MEMORANDUM

TO: Jennifer Teerlink, PhD,
Assistant Director and Deputy Science Advisor

FROM: Shelley DuTeaux, PhD MPH, Chief
Human Health Assessment Branch
On behalf of the Fipronil Risk Assessment Project Team: Leona D. Scanlan, PhD, (former member), Svetlana E. Koshlukova, PhD, Andrew L. Rubin, PhD DABT, Anna Kalashnikova, PhD, Puttappa Dodmane, PhD, Stephen Rinkus, PhD, Peter N. Lohstroh, PhD, Qiaoxiang Dong, PhD, Brendan Darsie, MPH, Shelley DuTeaux, PhD MPH

DATE: January 17, 2023

SUBJECT: Response to Comments by the Office of Environmental Health Hazard Assessment on DPR's 2021 Draft Risk Characterization Document for Fipronil

Background

At the request of the Department of Pesticide Regulation (DPR), the Office of Environmental Health Hazard Assessment (OEHHA) reviewed the January 2021 Draft Risk Characterization Document (RCD) for Fipronil. OEHHA was asked to respond to a series of charge questions covering the hazard identification, exposure assessment, risk characterization, and worker and home user margins of exposure, and provided comments to DPR on May 13, 2021.

This memorandum summarizes DPR's responses to OEHHA's comments on the draft RCD in an itemized fashion and is divided into the following sections: Detailed Comments; Response to Charge Statements; and Other Comments. Corresponding revisions were also made to the final RCD and its appendices as appropriate. Responses specific to the exposure assessment (other than dietary exposure assessment) are detailed in a separate memorandum.

Note that references cited in this memorandum are specific to OEHHA comments or DPR's response, and not necessarily duplications of those in the draft or final RCD. Likewise, every effort has been made to ensure that any references to tables found in the draft or final RCD are clear. Tables specific to this memorandum are numbered independently of the RCD. All OEHHA comments in this memorandum are direct quotes from the documents, which can be found at https://oehha.ca.gov/media/downloads/pesticides/document/fipronilcomments051321.pdf.

OEHHA Detailed Comments – Toxicity Evaluation and Risk Assessment

1. Non-cancer Toxicity Evaluation and Point of Departure Determination
a. Pharmacokinetics
OEHHA Comment: The absorption, distribution, metabolism, and excretion of fipronil are adequately addressed in the draft RCD …

DPR Response: Comments on this point are noted.

b. General Approaches
OEHHA Comment: The draft RCD derives critical toxicity endpoints for only the parent compound fipronil; OEHHA agrees that PODs based on the parent compound will be protective of the major metabolites as well. There is also limited information regarding the toxicity of fipronil through the dermal and inhalation routes, but the available data suggest these routes are not more toxic than the oral route. For this reason, OEHHA agrees with the use of the oral PODs to assess inhalation and dermal exposure pathways.

DPR Response: Comments on this point are noted.

c. Acute Toxicity
OEHHA Comment: The draft RCD selected the acute neurotoxicity study reported by Hughes (1997) as the critical study and the decreased hindlimb splay reported in male rats 7 hours post-dosing via oral gavage as the critical endpoint. The study NOAEL was 2.5 mg/kg-day with a Lowest-Observed-Adverse-Effect Level (LOAEL) of 7.5 mg/kg-day. The NOAEL was also based on decreased weight gain and food consumption in females during week 1 following treatment. DPR used Benchmark Dose (BMD) modeling with a 10% benchmark response (BMDL10) and derived a critical acute POD of 0.87 mg/kg-day. The critical acute POD is higher than other potential acute PODs discussed in the draft, but DPR's rationale for chosing [*sic*] the study over others included less uncertainty in the dose range between the NOAEL and LOAEL, the relevance of the critical effect to human health, and uncertainties in the other studies that limited their utility for acute POD derivation. OEHHA concurs with DPR's use of BMD modeling for this dataset, as BMD modeling can overcome some of the limitations of the NOAEL/LOAEL approach, and male rats exhibited a dose-dependent but non-statistically significant decrease in hindlimb splay at the study NOAEL. The draft RCD cites more confidence in this study over other potential PODs in large part due to it being amenable to BMD modeling. However, as discussed in the following paragraphs, OEHHA does not agree with the acute POD selected …

An acute neurotoxicity study in rats (Gill *et al.*, 1993) with a similar study design showed a lower NOAEL of 0.5 mg/kg-day for the same endpoint. The draft RCD cited the 10-fold difference between the NOAEL and LOAEL as a source of uncertainty and reasoning for not using this lower value to derive an acute POD. However, it should be noted that there are

uncertainties in the study design and NOAELs in both the Hughes (1997) and Gill (1993) studies because they might not have captured the peak effects of the treatment. Based on the Time Of Peak Effects (TOPE) probe study conducted by Gill (1993), at 50 and 80 mg/kg, neurotoxic effects were observed as early as 2 hours post dosing, with convulsions and tremors readily apparent at 4-5 hours post dosing. In a similar TOPE study conducted by Hughes (1997), at 25 mg/kg, neurotoxic effects were seen at 4 hours post dosing. It is possible that for both studies, neurotoxicity testing using a functional observation battery (FOB) at 7 hours post-dosing might have missed the most severe effects …

DPR Response: DPR did not use the Gill (1993) study to establish a critical acute POD for the following reasons:

1. Because of the study design dose selection, the no observed effect level (NOEL) of 0.5 mg/kg-day was 10-fold lower than the lowest observed effect level (LOEL), which was based on neurobehavioral signs (decreased hindlimb splay, rearing and approach response). Therefore, the true NOEL could be closer to the LOEL of 5 mg/kg/day. BMD modeling is preferred over the traditional NOEL/LOEL approach to establishing points of departure (PODs), especially when a large gap exists between the NOEL and LOEL. However, a statistically acceptable Benchmark Dose (BMD) model could not be identified for hindlimb splay data from the Gill study.

2. The dose gap in Gill (1993) triggered the second acute neurotoxicity study by Hughes (1997). This study featured more closely spaced doses and confirmed that hindlimb splay was the most sensitive endpoint. While non-significant variations in hindlimb splay were observed at the NOEL in both studies (0.5 mg/kg in Gill, 2.5 mg/kg in Hughes), modeling the Hughes dataset incorporated the effects at the study NOEL and generated a statistically acceptable BMDL.

3. The experimentally determined time to peak effect (TOPE) of 7 hours from both studies was based on the highest incidence of neurobehavioral changes (convulsions, chewing, licking and wet anogenital region). The Appraisal section of the draft RCD detailed the uncertainty regarding TOPE for hindlimb splay, specifically that the TOPE may have occurred at a different time than that for convulsions. Nevertheless, DPR selected Hughes (1997) as the most reliable study for the critical acute POD among the available studies with acute and short-term effects.

OEHHA Comment, continued: In a chronic oral study, Aughton (1993) observed significantly decreased thyroid hormone thyroxine (T4) levels after one week of fipronil exposure at 0.06 mg/kg-day in male rats and 1.6 mg/kg-day in female rats, with NOAELs of 0.02 mg/kg-day and 0.08 mg/kg-day for males and females, respectively. In addition to lower T4 levels, convulsions were observed in three males in the 0.06 mg/kg-day dose group during the first few weeks of treatment. There was discussion in the draft RCD surrounding the decision not to use this dataset to derive an acute POD, which includes the NOAEL being higher in females, and the reasoning that short-term changes in thyroid hormone levels are not likely deleterious to adults. OEHHA

disagrees with these statements. Placental transfer of maternal thyroid hormones is critical in early embryonic development and up until maturation of the fetal thyroid gland. A decrease in maternal serum T4 even for a short period can have detrimental effect on the neurodevelopment of the fetus (OEHHA, 2015; Miranda and Sousa, 2018). If decreased serum T4 were selected as the critical endpoint, an acute oral POD of 0.02 mg/kg-day or 0.08 mg/kg-day could be determined.

DPR Response: Convulsions were observed in the Aughton (1993) study in males at 0.06 mg/kg/day during weeks 23, 61 and 69. DPR considers these exposure durations to be subchronic. Other studies indicate that convulsions typically occur above 1 mg/kg/day following acute exposures (2–3 mg/kg/day in dogs following oral administration, 4.8 mg/kg/day in rats following inhalation administration, and 50 mg/kg/day in rats following oral administration) (Holmes, 1992; Gill *et al.*, 1993; Holmes, 1993; Adamo-Trigiani, 1999). In the acute oral neurotoxicity study by Gill, hindlimb splay was a more sensitive endpoint than convulsions (POD of 0.5 mg/kg/day for splay compared to 50 mg/kg/day for convulsions). Doses up to 25 mg/kg/day did not result in convulsions in the Hughes study (Gill *et al.*, 1993; Hughes, 1997). Acute neurotoxic effects have also been also observed in rodents at higher dose levels (5-25 mg/kg/day) (Freeborn *et al.*, 2015; Maeda *et al.*, 2021).

The thyroxine (T4) reductions observed in male and female CD rats after one week of exposure (NOEL/LOEL = 0.02/0.06 and 0.08/1.6 mg/kg-day, respectively) were not selected as the critical effect (Aughton, 1993). The 20-fold gap between the observed NOEL and LOEL, however, leaves the possibility that the true NOEL could have been higher in female rats than what was documented in the study. Reduced T4 levels in pregnant females, even for short periods of time, may result in developmental effects in the unborn child. However, thyroid dependent developmental toxicity is less likely in humans because of the presence of thyroid binding globulin which buffers fluctuations in circulating T4 levels.

OEHHA Comment, continued: The importance of protecting the fetus and developing neonatal brain is highlighted by a comparative thyroid assay (CTA) in pregnant rats and their offspring reported by Coder (2019). The study showed dosing at the LOAEL of 1 mg/kg-day fipronil in pregnant female rats had a non-significant effect on T4 in the dams at gestational day 20 yet caused a statistically significant 19% reduction in T4 in their fetuses at the same time point. The NOAEL for T4 effects in the fetus equated to a maternal dose of 0.3 mg/kg-day. This shows the rat fetus is more susceptible to thyroid hormone disruption caused by fipronil than the dam, and there is a potential hazard to the fetus at the LOAEL of 1 mg/kg-day which is close to the acute POD of 0.87 mg/kg-day.

The draft RCD cites uncertainty in the thyroid hormone measurements from this study as a basis for not considering it for POD selection, based on an ion ratio analysis requested by US EPA. However, the toxicological significance of the findings of this study are supported by statements in the draft RCD, stating that the changes in measured thyroid hormone levels following

treatment with fipronil in the CTA study were consistent with effects measured in similar dose groups in other animal toxicity studies, that many of the failed samples were just outside of the tolerable range for the ion ratio analysis, and the accompanying effects on thyroid weight and histopathology at higher doses in the study suggested that changes in thyroid hormone levels were representative of potential physiological or pathological change. While the draft RCD used the ion ration analysis to "preclude the use of the acute results from the CTA to derive a quantitative acute POD," this approach is inconsistent with US EPA who selected NOAELs for thyroid hormone disruption from Coder (2019) for both maternal (0.3 mg/kg-day) and offspring (1 mg/kg-day) as critical PODs for short and intermediate term assessments, depending on the population being assessed.

DPR Response: T4 levels in GD 20 fetuses as a percentage of controls were 100%, 84%, 92%, 81%*, and 70%** at 0, 0.1, 0.3, 1 and 3 mg/kg-day, respectively (Coder, 2019). Comparable T4 levels in GD 20 dams were 100%, 89%, 111%, 91% and 72%** (*, **$p < 0.05$, 0.01 respectively). While DPR assigned a lower fetal than maternal LOEL (as reflected by the statistically significant 19% T4 reduction at 1 mg/kg-day), the data did not clearly demonstrate increased fetal T4 sensitivity to fipronil.

With respect to the ion ratio determinations, DPR concluded that the T4 findings in the comparative thyroid assay were not reliable for quantitative use. As discussed in the draft RCD, 286 of 855 T4 measurements (33%) were outside the ion ratio tolerance established by the authors, with the number of failed measurements in different exposure groups ranging from 0 to 88.2%. Nonetheless, the changes in thyroid weight and histopathology in dams and pups at 1 and 3 mg/kg/day were likely due to disrupted thyroid homeostasis. All considered, the comparative thyroid assay data can be evidence of a general sensitivity in the 0.3 – 3 mg/kg-day range. It is worth noting that the US Environmental Protection Agency (US EPA) set maternal and fetal NOAELs at 0.3 and 1 mg/kg-day, respectively, which is opposite of DPR's designations. US EPA did not consider increased fetal susceptibility as an effect of fipronil exposure. In fact, US EPA also reduced the toxicodynamic portion of the default intraspecies uncertainty factor from 10 to 3, citing unique sensitivity in adult rats to thyroid hormone perturbations compared to juvenile rats and humans (US EPA, 2020).

Because of the similarity of the Coder (2019) fetal NOEL of 0.3 mg/kg-day and DPR's critical acute BMDL value derived from Hughes (1997), DPR's values combined with the appropriate intrahuman uncertainty factors would protect fetuses from any temporal fetal T4 reductions.

OEHHA Comment, continued: A teratology study in rabbits showed a decrease in maternal body weight gain within two days of treatment, with a NOAEL of 0.1 mg/kg-day (King, 1990). While the effect at 0.1 mg/kg-day was not statistically significant, it still represented a 33% reduction in body weight gain at that dose. The higher doses, at 0.2 mg/kg-day, 0.5 mg/kg-day, and 1.0 mg/kg-day, all caused statistically significant reductions in body weight gain over the

first 2 days of fipronil treatment. Maternal T4 was not measured in this study. While no teratogenicity from fipronil exposure was observed in the fetuses in this study, decrements in body weight gain in the pregnant dam suggest pregnancy may be an especially susceptible life stage to fipronil toxicity. Furthermore, severe effects on maternal body weight during pregnancy could lead to adverse developmental or neurodevelopmental effects of offspring.

DPR Response: DPR ultimately concluded that the bodyweight data from the King (1990) rabbit developmental toxicity study was unreliable for critical endpoint determination. First, deficits in maternal bodyweight gain during gestation, while consistently significant by pairwise comparison did not show a clear dose dependence, particularly at 0.1 and 0.2 mg/kg-day. In addition, bodyweight gains in any dose group did not exceed 10% of the total bodyweight of the does over the entire exposure period (gestation day (GD) 6–20). Control animals weighed 3.95 grams on GD 6 and 4.25 grams on GD 20 (a 7.6% increase). The equivalent values at the high dose were 3.89 grams and 3.98 grams, respectively (a 2.3% increase). The toxicological significance of such small differences is unclear. The gestational bodyweight gain deficits were likely due to changes in food consumption over the exposure period. This was particularly evident at the two highest doses, where the deficits gained pairwise significance over GD 13–19 (see Table 20 in the draft RCD). Nonetheless, the toxicological significance of slight effects on bodyweight gain associated with reduced food consumption is unclear, especially since no developmental effects were noted at any dose level in this study.

OEHHA Comment, continued: …OEHHA suggests including the results from the developmental neurotoxicity (DNT) study in rats (Mandella, 1995) when considering the health protectiveness of the acute oral POD. The study derived a NOAEL for developmental neurotoxicity of 0.05 mg/kg-day and a LOAEL of 0.9 mg/kg-day. Even though a repeated exposure protocol was used in the study, we cannot be certain that the developmental neurotoxicity observed in the offspring was not caused by a single or short-term exposure on a sensitive day (whichever day that may have been during the prenatal or lactation periods) for the observed outcomes. Because the acute oral POD of 0.87 mg/kg-day is so close to the LOAEL of 0.9 mg/kg-day, there is a concern that the POD is not sufficiently health protective …

DPR Response: The developmental NOEL/LOEL of 0.05/0.9 mg/kg-day in the Mandella (1995) study were based on decreased pup bodyweights, delayed preputial separation, and decreased maximum startle response. These effects are likely indicative of developmental toxicity, especially since they occurred at doses lower than the maternal toxicity endpoints (maternal NOEL/LOEL = 0.9/15 mg/kg-day). However, due to the extended exposure period of 25 days (GD 6 through lactation day 10), these were neither acute nor short-term in nature.

The delayed preputial separation was likely related to the reduced body weights of the male pups, and thus required repeated exposures (see page 117 of the draft RCD). Decreased maximum startle response may also be a function of fetal growth status, although this is less

supported. BMD analysis of the pup bodyweight data generated a $BMDL_{05}$ of 0.334 mg/kg/day, a value closer to the LOEL of 0.9 mg/kg-day and much higher than the study NOEL of 0.05 mg/kg-day. Any pup effect dependent on bodyweight decrements would likely require repeated exposures. DPR attempted to calculate BMDL values for other endpoints in this study. The startle response and preputial separation did not result in reliable models, and female pup brain weights on post-natal day (PND) 11 resulted in a BMDL similar to that for reduced pup bodyweight. Finally, the 18-fold dose gap between the developmental NOEL and LOEL places additional uncertainty into the NOEL determination.

To summarize, DPR considered the Mandella developmental NOEL to be relevant to subchronic scenarios. The critical subchronic endpoint of 0.02 mg/kg-day based on decreased T4 and convulsions in rats (Aughton, 1993) is expected to be protective of the developmental endpoints indicated by Mandella.

d. Subchronic and Chronic Toxicity
OEHHA Comment: OEHHA agrees that this critical POD is appropriate and health protective for assessing both subchronic and chronic exposures to fipronil.

DPR Response: Comments on this point are noted.

e. Reproductive and Developmental Toxicity
OEHHA Comment: The developmental study database for fipronil includes teratology studies in rats and rabbits, a developmental neurotoxicity (DNT) study in rats, and the CTA assay in pregnant rats. In general, OEHHA agrees with the interpretation of the major effects of these studies. However, OEHHA suggests that all developmental toxicity studies (including DNT) and the CTA assay (Coder, 2019) be considered quantitatively for acute and subchronic PODs, as we have outlined in the acute toxicity section (II.A.1.c) of this report. This is supported by section VII.A.5. of the draft fipronil RCD, where it is clearly stated that even short duration deficits in thyroid hormone during specific times in development can cause irreversible brain damage, and that damagingly low levels of thyroid hormone in the neonate can be associated with maternal levels appearing in the normal range (Bernal, 2015 and OEHHA, 2015, as cited in DPR, 2020).

DPR Response: Short duration deficits in thyroid hormone levels during development may present serious hazards to the fetus. However, both the subchronic and chronic risk evaluations are based on measurements of T4 reductions in male rats at 0.06 mg/kg/day, resulting in NOELs of 0.02 mg/kg/day for both exposure durations (Aughton, 1993). These NOELs are lower than any of the PODs established in the developmental toxicity studies. However, DPR did not base the acute/short term POD on this value or on other values derived from developmental studies for reasons detailed in above responses.

1. Genotoxicity

OEHHA Comment: OEHHA disagrees with the conclusion in the draft RCD that fipronil is not genotoxic. There are five in vivo studies that showed fipronil was genotoxic in mammals (four included in the draft RCD and one additional study identified by OEHHA below) causing DNA strand breaks, and some of the studies showed positive results in chromosomal aberration or micronuclei tests (Appendix I). In other in vivo studies, the chemical was also shown to be genotoxic in other species, such as bird, fish, and fruit fly.

In many in vitro test systems, fipronil caused DNA damage, DNA alterations, chromosomal aberration, micronuclei, and other chromosomal effects (Appendix 1). In particular, fipronil induced DNA strand breaks and chromosomal damage in human peripheral blood lymphocytes and laryngeal mucosal cells. These positive studies in primary human cells are important per IARC's Preamble, which states that in evaluating mechanistic data for carcinogenicity, "[s]tudies in exposed humans and in human primary cells or tissues that incorporate end-points relevant to key characteristics of carcinogens are emphasized when available." OEHHA found that no cytotoxicity or presence of oxidative stress markers were reported in most of these studies at the lowest doses that indicated positive results for genotoxicity. A summary table of the in vivo and in vitro genotoxicity tests as listed in the draft RCD and OEHHA's interpretation of them is included in Appendix 1 of this report. Using the weight of evidence approach, OEHHA determined there is evidence to show fipronil is genotoxic.

DPR Response: DPR reviewed all published studies with genotoxic endpoints and updated the genotoxicity section of the final RCD. Analysis of the five genotoxicity studies mentioned by OEHHA are described below. Three of the 5 studies were excluded because they lacked information on the purity of the test compound, used formulated pesticides, or used improper controls (de Oliveira *et al.*, 2012; Girgis and Yassa, 2013; Lovinskaya *et al.*, 2016). The remaining two studies (Khan *et al.*, 2015; Badgujar *et al.*, 2016) provided evidence that fipronil induced DNA damage and clastogenicity in rats and mice in the presence of oxidative stress and/or apoptosis (Khan *et al.*, 2015). However, DPR noted methodological deficiencies in these studies that limited the reliability of their overall conclusions. Evaluation of each study is detailed below.

1. **de Oliviera *et al.*, (2012).** DPR excluded this study from the weight of evidence analysis for genotoxicity because:
    a. The test compound was an 80% fipronil-formulated product and therefore contained 20% other ingredients that were not identified or tested separately for genotoxicity.
    b. Instead of using the solvent of the formulation as a negative control, the study's control animals were treated only with water.
    c. Deaths or animals in an agonal state were not mentioned in the study, despite the use of doses at 100%, 50% and 30% of the stated LD50.
    d. The descriptions of the methods and the results raise concerns. For example, the incidence of clouds, also called ghost cells observed in Comet assays was not

indicated, despite recommendations for Comet assay reporting (Kumaravel *et al.*, 2009).

2. **Girgis and Yassa (2013).** DPR excluded this study from the weight of evidence analysis for genotoxicity because:
    a. The test compound appeared to be a fipronil-formulated product with unknown percent purity. The identities and respective concentrations of other chemicals in the product were not described.
    b. The history of the product (including storage and preparation of dosing solutions) was not described.
    c. The description of the test animals and study conduct is inadequate. No information was provided concerning the age of rats, vehicle and method of administration, or whether the negative control was treated with the vehicle.
    d. The descriptions of the methods used for the cytogenetic analyses and micronuclei assays are inadequate. For example, references cited for the assays do not describe the nonfluorescent staining method indicated by the report's photograph of bone marrow cells. In addition, the micronucleus data raise concerns: the negative-control values are significantly greater than would be expected; and the maximum incidence occurred at 96 h post dosing whereas typically the maximum occurs at ~48 h post dosing following a single exposure to many known genotoxicants (US EPA, 2012b).
    e. The research was published in a journal dedicated to ecological research, which may not follow existing genotoxicity testing standards.

3. **Khan *et al.*, (2015).** This study met DPR's minimum data acceptance criteria. A decrease in sperm density, motility, viability, and acrosome integrity and a change in sperm morphology was reported in rats after 28 consecutive days of fipronil administration via gavage. Reactive oxygen species and lipid peroxidation occurred at all doses. It should be noted that this study was not designed to test fipronil's mutagenic potential to somatic cells in the testis, because spermatozoa were studied. However, it may provide evidence of reproductive toxicity (details are described in the Reproductive Studies Published in Literature section of the draft and final RCDs). The investigators proposed that fipronil caused male reproductive toxicity through oxidative stress-induced DNA damage and apoptosis in spermatozoa. This was based on assays showing formation of ROS and lipid peroxidation, and Annexin V binding as a measure of apoptosis in spermatozoa. DPR has concerns regarding the reliability of the study results because the Comet assay lacked a positive control, testicular histology did not appear to follow standard protocols (e.g., US EPA or OECD guidance), and because background rates or incidences of DNA breakage in spermatozoa were not noted as a natural phenomenon.

4. **Badgujar *et al.*, (2016).** This study met DPR's minimum data acceptance criteria. Rats and mice were exposed once by gavage to fipronil in corn oil and sacrificed 24 hours

later. Fipronil was positive in two species and in both sexes for clastogenicity: 1) induction of micronucleus formation in bone-marrow erythrocytes in mice; 2) cytogenetic changes in rat bone-marrow cells; and 3) DNA damage in WBCs in peripheral blood of rats (increased tail length in Comet assay). The clastogenicity occurred in the absence of significant cytotoxicity in the bone-marrow micronucleus assay. In the rat study, cytotoxicity was not investigated. Overall, this study did not show that fipronil acts directly on DNA, rather that DNA damage was mediated through oxidative stress (based on the finding that pretreatment with vitamin E decreased fipronil-induced cytogenic and DNA damage in both rats and mice). DPR has concerns regarding the reliability of this study, including the indicated small gavage volume (approximately 20–25 µL per mouse). Such small gavage volume may not deliver reliably the intended dose. In addition, the effects on the mitotic index were not investigated and the data for chromosomal aberrations were provided graphically in a pooled form and not by the individual categories of aberrations, which prevented more informative analysis of individual results.

5. **Lovinskaya *et al.*, (2016).** DPR excluded this study from the weight of evidence analysis for genotoxicity because the test material appeared to be a formulated product for which the fipronil concentration was not stated and the identities and respective concentrations of other chemicals in the product were not indicated, and water was used as a vehicle with no mention of solvents.

OEHHA Comment, continued: OEHHA identified several additional genotoxicity studies that are not in the draft RCD through a quick review of the literature. They are listed below. We suggest a thorough search to identify any additional genotoxicity studies be performed.

DPR Response: DPR reviewed all published studies with genotoxic endpoints including the studies identified by OEHHA, screened each study for appropriateness for use in human health risk assessment, and updated the genotoxicity section of the final RCD accordingly. Most of the publications cited by OEHHA used atypical test species (e.g., birds, fish, plants) that limited their utility for determination of genotoxicity for human health risk assessment. In addition, these studies had other deficiencies, such as not identifying the percent active ingredient and vehicle, not having appropriate controls, or testing commercial products that contained unidentified ingredients not tested separately or collectively for genotoxicity. None of the studies identified by OEHHA could be used in the weight of evidence for genotoxicity. The specific reasons for excluding each study are detailed below:

- **Girgis and Yassa (2013).** The investigators used a relevant test system (rats), however, the test material appeared to be a formulated product for which the fipronil concentration was not stated and the identities and respective concentrations of other chemicals in the product, including the vehicle were not described.

- **Mohammed *et al.*, (2016).** In addition to using an atypical test organism (Japanese quails), the control group in this study received only water, which was not an appropriate control for the treated groups that received 20% fipronil formulation.
- **Ardeshir *et al.*, (2019).** This study used Caspian white fish, which is not a relevant test species for human health risk assessment and used ground water as a control.
- **de Castilhos Ghisi Nde *et al.*, (2011).** In addition to using an atypical test organism (Caspian white fish), this study used a 9% fipronil formulation without proper formulation controls.
- **Karaismailoglu (2017).** The test species was onion and the test material appeared to be a formulated product for which the fipronil concentration was not stated and the identities and respective concentrations of other chemicals in the product, including the vehicle were not described.
- **Ucar *et al.*, (2021).** In addition to the test species (rainbow trout), and the test material appeared to be a formulated product for which the fipronil concentration was not stated and the identities and respective concentrations of other chemicals in the product were not described.
- **Ziliotto *et al.*, (2017).** A 9.8% fipronil formulation was used without a vehicle control (untreated dogs were the only controls) and the identities and respective concentrations of other chemicals in the product were not described. In addition, the formulation also contained methoprene, a growth regulator used as an insecticide which could confound the results. Finally, authors stated that the results are negative.

2. Mechanistic Data

OEHHA Comment: OEHHA suggests that the analysis of data for fipronil in ToxCast/Tox21 be updated. The draft RCD states that fipronil is active in 134 of 667 high-throughput screening assays in ToxCast mostly associated with metabolism, elimination, inflammation, cell cycle regulation, and fatty liver disease. However, as of March 25, 2021, fipronil is active in 292/957 ToxCast/Tox21 assays. The present data include active assays for DNA binding and other important biological endpoints. These mechanistic data should be reviewed and added to the weight of evidence in determining the carcinogenicity of fipronil.

DPR Response: DPR revised the fipronil ToxCast analysis with the latest version of the CompTox Dashboard. This analysis has been updated in the final RCD. Regarding ToxCast, fipronil was active in DNA binding assays that showed altered binding of transcription factors to DNA. These assays do not measure covalent binding to DNA, which would be indicative of mutagenicity. Therefore, the DNA binding assays in ToxCast did not show evidence of direct fipronil-DNA reactivity. Assays related to mutagenicity were positive only at exposure concentrations 2.5- to 7.2-times higher than the cytotoxic limit (AC_{50} > 11.72 μM) for fipronil.

3. Carcinogenicity

OEHHA Comment: OEHHA reviewed the cancer bioassay in rats (Aughton, 1993) and found fipronil caused thyroid follicular cell adenomas and carcinomas in male and female rats. OEHHA disagrees with the draft RCD that these tumors are not likely relevant to humans and can be evaluated using a threshold approach. The guidance from IARC (1999) discusses the induction of follicular cell tumors in rodents through various mechanisms (e.g., genotoxicity, thyroid hormone imbalance). Specifically, IARC noted all the following criteria must be met for identifying a chemical as causing thyroid follicular-cell neoplasia in rats 'solely through hormonal imbalance.

- There is a lack of genotoxic activity (agent and/or metabolite) based on an overall evaluation of in-vitro and in-vivo data.
- The presence of hormone imbalance has been demonstrated under the conditions of the carcinogenicity assay.
- The mechanism whereby the agent leads to hormone imbalance has been defined.'

As discussed in the genotoxicity section, OEHHA has determined that fipronil is genotoxic and this finding makes the first criterion not fulfilled. It is possible that multiple mechanisms are operative in the induction of thyroid tumors by fipronil, including genotoxic mechanisms, such as chromosomal changes, as well as mechanisms resulting in disruption of the hypothalamus-pituitary-thyroid axis. This possibility is strengthened by the observation of liver hepatocellular carcinomas in male mice in other cancer bioassays (Broadmeadow, 1993) and the data from ToxCast (see II.A.3) indicating direct DNA interacting mechanisms could be operative.

> **DPR Response:** The current genotoxicity database for fipronil consists of 6 registrant submitted studies and 20 studies published in the open literature (latest systematic literature review conducted on June 16, 2022). All 10 papers identified by OEHHA were captured in the DPR's literature search. In the registrant studies, fipronil was negative in all in vivo and in vitro assays, except for one test where in vitro chromosomal aberrations were seen at concentrations that also caused cytotoxicity. Of the 20 published studies with genotoxic endpoints, 17 had experimental, design or reporting issues that would preclude their use in human health risk assessment (see Appendix I. of this document). The 3 remaining publications that met DPR's minimum data acceptance criteria showed genotoxicity results in association with apoptosis, cytotoxicity, or indirect evidence of oxidative stress (Khan *et al.*, 2015; Badgujar *et al.*, 2016; Quesnot *et al.*, 2016). In no case mutagenicity was directly implicated. When applying the IARC criteria (1999), the overall evaluation of registrant-submitted and published toxicity studies and the ToxCast database did not show evidence for direct genotoxic activity.

OEHHA Comment, continued: The draft RCD does not conduct a linear dose response analysis of the rat thyroid tumor data, and instead states that "the critical chronic POD of 0.02 mg/kg/day based partly on the precursor event for tumors at 0.06 mg/kg/day will be protective of any possible tumor formation in humans." For the reasons provided, OEHHA recommends the

thyroid follicular cell adenoma and carcinoma data should be evaluated by a linearized multistage model.

DPR Response: DPR considered a threshold approach based on depressed T4 and elevated TSH levels in rats to be the most appropriate methodology for evaluating the risk of thyroid tumorigenesis in humans. This was based on a series of biological considerations outlined in the RCD indicating that disruption of the hypothalamus-pituitary-thyroid (HPT) axis is the main driver for oncogenesis. Application of linearized multistage modeling is not supported by the data from the Aughton study and other fipronil studies. First, mutagenicity as a guideline criterion for linearized modeling, was unlikely, as explained above and in the RCD. Second, there is evidence for a well-supported biological mode of action (e.g., disruption of the HPT axis) which preclude modeling. Third, a statistically appropriate linearization of the tumor incidence data would have been problematic because pairwise significance was evident only at the high dose. In conclusion, threshold analysis is the most scientifically defensible approach to oncogenic risk evaluation for fipronil.

OEHHA Comments, continued: OEHHA has additional concerns about the adequacy of the rat cancer bioassays to fully assess the carcinogenic potential of fipronil, as the study duration was only 89-91 weeks, which is shorter than the recommended 104 weeks for a rat cancer bioassay and considered a less-than-lifetime study. It is possible that more thyroid tumors might have been observed if the study duration had been extended to 104 weeks. This is particularly concerning as the LOAEL for thyroid hormone disruption, which was considered the precursor event in the draft RCD, is the same as the LOAEL for tumor formation (0.06 mg/kg-day).

DPR Response: As noted in the draft RCD, the oncogenicity phase was shortened from 104 weeks to 89 weeks (males) or 91 weeks (females) due to poor survival of both controls and treated rats in the Aughton (1993) study. DPR considers 89–91 weeks sufficient in CD rats to qualify as a lifetime cancer bioassay. The combined incidence of thyroid follicular adenomas and carcinomas was 0/49, 1/48, 5/50, 3/50, and 17/50 at 0, 0.02, 0.06, 1.3, and 13 ppm. The effects at the high dose (13 mg/kg/day) were clearly attributed to fipronil exposure. However, the incidence of adenomas in males at 0.06 and 1.3 mg/kg/day did not suggest a dose-response and could not clearly be attributed to fipronil. Nonetheless, the critical POD of 0.02 mg/kg/day based on T4 decreases would protect against potential effects of increased TSH, including development of thyroid tumors at higher doses.

OEHHA Comment, continued: OEHHA also reviewed the cancer bioassays in mice (Broadmeadow, 1993) and found fipronil caused hepatocellular carcinomas in male mice. OEHHA agrees with the determination in the draft RCD that these tumors were treatment related but has several issues with the analysis and interpretation of this dataset.

In its Table 17, the draft RCD only included mice that were killed after 78 weeks, and excluded animals that died before 78 weeks, resulting in a significant number of liver tumors observed

before week 78 not being included in the analysis. OEHHA believes all the liver tumors need to be considered, whether they were discovered at the 78-week sacrifice or earlier in the study. OEHHA re-analyzed the male CD-1 mouse bioassay data and determined that there were altogether 10, 3, 2, 6, and 5 hepatocellular adenomas and 1, 1, 2, 1, 5 hepatocellular carcinomas in the control, 0.1, 0.5, 10, and 30 ppm groups, respectively …

Because detailed data on individual animals are available, OEHHA calculated the number of tumor-bearing animals and compared them to the effective animal number, which is the number of animals alive at the first occurrence of the tumor. In male mice, the first occurrence of hepatocellular carcinoma was on day 409 (at 58 weeks). OEHHA's analysis identified a statistically significant dose-dependent trend in hepatocellular carcinomas in male mice ($p = 0.025$) …

DPR Response: In response to OEHHA's comment, DPR identified all animals with liver tumors in the study. The incidence of liver adenomas was 10, 3, 2, 6, and 6 in the 0, 0.1, 0.5, 10, and 30 ppm groups, respectively, from day 317 (the day that first adenoma was detected) onwards. The high dose group had 6 adenomas. The incidence of liver carcinomas was 1, 1, 2, 1, and 5 in the 0, 0.1, 0.5, 10, and 30 ppm groups, respectively, from day 409 (the day that first carcinoma was detected) onwards. The combined incidence for animals that survived until at least day 317 was 11, 4, 4, 7, and 10 at 0, 0.1, 0.5, 10, and 30 ppm, respectively (Table R.1). Note: One animal in the high-dose group had both an adenoma and a carcinoma, which is why only 10 tumors were totaled instead of 11.

Table R.1. Liver tumor incidence in male mice and at-risk animal numbers

Dose (ppm)	**0**	**0.1**	**0.5**	**10**	**30**
Dose (mg/kg/day)	**0**	**0.01**	**0.06**	**1.18**	**3.43**
N day 317	47	50	44	40	47
Adenomas[1]	10	3	2	6	6[a]
Percent Adenoma	21%	6%	5%	15%	13%
N day 409	41	39	34	32	42
Carcinomas[2]	1	1	2	1	5
Percent Carcinoma	2%	3%	6%	3%	12%
Adenoma + Carcinoma	11	4	4	7	10[a]
Percent Adenoma + Carcinoma[3]	23%	8%	9%	18%	21%

[1]Culumative incidence of adenomas from day 317 onward.
[2]Cumulative incidence of carcinomas from day 409 onward.
[3]N used here is from day 317.
[a]One male in the 30-ppm group developed both an adenoma and a carcinoma. Both are reported here.

DPR performed Fisher's exact tests and Cochran-Armitage trend tests on the combined adenoma and carcinoma data and for carcinomas alone. Trend tests were performed using two sets of N values: those adjusted for at-risk animals based on the first incidence of adenoma or carcinoma (above), or those derived from a Poly-3 survival-adjusted quantal-response test. No Cochran-Armitage trend tests on carcinomas and adenomas combined were

statistically significant. The trend test for carcinomas alone were statistically significant ($p < 0.05$), as observed by OEHHA. However, no pair-wise Fisher's tests showed that the incidence in treated groups was statistically significantly increased compared to controls for combined adenomas and carcinomas and for carcinomas alone. The statistical analyses are detailed in in the final RCD.

OEHHA Comments, continued: OEHHA also notes that the incidence rate of hepatocellular adenoma in the control group was unusually high; it is outside the range of historical controls of the laboratory, and over four times higher than the average historical control rate. It is unclear why there was such high incidence of hepatocellular adenoma in the control group, yet a very similar dose in the treated animals (0.01 mg/kg-day) had a much lower rate. This could pose a problem for linearized cancer risk model should DPR chose to model the combined incidence of hepatocellular adenoma and carcinoma. To overcome this problem, OEHHA suggests DPR evaluate the hepatocellular carcinoma data using a linearized multistage model. However, due to early mortality between controls and some treatment groups, it is possible that the regular linearized multistage model may not be suitable for cancer dose-response assessment. An alternative approach such as the multistage Weibull time-to-tumor model may be more appropriate for the cancer dose-response analysis. Similar to the cancer bioassay in the rat, another issue with the cancer bioassay in the mouse is that the study duration was only 78 weeks, also a less-than-lifetime study. This is particularly important because hepatocellular adenomas can progress to carcinomas over time. It is possible that if the study duration were 104 weeks, more hepatocellular carcinomas might have been observed.

DPR Response: DPR considered OEHHA's suggestion to use Weibull time-to-tumor modeling. Analysis of the data showed that the first carcinoma appeared in the control group on day 409 and all carcinomas in the treated mice occurred after day 409. The majority of carcinomas in the treated groups were observed only at the terminal sacrifice irrespective of dose (see the final RCD). Taken together, there is no evidence for treatment-related reduction in time-to-tumor occurrence, therefore the Weibull time-to-tumor model was not utilized. Instead, DPR performed a Poly-3 survival adjustment to account for different rates of mortality (see response above). With respect to the linearized multistage model suggested by OEHHA, the liver tumor data are not appropriate for modeling according to DPR or US EPA guidance or best practices. There was no consistent or sustained dose response observed in the carcinoma data that could be used in low dose linear extrapolation, regardless of whether total animals were counted or if mortality in at-risk animals was considered. Any multistage model analysis will be an artifact of the model and will not likely reflect the biology of the tumor. OEHHA's suggestion to apply linear extrapolation to hepatocellular carcinomas is also problematic. The meaning of any slope emerging from this dose-response data would be questionable because the only positive tumor response occurred at the high dose of 3.43 mg/kg/day, and that response failed to achieve pairwise statistical significance. There was an indication from this study that fipronil may have a role in hepatocellular tumor induction in male mice. However, the experimental results were compromised because of the high

incidence of adenomas in and low survival of the control males, which exceeded historical control data.

Regarding the definition of a lifetime study, FIFRA guidelines for mouse oncogenicity studies require 18 months (77 weeks) of exposure, which is approximately lifespan of the CD-1 mouse strain used in the Broadmeadow study. Exposure for greater than an 18-month duration may result in more tumors, but there is a high likelihood of excessive deaths due to other diseases of aging. DPR thus considers the 18-month duration of the Broadmeadow mouse study to be appropriate.

OEHHA Comment, continued: Collectively, OEHHA presents evidence that fipronil-induced thyroid follicular cell tumors in rats could be due to a genotoxic mechanism of action in addition to thyroid hormone disruption, and that there is concern that the threshold approach taken in the draft RCD may be inadequate to protect human health. When constructing a carcinogenicity mode of action network for fipronil, OEHHA suggests that the thyroid follicular cell tumors in rats and the liver carcinomas in mice be considered relevant for human cancer risk assessment. OEHHA also suggests that the genotoxicity (see II.A.4) and recent data from ToxCast (see II.A.3) be included in the mechanistic considerations.

DPR Response: As described in responses above, neither the ToxCast results nor the genotoxicity database support a mutagenic action for fipronil. In the presence of a well-supported biological mode of action (disruption of the HPT axis), DPR will continue to utilize the threshold approach for oncogenic risk, in this case with T4 reduction as the driver. As noted in the response to the previous comment, the mouse liver tumor data are severely limited and thus inadequate for use in risk analysis.

4. Extrapolation, Variability, and Uncertainty
a. Duration Extrapolation
OEHHA Comment: No duration extrapolations were used in the draft RCD. The chronic POD is protective of subchronic health effects and is used as the subchronic POD in the draft RCD.

DPR Response: Comments on this point are noted.

b. Intraspecies Extrapolation
OEHHA Comment: In the draft RCD, a default UF of 10-fold was applied to account for intraspecies variability within the human population (UFH). This is generally considered to be a factor of √10 for pharmacokinetics and √10 for pharmacodynamics. It is OEHHA's opinion that an intraspecies UF of 10 is insufficient as there are many factors affecting human variability in response to a chemical exposure (OEHHA, 2008; Zeise et al. 2013) … Thus, OEHHA recommends addressing these concerns by increasing the intraspecies pharmacokinetic UF to 10, resulting in a total UFH of 30.

DPR Response: Consistent with DPR's current practice (DPR, 2011), a default intraspecies UF of 10 consisting of a pharmacokinetic UF of 3 and a pharmacodynamic UF of 3 was applied to the acute, subchronic and chronic risk evaluations for fipronil.

c. Sensitive Populations and Life-Stages

OEHHA Comment: As discussed in the acute toxicity and reproductive and developmental toxicity sections above, infants and fetuses appear to be especially vulnerable to fipronil toxicity, and thyroid hormone disruption during critical development periods can cause neurological and developmental effects in offspring. The acute POD selected in the draft RCD is higher than multiple potential PODs derived from developmental studies, as described above. OEHHA suggests that the toxicity database be re-evaluated and a more health protective acute POD be selected.

However, if the acute POD based on hindlimb splay in adults is retained, OEHHA suggests an additional UF be applied for exposure scenarios that include infants, children, and women of childbearing age to protect them from potential developmental or neurodevelopmental effects resulting from in utero or early-life fipronil exposure.

DPR Response: Detailed responses to comments bearing on the issues of acute, reproductive, or developmental toxicity, as well as the appropriateness of the intrahuman uncertainty factor, appear elsewhere in this document.

d. Risk Characterization

OEHHA Comment: The Margin of Exposure (MOE) approach was used to evaluate non-cancer hazards. The draft RCD characterized whether an exposure is likely to cause adverse health effects using a target MOE of 100 for all age groups. OEHHA recommends a target MOE of 300 for all age groups, occupational and non-occupational, to take into account the recommended increase in the intraspecies pharmacokinetic UF from √10 to 10. An additional UF may also be warranted for exposure scenarios that include women of child-bearing age and children due to developmental and neurodevelopmental concerns following in utero and early life exposures if the acute POD based on hindlimb splay is retained, as described above.

DPR Response

Detailed responses to the issues of appropriate target MOE setting and intraspecies uncertainty factors appear elsewhere in this document.

Responses to Toxicity Charge Questions

Toxicity Charge Question 1. All critical points of departure (PODs) used in this assessment were established using the parent compound fipronil.

OEHHA Comment: OEHHA concurs with the use of the parent compound, fipronil, for the purposes of deriving critical PODs for human health risk assessment (see section II.A.1a).

DPR Response: Comment on this question is noted.

Responses to Hazard Identification Charge Questions

Hazard Identification Charge Question 1. The acute oral POD of 0.87 mg/kg-day was based on neurotoxic effects observed in the adult rat.

OEHHA Comment: The draft RCD chose a dose-dependent reduction in hindlimb splay in rats observed seven hours post-administration as the critical effect and estimated an acute oral POD of 0.87 mg/kg-day using BMD modeling. There is evidence that certain effects occurred earlier than seven hours, and there is uncertainty about whether a lower POD would be estimated if the optimal time were chosen. Furthermore, endpoints observed in several acute or short-term studies suggest a lower POD. OEHHA recommends the acute oral POD be re-evaluated. There is further discussion on this in the following charge statement response and under the detailed comments sections of this report (II.A.1.c and II.A.1.e).

DPR Response: After reviewing all relevant data, DPR maintains that the POD established through BMD modeling of hindlimb splay data generated by Hughes (1997) provides the most realistic and scientifically defensible approach to acute risk estimation for fipronil. Updating the BMD model resulted in a $BMDL_{10}$ of 0.77 mg/kg/day. Detailed responses regarding hindlimb splay and the possibility of lower PODs from other acute or short-term studies can be found above.

Hazard Identification Charge Question 2. Three repeated dose studies in rats identified PODs lower than the critical acute POD of 0.87 mg/kg-day for effects that could potentially result from acute to short-term exposures. However, DPR did not consider these PODs as appropriate critical values to characterize the risk from acute exposures to humans.

OEHHA Comment: The three repeat dose studies cited by DPR with PODs lower than the critical acute oral POD are Mandella (1995), Coder (2019), and Aughton (1993). The developmental neurotoxicity study (Mandella, 1995) identified several endpoints, the most sensitive being delayed preputial separation, decreased maximum startle response, and decreased body weight in male pups at a LOAEL of 0.9 mg/kg-day. The study NOAEL was 0.05 mg/kg-day. As discussed under the detailed comments sections, there is concern that acute POD of 0.87 mg/kg-day, similar to the LOAEL from Mandella (1995), is not sufficiently protective of the fetus.

The CTA study (Coder, 2019) reported decreased T4 hormone levels and decreased thyroid gland weight in fetuses at gestational day 20 from dams exposed to 1 mg/kg-day fipronil resulting in a NOAEL of 0.3 mg/kg-day. OEHHA disagrees that this study is inadequate for critical POD determination and recommends that this study be reconsidered. This would be

consistent with the most recent US EPA (2020) draft risk assessment on fipronil which found the study acceptable for quantitative POD determination.

The Aughton (1993) study showed effects in the range of 0.06 – 1.6 mg/kg-day, during the first week of treatment, significantly decreased T4 levels. Convulsions were also observed in 3 male animals during the first few weeks of treatment. OEHHA disagrees with the rationale presented in the draft RCD for not selecting this endpoint to characterize acute risk. Thyroid hormone disruption seems to be one of the most sensitive effects at any exposure duration, and an acute POD based on this endpoint would be more health protective for sensitive populations. DPR should reconsider these, as well as other studies outlined above, in the determination of an acute oral POD. Additional discussion of these points can be found in our detailed comments (section II.A.1).

DPR Response: Responses to these comments can be found earlier in this document.

Hazard Identification Charge Question 3. PODs from dermal and inhalation studies were not used to establish critical PODs.

OEHHA Comment: While route-specific studies are available for acute and subchronic inhalation and dermal exposures, OEHHA agrees that the oral studies are more suitable for POD derivation, and that the approach of route-to-route extrapolation is appropriate.

DPR Response: Comment on this question is noted.

Hazard Identification Charge Question 4. This RCD did not include a cancer risk estimate for fipronil.

OEHHA Comment: OEHHA disagrees with the draft RCD finding that thyroid follicular cell tumors are not relevant to humans and can be evaluated using a threshold approach. OEHHA suggests that the thyroid follicular cell tumors in male and female rats and liver tumors in male mice should be considered relevant for human cancer risk assessment, and the risk should be evaluated by the linearized cancer risk model. This approach is supported by the positive genotoxicity data (see II.A.4) and recent mechanistic data from ToxCast (see II.A.3).

DPR Response: Responses to OEHHA's recommendation that thyroid follicular cell tumors be subjected to linearized multistage analysis can be found above.

Responses to Exposure Charge Questions
Exposure comments are addressed in a separate document.

Responses to Risk Characterization Charge Questions

Risk Characterization Charge Question 1. The target margin of exposure (MOE) was set at 100, reflecting the default assumption that humans are 10-fold more sensitive than animals, and that a 10-fold range of sensitivity exists within the human population.

OEHHA Comment: OEHHA agrees with the use of 10-fold UF for interspecies extrapolation. However, as described in section (II.A.5.b), OEHHA generally uses a combined intraspecies UF of 30 to account for wide variability in pharmacokinetics in the human population, especially due to susceptible life-stages, health, immune, and genetic factors, and disproportionate pollution burden. Additionally, for acute exposure scenarios that include infants, children, and women of childbearing age, OEHHA recommends an additional UF if the acute oral POD of 0.87 mg/kg-day is retained due to concern for developmental and neurodevelopmental toxicities (see section II.A.5.c).

> **DPR Response:** As noted above, DPR practice is to apply a 10-fold total intraspecies uncertainty factor in all cases absent specific data to support a different or additional value. Following extensive review of the data, DPR opted to maintain the default of 10.

Responses to the remaining Risk Characterization Charge Questions are addressed in a separate document.

Other OEHHA Comments on the Draft Risk Characterization Document

A. Toxicity Evaluation and Risk Assessment

OEHHA Comment: The critical acute POD lists a $BMDL_{10}$ of 0.87 mg/kg-day. This value appears to be from modeling the hindlimb splay dataset using the Exponential4 model assuming non-constant variance, not assuming constant variance as listed in Table 2 (Appendix IV) of the draft RCD. OEHHA modeled the same dataset using a constant variance model and returned lower BMD and BMDL10 values of 2.09 mg/kg-day and 0.77 mg/kg-day, respectively, from the best-fit model—Exponential4. OEHHA recommends the BMD modeling be verified in the final RCD for accuracy and recommends using constant variance, which appears to be the most appropriate for this dataset.

> **DPR Response**: DPR re-modeled this data using the updated BMDS version 3.2. DPR agrees that the value derived with a constant variance model is most appropriate for this dataset and updated the POD and RCD accordingly.

References

Adamo-Trigiani, M. 1999. A 28-Day Inhalation Toxicity Study by Nose-Only Exposure of Fipronil Technical (micronized) in the Albino Rat. In *Sumitomo Chemical Company, LTD, Osaka, Japan, Project No. 91087*.

Badgujar, P. C., Selkar, N. A., Chandratre, G. A., Pawar, N. N., Dighe, V. D., Bhagat, S. T., Telang, A. G., and Vanage, G. R. 2016. Fipronil-induced genotoxicity and DNA damage in vivo: Protective effect of vitamin E. *Hum Exp Toxicol*.

Coder, P. 2019. Fipronil – an oral (dietary) comparative thyroid assay in pregnant, postnatal and fetal Sprague-Dawley rats. In *Fipronil Task Force, LLC, Raleigh, NC, Lab Project ID 00657506*.

de Oliveira, P. R., Bechara, G. H., Denardi, S. E., Oliveira, R. J., and Mathias, M. I. 2012. Genotoxic and mutagenic effects of fipronil on mice. *Exp Toxicol Pathol* 64:569-573.

DPR. 2011. DPR Risk Assessment Guidance.

Freeborn, D. L., McDaniel, K. L., Moser, V. C., and Herr, D. W. 2015. Use of electroencephalography (EEG) to assess CNS changes produced by pesticides with different modes of action: effects of permethrin, deltamethrin, fipronil, imidacloprid, carbaryl, and triadimefon. *Toxicol Appl Pharmacol* 282:184-194.

Gill, M., Wagner, C. L., and Driscoll, C. D. 1993. Single Exposure Peroroal (Gavage) Neurotoxicity Study In Sprague Dawley Rats. Bushy Run Research Center (BRRC). Union Carbide and Plastiscs Company Inc. (UCC&P). Export, PA, No. 91N0099. Bushy Run Research Center (BRRC). Union Carbide and Plastiscs Company Inc. (UCC&P). Export, PA, No. 91N0099. (DPR Vol. No. 52062-024, Record No. 137583).

Girgis, M. S., and Yassa, F. V. 2013. Evaluation of the Potential Genotoxic and Mutagenic Effects of Fipronil in Rats. *Journal of Mediterranean Ecology* 12:5-11.

Holmes, P. 1992. M&B 46030: Toxicity Study by Oral (Capsule) Administration to Beagle Dogs for 52 weeks. Life Science Research Limited Eye. Suffolk, UK. Study No. 92/RHA311/0464. (DPR Vol. No. 52062-029, Record No. 137588).

Holmes, P. 1993. M&B 46030: Toxicity Study by Dietary Administration to Beagle Dogs for 52 weeks. Pharmaco-Life Science Research Limited Eye. Suffolk, UK. Study No. 93/RHA465/0243. (DPR Vol. No. 52062-030, Record No. 137589).

Homburger, F., Russfield, A. B., Weisburger, J. H., Lim, S., Chak, S. P., and Weisburger, E. K. 1975. Aging changes in CD-1 HaM/ICR mice reared under standard laboratory conditions. *J Natl Cancer Inst* 55:37-45.

Hughes, E. 1997. Fipronil: Neurotoxicity to rats by acute oral administration (including a time to peak effect study). Huntingdon Life Sciences Ltd., Cambridgeshire, PE18 6ES, U.K., RNP 536/973345. (DPR Vol. No. 52062-0387, Record No. 235557).

Khan, S., Jan, M. H., Kumar, D., and Telang, A. G. 2015. Firpronil induced spermotoxicity is associated with oxidative stress, DNA damage and apoptosis in male rats. *Pestic Biochem Physiol* 124:8-14.

Kumaravel, T. S., Vilhar, B., Faux, S. P., and Jha, A. N. 2009. Comet Assay measurements: a perspective. *Cell Biol Toxicol* 25:53-64.

Lovinskaya, A. V., Kolumbayeva, S. Z., Kolomiets, O. L., and Abilev, S. K. 2016. [Genotoxic effects of pesticide fipronil in somatic and generative cells of mice]. *Genetika* 52:561-568.

Maeda, M., Yokoyama, T., Kitauchi, S., Hirano, T., Mantani, Y., Tabuchi, Y., and Hoshi, N. 2021. Influence of acute exposure to a low dose of systemic insecticide fipronil on locomotor activity and emotional behavior in adult male mice. *J Vet Med Sci* 83:344-348.

Papineni, S., Marty, M. S., Rasoulpour, R. J., LeBaron, M. J., Pottenger, L. H., and Eisenbrandt, D. L. 2015. Mode of action and human relevance of pronamide-induced rat thyroid tumors. *Regul Toxicol Pharmacol* 71:541-551.

US EPA 2020. Fipronil: Draft Risk Assessment for Registration Review. Document ID: EPA-HQ-OPP-2011-0448-0076. United States Environmental Protection Agency, Washington D.C.

APPENDIX I.

Published Studies Excluded from the Fipronil Genotoxicity Assessment

Appendix I. Published Studies Excluded from the Fipronil Genotoxicity Assessment

Introduction

Through systematic literature review the Department of Pesticide Regulation (DPR) identified 20 published studies in the open literature relating to genotoxicity of fipronil (latest systematic literature review conducted on June 16, 2022). DPR uses a screening process to identify journal articles that could be useful in human health risk assessment. To be eligible for consideration, published papers must meet a set of minimum study acceptance criteria (US EPA, 2012a). Seventeen of the 20 studies were excluded from use in assessment of genotoxicity because they did not meet one or more of the study acceptance criteria, specifically:

1. The toxic effects are in an appropriate test animal species.
2. Treatment(s) are compared to acceptable controls (studies which use a solvent vehicle should also include solvent vehicle controls).
3. Adequate data are provided on the chemical tested (i.e., test article characterization, exact nature and source of the pesticide; the percent active ingredient and/or the purity of the test compound).
4. The study results (findings) are adequately reported.
5. The study findings are relevant to assessing human health risks.

Table R.A.1. Published studies excluded from the fipronil genotoxicity assessment

	Study Reference	Study Design	Reason for Exclusion
1	Ghisi Nde *et al.*, 2011	**Test article:** 2.5 % Fipronil formulation Termidor (BASF) **Test system:** Caspian white fish (Rhamdia quelen) **N:** 15/dose **Exposure route:** test compound added to fish tanks **Dosing schedule:** Daily for 60 days **Doses:** 0, 0.05, 0.10 and 0.23 µg/L **Vehicle:** not specified **Endpoints:** Nuclear morphological alterations at 0.10 and 0.23 mg/ml	This study used a commercially formulated product with unknown formulation ingredients, the control vehicle was not described, and the test species is not relevant for human health risk assessment.
2	de Oliveira *et al*., 2012	**Test article:** 80% Fipronil formulation - Reagent 800 WG, (BASF) **Test system:** 5-6 weeks old female Swiss mice **N:** 5/dose **Exposure route:** intraperitoneal **Dosing schedule:** single injection **Doses:** 0, 15, 25, 50 mg/kg **Vehicle:** distilled water **Endpoints:** DNA damage (Comet assay of nucleated cells) and micronucleus induction in reticulocytes in peripheral blood 24 h after exposure at 50 mg/kg	This study used a commercially formulated product with unknown formulation ingredients and unacceptable vehicle controls (animals received distilled water).

Table R.A.1. Published studies excluded from the fipronil genotoxicity assessment

	Study Reference	Study Design	Reason for Exclusion
3	Girgis and Yassa, 2013	**Test article:** Fipronil product with unknown purity (Agrovetzaschita, S.P. Company) **Test system:** albino rats **N:** 10/dose **Exposure route:** oral **Dosing schedule:** single exposure **Doses:** 0, 25 and 50 mg/kg **Vehicle:** not specified **Endpoints:** Increases in chromosomal aberrations and micronuclei in bone marrow cells 24, 48 and 96 hours post dosing	This study used a fipronil product with unknown percent purity and the control vehicle was not described. The storage and preparation of dosing solutions were not detailed. The description of the test animals, study conduct including exposure (gavage or diet) and the methods used for the cytogenetic analyses and micronuclei assay were inadequate.
4	Celik *et al.*, 2014	**Test article:** 7.5% Fipronil formulation FIBREX 75 (described by authors as having 412.5 mg fipronil in 5.5 ml) **Test system:** Human peripheral blood lymphocytes **N:** 3 male donors **Dosing schedule:** fipronil added to cell cultures **Concentration:** 0, 0.1, 0.3, 0.7 µg/mL for 72 hours **Vehicle:** Not described **Endpoints:** Significant increase in sister-chromatid exchanges and micronucleus formation, and DNA damage in a Comet assay	This study used commercially formulated product with unknown formulation ingredients, the source of the fipronil product and the control vehicle was not described. Additional concerns with this study included the unknown exposure temperature, duration, and the test concentration in the Comet assay, as well as the use of X-ray contrast agent that may not be entirely inert to the cell.
5	de Morais *et al.*, 2016	**Test article:** 80% Fipronil formulation Reagent 800 WG (BASF) **Test system:** Drosophila melanogaster **N:** 40 flies/sex/dose **Exposure route:** larval immersion **Dosing schedule:** 48 hours **Concentrations:** 0, 0.3; 0.7; 1.5 or 3.0 x 10^{-5} mM **Vehicle:** not specified **Endpoints**: mutagenicity, recombinogenicity and carcinogenicity in somatic cells of D. melanogaster at all doses with cytotoxicity at 0.7x10^{-5} mM and higher concentrations	This study used a commercially formulated product with unknown formulation ingredients and unacceptable vehicle controls (water).
6	Lovinskaya *et al*., 2016	**Test article:** Fipronil product not specified **Test system:** 2–3 month-old male BALB/cYwal mice **N:** 5/dose **Exposure route:** intraperitoneal **Dosing schedule:** single and repeated (10 days) exposures **Doses:** 0, 4.75, 9.50, 19.00, and 31.70 mg/kg/day **Vehicle:** water **Endpoints:** Significant DNA damage in cells of liver and spleen at ≥ 9.50 mg/kg and in lung cells at all doses, chromosomal aberrations in bone marrow cells and structural abnormalities of spermatocytes at 19 and 31.7 mg/kg/day.	This study used fipronil of unknown source and purity, and unacceptable controls (water).

Table R.A.1. Published studies excluded from the fipronil genotoxicity assessment

	Study Reference	Study Design	Reason for Exclusion
7	Mohammed *et al.*, 2016	**Test article:** Fipronil formulation 20% (Yong-nong Bioscience Co, Ltd) **Test system:** Japanese quaill **N:** 10 birds/dose **Exposure route:** Oral gavage **Dosing schedule:** Single dose **Doses:** 0, 1.13, 2.26, 5.65. 11.3 mg/kg **Vehicle:** distilled water **Endpoints:** Dose-dependent increases of DNA strand breaks in liver cells of quails, liver histopathology, reduced body weight and food intake, and mortality	This study used a commercially formulated product with unknown formulation ingredients, unacceptable controls (animals were exposed to water) and the test species is not relevant for human health risk assessment.
8	Yildirim and Agar, 2016	**Test article:** Fipronil purity not described but was purchased from Sigma and likely was of high purity **Test system:** roots of Vicia faba seedlings **N:** 15 seeds/dose **Exposure route:** seeds soaked in unspecified fipronil solution **Dosing schedule:** 7 days **Concentrations:** 0, 0.5, 1, 2, 3, and 4 ppm **Vehicle:** Not specified **Endpoints:** Dose dependent changes in genomic DNA template stability, decreased amount of root length and increased level of protein	This study did not detail the storage, preparation of dosing solutions, the vehicle and the controls. In addition, the test organism is not appropriate for human health risk assessment.
9	Karaismailoglu, 2017	**Test article:** Fipronil purity and source not specified **Test system:** roots of plant Allium cepa (onion) **N:** 5 onion bulbs/dose **Exposure:** roots soaked in unspecified fipronil solution **Dosing schedule:** 6, 12 and 24 h **Doses:** 0, 1, 2.5, 5, and 10 ppm **Vehicle:** Not specified **Endpoints:** Statistically significant increases in chromosome aberrations and micronuclei in somatic cells of the plant	This study did not specify the purity of the test compound, the vehicle and the controls, and the test organism is not appropriate for human health risk assessment.
10	Ziliotto *et al.*, 2017	**Test article:** 9.8% Fipronil formulation Frontline plus® **Test system:** adult crossbred dogs **N:** 5/sex **Exposure route:** dermal **Dosing schedule:** single application **Doses:** 0, 6.7 mg/kg **Vehicle:** not described **Endpoints**: Negative for genotoxicity based on lack of increased DNA damage in peripheral blood nucleated cells at 3, 8 and 24 h, measured in a Comet assay	This study used a commercially formulated product with unknown formulation ingredients and unacceptable controls (untreated control dogs assayed just before they received a single dermal dose). The formulation also contained methoprene, a growth regulator used as an insecticide.
11	Ardeshir *et al.*, 2019	**Test article:** Fipronil 95% purity (Moshkfam Fars Chemical Co) **Test system:** Caspian white fish **N:** 12 fish/dose **Exposure route:** fipronil solutions added to fish tank **Dosing schedule:** 14 days **Doses:** 0, 0.1, 1, 5 and 10 µg/L) **Vehicle:** not specified **Endpoints:** Increased DNA strand breaks in livers measured by the Comet assay at all concentrations.	This study used unacceptable controls (ground water instead of solvent or vehicle) and the test species is not relevant for human health risk assessment.

Table R.A.1. Published studies excluded from the fipronil genotoxicity assessment

	Study Reference	Study Design	Reason for Exclusion
12	de Morais *et al.*, 2019	**Test article:** 80% Fipronil formulation Reagent 800 WG (BASF) **Test system:** plant Tradescantia pallida **N:** 25 stems /dose **Exposure:** fipronil added to water **Dosing schedule:** 8 h **Concentrations:** 0.025; 0.05; 0.1; 0.2; 0.4; 0.8 and 1.6 g/L **Vehicle:** distilled water **Endpoints**: Increased micronuclei in tetrads of T. pallida at 0.2 g/L and higher concentrations.	This study used a commercially formulated product with unknown formulation ingredients, unacceptable control (distilled water) and the test species is not a relevant for human health risk assessment.
13	Amaeze *et al.*, 2020	**Test article:** 2.5% Fipronil-EC formulation **Test system:** Catfish Clarias gariepinus **N:** 25 stems /dose **Exposure:** fipronil added to water **Dosing schedule:** 96 hours **Concentrations:** 0, 6.5, 7.5, 8.5, 9.5, 10.5 µg/L **Vehicle:** dechlorinated water **Endpoints**: Increased nuclear abnormalities in red blood cell in catfishes, no micronuclei formation observed.	This study used a commercially formulated product with unknown formulation ingredients, unacceptable controls (dechlorinated municipal water) and the test species is not relevant for human health risk assessment.
14	de Oliveira *et al.*, 2020	**Test article:** 80% Fipronil formulation - Reagent 800 WG, (BASF) **Test system:** Amazonian turtles (Podocnemis expansa) **N:** 5 eggs /dose **Exposure:** eggs incubated in fipronil solution **Dosing schedule:** 59 days (from beginning of egg incubation to hatching) **Doses:** 0, 4, 400 ppb **Vehicle:** distilled water mixed with sand **Endpoints:** Changes suggestive morphotoxicity and aneuploidogenicity in erythrocytes of pups from treated eggs at 4 but bot at 400 ppb, no micronuclei formation or DNA damage observed.	This study used a commercially formulated product with unknown formulation ingredients, unacceptable controls (distilled water) and the test species is not relevant for human health risk assessment.
15	Santos *et al.*, 2021	**Test article:** 80% Fipronil formulation - Reagent 800 WG, (form BASF) **Test system:** tadpoles **N:** 4 /dose **Exposure:** fipronil added to tank water **Dosing schedule:** 4, 8, 12, and 16 days **Doses:** 0.00, 0.04, 0.08, 0.4 mg/L, **Vehicle:** not specified **Endpoints:** Increases of anucleated erythrocyte cells.	This study used a commercially formulated product with unknown formulation ingredients, unacceptable controls (tank water only), and the test species is not relevant for human health risk assessment.
16	Uçar *et al.*, 2021	**Test article:** Fipronil purity not specified (obtained from Akdeniz Chemistry**)** **Test system:** Rainbow trout **N:** not specified, 160 fish were purchased **Exposure:** fipronil added to aquarium water **Dosing schedule:** 4 days **Doses:** 0, 0.05, 0.1 and 0.2 mg/L **Vehicle:** not specified **Endpoints:** Statistically significant increase of micronucleus formation in erythrocytes, DNA damage	This study did not describe the purity of fipronil, the preparation of dosing solutions, the vehicle and the controls. In addition, the test organism is not appropriate for human health risk assessment.

Table R.A.1. Published studies excluded from the fipronil genotoxicity assessment

	Study Reference	Study Design	Reason for Exclusion
17	Tisch *et al.*, 2007[a]	**Test article:** Fipronil 95.5% (obtained from BASF AG) **Test system:** tonsil mucosal epithelial cells isolated from 85 tonsillitis patients over unspecified time span **Exposure:** fipronil added to culture medium **Dosing schedule:** 1 h at 37°C **Doses:** 0 (DMSO), 0.05, 0.1, 0.5, 0.75 and 1.00 mM **Vehicle: DMSO (**concentration not specified) **Endpoints:** DNA damage measured by the Comet assay	Study quality is a concern. This study did not include methods section and provided only a cursory explanation of the experimental design. Methods for determining cytotoxicity and the software used to score comets were not described. The DMSO concentration was not indicated and fipronil concentrations may have exceeded its solubility in cell suspension medium. DPR could not determine if the test system was capable of detecting negative results. Finally, the study did not demonstrate that DNA breakage stemmed from genotoxicity as opposed to cell death (necrosis and/or apoptosis).

[a]Article originally published in German. HHA translated the paper into English with Google translator

Appendix References

Adamo-Trigiani, M. 1999. A 28-Day Inhalation Toxicity Study by Nose-Only Exposure of Fipronil Technical (micronized) in the Albino Rat. In *Sumitomo Chemical Company, LTD, Osaka, Japan, Project No. 91087*.

Amaeze, N. H., Komolafe, B. O., Salako, A. F., Akagha, K. K., Briggs, T. D., Olatinwo, O. O., and Femi, M. A. 2020. Comparative assessment of the acute toxicity, haematological and genotoxic effects of ten commonly used pesticides on the African Catfish, Clarias gariepinus Burchell 1822. *Heliyon* 6:e04768.

Ardeshir, R. A., Zolgharnien, H., Movahedinia, A., Salamat, N., Zabihi, E., and Rastgar, S. 2019. Measurement of DNA damage by CellProfiler software in the liver of Caspian white fish exposed to environmental concentrations of fipronil. *Computational Toxicology* 12:100105.

Badgujar, P. C., Selkar, N. A., Chandratre, G. A., Pawar, N. N., Dighe, V. D., Bhagat, S. T., Telang, A. G., and Vanage, G. R. 2016. Fipronil-induced genotoxicity and DNA damage in vivo: Protective effect of vitamin E. *Hum Exp Toxicol*.

Celik, A., Ekinci, S. Y., Guler, G., and Yildirim, S. 2014. In vitro genotoxicity of fipronil sister chromatid exchange, cytokinesis block micronucleus test, and comet assay. *DNA Cell Biol* 33:148-154.

Coder, P. 2019. Fipronil – an oral (dietary) comparative thyroid assay in pregnant, postnatal and fetal Sprague-Dawley rats. In *Fipronil Task Force, LLC, Raleigh, NC, Lab Project ID 00657506*.

de Morais, C. R., Bonetti, A. M., Carvalho, S. M., de Rezende, A. A. A., Araujo, G. R., and Spanó, M. A. 2016. Assessment of the mutagenic, recombinogenic and carcinogenic potential of fipronil insecticide in somatic cells of Drosophila melanogaster. *Chemosphere* 165:342-351.

de Morais, C. R., Pereira, B. B., Almeida Sousa, P. C., Vieira Santos, V. S., Campos, C. F., Carvalho, S. M., Spanó, M. A., de Rezende, A. A. A., and Bonetti, A. M. 2019. Evaluation of the genotoxicity of neurotoxic insecticides using the micronucleus test in Tradescantia pallida. *Chemosphere* 227:371-380.

de Oliveira, J. S. P., Vieira, L. G., Carvalho, W. F., de Souza, M. B., de Lima Rodrigues, A. S., Simões, K., de Melo De Silva, D., Dos Santos Mendonça, J., Hirano, L. Q. L., Santos, A. L. Q., and Malafaia, G. 2020. Mutagenic, genotoxic and morphotoxic potential of different pesticides in the erythrocytes of Podocnemis expansa neonates. *Sci Total Environ* 737:140304.

de Oliveira, P. R., Bechara, G. H., Denardi, S. E., Oliveira, R. J., and Mathias, M. I. 2012. Genotoxic and mutagenic effects of fipronil on mice. *Exp Toxicol Pathol* 64:569-573.

DPR. 2011. DPR Risk Assessment Guidance.

Freeborn, D. L., McDaniel, K. L., Moser, V. C., and Herr, D. W. 2015. Use of electroencephalography (EEG) to assess CNS changes produced by pesticides with different modes of action: effects of permethrin, deltamethrin, fipronil, imidacloprid, carbaryl, and triadimefon. *Toxicol Appl Pharmacol* 282:184-194.

Ghisi Nde, C., Ramsdorf, W. A., Ferraro, M. V., de Almeida, M. I., Ribeiro, C. A., and Cestari, M. M. 2011. Evaluation of genotoxicity in Rhamdia quelen (Pisces, Siluriformes) after sub-chronic contamination with Fipronil. *Environ Monit Assess* 180:589-599.

Gill, M., Wagner, C. L., and Driscoll, C. D. 1993. Single Exposure Peroroal (Gavage) Neurotoxicity Study In Sprague Dawley Rats. Bushy Run Research Center (BRRC). Union Carbide and Plastiscs Company Inc. (UCC&P). Export, PA, No. 91N0099. Bushy Run Research Center (BRRC). Union Carbide and Plastiscs Company Inc. (UCC&P). Export, PA, No. 91N0099. (DPR Vol. No. 52062-024, Record No. 137583).

Girgis, M. S., and Yassa, F. V. 2013. Evaluation of the Potential Genotoxic and Mutagenic Effects of Fipronil in Rats. *Journal of Mediterranean Ecology* 12:5-11.

Holmes, P. 1992. M&B 46030: Toxicity Study by Oral (Capsule) Administration to Beagle Dogs for 52 weeks. Life Science Research Limited Eye. Suffolk, UK. Study No. 92/RHA311/0464. (DPR Vol. No. 52062-029, Record No. 137588).

Holmes, P. 1993. M&B 46030: Toxicity Study by Dietary Administration to Beagle Dogs for 52 weeks. Pharmaco-Life Science Research Limited Eye. Suffolk, UK. Study No. 93/RHA465/0243. (DPR Vol. No. 52062-030, Record No. 137589).

Hughes, E. 1997. Fipronil: Neurotoxicity to rats by acute oral administration (including a time to peak effect study). Huntingdon Life Sciences Ltd., Cambridgeshire, PE18 6ES, U.K., RNP 536/973345. (DPR Vol. No. 52062-0387, Record No. 235557).

Karaismailoglu, M. C. 2017. Assessments on the potential genotoxic effects of fipronil insecticide on Allium cepa somatic cells. *Caryologia* 70:378-384.

Khan, S., Jan, M. H., Kumar, D., and Telang, A. G. 2015. Firpronil induced spermotoxicity is associated with oxidative stress, DNA damage and apoptosis in male rats. *Pestic Biochem Physiol* 124:8-14.

Kumaravel, T. S., Vilhar, B., Faux, S. P., and Jha, A. N. 2009. Comet Assay measurements: a perspective. *Cell Biol Toxicol* 25:53-64.

Lovinskaya, A. V., Kolumbayeva, S. Z., Kolomiets, O. L., and Abilev, S. K. 2016. Genotoxic effects of pesticide fipronil in somatic and generative cells of mice. *Russian Journal of Genetics,* 52:491-497.

Maeda, M., Yokoyama, T., Kitauchi, S., Hirano, T., Mantani, Y., Tabuchi, Y., and Hoshi, N. 2021. Influence of acute exposure to a low dose of systemic insecticide fipronil on locomotor activity and emotional behavior in adult male mice. *J Vet Med Sci* 83:344-348.

Mohammed, A. T., Imam, T. S., Farag, M. R., and Ghoneim, M. H. 2016. Assessment of the toxic impacts of acute exposure to fipronil insecticide on Japanese quails. *Japanese Journal of Veterinary Research* 64:S243-S249.

Quesnot, N., Rondel, K., Audebert, M., Martinais, S., Glaise, D., Morel, F., Loyer, P., and Robin, M. A. 2016. Evaluation of genotoxicity using automated detection of gammaH2AX in metabolically competent HepaRG cells. *Mutagenesis* 31:43-50.

Santos, A. T., Valverde, B. S. L., De Oliveira, C., and Franco-Belussi, L. 2021. Genotoxic and melanic alterations in Lithobates catesbeianus (anura) tadpoles exposed to fipronil insecticide. *Environ Sci Pollut Res Int* 28:20072-20081.

Tisch, M., Faulde, M., and Maier, H. 2007. Genotoxic effects of insecticides in current use on mucosal epithelial cells from human tonsil tissue. *Hno* 55 Suppl 1:E15-22.

Uçar, A., Parlak, V., Çilingir Yeltekin, A., Özgeriş, F. B., Çağlar, Ö., Türkez, H., Alak, G., and Atamanalp, M. 2021. Assesment of hematotoxic, oxidative and genotoxic damage potentials of fipronil in rainbow trout Oncorhynchus mykiss, Walbaum. *Toxicol Mech Methods* 31:73-80.

US EPA 2012a. Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment. Office of Pesticide Programs. United States Environmental Protection Agency, Washington, D.C.

US EPA 2012b. TSCA mammalian erythrocyte micronucleus test. Code of Federal Register 40: 799.9539. United States Environmental Protection Agency, Washington D.C.

US EPA 2020. Fipronil: Draft Risk Assessment for Registration Review. Document ID: EPA-HQ-OPP-2011-0448-0076. United States Environmental Protection Agency, Washington D.C.

Yildirim, N., and Agar, G. 2016. Determination of genotoxic effects of fipronil in Vicia faba using random amplified polymorphic DNA analysis. *Toxicol Ind Health* 32:1450-1455.

Ziliotto, L., Luna, S. P. L., Fihlo, D. A. A., Resende, L. O., Aun, A. G., and Braz, M. G. 2017. Genotoxicity assessment of fipronil (frontline plus®) in Canis familiaris. *Pesq. Vet. Bras.* 37:257-260.