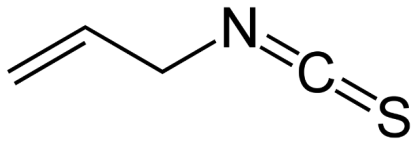
ALLYL ISOTHIOCYANATE

Risk Characterization Document

Occupational and Bystander Exposures



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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

List of Abbreviations

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

List of Abbreviations

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. Technical grade 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.

DPR's analysis of the potential human health risk of AITC was based on all available data as of April 2022. In April 2022, US EPA published its final work plan for evaluating AITC as a conventional pesticide. DPR will evaluate these new data as they become available and will update its occupational, bystander, and dietary risk assessments as appropriate.

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, and urinary bladder hyperplasia was observed between 2 and 104 weeks of oral AITC exposure. However, AITC by the inhalation route did not induce urinary bladder hyperplasia after 13 weeks of exposure at equivalent concentrations. This observation suggests that bladder effects were relevant for oral, but not inhalation exposures at the tested 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 plausible. However, a cancer potency analysis was not carried out because the apparent effect was observed only at a single high dose.

The selected chronic inhalation POD protects against the precursor lesions that are necessary for urinary bladder tumor development observed from the oral route. Protection of this upstream effect for both inhalation and oral routes further supports not assessing the cancer risk of AITC by the inhalation exposure.

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 relevant uncertainty factors. The most commonly used default uncertainty factors are 10x to account for differences in sensitivity between humans and laboratory animals (UF_A) and 10x to account for intraspecies (human) variability (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, 2012). 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, the product of the 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 variability 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. An 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).

Margin of Exposure (MOE) = POD (in ppb) / 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 the Exposure Assessment Document (DPR, 2022) and the Air Concentration Tables (Appendix A of this document).

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 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). US EPA announced in August 2021 its decision to consider AITC as a conventional fumigant rather than as a biopesticide (US EPA, 2021).

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: April 2022) (Appendix B). 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.

2020: DPR released the Draft Risk Characterization Document for Allyl Isothiocyanate (Occupational and Bystander Exposures) for pre-registrational risk assessment of AITC as a conventional fumigant use in California.

2021-22: US EPA published preliminary (June 2021) and final (April 2022) work plans for a registration review of AITC as a conventional pesticide.

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.

The Sentinel Event Notification System for Occupational Risk (SENSOR) program database, which is maintained by NIOSH with support from US EPA and with the participation of 13 states, collects pesticide illness reports mandated by FIFRA. The SENSOR database includes six pesticide illness reports concerning potential exposure to AITC-containing repellant products occurring between 2007 and 2014. These records mentioned self-reported clinical signs such as eye irritation and pain, cough, respiratory irritation, shortness of breath, and asthma attack/exacerbation. In 2017, an additional incident report was filed with US EPA, but mentioned no report of injury or environmental impact. No information in these reports could be used to determine a POD.

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 latest 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 oil of mustard OR allylisothiocyanate OR 3-isothiocyanatoprop-1-ene OR 57-06-7" on April 4, 2022. Further details of the systemic review process are described in Appendix B.

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 appear 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 metabolite 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 animal 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) bioassay, 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.

Muztar et al. (1979)

Study methods

AITC was administered through the diet for 31 days to five male Sprague-Dawley rats per group at 0 (control) or 0.1% AITC (w/v). Urine was collected every other day from day 21 to 31. Rats were sacrificed under anesthesia on day 31. Blood was analyzed for plasma glucose and uric acid levels. Neither AITC nor its metabolites were measured in urine or blood.

Urine volume

AITC increased urine volume by ~2-fold compared to controls. Control males excreted 119 ± 9 ml/kg/day and AITC-treated males excreted 231 ± 14 ml/kg/day.

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 preceded by a separate 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 rearing 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 (Goto *et al.*, 2010) and a book chapter that highlighted the invention (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

[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

identified the peak time of effect at 2 hours into the exposure period. Functional observational battery (FOB) measurements were made at the end of exposure, and motor activity assessments were initiated within 2 hours post-exposure, 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 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.

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.

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 within 2 hours of the end 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 vapor 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**
Motor Activity 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%)
FOB Measurements - Day 0 (mean ± SD)								
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. Considering the evidence that AITC is a dermal sensitizer, it is plausible that AITC might induce respiratory sensitization following inhalation exposure. However, no evidence could be found to support this. No NOELs were established in any of the dermal studies. The evaluation of these studies did not lead to the identification of any critical endpoints 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 endpoints 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 into 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. The study design was not adequate to test for statistical significance with respect to motor activity changes associated with AITC treatment. However, as the effect on motor activity trends with increasing dose, the possibility of biological significance exists. 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) (n = 16)[a]				9				8
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** (-12%)
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, and decreased total cholesterol in females exposed to analytical-grade AITC in drinking water for 13 weeks (Hasumura *et al.*, 2011).

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[b]	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 instead 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, although 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 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 *Drosophila 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 *Drosophila melanogaster*	Feeding: 0, 54 ppm	NA	Negative	Zimmering *et al.* (1989)
In vivo mutagenicity; induction of sex-linked recessive lethals in *Drosophila melanogaster*	Spray; pure spray, 10 second intervals for 9 minutes	NA	Positive (2.2% sex-linked lethals)	Auerbach and Robson (1946)
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)
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)

Table 9. Genotoxicity Studies of AITC

Test System(s)	Exposure Concentrations or Doses	S9 Fraction	Outcome(s)	Reference(s)
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)
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)

Table 9. Genotoxicity Studies of AITC

Test System(s)	Exposure Concentrations or Doses	S9 Fraction	Outcome(s)	Reference(s)
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; γH2AX; 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)
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	13	3	6	4
In vivo mutagenicity	3	1	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 two 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].

[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).

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 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.

[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).

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 ($p<0.05$) 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. The average intake of HRE in the low-, medium-, and high-dose groups was 2.7, 5.4, and 20.5 mg/kg/day, corresponding to an estimated AITC intake of 2.2, 4.4, and 16.8 mg/kg/day, respectively. 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	2.2	14***	3	5	0	ND
	0.01	15	4.4	15***	4*	1	0	ND
	0.04	15	16.8	15***	14***	9*	0	ND
32 weeks	0	15	0	11	7	7	0	3.7 ± 8.8
	0.005	15	2.2	13	12	12	0	7.7 ± 9.6
	0.01	15	4.4	14	13*	13*	0	35 ± 95
	0.04	15	16.8	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	Non-neoplastic 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 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]Acute POD is an estimated no-observed-effect level (ENEL) derived by dividing the acute lowest observed effect level (LOEL) by a dose extrapolation factor of 10; [b]Subchronic NOEL; [c]Chronic ENEL derived by dividing the subchronic no-observed effect level (NOEL) by a duration extrapolation factor of 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 of 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 C). 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 $BMDL_{10}$ of 4.9 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 7.1 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 = 4.9 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 = ppm x 4.06

(4.06 = molecular weight 99.15 / 24.45 liter of gas for 1 mole substance at 25°C, 1 atm)

POD = 4.9 mg/kg/day / 0.17 m^3/kg / 4.06 = 7.1 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, 2011).

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. Moreover, because the parental PODs (acute, subchronic) were lower than the corresponding developmental NOELs when compared after to route-to-route conversions, they are expected to be protective of both maternal and developmental effects.

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, 2012). 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, 2012; 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, 2012).

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 $DAF_{SYS \text{ or } POE}$

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, 2012)
DAF_{SYS}: dosimetric adjustment factor to account for inhalation absorption differences between rat and human respiratory tracts, equal to 1 (US EPA, 2012; 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 may be found in the Exposure Assessment Document (EAD) for AITC (DPR, 2022). 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 final evaluated exposure scenarios were based on the proposed label for Dominus® (96.3% 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 application method, 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). An 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. An 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 the EAD and Appendix A, respectively (DPR, 2022). 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 isothiocyanates, 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 lifetime). 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 114 (Table 18). Ten of 14 handler tasks generated short-term MOEs lower than the target of 30.

Under seasonal exposure conditions, handler MOEs ranged between 11 and 1875. Four of 14 handler tasks generated seasonal MOEs lower than the target of 30.

Under annual exposure conditions, handler MOEs ranged between 4 and 1875. Seven of 14 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 30 and 40 (Table 19). All four re-entry tasks generated short-term MOEs equal to or greater than the target of 30.

Under seasonal exposure conditions, all four re-entry tasks generated seasonal MOEs higher than the target of 30 (ranging between 104 and 341).

Under annual exposure conditions, re-entry worker MOEs ranged between 27 and 188. One of 4 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 15 and between 2 and 26, respectively (Table 21). At 25 and 100 feet from a 40-acre application, short-term MOEs ranged between <1 and 5 and between < 1 and 6, respectively. At 25 and 100 feet

from a 100-acre application, short-term MOEs ranged between < 1 and 4 and between < 1 and 5, 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 11 and between 2 and 23, 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 6, 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 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, broadcast, with tarp (Appendix A, Table 1)								
1250	32	39	3750	13	288	375	3	125
Shallow shank, bed/strip, with tarp (Appendix A, Table 1)								
1250	18	69	3750	2	1875	375	0.2	1875
Shallow shank, broadcast, without tarp (Appendix A, Table 2)								
1250	72	17	3750	20	188	375	5	75
Shallow shank, bed/strip, without tarp (Appendix A, Table 2)								
1250	283	4	3750	25	150	375	4	94
Deep shank, broadcast, with tarp (Appendix A, Table 3)								
1250	32	39	3750	13	288	375	3	125
Deep shank, broadcast, without tarp (Appendix A, Table 3)								
1250	72	17	3750	20	188	375	4	94
Drip application (Appendix A, Table 4)								
1250	11	114	3750	4	938	375	0.5	750
Loader, broadcast, shallow shank (Appendix A, Table 5)								
1250	1826	1	3750	351	11	375	91	4
Loader, bed/strip, shallow shank (Appendix A, Table 5)								
1250	1374	1	3750	104	36	375	17	22
Loader, broadcast, deep shank (Appendix A, Table 5)								
1250	1826	1	3750	351	11	375	72	5
Tarp cutter / remover / puncher, broadcast, shallow shank (Appendix A, Table 6)								
1250	401	3	3750	141	27	375	37	10
Tarp cutter / remover / puncher, bed/strip, shallow shank (Appendix A, Table 6)								
1250	301	4	3750	42	89	375	7	54
Tarp cutter / remover / puncher, broadcast, deep shank (Appendix A, Table 6)								
1250	401	3	3750	141	27	375	29	13
Tarp cutter / remover / puncher, broadcast, drip (Appendix A, Table 6)								
1250	301	4	3750	106	35	375	14	27

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**
Broadcast, shallow shank, re-entry at 4 days (Appendix A, Table 7)								
1250	41	30	3750	36	104	375	9	42
Bed/strip, shallow shank, re-entry at 4 days (Appendix A, Table 7)								
1250	31	40	3750	11	341	375	2	188
Broadcast, deep shank, re-entry at 4 days (Appendix A, Table 7)								
1250	41	30	3750	36	104	375	14	27
Drip, re-entry at 4 days (Appendix A, Table 7)								
1250	31	40	3750	27	139	375	4	94

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 A, Table 8)						
1250	81	15	242	5	303	4
Shallow shank, without tarp (Appendix A, Table 8)						
1250	785	2	2337	1	2924	< 1
Deep shank, without tarp (Appendix A, Table 8)						
1250	512	2	1525	1	1907	1
Drip, with tarp (Appendix A, Table 8)						
1250	441	3	1413	1	1762	1
Deep drip, without tarp (Appendix A, Table 8)						
1250	1031	1	3306	< 1	4123	< 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 A, Table 9)						
420	28	15	86	5	109	4
Shallow shank, without tarp, 25 feet (Appendix A, Table 9)						
420	261	2	844	< 1	1047	< 1
Deep shank, without tarp, 25 feet (Appendix A, Table 9)						
420	225	2	731	1	911	< 1
Drip, with tarp, 25 feet (Appendix A, Table 9)						
420	180	2	551	1	696	1
Deep drip, without tarp, 25 feet (Appendix A, Table 9)						
420	372	1	1175	< 1	1456	< 1
Shallow shank, with tarp, 100 feet (Appendix A, Table 9)						
420	16	26	70	6	91	5
Shallow shank, without tarp, 100 feet (Appendix A, Table 9)						
420	156	3	627	1	829	1
Deep shank, without tarp, 100 feet (Appendix A, Table 9)						
420	146	3	583	1	755	1
Drip, with tarp, 100 feet (Appendix A, Table 9)						
420	100	4	441	1	583	1
Deep drip, without tarp, 100 feet (Appendix A, Table 9)						
420	213	2	908	< 1	1205	< 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 A, Table 10)						
420	39	11	100	4	122	3
Shallow shank, without tarp, 25 feet (Appendix A, Table 10)						
420	343	1	933	< 1	1137	< 1
Deep shank, without tarp, 25 feet (Appendix A, Table 10)						
420	317	1	822	1	1008	< 1
Drip, with tarp, 25 feet (Appendix A, Table 10)						
420	247	2	639	1	785	1
Deep drip, without tarp, 25 feet (Appendix A, Table 10)						
420	497	1	1324	< 1	1624	< 1
Shallow shank, with tarp, 100 feet (Appendix A, Table 10)						
420	18	23	73	6	94	4
Shallow shank, without tarp, 100 feet (Appendix A, Table 10)						
420	167	3	645	1	848	< 1
Deep shank, without tarp, 100 feet (Appendix A, Table 10)						
420	158	3	615	1	782	1
Drip, with tarp, 100 feet (Appendix A, Table 10)						
420	114	4	461	1	604	1
Deep drip, without tarp, 100 feet (Appendix A, Table 10))						
420	234	2	950	< 1	1249	< 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 (DPR, 2022).

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 C 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 should be lower than acute PODs due to 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 of 6.6 mg/kg/day or $BMDL_{10}$ of 4.9 mg/kg/day (based on urothelial hyperplasia in Hasumura, et al., 2011; Appendix C) was converted to a human equivalent air concentration. The resulting value, 9.5 ppm or 7.1 ppm (for NOEL and $BMDL_{10}$, respectively), was close to the subchronic critical POD of 5 ppm[9] conferring a stronger degree of certainty in the latter value.

The subchronic inhalation NOEL of 5 ppm should protect against effects observed in both the subchronic oral (bladder hyperplasia) and inhalation (decreased motor activity and olfactory epithelial degeneration) studies. While the effects noted in inhalation and oral studies are qualitatively different, evaluation of the current dataset indicates that urinary bladder hyperplasia is the most sensitive non-acute endpoint and that protecting against this endpoint will protect against the occurrence of other subchronic or chronic effects, including urinary bladder tumors.

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, 2011; IPCS,

[9] *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 = 4.9 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; Therefore, POD = 4.9 mg/kg/day / 0.17 m^3/kg / 4.06 = 7.1 ppm. Similarly, a POD of 6.6 mg/kg/day results in 9.5 ppm.

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 produced 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 is likely induced by chronic oral exposure and is 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 the 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.

Repeated inhalation exposure to AITC does not present a risk for bladder tumors at the air concentrations tested. The route-to-route extrapolation herein showed that the critical chronic inhalation POD for decreased motor activity and olfactory epithelial degeneration will be protective of any systemic toxicity, including bladder hyperplasia and tumors. Because the weight of evidence supports urinary bladder hyperplasia as a precursor lesion for bladder tumors with a threshold dose response, a cancer potency was not calculated.

Undifferentiated leukemia

The incidence of undifferentiated leukemia[10] 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

[10] 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.

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:
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 studies 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 isothiocyanates. 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- to157-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, 2011). 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 scope of this assessment was the inhalation toxicity of AITC to align with its proposed use as a chemical fumigant. The RfCs herein are based on critical PODs derived from inhalation toxicity studies in rats using the current database. The most sensitive effects following acute inhalation exposure were decreased rearing counts and decreased motor activity in experimental animals. The most sensitive effects following subchronic inhalation exposure were metaplasia of the respiratory epithelium, degeneration of the olfactory epithelium, and decreased motor activity. No chronic inhalation study was available at this time.

This assessment did not include a cancer risk estimate for AITC. Undifferentiated leukemia was observed in one oral cancer bioassay. However, there is compelling evidence that the results were artifacts of the study design rather than AITC treatment. Urinary bladder hyperplasia was observed following subchronic oral AITC exposure, while urinary bladder tumors were observed in two oral cancer bioassays. There was a rapid induction of urinary bladder hyperplasia in the oral study. The weight of evidence analysis supported urinary bladder hyperplasia as the critical key event in the development of bladder tumors. However, there was no evidence of bladder hyperplasia following a 13-week inhalation study. Therefore, repeated inhalation exposure to AITC does not present a risk for bladder tumors at the air concentrations tested.

The acute inhalation POD and RfC should protect handlers and bystanders from the most sensitive effects noted in experimental studies. The subchronic inhalation POD and RfC should protect against effects observed in both the subchronic oral and inhalation studies. While the effects noted in inhalation and oral studies are qualitatively different, urinary bladder hyperplasia is the most sensitive non-acute endpoint. Protecting against this endpoint will protect handlers and bystanders against the occurrence of other subchronic or chronic effects, including urinary bladder tumors.

Non-oncogenic risks were calculated as 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}; derived from an interspecies uncertainty factor of 3, and an intraspecies uncertainty factor of 10). 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 114. Ten of 18 short-term MOEs were lower than the target of 30.
- Under seasonal exposure conditions, worker MOEs ranged between 11 and 1875. Four of 18 scenarios generated seasonal MOEs lower than the target of 30.

- Under annual exposure conditions, worker MOEs ranged between 4 and 1875. Eight of 18 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.

DPR's analysis of the potential human health risk of AITC was based on all available data as of April 2022. The analysis of exposure and risk included a systematic review and a comprehensive evaluation of guideline studies and open literature. However, gaps in the current database, especially for inhalation specific studies, resulted in the use of conservative estimates and assumptions. In April 2022, US EPA published its final work plan for evaluating AITC as a conventional pesticide. DPR will evaluate these new data as they become available and will update its occupational, bystander, and dietary risk assessments as appropriate.

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APPENDICES

APPENDIX A. AIR CONCENTRATION TABLES

Summary of Air Concentration Tables

Table 1. 8-hr time weighted average allyl isothiocyanate air concentrations for applicators using shallow shank applications with tarp

Application	Air concentration	Short-term	Intermediate	Annual	Life-time
Broadcast	µg/m^3	130	53	14	7
	ppb[a]	32	13	3	2
Bed/Strip	µg/m^3	72	7	1	0.6
	ppb[a]	18	2	0.2	0.1

a: assuming at 25 ºC and 1 atm.

Table 2. 8-hr time weighted average allyl isothiocyanate air concentrations for applicators using shallow shank applications without tarp

Application	Air concentration	Short-term	Intermediate	Annual	Life-time
Broadcast	µg/m^3	291	82	21	11
	ppb[a]	72	20	5	3
Bed/Strip	µg/m^3	1149	103	17	9
	ppb[a]	283	25	4	2

a: assuming at 25 ºC and 1 atm.

Table 3. 8-hr time weighted average allyl isothiocyanate air concentrations for applicators using broadcast deep shank applications with and without tarp

Application	Air concentration	Short-term	Intermediate	Annual	Life-time
with tarp	µg/m^3	130	53	11	6
	ppb[a]	32	13	3	1
without tarp	µg/m^3	291	82	17	9
	ppb[a]	72	20	4	2

a: assuming at 25 ºC and 1 atm.

Table 4. 8-hr time weighted average allyl isothiocyanate air concentrations for applicators using drip applications

Air concentration	Short-term	Intermediate	Annual	Life-time
µg/m^3	44	15	2	1
ppb[a]	11	4	0.5	0.2

a: assuming at 25 ºC and 1 atm.

Table 5. 8-hr time weighted average allyl isothiocyanate air concentrations for loaders using shallow and deep shank applications

Air concentration	Short-term	Intermediate	Annual	Life-time
Broadcast shallow				
µg/m^3	7408	1426	371	198
ppb[a]	1826	351	91	49
Bed/Strip shallow				
µg/m^3	5573	423	71	38
ppb[a]	1374	104	17	9
Broadcast deep				
µg/m^3	7408	1426	293	156
ppb[a]	1826	351	72	38

a: assuming at 25 ºC and 1 atm.

Table 6. 8-hr time weighted average allyl isothiocyanate air concentrations for tarp cutter, remover and puncher

Air concentration	Short-term	Intermediate	Annual	Life-time
Broadcast shallow shank				
µg/m^3	1625	573	149	80
ppb[a]	401	141	37	20
Bed/Strip shallow shank				
µg/m^3	1222	170	28	15
ppb[a]	301	42	7	4
Broadcast deep shank				
µg/m^3	1625	573	118	63
ppb[a]	401	141	29	16
Drip				
µg/m^3	1222	431	58	31
ppb[a]	301	106	14	8

a: assuming at 25 ºC and 1 atm.

Table 7. 8-hr time weighted average allyl isothiocyanate air concentrations for re-entry workers

Air concentration	Short-term	Intermediate	Annual	Life-time
Broadcast shallow shank				
µg/m^3	167	145	38	20
ppb[a]	41	36	9	5
Bed/Strip shallow shank				
µg/m^3	125	43	7	4
ppb[a]	31	11	2	1
Broadcast deep shank				
µg/m^3	167	145	56	30
ppb[a]	41	36	14	7
Drip				
µg/m^3	125	109	18	9
ppb[a]	31	27	4	2

a: assuming at 25 ºC and 1 atm.

Table 8. 8-hr time weighted average AITC air concentrations for occupational bystanders

Short-term, µg/m^3	1 ac	40 ac	100 ac
Shallow shank w/ tarp	330	982	1229
Shallow shank w/o tarp	3184	9481	11863
Deep shank w/o tarp	2077	6186	7739
Drip w/ tarp	1788	5731	7148
Deep drip w/o tarp	4185	13412	16728

Short-term, ppb	1 ac	40 ac	100 ac
Shallow shank w/ tarp	81	242	303
Shallow shank w/o tarp	785	2337	2924
Deep shank w/o tarp	512	1525	1907
Drip w/ tarp	441	1413	1762
Deep drip w/o tarp	1031	3306	4123

assuming at 25 ºC and 1 atm.

Table 9. 24-hr time weighted average AITC air concentrations for residential adult bystanders

Short-term, µg/m^3	1 ac	40 ac	100 ac
25ft			
Shallow shank w/ tarp	113	349	441
Shallow shank w/o tarp	1059	3425	4246
Deep shank w/o tarp	912	2965	3697
Drip w/ tarp	732	2235	2824
Deep drip w/o tarp	1510	4769	5906
100ft			
Shallow shank w/ tarp	66	282	368
Shallow shank w/o tarp	633	2542	3363
Deep shank w/o tarp	591	2367	3062
Drip w/ tarp	407	1788	2367
Deep drip w/o tarp	864	3683	4888

Short-term, ppb	1 ac	40 ac	100 ac
25ft			
Shallow shank w/ tarp	28	86	109
Shallow shank w/o tarp	261	844	1047
Deep shank w/o tarp	225	731	911
Drip w/ tarp	180	551	696
Deep drip w/o tarp	372	1175	1456
100ft			
Shallow shank w/ tarp	16	70	91
Shallow shank w/o tarp	156	627	829
Deep shank w/o tarp	146	583	755
Drip w/ tarp	100	441	583
Deep drip w/o tarp	213	908	1205

Assuming at 25 °C and 1 atm

Table 10. 24-hr time weighted average AITC air concentrations for residential child bystanders

Short-term, µg/m^3	1 ac	40 ac	100 ac
25ft			
Shallow shank w/ tarp	158	404	497
Shallow shank w/o tarp	1393	3786	4615
Deep shank w/o tarp	1288	3336	4091
Drip w/ tarp	1003	2591	3185
Deep drip w/o tarp	2016	5370	6590
100ft			
Shallow shank w/ tarp	75	296	381
Shallow shank w/o tarp	678	2617	3439
Deep shank w/o tarp	643	2495	3172
Drip w/ tarp	462	1869	2452
Deep drip w/o tarp	948	3855	5067

Short-term, ppb	1 ac	40 ac	100 ac
25ft			
Shallow shank w/ tarp	39	100	122
Shallow shank w/o tarp	343	933	1137
Deep shank w/o tarp	317	822	1008
Drip w/ tarp	247	639	785
Deep drip w/o tarp	497	1324	1624
100ft			
Shallow shank w/ tarp	18	73	94
Shallow shank w/o tarp	167	645	848
Deep shank w/o tarp	158	615	782
Drip w/ tarp	114	461	604
Deep drip w/o tarp	234	950	1249

Assuming at 25 °C and 1 atm

APPENDIX B. SYSTEMATIC REVIEW METHODS

Appendix B. Systematic Review Methods

Specific Aims

- Conduct literature searches and use systematic review methods to identify studies published through April 2022 (final search) pertaining to understanding the potential human health hazards of allyl isothiocyanate as outlined in the PECO criteria
- Track potentially relevant supplemental material, including mechanistic evidence informative for mode of action (MOA) analysis, ADME information, genotoxicity, and studies conducted in non-mammalian model systems.

Methods

Database Searches

The initial 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") in August 2019. The resultant studies (2133) were exported from PubMed as a .csv file and imported into Microsoft Excel. Additional searches are reported below.

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. Studies that didn't meet PECO criteria were tagged with one of the reasons for exclusion listed in Table 2.

Table B23 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 B2 **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 2308 studies identified by all searches, 51 were identified as meeting all PECO criteria. An additional 32 studies were identified as potentially relevant genotoxicity studies, 15 as potentially relevant ADME studies, and 324 were identified as potentially relevant supplementary mechanistic studies.

Stage 2: Secondary review and summary table

PECO Studies

The 51 publications identified as meeting PECO criteria proceeded to stage 2 screening. 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. Studies with data that would not be useful for deriving points of departure did not proceed to Stage 3 review.

Genotoxicity Studies

The 32 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.

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, 324 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.

Criteria for inclusion were:

1. Allyl isothiocyanate or NAC-AITC tested, not as a mixture
2. 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 January 2020

Identified publications where the AI name is spelled without a space (i.e., allylisothiocyanate). A PubMed search was conducted using this key word; non-duplicates were added to PubMed Results and screened for relevance.

31 July 2020

A PubMed search was conducted to identify any potentially relevant study articles published since the previous search. Key words: "AITC OR allyl isothiocyanate OR oil of mustard OR allylisothiocyanate OR 3-isothiocyanatoprop-1-ene OR 57-06-7." Non-duplicates were added to PubMed Results and screened for relevance. No newly identified publications were identified as relevant after screening.

30 July 2021

A PubMed search was conducted to identify any potentially relevant study articles published since the previous search. Key words: "AITC OR allyl isothiocyanate OR oil of mustard OR allylisothiocyanate OR 3-isothiocyanatoprop-1-ene OR 57-06-7." Non-duplicates were added to PubMed Results and screened for relevance. No newly identified publications were identified as relevant after screening.

4 April 2022

A PubMed search was conducted to identify any potentially relevant study articles published since the previous search. Key words: "AITC OR allyl isothiocyanate OR oil of mustard OR allylisothiocyanate OR 3-isothiocyanatoprop-1-ene OR 57-06-7." Non-duplicates were added to PubMed Results and screened for relevance. No newly identified publications were identified as relevant after screening.

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 C. BENCHMARK DOSE MODELING

Appendix C. 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:

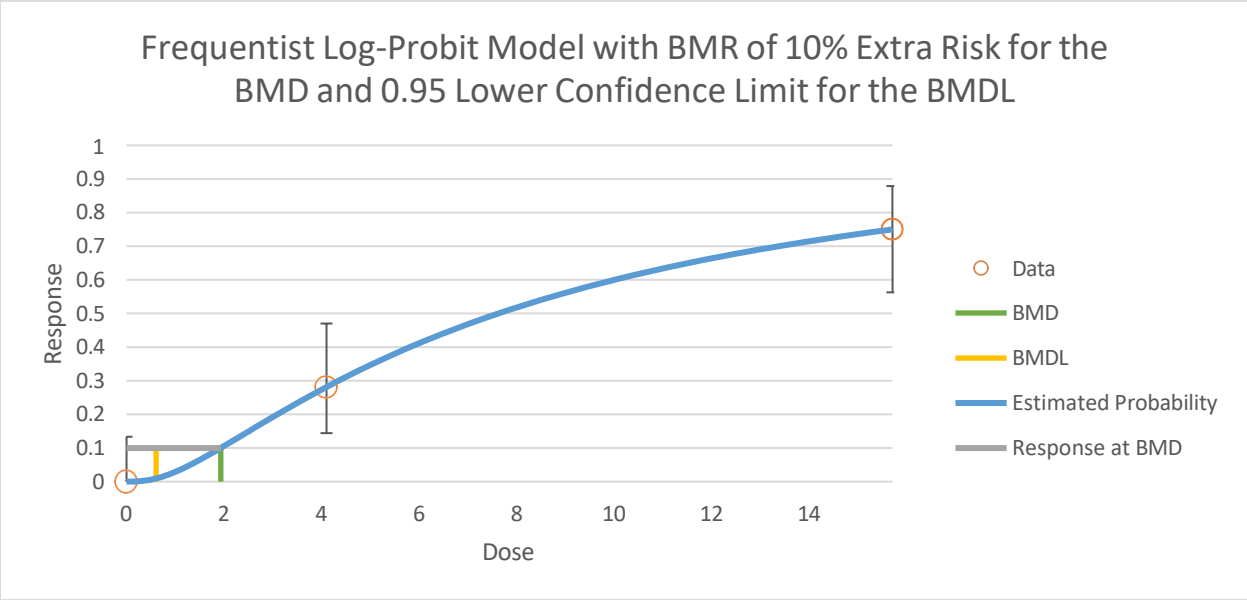

Table i. Summary results of models

Model	Analysis Type	Restriction	Risk Type	BMRF	BMD	BMDL	BMDU	P Value	AIC	Un-normalized 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	3.929402	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	2.990693 6	NA	80.01377 17	-	-0.00070222	-0.00070222	Questionable	BMDL 3x lower than lowest d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Log-Logistic	frequentist	Restricted	Extra Risk	0.1	1.788026	0.627538	3.103272 8	0.999442 9	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	2.734775 1	0.999436	78.01377 17	-	- 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	0.818560 5	78.06690 66	-	- 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	2.889205 2	NA	80.01377 307	-	- 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	5.046366 6	0.024868 2	85.19568 542	-	1.510914819	-1.618122687	Questionable	Goodness of fit p-value < 0.1
Log-Probit	**frequentist**	**Unrestricted**	**Extra Risk**	**0.1**	**1.932222**	**0.607506**	**3.206865 8**	**0.999443**	**78.01377 168**	**-**	**- 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	4.663025 4	0.030942 6	84.46677 139	-	1.516865883	-1.487672084	Questionable	Goodness of fit p-value < 0.1

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

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:

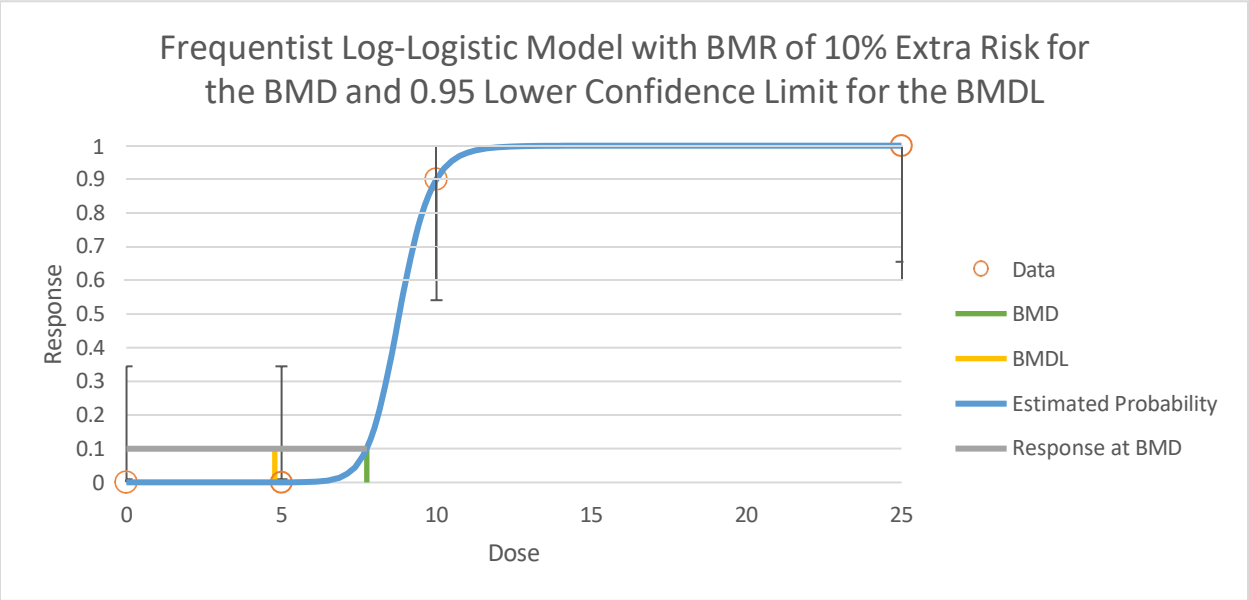

Table ii. Summary results of models

Model	Analysis Type	Restriction	Risk Type	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	0.999710 4	10.502817 2	-	0.000920 412	-0.000390256	Viable - Alternate	
Gamma	frequentist	Restricted	Extra Risk	0.1	5.667 452	4.459193	6.648790 3	0.761272 1	11.460905 42	-	-0.647464 327	-0.000390998	Viable - Alternate	
Log-Logistic	**frequentist**	**Restricted**	**Extra Risk**	**0.1**	**7.75134**	**4.783868**	**Infinity**	**0.999996 3**	**8.502816831**	**-**	**0.001069 007**	**-0.000390256**	**Viable - Recommended**	**Lowest AIC**
Multistage Degree 3	frequentist	Restricted	Extra Risk	0.1	4.030 274	2.415871	5.008107 4	0.414777 1	13.259055 1	-	-1.492797 582	-0.000390256	Viable - Alternate	
Multistage Degree 2	frequentist	Restricted	Extra Risk	0.1	2.871 976	1.58971	3.886251 1	0.068729 1	18.830749 15	-	-1.939644 938	-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	0.942 484	0.610428	1.496761 7	0.005299 3	25.803630 66	-	-0.000390 471	-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

Table ii. Summary results of models

Model	Analysis Type	Restriction	Risk Type	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
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	

3. Modeling urinary bladder simple hyperplasia in males from Hasumura (2011).

The incidence urinary bladder simple hyperplasia in male rats from Hasumura (2011) 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 April 2, 2018)

Input data:

Male Urinary Bladder Simple Hyperplasia from Hasamura (2011)		
[Add user notes here]		
Dose	N	Incidence
Dose	N	Incidence
0	10	0
10.7	10	2
16.3	10	3
30.6	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:

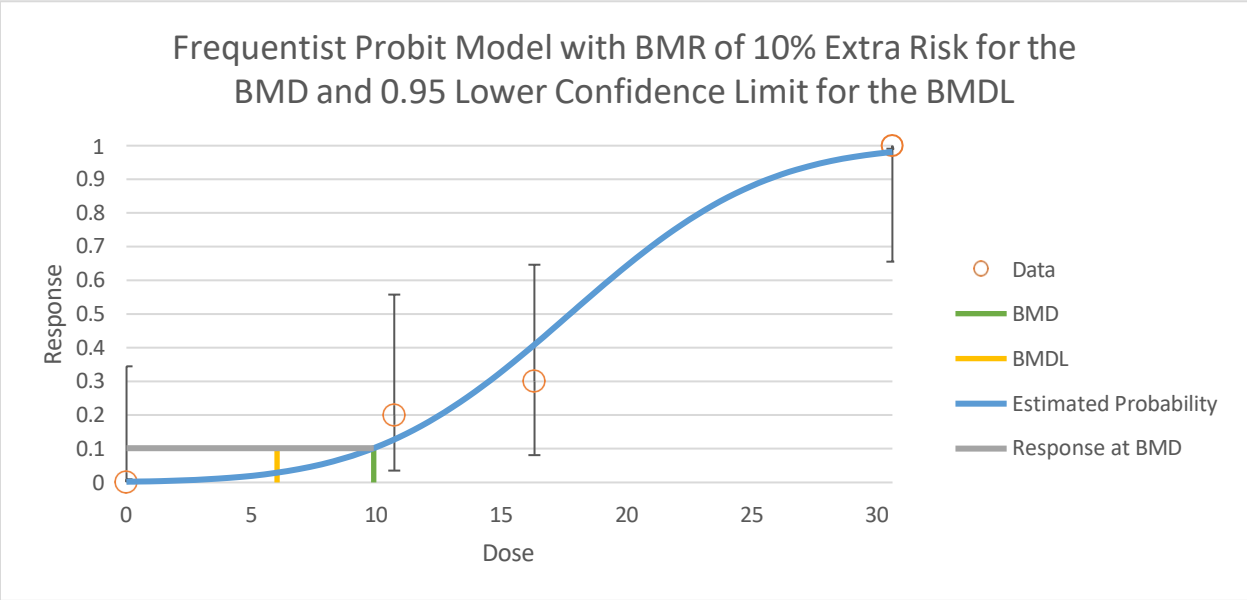

Table iii. Summary results of models

Model	Analysis Type	Restriction	Risk Type	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	10.51948	6.602297	13.28551	0.1121385	31.24332056	-	0.947565527	-0.000390256	Viable - Alternate	
Gamma	frequentist	Restricted	Extra Risk	0.1	10.25669	6.128283	12.923236	0.1612035	30.51094347	-	0.797035792	-0.000390325	Viable - Alternate	
Log-Logistic	frequentist	Restricted	Extra Risk	0.1	10.51955	6.602283	13.285501	0.1121349	31.2433203	-	0.947604926	-0.000390274	Viable - Alternate	
Multistage Degree 3	frequentist	Restricted	Extra Risk	0.1	9.288765	3.605986	11.462218	0.569852	27.63139655	-	0.473469587	-0.000390256	Viable - Alternate	
Multistage Degree 2	frequentist	Restricted	Extra Risk	0.1	6.661003	3.423869	8.5771894	0.4888708	27.77186797	-	-0.282518715	-0.000390256	Viable - Alternate	BMDL 3x lower than lowest non-zero dose
Multistage Degree 1	frequentist	Restricted	Extra Risk	0.1	2.471573	1.620089	3.9785561	0.0885583	33.52272083	-	-0.000390256	-0.000390256	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	10.08487	6.02626	13.264241	0.2689243	29.50930154	-	0.771150247	-0.000390315	Viable - Alternate	

Table iii. Summary results of models

Model	Analysis Type	Restriction	Risk Type	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
Logistic	frequentist	Unrestricted	Extra Risk	0.1	10.24382	6.472822	13.557919	0.5054838	27.82098173	-	0.795797325	-0.251814073	Viable - Alternate	
Log-Probit	frequentist	Unrestricted	Extra Risk	0.1	15.83177	6.78122	26.04086	0.1360374	31.22060498	-	3.46844E-07	-1.054093477	Viable - Alternate	
Probit	**frequentist**	**Unrestricted**	**Extra Risk**	**0.1**	**9.878488**	**6.012781**	**13.148768**	**0.5568187**	**27.55938247**	**-**	**0.686639484**	**-0.142724203**	**Viable - Recommended**	**Lowest AIC**
Quantal Linear	frequentist	Unrestricted	Extra Risk	0.1	2.471573	1.620087	3.9785563	0.038232	35.52272083	-	-0.000391086	-0.000391086	Questionable	Goodness of fit p-value < 0.1 BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose

3. Modeling urinary bladder simple hyperplasia in females from Hasumura (2011).

The incidence urinary bladder simple hyperplasia in female rats from Hasumura (2011) 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 April 2, 2018)

Input data:

Female Urinary Bladder Simple Hyperplasia		
[Add user notes here]		
Dose	N	Incidence
0	10	0
10.7	10	1
16.3	10	6
30.6	10	10

Model inputs:

User Input

Info	
Model	frequentist Multistage degree 3 v1.1
Dataset Name	Female Urinary Bladder Simple Hyperplasia
User notes	[Add user notes here]
Dose-Response Model	P[dose] = g + (1-g)*[1-exp(-b1*dose^1-b2*dose^2 - ...)]

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	4

Model Results

Benchmark Dose	
BMD	8.3474392
BMDL	3.644887697
BMDU	10.14238621
AIC	22.9242414
P-value	0.849702939
D.O.F.	3
Chi^2	0.799013657
Slope Factor	0.027435688

Model Parameters	
# of Parameters	4
Variable	Estimate
g	Bounded
b1	Bounded
b2	Bounded
b3	0.000181142

Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	1.523E-07	0	10	-0.00039
10.7	0.199009533	1.990095334	1	10	-0.784199
16.3	0.5436423	5.436423005	6	10	0.357803
30.6	0.994428996	9.944289961	10	10	0.23669

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-9.980946404	4	-	-	-
Fitted Model	-10.4621207	1	0.9623486	3	0.8103614
Reduced Model	-27.27418435	1	34.5864759	3	<0.0001

Plot of data selected model:

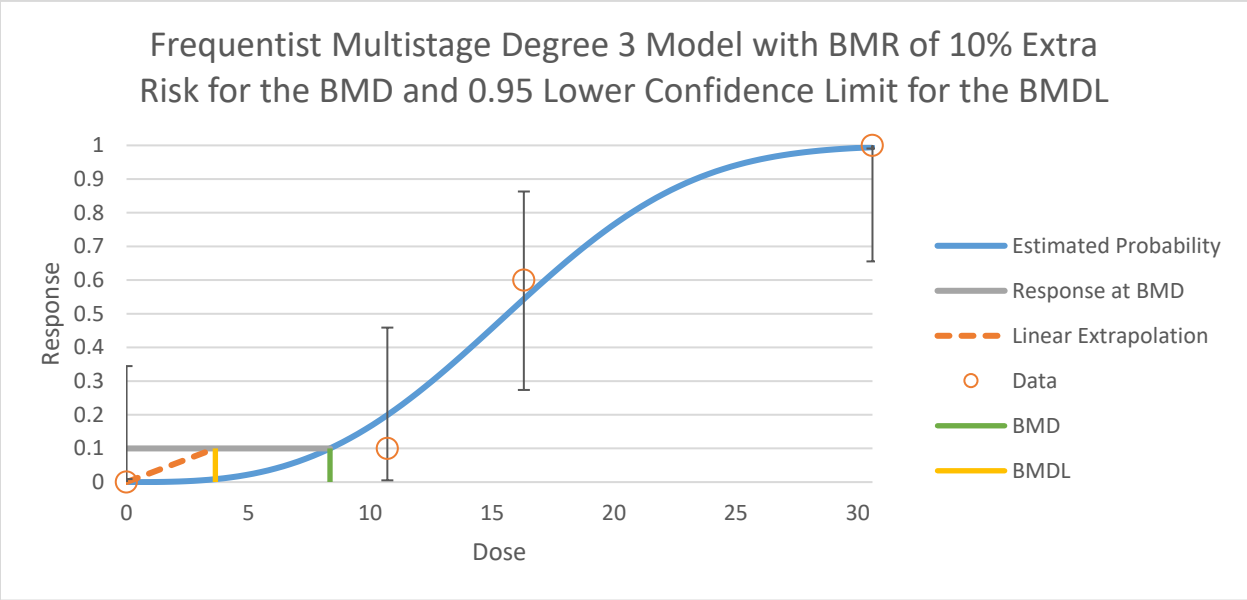

Table iv. Summary results of models

Model	Analysis Type	Restriction	Risk Type	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	10.94244	7.728883	13.18987	0.938691	24.17311932	-	0.143753585	-0.000390256	Viable - Alternate	
Gamma	frequentist	Restricted	Extra Risk	0.1	10.75614	7.200342	12.777064	0.9934777	23.98536133	-	0.033821312	-0.000390256	Viable - Alternate	
Log-Logistic	frequentist	Restricted	Extra Risk	0.1	10.94234	7.72888	13.189882	0.7220645	26.17311903	-	0.143692962	-0.000390429	Viable - Alternate	
Multistage Degree 3	**frequentist**	**Restricted**	**Extra Risk**	**0.1**	**8.347439**	**3.644888**	**10.142386**	**0.8497029**	**22.9242414**	**-**	**-0.784199208**	**-0.000390256**	**Viable - Recommended**	**Lowest AIC**
Multistage Degree 2	frequentist	Restricted	Extra Risk	0.1	5.855833	3.057627	7.4964021	0.4716208	25.45443277	-	-1.360916039	-0.000390256	Viable - Alternate	BMDL 3x lower than lowest non-zero dose
Multistage Degree 1	frequentist	Restricted	Extra Risk	0.1	1.994263	1.32516	3.1377803	0.0304091	33.76330689	-	-0.000397755	-0.000397755	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	10.69999	6.289064	13.653101	0.9999999	23.96189311	-	-3.70583E-06	-0.000390256	Viable - Alternate	
Logistic	frequentist	Unrestricted	Extra Risk	0.1	10.80383	6.718308	13.425161	0.9919389	23.9919678	-	0.04059503	-0.081147998	Viable - Alternate	

Table iv. Summary results of models

Model	Analysis Type	Restriction	Risk Type	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
Log-Probit	frequentist	Unrestricted	Extra Risk	0.1	10.82418	7.841542	12.767718	0.8230981	26.04461867	-	0.082667171	-0.000391396	Viable - Alternate	
Probit	frequentist	Unrestricted	Extra Risk	0.1	10.7037	6.259011	13.028785	0.9998622	23.96243925	-	0.001758918	-0.011098876	Viable - Alternate	
Quantal Linear	frequentist	Unrestricted	Extra Risk	0.1	1.994304	1.325168	3.1377919	0.0304097	33.76330687	-	-0.000391206	-0.000391206	Questionable	Goodness of fit p-value < 0.1 BMD 3x lower than lowest non-zero dose BMDL 3x lower than lowest non-zero dose

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