Appendix G: Ecological Toxicity Data

Toxicity testing required does not test all species of birds, fish, mammals, invertebrates, and plants. Only two surrogate species for birds (bobwhite quail and mallard) are used to represent all bird species (over 1000 in the US, including subspecies), three species of freshwater fish (rainbow trout, bluegill sunfish and fathead minnow) are used to represent all freshwater fish species (over 900 in the US), and one estuarine/marine fish species (sheepshead minnow) is used to represent all estuarine/marine fish (over 300 in the US). The surrogate species for terrestrial invertebrates is the honey bee, for freshwater invertebrates the surrogate species is usually the waterflea (*Daphnia magna*) and for estuarine/marine invertebrates the surrogate species are mysid shrimp and eastern oyster. These four species are used to represent all invertebrate species (over 10,000 in the US). For plants, there are ten surrogate species used for all terrestrial plants and five surrogate species used for all aquatic plants. There are over 20,000 plant species in the US which includes flowering plants, conifers, ferns, mosses, liverworts, hornworts and lichens with over 27,000 species of algae worldwide.

The surrogate species testing scheme used in this assessment assumes that a chemical's mechanism of action and toxicity found for avian species is similar to that in all reptiles (over 300 species in the US). The same assumption applies to amphibians (over 200 species in the US) and fish; the tadpole stage of amphibians is assumed to have the same sensitivity as a fish. Therefore, the results from toxicity tests on surrogate species are considered applicable to other member species within their class and are extrapolated to reptiles and amphibians. The US species numbers noted in this section were taken from the Natureserve website (www.natureserve.org NatureServe: An online encyclopedia of life [web application].2000) and the worldwide species number from Ecological Planning and Toxicology, Inc.1996.

In the following sections, the shaded values in the tables are the ones used in the current risk assessment.

a. Toxicity to Terrestrial Animals

i. Birds, Acute and Subacute

An acute oral toxicity study using the technical grade of the active ingredient (TGAI) is required to establish the toxicity of metam-sodium to birds. The avian oral LD_{50} is an acute, single-dose laboratory study designed to estimate the quantity of toxicant required to cause 50% mortality in a test population of birds. The preferred test species is either the mallard, a waterfowl, or bobwhite quail, an upland gamebird. The TGAI is administered by oral intubation to adult birds, and the results are expressed as LD_{50} milligrams (mg) active ingredient (a.i.) per kilogram (kg) of body weight. Toxicity category descriptions are the following:

If the LD_{50} is *less than 10 mg a.i./kg*, then the test substance is *very highly toxic*.
If the LD_{50} is *10-to-50 mg a.i./kg*, then the test substance is *highly toxic*.
If the LD_{50} is *51-to-500 mg a.i./kg*, then the test substance is *moderately toxic*.
If the LD_{50} is *501-to-2,000 mg a.i./kg*, then the test substance is *slightly toxic.*
If the LD_{50} is *greater than 2,000 mg a.i./kg*, then the test substance is *practically nontoxic.*

Table 1: Avian Acute Oral Toxicity – Technical

Species	% ai	LD_{50} (mg a.i./kg)	Toxicity Category	MRID/Accession (AC) No. Author/Year	Study Classification[1]
Mallard Duck *(Anas platyrhynchos)*	42.2	211	moderately toxic	41476402/Munk/1985	Core

[1] Core means study satisfies guideline. Supplemental means study is scientifically sound, but does not satisfy guideline.

The guideline (71-1a) is satisfied for metam-sodium (MRIDs 41476402). However, acute oral testing on MITC is needed for risk assessment.

Two dietary studies using the TGAI are usually required to establish the toxicity of pesticides to birds. These avian dietary LC_{50} tests, using the mallard and bobwhite quail, are acute, eight-day dietary laboratory studies designed to estimate the quantities of toxicant in the feed required to cause 50% mortality in the two respective test populations of birds. The TGAI is administered by mixture to juvenile birds' diets for five days followed by three days of "clean" diet, and the results are expressed as LC_{50} parts per million (ppm) active ingredient (a.i.) in the diet. Toxicity category descriptions are the following:

If the LC_{50} is *less than 50 ppm a.i.*, then the test substance is *very highly toxic*.
If the LC_{50} is *50-to-500 ppm a.i.*, then the test substance is *highly toxic.*
If the LC_{50} is *501-to-1,000 ppm a.i.*, then the test substance is *moderately toxic.*
If the LC_{50} is *1001-to-5,000 ppm a.i.*, then the test substance is *slightly toxic.*
If the LC_{50} is *greater than 5,000 ppm a.i.*, then the test substance is *practically nontoxic.*

Results of these tests are tabulated below.

Table 2: Avian Subacute Dietary Toxicity - Technical

Species	% ai	LC50(ppm)	Toxicity Category	MRID/Accession (AC) No. Author/Year	Study Classification[1]
Mallard Duck *(Anas platyrhynchos*)	42.2	1835.7	slightly toxic	41476403/Munk/1986	Suppl.
Mallard Duck *(Anas platyrhynchos*)	43	> 5000	practically non-toxic	42914001/Pederson & Slatycki/1993	Core
Mallard Duck *(Anas platyrhynchos*)	Tech	> 5000	practically non-toxic	00022923/USFWS/1975	Core
Northern Bobwhite Quail *(Colinus virginianus*)	43	> 5000	practically non-toxic	42914002/Pederson & Solatycki/1993	Core
Northern Bobwhite Quail *(Colinus virginianus*)	42.2	> 2110	slightly toxic or less	41476401/Munk/1986	Suppl.
Northern Bobwhite Quail *(Colinus virginianus*)	Tech	> 5000	practically non-toxic	00022923/USFWS/1975	Core

[1] Core (study satisfies guideline). Supplemental (study is scientifically sound, but does not satisfy guideline)

The guideline (71-2a,b) is satisfied. However, dietary exposure is not the expected route of avian exposure and the above data are not used in the current risk assessment. Inhalation toxicity data on MITC are needed to improve the certainty of the current risk assessment based on MITC inhalation.

iii. Mammalian Toxicity Data (from HED)

ACUTE TOXICITY

1. MITC

Acute Toxicity of Methyl Isothiocyanate (PC Code 068103)

Guideline No.	Study Type	MRID #(S).	Results	Toxicity Category
81-1	Acute Oral-Rat	162331	LD_{50} = 82 mg/kg ♂ 55 mg/kg ♀	II
81-2	Acute Dermal-Rat	16233042442501	LD_{50} = 136-436 mg/kg ♂ 181 mg/kg ♀	I
81-3	Acute Inhalation-Rat	16232742365605	LC_{50} = 0.54 mg/L	II
81-4	Primary Eye Irritation	162328	corrosion of the cornea and conjunctivae	I
81-5	Primary Skin Irritation	162329	all animals died within one hour	I
81-6	Dermal Sensitization	Not available		

SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

1. MITC

Summary of Toxicology Endpoint Selection for Methyl isothiocyanate (PC Code 068103)

Exposure Scenario	Dose Used in Risk Assessment	Special FQPA SF[b] and Level of Concern for Risk Assessment	Study and Toxicologial Effects
Acute Dietary general population including infants and children	Dietary exposure is not expected for MITC		
Chronic Dietary (All populations)	Dietary exposure is not expected for MITC		
Incidental Oral Short-Term (1 - 30 Days)	Incidental oral exposure is not expected for MITC		
Incidental Oral Intermediate-Term (1 - 6 Months)	Incidental oral exposure is not expected for MITC		

Exposure Scenario	Dose Used in Risk Assessment	Special FQPA SF[b] and Level of Concern for Risk Assessment	Study and Toxicologial Effects
Dermal Short-Term (1 - 30 days), Intermediate-Term (1 - 6 Months) Long-Term (> 6 Months)	No dermal hazard via typical dermal contact with MITC is expected. Unprotected skin could exposed to MITC vapor; however this exposure can not, at this time, be quantified.		
Inhalation Short-Term (1 - 30 days) Intermediate-Term (1 - 6 Months) Long-Term (>6 Months)	Inhalation NOAEL= 5.4 mg/kg/day	**Residential** LOC for MOE = 1000[h] **Occupational** LOC for MOE = 100[g]	**Subchronic inhalation toxicity- rat with *MITC* (MRID 45314802)** LOAEL = 27 mg/kg/day based on based on persistent clinical signs, body weight changes, and gross and histopathological lesions
Cancer	**Classification:** Probable human carcinogen (B2) Q1* =3.54 x10^{-1} in human equivalents converted from animals		

a Margin of Exposure (MOE) or Uncertainty Factors (UF) = 1000 [10x for interspecies extrapolation, 10x for intraspecies variations, 10x NOAEL to LOAEL factor and 1x special hazard-based FQPA safety factor.]; b FQPA SF = Special FQPA safety factor is not applicable, c LOC = level of concern; d NOAEL = no observed adverse effect level; e N/A = Not Applicable; f LOAEL = lowest observed adverse effect level; g Margin of Exposure (MOE) or Uncertainty Factors (UF) = 100 [10x for interspecies extrapolation, 10x for intraspecies variations.]; h Margin of Exposure (MOE) or Uncertainty Factors (UF) = 1000 [10x for interspecies extrapolation, 10x for intraspecies variations, 10x database uncertainty factor and 1x special hazard-based FQPA safety factor.].

b. Toxicity to Freshwater Aquatic Animals

i. Freshwater Fish, Acute

Two freshwater fish toxicity studies using the TGAI are required to establish the toxicity of metam-sodium to fish. It has been determined that data on metam-potassium satisfy the data requirement for metam-sodium (10/1/93 EFED Memorandum). The preferred test species are rainbow trout (a coldwater fish) and bluegill sunfish (a warmwater fish). Results of these tests are tabulated below. The toxicity category descriptions for freshwater and estuarine/marine fish and aquatic invertebrates, are defined below in parts per million (ppm).

If the LC_{50} is *less than 0.1 ppm a.i.*, then the test substance is *very highly toxic*.
If the LC_{50} is *0.1-to-1.0 ppm a.i.*, then the test substance is *highly toxic.*
If the LC_{50} is *greater than 1 and up through 10 ppm a.i.*, then the test substance is *moderately toxic.*
If the LC_{50} is *greater than 10 and up through 100 ppm a.i.*, then the test substance is *slightly toxic.*
If the LC_{50} is *greater than 100 ppm a.i.*, then the test substance is *practically nontoxic.*

The requirement for two freshwater fish acute toxicity studies has been satisfied.

Additionally, studies have been conducted on MITC, the principal degradate of metam-sodium.

This is the principal chemical to which fish are likely to be exposed, based on current modeling. The studies are summarized in the following table.

Table 3: Freshwater Fish Acute Toxicity - MITC

Species/ Flow-through or Static	% ai	LC_{50} (ppm)	Toxicity Category	MRID/Accession (ACC) No. Author/Year	Study Classification
Bluegill Sunfish *(Lepomis macrochirus)*/flow-through	94.9	0.142	highly toxic	44523412 (=42058001)/Schupner & Stachura/1991	Core
Rainbow Trout (Oncorhynchus sp.)/flow-through	94.9	0.094	very highly toxic	44523413 (=42058002)/Schupner & Stachura/1991	Core
Rainbow Trout/(Oncorhynchus sp.)/static renewal	99.6	0.0512	very highly toxic	45919420/Zok/2002	Suppl.

ii. Freshwater Fish, Chronic

No chronic exposure is anticipated based on the physio-chemcial properties of MITC. Fate studies submitted indicate no MITC residue detected after 14 days.

(iii) Freshwater Invertebrates, Acute

A freshwater aquatic invertebrate toxicity test using the TGAI is required to establish the toxicity of MITC to aquatic invertebrates. The preferred test organism is *Daphnia magna*, but early instar amphipods, stoneflies, mayflies, or midges may also be used. Results of this test are tabulated below.

[1]

Studies have been conducted on MITC, the principal degradate of metam-sodium and the focus of the present risk assessment. They are summarized in the following table.

Table 6: Freshwater Invertebrate Acute Toxicity – MITC

Species/ Flow-through or Static	% ai	LC_{50} (ppm)	Toxicity Category	MRID/Accession (ACC) No. Author/Year	Study Classification
Daphnid *(Daphnia magna)*/flow-through	95	0.055	very highly toxic	41819302/Schupner/1991	Core

Table 6: Freshwater Invertebrate Acute Toxicity – MITC

Species/ Flow-through or Static	% ai	LC_{50} (ppm)	Toxicity Category	MRID/Accession (ACC) No. Author/Year	Study Classification
Daphnid *(Daphnia magna)*/static renewal	99.6	0.076	very highly toxic	45919419/Dohmen/2002	Supplemental

[1] Core (study satisfies guideline). Supplemental (study is scientifically sound, but does not satisfy guideline).

With a lowest EC_{50} of 0.055 ppm, MITC is categorized very highly toxic to freshwater aquatic invertebrates on an acute basis. The guideline (72-2a) is satisfied.

iv. Freshwater Invertebrate, Chronic

No chronic exposure is anticipated based on the physio-chemcial properties of MITC. Fate studies submitted indicate no MITC residue detected after 14 days, however a 21 day guideline study was submitted.

Due to the rapid degradation of metam-sodium to MITC in the presence of water, the required test material is MITC. The preferred test species is *Daphnia magna*. Results of this test are tabulated below.

Table 7: Freshwater Aquatic Invertebrate Life-Cycle Toxicity- MITC

Species/Static Renewal or Flow-through	% ai	21-day NOAEC/LOAEC (ppm)	Endpoints Affected	MRID/Accession (AC) No. Author/Year	Study Classification[1]
Daphnid*(Daphnia magna*/ static renewal	NR	0.025/>0.025 0.025/0.050	Reproduction Parental mortality	45634001/Jatzek/2001	Supplemental

[1] Core (study satisfies guideline). Supplemental (study does not satisfy guideline)

The guideline (72-4b) is not fulfilled, since mean measured concentrations were not determined, the stability of the test substance under actual use conditions was not assessed, and terminal growth measurements were not obtained.

d. Toxicity to Plants

i. Terrestrial Plants

Terrestrial plant Tier I seedling emergence and vegetative vigor testing of a Typical End-Use product (TEP) is currently recommended for all pesticides having outdoor uses (EFED Policy, Keehner. July 1999). For seedling emergence and vegetative vigor testing, the following plant species and groups should be tested: (1) six species of at least four dicotyledonous families, one species of which is soybean (*Glycine max*) and the second is a root crop, and (2) four species of at least two monocotyledonous families, one of which is corn (*Zea mays*). Tier I tests measure the response of plants, relative to a control, at a test level that is equal to the highest use rate expressed as pounds active ingredeint per acre (lbs ai/A). Tier II studies are required if the Tier I studies indicate any of the test species, when exposed to the test material, displayed a ≥25% inhibition or over-enhancement of various growth parameters as compared to the control. This guideline has not been satisfied.

ii. Aquatic Plants

Aquatic plant testing is recommended for all pesticides having outdoor uses (EFED Policy, Keehner. July 1999). The tests are performed on species from a cross-section of the aquatic plant population. The preferred test species are duckweed (*Lemna gibba*), marine diatom (*Skeletonema costatum*), blue-green algae (*Anabaena flos-aquae*), freshwater green alga (*Selenastrum capricornutum*), and a freshwater diatom. Tier I aquatic plant testing is a maximum dose test designed to quickly evaluate the toxic effects to the test species in terms of growth and reproduction and to determine the need for additional aquatic plant testing. Tier II aquatic plant testing is a multiple dose test of the plants species that showed a phytotoxic effect to the pesticide being tested at the Tier I level. Tier II testing is designed to determine the detrimental effect levels of the chemical on the aquatic plants which showed a greater than 50% detrimental effect in Tier I testing.

For metam-sodium, four studies on the degradate MITC have been submitted. They are summarized in the following table.

Table 10: Aquatic Plant Toxicity (Tier II) - MITC

Species/duration	% A. I.	EC_{50}/NOAEC (ppm) (nominal or measured)	MRID No. Author/year	Classification
Vascular Plants				
Duckweed (*Lemna gibba)*	99.6	0.59/0.09 # fronds and growth (meas.)	45919421/Junker/2002	Core
Nonvascular Plants				
Blue-green algae *(Anabaena flos-aqua)*	99.6	1.5/5.0 cell density (meas.)	45919422/Kubitza/2002	Supplemental
Green algae *(Pseudokirchneriella subcapitata = Selenastrum capricornutum)*	99	0.28/0.207 biomass (meas.)	45919416/Kubitza/1998	Supplemental
Algae *Scenedesmus subspicatus*	95.7	0.254 cell density (nominal)	44588903/van Dijk/1990	Supplemental

The guideline is satisfied for *Lemna*. Core studies are needed for the remaining four species.

e. Toxicity to Non-target Insects

An acute contact study with the honey bee (141-1) is required, since the proposed uses are outdoors. Data are summarized in the following table.

No study has been submitted for MITC.

ATTACHMENT VI: ECOTOX Open Literature

A study (Birch and Prahlad, 1986, ECOTOX Ref. #12119) examines the developmental toxicity of MITC in the South African clawed frog (*Xenopus laevis*). Data from this study was not used due to the limitations of the study. No control versus solvent control mortality was reported for the tadpole study. No control mortality was reported. The loading (number of tadpoles per chamber) would impact water quality . There was no report of tadpoles being fed. No results for the mortality were provided in data for the tadpole study, therefore no dose-response effect was verified.. No measured concentrations were reported either initial or termination concentrations for the volatile pesticide MITC. No data was available for statistical review. Embryos were less sensitive to MITC than tadpoles for mortality. The LOAEL for embryo mortality is reported to be 0.01 µg/L at 10 days for MITC. Embryos demonstrated a 50% mortality effect at 0.05 µg/L at 10 days for MITC. The study reports "severely twisted" notochords in the developing embryos in concentrations above 50.0 µg/L. The MITC concentration at which malformations are reported is above the EFED peak aquatic EEC of 0.6 µg/L for strawberries. Only embryo data for survival and damage were reported for the control and for concentrations below 1 µg/L. No data was available for statistical review. No measured concentrations were reported for this volatile chemical. Based on no report of measured concentrations,no control mortality data, no report of feeding tadpoles, and loading issues impacting water quality this study is classified as invalid.

A study (Haendel, M, et. al. 2004; ECOTOX Ref. #80675) examines the developmental toxicity of both metam sodium and MITC in the zebrafish (*Danio rerio*). The data from this study was not used in this assessment. This study is classified as invalid based on no reported measured concentrations for initial or termination concentrations for a static toxicity test on a volatile chemical. The study does not identify concentrations or number of treatments used in the study for the mortality or developmental studies. No control mortality is reported at 48 h, test termination, for either mortality or developmental endpoints. It reports "severely twisted" notochords in the developing fish. The LOAEL for both notochord defects and decreased hatching rate is reported to be 26 µg/L for metam sodium (where 25% of the fish had malformations) and 29 µg/L for MITC. A 48 hour post fertilization (hpf) LC50 is reported to be 248 µg/L and 137 µg/L for MITC. The LOEL for notochord defects and hatching effects reported for MITC is lower the EFED peak aquatic EEC of 59.4 µg/L for strawberries using the irrogation application method. The LC50, 137 µg/L, effects reported for MITC is higher the EFED peak aquatic EEC of 59.4 µg/L for strawberries using the sprinkler irrigation application method