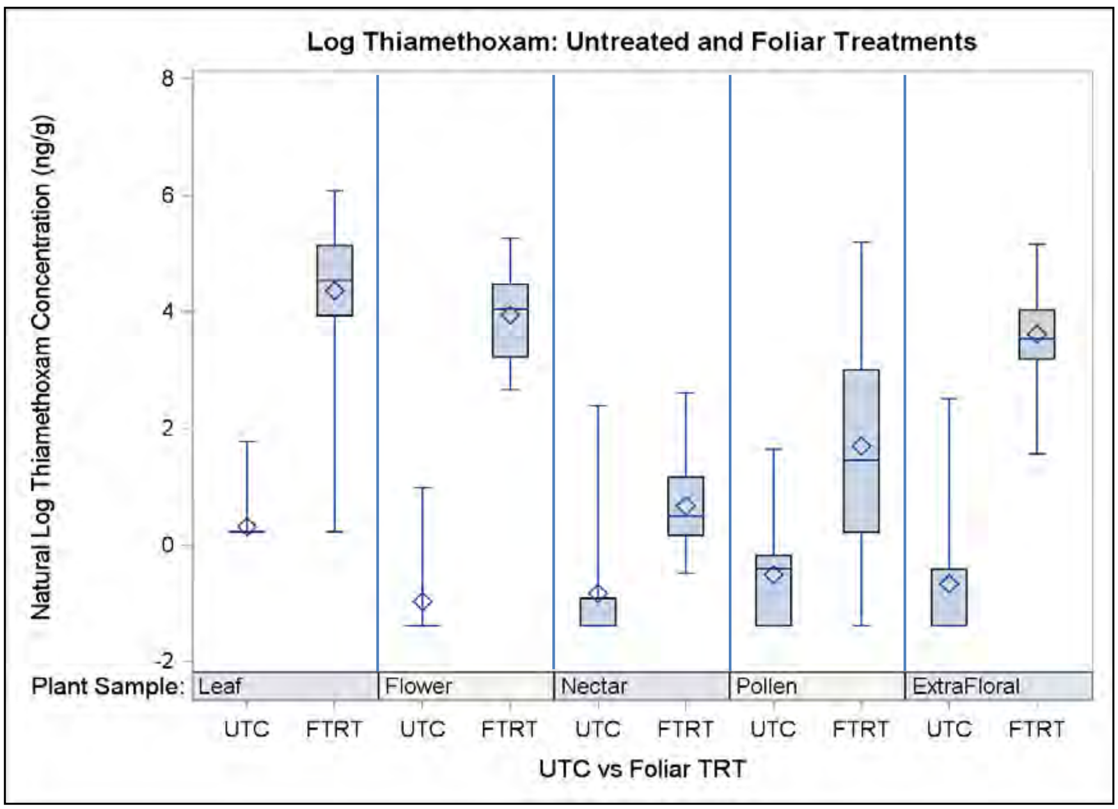

Figure 4. Foliar treated plants compared to untreated controls: Distribution of concentrations of CGA322704 metabolite residues measured in plant matrices sampled at bloom. Concentrations in foliar treated plants (FTRT) are compared to untreated control plants (UTC). Values were transposed to natural logarithms.

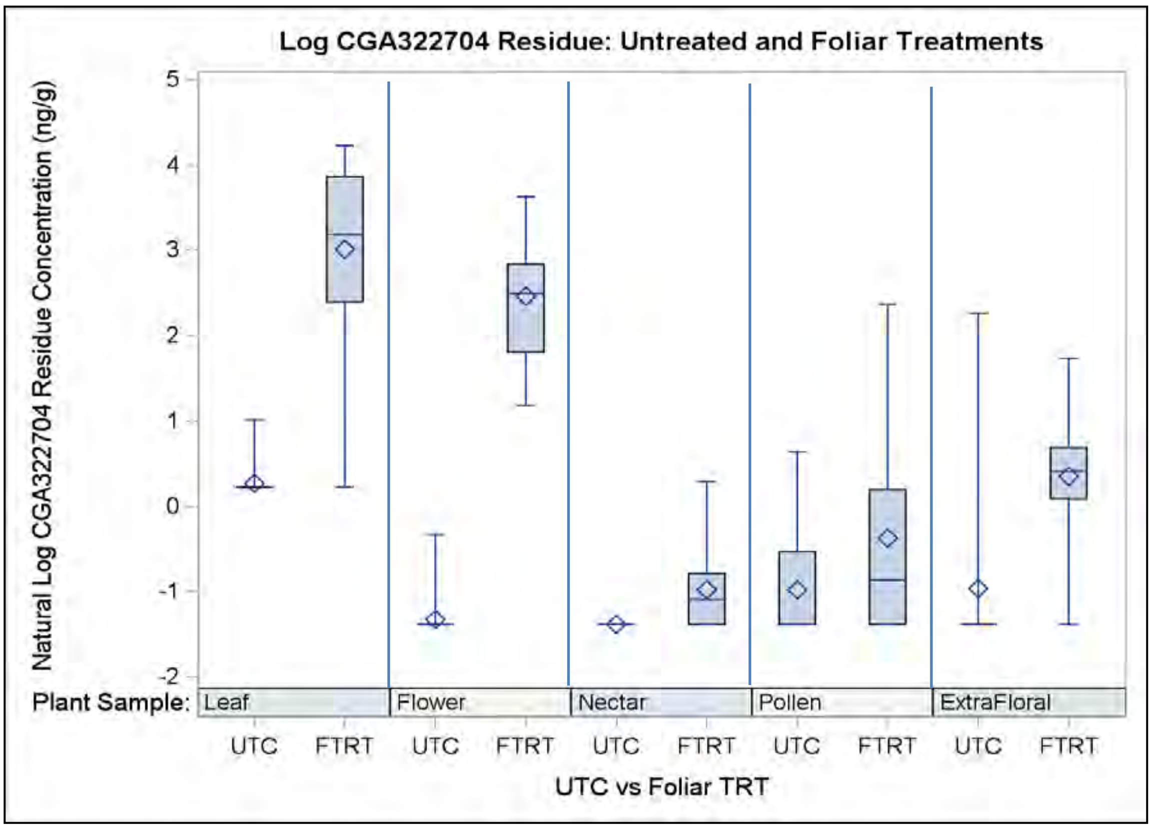

Figure 5. Soil treated plants compared to untreated controls: Distribution of concentrations of thiamethoxam residues measured in plant matrices sampled at bloom. Concentrations in seed treated plants (STRT) are compared to untreated control plants (UTC). Values were transposed to natural logarithms.

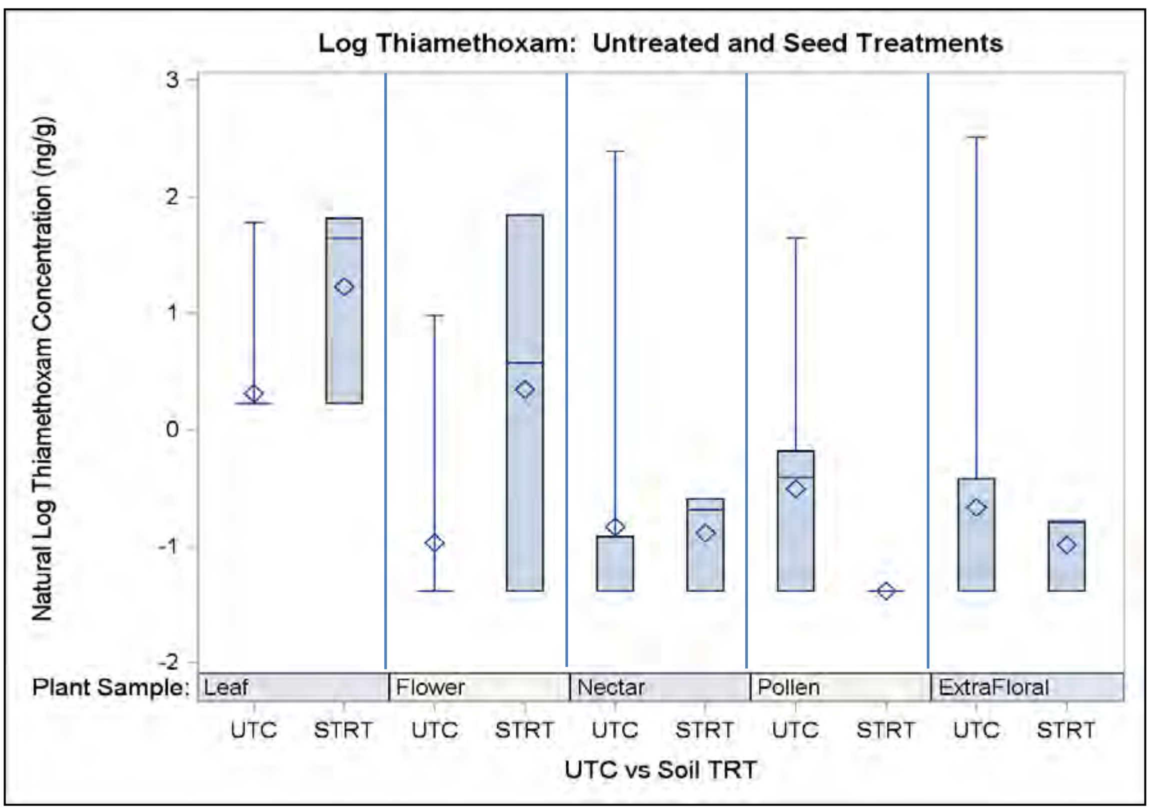

Figure 6. Soil treated plants compared to untreated controls: Distribution of concentrations of CGA322704 residues measured in plant matrices sampled at bloom. Concentrations in seed treated plants (STRT) are compared to untreated control plants (UTC). Values were transposed to natural logarithms.

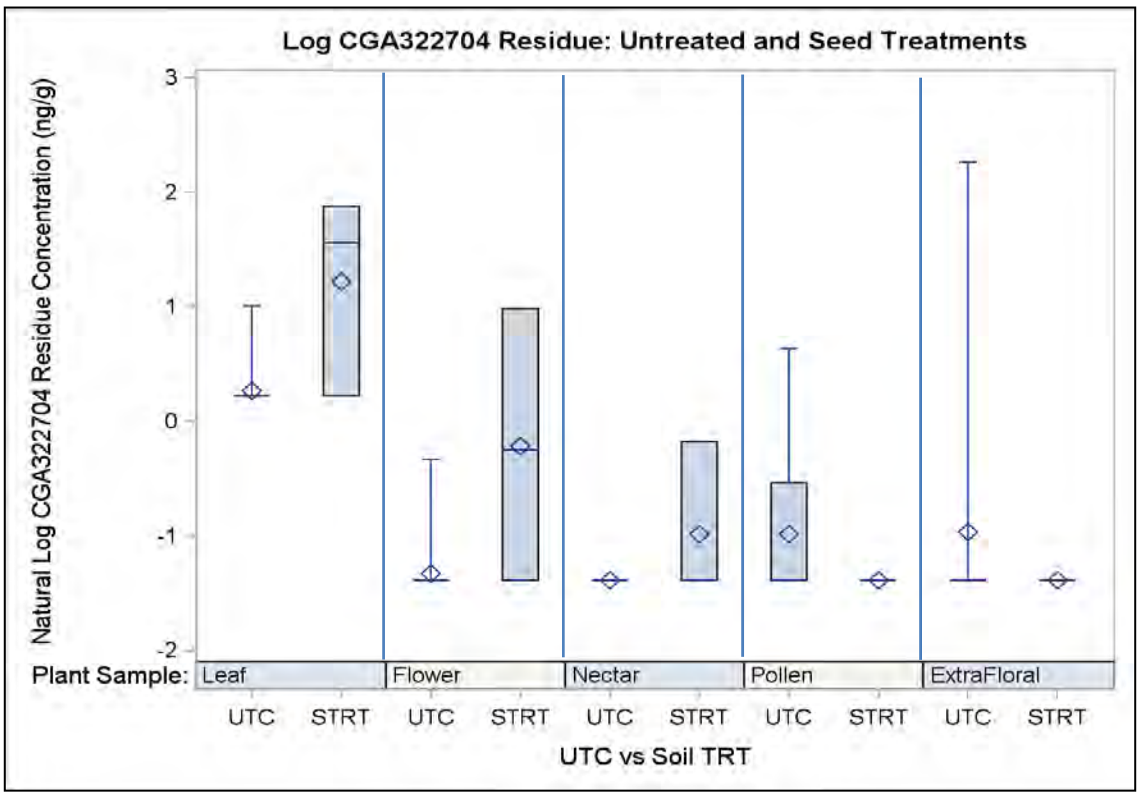

Figure 7. Foliar treated plants compared to soil treated plants: Distribution of concentrations of thiamethoxam residues measured in plant matrices sampled at bloom. Concentrations in foliar treated plants (FTRT) are compared to plants that received a soil treatment at planting (SFTR). Values were transposed to natural logarithms.

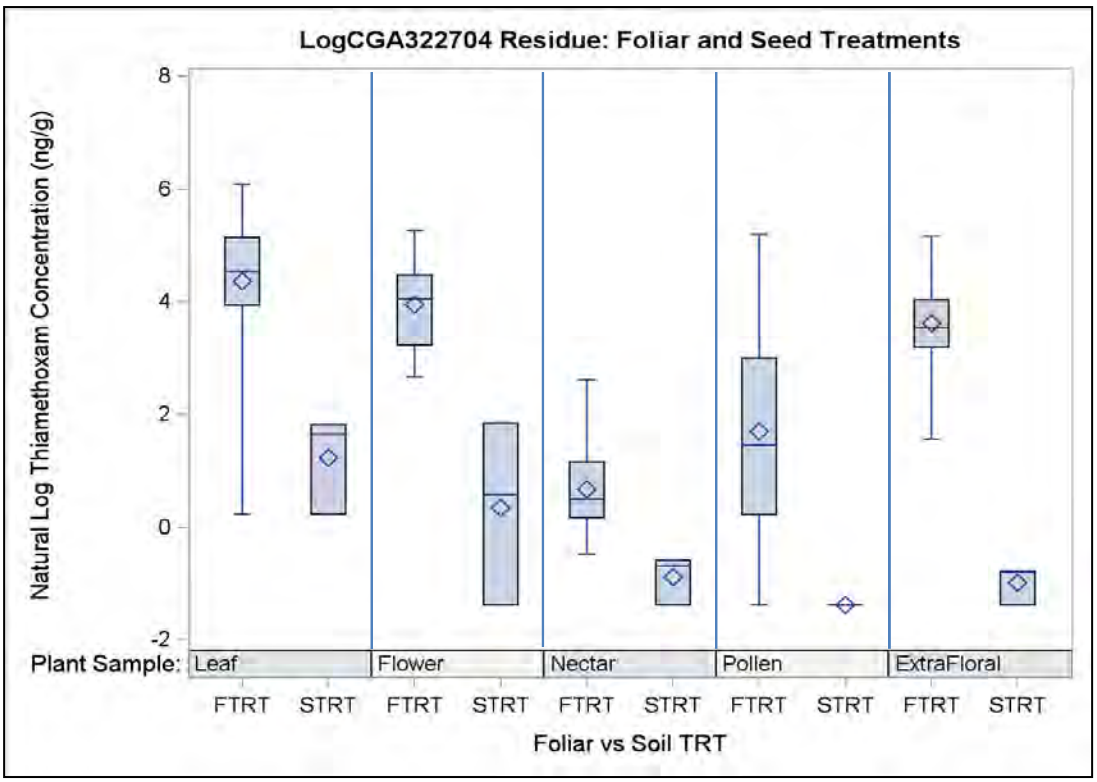

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Figure 8. Foliar treated plants compared to soil treated plants: Distribution of concentrations of CGA322704 metabolite residues measured in plant matrices sampled at bloom. Concentrations in foliar treated plants (FTRT) are compared to plants that received a soil treatment at planting (SFTR). Values were transposed to natural logarithms.

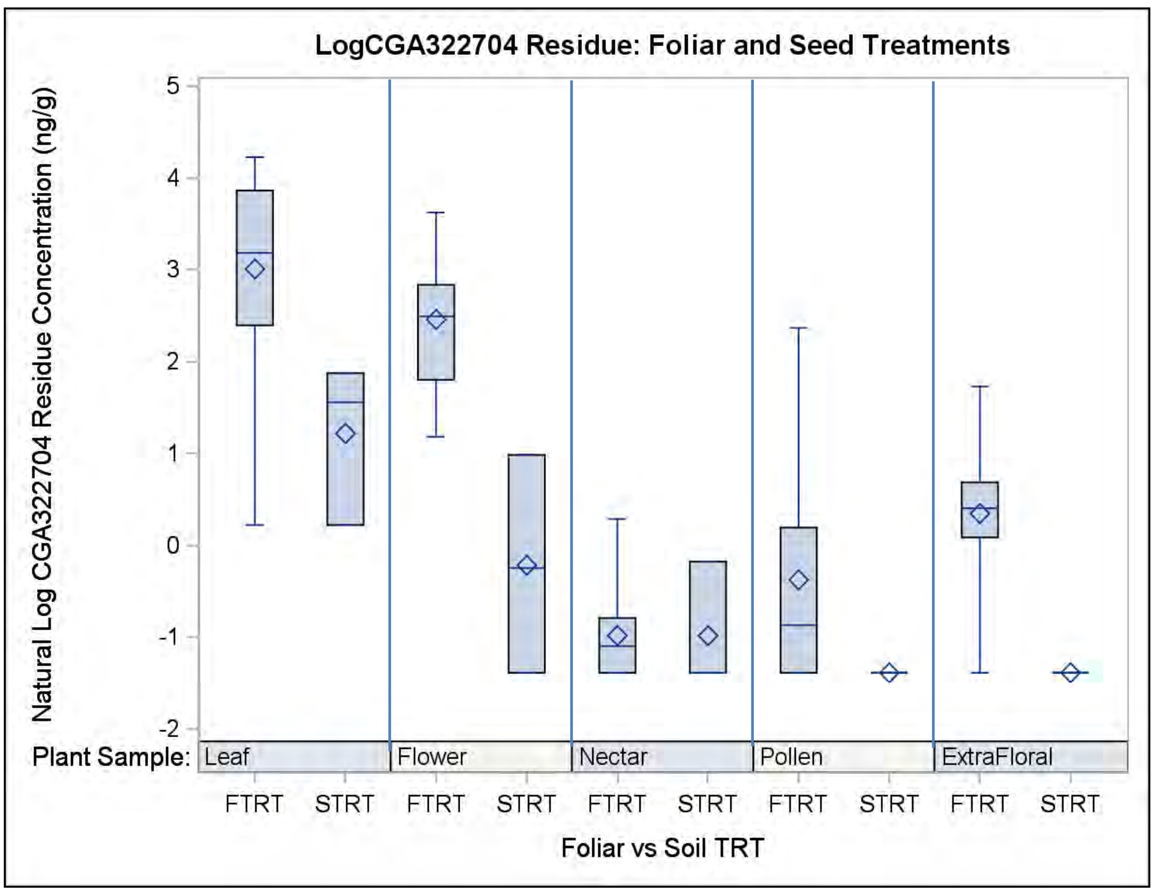


Figure 9. Soil comparison: Distribution of concentrations of thiamethoxam residues measured in plant matrices sampled at bloom. Concentrations in foliar treated plants (FTRT) grown in either coarse or medium textured soil. Values were transposed to natural logarithms.

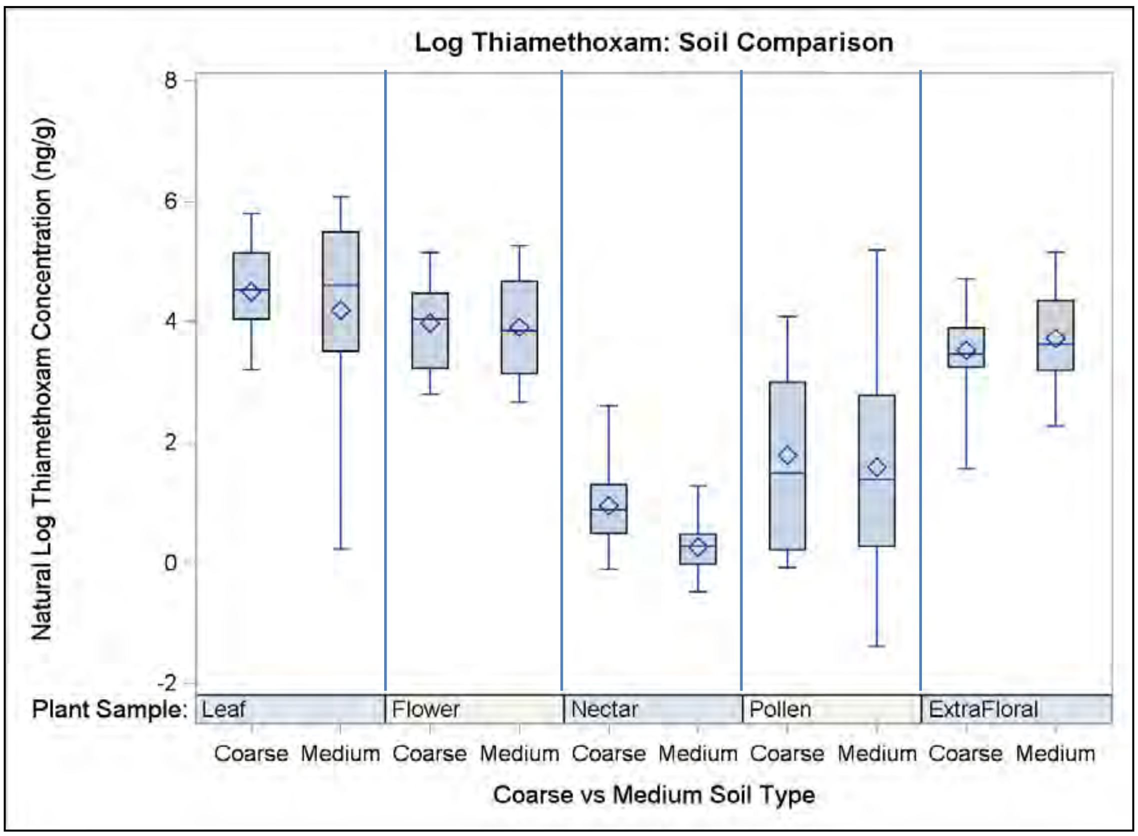


Figure 10. Soil comparison: Distribution of concentrations of CGA322704 metabolite residues measured in plant matrices sampled at bloom. Concentrations in foliar treated plants (FTRT) grown in either coarse or medium textured soil. Values were transposed to natural logarithms.

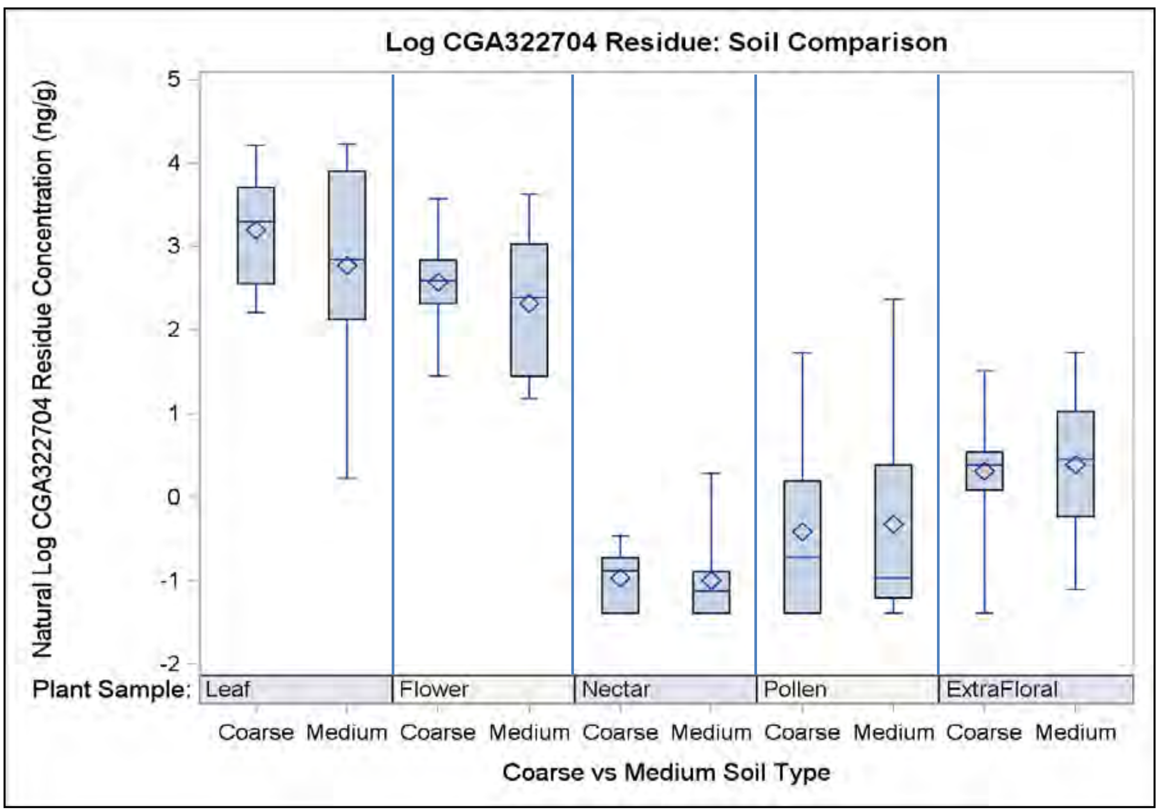
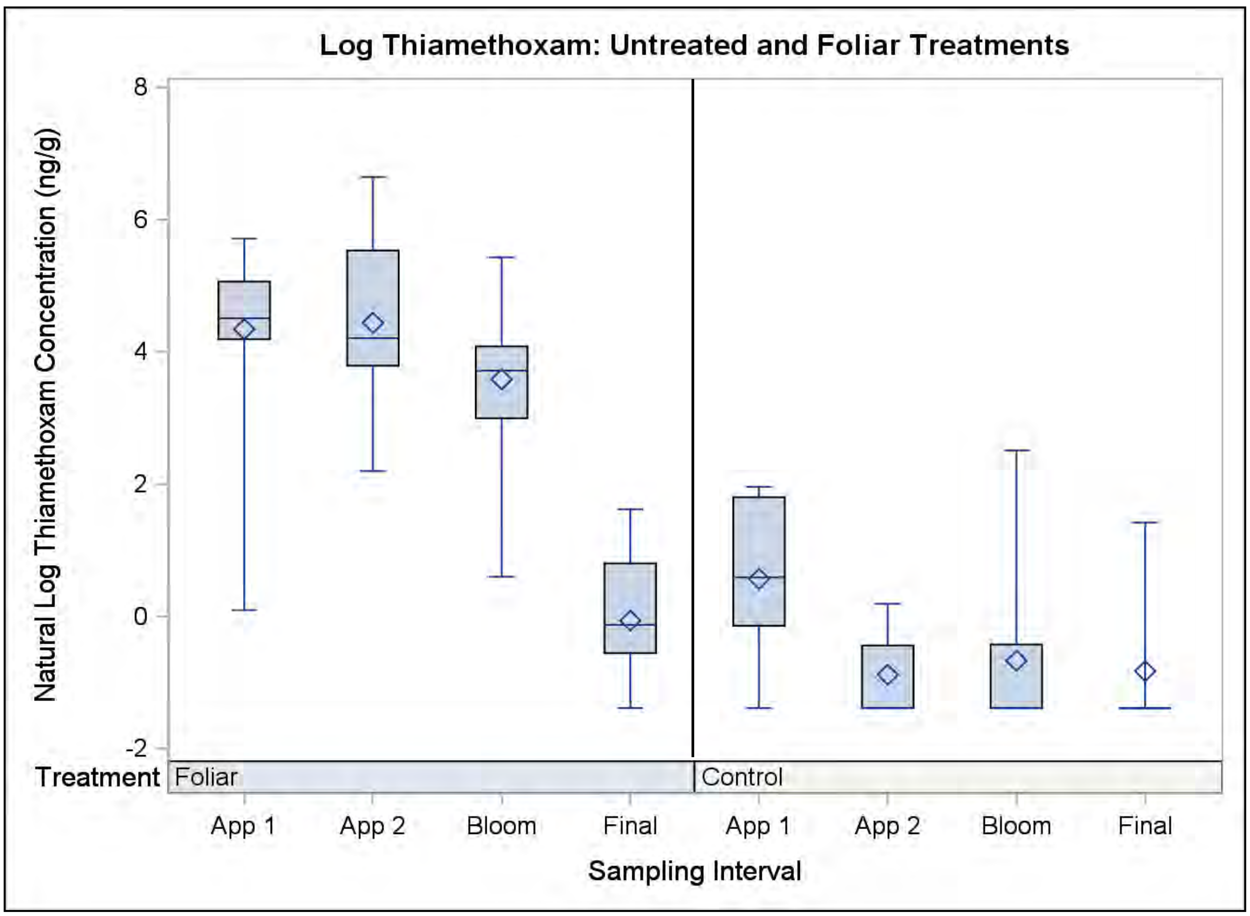
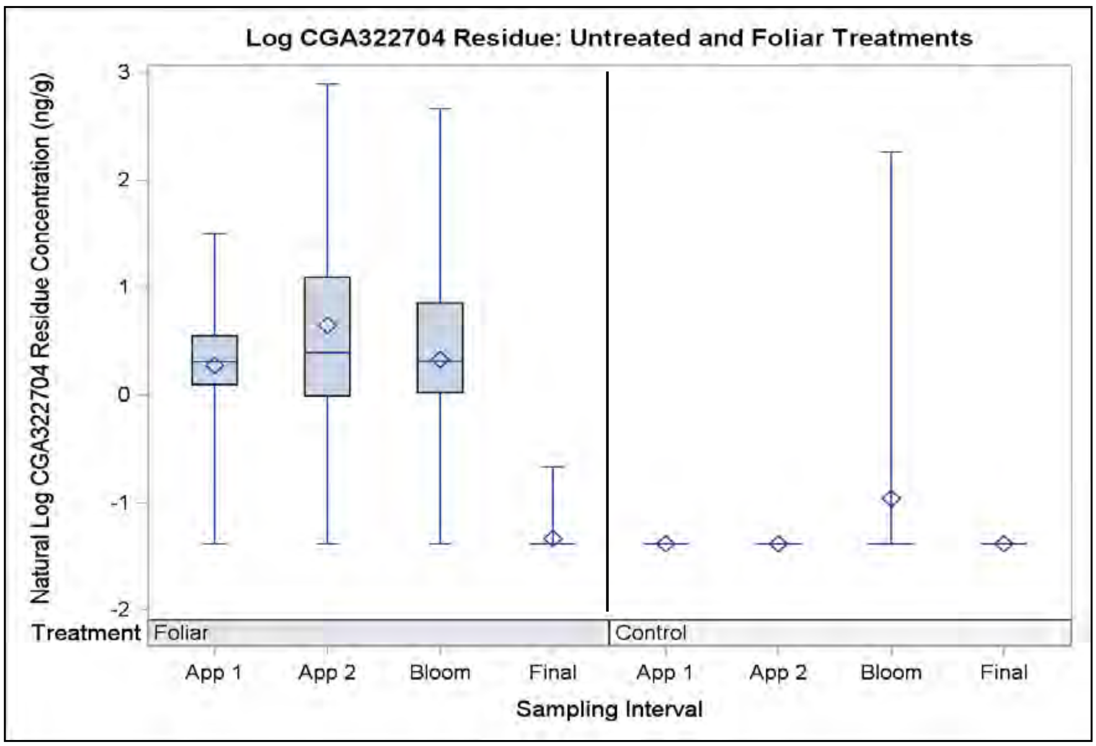


Figure 11. Extra floral nectar concentration over time: Distribution of concentrations of thiamethoxam residues measured in extra floral nectar directly after the first foliar application (App 1), directly after the second foliar application (App 2), at bloom, which was 7 days after the second application, and then at 24 days after the second application (Final). Concentrations in treated plants (Foliar) are compared to untreated control plants (Control). Values were transposed to natural logarithms.



MRID TBD CDPR THX Cotton

Figure 12. Extra floral nectar concentration over time: Distribution of concentrations of CGA322704 metabolic residues measured in extra floral nectar directly after the first foliar application (App 1), directly after the second foliar application (App 2), at bloom, which was 7 days after the second application, and then at 24 days after the second application (Final). Concentrations in treated plants (Foliar) are compared to untreated control plants (Control). Values were transposed to natural logarithms.



Appendix 10. Evaluations of Residue Studies Included in this Risk Determination Document

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Lange, B. 2017. Thiamethoxam (A18481A) - Determination of Residues in Leaves, Flowers, Anthers, Pollen, and Nectar of Soybean Plants After Foliar Application Lange Research Study Number: LR16192 Golden Pacific Study Number: 160670 Report Number: TK0250070	Non-Guideline field residue study on soybeans to establish thiamethoxam and metabolite concentrations in whole flowers, anthers, leaves and bee-collected nectar following foliar applications	This study quantified thiamethoxam and CGA322704 residues in soybean (*Glycine max*) grown in three locations: North Carolina (NC; sandy loam), Louisiana (LA; silt loam), and Iowa (IA; loam). Three replicate plots were used in each location. Each plot received foliar applications at 10 and 5 days before bloom at a nominal rate of 0.063 lbs. ai/A. Nectar was sampled at early-, mid-, and late-bloom. No pollen samples were collected. Anthers were analyzed in place of pollen. Samples of anthers and nectar were collected 10-20, 5-15, and 5-17 days after the last application in NC, LA, and IA, respectively. Analyses of fortified samples of anthers (83.7-91.8% thiamethoxam and 89.2-97.1 for CGA322704) and nectar (89.2-98.8% thiamethoxam and 103-112 for CGA322704) were all within acceptable limits. Nectar samples were collected by bees within tunnels. Mean thiamethoxam residues in nectar across all locations (3.60 ppb) were comparable to CGA322704 residues (3.21 ppb) in the early bloom samples but thiamethoxam residues were less than CGA322704 by the late-bloom samples (0.500 ppb vs. 4.45 ppb, respectively). Mean thiamethoxam residues (68.2 ppb) in anthers were notably greater than CGA322704 residues (9.38 ppb) in the early bloom samples and thiamethoxam residues remained greater than CGA322704 through the late-bloom samples (10.6 ppb vs. 3.40 ppb, respectively). Mean residues for thiamethoxam in nectar were greatest across all sample periods in LA with residues comparable in NC and IA. Mean residues for thiamethoxam in anthers were greatest across all sample periods in LA with residues then greater in IA than in NC. Mean concentrations of CGA322704 in nectar were greatest in LA and comparable in NC and IA. Mean concentrations of CGA322704 were comparable in anthers across all regions.	N/A

Appendix 10. Evaluations of Residue Studies Included in this Risk Determination Document

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Gilson, L. 2017. Thiamethoxam 75SG (A9549C) – Determination of Residues in Pollen, Flowers, and Leaves of Tomato After Soil Application with Platinum® 75SG. Syntech Research Laboratory Services Study Number 069SRUS16C087 Report Number: TK0242072	Non-Guideline field residue study on tomato to establish thiamethoxam and metabolite levels in whole flowers, leaves and manually-collected pollen, and following a soil application	This study quantified thiamethoxam and CGA322704 residues in tomato (*Solanum lycopersicum*) grown in three locations: Kansas (KS; silt loam), Illinois (IL; silt loam), and California (CA; sandy loam). Three replicate plots were used in each location. Each plot received a single soil application at transplanting at nominal rates of 0.172 lbs. ai/A or 0.125 lbs. ai/A. Pollen and whole flowers were sampled at early-bloom, and again at 10 and 20 days later. Samples of pollen and whole flowers were collected 40-60, 42-61, and 42-60 days post-application in KS, IL, and CA, respectively. Analyses of fortified samples of pollen (83.8-97.5% thiamethoxam and 97.0-105 for CGA322704) and whole flower (98.2-105% thiamethoxam and 97.3-101 for CGA322704) were all within acceptable limits. Pollen samples were manually extracted from whole flowers. At the maximum application rate, mean thiamethoxam residues (68.9 ppb) in whole flowers were notably less than CGA322704 residues (120 ppb) in the early bloom samples but were less different by the late-bloom samples (38.9 ppb vs. 55.7 ppb, respectively). Mean thiamethoxam residues (46.3 ppb) in pollen were also notably less than CGA322704 residues (93.9 ppb) in the early bloom samples but were comparable by the late-bloom samples (31.5 ppb vs. 27.1 ppb, respectively).	N/A.

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Louque, R. 2017. Thiamethoxam 75SG (A9549C) – Determination of Residues in Leaves, Flowers, Pollen, and Nectar of Pumpkin, Summer Squash, and Muskmelons After Soil Application. Smithers Viscient Study Number 1781.4148 Report Number: TK0222530	Non-Guideline field residue study on pumpkins, summer squash, and muskmelon to establish thiamethoxam and metabolite levels in manually-collected nectar, pollen, whole flowers and leaves following soil applications	This study quantified thiamethoxam and CGA322704 residues in pumpkin (*Cucurbita pepo*), muskmelon (*Cucumis melo*), and summer squash (*Cucurbita pepo*) grown in three locations: North Carolina (NC; sand), Missouri (MO; loamy sand), and California (CA; clay loam). Three replicate plots were used in each location. Each plot in NC received a single at-planting application at nominal rates of 0.172 lbs. ai/A or 0.125 lbs. ai/A in pumpkins, 0.172 lbs. ai/A or 0.0172 lbs. ai/A in squash, and 0.0858 or 0.172 lbs ai/A in muskmelon. The body of the report only indicates that rates of 0.125 lbs ai/A or 0.172 lbs ai/A were used; however, this is contradicted by a table in the appendix of the report indicating 0.0172 lbs ai/A was applied to squash and 0.0858 lbs ai/A to melon at one site. Each plot in MO or CA received a single at-planting application at nominal rates of 0.172 lbs. ai/A or 0.125 lbs. ai/A in pumpkins, 0.172 lbs. ai/A in squash and muskmelon. Pollen and nectar was sampled at bloom and subsequently at 5, 10, 15, and 20 days after bloom. Pumpkin samples were collected 58-79, 49-70, and 37-57 days post-application in NC, MO, and CA, respectively. Summer squash samples were collected 15-19 (only at bloom and a few 5 day post-bloom samples collected), 36-52, and 41-62 days post-application in NC, MO, and CA, respectively. Muskmelon samples were collected 48-68, 35-57, and 43-64 days post-application in NC, MO, and CA, respectively. Analyses of fortified samples of pollen (71.2-115% thiamethoxam and 73.5-120 for CGA322704) and nectar (77.3-118% thiamethoxam and 70.4-120 for CGA322704) were all within acceptable limits. Pollen and nectar samples were manually extracted from whole flowers. Reported mean thiamethoxam residues in pumpkin nectar (4.58 ppb) were notably greater than CGA322704 residues (1.33 ppb) in the early bloom samples and remained greater through the late-bloom samples (2.11 ppb vs. 0.554 ppb, respectively). Mean thiamethoxam residues (4.76 ppb) in pumpkin pollen were also notably greater than CGA322704 residues (2.76 ppb) in the early bloom samples but were comparable by the final samples (2.02 ppb vs. 2.31 ppb, respectively). Reported mean thiamethoxam residues in summer squash nectar (13.9 ppb) were notably greater than CGA322704 residues (1.06 ppb) in the early bloom samples and remained greater through the late-bloom samples (2.06 ppb vs. 0.696 ppb, respectively). Mean thiamethoxam residues in summer squash pollen (6.15 ppb) were also notably greater than CGA322704 residues (1.69 ppb) in the early bloom samples and remained greater through the late-bloom samples (2.72 ppb vs. 1.45 ppb, respectively). Reported mean thiamethoxam residues in muskmelon (21.4 ppb) were notably greater than CGA322704 residues (2.37 ppb) in the nectar in the early bloom samples and	A table on page 108 of the study report indicates different application rates (0.0172 lbs ai/A for squash in NC and 0.0858 lbs ai/A for melon in NC) than anywhere else in the report. It is uncertain if this is a typographical error, since all other application rates mentioned are 0.125 lbs ai/A or 0.172 lbs ai/A. The rates 0.0172 and 0.0858 lbs ai/A do not appear outside of this one table. Only data from the maximum rate allowed by the label were included in independent statistical analysis by DPR. MO squash samples were collected at 2 or 4 day intervals, rather than the 5-day interval specified.

		remained greater through the late-bloom samples (5.31 ppb vs. 0.832 ppb, respectively). Mean thiamethoxam residues in the muskmelon pollen (6.49 ppb) were also notably greater than CGA322704 residues (1.85 ppb) in the early bloom samples remained greater through the late-bloom samples (23.5 ppb vs. 6.14 ppb, respectively).	

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Loque, R. 2017. Thiamethoxam 75SG (A9549C) - Determination of Residues in Leaves, Flowers, Pollen, and Nectar of Pumpkin After Foliar Application Smithers Viscient Study Number 1781.4149 Report Number: TK0242074	Non-Guideline field residue study on pumpkin to establish thiamethoxam and metabolite concentrations in whole flowers, leaves and manually-collected nectar, pollen, and following foliar applications	This study quantified thiamethoxam and CGA322704 residues in pumpkin (*Cucurbita pepo*) grown in three locations: North Carolina (NC; sand), Missouri (MO; loamy sand), and California (CA; clay loam). Three replicate plots were used in each location. Each plot received foliar applications at 10 and 5 days before bloom at nominal rates of 0.086 lbs. ai/A or 0.023 lbs. ai/A. Pollen and nectar were sampled at early-bloom, and again at 5, 10, 15 and 20 days later. Samples of pollen and nectar were collected 5-26, 6-27, and 5-27 day [s after the last application in NC, MO, and CA, respectively. Analyses of fortified samples of pollen (70.2-109% thiamethoxam and 71.7-119 for CGA322704) and nectar (73.5-107% thiamethoxam and 70.3-119 for CGA322704) were all within acceptable limits. Pollen and nectar samples were manually extracted from whole flowers. At the higher application rate, mean thiamethoxam residues (10.6 ppb) in nectar were notably higher than CGA322704 residues (3.18 ppb) in the early bloom samples but thiamethoxam residues were less than CGA322704 by the late-bloom samples (0.691 ppb vs. 2.07 ppb, respectively). Mean thiamethoxam residues (15.9 ppb) in pollen were also notably greater than CGA322704 residues (3.99 ppb) in the early bloom samples but thiamethoxam residues were less than CGA322704 by the late-bloom samples (1.76 ppb vs. 2.31 ppb, respectively).	N/A.

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Lange, B. 2017. Thiamethoxam WG (A9584C) – Determination of Residues in Leaves, Flowers, Pollen, and Nectar of Sweet Orange After Foliar Application Lange Research Study Number: LR16203 Report Number: TK0250069	Non-Guideline field residue study on orange to establish thiamethoxam and metabolite concentrations in whole flowers, leaves and manually-collected nectar and pollen following foliar applications	This study quantified thiamethoxam and CGA322704 residues in sweet orange (*Citrus* x *sinensis*) grown in three locations: Florida (FL; sand; 2 sites) and California (CA; sandy clay loam). Three replicate plots were used in each location. One set of plots received two foliar applications in the fall, 7 days apart. A second set of plots at each location received foliar applications 7 days before pre-bloom and 7 days later. A third set of plots received a single application at pre-bloom. All applications were made at 0.086 lbs. ai/A. Nectar and pollen were sampled at early-, mid-, and late-bloom. Samples of pollen and nectar were collected 69-78, 56-88, and 104-117 days after the last of the fall applications in FL1, FL2, and CA, respectively. Samples of pollen and nectar were collected 34-43, 21-53, and 38-51 days after the last of the pre-bloom applications in the two remaining application scenarios in FL1, FL2, and CA, respectively. Analyses of fortified samples of pollen (73.1-113% thiamethoxam and 77.8-112 for CGA322704) and nectar (93.4-114% thiamethoxam and 88.0-110 for CGA322704) were all within acceptable limits. Nectar and pollen samples were manually collected from flowers. Mean thiamethoxam residues in nectar following fall applications across all locations (0.560 ppb) were comparable to CGA322704 residues (0.567 ppb) in the early bloom samples and through the late-bloom samples (0.506 ppb vs. 0.548 ppb, respectively). Mean thiamethoxam residues in pollen following fall applications across all locations (39.6 ppb) were notably greater than CGA322704 residues (4.51 ppb) in the early bloom samples but were less different by the late-bloom samples (5.60 ppb vs. 3.51 ppb, respectively). Mean thiamethoxam residues in pollen from plots receiving pre-bloom applications were comparable to CGA322704 residues in the early bloom samples but thiamethoxam residues became somewhat less than CGA322704 in the late-bloom samples. Mean residues for thiamethoxam in nectar following pre-bloom applications were somewhat greater than CGA322704 residues in early bloom samples becoming comparable to possibly greater by the late-bloom samples.	N/A

Appendix 10. Evaluations of Residue Studies Included in this Risk Determination Document

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Mitchell, J. 2017. Thiamethoxam 25WG (A9584C) - Magnitude of Residues in Leaves, Flowers, Pollen, and Nectar of Apple After Foliar Application Waterborne Study Number: 796.123 Battelle Report Number: 100078149 Report Number: TK0250071	Non-Guideline field residue study on apple to establish thiamethoxam and metabolite concentrations in whole flowers, leaves and manually-collected nectar and pollen following a foliar application	This study quantified thiamethoxam and CGA322704 residues in apple (*Malus domestica*) grown in three locations: New York (NY; loamy sand), Virginia (VA; sandy loam) and Washington (WA; loamy sand). Three replicate plots were used in each location. A single application was made 5 days before bloom at a nominal rate of 0.086 lbs. ai/A. Nectar and pollen were sampled at early-, mid-, and late-bloom. Samples of pollen and nectar were collected 9-14, 5-14, and 5-13 days post-application in NY, VA, and WA, respectively. Analyses of fortified samples of pollen (70-120% thiamethoxam and 81-109 for CGA322704) and nectar (74-109 for CGA322704) were within acceptable limits. Analyses of fortified samples of nectar (68-97% thiamethoxam) were slightly outside the acceptable limits of 70-120%. Nectar and pollen samples were manually collected from flowers. Mean thiamethoxam residues in pollen across all locations (1680 ppb) were notably greater than CGA322704 residues (56.4 ppb) in the early bloom samples and through the late-bloom samples (858 ppb vs. 108 ppb, respectively). Mean thiamethoxam residues in nectar across all locations (280 ppb) were notably greater than CGA322704 residues (6.74 ppb) in the early bloom samples but were less different by the late-bloom samples (35.6 ppb vs. 2.26 ppb, respectively).	The application rate is lower than the maximum rate allowed for pome fruit. Lower than acceptable recoveries in nectar for thiamethoxam raises the possibility that nectar residue values might be higher than reported.

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Lange, B. 2017. Thiamethoxam 25WG (A9584C) – Determination of Residues in Leaves, Flowers, Pollen, and Nectar of Blueberry After Foliar Application Lange Research Study Number: LR16191 Report Number: TK0250072	Non-Guideline field residue study on blueberry to establish thiamethoxam and metabolite concentrations in whole flowers, leaves and manually-collected nectar and pollen following foliar applications	This study quantified thiamethoxam and CGA322704 residues in blueberry (*Vaccinium corymbosum*) grown in three locations: California (CA; sand), Quebec (QC; loam), and Washington (WA; loamy sand). Three replicate plots were used in each location. One set of plots received three foliar applications at 19, 12, and 5 days before bloom at a nominal rate of 0.063 lbs. ai/A, and another set of plots at each location received a single foliar application 15 days before bloom at a nominal rate 0.063 lbs. ai/A. Nectar and pollen were sampled at early-, mid-, and late-bloom. Samples of pollen and nectar were collected 5-22, 5-11, and 12-24 days after the last of the three application in CA, QC, and WA, respectively. In those plots receiving a single application, pollen and nectar were collected 14-31, 19-25, and 22-34 days post-application in CA, QC, and WA, respectively. Analyses of fortified samples of pollen (75.1-101% thiamethoxam and 78.6-102 for CGA322704) and nectar (77.4-96.9% thiamethoxam and 85.8-101 for CGA322704) were all within acceptable limits. Nectar and pollen samples were manually collected from flowers. Mean thiamethoxam residues in nectar from plots receiving repeated applications across all locations (118 ppb) were less than CGA322704 residues (142 ppb) in the early bloom samples but thiamethoxam residues were comparable to CGA322704 by the late-bloom samples (51.2 ppb vs. 59.1 ppb, respectively). Mean thiamethoxam residues in pollen from plots receiving repeated applications (370 ppb) were notably greater than CGA322704 residues (60.2 ppb) in the early bloom samples and thiamethoxam residues remained greater than CGA322704 in the late-bloom samples (156 ppb vs. 48.4 ppb, respectively). Mean residues for thiamethoxam in nectar were greatest across all sample periods in QC with residues comparable in CA and WA. Mean residues for thiamethoxam in pollen were greatest across all sample periods in WA with residues greater in QC than in CA. Mean concentrations of CGA322704 in nectar were greatest in QC with residues comparable in CA and WA. Mean concentrations of CGA322704 in pollen were greatest across all sample periods in QC with residues greater in WA than in CA.	N/A

Appendix 10. Evaluations of Residue Studies Included in this Risk Determination Document

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Trask, J. 2017. Endigo® ZC (A13623Q), Endigo® ZCX (A18481A) and Cruiser® 5S (A9765N) – Magnitude of Residues in Pollen and Leaves of Corn Plants After Application as a Seed Treatment with Cruiser® 5S and After Foliar Application with Endigo® ZC or Endigo® ZCX Waterborne Study Number: 796.110 EPL Study Number: 110G1111 Report Number: TK0258214	Non-Guideline field residue study on corn to establish thiamethoxam and metabolite concentrations in leaves and manually-collected pollen following foliar applications and seed treatment	This study quantified thiamethoxam and CGA322704 residues in corn (*Zea mays*) grown in three locations: Pennsylvania (PA; loam), Iowa (IA; silty clay loam), and Oklahoma (OK; sandy loam). Three replicate plots were used for each treatment in each location. Seeds treated with Cruiser® 5S were planted in all test plots. One set of plots received no foliar applications. The remaining plots received two foliar applications at 0.086 lbs. ai/A of either Endigo® ZC, or Endigo® ZCX with the first application at either V8 growth stage or at first silk emergence. Each initial foliar application was followed 7 days later with the same formulated product. Pollen was sampled at pollen shed in all plots. Pollen was also collected in OK 2 days following the second application in those plots receiving the first application at initial silk emergence. Samples of pollen were collected 58, 57, and 58 days after planting in PA, IA, and OK, respectively. In those plots receiving the first application at V8 growth stage, pollen was collected 18, 18, and 15 days after the last application in PA, IA, and OK, respectively. In those plots receiving the first application at silk emergence, pollen was collected 1, 3, and 4 days after the first application in PA, IA, and OK, respectively. Analyses of fortified samples of pollen (88.4-119% thiamethoxam and 81.4-109 for CGA322704) were all within acceptable limits. Pollen samples were manually collected. Mean thiamethoxam residues from plots regardless of treatment were comparable to CGA322704 residues in pollen except in those treatments that received foliar applications at silk emergence where thiamethoxam residues were notably greater.	Foliar applications were made to corn grown from treated seeds.

. . E A Data Evaluation eports (Thiamethoxam)

U.S. EPA. (2017). Data evaluation report: hiametho am 25 WG (AC9584C) - magnitude of the residues in pollen, nectar, flowers, and leaves of cranberry after foliar application. Washington, D.C.: Author. Laboratory Report Number K0236307.

U.S. EPA. (2017). Data evaluation report: hiametho am 25 Wg (AC9584C) - magnitude of the residues in leaves, flowers, pollen, and nectar of cucumber after foliar application. Washington, D.C.: Author. Laboratory Report Number K0222532.

U.S. EPA. (2017). Data evaluation report: hiametho am - hiametho am 75 SG (A9549C) - magnitude of residues in pollen, flowers, and leaves of pepper after soil application: inal report. Washington, D.C.: Author. Laboratory Report Number K0236306.

U.S. EPA. (2017). Data evaluation report: hiametho am - hiametho am 25WG (A9584C) - magnitude of residues in pollen, flowers, and leaves of tomato after foliar application. Washington, D.C.: Author. Laboratory Report Number K0222531.

U.S. EPA (2017). Data evaluation report: hiametho am 75SG (A9549C) - determination of residues in leaves, flowers, pollen, and nectar of strawberry after soil application. Washington, D.C.: Author. Laboratory Report Number K0250068.

Dinotefuran Data Evaluations (begin on next page)

Appendix 10. Evaluations of Residue Studies Included in this Risk Determination Document

MRID 50198501 CDPR Dino Cotton

Reference
Hummel, R. (2017) Quantitation of Residues of Dinotefuran, DN and UF in Nectar, Extrafloral Nectar, Pollen and Leaves Following Foliar Treatment of Dinotefuran to Cotton. Study Number: 43411B104. Unpublished study prepared by Landis International, Inc. 307. MRID 50198501, CDPR Study ID 297894, Data Volume 52911-0490, Tracking ID# 280249

1. STUDY INFORMATION

Chemical:	Dinotefuran	**PC Code**	44312
Test Material:	Dinotefuran 20 SG	**Percent Active Ingredient:**	20%
1Study Type:	Residue study to measure the magnitude of Dinotefuran and its major metabolites, UF and DN, in cotton leaves, pollen, extrafloral nectar and nectar following foliar applications.		
Sponsor:	Landis International, Inc. P.O. Box 5126 3185 Madison Highway Valdosta, Georgia 31603-5126 USA	**Experiment Start and End Date:**	May 3, 2016 – November 30, 2016
Sponsor Study ID:	43411B104	**Study Locations:**	Six trial sites of cotton located in California.
Study Completion Date:	February 26, 2017		
GLP Status:	GLP Compliant; protocol reviewed by CDPR. [CDPR Study ID 297894, Data Volume 52911-0490, Tracking ID# 280249]		

2. REVIEWER INFORMATION

Study Reviewed by: California Department of Pesticide Regulation	Richard Bireley, Sr. Environmental Scientist (Specialist) John Troiano, Ph.D., Research Scientist III Alexander Kolosovich, Sr. Environmental Scientist Brigitte Tafarella, Environmental Scientist Denise Alder, Sr. Environmental Scientist (Specialist) Russell Darling, Sr. Environmental Scientist (Specialist)

3. EXECUTIVE SUMMARY

The purpose of this study was to determine the residue concentrations of dinotefuran and its major metabolites, DN and UF, in floral nectar, extra-floral nectar, pollen and leaves collected following foliar treatment applications of Dinotefuran 20 SG to cotton.

Six field trials were conducted during the 2016 growing season on cotton in California. Three treated plots and a non-treated plot were established at each test location. Trial CA 1 was planted with pima cotton (*Gossypium barbadense*), and the remaining five trials were planted with upland cotton (*G. hirsutum*). In general, pima cotton varieties are grown in western states *(e.g.*, California and Arizona), while upland cotton varieties are grown throughout the United States.

Dinotefuran 20 SG (containing 20% dinotefuran w/w) was applied to cotton plants in two broadcast foliar applications at a rate of 0.129 – 0.136 lb ai/Acre/application (144 - 152 g ai/ha/application). All applications were made in 14 – 17 gal/A of water (131-159 L/ha) using ground equipment. All sprays were calibrated prior to each application with the volume/time method and consisted of commercial or simulated commercial application equipment.

Samples were analyzed for residues of dinotefuran and its metabolites, UF and DN, using Eurofins analytical method No. RA046. Quantitation of residues in all samples was achieved using an external calibration curve calculated by linear regression of instrument responses for the reference substances at multiple concentrations. The performance of the instrument was evaluated during each injection set.

4. STUDY VALIDITY

Guideline Followed:	Protocol was reviewed and approved by CDPR
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	**ACCEPTABLE**
Rationale:	N/A
Reparability:	N/A

5. MATERIALS

Test Material Characterization for Foliar Application End Use Product			
Test item:	Dinotefuran 20 SG	**Percent A.I.:**	20% A.I.
Formulation Type:	Water Soluble Granule	**Date of Issue:**	April 15, 2015
CAS #:	165252-70-0	**Expiration Date:**	March 27, 2017

5A. STUDY DESIGN

This study requirement was part of the dinotefuran special review at the California Department of Pesticide Regulation (CDPR). The study design and protocol were approved by the CDPR. The study initiation date was May 3, 2016. The experimental start date was July 6, 2016 and the experimental end date was November 30, 2016 (last sample injection).

Six field trials were conducted during the 2016 growing season on cotton in California. Three treated plots and one non-treated plot were established at each test location. Dinotefuran 20 SG (containing 20% dinotefuran w/w) was applied to cotton plants in two broadcast foliar applications at a rate of 0.129 – 0.136 lb ai/A/application (144 - 152 g ai/ha/application). All applications were made in 14 – 17 gal/A of water (131-159 L/ha) using ground equipment. All sprays were made using commercial or simulated commercial application equipment, and all sprayers were calibrated prior to each application with the volume/time method.

Each trial included a non-treated control plot, from which non-treated samples of leaves, extrafloral nectar, pollen and floral nectar were collected to provide a relative indication of background levels of dinotefuran and to give an indication of possible analytical matrix interferences. Each non-treated plot was located at least 100 feet from the nearest treated plot and was not down-wind during foliar applications.

Commercially available varieties of cotton were used, and each crop was grown following local agronomic practices at each test site. Trial CA 1 was planted with pima cotton (*Gossypium barbadense*), and the remaining five trials were planted with upland cotton (*G. hirsutum*). The conditions at each test site are summarized in Table 1 and Table 2.

5B. Study Site Location and Characteristics

The crops were grown and maintained according to typical agricultural practices for each geographical region. The crop varieties selected were typical for commercial production in the area. The actual temperature and rainfall were within normal parameters during the residue study period with the noted exceptions. Irrigation was used to supply adequate moisture for vigorous crop growth, as needed. There were no meteorological abnormalities that occurred during the conduct of the study that had a significant effect on the cotton crops.

Table 1. Site Locations and Cotton Varieties

Trial Site	Site Identification	Nearest Town/County	EPA Region	Variety	Irrigation Type
1	CA 1	Madera, California/Madera County	10	Prima	Flood
2	CA 2	Porterville, California/Tulare County	10	Acala	Drip
3	CA 3	Porterville, California/Tulare County	10	Acala	Drip
4	CA 4	Zamora, California/Yolo County	10	ST 5115 GLT	Drip
5	CA 5	Pearson, California/Yolo County	10	ST 5115 GLT	Sprinkler
6	CA 6	Fresno, California/Fresno County	10	Acala	Drip

Table 2. Trial Site Conditions

Trial Site	Sand %	Silt %	Clay %	USDA Textural Class	CEC Meq/100g	Organic Matter %	Soil pH
CA 1	89	9	2	Sand	4.6	0.52	7.8
CA 2	80	16	4	Loamy Sand	7.2	0.57	7.4
CA 3	40	20	40	Clay Loam	33.2	1.2	8
CA 4	26	38	36	Clay Loam	23.2	1.2	7
CA 5	30	36	34	Clay Loam	18.8	2.3	6.7
CA 6	74	24	2	Loamy Sand	6.7	0.79	7.7

5C. Application Timing and Rates

Table 3. Study Use Pattern for Dinotefuran

Trial ID	Method	Application Timing (Crop Stage)	Timing	Application Volume		Rate (lb ai/ha)	Rate (g ai/ha)	Application Number	RTI[a]	Total Rate (lb ai/A)	Total Rate (g ai/ha)
				gal/A	L/ha						
CA 1	Broadcast	BBCH 60	9/16/16	17	159	0.134	150	1	n/a	0.268	300
		BBCH 61	9/23/16	17	159	0.134	150	2	7		
CA 2	Broadcast	BBCH 59	7/19/16	15	140	0.131	147	1	n/a	0.260	291
		BBCH 61	7/26/16	15	140	0.129	144	2	7		
CA 3	Broadcast	BBCH 55	7/13/16	15	140	0.130	146	1	n/a	0.261	293
		BBCH 60	7/20/16	15	140	0.131	147	2	7		
CA 4	Broadcast	BBCH 60	8/22/16	15	140	0.135	151	1	n/a	0.271	303
		BBCH 61	8/30/16	15	140	0.136	152	2	8		
CA 5	Broadcast	BBCH 60	8/23/16	14	131	0.134	150	1	n/a	0.268	300
		BBCH 61	8/31/16	14	131	0.134	150	2	8		
CA 6	Broadcast	BBCH 59	7/6/16	15	140	0.134	150	1	n/a	0.267	299
		BBCH 61	7/13/16	15	140	0.133	149	2	7		

[a] Re-treatment interval (Number of days between applications). Not applicable= n/a.

5D. Sample Collection, Handling, Processing

To prevent cross-contamination, the non-treated control plots were sampled first, or separate personnel sampled the non-treated plot independently from other samples. For each matrix, one sample was collected from each treated replicate plot A, B, and C, as well as from the control plot, at designated intervals.

Leaf Samples

Leaf samples were collected prior to the second application, and then at early, mid, and late bloom, and again following bloom (BBCH 69). Samples consisted of a minimum of 24 leaves collected from at least 12 plants. Target weights of 200 g from untreated and 50 g for treated leaves were collected directly into labelled sealable plastic bags. The samples were then held in separate ice chests labeled “control” and “treated” on substitute ice until placed into frozen storage.

Pollen Samples

Pollen samples were collected at early, mid, and late bloom. Flowers were collected from the untreated control and the treated sub-plots and either bagged or placed in trays to be transported to the field laboratory for pollen extraction. Pollen samples were extracted manually from flowers using a plastic filtered collection tip attached to a vacuum pump. The samples were vacuumed directly from the anthers or dislodged manually and then collected either into the filtered vacuum tip or placed directly into a small vial. The sample containers (tips or vials) were weighed before and after the pollen extraction where the net weight represented the sample size. Once the target weight of 100 mg was obtained (or when all flowers available for pollen sampling were used), the plastic tips containing pollen were wrapped in Parafilm and placed in labelled plastic bottles. The bottles were sealed, placed in re-sealable plastic bags, and transferred immediately into separate freezers for the treated and untreated samples.

Extra-Floral Nectar Samples

Extra-floral nectar samples were collected prior to the second application and then at early, mid, and late bloom, and again following bloom (BBCH 69). Flowers were collected from the untreated control and the treated sub-plots and then either bagged or placed in trays for transport to the field laboratory for extra-floral nectar extraction. Extrafloral nectar samples were collected from the outer parts of blossoms (sepals, bracts and pedicels) manually using glass micro capillary pipettes. Nectar was then transferred into a pre-weighed amber glass vial. Each vial was weighed before and after nectar collection, with the net weight representing the sample size. Once the target weight of 100 mg was obtained (or when all flowers available for nectar sampling were used), the vials containing nectar were sealed, placed into individual labelled secondary containers and then placed in re-sealable plastic bags for immediate transfer into separate freezers for the treated and untreated samples.

Floral Nectar Samples

Floral nectar samples were collected at early, mid, and late bloom. Flowers were collected from the untreated control and the treated sub-plots and then either bagged or placed in trays for transport to the field laboratory for extra-floral nectar extraction. Floral nectar samples were collected from the inner-blossom nectary structures manually using glass micro capillary pipette or spun out using a filtered centrifuge tube in a table-top centrifuge. Nectar was then transferred into a pre-weighed amber glass vial. Each vial was weighed before and after nectar collection, with the net weight representing the sample size. Once the target weight of 100 mg was obtained, (or when all flowers available for nectar sampling were used), the vials containing nectar were sealed, placed into individual labelled secondary containers and then placed in re-sealable plastic bags for immediate transfer into separate freezers for the treated and untreated samples.

Sample Storage.

All residue samples (leaf, pollen, extra-floral nectar, and floral nectar) were shipped from the test sites in separate treated and untreated boxes, or ice chests with dry ice, to Eurofins Agroscience Services, Inc. via ACDS trucking or Federal Express overnight service. All samples were received frozen from the field and were stored in freezers (approximately -20 °C) at Eurofins Agroscience Services, Inc.

The leaf samples were homogenized to a consistency appropriate for analysis using a bench-top industrial food processor with dry ice. Homogenized samples were stored frozen in plastic bags until analysis. Pollen samples were not homogenized due to their uniform powdery texture. Nectar samples were not homogenized prior to the extraction.

The maximum frozen storage interval from sample collection to extraction for analysis was 133 days for treated samples. Critical dates and storage intervals for each sample are presented in Appendix F of the study report. The available data from freezer storage stability studies on Dinotefuran and its metabolites show that each analyte is stable in nectar, pollen and leaf matrices for at least 274 days.

5E. ANALYTICAL METHODS

Samples were analyzed for residues of dinotefuran and its metabolites, UF and DN, using Eurofins analytical method No. RA046 entitled "Residue Analysis of Dinotefuran, DN and UF in Nectar, Pollen, Leaves and Soil by LC-MS/MS" (Reference 1, Reference 2).

The method performance was verified during sample analysis by determining the recoveries from control samples fortified with dinotefuran, UF, and DN at 1, 1, and 3.08 ppb and 100, 100, and 61.6 ppb for nectar; 2, 2, and 3.08 ppb and 100, 100, and 61.6 ppb for pollen; and 5, 5, and 3.08 ppb and 100, 100, and 61.6 ppb for leaves.

In brief, samples of pollen (0.1 g) were extracted with a mixture of methanol and water using a Fastprep-24 homogenizer. After centrifugation, the samples were subjected to SPE clean-up with a C18 (100mg/1mL) SPE cartridge. After elution, the samples were diluted and analyzed by LC-MS/MS.

To minimize the different matrix effect of cotton pollen and organic pollen (used to prepare curve and quality control samples), all cotton pollen samples were diluted using extract from control organic pollen and analyzed by LC-MS/MS.

Samples of nectar (0.1 g) were extracted with a mixture of methanol and water using a Fastprep-24 homogenizer. After centrifugation (if necessary), the samples were transferred to autosampler vials, diluted and analyzed by LC-MS/MS in a positive ionization mode.

The mass of several nectar samples was less than the minimum mass specified for standard analysis (0.1 g); these samples were extracted by adjusting the extraction solvent proportionally to the sample mass. Low weight quality control samples (sample mass = 0.05 g) were also prepared and tested for recovery.

Samples of leaves (2.5 g) were extracted with a mixture of methanol and water using a multi-tube vortexer. After centrifugation, the supernatant was decanted into a clean tube and the samples were subjected to a second extraction, combining the two supernatants. After the second extraction, sample extracts were then transferred to autosampler vials, diluted and analyzed by LC-MS/MS in a positive ionization mode.

Quantitation of all samples was achieved using calibration curves calculated by linear regression of instrument responses for the reference substances at multiple concentrations. The performance of the instrument was evaluated during each injection set. The correlation coefficient (r) for each calibration curve was required to be >0.990. The performance of the analytical method was evaluated during each sample set by fortifying control matrix with mixed standards of each analyte.

5F. QUALITY ASSURANCE RESULTS

The reference substances were used to generate data for both instrument and method performance. Quantitation of residues in all samples was achieved using an external calibration curve calculated by linear regression of instrument responses for the reference substances at multiple concentrations. The performance of the instrument was evaluated during each injection set.

All control samples of the various matrices were free from interferences above the limit of detection (LOD) at the mass transitions used for quantification of the analytes. The LOD for dinotefuran and UF correspond to 0.3 ppb in nectar, 0.6 ppb in pollen and 1.5 ppb in leaves. The LOD for DN corresponds to 0.185 ppb in nectar, 0.37 ppb in pollen, and 0.925 ppb in leaves.

The limit of quantitation (LOQ) corresponds to 1 ppb for dinotefuran and UF in nectar, 2 ppb for dinotefuran and UF in pollen, 5 ppb for dinotefuran and UF in leaves, and 3.08 ppb for DN in pollen, nectar and leaves.

The recoveries for dinotefuran in nectar were between 59% and 108% with an average and RSD of 95% ± 10%; the recoveries for dinotefuran in pollen were between 73% and 109% with an average and RSD of 87% ± 11%, the recoveries for dinotefuran in leaves were between 87% and 111% with an average and RSD of 100% ± 7%.

The recoveries for UF in nectar were between 72% and 107% with an average and RSD of 92% ± 11%; the recoveries for UF in pollen were between 61% and 85% with an average and RSD of 73% ± 12%, the recoveries for UF in leaves were between 95% and 109% with an average and RSD of 103% ± 4%.

The recoveries for DN in nectar were between 83% and 106% with an average and RSD of 97% ± 7%; the recoveries for DN in pollen were between 76% and 93% with an average and RSD of 85% ± 7%, the recoveries for DN in leaves were between 95% and 108% with an average and RSD of 102% ± 4%.

Detailed information regarding the reference substances, including the certificates of analysis, is presented in Appendix C (Reference Substance Information) of the study report.

Detailed analytical data such as supporting raw data for re-calculations, representative chromatograms, and example calculations are provided in Appendix E (Analytical Summary Report) of the study report.

Table 4: Summary of reported analytical LOQs and LODs for each analyte in each plant sample.

Laboratory	Matrix	Analyte	LOD (ppb, parent equivalents)	LOQ (ppb, parent equivalents)
Eurofins Agroscience Services, Inc.	**Nectar**	Dinotefuran and UF	0.3	1.0
	Pollen	Dinotefuran and UF	0.6	2.0
	Leaves	Dinotefuran and UF	1.5	5.0
	Nectar	DN	0.185	3.08
	Pollen	DN	0.37	3.08
	Leaves	DN	0.925	3.08

6. RESULTS:

Residue data for concentration of parent dinotefuran and UF and DN degradation products in cotton leaves, extra-floral nectar, floral nectar, and pollen are reproduced from the report in Tables 5-16. Residue values below the analytical limit of detection (LOD) were reported as ½ of the LOD value noted in Table 4. There was only 1 missing sample for nectar that occurred in Trial CA6 for replicate 1 at the late-bloom sampling interval (Tables 11, 12 and 13). Total dinotefuran residue determined during statistical analysis was the sum of the each value noted for the replicate associated at each trial site. For leaf and extra-floral nectar samples, sampling intervals occurred prior to and after the blooming period and they are noted as pre-bloom and post-bloom in Tables and graphs, respectively. Successive samples taken during bloom are noted as early-bloom, mid-bloom, and late-bloom. Plants at pre-bloom had received only 1 application of dinotefuran whereas plants at early-bloom had received the full 2 foliar spray treatments of dinotefuran. Sampling intervals occurred at approximately 7 day intervals, which was designated in regressions to determine dissipation half-life values for residues. Statistical procedures used in the Statistical Analysis System (SAS) software were PROC CAPABILITY to provide distribution statistics, PROC GLM for conducting Analysis of Variance (ANOVA) to test effects of soil type and sampling time on concentration of residues, PROC REG to produce coefficients for dissipation over time used to estimate half-lives, and PROC BOXPLOT to produce bar charts for comparing distributions between treatments and trial sites.

Each trial included a non-treated control plot, from which non-treated samples of leaves, extrafloral nectar, pollen and floral nectar were collected to provide a relative indication of background levels of dinotefuran and to give an indication of possible analytical matrix interferences. Each non-treated plot was located at least 100 feet from the nearest treated plot and was not down-wind during foliar applications. Non-treated samples of leaves, floral nectar and extra-floral nectar did not contain quantifiable levels of dinotefuran, UF or DN. However, non-treated pollen samples from selected trial locations (-01, -04 and -05) contained measurable amounts of dinotefuran, UF and DN. For trial -01, the dinotefuran residue in the non-treated pollen sample (LA16-12) was 782 ppb *vs*. a mean residue of 17,878 ppb in pollen from the treated plots; for trial -04, dinotefuran residue in the non-treated pollen sample (LA16-198) was 23.3 ppb *vs*. a mean residue of 428 ppb from pollen in the treated plots; and for trial -05, dinotefuran residue in the non-treated pollen sample (LA16-260) was 40.5 ppb *vs*. a mean residue of 792 ppb from pollen in the treated plots.

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Table 5. Dino Residues in Cotton Leaves

Trial	Cotton Variety	Total Rate (lb ai/A)	Sample Timing [a]	Dinotefuran Residue (ppb)			
				Replicate 1	Replicate 2	Replicate 3	Mean
CA 1	Prima	0.268	-2	4715	5450	4510	4892
			3	7700	8550	8700	8317
			10	2355	3350	1835	2513
			17	1115	1540	1215	1290
			24	600	600	409	536
CA 2	Acala	0.260	-1	2240	2550	1715	2168
			7	2035	1785	2330	2050
			15	415	625	575	538
			22	200	220	328	249
			29	167	103	230	167
CA 3	Acala	0.261	-1	2205	2430	2025	2220
			9	2675	2585	3975	3078
			16	590	605	1035	743
			22	152	146	191	163
			29	76.0	142	124	114
CA 4	ST 5115 GLT	0.271	-1	3210	3175	4155	3513
			6	5350	8550	6150	6683
			14	3310	3110	3215	3212
			20	1965	1580	2175	1907
			26	1485	1230	1260	1325
CA 5	ST 5115 GLT	0.268	-2	3430	4755	3000	3728
			7	6050	6150	6250	6150
			14	2425	2285	2210	2307
			20	565	2870	1525	1653
			26	765	1130	670	855
CA 6	Acala	0.267	-2	2075	3575	3570	3073
			5	3030	4025	3625	3560
			13	424	630	715	590
			20	190	164	74.0	143
			27	56.0	70.5	46.5	58

[a] **Number of days after final application.**

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Table 6. UF Residues in Cotton Leaves

Trial	Cotton Variety	Total Rate (lb ai/A)	Sample Timing [a]	UF Residue (ppb)			
				Replicate 1	Replicate 2	Replicate 3	Mean
CA 1	Prima	0.268	-2	1135	1680	1700	1505
			3	1870	2220	2360	2150
			10	2090	2325	1830	2082
			17	1155	1480	1895	1510
			24	705	760	545	670
CA 2	Acala	0.260	-1	2190	2235	1440	1955
			7	2315	2250	2510	2358
			15	1230	1385	1300	1305
			22	800	610	935	782
			29	510	465	530	502
CA 3	Acala	0.261	-1	2390	2545	2000	2312
			9	4935	5150	6650	5578
			16	3995	3970	4285	4083
			22	2245	1700	2120	2022
			29	1580	1300	1020	1300
CA 4	ST 5115 GLT	0.271	-1	1530	1880	2770	2060
			6	3545	6300	4765	4870
			14	5350	6600	6450	6133
			20	4735	6000	5700	5478
			26	4655	4855	4700	4737
CA 5	ST 5115 GLT	0.268	-2	140	1635	1290	1442
			7	2290	2175	1845	2103
			14	1985	2185	1950	2040
			20	1135	2195	1640	1657
			26	1690	2515	1765	1990
CA 6	Acala	0.267	-2	1925	2610	2800	2445
			5	4360	4485	3500	4115
			13	1350	1465	1995	1603
			20	625	510	458	531
			27	309	311	303	308

[a] **Number of days after final application**

Table 7. DN Residues in Cotton Leaves

Trial	Cotton Variety	Total Rate (lb ai/A)	Sample Timing [a]	DN Residue (ppb)			
				Replicate 1	Replicate 2	Replicate 3	Mean
CA 1	Prima	0.268	-2	865	1170	1000	1012
			3	1375	1725	1825	1642
			10	2005	2395	1725	2042
			17	1335	1625	1955	1638
			24	985	1145	775	968
CA 2	Acala	0.260	-1	2145	2205	1390	1913
			7	2720	2805	2980	2835
			15	1945	2470	2675	2363
			22	2050	1245	1990	1762
			29	1430	1955	1680	168
CA 3	Acala	0.261	-1	2500	2675	2210	2462
			9	5000	5050	6750	5600
			16	5450	5900	6100	5817
			22	3495	2930	3375	3267
			29	3430	3210	2740	3127
CA 4	ST 5115 GLT	0.271	-1	1525	2030	2575	2043
			6	3985	5500	4530	4672
			14	6700	7500	7400	7200
			20	6950	7750	7400	7367
			26	8200	7250	6950	7467
CA 5	ST 5115 GLT	0.268	-2	2300	2350	1990	2213
			7	3130	2730	2535	2798
			14	3695	4070	3075	3613
			20	2460	3505	2960	2975
			26	4105	5650	3845	4533
CA 6	Acala	0.267	-2	1255	2005	2105	1788
			5	4355	5350	4615	4773
			13	3300	4270	4495	4022
			20	3280	2675	1965	2640
			27	2660	2940	2375	2658

[a] **Number of days after final application.**

Table 8. Dinotefuran Residues in Extra-Floral Cotton Nectar.

Trial	Cotton Variety	Total Rate (lb ai/A)	Sample Timing [a]	Residue (ppb)[b]			
				Replicate 1	Replicate 2	Replicate 3	Mean
CA 1	Prima	0.268	-2	1740	1300	5080	2707
			3	15500	10500	4890	10297
			10	1920	1800	1580	1767
			17	647	197	163	336
			24	111	216	119	149
CA 2	Acala	0.260	-1	82.9	83.7	20.7	62
			7	105	173	203	160
			15	5.06	6.24	6.68	6
			22	1.93	1.96	2.43	2
			29	0.500	0.500	3.50	2
CA 3	Acala	0.261	-1	182	589	162	311
			9	164	220	153	179
			16	12.9	3.86	6.78	8
			22	1.76	1.41	1.17	1
			29	0.500	8.96	0.500	3
CA 4	ST 5115 GLT	0.271	-1	375	1530	866	924
			7	428	250	290	323
			14	60.3	44.2	29.4	45
			20	41.7	10.4	6.46	20
			26	0.500	2.42	1.69	2
CA 5	ST 5115 GLT	0.268	-2	117	75.5	59.2	84
			7	2880	569	2670	2040
			14	586	189	236	337
			20	7.51	3.32	5.06	5
			26	3.08	2.34	3.29	3
CA 6	Acala	0.267	-2	545	1070	1030	882
			5	1180	1510	986	1225
			13	490	319	233	347
			20	8.74	29.3	17.9	19
			27	0.500	4.21	0.500	2

[a] Number of days after final application

[b] For the purpose of calculating means, values below the LOQ (1.00 ppb) were set to half of the LOQ (0.500 ppb).

Table 9. UF Residues in Extra-Floral Cotton Nectar

Trial	Cotton Variety	Total Rate (lb ai/A)	Sample Timing [a]	UF Residue (ppb)[b]			
				Replicate 1	Replicate 2	Replicate 3	Mean
CA 1	Prima	0.268	-2	225	200	632	352
			3	1390	1360	996	1249
			10	481	586	444	504
			17	250	87.6	72.5	137
			24	73.2	154	72.2	100
CA 2	Acala	0.260	-1	38.1	41.8	10.2	30
			7	44.9	57.4	64.0	55
			15	5.69	7.27	5.76	6
			22	2.23	2.23	2.07	2
			29	0.500	1.11	1.02	1
CA 3	Acala	0.261	-1	57.7	110	44.1	71
			9	35.6	70.9	62.9	56
			16	7.06	4.48	17.2	10
			22	3.25	2.84	2.92	3
			29	1.51	11.3	1.13	5
CA 4	ST 5115 GLT	0.271	-1	140	297	276	238
			7	155	70.7	83.2	103
			14	28.7	26.0	24.9	27
			20	8.05	17.7	12.6	13
			26	1.81	4.17	2.62	3
CA 5	ST 5115 GLT	0.268	-2	37.8	20.1	18.0	25
			7	467	127	441	345
			14	459	52.7	130	214
			20	10.3	5.45	9.45	8
			26	3.05	3.08	3.31	3
CA 6	Acala	0.267	-2	250	536	395	394
			5	759	846	451	685
			13	518	487	266	424
			20	28.5	115	28.1	57
			27	3.87	17.7	3.71	8

[a] Number of days after final application

[b] For the purpose of calculating means, values below the LOQ (1.00 ppb) were set to half of the LOQ (0.500 ppb).

Table 10. DN Residues in Extra-Floral Cotton Nectar

Trial	Cotton Variety	Total Rate (lb ai/A)	Sample Timing [a]	DN Residue (ppb)[b]			
				Replicate 1	Replicate 2	Replicate 3	Mean
CA 1	Prima	0.268	-2	132	217	348	202
			3	1080	996	413	830
			10	292	438	257	329
			17	151	39.3	18.0	69
			24	17.3	33.8	10.1	20
CA 2	Acala	0.260	-1	11.9	10.2	5.3	9
			7	30.9	42.3	43.6	39
			15	5.99	5.77	5.70	6
			22	1.54	1.54	1.54	2
			29	1.54	1.54	1.54	2
CA 3	Acala	0.261	-1	77.1	93.1	52.2	74
			9	23.4	62.8	42.8	43
			16	6.23	4.25	26.4	12
			22	5.08	1.54	1.54	3
			29	1.54	6.58	1.54	3
CA 4	ST 5115 GLT	0.271	-1	113	232	202	182
			7	121	62.0	70.4	84
			14	26.7	24.4	22.8	25
			20	6.92	67.7	9.74	28
			26	1.54	6.89	5.01	4
CA 5	ST 5115 GLT	0.268	-2	58.3	14.3	11.2	28
			7	303	80.6	376	253
			14	795	55.4	197	349
			20	5.96	3.57	7.42	6
			26	1.54	1.54	1.54	2
CA 6	Acala	0.267	-2	104	281	208	196
			5	373	490	260	374
			13	294	282	171	249
			20	22.2	216	26.6	88
			27	6.15	14.7	3.94	8

[a] Number of days after final application
[b] For the purpose of calculating means, values below the LOQ (1.00 ppb) were set to half of the LOQ (0.500 ppb).

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Table 11. Dinotefuran Residues in Floral Cotton Nectar.

Trial	Cotton Variety	Total Rate (lb ai/A)	Sample Timing [a]	Dinotefuran Residue (ppb)			
				Replicate 1	Replicate 2	Replicate 3	Mean
CA 1	Prima	0.268	3	346	263	257	289
			10	55.0	54.0	44.5	51
			17	36.0	27.4	28.8	31
CA 2	Acala	0.260	7	47.9	90.7	109	83
			15	6.74	7.58	7.75	7
			22	6.09	6.21	6.57	6
CA 3	Acala	0.261	9	27.1	321	24.9	124
			16	3.84	3.86	5.39	4
			22	7.30	4.08	3.48	5
CA 4	ST 5115 GLT	0.271	7	141	27.5	73.8	81
			14	6.96	149	14.2	57
			20	2.73	2.51	3.30	3
CA 5	ST 5115 GLT	0.268	7	81.6	175	106	121
			14	9.09	18.5	13.9	14
			20	6.01	5.19	6.01	6
CA 6	Acala	0.267	5	328	274	309	304
			13	16.0	56.1	19.4	31
			20	--[b]	19.2	22.5	21

[a] Number of days after final application.
[b] The mass of this sample was too small for analysis. The mean is based on two replicates.

Table 12. UF Residues in Floral Cotton Nectar.

Trial	Cotton Variety	Total Rate (lb ai/A)	Sample Timing [a]	UF Residue (ppb)			
				Replicate 1	Replicate 2	Replicate 3	Mean
CA 1	Prima	0.268	3	49.6	48.1	36.5	45
			10	15.2	15.4	15.0	15
			17	11.5	10.7	12.0	11
CA 2	Acala	0.260	7	12.0	26.2	27.7	22
			15	2.81	3.06	3.13	3
			22	1.45	1.37	1.62	1
CA 3	Acala	0.261	9	16.7	99.4	10.5	42
			16	2.80	2.37	3.00	3
			22	2.97	3.07	2.97	3
CA 4	ST 5115 GLT	0.271	7	40.8	6.75	13.0	20
			14	5.53	73.4	7.04	29
			20	2.41	2.90	3.03	3
CA 5	ST 5115 GLT	0.268	7	10.1	18.5	11.4	13
			14	3.95	6.99	5.59	6
			20	4.45	4.95	3.89	4
CA 6	Acala	0.267	5	198	136	140	158
			13	25.7	45.0	19.2	30
			20	--[b]	16.9	23.3	20

[a] Number of days after final application.
[b] The mass of this sample was too small for analysis. The mean is based on two replicates.

Table 13. DN Residues in Floral Cotton Nectar.

Trial	Cotton Variety	Total Rate (lb ai/A)	Sample Timing [a]	DN Residue (ppb)[b]			
				Replicate 1	Replicate 2	Replicate 3	Mean
CA 1	Prima	0.268	3	10.1	6.90	8.86	9
			10	1.54	1.54	1.54	2
			17	1.54	1.54	1.54	2
CA 2	Acala	0.260	7	1.54	9.28	9.43	7
			15	1.54	1.54	1.54	2
			22	1.54	1.54	4.54	2
CA 3	Acala	0.261	9	4.80	69.7	5.17	27
			16	1.54	1.54	1.54	2
			22	1.54	1.54	1.54	2
CA 4	ST 5115 GLT	0.271	7	51.1	6.11	14.6	24
			14	5.24	44.1	9.61	20
			20	3.79	3.65	5.05	4
CA 5	ST 5115 GLT	0.268	7	7.84	35.3	13.6	19
			14	3.75	5.44	10.2	6
			20	3.49	3.42	4.12	4
CA 6	Acala	0.267	5	64.4	47.1	55.6	56
			13	9.26	16.6	6.92	11
			20	--[c]	6.43	5.97	6

[a] Number of days after final application.
[b] For the purpose of calculating means, values below the LOQ (3.08 ppb) were set to half of the LOQ (1.54 ppb).
[c] The mass of this sample was too small for analysis. The mean is based on two replicates.

Table 14. Dinotefuran Residues in Cotton Pollen.

Trial	Cotton Variety	Total Rate (lb ai/A)	Sample Timing [a]	Dinotefuran Residue (ppb)			
				Replicate 1	Replicate 2	Replicate 3	Mean
CA 1	Prima	0.268	3	14757	18200	20676	17878
			10	8610	5722	6073	6802
			17	8212	6968	5817	6999
CA 2	Acala	0.260	7	370	658	742	590
			15	114	92.0	13.8	73
			22	34.2	16.1	42.3	31
CA 3	Acala	0.261	9	71.3	314	374	253
			16	15.2	61.0	148	75
			22	42.2	39.4	26.3	36
CA 4	ST 5115 GLT	0.271	8	672	347	265	428
			15	686	545	419	550
			20	401	781	445	542
CA 5	ST 5115 GLT	0.268	8	886	641	849	792
			14	183	164	389	245
			20	23.3	82.1	50.8	52
CA 6	Acala	0.267	5	201	141	146	163
			13	132	31.6	83.1	82
			20	48.6	41.9	20.9	37

[a] **Number of days after final application.**

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Table 15. UF Residues in Cotton Pollen.

Trial	Cotton Variety	Total Rate (lb ai/A)	Sample Timing [a]	UF Residue (ppb)			
				Replicate 1	Replicate 2	Replicate 3	Mean
CA 1	Prima	0.268	3	2354	2802	3424	2860
			10	2759	1866	2105	2243
			17	3693	2991	2523	3069
CA 2	Acala	0.260	7	197	343	664	401
			15	146	122	25.2	98
			22	48.1	48.2	42.0	46
CA 3	Acala	0.261	9	38.2	93.5	130	87
			16	20.7	32.8	101	52
			22	49.9	48.2	31.7	43
CA 4	ST 5115 GLT	0.271	8	219	137	114	157
			15	473	287	175	312
			20	370	565	463	466
CA 5	ST 5115 GLT	0.268	8	124	116	113	118
			14	70.2	73.2	145	96
			20	18.5	42.9	34.3	32
CA 6	Acala	0.267	5	94.5	73.6	70.4	80
			13	200	46.3	80.7	109
			20	111	66.7	26.8	68

[a] Number of days after final application.

Table 16. DN Residues in Cotton Pollen.

Trial	Cotton Variety	Total Rate (lb ai/A)	Sample Timing [a]	DN Residue (ppb)			
				Replicate 1	Replicate 2	Replicate 3	Mean
CA 1	Prima	0.268	3	1718	2265	2336	2106
			10	2500	1753	1473	1909
			17	3017	2630	1540	2396
CA 2	Acala	0.260	7	268	605	488	454
			15	229	220	33.6	161
			22	56.8	79.9	68.5	68
CA 3	Acala	0.261	9	47.2	116	166	110
			16	40.8	78.0	253	124
			22	76.6	175	67.5	106
CA 4	ST 5115 GLT	0.271	8	288	166	114	189
			15	758	479	270	502
			20	537	877	803	739
CA 5	ST 5115 GLT	0.268	8	128	88.4	111	109
			14	96.1	75.7	167	113
			20	21.4	63.4	40.1	42
CA 6	Acala	0.267	5	52.3	42.3	46.5	47
			13	130	27.4	60.0	72
			20	101	56.4	24.0	60

[a] **Number of days after final application.**

7. STATISTICAL ANALYSIS

COMPARISON OF DISTRIBUTION BETWEEEN PARENT AND DEGRADATION PRODUCTS:
Comparison of the relative mean concentrations and associated distribution statistics for parent dinotefuran and degradation products are presented in Table 17 for leaf samples, Table 18 for extra-floral nectar samples, Table 19 for floral nectar, and Table 20 for pollen samples. The distributions were derived from all data that were collected as it reflects the actual distribution measured within and between fields. When values were below the analytical limit of detection (LOD) the authors reported values at one-half the respective LOD.

LEAVES: Mean parent dinotefuran residue at the first pre-bloom sampling interval was less than 50% of the mean total residue (Table 17 and Figure 1). At pre-bloom, the leaf samples were harvested after the first application of dinotefuran but prior to the second application. Theoretically, concentrations of UF and DN degradation products were low in the product that was applied, so their presence at this sampling interval indicates rather rapid dissipation of parent dinotefuran. At the post-bloom sampling interval, which was approximately 28 days after the second application, parent dinotefuran was only 9% of the total residue; DN degradation product accounted for 62% of the total residue and UF degradation product was at 29% of total residue. Although the degradation products represented a greater portion of total residue over time, the total amount was also decreasing where, at the post-bloom interval, the mean total residue was 45% of the residue value measured at early-bloom. The relative percentage of DN steadily increased throughout the sampling intervals that succeeded the early-bloom interval; DN residues were at 30% at early-bloom rising to 62% of the total residue at post-bloom sampling. This effect was due to the stability of DN residues as compared to parent dinotefuran and UF degradate concentrations that were decreasing over time (Figure 1).

EXTRA-FLORAL NECTAR: Mean concentration of parent dinotefuran at the pre- and early-bloom sampling times was measured at approximately 75% of the mean total residue, which was a greater percentage than measured in leaf samples (Table 18 and Figure 2). At the subsequent mid-bloom sampling time, the relative amount of parent dinotefuran dropped to approximately 50% of the total residue with UF and DN degradates at essentially equal percentages. This pattern was reflected throughout the rest of the sampling times. Total amount of residue decreased over time where at the post-bloom interval concentrations had decreased to 2% of the amount measured at the early-bloom sampling interval. Compared to leaves, the relative distribution of residues appeared stable between the sampling times, but the overall dissipation rate was faster. Similarly, relative concentration between residues from mid- to post-bloom indicated that all residues rapidly dissipated over time.

FLORAL NECTAR: Many of the analyses for the degradate DN were lower than the limit of detection at the mid- and late-bloom sampling intervals where all replicates for 3 of the 6 trials were assigned half the respective LOD values. Even though values were inserted into the data set, the resulting statistics should reflect the distribution and potential dissipation of residues over time. Parent dinotefuran at the first early-bloom sampling period was the major portion of residue measured at 69% of mean total residue (Table 19 and Figure 3). The relative amount dropped to approximately 55% of total residue at the mid- and late-bloom sampling periods. The UF degradation residue was nearly twice the concentration of the DN degradate residue throughout all sampling periods. Mean total residue concentration at late-bloom had decreased rapidly and was at only 9% of the value measured at early-bloom.

POLLEN: The pattern in pollen was similar to that observed in floral nectar where mean parent dinotefuran was the major portion of residue at 75% of mean total residue when sampled at early bloom (Table 20 and Figure 4). The portion of parent residue dropped to approximately 55% of the total residue at the mid- and late-bloom sampling periods. The pattern for UF and DN degradation products differed from that observed for leaves in that they were measured at essentially equal portions throughout the sampling intervals. The decrease of mean total residue concentration was slower than observed for nectar where the mean concentration at late-bloom was 55% of the value measured at early-bloom.

STATISTICAL ANALYSIS FOR EFFECT OF TIME AND SOIL TEXTURE CATEGORY:
The six trial sites were evenly split between coarse- and fine-texture soil categories. Analysis of variance was conducted to determine if there was an overall difference in concentration of residues between the two soil texture categories, to verify the dissipation of residues over time, and to determine if the dissipation rate was similar between plants grown in the two texture categories. Since plants from the pre-bloom sampling interval had been exposed to only 1 application of dinotefuran, this data was excluded from the analysis. Tests for normality of distributions in each plant sample indicated that transformation to natural logarithms was effective in providing a shift to a normal distribution. Estimates of dissipation half-lives were determined from the regression coefficient calculated for the significant linear effect of time. When data are expressed as natural logarithms, half-life values are determined according to Equation 1:

Equation 1: Half-life (days) = log(2)/R R=regression coefficient for linear effect of time

A general result from the analysis of variances was that there was no indication of interaction between soil type and dissipation rates. This means that the dissipation rate was similar between plant samples from coarse- and fine-textured soil, even when an effect of soil type was indicated. Furthermore, only the linear effect of time was consistently significant so dissipation half-lives were calculated from that estimated parameter, according to Equation 1. Only results for soil type and the linear effect of time are discussed (ANOVA results are given in Table 21A and estimated dissipation half-lives in Table 21B). The degradation of parent residues, the appearance of degradation products, and their subsequent degradation is complex requiring an understanding of the formation and breakdown rate of separate residues. For studies that cover short time intervals, such as this study, formation of degradation products generally indicates increases in concentration for a chemical that is relatively long-lived in the environment. Calculation of a dissipation half-life for DN and UF degradation products within the time span of this study is just an indication that these chemicals are relatively short-lived in the environment.

LEAVES: A significant effect of soil type was indicated for all residues except for parent dinotefuran where concentrations were greater in plants grown in fine-textured soils (Table 21A and Figure 1). This result appears opposite to previous reports for other neonicotinoids where plants grown in coarse-texture soil exhibited higher concentrations. Significant dissipation was indicated for parent dinotefuran and the degradate UF as well as for the total residue. Stability of DN residues was previously indicated where it was noted that its relative portion in total residue appeared to increase over the sampling periods. The half-life value for total residue was estimated at approximately 20 days with rates of 8 and 17 days and no dissipation for parent dinotefuran, UF ,and DN, respectively (Table 21B) .

EXTRA-FLORAL NECTAR: In contrast to leaves, there was no significant effect of soil type so concentrations were similar between plants grown in coarse- and fine-textured soils (Table 21A). Although a graphical comparison indicated potentially greater concentrations in coarse-textured soil, data generated for site CA1 were extremely high compared to the other sites (Tables 2, 8, 9 and 10 and Figure 2). Removal of trial 1 resulted in similar graphs between the soil types. Dissipation half-live values were rapid for all residues, which were measured at 5, 4, 5,and 5 days for total residue, parent dinotefuran, and UF and DN degradates, respectively (Table 21B).

FLORAL NECTAR: A significant difference in concentration for soil type was indicated only for parent dinotefuran residues where residues appeared greater in coarse-textured soil (Table 21A). Rapid dissipation was again measured in all residues with half-life values of 4, 3, 5, and 5 days for total residue, parent dinotefuran, and UF and DN degradates, respectively (Table 21B).

POLLEN: Results for pollen were in stark contrast to the other residues because no effect was indicated for soil type or for dissipation of residues (Table 21A). Again, the graphical comparison between soil type indicates potentially greater concentrations in coarse-textured soil, but as previously indicated, removal of data from trial CA1 results in similar plots (Tables 14, 15, and 16).

MAGNITUDE OF RESIDUES IN BEE-RELEVANT MATRICES

Concentration of total residues at early bloom at site CA1 were within the range measured at the other sites, indicating that exposure to the dinotefuran applications was not extraordinary at this site (Figure 5). The concentrations measured for extra-floral nectar and pollen did indicate greater concentrations at CA1 site than at the other 5 sites. These patterns were similar at subsequent sampling periods for each plant sample. Difference in distribution between plant samples could be due to normal variability of site variables, or as the authors indicate, could be due to the use of the Pima variety of cotton at site CA1 as it may have different phenotypical attributes than the highland varieties planted at the other trial sites. The distribution of residues collected from all sites will be discussed because the range in values reflect variance that may be caused by specific site environmental variables or by phenotypic differences due to plant variety. As indicated in the analysis for dissipation rate over time, the concentrations measured at early-bloom reflected the highest range in expected values for parent and degradation products in leaves, extra-floral nectar, and floral nectar. Analyses for pollen differed from the other plant samples because analyses indicated stability in residues over the time interval of this study. Figures 6 and 7 present a comparison of the distribution of the triplicate measures for floral nectar and pollen at each trial site. For nectar samples, a decrease in concentration of total residue is evident at each site whereas there is overlap of the bar charts overtime for pollen samples at each trial site. The range in values measured for each bee-relevant matrix will be indicated for the early-bloom sampling period. Potential exposure scenarios should factor-in the rapid dissipation previously observed for extra-floral and floral nectar samples.

EXTRAFLORAL NECTAR: The maximum, 90th, and median values measured for parent dinotefuran residues at early-bloom were 15500, 10500, and 499 ug/kg, respectively. Based on the mean values, parent dinotefuran was the predominant residue at this time where it was 73% of the total residue. Maximum, 90th and median values for the UF degradate were 1390, 1360, and 141 ug/kg, and for the DN degradate were 1080, 996, and 101, respectively. As indicated, the magnitude of residues in extra-floral samples decreased overtime, with the degradation products comprising a larger portion of the total residue. Residues were still measured in samples at post-bloom, which occurred 21 days after the sampling event at early-bloom.

FLORAL NECTAR: Concentrations of residues were much lower in floral nectar than in extra-floral nectar samples. The maximum, 90th, and median values measured for parent dinotefuran residues at early bloom were 346, 328, and 125 ug/kg, respectively. Based on the mean values, parent dinotefuran was the predominant residue at this time where it was 69% of the total residue. Maximum, 90th and median values for the UF degradate were 198, 140, and 27 ug/kg and for the DN degradate were 70, 64, and 10, respectively. The concentration and distribution in residues over time also reflected the pattern measured in extra-floral nectar where concentration decreased overtime, and where the degradation products comprised a greater portion of total residue over time.

POLLEN: Concentrations of residues measured in pollen samples ranged even higher than those measured for extra-floral nectar. The maximum, 90th, and median values measured for parent dinotefuran residues at early-bloom were 20676, 18200, and 508 ug/kg, respectively. Similar to the pattern noted in the other bee-relevant samples, parent dinotefuran was the predominant residue at this time where it was 75% of the total residue. Maximum, 90th and median values for the UF degradate were 3424, 2802, and 127 ug/kg and for the DN degradate were 2336, 2265, and 147, respectively. Concentrations in pollen were more stable overtime as reflected by nonsignificant regressions and the large amount of overlap in bar charts comparing the distribution of concentration at each trial over the sampling events (Table 21A and Figure 7). The pattern for distribution of residues did reflect reduction in the relative amount of parent dinotefuran over time where it was reduced to around 55% of the total residue at mid- and later-bloom sampling.

8. STUDY STRENGTHS, LIMITATIONS AND CONCLUSIONS

In the context of documenting the magnitude of dinotefuran residues in bee-related matrices of cotton plants, the following strengths are observed with this study.

1. Data provide quantitative values for parent dinotefuran and the UF and DN degradation products expected in pollen, extra-floral nectar, floral nectar, and leaves of various varieties of cotton.

2. Sequential sampling over the blooming period provided a basis to determine potential dissipation half-live rates for the residues.

3. Although data were generated from only 6 of 9 requested sites, the values indicate substantial exposure to dinotefuran residues in bee-relevant matrices.

4. Two soil types were evenly distributed among the sites where 3 were located in coarse-texture soil and 3 in fine-textured soil, allowing for a comparison of the effect of soil type on concentrations measured in plant samples.

Limitations noted in this study include:

1. Samples were taken from a mixture of plant varieties. Since the effect of different varieties on distribution of residues is unknown, the results reflect general observations made to all planted cotton crops.

2. Data for only 6 of 9 requested sites in the DCI were submitted.

3. Three of the six nontreated control pollen samples (trial locations -01, -04 and -05) contained measurable amounts of dinotefuran, UF and DN. For trial -01, the dinotefuran residue in the non-treated pollen sample (LA16-12) was 782 ppb *vs*. a mean residue of 17,878 ppb in pollen from the treated plots; for trial -04, dinotefuran residue in the non-treated pollen sample (LA16-198) was 23.3 ppb *vs*. a mean residue of 428 ppb from pollen in the treated plots; and for trial -05, dinotefuran residue in the non-treated pollen sample (LA16-260) was 40.5 ppb *vs*. a mean residue of 792 ppb from pollen in the treated plots. It is unclear if the nature of the control contamination may have had an effect on the magnitude of residues in the treated plots.

Overall, considering the strengths and limitations of this study, the following conclusions can be drawn:

Classification/Utility for Bee Risk Assessment. This study is classified as acceptable. It provides the dinotefuran, UF and DN residues in nectar, pollen, extrafloral nectar and leaves collected from cotton that have received two foliar applications of Dinotefuran 20SG (active ingredient dinotefuran).The residue values presented should be considered to be fully reliable.

Magnitude of Residues in Bee-relevant Matrices. Significant concentration of parent dinotefuran and UF and DN degradation products were measured in bee-relevant matrices directly following a second foliar application to cotton. In floral nectar, the maximum, 90th, and median values measured for parent dinotefuran residues at early bloom were 346, 328, and 125 ug/kg, respectively. In pollen, the maximum, 90th, and median values measured for parent dinotefuran residues at early-bloom were 20676, 18200, and 508 ug/kg, respectively.

Temporal Variability in Residues. Significant dissipation of residues over the sampling time of the study indicated relatively rapid dissipation half-lives in extra-floral nectar, floral nectar, and leaves estimated at 5, 4, and 20 days, respectively. Dissipation was not indicated for residues in pollen samples.

Spatial Variability in Residues. There was no consistent effect of soil type on measured concentrations in plant samples.

Pesticide Carryover. In this study, samples were collected during a single flowering period, and thus, was not designed to measure pesticide carryover year to year.

9. STUDY VALIDITY/CLASSIFICATION

The study is classified as ACCEPTABLE for quantitative use in risk assessment. The data from this study provide an expected distribution of the concentrations of dinotefuran residues that bees are exposed to in extra-floral nectar, nectar, and pollen of cotton plants grown under actual agronomic practices in California. Relating concentrations measured in flower parts to bee health is possible by comparing the concentrations measured in bee relevant plant parts to target values that define acute or chronic exposure scenarios. Relatively rapid dissipation rates were estimated for residues in extra-floral nectar and floral nectar, which could be factored into potential exposure scenarios. Dissipation of residues was not measured in pollen samples. The study is considered scientifically sound and useful for risk assessment purposes.

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Table 17. Leaves: Distribution of dinotefuran residues in leaves of cotton compared between the sampling intervals. A second foliar application of dinotefuran occurred in between the pre bloom and early bloom sampling intervals. Sampling intervals were approximately 7 days apart.

Leaves																				
	Pre-Bloom				Early-Bloom				Mid-Bloom				Late-Bloom				Post-Bloom			
Statistic	UF	DN	Parent	**Total**	UF	DN	Parent	**Total**	UF	DN	Parent	**Total**	UF	DN	Parent	**Total**	UF	DN	Parent	**Total**
N	18	18	18	**18**	18	18	18	**18**	18	18	18	**18**	18	18	18	**18**	18	18	18	**18**
Mean (ug/kg)	1883	1905	3266	**7054**	3529	3720	4973	**12222**	2874	4176	1651	**8701**	1997	3275	901	**6172**	1584	3407	509	**5500**
SD (ug/kg)	664	560	1097	**1266**	1559	1506	2387	**3330**	1780	1917	1133	**4201**	1719	2016	868	**4241**	1573	2239	484	**4172**
CV (%)	35	29	34	**18**	44	41	48	**27**	62	46	69	**48**	86	62	96	**69**	99	66	95	**76**
Min (ug/kg)	140	865	1715	**4545**	1845	1375	1785	**6840**	1230	1725	415	**3590**	458	1245	74	**2075**	303	775	47	**1729**
Median (ug/kg)	1903	2068	3193	**7038**	3005	3558	4688	**12120**	2043	3883	1435	**7653**	1560	2803	447	**4711**	890	2840	320	**3603**
75th (ug/kg)	2390	2300	4155	**8190**	4765	5000	6250	**12885**	3995	5900	2425	**10475**	2195	3495	1540	**6125**	1765	4105	765	**6560**
90th (ug/kg)	2770	2575	4755	**8740**	6300	5500	8550	**17375**	6450	7400	3310	**17065**	5700	7400	2175	**15275**	4700	7250	1260	**13335**
95th (ug/kg)	2800	2675	5450	**9500**	6650	6750	8700	**20350**	6600	7500	3350	**17210**	6000	7750	2870	**15330**	4855	8200	1485	**14340**
Max (ug/kg)	2800	2675	5450	**9500**	6650	6750	8700	**20350**	6600	7500	3350	**17210**	6000	7750	2870	**15330**	4855	8200	1485	**14340**
% of Total	**27**	**27**	**46**		**29**	**30**	**41**		**33**	**48**	**19**		**32**	**53**	**15**		**29**	**62**	**9**	

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Table 18. Extra floral-nectar: Distribution of dinotefuran residues in extra floral nectar of cotton plants compared between the sampling intervals. A second foliar application of dinotefuran occurred in between the pre-bloom and early bloom sampling intervals. Sampling intervals were approximately 7 days apart.

	Extra-Floral Nectar																			
	Pre-Bloom				Early-Bloom				Mid-Bloom				Late-Bloom				Post-Bloom			
Statistic	UF	DN	Parent	**Total**	UF	DN	Parent	**Total**	UF	DN	Parent	**Total**	UF	DN	Parent	**Total**	UF	DN	Parent	**Total**
N	18	18	18	**18**	18	18	18	**18**	18	18	18	**18**	18	18	18	**18**	18	18	18	**18**
Mean (ug/kg)	185	121	828	**1134**	416	271	2371	**3057**	197	162	418	**777**	37	33	64	**133**	20	7	27	**53**
SD (ug/kg)	186	104	1197	**1441**	463	319	4160	**4871**	228	209	647	**991**	63	59	156	**255**	40	8	60	**107**
CV (%)	101	87	145	**127**	111	118	176	**159**	115	130	155	**128**	171	179	245	**191**	202	126	224	**202**
Min (ug/kg)	10	5	21	**36**	36	23	105	**181**	4	4	4	**13**	2	2	1	**6**	1	2	1	**3**
Median (ug/kg)	125	99	460	**710**	141	101	499	**740**	41	41	125	**206**	10	7	7	**26**	3	3	2	**8**
75th (ug/kg)	276	208	1070	**1717**	759	376	2670	**3487**	459	282	490	**1302**	29	27	29	**96**	11	7	4	**27**
90th (ug/kg)	536	281	1740	**2097**	1360	996	10500	**12856**	518	438	1800	**2693**	115	151	197	**360**	73	17	119	**202**
95th (ug/kg)	632	348	5080	**6060**	1390	1080	15500	**17970**	586	795	1920	**2824**	250	216	647	**1048**	154	34	216	**404**
Max (ug/kg)	632	348	5080	**6060**	1390	1080	15500	**17970**	586	795	1920	**2824**	250	216	647	**1048**	154	34	216	**404**
% of Total	**16**	**11**	**73**		**14**	**9**	**78**		**25**	**21**	**54**		**28**	**25**	**48**		**38**	**12**	**50**	

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Table 19. Floral Nectar: Distribution of dinotefuran residues in extra floral nectar of cotton plants compared between the sampling intervals. Plants had received two foliar sprays of dinotefuran prior to bloom. Sampling intervals were approximately 7 days apart.

Floral Nectar												
	Early-Bloom				Mid-Bloom				Late-Bloom			
Statistic	UF	DN	Parent	**Total**	UF	DN	Parent	**Total**	UF	DN	Parent	**Total**
N	18	18	18	**18**	18	18	18	**18**	17	17	17	**17**
Mean (ug/kg)	50	23	167	**240**	14	7	27	**48**	6	3	11	**21**
SD (ug/kg)	56	23	117	**182**	18	10	36	**62**	6	2	11	**16**
CV (%)	111	100	70	**76**	130	147	130	**128**	98	55	95	**78**
Min (ug/kg)	7	2	25	**40**	2	2	4	**8**	1	2	3	**8**
Median (ug/kg)	27	10	125	**187**	6	3	14	**30**	3	3	6	**13**
75th (ug/kg)	50	47	274	**406**	15	9	45	**61**	11	4	19	**40**
90th (ug/kg)	140	64	328	**505**	45	17	56	**118**	17	6	29	**49**
95th (ug/kg)	198	70	346	**590**	73	44	149	**267**	23	6	36	**52**
Max (ug/kg)	198	70	346	**590**	73	44	149	**267**	23	6	36	**52**
% of Total	**21**	**10**	**69**		**29**	**14**	**56**		**31**	**15**	**54**	

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Table 20. Pollen: Distribution of dinotefuran residues in pollen of cotton flowers compared between the sampling intervals. Plants had received two foliar sprays of dinotefuran prior to bloom. Sampling intervals were approximately 7 days apart.

Pollen												
	Early-Bloom				Mid-Bloom				Late-Bloom			
Statistic	UF	DN	Parent	**Total**	UF	DN	Parent	**Total**	UF	DN	Parent	**Total**
N	18	18	18	**18**	18	18	18	**18**	18	18	18	**18**
Mean (ug/kg)	617	503	3351	**4470**	485	480	1305	**2270**	621	569	1283	**2472**
SD (ug/kg)	1058	762	6767	**8569**	832	705	2594	**4115**	1156	919	2670	**4720**
CV (%)	171	152	202	**192**	172	147	199	**181**	186	162	208	**191**
Min (ug/kg)	38	42	71	**157**	21	27	14	**73**	19	21	16	**63**
Median (ug/kg)	127	147	508	**840**	134	194	156	**476**	49	78	45	**179**
75th (ug/kg)	343	488	849	**1606**	287	479	545	**1311**	463	803	445	**1711**
90th (ug/kg)	2802	2265	18200	**23267**	2105	1753	6073	**9651**	2991	2630	6968	**12589**
95th (ug/kg)	3424	2336	20676	**26436**	2759	2500	8610	**13869**	3693	3017	8212	**14922**
Max (ug/kg)	3424	2336	20676	**26436**	2759	2500	8610	**13869**	3693	3017	8212	**14922**
% of Total	**14**	**11**	**75**		**21**	**21**	**57**		**25**	**23**	**52**	

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Table 21. Results of regression analyses measuring the linear effect of time and soil type on concentration of dinotefuran residues in cotton plant samples. Data from the pre-bloom samples were not included because plants had not received full foliar treatments. Table A reports the statistical significance measured for each effect and Table B reports the estimated half-lives determined from the regression coefficient for the linear effect of time, according to Equation 1.

A.

Plant Sample and Effect	Regression Probability Levels for Effect			
	Total Residue	Dinotefuran	UF Degradate	DN Degradate
Leaves				
Soil Type	0.001	NS	0.001	NS
Time - Linear	0.001	0.001	0.001	0.001
Extra-Floral Nectar				
Soil Type	NS	NS	NS	NS
Time - Linear	0.001	0.001	0.001	0.001
Floral Nectar				
Soil Type	NS	0.04	NS	NS
Time - Linear	0.001	0.001	0.001	0.001
Pollen				
Soil Type	NS	NS	NS	NS
Time - Linear	NS	NS	NS	NS

B.

Plant Sample	Estimated Dissipation Half-life (Days):			
	Total Residue	Dinotefuran	UF Degradate	DN Degradate
Leaves	19.8	7.5	16.6	NS
Extra-Floral Nectar	4.6	3.8	5.3	5.3
Nectar	4	3.4	5	5.3
Pollen	NS	NS	NS	NS

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Figure 1. Leaves: Distribution of dinotefuran residues in cotton leaves measured at 5 sampling intervals and in plants grown in coarse or fine-textured soil. Total residue is the summation of parent dinotefuran and UF and DN degradation products. A second foliar application of dinotefuran occurred in between the pre-bloom and early-bloom sampling intervals. Sampling occurred at approximately 7 day intervals.

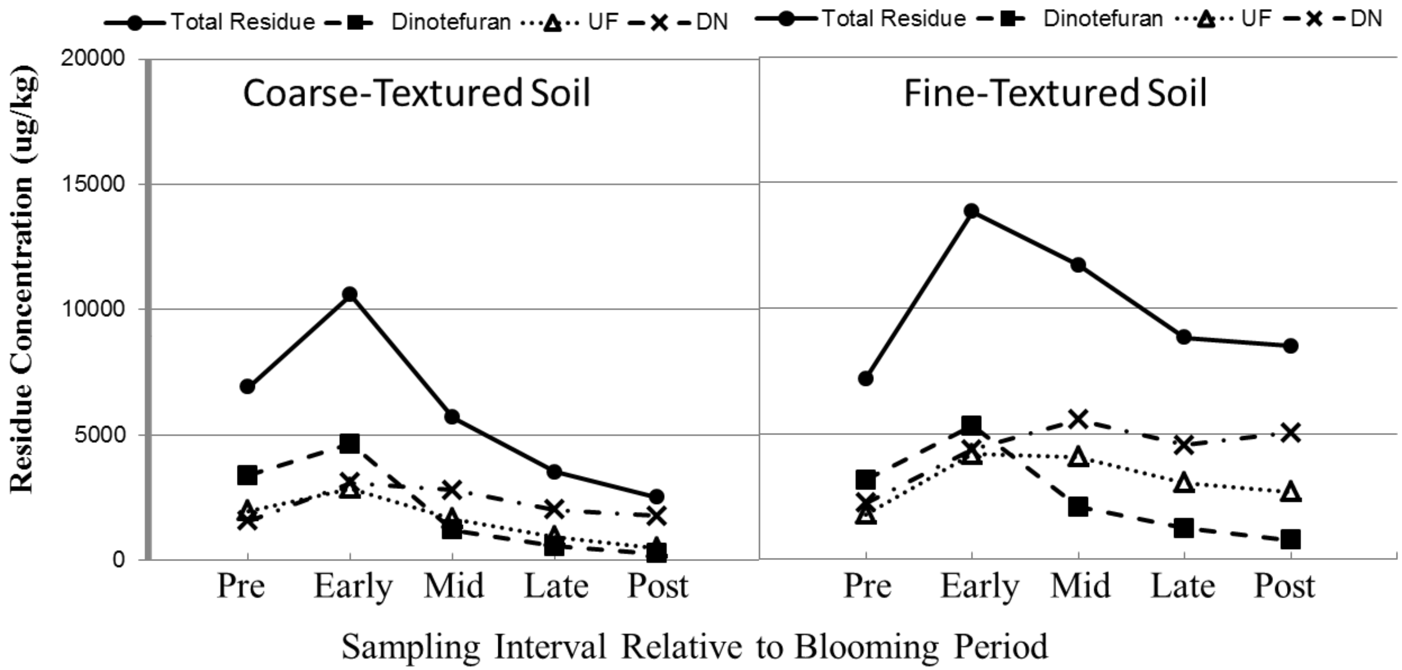

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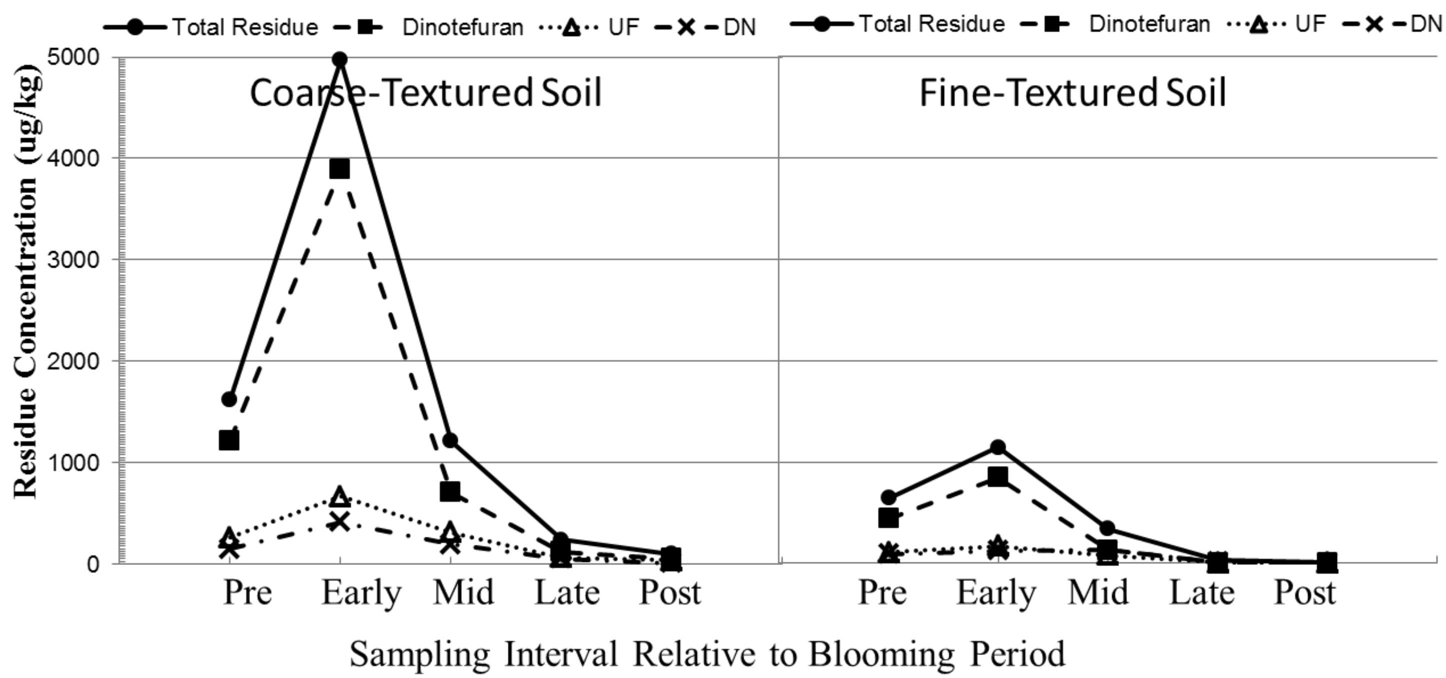
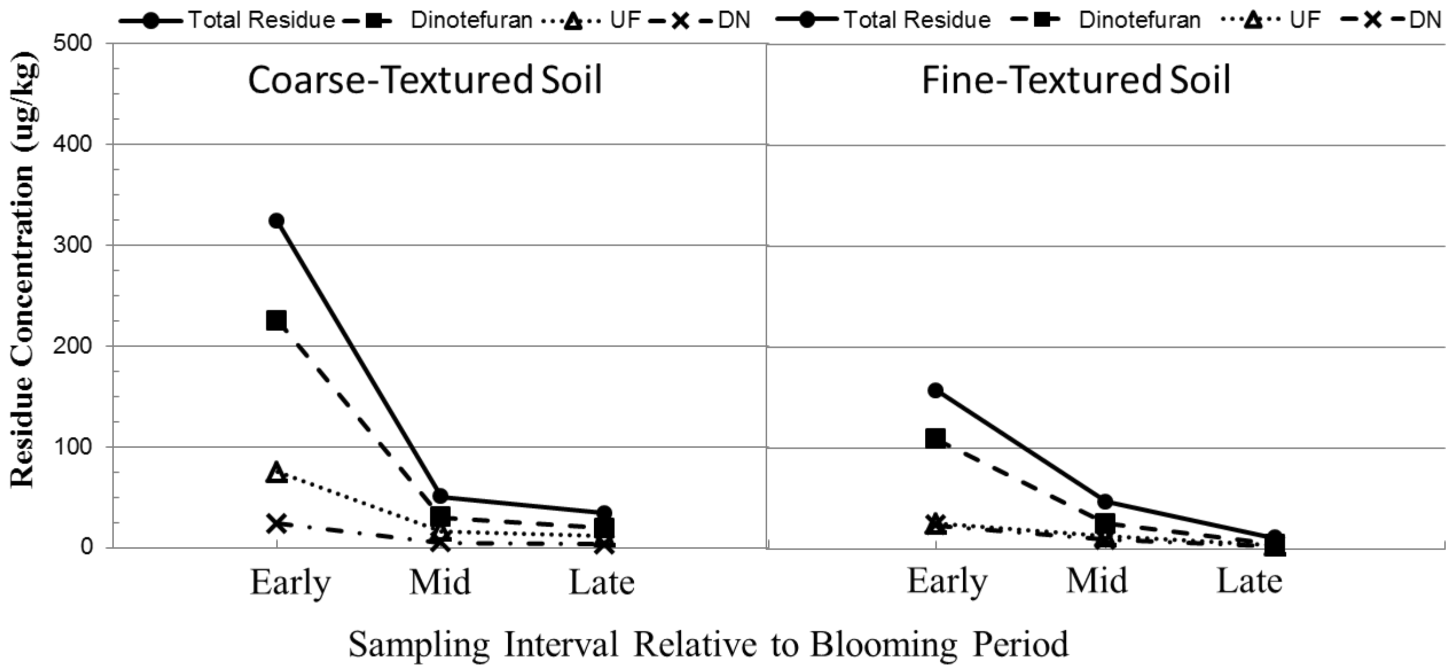
Figure 2. Extra-Floral nectar: Distribution of dinotefuran residues in extra-floral nectar of cotton plants measured at 5 sampling intervals and in plants grown in coarse or fine-textured soil. Total residue is the summation of parent dinotefuran and UF and DN degradation products. A second foliar application of dinotefuran occurred in between the pre-bloom and early-bloom sampling intervals. Sampling occurred at approximately 7 day intervals.



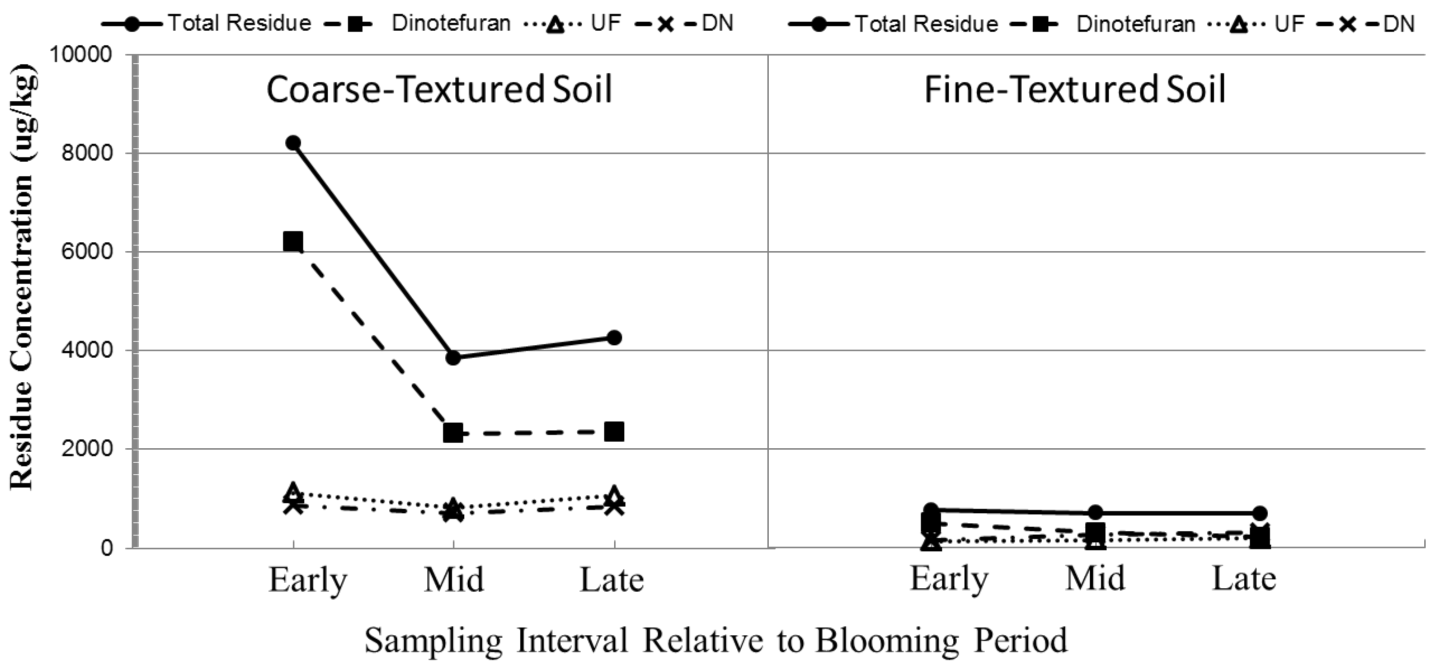
MRID 50198501 CDPR Dino Cotton

Figure 3. Floral nectar: Distribution of dinotefuran residues in floral nectar of cotton plants measured at 5 sampling intervals and in plants grown in coarse or fine-textured soil. Total residue is the summation of parent dinotefuran and UF and DN degradation products. Plants had received two foliar sprays of dinotefuran prior to bloom. Sampling occurred at approximately 7 day intervals.



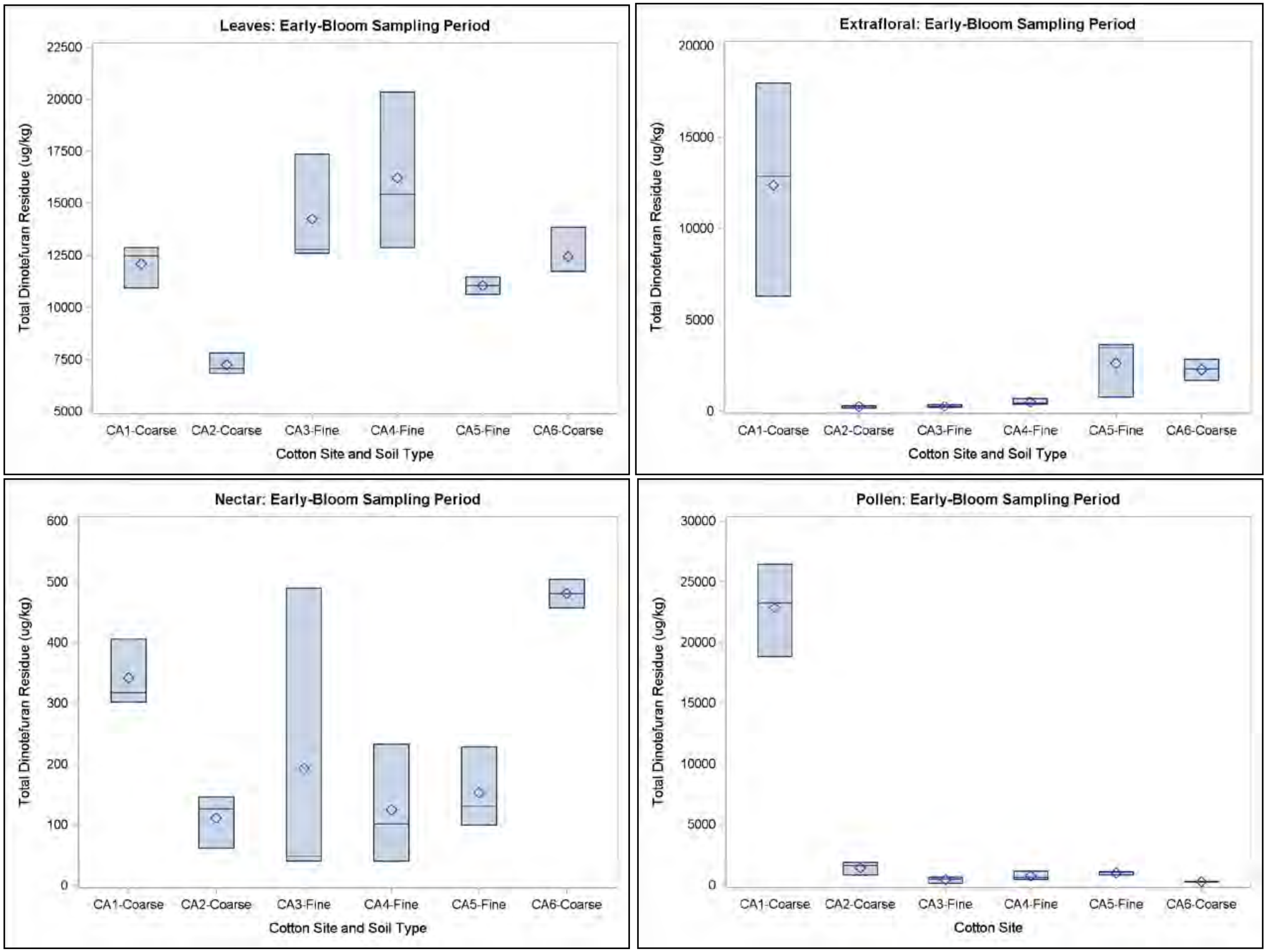
MRID 50198501 CDPR Dino Cotton

Figure 4. Pollen: Distribution of dinotefuran residues in pollen of cotton plants measured at 5 sampling intervals and in plants grown in coarse or fine-textured soil. Total residue is the summation of parent dinotefuran and UF and DN degradation products. Plants had received two foliar sprays of dinotefuran prior to bloom. Sampling occurred at approximately 7 day intervals.



MRID 50198501 CDPR Dino Cotton

Figure 5. Comparison of the distribution of total dinotefuran residue between plant samples taken at early-bloom. Each site had 3 replicate samples so the extremes in the bar chart indicate the minimum and maximum values and within the box the diamond is the mean and the line is the median of the 3 values.



MRID 50198501 CDPR Dino Cotton

Figure 6. Nectar: Comparison of the distribution of total dinotefuran residue measured at each sampling interval for each trial site. Data are express on natural logarithm scale. Each site had 3 replicate samples so the extremes in the bar chart indicate the minimum and maximum values and within the box the diamond is the mean and the line is the median of the 3 values.

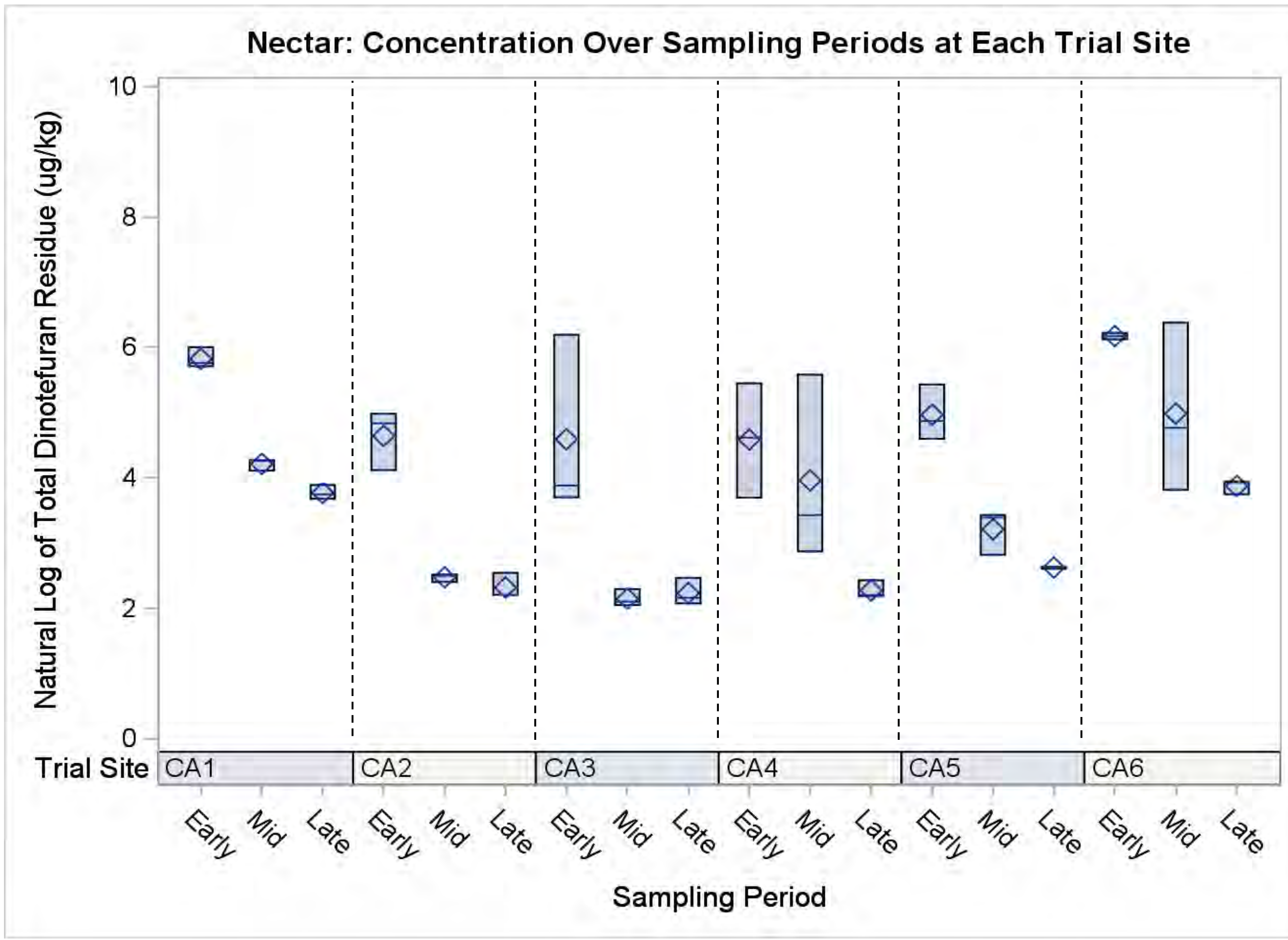
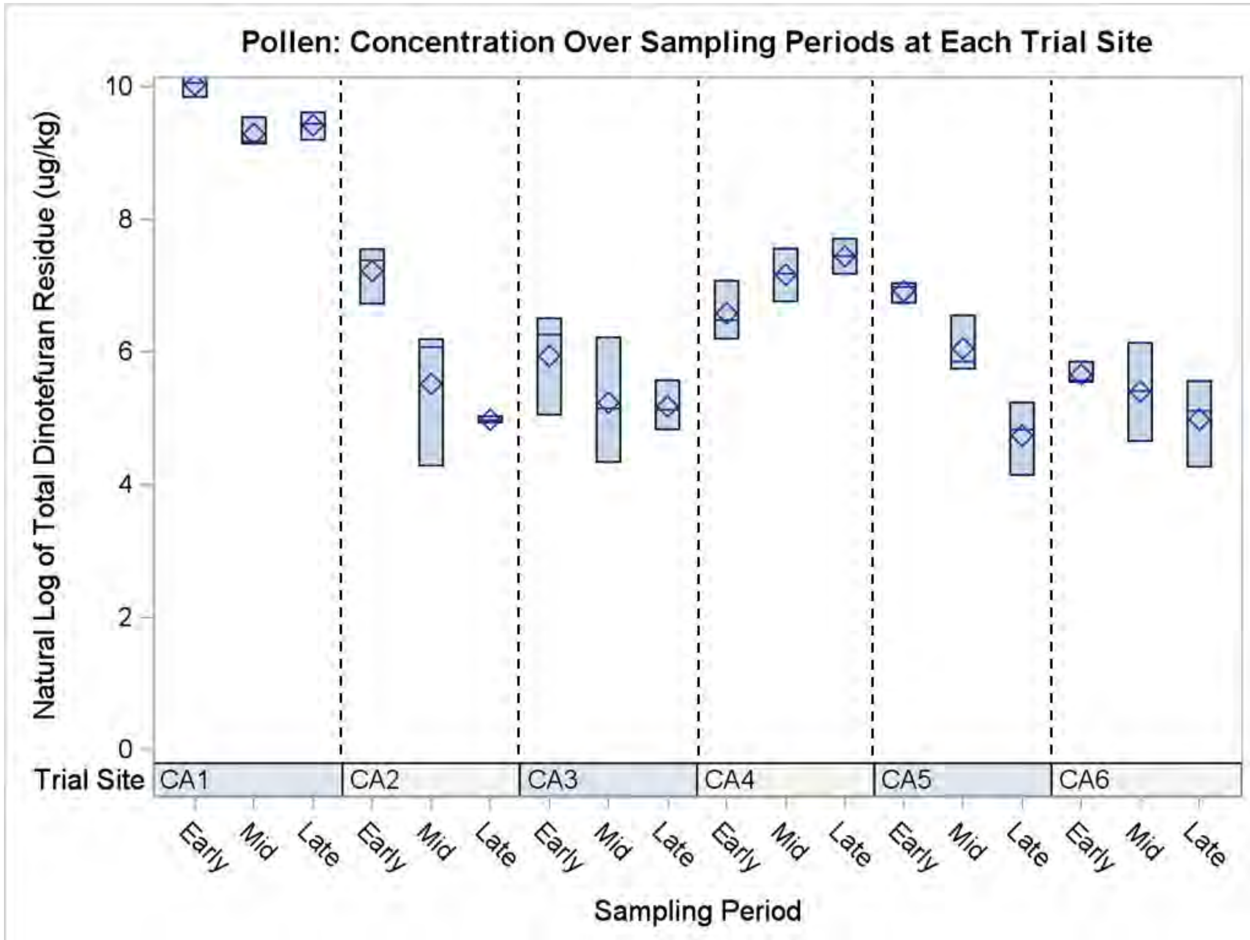


Figure7. Pollen: Comparison of the distribution of total dinotefuran residue measured at each sampling interval for each trial site. Data are express on a natural logarithm scale. Each site had 3 replicate samples so the extremes in the bar chart indicate the minimum and maximum values and within the box the diamond is the mean and the line is the median of the 3 values



10. REFERENCES

1. Daorong Guo, "Residue Analysis of Dinotefuran, DN and UF in Nectar, Pollen, Leaves and Soil by LC-MS/M", Method No. RA046, Eurofins Agroscience Services, Inc. Lancaster, PA, 20 Dec 2016.

2. Daorong Guo, "Method Validation of Residues of Dinotefuran, DN and UF in Nectar, Pollen, Leaves and Soil Matrices", Study No. S16-05692, Eurofins Agroscience Services, Inc. Lancaster, PA, 20 Dec 2016

Appendix 10. Evaluations of Residue Studies Included in this Risk Determination Document

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Lamore M. 2016. Quantification of Dinotefuran and its Metabolites in Bee-collected Nectar, Pollen, Leaves and Soil Following Soil Application of Dinotefuran 20 SG to Bell Pepper. (S16-01167)	Non-Guideline field residue study on bell peppers to establish dinotefuran and metabolite levels in bee-collected nectar, pollen, leaves, and soil following soil applications	This study quantified Dinotefuran residues in bell pepper plants (*Capsicum anuum*) grown in three locations: North Carolina (NC; Sandy Loam), Georgia (GA; Sand), and California (CA; Sandy Loam). Three replicate plots were used in each location and each plot received two soil applications: one application at a rate of 0.206 lbs ai/A and the second application, seven days later, at a rate of 0.330 lbs ai/A. The label for Dinotefuran 20SG prohibits soil and foliar applications to the same plot. In all three locations, soil and foliar application rates and reapplication intervals were sufficient to represent a worst case scenario. Analyses of fortified samples of pollen (80-127%) and nectar (93-106%) were all within acceptable limits. Pollen and nectar samples were collected by bumble bees. Bumble bees were caught using a net and placed in a jar with dry ice. Nectar was harvested via dissection of the bumble bees honey stomachs. Pollen was collected from bumble bee pollen baskets. If pollen samples collected from bees were less than 300mg, then those samples were supplemented with anthers collected from fresh flowers. Mesh tunnel tents were used to ensure bumble bees did not forage on other crops and that other pollinators did not contaminate the bell peppers.	Uncertainties: None that would affect the integrity of the study

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Louque, R. 2016. Quantitation of Residues of Dinotefuran in Nectar, Pollen and Leaves Following Foliar Application and Trunk Injection of Dinotefuran 20 SG to Cherry Trees. Smithers Viscient Study Number 10934.4105	Non-Guideline field residue study on cherry to establish dinotefuran and metabolite levels in nectar, pollen, and leaves following foliar applications and trunk injections	This study quantified Dinotefuran residues in cherry (*Prunus spp.*) grown in three locations: California (CA; clay loam), New York (NY; sandy loam), and Oregon (OR; sandy loam). Three replicate plots were used for each type of application in each location and each plot received either two foliar applications, or one trunk injection, of Dinotefuran 20SG. The foliar applications were conducted at nominal rates of 0.232 lbs ai/A and 0.304 lbs ai/A. The first foliar application was scheduled for late in the season (September), before leaf drop, and the second foliar application was made seven days after the first. Dinotefuran 20SG is not currently registered for use on cherry trees. The closest related plant that is registered on the label for Dinotefuran 20SG is peach, which is in the same genus as cherry (*Prunus spp.*). The maximum single foliar application rate for peaches is 0.180 lbs ai/A and the maximum seasonal foliar application rate is 0.270 lbs ai/A/season. In the section for peaches, the label also contains the following note: “Regardless of application method, do not apply more than a total of 1.8 lbs. of DINOTEFURAN 20 SG (0.360 lb. a.i.) per acre per season.” Trunk injection applications were also conducted late in the season (September), before leaf drop, at a rate of 2 g of product per inch of trunk diameter either at breast height or right below the first trunk bifurcation. Samples of pollen and nectar were collected 165-170, 190-199, and 236-243 days after the last application in OR, CA, and NY, respectively. Analyses of fortified samples of pollen (94.3-118%) and nectar (76.2-113%) were all within acceptable limits except for one pollen sample in which the analytical results were only 4.72% of the fortification level. The maximum measured dinotefuran residues resulting from foliar applications were 200 ppb in pollen (237 days after the last application) and 25 ppb in nectar (238 days after the last application). The maximum measured dinotefuran residues resulting from tree injection applications were 31,688 ppb in pollen (201 days after application) and 17,484 ppb in nectar (237 days after application). This study is acceptable, but the low recoveries suggest that some of the residues in pollen might be as much as 20x higher than reported.	The extremely low recovery during analysis of one of the fortified pollen samples presents uncertainty in terms of the accuracy of the analyses conducted. This low recovery was noted in one of the nine fortified pollen samples analyzed as part of the quality control procedures. The pollen samples was fortified to a level of 99.7 ppb, but the analysis resulted in a measurement of 4.70 ppb (4.72%). If a similar error occurred during analysis of samples then the values presented for Dinotefuran residues in pollen might be 20x higher than reported.

Year/Authors/Title	Study Type	Summary	Notes/Uncertainties
Lange, B. 2017. Thiamethoxam 25WG (A9584C) – Determination of Residues in Leaves, Flowers, Pollen, and Nectar of Blueberry After Foliar Application Lange Research Study Number: LR16191 Report Number: TK0250072	Non-Guideline field residue study on blueberry to establish thiamethoxam and metabolite concentrations in whole flowers, leaves and manually-collected nectar and pollen following foliar applications	This study quantified thiamethoxam and CGA322704 residues in blueberry (*Vaccinium corymbosum*) grown in three locations: California (CA; sand), Quebec (QC; loam), and Washington (WA; loamy sand). Three replicate plots were used in each location. One set of plots received three foliar applications at 19, 12, and 5 days before bloom at a nominal rate of 0.063 lbs. ai/A, and another set of plots at each location received a single foliar application 15 days before bloom at a nominal rate 0.063 lbs. ai/A. Nectar and pollen were sampled at early-, mid-, and late-bloom. Samples of pollen and nectar were collected 5-22, 5-11, and 12-24 days after the last of the three application in CA, QC, and WA, respectively. In those plots receiving a single application, pollen and nectar were collected 14-31, 19-25, and 22-34 days post-application in CA, QC, and WA, respectively. Analyses of fortified samples of pollen (75.1-101% thiamethoxam and 78.6-102 for CGA322704) and nectar (77.4-96.9% thiamethoxam and 85.8-101 for CGA322704) were all within acceptable limits. Nectar and pollen samples were manually collected from flowers. Mean thiamethoxam residues in nectar from plots receiving repeated applications across all locations (118 ppb) were less than CGA322704 residues (142 ppb) in the early bloom samples but thiamethoxam residues were comparable to CGA322704 by the late-bloom samples (51.2 ppb vs. 59.1 ppb, respectively). Mean thiamethoxam residues in pollen from plots receiving repeated applications (370 ppb) were notably greater than CGA322704 residues (60.2 ppb) in the early bloom samples and thiamethoxam residues remained greater than CGA322704 in the late-bloom samples (156 ppb vs. 48.4 ppb, respectively). Mean residues for thiamethoxam in nectar were greatest across all sample periods in QC with residues comparable in CA and WA. Mean residues for thiamethoxam in pollen were greatest across all sample periods in WA with residues greater in QC than in CA. Mean concentrations of CGA322704 in nectar were greatest in QC with residues comparable in CA and WA. Mean concentrations of CGA322704 in pollen were greatest across all sample periods in QC with residues greater in WA than in CA.	Uncertainties: None that would affect the integrity of the study

U.S. EPA Data Evaluation Reports (Dinotefuran):

U.S. EPA. (2016). Data evaluation report: amended final report - quantitation of residues of dinotefuran in nectar, pollen, and laves after foliar treatment application of Dinotefuran 20 SG to cranberries. Washington, D.C.: Author. Laboratory Report Number 10934.4101.

U.S. EPA. (2016). Data evaluation report: amended final report - quantitation of residues of dinotefuran in pollen and leaves following soil application of Dinotefuran 20 SG to potato. Washington, D.C.: Author. Laboratory Report Number 10934.4100.

U.S. EPA. (2016). Data evaluation report: quantitation of residues of dinotefuran in nectar, pollen and leaves following soil application of Dinotefuran 20 SG to pumpkin. Washington, D.C.: Author. Laboratory Report Number 10934.4104.

U.S. EPA. (2016). Data evaluation report: amended final report - quantitation of residues of dinotefuran in pollen and leaves following soil and foliar application of Dinotefuran 20 SG to Tomato. Washington, D.C.: Author. Laboratory Report Number 10934.4103.

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

The following tables present descriptive statistics derived from the residue studies used to characterize exposure. Study identification numbers are provided, along with the crop, in the header of each table. For Imidacloprid and Thiamethoxam, the total concentration values (in the "Total" column) are not simply the sum of the parent molecule and metabolites. Rather, the total values were determined from the summation of the concentrations of the parents and metabolites measured on each individual sample. Since exact values, such as the maximum value, may not be measured on the same individual sample for each chemical, the distribution for total concentration will not necessarily match up to simple addition of concentrations noted for each individual chemical. Section 2.3 of the main document provides additional information regarding the toxicity of various metabolites. In addition, for a review of each study, please refer to Appendix 10.

Imidacloprid (IMI):

Tomato (EBNTN012)				
	Pollen			
Statistic	**5-OH**	**Olefin**	**IMI**	**Total**
N	113.0	113.0	113.0	**113.0**
Mean (ng/g)	8.4	2.8	166.4	**177.7**
SD (ng/g)	12.1	3.7	268.5	**282.8**
CV (%)	143.6	129.2	161.3	**159.0**
Min (ng/g)	0.3	0.2	1.4	**1.8**
Median (ng/g)	3.6	1.0	57.7	**65.2**
75th (ng/g)	9.8	4.1	162.3	**174.5**
90th (ng/g)	24.1	7.7	449.8	**476.9**
95th (ng/g)	37.2	9.6	632.8	**679.3**
Max (ng/g)	63.5	24.1	1679.7	**1762.5**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Citrus (EBNTY007)								
	Nectar				Pollen			
Statistic	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total
N	67	67	67	**67**	53	53	53	**53**
Mean (ng/g)	7.1	4.4	84.3	**95.9**	117.4	150.9	1751.6	**2020**
SD (ng/g)	3.9	2.4	83.7	**89.3**	53.5	68.4	940.2	**1056**
CV (%)	54.5	54.9	99.2	**93.1**	45.5	45.3	53.6	**52.2**
Min (ng/g)	1.4	1.1	7.6	**10.1**	7.4	9.5	67.4	**86.5**
Median (ng/g)	6.1	3.8	50.4	**59.4**	132.4	169.6	1752	**2061.3**
75th (ng/g)	9	6	107.8	**119.8**	148.3	196	2324	**2629.2**
90th (ng/g)	13.9	8.7	243	**267.1**	177.6	232.7	2846	**3257.9**
95th (ng/g)	15	9.5	253.9	**280.9**	199.1	238.8	3556	**3973.2**
Max (ng/g)	19.9	10	408.7	**431.2**	210.4	252.9	3705	**4142.1**

Citrus (EBNTL056-07)				
	Nectar			
Statistic	5-OH	Olefin	IMI	Total
N	95	95	95	**95**
Mean (ng/g)	1.6	1.6	7.3	**10.6**
SD (ng/g)	1.8	1.8	7.5	**10.4**
CV (%)	111.2	111.4	103.1	**98.9**
Min (ng/g)	0	0.1	0.1	**0.3**
Median (ng/g)	0.4	0.8	3.3	**4.7**
75th (ng/g)	3.2	2.5	12.7	**19.4**
90th (ng/g)	4.3	4.3	18.6	**25**
95th (ng/g)	5.4	5.6	20.9	**29.1**
Max (ng/g)	6.4	6.9	33.8	**39.9**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Apple (EBNTN014)								
	Nectar				Pollen			
Statistic	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total
N	33	33	33	**33**	34	34	34	**34**
Mean (ng/g)	1.1	0.6	0.8	**2.6**	1.1	4.2	18.7	**24.1**
SD (ng/g)	4.4	0.5	1.5	**6.1**	2.3	9.4	22.2	**26.5**
CV (%)	381.7	90.1	184.8	**229.2**	200.2	224	118.6	**109.8**
Min (ng/g)	0.35	0.3	0.15	**0.8**	0.25	0.15	0.7	**1.1**
Median (ng/g)	0.35	0.3	0.4	**1.25**	0.5	0.45	11.7	**15.18**
75th (ng/g)	0.35	0.8	0.9	**2.05**	1.1	4.2	20.5	**30.05**
90th (ng/g)	0.7	1.6	1.2	**3.45**	2.4	9.5	45.9	**58.5**
95th (ng/g)	0.9	2	2.8	**4.05**	3.6	14.7	91.3	**102.7**
Max (ng/g)	26	2.5	8.9	**36.3**	13.9	52.1	92.8	**103.4**

Stone Fruit (EBNTN013)								
	Nectar				Pollen			
Statistic	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total
N	34	34	34	**34**	30	30	30	**30**
Mean (ng/g)	2.7	0.6	1.6	**4.8**	1.8	3.2	44.8	**49.7**
SD (ng/g)	6.8	0.6	2.4	**7.7**	1.9	7.5	66.8	**71.7**
CV (%)	255	104	153	**161**	105	236	149	**144**
Min (ng/g)	0.4	0.3	0.2	**0.8**	0.3	0.2	1.9	**2.3**
Median (ng/g)	0.4	0.3	0.4	**1.7**	1.3	1.3	23	**26.9**
75th (ng/g)	1.2	0.7	1.2	**6.8**	2.6	2.7	52.3	**54.9**
90th (ng/g)	5.5	0.8	5.9	**9.5**	3.8	4	124.9	**136.2**
95th (ng/g)	28.7	1.4	7.1	**32**	4	14.3	144.7	**187.8**
Max (ng/g)	28.8	3.5	8.9	**33.6**	9.4	40.3	328	**341.3**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Blueberry (EBNTY006)												
	Nectar				Pollen-Blueberry Flowers				Pollen-Bumble Bee			
Statistic	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total
N	87	87	87	**87**	51	51	51	**51**	17	17	17	**17**
Mean (ng/g)	0.5	0.4	1.1	**2**	0.4	0.7	7.8	**9**	0.3	0.2	1.4	**1.8**
SD (ng/g)	0.3	0.2	2.3	**2.6**	0.4	0.8	7	**7.9**	0	0.1	0.9	**1**
CV (%)	67.2	62.6	203.8	**130.9**	85.3	104.4	89.9	**88.1**	0	46.1	64.8	**52.6**
Min (ng/g)	0.35	0.3	0.15	**0.8**	0.25	0.15	0.2	**0.6**	0.25	0.15	0.2	**0.6**
Median (ng/g)	0.35	0.3	0.15	**0.8**	0.25	0.4	5.2	**5.8**	0.25	0.15	1.4	**1.8**
75th (ng/g)	0.35	0.3	0.8	**1.95**	0.25	1.1	11.9	**14.5**	0.25	0.15	1.6	**2**
90th (ng/g)	0.8	0.7	3.2	**4.6**	0.9	1.9	15	**17.5**	0.25	0.4	3	**3.65**
95th (ng/g)	1.3	0.9	6.2	**7.45**	1.3	2.5	19	**22.2**	0.25	0.4	3.5	**4.05**
Max (ng/g)	2.1	1.5	13.8	**16.4**	1.6	2.8	38.5	**42.4**	0.25	0.4	3.5	**4.05**

Cherry (EBNTY008)								
	Nectar				Pollen			
Statistic	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total
N	65	65	65	**65**	53	53	53	**53**
Mean (ng/g)	0.9	0.5	1.5	**2.8**	5.1	5.2	108.2	**118.5**
SD (ng/g)	0.9	0.5	1.3	**2**	5.6	7.3	182	**193.5**
CV (%)	106.7	155.3	87.6	**70.2**	107.9	139.7	168.2	**163.2**
Min (ng/g)	0.35	0.3	0.15	**0.15**	0.25	0.15	0.9	**1.3**
Median (ng/g)	0.35	0.3	1.2	**2.05**	2.2	1.8	15.8	**24.2**
75th (ng/g)	0.9	0.3	2	**3.55**	8.9	7.1	131.7	**147.5**
90th (ng/g)	2.3	1.1	2.8	**5.05**	13.8	17.7	361.6	**393.8**
95th (ng/g)	2.8	1.2	3.7	**6.8**	16	21.9	523.5	**560.9**
Max (ng/g)	4.4	4	7.8	**10.5**	22.7	32.4	965.4	**1004.4**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Strawberry (EBNTL056-04)												
	Anther				Blossoms				Pollen			
Statistic	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total
N	14	14	14	**14**	14	14	14	**14**	14	14	14	**14**
Mean (ng/g)	20.7	6.1	66	**92.8**	18	5.5	139.3	**162.7**	15.1	7.5	67.9	**90.5**
SD (ng/g)	10.2	4.7	84.2	**97.1**	16.7	3.7	179.5	**197.6**	13.3	4.3	91.1	**108.4**
CV (%)	49	78.1	127.5	**104.6**	93	67.5	128.9	**121.4**	88	57.7	134.1	**119.7**
Min (ng/g)	7.6	2.5	2.5	**12.6**	2.5	2.5	2.5	**7.5**	5	5	5	**15**
Median (ng/g)	21	4	20.25	**47.4**	7.2	2.5	12.25	**21.95**	5	5	5	**15**
75th (ng/g)	26	8.8	160	**191.4**	33	8.7	340	**379.7**	25	10	150	**185**
90th (ng/g)	35	9.5	180	**224.5**	40	10	420	**465**	33	14	200	**247**
95th (ng/g)	43	19	240	**302**	46	12	470	**526**	42	17	260	**319**
Max (ng/g)	43	19	240	**302**	46	12	470	**526**	42	17	260	**319**

Cotton (EBNTY010)																
	Extrafloral Nectar				Floral Nectar				Pollen				Blossoms			
Statistic	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total	5-OH	Olefin	IMI	Total
N	192	192	192	**192**	211	211	211	**211**	212	212	212	**212**	215	215	215	**215**
Mean (ng/g)	0.5	0.4	4.9	**5.8**	0.5	0.5	7.9	**8.9**	0.3	0.2	3.4	**3.8**	1.4	1.3	6.6	**9.3**
SD (ng/g)	0.4	0.3	4.8	**5.1**	0.3	0.4	7.2	**7.6**	0.1	0.1	6.9	**7.1**	1.0	0.7	6.4	**7.9**
CV (%)	93.4	76.7	98.3	**88.8**	56.3	78.8	90.2	**85.7**	49.6	77.3	206.7	**186.0**	75.7	55.2	96.8	**84.7**
Min (ng/g)	0.4	0.3	0.2	**1.1**	0.4	0.3	1.1	**1.8**	0.3	0.2	0.3	**0.7**	1.0	1.0	0.9	**2.8**
Median (ng/g)	0.4	0.3	3.3	**4.0**	0.4	0.3	5.5	**6.2**	0.3	0.2	1.2	**1.6**	1.0	1.0	4.8	**7.0**
75th (ng/g)	0.4	0.3	6.5	**7.3**	0.4	0.3	10.4	**11.5**	0.3	0.2	2.8	**3.3**	1.0	1.0	8.8	**11.0**
90th (ng/g)	0.8	0.7	12.1	**13.3**	0.9	1.0	17.4	**18.4**	0.3	0.3	6.2	**6.6**	3.1	2.5	15.6	**20.0**
95th (ng/g)	1.3	1.1	14.8	**16.0**	1.1	1.3	25.2	**27.9**	0.3	0.5	16.4	**16.8**	4.0	2.9	20.5	**26.5**
Max (ng/g)	3.6	2.4	27.0	**30.3**	1.7	2.4	36.9	**39.4**	2.1	1.6	53.0	**56.7**	5.4	4.3	35.8	**45.1**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Cotton (EBNTN011)												
	Extrafloral Nectar				**Floral Nectar**				**Pollen**			
Statistic	**5-OH**	**Olefin**	**IMI**	**Total**	**5-OH**	**Olefin**	**IMI**	**Total**	**5-OH**	**Olefin**	**IMI**	**Total**
N	94	94	94	**94**	98	98	98	**98**	102	102	102	**102**
Mean (ug/kg)	6	1.3	205.5	**213**	1.3	1	43.3	**45.6**	5.6	0.8	102	**104.6**
SD (ug/kg)	11.8	2.2	433.7	**447.4**	1	0.8	28.2	**39.7**	6.3	2	343.9	**351.8**
CV (%)	195.8	166.7	210.9	**210**	78	78.9	88.3	**87**	329.9	253.1	337	**336.1**
Min (ug/kg)	0.35	0.3	0.15	**0.8**	0.35	0.3	0.3	**0.95**	0.25	0.15	0.2	**0.6**
Median (ug/kg)	1.6	0.7	30.2	**32.8**	1.05	0.8	35.55	**36.87**	0.25	0.15	11.55	**11.95**
75th (ug/kg)	5.9	1.3	186.2	**191.1**	1.8	1.4	62.4	**66**	1.1	0.5	63.7	**64.8**
90th (ug/kg)	14.4	2.7	565.3	**578.6**	3	1.9	103.2	**107**	2.9	1.9	175.9	**182.2**
95th (ug/kg)	34.3	5.7	1338.4	**1377.6**	3.5	2.8	125.6	**134**	4	3.1	319.9	**326.2**
Max (ug/kg)	77	17.5	2680	**2774.5**	4.7	4.5	164	**170.6**	44.4	15.5	2846.3	**2906.2**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

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Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Thiamethoxam:

Cotton (TK0177223) - Foliar									
	Nectar			**Pollen**			**Extra Floral Nectar**		
Statistic	**Thiamethoxam**	**CGA322704**	**Total**	**Thiamethoxam**	**CGA322704**	**Total**	**Thiamethoxam**	**CGA322704**	**Total**
N (#)	50	50	**50**	54	54	**54**	53	53	**53**
Mean (ng/g)	3.3	0.4	**3.7**	27.8	1.9	**29.8**	53.4	2	**55.4**
SD (ng/g)	6.5	0.3	**6.6**	58.7	3.3	**61.9**	50	2.3	**51.8**
CV (%)	201	69	**178**	211	170	**208**	94	112	**94**
Min (ng/g)	0.3	0.3	**0.8**	0.3	0.3	**0.5**	1.8	0.3	**2.1**
Median (ng/g)	1.7	0.3	**2.4**	3.8	0.3	**4.3**	41.1	1.4	**42.8**
75th (ng/g)	2.9	0.6	**3.4**	17	2.2	**18.5**	59.3	2.4	**61.9**
90th (ng/g)	5.3	0.8	**5.8**	96.4	6.1	**102.5**	123	3.8	**125.9**
95th (ng/g)	6.9	1	**7.3**	141	10.2	**151**	178	5	**186.4**
Max (ng/g)	46.2	1.5	**47**	351	15.4	**366.4**	228	14.3	**242.3**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Citrus (TK0177221)						
	Nectar			Pollen		
Statistic	**Thiamethoxam**	**CGA322704**	**Total**	**Thiamethoxam**	**CGA322704**	**Total**
N (#)	52	52	**52**	54	54	**54**
Mean (ng/g)	2.6	0.6	**3.2**	14.4	6.6	**21.1**
SD (ng/g)	5.1	1.2	**6.3**	22.3	12.2	**33**
CV (%)	201.5	192	**196.9**	154.4	185.3	**156.8**
Min (ng/g)	0.1	0.1	**0.3**	0.5	0.3	**1.4**
Median (ng/g)	0.3	0.1	**0.4**	3.6	1.3	**5.5**
75th (ng/g)	1.3	0.7	**2**	13.3	3.6	**23.1**
90th (ng/g)	8.7	1.4	**10.2**	51.1	21.2	**62.3**
95th (ng/g)	16.9	3.2	**21.6**	67.2	36	**107.8**
Max (ng/g)	22.5	7	**27.8**	104	61.5	**135.1**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Cucumber (TK0024668)						
	Nectar			Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	9	9	**9**	9	9	**9**
Mean (ng/g)	6.0	2.6	**8.6**	4.5	1.2	**5.7**
SD (ng/g)	3.8	1.8	**2.3**	2.4	0.8	**3.0**
CV (%)	62.5	71.8	**26.7**	52.2	66.9	**53.5**
Min (ng/g)	1.3	1.0	**6.3**	1.3	0.2	**1.5**
Median (ng/g)	7.5	1.6	**8.5**	4.1	0.9	**5.7**
75th (ng/g)	7.8	5.0	**9.4**	5.2	1.7	**5.9**
90th (ng/g)	11.5	5.0	**13.2**	8.2	2.6	**10.8**
95th (ng/g)	11.5	5.0	**13.2**	8.2	2.6	**10.8**
Max (ng/g)	11.5	5.0	**13.2**	8.2	2.6	**10.8**

Strawberry (TK0177224)						
	Nectar			Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	27	27	**27**	27	27	**27**
Mean (ng/g)	191.6	4.0	**195.6**	2023.0	25.0	**2048.0**
SD (ng/g)	113.6	2.6	**115.5**	2432.0	18.5	**2449.2**
CV (%)	59.3	64.8	**59.0**	120.0	74.2	**119.5**
Min (ng/g)	51.2	0.1	**52.1**	102.0	4.1	**107.9**
Median (ng/g)	177.5	4.0	**182.8**	861.0	19.9	**875.1**
75th (ng/g)	214.7	5.0	**215.3**	2486.0	36.6	**2522.7**
90th (ng/g)	296.2	5.4	**300.5**	7349.0	62.0	**7411.4**
95th (ng/g)	376.0	9.9	**381.2**	7445.0	62.3	**7492.6**
Max (ng/g)	647.3	12.8	**660.0**	7473.0	66.1	**7539.6**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Cranberry (TK0236307)						
	Nectar			Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	27	27	**27**	27	27	**27**
Mean (ng/g)	312.0	18.1	**330.1**	316.0	10.4	**326.4**
SD (ng/g)	527.2	31.5	**555.8**	564.2	16.6	**575.9**
CV (%)	168.9	174.2	**168.3**	178.5	159.6	**176.4**
Min (ng/g)	28.0	1.3	**30.1**	11.2	0.3	**11.5**
Median (ng/g)	112.1	5.5	**120.6**	49.1	3.4	**52.5**
75th (ng/g)	293.5	12.3	**301.5**	331.0	13.1	**353.6**
90th (ng/g)	834.9	87.0	**921.9**	1149.9	26.0	**1226.4**
95th (ng/g)	1580.0	92.8	**1698.8**	1564.6	44.2	**1608.8**
Max (ng/g)	2353.2	118.8	**2446.0**	2226.7	76.5	**2252.7**

Cucumber (TK0222532)						
	Nectar			Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	27	27	**27**	27	27	**27**
Mean (ng/g)	87.4	6.9	**94.3**	314.6	17.7	**332.3**
SD (ng/g)	88.5	7.9	**95.7**	355.1	18.4	**365.2**
CV (%)	101.2	113.5	**101.4**	112.8	103.7	**109.9**
Min (ng/g)	8.8	0.5	**9.3**	3.0	1.5	**4.6**
Median (ng/g)	51.4	3.2	**52.4**	173.0	12.9	**186.7**
75th (ng/g)	134.0	9.4	**144.9**	389.0	21.7	**436.5**
90th (ng/g)	263.0	22.0	**288.6**	1050.0	34.6	**1079.9**
95th (ng/g)	281.0	25.6	**303.0**	1060.0	47.5	**1142.7**
Max (ng/g)	317.0	25.8	**342.8**	1410.0	92.7	**1431.3**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Strawberry (TK0250068)						
	Nectar			Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	53	53	**53**	54	54	**54**
Mean (ng/g)	23.5	2.9	**26.4**	202.0	30.7	**202.0**
SD (ng/g)	37.0	4.7	**41.4**	364.1	32.2	**364.1**
CV (%)	157.2	162.5	**156.7**	180.2	105.2	**160.1**
Min (ng/g)	0.7	0.1	**0.9**	8.3	1.3	**12.7**
Median (ng/g)	10.0	0.9	**13.1**	57.0	19.0	**78.5**
75th (ng/g)	30.0	4.3	**33.8**	222.0	41.0	**261.0**
90th (ng/g)	48.0	6.1	**52.3**	491.0	73.0	**541.0**
95th (ng/g)	134.0	10.0	**145.0**	807.0	99.0	**848.0**
Max (ng/g)	188.0	25.0	**213.0**	1930.0	158.0	**1947.0**

Pepper (TK0236306)						
	Nectar			Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	15	15	**15**	17	17	**17**
Mean (ng/g)	52.5	78.7	**131.3**	30.7	110.5	**141.2**
SD (ng/g)	136.6	234.5	**370.8**	32.4	80.8	**92.1**
CV (%)	259.9	297.7	**282.4**	105.6	73.0	**65.1**
Min (ng/g)	3.0	0.6	**3.6**	1.1	8.5	**9.6**
Median (ng/g)	11.6	10.3	**19.9**	25.5	119.4	**168.5**
75th (ng/g)	29.0	29.4	**53.7**	38.6	173.0	**201.6**
90th (ng/g)	66.8	114.1	**180.9**	45.9	224.5	**259.9**
95th (ng/g)	542.1	920.4	**1462.4**	142.7	231.6	**274.3**
Max (ng/g)	542.1	920.4	**1462.4**	142.7	231.6	**274.3**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Tomato (TK0222531)						
	Pollen			Bee Collected Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	21	21	**21**	6	6	**6**
Mean (ng/g)	2292.6	423.0	**2715.6**	0.8	3.6	**4.5**
SD (ng/g)	3939.7	417.8	**4085.0**	0.4	1.5	**1.6**
CV (%)	171.8	98.7	**150.4**	43.0	40.7	**36.3**
Min (ng/g)	22.6	22.9	**45.6**	0.5	2.1	**2.6**
Median (ng/g)	651.4	308.8	**117.0**	0.8	3.3	**4.2**
75th (ng/g)	2311.6	558.2	**3152.6**	1.2	4.8	**5.3**
90th (ng/g)	6116.1	834.2	**6519.7**	1.2	6.0	**7.2**
95th (ng/g)	9151.5	135.2	**9637.3**	1.2	6.0	**7.2**
Max (ng/g)	15969.0	1524.9	**16803.2**	1.2	6.0	**7.2**

Apple (TK0250071)						
	Nectar			Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	44	36	**44**	43	43	**43**
Mean (ng/g)	95.5	2375.6	**113.1**	828.4	71.3	**899.7**
SD (ng/g)	149.4	2350.9	**153.5**	758.8	64.6	**785.0**
CV (%)	156.3	184.2	**135.7**	91.5	90.6	**87.2**
Min (ng/g)	0.0	0.1	**0.2**	2.8	1.9	**4.7**
Median (ng/g)	57.3	3.6	**79.0**	928.0	57.0	**964.8**
75th (ng/g)	89.2	9.9	**169.5**	1430.0	88.5	**1599.0**
90th (ng/g)	216.0	86.0	**225.4**	1880.0	190.0	**1954.7**
95th (ng/g)	354.0	96.0	**361.5**	2130.0	213.0	**2153.3**
Max (ng/g)	756.0	118.0	**769.0**	2410.0	222.0	**2471.3**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Corn (TK0258214) [Corn Seed + Foliar Treatment]			
	Pollen		
Statistic	Thiamethoxam	CGA322704	Total
N (#)	24	24	**24**
Mean (ng/g)	216.8	6.4	**223.2**
SD (ng/g)	249.6	3.6	**251.3**
CV (%)	115.1	56.2	**112.0**
Min (ng/g)	5.3	1.3	**6.6**
Median (ng/g)	84.6	5.5	**97.0**
75th (ng/g)	394.5	8.1	**401.6**
90th (ng/g)	528.0	12.5	**538.9**
95th (ng/g)	559.3	12.8	**565.6**
Max (ng/g)	993.6	13.0	**1006.6**

Muskmelon (TK0222530)						
	Nectar			Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	45	45	**45**	45	45	**45**
Mean (ng/g)	11.4	1.6	**13.0**	12.9	13.0	**38.2**
SD (ng/g)	12.2	1.1	**12.9**	36.7	12.9	**120.2**
CV (%)	106.9	69.7	**99.1**	284.0	408.5	**315.5**
Min (ng/g)	0.6	0.1	**1.3**	0.3	0.5	**0.8**
Median (ng/g)	8.4	1.3	**9.1**	3.4	1.4	**5.0**
75th (ng/g)	13.3	2.0	**14.5**	5.5	3.4	**15.1**
90th (ng/g)	25.0	3.5	**27.9**	11.2	26.9	**119.7**
95th (ng/g)	37.4	3.7	**39.5**	91.8	113.2	**157.6**
Max (ng/g)	61.5	5.1	**66.4**	192.9	676.0	**767.8**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Squash (TK0222530)						
	Nectar			Pollen		
Statistic	**Thiamethoxam**	**CGA322704**	**Total**	**Thiamethoxam**	**CGA322704**	**Total**
N (#)	34	34	**34**	34	34	**34**
Mean (ng/g)	8.9	1.0	**9.9**	5.4	1.5	**6.9**
SD (ng/g)	10.8	1.0	**11.5**	6.3	0.9	**6.9**
CV (%)	120.1	101.4	**115.1**	116.0	55.2	**99.4**
Min (ng/g)	0.3	0.1	**0.4**	0.3	0.3	**0.5**
Median (ng/g)	3.3	0.7	**4.3**	3.0	1.6	**4.9**
75th (ng/g)	15.1	1.4	**17.1**	7.1	2.0	**8.6**
90th (ng/g)	29.2	2.9	**31.7**	14.1	2.4	**16.1**
95th (ng/g)	31.9	3.2	**33.7**	22.0	3.4	**24.7**
Max (ng/g)	32.4	3.3	**33.8**	27.5	4.4	**31.9**

Pumpkin (TK0222530)						
	Nectar			Pollen		
Statistic	**Thiamethoxam**	**CGA322704**	**Total**	**Thiamethoxam**	**CGA322704**	Total
N (#)	44	44	**44**	45	45	**45**
Mean (ng/g)	3.0	0.7	**3.7**	3.3	2.6	**5.9**
SD (ng/g)	2.4	0.9	**3.1**	3.5	1.8	**4.5**
CV (%)	80.9	130.5	**84.0**	105.4	70.7	**76.4**
Min (ng/g)	0.3	0.1	**0.4**	0.3	0.3	**0.5**
Median (ng/g)	1.8	0.3	**2.1**	2.1	1.9	**4.4**
75th (ng/g)	4.3	0.8	**5.1**	4.7	3.1	**7.4**
90th (ng/g)	5.8	1.6	**8.1**	6.9	5.0	**12.2**
95th (ng/g)	8.3	2.8	**10.4**	10.6	5.9	**15.5**
Max (ng/g)	10.5	3.9	**13.8**	17.0	10.6	**22.7**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Pumpkin (TK0242074)						
	Nectar			Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	45	45	**45**	45	45	**45**
Mean (ng/g)	4.1	3.4	**7.5**	6.0	2.4	**8.4**
SD (ng/g)	5.2	3.5	**6.8**	8.4	1.5	**9.6**
CV (%)	124.3	104.3	**90.6**	140.7	61.8	**115.1**
Min (ng/g)	0.1	0.1	**1.0**	0.3	0.1	**1.0**
Median (ng/g)	2.3	2.2	**5.2**	2.4	2.4	**5.1**
75th (ng/g)	5.1	3.7	**9.8**	8.5	2.9	**10.9**
90th (ng/g)	8.6	10.1	**15.0**	14.7	3.7	**18.0**
95th (ng/g)	13.5	10.2	**21.7**	20.4	4.1	**24.5**
Max (ng/g)	24.9	12.6	**29.6**	45.4	7.4	**52.3**

Tomato (TK0242072)			
	Pollen		
Statistic	Thiamethoxam	CGA322704	Total
N (#)	27	27	**27**
Mean (ng/g)	32.5	49.9	**82.4**
SD (ng/g)	24.0	50.6	**69.2**
CV (%)	73.9	101.4	**83.9**
Min (ng/g)	3.8	15.2	**19.7**
Median (ng/g)	27.0	34.4	**57.9**
75th (ng/g)	53.5	47.7	**108.5**
90th (ng/g)	66.6	87.9	**157.2**
95th (ng/g)	73.0	187.5	**251.7**
Max (ng/g)	85.4	233.4	**318.8**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Blueberry (TK0250072)						
	Nectar			Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	27	27	**27**	27	27	**27**
Mean (ng/g)	89.9	106.2	**196.1**	247.6	58.0	**305.6**
SD (ng/g)	128.9	135.8	**247.2**	264.6	62.3	**319.2**
CV (%)	143.3	127.9	**126.1**	106.5	107.5	**104.4**
Min (ng/g)	1.4	3.4	**4.8**	2.6	0.5	**3.1**
Median (ng/g)	15.5	30.9	**51.6**	75.0	33.8	**124.4**
75th (ng/g)	186.0	184.0	**389.0**	462.0	92.8	**635.0**
90th (ng/g)	267.0	375.0	**613.0**	757.0	172.0	**836.4**
95th (ng/g)	431.0	381.0	**708.0**	779.0	173.0	**947.0**
Max (ng/g)	459.0	421.0	**713.0**	828.0	174.0	**987.0**

Sweet Orange (TK0250069)						
	Nectar			Pollen		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	54	54	**54**	54	54	**54**
Mean (ng/g)	0.6	0.6	**1.5**	37.2	32.5	**69.7**
SD (ng/g)	0.8	0.8	**2.5**	88.7	89.0	**175.7**
CV (%)	188.6	135.8	**166.9**	238.6	273.5	**251.9**
Min (ng/g)	0.1	0.1	**0.3**	2.2	0.5	**2.9**
Median (ng/g)	0.5	0.3	**0.9**	9.3	6.2	**18.2**
75th (ng/g)	0.9	0.7	**1.6**	14.5	21.8	**36.8**
90th (ng/g)	1.3	0.9	**2.1**	91.2	48.6	**126.7**
95th (ng/g)	4.8	2.6	**7.4**	276.0	289.0	**565.0**
Max (ng/g)	9.2	4.3	**13.4**	497.0	457.0	**948.0**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Soybean (TK0250070)						
	Nectar			Anther		
Statistic	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	27	27	**27**	35	35	**35**
Mean (ng/g)	0.5	1.2	**1.7**	13.5	3.0	**16.5**
SD (ng/g)	0.8	1.7	**1.9**	16.0	2.5	**18.3**
CV (%)	142.5	149.5	**115.5**	118.6	84.7	**110.9**
Min (ng/g)	0.1	0.1	**0.3**	0.5	0.3	**1.0**
Median (ng/g)	0.3	0.3	**0.9**	8.9	2.5	**11.3**
75th (ng/g)	0.3	1.5	**2.1**	16.5	4.6	**22.9**
90th (ng/g)	1.3	3.2	**4.7**	34.3	6.9	**41.2**
95th (ng/g)	2.2	4.5	**5.8**	51.1	7.4	**58.3**
Max (ng/g)	3.6	7.7	**8.0**	68.2	9.4	**77.6**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Stone Fruit (TK0177222)									
Statistic	Nectar			Pollen			Anthers		
	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total	Thiamethoxam	CGA322704	Total
N (#)	54	54	**54**	50	50	**50**	54	54	**54**
Mean (ng/g)	0.4	0.2	**0.6**	54.2	1.1	**55.3**	2.9	1.6	**4.5**
SD (ng/g)	0.5	0.3	**0.6**	75.5	1.2	**75.5**	12	2.9	**13.1**
CV (%)	135.7	117	**98.8**	139.3	104.7	**136.5**	420.4	175.2	**290.9**
Min (ng/g)	0.1	0.1	**0.3**	0.4	0.2	**0.5**	0.3	0.3	**0.5**
Median (ng/g)	0.2	0.1	**0.4**	29.6	0.9	**30.5**	0.3	0.3	**0.8**
75th (ng/g)	0.3	0.3	**0.5**	63.3	1.6	**63.6**	0.5	2.2	**3.5**
90th (ng/g)	1	0.5	**1.6**	132	2.4	**133.2**	4.4	4.4	**7.9**
95th (ng/g)	1.8	0.7	**2**	181.6	2.6	**182.1**	7.2	5.8	**11.7**
Max (ng/g)	2.4	1.7	**2.6**	382	5.9	**383**	87.5	15.8	**91.7**

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

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Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Clothianidin:

Potato (VP-38985)		
Statistic	Clothianidin	
	Pollen	Anthers
N	9	27
Mean (ng/g)	61.9	4.7
SD (ng/g)	25.9	5.3
CV (%)	41.8	111.6
Min (ng/g)	27.8	0.3
Median (ng/g)	61.0	2.9
75th (ng/g)	70.3	5.7
90th (ng/g)	113.9	15.1
95th (ng/g)	113.9	15.7
Max (ng/g)	113.9	21.2

Pumpkin (VP-38263)		
Statistic	Clothianidin	
	Nectar	Pollen
N	18	18
Mean (ng/g)	3	8.1
SD (ng/g)	2.4	6.3
CV (%)	78.3	77.4
Min (ng/g)	0.7	1.6
Median (ng/g)	2.4	6.8
75th (ng/g)	3.8	11.1
90th (ng/g)	6.3	17
95th (ng/g)	9.6	25.8
Max (ng/g)	9.6	25.8

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Pumpkin (VP-38971)		
Statistic	Clothianidin	
	Nectar	Pollen
N	38	45
Mean (ng/g)	3.8	7.0
SD (ng/g)	3.8	8.5
CV (%)	98.9	121.0
Min (ng/g)	0.2	0.6
Median (ng/g)	1.5	4.0
75th (ng/g)	7.4	7.9
90th (ng/g)	9.9	20.3
95th (ng/g)	10.6	31.3
Max (ng/g)	11.3	31.9

Cucumber (VP-38938)		
Statistic	Clothianidin	
	Nectar	Anthers
N	5	12
Mean (ng/g)	18.3	20.9
SD (ng/g)	14	8.3
CV (%)	76.7	39.4
Min (ng/g)	7.5	12.2
Median (ng/g)	10.7	18.7
75th (ng/g)	25.5	29.3
90th (ng/g)	39.6	32
95th (ng/g)	39.6	34.2
Max (ng/g)	39.6	34.2

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Melon (VP-38938)			
	Clothianidin		
Statistic	Nectar		Anthers
N	4		15
Mean (ng/g)	7.9		11.1
SD (ng/g)	4.6		4.6
CV (%)	58.5		41.7
Min (ng/g)	4.7		4.9
Median (ng/g)	6.0		10.7
75th (ng/g)	10.8		12.0
90th (ng/g)	14.6		18.7
95th (ng/g)	14.6		20.7
Max (ng/g)	14.6		20.7

Pumpkin (VP-38938)			
	Clothianidin		
Statistic	Nectar	Pollen	Anthers
N	15	15	12
Mean (ng/g)	3.1	9.5	5.2
SD (ng/g)	2	10.1	3.3
CV (%)	65	106.3	63.6
Min (ng/g)	0.8	2	1.8
Median (ng/g)	2.1	4.8	3.8
75th (ng/g)	4.3	11.8	7.1
90th (ng/g)	6.6	21	8.9
95th (ng/g)	7.2	40.1	13.2
Max (ng/g)	7.2	40.1	13.2

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Pumpkin (VP-38313)		
	Clothianidin	
Statistic	Nectar	Pollen
N	20	20
Mean (ng/g)	2.5	27.1
SD (ng/g)	1.7	33.1
CV (%)	68.4	122.4
Min (ng/g)	0.7	1.6
Median (ng/g)	14.8	17
75th (ng/g)	4	70.4
90th (ng/g)	5	71
95th (ng/g)	6	107.9
Max (ng/g)	6.5	123

Squash (VP-38938)			
	Clothianidin		
Statistic	Nectar	Pollen	Anthers
N	15	15	12
Mean (ng/g)	2.8	6.3	5.3
SD (ng/g)	1.1	3.5	2.0
CV (%)	39.3	56.1	38.7
Min (ng/g)	1.4	2.3	2.4
Median (ng/g)	2.6	5.6	5.5
75th (ng/g)	4.0	8.0	6.7
90th (ng/g)	4.4	10.7	7.2
95th (ng/g)	4.5	14.7	8.7
Max (ng/g)	4.5	14.7	8.7

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Peach (VP-38563)		
	Clothianidin	
Statistic	Nectar	Pollen
N	18	17
Mean (ng/g)	0.1	10.6
SD (ng/g)	0.1	30.9
CV (%)	48.5	292.5
Min (ng/g)	0.1	0.3
Median (ng/g)	0.1	2.0
75th (ng/g)	0.1	5.3
90th (ng/g)	0.3	10.0
95th (ng/g)	0.3	130.1
Max (ng/g)	0.3	130.1

Grape Soil (VP-38992)	
	Clothianidin
Statistic	Pollen
N	24
Mean (ng/g)	55.4
SD (ng/g)	58.3
CV (%)	105.3
Min (ng/g)	3.7
Median (ng/g)	28.3
75th (ng/g)	86.9
90th (ng/g)	157.3
95th (ng/g)	168
Max (ng/g)	205.9

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Almond (VP-38473)			
	Clothianidin		
Statistic	Nectar	Pollen	Anthers
N	54	41	12
Mean (ng/g)	0.3	5.62	16.8
SD (ng/g)	0.4	4.7	24.4
CV (%)	123	83.6	145
Min (ng/g)	0.1	0.55	0.35
Median (ng/g)	0.1	4.32	11.62
75th (ng/g)	0.37	8.26	21.15
90th (ng/g)	0.84	12.7	27
95th (ng/g)	1.15	13.8	88.1
Max (ng/g)	2.04	20	88.1

Grape Foliar (VP-38992)	
	Clothianidin
Statistic	Pollen
N	24
Mean (ng/g)	632.7
SD (ng/g)	382.2
CV (%)	60.4
Min (ng/g)	116.9
Median (ng/g)	540.9
75th (ng/g)	886
90th (ng/g)	1229.8
95th (ng/g)	1246.8
Max (ng/g)	1563.9

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Apple (VP-38552)		
	Clothianidin	
Statistic	Nectar	Pollen
N	9	9
Mean (ng/g)	0.4	11.7
SD (ng/g)	0.3	18.7
CV (%)	72.3	159.2
Min (ng/g)	0.1	0.1
Median (ng/g)	0.4	3
75th (ng/g)	0.6	18.1
90th (ng/g)	0.7	57.4
95th (ng/g)	0.7	57.4
Max (ng/g)	0.7	57.4

Cotton (VP-38259)			
	Clothianidin		
Statistic	Nectar		Extrafloral Nectar
N	27		27
Mean (ng/g)	29.5		448.5
SD (ng/g)	43.2		946.9
CV (%)	146.3		211.1
Min (ng/g)	0.2		9.9
Median (ng/g)	17.4		104.0
75th (ng/g)	32.0		213.0
90th (ng/g)	79.4		1692.0
95th (ng/g)	142.0		2624.0
Max (ng/g)	182.0		4163.0

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

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Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Dinotefuran:

Cotton (43411B104)			
	Dinotefuran		
Statistic	Floral Nectar	Pollen	Extrafloral Nectar
N	53	54	90
Mean (ng/g)	69.6	1979.3	741.49
SD (ng/g)	99.7	4482.1	2102.3
CV (%)	143.3	226.4	283.5
Min (ng/g)	2.51	13.8	0.5
Median (ng/g)	22.5	233	108
75th (ng/g)	81	686	545
90th (ng/g)	81.6	6968	1660
95th (ng/g)	321	14757	2880
Max (ng/g)	346	20676	15500

Pumpkin (10934.4104)		
	Dinotefuran	
Statistic	Floral Nectar	Pollen
N	27	27
Mean (ng/g)	17.9	32.1
SD (ng/g)	17.6	28.5
CV (%)	98.2	88.6
Min (ng/g)	0.5	4.8
Median (ng/g)	15.7	17.7
75th (ng/g)	21.2	48.9
90th (ng/g)	39	88.3
95th (ng/g)	50.8	92.9
Max (ng/g)	84.4	105.3

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Tomato Foliar (10934.4103)	
	Dinotefuran
Statistic	Pollen
N	24
Mean (ng/g)	2421
SD (ng/g)	5477
CV (%)	226
Min (ng/g)	5
Median (ng/g)	58
75th (ng/g)	1260
90th (ng/g)	10439
95th (ng/g)	12210
Max (ng/g)	22839

Tomato Soil (10934.4103)	
	Dinotefuran
Statistic	Pollen
N	24
Mean (ng/g)	1307
SD (ng/g)	2667
CV (%)	204
Min (ng/g)	4
Median (ng/g)	33
75th (ng/g)	652
90th (ng/g)	5532
95th (ng/g)	7208
Max (ng/g)	9813

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Cranberry (10934.4101)		
	Dinotefuran	
Statistic	Floral Nectar	Pollen
N	27	27
Mean (ng/g)	340.8	370
SD (ng/g)	275.6	323.3
CV (%)	80.8	387.3
Min (ng/g)	69.3	37.1
Median (ng/g)	220.3	256.5
75th (ng/g)	438.5	581.7
90th (ng/g)	780.9	763.5
95th (ng/g)	1010.7	1247.6
Max (ng/g)	1159.2	1268.7

Potato (10934.4100)	
	Dinotefuran
Statistic	Pollen
N	27
Mean (ng/g)	22.9
SD (ng/g)	27.3
CV (%)	119.3
Min (ng/g)	0.5
Median (ng/g)	9.3
75th (ng/g)	42.2
90th (ng/g)	56.9
95th (ng/g)	78.3
Max (ng/g)	103.6

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Cherry Foliar (10934.4105)		
	Dinotefuran	
Statistic	Floral Nectar	Pollen
N	27	26
Mean (ng/g)	8.4	49.8
SD (ng/g)	4.6	51.5
CV (%)	54.7	103.5
Min (ng/g)	1.2	5.6
Median (ng/g)	7.2	28.7
75th (ng/g)	10.4	95.8
90th (ng/g)	12.5	130.5
95th (ng/g)	15.7	153.7
Max (ng/g)	25.5	171.7

Cherry Trunk (10934.4105)		
	Dinotefuran	
Statistic	Floral Nectar	Pollen
N	27	27
Mean (ng/g)	5403.3	9321.9
SD (ng/g)	4968	8500.3
CV (%)	91.9	91.1
Min (ng/g)	118.4	368.3
Median (ng/g)	5233.8	8699
75th (ng/g)	8456.5	13626
90th (ng/g)	12090.3	21822
95th (ng/g)	16604.5	23697.6
Max (ng/g)	17483.8	31688.5

Appendix 11. Descriptive Statistics Derived from the Residue Studies Included in this Risk Determination Document

Blueberry (10934.4107)		
	Dinotefuran	
Statistic	Floral Nectar	Pollen
N	27	27
Mean (ng/g)	231.5	183.2
SD (ng/g)	163.9	188.4
CV (%)	70.7	102.8
Min (ng/g)	30	24.7
Median (ng/g)	198.9	110.1
75th (ng/g)	395.5	233.3
90th (ng/g)	470.8	468.9
95th (ng/g)	484.6	581.7
Max (ng/g)	484.7	770.6

Bell Pepper (S16-01167)			
	Dinotefuran		
Statistic	Floral Nectar	Pollen	Anthers
N	26	24	15
Mean (ng/g)	1.8	59.4	93.4
SD (ng/g)	1.7	87.5	93.8
CV (%)	95.2	147.2	100.4
Min (ng/g)	0.1	5.6	17.1
Median (ng/g)	1.3	24	49.9
75th (ng/g)	2.7	67.9	141
90th (ng/g)	4.5	183	238
95th (ng/g)	4.8	212	344
Max (ng/g)	6.5	387	344

Department of Pesticide Regulation

Mary-Ann Warmerdam
Director

Arnold Schwarzenegger
Governor

<Contact Name>
<Company Name>
<Address>
<City, State Zip>

Dear <Salutation>:

Pursuant to Title 3, California Code of Regulations, section 6220, et seq., the Director of the Department of Pesticide Regulation (DPR) notices her decision to initiate a reevaluation of certain pesticide products within the nitroguanidine insecticide class of neonicotinoids containing the following active ingredients: imidacloprid, clothianidin, dinotefuran, and thiamethoxam, including the following product(s):

Product Brand Name, EPA Reg. No.	Active Ingredient
<Product Name, EPA Reg. No. 999-88-AA>	Imidacloprid

DPR is required to investigate all reported pesticide episodes and information received indicating that a pesticide may have caused, or is likely to cause, a significant adverse impact. If the Director finds from the investigation that a significant adverse effect has occurred or is likely to occur; the pesticide involved shall be reevaluated. Therefore, certain products within the nitroguanidine insecticide class of neonicotinoids, including the above product(s), are being reevaluated.

BASIS FOR REEVALUATION

In 2008, DPR received an adverse effects disclosure pursuant to Federal Insecticide Fungicide and Rodenticide Act (FIFRA) section 6(a)(2) and Food and Agricultural Code section 12825.5 regarding the active ingredient imidacloprid. The disclosure included twelve residue and two combination residue, honey, bumble bee studies of imidacloprid use on a number of ornamental plants. DPR's evaluation of the data noted two critical findings. One, high levels of imidacloprid in leaves and blossoms of treated plants, and two, increases in residue levels over time.

Imidacloprid levels in leaves and blossoms varied depending on the application rate and the type of plant, but the data indicate that residues in some plants measured higher than 4 parts per million (ppm). The data also indicate that when using soil application methods, imidacloprid residues remained relatively low for the first six months after application, followed by a dramatic increase that remained stable in some cases for more than 500 days after treatment. Where imidacloprid was applied to the soil, no significant decline in residue levels was observed in any of the studies, even in studies where residues were tested at 540 days after treatment. DPR found

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that the treatment rates used in the studies where high imidacloprid residue levels were found in leaves and blossoms, were comparable to application rates found on currently registered labels for orchards, assuming the orchards were planted at a density of 200 trees per acre or fewer. The data indicate that use of imidacloprid on an annual basis may be additive, in that significant residues from the previous use season appear to be available to the treated plant. DPR also received preliminary information from a University of California at Riverside researcher who is investigating imidacloprid residues in eucalyptus nectar and pollen. The researcher's preliminary results indicate imidacloprid residues in eucalyptus nectar at levels of up to 550 parts per billion (ppb).

Based upon data on file, DPR estimates the lethal concentration of imidacloprid needed to kill 50 percent of a test population (LC_{50}) of honey bees is 185 ppb[1]. In their everyday foraging and pollination activities, honey bees collect both nectar and pollen from flowering plants. If the imidacloprid residue levels in a plant's nectar and pollen are similar to those found in the leaves and blossoms of the plants described in the adverse effects data, the levels are well above the estimated LC_{50} for honey bees. The levels found in some of the plants were more than twenty times the estimated honey bee LC_{50} of 185 ppb.

All of the neonicotinoids share many of the same characteristics as imidacloprid. However, the three other neonicotinoids included in this reevaluation, clothianidin, dinotefuran, and thiamethoxam, are in the same chemical family (nitroguanidines) as imidacloprid. These three other active ingredients, in particular, have soil mobility characteristics and half-lives that are very similar to imidacloprid. Based on available data, DPR scientists believe these active ingredients would have the same potential residue concerns as imidacloprid. Data also indicate that these active ingredients are similar to imidacloprid in toxicity to honey bees. Due to the chemical and toxicological similarities between imidacloprid and the other neonicotinoids, DPR is providing those registrants with the option of generating data on their own chemicals or providing/relying upon data generated using a surrogate nitroguanidine.

DPR exempted the following formulation categories and product types from the reevaluation:

1. Formulated as a gel or impregnated in a strip;
2. Termiticide;
3. Flea control products combined with rodenticide;
4. Pet spot applications;
5. Ant and roach baits;
6. Premise application for control of nuisance pests; or,
7. Manufacturing use only products.

[1] The LC_{50} was estimated by converting the acute oral LD_{50} (the amount of a material that causes the death of 50 percent of a test population) to a concentration in nectar using the standard consumption model used in bee feeding studies.

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DPR exempted the above types of products from the reevaluation because the manner in which the products are formulated or applied makes it unlikely that the neonicotinoid will move into plants that bloom or be a source of forage for honey bees and pollinators.

DPR plans to work closely with the United States Environmental Protection Agency's (U.S. EPA's) Office of Pesticide Programs throughout the reevaluation process. U.S. EPA's Registration Review docket for imidacloprid <http://www.epa.gov/oppsrrd1/registration_review/imidacloprid/index.htm> opened in December 17, 2008, and the dockets for other neonicotinoids were previously scheduled to open between 2013 and 2015. In order to better ensure a "level playing field" for the neonicotinoid class as a whole, and to best take advantage of new research as it becomes available, U.S. EPA moved the docket openings for the remaining neonicotinoids on the registration review schedule (acetamiprid, clothianidin, dinotefuran, nitrapyrin, thiacloprid and thiamethoxam) to fiscal year 2012.

DATA REQUIREMENTS

DPR will inform you of the data required pursuant to this reevaluation in a separate letter. The data requirements will be finalized and announced after the April 17, 2009 deadline listed below.

MEET WITH DPR STAFF

DPR has scheduled a meeting with registrants to discuss the neonicotinoid reevaluation data requirements at the time and place noted below. Enclosed are directions for traveling to the Cal/EPA Headquarters building and a proposed agenda.

DATE:	April 1, 2009
TIME:	10:00 a.m.
PLACE:	California Environmental Protection Agency (Cal/EPA Headquarters) Coastal Hearing Room, 2nd Floor 1001 I Street Sacramento, CA 95814

As part of the reevaluation process, DPR intends to require field-based data on neonicotinoids in order to better understand their impact on honey bees. DPR plans to require registrants to analyze residues from nectar and pollen of a representative number of crops grown in California. In addition, DPR plans to require acute [laboratory] toxicity studies on various honey bee life stages. Attachment 1 contains the details of DPR's data requirement proposal.

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DPR will consider written comments on the proposed data requirements received no later than the close of business April 17, 2008. Please address all correspondence regarding this reevaluation as follows:

> Neonicotinoid Reevaluation
> Attn: Denise Webster
> Department of Pesticide Regulation
> 1001 I Street, P.O. Box 4015
> Sacramento, California 95812-4015

<u>CONTACTS</u>

For information regarding the reevaluation process, please contact either Ms. Denise Webster, by e-mail at <dwebster@cdpr.ca.gov> or by telephone at (916) 324-3522, or Ms. Alveena Prasad, by e-mail at <aprasad@cdpr.ca.gov> or by telephone at (916) 324-3905.

Sincerely,

Original signed by — *February 26, 2009*
Ann M. Prichard, Chief — Date
Pesticide Registration Branch
(916) 324-3931

Enclosures

cc: Ms. Denise Webster, Program Specialist
Ms. Alveena Prasad, Environmental Scientist
<Regulatory Scientist>