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MEMORANDUM

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DATE: February 9, 2022

SUBJECT: Response to comments by ISAGRO USA, Inc. on DPR's 2020 Allyl Isothiocyanate Draft Risk Characterization Document

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Background

The registrant, ISAGRO USA, Inc., reviewed the draft Risk Characterization Document (RCD) for allyl isothiocyanate (AITC) (July 31, 2020) prepared by the Human Health Assessment Branch (HHA) of the Department of Pesticide Regulation (DPR). Their comments were submitted to HHA on October 5, 2020. This memorandum contains HHA's responses to comments by ISAGRO USA specific to the toxicology, hazard identification, and risk characterization sections of the RCD. Responses to comments pertaining to the Exposure Assessment Document are covered in a separate memorandum.

This memorandum is divided into three parts to correspond to comments received from ISAGRO on the Executive Summary, Toxicology Profile, and Risk Assessment sections of the RCD. Note that references cited in the ISAGRO review are not included in the reference section of this memorandum. Likewise, every effort has been made to ensure that any references to tables in the draft or final RCD are clear.

DPR sincerely appreciates the efforts taken made by the Registrant and its representatives to review the draft RCD. When appropriate, ISAGRO's comments were taken into account in the final AITC RCD. Responses to specific comments are detailed below.

Comments on the Executive Summary

ISAGRO Comment 2.1:

Page 1, paragraph 1, last sentence: "*DPR initiated the risk assessment process for AITC in 2018 due to its proposed use and based on evidence that* ***it may cause reproductive toxicity*** [emphasis added by Registrant]*, genotoxicity, and oncogenicity in animal studies (DPR, 2018).*"

There may have been an indication in the literature that AITC could cause developmental toxicity, but we don't [sic] know of any evidence in the literature that AITC could cause reproductive toxicity.

> **DPR Response:** AITC caused developmental and/or reproductive toxicity in oral studies with rodents. Increases in total resorptions and dead fetuses were noted in a developmental study in mice. In the rat 2-generation reproductive toxicity study, a treatment-related reduction of pup survival and pup weight, and an increased incidence of bilateral cataracts and retinal dysplasia, were evident in the F1generation.

ISAGRO Comment 2.2:

Page 1, paragraph 3, last sentence: "*AITC is also corrosive at the point of contact.*"

We suggest clarification of the sentence to say something like: "At high concentrations AITC is also corrosive at the point of contact."

> **DPR Response:** DPR classifies compounds as corrosive or non-corrosive based on chemical characteristics of the pure active ingredient, the results of the required FIFRA dermal irritation studies, and the US EPA Toxicity Categories found in the Review Manual (see https://www.epa.gov/pesticide-registration/label-review-manual). Technical grade AITC was shown to be corrosive in rabbits. The RCD was updated to specify technical grade AITC is corrosive at point of contact.

ISAGRO Comment 2.3:

Page 2, Paragraph 3: "*Because the lowest tested concentration in the study represented the LOEL, a default factor of 10 was applied to estimate the acute POD of 2.5 ppm, which was then used to estimate the human risk from acute/short term inhalation exposures to AITC.*"

We believe that the default factor is too high given the mild effect in the 25-ppm group and suggest a factor of 2 or 3 instead. See elaboration and support for this perspective in response to Section D.

> **DPR Response:** Decreased motor activity (~33-57% compared to control), and decreased rearing counts were used as the critical endpoints for selecting the LOEL of 25

ppm from the study of Herberth (2017). When a study does not include an experimental NOEL or the dataset could not be modeled, DPR's current practice for dose-extrapolation is to use a default factor of 10 (DPR, 2011). DPR will apply appropriate adjustments if data are available to address severity of the critical effect of interest, such as objective severity grading scores for the critical effect. Since such information was not provided in this study, DPR applied a default extrapolation factor of 10 to the LOEL from Herberth (2017) to derive the acute inhalation POD.

ISAGRO Comment 2.4:

Page 2, paragraph 4: "*The effects at the LOEL included metaplasia of the respiratory epithelium, degeneration of the olfactory epithelium* ***and decreased motor activity*** [emphasis added by Registrant]."

Significantly lower total motor activity and ambulatory counts were reported only in the high dose group, not in the LOEL group in this subchronic inhalation study.

DPR Response: The study design used by Randazzo (2017) was inadequate to exclude the non-statistically significant changes in motor activity at 10 ppm due to the high inter-individual variability in the data (changes in ambulatory counts and total motor activity counts did achieve statistical significance in 25 ppm males). A sample size greater than 10 animals per group would have been be required to capture a statistically significant treatment effect at the mid dose (10 ppm). Nonetheless, DPR concluded that the >20% decrease in average motor activity observed at both 10 ppm and 25 ppm was sufficient to support a LOEL designation, despite the lack of statistical significance at the former dose.

ISAGRO Comment 2.5:

Page 2, paragraph 5: "*The critical chronic inhalation POD of 0.5 ppm was based on the critical subchronic inhalation POD of 5 ppm for metaplasia of the respiratory epithelium, degeneration of the olfactory epithelium,* ***and decreased motor activity in rats*** [emphasis added by Registrant]."

Slightly lower total motor activity and ambulatory counts were reported only in the high dose group (25 ppm), not in the LOEL group (10 ppm) in this study. We suggest a clearer sentence would be something like: "The critical chronic inhalation POD of 0.5 ppm was based on the critical subchronic inhalation POD of 5 ppm for metaplasia of the respiratory epithelium and degeneration of the olfactory epithelium, at the LOEL exposure of 10 ppm.

DPR Response: See the response to ISAGRO Comment 2.4.

ISAGRO Comment 2.6:

Page 4, Summary Table 1: It is confusing to have a "Summary Table 1" on page 4 of a document and a Table 1 on page 12 of the same document.

> **DPR Response:** The table header in the Executive Summary has been removed to avoid confusion.

ISAGRO Comment 2.7:

Page 4, last paragraph: "*A summary of the MOE calculations* ***is found in the Risk Characterization section*** *and the supporting technical documentation is found in Exposure Assessment and the Air Concentration Tables* ***found in Appendix 1 and Appendix 2, respectively*** [emphasis added by Registrant]*."*

Please specify that the Risk Characterization section is a subsection of Section D (Risk Assessment) to make it easier to find. Also note that there are two Appendix 1's (page 87 of the RCD, and pg 138 of the full pdf), and two Appendix 2's (pgs 166, and 191 of the full pdf).

> **DPR Response:** Appendices of the Risk Characterization Document have been renamed. Instead of Appendices 1 – 3, those sections will be referred to as Appendices A – C. This should avoid any confusion with the numbered Appendices from the Exposure Assessment Document. In addition, the final Exposure Assessment Document will be issued as a separate document.

Comments on the Toxicological Profile

ISAGRO Comment 3.1:

Page 15, Paragraph 3: "*Herberth (2017) evaluated the neurotoxic potential of AITC vapor in rats following a single 4- hour whole-body inhalation exposure. This was followed up with a second rat study that tested the lethality of AITC as an aerosol following a single 4-hour exposure using a nose-only exposure apparatus in rats (Lowe, 2012)."*

The sentence states that the acute neurotoxicity study that exposed rats to AITC vapor for 4 hours was followed up with an acute median Lethal Concentration (LC50) study that exposed rats to an AITC aerosol for 4 hours. While it is true that both studies were done, the aerosol study was done first. The intent of the LC50 study is always to identify the median lethal concentration with a 4- hour exposure (concentration that results in death of 50% of the rats exposed). The acute neurotoxicity study is a very different study, intended to characterize any behavioral or structural changes in the central or peripheral nervous systems of the exposed rats.

DPR Response: The RCD has been updated in section C.2.1 to clarify that Lowe (2012) preceded Herberth (2017).

ISAGRO Comment 3.2.1:

Page 15, Paragraph 4: "*The major effects of acute inhalation exposure to AITC in rats were mortality, weight loss, decreased motor activity and neuromuscular performance, point of contact effects (i.e., crusty nasal / oral deposits, ocular and/or nasal discharge), decreased respiratory rate, and decreased body temperature. Higher exposure concentrations led to greater severity of the observed effects. Decreased motor activity and* ***neuromuscular performance were observed at the lowest tested concentration****, leading to an acute inhalation LOEL at 25 ppm (Herberth, 2017).* ***NOEL values were not set for either study*** [emphasis added by Registrant]. *Nose-only exposure to comparable air concentrations of aerosolized AITC (Lowe, 2012) appeared to induce more severe toxic effects than those noted after whole body inhalation exposure to vaporized AITC (Herberth, 2017). For example, mortality is seen with aerosolized AITC by nose-only exposure at 51 ppm, whereas no mortality was observed even at 125 ppm vaporized AITC by whole-body exposure. The studies are summarized below."*

Paragraph 4 discusses the acute neurotoxicity study and the LC50 study as though the studies are comparable, but they are very different studies conducted for different purposes, with different effects. We suggest that paragraph 4 be separated into 2 paragraphs, one to summarize the LC50 study and one to describe the acute neurotoxicity study.

DPR Response: DPR examined both Herberth (2017) and Lowe (2012) to determine the acute effects of AITC. Although the two studies are presumably designed for different purposes, the effects seen in these studies were the result of acute exposure to AITC (aerosol or vapor). Consequently, it was appropriate to discuss both acute inhalation studies in the same paragraph.

ISAGRO Comment 3.2.2:

There was no impact of AITC exposure at the lowest concentration on neuromuscular performance in the acute neurotoxicity study, so that sentence (bolded above) should be reviewed and revised.

DPR Response: The sentence has been revised in the RCD in section C.2.1 to more precisely reflect the measured parameter.

ISAGRO Comment 3.2.3:

The statement that NOEL values were not set for either study should be removed or redirected to include only the acute neurotoxicity study because NOELs are never identified for an LC50 study.

> **DPR Response:** DPR stands by the conclusion that NOEL values were not set for either study. DPR notes that while LC50 studies are not specifically designed for POD determination, they can include a NOEL dose. For example, the DPR 2006 RCD for sulfuryl fluoride established NOELs from several LC50 studies that were in the same range as the critical acute POD.

ISAGRO Comment 3.3:

Page 15, last paragraph: "*The details for this non-peer-reviewed study were sourced both from the **patent application and a book chapter** that highlighted the invention…*[emphasis added by Registrant]"

Citing a book chapter and a patent to support a scientific RCD seems unusual. I would expect the primary citation from the book to be included. This has always been the requirement for registrants submitting to DPR.

> **DPR Response:** Both the book and patent application citations have been cited in the final RCD.

ISAGRO Comment 3.4:

Page 16, paragraph 3: *"Investigators identified the peak time of effect occurred at 2 hours into the exposure period. Functional observational battery **(FOB) measurements and motor activity assessments were initiated mid-exposure (approximately 2 hours after the start)*** [emphasis added by Registrant]*, and on days 7 and 14."*

The FOB measurements were taken within 2 hours after exposure, not in the middle of the exposures. This error should be corrected throughout the RCD document.

> **DPR Response:** The correction has been included in the final RCD.

ISAGRO Comment 3.5:

Page 17, Paragraph 4: "*On Day 0, recording of* ***motor activity counts was initiated approximately 2 hours after beginning of exposure*** [emphasis added by Registrant]*, i.e., at the midpoint of exposure."*

Recording of the motor activity counts was conducted within 2 hours after exposure. This error in study design understanding is repeated throughout the RCD.

> **DPR Response:** Corrections have been included in the final RCD at all appropriate points.

ISAGRO Comment 3.6:

Page 18, paragraph 3: "*Whole-body inhalation exposure to AITC in rats showed concentration-dependent effects…*"

We suggest the word vapor be inserted in the sentence to distinguish this acute neurotoxicity study from the LC50 aerosol study, for example, "…exposure to AITC vapor in rats…"

> **DPR Response:** The word "vapor" has been added to the quoted sentence to more precisely describe the exposure paradigm.

ISAGRO Comment 3.7:

Page 32, Table 6, Randazzo study summary: "*Mild-to-moderate degeneration of olfactory epithelium in males and females; mild metaplasia of respiratory epithelium in males;* ***decreased motor activity in both sexes*** [emphasis added by Registrant]."

The apparent decrease in motor activity was not statistically significant in the LOEL group compared to the control group. There was high variability in the control group motor activity reflected in very high standard deviations. The standard deviations in the total and ambulatory motor activity counts of the control males were 42% and 53% of the total number of counts, and the standard deviations in the total and ambulatory motor activity counts of the control females were 54% and 66% of the total number of counts. Given this variability in behavior of control rats, a difference between control group and LOEL treated group motor activity was not determined.

> **DPR Response:** As indicated by the Registrant, the study design lacked adequate power to statistically differentiate control and test motor activities due to the high variability of the control data. As such, additional text has been added to the final RCD to clarify the study's limitations. In any case, DPR notes that while not statistically significant, the possibility of biological significance exists, because the effect on motor activity trends with increasing dose. This apparent dose dependence of motor activity along with the

statistically significant endpoints (i.e., metaplasia of respiratory epithelium and degeneration of olfactory epithelium; Table 6 of the draft RCD) provide additional support for setting the study POD at 5 ppm.

Comments on the Risk Assessment

ISAGRO Comment 4.1:

Page 52. Table 15: Footnotes a and b don't seem to refer to the correct text.

DPR Response: The footnotes for Table 15 have been updated for correctness.

ISAGRO Comment 4.2:

Page 52, Paragraph 1: "*The LOEL from this study was 25 ppm based on statistically significant decrements in total motor activity (males and females), ambulatory activity (males and females), and rearing (females). As this was the lowest dose tested,* ***the critical ENEL (estimated no effect level) of 2.5 ppm was calculated by invoking a UF of 10*** [emphasis added by Registrant]*."*

The LOEL effects in the Herberth (2017) acute neurotoxicity study were very subtle and consequently, the standard default conversion (uncertainty) factor of 10 should be reduced to a factor of 2 or 3. The subtle effects were a transient decrease in motor activity counts in the first 10 to 20 minutes of the one-hour testing period, and a decrease in rearing incidence during the same testing period that was performed within 2 hours after the inhalation exposure to AITC vapor.

Criteria used to reduce the default LOEL to NOEL conversion factor have been identified by the US EPA and in the public literature and include severity of the effect, the shape of the dose-response curve, and the relationship of endpoints (US EPA, 1994; Dankovic et al., 2015). In this case, the LOEL from the Herberth (2017) acute neurotoxicity study was based on minor motor effects of reduced rearing and motor activity, compared to the more severe neuromuscular effects reported in the high dose, 75 ppm group. That said all effects noted at all dose levels in the study were transient; no behavioral or other effects were reported 7 or 14 days after exposure. The result of reducing the conversion factor from 10 to 2 or 3 would be a POD of between 8 ppm and 12.5 ppm. Furthermore, the acute duration ENEL of 2.5 ppm does not make sense in the context of the subchronic NOEL of 5 ppm. Normally a short-term NOEL (or POD) is higher than a longer term NOEL. The shorter term NOEL would be divided by an uncertainty factor to derive an ENEL for a longer duration, as presented on page 51, paragraph 3: "*Likewise, if a chronic POD was not available, it was derived by dividing the subchronic POD by a factor of 10 to provide a duration extrapolation*." Based on this information, we would expect the acute duration ENEL to be higher than the subchronic NOEL of 5 ppm.

> **DPR Response:** See our response to ISAGRO Comment 2.4. Consistent with DPR's current practice, a LOEL-to-NOEL extrapolation factor of 10 was applied to derive the acute inhalation POD of 2.5 ppm, which is lower than the experimentally derived subchronic inhalation POD is 5 ppm. The real difference between acute and subchronic inhalation PODs will only be known upon further experimentation. For further discussion see Risk Appraisal, Section E.1.3 Subchronic Inhalation POD in the final RCD.

ISAGRO Comment 4.3:

Page 52, Paragraph 2: "*In an acute inhalation study conducted by Lowe (2012), Sprague-Dawley* ***rats sustained mortality****, tremors, irregular respiration, hypoactivity, nasal and/or ocular discharge* ***at the low dose of 51 ppm, resulting in an ENEL of 5.1 ppm (i.e., LOEL divided by 10)*** [emphasis added by Registrant]."

The study described is an acute toxicity study intended to identify the median lethal concentration (LC50) of the test substance and is not intended for risk assessment. The LC50 study is required for registration to support pesticide product label language only. In this study the AITC was aerosolized as is customary for acute inhalation studies conducted with liquid test substances. The AITC aerosol liquid particles or droplets in this study were sized to be deposited on the rat lung cell surfaces. Humans never will be exposed to aerosolized AITC from the proposed use of the product.

> **DPR Response:** DPR evaluates all available information to understand the toxicity of any compound, including AITC. Therefore, effects observed in Lowe (2012) were evaluated and compared to Herberth (2017) for similarities and differences in both study design and effects. Because other acute data were lacking, DPR selected Herberth (2017) to derive the acute inhalation POD.

ISAGRO Comment 4.4:

Page 52. Paragraph 2: "*However, it also reported more severe effects (e.g., tremors and death) at lower concentrations than observations reported in the Herberth (2017) study. This could be attributed to the nature of the test article (AITC vapor in Herberth versus aerosol in Lowe) or the mode of exposure (whole-body in Herberth versus nose-only in Lowe).*"

The greater toxicity reported in the Lowe (2012) study compared to the Herberth (2017) study is certainly because the Lowe study exposed rats to an aerosol of AITC and the Heberth study exposed rats to a vapor (gas) of AITC. The Herberth (2017) acute neurotoxicity study exposed the rats to AITC vapor, which is not deposited on the lung surfaces, while the aerosol of the Lowe study is deposited on the lung surfaces. The only way humans could be exposed to AITC by the inhalation route from the proposed use of the product is as a vapor. As the RCD acknowledges in the discussion of blood:gas (air) partition coefficients (pg 57), vapor travels

through the respiratory tract and is absorbed with different characteristics than particulates, including aerosols (EPA, 1994).

DPR Response: No response required.

ISAGRO Comment 4.5:

Page 52. Paragraph 2: *"Regardless, the ENEL of 5.1 ppm established by Lowe (2012) was supportive the critical ENEL of 2.5 ppm, even considering the differences in these two studies."*

Extrapolation from an aerosol exposure lethality study to a vapor exposure for risk assessment is of questionable utility. Just because the numbers are in the same general range does not mean there is a real relationship between the two.

DPR Response: DPR considers the proximity of the two endpoints to be mutually supportive.

ISAGRO Comment 4.6:

Page 58, Acute HECs Section:

"POD_{ADJ} *(ppm) = POD (ppm) x* (H_a/H_h)
0.42 *ppm = 2.5 ppm x (4 hours/day*$_{rat}$ */ 24 hours/day*$_{human}$*)"*

We would expect the acute exposure POD to be greater than 2.5 ppm based on the information presented above, including the fact that the subchronic POD is 5 ppm.

DPR Response: Due to the lack of an experimentally derived acute inhalation NOEL, DPR applied a LOEL-to-NOEL extrapolation factor of 10 to convert the study LOEL from Herberth (2017) into an estimated acute NOEL, as discussed above.

References

DPR. 2011. Default "Uncertainty Factors" for noncancer endpoints. 1-4.

Herberth, M. T. 2017. Acute Inhalation Neurotoxicity Study of IR9804 in Sprague-Dawley Rats. Product Safety Labs, Dayton, Nj (5940): Isagro Usa, Inc. (DPR Vol. No. 50544-0025, Record No. 298558) 1400.

Lowe, C. 2012. Acute Inhalation Toxicity Study In Rats. Product Safety Labs, Dayton, Nj (5940): Isagro Usa, Inc. MRID 48824105. (DPR Vol. No. 50544-0009, Record No. 279508) 60.

Randazzo, J. 2017. A 13-Week Whole-Body Inhalation Combined Subchronic Neurotoxicity/Toxicity Study of IR9804 in Sprague-Dawley Rats. Product Safety Labs, Dayton, Nj (5940): Isagro Usa, Inc. (DPR Vol. No. 50544-0026, Record No. 298559) 2597.