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DATE: October 19, 2021

SUBJECT: RESPONSE TO THE EXTERNAL SCIENTIFIC REVIEW OF THE DEPARTMENT OF PESTICIDE REGULATION'S RISK CHARACTERIZATION DOCUMENT FOR ALLYL ISOTHIOCYANATE (AITC)

Introduction

In accordance with Health and Safety Code section 57004, the California Department of Pesticide Regulation (DPR) requested external peer review of its draft risk and exposure assessment document entitled "Allyl Isothiocyanate: Draft Risk Characterization Document: Occupational and Bystander Exposures." The intent of the external peer review of the human health assessment of allyl isothiocyanate (AITC) was to review the science that will serve as the basis of the designation of this specific pesticidal active ingredient as a restricted material, and in particular, the toxicological evaluation that resulted in the recommendation of a 14 parts per billion (14 ppb) reference concentration for bystander acute exposures. The documents that underwent external scientific review may also provide scientific basis for registration of future product formulations and the development of any mitigation strategies relative to AITC use.

DPR requested reviewer expertise in human health risk assessment, inhalation toxicology, and exposure assessment/air dispersion modeling. DPR received responses from the reviewers listed below.

Alison Elder, PhD, Associate Professor [Responded to Charge Questions 1, 2 and 7]
Dept. of Environmental Medicine
School of Medicine and Dentistry
University of Rochester Medical Center
601 Elmwood Ave., Box EHSC
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Judith T. Zelikoff, PhD, Professor [Responded to Charge Questions 1, 2, 3 and 4]
Dept. of Environmental Medicine
NYU Grossman School of Medicine
341 E. 25th Street
New York, NY 10010

Thomas O. Spicer, PhD, Professor [Responded to Charge Questions 6, 8 and 9]
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This memorandum contains DPR's responses to the external scientific review of the AITC Risk Characterization Document (RCD). Please note that table numbers refer to those within the draft RCD or its appendices. The references listed at the conclusion of this document are those that are new to the final version of the RCD, currently in preparation; all remaining references cited herein can be found in both the draft and final versions of the RCD. All comments are direct quotes from reviewers.

The Peer Reviewers were asked to determine whether the RCD is based upon sound scientific knowledge, methods, and practices. In addition, they were requested to make determinations on the following scientific assumptions, findings, and conclusions:

1. **A default 10x extrapolation factor was used to establish the critical acute point of departure (POD) of 2.5 ppm.**

Dr. Elder Comment 1-1: The document describes an approach where the rodent lowest-observed-effect-level (LOEL) of 25 ppm AITC was adjusted by a factor of 10 to derive a POD of 2.5 ppm. This ***may be too stringent*** [emphasis by author] given the findings of Goto et al. (2010). While the study was not peer-reviewed, it is in the 'right species' and can provide a guidepost for the POD considerations. Specifically, the study showed that people could be awoken 'with less discomfort' when AITC concentrations were kept below 15 ppm than when they were higher, suggesting that 15 ppm could be a LOEL. AITC has decent warning properties in that the odor threshold is lower than the concentration at which sensory irritant responses begin and these responses would likely lead to exposure avoidance behavior. Indeed, the decreased motor activity and irregular respiration that was observed in animals upon two-week exposures for several hours a day could be indicative of sensory irritant responses mediated via the TRPA receptor system. The main reason for raising this point is that it seems odd to promote reference concentrations (RfC) for acute exposures (Summary Table 1) that are similar to or lower than those for subchronic and chronic exposures. If the Goto study findings are considered, then a smaller adjustment factor could be used given that the interspecies component would no longer be needed, resulting in a POD that is somewhat higher than 2.5 ppm AITC as the starting point for deriving estimates for bystander and occupational acute exposures. This would also obviate the need for further adjustment related to interspecies variability when calculating the RfC.

DPR Response: Goto et al. (2010) was reported as part of a patent application by the inventors of an AITC-based fire alarm system for people with impaired hearing. The patent application stated that human volunteers were able to detect the odor of AITC in air at concentrations as low as ~1 ppm. The study's finding was that sleeping human subjects woke with less discomfort when AITC air concentrations were between 5 – 15 ppm than when concentrations were > 15 ppm. In general, studies conducted for purposes of patent applications do not incorporate the same scientific standards as those conducted for toxicity testing of pesticides. To be sure, human data can be of critical importance in the evaluation of a pesticide's effects on human populations if they are collected using rigorous safety protocols and the study utilized adequate statistical design. Although the Goto et al. (2010) study was conducted in humans, it was not selected as the critical study for acute AITC exposure for several reasons: (1) No detailed study data were available, making it impossible to evaluate the AITC air concentrations reported to have an effect on the subjects; (2) the number of adults tested (n=14) may not adequately represent the range of responses expected in a population, including those individuals considered to be most sensitive; (3) the response metric (awakening) is poorly understood and its toxicological significance unknown; (4) the study protocol, methods, and results were not peer reviewed; and, (5) a comprehensive toxicity evaluation designed to capture all effects of AITC in humans at a reasonable range of doses was not done. The details presented in the draft RCD

were sourced from the minimal study information provided in the patent application (Goto et al., 2010) and in the book chapter that described the invention (Brand, 2019). Multiple efforts to contact the study authors to obtain further study details were unsuccessful.

The acute RfC for AITC was derived from Herberth (2017). However, a study no-observed-effects level (NOEL) could not be established because toxicological effects were observed at the lowest dose tested (25 ppm). So, consistent with DPR's current practice, a default (LOEL)-to-NOEL extrapolation factor of 10 was applied to derive the acute inhalation POD of 2.5 ppm. DPR concurs that it is not commonplace for an acute point of departure (POD) to be a smaller value than a subchronic POD, in that the latter would generally result from longer durations of exposure to lower concentrations. Nonetheless, the Goto et al. (2010) study was of inadequate quality to be used to derive the acute POD. Further experimentation is needed to better characterize the difference between acute and subchronic inhalation PODs, as discussed in the Risk Appraisal section of the RCD. Additionally, more detail concerning each of the above issues has been added to the final RCD.

Dr. Zelikoff Comment 1-1: For the risk determination of Allyl isothiocyanate (AITC), only three papers were available for review by the DPR that utilized inhalation as the exposure scenario. Two studies were carried out in rats and submitted as FIFRA guideline studies and the other one a human patent study that tested an alarm device for aerosolized AITC in a contained room. The two rodent studies utilized a well-thought out design that evaluated the neurotoxic potential of AITC in rats following either a single 4- hour whole-body vapor or nose-only aerosol inhalation exposure. Both studies used valid and measurable health outcomes for setting the POD value, and excluded the point-of-contact endpoints such as nasal discharge, which were less valid for determining risk.

DPR Response: By way of clarification, there were three experimental studies conducted in rats (two acute and one subchronic). The fourth study was the human patent application mentioned in Dr. Zelikoff's comment. Text in the final RCD will be revised to ensure that this point is clear to the reader.

Dr. Zelikoff Comment 1-2: I agree with the DPR that the use of decreased motor activity and neuromuscular performance were excellent endpoints from which to derive the LOEL of 25 ppm. I also agree that: NOEL values could not be established from the aforementioned rodent studies; the two rat inhalation studies are valid for use in setting short term guidelines; and an uncertainty factor of 10 was a conservative UF to be used in this case.

DPR Response: No response required.

Dr. Zelikoff Comment 1-3: There were different outcomes between the two inhalation studies as was mentioned in the Draft Risk Characterization Document which were most likely due to AITC vapor vs. aerosol and nose-only vs. whole body exposure. Nose-only is likely to lead to a

higher tissue both locally and systemically tissues than whole body and could have led to the increased mortality seen with nose-only exposure. Also, more information should be provided in the document regarding the chemicals that may differ between AITC vapor and the gas/particle aerosol, particularly since the vapor was selected for POD.

DPR Response: DPR has reexamined the implications of nose-only versus whole-body the inhalation exposure, as well as those of vapor versus aerosol exposures. The findings are summarized in the following paragraphs and have been added to the final RCD in the Risk Appraisal section.

Whole-body vs nose-only exposure: Several studies in rats have examined the influence on respiratory uptake of whole-body versus nose-only inhalation exposure to titanium dioxide (TiO_2) and cigarette smoke (Yeh et al., 1990; Kogel, 2021; Mouderly 1989, Oyabu et al., 2015). While body burdens were similar between the two exposure methods, higher lung deposition and correspondingly higher lung inflammation were noted after nose-only exposure. These observations are consistent with Dr. Zelikoff's suggestion that AITC delivered through nose-only exposure might contribute to a greater lung burden, and thus greater lethality, than what is produced after whole-body exposure. In addition, as Dr. Elder notes, more severe outcomes may result from the significant stress to the animals produced by their insertion the restrictive nose-only devices. For example, higher ventilation rates resulting in higher regional or systemic uptake are one likely consequence of nose-only exposure. In any case, a critical endpoint based on whole-body exposure is appropriate, as it is likely to represent a physiologically realistic inhalation scenario.

Vapor vs aerosol exposure: In a nose-only exposure study by Marshall and Cheng (1983), rat body burdens resulting from inhalation of radiolabeled ethylene glycol were similar regardless of vapor or aerosol administration, although the aerosolized form was deposited locally in lung tissue at 6-fold higher levels than vapor. Moreover, 1.7-fold higher levels of the vapor form deposited in the nares, suggesting that the methodological differences in deposition to the respiratory system could be toxicologically consequential. Salem and Cullumbine (1960) compared the effects of vapors and aerosols of multiple aldehydes in mice, rabbits, and guinea pigs following whole-body inhalation exposure. Mean lethal concentrations were similar for both vapors and aerosols, suggesting that systemic toxicity was similar for the two forms. Based on the evidence for greater lung deposition of aerosolized ethylene glycol, it is possible that, as Dr. Zelikoff stated, similarly aerosolized AITC would be more toxic than the vapor form. This consideration has been added to the Risk Appraisal section in the final RCD.

Dr. Zelikoff Comment 1-4: Selection of inhalation endpoints for setting the POD should be one that is positive for both sexes, as was selected for the acute rodent studies by relying on ambulatory activity and total motor activity. If a LOEL or NOEL is only established in a single sex then it is my belief that two different PODs could be used for each sex. Alternatively, the

lowest value observed between the two sexes for a given endpoint could be used as the POD, but this choice should be thoroughly explained in the Document.

DPR Response: DPR sets regulatory PODs and reference values based on the lowest scientifically defensible dose or concentration regardless of gender. This is based on the principle that protecting the more sensitive sex will protect both sexes. Unfortunately, the AITC database currently lacks the depth of supporting evidence required to determine the existence of any sex-based differences at this time.

Dr. Zelikoff Comment 1-5: I agree with the selection of the critical study for this Risk Assessment Document being the whole body inhalation toxicity study in rats exposed for 4 hours to vaporized AITC. The nose-only exposure experiment should be discounted due to the high rate of mortality. The effects at the lowest tested dose (LOEL of 25 ppm) for both sexes included decreased ambulatory and decreased motor activity in both sexes. I also agree that a benchmark dose (BMD) modeling approach should not be used to establish the acute POD for AITC because of the high variability in the data. I also agree that a default dose extrapolation factor of 10 was appropriate and conservative for setting the POD, in this case.

DPR Response: No response required.

Dr. Zelikoff Comment 1-6: As the most relevant route of exposure for assessing risk for AITC are via inhalation, it makes perfect sense to me to use the DPR-selected whole-body exposure to set the POD, rather than dermal or drinking water studies. This study also revealed concentration dependent effects which go towards weight-of-evidence for the selection of the whole-body AITC inhalation study.

DPR Response: No response required.

2. **The critical chronic inhalation POD was estimated from the subchronic critical POD by applying a default duration extrapolation factor of 10. This was necessitated by the lack of chronic inhalation studies.**

Dr. Elder Comment 2-1: Because no chronic inhalation exposure studies were available for review, the document describes the findings from a single subchronic inhalation study to serve as a starting point for considering risks from longer-duration exposures. The reported NOEL from Randazzo (2017) was 5 ppm AITC. Responses at higher concentrations included degenerative and metaplastic changes in the nasal epithelium, as well as decreased motor activity. Subchronic oral studies showed effects related to preneoplastic changes in the urinary bladder; while these effects were not reported upon inhalation exposure, it is likely that systemic absorption would occur via this route and, so, the findings should not be completely discounted. Nevertheless, dosimetric extrapolation suggests that the proposed POD from inhalation exposure is likely to be

protective with respect to the documented responses following oral exposures. […] Accounting for differences in exposure duration, as well as the points regarding oral exposures and the fact that only a single subchronic inhalation exposure was available, ***the factor of 10 to derive the chronic exposure POD of 0.5 ppm AITC is warranted*** [emphasis by author].

> **DPR Response:** DPR recognizes that the limited inhalation database constituted the main toxicological uncertainty in this risk assessment. Although there were indications that effects seen in the oral studies were specific to this route, all oral studies (acute, subchronic, and chronic) were evaluated in parallel with inhalation studies in order to identify the most sensitive effect. DPR concurs with Dr. Elder that it is likely that AITC is absorbed systemically when administered by inhalation route. To ensure that the effects in the oral studies are accounted for, DPR employed a route-to-route extrapolation to generate equivalent external air concentrations from the oral NOELs and compared them to the subchronic and chronic inhalation PODs. As the actual inhalation PODs were lower than those generated by extrapolation, the former were considered protective of effects observed in both the oral and inhalation studies.

Dr. Zelikoff Comment 2-1: No studies evaluating the toxicity of AITC via the inhalation route were available, as the DPR points out. That being said, two long-term studies were reviewed in mice and rats exposed orally by gavage with either AITC or AITC rich substances (horseradish extract (HRE) for 103-wk, and evaluated for chronic toxicity in this risk assessment. The third oral study was focused on the ability of AITC to serve as a promoter for NNK-mediated carcinogenesis. I concur with the DPR's decision not to use the urinary oncology studies to derive the POD, because: the study design uses an historical basis for the control values; and the sex differential effects.

> **DPR Response:** By way of clarification, the NNK (4-(methylnitrosamino)-1-(3-pyridyl)butanone) study was designed to test whether a single dose of AITC had a protective effect against NNK-induced lung adenomas (Jiao et al., 1994a; draft RCD, p. 50). Tumor incidence was 100% in the presence of NNK regardless of the presence or absence of AITC, therefore a protective effect was not demonstrated. Text in the final RCD will be revised to ensure that this point is clear to the reader.

Dr. Zelikoff Comment 2-2: I agree with the DPR that the three rodent studies using an oral exposure paradigm should not be used to set a POD, as they differ substantially by exposure route which will likely result in different effects than by inhalation, and because of study design weaknesses in the NTP studies, including the low survival rate (58 – 74%) in the controls and possible infection in the mouse studies which will/could skew the outcomes.

> **DPR Response:** No response required.

Dr. Zelikoff Comment 2-3: As the subchronic LOEL of 10 ppm included degenerative lesions

in the olfactory epithelium and metaplasia of the respiratory epithelium and systemic effects at the same concentration (i.e., activity decrements), I agree with the use of the 13-wk inhalation study to derive the POD of 0.5 ppm from the subchronic POD of 5 ppm and using the full default value of 10, rather than 3 due to the limited inhalation database.

DPR Response: No response required.

3. PODs from oral studies were not used to establish critical PODs.

Dr. Zelikoff Comment 3-1: I agree with the DPR's conclusion that all critical points of departure (PODs) for this risk assessment are established from inhalation studies in rats. These studies were well designed and provided crucial information on multiple toxicologically relevant parameters, including motor activity, functional observational battery behaviors, and organ and tissue histopathology. My agreement for using the inhalation studies rather than the acute, sub-chronic or chronic oral studies are primarily based on the observed differences in physiological outcomes between oral and inhalation exposures and the fact that outcomes in oral studies are more inclined to be due to exposure route rather than treatment-specific.

DPR Response: No response required.

Dr. Zelikoff Comment 3-2: Sex differentials for ADME are important and should be discussed in the Risk Characterization Document, along with the fact that different amounts of urine are stored/excreted between the sexes and could account for differentially-observed effects.

DPR Response: In a pharmacokinetic study in rats with AITC by oral gavage or intravenous injection, significant sex differences were noted in: (*1*) the urine volume with females exhibiting increased (~2-fold) urine volume, and (*2*) the retention and concentration of AITC-derived radioactivity in urinary bladder at early time-points, with male rats having up to 16 fold higher levels of radiolabel, at 45 minutes (Ioannou et al. 1984). The authors attributed the latter to the larger volume of urine excreted by female rats resulting in dilution of metabolites. The inherent sex differences in urine volume production in rats, combined with potential sex differences in the diuretic effects of AITC, likely contributed to the reported sex differences in both the urine metabolite concentration and the urinary bladder lesions observed in the chronic oral study (NTP, 1982). Dietary AITC also generated a diuretic effect in male rats, although female rats were not tested in the study (Muztar et al., 1979). A discussion of the results of Muztar et al. (1979) has been added to the final RCD. It is noteworthy that the sex differences in the urinary bladder effects were not evident when AITC was administered in drinking water or by the inhalation route (Cho et al., 2017, Hasumura et al., 2010). In conclusion, the available data from the oral studies could not definitively establish sex differences in AITC metabolism and excretion. There are no data to inform on sex differences via the inhalation route.

Dr. Zelikoff Comment 3-3: Use of oral studies that employ extracts from horseradish or other cruciferous vegetables are not appropriate to set PODs for AITC inhalation exposure as they contain small amounts of other ingredients that could also be playing a role in observed toxicity, depending on the toxic potential of these minor ingredients.

DPR Response: No response required.

Dr. Zelikoff Comment 3-4: More information regarding the differences between nose-only and whole body, as well as between vapor and aerosols should be described in the Risk Assessment Document in greater detail as the differences observed in the studies may be due to these exposure variables.

DPR Response: See the response to Dr. Zelikoff's Comment 1-3.

Dr. Zelikoff Comment 3-5: In the Lowe study (2012) description in the Draft Risk Document, the Standard deviation of the AITC particles, as well as the MMAD should be provided.

DPR Response: Lowe et al., (2012) reported a mean MMAD of 1.93 to 2.29 µm with a geometric standard deviation of 2.06-2.07 for the 0.05 mg/L exposure concentration, and a mean MMAD of 2.88 to 3.03 µm with a geometric standard deviation of 2.48-2.53 for the 0.5 mg/L exposure concentration. These values have been added to the revised RCD.

Dr. Zelikoff Comment 3-6: Due to loss of body weight and changes in eating patterns, some oral study induced effects may be due to dehydration, nutritional deficit and/or body weight loss, and thus confirm that results should not be used for setting PODs.

DPR Response: No response required.

Dr. Zelikoff Comment 3-7: More information should be provided in the Risk Document for the nose-only study regarding the high incidence of mortality in the controls.

DPR Response: To clarify, Lowe (2012) was a LD50 study which did not include a control group. However, an expanded description of the study has been included in the Toxicological Profile section of the final RCD. In addition, DPR has reexamined the results from nose-only inhalation exposures and our findings have been added to the Risk Appraisal Section of the final RCD. For more on that topic, see the response to Dr. Zelikoff's Comment 1–3, above.

4. This RCD did not include a cancer risk estimate for AITC.

Dr. Zelikoff Comment 4-1: Chronic inhalation studies using AITC were not available for assessing a cancer risk estimate in humans or rodents. However, several long-term oral studies in rodents were available for review, but were not considered by DPR as they were thought to be either species-specific or associated only with the oral exposure route. Thus, these studies were deemed inappropriate for basing an inhalation POD.

I disagree with the statement/conclusion that because no carcinogenic effects were observed after a 13-wk inhalation exposure, the idea of urinary bladder tumors by inhalation was completely ruled out. The fact that bladder cancers did not appear after a 13-wk inhalation exposure is of no surprise given the extensive exposure time (i.e., 1 year) required for chemical exposures to produce cancers in exposed mice or rats. This, however, does not rule out using the 13-wk inhalation study as a POD, but the aforementioned statement is not accurate and at best uncertain and should be removed.

DPR Response: Dr. Zelikoff is correct to point out that 13 weeks is insufficient to allow for bladder tumor development. For this reason, the ability of inhaled AITC to induce such tumors was evaluated using the cancer mode of action evaluation framework developed by US EPA (USEPA, 2005). Using this framework, DPR concluded that urinary bladder hyperplasia was a necessary precursor for urinary bladder tumor formation. Accordingly, exposures that do not result in hyperplastic lesions in the urinary bladder would not be expected to cause urinary bladder tumors. The draft RCD explicitly recognized the uncertainty in this conclusion, particularly as route-specific chronic exposure studies were not available for evaluation. The data showed that hyperplasia was evident after just 2 weeks of oral dosing in the initial study of Cho et al. (2017) at a LOEL dose of 19 mg/kg/day AITC (NOEL = 6.1 mg/kg/day). Bladder papillomas and carcinomas were observed after 2 years of exposure at 15.7 mg/kg/day in the follow-up study (no tumors at 4.7 mg/kg/day), accompanied by bladder hyperplasia at the same and at the lower dose.

Two further observations are worth mentioning:

1. Bladder epithelial hyperplasia was coincident with tumors in both the 2-year rat drinking water study (Cho et al., 2017) and the AITC promotion study with the initiator BBN (N-butyl-N-(4-hydroxybutyl)nitrosamine) (Cho et al., 2017). This is consistent with the hyperplasia-to-tumor mode of action under discussion.

2. Hasumura et al. (2011) also noted induction of bladder epithelial hyperplasia after 13 weeks of oral exposure to AITC, although the study was terminated at that point, which did not allow sufficient time for tumor development.

Taken together, the observations of bladder epithelial hyperplasia and tumor formation in

rats supports the proposed mode of action. The rapid induction of hyperplasia noted by Cho et al. (2017) suggests that this organ responds within a matter of weeks to AITC or to its primary metabolite, N-acetyl cysteine allyl isothiocyanate (NAC-AITC). Since there was no evidence of bladder hyperplasia even at the end of the 13-week the inhalation study (Randazzo, 2017), we concluded that repeated inhalation exposure to AITC does not present a risk for bladder tumors at the concentrations tested. Nevertheless, in recognition of the limited inhalation database for AITC, and specifically the lack of a 2-year inhalation study to inform on tumor formation, we applied a full extrapolation factor of 10 to the subchronic inhalation POD to estimate the chronic inhalation POD. Typically, a factor of 3 is recommended when a 13-week subchronic study is used for duration extrapolation because the study covers about 13% of the 2-year rat lifetime (OEHHA, 2008, IPCS, 2014). The route-to-route extrapolation showed that the critical chronic inhalation POD for decreased motor activity and olfactory epithelial degeneration will be protective of any systemic toxicity, including bladder hyperplasia and tumors.

Dr. Zelikoff Comment 4-2: Given the study design shortcomings identified above in the chronic drinking studies performed by NTP, these rodent studies are not reliable for use to set a cancer risk estimate for AITC, not the least of which is the low survival rates of the control rats and the report of an infection in the chronically-exposed mice which can impact such factors as nutrition, behavior and immune response which is critical for tumor surveillance, all of which can lead to unreliable outcomes.

DPR Response: DPR carefully evaluated all available chronic studies including the rat and mouse chronic oral gavage study (NTP, 1982) and the drinking water studies (Cho et al., 2017), and concurs with Dr. Zelikoff's conclusion that they are not adequate to determine risk.

Dr. Zelikoff Comment 4-3: It is my contention that based on the chamber variables and uneven distribution of AITC in the chambers, the LOEL for non-neoplastic effects should not be based on cataract data.

DPR Response: Cataracts were not observed in the inhalation toxicity studies. Although cataracts were noted in a few oral studies, they did not represent the most sensitive effects. Instead, the most sensitive effects included decreased rearing counts and decreased motor activity following acute inhalation exposure, and metaplasia of the respiratory epithelium and decreased motor activity following subchronic exposure. These studies were the basis of the critical acute, subchronic, and chronic PODs. Based on the route-to route extrapolation results, DPR considers the critical inhalation PODs to be protective of all effects observed in the oral studies, including cataracts.

Dr. Zelikoff Comment 4-4: Cytoplasmic vacuolization is considered by many human pathologists as a "morphological phenomenon" that can be transient and form as a general

response that can occur with infections and/or a variety of natural and artificial low molecular weight compounds, and therefore not recognized as a specific pathologic endpoint. For these reasons, cataracts should not be considered an endpoint for POD consideration.

DPR Response: See response above (4-3).

5. Due to a lack of AITC exposure monitoring data, worker exposures to AITC were estimated using exposure monitoring data from 1,3-D and chloropicrin.

No external review comments were received on this charge question.

6. Due to a lack of AITC exposure monitoring data, worker exposures to AITC were estimated using exposure monitoring data from 1,3-D and chloropicrin.

Dr. Spicer Comment 6-1: In the Emission Memorandum, [a] review of AITC emission study data was summarized, and previously identified issues were resolved satisfactorily so that this data was used (after normalization to the maximum AITC application rate) in the summarized emission rates. The normalization process uses different rates which makes direct comparison of the reported values difficult (e.g., Table 5 is normalized to 340 lb/ac for drip and shallow shank application methods, but Table E1 normalizes drip to 245 lb/ac and 340 lb/ac for shallow shank). Furthermore, the differences between Table 5 and Table E1 are not clear (e.g., the maximum emission rate in Table 5 for shallow shank application with a tarp is not consistent with the value reported in Table E1 even though both are based on the same application rate). Finally, the AITC study considered both totally impermeable film (TIF) and polyethylene film (PE), but Table 1 does not make clear which of these films (or an average) were used in determination of the reported emission rate. […] My understanding is that I am asked to determine whether the scientific work product in support of Assumption 6 is based on sound knowledge, methods, and practices, and in response, I have determined that this assumption is based on sound knowledge, methods, and practices provided that the supporting conclusions are confirmed in the review process. The reports considered here provide justification for the use of AERMOD by DPR to estimate bystander exposures to AITC. There are aspects of the Emissions Memorandum that could be clearer, but the validity of the conclusions will likely be unchanged.

DPR Response: DPR appreciates Dr. Spicer's and other reviewers' comments to this point, and as such the emission rates in all the tables of the revised emission memo (Appendix 1 of the AITC Exposure Assessment Document (EAD)) are now normalized to the same application rates (327 lb/ac for broadcast shank, or 246 lb/ac for drip and bed/strip shank). For all the analyzed application methods, the emission rates and the source of these rates have been provided in the newly added Table 8 of the revised emission memo. Also as

indicated in Table 8, for tarp applications, emission rates were obtained from polyethylene (PE) tarped fields.

7. Dosimetric adjustments of air concentrations to account for pharmacokinetic differences between laboratory animals and humans were used to calculate reference calculations *[sic]* **(RfCs) and risk targets (i.e., target Margins of Exposure).**

Dr. Elder Comment 7-1: In general, the approach that is described in the document is sensible. It aligns with the US EPA's approach and assumes that interspecies differences are minimized based on an assumption of similar respiratory tract absorption rates (thus reducing the UFA from 10 to 3). The RfCs that were derived for subchronic and chronic exposure scenarios for workers should be amply protective. As mentioned above, though, ***the acute exposure RfCs are somewhat out of alignment with those for longer-duration exposures*** [emphasis by author]. Specifically, they are an order of magnitude lower than the subchronic RfC, which is odd. It is acknowledged that the single human study (Goto et al., 2010) was limited, but further consideration could be helpful in reducing uncertainties related to species-specificity and response threshold, thus producing both a less stringent POD and further reducing the UFA from 3 to 1.

DPR Response: As Dr. Elder observed, the RfC values were calculated from the critical PODs using dosimetry and exposure time adjustments to derive HECs, which were then divided by appropriate uncertainty factors. The acute POD is 2-fold lower than the subchronic POD (2.5 ppm and 5 ppm, respectively). The acute HEC and RfC for workers are both 3-fold lower than the respective subchronic values (HEC workers - 1.25 ppm and 2.75 ppm; RfC workers - 42 ppb and 125 ppb, respectively), which are less than an order of magnitude difference (see summary Table 1). The difference in the ratios between the subchronic and acute values results from the different exposure durations (4 hr/8 hr for acute, 6 hr/8hr for subchronic).

The acute RfC for workers (42 ppb) is lower than the longer duration subchronic RfC (125 ppb). The apparent discrepancy between the acute and subchronic RfCs results from the derivation of the PODs. The subchronic RfC is based on an experimental NOEL. In the case of the acute RfC, there was no experimental NOEL. Instead, the acute POD was based on the estimated NOEL, which resulted in a lower value (estimated NOEL 2.5 ppm = LOEL 25 ppm ÷ UF_L of 10). For further discussion see Risk Appraisal, Section E.1.3 Subchronic Inhalation POD in the final RCD. In regards to using Goto et al. (2010) to reduce uncertainties related to species-specificity and response threshold for the acute POD, DPR did not consider this study to be conducted under sufficiently controlled conditions to be used for POD or UF establishment (also see DPR's response to Dr. Elder Comment 1-1).

Summary Table 1. Points of Departure (PODs) and Reference Concentrations (RfCs) for Workers and Residential and Occupational Bystanders for Inhalation Exposure to Allyl Isothiocyanate

Duration/ Route	Acute Inhalation			Subchronic Inhalation	Chronic Inhalation
Parameter	Residential Bystander (child and adult)	Worker	Occupational Bystander	Worker	Worker
POD (ppm)	2.5	2.5	2.5	5	0.5
POD_{HEC} (ppm)	0.42	1.25	1.25	3.75	0.375
UF_A	3	3	3	3	3
UF_H	10	10	10	10	10
UF_{TOTAL}	30	30	30	30	30
RfC (ppm)	0.014	0.042	0.042	0.125	0.0125
RfC (ppb)	14	42	42	125	13

Abbreviations: POD, point of departure; POD_{HEC}, human equivalent concentration; ppb, parts per billion; ppm, parts per million; RfC, reference concentration; UF_A, uncertainty factor to account for interspecies variability; UF_H, uncertainty factor to account for intraspecies sensitivity. *Additional detail regarding the derivation of the above values can been found in the RCD.*

8. Risks to workers were estimated for acute (short term), subchronic (seasonal), and chronic (annual, lifetime) exposures.

Dr. Elder Comment 8-1: The risk appraisal involved comparisons of the acute, subchronic, and chronic PODs with AITC exposures in 88 different modeled conditions to derive margins of exposure (MOEs). The target MOE was defined to be equivalent to the total uncertainty factor of 30 that was used in the RfC calculations for different exposure categories. For many of the 88 scenarios, the MOEs were lower than the target of 30, suggesting risks for adverse health outcomes. The exposures were all modeled due to the lack of data regarding real-world applications with AITC. My expertise is not related to exposure modeling, but it was curious that bystander exposures were often modeled to be much higher than occupational ones. Another consideration, as mentioned above, is that the POD for acute exposures may be too low, which would impact the MOE for this category of exposure. ***Nonetheless, in the absence of information, additional precautions are sensible*** [emphasis by author]. The document does not describe how the findings regarding the MOEs would change practices, if at all, with respect to AITC application in agricultural settings. As pointed out, applicators are instructed to wear respirators, for example. The use of respirators by other types of workers may be considered.

DPR Response: In regards to the exposure assessment, AITC inhalation exposures were estimated to be higher for bystanders than for handlers because the AITC product label requires handlers to use respirators. As shown in the exposure estimate tables in the section V. Exposure Assessment of the EAD, this assessment assumed respirators provided 90%

protection (Thongsinthusak et al., 1993). Use of respirators is not required for bystanders, therefore their inhalation exposure is estimated to be higher. In addition, it is worth noting that air dispersion modeling was only used to estimate bystander exposures. Due to the lack of AITC-specific data, handler exposures in this assessment had to be estimated with surrogate data from field studies that monitored worker exposures to chloropicrin and 1,3-dichloropropene. In regards to how the MOEs would change AITC applications in agricultural settings, DPR has initiated the mitigation of potential risks for this new active ingredient. The department has drafted a rulemaking package to make AITC a restricted material in California, which will restrict the types of applications allowed as well as require special use authorizations to protect both workers and downwind communities. The estimation of the MOEs and RfCs, as well as technical details found in the RCD and EAD, provide the scientific justification for some of those mitigation and registration decisions.

9. Risk to occupational and residential bystanders were estimated for acute exposures.

Dr Spicer Comment 9-1: My understanding is that I am asked to determine whether the scientific work product in support of Conclusion 9 is based on sound knowledge, methods, and practices, and in response, I have determined that this conclusion is based on sound knowledge, methods, and practices provided that the supporting conclusions are confirmed in the review process. […] Furthermore, the risk to occupational and residential bystanders were properly estimated for acute exposures.

DPR Response: No response necessary.

Responses to Additional Comments

DPR also received comments from reviewers on the RCD in general. Those comments, and DPR's responses, are listed below.

Dr. Elder Additional Comment 1: Table 3 reporting of the Goto et al. (2010) study could be modified to include a description of the findings regarding responses around a threshold of 15 ppm.

DPR Response: Details of effects observed in the Goto et al. (2010) have been added in the Acute Inhalation Toxicity section (C.2.1) of the RCD.

Dr. Elder Additional Comment 2: The study by Herberth (2017) was said to have been done via whole-body inhalation of AITC vapors, which could have interacted with structures throughout the entire respiratory tract. The Lowe study (2012) was done via nose-only inhalation exposure to an atomized mist (respirable droplets). With the data available, it seems that the aerosol deposition patterns and, therefore, the dosimetry may have been very different in these

two studies, possibly explaining the differences in outcomes. The nose-only exposure method of Lowe may have also contributed to more severe outcomes given the significant stress to the animals (no mention of adaptation phase prior to start of study), thus producing higher ventilation rates. These points could be added for clarity to help the reader understand the differences in findings between the two studies.

DPR Response: See response to Dr. Zelikoff's Comment 1-3.

Dr. Elder Additional Comment 3: Agree with the conclusion that the 25 ppm level is appropriately interpreted as being the acute exposure LOEL from rat inhalation studies. The main critical effects in these studies related to motor and ambulatory behavior. The reductions in respiratory rates at the higher concentrations are consistent with the sensory irritation potential of AITC.

DPR Response: No response necessary.

Dr. Elder Additional Comment 4: Agree with the conclusion that the 5 ppm level is appropriately interpreted as being the 13- week study NOEL in male and female rats that were exposed via whole-body inhalation to AITC. The main critical effects in these studies (at higher airborne AITC concentrations) included degeneration of the olfactory and respiratory epithelium in the nose, changes in motor activity, and losses in body weight. Interestingly, there were no reported changes in urinary bladder histology in this study, even at the highest concentration of 25 ppm in air.

DPR Response: See the response to Dr. Zelikoff's Comment 4-1 regarding the bladder histopathology.

Dr. Elder Additional Comment 5: The report is largely dominated by findings from oral exposures, i.e., drinking water, corn oil gavage, and feeding studies in rats and mice over various time frames using either AITC itself or horseradish extract, which contains a large fraction of AITC. Many of these studies are consistent in their reporting of urinary bladder epithelial preneoplastic (hyperplasia) or neoplastic changes (papilloma). There is also some evidence of other lesions such as cytoplasmic vacuolization of liver, cataract formation, and subcutaneous fibrosarcoma. AITC may also function as a tumor promoter in the urinary bladder, as evidenced by increased incidence of neoplastic changes, tumor volumes, and acceleration of pathology in rats that were pretreated with a nitrosamine. I performed my own calculations using a slightly different approach than the one described in the report in section D.1.2 to estimate the airborne concentrations to which humans would have to be exposed to reach similar whole-body doses (assuming 100% absorption via the respiratory tract) as those described for oral exposures. The airborne concentrations that I derived from these calculations were of a similar order as the NOEL of 5 ppm from the 13-week inhalation study (range of 2-18 ppm depending on exposure duration).

DPR Response: No response necessary.

Dr. Elder Additional Comment 6: While the oral studies contribute to a more thorough understanding of AITC toxicity, they are limited by the failure to define NOELs, as pointed out in the document, making it difficult to consider adjustments to the overall NOEL. The findings via the oral route also contradict those from the subchronic inhalation studies, where no urinary bladder pathological changes were found. This may suggest that the biodistribution and/or metabolism of AITC differ(s) as a function of route of exposure or that the means of delivery is a confounding factor (i.e., bolus). This analysis points to a suggested revision of the draft document, specifically the summary of findings from the chronic oral exposure studies as presented in Table 14 on page 49. Here, the conclusion from the NTP study in rats is incomplete, in that there was a finding of a positive association between exposure and urinary bladder papilloma incidence (conclusion included at the top of page 45, data presented in Table 11).

DPR Response: Table 14 has been updated to clarify that it only contains non-neoplastic endpoints. The evaluation and decision on the urinary bladder papilloma findings is described in the Oncogenicity section (E.1.7). In addition, see the response to Dr. Zelikoff Comment 4-1.

Dr. Elder Additional Comment 7: The route to route extrapolation that is presented on page 54 may need to be modified. First, there is an error, I believe, in the AITC milligram per cubic meter to ppm conversion factor. By my calculation, 1 mg/m^3 AITC is equivalent to 0.25 ppm AITC (mg/m^3 x 24.45/molecular weight). Also, the rationale for including an adjustment for weekly intake does not make sense when the starting value of 6.6 mg/kg/day represents daily intake. These things do not affect the interpretation regarding the utility of the oral exposures at all, so the comment is made for the sake of clarity and completeness. Showing more detail for the calculations in their entirety may be helpful.

DPR Response: Further details of the mg/kg/day to ppm conversion factor have been added to the RCD, section D.1.2.

The weekly intake adjustment was necessary for the route to route extrapolation. The rat oral POD was converted to a duration-adjusted air concentration by applying a time adjustment to the rat default breathing rate to account for the exposure regimen used in the rat study (6 hr/day, 5 days/week). Thus the rat 24-hour default breathing rate of 0.96 m^3/kg was multiplied by 6 hr/24 hr and 5 days/7 days to derive the rat duration-adjusted breathing rate of 0.17 m^3/kg. The rat equivalent inhalation POD (9.5 ppm) was then calculated by dividing the oral POD (6.6 mg/kg/day) by the duration-adjusted breathing rate and by the AITC conversion factor for mg/m^3 to ppm of 4.06.

Dr. Elder Additional Comment 8: It is unclear as to why the 2-week oral gavage studies from

NTP (1982) and Hasamura et al. (2011) are included in Table 6 with subchronic studies, as opposed to being grouped with other 2-week studies in Table 3 (a 2-week exposure is typically considered to be acute in nature).

> **DPR Response:** DPR considers effects resulting from a single treatment, as well as those resulting from exposures of up to 7 consecutive days, to be appropriate for purposes of acute and short term NOEL designation, excepting dermal sensitization studies, which involve repeated treatment to prime the immune response in order to detect an acute sensitizing effect. Table 3 lists all available acute studies, which for AITC includes exposure durations of 1 to 3 days. As such, the sensitization studies are part of the acute toxicity testing protocol and thus were appropriately placed in Table 3. The oral gavage studies from NTP (1982) and Hasamura et al. (2011) employed daily exposures for two weeks. Since this was longer than 7 days, DPR placed these studies in Table 6 (subchronic duration) in order to differentiate them from the acute and short term studies. DPR recognizes that the 2-week duration differs from the more convention 1-12 month duration for a subchronic oral study, but DPR considers the subchronic classification (i.e., Table 6) to be more appropriate.

References

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