Allyl Isothiocyanate

Crops

Identification of Petitioned Substance

Chemical Name:
Allyl isothiocyanate

Other Names:
2-propenyl isothiocyanate
3-isothiocyanato-1-propene
Allyl isosulfocyanate

CAS Number:
Trade Names:
Oil of mustard
Allyl isothiocyanate (AITC)

57-06-7

Other Codes:
200-309-2 (EINECS No.)
24862709 (PubChem ID)

Summary of Petitioned Use

The petition before the National Organic Standards Board (NOSB) is to add allyl isothiocyanate (AITC, oil of mustard) as an allowed synthetic substance in organic crop production (§205.601) as a pre-plant fumigant. Specifically, AITC produced through chemical synthesis is petitioned for use. There is no related ruling offered by the National Organic Program (NOP) regarding the use of AITC in organic crop or livestock production from which comparisons may be drawn.

Although AITC is naturally generated through the composting and decomposition of mustard greens, the use of synthetic AITC as a pre-plant fumigant for organic crop production necessitates consideration of the chemistry of the concentrated substance in the terrestrial environment at the proposed application rates. Use of synthetic AITC must be evaluated against the criteria in the Organic Foods Production Act (OFPA), with consideration of the potential toxicity to beneficial soil microorganisms and terrestrial animals as well as alternative substances and practices available to organic crop producers.

Characterization of Petitioned Substance

Composition of the Substance:

The compositions of allyl isothiocyanate (AITC) formulations differ depending on the source of AITC and intended purpose of the product. At the molecular level, allyl isothiocyanate, with a molecular formula of C_4H_5NS, is a volatile organic compound composed of carbon, hydrogen, nitrogen and sulfur atoms (Chemical Book, 2010). Synthetic sources of AITC may contain traces of residual reagents and solvents used during synthesis, extraction, and/or purification of the substance. The synthetic sources being considered for pre-plant fumigation are typically greater than 95 percent pure (Isagro USA, 2013). Natural sources of AITC may contain small amounts of other plant-derived chemicals and solvent residues depending on the plant source and extraction technique employed to isolate AITC.

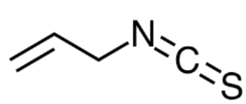


Figure 1. Allyl isothiocyanate (AITC) structural formula

Source or Origin of the Substance:

Both solvent extraction from natural plant sources and chemical synthetic procedures are used in the commercial production of allyl isothiocyanate (AITC). Historically, AITC has been extracted from the dried seeds of *Brassica nigra* (black mustard) for various industrial and therapeutic applications (Merck, 2006). Before being extracted, AITC is liberated from the glucosinolate sinigrin through reaction with myrosinase, an enzyme released when black mustard seeds are crushed (Romanowski, 2000). Chemical synthetic methods for AITC production from allyl iodide and potassium thiocyanate were published in the 1920s and variants of this process currently remain in use (Fan, 2012).

In addition to mustard seeds and foliage, a number of other plants (e.g., cabbage, kale, horseradish) naturally produce AITC. Likewise, synthetic AITC is added to processed foods as a flavoring agent and/or preservative. Table 1 below provides additional information on the occurrence of AITC in common food items. AITC concentrations observed in processed foods may represent naturally formed AITC released from glucosinolates and/or synthetic AITC intentionally added during food production.

Table 1. Occurrence of AITC in Common Foods

Product	AITC concentration (mg/kg)
Brussels sprouts	0.10
Cabbage	3.00
Cauliflower	0.08
Horseradish	1,350
Mustard	400–15,000
Baked goods	25–100
Condiments	700–5,000
Fats, oils	50
Fish products	0.05–0.07
Gelatins, puddings	1.00–2.00
Meat products	35–60
Seasonings, flavorings	6–30
Snack foods	48–100

Data Sources: Stofberg 1987; Velisek, 1995; Burdock, 2010
mg/kg = milligrams per kilogram (equivalent to parts per million, ppm)

<u>Properties of the Substance:</u>

Allyl isothiocyanate (AITC) is a colorless to light amber oily liquid with pungent odor. A summary of the chemical and physical properties of pure AITC is provided below in Table 2.

Table 2. Chemical and Physical Properties for AITC

Property	Value/Description
Color	Clear, colorless to light amber
Physical State	Oily liquid
Molecular Formula	$CH_2=CHCH_2N=C=S$ (C_4H_5NS)
Molecular Weight, g/mol	99.15
Freezing Point, ºC	–80; –102.5
Boiling Point, ºC	150–154
Density, g/mL	1.0126
Solubility in water at 20 ºC, mg/L	2,000 (soluble)
Solubility in organic solvents	Miscible in many organic solvents, including ethanol, ethyl ether, chloroform and benzene
Soil Organic Carbon-Water Partition Coefficient (K_{oc}), mL/g	260 (Moderately mobile in soils)
Aerobic Soil Half-life (DT_{50})	Literature suggests DT_{50} is 2 days
Hydrolysis	Facile (fully degraded within 80 minutes at pH 8)
Photodegradation	Photolysis not expected due to lack of chromophores; degraded in the atmosphere by photochemically produced hydroxyl radicals (half-life = 2.4 hours at 25 ºC).
Octanol/Water Partition Coefficient (K_{ow})	141

Vapor Pressure at 25 °C, mm Hg	3.7
Henry's Law Constant, atm•m^3/mol	5 x 10^{-6}

Data Sources: HSDB, 2013; US EPA, 2013a; Chemical Book, 2010.

Specific Uses of the Substance:

Synthetic allyl isothiocyanate (AITC) generally is used as an insecticide, bacteriocide, nematicide for certain crop protection applications, while synthetic and natural forms of AITC (i.e., volatile oil of mustard) are commonly used for the flavoring and preservation of foods (EFSA, 2010). The current review is focused on the US EPA-registered uses of AITC for pre-plant soil fumigation.

According to US EPA, AITC is a biochemical pesticide used as an "insect and animal repellent, feeding suppressant, insecticide, fungicide, herbicide and nematicide" (US EPA, 2013a). AITC is used heavily in the sugar industry due to its potent fungicidal activity. In this context, the substance protects sugar beets from fungi during storage (Romanowski, 2000). AITC has also been used for combatting *Hylemya brassicae* (the cabbage maggot fly) and other plant pests.

Numerous small-scale uses of AITC have also been reported in the available literature. For example, AITC may be used as a chemical feedstock in the production of war gases (Merck, 2006), a counter-irritant in medicine, a repellent for cats and dogs, a deterrent in some model airplane cements, and externally as a rubefacient (i.e., a substance for topical application that produces redness of the skin) (Gosselin, 1984).

Approved Legal Uses of the Substance:

The United States Food and Drug Administration (FDA) regulations allow the use of allyl isothiocyanate (AITC) as a food additive and active ingredient in certain drugs. According to FDA regulations, AITC may be added to food as a synthetic flavoring substance or adjuvant if the substance is used in the minimum quantity to produce the intended effects and in accordance with the principles of good manufacturing practice (21 CFR 172.515). FDA acknowledges that some over-the-counter drug products contain AITC as the active ingredient, although inadequate data are available to establish general recognition of safety and effectiveness for these products. Specifically, AITC may be used in nasal decongestant drug products (21 CFR 310.545(a)(6)(ii)) as well as commercially available fever blister and cold sore treatments (21 CFR 310.545(a)(10)(v)).

The United States Environmental Protection Agency (US EPA) regulates all non-food applications of AITC, including its use as a fungicide, insecticide and animal repellent. Although US EPA first registered oil of mustard for pesticidal use in 1962, AITC is the active ingredient in only six EPA-registered products (EPA, 2013a; US EPA, 2014). Currently registered products include outdoor animal repellants and broad spectrum pre-plant soil biofumigants for control of certain soil-borne fungi, nematodes, weeds and insects (EPA, 2014). According to EPA regulation, AITC is exempt from the requirement of a tolerance for residues when used as a component of food grade oil of mustard, in or on all raw agricultural commodities (40 CFR 180.1167). The petitioned non-food use of AITC as a pre-plant fumigant would not lead to residues on food due to the prescribed use pattern and rapid dissipation of the substance in the environment.

Action of the Substance:

Allyl isothiocyanate (AITC) controls soil-borne pathogens, nematodes and weeds by acting as a general irritant and/or desiccant that may alter respiration in target diseases and pests. Following injection into the soil using a drip irrigation system or tractor for shank application, AITC acts to reduce the populations of soil-borne plant diseases and pests (Isagro USA, 2013).

Research involving exposure of bacterial species to AITC has provided insight into the toxic mode of action of pesticides containing AITC toward microbes. Reduced oxygen uptake and inhibition of some enzymatic activities were observed in gram-positive bacteria exposed to AITC. In the bacterium *Escherichia coli*, AITC exposure leads to disruption of the cellular membrane with concomitant leakage of intracellular metabolites. In particular, treatment of *E. coli* with AITC results in significant loss of intracellular adenosine triphosphate (ATP), an energy carrier for numerous metabolic processes. Experiments in another gram-positive bacterium suggest that AITC alters bacterial proteins by oxidative cleavage of disulfide bonds and attack of free amino groups (Hyldgaard, 2012; Faleiro, 2011).

In addition to the toxic mode of action described above, AITC also acts as a potent animal repellent owing to its very pungent, irritating odor (US EPA, 2013a).

Combinations of the Substance:

Formulated pesticide products may contain more than one active ingredient, as well as surfactants, carriers and other adjuvants. The Isagro USA products included in the current petition contain synthetic allyl isothiocyanate (AITC) at 99.8% and 96.3% with no other active ingredients listed on the label (Isagro USA, 2013). Alternatively, a related insect control concentrate contains a mixture of AITC (3.7%) and capsicum oleoresin (0.42%) as the active ingredients (Champon, 2012). No other ingredients are listed on the label for this product. Dog and cat repellent products contain a complex mixture of essential oils and synthetic active ingredients, including oil of lemongrass (2.0%), oil of citronella (1.2%), AITC (0.20%), oil of orange (0.02%), methyl salicylate (0.02%), geraniol (0.04%), ionone alpha (0.01%), and oil of bergamot (0.11%). However, the manufacturer does not disclose the identity of other formulation ingredient on the label (Bakers, 2008). Overall, product formulations are considered confidential business information, and companies may reformulate products at any time.

Status

Historic Use:

Mustard oils produced through the pressing of black mustard seeds consist mostly of fatty acids as well as small amounts of allyl isothiocyanate (AITC). In fact, it is the AITC component of mustard oil that imparts its characteristic fragrance. Pressed mustard oil has been used for cooking and other cultural purposes for centuries, especially in northern India (Shiva, 2000). However, the available literature suggests that it is the fatty acid composition, and not the AITC content, that is responsible for its historical uses in Indian culture.

The process of biofumigation or 'green manuring' utilizes *Brassica* plants (e.g., the mustard plant) as cover crops. The biofumigation process takes advantage of the naturally occurring volatile compounds (allelochemicals such as AITC) that are specific to the *Brassicaceae* genus and are released from damaged plant tissues when the cover crop is plowed under before reaching full maturity. It has been found that volatile chemicals like AITC are useful in the control of soil-borne pests and pathogens. In situations where green manuring or plow down crops are not practical, growers may utilize de-oiled mustard seed meals and powders in which the fatty acids have been removed from the seed through extraction. Noticeable differences in the amount of AITC produced from these meals is observed depending on how the mustard was grown, handled and processed (MPT, 2011).

US EPA first registered naturally occurring AITC as a component of oil of mustard in 1962 (US EPA, 2013a). As the key component of Oil of Mustard, EPA determined that AITC was the residue of concern and characterized the hazards to human health and the environment in the Reregistration Eligibility Decision for Flower Oils and Vegetable Oils (US EPA, 1993), the Biopesticides Registration Action Document for Oriental Mustard Seed (US EPA, 2008), and the Vegetable and Flower Oil Summary Document for Registration Review (US EPA, 2010). Products containing synthetic AITC are currently registered as pre-plant soil biofumigants and animal repellents. The biofumigation products included in the current petition are registered for use as insecticides, fungicides, herbicides and nematicides, and are applied by drip or shank injection (US EPA, 2013a; Isagro USA, 2013).

Organic Foods Production Act, USDA Final Rule:

Neither of the terms "allyl isothiocyanate" or "oil of mustard" are mentioned in the Organic Foods Production Act of 1990 (OFPA). However, the OFPA states that handlings operators shall not "use any packaging materials, storage containers or bins that contain synthetic fungicides, preservatives, or fumigants." None of the National List sections for organic crop production (7 CFR 205.601 and 205.602), organic livestock production (7 CFR 205.603 and 205.604), or organic handling (7 CFR 205.605 and 205.606) mention the use of AITC, oil of mustard, or fumigants. The current petition represents the first consideration of synthetic AITC biofumigants in any form of organic production in the United States.

International

Guidelines and regulations from a number of international organizations and regulatory bodies indicate that allyl isothiocyanate (AITC) is not permitted for use in organic production. Below, international standards and regulations regarding the use of chemical fumigants in any form of organic production are summarized.

Canadian General Standards Board

Canadian organic production standards forbid the use of "equipment, packaging materials and store containers, or bins that contain a synthetic preservative or fumigant" (CAN, 2011a). In addition, allyl isothiocyanate and oil of mustard are not listed on the Canadian Organic Production Systems Permitted Substances List (CAN, 2011b).

Codex Alimentarius

Allyl isothiocyanate and oil of mustard are not allowed for use in organic production under the Codex guidelines. Although pre-plant soil fumigation is not specifically mentioned, item six of Annex 1states that steam sterilization may be used for the control of soil diseases and pests when proper rotation of soil renewal cannot take place (Codex, 2013). It is further noted in item seven that "only in cases of imminent or serious threat to the crop and where the measures identified in 6 (above) are, or would not be effective, recourse may be had to products referred to in Annex 2." Synthetic allyl isothiocyanate is not currently included in Annex 2 as a permitted substance for plant pest and disease control (Codex, 2013).

European Economic Community Council

Commission Regulations (EC) No 834/2007 and 889/2008 do not permit the use of allyl isothiocyanate, oil of mustard or any other synthetic substance for pre-plant soil fumigation. As stated in EC 889/2008:

> *Where plants cannot be adequately protected from pests and diseases by measures provided for in Article 12 (1)(a), (b), (c) and (g) of Regulation (EC) No 834/2007, only products referred to in Annex II to this Regulation may be used in organic production. Operators shall keep documentary evidence of the need to use the product.*

Neither "allyl isothiocyanate" nor "oil of mustard" is listed in Annex II of EC 889/2008.

Japan Ministry of Agriculture, Forestry, and Fisheries

According to the Japanese standard, allyl isothiocyanate and oil of mustard are not listed as allowed substances for any purpose in organic plant production. Carbon dioxide is the only synthetic substance allowed for plant pest and disease control, and is limited to use in storage facilities (JMAFF, 2005a). This allowance is also listed in the Japanese standards for organic livestock products (JMAFF, 2005b). No mention of allyl isothiocyanate, oil of mustard, or fumigation was identified in the Japanese standards for organic feeds (JMAFF, 2005c) and organic processed foods (JMAFF, 2005d).

International Federation of Organic Agricultural Movements

Under the IFOAM Norms, fumigation with ethylene oxide, methyl bromide, aluminum phosphide or other substance not contained in Appendix 4 of the Norms is a prohibited pest control practice (IFOAM, 2014). Neither "oil of mustard" nor "allyl isothiocyanate" is listed in Appendix 4, and therefore AITC is not allowed for use in any form of organic production.

United Kingdom Soil Association

According to section 4.13.3 of the UK Soil Association organic crop production guide, growers may not use chemical fumigants in stores or on premises where organic crops are stored (Soil Association, 2014). There is no mention of AITC as a permitted pre-plant soil fumigant under the UK Soil Association standards.

Evaluation Questions for Substances to be used in Organic Crop or Livestock Production

<u>Evaluation Question #1: Indicate which category in OFPA that the substance falls under:</u> (A) Does the substance contain an active ingredient in any of the following categories: copper and sulfur

compounds, toxins derived from bacteria; pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals; livestock parasiticides and medicines and production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleansers? (B) Is the substance a synthetic inert ingredient that is not classified by the EPA as inerts of toxicological concern (i.e., EPA List 4 inerts) (7 U.S.C. § 6517(c)(1)(B)(ii))? Is the synthetic substance an inert ingredient which is not on EPA List 4, but is exempt from a requirement of a tolerance, per 40 CFR part 180?

(A) As indicated in its chemical name and molecular formula (C_4H_5NS), allyl isothiocyanate (AITC) contains a single sulfur atom; therefore, AITC may be considered a sulfur compound.

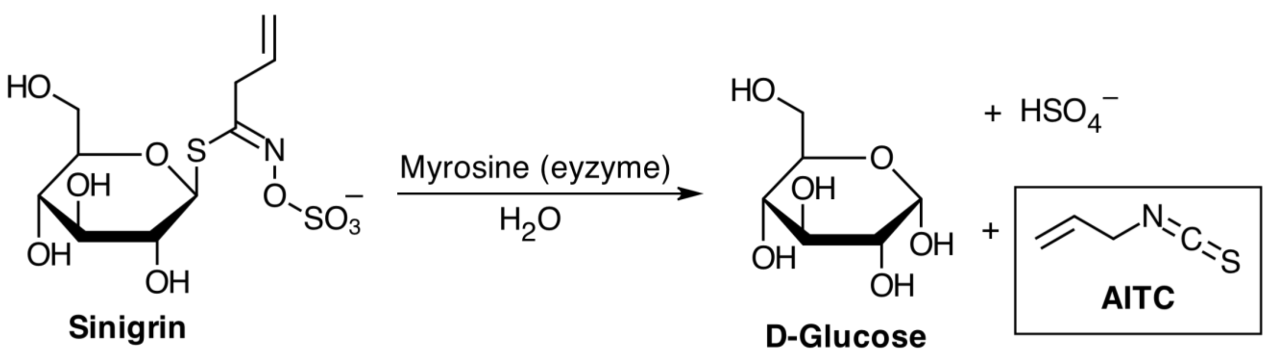
(B) AITC is an active ingredient; it is not considered an inert ingredient when used in pesticide products. According to EPA regulation, AITC is exempt from the requirement of a tolerance for residues when used as a component of food grade oil of mustard, in or on all raw agricultural commodities (40 CFR 180.1167). The petitioned non-food use of AITC as a pre-plant fumigant and rapid dissipation of AITC in the environment precludes the occurrence of AITC residues on food.

Evaluation Question #2: Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).

A variety of preparatory techniques are available for allyl isothiocyanate (AITC), ranging from the *in situ* generation of AITC in agricultural fields using *Brassica* cover crops and mustard seed meal to synthetic production processes such as extraction of AITC from natural plant sources and industrial production techniques. The sections below provide details regarding three general strategies of producing AITC as a soil biofumigant.

Natural Formation from Plant Materials

Growers seeking to reduce the application of chemical inputs commonly utilize specialized cover crops for soil quality improvement and pre-plant pest management. In particular, cover crops consisting of mustard plants and related *Brassica* species (i.e., cole crops) are capable of naturally producing AITC for soil biofumigation (Haramoto, 2004). Mustards and related plants contain elevated amounts of glucosinolates[a] and the hydrolase enzyme, myrosinase (Borek, 1995). The glucosinolate sinigrin and enzyme myrosinase remain in separate compartments of the plant cell under typical growing conditions (Romanowski, 2000). Once the plant tissue is damaged, however, the enzyme myrosinase is released and liberates AITC from the glucosinolate sinigrin through enzymatic hydrolysis (bond cleavage with water) (Scheme 1). Therefore, flailing and plowing under mustard and related cover crops is a natural way of generating AITC in soil for pre-plant soil fumigation.



Scheme 1. AITC is naturally produced through the enzymatic reaction of myrosinase with the glucosinolate sinigrin under moist conditions.

[a] Glucosinolates are organic anions containing a D-thioglucose moiety, a sulfonated oxime (N-O bonded group) and a unique side chain.

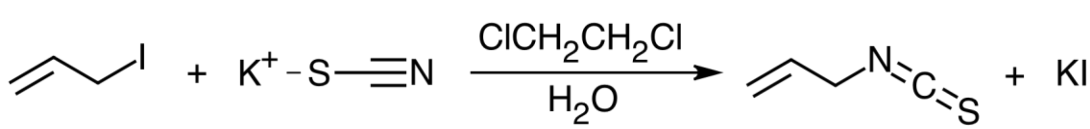
When living plant tissues containing the glucosinolate sinigrin and the enzyme myrosinase (e.g., mustard plants) are crushed, water within the plant material is available to facilitate AITC formation. Alternatively, crushing dried mustard seed in the absence of water does not lead to an immediate reaction. Commercial mustard meals prepared through the crushing of mustard seeds followed by removal of fatty acids using a hexane wash are marketed as sources of AITC for biofumigation (US EPA, 2008). Mincing mustard seed brings the key reaction components into physical proximity, but the enzymatic reaction resulting in liberation of AITC from the sinigrin precursor is initiated only through the introduction of water. AITC is released when mustard seed meal is wetted, and therefore incorporation of mustard seed meal into moist soil represents a natural approach to generating AITC on-site for soil biofumigation (Johnson, 2011). With the typical application rate of 1 ton/acre (Farm Fuel Inc., 2013b) and AITC content of mustard seed meal ranging from 2–17 g/kg (Dai and Lim, 2014), the equivalent application rate of AITC is 4–33 lb/acre. The available resources indicate that some organic growers, including organic strawberry producers, are adopting mustard seed meal as a natural option for soil pest control.

Extraction from Natural Sources

Chemically pure AITC was first produced through the extraction of the appropriate plant materials (e.g., mustard leaves and seeds) followed by distillation of the resulting extract residue. Much like the natural process described above, extraction of AITC involves the initial liberation of AITC from the glucosinolate sinigrin through reaction with myrosinase, an enzyme released when black mustard seeds and plant tissues are crushed (Romanowski, 2000). The original and more recent patent literature describes processes in which mustard seed is cracked and then combined with water to activate the enzyme myrosinase for AITC production (Mustakas, 1963; Sakai, 2005a and 2005b). This "activated mustard slurry" is allowed to react for a specified period of time at slightly elevated temperatures (e.g., 50 ºC) before the AITC generated through enzymatic hydrolysis of sinigrin is separated from the bulk mustard seed residue. The ground mustard seed powders used in these processes are commonly defatted (devoid of fatty acids) through washing with hexanes to accelerate the hydrolysis reaction. Isolation of the resulting AITC from mustard slurries typically involves solvent (e.g., hexane, ethanol, diethyl ether) extraction and/or steam distillation (Sharma, 2012; Li, 2010).

Chemical Synthesis

Commercial sources of AITC are primarily produced using chemical synthetic methods. Specifically, AITC is produced on an industrial scale by reaction of allyl chloride, bromide or iodide ($CH_2=CH–CH_2X$, where X = Cl, Br or I) with alkali rhodanides (e.g., potassium thiocyanate) in a two-phase solvent system comprised of water and 1,2-dichloroethane (Scheme 2) (Romanowski, 2000). Numerous variants of this basic chemical reaction have been published in the scientific and patent literature. As an example, catalytic amounts of methyl trioctyl ammonium chloride [$(CH_3)(C_8H_{17})_3NCl$] were used in the reaction between allyl bromide ($CH_2=CH–CH_2Br$) and potassium thiocyanate in acetonitrile solvent (Patent CN102452967 A). Alternatively, a method involving the initial reaction of allyl amine ($CH_2=CH-CH_2-NH_2$) and carbon disulfide (CS_2) followed by oxidation of the reaction intermediate using a peroxide to form AITC recently appeared in the published patent literature (Patent CN101735128 B). This method is not currently employed in the industrial production of AITC.



Equation 2. AITC can be industrially produced through treatment of allyl halides such as allyl iodide with alkali rhodanides such as potassium thiocyanate in a mixture of water and 1,2-dichloroethane.

<u>Evaluation Question #3:</u> Discuss whether the petitioned substance is formulated or manufactured by a chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).

Allyl isothiocyanate (AITC) may be considered synthetic or natural (nonsynthetic) depending on the method utilized for its production. Under the USDA organic regulations, the NOP defines synthetic as "a substance that is formulated or manufactured by a chemical process or by a process that chemically changes a substance extracted from naturally occurring plant, animal, or mineral sources, except that such term shall not apply to substances created by naturally occurring biological processes" (7 CFR 205.2). According to this definition, *in situ* production of AITC from mustard and related cover crops or mustard seed meals constitutes a natural (nonsynthetic) process. In contrast, industrial sources of AITC are produced through chemical synthesis, and would therefore be considered synthetic due to the application of synthetic chemicals (reagents and solvents) in both the production as well as the purification/processing of crude AITC. It is unlikely that residues of chemical precursors will persist in the petitioned form of the substance, synthetic AITC.

<u>Evaluation Question #4:</u> Describe the persistence or concentration of the petitioned substance and/or its by-products in the environment (7 U.S.C. § 6518 (m) (2)).

This section summarizes technical information related to the persistence of allyl isothiocyanate (AITC) in soil, water, and the atmosphere. The compiled data indicate that AITC is readily biodegradable in all three environmental compartments. Production and use of AITC as a flavoring agent and ingredient in ointments may result in its release to the environment through waste streams, while its use as a soil fumigant and animal repellent will necessarily result in direct release to the environment. Because AITC is a volatile organic compound and has the potential to cause irritation and systemic toxicity, exposure of and potential adverse effects on non-target receptors (humans and wildlife) is likely considering its proposed use pattern as a pre-plant soil biofumigant at the application rates proposed (85–340 lbs/acre). In addition to synthetic sources, AITC is also present in the seeds and leaves of plants such as mustards, horseradish and broccoli (HSDB, 2013; US EPA, 2013a).

Soil incorporation of AITC is most relevant as the petitioned use involves addition of AITC to soils as a pre-plant biofumigant. AITC released to soil is expected to have moderate mobility based on the calculated K_{oc} of 260 mL/g. Significant volatilization from moist and dry soils is expected for AITC based on its Henry's Law constant and vapor pressure that are on the same order of magnitude as these same parameters for conventional fumigants. Decomposition half-lives for AITC in soil range from 20 to 60 hours. The mean soil half-life of 47 ± 27 hours (approximately two days) was determined based on dissipation studies in six different soil types, with the greatest AITC degradation rates observed in soils that have high organic carbon and total nitrogen contents. Comparison of aerobic (with oxygen) and anaerobic (without oxygen) soil dissipation studies indicates that biodegradation from soil microbial activity is not an important fate process for AITC (HSDB, 2013; US EPA, 2013a, 2013b).

Although AITC is not intended to be applied directly to water, runoff from treated fields may lead to releases of the substance to neighboring water bodies. When released to water, AITC is expected to adsorb to suspended solids and sediment based on its estimated organic carbon partition coefficient (K_{oc}). Half-lives for volatilization of AITC from a model river (6.5 hours) and model lake (5 days) are relatively short; however, adsorption of AITC to suspended solids and sediment in the water column may diminish volatilization from water surfaces. Adsorption may increase the half-life of volatilization from a model pond to an estimated 30 days. With a bioconcentration factor (BCF) of 12, it is unlikely that AITC will bioaccumulate in aquatic organisms. Hydrolysis is expected to be an important environmental fate process since isocyanates readily hydrolyze at environmentally relevant pH levels of five to nine (HSDB, 2013).

At environmentally relevant pH ranges (pH between six and eight), AITC will degrade completely. Within this pH range, the primary degradates identified include allyl thiocyanate (ATC), allyl amine (AA) and carbon disulfide (CDS). The profile of decomposition products for AITC in water is largely dependent on the temperature and pH of the aqueous medium. AITC and its isomerization product ATC are typically observed under environmental conditions. Under basic (high pH) conditions, AA, CDS, allyl dithiocarbamate (ADTC) and diallylthiourea (DATU) were the major reaction products identified. AA and CDS were also the primary degradates of AITC in neutral (pH 6) and slightly acidic (pH 4) media. Traces of other minor degradation products have also been observed in published decomposition studies (Pecháček, 1997). AA is expected to biodegrade quickly in the environment, making human and animal exposure to AA unlikely following AITC application to soils (US EPA, 2013a). Background levels of CDS are found naturally in the environment (US EPA, 2013a). However, assuming an AITC application rate of 300 lbs/acre (Isagro USA, 2013) and 25% transformation to CDS (Pecháček, 1997), it is conceivable that approximately 60 lbs/acre of CDS would be released to the environment from a single application of synthetic AITC. This concentration of CDS in the environment is not representative of naturally occurring background levels.

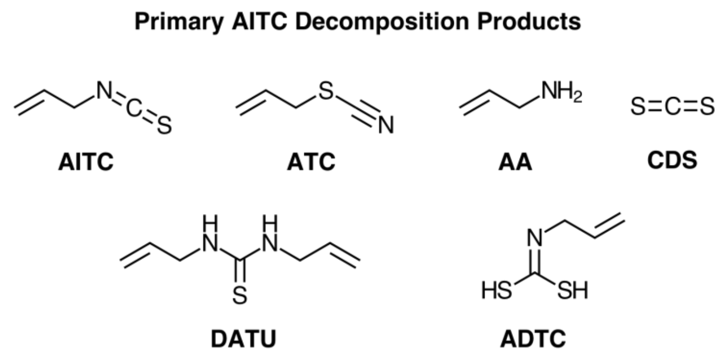


Figure 2. AITC readily isomerizes to ATC and forms a variety of decomposition products in water.

AITC released to the air will exist primarily in the vapor form considering the relatively high vapor pressure of 3.7 mm Hg at 25 ºC. Direct photolysis of AITC by sunlight will not occur due to the absence of chromophores in the AITC chemical structure that would absorb radiation at wavelengths greater than 290 nm. However, vapor-phase AITC undergo facile degradation in the atmosphere through reaction with photochemically produced hydroxyl radicals (half-life = 2.4 hours) (HSDB, 2013).

Evaluation Question #5: Describe the toxicity and mode of action of the substance and of its breakdown products and any contaminants. Describe the persistence and areas of concentration in the environment of the substance and its breakdown products (7 U.S.C. § 6518 (m) (2)).

This section summarizes allyl isothiocyanate (AITC) toxicity to four taxa groups, including mammals, fish, aquatic invertebrates and soil microorganisms. Overall, it can be concluded that the toxicity rating of AITC ranges from toxic to practically non-toxic to the few non-target taxa groups evaluated in the literature.

The risk of toxicity associated with mammalian exposure to AITC is variable depending on the source and concentration of AITC used in toxicity testing. According to US EPA, oil of mustard containing AITC at a concentration of 4.43% is practically non-toxic (Category IV) via the acute oral and inhalation routes of exposure. In addition, oil of mustard is not an acute dermal irritant (Category IV) or sensitizing agent. Studies further suggest that AITC is slightly toxic via the dermal route of exposure (Category III) and is a slight eye irritant (Category III) (US EPA, 2010). In contrast, acute oral toxicity testing for a product containing 99.8% AITC using rats as test subjects provided an LD_{50} value of 425.4 mg/kg (US EPA, 2013b). US EPA classifies pure AITC as moderately toxic for acute oral and inhalation exposure (Category II). Likewise, highly concentrated AITC is categorized as highly toxic (Category I) for primary eye and dermal irritation because the substance is highly corrosive. US EPA classifies pure AITC as a dermal sensitizer based on a dermal sensitization test in guinea pigs (US EPA, 2013b). The European Food Safety Authority (EFSA) concluded that AITC may cause hypersensitivity, based on the occurrence of allergies to mustard and reports of allergic contact dermatitis in humans (EFSA, 2010).

Inhalation toxicity data for AITC and its degradates are not available. US EPA waived data requirements for the 90-day subchronic inhalation toxicity study despite the high volatility of AITC and the fact that the label Personal Protective Equipment requirements for registered AITC products indicates concerns about inhalation exposure (Isagro USA, 2013). The structural similarity of AITC to the conventional fumigant methyl isothiocyanate (MITC) derived from metam-based fumigant pesticides raises additional concerns regarding inhalation toxicity, since respiratory irritation from inhalation exposure is the risk driver for MITC.

The physical properties of AITC are very similar to those of the conventional soil fumigant MITC (vapor pressure = 16 mm Hg at 25 ºC, application rate = 40–300 lbs/acre), for which a great deal of environmental fate and air monitoring data are available (CDPR, 2002a; CDPR, 2002b; US EPA, 2009a). Air monitoring studies for MITC conducted near application sites demonstrate high air concentrations of MITC in the first

24 hours after the application, tapering off over the course of a week. Indeed, MITC has been responsible for a number of poisoning incidents in which hundreds of people were evacuated from their homes in response to MITC drift from applications up to 0.5 miles distant (CDPR, 2014). Based on the similar physical properties of AITC to MITC, it is thus possible to predict that use of AITC will result in exposure via inhalation for pesticide applicators and residential bystanders due to the proposed use pattern in soil biofumigation. The impact of these exposures is unknown because inhalation toxicology studies are not available; however, products labels for conventional fumigant products containing AITC indicate high inhalation hazards and require applicators to utilize respirators (Isagro USA, 2014).

AITC has been evaluated for developmental and reproductive effects, carcinogenicity and mutagenicity potential in mammals. One study evaluating the developmental toxicity of AITC and related compounds found no difference in the percentage of abnormal fetuses in AITC-treated offspring compared to control groups (US EPA, 2013a). The authors concluded AITC did not demonstrate teratogenic potential at the no observed adverse effect level (NOAEL) of 60 mg/kg, an amounts equivalent to 4.2 grams of AITC for a 150 pound person. AITC was found to cause transitional-cell papillomas of the urinary bladder in male rats, but the evidence of carcinogenicity in female rats was ambiguous and AITC demonstrated no carcinogenic effects in mice (Dunnick, 1982; NTP, 1982). Taken together, the results of several reverse mutation studies, *in vitro* mammalian gene mutation studies using mouse lymphoma cells, and an *in vivo* mammalian chromosome aberration study suggest that AITC is not likely to be a mutagen. Increases in mutant frequency were observed even at lower test concentrations (e.g., 0.4 to 0.8 mg/mL); however, these tests were conducted without S9 activation (i.e., no mammalian enzymes for substrate metabolism were present) and the tests were complicated by cytotoxicity at higher doses (US EPA, 2013a). Nevertheless, AITC is included on Columbia University's list of carcinogens, mutagens, and reproductive poisons commonly used in research laboratories (Columbia, 2008).

One of the degradation products of AITC is carbon disulfide, CS_2 (CDS). There are concerns regarding exposure to CDS because it is listed by the State of California on the Proposition 65 list as a developmental toxicant (OEHHA, 2014) and is known to induce neuropathological changes and other toxic effects in rodents exposed through inhalation over an intermediate during of less than one year (OEHHA, 2001). As discussed in Evaluation Question #4, AITC biodegrades in the environment to form a variety of breakdown products, including CDS at approximately 20–30% transformation. Because CDS is a major degradate of AITC, the human and environmental toxicity of CDS should be considered as part of the evaluation of AITC for use in organic crop production. Please see Evaluation Question #10 for additional information on the human toxicity potential of CDS.

In reviewing pesticide products containing AITC as the active ingredient, US EPA waived the data requirements for birds, freshwater fish, freshwater invertebrates, non-target plants and non-target insects (US EPA, 2013a). Details regarding the rationale for these data waivers are provided below in Table 3.

Table 3. US EPA Waiver of Non-Target Organism Data Requirements for AITC.

Study Description	Rationale Statement
Avian Acute Oral	No acute oral exposure anticipated based on the application method and rapid environmental degradation.
Avian Dietary	No dietary exposure anticipated based on the application method and rapid environmental degradation.
Freshwater Fish LC50	Very Highly Toxic (96-hour LC_{50} = 0.077 ppm), but no aquatic exposure anticipat based on the application method and rapid environmental degradation.
Freshwater Invertebrate	Very Highly Toxic (48-hour EC_{50} = 0.73 ppm), but no aquatic exposure anticipate based on the application method and rapid environmental degradation.
Non-target Plants	No non-target exposure anticipated based on the application method and rapid environmental degradation.
Non-target Insects	No non-target exposure anticipated based on the application method and rapid environmental degradation.

LC_{50} = Concentration of AITC lethal to 50 percent of test organisms
EC_{50} = Effective concentration at which 50 percent of test organisms experience adverse effects, excluding death

Very few peer-reviewed papers on the ecological toxicity of AITC are available. The aquatic toxicity of AITC was evaluated for Japanese rice fish (*Oryzais latipes*) using a continuous-flow-mini-diluter system and five concentrations of AITC. Significant mortality was observed in *O. latipes* exposed to AITC on an acute basis (96-hour LC_{50} = 0.077 mg/L), and the maximum allowable toxicant concentration (MATC) for chronic (28-day) exposure to AITC was 0.013 mg/L (Holcombe, 1995). Another study found that pure AITC and essential oil extracts containing AITC are completely larvicidal in mosquitoes (*A. aegypti*) even at the lowest concentration tested (0.1 mg/mL); however, this measurement indicates that AITC is significantly less toxic compared to some synthetic pesticides. In addition, AITC was toxic to the freshwater water flea (*Daphnia magna*) with a 50% effective concentration value of 0.735 mg/L based on combined mortality and immobility measurements (Park, 2011). As expected, AITC is also highly toxic to soil microorganisms and nematodes, such as the non-parasitic free-living soil nematode *Caenorhabditis elegans* (Donkin, 1995). See Evaluation Question #8 for additional information on the toxicity of AITC to soil organisms.

<u>Evaluation Question #6:</u> Describe any environmental contamination that could result from the petitioned substance's manufacture, use, misuse, or disposal (7 U.S.C. § 6518 (m) (3)).

Considering its moderately high volatility (3.7 mm Hg at 25°C), high application rates (85–340 lbs/acre), and agricultural use as a soil biofumigant, releases of allyl isothiocyanate (AITC) to the environment are inevitable. AITC is both flammable and potentially toxic to nontarget organisms such as mammals and fish (Sigma Aldrich, 2014a). Aquatic wildlife may be exposed to AITC through spills and/or irrigation runoff. As with conventional fumigants, measures such as the use of plastic tarps on treated fields or application of AITC through a drip system could be taken to further protect humans (bystanders and workers) and nontarget terrestrial organisms from exposure to AITC following soil biofumigation. The rapid breakdown and dissipation of AITC in the environment reduces the probability of contamination of groundwater and surface water due to agricultural applications of the substance.

In the absence of accidental spills, the risk of water contamination from the use of AITC as a soil biofumigant is considered to be minimal. The release of chemical reagents (e.g., allyl iodide and potassium thiocyanate) and highly toxic, flammable and hazardous solvents (e.g., 1,2-dichloroethane) used in the production of AITC due to improper handling/disposal could lead to serious environmental impairments and ecotoxicity in both terrestrial and aquatic environments (Sigma Aldrich, 2014b). No incidents involving the release of these chemical feedstocks from AITC production facilities have been reported to date. Although possible, it is unlikely that large-scale spills and associated environmental contamination will occur when AITC soil biofumigation products are used in accordance with label instructions.

It must be noted that the application rates and the emission rates of AITC are very different between mustard cover crops or seed meals (effective application rate 4–33 lbs/acre) and >95% pure AITC applied at 85–340 lbs/acre. The rate of dissipation of AITC into the environment from mustard cover crops or seed meals is slower than that of AITC applied as a pure substance because the rate of generation is dependent on exposure of the shredded leaves or mustard meal to water, the action of the enzyme, and the rate of escape of AITC from the organic matrix. Thus, while AITC is naturally produced from mustard cover crops or seed meals, as well as other *Brassica* crop varieties in the agricultural environment without apparent impacts, it is not at all clear that higher application rates of pure AITC will be equally without impact; in fact, the high volatility and high proposed application rates suggest exposure patterns similar to conventional fumigants. The fact that structurally related isothiocyanates such as methyl isothiocyanate (MITC, the active fumigant from application of metam sodium) are strong respiratory sensitizers suggests that AITC may pose similar risks. Because the inhalation toxicity data are not a part of the data package submitted by the registrant, it is difficult to know precisely how toxic AITC is by the inhalation route.

<u>Evaluation Question #7:</u> Describe any known chemical interactions between the petitioned substance and other substances used in organic crop or livestock production or handling. Describe any environmental or human health effects from these chemical interactions (7 U.S.C. § 6518 (m) (1)).

Limited technical information is available regarding the potential for chemical interactions between allyl isothiocyanate (AITC) and other substances used in organic livestock production. One possible interaction between the petitioned substance and other materials used in organic crop production involves the reaction of AITC with free amino acids, peptides and proteins contained in organic composts and fertilizers. Specifically, electrophilic (electron deficient) AITC is capable of reacting with the nucleophilic (electron rich) amino groups of the free amino acids alanine and glycine (Cejpek, 2000), as well as cysteine, lysine and arginine residues of intact proteins (Kawakishi, 1987). Diminished enzymatic digestibility was documented for some of the resulting protein-AITC adducts; however, it is uncertain how these chemical transformation products might affect the absorption and metabolism of amino acid building blocks in plants. Related technical information on the effect of AITC on the beneficial soil organisms that facilitate uptake of organic nutrients through plant roots is provided below in Evaluation Question #8.

Evaluation Question #8: Describe any effects of the petitioned substance on biological or chemical interactions in the agro-ecosystem, including physiological effects on soil organisms (including the salt index and solubility of the soil), crops, and livestock (7 U.S.C. § 6518 (m) (5)).

The current technical evaluation report concerns the use of allyl isothiocyanate (AITC) as a pre-plant soil biofumigant for control of soil microorganisms and nematodes, insects and weeds in organic crop production. When used for this purpose, it is understood that AITC will interact with multiple components of the terrestrial agro-ecosystem (i.e., agricultural land). Although limited technical information is available regarding non-target effects of AITC application on livestock and wildlife, the available literature suggests the risk of impairment is minimal when label instructions and precautions are followed. Leakage of AITC, particularly large-scale spills, near the agro-ecosystem will result in the destruction to soil organisms (plants, fungi, etc) and may be hazardous to non-target wildlife in the area.

Toxicity of AITC to soil-dwelling organisms is well documented in the scientific literature due to use of the substance as a pre-plant soil biofumigant. The primary targets of AITC biofumigants are deleterious soil microorganisms, and a significant body of research has been conducted on the efficacy of synthetic AITC in addition to plant materials that naturally infuse AITC into the soil for plant pathogen control (Weerakoon, 2012). One study demonstrated inhibition of the plant pathogenic fungi *Pythium ultimum* and *Rhizoctonia solani* using shredded leaves of different *Brassica* species. It should be noted that AITC comprised greater than 90% of the volatile chemicals measured from these leaves (Charron, 1999). Another study investigated Indian mustard and pure AITC suppression of mycelial growth and sclerotial germination of *Atherlia rolfsii*, a soil-borne plant pathogen, which causes southern blight in crops. It was shown that intact Indian mustard, as opposed to pure AITC, exhibited the strongest antimicrobial action at a concentration of one gram per liter (Harvey, 2002).

Other studies have demonstrated that AITC released from mustard plants can disrupt mutualistic fungal associations (i.e., arbuscular mycorrhiza) with certain plants species. For example, even low levels of AITC (i.e., approximately 0.001 millimolar) infused in soil by invasive garlic-mustard plants have the ability to significantly suppresses fungal growth and spore germination of the beneficial soil fungus *Glomus clarum* (Cantor, 2011). In another study, it was also found that AITC emitted from garlic mustard adversely impacts the abundance of entomopathogenic fungi (i.e., fungal parasite of pest insects) in forest soils (Vaicekonyte, 2012). These reports provide direct evidence that AITC does not specifically target soil pests; rather, AITC is a broad-spectrum antimicrobial compound that effectively kills both plant pathogens and beneficial soil microorganisms. Additionally, it is known that certain species of soil fungi enhance the bioavailability of organic soil nutrients and mediate the uptake of these nutrients by their mycorrhiza host plants (Näsholm, 2009). AITC drift would therefore be problematic for both the beneficial soil fungi and associated plants.

In addition to soil microorganisms, plants, insect pests and animals have demonstrated varying responses to AITC soil treatments. Phytotoxicity studies of various seed meals demonstrated that mustard seed meal, which releases AITC in soil, prevented or significantly diminished germination of lettuce seeds within the first week after application (Meyer, 2011). Larvae of the pest *Cyclocephala spp.* (masked chafer beetle) were well controlled when macerated *Brassica* tissue was applied as four to eight percent of the soil, giving an average AITC concentration of 11.4 mg per liter of soil atmosphere (Noble, 2002). AITC extracted from horseradish was tested as a fumigant against four major pest species of stored rice, including *Sitophilus zeamais* (maize weevil), *Rhizopertha dominica* (lesser grain borer), *Tribolium ferrugineum* and *Liposcelis entomophila* (book louse). Adult mortality of 100% of all four pest species after 72 hour exposure to AITC fumes at an atmospheric concentration of 3 mg/mL showed no significant difference in insecticidal activity compared to insects exposed to phosphine (PH_3; a stored commodity fumigant) at 5 mg/mL (Wu, 2009).

Improper use or disposal of chemical reagents (e.g., potassium thiocyanate and allyl iodide) and highly toxic solvents (e.g., 1,2-dichloroethane) during the production of AITC would likely result in adverse effects to soil organisms. However, based on the chemical composition of potential contaminants, spills of AITC and precursors are unlikely to alter pH and chemical composition of the soil. Improper treatment and subsequent release of extraction mixtures containing volatile mustard seed meal and volatile solvents (e.g., hexane) may also impair soil populations. Although possible, these types of spill scenarios are unlikely due to manufacturing safeguards.

Technical information regarding the potential impacts of AITC on endangered species, populations, viability or reproduction of non-target organisms and the potential for measurable reductions in genetic, species or ecosystem biodiversity, is not readily available.

<u>Evaluation Question #9:</u> Discuss and summarize findings on whether the use of the petitioned substance may be harmful to the environment (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i)).

Allyl isothiocyanate is a naturally occurring essential oil and is not persistent or bioaccumulative in the environment. Both synthetic and natural sources of the substance are readily biodegradable in all three environmental compartments. Similar to other soil fumigants such as MITC, soil decomposition half-lives for AITC range from 20 to 60 hours, with higher rates of AITC degradation in soils with high organic carbon and total nitrogen contents. Although AITC has the potential to adsorb to suspended solids and sediments, it rapidly dissipates in water due to facile hydrolysis and volatilization from the water surface. Photochemically produced hydroxyl radicals degrade atmospheric AITC with a half-life of 2.4 hours. Allyl amine and carbon disulfide, a naturally occurring sulfur compound, are the primary byproducts of AITC under environmentally relevant conditions (HSDB, 2013; US EPA, 2013a; US EPA, 2013b).

Based on the available literature, it can be concluded that pure AITC ranges from highly toxic to practically non-toxic to various taxa groups. AITC is classified as an eye and skin irritant and is moderately acutely toxic (Category II) to mammals via the oral route of exposure. Data are lacking on inhalation toxicity; however, the structural similarity of AITC to methyl isothiocyanate (MITC; $CH_3N{=}C{=}S$) and known irritant properties of AITC (see Evaluation Question #10 below) would indicate that inhalation toxicity may be a concern. The bulk of the available literature for extended dosing studies suggests that AITC is not a developmental or reproductive toxicant, and is unclassifiable as to its carcinogenicity (US EPA, 2013a; IARC, 1999). In comparison to moderate acute oral toxicity in mammals, AITC is highly toxic to aquatic organisms, such as fish and aquatic invertebrates (US EPA, 2013a). Exposure of aquatic organisms to AITC may occur from spills and short-term runoff following irrigation or heavy rain. As a potent soil fumigant, AITC is highly toxic to pathogenic soil organisms as well as non-parasitic free-living soil nematodes (Donkin, 1995) and symbiotic soil fungi (Cantor, 2011).

The release of chemical reagents (e.g., allyl iodide and potassium thiocyanate) and highly toxic, flammable and hazardous solvents (e.g., 1,2-dichloroethane) used in the production of AITC due to improper handling/disposal could lead to serious environmental impairments and ecotoxicity in both terrestrial and aquatic environments (Sigma Aldrich, 2014b). No incidents involving the release of these chemical feedstocks from AITC production facilities have been reported. In addition to targeting soil pathogens, insects and weeds, AITC is also toxic to fungi that produce mutualistic relationships with plants and prey on pest insects (Cantor, 2011; Vaicekonyte, 2012). Therefore, non-target plants and beneficial microorganisms would be damaged in treatment plots and neighboring areas due AITC drift.

<u>Evaluation Question #10:</u> Describe and summarize any reported effects upon human health from use of the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518 (m) (4)).

Natural sources of allyl isothiocyanate (AITC) contained in natural vegetable oils (e.g., mustard oil) are generally non-toxic to humans via the oral route of exposure. This observation is not surprising considering the high concentrations of AITC (3 mg/kg to 15 g/kg) generally found in popular food items such as kale, broccoli, mustard and horseradish. However, moderate doses of concentrated AITC are considered toxic to mammals based on laboratory studies in animals.

Acute, sub-chronic and even chronic (long-term) exposure to AITC is likely for humans living and working near AITC application sites. Studies investigating the time-course of sensitization and desensitization to AITC nasal stimuli in healthy human subjects found that short-term sensitization occurred but markedly decreased in intensity with increasing time between nasal stimulation with AITC (Brand, 2002). AITC vapor is lacrimatory (causes tears to form), and can causes keratitis in which the front part of the eye becomes inflamed and eyesight is temporary impaired (HSDB, 2013). Allyl isothiocyanate is known to irritate the mucous membranes and induce inflammatory skin conditions (eczema) or skin lesions (vesicles). Indeed, patch tests for irritant contact dermatitis with radishes and AITC produced positive reactions (IARC, 1999). Other studies have concluded that contact dermatitis from AITC occurs in only a limited number of cases, despite frequent exposure to the substance in fresh foods and various condiments (Lerbaek, 2004). There are no reports of acute systemic toxicity in humans related to ingestion of AITC found naturally or artificially in foods. A 90-day (sub-chronic) oral toxicity study conducted by the National Toxicology Program in rats determined a No Observed Adverse Effect Level (NOAEL) of 25 mg AITC/kg-body weight/day, the highest dose tested in the study (US EPA, 2013a).

Inhalation toxicity data for AITC and its degradates are not available. Data requirements for the 90-day subchronic inhalation toxicity study were waived by US EPA, which is unusual, considering the high volatility of AITC and the fact that the label Personal Protective Equipment requirements for registered AITC products indicates concerns about inhalation exposure (Isagro USA, 2013):

> *Where liquid contact is a potential all handlers (including mixers, loaders and applicators) in addition to the above listed PPE must wear an air purifying respirator with an organic-vapor removing cartridge with pre-filter approved for pesticides (MSHA/NIOSH approved number prefix TC-23C), or a canister approved for pesticides (MSHA/NIOSH) approval number prefix TC-14G), or a NIOSH approved respirator with an organic vapor (OV) cartridge or canister with any N, R, P, or HE pre-filter.*

The structural similarity of AITC to the conventional fumigant MITC derived from metam-based fumigant pesticides raises additional concerns regarding inhalation toxicity, since respiratory irritation from inhalation exposure is the risk driver for MITC. Because the inhalation toxicity data were not required by US EPA, this remains as a significant data gap.

When taken together, the bulk of the available literature suggests that AITC is unclassifiable as to carcinogenicity and mutagenicity. The International Agency for Research on Cancer (IARC) categorized AITC in Group 3, "not classifiable as to its carcinogenicity to humans," based on inadequate evidence in humans and limited evidence in experimental animals for carcinogenicity of AITC (IARC, 1999). AITC was initially tested for carcinogenicity as part of a 2-year carcinogenesis bioassay of food grade AITC (greater than 93% pure) administered to one strain of mice and one strain of rats in corn oil five times per week for 103 weeks. No incidence of tumors was observed in mice; however, a statistically significant increased incidence of epithelial hyperplasia (proliferation of skin cells) and transitional-cell papillomas (benign epithelial tumor) of urinary bladder was observed in male rats (US EPA, 2013a; IARC, 1999; NTP 1982). Subsequent studies confirmed the absence of carcinogenicity in mice treated with AITC via gavage administration (IARC, 1999). Despite the carcinogenic response in male rates exposed to AITC via gavage, a number of studies have demonstrated the potential AITC at lower dietary exposure levels (<1 mg/kg) to protect against and in some cases reverse the development of colorectal (Musk, 1993), bladder (Zhang, 2010), and presumably other cancer cell lines (Wang, 2010).

National Toxicology Program (NTP) studies on AITC show inconsistent results for gene mutation studies in the bacterium *Salmonella typhimurium* (AMES test) with and without exogenous metabolic activation using extracts containing mammalian enzymes. AITC did not induce gene mutation in several *Salmonella* strains in the absence of metabolic activation. A negative response was also observed in one trial using mouse lymphoma cells without activation at concentrations ranging from 0.05 to 0.8 mg/mL; however, two other trials without activation demonstrated a significant increase in average mutant frequency and reduction in total growth at concentrations between 0.4 and 1.4 mg/mL. The authors noted that the positive results were observed without metabolic activation, thus leading to considerably different experimental conditions compared to natural biological (*in vivo*) conditions. The results of these studies are also compromised by the high degree of cytotoxicity observed at moderate to high doses. An *in vivo* mammalian chromosome aberration study conducted using mice dosed via direct injection of AITC into the body cavity revealed no differences between treatment and control mice (US EPA, 2013a; IARC, 1999). Accordingly,

> *The [US Environmental Protection] Agency has determined that the weight of evidence demonstrates that AITC is not likely to be a mutagen. In addition, the method of application and rapid degradation rate for the proposed pre-plant soil treatment, together with appropriate PPE, mitigates exposure to humans.*

In comparison to AITC, the related chemical MITC has shown limited evidence of carcinogenicity in animal studies. US EPA determined that the current data set is insufficient to characterize the cancer risk of MITC and requested inhalation carcinogenicity studies with MITC in rats and mice (US EPA, 2009). On the contrary, the parent compound (metam-sodium) and breakdown product (methyl isocyanate, MIC) of MITC are considered to be carcinogenic and mutagenic based on the results of tissue cultures (*in vitro*) and lifetime animal dosing studies (US EPA, 2009; CDPR, 2003). In light of the health concerns for these related chemicals (MITC and MIC), it will be necessary to update the literature review on the carcinogenic potential of AITC as new scientific insights become available.

One of the major degradation products of AITC is carbon disulfide, CS_2 (CDS). There are concerns regarding exposure to CDS because it is listed by the State of California on the Proposition 65 list as a developmental toxicant (OEHHA, 2014) and is a known human neurotoxin. In addition to animal studies, CDS has been found to cause reproductive toxicity in males and females through occupational exposure. Specifically, significant adverse effects on spermatogenesis, sex hormone levels and libido in men, as well as menstrual disturbances in women were observed in workers exposed to CDS levels of 3.1–14.8 mg/m^3 (OEHHA, 2001). Studies have also identified alterations in the nerve conduction of workers exposed to lower levels of CDS over an extended period of time (chronic exposure). A NIOSH occupational study in male factory workers exposed to AITC air concentrations of 0.6 to 16 ppm for a mean duration of 12 years resulted in a lowest observed adverse effect level (LOAEL) of 7.6 ppm based on minor neurological effects (OEHHA, 2001). In another study, male workers exposed to CDS for an average of 14 years had higher rates (42%) of 24-hour electrocardiogram abnormalities than non-exposed workers (OEHHA, 2001).

<u>Evaluation Question #11:</u> Describe all natural (non-synthetic) substances or products which may be used in place of a petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (ii)). Provide a list of allowed substances that may be used in place of the petitioned substance (7 U.S.C. § 6518 (m) (6)).

A variety of alternative substances are available to organic producers for controlling insect pests, weeds and other soil-borne pests. These substances include natural materials for biofumigation, microbial biopesticides, and naturally derived chemicals that alter soil pH. The following paragraphs describe how these substances may be used in organic production, as well as their efficacy and the availability of commercial products containing these substances.

Biofumigation using soil amendments or cover crops is a natural alternative to the use of commercially available chemical fumigants (including methyl bromide, chloropicrin, 1,3-dichloropropene, metam-sodium and metam-potassium) for controlling soil-borne pathogens, nematodes, insects and weeds prior to planting. Conventional soil fumigants are not allowed in the production of organic crops. In addition to allyl isothiocyanate (AITC), other naturally occurring isothiocyanates such as methyl isothiocyanate (MITC) and phenyl isothiocyanate exhibit nematocidal, bactericidal, fungicidal and herbicidal properties. These related isothiocyanates are generated by enzymatic degradation of the corresponding glucosinolate contained in cruciferous vegetables much like the formation of AITC. For example, MITC is enzymatically released from glucocapparin (i.e., methyl glucosinolate) naturally contained within the caper plant. MITC is primarily used in conventional agriculture as the active pesticidal substance released from degradation of metam-sodium and metam-potassium, which are highly toxic and widely used chemical fumigants (Johnson, 2009; Romanowski, 2000).

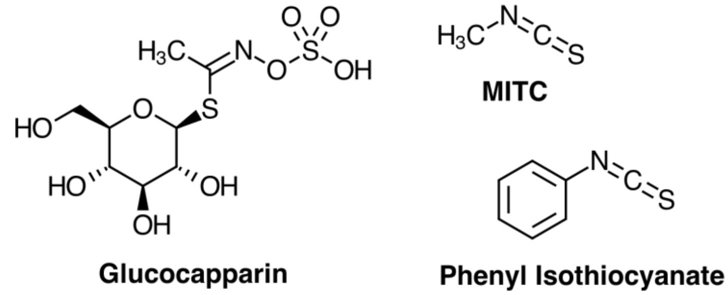


Figure 3. Chemical structures of glucocapparin, methyl isothiocyanate (MITC) and phenyl isothiocyanate.

Meals that are produced when mustard seeds are pressed to extract natural oils have been shown to suppress weeds and soil-borne pathogens. It is recommended that mustard seed meals be applied at a rate of 1,000–4,000 pounds per mulched acre and that the grower observe a waiting period of 20 days before planting (Johnson, 2011; Farm Fuel Inc, 2013). While high application rates are required to generate sufficient amounts of AITC for biofumigation, the excess seed meal fertilizes the soil with nitrogen, carbon and other nutrients that generally accompany organic material additions to soils (Johnson, 2011). Regarding biofumigation, the compiled data indicate an increased rate of AITC release to soil with increasing relative humidity and temperature (Dai, 2014). Particle size and oil content of the mustard meal powder also affects the release rate. The available literature suggests that mustard seed meal biofumigants can lead to extended protection against deleterious soil pathogens (Weerakoon, 2012). Indeed, the incorporation of AITC using intact mustard products (e.g., mustard seed meals or soil incorporation of mustard cover crops) may alter the composition of the soil fungal community. For example, seed meal-treated soils exhibited preferential proliferation of *Trichoderma spp.*, a genus of fungi that forms mutualistic relationships with several plant species, which may contribute to long-term control of pathogenic fungi such as *Pythium abappressorium* (Weerakoon, 2012).

A number of field trials have been conducted using mustard green manures (plowed cover crops) and seed meals for the biofumigation of agricultural fields. For example, one study found that soil incorporation of 2,240 kg/ha to 4,480 kg/ha mustard seed meal can increase yields of plasticulture-grown strawberries when compared to control plots. In addition to the partial control of soil-borne anthracnose, soil incorporation of mustard seed meal can greatly decrease competition from broadleaf weeds for strawberry plants established in the fall (Deyton, 2010). Extension specialists and industry groups have also reported yield improvement for strawberries and other crops grown in soils pre-treated with mustard meals (Farm Fuel, 2013a; Johnson, 2011). Although mustard seed meals have shown potential, specific meals or blends of seed meals must be used at high application rates in combination with other practices since results vary due to field activity (CDPR, 2013; Mazzola, 2010). In addition, some natural substances and practices are not compatible with the use of mustard meals for biofumigation. Green manures and seed meals that naturally produce AITC may be harmful to certain beneficial soil nematodes responsible for biologically controlling deleterious soil pathogens, indicating incompatibility of mustard meals and certain biocontrol agents (Henderson, 2009). See also Evaluation Question #11 for details regarding the use of beneficial nematodes as an alternative to soil fumigation.

Biologically based pesticides are also available for the management of soil-borne pests. These include both microbial biopesticides, including products derived from microbes and their metabolites, and biochemical biopesticides, which are naturally occurring or naturally inspired synthetic chemicals. For example, the OMRI approved Regalia® product is formulated with extract of giant knotweed (*Reynoutria sachalinensis*, 20%) to induce systemic resistance to certain fungi in strawberry and other treated plants. An insufficient number of large-scale, on-farm demonstrations have been conducted to determine the potential of this and related biopesticides as fumigant alternatives (CDPR, 2013).

Microbial biopesticides are also being investigated as viable fumigant alternatives. These pesticides may include the entire microorganisms and/or chemical products they produce as metabolites. For example, *Streptomyces lydicus* strain WYEC 108 is a naturally occurring bacterium commonly found in soil and recently formulated in commercial biopesticide products (CDPR, 2013). It is thought that the bacterium exerts its antimicrobial properties by colonizing the growing root tips of plants and parasitizing root decay fungi such as *Fusarium*, *Pythium*, and other species (US EPA, 2009b). When used in strawberry production, the Actinovate® (*S. lydicus*) product showed good yields compared to untreated controls in field trials. No adverse environmental or human health effects are expected from use of this bacterial strain in agriculture. Fungal species belonging to the *Muscador* genus produce volatile compounds that can kill nematodes, insects and plant pathogens. Other examples of microbial biopesticides include Serenade® (*Bacillus subtilis* strain 713), Bionematicide Melocon® (*Paecilomyces lilacinas* and *Gliocladium*), and fungal biocontrol SoilGard® (*Trichoderma virens*) for control of soil-borne diseases caused by *Pythium*, *Rhizoctonia* and *Fusarium* (CDPR, 2013; Certis USA, 2014). Some species of nematodes are also effective for pest control. Specifically, the beneficial nematode *Heterorhabditis bacteriophora* is commercially available and effectively controls pest through production of a toxic bacterial during its development in the host insect (Buglogical, 2014; Arbico Organics, 2014).

Soil pH is an important factor influencing the development of certain soil-borne diseases. The classic example of this phenomenon is clubroot disease of crucifers caused by *Plasmodiophora brassicae*. Symptoms of clubroot include aboveground stunting, severely swollen and deformed roots, root rot, and plant death. This condition is a major problem in acidic soils (pH of 5.7 or lower); the disease is dramatically reduced when the pH rises from 5.7 to 6.2 and is practically eliminated at soil pH values greater than 7.3 or 7.4 (Koike, 2003). Once posing a major threat in the Salinas Valley of Central California, this disease has been largely managed in recent decades by liming the soil (i.e., adding calcium hydroxide) to raise the pH (Koike, 2003). According to the National List, "hydrated lime," which is primarily composed of calcium hydroxide [$Ca(OH)_2$], is only approved for use as a component of foliar sprays for plant disease control in organic crop production (7 CFR 205.601(i)(4)). Organic crop producers may use naturally mined minerals, such as calcium carbonate ($CaCO_3$), as alternatives to raise soil pH.

<u>Evaluation Question #12:</u> Describe any alternative practices that would make the use of the petitioned substance unnecessary (7 U.S.C. § 6518 (m) (6)).

Organic farmers are generally dependent upon preventative cultural practices and physical controls for suppressing pest insects, weeds and soil-borne pathogens. The "Crop pest, weed, and disease management practice standard" in the NOP rule states that producers must use the following management practices to prevent crop pests, weeds and diseases (7 CFR 205.206(a)):

- Crop rotation and soil and crop nutrient management practices;
- Sanitation measures to remove disease vectors, weed seeds and habitat for pest organisms;
- Cultural practices that enhance crop health, including selection of plant species and varieties with regard to suitability to site-specific conditions and resistance to prevalent pests, weeds and diseases.

Pest problems may be controlled through mechanical or physical methods (7 CFR 205.206(b)):

- Augmentation or introduction of predators or parasites of the pest species;
- Development of habitat for natural enemies of pests;
- Nonsynthetic controls such as lures, traps and repellents.

Organic producers may control weed problems using the following activities (7 CFR 205.206(c)):

- Mulching with fully biodegradable materials;
- Mowing;
- Livestock grazing;
- Hand weeding and mechanical cultivation;
- Flame, heat or electrical means;
- Plastic or other synthetic mulches: *Provided* that, they are removed from the field at the end of the growing or harvest season.

Lastly, the standard allows for the following activities to control plant disease problems (7 CFR 205.206(d)):

- Management practices which suppress the spread of disease organisms;

- Application of nonsynthetic biological, botanical or mineral inputs.

While some conventional farms rely heavily on chemical fumigation of soil, organic producers must develop a diverse tool kit for effective pre-plant pest, weed and plant disease management that ensures acceptable yields. Grower experience and continued research has led to current practices such as soil inversion by deep plowing, the application of *Brassica* seed meals or other antimicrobial crop residues (Evaluation Question #11), crop rotations and anaerobic soil disinfestation. Crop rotation remains the primary method of combating soil pests. The following paragraphs describe currently developed and experimental practices that may serve as alternatives to chemical fumigants such as AITC in organic crop production.

Over the past several millennia, farmers have developed various crop rotation methods to increase yields by improving soil fertility and better controlling pests, weeds and plant diseases. Organic farmers base their crop rotations on whether various plants in their rotational lineup are considered light or heavy feeders and on the suite of pests that attack similar crops. Soil-depleting crops, including row crops like corn, soybeans, vegetables and potatoes, are typically rotated with crops that incorporate nutrients into the soil, such as the legume sods—alfalfa and clover—and various grasses (Baldwin, 2006). In addition to soil fertility, crop rotations are critical for reducing the adverse impacts of insects, weeds and pathogens. By changing the environmental conditions in the field and removing food sources to prevent pest buildup, crop rotations can enable farmers to effectively reduce pest populations (McGuire, 2003). Crops of the same family should not follow one another in the field, and should typically be separated by at least two years and as much as five years to minimize the occurrence of pests and pathogens in the soil (Baldwin, 2006). A rotation of crop families might include *Brassicaceae* (cole crops), followed by *Asteraceae* (lettuce, cut flowers), followed by *Solanaceae* (tomatoes, potatoes, peppers, eggplants), followed by *Curbitaceae* (squashes, cucumbers and melons). Specific plant diseases will require tailored crop rotations; for example, detection of *Sclerotium rolfsii* (southern blight) in vegetable crops may require a rotation of corn, grass, hay or pasture crop for two or three years (Baldwin, 2006). Crop rotations are most effective when combined with such practices as composting, cover cropping, green manuring and short pasturing cycles.

Planting cover crops for biological fumigation prior to planting has the potential to significantly reduce the need for chemical fumigation in conventional crop production and is a commonly used approach in organic agriculture. Specifically, certain varieties of mustard cover crops (e.g., Ida Gold, Mighty Mustard and Pacific Gold) planted in a resting field are grown for a certain period of time and then plowed under before reaching full maturity in order to maximize the concentration of nutrients and allelochemicals (e.g., AITC and glucosinolates) available from the mustard crop (Johnson, 2009). The damaged plant tissues naturally release AITC for biofumigation, as discussed in previous sections of this report. Cover crops of wheat, barley, oats, rye, sorghum and sudangrass have been shown to suppress weeds and in some cases nematodes and insect pests (Baldwin, 2006). Some cover crops, such as vetches and clovers, encourage populations of beneficial insects like ladybugs that prey on pest insects (Baldwin, 2006). Green manures from various cover crops may also serve as energy sources for beneficial microorganisms that out-compete plant pathogens and potentially confer disease resistance to crops (McGuire, 2003). In the larger context of sustainable agriculture, planting cover crops between production cycles can help minimize soil erosion, naturally enhance soil fertility without the use of synthetic fertilizers, and improve weed, insect and disease management in fields (Baldwin, 2006).

Non-chemical methods including anaerobic soil disinfestation (ASD), steam sterilization and soil solarization are being further developed as alternatives to chemical fumigation. ASD is a method that creates anaerobic (without oxygen) conditions in the soil profile by incorporating readily available carbon sources into topsoil that irrigated to field capacity and covered by a tarp. The tarp is left covering the soil for a certain period of time to maintain the high soil moisture level and oxygen-free conditions. Anaerobic organisms produce byproducts that are toxic to soil pathogens through their metabolisms of the added carbon (UCANR, 2014). The typical procedure involves the following steps: 1) spread carbon source such as rice bran, 2) incorporate in soil, 3) form beds and lay drip tape, 4) cover with plastic tarp, 5) irrigate and keep at field capacity, 6) leave for three weeks, 7) punch holes in plastic, 8) plant fruit or vegetable crop (e.g., strawberries) a few days later (Shennan, 2012). Rice bran is the primary carbon source used to date; other potential sources include molasses, grape pommace and ethanol (used in Japan) (CDPR, 2013).

Researchers are currently experimenting with application rates of organic matter and ways of managing nitrogen runoff before the technique is adopted in large-scale agricultural systems.

Steam treatments effectively manage pathogens and weeds in soil directly contacted by the steam. While steam application to static soil may take hours to heat, physically mixing steam and soil results in rapid heating of the soil within approximately 90 seconds. Trials indicate strawberry yields in steamed soils are equal to yields from fumigated soils, and weed and pathogen management using this method is equivalent to fumigation in the soil zone where steam is applied (CDPR, 2013). Because of the labor intensive and expensive nature of steam treatments, questions remain about the economic and environmental practicality of this approach. Steam treatments could be combined with alternative substances such as biopesticides to reduce cost and other limitations, but these combinations must be investigated before implementation in agriculture (CDPR, 2013).

A third non-chemical approach involves the use of plastic sheets to trap solar energy and kill soil-borne organisms with heat. Known as soil solarization, the heat produced using this method kills soil-borne seeds and microorganisms near the surface, but fails to reach organisms deeper in the root zone (CDPR, 2013). This technique is limited to growing regions where solarization temperatures are high enough to be effective. Although additional trials are needed, the combination of soil solarization with biofumigants such as mustard seed meal may improve control of soil pests (CDPR, 2013).

A significant amount of funding has been made available for research into biofumigation and non-chemical approaches to soil disinfestation in light of the methyl bromide phase-out and environmental impacts of related chemical fumigants. While some of the methods described above are ready for implementation in crop production, research efforts aimed at improving existing techniques and developing new strategies to eliminate the use of fumigants are ongoing. In addition to traditional crop rotation, the available information suggests that the variety of available management techniques preclude the application of synthetic biofumigants such as AITC in organic crop production.

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