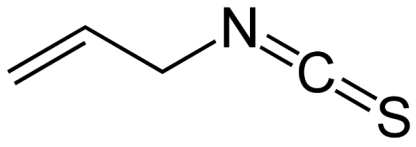
ALLYL ISOTHIOCYANATE

Draft Risk Characterization Document

Occupational and Bystander Exposures



July 2020

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Acknowledgment: We wish to thank Drs. Thomas Moore and Peter Leung from the Active Ingredient Section and Dr. Neelima Verma from the Product Formulation Section within the Department of Pesticide Regulation's Human Health Assessment Branch for their review of the toxicology data submitted in support of allyl isothiocyanate registration.

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List of Abbreviations

^{131}I	Iodine 131
AC50	Concentration at 50% bioactivity
ADI	Acceptable Daily Intake
ADME	Absorption, distribution, metabolism and excretion
AIHA	American Industrial Hygiene Association
ALT	Alanine aminotransferase
AITC	Allyl isothiocyanate
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BBN	N-butyl-N-(4-hydroxybutyl)nitrosamine
BMC	Benchmark concentration
$BMCL_{10}$	Lower confidence limit of the 10% benchmark concentration
BMD	Benchmark dose
$BMDL_{10}$	Lower confidence limit of the 10% benchmark dose
BMR	Benchmark response
BPM	Breaths per minute
BrdU	5-Bromo-2′-Deoxyuridine
BUN	Blood urea nitrogen
BW or bw	Body weight
CalPIQ	California Pesticide Illness Query
C_{max}	Maximum serum concentration of compound after dosing
DAF	Dosimetric adjustment factor
DAF_{POE}	DAF for portal of entry
DAF_{SYS}	DAF for systemic
DMA	Dimethylarsinic Acid
DNA	Deoxyribonucleic acid
DPR	Department of Pesticide Regulation
EAD	Exposure Assessment Document

EFSA	European Food Safety Administration
ENEL	Estimated no effect level
EPA	Environmental Protection Agency
F	Female
FDA	United States Food and Drug Administration
FDRL	Food and Drug Research Laboratories
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FOB	Functional observational battery
GD	Gestational day
GSH	Glutathione
HEC	Human equivalent concentration
HRE	Horseradish extract
LC_{50} or LD_{50}	Median lethal concentration or dose
LLNA	Local Lymph Node Assay
LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
M	Male
MMAD	Mass Median Aerodynamic Diameter
MOA	Mode of action
MOE	Margin of exposure
MW	Molecular weight
NAC	N-acetyl cysteine
NCI	National Cancer Institute
ND	Not determined
NNK	4-(methylnitrosamino)-1-(3-pyridyl)butanone
NOEL	No observed effect level
NTP	National Toxicology Program
OEHHA	Office of Environmental Health Hazard Assessment
PBPK	Physiologically based pharmacokinetic
PEITC	Phenyethyl isothiocayanate
PGR	Progesterone receptor
PND	Postnatal day
POD	Point of departure
POD_{ADJ}	POD duration adjusted
POD_{HEC}	POD human equivalent concentration
POE	Portal of entry
ppm or ppb	Parts per million or parts per billion
RANOVA	Repeated measures analysis of variance
RCD	Risk Characterization Document
RED	Reregistration Eligibility Decision

RfC	Reference concentration
RXRB	Retinoid X Receptor Beta
S9	Liver homogenate fraction that contains Phase I and II enzymes
SD	Standard deviation
TRPA1	Transient Receptor Potential Ankyrin 1
UDS	Unschedules DNA sysnthesis
UF	Uncertainty factor
UF_A	Interspecies UF
UF_H	Intraspecies UF
UF_L	UF for LOEL-to-ENEL conversion
UF_S	UF for subchronic-to-chronic duration extrapolation
UF_{TOTAL}	UF composite
US EPA	United States Environmental Protection Agency

A. EXECUTIVE SUMMARY

The purpose of this Risk Characterization Document is to evaluate the risk to human health resulting from inhalation of the fumigant allyl isothiocyanate (AITC). In 2017, Isagro USA Inc. submitted a registration application to the Department of Pesticide Regulation (DPR) for the use of AITC as a pre-plant soil fumigant for food and non-food crops. According to DPR policy, a comprehensive pre-registrational risk assessment is conducted for all fumigants under consideration for use in California. 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, genotoxicity, and oncogenicity in animal studies (DPR, 2018).

Background

Allyl isothiocyanate (AITC) (3-isothiocyanatoprop-1-ene; CAS 57-06-7) is a naturally occurring plant compound. It is produced by mustard, horseradish, wasabi, and other cruciferous vegetables when the enzyme myrosinase reacts with glucosolinate sinigrin in the presence of water. AITC is federally registered as a biopesticide for use on food and non-food crops to control microbial pathogens, nematodes, and weeds (US EPA, 2013). US EPA has also approved the use of AITC in insect and animal repellants and as a feeding suppressant (US EPA, 2013).

The pesticidal mode of action of AITC is based on its ability to disrupt cellular integrity by damaging cell membranes and enzymes involved in intracellular processes, especially those related to respiration and energy production (USDA, 2014). In mammals, AITC activates sensory nerve fibers by acting as an agonist of the transient receptor potential ankyrin 1 (TRPA1) cation channel, which mediates the cellular influx of calcium and other cations. This interaction ultimately causes acute pain and local inflammation. AITC is also corrosive at the point of contact.

Scope of Risk Assessment

This assessment is focused on the inhalation toxicity of AITC to align with its proposed use as a chemical fumigant. Risks to workers, occupational bystanders, and residential bystanders, including vulnerable subpopulations, were estimated for acute exposures. Risks to workers were also estimated for subchronic (seasonal) and chronic (annual, lifetime) exposures. Both reference concentrations (e.g., air concentrations that are likely to be without appreciable risk of deleterious effects) and margins of exposure have been estimated from the available data.

However, the AITC inhalation toxicity database was limited, consisting of three inhalation studies in rats (two acute and one subchronic). In addition, no relevant human inhalation studies were located by systematic review and no inhalation studies were available to determine toxicokinetics, chronic toxicity, reproductive or developmental toxicity, or oncogenicity. A number of oral toxicity studies in laboratory animals were available. When possible, points of

departure (PODs) from subchronic and chronic studies oral studies were converted to inhalation PODs using route to route extrapolation. These extrapolated PODs were not used to establish critical PODs because of concerns about route specificity of observed effects and to avoid the introduction of additional levels of uncertainty. However, the values were helpful in determining if equivalent external air concentrations from the oral studies could generate effects at concentrations similar to those in the inhalation studies. Oral studies were also used to inform toxicokinetics, oncogenicity, and developmental toxicity.

Findings

Acute PODs were calculated to assess the inhalation risks posed by AITC for adult workers, occupational bystanders, and child and adult residential bystanders under short-term exposure scenarios. In addition, subchronic and chronic points of departure were calculated to assess the inhalation risks for workers under seasonal and annual exposure scenarios. Due to the lack of both air monitoring data and AITC use information in California, this analysis only assessed short-term exposures and associated risk for both occupational and residential bystanders.

Acute Toxicity: The critical acute inhalation POD of 2.5 ppm was selected from a whole body inhalation toxicity study in rats exposed to vaporized AITC. The effects at the lowest observed effect level (LOEL) of 25 ppm included decreased rearing counts and decreased motor activity. 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.

Subchronic Toxicity: The no observed effect level (NOEL) of 5 ppm from a 13 week inhalation toxicity study in rats was established as the critical subchronic inhalation POD. The effects at the LOEL included metaplasia of the respiratory epithelium, degeneration of the olfactory epithelium and decreased motor activity. The subchronic critical POD of 5 ppm was used to evaluate the human risks from seasonal inhalation exposures to AITC.

Chronic Toxicity: 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. A subchronic-to-chronic duration extrapolation was performed by dividing the subchronic inhalation POD by a factor of 10.

Oncogenicity: This draft Risk Characterization Document does not include a cancer risk estimate for AITC. Undifferentiated leukemia was observed in one oral oncogenicity bioassay. However, there is compelling evidence that the observations were artifacts of the study design and the selected rat stain (F344/N), rather than AITC treatment. Urinary bladder tumors were observed in two oral oncogenicity bioassays in rats. However, AITC by the inhalation route did not induce urinary bladder hyperplasia after 13 weeks of exposure. This

observation suggests that bladder effects were relevant for oral, but not inhalation exposures. Consequently, urinary bladder epithelial hyperplasia and bladder tumors induced by chronic oral exposure were unlikely to result from inhalation exposure. As a result, bladder tumor data were not used to calculate a cancer potency value. Likewise, fibrosarcomas were observed in a two year oral gavage study using rats. A role for AITC in fibrosarcoma induction was considered plausible. However, cancer potency analysis was precluded by the fact that the apparent effect was observed only at a single high dose.

It is important to note that an analysis of precursor lesions from both the inhalation and the oral studies suggested that precursor effects required for tumor development were specific to the oral route. This lack of experimental support is the primary justification for not assessing the cancer risk of AITC by the inhalation route.

Reference concentrations (RfCs) are target air concentrations that are likely to be without appreciable risk of deleterious effects. These values are calculated by dividing the critical endpoint concentrations by the uncertainty factors. Commonly used default uncertainty factors are 10x to account for interspecies variability (UF_A) and 10x to account for intraspecies (human) sensitivity (UF_H). Both uncertainty factors are themselves products of two separate components, a pharmacokinetic uncertainty factor of 3x and a pharmacodynamic uncertainty factor of 3x.

For AITC, DPR converted the critical PODs from the selected animal studies to human equivalent concentrations (POD_{HEC}) using dosimetric adjustment factors based on US EPA reference concentration (RfC) methodologies (US EPA, 1994; US EPA, 2012a). Because the dosimetric adjustment accounts for physiological and anatomical differences between humans and animals, the pharmacokinetic component in the UF_A can be reduced from 3x to 1x. Therefore, the total uncertainty factor for AITC is 30, a product of UF_A of 3 to account for pharmacodynamic differences between laboratory animals and humans and UF_H of 10 to account for an assumed 10-fold range of sensitivity within the human population. RfCs are calculated by dividing the critical human equivalent concentration (POD_{HEC}) by the total uncertainty factor. All values are summarized in Summary Table 1, below.

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[a] (ppm)	2.5	2.5	2.5	5	0.5
POD_{HEC}[b] (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[c] (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.

[a] Point of Departure (PoD): The critical acute PoD is an extrapolated no-effect level from the LOEL of 25 ppm for decreased rearing counts in females and decreased motor activity both sexes (rats) (Herberth, 2017). The critical subchronic POD of 5 ppm is for degenerative lesions in olfactory epithelium, metaplasia of respiratory epithelium, and decreased motor activity in males (rats) (Randazzo 2017). The critical chronic POD of 0.5 ppm is the duration-extrapolated subchronic POD (Randazzo 2017).

[b] The critical POD is adjusted by ratio of the experimental animal exposure duration to estimated human exposure duration (24 hours/day 7 days/week for residential bystanders, and 8 hours/day for 5 days/week for occupational exposures). The resulting duration-adjusted POD (POD_{ADJ}) is converted to a human equivalent concentration (POD_{HEC}) using a dosimetric adjustment factor (DAF) for either portal of entry effects (US EPA 2012) or systemic effects (US EPA 1994).

[c] Reference Concentration (RfC): Derived by dividing the POD_{HEC} by the total uncertainty factor (UF_{TOTAL}). Detailed equations are found in the Hazard Identification section.

The margin of exposure (MOE) is a quantitative tool used by DPR to determine the potential risk arising from exposure to a pesticidal active ingredient. A MOE is defined as the ratio of the POD value derived from the definitive acute, subchronic, or chronic studies to the estimated human exposure. The resulting value is compared to the acceptable or target MOE which, for purposes of this risk assessment, is equivalent to the total uncertainty factor (UF_{TOTAL}) of 30. Values at or above the target MOE are generally considered protective against the toxicity of AITC. Because this analysis is focused on risks from inhaling AITC, both the POD and the exposure values are expressed as air concentrations (in units of ppm or ppb).

$$\text{Margin of Exposure (MOE)} = \text{POD (in ppb)} / \text{Exposure concentration (in ppb)}$$

There are numerous exposure scenarios in this assessment that may carry risk for workers and bystanders. 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.

B. INTRODUCTION

Allyl isothiocyanate (AITC) is a naturally occurring plant self-defense compound that is proposed for use in California as a fumigant. It is approved for use as a food additive by the US Food and Drug Administration (FDA) (US FDA, 2018), and is registered for use by the US Environmental Protection Agency (US EPA) as an insect and animal repellent, insecticide, fungicide, herbicide, and nematicide for use prior to planting. It also has non-food uses (US EPA, 2013). In California, AITC has previously been registered as an animal repellent for formulations containing less than 5% AITC, but not as a fumigant or at concentrations greater than 5%. There are no current registrations of AITC in California.

B.1 Chemical Identification

AITC is a degradation product of sinigrin, a glucosinolate naturally produced by mustard, horseradish, wasabi, broccoli, and various other Brassicaceae. Glucosinolates break down to their respective isothiocyanates when the plant is damaged, creating a biochemical defense against herbivore attack. When sinigrin is exposed to the plant enzyme myrosinase and water, it degrades to AITC and glucose. Most Brassicaceae produce multiple glucosinolates that generate various isothiocyanates, although individual plant species often contain higher concentrations of one or two over the others. For example, horseradish contains at least nine isothiocyanates (in order of abundance): allyl isothiocyanate, 2-phenethyl isothiocyanate, *n*-butyl isothiocyanate, 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate, 5-hexenyl isothiocyanate, 5-methylsuphinylpentyl isothiocyanate, 6-methylsulphinylhexyl isothiocyanate, and 7-methylsuplinylheptyl isothiocyanate (Nguyen *et al.*, 2013). AITC can constitute as much as 78 to 86% of the isothiocyanates in horseradish (Nguyen *et al.*, 2013; Cho *et al.*, 2017). Other degradation products of glucosinolates potentially include goitrin, thiocyanate, nitrile, and epithionitrile, although these occur at much lower concentrations than the isothiocyanate products (Ishida *et al.*, 2014). AITC can also be synthetically manufactured.

AITC's effectiveness as a fumigant is likely based on its induction of oxidative stress, resulting in direct damage to cell membranes and proteins, including those related to respiration and energy production (Luciano and Holley, 2009); Dufour *et al.* (2015). AITC is considered a chemical irritant in mammals. This is attributed to it being an agonist of the transient receptor potential ankyrin 1 (TRPA1) cation channel, which mediates the influx of calcium and other cations in sensory neurons and controls cold and exogenous chemical induced pain and inflammatory responses (Bautista *et al.*, 2013). This mechanism of action has led to AITC's frequent use as a model compound to study itch, pain, inflammation, and cough.

Technical-grade AITC is a moderate acute mammalian toxicant by the oral, inhalation, and dermal routes. It is designated as a Category II toxicant or "moderate" acute mammalian toxicant by the oral, inhalation, and dermal routes based on the dose or concentration in air which was lethal to 50% of the experimental animals (LD_{50} and LC_{50} values, respectively). The acute oral,

inhalation and dermal studies used for LD_{50}/LC_{50} determinations used technical grade AITC of 99.8%. In addition, AITC is categorized as Toxicity Category I due to skin and eye irritation, corrosivity, and its ability to act as a dermal sensitizer. AITC does not currently have a cancer classification by US EPA (US EPA, 2013). In humans, the odor threshold has been reported as approximately 1 ppm (AIHA, 2013) and the irritation threshold has been reported as approximately 4.2 ppm (Ruth, 1986).

B.2 Regulatory History and Scope of Assessment

As noted above, AITC is registered for use by the US EPA as an insect and animal repellent, insecticide, fungicide, herbicide and nematicide for use prior to planting for food and non-food crops (US EPA, 2013). Three soil fumigant products are federally registered as biopesticides (US EPA, 2013). There are no active registrations for products containing AITC in California. In 2017, the registrant, Isagro USA Inc., submitted applications for two products, Dominus® and Dominus® 100, containing 96.3 and 99.8% AITC, respectively, to be used as pre-plant soil fumigants for food and non-food crops (DPR, 2020b).

In October 2018, the Department of Pesticide Regulation (DPR) initiated the risk assessment process for AITC due to its proposed registration as a new fumigant active ingredient, as well as its potential for reproductive toxicity, genotoxicity and oncogenicity (DPR, 2018). DPR defined the scope for the risk assessment by developing a problem formulation document (DPR, 2018). The problem formulation was based on the evaluation of information regarding toxicology, proposed uses, relevant exposure scenarios (including routes and durations), regulatory documents from US EPA, and the potential need for mitigation. The scope of this assessment is based on its proposed use in California as a conventional pre-plant soil fumigant and will include occupational and bystander exposure scenarios that are limited to the inhalation route.

The studies evaluated to determine critical points of departure for risk assessment included guideline studies submitted to fulfill data requirements for registration, as well as those required under the California Birth Defect Prevention Act of 1984 (SB 950). Additionally, a systematic review of the open literature and regulatory agency reports was conducted (most recent search: July 2020) (Appendix 3). Data found in open literature or guideline studies that were not used to define critical points of departure were analyzed as part of a weight-of-evidence approach.

1962: US EPA approved registration of Oil of Mustard as a dog repellant (<5% AITC).

1981: DPR approved registration of Oil of Mustard as an insecticide and animal repellant (<5% AITC).

1992: As of December 31, 1992, all registrations for products containing Oil of Mustard in California are inactivated (mostly animal repellents with Oil of Mustard concentration of less than 5%).

1993: US EPA published Reregistration Eligibility Decision (RED) document for Flower and Vegetable Oils. Low risk from Oil of Mustard attributed to low concentrations in registered products (<5% AITC).

2009: US EPA approved registration of CA-1 as a biopesticide for turf, ornamental plant, and nematicide/fungicide use with a concentration of 98% oriental mustard seed. This was the first registration by US EPA of an AITC-containing product with allyl isothiocyanate listed an active ingredient greater than 5% concentration.

2012: Isagro USA Inc. submitted application to register AITC products as a biofumigant to US EPA.

2013: US EPA approved registration of AITC as a biofumigant (Dominus® and Dominus® 100, 96.3 and 99.8% AITC respectively).

2013: As of December 31, 2013, all registrations for products containing AITC in California are inactivated (mostly animal repellents with AITC concentrations of less than 1%).

2017: Isagro USA Inc. submitted applications to DPR to register products containing AITC as pre-plant fumigants for use on food and non-food crops (Dominus® and Dominus® 100, 96.3 and 99.8% AITC, respectively).

2018: DPR published the Problem Formulation Document for Allyl Isothiocyanate and initiates the pre-registrational risk assessment process for the use of AITC as a conventional fumigant in California.

B.3 Illness Reports

There were no AITC-related illnesses reported by California Pesticide Illness Query (CalPIQ) between 1992 and 2016 (DPR, 2020a) (last access: May 2020). A systematic review of open literature did not identify human poisoning cases or illnesses that could be attributed to AITC.

C. TOXICOLOGICAL PROFILE

The database used to develop the toxicological profile of AITC consisted of registrant-submitted studies and scientific publications in the open literature. A systematic review approach was used to identify relevant studies in the open literature and in other regulatory documents. The database search was conducted in PubMed (www.ncbi.nlm.nih.gov) using the common compound names as the key words ("AITC OR allyl isothiocyanate OR allylisothiocyanate OR oil of mustard") on July 31, 2020. Further details of the systemic review process are described in Appendix 3.

C.1 Summary of Metabolism and Toxicokinetics

Seven open literature studies on the toxicokinetics of AITC, two in humans and five in animals, were identified through systematic review or submitted as part of the registration package to DPR. All seven studies used an oral route of exposure of AITC. Taken together, these studies provide a picture of the absorption, distribution, metabolism, and excretion (ADME) of AITC after oral exposure. Pharmacokinetic studies were not available for a direct determination of the rate of absorption following inhalation. However, elicitation of toxic effects after inhalation exposure in animals indicates absorption by that route.

Based on a comparison of the ADME data for humans, rats, and mice, rats appears to be the closest match to humans with respect to internal exposures to metabolic species that may play a role in AITC's toxicity. The oral absorption in rats and mice was estimated to be > 90%. DPR considers oral absorption > 90% as complete (100%). In the absence of data for inhalation uptake, DPR assumes a default inhalation absorption of 100%.

C.1.1 Absorption and distribution

The toxicokinetics of AITC was based on oral studies using either pure AITC or AITC-containing plant derivatives. These studies show ~10% of orally administered AITC is excreted in feces in rats and mice, suggesting > 90% absorption of the labeled AITC by the oral route. In rodents, 50 – 81% of labeled AITC was excreted in urine (Borghoff and Birnbaum, 1986; Bollard *et al.*, 1997). In humans, > 50% of AITC was recovered in urine as dithiocarbamate within 12 hours of ingestion (Jiao *et al.*, 1994b).

Within 6 hours of oral or intravenous AITC administration, higher levels of AITC were found in urinary bladder, particularly in the males (approximately 10-fold greater) than in any other tissue in the rodent models. While male rats exhibited 6-17 times higher levels in urinary bladder than females at early time points, by 24 hours the gender differences had resolved (Bollard *et al.*, 1997). It should be noted that regardless of treatment, male rats and mice tend to have about 2-fold lower urine volume than females. The tendency of males to accumulate more AITC in the bladder maybe associated with their more concentrated urine (Ioannou *et al.*, 1984).

AITC is subject to enterohepatic recirculation in rats (Ioannou *et al.*, 1984). In rodent studies, 73 – 87% of radioactivity from labeled-AITC was cleared from the body by 3 days post-exposure, regardless of sex.

C.1.2 Metabolism and excretion

The isothiocyanic functional group (–N=C=S) on AITC reacts with electrophilic agents, including amino acids, hydroxyl thiol and carboxylic acid moieties, and water (Zhang *et al.*, 1996). Studies on AITC metabolism in humans, rats, and mice show that metabolism and excretion in humans is more similar to rats than mice (Jiao *et al.*, 1994b; Bollard *et al.*, 1997). In both rats and humans, AITC is primarily metabolized by glutathione (GSH) conjugation. The -N=C=S group is conjugated with the cysteine thiol group of glutathione resulting in the formation of GSH-AITC, which is further metabolized to an allyl thiocarbamoylmercapturate (N-acetyl-S-(N-allylthiocarbamoyl)cysteine). This is the major metabolite in urine in both humans and rats. In mice, the allyl moiety is cleaved by a hydrolytic cleavage from the isothiocyanic group. Subsequent rearrangement of the isothiocyanic group to a thiocyanic group (–S–C≡N) results in production of thiocyanate, the major mouse urinary metabolite (Bollard *et al.*, 1997; Pechacek, 1997).

In rats, the maximum concentration (C_{max}) for the major metabolite in plasma (allyl thiocarbamoylmercapturate) was achieved within 30 minutes of exposure. The corresponding C_{max} in urine occurred between 1 and 4 hours (Jiao *et al.*, 1994b; Kim *et al.*, 2015). The half-life in rats was less than 4 hours based on the finding that all of the metabolite was excreted within 8 hours. In humans, the urinary C_{max} for the same metabolite was reached between 1 and 2 hours. The half-life of AITC in humans was 2 hours based on thiocarbamate determinations in urine (Shapiro *et al.*, 1998). The major metabolite in humans, N-acetyl-S-(allylthiocarbamoyl)-L-cysteine, was not detected after 12 hours post-ingestion (Jiao *et al.*, 1994b). These data showed similar metabolism and excretion between humans and rats. These comparisons were based on analyses of studies in which rats and humans were exposed to unlabeled AITC and the thiocarbamate methabolite was followed in blood and urine.

Rodent excretion of radiolabeled AITC showed an initial rapid phase (rats – 37 h, mice – 15 h) and a later slower phase (rats – 140 h, mice – 56 h). The tendency for radiolabel AITC to take longer to be excreted is due to quantification of the radiolabel and not the thiocarbamate metabolite, as in the studies above (Bollard *et al.*, 1997). Individual human and animals studies are detailed below.

C.1.3 Human Studies

Shapiro et al. (1998)

Study methods

Ten healthy human volunteers, ages 25 - 72 years, were given a single 74 µmol dietary dose of AITC (20 ml of horseradish supernatant fluid containing 3.7 µmol isothiocyanate/ml). Subjects were instructed to avoid dietary sources of glucosinolates and isothiocyanates for 2 days before dosing. Eleven 1-h urine samples, starting 1 hour before dosing were collected.

Excretion

Peak dithiocarbamate excretion occurred at 1.4 ± 0.2 h after dosing. By 10 hours post feeding, 42 ± 5% of the ingested dose had been recovered. A smaller secondary rise in excretion occurred at approximately 6 hours after dosing, which may be the result of enterohepatic recycling of metabolites. In a typical volunteer, a brisk excretion that was first order with a 2-h half-life, and largely complete excretion by 10 hours after dosing was observed.

Jiao et al. (1994b)

Study methods

Two male and two female volunteers, age 20 – 45 years, were advised to avoid cruciferous vegetables, mustard, and mustard flavored foods. Brown mustard ("Grey Poupon Dijon") containing 453 parts per million (ppm; 0.453 mg of AITC/g of mustard) was used as the source of AITC. In the first experiment, each participant ingested 10 grams of brown mustard with bread. In the second experiment, each participant ingested 20 grams of brown mustard with a turkey or chicken sandwich. In both experiments, urine samples were collected at intervals of 0 – 2, 2 – 4, 4 – 8, 8 – 12, 12 – 24, 24 – 36, and 36 – 48 hours following consumption. Urine samples were stored at -20°C analyzed immediately.

Excretion

The N-acetyl cysteine (NAC) conjugate of allyl isothiocyanate (N-acetyl-S-(allylthiocarbamoyl)-L-cysteine) was identified in urine after, but not before, mustard ingestion. NAC-AITC was quantified to calculate the percent conversion of AITC to NAC-AITC, yielding an average of 53.5 ± 8.1%. This finding suggested that (1) N-acetyl-S-(allylthiocarbamoyl)-L-cysteine is the major metabolite (> 50%) of AITC in humans and (2) AITC derivatives are mainly excreted in the urine. Thus, the metabolism of AITC in humans resembles that in rats more than in mice. N-acetyl-S-(allylthiocarbamoyl)-L-cysteine was not detected in urine after 12 hours post-ingestion.

C.1.4 Animal Studies

Bollard et al. (1997)

Study methods

Fischer 344 rats and B6C3F1 mice of both sexes (N= 4 – 6 animals/sex/dose) were treated by gavage with 2.5 or 25 mg/kg of [^{14}C]allyl isothiocyanate to study absorption, metabolism and excretion patterns. The radiolabel was located within the isothiocyanate moiety. Urine and feces were collected daily for 4 days. Urine was collected into tubes on ice containing 0.1 M citric acid/phosphate buffer to prevent loss of volatile metabolites. In separate experiments, exhaled CO_2 was collected into trapping fluid for up to 4 days. The trapping fluid was sampled every 2 hours to determine percent labeled CO_2. Biliary excretion was measured every 30 minutes for 6 hours by cannulating the common bile duct in rats dosed with 2.5 mg/kg [^{14}C]AITC intravenously. A time-course experiment was conducted to study tissue distribution after oral dosing of 2.5 or 25 mg/kg [^{14}C]AITC. Three animals/sex/dose were sacrificed at 20 and 40 minutes, and at 1.0, 1.5, 2, 6, 12, 18 and 24 hours after dosing.

Excretion kinetics

Rats metabolized and excreted AITC more slowly than mice. In both species, the levels of [^{14}C]AITC-derived radioactivity in blood peaked within 3 hours of administration. The kinetic profile shows that the plasma half-life of AITC in rats was twice that of mice. The excretion of AITC was biphasic, with an initial rapid phase (37 h in rats; 15 h in mice) and a later slower phase (140 h in rats; 56 h in mice). A major portion of the administered dose was excreted in urine in rats (50 - 57%) and mice (79 - 81%). Rats retained 19 - 24% of AITC in the tissues compared to 2 - 5% in mice after 4 days. Both species excreted between 6 - 12% in feces and 5 - 7% in expired air. In rats, biliary excretion constituted 13% and 8% of the dose in males and females, respectively, up to 6 hours post-dose. Up to 6 hours post-dose, the urinary bladder contained the highest levels of [^{14}C]AITC-derived radioactivity compared to other organs (~10 fold higher than liver and kidneys, 20 fold higher than spleen and 100 fold higher than brain).

Metabolite identification

Three major metabolites were identified in urine: thiocyanate, allyl thiocarbamoylcysteine and allyl thiocarbamoylmercapturic acid (Table 1). Thiocarbamoylmercapturic acid was the predominant metabolite in rats, while thiocyanate constituted the majority of the radiolabel in mice. Allyl thiocarbamoylcysteine was detected in the urine of mice but not in rats.

Table 1. Relative Quantities of [^{14}C]AITC Metabolites in Urine Collected Over 24 hour in Rats and Mice

				Metabolites, percent of radioactivity in urine		
Species	**Sex**	**Dose**	**Number of animals**	**Thiocyanate**	**Allyl thiocarbamoyl-cysteine**	**Allyl thiocarbamoyl-mercapturate**
Rat	M	2.5	6	18.1 ± 7	ND	81.9 ± 7
	F	2.5	6	14.7 ± 5.3	ND	85.3 ± 5.3
	M	25	6	27.8 ± 11.9	ND	72.2 ± 11.9
	F	25	5	31.1 ± 8.5	ND	66.9 ± 8.5
Mouse	M	2.5	4	75.6 ± 6.1	12.1 ± 1	12.3 ± 1.9
	F	2.5	6	47.6 ± 12.8	52.4 ± 12.8	ND
	M	25	6	84.7 ± 7.4	7.3 ± 2.8	8 ± 1.9
	F	25	6	78.1 ± 3.1	21.9 ± 3.1	ND

ND = Not Detected

Borghoff and Birnbaum (1986)

Study methods

The objective of this study was to determine the influence of age on glutathione conjugation following oral administration of AITC in Fischer 344 rats. The test compound was synthesized with radiolabel at all carbon positions in a uniform pattern, i.e., [U-^{14}C]AITC. Males aged 3, 16 and 27 months, 3 – 4 animals/group were gavaged with 25 mg/kg of labeled AITC. Urine samples were collected at 4, 8, 12, 24, 48 and 72 hours after dosing, fecal samples at 24, 48 and 72 hours, and expired $^{14}CO_2$ at 2, 4, 6, 8, 10, 12, 24, 48 and 72 hours. In a second experiment, bile duct cannulations were carried out under anesthesia in rats treated intravenously with 10 mg/kg labeled AITC. Bile was collected at 15, 30, 45, and 60 minutes, and at 1.5, 2, 2.5, 3, 4, 5 and 6 hours after dosing. To assess how age affects the ability to conjugate AITC with GSH, GSH levels were measured in livers of untreated rats at 2.5, 3, 6, 12, 18, 24 and 27 months of age.

Excretion kinetics

Radiolabeled AITC was primarily excreted in urine. The percentage of administered dose excreted in urine increased slightly with age: 67, 72, and 79% in rats aged 3, 6, and 27 months, respectively. The remaining label was distributed between feces, exhaled CO_2, and volatile components in the expired air, with some variation depending on age. The radiolabel recovered in bile up to 6 hours post-dose ranged between 14.5% and 36.7% of the administered dose. These percentages were greater than the percentages of radiolabel recovered in the feces of the uncannulated rats, indicating enterohepatic recirculation of AITC.

Metabolite identification

The predominant urinary metabolite was allyl thiocarbamoylmercapturate (N-acetyl-S-(N-allylthiocarbamoyl)-L-cysteine), which accounted for 68-72% of the recovered radiolabel at 24 hours post-dose. In addition, five minor metabolites were isolated but not identified. Three of these were also present in the bile at 30 minutes post-dose.

The authors concluded that (1) urine was the major route of excretion for orally administered AITC, and (2) AITC-GSH conjugation was not greatly affected by the age of the animals.

Ioannou et al. (1984)

Study methods

In a comparative study of the disposition of AITC in rats and mice, Ioannou et al. (1984) dosed Fischer 344 (F344) rats and B6C3F mice of both sexes with a single dose of 25.2 or 252 µmol/kg of [U-^{14}C]AITC (uniformly labeled) by gavage, or with 252 µmol/kg by intravenous injection. These were equivalent to doses of 2.5 and 25 mg/kg used in 2-year National Toxicology Program (NTP) bio-assay, respectively. Animals were housed in metabolic cages for up to 3 days for collection of urine and feces. Urine was collected in vessels packed with dry ice to minimize loss of volatile metabolites. The animals were sacrificed at various time points from 15 min to 3 days after treatment. At necropsy, major tissues were removed, weighed, and stored at -20 °C until assayed. Expired CO_2 was collected for up to 24 hours from a separate group of rats dosed orally with 252 µmol/kg labeled AITC. In a third group, excretion of radiolabel into bile was determined in bile duct-cannulated rats dosed intravenously with 252 µmol/kg labeled AITC. Bile samples were serially collected for 6 hours after dosing.

Absorption and distribution

Results indicate nearly complete gastrointestinal absorption of AITC. Neither tissue distribution nor excretion pattern was significantly different between oral and intravenous routes. Following intravenous administration, AITC was distributed to all tissues examined between 15 min and 3 days, appearing predominantly in the urinary bladder. This concentration in the urinary bladder was pronounced in males, where levels were 6 – 17 times higher compared to females up to 6 hours after treatment.

Excretion kinetics

Radiolabel levels in excreta were comparable in rats and mice, though blood levels were more persistent in rats than mice. Urine comprised the predominant route of excretion. By day 3 post-dose, 73 – 87% and 74 – 80% of the dose appeared in the urine of rats and mice, respectively, regardless of sex. Recovery in the feces constituted less than 6% in both species. The radiolabel

recovered as exhaled CO_2 constituted 12.6 – 14.5% of the administered dose at 24 hours for the 252 µmol/kg dose group.

Female rats excreted approximately twice the amount of urine volume compared to males. The investigators indicated that females must have excreted a more dilute urine with respect to AITC, which may have contributed to sex differences in the retention and concentration of AITC-derived radioactivity in urinary bladder. Compared to rats, mice had lower levels of radiolabel in urinary bladder and smaller male to female tissue concentration ratios. By 24 hours, levels were comparable in all assayed tissues in both rats and mice.

Metabolite identification

Three major metabolites were identified in rat urine, while four were detected in mouse urine. Species-related differences were observed in the amounts of most metabolites excreted in urine. Allyl thiocarbamoylmercapturate (N-acetyl-S-(N-allylthiocarbamoyl)-L-cysteine) was the predominant metabolite in rat, and the only chemically-identified metabolite. The other metabolites were not identified by the authors.

Kim et al. (2015)

Study methods

To study the kinetic parameters of AITC, a single gavage dose of 25 mg/kg AITC was administered to male Sprague-Dawley rats followed by sacrifice at graded time points through 8 hours post-administration. Urine, blood, liver, heart spleen, kidney, and lung were collected. AITC was extracted from plasma and tissues and subjected to chromatographic analysis.

Metabolite identification and handling

NAC-AITC (N-acetyl-S-(N-allylthiocarbamoyl)-L-cysteine) and GSH-AITC (glutathione-AITC) were identified as the primary metabolites in rat plasma. The T_{max} values in plasma for GSH-AITC and NAC-AITC were both determined to be 0.5 hours. The plasma C_{max} values for GSH-AITC and NAC-AITC were 1.47 and 14.03 µg/ml, respectively. The area under the curve of the concentration-time profile ($AUC_{0\text{-}8\ h}$) of GSH-AITC and NAC-AITC were 1.3 and 12.33 µg/ml, respectively. In urine samples, NAC-AITC was detected and quantified but GSH-AITC was not detected. This is plausibly due to conversion of GSH-AITC to NAC-AITC in the renal tissue, which contains enzymes that efficiently breakdown GSH conjugates (Ballatori, 2019). The maximum concentration of NAC-AITC in urine occurred between 1 – 4 hours following administration of AITC, with a C_{max} of 2.26 µg/ml. Most urinary NAC-AITC was excreted within 8 hours of administration. Tissue analysis showed that the metabolites underwent rapid and wide distribution. GSH-AITC deposition was greatest in liver, followed by kidney, spleen, heart, and lung, whereas the greatest NAC-AITC deposition occurred in the kidney.

C.2 Acute Toxicity

AITC induces acute toxicity after inhalation, oral, and dermal exposure. Table 3 summarizes the health effects resulting from acute to short-term exposure to AITC or AITC-rich substances in animals and humans.

C.2.1 Acute inhalation toxicity

Three reports examining the effects of AITC by the inhalation route were evaluated for this risk assessment (Goto *et al.*, 2010; Lowe, 2012; Herberth, 2017). Two were registrant-submitted FIFRA guideline studies in rats, and one was an exposure study in humans in a patent application (Table 3). In the latter, investigators invented and tested an alarm device to spray aerosolized AITC inside a room. The effect measured was awakening a sleeping human subject. They concluded that 5 – 15 ppm was sufficient to safely awaken sleeping human subjects (Goto *et al.*, 2010).

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 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. 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.

Goto et al. (2010)

Study methods

The details for this non-peer-reviewed study were sourced both from the patent application and a book chapter that highlighted the invention (Goto *et al.*, 2010; Brand, 2019). As part of a patent application, the inventors tested an AITC-spraying alarm device in two exposure scenarios on a total of 14 human subjects, including one person with deafness. In exposure scenario 1, three AITC-loaded devices, one control (filtered-air), and four AITC sensors were set up in a room with 9.51 m^3 space. Firing of all three sprays at once increased AITC levels in the room by 2

ppm. With one person asleep inside the room, concentrations from 0 to >24 ppm were generated to test the device. In the exposure scenario 2, the room was smaller with a 7.92 m^3 volume; firing of one AITC-loaded device increased the AITC concentration in the room starting at 0 ppm and increasing by increments of 5 ppm up to concentrations of > 15 ppm. The sleeping subject was observed for level of discomfort and the time taken to wake up.

Results

Based on the test results, the authors concluded that it was possible to safely awaken a sleeping human subject with less discomfort when AITC concentrations in air were between 5 – 15 ppm, than when concentrations were > 15 ppm. The report lacked many experimental details, including detection method, selection and testing regimen for each subject, symptomology and recovery of subjects, sample concentration verification, or ambient air concentrations measurements or validation.

Herberth (2017)

Study methods

Sprague-Dawley rats (10/sex/group) were exposed to a single dose of AITC vapor (99.9% purity) for 4 hours in a whole-body chamber followed by a 14-day observation period before sacrifice. AITC air concentrations were 0 (filtered air), 25, 74 or 124 ppm AITC[1]. 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), and on days 7 and 14. Macro- and microscopic neuropathological observations were made following sacrifice on day 15. Major treatment-related effects included changes to FOB parameters, motor activity, body weight, and body temperature. Study results are summarized in Table 2.

Results

Clinical observations

Significantly decreased respiratory rate (< 80 breaths per minute (BPM) at midpoint of exposure), gasping and crusty deposits on the nose and mouth in both males and females were observed at 74 and 124 ppm. In addition, there was a concentration-dependent decrease in body

[1] Whole-body exposure methodology. Inhalation exposures were carried out in whole-body inhalation exposure chambers. One chamber was dedicated for each group. Vapors of AITC were generated using a glass-bead column-type vaporization system. The column was filled with various-sized glass beads and heated to ~140 – 160°C. Vaporization occurred as AITC flowed over the surface of the heated beads while compressed air flowed up through the column. The vapors were directed into chamber to achieve target concentrations. The control exposure chamber was supplied with air delivered to a whole-body chamber. The chamber concentrations were analyzed at ~45-minute intervals. According to authors, during the method development, it was made sure there was no aerosol development, and temporal stability and homogeneity was achieved in the chamber.

temperature in both males and females in all dose groups. However, statistical significance was not achieved at the lowest concentration (25 ppm).

Body weight determinations

A concentration-dependent reduction in mean body weight was observed at all concentrations in males on days 7 and 14 that reached statistical significance at the mid and high concentrations. Compared to the untreated controls, the decreases were 2.5, 5.5, and 9.0% in males and 2.0, 4.7, and 7.4% in females at ascending concentrations. Similarly, the rate of body weight gain was lower than controls in a concentration-dependent manner on day 7 but not on day 14 in males, suggesting that the decline in rate of gain was had reversed by that time. Body weight changes in females were unremarkable.

Neuromuscular performance tests

Both rotarod performance and hind limb foot splay were decreased in males compared to controls on day 0, although the former achieved significance only at high concentration, while the latter was significant at both the mid and high concentrations. In females, hind limb foot splay was significantly reduced at the mid and high concentrations, while no effect was detected on the rotarod.

Open field-rearing counts

Open field-rearing counts decreased on day 0 in a concentration-dependent manner in both sexes, achieving statistical significance for both sexes at 74 and 124 ppm and in females at the low dose of 25 ppm as well (Table 2).

Motor activity measurement and analysis by the study authors

Open field motor activity was measured as the interruption of infrared photobeams during movement of rats in cages. Interruption of one photobeam was counted for total motor activity (e.g., fine motor skills such as grooming), while interruption of two or more consecutive photobeams was counted as ambulatory motor activity. Recordings were done before exposure to AITC (pretest), and on days 0, 7, and 14. On Day 0, recording of motor activity counts was initiated approximately 2 hours after beginning of exposure, i.e., at the midpoint of exposure. Each animal was tested separately. Data were collected in 5-minute epochs, and the test session duration was 60 minutes. These data were compiled as six 10-minute subintervals for tabulation. Within-session repeated measures analysis of variance was conducted by the authors across the subintervals of each test session to determine total and ambulatory motor activity. Overall interval means (representing the entire 60-minute session activity) were also determined.

A concentration-dependent decrease in both total and ambulatory motor activity was observed in both sexes on day 0. Females showed a sharper decrease than males when compared across the

range of concentrations. The overall mean counts of both ambulatory and total motor activity for the 60 minute sessions were statistically reduced in all three treated groups compared to controls ($p < 0.01$). At the subinterval level, mean counts were significantly reduced at 0 – 10 minutes (males and females), 1 – 20 minutes (females only), and 21 – 30 minutes (females at 124 ppm, ambulatory only). Statistically, there was no difference in mean motor activity counts (overall or subinterval) at pretest, day 7 or 14 post-exposure.

Histopathology

Macroscopic and microscopic neuropathology conducted on tissues collected at day 15 sacrifice were unremarkable for all treatment groups. Brain morphometric measurements were not altered compared to controls.

Conclusion

Whole-body inhalation exposure to AITC in rats showed concentration-dependent effects, including decreased FOB activities, increased respiratory system irritation (decreased respiration rate and crusty deposits in the nose and mouth), decreased core body temperature, and decreased absolute body weight in males. These effects suggested that AITC induces effects both at the point of entry and systemically. The neurobehavioral effects (decreased motor activity in both sexes, and decreased open field-rearing counts in females) were statistically significant at the lowest tested dose of 25 ppm. Based on these observations, the lowest tested dose of 25 ppm was the LOEL for this study. A NOEL could not be established.

Table 2. Findings in an Acute Whole-body AITC Vapor Inhalation Toxicity Study Using Rats

End points	**Males (10/concentration)**				**Females (10/ concentration)**			
	0 ppm	**25 ppm**	**74 ppm**	**124 ppm**	**0 ppm**	**25 ppm**	**74 ppm**	**124 ppm**
Clinical observations (Incidence/10)								
Reduced respiratory rate (<80 BPM)[a]	0	0	9*	10*	0	1	8*	10*
Crusty deposits - nose[a]	0	1	7*	6*	0	0	6*	7*
Crusty deposits - mouth[a]	0	2	5*	4	0	2	8*	6*
Body weight (g mean ± SD)[b]								
Day 0	237 ± 18	238 ± 13	235 ± 10	231 ± 15	157 ± 10	160 ± 14	159 ± 10	157 ± 10
Day 7	298 ± 13	291 ± 10	282 ± 8**	271 ± 12*	178 ± 13	187 ± 10	180 ± 10	181 ± 10
Day 14	350 ± 17	343 ± 7.5	334 ± 12**	324 ± 14**	202 ± 14	209 ± 12	204 ± 11	204 ± 14
Body weight gain on Day 7 (g mean ± SD)[d]	61 ± 14	53 ± 5	47 ± 10**	40 ± 7**	21 ± 5	26 ± 8	21 ± 5	24 ± 7
Body temperature[b] (°C; mean ± SD) on Day 0	38 ± 0.4	37.2 ± 0.4	34.4 ± 0.4**	32.3 ± 1.3**	37.9 ± 0.3	37.6 ± 0.5	36.1 ± 0.6**	33.2 ± 1.4**
FOB Measurement - Day 0 (mean ± SD)								
Ambulatory activity counts (0-60 min)[c]	317 ± 73 (100%)	211 ± 60* (67%)	80 ± 48* (25%)	56 ± 43* (18%)	596 ± 225 (100%)	260 ± 109* (44%)	179 ± 78* (30%)	74 ± 62* (12%)
Total motor activity counts (0-60 min)[c]	1451 ± 208 (100%)	1005 ± 359* (69%)	618 ± 299* (43%)	886 ± 531* (61%)	2177 ± 827 (100%)	939 ± 370* (43%)	896 ± 344* (41%)	749 ± 272* (34%)
Rearing counts – open field[b]	3.8 ± 3.4	2.3 ± 2	1.2 ± 1.03*	0.7 ± 0.95**	6.2 ± 2.9	3.5 ± 2.76*	0.8 ± 1.32*	0.6 ± 0.84**
Rotarod performance (sec)[b]	92 ±45	86 ± 43	50 ± 48	36 ± 45*	76 ± 48	109 ± 36	89 ± 51	65 ± 49
Hindlimb foot splay (mm)[b]	63 ± 11	54 ± 10	50 ± 16**	37 ± 9**	65 ± 14	58 ± 14	48 ± 10*	36 ± 11**

Reference: Herberth (2017)***;*** Significantly different from control * $p < 0.5$ level; ** $p < 0.01$ level; [a]Fisher's Exact Test - conducted by the study authors; [b]One-Way ANOVA followed by Dunnett's test - conducted by the authors; [c]Repeated measures analysis of variance (RNOVA), with sequential linear trend test for monotonic exposure response, or pair-wise comparisons for nonmonotonic responses – conducted by the study authors; [d]One-Way ANOVA followed by Dunnett's test – Conducted by the risk assessor

Lowe (2012)

Study methods

Sprague-Dawley rats (5/sex/group) were exposed to AITC aerosol at concentrations of 0.206 and 0.508 mg/L (51 and 126 ppm, respectively) by nose-only inhalation for 4 hours to determine the concentration which was lethal to 50% of the animals (LC_{50}). The experimental design did not include an untreated control group. A desired distribution of AITC aerosol particles with a Mass Median Aerodynamic Diameter (MMAD) of 1 – 4 µm was achieved. Particles with an MMAD of < 5 µm deposit in the bronchial region and the deeper lung airways of rats (Raabe *et al.*, 1988; SOT, 1992; Pauluhn, 2003). Particles with MMAD of > 5 – 10 µm deposit predominantly in the nasopharyngeal region (head airway region) in rats. It should be noted that this study utilized only particle sizes < 5 µm. For inhalation toxicants causing systemic effects (as opposed to local toxicity), particles that deposit to any region of the respiratory tract (i.e. MMAD of ≤ 10 µm) may be considered as bioavailable (Raabe *et al.*, 1988). Survivors were observed for 14 days prior to sacrifice. Cage-side observations were carried out daily. Body weights were measured prior to exposure and on days 1, 3, 7 and 14. Necropsies were conducted on day 14. The findings appear below by concentration.

Results

51 ppm (lowest) exposure concentration

The time weighted average and nominal chamber concentrations[2] were 0.206 mg/L and 1.31 mg/L, respectively. The MMAD was calculated to be 2.1 µm. At this MMAD, approximately 81% and 97.5% of particles had < 5 µm and < 9 µm diameter, respectively. Rats exhibited irregular respiration, hypoactivity, nasal and/or ocular discharge, ano-genital staining, and tremors. Two animals died, one male on day 1 and one female on day 2. One male and one female showed superficial nasal eschar between days 3 and 12, and alopecia in the same area on days 13 and 14. The superficial nasal effects were not observed in the whole-body inhalation study. All survivors had recovered from symptoms by day 10. All rats lost body weight by day 1, with two males continuing through day 3. All survivors showed weight gains thereafter. No gross abnormalities were noted in surviving rats on day 14, though discoloration of lung, distention of stomach and/or intestines, and/or mottled liver were observed in decedents.

126 ppm (highest) exposure concentration

The time weighted average and nominal chamber concentrations for this exposure concentration were 0.508 mg/L and 3.97 mg/L, respectively. The MMAD was calculated to be 3.0 µm. At this MMAD, approximately, 71% and 90% of particles had diameters of < 5 µm and < 9 µm,

[2] Nominal concentration = Total test substance used in mg / (average airflow x total time)

respectively. All five males, and 4 of 5 females died at this exposure concentration. Four males and one female were found dead upon removal from the exposure tubes. Survivors exhibited abnormal respiration, tremors, hypoactivity, and/or facial alopecia. On day 1, one surviving female rat died. On day 2, one male and two female surviving rats died. By day 2, only one female was still living. None of the males survived beyond day 2. Gross necropsy findings on the decedents were similar at both concentrations (51 and 126 ppm), including discolored lungs, distended stomach and intestines, and mottled or darkened liver. No gross abnormalities were seen in the female that survived to day 14.

Conclusion

Nose-only inhalation exposure to aerosolized AITC for 4 hours resulted in LC_{50} values between 51 and 126 ppm. The LOEL for mortality and clinical signs was 0.206 mg/L (51 ppm).

C.2.2 Acute dermal toxicity (including dermal irritation)

One registrant-submitted acute dermal toxicity study of AITC using rats was evaluated for this risk assessment (Durando, 2012a). One animal dermal irritation (Durando, 2012d), two dermal sensitization (Landsteiner and Di Somma, 1938; Durando, 2012c), and two human dermal sensitization studies (Landsteiner and Di Somma, 1938; Andersen *et al.*, 2017) were also evaluated. Contact irritation, sensitization, hypoactivity, weight loss, and death were observed in animals while human studies reported pain and somatosensory sensitization. No NOELs were established in any of the dermal studies. The evaluation of these studies did not lead to the identification of any critical end-points or PODs for assessing the inhalation risks of AITC exposure and were not included in the Hazard Identification section for AITC.

C.2.3 Acute oral toxicity

Multiple acute single dose exposure studies of AITC in rats and mice were identified. Based on the studies by Durando (2012b) in rats, NTP (1982) in rats and mice, and Lewerenz *et al.* (1988b), acute oral administration of AITC resulted in decreased body weight, increased liver weight, inactivity, drooping of eye lids, necrotic and thickened stomach mucosa, and death (Table 3). The LOEL was 100 mg/kg/day based on decreased body weight and increased liver weight observed in rats by Lewerenz *et al.* (1988b), and inactivity, drooping eyelids, and ruffled fur in mice by NTP (1982). The NOEL was 50 mg/kg/day from both studies. The evaluation of these studies did not lead to the identification of any critical end-points or PODs for assessing the inhalation risks of AITC exposure.

Table 3. Acute Toxicity Studies of AITC or AITC-Rich Substances

Study	***Study Design***	**Effects at LOEL**	**NOEL**	**LOEL**
Inhalation route				
Goto *et al.* (2010)	Inhalation (human); AITC aerosolized in to a room with one sleeping human subject at a time. Exposure scenario 1: 9.51 m^3 room; 1 dummy device; 3 AITC-loaded devices; 4 concentration sensors; 14 men and women 20 to 40 years old; AITC concentrations increased from 0 to 24 ppm or more at 2 ppm increments. Exposure scenario 2: 7.92 m^3 room; AITC levels were 5 ppm, 10 ppm, 15 ppm, and higher.	Not reported	ND[a]	ND
Herberth (2017)	Inhalation (whole-body); AITC vapor, 4 h, single whole-body exposure; post treatment observations for 14 days; rats, 10/sex/group; 0, 25, 74, 124 ppm;	Decreased motor activity (M/F); Decreased rearing counts (F)	ND[a]	25 ppm (0.1 mg/L)[b]
Lowe (2012)	Inhalation (nose-only); AITC aerosol, 4 h, single exposure; post treatment observation for 14 days; rats, 5/sex/group; 0.206 or 0.508 mg/L	Mortality, hypoactivity, irregular respiration, rales, tremors, ocular and/or nasal discharge, (M/F)	ND	0.206 mg/L (51 ppm)[b]
Oral route				
Durando (2012b)	Oral gavage; single dose of AITC; observed for 14 days; number of female rats and dose levels (n/(mg/kg/day)):1/55, 2/ 175, 3/550, 2/ 2000	Discolored intestines, distension of stomach and intestines, and death	55	175
NTP (1982)	Oral gavage; single dose of AITC, observed for 16 days; rats, 5/sex/dose; 25, 50, 100, 200, 400 mg/kg/day of AITC	Decreased body weight, inactivity, watery eyes; no mortality observed	100	200
NTP (1982)	Oral gavage; single dose, observed for 16 days; mice, 5/sex/dose; 50, 100, 200, 400, 800 mg/kg/day of AITC,	Inactivity, drooping eyelids	50	100
Lewerenz *et al.* (1988b)	Oral gavage; male rats, 12 rats/dose; 0, 50, 100, 150 mg/kg/day of AITC for 3 days	Decreased BW (>10%) and increased rel. liver weight	50	100
Langer and Stolc (1965)	Oral gavage; single dose; male rats fed iodine-deficient diet; 3-4 rats/dose; 0, 2, 4 mg/animal of AITC	Decreased labeled-iodine uptake	--	10 (or 2 mg/animal[c])

Table 3. Acute Toxicity Studies of AITC or AITC-Rich Substances

Study	***Study Design***	**Effects at LOEL**	**NOEL**	**LOEL**
Dermal route – Skin sensitization studies				
Landsteiner and Di Somma (1938)	Dermal; human, 6 persons treated 6 days/week for 3 weeks; 1 drop of synthetic mustard oil onto forearm.	One person developed distinct hypersensitive reaction – appeared 12 hours after 13th application. Severity increased next day; 2 persons developed slight transient delayed reactions indicating low-grade sensitization. Sensitization was present when oil of mustard was applied to other sites in these individuals	ND	ND
Landsteiner and Di Somma (1938)	Dermal; Guinea pigs, monkeys and rabbits – repeated superficial application, and intracutaneous injection of mustard oil diluted in olive oil for 3 weeks	No definite positive effect	ND	ND
Landsteiner and Di Somma (1938)	Dermal; Chester Whites hogs (3); mustard oil was applied for 3 weeks on the same site on skin	Two hogs showed distinctive hypersensitive reaction similar to humans	ND	ND
Andersen *et al.* (2017)	Dermal; human; double-blinded; 1 ml/concentration on cotton pad in polylpropylene chamber that was fixed to two pre-marked 9-cm^2 areas on skin for 5 minutes; 10 males/4 females; AITC – 0, 10%, 50%, 90% (v/v) in paraffin	Pain, somatosensory sensitization in a dose-dependent manner**;** Hyperalgesia, Allodynia, and neurogenic pain was present at all doses	ND	ND
Durando (2012c)	LLNA female mice, 2/group; 25 µl of 0, 2.5%, 5%, 10% of AITC applied on both ears for 3 days; ^{3}H-methyl thymidine was injected intravenously 5 hours before sacrifice on Day 6	Positive dermal sensitizer	ND	ND
Dermal route – Skin irritation and toxicity				
Durando (2012a)	Dermal; rats, 5/sex/per dose; 200, 2000 mg/kg for 24 h; observed for 14 days	Dermal irritation, nasal and/or ocular discharge at low dose; Ano-genital staining, hypoactivity, and death at high dose	ND	200
Durando (2012d)	Dermal Irritation; rabbits, AITC applied for 4 h on 6-cm^2 skin area	Positive skin irritation with presence of edema and edema; Category I (Corrosive)	ND	ND

[a]Not determined; [b]Conversion: X mg/L = (X ppm * AITC MW 99.1565 g/mol)/(24.45*1000) (http://www.aresok.org/npg/nioshdbs/calc.htm); [c]Calculated using rat body weight of 200 g reported by the authors, Langer and Stolc (1965): 2 mg total dose/0.2 kg = 10 mg/kg;

C.3 Subchronic toxicity

C.3.1 Subchronic inhalation toxicity

Randazzo (2017)

Study methods

One registrant-submitted study was available in laboratory animals for evaluation of effects from subchronic inhalation exposure to AITC (Randazzo, 2017). In this study, rats (16/sex/group) were exposed to 0 (control), 5, 10 or 25 ppm AITC vapor (97.9% purity) by whole-body inhalation exposure, 6 hours/day, 5 days/week for 13 weeks[3]. Several parameters were evaluated including clinical signs, clinical pathology, ocular pathology, body weight, and food consumption. On Week 12, 10 rats/sex/group were subjected to functional observational batteries (FOB) and motor activity determinations. At the end of treatment, 10 rats/sex/group were sacrificed for macro- and microscopic pathological observations. An additional 6 rats/sex/group were perfused with 4% paraformaldehyde buffered solution *in situ* to evaluate brain tissue. Important findings related to critical endpoints included metaplasia of respiratory epithelium, degenerative lesions of olfactory epithelium, and decrease in motor activity.

Motor activity measurements were conducted twice (pretreatment and during week 12 prior to daily exposure) using a computer-controlled system that employs a series of infrared photobeams surrounding an amber plastic rectangular cage to quantify activity of the animal inside. Data were collected in 5-min epochs during a 60-min session and compiled as six 10-minute subintervals for tabulation. Total motor activity, also defined as a combination of fine motor skills (e.g., grooming), was recorded as interruption of one photobeam. Ambulatory activity was defined as animals moving, and recorded as interruption of two or more consecutive photobeams. Statistical analysis was carried out with RNOVA[4]. The results from this study are summarized in Table 4 and Table 5.

[3] Whole-body exposure methodology: Rats were exposed in a whole-body exposure chamber, with one dedicated chamber for each group. Vapors of AITC were generated using a glass-bead column-type vaporization system by heating the beads to ~140-160°C. Vaporized AITC was carried through the column into the exposure chamber by compressed air. The chamber concentrations were analyzed at ~45-minute intervals. During the method development concentration-exposure atmosphere was evaluated for temporal stability, homogeneity, and that no aerosol was developed in the chamber.

[4] BioSTAT Consultants Inc. (Portage, MI) performed statistical analysis using repeated measures of analysis of variance (RANOVA). Factors in the model included treatment group, time interval, and the interaction of time interval and treatment group. Monotonic dose-response relationship was evaluated using sequential linear trend tests based on dose levels. If the linear dose by time interaction was significant at 0.05, trend tests on treatment means were performed at the 0.05 level for each interval. If the linear dose by time interaction was not significant the trend test was conducted across the pooled time intervals for the entire session. Nonmonotonic dose responses were evaluated whenever no significant linear trends were detected but treatment and/or treatment-time interaction was significant at the 0.01 level. Pairwise comparisons were made between control and treatment groups.

Results

Effects at 5 ppm

Minimal squamous cell metaplasia of the respiratory epithelium in males (1/10) and minimal degeneration of the olfactory epithelium in females (1/10) were present at the lowest tested dose. These effects increased in incidence and severity with increasing concentrations. No other histologic changes were observed at this concentration. Effects on the nasal epithelium due to inhalation exposure to chemicals that are described as "minimal" grade are generally not considered significant by pathologists (Hardisty *et al.*, 1999), nor in a framework for determining level of adversity (Palazzi *et al.*, 2016). No histopathological lesions were observed in controls. Additionally, a non-statistically significant 7 – 16% reduction in mean motor activity (total and ambulatory) was observed in both sexes. Although the decrease in mean terminal body weight showed concentration dependency, it did not attain statistical significance compared to controls.

Effects at 10 ppm

All 10 males, and 6/10 females showed minimal-to-moderate olfactory epithelial degeneration. For males, the epithelial degeneration in 1/10 animals was graded as minimal, 2/10 as mild, and 7/10 as moderate. One male also exhibited mild squamous cell metaplasia of the respiratory epithelium. For females, 4/10 showed minimal and 2/10 mild olfactory epithelial degeneration. One female also had a mild mixed-cell inflammatory lesion in the nasal epithelial tissue. Additionally, motor activity was decreased by 21 – 45%, although the effect was not statistically significant. Body weight decreases of 5% were reported for the males in this group, but this was not statistically significant.

Effects at 25 ppm

Statistically significant decreases in body weights, motor activity, and a spectrum of effects on the upper respiratory epithelia were reported at this dose. The severity of the olfactory epithelial degeneration increased with increasing concentration, with most graded from mild to marked. Additional histopathological lesions included degeneration, erosion, or atrophy of respiratory, transitional, and olfactory epithelia, olfactory nerve bundles, and olfactory bulbs in the brain. In many of the animals these lesions were graded as moderate-to-marked. Statistically significant reductions in mean total motor activity (37%) and ambulatory activity (42%) were reported in males. Other effects at this concentration included red material around the nose (more frequent in females), changes in absolute and relative weights of several organs, and clinical pathology parameters (minimal but statistically significant decreases in blood urea nitrogen, sorbitol dehydrogenase, higher prothrombin time, lower reticulocyte percentage and lower urine pH, and higher hemoglobin distribution width in either males and/or females). Ophthalmic examinations revealed no ocular changes at any concentrations on day 88. Similarly, histology revealed no changes in urinary bladder.

Conclusion

Metaplasia of respiratory epithelium, degeneration of olfactory epithelium, and decreased motor activity displayed concentration dependence for incidence and/or severity. These were the most sensitive endpoints and were observed at both 10 ppm and 25 ppm at significant levels. Animals in the 25 ppm group showed several additional effects. The study NOEL was 5 ppm based on mild metaplastic lesions in respiratory epithelium, mild-to-moderate degenerative changes in the nasal olfactory epithelium, and decreased motor activity at the LOEL (10 ppm).

Table 4. Histopathological Findings Following Subchronic Inhalation of AITC Vapor in Rats

Exposure groups (10 rats /sex/group)	**Male**				**Female**			
Vapor concentration (ppm)	**0**	**5**	**10**	**25**	**0**	**5**	**10**	**25**
Olfactory Epithelium Degeneration (Total)			10	10		1	6	10
Minimal			1			1	4	
Mild			2	1			2	
Moderate			7	7				7
Marked				2				3
Respiratory Epithelium Degeneration (Total)				3				7
Minimal								1
Mild								4
Moderate				3				2
Respiratory Epithelium Atrophy (Total)				2				0
Minimal								
Mild				2				
Squamous Cell Metaplasia (Total)		1	1	6				7
Minimal		1		3				
Mild			1	1				6
Moderate				2				1
Nasal Epithelial Ulceration / Erosion (Total)				7				7
Minimal				2				4
Mild				3				3
Moderate				2				
Nasal Epithelial Mixed-Cell Inflammation (Total)				9			1	9
Minimal				2				
Mild				6			1	7
Moderate				1				2
Transitional Epithelium Degeneration (Total)				0				4
Mild								3
Moderate								1
Olfactory Nerve Bundle Atrophy (Total)				6				6
Mild				5				3
Moderate				1				3
Olfactory Bulb Atrophy in brain (Total)				9				8

Table 4. Histopathological Findings Following Subchronic Inhalation of AITC Vapor in Rats

Exposure groups (10 rats /sex/group)	**Male**				**Female**			
Vapor concentration (ppm)	**0**	**5**	**10**	**25**	**0**	**5**	**10**	**25**
(n = 16)[a]								
Minimal				2				3
Mild				7				5

Reference: Randazzo (2017); [a]10 animals/sex were used for regular necropsy observations. Another 6 animals/sex were anesthetized and perfused *in situ* to specifically investigate neuropathology.

Table 5. Miscellaneous Toxicological Endpoints Following Subchronic Inhalation of AITC Vapor in Rats

Exposure groups (n= 10 or 16)[a]	**Male**				**Female**			
Vapor concentration (ppm)	**0**	**5**	**10**	**25**	**0**	**5**	**10**	**25**
Total Motor Activity (% change)	2966±1246	2702±1128 (-9%)	2340±622 (-21%)	1865±366* (-37%)	3597±1949	3362±1647 (-7%)	2504±1032 (-30%)	2807±867 (-22%)
Ambulatory Motor Activity (% change)	536±286	448±237 (-16%)	371±158 (-31%)	311±107* (-42%)	879±579	798±421 (-9%)	481±219 (-45%)	634±159 (-28%)
Body weight: (g; n=16) (% change)	549±57	538±50 (-2%)	521±43 (-5%)	441±35** (-20%)	312±24	299±20 (-4%)	304±24 (-3%)	275±16** (-125)
Liver weight (g)	12.9±1.7	11.9±1.5	11.5±1.5	9.3±1.0**	7.47±0.8	7.43±0.9	7.04±0.7	6.41±0.9*
Liver weight relative to BW	2.42±0.16	2.37±0.15	2.34±0.15	2.24±0.14	2.51±0.16	2.64±0.33	2.51±0.2	2.54±0.21
Total bilirubin (mg/dl)	0.03±0.048	0.07±0.048	0.09±0.032*	0.08±0.042*	0.1±0.047	0.1±0	0.11 ±0.6	0.1±0

Values taken directly from Randazzo (2017); [a]Unless mentioned, number of animals = 10; Values are Mean±SD; * Significantly different from controls at * $p < 0.05$ or ** $p < 0.01$ using Dunnett's test

C.3.2 Subchronic oral toxicity

Twelve studies in which AITC or AITC-rich substances were orally administered to rats or mice for 2 to 26 weeks were evaluated for this risk assessment (Table 6). The experimental animals were exposed by oral gavage, drinking water or diet. Because of the volume of studies and because similar effects were reported under numerous exposure times and doses, the studies were grouped by effect for clarity:

1) Portal-of-entry effects in the stomach, mainly with gavage administration
2) Urinary bladder epithelial lesions
3) General toxicity, body weight and organ weights changes
4) Alterations in hematological and serum chemistry parameters at higher doses
5) Effects on thyroid glands in one human case, and in rats fed with diet containing low on iodine
6) Death at higher doses.

The observed effects depended on the mode of administration (i.e. water, diet, or gavage). For example, urothelial effects were evident in animals that received AITC or horseradish extract (HRE) in drinking water and by gavage, but not in the diet. Similarly, hyperplastic lesions in the non-glandular forestomach were seen in gavage studies but not in diet or drinking water studies.

Portal of entry effects in stomach

Rats and mice exposed to AITC by gavage showed effects at the point of contact (Hagan *et al.*, 1967; NTP, 1982) in stomach. These effects included thickened non-glandular mucosal epithelium, stomach mucosal hyperplasia, and ulceration of the stomach epithelium. A 14-day gavage study in mice generated a NOEL of 25 mg/kg/day for portal of entry effects in stomach (NTP, 1982). A 20-day gavage study in rats generated a LOEL of 20 mg/kg/day (lowest tested dose) for thickened non-glandular mucosa and ulceration of stomach mucosa (Hagan *et al.*, 1967). Administration of AITC in drinking water for 2 or 13 weeks in rats produced a low incidence of stomach mucosal hyperplasia of the glandular region. Unlike the case in gavage studies, drinking water studies did not induce changes the non-glandular region of the stomach. However, AITC in drinking water did induce mucosal hyperplasia in limiting ridge (line separating glandular and non-glandular stomach) at doses >24 mg/kg/day AITC after 2 weeks. At 13 weeks, 2/10 animals exhibited mucosal hyperplasia and erosion of the pyloric glandular region of stomach at 22.5 mg/kg/day AITC (Hasumura *et al.*, 2011).

Urinary bladder epithelial lesions

Urothelial hyperplastic changes were observed in rats exposed to AITC or AITC-rich HRE in drinking water for either 2 or 13 weeks (Hasumura *et al.*, 2011; Cho *et al.*, 2017) and in mice exposed by gavage for 14 days (NTP, 1982). In mice, the urothelial effects included thickened urothelium in males with a study LOEL of 50 mg/kg/day AITC and a NOEL of 25 mg/kg/day

AITC. In rats, simple and/or papillary/nodular hyperplasia of urothelium was reported by Hasumura *et al.* (2011) at all tested doses, but statistically significant only at doses 20.5 mg/kg/day HRE (18.5 mg/kg/day AITC equivalent) and above after 13 weeks of treatment. A NOEL of approximately 8 mg/kg/day HRE (or 6.6 mg/kg/day AITC equivalent) for urothelial effects was reported by Hasumura *et al.* (2011). Almost identical results were obtained by the same group after a 2-week administration of HRE in drinking water (Cho *et al.*, 2017). The LOEL was 23.1 mg/kg/day (19 mg/kg/day AITC equivalent) for urothelial hyperplasia and the NOEL was 7.4 mg/kg/day (6.1 mg/kg/day AITC equivalent) (Cho *et al.*, 2017).

Body weight, food consumption and organ weights

Multiple studies (NTP, 1982; Lewerenz *et al.*, 1988a; Hasumura *et al.*, 2011) reported changes in body weight and other organ weights due to subchronic oral exposure to AITC or HRE. Specifically, NTP (1982) reported lower body weight gain with increasing doses after 2-weeks of oral exposure to AITC in rats.

In a 2-week study in rats exposed to AITC or HRE, Hasumura *et al.* (2011) reported a dose-dependent decrease in body weight, accompanied by dose-dependent decreases in food and water intake. However, the decrease was significant only at the high dose of 83 mg/kg/day HRE (68 mg/kg/day AITC equivalent). Relative kidney weight (relative to body weight) was increased significantly at the high dose of 83 mg/kg/day.

Lewerenz *et al.* (1988a) exposed rats to AITC by oral gavage for 6 weeks, generating a dose-dependent increase in absolute liver and adrenal weight after 3 weeks, including at the lowest tested dose (10 mg/kg/day). The increase in liver weight was accompanied by hepatocellular hypertrophy. At the high dose, a decrease in thymus weight at 1 and 2 weeks, reduced body weight (~20%) that returned to control level after 4 weeks of treatment, decreased food intake, and a transitory increase in weight of adrenals were additionally observed.

In a 13-week drinking water study (Hasumura *et al.*, 2011), rats exposed to HRE or AITC exhibited decreased (<10%) body weight at the high dose of 0.1% HRE and with 40 mg/kg analytical-grade AITC (97% purity). The authors attributed small changes in organ weights to changes in total body weight.

Serum chemistry

AITC did not induce changes to hematological and clinical chemistry at the doses employed in the majority of available studies. However, a 6-week oral gavage study in rats by Lewerenz *et al.* (1988a) and a 13-week drinking water study in rats by Hasumura *et al.* (2011) reported changes in serum chemistry parameters in treated animals. Effects included decreased blood glucose, decreased serum globulin, and increased urinary aspartate aminotransferase (ASAT) at 40 mg/kg/day AITC (Lewerenz *et al.*, 1988a), dose-dependent (< 30%) increase in blood urea

nitrogen (BUN) in HRE treated males and females (Hasumura *et al.* (2011)), and decreased total cholesterol in females exposed to analytical-grade AITC in drinking water for 13 weeks (Lewerenz *et al.*, 1988a).

Effects on the thyroid gland

No goitrogenic effects were observed in rats or mice exposed to AITC and fed with regular diet that contained normal levels of iodine (NTP, 1982; Lewerenz *et al.*, 1988a). However, rats fed low-iodine diet and exposed to AITC for 20 to 60 days demonstrated thyroid effects (Langer, 1964; Langer and Stolc, 1965). These effects included increased weight of thyroid glands, increased serum thiocyanate levels, and decreased protein-bound iodine in plasma. Protein-bound iodine levels in plasma were decreased only in studies with longer periods of exposure to AITC (Langer, 1964).

Death at higher doses

Only one study reported death due to AITC treatment All rats treated at doses of 200 mg/kg/day or higher died between days 2 and 9 (NTP, 1982). Pathology examination revealed stomach mucosal thickening and adhesion of stomach to the peritoneum in rats administered 50-400 mg/kg/day.

Table 6. Subchronic Toxicity Studies of AITC and AITC-Rich Substances

Author	Study Design Species, Route, Dose and Duration	Effects at LOEL	NOEL	LOEL
Inhalation route				
Randazzo (2017)	Inhalation; 13-week, whole-body Inhalation exposure; rats, 16M/16F per group; 0, 5, 10, 25 ppm; FOB and motor activity measurements on day 0 and week 12	Mild-to-moderate degeneration of olfactory epithelium in males and females; mild metaplasia of respiratory epithelium in males; decreased motor activity in both sexes	5 ppm or 0.02 mg/L[b]	10 ppm or 0.041 mg/L
Oral route (listed order of increasing study duration)				
NTP (1982)	Oral gavage; 14-day; F344/N rats, 5M/5F rats; 25, 50, 100, 200, 400 mg/kg/day; No controls group was used	Inactivity and ruffled feather in all treated animals, decreased body weight gain	ND	25
NTP (1982)	Oral gavage; 14-day; B6C3F1 mice, 5M/5F; 3, 6, 12, 25, 50 mg/kg/day	Thickened stomach nonglandular mucosa, thickened urothelium in males	25	50
Hasumura *et al.* (2011)	Oral drinking water; AITC; 2-weeks; F344/DuCrj rats, 5M; AITC - 0, 0.025%, 0.05%, 0.1% (0, 24.2, 47.9, 83 mg/kg/day, respectively)	Urinary bladder simple, and papillary/nodular hyperplasia	ND	24
	Oral drinking water; horseradish extract (HRE); 2-weeks; F344/DuCrj rats, 5M; HRE – 0, 22.8, 46.5, 69.8 mg/kg/day		ND	22.8 (or 18.7 for AITC[a])
Cho *et al.* (2017)	Oral drinking water; BrdU incorporation; horseradish extract (HRE); 2-wks; rats; HRE doses – 0, 0.005%, 0.01%, 0.04% in drinking water (or 0, 3.2, 6.1, 18.9 mg/kg/day of AITC) Sacrifices after day 1, day 3, week 1, or week 2 of treatment BrdU was injected 1 hour before sacrifice	Increased incidence of simple and papillary/nodular hyperplasia.	7.4 (or 6.1 for AITC[a])	23.1 (or 18.9 for AITC[a])
Hagan *et al.* (1967)	Oral gavage; 20-day; weanling Osborne-Mendel rats, 5M/5F; 0, 20, 50 mg/kg/day	Thickened nonglandular mucosa, ulceration of stomach mucosa	ND	20
Langer (1964)	Oral gavage; 20 or 50-day; Wistar offspring, Dobra Voda breed rats; 6M rats at 6 mg AITC for 20 days; 11M rats at 2 mg AITC for 50 days; rats given low-iodine feed	Increased weight of thyroid gland No change in iodine level in thyroid gland, or the serum-protein-bound iodine; serum thiocyanate levels increased significantly	15[2]	45

Table 6. Subchronic Toxicity Studies of AITC and AITC-Rich Substances

Author	Study Design Species, Route, Dose and Duration	Effects at LOEL	NOEL	LOEL
Lewerenz *et al.* (1988a)	Oral gavage; 6-weeks; WIST Rats, M (number not reported); 0, 10, 20, 40 mg/kg/day, 5 day/week	From weeks 1 to 3, increased weight of liver, and adrenals was present at all doses	ND	10
Langer and Stolc (1965)	Oral gavage; 60 days; Wistar rats, 5 – 6M; 0, 2.5 mg, and 5 mg; rats given low-iodine feed	Decreased serum-protein-bound iodine; Increased trend in thyroid weight, only significant at high dose	ND	6.7 mg/kg/day[c]
Hasumura et al. (2011)	Oral drinking water; AITC and HRE; 13-weeks; F344/DuCrj rats, 10F/10M; HRE – 0.0125%, 0.025%, 0.05% (estimated AITC intake in males – 10.7, 16.3, 30.6 mg/kg/day, respectively; in females – 9.1, 17.2, 30.7 mg/kg/day, respectively); AITC – 0.0425% (40 and 37.9 mg/kg/day in males & females, respectively)	Simple bladder mucosal hyperplasia in both sexes for HRE and AITC. BUN levels were increased in a statistically significant and dose-dependent manner, but with a high of < 30% increase.	8 mg/kg/ day[d] HRE (or 6.6 mg/kg/day AITC)	20.5 mg/kg/day HRE (or 17.2 mg/kg/day AITC)
NTP (1982)	Oral gavage; 13-week; F344/N rats; 10M/10F; 0, 1.5, 3, 6, 12, 25 mg/kg/day (5 days per week)	No treatment related effects observed	25	ND
NTP (1982)	Oral gavage;13-week; B6C3F1 mice, 10M/10F; 0, 1.5, 3, 6, 12, 25 mg/kg/day (5 days per week)	No treatment related effects observed	25	ND
Hagan *et al.* (1967)	Oral dietary; 26-week; weanling Osborne-Mendel rats, 5M/5F rats; 0, 1000, 2500, 10,000 ppm (0 – 700 mg/kg/day equivalent)	No treatment related effects observed	700[e],	ND

Abbreviations: 5-Bromo-2′-Deoxyuridine: BrdU; Blood Urea Nitrogen: BUN; females: F; Horseradish extract: HRE; males: M; Not determined: ND

[a]Calculated based on adjustment of HRE for AITC content of 82% (Hasumura et al., 2011 or Cho et al., 2017); [b]Calculated using mean BW of 133 g at Day 0 of the experiment (Langer 1964): 2 mg/d AITC divided by 0.133 g BW = 15 mg/kg/day; [c]Calculated using BW at the end of study : 2 mg AITC/0.299 = 6.7 mg/kg/day; [d]After accounting for the stability of HRE-mixed drinking water, 9.1 mg/kg/day HRE in females was converted to 8 mg/kg/day HRE by the authors (Hasumura et al., 2011); [e]Daily dose in mg/kg/day was calculated using default values for a chronic study for Osborne-Mendel strain of female and male rats (US EPA, 1998 Recommendations for and Documentation of Biological Values for Use in Risk Assessment) – Food intake factor 0.07 and 0.77 kg/day/kg BW used to derive 700 and 770 mg/kg/day for male and female, respectively.

C.4 Reproductive and Developmental Toxicity

Tanner (2017)

Study Methods

Tanner (2017) studied the effects of 0 (corn oil vehicle), 20, 40 or 60 mg/kg/day AITC (99.9% purity) on reproductive performance in parental and offspring Crl:CD(SD) rats. Doses were based on results from a 2-generation range-finding study. Parental generation (F0) rats (25 rats/sex/dose) were treated via gavage for a total of 127 – 132 days, including 70 days prior to mating and throughout mating and lactation. Similarly, F1 rats (25/sex/dose) were treated for a total of 139 – 148 days, including in utero exposures and via milk until weaning. F1 parents were selected after randomly culling litters on post-natal day (PND) 21. F2 animals were not gavaged, but were exposed through milk from dams. F2 pups were sacrificed on PND21 for examination.

In addition to clinical observations, F0 and F1 adults were assessed for reproductive performance indicators, including estrous cycles, parturition, and breeding indices (mating, fertility, copulation, and conception). F1 and F2 litters were assessed for viability and mortality, litter size, clinical observations of pups, body weights, and litter sex ratios. Developmental landmarks (balanopreputial separation and vaginal patency) and ophthalmic examinations were conducted in F1 animals only. Spermatogenic endpoint evaluations were conducted in F0 and F1 males. A complete necropsy was conducted on all parental animals (F0 and F1) found dead, euthanized or at termination. Gross necropsies with emphasis on developmental morphology and reproductive organs were performed on select euthanized F1 and F2 weanlings. Histological examination was performed on multiple tissues, including eyes, from all F0 and F1 parental animals found dead or euthanized. The results from this study are summarized in Table 7 and Table 8.

Results

Parental Effects

Urinary bladder epithelial hyperplasia was observed in both F0 and F1 parents at a high incidence compared to control. The incidence was at or near maximal response in all dose groups in both F0 and F1 rats. The severity of this lesion also increased with dose.

Hyperplasia of non-glandular stomach mucosa was noted at higher incidence in all treated F0 parents compared to controls. All doses had near or at maximal response, and severity increased with dose. In contrast to F0 parents, fewer F1 parents showed stomach mucosal hyperplasia at 20 mg/kg/day,

Eye examinations revealed dose responsive corneal opacity and enophthalmus[5], at 40 and 60 mg/kg/day in F1 parents. These lesions were neither observed at 20 mg/kg/day nor in F1 controls (except 1/25 male), and in none of the F0 parents. Detailed ophthalmic examination prior to euthanasia in F1 parents showed increased incidence of unilateral and/or bilateral dose-dependent cataracts in both males (3/25, 3/24, 10/24, 24/24) and females (0/25, 1/25, 7/24, 21/21). Ocular histology revealed a dose-dependent increase in the incidence of retinal dysplasia in both males (2/25, 4/25, 8/24, 24/24) and females (1/25, 2/25, 9/24, 21/21). The incidence of retinal dysplasia (both sexes) and cataracts (females) at 40 and 60 mg/kg/day were statistically significant compared to controls ($p < 0.05$), but not at 20 mg/kg/day. These lesions were not observed in the F0 parents, suggesting that they are developmental effects.

Liver weight relative to body weight increased in a dose-related manner and was statistically significant in all treatment groups at termination after analysis by study authors. This is in contrast to the terminal body weight, which decreased at 40 and 60 mg/kg/day. However, no microscopic changes in the liver correlated with increased relative liver weight (Table 8).

Additional effects were observed in parental males at 40 and 60 mg/kg/day included decreases in terminal body weights. Forty and 60 mg/kg/day F0 parents also showed dose dependent increases in incidence of adrenal cortical hypertrophy and relative weight at these doses. Relative brain weights were also increased at 40 and 60 mg/kg/day, but showed no histopathologic parallel.

Estrous cycle and gestation length were unaffected. Although relative testis weights were slightly increased at 40 and 60 mg/kg/day doses, there were no toxicologically significant changes in spermatogenic parameters. Reproductive performance indices were not altered in any of the parental dose groups.

F1 and F2 Pup Effects

A dose response in postnatal pup survival and decrements in pup body weights were observed at 40 and 60 mg/kg/day. The majority of pup losses occurred between PND0 to PND4. These parameters were unaffected at 20 mg/kg/day dose. Except for spleen weight in both F1 and F2 pups at termination, other organ weights were observed to be proportional to the body weight. Both absolute and relative spleen weight (relative to body weight) were decreased in a dose related manner at 40 and 60 mg/kg/day.

Developmental Effects

Neither balanopreputial separation nor vaginal patency were affected by treatment. As noted above, retinal dysplasia usually accompanied by cataract formation was noted in F1 animals at

[5] Enophthalmos can be defined as a retrodisplacement of the globe within the bony confines of the eye socket. Source: www.sciencedirect.com/topics/medicine-and-dentistry/enophthalmos; Accessed on July 23, 2020.

termination in dose-dependent manner in both males (2/25, 4/25, 8/24, 24/24) and females (1/25, 2/25, 9/24, 21/21).

Conclusion

A study LOEL of 20 mg/kg/day based on hyperplasia of non-glandular stomach and urinary bladder epithelium in F0 and F1 animals. As this was the low dose, a NOEL was not established.

Table 7. Findings in a Two-Generation Reproductive and Developmental Toxicity Study of AITC in Rats

Sex	**Male**				**Female**			
Dose (mg/kg/day)	**0**	**20**	**40**	**60**	**0**	**20**	**40**	**60**
F0								
Stomach, squamous cell hyperplasia	0/24	24/25*	25/25*	25/25*	0/22	25/25*	22/23*	23/23*
Urinary bladder, hyperplasia	0/24	24/25*	25/25*	23/25*	0/22	24/25*	22/23*	23/23*
Adrenal cortex, hypertrophy	0/24	0/25	4/25	5/25	0/22	0/25	9/23*	7/23*
Opacity – eye (left or right; n=25)	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25
Enophthalmus (left or right; n=25)	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25
F1								
Urinary bladder, hyperplasia	0/25	24/25*	24/24*	24/24*	0/25	25/25*	24/24*	21/21*
Stomach, hyperplasia, squamous cell	0/25	16/25*	24/24*	24/24*	0/25	10/25*	24/24*	21/21*
Eyes, retinal dysplasia	2/25	4/25	8/24*	24/24*	1/25	2/25	9/24*	21/21*
Eyes, cataract (unilateral or bilateral)	3/25	3/25	10/24	24/24*	0/25	1/25	7/24	21/21*
Opacity – Left eye	0/25	0/25	7/25*	23/25*	0/25	0/25	6/25*	22/25*
Opacity – Right eye	0/25	0/25	6/25*	24/25*	0/25	0/25	7/25*	22/25*
Enophthalmus – Left eye	1/25	0/25	6/25*	25/25*	0/25	0/25	5/25*	25/25*
Enophthalmus – Right eye	0/25	0/25	6/25*	25/25*	0/25	0/25	7/25*	25/25*

Reference: Tanner (2017); * = Significant, $p < 0.05$. Analysis by investigators.

Table 8. Effects of AITC in a Two-generation Reproductive and Developmental Toxicity Study in rats

Sex	Male	Male	Male	Male	Female	Female	Female	Female
Dose (mg/kg/day)	*0*	*20*	*40*	*60*	*0*	*20*	*40*	*60*
F0								
N	24	25	25	25	22	25	23	23
Final Body Weight (g ± SD)	701 ± 106	712 ± 93	673 ± 83	585 ± 88**	314 ± 26	325 ± 22	330 ± 28	335 ± 38
Liver (g per 100 g body weight ± SD)	3.2 ± 0.2	3.5 ± 0.3**	3.7 ± 0.3**	4.0 ± 0.3**	3.7 ± 0.5	3.8 ± 0.4	3.9 ± 0.2	4.2 ± 0.3**
F1								
N (unless otherwise noted)	25	25	24	24	25	25	24	21
Pup Litter Initial Weight (PND 1) (g ± SD)	7.1 ± 0.7[d]	7.1 ± 0.9[g]	6.3 ± 1.0 **[g]	5.5 ± 0.8**[f]	6.7 ± 0.7[d]	6.7 ± 0.8[g]	6.0 ± 0.9**[g]	5.3 ± 0.8**[e]
Pup Litter Weaning Weight (PND 21) (g ± SD)	57.3 ± 4.9[c]	57.7 ± 5.8[g]	52.0 ± 5.1*[f]	43.5 ± 7.9**[b]	55.0 ± 3.8[c]	55.5 ± 5.6[g]	50.3 ± 4.7*[f]	43.1 ± 5.6**[a]
Pup Survival (PND 4) (% ± SD)	NA	NA	NA	NA	87 ± 26	94 ± 17	78 ± 32	59 ± 38*
Final Body Weight (g ± SD)	724 ± 65	736 ± 90	642 ± 93**	526 ± 66**	314 ± 23	333 ± 30	331 ± 33	312 ± 36
Adrenal Glands (g per 100 g body weight ± SD)	0.0090 ± 0.0011	0.0090 ± 0.0018	0.010 ± 0.0013*	0.013 ± 0.0023**	0.023 ± 0.0043	0.024 ± 0.0038	0.024 ± 0.0029	0.029 ± 0.0053**
Liver (g per 100 g body weight ± SD)	3.33 ± 0.32	3.56 ± 0.41	3.79 ± 0.37**	3.94 ± 0.32**	3.78 ± 0.30	3.97 ± 0.30	4.06 ± 0.30**	4.17 ± 0.28**
F2								
N	24	23			24	23		
Pup Litter Initial Weight (PND 1) (g ± SD)	7.2 ± 0.8	7.2 ± 0.9	6.6 ± 0.7[f]	5.7 ± 0.8[c]	6.9 ± 0.7[h]	6.8 ± 0.7	6.1 ± 0.6[e]	5.1 ± 0.8[f]
Pup Litter Weaning Weight (PND 21) (g ± SD)	54.2 ± 6.9	58.4 ± 5.4	52.1 ± 5.0[b]	40.3 ± 10.2[i]	51.8 ± 5.4	55.9 ± 4.9	49 ± 7.2[b]	41.1 ± 4.9[j]
Final Body Weight (g ± SD)	55 ± 8.4	57 ± 6.9	52 ± 5.7	41 ± 10.4**	52 ± 6.5	56 ± 4.8	49 ± 7.7	41 ± 4.6**

Reference: Tanner (2017); all statistical test results are as reported by the study authors *p < 0.05 **p < 0.01; [a]n=16 [b]n=17 [c]n=18 [d]n=19 [e]n=20 [f]n=21 [g]n=22 [h]n=25 [i]n=11 [j]n=10

Food and Drug Research Laboratories, FDRL (1973)

Study Methods

The developmental toxicity of Oil of Mustard (designated as FDA 71-26) was evaluated in mice, rats, hamsters, and rabbits. Oil of mustard was diluted in corn oil and administered by gavage for several days depending on the species. Aspirin was used as the positive control. Albino CD-1 mice (22 – 25 animals/dose) were treated with 0, 0.3, 1.3, 6.0 or 28 mg/kg/day from gestation day (GD) 6 to 15 and pups were delivered on GD17. Wistar rats (20 – 24 animals/dose) were treated 0, 0.2, 0.85, 4, or 18.5 mg/kg/day from GD6 to GD15 and pups were delivered GD20. Outbred golden hamsters (25 – 27 animals/dose) were treated with 0, 0.2, 1.1, 5.1, or 23.8 mg/kg/day from GD6 to GD10 and pups were delivered on GD14. Dutch-belted rabbits (11 – 13 animals/dose) were treated with 0, 0.123, 0.6, 2.8, or 12.3 mg/kg/day from GD0 to GD14 and pups were delivered on GD29. The number of implantation sites, resorption sites, and live and dead fetuses were recorded at cesarean section. Each pup was examined for weight and visceral and skeletal defects.

Results

Neither fetal nor maternal effects were observed at the highest tested doses in rats, rabbits and hamsters, resulting in species-specific NOELs of 18.5, 12.3, and 23.8 mg/kg/day in rat, rabbits, and hamsters, respectively. Although hamsters exhibited a slight increase in litter and fetal incidences of incomplete ossification of sternebrae, and missing sternebrae, they were not statistically significant compared to control based on analysis by DPR. Mice exhibited increased litters with resorption sites, and numbers of dead fetuses at a LOEL of 28 mg/kg/day, and a NOEL of 6 mg/kg/day. The authors established the maternal and developmental NOEL at 6 mg/kg/day for this study.

C.5 Genotoxicity

The entire genotoxicity database for AITC was identified in a systematic review of the open literature. Various genotoxicity endpoints were examined, including mutagenicity, DNA alkylation, DNA damage, and clastogenicity. AITC was negative for mutagenicity under the conditions of two *in vivo* and three *in vitro* studies. On the other hand, AITC was weakly positive for point mutations in six other studies, mostly at or near cytotoxic concentrations. AITC was DNA reactive in alkylation tests but was regarded as having “poor or borderline” activity by the study authors. Cytotoxicity and DNA damage were observed in several studies. Both negative and positive evidence for clastogenicity was reported. The studies consistently demonstrated that AITC induced DNA damage in the test systems. Studies and their results are summarized in Table 9 and Table 10.

C.5.1 Mutagenicity

Two *in vivo*, and ten *in vitro* mutation studies for AITC were identified in a systematic review of the open literature. AITC was not mutagenic in either *in vivo* study that used the *Drosophila melanogaster* model. Bactericidal activity was consistently demonstrated in the *in vitro* studies.

In an *in vitro* mouse lymphoma thymidine kinase mutation assay, a positive result was observed in the presence of cytotoxicity (McGregor *et al.*, 1988). AITC was negative for inducing mutations in TA97, TA1535, TA1536, TA1537, and TA1538 Salmonella strains and in the WP67 *E. coli* strain. Inconsistent results were reported in TA100 and TA98 Salmonella strains for mutagenicity and requirement of adding the S9 supernatant fraction of liver homogenate. AITC ± S9 was negative in inducing point mutations in TA98 and/or TA100 in multiple studies (Eder *et al.*, 1980; Kasamaki *et al.*, 1982; Azizan and Blevins, 1995). Similarly, multiple studies published weakly positive (~2-3 fold compared to control) outcomes for the same TA98 and/or TA100 salmonella strains, usually at or near concentrations that caused cellular toxicity (Yamaguchi, 1980; Rihova, 1982; Neudecker and Henschler, 1985; Mortelmans *et al.*, 1986; Kassie and Knasmuller, 2000).

C.5.2 DNA reactivity

Two publications were identified that tested DNA reactivity and bacterial mutagenicity *in vitro* (Eder *et al.*, 1980; Eder *et al.*, 1982). Specifically, the authors tested alkylating property of AITC, and other allyl and allylic compounds using a standard alkylation test (4-(p-nitrobenzyl)-pyridine or NBP test). This method is based on the formation of a chromophore in the reaction between an alkylating agent and the nucleophile NBP that is measured spectrophotometrically. AITC's ability to react with NBP was tested in the solvents ethyl methyl ketone and ethyl glycol. AITC was negative for alkylation readings with ethyl methyl ketone as the solvent and was borderline reactive with ethylene glycol as the solvent. In the same assays, multiple other chemicals were positive (Eder *et al.*, 1980; Eder *et al.*, 1982). Taken together the authors concluded that AITC was a poor or borderline alkylating agent.

C.5.3 DNA damage and clastogenicity

Charron *et al.* (2013) used a randomized crossover study with 46 human volunteers in USA (40-79 years age, 34 female and 12 female) to evaluate the ability of AITC to cause DNA damage by employing comet assay. The study protocol was approved by MedStar Health Research Institute (Hyattsville, MD) and written, informed consent was obtained from each individual. Volunteers were fed 114.7 µmol/day (11.4 mg/day) food-grade AITC in the evening diet for 11 days, followed by a washout period (no treatment) of 17 days. On day 11 volunteers consumed a bolus dose of AITC in morning. Urine and blood samples were collected before and after the bolus dose and DNA strand breaks were analyzed in peripheral blood mononuclear cells and level of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), a marker of DNA oxidation, was estimated in urine samples. No DNA strand breaks or DNA oxidation products were observed after 10 days of AITC administration. No DNA oxidation was detected, but a small increase in DNA strand breaks (8% in treated compared to 3% in controls) was observed after the acute bolus dose on day 11. The DNA breaks were no longer present 6 hours after administration.

In an *in vivo* rat test system, Bechtel *et al.* (1998) showed that AITC did not induce unscheduled DNA synthesis (UDS) in liver. The rats were treated with a single dose of 0 (corn oil), 3.75, 125,

250 or 500 mg/kg/day AITC by gavage in 4 rats/dose. The rats were killed, hepatocytes were isolated and cultured, and incubated with (methyl-3H)thymidine for 4 hours. The cell were washed off (methyl-3H)thymidine and cultured 24 hours to chase the radiolabel. Radiolabel incorporation into DNA was visualized observed by autoradiography to count of cell containing "nuclear grain" structures as evidence of unscheduled DNA synthesis. The authors concluded that AITC did not induce UDS, although it was evident in the parallel positive controls (2-acetylaminoflurene or DMA). Mortality was observed in both 250 and 500 mg/kg/day groups.

Multiple reports on testing AITC for *in vitro* DNA damage and/or clastogenicity were identified. When tested in the absence but not in the presence of the S9 fraction, AITC induced repairable DNA damage in the bacterial systems. All *in vitro* tests detecting DNA damage by comet assay were positive (Table 9). Both positive and negative outcomes have been reported for chromosomal aberrations in multiple studies (Table 9). However, micronucleus assays, including at AITC concentrations that induced DNA strand breaks by comet assay, were negative for micronuclei formation (Shelby *et al.*, 1993; Kassie and Knasmuller, 2000; Savio *et al.*, 2014).

Therefore, *in vivo* data show that AITC induced damage only at bolus dose and did not induce DNA oxidation products in urine. *In vitro* data show that AITC induced DNA damage, and clastogenicity but not micronucleus formation.

Table 9. Genotoxicity Studies of AITC

Test System(s)	**Exposure Concentrations or Doses**	**S9 Fraction**	**Outcome(s)**	**Reference(s)**
Mutagenicity				
In vivo mutagenicity; Induction of sex-linked recessive lethals in *Drosophilia melanogaster*	Dietary: 0, 650 ppm; Injection: 0, 700 ppm	NA	Negative	Valencia *et al.* (1985)
In vivo mutagenicity; Induction of sex-linked recessive lethals in *Drosophilia melanogaster*	Feeding: 0, 54 ppm	NA	Negative	Zimmering *et al.* (1989)
In vitro mutagenicity; L5178Y tk+/tk- mouse lymphoma cell; forward mutation assay	0.2 to 1.6 µg/mL	Absent	Positive (2.4-fold) (at 15% growth of control)	McGregor *et al.* (1988)
In vitro mutagenicity; reverse mutation; *Salmonella typhimurium* (TA1535, TA1536, TA1537, TA1538, TA98, and TA100)	0 to 100 µg/plate	Both	Positive (2.5-fold)	Yamaguchi (1980)
In vitro mutagenicity; reverse mutation; *E. coli* (TA1535, TA1537, TA1538, TA98)	0.1 to 5 mM	Present Absent	Positive (at ≥ 3 mM, > 3-fold) Negative	Rihova (1982)
In vitro mutagenicity; reverse mutation; *Salmonella typhimurium* (TA1535, TA1537, TA97, TA98, and TA100)	1 to 1000 µg/plate	Present Absent	Weakly positive (< 2-fold) Negative (< 1.5-fold)	Mortelmans *et al.* (1986)

Table 9. Genotoxicity Studies of AITC

Test System(s)	Exposure Concentrations or Doses	S9 Fraction	Outcome(s)	Reference(s)
In vitro mutagenicity; reverse mutation; *Salmonella typhimurium* (TA97, TA98, TA100); extended incubation time (60 minutes); identical to Yamaguchi, 1980)	~10 to 200 µg/plate	Present Absent	Negative Weakly positive (~2-fold)	Kassie and Knasmuller (2000)
In vitro mutagenicity; reverse mutation; *Salmonella typhimurium* (TA100); extended incubation time (120 minutes)	Up to 0.5 µL/plate	Present Absent	Weakly positive (~2-fold; and mitigated by S9) Negative	Neudecker and Henschler (1985)
In vitro mutagenicity; reverse mutation; *Salmonella typhimurium* (TA98, TA100)	0.05 to 500 µg/plate	Both	Negative	Kasamaki *et al.* (1982)
In vitro mutagenicity; reverse mutation; *Salmonella typhimurium* (TA97, TA98, TA100); extended incubation time (120 minutes)	1 mg/mL	Both	Negative	Azizan and Blevins (1995)
In vitro mutagenicity; reverse mutation; *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98); modified liquid suspension	Not Specified	Present Absent	Negative Negative	Eder *et al.* (1982)
In vitro mutagenicity; reverse mutation; *Salmonella typhimurium* (TA100)	0.0003 to 0.1 µL/2mL	Both	Negative (<1-fold)	Eder *et al.* (1980)
In vitro DNA reactivity				
NBP-Test for alkylation of DNA: AITC and 4-(p-nitrobenzyl)-pyridine were reacted to identify alkylation of NBP by spectrophotometer	Ethyl methyl ketone solvent	NA	Borderline positive	Eder *et al.* (1982)
NBP-Test for alkylation of DNA: AITC and 4-(p-nitrobenzyl)-pyridine were reacted to identify alkylation of NBP by spectrophotometer	Ethyl methyl ketone solvent Ethylene glycol solvent	NA	Negative Borderline positive	Eder *et al.* (1980)
In vivo unscheduled DNA Synthesis				
In vivo unscheduled DNA synthesis; Sprague-Dawley Rats (Hsd/Ola), hepatocytes; 2 hr and 12 hr	Oral: 37.5 to 125 mg/kg	NA	Negative	Bechtel *et al.* (1998)
In vivo DNA Damage				
In vivo DNA damage; strand breaks; Comet assay; humans 40-79 years old; lymphocytes	Dietary: 114.7 µmol/person/d	NA	Positive	Charron et al., 2013
In vivo DNA damage; micronucleus test; mouse (B6C3F1) bone marrow; 9-14 weeks	37.5 to 150 mg/kg	NA	Negative	Shelby *et al.* (1993)

Table 9. Genotoxicity Studies of AITC

Test System(s)	Exposure Concentrations or Doses	S9 Fraction	Outcome(s)	Reference(s)
In vivo repairable DNA damage; differential DNA repair assay; *E. coli* injected to mice simultaneously with AITC	Gavage: 90 or 270 AITC mg/kg/day	NA	Positive	Kassie and Knasmuller (2000)
In vitro DNA damage				
In vitro repairable DNA damage; differential DNA repair assay; *E. coli* cultured with AITC	0, 12.5, 25, 50, 100, 200 µg/plate	Present Absent	Negative Positive at higher conc.	Kassie and Knasmuller (2000)
In vitro DNA damage; Comet assay; human breast cancer cells (MCF-7, MDA-MB-231)	1 to 25 µM	Absent	Positive	Bo *et al.* (2016)
In vitro DNA damage; strand breaks; Comet assay; human hepatocellular carcinoma (HepG2); 24 hour exposure	0.1 to 1 µM	Absent	Positive	Garcia *et al.* (2008)
In vitro DNA damage; strand breaks; Comet assay; human hepatoblastoma (HepG2)	0.1 to 50 µM	Absent	Positive (at >25 µM)	Laky *et al.* (2002)
In vitro DNA damage; strand breaks; Comet assay; human hepatoblastoma (HepG2)	2.5 to 20 µM	Absent	Positive	Liu *et al.* (2018)
In vitro DNA damage; Comet assay; human urothelial carcinoma	0.005 to 0.25 µM	Absent	Positive	Savio *et al.* (2014)
In vitro DNA damage; p53 gene DNA fragments; outside of cell environment in the presence of copper	0.2 to 2.0 mM	NA	Positive	Murata *et al.* (2000)
In vitro DNA damage; DNA damage response; phospho-Chk1; non-small cell lung cancer cells (A549)	20 µM	Absent	Positive	Tripathi *et al.* (2015)
In vitro chromosomal aberrations				
In vitro sister chromatid exchange; Chinese hamster ovary cells (B241)	0.1 to 1.6 µg/mL	Present Absent	Positive Negative	Galloway *et al.* (1987)
In vitro chromosomal aberrations; Chinese hamster ovary cells (B241)	0.1 to 1.6 µg/mL	Both	Weakly positive	Galloway *et al.* (1987)
In vitro chromosomal aberrations; Chinese hamster ovary cells (B241); 5-day exposure	1 to 10 nM	Absent	Positive	Kasamaki and Urasawa (1985)
In vitro chromosomal aberrations; bovine artery endothelial (CPAE CCL 209) and human fibroblast (HAIN-55)	20 nM	Absent	Positive	Kasamaki and Urasawa (1993)
In vitro chromosomal aberrations; Chinese hamster cells (B241)	5 nM	Both	Positive	Kasamaki *et al.* (1982)
In vitro chromosomal aberrations; SV-40-transformed Indian muntjac	0.2 to 0.8 µg/mL	Absent	Negative	Musk and Johnson (1993)

Table 9. Genotoxicity Studies of AITC

Test System(s)	Exposure Concentrations or Doses	S9 Fraction	Outcome(s)	Reference(s)
In vitro chromosomal aberrations; Chinese hamster ovarian cells	2.4 to 3.0 µg/mL	Absent	Negative	Musk *et al.* (1995)
In vitro clastogenicity; micronucleus assay; human urothelial carcinoma	0.005 to 0.25 µM	Absent	Negative	Savio *et al.* (2014)
In vitro clastogenicity; micronucleus assay; Human Hep G2 cells	0 to 4 µg/ml	NA	Negative	Kassie and Knasmuller (2000)
In vitro neoplastic transformation				
In vitro neoplastic transformation; Chinese hamster and human diploid fibroblast (HAIN-55); transplanted into 5 week old male mice (BALB/c, JCL, NuNu)	Chinese hamster: 5 nM HAIN-55: 20 nM	Absent	Positive Negative	Kasamaki *et al.* (1987)

Table 10. Genotoxicity Study Results Summary

Study Type	Number of Studies	Number with Positive Results	Number with Negative Results	Number with Mixed Results
Mutagenicity	12	2	6	4
In vivo mutagenicity	2	0	2	0
In vitro mutagenicity	10	2	4	4
DNA reactivity	2	0	2	0
Unscheduled DNA synthesis	1	0	1	0
DNA damage	11	9	1	1
In vivo DNA damage	3	2	1	0
In vitro DNA damage	8	7	0	1
Chromosomal aberrations	9	4	4	1

C.6 Chronic Toxicity and Carcinogenicity

C.6.1 Chronic inhalation toxicity

No studies evaluating the toxicity of AITC via the inhalation route were available.

C.6.2 Chronic oral toxicity

Three studies in which AITC or AITC-rich substances were orally administered to rats or mice for 103 weeks were evaluated for chronic toxicity in this risk assessment (Table 14).The chronic oral toxicity and carcinogenicity of AITC or AITC-rich horseradish extract (HRE) was evaluated in 2 studies using rats and mice (NTP, 1982; Cho *et al.*, 2017). NTP (1982) administered AITC by gavage with corn oil as vehicle for 2 years in rats and mice (both sexes). Cho *et al.* (2017)

administered HRE in drinking water for 2 years to male rats. A third study evaluating the ability of AITC to protect mice from 4-(methylnitrosamino)-1-(3-pyridyl)butanone (NNK) mediated adenomas was also reviewed (Jiao *et al.*, 1994a).

NTP (1982)

This study is comprised two separate 103-week oral oncogenicity bioassays with similar protocols, one in rats and one in mice.

Rat Study

Study Methods

Fifty F344/N rats/sex/dose were exposed to 0 (corn oil vehicle), 12 or 25 mg/kg/day AITC (>93% purity) by oral gavage, 5 days/week for 103 weeks. The mixture of corn oil and AITC was prepared once a week and stored at 5°C. The animals were regularly observed for clinical manifestations, morbidity, and mortality. Body weight and food / water intake were also recorded on a regular basis. Necropsies were conducted on terminal sacrifices (weeks 104 – 106) and deceased animals and included both macro- and microscopic examinations of multiple organs. The results from this study are summarized in Table 11.

Results

Over the course of the study, one male and two females in the low-dose group and one male in the high-dose group were accidentally killed. The survival rate of 58 – 74% was comparable among all groups, including controls. The authors reported that this survival rate was lower than that usually observed in their laboratory. Consistently higher body weights compared to controls were observed in all treated females and low-dose males. Lower male mean body weights at the high dose occurred throughout the study, with the greatest deficit (13%) at 26 weeks.

Retinopathy and cataract formation were observed at higher rates than controls in high-dose males and both low- and high-dose females. Among females, low-dose animals had the highest incidence of retinopathy and cataracts. The authors reported that higher incidence of these lesions occurred most frequently in animals occupying the two top levels of the racks (i.e., high-dose males, and low- and high-dose females), a position that resulted in maximum light exposure. According to the authors, this correlation was also observed with other chemicals undergoing similar assessments, although not all (e.g., stannous chloride). A causative relationship between AITC exposure and ocular effects was unclear.

Dose-dependent pre-neoplastic and neoplastic lesions were present in the urinary bladder of males. The incidence of urinary bladder epithelial hyperplasia at ascending doses was 0/49, 1/49, 7/49 in males and 0/49, 0/49, 1/50 in females. Similarly, the incidence of urinary bladder epithelial papilloma was 0/49, 2/49, 4/49 in males and 0/49, 0/49, 1/50 in females. According to the investigators, urinary bladder papillomas were not observed in 568 untreated male control F344/N rats in their laboratory. They also reported that the incidence of urinary bladder

papillomas in male controls in all laboratories in the NCI/NTP bioassay program was 1/994 (0.1%). Based partly on these data, they concluded that urinary bladder epithelial tumors may have been induced by AITC.

The incidence rate for fibrosarcomas was 3/50 in high-dose females, with none in controls, low dose females, or males at any dose. According to the investigators, the incidence rate of 3/50 (6%) in high-dose females was greater than the historical incidence rate of 9/999 (0.9%) for this tumor in vehicle-treated females in all laboratories in the NCI/NTP bioassay program. They concluded that the evidence for AITC-induced fibrosarcomas was "equivocal" based on the NTP's cancer evaluation criteria after comparing against historical background data[6].

The incidence of undifferentiated leukemia[7] increased with a statistically significant trend in males (Cochran Armitage trend test, $p < 0.05$), though there was no significant trend in females (Table 11). The incidence rate in high-dose males was also significant by pairwise comparison (Fisher's Exact test and Incidental Tumor test, $p < 0.05$). However, the observed incidence was not statistically different when compared to the historical incidence rate in male gavage controls in all laboratories in the Bioassay Program (96/999, 10%). Consequently, the authors considered the observed leukemias to be unrelated to AITC treatment.

In conclusion, both non-neoplastic and neoplastic effects were observed at the lowest tested dose of 12 mg/kg/day. Based on cataracts in females and urothelial hyperplasia in males, 12 mg/kg/day was established as the LOEL for non-neoplastic effects. A study NOEL could not be established.

Mouse Study

Study Methods

Fifty B6C3F1 mice/sex/dose were treated with corn oil (vehicle control), 12 or 25 mg/kg/day AITC by oral gavage, 5 days/week for 103 weeks. The study protocol was similar to the one for the corresponding study using rats (see above).

Results

Survival at the end of the 103 weeks was similar to controls in both males and females, but lower than that usually observed in the laboratory. The investigators indicated that an infection may have contributed to the lower survival rate. High dose males and females showed higher mean body weights than controls throughout the study. Hepatocytic cytoplasmic vacuolization increased with dose in males (0/49, 4/49, and 10/50 at ascending doses). Alveolar/bronchiolar carcinoma incidence was higher in high-dose mice of both sexes than controls, though statistical

[6] The criteria designates cancer evidence into four categories based on the strength: "clear evidence", "some evidence", "equivocal evidence" and "no evidence" (Public Health Service, 1986; Federal register 51(66):p-11843).
[7] Terminology for this tumor continues to evolve. Currently it is called mononuclear cell leukemia (MCL) or large granular lymphocytic leukemia (LGL) (Thomas *et al*., 2007).

significance was not achieved. In general, the data provide only weak support for AITC-driven oncogenesis in mice. The LOEL of 12 mg/kg/day was based on cytoplasmic vacuolization in liver. As this was the lowest dose tested, a corresponding NOEL was not established.

Table 11. Neoplastic and Non-Neoplastic Lesions in Chronic Toxicity Studies of Rats and Mice

Sex	**Male**			**Female**		
Dose (mg/kg/day)	***0***	***12***	***25***	***0***	***12***	***25***
Duration adjusted dose (mg/kg/day)	***0***	***8.6***	***17.9***	***0***	***8.6***	***17.9***
Number of animals (unless indicated‡)	***50***	***50***	***50***	***50***	***50***	***50***
RAT						
Eye						
Cataract	7	6	13	2	33*	9*
Retinopathy	9	6	39*	4	35*	11*
Urinary bladder						
Hyperplasia, Epithelial and/or Nodular	0‡	1‡	7‡	0‡	0‡	1
Hyperplasia, Epithelial	0‡	1‡	6‡	0‡	0‡	1
Hyperplasia, Nodular	0‡	0‡	1‡	0‡	0‡	0
Transitional-cell papilloma	0‡	2‡	4*‡	0	0	1
Hematopoietic						
Undifferentiated leukemia	2	6	8*	7	9	11
Skin						
Subcutaneous fibrosarcoma	5	5	1	0	0	3
MOUSE						
Lung						
Alveolar/bronchiolar carcinoma	0	1	3	0	2	3
Alveolar/bronchiolar adenoma	4	3	5	2	1	0
Alveolar/bronchiolar adenoma or carcinoma	4	4	8	2	3	3
Liver						
Cytoplasmic vacuolization	2‡	8‡	13	0	1‡	1‡

Reference: NTP (1982)*;* in females for subcutaneous fibrosarcoma; *significant ($p<0.5$) overall trend and difference compared to control by Cochran-Armitage Trend, and Fisher Exact Tests (one-sided); †Sample size unless otherwise noted ‡N= 49

Cho et al. (2017)

The overall study evaluated here was comprised of 2 separate sub-studies using rats: a 104-week oncogenicity bioassay and a 32-week bioassay focused on promotion. Evaluations for both follow.

104-week study

Study Methods

In a full oncogenicity bioassay conducted by Biological Safety Research, National Institute of Health Sciences, Tokyo, Japan, the authors tested the safety of horseradish extract (HRE)[8] in F344/DuCrj rats. AITC was the major (82-86%), and phenethyl isothiocyanate (PEITC, 9%), and butenyl isothiocyanate (3%) and pentenyl isothiocyanate (1%) were the minor components of HRE.

Thirty two F344/DuCrj male rats/group were administered 0% (0.03% Tween80 as vehicle control), 0.01% (low dose), or 0.04% (high dose) HRE in drinking water for 104 weeks. Individual bottles were used. The average intake of HRE in the low- and high-dose groups was 5.0 and 19.2 mg/kg/day, with an estimated AITC intake of 4.1 and 15.7 mg/kg/day, respectively. Dosing was based on a 13-week study in male rats which recorded body weight deficits and hyperplasia in urinary bladder epithelium (Hasumura *et al.*, 2011). Animals were monitored daily for clinical signs. Body weights and food and water intake were recorded regularly. At the end of the study, complete necropsies, including histological examination of eye and urinary bladder, were conducted. Urinary bladders were handled at necropsy by inflating each with 10% neutral-buffered formalin before immersion in fixative. Bladders were split in two; one half was used for gross examination, the other for histological examination. The findings from this study appear in Table 12.

Results

Body weight and water/food consumption decreased with increasing doses of HRE. Mean body weights decreased by 5%, and 12% in low and high-dose animals, respectively. Decreased absolute brain, heart, and liver weights, and increased relative brain, spleen, and kidney weights were observed at the high dose (15.7 mg/kg/day). No histological changes were observed in eye structures. In addition, there were no statistically significant increases in the incidence of neoplastic lesions in any organ, though a non-statistically significant increase in bladder papillomas was noted at the high dose, as discussed below. Finally, a dose-related increase in incidence of pre-neoplastic lesions in the urinary bladder epithelium was observed.

[8] Horseradish extract (HRE) is distilled with steam from milled horseradish (*Armoracia Rusticana*) roots. Its principal component, AITC, comprises 82-86% of the HRE. HRE also contains other isothiocyanates, such as phenethyl isothiocyanate (9%), butenyl isothiocyanate (3%), and pentenyl isothiocyanate (1%) (Cho et al., 2017).

The incidence of simple hyperplasia of urinary bladder epithelium increased in a dose-dependent, statistically significant manner. High dose animals also showed papillary/nodular hyperplasia, papilloma and one animal with urothelial carcinoma. The control group evidenced single incidences of nodular hyperplasia and bladder papilloma. The LOEL for non-neoplastic effects was 4.1 mg/kg/day (0.01% HRE) based on simple hyperplasia of urinary bladder epithelium. Neither the incidence of papilloma nor carcinoma achieved statistical significance.

Table 12. Urinary bladder lesions in male rats following HRE administration for 104 weeks

Dose of HRE (or AITC) in drinking water	**Control**	**0.01% HRE (AITC = 4.1 mg/kg/day)**	**0.04% HRE (AITC = 15.7 mg/kg/day)**
Number of animals / dose	**32**	**32**	**32**
Simple Hyperplasia	0	9**	24**
Papillary/nodular hyperplasia	1	0	5
Papilloma	1	0	3
Urothelial carcinoma	0	0	1

Reference: Cho et al. (2017); **Significantly different from the Control at $p < 0.01$ by Fisher's Exact test conducted by the study authors

32-week promotion study

Study Methods

In a 32-week medium-term promotion bioassay study, 120 F344/DuCrj males were treated with 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN, an initiator) in drinking water for 4 weeks, followed by a 1-week of washout period (no treatment period). The BBN-administered rats were then divided into 4 groups and exposed to 0 (0.03% Tween80), 0.005%, 0.01%, or 0.04% HRE in drinking water for 13 or 32 weeks to examine early and late stage tumor promotion. Body weight and food and water consumption were measured. At the end of treatment periods, 15 rats/group were sacrificed and urinary bladders were inflated for macroscopic and histopathological examination. The results from this study are summarized in Table 13.

Results

Although not statistically significant, a dose-dependent decrease in water consumption was observed during HRE treatment. Results demonstrated that AITC augmented BBN's effect on the urothelium. Specifically, the authors concluded that AITC increased the incidence of urothelial pre-neoplastic and neoplastic lesions, augmented the tumor volume, and decreased the time-to-effect. Moreover, AITC promoted BBN effects at doses that did not induce papillomas in a 2-year bioassay, suggesting a tumor promotional mode of action for AITC.

Table 13. Urinary Bladder Lesions in Male Rats administered BBN and HRE for 13 or 32 weeks

Duration of treatment	HRE dose (%)	Number of animals	Estimated AITC Dose (mg/kg/day)	Papillary or nodular hyperplasia	Papilloma	Urothelial carcinoma	Squamous cell carcinoma	Total tumor volume (mm^3)
13 weeks	0	15	0	5	0	2	0	ND
	0.005	15	2.7	14***	3	5	0	ND
	0.01	15	5.4	15***	4*	1	0	ND
	0.04	15	20.5	15***	14***	9*	0	ND
32 weeks	0	15	0	11	7	7	0	3.7 ± 8.8
	0.005	15	2.7	13	12	12	0	7.7 ± 9.6
	0.01	15	5.4	14	13*	13*	0	35 ± 95
	0.04	15	20.5	15*	14*	15*	3	531 ± 1495**

Reference: Cho et al. (2017); *,**,*** significantly different from the control at $p < 0.05$, 0.01, 0.001, respectively; ND, Not determined

Table 14. Chronic Toxicity Studies of AITC and AITC-Rich Substances

Author	Study Design Species, Route, Dose and Duration	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)
NTP (1982)	Oral gavage; AITC in corn oil; 103 weeks; F344/N rats; 50M/50F; 0, 12, 25 mg/kg/day	Cataracts in females; hyperplasia of urinary bladder epithelium in males	ND	12
NTP (1982)	Oral gavage; AITC in corn oil; 103 weeks; B6C3F1 mice; 50M/50F; 0, 12, 25 mg/kg/day	Hepatocytic cytoplasmic vacuolization	ND	12
Cho *et al*. (2017)	Oral: horseradish extract (HRE) in drinking water; 104 weeks; F344/DuCrj rats, 32M; 0, 4.1, 15.7 mg/kg/day	Simple hyperplasia of urinary bladder epithelium.	ND	4.1

C.7 *In vitro* high throughput toxicity screening (ToxCast)

Toxicity Forecaster (ToxCast™) is a federal program that aims to systematically reduce the number of animals used in toxicity testing by validating high-throughput screening technologies and toxicity data. DPR includes ToxCast™ data in its RCDs to help inform on chemical hazards. The ToxCast data on AITC was accessed on US EPA's Chemistry Dashboard (https://comptox.epa.gov/dashboard) on July 17, 2020. AITC was borderline active and only at high concentrations (with AC50 > 25 µM) for the bioactivity of nuclear receptors; retinoid X receptor beta (RXRB) and progesterone receptor (PGR). These receptors are involved various cellular functions, including cell signaling and metabolism.

C.8 Other studies

Jiao et al. (1994a)

Study Methods

This single-dose study was designed to test the ability of AITC (and other isothiocyanate) to protect against 4-(methylnitrosamino)-1-(3-pyridyl)butanone (NNK) induced lung adenomas. Multiple groups of at least 20 female A/J mice were selected. Each group received a single dose of corn oil vehicle, 1 or 5 µmol of AITC by gavage. Two hours later, a single dose of 10 µmol NNK or saline was administered by intraperitoneal injection. The treatment groups were corn oil + saline, 5 µmol AITC + saline, corn oil+ NNK, 1 µmol AITC + NNK, and 5 µmol AITC + NNK. The mice were left untreated for 16 weeks before the scheduled sacrifice. Tumor incidence and multiplicity of pulmonary adenomas were calculated for each group.

Results

In the absence of NNK, the tumor incidence was 20% in saline control and 10% in the 5 µmol AITC group. In the presence of NNK, the tumor incidence was 100% in absence or presence of AITC, suggesting that AITC did not reduce the tumor-inducing effect of NNK. Tumor multiplicity of NNK was not changed by prior administration of AITC.

D. RISK ASSESSMENT

D.1 Hazard Identification

Data from toxicity studies submitted either by the registrant or identified through a comprehensive systematic review of the open literature were used to establish critical points-of-departure (PODs). For purposes of this risk assessment, PODs are air concentrations that do not produce toxicologically significant effects upon inhalation exposure. They were used to delineate threshold concentrations for non-carcinogenic effects. Cancer risk was not calculated for this assessment, either by linear extrapolation or by threshold determination (see discussion below).

PODs can either be experimentally-determined (i.e., no observed effects levels or NOELs) or data-derived. Data-derived POD values were used (a) when toxicologically significant effects were observed at the lowest treatment level in a study, (b) when dose extrapolation could be used to provide a more accurate no-effect level than relying on a study's pre-determined treatment levels, or (c) when there were no route- or duration-specific study data on which to base a POD. DPR established both experimentally determined and data-derived PODs for this RCD.

Data-derived POD values for AITC were calculated using dose or duration extrapolation factors. If there was no study derived NOEL, then the acute lowest observed effect level (LOEL) was divided by a factor of 10 to estimate a no effect level or ENEL. 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. Benchmark dose or concentration (BMD or BMC) modeling was also used to derive PODs from datasets that were amenable for modeling. However, no BMD-derived POD was considered critical or used to calculate risk for any AITC exposures in this assessment.

Critical PODs were used to estimate the risks posed by AITC exposures. PODs were evaluated as critical based on their corresponding route and duration, relative value, and toxicological considerations. All of the critical PODs for AITC were based on effects observed in inhalation toxicity studies using laboratory rats (Table 15). These studies utilized technical grade AITC (97.9 to 99.9%) and thus the observed effects were not confounded by impurities or presence of other isothiocyanates.

Table 15. Summary of Critical Points of Departure (PODs) for AITC

Duration	Route	Critical Endpoint	POD (ppm)	Reference
Acute	Inhalation	Decreased motor activity in males and females, and rearing counts in females	2.5 ppm[a]	Herberth (2017)
Subchronic	Inhalation	Mild-to-moderate degenerative changes in the nasal olfactory epithelium in both sexes, and mild metaplastic lesions in respiratory epithelium and decreased motor activity in males	5 ppm[b]	Randazzo (2017)
Chronic	Inhalation	Mild-to-moderate degenerative changes in the nasal olfactory epithelium in both sexes, and mild metaplastic lesions in respiratory epithelium and decreased motor activity in males	0.5 ppm[c]	Randazzo (2017)

Abbreviations: POD, point of departure; as defined by US EPA (2012b), a point of departure (POD) is the dose-response point that marks the starting point for low-dose extrapolation, and generally corresponds to a select, estimated, low-level of response. [a]NOEL, no-observed-effect level; LOEL, lowest observed effect level; ENEL, estimated no-effect level; LOEL was divided by a factor of 10 to derive the ENEL ;, The chronic POD was estimated by dividing the subchronic POD by a factor of 10; [b]ENEL, LOEL÷ 10; [c]ENEL, NOEL÷ 10

D.1.1 Points of Departure for Acute Inhalation Exposure

The available acute inhalation toxicity database for AITC was limited (Table 16). Two guideline studies in rats and one human exposure study (non-peer reviewed) were available for evaluation. The acute inhalation neurotoxicity study (Herberth, 2017) was considered the most appropriate for evaluating acute inhalation risk. 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.

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). The usefulness of this study for risk assessment was reduced because it did not include a control group and the evaluations were limited to clinical observations. 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). 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.

Table 16. Summary of acute inhalation toxicity studies

Species/Duration	Effects at LOEL	LOEL (ppm)	NOEL (ppm)	References
Rat, 4 hrs once (AITC vapor)	Decreased motor activity (ambulatory and total) in males and females, and rearing counts in females	25	ND	Herberth (2017)
Rat, 4 hrs once (AITC aerosol)	Mortality, tremors, irregular respiration, hypoactivity, nasal and/or ocular discharge	51	ND	Lowe (2012)

Abbreviations: hr(s), hour(s); ND, not determined (LOEL was the lowest dose tested). All studies evaluated effects in adult animals.

D.1.2 Points of Departure for Subchronic Inhalation Exposure

One subchronic inhalation toxicity study was available for analysis. Sprague-Dawley rats were exposed to 0, 5, 10 or 25 ppm AITC vapor for 13 weeks, 6 hr/day, 5 days/week (Randazzo, 2017). The critical inhalation POD of 5 ppm was based both on portal-of-entry effects (degenerative lesions in olfactory epithelium and metaplasia of respiratory epithelium) and on systemic effects (decrements in motor activity) at the LOEL of 10 ppm. The motor activity data were not amenable to BMC modeling. Modeling of the olfactory epithelial degeneration in males produced a $BMCL_{10}$ of 4.78 ppm (log-logistic model and benchmark response of 10%, see Appendix 4). The quantitative equivalence of the BMCL (4.78 ppm) and the NOEL (5 ppm) provided ample support for use of the latter to estimate seasonal or subchronic risk.

While no other subchronic inhalation studies were available, subchronic oral studies evidenced portal of entry (thickened stomach mucosa in rats and mice) and systemic (urinary bladder hyperplasia in rats) effects (NTP, 1982; Hasumura *et al.*, 2011) (Table 6, Toxicity Profile**)**. These effects were not observed in the subchronic inhalation study in rats, and therefore appeared to be specific to the oral route of exposure. DPR did not use the oral subchronic studies to establish critical NOELs, because (a) inhalation is the most relevant route of exposure for AITC and (b) a critical subchronic POD could be determined based on effects identified in the 13-week inhalation study in rats. Nevertheless, the equivalent external air concentrations in the oral studies were calculated using route-to route extrapolation to see if they generated effects at similar concentrations as the inhalation study. For this analysis, the NOEL of 6.6 mg/kg/day for urinary bladder hyperplasia in rats in the 13-week drinking water study of Hasumura *et al.* (2011) was divided by a default rat breathing rate of 0.17 m^3/kg (see full calculation below). This breathing rate was derived from the 24-hour default rat breathing rate of 0.96 m^3/kg (Andrews and Patterson, 2000) by adjusting for duration to match the exposure regimen employed in the 13-week subchronic inhalation study (6 hours per day, 5 days per week). The resulting equivalent external air concentration was 9.5 ppm. This value is similar to the estimated critical subchronic inhalation POD of 5 ppm in rats for motor activity decrements. Because urinary bladder hyperplasia was the most sensitive systemic endpoint, this analysis shows that the critical inhalation POD will be protective of any of the effects of systemic toxicity observed for AITC.

Route to route extrapolation, internal dose to equivalent air concentration:

Inhalation POD ppm = Rat Oral POD (mg/kg) / rat BR (m^3/kg) / AITC conversion factor:

Subchronic oral POD = 6.6 mg/kg/day

Default rat breathing rate (BR) = 0.17 m^3/kg, derived from the 24-hour default breathing rate of 0.96 m^3/kg adjusted by duration of inhalation exposure (6 hours per day; 5 days per week), as follows:

0.96 m^3/kg x 6h/24h x 5 days/7 days

AITC conversion factor, mg/m^3 to ppm = 4.06

POD = 6.6 mg/kg/day / 0.17 m^3/kg / 4.06 = 9.5 ppm

D.1.3 Points of Departure for Chronic Inhalation Exposure

No chronic inhalation toxicity studies for AITC were available. The critical chronic inhalation POD of 0.5 ppm was based on the rat critical subchronic inhalation POD. As noted above, portal of entry effects at the subchronic LOEL of 10 ppm included degenerative lesions in the olfactory epithelium and metaplasia of the respiratory epithelium. Systemic effects at the same concentration included motor activity decrements. A subchronic-to-chronic extrapolation was performed by dividing the subchronic inhalation POD of 5 ppm by a factor of 10 (DPR, 2014).

Three chronic oral toxicity studies with AITC were also evaluated, including two in rats and one in mice. Non-oncogenic effects in the chronic studies included hyperplasia of urinary bladder epithelium, cytoplasmic vacuolization in hepatocytes of mice and cataracts and retinopathy in rats (NTP, 1982). None of the endpoints in the oral studies were observed in the subchronic inhalation study, suggesting that these effects were specific to the oral route. The lowest chronic oral POD of 0.6 mg/kg/day was estimated using benchmark dose modeling based on of urinary bladder hyperplasia in rats exposed for 2 years to drinking water containing horseradish extract normalized for AITC (Cho *et al.*, 2017). The same route-to-route extrapolation described above was performed by dividing the POD of 0.6 mg/kg/day by the duration adjusted default rat breathing rate. The resultant rat external air concentration was 0.9 ppm. This value is similar to the estimated critical chronic inhalation POD of 0.5 ppm in rats for motor activity decrements.

In conclusion, because the urinary bladder hyperplasia was the most sensitive systemic endpoint, the critical chronic inhalation POD will be protective of any systemic toxicity. DPR does not consider oncogenic effects when designating non-oncogenic PODs (see Section D.1.5 below for further discussion of oncogenicity). Tumor incidence data are either directly used to estimate oncogenic potency or, when appropriate (as in the present case), includes the incidence of a preneoplastic lesion among the critical effects.

D.1.4 Genotoxicity

Thirteen *in vitro* and 5 *in vivo* genotoxicity studies were evaluated for this assessment. AITC generated positive results in 4 *in vitro* mutagenicity studies, 5 chromosomal aberration studies, and 1 DNA damage study. The positive results were generally regarded by the study authors as

"weak" and were often coincident with high levels of cytotoxicity. The results for 4 of the 5 *in vivo* studies conducted in mice, rats, and Drosophila were negative. In the fifth study, human volunteers were exposed to dietary AITC for 10 consecutive days, then to a single bolus dose following a 17-day washout period (Charron *et al.*, 2013). DNA damage was observed in a blood sample collected 3 hours after the bolus dose, but not in the 6-hour sample (Charron *et al.*, 2013). Two studies testing the ability of AITC to alkylate DNA showed possible borderline DNA reactivity (Eder *et al.*, 1980; Eder *et al.*, 1982). Overall, the genotoxicity results for AITC were mixed and often confounded by cytotoxicity. They also suggested that any positive results for AITC may not have been mediated by direct DNA-reactivity. Therefore, AITC is not likely to act as a mutagen at physiologically relevant concentrations.

D.1.5 Oncogenicity

No studies evaluating oncogenicity by the inhalation route were available. However, three long-term oral studies were available, two in rats and one in mice (NTP, 1982; Cho *et al.*, 2017). Three different cancers were observed: subcutaneous fibrosarcomas, leukemia, and urinary bladder tumors (papillomas and carcinomas).

Fibrosarcomas were observed in a 2-year oral gavage study in F344/N rats (NTP, 1982). Dose responsiveness was not apparent in males, while in females, tumors were observed only at the high dose. Undifferentiated leukemia was also observed in male and female rats from the same study. However, this strain has a high and variable background rate for leukemia (King-Herbert and Thayer, 2006; Thomas *et al.*, 2007). For this reason, DPR concurs with the National Toxicology Program's (NTP) conclusion that leukemia incidence was unlikely to be related to AITC treatment (NTP, 1982).

Urinary bladder tumors and corresponding precursor lesions were observed in two studies (NTP, 1982; Cho *et al.*, 2017). In the first, male F344/DuCrj rats exposed to AITC for 2 years through drinking water exhibited increased papillomas and carcinomas at the high dose (15.7 mg/kg/day estimated AITC) (Cho *et al.*, 2017). Precursor lesions in males included a statistically significant ($p < 0.01$) and dose responsive increase in simple hyperplasia in all dose groups (≥ 4.1 mg/kg/day estimated AITC) and an increase in papillary/nodular hyperplasia at the high dose (Cho *et al.*, 2017). In the second, a 2-year oral gavage study, male F344/N rats exhibited a dose-responsive increase in urinary bladder papillomas that reached statistical significance at the high dose ($p < 0.05$ at 17.9 mg/kg/day). Females showed a single incidence at the high dose (NTP, 1982). Precursor lesions in males included a dose-responsive increase in bladder epithelial hyperplasia in all dose groups (≥ 8.6 mg/kg/day) and a single incidence of nodular hyperplasia at the high dose. Precursor lesions in females included a single incidence of nodular hyperplasia at the high dose (NTP, 1982).

A mode of action (MOA) involving sustained, high levels of key AITC metabolite(s) in urine leading to bladder epithelial hyperplasia and eventual tumors is likely to be operative in this case. Relevance to humans is supported by a prior US EPA analysis (US EPA, 2006). Support for this

MOA can be summarized as follows: (a) the epithelial proliferator NAC-AITC is the major urinary metabolite of AITC in both rats and humans (Jiao *et al.*, 1994b; Bollard *et al.*, 1997; Shapiro *et al.*, 1998); (b) oral AITC causes dose-dependent urinary bladder epithelial cell proliferation and hyperplasia in rats (NTP, 1982; Hasumura *et al.*, 2011; Cho *et al.*, 2017); (c) papillomas and carcinomas were also observed in rat bladders (NTP, 1982; Cho *et al.*, 2017); and (d) AITC can act as a tumor promoter on these tissues (Cho *et al.*, 2017).

As with the bladder tumors, bladder hyperplasia was only observed following oral exposures; no bladder effects were noted following inhalation exposures. For purposes of this risk assessment, the establishment of the chronic inhalation POD was based on a duration extrapolation from the critical subchronic inhalation study. Because the finding that critical effects were route specific, a route-to-route extrapolation of oral to inhalation exposures was not used. The observed route of exposure differences in effect is also the rationale for not quantifying the cancer risk of AITC herein, whether by a threshold approach based on a POD for an oral route-specific precursor lesion or by low-dose linear extrapolation. The implications of this decision are examined in the Risk Appraisal section of this document. A comparison of resulting reference concentrations generated by exposure duration extrapolation versus exposure route extrapolation is also found in the Risk Appraisal.

D.1.6 Reproductive and Developmental Toxicity

Neither reproductive nor developmental toxicity studies by the inhalation route were available for evaluation. However, one 2-generation reproductive toxicity study in rats and a series of developmental toxicity studies in rats, mice, rabbits, and hamsters were available (FDRL, 1973; Tanner, 2017). In the reproductive toxicity study, the parental LOEL (20 mg/kg/day) was based on hyperplasia of the stomach and urinary bladder epithelium, and cataracts in F1 males (Tanner, 2017). None of these effects were observed in animals exposed by inhalation. As this was the lowest dose tested, the parental NOEL was < 20 mg/kg/day. The offspring NOEL of 20 mg/kg/day was based on decrements in bodyweight and survival of F1 and F2 pups at 40 mg/kg/day (Tanner, 2017). In the rat and rabbit developmental toxicity studies, no effects were observed at the highest tested dose, resulting in a NOEL of 12.3 mg/kg/day for both species. In the mouse developmental toxicity study, the NOEL of 6 mg/kg/day was based on increased resorptions and an increased number of dead fetuses at 28 mg/kg/day. Based on the available data, it was not possible to discern if these effects were reflections of maternal or fetal toxicity. In the hamster developmental toxicity study, there was a rise in incidence of incomplete sternebral ossification in fetuses at the highest tested dose (23.8 mg/kg/day) (FDRL, 1973). Ossification delays are generally regarded as evidence of slowed fetal growth, thus are not specific developmental effects. In any case, this effect was not statistically significant, so was not accorded toxicological significance. In conclusion, with the exception of the mouse (for which there were insufficient data to make a decision), fetal and pup effects of AITC were plausibly secondary to maternal toxicity.

D.1.7 Human Equivalent Concentrations

For inhalation risk assessments, DPR currently uses US EPA's RfC methodology to derive human equivalent concentrations (HECs) (US EPA, 1994; US EPA, 2012a). The HEC is the external air concentration that produces the same internal target tissue dose in humans as that achieved in laboratory animals. Traditionally, HEC calculation involves two steps. First, the critical POD from the selected animal study is adjusted by the estimated human exposure duration (e.g. 24 hours/day and 7 days/week for residential bystanders, etc.). This results in a duration-adjusted POD (POD_{ADJ}). Then the POD_{ADJ} is converted to an HEC (POD_{HEC}) using a dosimetric adjustment factor (DAF) for either portal of entry effects, depending on regional anatomic differences between rat and human respiratory tracts, or for systemic effects, based on relative inhalation absorption capacities between the two species.

For systemic effects, DPR adopted US EPA's 1994 RfC methodology with a default DAF = 1. This was based on the assumption that chemical-specific blood:gas (air) partition coefficients (Hb/g) are equivalent in animals and humans (US EPA, 1994). For portal of entry effects occurring in the extra-thoracic region, DPR again uses a default DAF of 1. This was based on US EPA's 2012 RfC update showing that PBPK model-derived DAFs were ≥ 1 for most chemicals when data relevant to local effects were available (US EPA, 2012a; Kuempel *et al.*, 2015). For portal of entry effects occurring in the tracheobronchial or pulmonary regions, DPR uses the species-specific DAFs based on the animal:human ratios of overall minute ventilation and the overall surface area for the affected respiratory tract region (US EPA, 1994).

The critical acute and subchronic endpoints for AITC of decreased motor activity and rearing counts were assumed to be systemic effects that occurred after AITC and its metabolites after entered the blood stream and were distributed to target tissues. In contrast, nasal olfactory degeneration and mild metaplasia of the rat respiratory epithelium observed the subchronic inhalation study were considered to be portal of entry (POE) effects occurring the initial point of contact. These designations were necessary as part of the process for converting the critical animal PODs to human equivalent concentrations (HEC or POD_{HEC}). A DAF of 1 will be applied for calculating HEC in both scenarios based on US EPA's methodologies, as noted above (US EPA, 1994; US EPA, 2012a).

Acute, subchronic, and chronic HECs were calculated for workers to assess the inhalation risks under short-term, seasonal, and annual exposure scenarios (Table 17). Acute HECs were also calculated for occupational bystanders and child and adult residential bystanders in order to assess the inhalation risks posed by AITC under short-term exposure scenarios. At present, longer term occupational and residential bystander risks due to AITC exposure cannot be estimated due to lack of information. For fumigants with more extensive databases, such as 1,3-dichloropropene, intermediate and long-term exposures estimates for bystanders can be calculated. As additional data become available, additional exposure scenarios could be evaluated. However, at this time the analysis of bystander risk is limited to short-term scenarios.

The general formulas used to calculate HECs for AITC are as follows:

POD_{ADJ} (ppm) = POD ppm x (H_a/H_h) x (D_a/D_h)
POD_{HEC} (ppm) = POD_{ADJ} ppm x $DAF_{SYS\ or\ POE}$

Parameters and definitions:

$POD_{ADJ:}$ adjusted POD to account for difference between experimental and likely human scenario exposure durations.
H_a: duration of animal exposure (hours/day)
H_h: duration of anticipated human exposure (hours/day)
D_a: duration of animal exposure (days/week)
D_h: duration of anticipated human exposure (days/week)
DAF_{POE}: dosimetric adjustment factor to account for regional differences between rat and human respiratory tracts, equal to 1 (US EPA, 1994; US EPA, 2012a)
DAF_{SYS}: dosimetric adjustment factor to account for inhalation absorption differences between rat and human respiratory tracts, equal to 1 (US EPA, 2012a; Kuempel *et al.*, 2015)

Acute HECs

Acute POD = 2.5 ppm; ENEL based on decreased rearing motor activity counts in rats (Herberth, 2017).

Non-occupational (child and adult residential bystander)

POD_{ADJ} (ppm) = POD ppm x (Ha/Hh)
0.42 ppm = 2.5 ppm x (4 $hours/day_{rat}$ / 24 $hours/day_{human}$)

POD_{HEC} (ppm) = POD_{ADJ} ppm x DAF_{SYS}
0.42 ppm = 0.42 ppm x 1

Occupational (workers and occupational bystander)

POD_{ADJ} (ppm) = POD ppm x (Ha/Hh)
1.25 ppm = 2.5 ppm x (4 $hours/day_{rat}$/8 $hours/day_{human}$)

POD_{HEC} (ppm) = POD_{ADJ} ppm x DAF_{SYS}
1.25 ppm = 1.25 ppm x 1

Subchronic HECs

Subchronic POD: 5 ppm; NOEL based on mild-to-moderate degenerative lesions in olfactory epithelium, mild metaplasia of respiratory epithelium, and decreased motor activity in rats (Randazzo, 2017).

Occupational

POD_{ADJ} (ppm) = POD ppm x (Ha/Hh) x (Da/Dh)

3.75 ppm = 5 ppm x (6 hours/day_{rat}/8 hours/day_{human}) x (5 $days_{rat}$/5 $days_{human}$)

POD_{HEC} (ppm) = POD_{ADJ} ppm x $DAF_{SYS\ or\ POE}$

3.75 ppm = 3.75 ppm x 1

Chronic HECs

Chronic POD: 0.5 ppm; UF of 10 for subchronic-to-chronic extrapolation based on mild-to-moderate degenerative lesions in olfactory epithelium, mild metaplasia of respiratory epithelium, and decreased motor activity in rats (Randazzo, 2017).

Occupational

POD_{ADJ} (ppm) = POD ppm x (Ha/Hh) x (Da/Dh)

0.375 ppm = 0.5 ppm x (6 hours/day $_{rat}$/8 hours/day $_{human}$) x (5 days $_{rat}$/5 days $_{human}$)

POD_{HEC} (ppm) = POD_{ADJ} ppm x $DAF_{SYS\ or\ POE}$

0.375 ppm = 0.375 ppm x 1

D.1.8 Derivation of Reference Concentrations

Reference concentrations (RfCs) are air concentrations that are likely to be without appreciable risk of deleterious effects. RfCs are calculated by dividing the critical HEC by the total uncertainty factor (UF_{TOTAL}). The default UF_{TOTAL} (100) is the product of a default UF (10) to account for interspecies variability (UF_A) and a default UF (10) to account for intraspecies (human) sensitivity (UF_H). The UF_A and UF_H are themselves products of separate pharmacokinetic and pharmacodynamic uncertainty factors. When a POD_{HEC} is used for RfC derivation, the pharmacokinetic component in the UF_A is typically reduced from 3 to 1 because DAF adjustments account for physiological and anatomical differences between humans and animals. As a result, the UF_{TOTAL} for AITC is 30.

PODs, HECs, and RfCs appear in Table 17.

Table 17. PODs, HECs, Total UFs, and Reference Concentrations (RfCs) for Workers and Residential and Occupational Bystanders

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_{ADJ}, POD adjusted by duration. POD_{HEC}, human equivalent concentration; ppm, parts per million; RfC, reference concentration; UF_A, uncertainty factor to account for interspecies variability; UF_H, uncertainty factor to account for intraspecies (human) sensitivity

D.2 Exposure Assessment

DPR's comprehensive Exposure Assessment Document (EAD) for AITC is found in Appendix 1. The EAD provides human exposure estimates for two general scenarios: workers (including applicators, tarp cutters, and re-entry workers) and bystanders (occupational and residential). The exposure scenarios were based on the proposed labels for Dominus® (96.3% AITC) and Dominus 100® (99.8% AITC).

D.2.1 Worker Exposure

The EAD contains detailed estimates for occupational handler and re-entry worker exposures (short-term, seasonal, annual, and lifetime), along with a complete description of the methods used to arrive at those estimates (including input data, formulae, soil emission rates, assumptions, re-entry intervals, presence or absence of personal protective equipment, etc.). As no worker exposure monitoring has been done for AITC, handler and re-entry worker exposures were estimated using surrogate data from two other soil fumigants, chloropicrin and 1,3-dichloropropene.

D.2.2 Bystander Exposure

As with occupational exposures, a detailed description of the bystander scenarios and methods used to estimate exposures (e.g. input data, formulae, assumptions, etc.) appear in the EAD. Both occupational and residential bystander exposures were estimated using AERMOD computer simulations. Residential bystander estimates were generated for both adults and children.

D.3 Risk Characterization

The potential for non-oncogenic health effects resulting from exposure to AITC was expressed as the margin of exposure (MOE). A MOE is the ratio of the POD value derived from the definitive acute, subchronic, or chronic studies divided by the estimated human exposure. As this assessment is focused on risks from inhalation exposure to AITC, both the POD and the exposure values are expressed as air concentrations (in units of ppm or ppb) rather than as internal doses (in units of mg/kg BW).

Margin of Exposure (MOE) = POD (in ppb) / Exposure concentration (in ppb)

Calculated MOEs are compared to a corresponding target MOEs. Calculated MOEs that are lower than the target MOE indicate a potential health concern.

D.3.1 Target and Calculated MOEs

The margin of exposure (MOE) is a quantitative tool used by DPR to determine the potential risk arising from exposure to a pesticidal active ingredient. A MOE is defined as the ratio of the POD value derived from the definitive acute, subchronic, or chronic studies to the estimated human exposure. The resulting value is compared to the acceptable or target MOE which, for purposes of this risk assessment, is equivalent to the total uncertainty factor (UF_{TOTAL}) of 30. Values at or above the target MOE are generally considered protective against the toxicity of AITC for all populations, regardless of exposure conditions. Because this analysis is focused on risks from inhaling AITC, both the POD and the exposure values are expressed as air concentrations (in units of ppm or ppb).

Margin of Exposure (MOE) = POD (in ppb) / Exposure concentration (in ppb)

The risk estimates are based on exposure scenarios and air concentration data found in Appendices 1 and 2, respectively. Due to the lack of AITC use information and exposure monitoring data, as well as limited information on AITC soil emission rates, other soil fumigants were used as surrogates to conduct the exposure analysis either directly, as in the air concentration and soil emission data for 1,3-dichloropropene and chloropicrin, or indirectly, such as methyl bromide and methyl isothiocyantes, which were used to identify use regions. A total of 88 exposure scenarios were assessed, and AITC inhalation exposures were estimated for four different exposure periods (short-term, seasonal, annual and life-time). Applicators were assumed to be wearing personal protective equipment as currently described on the proposed label. Accordingly, the exposure estimates for these categories were reduced by 90%. Personal protective equipment was not assumed for any re-entry worker or bystander category (occupational, adult residential and child residential).

D.3.2 Worker Exposure Scenarios

Handlers

Under short-term exposure conditions, handler MOEs ranged between 1 and 96 (Table 18). Nine of 10 handler tasks generated short-term MOEs lower than the target of 30.

Under seasonal exposure conditions, handler MOEs ranged between 10 and 938. Four of 10 handler tasks generated seasonal MOEs lower than the target of 30.

Under annual exposure conditions, handler MOEs ranged between 4 and 750. Five of 10 handler tasks generated annual MOEs lower than the target of 30.

Re-Entry Workers

Under short-term exposure conditions, re-entry worker MOEs ranged between 29 and 40 (Table 19). Two of 3 re-entry tasks generated short-term MOEs lower than the target of 30.

Under seasonal exposure conditions, all 3 re-entry tasks generated seasonal MOEs higher than the target of 30 (ranging between 101 and 139).

Under annual exposure conditions, re-entry worker MOEs ranged between 25 and 125. One of 3 re-entry tasks generated annual MOEs lower than the target of 30.

D.3.3 Bystander Scenarios

Occupational Bystanders

Under short-term exposure conditions, all occupational bystander scenarios generated MOEs lower than the target of 30 (Table 20).

For a 1-acre application, the MOEs ranged between 1 and 15.

For a 40-acre application, short-term occupational bystander MOEs ranged between < 1 and 5.

For a 100-acre application, short-term occupational bystander MOEs ranged between < 1 and 4.

Adult Residential Bystanders

For adult residential bystanders, all MOEs were below the target of 30.

At 25 and 100 feet from a 1-acre application, the short-term MOEs ranged between 1 and 14 and between 2 and 21, respectively (Table 21). At 25 and 100 feet from a 40-acre application, short-term MOEs ranged between <1 and 4 and between < 1 and 5, respectively. At 25 and 100 feet

from a 100-acre application, short-term MOEs ranged between < 1 and 3 and between < 1 and 4, respectively.

Child Residential Bystanders

For child residential bystanders, all MOEs were below the target of 30.

At 25 and 100 feet from a 1-acre application, short-term MOEs ranged between 1 and 9 and between 2 and 18, respectively (Table 22). At 25 and 100 feet from a 40-acre application, short-term MOEs ranged between < 1 and 4 and between < 1 and 5, respectively. At 25 and 100 feet from a 100-acre application, short-term MOEs ranged between < 1 and 3 and between < 1 and 4, respectively.

Note: A target MOE of 30 applies to all of the following tables. Values that fall below this target are shaded.

Table 18. Estimated handler air concentrations and Margins of Exposure (MOE)

Short-term			Seasonal			Annual		
HEC (ppb)	**Air conc. (ppb)**	**MOE**	**HEC (ppb)**	**Air conc. (ppb)**	**MOE**	**HEC (ppb)**	**Air conc. (ppb)**	**MOE**
Shallow shank, with tarp (Appendix 2, Table 1)								
1250	43	29	3750	12	313	375	2	188
Shallow shank, without tarp (Appendix 2, Table 2)								
1250	452	3	3750	42	89	375	9	42
Deep shank, with tarp (Appendix 2, Table 3)								
1250	43	29	3750	11	341	375	3	125
Deep shank, without tarp (Appendix 2, Table 3)								
1250	452	3	3750	41	91	375	12	31
Drip application (Appendix 2, Table 4)								
1250	13	96	3750	4	938	375	0.5	750
Loader, shallow shank (Appendix 2, Table 5)								
1250	1898	1	3750	365	10	375	78	5
Loader, deep shank (Appendix 2, Table 5)								
1250	1898	1	3750	353	11	375	101	4
Tarp cutter / remover / puncher, shallow shank (Appendix 2, Table 6)								
1250	1015	1	3750	147	26	375	31	12

Tarp cutter / remover / puncher, deep shank (Appendix 2, Table 6)								
1250	1015	1	3750	142	26	375	41	9
Tarp cutter / remover / puncher, drip (Appendix 2, Table 6)								
1250	734	2	3750	106	35	375	13	29

Table 19. Estimated re-entry worker air concentrations and Margins of Exposure (MOE)

Short-term			**Seasonal**			**Annual**		
HEC (ppb)	**Air conc. (ppb)**	**MOE**	**HEC (ppb)**	**Air conc. (ppb)**	**MOE**	**HEC (ppb)**	**Air conc. (ppb)**	**MOE**
Shallow shank, re-entry at 4 days (Appendix 2, Table 7)								
1250	43	29	3750	37	101	375	10	38
Deep shank, re-entry at 4 days (Appendix 2, Table 7)								
1250	43	29	3750	36	104	375	15	25
Drip, re-entry at 4 days (Appendix 2, Table 7)								
1250	31	40	3750	27	139	375	3	125

Table 20. Occupational bystander air concentrations and Margins of Exposure (MOE)

HEC (ppb)	**Air conc. (ppb)**	**MOE**	**Air conc. (ppb)**	**MOE**	**Air conc. (ppb)**	**MOE**
	1 acre		**40 acres**		**100 acres**	
Shallow shank with tarp (Appendix 2, Table 8)						
1250	86	15	256	5	318	4
Shallow shank, without tarp (Appendix 2, Table 8)						
1250	828	2	2460	1	3056	< 1
Deep shank, without tarp (Appendix 2, Table 8)						
1250	542	2	1609	1	1999	1
Drip, with tarp (Appendix 2, Table 8)						
1250	454	3	1436	1	1791	1
Deep drip, without tarp (Appendix 2, Table 8)						
1250	1062	1	3361	< 1	4192	< 1

Table 21. Adult residential bystander air concentrations and Margins of Exposure (MOE)

HEC (ppb)	Air conc. (ppb)	MOE	Air conc. (ppb)	MOE	Air conc. (ppb)	MOE
	1 acre		40 acres		100 acres	
Shallow shank, with tarp, 25 feet (Appendix 2, Table 9)						
420	31	14	99	4	124	3
Shallow shank, without tarp, 25 feet (Appendix 2, Table 9)						
420	309	1	973	< 1	1225	< 1
Deep shank, without tarp, 25 feet (Appendix 2, Table 9)						
420	200	2	629	1	792	1
Drip, with tarp, 25 feet (Appendix 2, Table 9)						
420	199	2	627	1	789	1
Deep drip, without tarp, 25 feet (Appendix 2, Table 9)						
420	355	1	1120	< 1	1411	< 1
Shallow shank, with tarp, 100 feet (Appendix 2, Table 9)						
420	20	21	81	5	104	4
Shallow shank, without tarp, 100 feet (Appendix 2, Table 9)						
420	199	2	795	1	1027	< 1
Deep shank, without tarp, 100 feet (Appendix 2, Table 9)						
420	129	3	514	1	664	1
Shallow shank, with tarp, 100 feet (Appendix 2, Table 9)						
420	128	3	512	1	661	1
Deep drip, without tarp, 100 feet (Appendix 2, Table 9)						
420	229	2	915	< 1	1182	< 1

Table 22. Child residential bystander air concentrations and Margins of Exposure (MOE)

HEC (ppb)	Air conc. (ppb)	MOE	Air conc. (ppb)	MOE	Air conc. (ppb)	MOE
	1 acre		40 acres		100 acres	
Shallow shank, with tarp, 25 feet (Appendix 2, Table 10)						
420	45	9	114	4	139	3
Shallow shank, without tarp, 25 feet (Appendix 2, Table 10)						
420	448	1	1120	< 1	1374	< 1
Deep shank, without tarp, 25 feet (Appendix 2, Table 10)						
420	290	1	724	1	889	< 1
Drip, with tarp, 25 feet (Appendix 2, Table 10)						
420	289	1	721	1	885	< 1
Deep drip, without tarp, 25 feet (Appendix 2, Table 10)						
420	516	1	1289	< 1	1582	< 1
Shallow shank, with tarp, 100 feet (Appendix 2, Table 10)						
420	23	18	85	5	108	4
Shallow shank, without tarp, 100 feet (Appendix 2, Table 10)						
420	224	2	837	1	1062	< 1
Deep shank, without tarp, 100 feet (Appendix 2, Table 10)						
420	145	3	541	1	687	1
Drip, with tarp, 100 feet (Appendix 2, Table 10)						
420	144	3	539	1	684	1
Deep drip, without tarp, 100 feet (Appendix 2, Table 10))						
420	258	2	963	< 1	1222	< 1

E. RISK APPRAISAL

Risk assessment is the process by which the toxicity of a chemical is compared to the potential for human exposure under specific conditions in order to estimate the risk to human health. Every risk assessment has inherent limitations relating to the relevance and quality of the toxicity and exposure data. Assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment and exposure-assessment processes, resulting in uncertainty in the risk characterization, which integrates the information from those three processes. Qualitatively, risk assessments for all chemicals have similar uncertainties. However, the magnitude of those uncertainties varies with the availability and quality of the toxicity and exposure data, and with the relevance of that data to the anticipated exposure scenarios.

In the following sections, the uncertainties associated with characterization of health risks from exposure of workers and the general public to inhaled AITC are described. The exposure scenarios examined include inhalation exposure to workers and to occupational and residential bystanders. Uncertainties pertaining to the exposure assessment are delineated in the Exposure Assessment Document (Appendix 1).

E.1 Hazard Identification

While inhalation studies in humans would provide the most appropriate toxicity data for evaluating the health implications of inhaled AITC, such studies were not available. However, inhalation toxicity data from studies in laboratory animals were available and considered adequate to this purpose once the inherent uncertainties were identified and accounted for. In the following sections, the uncertainties associated with characterization of health risks from exposure of workers and the general public to AITC are described.

E.1.1 Study Database

The scope of this risk assessment was limited to the evaluation of health risks associated with exposure to AITC by the inhalation route. Confidence in the calculated risk estimates was impacted by the availability of only three inhalation toxicity studies (two acute, one subchronic), from which those estimates were based. This was compounded by the absence of any inhalation toxicokinetic, chronic, reproductive, or developmental toxicity study, necessitating substitution with oral studies in each of those cases. Thus the major toxicologic uncertainty overshadowing this assessment stemmed from a lack of primary and supporting studies by the inhalation route. Even so, the two inhalation studies used to derive the critical acute, subchronic, and chronic PODs were well designed and provided crucial information on multiple toxicologically relevant parameters, including motor activity, functional observational battery behaviors, and organ and tissue histopathology. Furthermore, conversion of the oral subchronic and chronic NOELs to equivalent air concentrations by a well-established route extrapolation technique provided strong support for the respective critical subchronic and chronic human equivalent air inhalation air concentration values. Finally, the assessment of AITC's reproductive, developmental, and oncogenic risks (or lack thereof) was highly dependent on analysis of the oral toxicity database

due to the absence of any inhalation studies strictly relevant to these effects. The uncertainties arising from this situation are detailed below.

E.1.2 Acute Inhalation POD

The risk from acute inhalation exposure to AITC vapor was evaluated using an ENEL of 2.5 ppm established in a 4-hour whole-body study in rats (Herberth, 2017). The ENEL was based on decreased motor activity and decreased rearing counts measured 2 hours after exposure began. The study was designed to examine the acute toxicology of AITC using several behavioral and anatomical parameters. The critical endpoints exhibited a concentration-dependent response and were significantly different from controls even at the lowest tested dose, obviating designation of a study NOEL. DPR generally prefers to use a BMD approach to establish PODs, especially when relevant effects occur at the low dose and when data are amenable to modeling. However, modeling was not used to establish the acute POD for AITC because data for the critical endpoint (decreased ambulatory, total motor, and rearing activity) could not be modeled due to high variability. Instead, a default 10 LOEL-to-NOEL extrapolation factor was used to establish the critical POD.

E.1.3 Subchronic Inhalation POD

A NOEL of 5 ppm from a 13-week whole-body vapor exposure study in rats was the critical POD for evaluating the risk from seasonal inhalation exposure to AITC (Randazzo, 2017). The strength of the study was its rigorous design, which included evaluations of clinical signs, clinical pathology, ocular pathology, body weight, food consumption, and FOB and motor activity determinations. The POD was based both on portal of entry effects (degenerative changes in the nasal olfactory epithelium in both sexes and metaplastic lesions in respiratory epithelium) and systemic effects (decreased ambulatory and total motor activity in male rats) at a LOEL of 10 ppm.

Both incidence rate and severity of the portal of entry effects increased with increasing AITC air concentration. While low levels of olfactory epithelial degeneration and squamous metaplasia of respiratory epithelium were observed at the POD of 5 ppm, these observations were insufficiently robust to be determining factors in the subchronic risk evaluation. In support of this position, nasal epithelial lesions of a "minimal" grade have not generally been considered significant by pathologists (Hardisty *et al.*, 1999), nor are they serious enough to qualify for any level of "adversity" designation (Palazzi *et al.*, 2016).

As noted above, a BMD approach is preferable to a traditional NOEL / LOEL approach in establishing a POD when the data are suitable for modeling. BMD modeling of the most sensitive portal of entry effect (olfactory epithelial degeneration in males) yielded a $BMCL_{10}$ of 4.78 ppm using the log-logistic algorithm and a benchmark response rate (BMR) of 10% (see Appendix 4 for BMD outputs). This value was essentially equivalent to the study NOEL of 5 ppm. Although the motor activity data showed concentration responsivity from the standpoint of group means, it was not amenable to modeling due to the high variability of the data and the

small group sizes. Nonetheless, decreased motor activity was considered to be biologically significant and toxicologically relevant at 10 ppm (> 20% decrease compared to control), particularly as similar effects were observed at 50 ppm in the acute inhalation toxicity study (Herberth, 2017).

The subchronic POD (5 ppm) exceeded the acute POD (2.5 ppm). This is counter to the expectation that subchronic PODs are generally lower then acute PODs, largely because of the enhanced sensitivity conferred by repeated exposures. In this assessment, however, the critical subchronic POD for AITC was derived from experimental results, whereas the critical acute POD was an estimated NOEL. Applying the default 10 LOEL-to-NOEL factor may therefore have resulted in an artificially low acute POD.

The subchronic critical inhalation POD was supported by the results of a route extrapolation analysis by which the subchronic oral NOEL (6.6 mg/kg/day based on urothelial hyperplasia) was converted to a human equivalent air concentration. The resulting value, 9.5 ppm, was close to the subchronic critical POD of 5 ppm conferring a stronger degree of certainty in the latter value.

E.1.4 Chronic Inhalation POD (non-oncogenic)

A critical POD of 0.5 ppm was used to estimate the risks of chronic inhalation exposure to AITC. It was derived from the subchronic critical POD of 5 ppm by applying a default duration extrapolation factor of 10 due to the lack of chronic inhalation studies. The POD was based on both portal of entry and systemic effects which resulted in a LOEL of 10 ppm (Randazzo, 2017).

Using the subchronic study to estimate chronic risk added uncertainty to the analysis. When a 13 week subchronic study is used for duration extrapolation, a factor of 3 could be considered because the study covers about 13% of the 2-year rat lifetime (OEHHA, 2008; DPR, 2014; IPCS, 2014). However, this analysis applied the full extrapolation factor of 10 due to the limited inhalation database for AITC. The possibility of using chronic oral toxicity studies (two in rats and one in mice; (NTP, 1982; Cho *et al.*, 2017) to identify a critical chronic oral endpoint and then derive the critical inhalation POD by route-to-route extrapolation was explored. However, this option was not pursued due to the evidence for route-specific effects:

a) AITC by the inhalation route induced both portal of entry and systemic effects upon subchronic (and presumably chronic) exposure (Randazzo, 2017). However, oral administration for any duration producsed dissimilar effects. Portal of entry effects in the upper respiratory tract were specific to AITC exposure by inhalation route.
b) Oral administration of AITC induced urinary bladder epithelial hyperplasia after both subchronic and chronic exposures. However, AITC by inhalation route did not induce urinary bladder hyperplasia after 13 weeks of exposure. This observation suggests that bladder effects were relevant for oral, but not inhalation, exposures.

Consequently, urinary bladder epithelial hyperplasia are likely induced by chronic oral exposure and are unlikely to result from inhalation exposures.

As such, the subchronic inhalation toxicity study of Randazzo (2017) was used to derive the chronic inhalation POD. For purposes of comparison, the external air concentration equivalent to the chronic oral $BMDL_{10}$ of 0.6 mg/kg/day (based on urothelial hyperplasia from Cho *et al.* (2017) was estimated to be 0.9 ppm by applying a default 24-hour rat inhalation rate of 0.96 m^3/kg and duration adjustments appropriate to the study in question. The air concentration equivalent was within two-fold of the selected critical chronic inhalation POD of 0.5 ppm. Based on these results, there was less uncertainty associated with a POD derived from a route specific subchronic inhalation endpoint than one derived from a chronic oral endpoint converted to an air concentration by route-to-route extrapolation.

E.1.5 Reproductive and Developmental Toxicity

Altogether, the PODs for effects in the reproductive and developmental toxicity studies ranged from 6 to 40 mg/kg/day. As these NOELs were higher than POD for urinary bladder hyperplasia in the subchronic oral study (Hasumura *et al.*, 2011), we consider them to be protective of downstream effects. Nonetheless, we recognize the uncertainty imparted by the absence of inhalation route specific studies.

E.1.6 Genotoxicity

AITC was negative in all *in vivo* mutagenicity studies, negative or weakly positive (at cytotoxic concentrations) in the standard bacterial mutagenicity assays, and exhibited poor DNA reactivity. Other genotoxicity tests showed that AITC induced chromosomal aberrations and DNA damage (but not micronucleus formation). Based on these and other observations, and according to US EPA's Guidelines for Carcinogen Risk Assessment (US EPA, 2005), DPR concluded that AITC is unlikely to be mutagenic. A similar conclusion was reached by other independent agencies US EPA and EFSA in their risk assessments of AITC (EFSA, 2010; US EPA, 2013).

E.1.7 Oncogenicity

As no chronic inhalation studies were available, two oral 2-year bioassays in rats (NTP, 1982; Cho *et al.*, 2017) and one in mice (NTP, 1982) were used to evaluate AITC's potential for oncogenicity. Orally administered AITC appeared to increase the incidence of three types of tumors in rats: urinary papilloma, undifferentiated leukemia, and fibrosarcoma. The use of the oral exposure route to address oncogenicity, as well as the absence of any chronic inhalation studies, added substantial uncertainty to the assessment, as plausible differences in internal exposures to target tissues may render tumors produced by one route of exposure irrelevant to other routes.

Undifferentiated leukemia

The incidence of undifferentiated leukemia[9] in F344/N male rats appeared to increase with dose in a study performed by the National Toxicology Program (NTP, 1982), achieving statistical significance at the high dose (incidence at 0, 12, and 25 mg/kg/day: 2/50, 6/50, and 8/50*; *p = 0.05). There was also a suggestion of dose responsiveness in females, though statistical significance was not achieved (7/50, 9/50, and 11/50). The investigators reported that the high incidence in males (8/50 = 16%) did not exceed the mean historical control rate of 20.8% (123/591; range: 0/15 [0%] – 20/50 [40%]) for F344/N males in their NTP laboratory (Dunnick *et al.*, 1982; NTP, 1982). Additionally, the incidence in high dose males was not statistically different from the male mean historical control rate of 10% in all NCI/NTP laboratories. In fact, the F344/N rat strain is known for its high background leukemia rate and study-to-study variability (Thomas *et al.*, 2007). These are the main reasons why NTP convened in 2005 a workshop on animal models used in the NTP rodent cancer bioassay (King-Herbert and Thayer, 2006). Following the recommendations of the panel members, NTP discontinued using F344/N rat for its bioassays and adopted Sprague Dawley rat as its default rat strain in 2006. Furthermore, re-analysis of the NTP database revealed 34 out of 500 substances that were possibly associated with undifferentiated leukemia, but AITC was not one of them (Thomas *et al.*, 2007).

It should also be noted that leukemia rates in F344/DuCrj rats did not increase in response to exposure to AITC-rich horseradish extract (HRE) (Cho *et al.*, 2017). While the rat substrains, exposure methodology, and test article identity varied between NTP (1982) and Cho *et al.* (2017), the failure to reproduce leukemias in the later study strongly suggested that AITC exposure was not a leukemogen.

Subcutaneous fibrosarcoma

An increased incidence of subcutaneous fibrosarcomas was observed in high-dose female rats (3/50 or 6%), but not in low-dose (0/50) or in control females (0/50) in the NTP (1982) study. Moreover, males showed no increase in these tumors in NTP (1982) study, nor was there an effect in males in the Cho *et al.* (2017) study. The female high-dose incidence of 6% exceeded NTP's background incidence of 0.2% (1/591) for this F344/N substrain, as well as the rate of 0.9% (9/999) observed in all other laboratories at the time of publication (NTP, 1982). A role for AITC in fibrosarcoma induction was therefore considered plausible. However, further analysis was not conducted because:

[9] NTP has changed terminology for this tumor type over the years. In current usage this cancer is referred to as "mononuclear cell leukemia" (Thomas *et al.*, 2007; Maronpot *et al.*, 2016). However, for this document the term "undifferentiated leukemia" is used in order to avoid confusion, as the latter was the term used by the study authors.

The apparent effect did not apply to F344/N males in the NTP study or in another substrain F344/ DuCrj males (Cho *et al.*, 2017).

1. A valid cancer potency analysis was precluded by the fact that the apparent effect was observed only at a single high dose.
2. A route-specific chronic inhalation study was not available. Further study using route and duration specific methodologies may alter this conclusion.

Urinary bladder tumors

AITC induced urinary bladder epithelial tumors in studies by NTP (1982) (papilloma) and Cho *et al.* (2017) (papilloma and carcinoma). Mice did not show the effect (NTP, 1982). While tumor induction in rats was independent of route of administration (gavage *vs.* drinking water), dose dependency was observed in both studies. In the Cho study, bladder papilloma incidence at 0, 4.1 and 15.7 mg/kg/day was 1/32, 0/32 and 3/32, respectively, though pairwise significance was not achieved. In the NTP study, papilloma incidence at 0, 12 and 25 mg/kg/day was 0/50, 2/50 and 4/50, respectively, and positive for trend. Both sets of investigators concluded that urinary bladder papillomas were indeed induced by AITC. DPR concurs with their conclusion.

The weight of evidence was analyzed for establishing a mode of action (MOA) for AITC induction of urinary bladder tumors through a preliminary hyperplastic step using the framework prescribed by US EPA in its Guidelines for Carcinogen Risk Assessment (US EPA, 2005). The analysis showed that the urinary bladder tumors in rats likely required sustained excretion of high levels of AITC metabolite(s) in urine, resulting in urothelial cell proliferation, hyperplasia (evident in both studies), and ultimately, tumors. The European Food Safety Authority (EFSA) arrived at a similar conclusion in its AITC risk assessment (EFSA, 2010). Based on this stepwise MOA, exposure to doses at that do not result in sustained hyperplasia were not expected to result in urinary bladder tumors.

Even in this light, careful consideration of the weight of evidence led to the conclusion that development of urinary bladder tumors was specific to the oral route of exposure. This was based on the observation that urothelial hyperplasia was absent in all available inhalation toxicity studies, and in particular, the subchronic inhalation study of Randazzo (2017). Nonetheless, substantial uncertainty remains, as route specific studies were not available at an appropriate chronic duration. However, the critical chronic inhalation PODs are expected to be protective of urothelial hyperplasia and urinary bladder tumors regardless of route-specific factors.

E.2 Horseradish Extract versus Technical Grade AITC

The available toxicity database for AITC consists of three inhalation studies in rats and numerous oral study in rodents. The inhalation studies utilized technical grade AITC (97.9 to 99.9%) and thus the observed effects were not confounded by impurities or presence of other isothiocyantes. The oral studies used 93% to 99.9% pure AITC administered to the laboratory

animals via gavage, diet or drinking water. In addition, several subchronic and chronic drinking water studies used HRE as a source of AITC. The HRE in these studies contained 82-86% AITC.

Among cruciferous vegetables, horseradish has the highest concentration of AITC (1350 mg/kg (USDA, 2014)). In addition, horseradish contains at least nine other isothiocyanates: 2-phenethyl isothiocyanate (PEITC), n-butyl isothiocyanate, 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate, 5-hexenyl isothiocyanate, 5-methylsuphinylpentyl isothiocyanate, 6-methylsulphinylhexyl isothiocyanate, and 7-methylsuplinylheptyl isothiocyanate. The second most abundant isothiocyanate in horseradish extract is PEITC, comprising up to 9% of HRE. Similar to AITC, PEITC is known to induce and promote urinary bladder hyperplasia and tumors in animal models (Hirose *et al.*, 1998; Akagi *et al.*, 2003). The influence of PEITC in the HRE induced effects cannot be ruled out; however, if it does influence these effects, it likely plays a minor role. No evidence of urinary bladder effects could be located for other minor components. Except for PEITC, no epidemiological studies or chronic animal bioassays were identified showing any association between the minor components of HRE and incidence of human cancer or oncogenic effects, respectively.

E.3 Antitumor Effects

Evidence accruing over the past 30 years points to cruciferous vegetables as prominent dietary components that may reduce the risk of cancer (Abbaoui *et al.*, 2018). The isothiocyanates, including AITC, that are abundant in broccoli, Brussels sprouts, cabbage, cauliflower, horseradish, kale and mustard seeds are thought to contribute to the cancer chemopreventive activity of these vegetables (Wu *et al.*, 2009). Investigations are currently in progress to strengthen this association.

In animal models, isothiocyanates are effective in preventing or reducing the risk of cancer induced by carcinogens in several target organs including lung, liver, forestomach, mammary gland, esophagus, small intestine, colon, and bladder (Hecht, 1995). The mode of action of isothiocyanates is postulated to involve induction of Phase II metabolizing enzymes leading to decreased activation and/or increased detoxification of carcinogens (Xiao *et al.*, 2003). *In vitro*, isothiocyanates inhibit growth of various types of cancer cells by affecting cell processes including apoptosis, the MAPK pathway, oxidative stress, and the cell cycle machinery (Wu *et al.*, 2009). However, it is of interest that AITC, which is present in the cruciferous vegetables, has also been shown to induce cancer in laboratory rats, where long-term dietary intake resulted in urinary bladder papillomas and possibly dermal fibrosarcomas (NTP, 1982; Cho *et al.*, 2017).

Several epidemiology studies reported that high intake of cruciferous vegetables was associated with a decreased risk for urinary bladder tumors and prostate cancer. Specific to urinary bladder tumors, as reviewed by Abbaoui *et al.* (2018), there are several large prospective studies (Michaud *et al.*, 2000; Michaud *et al.*, 2001) and retrospective case-control studies (Zhao *et al.*, 2007), meta-analyses of cohort and case-control studies (Liu *et al.*, 2013), a hospital-based case-controlled study with individuals with bladder cancer (Tang *et al.*, 2008) and a multi-ethnic cohort study that address this issue directly (Park *et al.*, 2013). Strong inverse correlations were

found between urinary bladder cancer and mortality on the one hand, and broccoli intake on the other (Michaud *et al.*, 2002; Tang *et al.*, 2010). Within the cruciferous vegetables, mustard, horseradish, and broccoli have the highest concentrations of AITC (USDA, 2014). Although, AITC-generating sinigrin is one of the common glucosinolate found in broccoli, it makes up only 0.8% of all the glucosinolates in broccoli (Jones *et al.*, 2006). Consequently, although these studies suggested that increased consumption of cruciferous vegetables is protective against urinary bladder cancer, no specific conclusions can be drawn for AITC in this regard.

The effects of AITC have been examined in several in animal cancer models. In a rat orthotopic model, where cancer cells are injected directly into the wall of the urinary bladder, AITC at low doses (~1 mg/kg/day) significantly reduced invasion of the muscle tissue by the bladder cancer xenograft (Bhattacharya *et al.*, 2010a; Bhattacharya *et al.*, 2010b; Bhattacharya *et al.*, 2012). AITC injected into a prostate cancer xenograft in mice decreased the volume of prostate tumors and reduced the levels of anti-apoptotic proteins (Srivastava *et al.*, 2003). In a rat model of diethynitrosamine-induced hepatocarcinogenesis, sinigrin administered in diet for 7 weeks reduced tumor multiplicity (Tanaka *et al.*, 1990). In several in vitro studies AITC inhibited growth and induced apoptosis in human prostate cancer cells, human leukemia and myeloblastic leukemia cells (Xu and Thornalley, 2001).

It is thus possible that low doses of AITC have anti-tumor activity. However, in the chronic gavage and drinking water studies in rats, AITC at doses of 12-25 mg/kg/day induced urinary bladder and fibrosarcomas (NTP, 1982; Cho *et al.*, 2017). Those doses are 600-1250 fold higher than the acceptable daily intake (ADI) of 0.02 mg/kg/day established by (EFSA, 2010). EFSA's ADI was based on a LOEL of 9 mg/kg/day for transitional cell papillomas of the urinary bladder observed in male rats in the NTP chronic gavage study. The total UF was 500, including 10 each for interspecies and intraspecies extrapolation and 5 for LOEL-to-NOEL extrapolation and uncertainties related to the absence of data on reproductive toxicity. EFSA also reported that the daily total exposure of AITC from all sources, including natural occurrence in food, use as a flavoring substance and application as an antispoilage agent, may exceed the ADI by 5 to 8 fold in children and adults, assuming the 95th percentile of consumption. Even so, an 8-fold higher daily dose of AITC (0.16 mg/kg/day) is still significantly lower (75-157 fold) than the tumorigenic doses in rats.

E.4 Reference Concentrations, Uncertainty Factors, and Margins of Exposure

Default uncertainty factors for deriving RfCs are conventionally set at 10 to account for interspecies variability (UF_A) and 10x for intraspecies (human) sensitivity (UF_H). Both UFs are themselves products of two separate components, a pharmacokinetic uncertainty factor of 3x and a pharmacodynamic uncertainty factor of 3x (US EPA, 2002; DPR, 2014). As for any risk characterization utilizing this approach, selection of default factors is itself associated with uncertainty which can only be reduced with targeted experiments.

For RfCs calculated from HECs, the conventional interspecies uncertainty factor of 10 was reduced to 3x because the interspecies pharmacokinetic differences were considered resolved by

the HEC conversion, regardless of whether the effects were portal of entry or systemic. The remaining default interspecies pharmacodynamic UF of 3x was retained because data relating to tissue level interactions were insufficient to quantitatively resolve potential animal-to-human differences (U.S. EPA, 1994). The full 10-fold intraspecies (UF_H) factor was also retained to reflect the range of sensitivity within the human population. These defaults carry their own uncertainty since their proximity to the actual values are not known.

The target MOE for AITC is equivalent to the UF_{TOTAL} of 30. This target MOE is considered adequate to protect human health for all potentially exposed populations (handlers, re-entry workers, occupational bystanders, and residential bystanders).

F. CONCLUSION

The purpose of this risk assessment was to evaluate the human health risks associated with inhalation exposure to AITC. Risk was quantitatively estimated for short-term, seasonal, and annual exposure scenarios for workers (handlers, and re-entry workers), and short-term exposure scenarios for occupational and residential bystanders according to the proposed labels.

All critical points of departure (PODs) for AITC were based on effects observed in inhalation toxicity studies in rats. Animal PODs were extrapolated to human equivalent concentrations (HECs) using dosimetric adjustment factors that account for physiological differences between laboratory rats and humans. Reference concentrations (RfCs) for AITC were calculated for all exposure scenarios by dividing the critical HEC by a total uncertainty factor (UF_{TOTAL}) of 30, which is the product of an interspecies uncertainty factor (UF_A) of 3 and an intraspecies uncertainty factor (UF_H) of 10. Acute RfCs for workers, occupational bystanders, and residential bystanders are 42, 42 and 14 ppb, respectively. The seasonal RfC for workers is 125 ppb. The annual RfC for workers is 13 ppb.

Non-oncogenic risks were calculated as margin of exposure (MOE), a quotient of the HEC and the estimated human exposure level. An analysis of the uncertainties inherent in the risk characterization resulted in designation of 30 as the target MOE for all analyzed scenarios. The target MOE is equivalent to the total uncertainty factor (UF_{TOTAL}). Estimated MOEs lower than the target MOE of 30 were, therefore, considered to pose a potential health risk.

Risk to Workers

- Under short-term exposure conditions, worker MOEs ranged between 1 and 96. Eleven of 13 short-term MOEs were lower than the target of 30.
- Under seasonal exposure conditions, worker MOEs ranged between 10 and 938. Four of 13 scenarios generated seasonal MOEs lower than the target of 30.
- Under annual exposure conditions, worker MOEs ranged between 4 and 750. Six of 13 scenarios generated annual MOEs lower than the target of 30.

Risk to Bystanders

- Under short-term exposure conditions, occupational bystander MOEs ranged between <1 and 15. All of the 15 evaluated scenarios generated MOEs lower than the target of 30.
- For residential bystanders, including sensitive subpopulations, all resulting MOEs calculated under short-term conditions were lower than the target of 30, indicating risks for all exposure scenarios for these groups

The critical acute, subchronic, and chronic PODs were derived from two inhalation studies. These studies were well designed and provided crucial information on multiple toxicologically relevant parameters. Furthermore, conversion of subchronic and chronic rat PODs from oral studies to equivalent rat air concentrations provided strong support for the respective critical subchronic and chronic inhalation air concentration values. However, the overall confidence in the calculated risk estimates was impacted by the availability of only three inhalation toxicity studies (two acute, one subchronic), from which those estimates were based. This was compounded by the absence of any inhalation toxicokinetic, chronic, reproductive, or developmental toxicity study, necessitating substitution with oral studies in each of those cases. Finally, considerations regarding the oncogenic potential of AITC via the inhalation route were highly dependent on analysis of the oral toxicokinetic and oral chronic toxicity database. In the end, oncogenic risk by the inhalation route was not calculated due to lack of experimental support.

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APPENDICES

APPENDIX 1. HUMAN EXPOSURE ASSESSMENT FOR ALLYL ISOTHIOCYANATE AS SOIL FUMIGANT

HUMAN EXPOSURE ASSESSMENT FOR ALLYL ISOTHIOCYANATE AS SOIL FUMIGANT

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July, 2020

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Acknowledgements:

The author thanks Dr. Lucia Graham and Michel Oriel from Worker Health and Safety branch (WHS) of DPR for collecting human illness information from Sentinel Event Notification System for Occupational Risk (SENSOR) program. The author also thanks Bernie Hernandez, Harvard Fong, Donald Richmond and Diana Le from WHS of DPR for sharing their valuable field experience.

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ACRONYMS

1,3-D	1,3-dichloropropene
AADD	Annual average daily dose
AITC	Allyl isothiocyanate
CalPIP	California Pesticide Information Portal
CalPIQ	California Pesticide Illness Query
DPR	California Department of Pesticide Regulation
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
Isagro	Isagro USA, Inc
Kow	Octanol-water partitioning coefficient
LADD	Life-time average daily dose
NRC	National Research Council
PC code	Pesticide chemical code
PE	Polyethylene
Pic	Chloropicrin
PPE	Personal protective equipment
REI	Restricted entry interval
MITC	Methyl isothiocyanate
MITC-K	Metam-potassium
MITC-Na	Metam-sodium
PISP	Pesticide Illness Surveillance Program
PUR	Pesticide use reporting
SADD	Seasonal average daily dose
STADD	Short-term absorbed daily dose
TIF	Totally impermeable film
U.S. EPA	U.S. Environmental Protection Agency

I. EXECUTIVE SUMMARY

Isagro USA, Inc (Isagro) submitted two product labels to the California Department of Pesticide Regulation (DPR) to register allyl isothiocyanate (AITC) for use as soil fumigant. As fumigant use of AITC has not been permitted in California, there is no use data available or human illness cases recorded. The primary exposure route is through inhalation.

Listed below are ranges of handler, re-entry worker, occupational and residential bystander exposures for short-term, seasonal, annual and life-time exposure scenarios:

- The estimated short-term absorbed daily doses (STADDs) for handlers range from 9 µg/kg/d for applicators in drip application to 1373 µg/kg/d for loaders in both shallow and deep shank applications;
- The estimated seasonal average daily doses (SADDs) for handlers range from 3 µg/kg/d for applicators in drip application to 264 µg/kg/d for loaders in shallow shank application;
- The annual average daily dose (AADDs) for handlers range from 0.3 µg/kg/d for applicators in drip application to 73 µg/kg/d for loaders in deep shank application;
- The life-time average daily dose (LADDs) for handlers range from 0.2 µg/kg/d for applicators in drip application to 39 µg/kg/d for loaders in deep shank application;
- The STADDs for re-entry workers range from 22 µg/kg/d for drip application to 31 µg/kg/d for both shallow and deep shank applications;
- The SADDs for re-entry workers range from 19 µg/kg/d for drip application to 27 µg/kg/d for shallow shank application;
- The AADDs for re-entry workers range from 3 µg/kg/d for drip application to 11 µg/kg/d for deep shank application;
- The LADDs for re-entry workers range from 1 µg/kg/d for drip application to 6 µg/kg/d for deep shank application;
- The STADDs for occupational bystanders at the edge of a 40 ac treated field range from 185 µg/kg/d for the field using shallow shank application and tarp-covered to 2431 µg/kg/d for the field using drip application without tarp cover;
- The STADDs for residential adult bystanders at 25 ft away from a 40 ac treated field range from 112 µg/kg/d for the field using shallow shank application and tarp-covered to 1272 µg/kg/d for the field using drip application without tarp cover;
- The STADDs for residential child bystanders at 25 ft away from a 40 ac treated field range from 272 µg/kg/d for the field using shallow shank application and tarp-covered to 3087 µg/kg/d for the field using drip application without tarp cover.
- Due to the lack of both use and ambient air monitoring data, SADDs, AADDs and LADDs of occupational and residential bystander exposures to AITC were not estimated in this assessment.

II. INTRODUCTION

In 2017, Pesticide Registration Branch of the California Department of Pesticide Regulation (DPR) received two applications for FIFRA Section 3 registration from Isagro USA, Inc (Isagro) to allow Dominus® and Dominus® 100 products for use in California. Allyl isothiocyanate (AITC) is the only active ingredient in both products, and the AITC content is 96.3% and 99.8% for Dominus® and Dominus® 100, respectively. This section provides background information of AITC and its current regulation status with both the U.S. Environmental Protection Agency (U.S. EPA) and DPR.

A. AITC and the submitted products

The chemical structure of AITC is shown in Figure 1, together with some key physiochemical properties (Jones, 2013). At room temperature, AITC is liquid with very pungent odor.

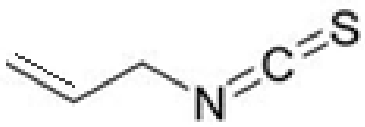


Figure 1. Chemical structure of AITC

- CAS No.: 56-06-7
- Molecular formula: C_4H_5NS
- Molecular weight: 99.2 g/mol
- Relative density: 1.0
- Boiling point: 148-154 °C
- Vapor pressure: 0.493 kPa at 20 °C
- Henry's Law constant: 3.7 x 10^{-5} Pa/m^3/mol
- Solubility (g/L, 20ºC): 0.002 in distilled water, 545.9 in acetone, 3.0 in toluene

Based on the two submitted product labels, the proposed use of Dominus® and Dominus® 100 is a pre-plant soil fumigant to control various nematodes, fungi, insects and weeds. In addition, Dominus® may be used in post-plant crop termination application. Both products can be applied via broadcast shank or bed/strip shank applications, and Dominus® may also be applied through drip irrigation (i.e., chemigation).

B. Regulation status

U.S. EPA. The fumigation use of AITC has been approved by the U.S. EPA, and as of June 2019, there are three actively registered AITC fumigant products, including the two submitted to DPR. Instead of designating as a "new" active ingredient, AITC is registered under oil of mustard (chemical code [PC code] 004901), which also contains AITC. The oil of mustard was first registered with the U.S. EPA in 1962 as dog repellant, and is currently registered as biochemical pesticide (USEPA, 1993; USEPA, 2013). De-oiled oriental mustard seed (Brassica juncea) is another pesticide that can produce AITC when in contact with water. Akin to the oil of mustard, it is registered with U.S. EPA as biochemical pesticide but with a different PC code 014921 (USEPA, 2008).

DPR. In California, currently there is no active registration of products containing either oil of mustard or AITC. In addition, AITC has never been registered as soil fumigant in California.

III. FACTORS CONSIDERED TO DEVELOP EXPOSURE SCENARIOS

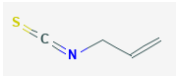
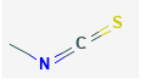
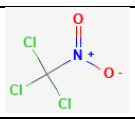
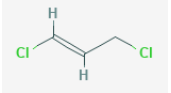
National Research Council (NRC) recommends DPR include a problem formulation/scoping step in the risk assessment process. NRC envisions the problem formulation as a phase to determine "*the major factors to be considered, the decision-making context, and the timeline and depth needed to ensure that the right questions are being asked in the context of the assessment*" (NRC, 2009). NRC suggested DPR that "*risk managers should be consulted in the problem-formulation stage so that a risk assessment can be designed to address the decisions that need to be made by managers and other stakeholders. Consideration should be given to whether a general set of problems and risk-management options could be formulated to use as a starting point in problem formulation*" (NRC, 2015).

DPR accepted this recommendation and during the problem formulation phase, reviewed exposure information and data relevant to AITC, especially the California-specific data (DPR, 2018). This section describes factors considered in the development of the exposure scenarios of AITC. Due to scarcity of AITC-specific data, this assessment used data from various sources including those from other soil fumigants (i.e., surrogates). The rationale for selecting these surrogates is explained below.

A. Physiochemical properties

The chemical structure of AITC is similar to methyl isothiocyanate (MITC), which is a soil fumigant produced from metam-sodium (MITC-Na) or metam-potassium (MITC-K). Table 1 compared some key physiochemical properties of AITC with MITC and two other fumigants commonly used in California: chloropicrin (Pic) and 1,3-dichloropropene (1,3-D). Both MITC and AITC are liquid at room temperature but readily volatize because of their high vapor pressure and low boiling points. AITC has lower water solubility and higher octanol-water partitioning coefficient (K_{ow}) than MITC, implying its higher potential of sorption to soil organic matter and lower transfer from soil surface to water. The water solubility and *K*ow values of AITC are similar to Pic and 1,3-D, indicating a similar partitioning and transport behavior among these fumigants in soil. AITC has higher boiling point and lower vapor pressure than Pic and 1,3-D, suggesting that at the soil surface AITC may be less ready to volatize into air.

Table 1. Comparisons of physiochemical properties between allyl isothiocyanate (AITC) and other soil fumigants

Property[a]	AITC	MITC	Pic	1,3-D
Molecular formula				
Density (g/cm^3)	1.0	1.1	1.6	1.2

Property[a]	AITC	MITC	Pic	1,3-D
Molecular weight (g/mol)	99.2	73.3	164.4	111.0
Boiling point (°C)	148-154	119	112	108
Solubility in water (mg/mL)	2 (at 20 °C)	7.6 (at 20 °C)	1.9 (at 20 °C)	2 (at 20 °C)
Vapor pressure (mmHg)	3.7 (at 30°C)	3.54 (at 25°C)	24 (at 25 °C)	34 (trans), 23 (cis) (at 25 °C)
logK_{ow}	2.1	0.94	2.1	2.1

a: data obtained from Jones (2013); NIH (2019);
b: AITC=allyl isothiocyanate, MITC=methyl isothiocyanate, Pic=chloropicrin, 1,3-D=1,3-dichloropropene.

B. Application method

According to the two product labels submitted to DPR, the application methods of AITC include shank injection and chemigation. Detailed application methods and tarp requirements for each product are summarized in Table 2 and 3 below.

Table 2. Application method, injection depth, and tarp requirement for Dominus® 100 (EPA Registration No. 89285-3)

Application method	Injection depth (in)	Tarp[a]	Comment
Broadcast shank	5-15	Yes	PE, VIF, TIF[b]
		No[c]	Overhead sprinkler, water cap and/or roller/packer, close chisel traces
	>17	No	Roller/packer
Bed shank or strip	8-15	Yes	PE, VIF, TIF
		No	Overhead sprinkler, water cap and/or roller/packer, close chisel traces

a: whether tarp is required by the product label;
b: PE=polyethylene, VIF=virtually impermeable film, TIF=totally impermeable film;
c: tarp is not required if alternative methods described in the comment column are used.

Table 3. Application method, injection depth and tarp requirement for Dominus® (EPA Registration No. 89285-2)

Application method	Injection depth (in)	Tarp	Comment
Broadcast shank	4-15	Yes	PE, VIF, TIF[a]
		No[b]	Overhead sprinkler, water cap and/or roller/packer, close chisel traces
	>17	No	Roller/packer
Bed shank or strip	4-15	Yes	PE, VIF, TIF
		No	Overhead sprinkler, water cap and/or roller/packer, close chisel traces
Drip	subsurface[c]	Yes	N/A[d]
		No	>1 in buried drip tape

a: PE=polyethylene, VIF=virtually impermeable film, TIF=totally impermeable film;
b: tarp is not necessary, if alternative methods as described in the comment column are used;
c: drip emitters are placed at shallow subsurface positions;
d: tarp materials are not specified on the product label.

C. Label precaution and PPE requirement

Pesticide labels use three signal words, i.e., Danger, Warning, or Caution, to categorize how dangerous a product may be to humans. Both AITC products carry the signal word "DANGER" due to their "*corrosive*" property that "*causes irreversible eye damage and skin burns.*" Other language on product labels also include "*keep out of reach of children*" and "*…causes irreversible eye damage and skin burns. Maybe fatal if swallowed, absorbed through skin, or inhaled. Do not get in eyes, on skin or on clothing. Do not breathe vapor. Prolonged or frequently repeated skin contact may cause allergic reactions in some individuals. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before use.*"

Handlers. Both AITC products require personal protective equipment (PPE) for handlers "*when performing activities with the potential for liquid contact.*" The required PPE include "*coveralls worn over long-sleeved shirt and long pants, chemical-resistant footwear plus socks, chemical resistant gloves, protective eyewear and respirator.*" For tarp cutters and removers, both product labels requires "*long-sleeved shirt, long pants and gloves when removing tarps following*

application prior to plants" and a minimum 5-day restricted entry interval (REI). Respirators are not required for tarp cutters and removers.

Re-entry workers. Both product labels require a minimum 5-day REI.

Occupational and residential bystanders. Both product labels requires a minimum 25 feet buffer zone from "*any occupied structure, such as a school, daycare, hospital, retirement home, business or residence.*" Occupational bystanders are not subject to this 25 ft buffer zone requirement. In addition, in the training materials prepared by Isagro for the applicators, it is suggested the application should not be within 100 ft of any "*sensitive sites*" which include "*…occupied nursing homes, hospitals, or prisons, and occupied licensed schools, state licensed day care centers (any childcare facility other than a family day care home, including infant centers, preschools, extended day care facilities and school age child care centers) playgrounds, and licensed assisted living facilities (licensed by state or local governments)*" (Isagro, 2015).

D. Projected AITC use in California

Use information of AITC as soil fumigant is not available as this use has not been registered in California. Based on the submitted product labels, some application methods of AITC are similar to other soil fumigants that are already registered in California, including 1,3-D, Pic, methyl bromide (MeBr), MITC-Na and MITC-K. Accordingly, use data of these fumigants was obtained from DPR's pesticide use reporting (PUR) database, i.e., the California Pesticide Information Portal (CalPIP), and analyzed to project potential AITC use regions (DPR, 2019).

This analysis used fumigant use data from 2012-2017. In 2012-2017, the above five fumigants were used in different counties around the entire state. Seventeen counties had fumigant use >1% of total state use in any of the six years (2012-2017). These counties are located in several geological regions of California, including Central Coast (Santa Cruz, Monterey, San Luis Obispo), Central Valley (San Joaquin, Stanislaus, Merced, Fresno, Kings, Tulare, Kern, Madera), Inland Empire (Riverside, Imperial), South Coast (Santa Barbara, Ventura, Los Angeles), and the northern region (Siskiyou). Fields in these counties were fumigated for planting different crops (strawberry in Monterey *vs.* grape in Tulare), which implies the application methods can be different (shank *vs.* drip, deep *vs.* shallow injection).

Some fumigant use information, such as the application method and name of the company that performed the applications, is not available from CalPIP. Among the five soil fumigants above, 1,3-D has the highest annual use for six years (2012-2017). AGRIAN® is a proprietary pesticide-use database that includes some 1,3-D application information not available from CalPIP. Therefore, this analysis used AGRIAN 1,3-D data to collect information that is not available from CalPIP. This database has been previously used in the 1,3-D risk characterization document (DPR, 2015). As of May 2019, the AGRIAN database was updated with 2018 1,3-D-use data. Table 4 compares annual 1,3-D use recorded by CalPIP or AGRIAN and shows the similarities between their records (<7% difference).

Table 4. 1,3-Dichloropropene (1,3-D) use (lbs) recorded by CalPIP and AGRIAN

Year	CalPIP	AGRIAN
2012	11,928,106	11,153,954
2013	12,930,424	13,188,984
2014	13,584,325	13,957,997
2015	15,689,571	15,893,927
2016	14,128,700	14,366,348
2017	12,581,936	12,584,993
2018	No data[b]	12,828,742

CalPIP records pesticide use in California by licensed applicators. CalPIP is maintained by California Department of Pesticide Regulation (DPR) and available to the public. AGRIAN database only records 1,3-D use in California and the proprietary database is not publicly available; b: pesticide use data in 2018 is not available as of November, 2019.

Figure 2 shows 1,3-D use from different application methods from 2012-2018. According to AGRIAN, deep shank injection accounted for most of the 1,3-D uses (~80%) in California. However, the 1,3-D use pattern varies greatly among different counties. In central valley counties such as Fresno, Merced and Kern, 1,3-D was almost exclusively applied (>90%) via deep shank injections for deep root-zone crops, such as almonds and grapes. However, for coastal counties such as Monterey, Santa Barbara and Ventura, deep shank applications were used much less and only accounted for <20% of the total 1,3-D uses. For instance, in 2018, over 80% of 1,3-D use in Santa Cruz was applied using shallow shank equipment. In San Luis Obispo and Santa Barbara, around half of 1,3-D use was applied via drip tubes.

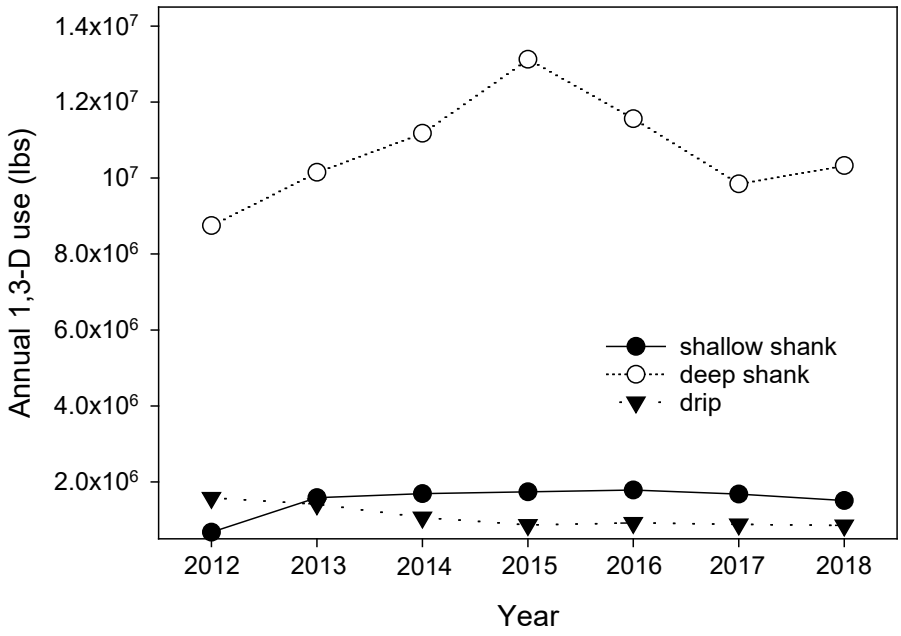


Figure 2. 1,3-Dichloropropene (1,3-D) uses from different application methods in from 2012-2018. Raw data was obtained from AGRIAN 1,3-D use database

Fumigant seasonal and annual use information, i.e., seasonal application rate and the number of applications per year, is needed to assess the intermediate- (seasonal) and long-term (annual and life-time) exposures. For re-entry workers, use information was summarized for the highest use county in California. For handlers, use information was summarized for the company that used the greatest amount of fumigant and in the highest use county. As AITC has no chemical-specific use data available, this analysis assumed that the use patterns (e.g., season, seasonal application rate, number of applications per year) would be the similar to 1,3-D. In addition, it was noted that 1,3-D and Pic are combined in many fumigant applications. For these applications, the total application rates of 1,3-D and Pic were combined to estimate AITC application rates. This is considered a reasonable estimate for AITC application rates because AITC is listed as the sole fumigant active ingredient in both Dominus® and Dominus® 100 products. Table 5 summarizes the estimated AITC seasonal and annual use information based on the 1,3-D data from the AGRIAN database.

In addition to the label requirements, 1,3-D use in California is subjected to permit condition regulations which specify the specific months of applications for different geographic regions as well as township caps; however, similar regulations do not exist for AITC since it is not yet registered for use in California (DPR, 2017). As shown in Table 5, the application rates used for seasonal exposure estimations are similar or equal to the maximum application rates listed on AITC product labels. AITC intermediate- and long-term exposures are not expected to be underestimated with the use of 1,3-D use information as described in detail above.

Table 5. Estimated AITC seasonal and annual use information based on 1,3-D use data retrieved from AGRIAN database in 2014-2018

Application method	Highest-county	Application days in year	Seasonal application rate[a] (lbs/ac)
Handler exposure scenarios (including tarp cutter, puncher and remover)			
Shallow shank	Monterey	78	340[b]
Deep shank	Fresno	105	328
Drip	Santa Barbara	46	246[b]
Re-entry worker			
Shallow shank	Monterey	100	340[b]
Deep shank	Fresno	157	328
Drip	Santa Barbara	47	246[b]

a: for applications when chloropicrin was applied together with 1,3-dichloropropene, the application rates of both compounds were combined to represent the total application rate;
b: the estimated application rate is over AITC maximum application rate (340 lb/ac for shank application and 246 lb/ac for drip application) hence the AITC maximum application rate is used instead.

E. Reported Illnesses

California. There are no AITC soil fumigant products registered in California, therefore the Pesticide Illness Surveillance Program (PISP) managed by DPR, i.e., California Pesticide Illness Query (CalPIQ), does not have any illness records associated with AITC. As of September 2019, CalPIQ did not have any illness records associated with use of oil of mustard, either.

F. Environmental concentrations

Occurrences of AITC in different environmental media (air, surface water, etc.) and their respective concentrations will be detailed in the "Environmental Fate of AITC" document developed by the Environmental Monitoring Branch of DPR (https://www.cdpr.ca.gov/docs/emon/pubs/envfate.htm).

G. Significant exposure scenarios

The assessed exposure scenarios can be grouped into four categories as listed below:

Occupational handler exposure. This group of scenarios includes occupational exposures occurring at the time of AITC application, such as loader and applicator (driver and co-pilot). The product labels require all handlers wear "*coveralls over long-sleeved shirt and long pants, chemical-resistant footwear plus socks, chemical-resistant gloves, protective eyewear and respirator.*" For applications with tarp, this group of scenarios also includes AITC exposures for tarp cutter, remover and puncher who enter the treated field after the REI (5 days) expires. The product labels state that workers performing tarp cutting, punching and removing are required to "*wear long-sleeved shirt, long pants and gloves.*" Protective respirator is not required for tarp cutter, remover, and puncher.

Occupational re-entry worker exposure. This group of scenarios covers post-application occupational exposures for workers preparing the treated field for next planting, such as soil shaper and pipe layer. The product labels do not specify the PPEs required for re-entry workers.

Occupational bystander exposure. This group of scenarios covers occupational exposures for workers in areas near the AITC treated field. Occupational bystanders are not subject to the 25 feet buffer zone requirement.

Residential bystander exposure. This group of scenarios covers non-occupational exposures for adults and children that reside near the AITC-treated field. The product labels specify AITC treatment should be a minimum 25 feet from "*any occupied structure, such as a school, daycare, hospital, retirement home, business or residence.*"

The exposure conceptual model is shown in Figure 3.

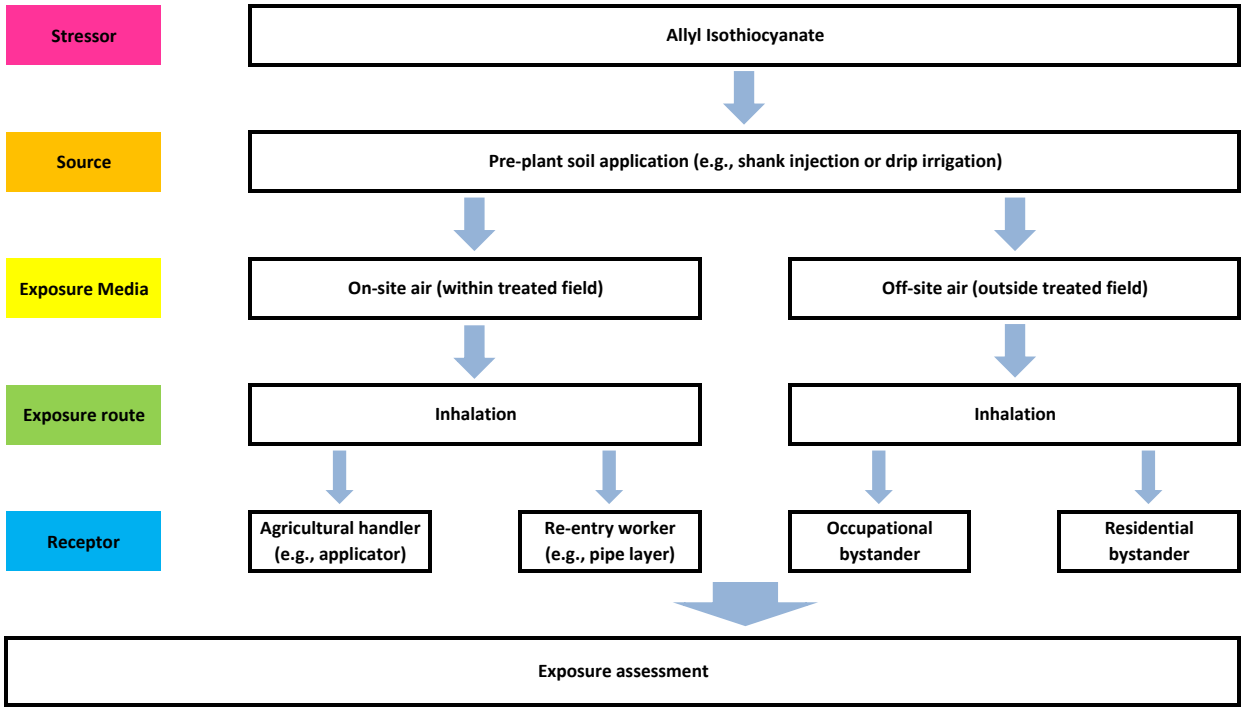


Figure 3. Exposure conceptual model for allyl isothiocyanate

IV. DERMAL/INHALATION ABSORPTION

This analysis only assesses human exposure through inhalation, based on the volatile property of AITC and PPE requirements of the two product labels submitted to DPR for handlers. In the absence of experimental data, the inhalation exposure was characterized using a default inhalation absorption rate of 100% (Frank, 2008).

V. EXPOSURE ASSESSMENT

A. Exposure duration

For occupational handlers and re-entry workers, this analysis assessed the AITC exposures for four periods: short-term, seasonal, annual and lifetime. Short-term exposure represents the highest exposure an individual may realistically experience while performing a label-permitted activity, and is assessed using the "upper-bound" estimate of exposure, such as the estimated 95th percentile of daily exposure or the maximum of monitored values (Frank, 2009). In addition, to assess short-term exposures for re-entry workers, it was also assumed the workers enter the fumigated areas immediately after the REI (5 days) expires. For assessing seasonal, annual and lifetime exposures, this assessment used the arithmetic mean instead of 95th percentile exposure value, as continuous daily exposure at the upper-bound level is unlikely.

Due to the lack of both air monitoring data and AITC use information in California, this analysis only assessed short-term exposures for both occupational and residential bystanders.

B. Occupational handler exposure

This assessment identified no registrant submitted studies that monitored handler exposure to AITC during soil applications, nor any data from open literature related handler exposure to AITC from soil fumigation. Therefore, studies that monitored handler exposure to other soil fumigants were used as surrogate data. The potential uncertainties associated with using surrogate data for AITC handler exposure assessment will be discussed in the appraisal section.

Applicator, shallow shank w/ tarp. Applicator exposure for this scenario was assessed based on two studies on Pic which monitored the applicator exposures during 11 shank applications (Beard *et al.*, 1996; Rotondaro, 2004). Both studies have been reviewed by DPR and determined to be of acceptable study quality for use in exposure assessment (Beauvais, 2005; Beauvais, 2010). Information on these applications is summarized in Table 6. In both studies, Pic exposure of each applicator was monitored by placing a XAD-4 tubes close to the collar area (i.e., breathing zone) and drawing air through the tube using an air pump at the flow rate of 50 mL/min. Monitored workers included 1) tractor drivers who loaded and connected Pic cylinders before application, operated the application tractors, and disconnected and removed cylinders when applications were complete, 2) co-pilots who worked closely with tractor drivers and assisted Pic application and tarp-laying, and 3) tarpers who drove tarp laying tractor following the Pic application tractor.

Table 6. Summary of application events used for applicator exposure assessments. These applications used shank applications with polyethylene tarp

Application[a]	Application rate (lb/ac)	Occupation[b]
Broadcast	343	Driver, co-pilot
Broadcast	360	Driver, co-pilot
Broadcast	351	Driver, co-pilot
Broadcast	346	Driver, co-pilot
Broadcast	345	Driver, co-pilot
Broadcast	334	Driver, co-pilot
Broadcast	332	Driver, co-pilot
Bed	189	Driver, co-pilot, tarper
Bed	116	Driver, co-pilot
Bed	210	Driver, co-pilot
Bed	45	Driver, co-pilot

a: information was summarized from (Beard *et al.*, 1996; Rotondaro, 2004). Each row represents one specific application event monitored. The application rate is pound active ingredient per broadcast acre.
b: occupations of the workers monitored for exposure.

These applications were conducted in four different states (CA, WA, AZ and FL). Among these 11 applications, seven used broadcast shank applications and the rest used bed shank applications. After normalized with the same 340 lb/gross ac application rate, the monitored air concentrations showed variability (up to 55 fold) among 45 different applicators (Table 7). Statistical analysis was conducted to calculate the air concentration estimates (i.e., average and 95th percentile values) needed for assessing the exposures of different time periods (Table 7). To assess seasonal, annual, and lifetime exposures, this analysis used 1,3-D use data from 2014-2018 as a surrogate to estimate AITC use patterns (i.e., seasonal application rate and number of applications per year). The estimated AITC exposures for applicators using shallow shank w/ tarp are summarized in Table 8.

Table 7. Summary statistics of chloropicrin air concentrations (µg/m^3) measured from applicators using broadcast and bed shank applications with tarp

Application[a]	*N*[b]	Average	Std. Dev.[c]	95th %ile[d]	Range
Broadcast & Bed	45	467	348	1759	25-1383
Broadcast	32	554	341	1352	99-1383
Bed	13	253	263	994	25-856

a: Information was summarized from Beard *et al.* (1996); Rotondaro (2004). Chloropicrin was applied through shallow shank and the treated field was covered with polyethylene tarp. Air concentrations were normalized to the same application rate of 340 lbs/ac;
b: Number of applicator replicates;
c: Standard deviation;
d: 95th percentile value was calculated based on the method from Frank (2009).

Table 8. Estimated applicator exposure to allyl isothiocyanate using shallow shank applications with tarp

	STADD[a]	SADD[b]	AADD[c]	LADD[d]
Exposure (µg/kg/d)	31	8	2	1

a: short-term absorbed daily dose (STADD) = air concentration (1759 µg/m^3, normalized to 340 lbs/ac application rate) × protection factor (0.1) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
b: seasonal average daily dose (SADD) = air concentration (467 µg/m^3, normalized to 340 lbs/ac application rate) × protection factor (0.1) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
c: annual average daily dose (AADD) = SADD × 78 d/yr ÷ 365 d/yr. See Table 5 for more details;
d: life-time average daily dose (LADD) = AADD × 40 yrs ÷ 75 yrs.

Applicator, shallow shank w/o tarp

Applicator exposure for this scenario was assessed based on three studies with 11 fumigant applications and a total of 34 applicators monitored (Houtman, 1993; Beard *et al.*, 1996; Rotondaro, 2004). Detailed application information of these studies are summarized in Table 9 below. As mentioned above, two of these studies monitored Pic exposures, and the other study monitored 1,3-D exposures (Beard *et al.*, 1996; Beauvais, 2005; Beauvais, 2010). Data from this 1,3-D study has been used in the previous 1,3-D risk assessment (DPR, 2015a).

Table 9. Information for applications used to assess applicator exposure using shank applications without tarp

A.I.[a]	Bed or Broadcast	Application rate (lbs/ac)	Occupation[b]
Pic	Broadcast	171	Driver, soil sealer
	Bed	86	Applicator

A.I.[a]	Bed or Broadcast	Application rate (lbs/ac)	Occupation[b]
Pic	Broadcast	191	Driver, soil sealer
	Broadcast	169	Driver, soil sealer
	Broadcast	174	Driver, soil sealer
	Bed	86	Applicator
	Bed	148	Applicator
	Bed	72	Applicator
1,3-D	Broadcast	235	Applicator
	Broadcast	161	Applicator
	Bed	113	Applicator

Information was summarized from Houtman (1993); Beard *et al.* (1996); Rotondaro (2004). The application rate is pound active ingredient per broadcast acre.
a: A.I.= active ingredient, Pic=chloropicrin, 1,3-D=1,3-dichloropropene;
b: occupations of the workers monitored for exposure.

This assessment considered both broadcast and bed shank applications to include more monitored handlers. Statistical analysis based on available data showed the applicator exposures were similar between bed and broadcast shank applications (Table 10). As the estimated 95th percentile air concentration is smaller than the maximum value monitored, the maximum value (18328 $\mu g/m^3$) was used to calculate STADD according to standard DPR process (Andrews and Patterson, 2000; Frank, 2009). The estimated applicator exposures are summarized in Table 11.

Table 10. Summary statistics of air concentrations ($\mu g/m^3$) measured from applicator breathing zones using broadcast and bed shank applications

Application	A.I.[a]	*N*[b]	Average	Std. Dev.[c]	95th %ile[d]	Range
Broadcast & Bed	1,3-D & Pic	32	1712	3462	6162	27-18328
	1,3-D	15	3238	4572	12568	124-18328
	Pic	17	366	494	1095	27-2143
Broadcast	1,3-D & Pic	22	855	1318	3024	27-5294

Application	A.I.[a]	*N*[b]	Average	Std. Dev.[c]	95th %ile[d]	Range
	1,3-D	10	1676	1605	5072	300-5294
	Pic	12	170	100	438	27-379
Bed	1,3-D & Pic	10	3599	5418	15883	124-18328
	1,3-D	5	6363	6552	42986	124-18328
	Pic	5	834	704	2579	138-2143

Information was summarized from Houtman (1993); Beard *et al.* (1996); Rotondaro (2004). Chloropicrin or 1,3-dichloropropene was applied through shallow shank without tarp use. Air concentrations were normalized to the same application rate of 340 lbs/ac.
a: A.I.=active ingredient, 1,3-D=1,3-dichloropropene, Pic=chloropicrin;
b: Number of applicator replicates;
c: Standard deviation;
d: 95th percentile value was calculated based on the method from (Frank, 2009). The maximum air concentration value (18328 µg/m^3) will be used to calculate short-term absorbed daily dose following the guideline (Frank, 2009).

Table 11. Estimated applicator exposure to allyl isothiocyanate using shallow shank applications without tarp

	STADD[a]	SADD[b]	AADD[c]	LADD[d]
Exposure (µg/kg/d)	327	31	7	3

a: short-term absorbed daily dose (STADD) = air concentration (18328 µg/m^3, normalized to 340 lbs/ac application rate) × protection factor (0.1) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
b: seasonal average daily dose (SADD) = air concentration (1712 µg/m^3, normalized to 340 lbs/ac application rate) × protection factor (0.1) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
c: annual average daily dose (AADD) = SADD × 78 d/yr ÷ 365 d/yr. See Table 5 for more details;
d: life-time average daily dose (LADD) = AADD × 40 yrs ÷ 75 yrs.

Applicator, deep shank w/ and w/o tarp

CalPIP data indicates deep shank applications accounted for the greatest portion of soil fumigant use in California especially in the Central Valley areas (Fig. 2). Because there is no study that monitored applicator exposures during deep shank applications, applicator exposures for deep shank applications w/ and w/o tarp were assessed using monitoring data from shallow shank applications as surrogate. To assess seasonal, annual and life-time exposures, this surrogate data was also used together with deep shank-specific use information, i.e., seasonal application rate and number of applications per year, as summarized in Table 5. The estimated applicator exposures for deep shank applications w/ and w/o tarp are summarized in Table 12.

Table 12. Estimated applicator exposure to allyl isothiocyanate using deep shank applications with and without tarp

Exposure (µg/kg/d)	STADD[a]	SADD[b]	AADD[c]	LADD[d]
w/ tarp	31	8	2	1
w/o tarp	327	29	8	5

a: data from shallow shank application were used as surrogate;
b: seasonal average daily dose (SADD) = air concentration (451 and 1652 µg/m^3 for w/ and w/o tarp respectively, normalized to 328 lbs/ac application rate) × protection factor (0.1) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
c: annual average daily dose (AADD) = SADD × 105 d/yr ÷ 365 d/yr. See Table 5 for more details;
d: life-time average daily dose (LADD) = AADD × 40 yrs ÷ 75 yrs.

Applicator, drip application w/ and w/o tarp

Applicator exposures for drip applications was assessed using a study that monitored 12 applicator exposure to Pic in 6 applications (Table 13) (Rotondaro, 2004). Due to the small number of applicators monitored and because the applicator exposures were comparable between tarped and non-tarped applications based on the available data, this assessment used monitoring data from both tarp types (Table 14). The exposure estimates are summarized in Table 15.

Table 13. Information for studies used to assess applicator exposure using drip applications

Tarp[a]	Application rate (lbs/ac)	Occupation[b]
PE	156	Applicator
PE	148	Applicator
PE	164	Applicator
No	120	Applicator
No	112	Applicator
No	158	Applicator

Information was summarized from Rotondaro (2004). The application rate is pound active ingredient per broadcast acre.
a: tarp material, PE=polyethylene;
b: occupations of the workers monitored for exposure.

Table 14. Statistics of air concentrations (µg/m^3) measured from applicator breathing zones using drip applications

Tarp[a]	*N*[b]	Average	Std. Dev.[c]	95th %ile[d]	Range
PE & no-tarp	12	146	157	438	21-525
PE	6	105	48	226	41-176
No-tarp	6	188	209	686	21-525

Information was summarized from Rotondaro (2004). Air concentrations were normalized to the same application rate of 246 lbs/ac.
a: tarp material, PE=polyethylene;
b: number of applicator replicates;
c: standard deviation;
d: 95th percentile value was calculated based on the method described elsewhere (Frank, 2009). The maximum air concentration value (525 µg/m^3) will be used to calculate short-term absorbed daily dose following the guideline (Frank, 2009).

Table 15. Estimated applicator exposure to allyl isothiocyanate using drip applications

	STADD[a]	SADD[b]	AADD[c]	LADD[d]
Exposure (µg/kg/d)	9	3	0.3	0.2

a: short-term absorbed daily dose (STADD) = air concentration (525 µg/m^3, normalized to 246 lbs/ac application rate) × protection factor (0.1) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
b: seasonal average daily dose (SADD) = air concentration (146 µg/m^3, normalized to 246 lbs/ac application rate) × protection factor (0.1) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
c: Annual average daily dose (AADD) = SADD × 46 d/yr ÷ 365 d/yr. See Table 5 for more details;
d: life-time average daily dose (LADD) = ADD × 40 yrs ÷ 75 yrs.

Loader

Loader exposure was assessed based on a monitoring study of 1,3-D during shallow shank applications without tarp (Houtman, 1993). The breathing zone concentrations were monitored during three different application conditions: 1) no mitigation used, 2) using dry disconnects, and 3) using both dry disconnects and vapor recovery. This study described "*dry disconnects*" as a technique utilized "*during detachment of the product loading line from the applicator rig following completion of product loading*". In the same study, "*vapor recovery*" was described as "*a vapor return line was installed between the applicator tank and the nurse truck… to exchange the product leaving the nurse tank with an equal volume of displaced vapor from the applicator tank during the loading process.*" This analysis used data from the first condition (no mitigation), as neither dry disconnects nor vapor recovery is required in the submitted product labels. Detailed information of the selected applications is summarized in Table 16. Due to the lack of monitoring data, loader exposures for deep shank applications was estimated using the air concentration from shallow shank applications as surrogate combined with deep shank-specific use information (Table 5). The calculated loader exposures for both shallow and deep shank applications are summarized in Table 17.

Table 16. Information for studies used to assess loader exposure using shank applications

Bed or Broadcast	Tarp	Application rate (lbs/ac)
Broadcast	No	161
Bed	No	113

Information was summarized from elsewhere Houtman (1993). The application rate is pound active ingredient per broadcast acre. 1,3-Dichloropropene exposures were monitored in this study.

Table 17. Estimated loader exposure to allyl isothiocyanate using shallow and deep shank applications

Exposure (µg/kg/d)	STADD[a]	SADD[b]	AADD[c]	LADD[d]
Shallow	1373	264	56	30
Deep	1373	255	73	39

Information was summarized from elsewhere by Houtman (1993).
a: short-term absorbed daily dose (STADD) = air concentration (77021 µg/m^3, normalized to 340 lbs/ac application rates) × protection factor (0.1) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
b: seasonal average daily dose (SADD) = air concentration (14828 µg/m^3 (normalized to 340 lbs/ac for shallow shank) or 14305 µg/m^3 (normalized to 328 lbs/ac for deep shank) × protection factor (0.1) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
c: annual average daily dose (AADD) = SADD × 78 and 105 d/yr for shallow and deep shank respectively ÷ 365 d/yr. See Table 5 for more details;
d: life-time average daily dose (LADD) = AADD × 40 yrs ÷ 75 yrs.

Tarp cutter, remover and puncher

This scenario includes AITC handlers that cut and remove tarps (for shank applications) or perforate on tarps (for drip applications). According to the submitted product labels, respiratory protections are not required for tarp cutters, removers and punchers, and the REI is 5 days.

There is no AITC-specific study that monitored tarp cutter/remover/puncher exposures; hence, the exposures for this scenario were assessed based on a study that monitored Pic exposures (Beard *et al.*, 1996). Information of Pic applications in this study are detailed in Table 6. Pic was applied via shallow shank applications with polyethylene (PE) tarps. Among these applications, the shortest time interval between applications and worker re-entry was 6 days. Therefore, monitoring data from those 6th-day re-entry workers were used to assess AITC exposures for this scenario. Tarp cutter, remover and puncher exposures for deep shank and drip applications were also assessed using the same set of data combined with deep shank- or drip-specific use information as summarized in Table 5. The estimated exposure values for shallow shank, deep shank and drip applications are summarized in Table 18.

Table 18. Estimated allyl isothiocyanate exposure of tarp cutter, remover and puncher

Exposure (µg/kg/d)	STADD[a]	SADD[b]	AADD[c]	LADD[d]
Shallow shank	734	106	23	12
Deep shank	734	103	29	16
Drip	531	77	10	5

Information was summarized from elsewhere by Beard et al. (1996).
a: short-term absorbed daily dose (STADD) = air concentration (4117 µg/m^3 (for both shallow and deep shank applications, normalized to 340 lbs/ac application rate) or 2979 µg/m^3 (for drip application, normalized to 246 lbs/ac application rate) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
b: seasonal average daily dose (SADD) = air concentration (596 µg/m^3 (for shallow shank application, normalized to 340 lbs/ac), 575 µg/m^3 (for deep shank application, normalized to 328 lbs/ac) or 431 µg/m^3 (for drip application, normalized to 246 lbs/ac)) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
c: annual average daily dose (AADD) = SADD × 78 (for shallow shank), 105 (for deep shank) or 46 (for drip) d/yr ÷ 365 d/yr. See Table 5 for more details;
d: life-time average daily dose (LADD) = AADD × 40 yrs ÷ 75 yrs.

C. Occupational re-entry worker

There is no AITC-specific study that monitored re-entry worker exposures. Hence, this assessment used data from 1,3-D and Pic as surrogates for estimating the exposure. The selected data is from workers entering the treated field 4 days after fumigation, which represents the best available data with the time interval closest to AITC's REI at 5 days (Houtman, 1993). The field was applied with 1,3-D using broadcast shank equipment and the workers were performing winterization activities. As monitoring data is not available for deep shank and drip applications, 1,3-D data from shallow shank applications was used as surrogate. To assess seasonal, annual and life-time exposures, the deep shank- or drip-specific use information, i.e., seasonal application rates and number of applications per year, was also used (Table 5). The estimated AITC exposures for different application types are summarized in Table 19.

Table 19. Estimated allyl isothiocyanate exposures for re-entry workers

Exposure (µg/kg/d)	STADD[a]	SADD[b]	AADD[c]	LADD[d]
Shallow shank	31	27	7	4
Deep shank	31	26	11	6
Drip	22	19	3	1

Information was summarized from elsewhere by Houtman (1993).
a: short-term absorbed daily dose (STADD) = air concentration (173 µg/m^3 (for both shallow and deep shank applications, normalized to 340 lbs/ac application rate), or 125 µg/m^3 (for drip application, normalized to 340 lbs/ac application rate)) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
b: seasonal average daily dose (SADD) = air concentration (150 µg/m^3 (for shallow shank application, normalized to 340 lbs/ac), 145 µg/m^3 (for deep shank application, normalized to 328 lbs/ac), or 109 µg/m^3 (for drip application, normalized to 246 lbs/ac)) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg (Andrews and Patterson, 2000);
c: annual average daily dose (AADD) = SADD × 100 (for shallow shank), 157 (for deep shank) or 47 (for drip) d/yr ÷ 365 d/yr. See Table 5 for more details;
d: life-time average daily dose (LADD) = AADD × 40 yrs ÷ 75 yrs.

D. Occupational and residential bystander

This assessment identified no registrant-submitted study that monitored bystander exposures to AITC. Thus, the exposure assessment for both occupational and residential bystanders used computer software to simulate the air concentrations. The software used in this simulation is AERMOD View™ version 9.6.5, and the modeling engine integrated in this software is AERMOD (version 18081) developed by American Meteorological Society and U.S. EPA (Lakes Environmental, 2019). In addition to meteorological data, the simulation requires soil emission rates as model inputs. For shallow shank and drip applications with the use of tarp, AITC-specific emission data was used to generate the needed emission rates, and for application scenarios with no-AITC specific emission data, the emission rates were derived from the most appropriate surrogate (1,3-D or Pic). Detailed descriptions on preparing soil emission profiles for different application types and tarp methods, and simulating breathing zone air concentrations using the prepared emission profiles, have been detailed in two memorandums by (Jiang, 2019b; Jiang, 2019a). With the simulated air concentrations, STADD values were calculated for both residential (adult and child) and occupational (adult only) bystanders and are summarized in Table 21-23.

Table 20. Model estimated allyl isothiocyanate exposures for occupational bystanders

STADD[a] (µg/kg/d)	1 ac[b]	40 ac	100 ac
Shallow shank w/ tarp	62	185	230
Shallow shank w/o tarp	599	1779	2210
Deep shank w/o tarp	392	1164	1446
Drip w/ tarp	328	1039	1296
Deep drip w/o tarp	768	2431	3032

a: STADD = short-term absorbed daily dose. Exposures were assessed using air concentrations at the treated field edge. 8-hr time-weighted average emission rates were used and normalized to the maximum application rates as described on the submitted product labels; STADD = air concentration (µg/m^3) × inhalation rate (1.6 m^3/hr) × 8 hr/d ÷ 71.8 kg.
b: size of the treated field.

Table 21. Model estimated allyl isothiocyanate exposures for residential adult bystanders

STADD[a] (µg/kg/d)	1 ac[b]	40 ac	100 ac
25ft[c]			
Shallow shank w/ tarp	36	112	141
Shallow shank w/o tarp	351	1105	1392
Deep shank w/o tarp	227	715	900

STADD[a] (µg/kg/d)	1 ac[b]	40 ac	100 ac
Drip w/ tarp	226	712	896
Deep drip w/o tarp	404	1272	1603
100ft			
Shallow shank w/ tarp	23	92	118
Shallow shank w/o tarp	226	903	1167
Deep shank w/o tarp	146	584	755
Drip w/ tarp	145	582	751
Deep drip w/o tarp	260	1040	1343

a: STADD = short-term absorbed daily dose; 24-hr time-weighted average emission rates were used and normalized to the maximum application rates as described on the submitted product labels; STADD = air concentration at 5 ft above ground (µg/m^3) × inhalation rate (0.28 m^3/kg/d);
b: size of treated fields;
c: distance from the treated field edge, based on the 25 ft buffer zone as specified on the product labels and 100 ft as described in the Isagro applicator training materials (Isagro, 2015).

Table 22. Model estimated allyl isothiocyanate exposures for residential child bystanders

STADD[a] (µg/kg/d)	1 ac[b]	40 ac	100 ac
25ft[c]			
Shallow shank w/ tarp	109	272	333
Shallow shank w/o tarp	1073	2681	3289
Deep shank w/o tarp	694	1734	2127
Drip w/ tarp	691	1727	2118
Deep drip w/o tarp	1235	3087	3786
100ft			
Shallow shank w/ tarp	54	203	258
Shallow shank w/o tarp	537	2003	2542
Deep shank w/o tarp	347	1296	1644
Drip w/ tarp	346	1290	1637
Deep drip w/o tarp	618	2306	2926

a: STADD = short-term absorbed daily dose; 24-hr time-weighted average emission rates were used and normalized to the maximum application rates as described on the submitted product labels; STADD = air concentration at 1.7 ft above ground (µg/m^3) × inhalation rate (0.59 m^3/kg/d);
b: size of treated fields;
c: distance from the treated field edge, based on the 25 ft buffer zone as specified on the product labels and 100 ft as described in the Isagro applicator training materials (Isagro, 2015).

VI. EXPOSURE APPRAISAL

This section evaluates uncertainties associated with the exposure assessment process. This analysis attempted to use AITC-specific information to assess the exposures, but the needed information in some instances was not be available. Hence, this section discusses the data gaps identified and their impact on the exposure assessment.

A. Occupational handler exposure

Applicator. Applicator exposures to AITC were assessed for three application methods (shallow shank, deep shank and drip) combined with 2 tarp conditions (tarp and non-tarp), and the assessment used human exposure monitoring data, i.e., data from collecting and analyzing air samples from worker breathing zones. Due to the lack of AITC-specific exposure data, applicator exposures were assessed using surrogate data from 1,3-D and Pic (Houtman, 1993; Beard *et al.*, 1996; Rotondaro, 2004). This is because the physiochemical properties of AITC that determine its movements in the soil environment, such as boiling point, water solubility and lipophilicity, are similar to 1,3-D and Pic (Table 1). Although AITC is structurally similar to MITC, applicator exposure data from MITC was not used because of the following reasons. First, unlike AITC, MITC is not a directly applied pesticide but produced in soils after the applications of other active ingredients such as MITC-Na and MITC-K. Second, the application techniques of MITC-Na and MITC-K, such as using rotary tillers and sprinklers, are different from shank and drip methods for AITC (Meyers, 1992; Meyers, 1993). Between AITC and MITC, only two of the nine MITC application methods (i.e., drip and shank) are allowable for AITC, suggesting that emission profiles and the associated pattern of human exposure to these two fumigants are rather different (DPR, 2015b). There was no MITC exposure monitoring data available for deep shank and drip applications. By contrast, almost all application methods for 1,3-D and Pic are also allowable for AITC. In addition, 1,3-D and chloropicrin exposure monitoring data is available for applicator, loader, tarp remover and re-entry workers. There was one study that monitored applicator exposures to MITC during shank applications w/o tarp (Meyers, 1992). While the estimated STADD of 130 µg/kg/d (n = 10) from this MITC monitoring study is in the same order of magnitude of the STADD value (327 µg/kg/d, n = 32) determined in this analysis using 1,3-D and Pic data, no MITC use data are currently available for estimating the intermediate- and long-term exposures for shank applications w/o tarp. Therefore, to ensure an internal consistency in data quality and coverage, 1,3-D and Pic data (instead of MITC data) was used for all application scenarios in this assessment.

For exposure scenarios without monitoring data, this assessment adopted the approach of using surrogate data to fill in data gaps that appeared in the 1,3-D exposure assessment document (DPR, 2015a). In that document, 1,3-D exposure data were only available for the applicator scenario using shallow shank w/o tarp. For estimating exposures for other scenarios, such as

applicator using shallow shank w/ tarp, Pic exposure data was used as surrogate. To account for the differences in the physiochemical properties between 1,3-D and Pic, an exposure adjustment ratio was calculated by dividing 1,3-D exposure from shallow shank w/o tarp applicators to Pic exposure of the same scenario. The resulting adjustment ratio was applied for scenarios when Pic data was used (e.g., applicator exposure using shallow shank w/ tarp). An example of using this exposure adjustment ratio is demonstrated using the equation below:

$$\text{Applicator, shallow shank w/ tarp (1,3-}D\text{)} = \text{Applicator, shallow shank w/ tarp (Pic)} \times \frac{\text{Applicator, shallow shank w/o tarp (1,3-}D\text{)}}{\text{Applicator, shallow shank w/o tarp (Pic)}}$$

However, this adjustment method used in the 1,3-D exposure assessment document was not used in this exposure assessment because there is no AITC exposure monitoring data were available to derive the adjustment ratio. There was one study that measured AITC emissions from four different application and tarp methods, however this study cannot be used to estimate handler exposures as human AITC exposures were not monitored (Ajwa *et al.*, 2014). Instead, exposure data from surrogate fumigants (1,3-D and Pic) was only corrected for appropriate application rates assuming at the same application rate, application method, and handler exposures are similar to AITC. Accordingly, factors that may contribute to different handler exposures between AITC and 1,3-D/Pic, such as different soil emission rates at the time of applications, were not considered. Table 23 compares the emission rates for the application periods between AITC and 1,3-D or Pic. The comparisons were done for four application types with available AITC emission data, i.e., shallow shank w/ totally impermeable film (TIF) tarp, shallow shank w/ PE tarp, drip w/ TIF tarp and drip with PE tarp. Table 24 also compares AITC air concentrations measured at adult breathing heights with those of 1,3-D or Pic concentrations in available studies. AITC data is available for two application types, i.e. drip w/ TIF and drip w/ PE tarp. Both tables indicate that, at the time of applications, AITC emission rates and air concentrations are comparable to 1,3-D and Pic. This further demonstrates that using 1,3-D and Pic data to assess applicator exposures to AITC is appropriate and represents the best available information available during the development of this exposure assessment.

Table 23. Comparison of fumigant emission rates during the time of applications

Fumigant[a]	Application duration (hr)	Sampling duration (hr)	Emission[c] (µg/m^2/s)
Broadcast shallow shank w/ TIF[b] tarp			
AITC	4.0	6	9.0
Pic	4.3	7	1.9
1,3-D	4.3	7	1.8
Pic	1.2	7	0.1
1,3-D	1.2	7	0.2
Pic	1.6	7	0.4
1,3-D	1.6	7	0.4

Fumigant[a]	Application duration (hr)	Sampling duration (hr)	Emission[c] (µg/m^2/s)
Pic	1.2	5	29.8
1,3-D	1.3	6	2.5
Pic	1.3	6	11.6
Broadcast shallow shank w/ PE tarp			
AITC	5.1	5	0.7
Pic	2.4	6	12.5
1,3-D	2.4	6	17.9
Pic	0.6	7	28.9
1,3-D	0.6	7	59.2
Pic	1.0	4	6.5
1,3-D	1.0	4	15.9
Pic	0.6	6	5.4
Pic	1.1	5	31.3
Shallow drip-TIF tarp			
AITC	1.8	6	7.7
Pic	3.0	6	5.7
1,3-D[d]	2.6	6	12.5
Pic[d]	2.6	6	19.5
1,3-D	2.5	6	5.3
Shallow drip-PE tarp			
AITC	3.9	4	54.9
Pic	1.0	4	23.0
Pic	3.0	6	86.8
Pic	4.7	5	48.8
1,3-D	4.7	5	73.6

a: AITC=allyl isothiocyanate, Pic=chloropicrin, 1,3-D=1,3-dichloropropene. Data was obtained from various sources (Knuteson and Dolder, 2000; van Wesenbeeck and Phillips, 2000; Rotondaro, 2004; Ajwa, 2008; Ajwa, 2009; Ajwa, 2010; Ajwa and Sullivan, 2010; Sullivan and Chellemi, 2010; Sullivan, 2012; Ajwa *et al.*, 2014; Ajwa, 2015);

b: TIF=totally impermeable film, PE=polyethylene;

c: the emission rates were normalized to 340 and 246 lbs/ac for shank and drip application respectively;

d: virtually impermeable tarp was used.

Table 24. Comparison of fumigant air concentrations measured near the breathing heights of applicators during the time of applications

Fumigant[a]	Application duration (hr)	Sampling duration (hr)	Concentration[b] (µg/m^3)
Drip w/ TIF tarp[c]			
AITC	1.8	6	28.7
Pic[d]	3.0	6	59.7
1,3-D[e]	2.6	6	97.4
Pic[e]	2.6	6	49.4
Drip w/ PE tarp			
AITC	3.9	4	317.9
Pic[d]	3.0	6	254.5
Pic	4.7	5	687.2
1,3-D	4.7	5	996.4

a: AITC=allyl isothiocyanate, Pic=chloropicrin, 1,3-D=1,3-dichloropropene. Data was obtained from various sources (Knuteson and Dolder, 2000; van Wesenbeeck and Phillips, 2000; Ajwa, 2010; Ajwa *et al.*, 2014);
b: TIF=totally impermeable film, PE=polyethylene;
c: the air concentrations were normalized to 246 lbs/ac;
d: the air concentration values may not be accurate as the actual sampling rates were not provided. Instead the target rate of 1000 mL/min was used for calculation;
e: virtually impermeable tarp was used.

Loader. Available MITC data did not support loader exposure assessment to AITC, as monitored loaders were either from different application types (sprinkler applications), or only monitored for very short periods of time (4-17 min) during their workdays (Meyers, 1992). Loader exposures were assessed using monitoring data from 1,3-D as surrogate (Houtman, 1993). In this study, 1,3-D breathing zone air concentrations were measured from loaders for their "*work period (or daily) exposures*" not just "*the period directly involved in product handling.*" In actual practice, applicators may also assist loading, connecting and unloading fumigant cylinders onto tractors, but they are not required to do so and they are not always around fumigant cylinders. This assessment considered loader exposures as a separate scenario assuming that they might experience great fumigant exposures, especially during their handling of fumigant cylinders. This assumption is consistent with the results in this assessment that loaders experienced higher AITC exposures than applicators and the periods of handling and loading cylinders accounted for a great portion of the loader exposures (median: 69%, *N*=15) during their work days (Houtman, 1993).

Tarp cutter/remover/puncher. There was no MITC available to support tarp remover exposure assessments. Exposures of this scenario were based on data that monitored tarp cutter exposures to Pic on the 6th day after applications (Beauvais, 2010). As AITC has a REI of 5 days, values in Table 18 may underestimate the exposures for this scenario as entry of workers at shorter post-application intervals is expected to result in greater exposures. Nevertheless, the use of Pic monitoring data on the 6th day is because of the following reasons. First, the Pic data represent the best information available with the post-application entry interval closest to AITC's REI. Second, emission rates of AITC on the day of tarp-cutting and those the following day are comparable (0.25 *vs* 0.20 $\mu g/m^2/s$ for broadcast shallow shank w/ TIF tarp, and 6.0 *vs* 4.0 $\mu g/m^2/s$ for broadcast shallow shank w/ PE tarp, 12-hr TWA) (Ajwa *et al.*, 2014). This assessment also compared Pic exposures between tarp cutters and tarp removers who entered the treated field one day after the tarp cutting; their exposures were also similar (Table 25) (Beard *et al.*, 1996; Rotondaro, 2004). Therefore, we conclude that this one-day difference will not cause significant underestimation of tarp cutter exposures.

Table 25. Statistics of chloropicrin air concentrations ($\mu g/m^3$) measured from tarp cutters and tarp removers from fields with broadcast shallow shank applications with tarp

Occupation[a]	Day[b]	*N*[c]	Average	Std. Dev.[d]	95th %ile[e]
Tarp cutter	6	14	596	1035	1689
Tarp remover	7	27	640	747	2404

a: Information was summarized from Beard *et al.* (1996); Rotondaro (2004). The application rate was normalized to the same 340 lbs/ac;
b: Number of days after application;
c: Number of applicator replicates;
d: Standard deviation;
e: 95th percentile value was calculated based on the method described elsewhere (Frank, 2009).

B. Occupational and residential bystanders

This analysis assessed occupational and residential bystander exposures for five application and tarp types, i.e., shallow shank w/tarp, shallow shank w/o tarp, deep shank w/o tarp, drip w/ tarp and buried drip w/o tarp. Among these five application types, the bystander exposures from three application types were assessed previously in the 1,3-D exposure assessment (DPR, 2015a). Bystander exposures from shallow shank w/ tarp application were assessed because AITC-specific soil emission data is available for this application method and then used in this document. Lastly, but not the least, available data on buried drip w/o tarp indicates that this application type may cause greater bystander exposures than drip applications w/ tarp (Jiang, 2019b). Of the five assessed application types mentioned above, two (shallow shank w/ tarp, and drip w/ tarp) used AITC-specific emission data (Ajwa *et al.*, 2014). For the remaining three application types, there was no MITC emission data available.

As there was only one set of AITC emission data for each of the two application types, the variability of emission rates caused by different field conditions (soil type, weather, application equipment, etc.) is unknown. This implies that there is a possibility of underestimating bystander exposure. As shown in Table 27, the emission rates of AITC used in this assessment are within the range of 1,3-D and Pic emission rates, but it may not represent field conditions with high fumigant emission potential. This assessment used the AITC emission data as they represent chemical-specific information. However, a few measures were employed to minimize the likelihood of underestimating bystander exposures including conducting air dispersion modeling using 5 year meteorological data in six different regions within California. Only the highest estimated air concentrations from these modeling were used for bystander exposure assessment in order to represent the reasonable worst-case exposure scenario.

Table 26. Maximum emissions of 1,3-dichloropropene (1,3-D), chloropicrin (Pic) and allyl isothiocyanate (AITC) for different time-weighted average (TWA) periods

Fumigant[a]	Maximum TWA emission ($\mu g/m^2/s$)		
	4 hr	8 hr	24 hr
Broadcast shank with polyethylene (PE) tarp			
AITC	12.6	8.4	17.8
1,3-D	106.0	66.1	129.8
Pic	14.2	8.7	17.4
1,3-D	110.3	83.9	135.1
Pic	68.1	46.4	83.4
1,3-D	114.3	86.1	140.0
Pic	71.0	48.8	86.9
Pic	23.1	11.4	28.3
Pic	56.8	29.0	69.6
Pic	71.3	41.8	87.4
Pic	55.8	30.3	63.9
Drip with PE tarp			
AITC	99.5	79.9	53.5
Pic	116.0	116.0	36.8
Pic	117.1	95.6	55.5
Pic	31.9	26.0	16.1

Fumigant[a]	Maximum TWA emission ($\mu g/m^2/s$)		
	4 hr	8 hr	24 hr
Pic	76.0	62.0	34.7
Pic	54.6	48.8	18.0

a: emission data was collected from various sources and normalized to the maximum application rates as specified on the product labels (Beard *et al.*, 1996; van Wesenbeeck and Phillips, 2000; Rotondaro, 2004; Ajwa, 2008; Ajwa, 2009; Ajwa, 2010; Ajwa and Sullivan, 2010; Sullivan and Chellemi, 2010; Ajwa *et al.*, 2014).

VII. CONCLUSION

This analysis assessed AITC exposures for occupational handlers, re-entry workers, occupational bystanders and residential bystanders. Based on the two submitted product labels from Isagro (Dominus and Dominus 100), the primary route of AITC exposures is through inhalation. Due to the lack of AITC use information and exposure monitoring data as well as limited information on AITC soil emission rates, other soil fumigants (1,3-D, Pic, MeBr, MITC-Na and MITC-K) were used as surrogate to collect data for this assessment. A total of 88 exposure scenarios were assessed, and AITC inhalation exposures were estimated for four different exposure periods (short-term, seasonal, annual and life-time). These exposure values are calculated for the development of Risk Characterization Document of AITC.

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APPENDIX 1.

Using allyl isothiocyanate-specific and surrogate data to determine AITC soil emissions for residential and occupational bystander exposure assessments

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MEMORANDUM

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SUBJECT: USING ALLYL ISOTHIOCYANATE-SPECIFIC AND SURROGATE DATA TO DETERMINE AITC SOIL EMISSIONS FOR RESIDENTIAL AND OCCUPATIONAL BYSTANDER EXPOSURE ASSESSMENTS

Executive summary:

This memorandum is prepared in response to a request for registering two products containing allyl isothiocyanate (AITC) for use in California as a soil fumigant. According to the submitted product labels, AITC may be applied through shallow shank injection, deep shank injection, or drip chemigation, and the treated fields can be covered with or without tarp. Currently AITC soil emission data is only available for shallow shank w/ tarp and drip w/ tarp. Therefore, this analysis estimated AITC emissions for other application scenarios using 1,3-dichloropropene (1,3-D) and chloropicrin (Pic) data as surrogates due to similar physiochemical properties, application methodologies and emission profiles, as well as availability of data for application scenarios without AITC-specific data. The calculated maximum AITC soil emissions for three different averaging periods (4, 8, or 24 hr) are summarized in Table E1. These emission values will be further used to estimate short-term residential and occupational bystander exposure from AITC applications.

Table E1. Time-weighted average (TWA) maximum allyl isothiocyanate (AITC) emissions in different application and tarp scenarios.

Application[a]	Source of data[b]	Maximum TWA emission[c] ($\mu g/m^2/s$)		
		4 hr	8 hr	24 hr
Shallow shank w/ tarp	AITC	17.8	12.6	8.4
Shallow shank w/o tarp	1,3-D	128.2	121.1	82.9
Deep shank w/o tarp	Pic	97.0	79.2	53.6
Drip w/ tarp	AITC	99.3	79.7	53.4
Deep drip w/o tarp	1,3-D	208.7	186.6	95.4

a: shank injection < 17 in is considered as shallow; Drip tape buried >1 inch is considered as deep; b: source of emission data, AITC, Pic and 1,3-D respectively represent allyl isothiocyanate, chloropicrin and 1,3-dichloropropene; c: the emission rates have been normalized to 340 (for shank applications) or 245 (for drip applications) lbs/ac application rates, except for shallow shank w/o tarp application where the emission rates were summarized from a shallow bed shank application study. Accordingly, the emission rates were normalized to 255 lb/ac application rate. 340, 255 and 245 lb/ac respectively represent the maximum application rates allowed in AITC product labels for broadcast shank, bed shank and drip applications.

Background:

In 2017, the Registration Branch of the California Department of Pesticide Regulation (DPR) received two application packages from Isagro USA, Inc (Isagro). These packages, with DPR track ID numbers 280548-N and 280549-N respectively, request to register the following two products for use in California under Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act:

DPR track ID: 280548-N
Product name: Dominus®
EPA Registration No. 89285-2
Active ingredient: allyl isothiocyanate (96.3%)

DPR track ID: 280549-N
Product name: Dominus® 100
EPA Registration No. 89285-3
Active ingredient: allyl isothiocyanate (99.8%)

Per the proposed label language, these products are both broad-spectrum pre-plant soil fumigants to control soil fungi, nematodes, and insects. Selected crops identified in the submitted labels include leafy vegetables (e.g., lettuce), root and tuber vegetables (e.g., carrot), fruiting vegetables (e.g., eggplant), strawberries, vineyards and nut crops, among others. Application methods for both products include broadcast and bed shank injections. In addition, Dominus® (89285-2) may be used in post-plant crop termination applications and raised plant beds using drip irrigation systems. Details on application methods and tarp requirements for each product are summarized in Tables 1 and 2.

Table 1. Application methods, injection depths, and tarp requirements for Dominus® 100 (EPA Registration No. 89285-3)

Application method	Injection depth (in)	Tarp[a]	Comment
Broadcast shank	5-15	Yes	PE, VIF, TIF[b]
		No[c]	Overhead sprinkler, water cap and/or roller/packer, close chisel traces
	>17	No	Roller/packer
Bed shank or strip	8-15	Yes	PE, VIF, TIF
		No	Overhead sprinkler, water cap and/or roller/packer, close chisel traces

a: whether tarping is required by the product label; b: PE=polyethylene, VIF=virtually impermeable film, TIF=totally impermeable film; c: tarp is not required if alternative methods described in the comment column are used.

Table 2. Application methods, injection depths and tarp requirements for Dominus® (EPA Registration No. 89285-2)

Application method	Injection depth (in)	Tarp	Comment
Broadcast shank	4-15	Yes	PE, VIF, TIF[a]

		No[b]	Overhead sprinkler, water cap and/or roller/packer, close chisel traces
	>17	No	Roller/packer
Bed shank or strip	4-15	Yes	PE, VIF, TIF
		No	Overhead sprinkler, water cap and/or roller/packer, close chisel traces
Drip	subsurface[c]	Yes	N/A[d]
		No	>1 in buried drip tape

a: PE=polyethylene, VIF=virtually impermeable film, TIF=totally impermeable film; b: tarp is not necessary, if alternative methods as described in the comment column are used; c: drip emitters are placed at shallow subsurface positions; d: tarp materials are not specified on the product label.

It is DPR's practice that all fumigants undergo comprehensive human health risk assessment before being registered for use in California. Occupational and bystander exposures to fumigant applications are evaluated in this process, necessitating fumigant emission data that quantify the rate and amount of fumigant escaping from treated soil. At present, DPR has identified one AITC emission study submitted by Isagro (Document No. 50544-0008). In this study, AITC emissions were measured for broadcast shallow application with totally impermeable film (TIF) tarp, broadcast shallow shank application with polyethylene (PE) tarp, shallow drip application with TIF tarp, and shallow drip application PE tarp (Ajwa *et al.*, 2014). However, AITC-specific emission data for other application and tarp conditions are not available. Therefore, fumigants with similar physiochemical properties as AITC are proposed for use as surrogates to bridge the data gap in order to complete the risk assessment and registration. This memorandum describes the method employed by the Exposure Assessment Section (EAS) of the Human Health Assessment (HHA) Branch to determine AITC emission values for bystander exposure assessment, using both AITC-specific and surrogate fumigant field emission studies.

Determination of averaging periods for human exposure assessment:

AITC emission data from field studies needs to be constructed based on the time periods used in bystander exposure assessment or the exposure periods from AITC toxicity studies, if warranted. For occupational and residential bystanders, the averaging periods are developed to match default bystander exposure times of 8 and 24 hrs/day respectively. Also, available information from the toxicology summary indicates that rats exhibited acute neurotoxic effects 4 hours after the inhalation exposure ceased (Herberth, 2017). Hence, in addition to 8 and 24 hours, a 4 hour time period is also included in this assessment.

Review of AITC emission study:

Data volume 50544-0008, submitted by Isagro, is the only available AITC emission study (Ajwa *et al.*, 2014). This study was conducted in the central coast area of California. AITC was applied to four fields in this study using two different application methods (shallow shank and drip) and two different tarp materials (totally impermeable (TIF) or polyethylene (PE) film) (Table 3). AITC emission rates were calculated from on-field measurements of air concentrations except for the first period of the two shank applications where off-field air concentrations were used by AERMOD to back-calculate the emissions. AITC air concentrations were continuously monitored starting from the application, and the air sampling tubes were replaced every 6 hours within the first 48 hours. After that and before tarp cutting (for shank applications) or tarp punching (for drip applications), the tube was replaced every 12 hours.

Table 3. Field layouts, application methods and tarp conditions of the four fields treated with allyl isothiocyanate[a]

Field layout	Treated acre	Application[b]	Tarp[c]	Gross application rate[d] (lbs/ac)
Bed	2.0	Drip (1 in)	TIF	209
Broadcast	1.9	Shank (8-10 in)	TIF	335
Bed	1.1	Drip (1 in)	PE	202
Broadcast	0.9	Shank (8-10 in)	PE	326

a: data were obtained from Document 50544-0008 (Ajwa *et al.*, 2014); b: number in brackets indicates the application depth; c: TIF= totally impermeable film, PE= polyethylene; d: actual AITC application amount per gross field acreage.

An initial review of the submitted study report was conducted in 2015, and concerns were raised especially towards some findings in the Quality Control section (Barry, 2015). These concerns

are (1) low recoveries were reported for quality control spike samples in Phase 1 (shank or drip applications with TIF tarp), (2) one field spike sample in Phase 2 (shank or drip applications with PE tarp) was reported with 51.5% recovery, but it was labeled as "Lost" without any explanation, and (3) field spike samples in both Phase 1 and 2 showed wide ranges of recoveries (Phase 1: 65.7-127.4%; Phase 2: 67.1-161.4%). Nevertheless, reevaluation of these concerns in this memorandum concluded that the emission data can be used for short-term bystander exposure assessment based on the following:

1) Low recoveries of quality control spike samples in Phase 1:

This refers to four 10 µg lab spike samples, labeled as QC (10µg), on Pages 138-139 of the study report. They had the same dates of preparation, extraction and analysis, and all showed similar AITC detections (0.9409, 1.1509, 1.2503 and 0.9098 µg/tube), that were significantly lower than the target 10 µg/tube spiked amount (about 10%). One most common cause for this low recovery in quality control spike samples is a different amount of AITC was spiked (e.g., 1µg), through either using a wrong standard solution or spiking a different volume. Considering the fact that all other QC samples extracted and analyzed on the same dates, including both lab and field spiking samples, showed acceptable recoveries (average: 100.4%, range: 76.1-127.4%, *N*=16), this analysis determined the results analyzed on 10/12/2013 are still acceptable.

2) One field spike sample in Phase 2 was reported with 51.5% recovery, but it was labeled as "Lost" without any explanation:

This refers to a 0.2 µg field spike sample in Phase 2 that was prepared and analyzed together with AITC emission samples collected from Periods 23-28, but data from Periods 23-28 will not be used in bystander exposure assessments as AITC emissions during those periods are low. The maximum AITC emissions for drip-PE and shank-PE applications were from Periods 2 and 11 respectively, and recoveries of field spike samples for these two periods are all acceptable (>70%) as shown in Table 4.

Table 4. Summary of field spike recoveries in study 50544-0008 submitted by Isagro[a]

Application and tarp type[b]	Periods with highest AITC emission	Length of period[c] (hr)	Recoveries of field spikes for that period[d]	Comment
TIF-Drip	3[e]	5	80.4-120.0%	Field spikes were received, extracted and analyzed on the same dates as Period 3 samples

TIF-Shank	1	6	80.4-120.0%	Field spikes were received, extracted and analyzed on the same dates as Period 1 samples
PE-Drip	2	6	71.0-161.4%	Field spikes were received on the same date, but extracted and analyzed 1 day prior to the dates of Period 2 samples.
PE-Shank	11	12	84.4-108.9%	Field spikes were received, extracted and analyzed on the same dates as Period 11 samples

a: data were obtained from Document 50544-0008 (Ajwa *et al.*, 2014); b: TIF=totally impermeable film, PE=polyethylene film; c: rounded to the closest whole hour; d: field spikes are not available for every period. The range here represents recoveries of field spike samples that are closest to the periods when the highest AITC emissions were measured; e: the period numbering system for TIF-Drip field starts with Period 2.

3) Field spike samples showed wide ranges of recoveries in both Phase 1 and 2:

As discussed above, only periods with the highest AITC emissions will be used for bystander exposure assessment as they represent the greatest exposure potential. As shown in Table 4, field spike recoveries of the periods with the highest AITC emissions were all >70%.

The emission profile within the first 5 days of applications, as well as the highest emissions of each treated field are summarized in Figure 1 and Table 5.

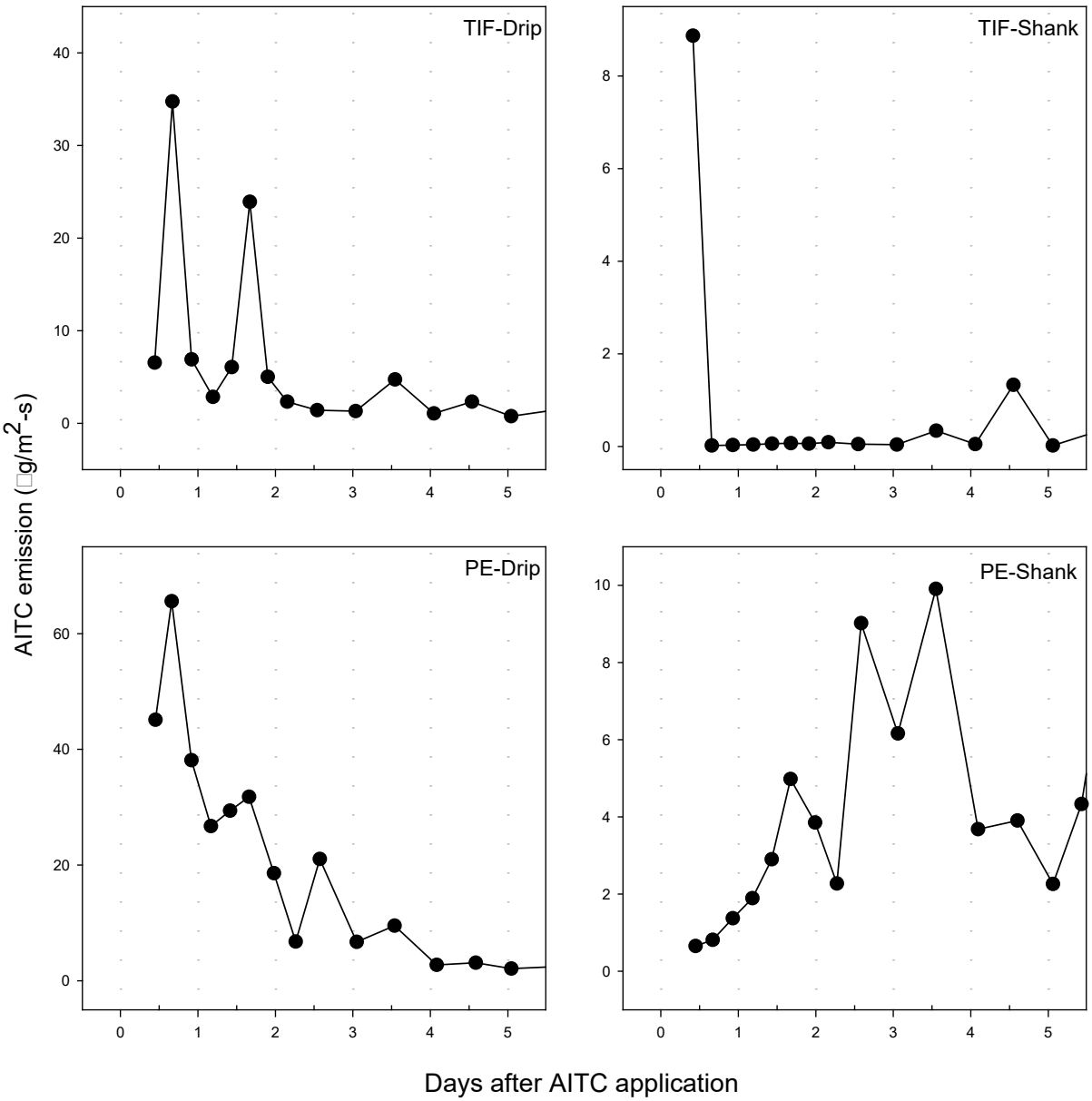


Figure 1. Allyl isothiocyanate (AITC) emissions from soil after different application methods and tarp conditions. AITC is applied through broadcast shank injection or drip irrigation, and the treated fields were covered with totally impermeable (TIF) or polyethylene (PE) film. Data were obtained from Document 50544-0008 (Ajwa *et al.*, 2014). The figure only shows the emissions within the first 5 days from application, and original emissions without application rate adjustment were used.

Table 5. Maximum allyl isothiocyanate soil emissions and the time when the maximum emissions were measured

Application method	Tarp type[a]	Maximum measured emission[b]	
		$\mu g/m^2/s$	Time[c]
Drip	TIF	56.5	1300-1900 (1st Day)
Broadcast shank	TIF	9.0	0900-1300 (1st Day)
Drip	PE	110.4	1300-1900 (1st Day)
Broadcast shank	PE	10.3	0700-1900 (4th Day)

a: the treated fields were covered with totally impermeable (TIF) or polyethylene (PE) film; b: data were obtained from Document 50544-0008 (Ajwa *et al.*, 2014). The emissions were normalized to 340 lbs/ac application rate; c: rounded to the closest whole hour.

For other application and tarp scenarios described on AITC product labels, such as deep shank without tarp, data from other soil fumigants were used as surrogates to estimate the emissions.

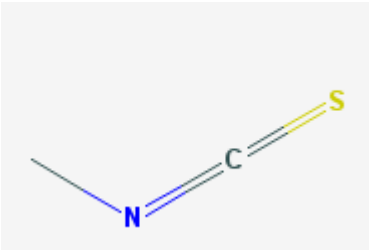
Selecting surrogate emission data:

This memorandum analyzed all fumigants registered in California and chosen five of them to consider as potential candidates of surrogate emission data. Based on DPR's Pesticide Use Reporting (PUR) database, these five fumigants, i.e., 1,3-dichloropropene (1,3-D), chloropicrin (Pic), methyl bromide (MeBr), metam-sodium (M-Na), potassium N-methyldithiocarbamate (M-K) and sulfuryl fluoride (SF), were the top 5 most used organic fumigant compounds in California, implying their extensive use data available in PUR and the likelihood of finding available emission data compatible with AITC application methods (DPR, 2019).

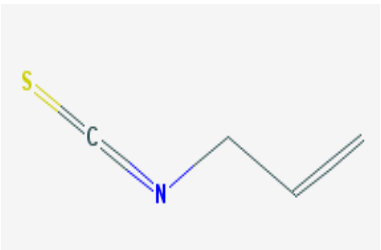
Sulfuryl fluoride. SF is primarily used for structural fumigation in California, thus was removed from being considered as surrogate data.

Methyl isothiocyanate (MITC). Similar to AITC, M-Na and M-K are both used as soil fumigants. After application, M-Na and M-K degrade and produce methyl isothiocyanate (MITC, Figure 2), which is responsible for the fumigation property. MITC is structurally similar to AITC and the physiochemical properties of MITC (boiling point, water solubility and

hydrophobicity) are also similar (Table 6). Thus MITC was first considered as an ideal source of surrogate data.



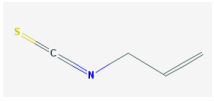
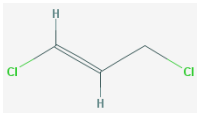
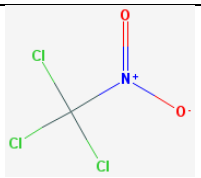
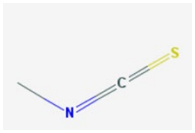
(a) MITC



(b) AITC

Figure 2. Structure of methyl isothiocyanate (MITC, a) and allyl isothiocyanate (AITC, b). Images are copied from PubChem (USNIH, 2019).

Table 6. Physiochemical properties of allyl isothiocyanate (AITC), methyl bromide (MeBr), 1,3-dichloropropene (1,3-D) and chloropicrin (Pic)

	AITC	MeBr	1,3-D	Pic	MITC
Structure					
Formula	C_4H_5NS	CH_3Br	$C_3H_4Cl_2$	CCl_3NO_2	C_2H_3NS
Molecular weight	99.2	94.9	111.0	164.4	73.1
Boiling point (°C)	152	3.5	108	112	119
Water solubility (g/L, at 20 °C)	2	18.5	2	1.9	7.6
K_{ow} (log)	2.2	1.2	2.0	2.1	0.94

Data obtained from PubChem (USNIH, 2019).

As shown in Table 7, applications of MITC products include methods such as soil-drenching or using overhead sprinklers, which are not allowed for AITC products (DPR, 2015). MITC emission studies also often used "water cap" by irrigating the treated field once or multiple times, which was supposed to decrease MITC emissions. However "water cap" is not a required

practice on AITC labels. For application methods with no AITC emission data and in need of surrogate data (e.g., deep shank w/o tarp), there is no MITC emission data available. In addition, available MITC data often showed maximum MITC emissions at night, which is different from available AITC emission data which show maximum emissions during the day (Figure 3).

Table 7. Application methods of metam sodium and metam potassium that produce methyl isothiocyanate after field applications

Application method #	Application method
2	metam sodium and metam potassium field soil fumigation recommended permit conditions for **drench** applications
3	metam sodium and metam potassium field soil fumigation recommended permit conditions for **drip** applications
4	metam sodium and metam potassium field soil fumigation recommended permit conditions for **flood** applications
5	metam sodium and metam potassium field soil fumigation recommended permit conditions for **power mulcher** and **rotary tiller (rototiller)** applications
6	metam sodium and metam potassium field soil fumigation recommended permit conditions for **rod bar** applications
7	metam sodium and metam potassium field soil fumigation recommended permit conditions for **shank** applications
8	metam sodium and metam potassium field soil fumigation recommended permit conditions for **spray blade with soil cap** applications
9	metam sodium and metam potassium field soil fumigation recommended permit conditions for **sprinkler** applications

This table was copied DPR (2015). The application methods are highlighted in bold.

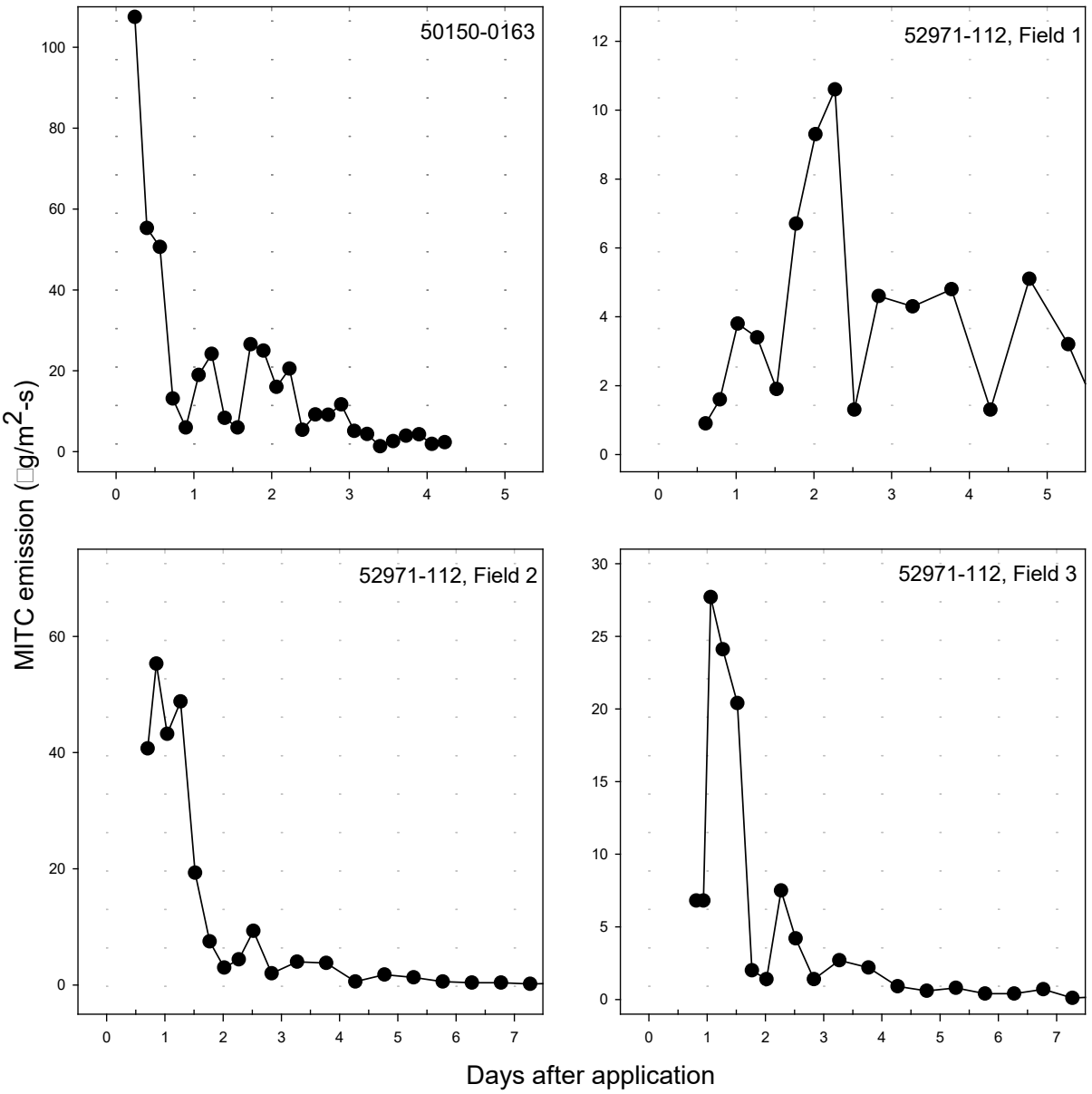


Figure 3. Methyl isothiocyanate (MITC) emissions from soil. Each figure is labeled with the study document number (Ajwa *et al.*, 2011; Sullivan, 2011). Application methods included shank injection and chemigation, and tarp materials included virtually impermeable film, polyethylene film, or non-tarp. The figure only shows the emissions within the first 5 days from the day of application. Original emissions without application rate adjustments were used.

MeBr, 1,3-D and Pic. MeBr, 1,3-D and Pic are the other three soil fumigants with significant use in California. Table 6 shows their respective structures, and compared to MeBr, physiochemical properties of 1,3-D and Pic are more similar to AITC: 1). The octanol-water partition coefficient (K_{ow}) of 1,3-D andPic are close to AITC, suggesting their similar affinity to soil particulates especially soil organic matter, 2). The water solubility of 1,3-D, Pic and AITC are similar, implying their similar transport potential to soil surface via soil water, and 3). 1,3-D and Pic have lower boiling points and higher vapor pressure than AITC, which means 1,3-D and Pic have greater volatility and higher emission potential from soil. All these reasons imply that compared to MeBr, 1,3-D and Pic are better surrogates of AITC emission data. In addition, For the application scenarios with available AITC data, we found similar emission rates between AITC and 1,3-D/Pic, which further supports using 1,3-D/Pic data as surrogate for applications without AITC data. Details on these comparisons will be provided in the latter appraisal section.

This assessment identified a total of 44 Pic and 1,3-D applications with complete soil emission data. Table A1 in the Appendix summarizes these applications and groups them based on their application methods and tarp conditions.

There were ten applications that treated soil simultaneously with both 1,3-D and Pic (Knuteson and Dolder, 2000; van Wesenbeeck and Phillips, 2000; Ajwa, 2009; Ajwa and Sullivan, 2010; Sullivan and Chellemi, 2010; Sullivan, 2012). These applications included broadcast shank with TIF-tarp, broadcast shank with PE-tarp, drip with virtually impermeable film (VIF)-tarp and drip with PE-tarp (Table 8). Most of these studies showed 1,3-D and Pic had comparable emission rates. There is not enough evidence to choose 1,3-D over Pic, and vice versa. Therefore emission data from both 1,3-D and Pic are considered in this analysis.

Table 8. Maximum emissions of 1,3-dichloropropene (1,3-D) and chloropicrin (Pic) from applications using both fumigants[a]

Application method	Tarp type[b]	Document No.	Field	Maximum measured emission[c] (µg/m^2/s)	
				1,3-D	Pic
Broadcast shank	TIF	50046-0198	1	9.6	6.4
			2	8.2	5.3
			3	6.5	4.2
			4	6.1	10.3

		199-0142	2	16.1	11.6
	PE	199-0143	1	110.3	68.1
			2	114.3	71.0
		199-0142	1	106.0	14.2
Drip	VIF	50046-0153	1	38.3	71.9
	PE	50046-0152	1	101.7	67.4

a: data were obtained from various sources (Knuteson and Dolder, 2000; van Wesenbeeck and Phillips, 2000; Ajwa and Sullivan, 2010; Sullivan and Chellemi, 2010; Sullivan, 2012); b: the treated fields were covered with totally impermeable (TIF), virtually impermeable (VIF), or polyethylene (PE) film; c: the emissions were normalized to 340 lbs/ac application rate.

Limited 1,3-D and Pic emission data was found for VIF-tarp applications. There were only one bed shank and one drip application with VIF-tarp, and no broadcast shank with VIF-tarp (Knuteson and Dolder, 2000; Rotondaro, 2004). The lack of data makes it difficult to analyze the emission variability of VIF-tarp applications among different studies. For instance, Pic emissions from a drip VIF-tarp field are about eight times that of Pic emissions from a drip TIF-tarp field. However, Pic emissions from a bed shank VIF-tarp field are comparable to the emissions from the TIF-tarp field. The U.S. Environmental Protection Agency (US EPA) often grouped VIF and TIF films together and assigned them the same buffer zone reduction credits (US EPA, 2018). For instance, for products containing 1,3-D and Pic, both Klerks VIF (1.30 mil) and Ginegar Ozgard T-Plus TIF (1.5 mil) were granted a 60% reduction in buffer zone distance. Considering the low number of VIF studies and US EPA's practice, emissions from VIF- tarp studies were grouped with TIF-tarp ones and used to generate emission rates for TIF-tarp scenarios.

Non-tarp applications are not common in California, but are allowed on AITC labels for both shank and drip applications (Spurlock, 2013). With the same application methods, non-tarp fields usually generate higher emissions than tarp fields, as the emitted fumigants can freely escape from the soil surface to the air. Therefore, to be consistent with label-permit conditions, emissions under non-tarp conditions were estimated in this analysis. AITC labels only allow deep (>1 in) drip non-tarp scenarios, but permit both shallow and deep (>17 in) injections for shank applications. Therefore, emissions of shank injection with both injection depths will be developed.

Most maximum emissions in Table A1 were measured from periods longer than 4 hours (range: 4-12 hours), within which higher emissions lasting shorter periods of time are possible.

Therefore, to estimate the 4 hr time-weighted average (TWA) emissions from measurements of longer periods, a peak-to-mean adjustment method was employed. Details of this method and the calculation equation have been discussed previously (Barry, 2000). This analysis used the maximum measured emissions as a conservative surrogate to represent the 8 hr-TWA emissions, except for applications with maximum emissions measured from periods longer than 8 hours. For these exceptions, the same peak-to-mean adjustment as mentioned above was used to calculate 8 hr-TWA emissions. This analysis also calculated 24 hr-TWA emissions by averaging emissions for any rolling 24-hr period. The calculated maximum 4, 8 and 24 hr-TWA emissions are summarized in Tables 9 and 10. For each application scenario without AITC data, the 1,3-D or Pic study with the highest emission was selected as the surrogate data. Emission profiles of those applications are presented in Figure 4.

Table 9. Maximum emission rates of allyl isothiocyanate (AITC) for different time-weighted average (TWA) periods[a]

Application method	Field	Maximum TWA emission[b] (µg/m^2/s)		
		4 hr-TWA	8 hr-TWA	24 hr-TWA
Shallow[c] shank w/ tarp	2	10.9	9.0	2.2
	4	17.8	12.6	8.4
Shallow[d] drip w/ tarp	1	46.7	40.8	13.8
	3	99.3	79.7	53.4

a: data is generated based on Document 50544-0008 (Ajwa *et al.*, 2014); b: The emission rates were normalized to 340 and 245 lb/ac application rate for shank and drip applications respectively; c: shank injection < 17 in is considered as shallow; d: drip tape buried ≤1 inch is considered as shallow.

Table 10. Maximum emission rates of 1,3-dichloropropene (1,3-D) and chloropicrin (Pic) for different time-weighted average (TWA) periods[a]

Application[b]	Source of data[c]	Maximum TWA emission[d] (µg/m^2/s)		
		4 hr	8 hr	24 hr
Deep shank w/o tarp	Pic	97.0	79.2	53.6

Shallow shank w/o tarp	1,3-D	128.2	121.1	82.9
Deep drip w/o tarp	1,3-D	208.7	186.6	95.4

a: data is cited from various sources (Gillis, 1998; van Wesenbeeck, 1998; Ajwa, 2008); b: shank injection < 17 in is considered as shallow; Drip tape buried >1 inch is considered as deep; c: source of emission data, Pic and 1,3-D respectively emission data of chloropicrin and 1,3-dichloropropene; d: the emission rates have been normalized to 340 (for deep shank w/o tarp), 255 (for shallow shank w/o tarp) or 245 (for deep drip w/o tarp) lbs/ac application rates. For shallow shank w/o tarp application, the emission rates were summarized from a study using bed applications. 340, 255 and 245 lb/ac respectively represent the maximum application rates allowed in AITC product labels for broadcast shank, bed shank and drip applications.

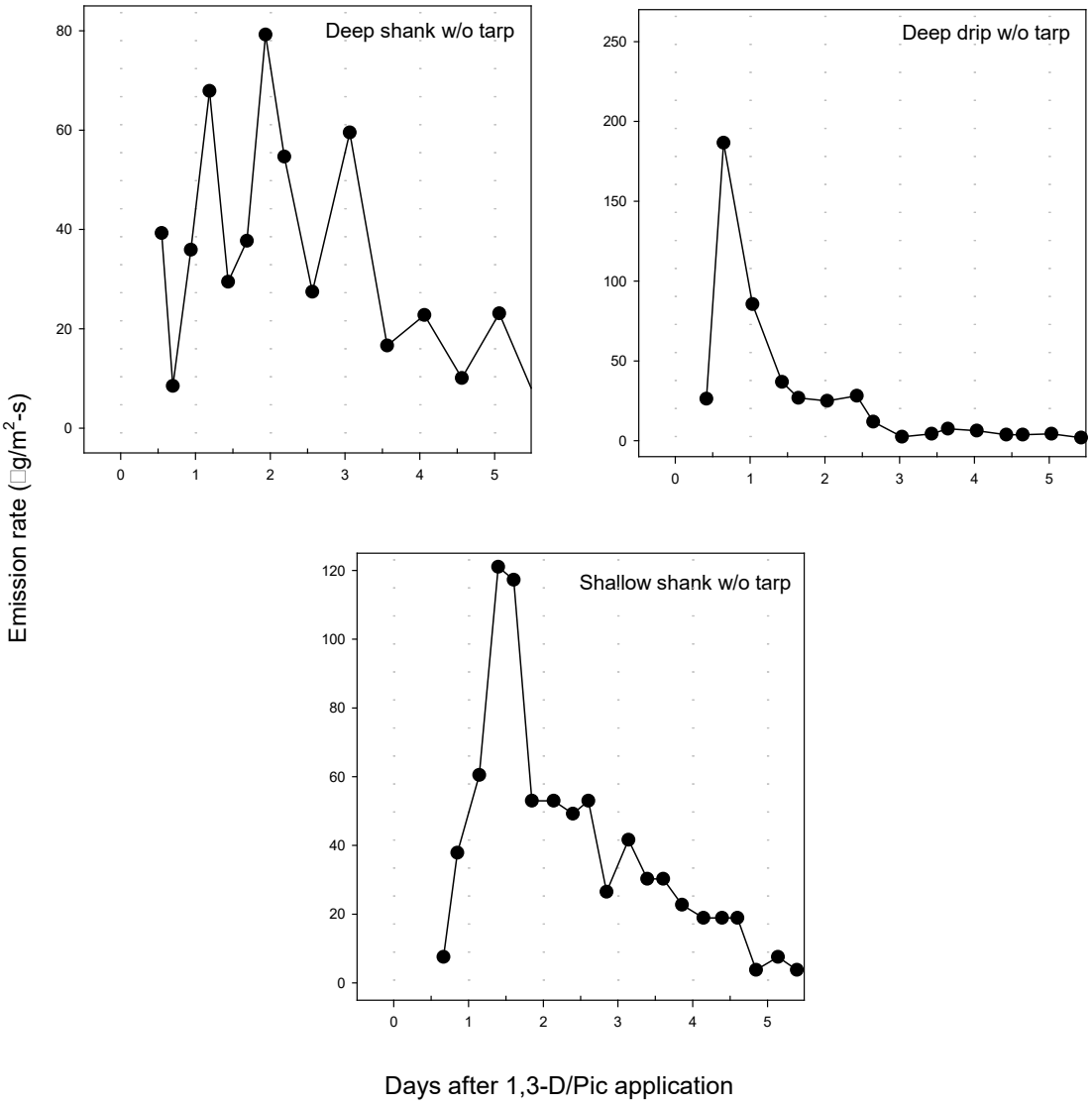


Figure 4. 1,3-Dichloropropene (1,3-D) and chloropicrin (Pic) emissions from soil. Data were obtained from various studies (Gillis, 1998; van Wesenbeeck, 1998; Ajwa, 2008). These applications were selected as surrogate data for scenarios without allyl isothiocyanate (AITC) emission data. The figure only shows the emissions within the first 5 days from the day of application. The emission rates have been normalized to 340 (for deep shank w/o tarp), 255 (for shallow shank w/o tarp) or 245 (for deep drip w/o tarp) lbs/ac application rates. For shallow shank w/o tarp application, the emission rates were summarized from a study using bed

applications. 340, 255 and 245 lb/ac respectively represent the maximum application rates allowed in AITC product labels for broadcast shank, bed shank and drip applications.

Methodology appraisal:

This analysis employs the best available information to estimate AITC emission data for bystander exposure assessment, and during this process, uncertainties have been identified. The section below addresses all uncertainties and the rationales underlying the selection and development of the proposed method.

Use of AITC emission study. This analysis reviewed and used the only AITC soil emission study submitted by the registrant, as this is the best available soil emission data specifically conducted using AITC (Ajwa *et al.*, 2014). However, this study only collected one set of emission data for each application and tarp scenario, so the variability of AITC emissions among different fields and weather conditions is unknown. This concern is addressed in a companion memorandum in which emission data are used to estimate bystander exposure. The companion memorandum takes possible measures to minimize potential underestimation of bystander exposure, including the use of maximum label-permit rate, using weather data from multiple-years and different locations throughout California, and using the maximum estimate of the air concentration modeled from the emission value.

Using 1,3-D and Pic emissions as surrogate data. 1,3-D and Pic were selected as surrogate data to estimate AITC emissions for scenarios without AITC-specific data. Based on currently available information, 1,3-D and Pic are considered to be the best surrogates for estimating potential bystander exposure because of similar physicochemical properties, emission profiles, and application methodologies, as well as availability of emission data for application scenarios without AITC emission data. As shown in Table 6, 1,3-D and Pic have water solubility and hydrophobicity values close to AITC, implying their similar sorption potential to soil particles and upward transport to the soil surface via soil water. The vapor pressure of 1,3-D and Pic is greater than AITC, implying their emissions from soil could be greater than AITC.

AITC emission rates could be different from 1,3-D and Pic; however, this difference cannot be quantified by directly comparing AITC and 1,3-D (or Pic) emissions as there is only one set of AITC emission data for each application and tarp scenario and the variability of AITC field emissions is unknown. As shown in Table 11, for the four application and tarp scenarios with

AITC data, AITC emissions are always within the range of Pic and 1,3-D emissions. Therefore, this analysis assumes the emission potential of AITC is the same as 1,3-D and Pic, and for the application scenarios without AITC data, the highest emission data of 1,3-D and Pic were selected as the surrogate.

Table 11. Maximum emissions of 1,3-dichloropropene (1,3-D), chloropicrin (Pic) and allyl isothiocyanate (AITC) for different time-weighted average (TWA) periods[a]

Document No.	Field	Fumigant	Maximum TWA emission[b] (μg/m^2/s)		
			4 hr	8 hr	24 hr
Broadcast shank with TIF or VIF tarp[c]					
50544-0008	2	AITC	10.9	9.0	2.2
50046-0198	1	1,3-D	11.7	9.6	6.0
50046-0198	1	Pic	11.1	7.8	3.6
50046-0198	2	1,3-D	10.0	8.1	7.3
50046-0198	2	Pic	9.2	6.5	3.7
50046-0198	3	1,3-D	7.9	6.5	3.0
50046-0198	3	Pic	7.2	5.1	1.8
50046-0198	4	1,3-D	10.5	7.4	3.2
50046-0198	4	Pic	17.9	12.6	9.6
199-0142	2	1,3-D	27.9	19.7	8.8
199-0142	2	Pic	14.2	11.6	6.5
123-0220	2	Pic	33.3	29.8	9.1
123-0220	5	Pic	15.2	11.1	5.4
Broadcast shank with PE tarp[d]					
50544-0008	4	AITC	17.8	12.6	8.4
199-0142	1	1,3-D	129.8	106.0	66.1
199-0142	1	Pic	17.4	14.2	8.7

199-0143	1	1,3-D	135.1	110.3	83.9
199-0143	1	Pic	83.4	68.1	46.4
199-0143	2	1,3-D	140.0	114.3	86.1
199-0143	2	Pic	86.9	71.0	48.8
199-0072	1	Pic	28.3	23.1	11.4
199-0072	2	Pic	69.6	56.8	29.0
199-0130	1	Pic	87.4	71.3	41.8
123-0220	1	Pic	63.9	55.8	30.3
Drip with TIF or VIF tarp					
50544-0008	1	AITC	64.6	56.5	19.1
199-0136	2	Pic	11.4	9.3	6.0
50046-0153	1	1,3-D	46.8	38.3	20.8
50046-0228	1	1,3-D	18.0	14.7	7.5
50046-0153	1	Pic	88.0	71.9	27.5
Drip with PE tarp					
50544-0008	3	AITC	137.5	110.4	73.9
199-0112	1	Pic	160.3	160.3	50.9
199-0136	1	Pic	161.8	132.1	76.7
199-0136	3	Pic	44.1	36.0	22.2
199-0136	4	Pic	105.0	85.7	47.9
50046-0152	1	Pic	75.4	67.4	24.9

a: emission data were collected from various sources (Gillis, 1998; Knuteson and Dolder, 2000; van Wesenbeeck and Phillips, 2000; Rotondaro, 2004; Ajwa, 2009; Ajwa, 2010a; Ajwa and Sullivan, 2010; Sullivan, 2012; Ajwa *et al.*, 2014; Ajwa, 2015); b: emissions from different studies were normalized to the same 340 lbs/ac application rate; c: TIF=totally impermeable film, VIF= virtually impermeable film; d: PE=polyethylene film.

Peak-to-mean adjustment. The peak to mean adjustment method was originally developed to "*estimate peak concentrations of varying duration from any mean concentration of 5 hours or*

less", and this method is expected to "*result in conservative estimates of peak concentrations*" (Barry, 2000). As shown in Table 12, most of the adjustments were applied to measurements lasting 5-6 hours. However, the broadcast shallow shank w/ tarp scenario used the emissions from an almost 12 hr-long measurement. Because this study provides the only available AITC emission data, and to be consistent with the adjustment used for other scenarios, the same peak-to-mean adjustment was still used with this particular scenario.

Table 12. Durations of measured maximum emissions for different studies selected as allyl isothiocyanate (AITC) emission surrogate data[a]

Application[b]	Fumigant[c]	Duration of measured max emission[d] (hr)
Shallow shank w/ tarp	AITC	12
Shallow shank w/o tarp	1,3-D	5
Deep shank w/o tarp	Pic	6
Drip w/ tarp	AITC	6
Deep drip w/o tarp	1,3-D	5

a: data were obtained from various sources (Gillis, 1998; van Wesenbeeck, 1998; Ajwa, 2008; Ajwa *et al.*, 2014); b: shank injection < 17 in is considered as shallow; Drip tape buried >1 inch is considered as deep; c: Pic=chloropicrin, 1,3-D=1,3-dichloropropene; d: rounded to the closest whole hour.

Injection depth and other emission control practices. Increasing injection depth was considered as an emission mitigation measure and this is supported by previous modeling efforts using HYDRUS (Spurlock, 2013). However, this could not be verified using 1,3-D and Pic field emission data. For instance, for broadcast shank TIF-tarp scenarios, Pic emission from deep shank injection was comparable to shallow shank emissions (Ajwa, 2009; Ajwa and Sullivan, 2010; Sullivan, 2012). To be consistent with the label-permitted application scenarios for AITC, this analysis determined emissions for both shallow and deep shank un-tarp scenarios. However, the variabilities of the emission rates for those scenarios cannot be assessed in this analysis, as there are not enough 1,3-D and Pic non-tarp emission studies to conduct a statistical analysis.

AITC labels contain several "*non-tarped type sealing*" methods, including the use of overhead sprinklers to irrigate treated fields, and the use of roller/packers to compact soil surface and

remove shank chisel traces. However, the efficacy of those methods on decreasing emissions is not well categorized or quantified under field conditions, and there is not enough 1,3-D or Pic emission data that used these practices. One study used a roller to compact soil surface after broadcast shallow shank injections and before PE-tarp, but the 1,3-D and Pic emissions from this application are comparable to other broadcast shallow shank PE-tarp applications (Sullivan and Chellemi, 2010). Therefore, those non-tarped sealing methods are not considered in this analysis.

Conclusion:

This memorandum describes a methodology of using 1,3-D and Pic soil emission data to determine AITC emissions for application and tarp conditions where AITC emission data is not available. This memorandum also describes why other surrogates are not appropriate for use in place of AITC, including metam sodium and metam potassium that generate MITC. 1,3-D and Pic were selected as surrogates because: 1) they have similar physiochemical properties to AITC, 2) their application methods are similar to AITC, 3) for application scenarios without AITC data, Pic and 1,3-D emission data are available, and 4) for application scenarios with available AITC emission data, 1,3-D and Pic showed similar emission patterns and comparable emission rates to AITC. The developed AITC emission data, as summarized in Table E1, will be further used for short-term bystander exposure assessment.

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Appendix:

Table A1. Summary of maximum chloropicrin (Pic) and 1,3-dichloropropene (1,3-D) soil emissions from studies used in this analysis[a]

Document No.	Field	Fumigant	Other application condition	Maximum measured flux[b]	
				µg/m^2/s	Time
Broadcast shank with totally impermeable film (TIF) tarp					
50046-0198	1	1,3-D		9.6	1245-1845 (2nd Day)[c]
		Pic		6.4	0645-1845 (3rd day)
	2	1,3-D		8.1	1245-1845 (2nd day)
		Pic		5.3	0645-1845 (4th day)
	3	1,3-D		6.5	1300-1900 (2nd day)
		Pic		4.2	1900-0700 (5th day)
	4	1,3-D	KTS[d]	6.1	0700-1900 (3rd day)
		Pic	KTS	10.3	0700-1900 (3rd day)
199-0142	2	1,3-D		16.1	0630-1830 (3rd day)
		Pic		11.6	1230-1830 (1st day)
123-0220	2	Pic		29.8	0830-1330 (1st day)
	5	Pic	Deep[e]	11.1	0030-0800 (3rd day)
Broadcast shank with polyethylene film (PE) tarp					
199-0142	1	1,3-D		106.0	1230-1830 (2nd day)
		Pic		14.2	1230-1830 (2nd day)
199-0143	1	1,3-D	Low disturbance[f]	110.3	1230-1830 (2nd day)
		Pic	Low disturbance	68.1	1230-1830 (2nd day)
	2	1,3-D		114.3	1230-1830 (2nd day)
		Pic		71.0	1230-1830 (2nd day)
199-0072	1	Pic		23.1	1230-1830 (3rd day)

	2	Pic		56.8	1200-1200 (2nd day)
199-0130	1	Pic		71.3	1330-1930 (2nd day)
123-0220	1	Pic		55.8	1330-1845 (1st day)
Broadcast shank without tarp					
50046-0067	1	1,3-D		27.4	1900-0700 (3rd Day)
50046-0127	1	1,3-D		103.5	1200-1720 (2nd Day)
199-0130	3	Pic		104.4	1930-0130 (2nd Day)
	4	Pic	Deep	79.2	1930-0130 (2nd Day)
Bed shank with totally impermeable (TIF) or virtually impermeable film (VIF) tarp					
199-0140	1	Pic	TIF	35.6	0700-1200 (1st Day)
123-0220	4	Pic	TIF, Deep	9.7	0800-1830 (3rd Day)
52971-0112	1	Pic	VIF	9.4	0330-0930 (3rd Day)
Bed shank with PE tarp					
52971-0112	2	Pic		65.6	1530-2130 (2nd Day)
	3	Pic		72.9	1530-2130 (2nd Day)
Bed shank without tarp					
50046-0088	1	1,3-D	Deep	49.6	1330-1930 (3rd Day)
50046-0127	2	1,3-D		156.3	1200-1700 (2nd Day)
Drip with TIF or VIF tarp					
50046-0153	1	1,3-D	VIF	38.3	1300-1900 (1st Day)
		Pic	VIF	71.9	1300-1900 (1st Day)
199-0136	2	Pic	TIF	9.3	1900-0100 (1st Day)
50046-0228	1	1,3-D	TIF	14.7	1300-1900 (1st Day)
Drip with PE tarp					
199-0112	1	Pic		160.3	1130-1530 (1st Day)
199-0136	1	Pic		132.1	1900-0100 (1st Day)

APPENDIX 2.

Determination of allyl isothiocyanate air concentrations around fields fumigated using shank or drip applications

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MEMORANDUM

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SUBJECT: DETERMINATION OF ALLYL ISOTHIOCYANATE AIR CONCENTRATIONS AROUND FIELDS FUMIGATED USING SHANK OR DRIP APPLICATIONS

Executive summary:

The California Department of Pesticide Regulation (DPR) has received a request for the registration of allyl isothiocyanate (AITC) as a soil fumigant in California. This memorandum describes the use of the air dispersion model AERMOD to estimate AITC air concentrations around a treated field using application-specific emission information. AITC has not previously been used as a fumigant in California. Therefore, the pesticide use data needed for the modeling is not available. This analysis assumes that AITC use areas will be similar to other fumigants including 1,3-dichloropropene, chloropicrin, methyl bromide, metam-sodium and potassium N-methyldithiocarbamate. AITC air concentrations were modeled from emissions of five different application scenarios, i.e., shallow shank w/ tarp, shallow shank w/o tarp, deep shank w/o tarp, drip w/ tarp and deep drip w/o tarp, and three emission average time periods (4, 8 and 24 hr), using emission rates detailed in another memorandum (Jiang, 2019). This analysis also used 2013-2017 meteorological data from six counties (Merced, Kern, Santa Cruz, Ventura, Riverside and Siskiyou) with three different field sizes (1, 40, and 100 acre) and at eight distances from the treated field edge (0, 25, 50, 100, 250, 500, 1000 and 2000 ft). At each distance, AITC concentrations were estimated at two heights corresponding to the breathing zone of adults and children (i.e., 5 and 1.7 ft). Estimated AITC air concentrations for the five modeled application scenarios can be found in the Results section, Table 7 through 16. Values in these tables can be used to assess occupational and residential bystander exposures.

Background:

Background information of this analysis has been detailed in a previous memorandum (Jiang, 2019). Briefly, in 2017 DPR received application packages from Isagro USA, Inc. (Isagro) to register two products for use in California:

DPR track ID: 280548-N
Product name: Dominus®
EPA Registration No. 89285-2
Active ingredient: allyl isothiocyanate (96.3%)

DPR track ID: 280549-N
Product name: Dominus® 100
EPA Registration No. 89285-3
Active ingredient: allyl isothiocyanate (99.8%)

Both products are soil fumigants, and can be applied through broadcast and bed shank injections. Dominus® (89285-2) can also be applied to soil beds via drip irrigation systems.

DPR conducts a comprehensive human health risk assessment prior to the registration of a new fumigant. The health risk assessment includes bystander exposure to fumigants that have escaped from the soil of treated fields. As of April 2019, DPR had not received any studies from Isagro that monitored bystander exposure to AITC emissions or identified AITC air concentration data from open literature that can be used for the bystander exposure assessment.

A previous companion memorandum determined AITC soil emission rates under 5 different application and tarp conditions based on AITC specific and suitable surrogates identified (i.e., 1,3-dichloropropene and chloropicrin) data. In that memorandum, the AITC-specific data was used to determine the emission rates for shallow shank application w/ tarp and drip application w/ tarp (Jiang, 2020). For the remaining three application scenarios when AITC-specific data were not available (shallow shank w/o tarp, deep shank w/o tarp and deep drip w/o tarp), 1,3-dichloropropene (1,3-D) and chloropicrin (Pic) emission data were used as surrogate. In addition, that memo also describes in detail why 1,3-D and Pic emission data were selected as appropriate surrogates compared to other active ingredients, such as methyl isothiocyanate (MITC), because of similar physiochemical properties, application methodologies and emission profiles, as well as availability of data for application scenarios in the absence of AITC-specific data. The time-

weighted average emission rates of AITC were determined for three periods, 4, 8 and 24 hr, which are consistent with the time periods used in toxicity tests and the defaults for occupational and residential exposure assessment.

In the current analysis, these emission rates were input into an air dispersion model (AERMOD) to estimate AITC air concentrations around a fumigated field. The resulting AITC air concentrations will be used in the exposure assessment document to assess both the occupational and residential bystander exposures through inhalation.

Method:

AITC use in California

Soil fumigant products containing AITC have not been registered in California. In the absence of information on the use pattern, this analysis assumes the use of AITC will be similar to other soil fumigants: 1,3-D, Pic, methyl bromide (MeBr), metam-sodium (M-Na) and potassium N-methyldithiocarbamate (M-K). Accordingly, the use data of these five fumigants were analyzed to project the AITC use regions and application acreage.

According to DPR's Pesticide Use Reporting (PUR) database, 35 counties in California reported agricultural use of at least one of the five fumigants from 2012-2017 (DPR, 2018). The highest use counties are Kern, Fresno, Monterey, Ventura, Merced and Santa Barbara, which account for >60% of total use in the entire state. The other counties that accounted for > 1% of the entire state fumigant use include Stanislaus, Siskiyou, San Joaquin, Santa Cruz, Tulare, San Luis Obispo, Kings, Madera, Imperial, Riverside, and Los Angeles.

These counties represent different geographic regions of California and have distinctive meteorological conditions. This analysis selected six counties to represent the regions where AITC may be possibly used (Table 1). For each selected county, this analysis modeled AITC air concentration for 1, 40 or 100 acre applications, based on the use data of aforementioned fumigants from the PUR database (DPR, 2018).

Table 1. Counties selected for air concentration modeling and the fumigant use acreage in 2012-2017

County	Region	Application acreage per use[a]

		Median	Average	95th %ile
Merced	Central Valley	18	28	93
Kern	Central Valley	45	52	105
Santa Cruz	Central coast	10	13	34
Riverside	Desert	27	28	71
Ventura	South Coast	22	30	92
Siskiyou	Northern	18	20	56

a: This information is retrieved from the California Department of Pesticide Regulation (DPR) Pesticide Use Reporting (PUR) database, and is based on the use of five fumigants in 2012-2017, i.e., 1,3-dichloropropene, chloropicrin, methyl bromide, metam-sodium and potassium N-methyldithiocarbamate (DPR, 2018). Each use record in PUR may contain multiple-day applications, for which the acreage number below may reflect the total area fumigated on these days.

Model setup

The algorithm to model AITC air concentrations around application fields is shown in Figure 1. This analysis used AERMOD View™ version 9.6.5, and the modeling engine integrated in this software is AERMOD (version 18081) developed by American Meteorological Society and U.S. Environmental Protection Agency (Lakes Environmental, 2019a; Lakes Environmental, 2019b). This analysis also used AERMET View™ (version 9.6.5) to prepare input files (surface and profile files) required by AERMOD (Lakes Environmental, 2019a). Required inputs for AERMET and AERMOD, are shown in square shapes in Figure 1 and described in detail below:

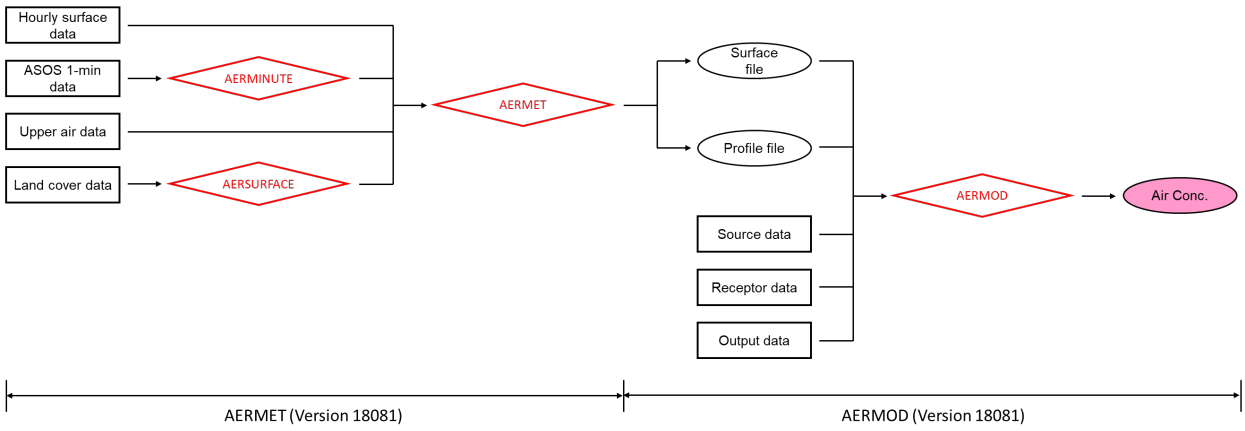

Figure 1. Modeling algorithm to estimate allyl isothiocyanate air concentration. Square, diamond and oval shapes respectively represent data inputs, processing models/tools and outputs.

AERMINUTE and AERSURFACE are tools incorporated in AERMET. Surface and profile files, as outputs of AERMET, also serve as input files for AERMOD.

Hourly surface and *ASOS 1-minute data.* Hourly surface and automated surface observing system (ASOS) 1-minute data include information such as wind speed, wind direction, and cloud cover recorded from ground stations which are usually located at airports. Data used in this analysis was obtained from six airports located in the selected counties (Table 2). These airports were selected based on their distance to agricultural fields and whether ASOS 1-minute data were available. This analysis used 5-year meteorological data (2013-2017) which is the same practice done by other agencies such as California Air Resources Board and local air pollution control districts (ARB, 2019; SCAQMD, 2019).

Upper air data. Upper air data is radiosonde soundings that measure meteorological parameters (e.g., temperature lapse rate, wind speed and direction, etc.) at different vertical layers of the atmosphere (USEPA, 2019). The measurements are only available from three locations in California. So depending on the location, each surface data station was paired with one of the three upper air stations to obtain the required data (Table 2).

Table 2. Sources of surface and upper air data

County	Surface data[a]	Latitude	Longitude	Elevation (m)	Upper air[b]
Merced	KMCE	37.285N	120.512W	48	Oakland
Kern	KBFL	35.433N	119.050W	150	Vandenberg
Santa Cruz	KWVI	36.936N	121.788W	49	Oakland
Ventura	KCMA	34.217N	119.100W	23	Vandenberg
Riverside	KTRM	33.627N	116.159W	-35	Miramar
Siskiyou	KSIY	41.781N	122.468W	805	Oakland

a: using airport 4-letter ICAO code; b: Upper air data is obtained from Oakland International Airport (Oakland), Miramar naval station (Miramar) or Vandenberg air force base (Vandenberg).

This analysis used AERMET to process the surface and upper air data and generate meteorological (surface and profile) files used in AERMOD to estimate AITC air concentrations. In California, AERMOD-ready meteorological files for certain areas are also available from some state and local government agencies including California Air Resources Board and local air

districts. However, we did not use the meteorological files from these sources, as they either did not use the latest AERMET version for the data processing, did not have available files for analyzed areas (e.g., Siskiyou County), or did not use the most recent 5-year (2013-2017) meteorological data.

Two locations (Merced County and Riverside County) were selected to validate our meteorological data processing. At each location, air concentrations were modeled using self-processed meteorological files and then compared to the ones modeled using air pollution control district meteorological files. Some key settings in AERMET for meteorological data processing are summarized in Table 3.

Table 3. AERMET settings to prepare AERMOD-ready meteorological files

County	Merced		Riverside	
Source	DPR[a]	ValleyAir[b]	DPR	South Coast AQMD[c]
AERMET Version	18081	18081	18081	16216
Year range	2013-2017	2013-2017	2012-2016	2012-2016
Surface data	KMCE[d]	KMCE	KTRM[e]	KTRM
ASOS station?[f]	Yes	Yes	Yes	Yes
Upper Air	Oakland[g]	Oakland	Miramar[h]	Miramar
Use of Adj_U*?	Yes	Yes	Yes	Yes
AERSURFACE setting	Airport site, average moisture, 12 sectors	unknown[i]	Airport site, average moisture, 12 sectors	unknown

a: AERMOD-ready meteorological files were generated in this analysis; b: AERMOD-ready meteorological files were obtained from San Joaquin Valley Air Pollution Control District; c: AERMOD-ready meteorological files were obtained from South Coast Air Quality Management District (AQMD); d: Surface data is obtained from Merced Regional Airport; e: Surface data is obtained from Jacqueline Cochran Regional Airport; f: Whether this surface station has 1-min ASOS data; g: Upper air data is obtained from Oakland International Airport; h: Upper air data is obtained from Miramar Naval Air Station; i: AERSURFACE settings were not disclosed.

Air concentrations were modeled for emissions occurring at different times and for different durations (4, 8 and 24 hr). As shown in Figures 2 and 3, the plots followed a one-to-one correlation for both selected counties, indicating air concentrations estimated from using self-

processed meteorological files are close to those from using air district AERMOD-ready files. This validates our meteorological processing method for modeling fumigant air dispersion.

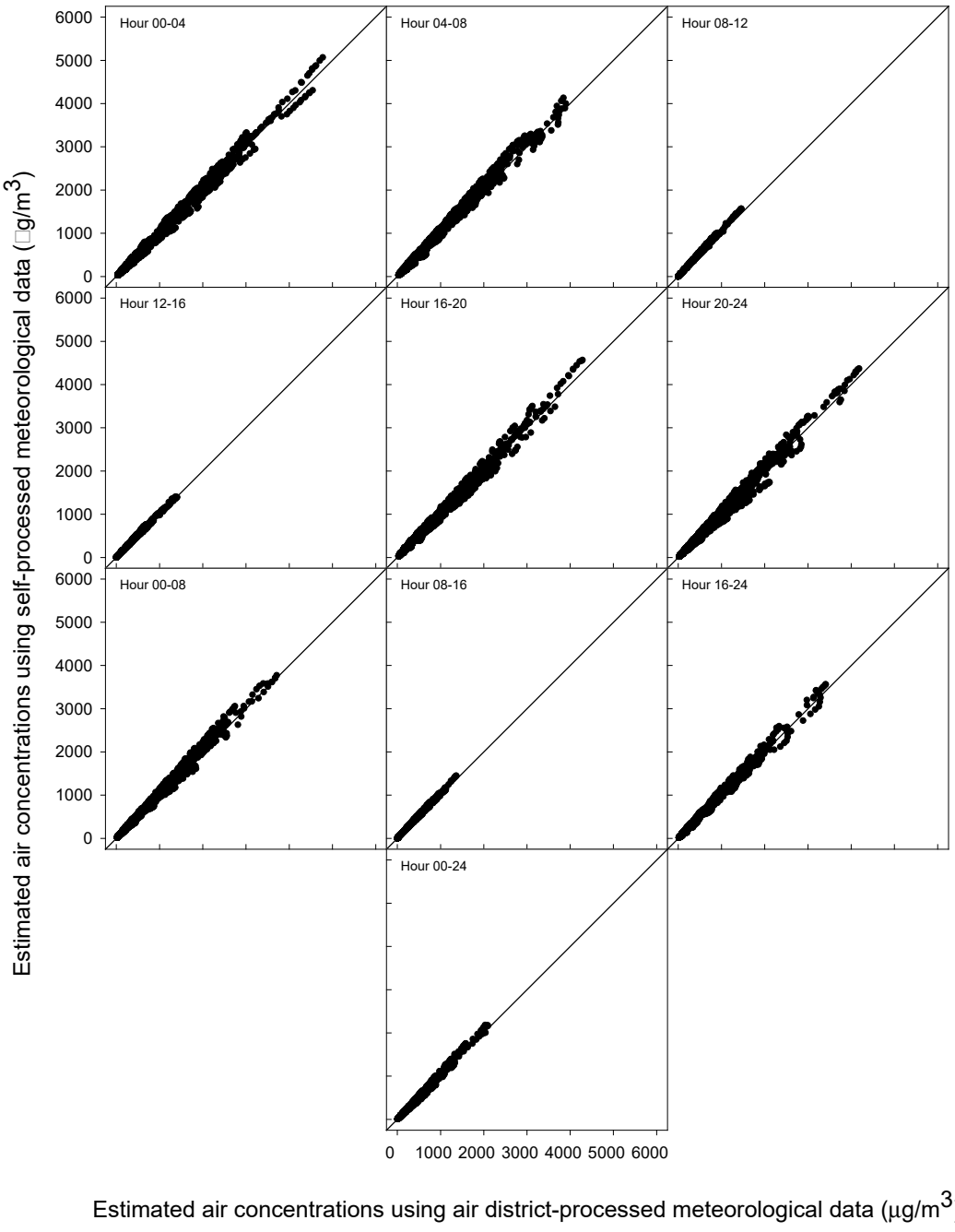

Figure 2. Correlation of estimated air concentrations in Riverside County from using self-processed or air district-provided meteorological files. The diagonal straight line represents a

one-to-one correlation. Each dot represents the maximum AITC air concentration measured at one receptor around a 1 acre fumigated field with 2012-2016 meteorological data. The AITC emission rate is 100 µg/m^2/s.

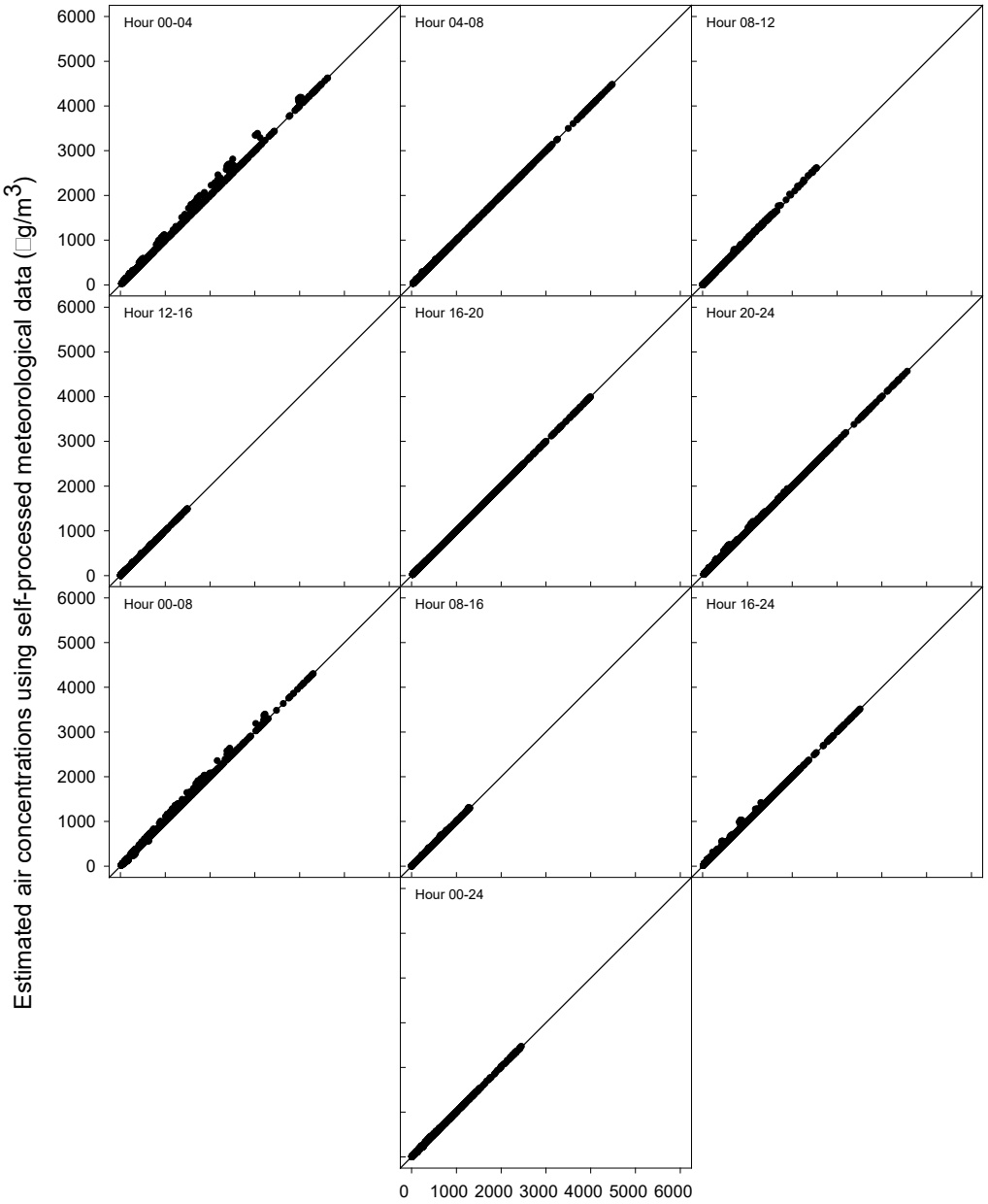

Figure 3. Correlation of estimated air concentrations in Merced County from using self-processed or air district-provided meteorological files. The diagonal straight line represents a

one-to-one correlation. Each dot represents the maximum AITC air concentration measured at one receptor around a 1 acre fumigated field with 2013-2017 meteorological data. The AITC emission rate is 100 $\mu g/m^2/s$.

Land cover data. Land cover data is used to determine ground characteristics of the target area, i.e., surface roughness, albedo and Bowen ratio. This analysis used California data from National Land Cover Dataset 1992 (NLCD1992), which is the data format accepted by AERMET.

Source data. This includes data that define the time and rate of fumigant emissions from soil, as well as the size of the fields that emit fumigants. For each county mentioned above, this analysis modeled AITC air concentrations from 30 different emission scenarios (Table 4). These include three different emission durations (4, 8 and 24 hr), six different emission start times (Hour 00, 04, 08, 12, 16, and 20), and three different application acreages (1, 40, and 100 ac).

Table 4. Summary of modeled emission scenarios

Emission period (hr)	Emission period (Hour)	Treated area (ac)
4	00-04, 04-08. 08-12, 12-16, 16-20, 20-24	1, 40, 100
8	00-08, 08-16, 16-24	1, 40, 100
24	00-24	1, 40, 100

Receptor data. This defines the locations and heights where AITC air concentrations will be modeled. Both proposed AITC product labels require a 25 ft buffer zone, but the buffer zone requirement does not apply to occupational bystanders that may work in a field adjacent to the fumigated area. To understand potential bystander impacts, this analysis placed receptors at eight different distances from the edge of a treated field, i.e., 0, 25, 50, 100, 250, 500, 1000 and 2000 ft. At each distance, the receptors were placed about every 16 ft around the field, except at the four corners where the receptors were placed every 5 degrees (Figure 4). Receptors are also placed at two heights (1.7 and 5 ft), which respectively represent the breathing zones for children and adults.

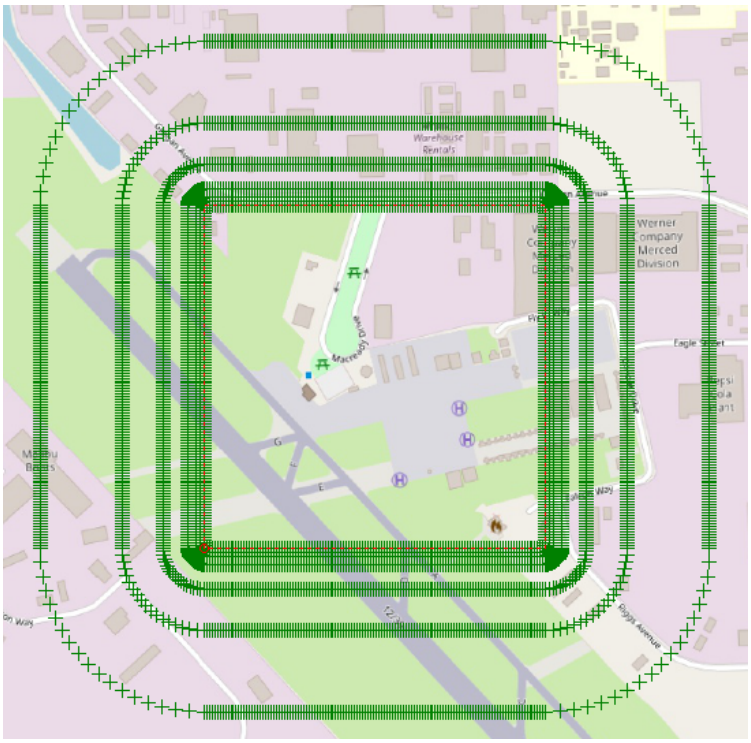

Figure 4. Demonstrative illustration of receptor placement.

Output data. This analysis used 2013-2017 meteorological data, and for each receptor in each modeled scenario, AERMOD was set to only produce the highest air concentration from all modeled days. After that, all the receptors at the same height and distance from the treated field were pooled together and only the highest value among them was used for bystander exposure assessment.

Results:

Effects of emission occurrence time and counties

Emission time is critical for determining the air concentrations. This analysis modeled AITC air concentrations from emissions at different times of a day. For instance, for a 4-hr emission, this analysis modeled the same 100 $\mu g/m^2/s$ emission occurring at six different time intervals (Hour 00-04, 04-08, 08-12, 12-16, 16-20, and 20-24). The assumption is that daytime solar radiation increases air turbulence which favors pesticide dispersion and decreases the air concentration. This assumption is supported by modeling results that showed AITC air concentrations were higher from nighttime emissions than from daytime emissions when the same emission rate and

duration were used (Figure 5). Considering this diurnal emission pattern, AERMOD modeling inputs preserved the time when the maximum emission was observed. Details and benefits of this method are discussed below and the modeled time-ranges for different application and tarp conditions are summarized in Table 5.

The emission profiles used in this analysis were prepared in another memorandum and the rates were extracted from field monitoring studies (Jiang, 2019). As short-term exposure is generally associated with the highest emissions, only the period with the maximum emission rate was used for each field application. However, because weather conditions also affect fumigant air dispersion, modeled daytime emissions have to be approximately 5 times higher than nighttime emissions to generate the same air concentrations (Fig. 5). After reviewing the emission profiles used in this analysis, we concluded that nighttime emissions (sunset to sunrise) have already been modeled in the current method (Table 5).

Instead of using the exact time when maximum emissions were observed in the field studies, this analysis incorporated wider time intervals within the modeling (Table 4). For example, by using data from a 4-hr emission observed at Hour 09-12, two emission scenarios could be modeled for Hour 04-08 and 08-12. Whichever scenario resulted in the higher air concentration could then be selected for the exposure assessment, thereby providing a reasonable worst case exposure estimate. Emission data are dependent on a fixed set of field conditions (e.g., application time, injection depth, etc.) for each application scenario. However, actual fumigations can employ a variety of application settings and can occur under different field conditions. Our method preserved not only the diurnal pattern of AITC emissions, but also allowed us to model the time when maximum emissions may occur and under diverse field use conditions.

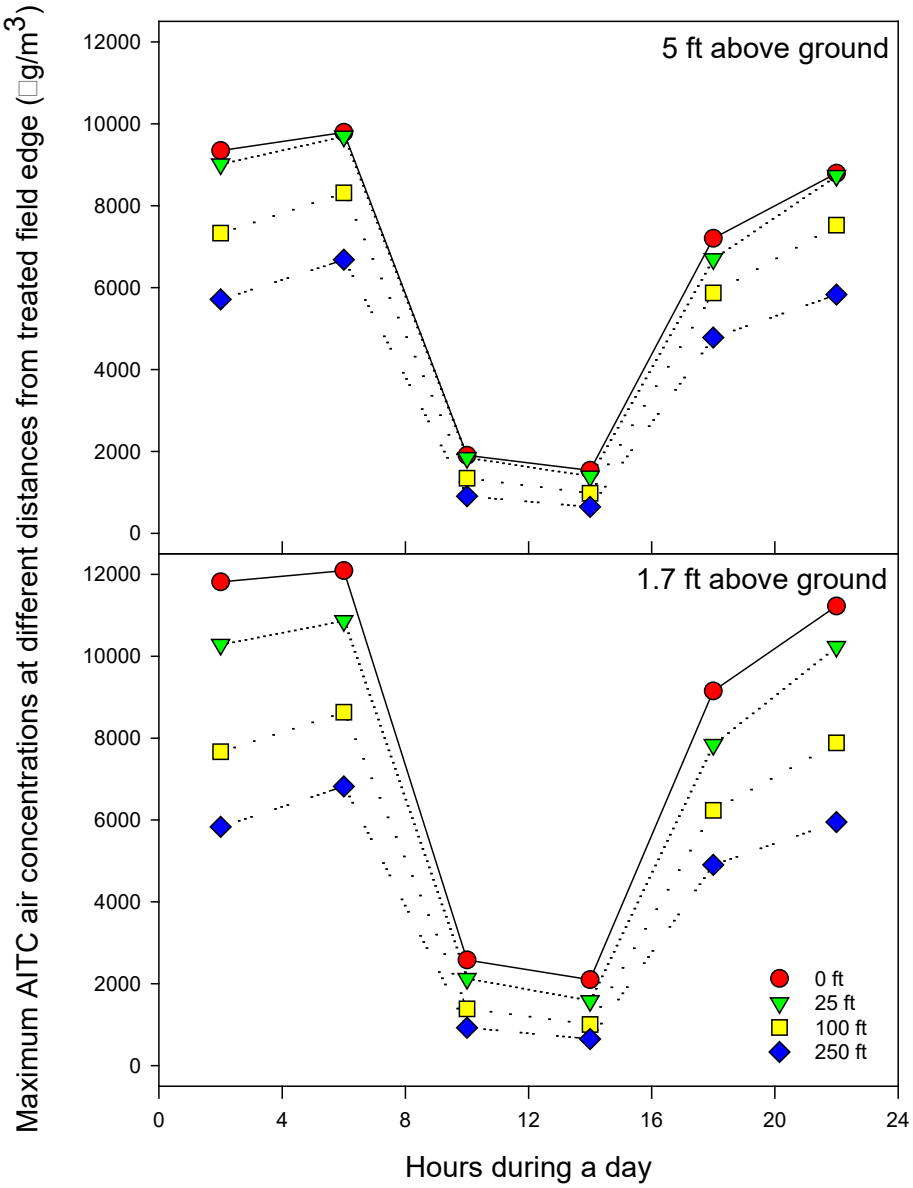

Figure 5. 4-hr averaging AITC air concentrations with different emission times. The modeled field is 40 acres and is located in Kern County. The emission rate is 100 µg/m^2/s flux, and the emissions occur at six different times of a day (Hour 00-04, 04-08, 08-12, 12-16, 16-20 and 20-24).

Table 5. Summary of time periods modeled for air concentrations for different application and tarp conditions

Application & Tarp type	Time[a]	4-hr						8-hr			24-hr
		00-04	04-08	08-12	12-16	16-20	20-24	00-08	08-16	16-24	00-24
Shallow shank w/ tarp	07-19		X	X	X	X		X	X	X	X
Deep shank w/o tarp	19-02	X				X	X	X		X	X
Shallow shank w/o tarp	07-17[b]		X	X	X	X		X	X	X	X
Shallow drip w/ tarp	13-19				X	X			X	X	X
Deep drip, no	13-18				X	X			X	X	X

a: Sampling intervals (in hrs) when the maximum emission rates were measured in the field emission studies; b: two periods (Hour 07-12 and 12-17) showed similar emissions, therefore both periods were considered for modeling.

This analysis modeled air concentrations using meteorological data from six counties. Using the same set of modeling conditions (i.e., 40-ac application, emission of 100 $\mu g/m^2/s$, and a time interval of Hour 04-08), Table 6 shows the highest modeled air concentrations among various counties at a given distance from the treated field. In general, at 0-250 ft from the treated field, Kern County exhibited the highest air concentrations, whereas at >250 ft, Santa Cruz County exhibited the highest concentrations. For this reason, for a certain treated area (1, 40 or 100 ac), distance from the field (0, 25, 50, 100, 250, 500, 1000, or 2000ft), and breathing zone height (1,7 or 5 ft), this analysis only used the highest air concentrations modeled from the six counties for assessing the highest possible bystander exposure to AITC within all of California.

Table 6. Maximum 4-hr time weighted average (TWA) air concentrations ($\mu g/m^3$) for six different counties[a]

Distance[b]	Kern	Ventura	Siskiyou	Santa Cruz	Merced	Riverside
			Adult			
0 ft	9792[c]	8054	9588	9750	7570	7472
25 ft	9698	7721	9484	9390	7254	7205
50 ft	9194	7232	9129	8722	6602	6709
100 ft	8315	6314	8287	7988	5753	5867
250 ft	6682	4980	6670	6551	4460	4636
500 ft	5263	3965	5170	5264	3445	3603
1000 ft	3796	2912	3760	3919	2573	2515

2000 ft	2293	1917	2397	2626	1747	1707
			Child			
0 ft	12090	10281	11920	11907	9191	9310
25 ft	10860	8957	10861	10278	7747	8057
50 ft	9850	7907	9824	9365	6863	7140
100 ft	8636	6620	8594	8283	5864	6051
250 ft	6823	5044	6769	6652	4513	4696
500 ft	5321	3992	5210	5307	3466	3627
1000 ft	3814	2922	3777	3935	2579	2524
2000 ft	2299	1922	2402	2632	1749	1710

a: The treated field is 40 ac, and the emission was 100 µg/m^2/s at Hour 04-08; b: distance from the treated field edge; c: shaded values represent the highest air concentrations among the six counties. AERMOD modeling used 2013-2017 meteorological data for each county.

Estimated air concentrations for emissions from different application and tarp methods

Tables 7 through 16 below summarize the estimated AITC air concentrations for 5 different application scenarios. If more than one emission period was modeled for each application scenario and for each of the averaging times, only the time period with the highest air concentrations was listed in these tables. Information on the time period selections is noted in the footnote of each table.

Table 7. Estimated AITC air concentration (µg/m^3) at 5 ft above ground around field fumigated using shallow shank application with tarp

Distance[a]	1 ac[b]			40 ac			100 ac		
	4hr[c]	8hr[d]	24hr	4hr	8hr	24hr	4hr	8hr	24hr
0 ft	620[e]	350	140	1743	1039	422	2139	1290	520
25 ft	608	326	127	1726	993	400	2131	1241	504
50ft	554	295	111	1636	949	372	2037	1191	475

100 ft	435	235	82	1480	856	327	1875	1093	422
250 ft	274	153	46	1189	673	251	1564	900	337
500 ft	163	83	24	937	513	180	1293	722	263
1000 ft	80	42	11	698	381	120	1002	540	180
2000 ft	36	17	5	467	255	72	724	397	120

a: Distance from the edge of the fumigated field; b: Size of the fumigated field; c: Emission occurs between Hour 04-08, as emissions occurring at this time generated the highest 4-hr time-weighted average air concentrations; d: Emissions between Hour 00-08, as emissions occurring at this time generated the highest 8-hr time-weighted average air concentrations; e: Application rate at 340 lb/ac.

Table 8. Estimated AITC air concentration (µg/m^3) at 1.7 ft above ground around field fumigated using shallow shank application with tarp

Distance[a]	1 ac[b]			40 ac			100 ac		
	4hr[c]	8hr[d]	24hr	4hr	8hr	24hr	4hr	8hr	24hr
0 ft	988[e]	582	246	2152	1305	534	2562	1557	638
25 ft	801	459	184	1933	1142	461	2339	1386	565
50 ft	648	361	141	1753	1028	404	2155	1271	507
100 ft	477	266	92	1537	891	344	1931	1130	437
250 ft	284	161	48	1214	684	256	1591	913	343
500 ft	165	84	25	947	520	182	1305	727	265
1000 ft	81	42	11	700	383	120	1002	543	181
2000 ft	36	17	5	468	256	73	724	398	120

a: Distance from the edge of the fumigated field; b: Size of the fumigated field; c: Emission occurs between Hour 04-08, as emissions occurring at this time generated the highest 4-hr time-weighted average air concentrations; d: Emissions between Hour 00-08, as emissions occurring at this time generated the highest 8-hr time-weighted average air concentrations; e: Application rate at 340 lb/ac.

Table 9. Estimated AITC air concentration (µg/m^3) at 5 ft above ground around field fumigated using deep shank application without tarp

Distance[a]	1 ac[b]			40 ac			100 ac		
	4hr[c]	8hr[d]	24hr	4hr	8hr	24hr	4hr	8hr	24hr

0 ft	3448[e]	2197	893	9468	6530	2691	11840	8111	3316
25 ft	3118	2050	810	8954	6243	2553	11206	7799	3215
50 ft	2642	1856	707	8212	5967	2375	10474	7485	3031
100 ft	2206	1475	522	7241	5379	2086	9360	6872	2695
250 ft	1370	965	291	5939	4230	1599	7781	5660	2154
500 ft	782	519	154	4775	3226	1148	6473	4537	1678
1000 ft	398	263	71	3565	2392	764	5091	3395	1150
2000 ft	181	106	30	2310	1604	462	3660	2494	766

a: Distance from the edge of the fumigated field; b: Size of the fumigated field; c: Depending on the size of the treated area and the distance from the treated area, the highest 4-hr time-weighted average air concentrations were seen from emissions at Hour 00-04 or Hour 20-24. Values present here are from emissions at Hour 00-04; d: Depending on the size of the treated area and the distance from the treated area, the highest 8-hr time-weighted average air concentrations were seen from emissions at Hour 00-08 or Hour 16-24. Values present here are from emissions at Hour 00-08; e: Application rate at 340 lb/ac.

Table 10. Estimated AITC air concentration ($\mu g/m^3$) at 1.7 ft above ground around field fumigated using deep shank application without tarp

Distance[a]	1 ac[b]			40 ac			100 ac		
	4hr[c]	8hr[d]	24hr	4hr	8hr	24hr	4hr	8hr	24hr
0 ft	5221[e]	3658	1567	11687	8200	3406	13975	9786	4070
25ft	3965	2886	1176	9976	7176	2939	12273	8714	3605
50 ft	3269	2270	900	8823	6463	2576	11105	7990	3236
100 ft	2493	1673	588	7635	5601	2196	9654	7100	2786
250 ft	1429	1011	304	6069	4302	1634	7918	5737	2192
500 ft	798	530	156	4829	3266	1161	6533	4570	1694
1000 ft	402	265	72	3586	2406	768	5116	3413	1155
2000 ft	181	106	30	2316	1609	463	3669	2500	768

a: Distance from the edge of the fumigated field; b: Size of the fumigated field; c: Depending on the size of the treated area and the distance from the treated area, the highest 4-hr time-weighted average air concentrations were seen from emissions at Hour 00-04 or Hour 20-24. Values present here are from emissions at Hour 00-04; d: Depending on the size of the treated area and the distance from the treated area, the highest 8-hr time-weighted average air concentrations were seen from emissions at Hour 00-08 or Hour 16-24. Values present here are from emissions at Hour 00-08; e: Application rate at 340 lb/ac.

Table 11. Estimated AITC air concentration (µg/m^3) at 5 ft above ground around field fumigated using shallow shank application without tarp

Distance[a]	1 ac[b]			40 ac			100 ac		
	4hr[c]	8hr[d]	24hr	4hr	8hr	24hr	4hr	8hr	24hr
0 ft	4461[e]	3358	1381	12551	9981	4161	15403	12397	5127
25 ft	4378	3133	1253	12430	9541	3947	15348	11920	4971
50 ft	3986	2837	1093	11784	9120	3673	14668	11439	4687
100 ft	3132	2255	807	10658	8222	3226	13500	10503	4167
250 ft	1977	1475	450	8564	6465	2472	11265	8651	3330
500 ft	1170	794	238	6747	4930	1774	9311	6935	2594
1000 ft	576	402	110	5023	3656	1181	7216	5189	1777
2000 ft	257	162	46	3366	2451	714	5214	3811	1184

a: Distance from the edge of the fumigated field; b: Size of the fumigated field; c: Emission occurs between Hour 04-08, as emissions occurring at this time generated the highest 4-hr time-weighted average air concentrations; d: Emissions between Hour 00-08, as emissions occurring at this time generated the highest 8-hr time-weighted average air concentrations; e: Application rate at 255 lb/ac, assuming 75% of the field is fumigated beds.

Table 12. Estimated AITC air concentration (µg/m^3) at 1.7 ft above ground around field fumigated using shallow shank application without tarp

Distance[a]	1 ac[b]			40 ac			100 ac		
	4hr[c]	8hr[d]	24hr	4hr	8hr	24hr	4hr	8hr	24hr
0 ft	7113[e]	5591	2423	15496	12533	5266	18450	14957	6293
25 ft	5767	4412	1818	13921	10968	4544	16845	13319	5574
50 ft	4667	3469	1392	12625	9879	3983	15517	12211	5003
100 ft	3433	2557	910	11069	8560	3395	13908	10852	4308
250 ft	2046	1545	469	8745	6576	2526	11457	8769	3389
500 ft	1189	810	242	6820	4991	1795	9394	6985	2619
1000 ft	581	405	111	5044	3678	1187	7216	5216	1786
2000 ft	258	163	46	3373	2458	716	5214	3821	1187

a: Distance from the edge of the fumigated field; b: Size of the fumigated field; c: Emission occurs between Hour 04-08, as emissions occurring at this time generated the highest 4-hr time-weighted average air concentrations; d: Emissions between Hour 00-08, as emissions occurring at this time generated the highest 8-hr time-weighted average air concentrations; e: Application rate at 255 lb/ac, assuming 75% of the field is fumigated beds.

Table 13. Estimated AITC air concentration (µg/m^3) at 5 ft above ground around field fumigated using drip application with tarp

Distance[a]	1 ac[b]			40 ac			100 ac		
	4hr[c]	8hr[d]	24hr	4hr	8hr	24hr	4hr	8hr	24hr
0 ft	2585[e]	1841	890	8154	5826	2680	10146	7267	3302
25 ft	2599	1862	807	7984	5786	2542	9946	7204	3202
50 ft	2327	1669	704	7519	5527	2365	9473	6925	3018
100 ft	1845	1310	520	6617	4957	2078	8555	6353	2684
250 ft	1107	724	290	5166	3804	1592	6902	5172	2144
500 ft	683	429	153	3931	2822	1143	5551	4010	1671
1000 ft	349	203	71	2833	1961	761	4150	2959	1145
2000 ft	152	90	30	1813	1209	460	2885	1951	763

a: Distance from the edge of the fumigated field; b: Size of the fumigated field; c: Emission occurs between Hour 16-20, as emissions occurring at this time generated the highest 4-hr time-weighted average air concentrations; d: Emissions between Hour 16-24, as emissions occurring at this time generated the highest 8-hr time-weighted average air concentrations; e: Application rate at 246 lb/ac, assuming 75% of the field is fumigated beds.

Table 14. Estimated AITC air concentration (µg/m^3) at 1.7 ft above ground around field fumigated using drip application with tarp

Distance[a]	1 ac[b]			40 ac			100 ac		
	4hr[c]	8hr[d]	24hr	4hr	8hr	24hr	4hr	8hr	24hr
0 ft	4536[e]	3239	1560	10230	7332	3392	12241	8769	4053
25 ft	3479	2653	1171	9031	6646	2927	11008	8071	3590
50 ft	2731	2025	896	8024	5982	2565	9996	7397	3222
100 ft	2004	1422	586	6838	5162	2187	8782	6561	2774

250 ft	1144	756	302	5238	3867	1627	6902	5241	2182
500 ft	697	439	156	3961	2843	1156	5551	4039	1687
1000 ft	353	205	71	2845	1968	765	4150	2959	1150
2000 ft	152	90	30	1818	1212	461	2885	1951	764

a: Distance from the edge of the fumigated field; b: Size of the fumigated field; c: Emission occurs between Hour 16-20, as emissions occurring at this time generated the highest 4-hr time-weighted average air concentrations; d: Emissions between Hour 16-24, as emissions occurring at this time generated the highest 8-hr time-weighted average air concentrations; e: Application rate at 246 lb/ac, assuming 75% of the field is fumigated beds.

Table 15. Estimated AITC air concentration (µg/m^3) at 5 ft above ground around field fumigated using deep drip application without tarp

Distance[a]	1 ac[b]			40 ac			100 ac		
	4hr[c]	8hr[d]	24hr	4hr	8hr	24hr	4hr	8hr	24hr
0 ft	5432[e]	4309	1590	17132	13635	4790	21318	17009	5902
25 ft	5461	4357	1442	16776	13542	4544	20897	16863	5723
50 ft	4890	3907	1259	15797	12936	4228	19904	16208	5396
100 ft	3877	3066	929	13903	11603	3714	17975	14869	4797
250 ft	2327	1694	518	10853	8903	2846	14501	12107	3833
500 ft	1434	1005	274	8259	6604	2043	11664	9386	2987
1000 ft	733	475	126	5953	4590	1360	8720	6925	2046
2000 ft	318	210	53	3809	2830	822	6061	4567	1363

a: Distance from the edge of the fumigated field; b: Size of the fumigated field; c: Emission occurs between Hour 16-20, as emission occurring at this time generated the highest 4-hr time-weighted average air concentrations; d: Emissions between Hour 16-24, as emission occurring at this time generated the highest 8-hr time-weighted average air concentrations; e: Application rate at 246 lb/ac, assuming 75% of the field is fumigated beds.

Table 16. Estimated AITC air concentration (µg/m^3) at 1.7 ft above ground around field fumigated using deep drip application without tarp

Distance[a]	1 ac[b]			40 ac			100 ac		
	4hr[c]	8hr[d]	24hr	4hr	8hr	24hr	4hr	8hr	24hr
0 ft	9530[e]	7582	2789	21495	17161	6063	25719	20525	7245

25 ft	7309	6211	2093	18975	15555	5231	23130	18891	6417
50 ft	5738	4740	1602	16860	14001	4585	21003	17313	5760
100 ft	4210	3328	1047	14366	12082	3909	18451	15357	4959
250 ft	2404	1770	540	11006	9052	2908	14501	12268	3901
500 ft	1465	1026	278	8323	6653	2067	11664	9453	3015
1000 ft	741	480	127	5977	4607	1367	8720	6925	2056
2000 ft	320	211	54	3820	2837	824	6061	4567	1366

a: Distance from the edge of the fumigated field; b: Size of the fumigated field; c: Emission occurs between Hour 16-20, as emissions occurring at this time generated the highest 4-hr time-weighted average air concentrations; d: Emissions between Hour 16-24, as emissions occurring at this time generated the highest 8-hr time-weighted average air concentrations; e: Application rate at 246 lb/ac, assuming 75% of the field is fumigated beds.

Methodology appraisal:

Inclusion of 2018 meteorological data

This analysis was started in late-2018 when complete 2018 meteorological data was not available, thus the meteorological data from 2013-2017 was used. To determine whether using 2018 meteorological data generates greater air concentrations, this analysis selected Ventura County and modeled the air concentrations for 2018. The maximum concentrations in 2018 were then compared with those using 2013-2017 meteorological data. As shown in Table 17, maximum AITC air concentrations in 2013-2017 are comparable to those in 2018. Therefore, using 2013-2017 meteorological data is not expected to underestimate the AITC air concentrations.

Table 17. Comparison of maximum AITC air concentrations (µg/m^3) in Ventura County between 2013-2017 and 2018

	Maximum time-weighted average (TWA) air concentration[a] (µg/m^3)					
Distance[b]	4-hr[c]		8-hr[d]		24-hr	
	2013-2017	2018	2013-2017	2018	2013-2017	2018
			Adult			
0 ft	16601	12670	12900	11643	4134	3528

25 ft	16218	12499	11817	11307	4038	3350
50 ft	15108	11880	10995	10504	3827	3062
100 ft	13107	10489	9709	8976	3400	2565
250 ft	9388	7658	7263	6586	2624	1760
			Child			
0 ft	20809	16339	16336	15141	5207	4635
25 ft	18211	14677	13312	13115	4638	3895
50 ft	16059	13023	11770	11448	4133	3339
100 ft	13510	10984	10040	9389	3532	2685
250 ft	9501	7801	7366	6715	2665	1791

a: The treated field is 40 ac and fumigated using deep drip application without tarp. The modeling used the same 245 lb/ac application rate; b: distance from edge of treated field; c: emission occurs between Hour 16-20, as emissions occurring at this time generated the highest 4-hr time-weighted average air concentrations; d: maximum air concentrations occur with emissions at Hour 16-24, as emissions occurring at this time generated the highest 8-hr time-weighted average air concentrations.

Representativeness of weather stations

AERMOD relies on a single meteorological station to model air dispersion, thus selecting the representative station is critical to estimate AITC air dispersion after emissions from soil. All the selected stations in this analysis have automatically collected all available 1min-ASOS data, which can help decrease the percentage of hours with missing data. These selected stations are also close to agricultural/unpaved fields where the surface characteristics are similar to areas where AITC may be applied. Among them, meteorological data measured from Bakersfield Airport in Kern County may be influenced by the nearby urban/residential areas.

San Joaquin Valley Air Pollution Control District (SJVAPCD) provided modeled meteorological data for several agricultural locations within the Central Valley. The meteorological model used by SJVAPCD is the Fifth-Generation Penn State/National Center for Atmospheric Research (NCAR) Mesoscale Model (MM5), which is a weather-forecast mesoscale model. This analysis did not use this MM5 data as the latest available five-year data is 2007-2011. To assess the representativeness of the Bakersfield Airport data selected in this study, we modeled and compared 2007-2011 AITC air concentrations using either self-processed, airport meteorological data, or MM5-modeled meteorological data provided by SJVAPCD. As shown in Tables 18 and

19, using airport and MM5-modeled meteorological data generated comparable air concentration estimates at the same order of magnitude for all three averaging periods (4, 8, and 24 hr). Higher 24-hr time-weighted average (TWA) air concentrations are seen from using the MM5-modeled meteorological data, but they are on the same order of magnitude as those from the Bakersfield Airport meteorological data. Considering available data, this analysis concludes that using meteorological data measured from the Bakersfield Airport is appropriate for modeling air dispersions in agricultural areas of Kern County.

Table 18. Comparison of maximum AITC air concentrations (µg/m^3) in Kern County using either MM5-modeled (MM5) or self-processed airport (KBFL) meteorological data in 2007-2011

	Maximum time-weighted average (TWA) air concentration[a] (µg/m^3)					
Distance[b]	4-hr[c]		8-hr[d]		24-hr	
	KBFL	MM5	KBFL	MM5	KBFL	MM5
			Adult			
0 ft	12793	12944	10752	11114	3728	5044
25 ft	12615	12355	10590	10561	3583	4793
50 ft	12125	11448	9972	9840	3423	4371
100 ft	11024	10047	8948	8734	3068	3773
250 ft	8919	7911	7069	6924	2400	2840
			Child			
0 ft	16032	15517	13234	13337	4770	6248
25 ft	14449	13327	11942	11555	4223	5180
50 ft	13048	11803	10723	10286	3751	4539
100 ft	11433	10182	9221	8903	3204	3842
250 ft	9050	7950	7148	6974	2441	2864

a: The treated field is 40 ac and fumigated using shallow shank application without tarp. The modeling used 255 lb/ac application rate; b: distance from edge of treated field; c: Emission occurs between Hour 04-08; d: Emissions between Hour 00-08.

Table 19. Comparison of maximum AITC air concentrations (µg/m^3) in Kern County using either MM5-modeled (MM5) or self-processed airport (KBFL) meteorological data in 2007-2011

	Maximum time-weighted average (TWA) air concentration in[a] (µg/m^3)					
Distance[b]	4-hr[c]		8-hr[d]		24-hr	
	KBFL	MM5	KBFL	MM5	KBFL	MM5
			Adult			
0 ft	17944	19316	12783	17587	4292	5807
25 ft	17786	18281	11684	16639	4125	5518
50 ft	17274	16572	10886	15212	3941	5033
100 ft	15713	14623	9687	13317	3532	4343
250 ft	12267	11601	7528	10307	2763	3270
			Child			
0 ft	23068	23143	15752	21221	5491	7193
25 ft	21272	19293	13471	17634	4862	5963
50 ft	19213	17231	11910	15636	4318	5226
100 ft	16612	14941	10131	13481	3688	4423
250 ft	12560	11696	7665	10358	2810	3297

a: The treated field is 40 ac and fumigated using deep drip application without tarp. The modeling used 246 lb/ac application rate; b: distance from edge of treated field; c: Emission occurs between Hour 16-20; d: Emissions between Hour 16-24.

Conclusion:

AITC has not been used in California for soil fumigations, so its use pattern is unknown, and current knowledge on its soil emission profiles is limited. To address the lack of data, we developed the current method and conducted modeling under various use scenarios, including different use areas in California, sizes of fields, application methods and tarp conditions. The breathing-zone air concentrations were estimated for both adults and children. The estimated air concentrations are summarized in Tables 7 through 16 and can be further used for assessing short-term bystander exposure.

References:

ARB 2019. California Air Resources Board (ARB) Meteorological files. California Air Resources Board.

DPR 2018. California Department of Pesticide Regulation (DPR) Pesticide Use Reporting. California Department of Pesticide Regulation.

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Lakes Environmental. 2019a. AERMET View.

Lakes Environmental. 2019b. AERMOD View.

SCAQMD 2019. South coast Air Quality Management District (SCAQMD) meteorological data for AERMOD applications. South Coast Air Quality Management District.

USEPA 2019. U.S. Environmental Protection Agency (USEPA) surface and upper air databases. U.S. Environmental Protection Agency.

APPENDIX 2. AIR CONCENTRATION TABLES

Table 1. Estimated applicator exposure (i.e., 8-hr time-weighted average air concentrations) to allyl isothiocyanate using shallow shank applications with tarp

Exposure Duration	Short-term	Intermediate	Annual	Life-time
AITC ($\mu g/m^3$)	176	47	10	5
AITC (ppb)[a]	43	12	2	1

a: assuming at 25 degree C and 1 atm.

Table 2. Estimated applicator exposure (i.e., 8-hr time-weighted average air concentrations) to allyl isothiocyanate using shallow shank applications without tarp

Exposure Duration	Short-term	Intermediate	Annual	Life-time
AITC ($\mu g/m^3$)	1833	171	37	20
AITC (ppb[a])	452	42	9	5

a: assuming at 25 degree C and 1 atm.

Table 3. Estimated applicator exposure (i.e., 8-hr time-weighted average air concentrations) to allyl isothiocyanate using deep shank applications with and without tarp

Exposure Duration	Short-term	Intermediate	Annual	Life-time
AITC ($\mu g/m^3$, with tarp)	176	45	13	7
AITC (ppb[a], with tarp)	43	11	3	2
AITC ($\mu g/m^3$, without tarp)	1833	165	48	25
AITC (ppb[a], without tarp)	452	41	12	6

a: assuming at 25 degree C and 1 atm.

Table 4. Estimated applicator exposure (i.e., 8-hr time weighted average air concentrations) to allyl isothiocyanate using drip applications

Exposure Duration	Short-term	Intermediate	Annual	Life-time
AITC ($\mu g/m^3$)	53	15	2	1
AITC (ppb[a])	13	4	0.5	0.2

a: assuming at 25 degree C and 1 atm.

Table 5**.** Estimated loader exposure (i.e., 8-hr average air concentrations) to allyl isothiocyanate using shallow and deep shank applications

Exposure Duration	Short-term	Intermediate	Annual	Life-time
AITC (µg/m^3, shallow shank)	7702	1483	317	169
AITC (ppb[a], shallow shank)	1898	365	78	42
AITC (µg/m^3, deep shank)	7702	1431	412	219
AITC (ppb[a], deep shank)	1898	353	101	54

a: assuming at 25 degree C and 1 atm.

Table 6**.** Estimated allyl isothiocyanate exposure (i.e., 8-hr time-weighted average air concentrations) of tarp cutter, remover and puncher

Exposure Duration	Short-term	Intermediate	Annual	Life-time
AITC (µg/m^3, shallow shank)	4117	596	127	68
AITC (ppb[a], shallow shank)	1015	147	31	17
AITC (µg/m^3, deep shank)	4117	575	165	88
AITC (ppb[a], deep shank)	1015	142	41	22
AITC (µg/m^3, drip)	2979	431	54	29
AITC (ppb[a], drip)	734	106	13	7

a: assuming at 25 degree C and 1 atm.

Table 7. Estimated allyl isothiocyanate exposure (i.e., 8-hr time-weighted average air concentrations) for re-entry workers

Exposure Duration	Short-term	Intermediate	Annual	Life-time
AITC (µg/m^3, shallow shank)	173	150	41	22
AITC (ppb[a], shallow shank)	43	37	10	5
AITC (µg/m^3, deep shank)	173	145	62	33
AITC (ppb[a], deep shank)	43	36	15	8

AITC (µg/m^3, drip)	125	109	14	7
AITC (ppb[a], drip)	31	27	3	2

a: assuming at 25 degree C and 1 atm.

Table 8**.** Model estimated short term allyl isothiocyanate exposures (i.e., 8-hr time-weighted average air concentrations) for occupational bystanders

Field size	1 ac	40 ac	100 ac
AITC (µg/m^3, shallow shank w/ tarp)	350	1039	1290
AITC (ppb[a], , shallow shank w/ tarp)	86	256	318
AITC (µg/m^3, shallow shank w/o tarp)	3358	9981	12397
AITC (ppb[a], , shallow shank w/o tarp)	828	2460	3056
AITC (µg/m^3, deep shank w/o tarp)	2197	6530	8111
AITC (ppb[a], , deep shank w/o tarp)	542	1609	1999
AITC (µg/m^3, drip w/ tarp)	1841	5826	7267
AITC (ppb[a], drip w/ tarp)	454	1436	1791
AITC (µg/m^3, deep drip w/o tarp)	4309	13635	17009
AITC (ppb[a], deep drip w/o tarp)	1062	3361	4192

a: assuming at 25 degree C and 1 atm.

Table 9. Model estimated short term allyl isothiocyanate exposures (i.e., 24-hr time-weighted average air concentrations) for residential adult bystanders

Field size	1 ac	40 ac	100 ac
25ft			
AITC (µg/m^3, shallow shank w/ tarp)	127	400	504
AITC (ppb[a], , shallow shank w/ tarp)	31	99	124
AITC (µg/m^3, shallow shank w/o tarp)	1253	3947	4971
AITC (ppb[a], , shallow shank w/o tarp)	309	973	1225
AITC (µg/m^3, deep shank w/o tarp)	810	2553	3215
AITC (ppb[a], , deep shank w/o tarp)	200	629	792

AITC (µg/m^3, drip w/ tarp)	807	2542	3202
AITC (ppb[a], drip w/ tarp)	199	627	789
AITC (µg/m^3, deep drip w/o tarp)	1442	4544	5723
AITC (ppb[a], deep drip w/o tarp)	355	1120	1411
100ft			
AITC (µg/m^3, shallow shank w/ tarp)	82	327	422
AITC (ppb[a], , shallow shank w/ tarp)	20	81	104
AITC (µg/m^3, shallow shank w/o tarp)	807	3226	4167
AITC (ppb[a], , shallow shank w/o tarp)	199	795	1027
AITC (µg/m^3, deep shank w/o tarp)	522	2086	2695
AITC (ppb[a], , deep shank w/o tarp)	129	514	664
AITC (µg/m^3, drip w/ tarp)	520	2078	2684
AITC (ppb[a], drip w/ tarp)	128	512	661
AITC (µg/m^3, deep drip w/o tarp)	929	3714	4797
AITC (ppb[a], deep drip w/o tarp)	229	915	1182

a: assuming at 25 degree C and 1 atm.

Table 10. Model estimated short term allyl isothiocyanate exposures (i.e., 24-hr time-weighted average air concentrations) for residential child bystanders

Field size	1 ac	40 ac	100 ac
25ft			
AITC (µg/m^3, shallow shank w/ tarp)	184	461	565
AITC (ppb[a], , shallow shank w/ tarp)	45	114	139
AITC (µg/m^3, shallow shank w/o tarp)	1818	4544	5574
AITC (ppb[a], , shallow shank w/o tarp)	448	1120	1374
AITC (µg/m^3, deep shank w/o tarp)	1176	2939	3605
AITC (ppb[a], , deep shank w/o tarp)	290	724	889
AITC (µg/m^3, drip w/ tarp)	1171	2927	3590
AITC (ppb[a], drip w/ tarp)	289	721	885

AITC (µg/m^3, deep drip w/o tarp)	2093	5231	6417
AITC (ppb[a], deep drip w/o tarp)	516	1289	1582
100ft			
AITC (µg/m^3, shallow shank w/ tarp)	92	344	437
AITC (ppb[a], , shallow shank w/ tarp)	23	85	108
AITC (µg/m^3, shallow shank w/o tarp)	910	3395	4308
AITC (ppb[a], , shallow shank w/o tarp)	224	837	1062
AITC (µg/m^3, deep shank w/o tarp)	588	2196	2786
AITC (ppb[a], , deep shank w/o tarp)	145	541	687
AITC (µg/m^3, drip w/ tarp)	586	2187	2774
AITC (ppb[a], drip w/ tarp)	144	539	684
AITC (µg/m^3, deep drip w/o tarp)	1047	3909	4959
AITC (ppb[a], deep drip w/o tarp)	258	963	1222

a: assuming at 25 degree C and 1 atm.

APPENDIX 3. SYSTEMATIC REVIEW METHODS

Appendix 3. Systematic Review Methods

Specific Aims

- Conduct literature searches and use systematic review methods to identify studies published through July 2020 pertaining to understanding the potential human health hazards of allyl isothiocyanate as outlined in the PECO
- Track potentially relevant supplemental material, including mechanistic evidence informative for mode of action (MOA) analysis, ADME information, and studies conducted in non-mammalian model systems.

Methods

Database Searches

The database search was conducted by DPR Human Health Assessment (HHA) staff in PubMed (www.ncbi.nlm.nih.gov) using the common compound names as the key words ("AITC OR allyl isothiocyanate OR oil of mustard") on July 28, 2020. The resultant studies (2133) were exported from PubMed as a .csv file and imported into Microsoft Excel.

See file: AIT_Pubmed_Result

Stage 1: PECO criteria screening and supplemental material tagging

Abstracts and full texts (as necessary) were screened for fulfillment of *all* PECO criteria listed in Table 1. Date of screening decision, result, and reason were noted in excel file "AIT_Pubmed_Result". Studies that didn't meet PECO criteria were tagged with one of the reasons for exclusion listed in Table 2.

Table 23 PECO Criteria established for allyl isothiocyanate systematic literature review

PECO Element	Evidence
P	**Human:** Any population and lifestage (occupational or general population, including children and other sensitive populations). **Animal:** Nonhuman mammalian animal species (whole organism) of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages).
E	**Relevant forms and synonyms:** • Allyl isothiocyanate (57-06-7) • Allylisothiocyanate • N-acetyl-S-(N-allylthiocarbamoyl)cysteine • Oil of mustard • Horseradish extract
C	**Human:** A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits), or exposure for shorter periods of time. *Case reports and case series will be tracked as "potentially relevant supplemental information."* **Animal and Other:** A concurrent control group exposed to vehicle-only treatment or untreated control.
O	All health outcomes (both cancer and noncancer), excluding studies in which the compound is used as a positive control, or as a model to evoke itch, pain, irritant or inflammatory responses.
Mechanistic	Study was performed *in vitro* or in a relevant *in vivo* model, but does not meet PECO criteria
Genotoxicity	Studies describing genotoxicity or mutagenicity
ADME/PBPK	Studies describing physiologically-based pharmacokinetic (PBPK) models and/or metabolic products will be included.

Table 2 **Reasons for exclusion established for allyl isothiocyanate systematic literature review**

Reason for Exclusion	**Description of reason**
Case Study/Series	Case study or series of case studies
Conference Proceedings	Paper resulting from conference discussion
Environmental Fate/Occurrence	Studies focused on environmental fate or natural occurrence of AI
Exposure Route	Exposure through injection or other route not relevant to risk assessment
Foreign Language	Study was not available in English
Inflammation/Irritation/Itch/Pain	Study was focused on properties of AI as an inflammatory agent or use as an irritant, itch or pain inducer
Mixture	Active ingredient was only tested as part of a mixture of compounds
No Health Outcome	Health effects were not tested or observed
Non-Mammalian	Model organism was not mammalian
Review/Editorial/Commentary	Publication without original data or analysis
Target Organism	Study of effects on target organisms of AI (i.e. insects, nematodes, or fungi)
Used as Control	Active ingredient was used only as positive control to induce inflammation, itch, pain, or TRP1A activation, without further investigation into mechanism of effect or other health endpoints
Not AI	Compound studied was not allyl isothiocyanate
Withdrawn/Retracted	Study was withdrawn or retracted by publisher
Other	Other categories do not apply

Of the initial 2133 studies, 31 were identified as meeting all PECO criteria. An additional 19 studies were identified as potentially relevant genotoxicity studies, and 163 were identified as potentially relevant supplementary mechanistic studies.

Stage 2: Secondary review and summary table

PECO Studies

The 26 publications identified as meeting PECO criteria proceeded to stage 2 screening (*see file: AIT_SUM_TABLE_OPENPUB_REVIEW*). This included full text evaluation for meeting of PECO criteria, and summaries of model, sex, exposure route, doses tested, key endpoints/findings, and NOEL/LOEL values where relevant. Studies found to not meet PECO criteria after this level of review were noted and tagged with reason for exclusion (*see file: AIT_Pubmed_Result*). Studies with data that would not be useful for deriving points of departure did not proceed to Stage 3 review.

See File: AIT_SUM_TABLE_OPENPUB_REVIEW

Genotoxicity Studies

The 19 studies identified as potentially relevant genotoxicity studies were reviewed in a similar manner to the PECO studies, briefly summarizing model, exposure route, exposure concentrations and key findings. These studies were not included in Stage 3 review.

See File: AIT_SUM_TABLE_OPENPUB_GENOTOX

Mechanistic study evaluations and categorizations

Here, the goal was to identify studies tagged as mechanistic that may be informative to mechanisms of toxicity identified by the PECO above. Following the PECO screening, 158 abstracts were tagged as "Mechanistic". These studies were screened, evaluated, and summarized for lowest concentration tested, main topics, model used, NOEL/LOEL if applicable, and measured endpoints.

See File: AIT_SUM_TABLE_OPENPUB_MECHANISM

Criteria for inclusion were:

1. Concentrations below LD50 OR where evidence shows tissue concentrations are likely to be similar to those tested in normal exposure paradigms
2. Allyl isothiocyanate or NAC-AITC tested, not as a mixture
3. Endpoints related to allyl isothiocyanate mechanism of action

Studies evaluated at this level were not included in Stage 3 review.

Stage 3: Full Text Review and In-Depth Description of Findings

The 7 studies that made it through the Stage 2 review stage were given full text reviews. In-depth summaries of the methodologies and findings of each individual study were prepared, and data suitable for BMDS modeling were identified.

Additional Literature Searches Conducted

13 August 2019

Review of Hooper et al 2016 suggested cardiac effects may be related to calcium flux induced by AITC. Searched Pubmed for papers describing the effect of calcium flux on cardiac tissue.
Search Term: calcium atrioventricular block

23 October 2019

Urinary bladder carcinogenesis appears to be a critical endpoint for AITC. Searched Pubmed for papers describing mechanisms and/or modes of action for rat urinary bladder carcinogenesis.
Search Term: rat urinary bladder

13 January 2020

Discovered there are a handful of publications that spell the AI without a space (allylisothiocyanate). Searched for this term in PubMed and added the non-duplicates to PubMed_Results

Quality Control

Following completion of screening phases, all numbers and findings in prepared review documents were compared to the original document to ensure quality of data presented in them. Reviewer and date were noted in the QC column of tables.

APPENDIX 4. BENCHMARK DOSE MODELING

Appendix 4. Benchmark Dose Modeling

DPR used a benchmark dose (BMD; BMC for benchmark concentration) approach to derive points of departure (PODs) for all data that were amenable for modeling for this risk assessment. The US EPA Benchmark Dose Software (BMDS; version 3.1.2) was used to estimate the threshold of toxicity for a corresponding endpoint. Urinary bladder epithelial hyperplasia data from Cho et al. (2017) and nasal epithelial lesions from Randazzo (2017) were modeled. Quantal or dichotomous response data are reported as either the presence or absence of an effect (incidence). DPR's default threshold response level (the benchmark response or BMR) for quantal data is 10% (US EPA, 2012). Each model resulted in the generation of a corresponding benchmark dose or concentration (BMD or BMC) value as well as a value representing a 95% lower bound of the BMD/BMC (BMDL/BMCL) and a POD for the observed effect.

In the BMD approach, the data for each endpoint were used to generate a family of models. The goodness-of-fit was then evaluated for each model over the full dose range to select a "best" model for each effect's data set. The evaluation process was based on a hierarchical examination of (a) the results for statistical tests for goodness-of-fit, (b) the lowest Akaike Information Criteria (AIC) score for relative goodness-of-fit, (c) closeness of BMD/BMC and BMDL/BMCL to each other and to nearest dose levels for goodness-of-fit and model dependence, (d) visual inspection of lines over data points for goodness-of-fit and toxicological plausibility, (e) the magnitude of residuals for goodness-of-fit, and (f) considerations of sample size, variability, and whether there is maximum response at high dose.

The "best" models for the endpoint were next evaluated as part of the hazard identification process for their fitness to provide PODs for risk assessment. This evaluation reconsidered factors that included the toxicological plausibility and relevance of the effect, the quality of the data, as well as the relative magnitude of the threshold of toxicity represented by the BMDL/BMCL.

1. Modeling Urinary Bladder Epithelial Hyperplasia Endpoint from Cho *et al.* (2017) study.

The incidences of urinary bladder epithelial hyperplasia (simple) induced by horseradish extract (HRE) in male rats from Cho et al. (2017) were modelled as described above. The output report summarizing the results of the analysis using BMD modeling software (BMDS 3.1.2) is provided below.

Analysis Report (Generated on July 19, 2020)

Input data:

Male Urothelial Simple Hyperplasia Cho et al 2017		
[Add user notes here]		
Dose	N	Incidence
Dose	N	Incidence
0	32	0
4.1	32	9
15.7	32	24

Inputs for the selected model:

User Input

Info	
Model	frequentist Log-Probit v1.1
Dataset Name	Male Urothelial Simple Hyperplasia Cho et al 2017
User notes	[Add user notes here]
Dose-Response Model	P[dose] = g+(1-g) * CumNorm(a+b*Log(Dose))

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	Dose
Independent Variable	Incidence
Total # of Observations	3

Output from the selected model:

Model Results

Benchmark Dose	
BMD	1.932222489
BMDL	0.607506426
BMDU	3.206865846
AIC	78.01377168
P-value	0.999442988
D.O.F.	1
Chi^2	4.87359E-07

Model Parameters	
# of Parameters	3
Variable	Estimate
g	Bounded
a	-1.89653666
b	0.933675838

Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	4.87359E-07	0	32	-0.000698
4.1	0.281249993	8.999999781	9	32	8.592E-08
15.7	0.750000024	24.00000076	24	32	-3.11E-07

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-37.00688535	3	-	-	-
Fitted Model	-37.00688584	2	9.7472E-07	1	0.9992123
Reduced Model	-61.77518909	1	49.5366075	2	<0.0001

Plot of data selected model:

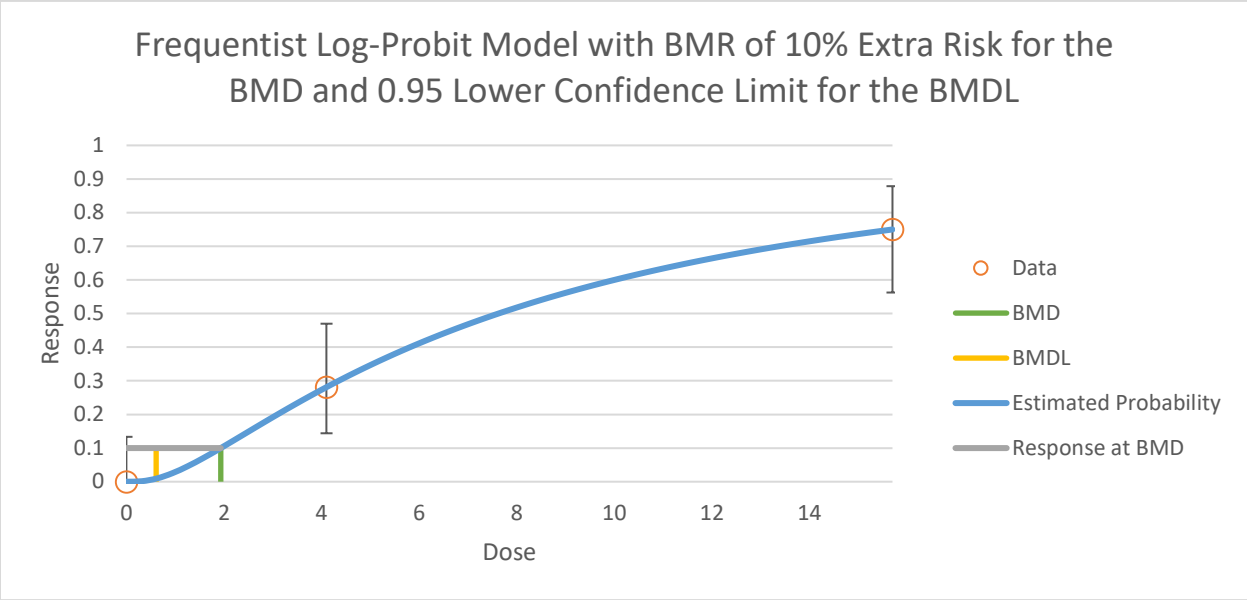

Table i. Summary results of models

Model	Analysis Type	Restriction	RiskType	BMRF	BMD	BMDL	BMDU	P Value	AIC	Unnormalized Log Posterior Probability	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	BMDS Recommendation	BMDS Recommendation Notes
Dichotomous Hill	frequentist	Restricted	Extra Risk	0.1	2.074755	0.622354	3.9293402	65535	82.01377168	-	3.60691E-09	-0.00069819	Viable - Alternate	BMD/BMDL ratio > 3 BMDL 3x lower than lowest non-zero dose
Gamma	frequentist	Restricted	Extra Risk	0.1	1.434706	0.92049	2.9906936	NA	80.0137717	-	-0.00070222	-0.00070222	Questionable	BMDL 3x lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Log-Logistic	frequentist	Restricted	Extra Risk	0.1	1.788026	0.627538	3.1032728	0.9994429	78.01377168	-	-0.000698111	-0.000698111	Viable - Alternate	BMDL 3x lower than lowest non-zero dose
Multistage Degree 2	frequentist	Restricted	Extra Risk	0.1	1.338675	0.920356	2.7347751	0.999436	78.0137717	-	-0.000698111	-0.000698111	Viable - Alternate	BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose
Multistage Degree 1	frequentist	Restricted	Extra Risk	0.1	1.22792	0.917916	1.682672	0.8185605	78.0669066	-	-0.000698353	-0.000698353	Viable - Alternate	BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose
Weibull	frequentist	Restricted	Extra Risk	0.1	1.407379	0.920433	2.8892052	NA	80.01377307	-	-0.001086484	-0.001086484	Questionable	BMDL 3x lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)

Logistic	frequentist	Unrestricted	Extra Risk	0.1	3.78415	2.826963	5.0463666	0.0248682	85.19568542	-	1.510914819	-1.618122687	Questionable	Goodness of fit p-value < 0.1
Log-Probit	**frequentist**	**Unrestricted**	**Extra Risk**	**0.1**	**1.932222**	**0.607506**	**3.2068658**	**0.999443**	**78.01377168**	**-**	**-0.000698111**	**-0.000698111**	**Viable - Recommended**	**Lowest AIC** **BMD/BMDL ratio > 3** **BMDL 3x lower than lowest non-zero dose**
Probit	frequentist	Unrestricted	Extra Risk	0.1	3.543038	2.706008	4.6630254	0.0309426	84.46677139	-	1.516865883	-1.487672084	Questionable	Goodness of fit p-value < 0.1

2. Modeling olfactory epithelial degeneration in male rats from Randazzo (2017) study.

The incidences nasal olfactory epithelia degeneration (mild, moderate, and marked combined) in male rats from Randazzo (2017) were modelled as described above. The output report summarizing the results of the analysis using BMD modeling software (BMDS 3.1.2) is provided below.

Analysis report: (generated on May 18, 2020)

Input data:

Olfactory epithelial degeneration in male rats from Randazzo (2017)		
[Add user notes here]		
Dose	N	Incidence
Dose	N	Effect
0	10	0
5	10	0
10	10	9
25	10	10

Model inputs:

User Input

Info	
Model	frequentist Log-Logistic v1.1
Dataset Name	DataSet Name1
User notes	[Add user notes here]
Dose-Response Model	P[dose] = g+(1-g)/[1+exp(-a-b*Log(dose))]

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	Dose
Independent Variable	Effect
Total # of Observations	4

Model Results

Benchmark Dose	
BMD	7.751339698
BMDL	4.783868355
BMDU	Infinity
AIC	8.502816831
P-value	0.999996293
D.O.F.	3
Chi^2	0.00057927

Model Parameters	
# of Parameters	3
Variable	Estimate
g	Bounded
a	Bounded
b	17.24769412

Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	1.523E-07	0	10	-0.00039
5	5.77789E-05	0.000577789	0	10	-0.024038
10	0.899898539	8.998985393	9	10	0.001069
25	0.999999985	9.999999848	10	10	0.0003903

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-3.250829734	4	-	-	-
Fitted Model	-3.251408416	1	0.00115736	3	0.9999895
Reduced Model	-27.67586637	1	48.8500733	3	<0.0001

Plot of data selected model:

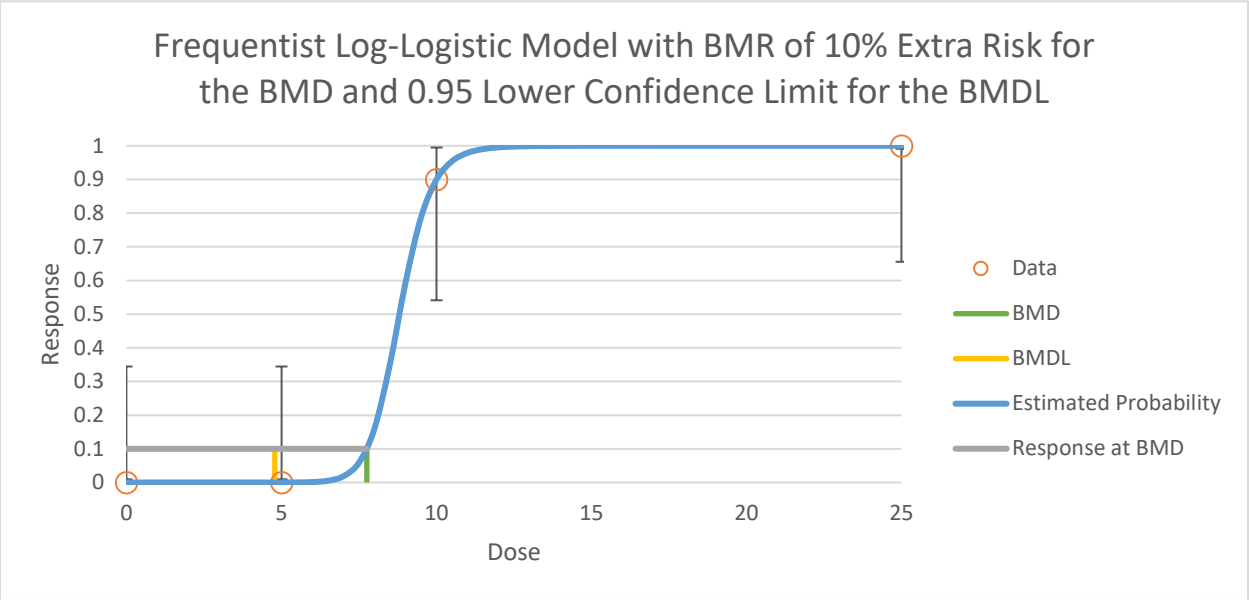

Table ii. Summary results of models

Model	Analysis Type	Restriction	RiskType	BMRF	BMD	BMDL	BMDU	P Value	AIC	Unnormalized Log Posterior Probability	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	BMDS Recommendation	BMDS Recommendation Notes
Dichotomous Hill	frequentist	Restricted	Extra Risk	0.1	7.75125	4.783882	Infinity	0.9997104	10.5028172	-	0.000920412	-0.000390256	Viable - Alternate	
Gamma	frequentist	Restricted	Extra Risk	0.1	5.667452	4.459193	6.6487903	0.7612721	11.46090542	-	-0.647464327	-0.000390998	Viable - Alternate	
Log-Logistic	**frequentist**	**Restricted**	**Extra Risk**	**0.1**	**7.75134**	**4.783868**	**Infinity**	**0.9999963**	**8.502816831**	**-**	**0.001069007**	**-0.000390256**	**Viable - Recommended**	**Lowest AIC**
Multistage Degree 3	frequentist	Restricted	Extra Risk	0.1	4.030274	2.415871	5.0081074	0.4147771	13.2590551	-	-1.492797582	-0.000390256	Viable - Alternate	
Multistage Degree 2	frequentist	Restricted	Extra Risk	0.1	2.871976	1.58971	3.8862511	0.0687291	18.83074915	-	-1.939644938	-0.000397516	Questionable	Goodness of fit p-value < 0.1 BMDL 3x lower than lowest non-zero dose

Multistage Degree 1	frequentist	Restricted	Extra Risk	0.1	0.942484	0.610428	1.4967617	0.0052993	25.80363066	-	-0.000390471	-0.000390471	Questionable	Goodness of fit p-value < 0.1 BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose
Weibull	frequentist	Restricted	Extra Risk	0.1	5.260886	0	Infinity	0.5434992	12.57461815	-	-0.93749593	-0.000390366	Unusable	BMD computation failed; lower limit includes zero BMDL not estimated
Logistic	frequentist	Unrestricted	Extra Risk	0.1	7.825026	4.725162	8.4203421	0.9999401	8.509059192	-	0.001946958	-0.000390256	Viable - Alternate	
Log-Probit	frequentist	Unrestricted	Extra Risk	0.1	8.29225	4.804618	Infinity	0.9999998	10.50166008	-	-3.76877E-09	-0.000390256	Viable - Alternate	
Probit	frequentist	Unrestricted	Extra Risk	0.1	6.532854	4.631007	7.60229	0.9917079	8.688585682	-	-0.293397893	-0.000103951	Viable - Alternate	

Reference:

Cho, Y. M., Hasumura, M., Imai, T., Takami, S., Nishikawa, A., and Ogawa, K. 2017. Horseradish extract promotes urinary bladder carcinogenesis when administered to F344 rats in drinking water. Journal of applied toxicology : JAT 37:853-862.

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.

US EPA. 2012. Benchmark Dose Technical Guidance. (June 2012). 1-99. EPA/100/R-12/001.