Data Supplement

MI

CIR EXPERT PANEL MEETING
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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Jinqiu Zhu, PhD, DABT, ERT, Toxicologist, CIR
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Date: May 29, 2020
Subject: Draft Amended Safety Assessment on Methylisothiazolinone – Wave 3

Since the Draft Amended Safety Assessment on Methylisothiazolinone (MI) was prepared by CIR staff, the US Environmental Protection Agency (EPA) has released a draft risk assessment for Methylchloroisothiazolinone (MCI) and MI,[1] and a hazard characterization of isothiazolinones[2] (*MI062020wave3_epa1* and *MI062020wave3_epa2*, respectively). The documents have been reviewed by CIR staff and the following notes have been prepared for the Panel's review.

1. The Panel reopened MI *based, in-part, upon the adverse effects on the inhalation of humidifier disinfectants containing MCI/MI*. The following summaries of inhalation data from the EPA reports are relatively new:[1]

- Residential and occupational handler risks were assessed using the MI maximum application rate of 400 ppm by weight. The inhalation margins of exposure (MOEs) for residential aerosol exposures range from 15 to 14,000 and are not of concern because they are greater than the level of concern (LOC) of 10. The inhalation MOE of 1.0 for the residential handler applying paint, however, is of toxicological concern (Table 20 in *MI062020wave3_epa1*). The MOE for post application exposure to the MI vapors is 1.9 on the day after painting and is of concern; the exposures to the paint vapors decline over time, and, by day 12 after painting, the MOE is 11 which is not of concern (Table 21 in *MI062020wave3_epa1*).
- The inhalation MOEs for occupational aerosol exposures range from 4.4 to 5,800. The MOE of 4.4 for the airless spray application of paint is of concern because it is less than the LOC of 10 (Table 28 in *MI062020wave3_epa1*). The occupational inhalation MOE for MI vapors emitted from MI preserved paints is 0.5 and is of concern (Table 30 in *MI062020wave3_epa1*).
- The inhalation route of exposure, to be aggregated for the residential use of MI-treated cleaners, includes the co-occurrence of a handler applying cleaners using a trigger spray & wipe, plus mopping floors. Table 26 presents the aggregate inhalation MOE of 170 for the daily application of the cleaners, which is not of toxicological concern (above the LOC of 10).

The EPA also assessed incidental oral and dermal post-application exposures for MI in textiles and floor cleaners. Though these assessments are not relevant to cosmetic exposures, the following results from the cumulative risk characterization are interesting:

- Because the toxic effects seen with the dermal exposure pathway differ from the effects seen with the oral pathway, the dermal exposures are not additive to the effects resulting from dietary exposure; therefore, these would not be aggregated in a cumulative risk assessment.[2] In other words, each of the three routes of exposure (oral, dermal, and inhalation) are based on different toxicological endpoints; thus, exposures across routes are not aggregated.[1]
- The EPA has determined that although the isothiazolinones share some chemical and/or toxicological characteristics (e.g., chemical structure or apical endpoint), the toxicological database does not support a testable hypothesis for a common mechanism of action. No further cumulative evaluation is necessary for the isothiazolinones.[1]

2. The novelty of the case study in the documents is that the EPA has used the Artificial Neural Network (ANN) model that combines multiple non-animal methods to predict local lymph node assay (LLNA) EC3 values to extrapolate dermal risk for the currently registered isothiazolinones, as part of registration review. The data generated from *in vitro* and *in chemico* assays have been used to derive concentrations of the isothiazolinones that can induce skin sensitization (concentrations that can cause sensitization in persons not previously exposed). In addition, the EPA has determined that the *in vitro* and *in chemico* studies provide information that is more reliable and relevant to humans than the information obtained with the LLNA.[2] This use of the *in vitro* and *in chemico* assays, along with the ANN-based defined approach, are the first use of such information in regulatory risk assessment.

3. In line with the findings in the Draft Amended Report, the EPA concluded that isothiazolinones do not present a mutagenic or carcinogenic concern, and developmental and reproductive toxicity is not observed. The available isothiazolinone databases indicate that these chemicals are not neurotoxic. However, all the isothiazolinones are positive skin sensitizers.

References

1. US Environmental Protection Agency (EPA). Registration Review Draft Risk Assessment for 2-methyl-4-isothiazolin-3-one (MIT) and 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT). Washington, DC April 6, 2020. Pages 94. (Accessed May 27, 2020.)

2. US Environmental Protection Agency (EPA). Hazard Characterization of Isothiazolinones in Support of FIFRA Registration Review. Washington, DC April 6, 2020. Pages 84. (Accessed May 27, 2020.)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: **April 6, 2020**

SUBJECT: **Registration Review Draft Risk Assessment for 2-methyl-4-isothiazolin-3-one (MIT) and 5-chloro-2-methyl-4-isothiasolin-3-one (CMIT)**

PC Codes: 107103 and 107104	DP Barcode: 455497,455498, 456952
Decision No.: 558140, 455498, 558144	Docket No: EPA-HQ-OPP-2013-0605
Regulatory Action: Registration Review	Case No.: 3092
Risk Assessment Type: Draft Risk Assessment (DRA)	CAS No.: 2682-20-4, 26172-55-4

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This document provides the preliminary human health and ecological risk assessment conducted in support of 2-methyl-4-isothiazolin-3-one (MIT) and 5- chloro-2-methyl-4-isothiasolin-3-one (CMIT) for registration review.

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1.0 EXECUTIVE SUMMARY

2-methyl-4-isothiazolin-3-one (MIT) and 5- chloro-2-methyl-4-isothiasolin-3-one (CMIT) are two of several cyclic compounds which belong to the isothiazolinone chemical group. The isothiazolone biocides are commonly used to control bacteria, fungi, and/ or algae in a variety of materials and processes. These include plastics, paints, household cleaning products, metalworking fluids, textiles, pesticide formulations as an inert, leather production, paper mill water systems, cooling water systems, oil recovery injection water, drilling muds and packer fluids and wood treatments. There are 140 products that contain a mixture of MIT/CMIT, MIT or CMIT as active ingredients.

Human Health Risk Summary

Dietary Risk Summary

Dietary risk estimates and exposures for MIT/CMIT used to preserve consumer and commercial products such as cleaners and dish detergents are not of concern. Other dietary uses such as adhesives, paper coatings and slimicides were also not of concern. Further, there are no risks associated with MIT/CMIT when used as an inert within the tolerance exemption allowance. In cases where MIT is found in cleaning products and dish detergents as a single active ingredient, risk estimates were below 30% of the population adjusted doses (PADs). Co-occuring dietary exposures of MIT/CMIT mixtures were below 30% of the cPAD for the highest exposed population subgroup (children 1 to 2 years old). Because, MIT exposures alone does not exceed co-occurring exposures of MIT/CMIT mixtures, it is determined that MIT/CMIT risk estimates are protective of MIT only uses and therefore are not added as total exposures. Combined risk estimates for MIT/CMIT included potential drinking water exposure caused by use and discharge from industrial sources.

Residential Risk Summary

Residential handler risks were assessed using the MIT maximum application rate of 400 parts per million by weight (ppm) for paints and household cleaners. Although products that contain MIT/CMIT are also used to preserve paints and household cleaners, these products with combined MIT/CMIT are applied at a lower rate of 135 ppm. The inhalation margins of exposure (MOEs) for aerosol exposures range from 15 to 14,000 and are not of concern because they are greater than the level of concern (LOC) of 10. The dermal MOEs for induction range from 110 to 1600 and are not of concern because they are greater than the LOC of 100. The dermal MOEs for elicitation range from 0.001 to 0.08 and are of concern because they are less than the LOC of 10.

Residential handler and post application exposures were assessed for MIT vapors emitted from MIT preserved paints using the Wall Paint Exposure Model (WPEM). The inhalation MOE of 1.0 for the handler applying the paint is of concern because it is less than the LOC of 10. The MOE for post application exposure to the MIT vapors is 1.9 on the day after painting and is of concern. The exposures to the paint vapors decline over time and by day 12 after painting the MOE is 11 which is not of concern.

Incidental oral and dermal post application exposures were assessed for MIT in textiles and floor cleaners. The incidental oral MOEs of 120 and 400, for textiles and floor cleaners, respectively, are greater than the LOC of 10 and are not of concern. The corresponding dermal MOEs for induction are 26 and 130. The dermal MOE of 26 is of concern because it is less than the LOC of 100. The dermal MOEs for elicitation of 0.001 for textiles and 0.007 for floor cleaners are of concern.

Aggregate Risk Summary

The acute and chronic aggregate assessments are based on the total dietary exposures as the residential exposures are expected to be best represented by the short- and intermediate-term (ST/IT) durations. The acute total dietary exposures are minimal for all of the exposed subpopulations (<1% aPAD). The chronic total dietary exposures do not show any risks for any of the subpopulation; highest estimated risk was for children (1-2 years old) at 30% cPAD equivalent to an MOE of 34 with a LOC of 10. For the ST/IT aggregate, the residential uses and inhalation, dermal, and oral routes of exposure are considered. Each route of exposure results in different toxicological endpoints of concern, and therefore, exposures are not aggregated across routes. For the inhalation route, the exposure scenarios that may co-occur are based on the residential use of treated cleaners. It is highly likely that one would apply the cleaners using the trigger spray & wipe plus mop floors on the same day. The combined inhalation exposure for these events results in an inhalation MOE of 170 which is not of concern (LOC of 10). For the dermal route, for those individuals already sensitized, the use of MIT/CMIT in paints, cleaners, and impregnated clothing would likely result in an allergic contact dermatitis (ACD) reactions based on the assumptions in this assessment. Therefore, the individual uses would need to be mitigated before they could be considered in an aggregate (*i.e.*, if individual uses have MOEs of concern by themselves, they are not considered in the aggregate as they fill the risk cup). For the oral route, there is one residential use of MIT/CMIT, children's incidental oral exposure for mouthing/sucking on treated clothing, to be combined with the total dietary exposure (food + drinking water). The ST/IT oral aggregate MOE for children (1 to <2 years old) is 66 for the combined exposures of total diet and incidental ingestion mouthing/sucking on treated clothing which is not of concern (*i.e.*, above the LOC of 10).

Occupational Risk Summary

Occupational handler inhalation and dermal exposures to MIT as an aerosol were assessed for open pouring liquids for material preservation and using paints and household cleaners preserved with MIT. The inhalation MOEs range from 4.4 to 5,800 and the MOE of 4.4 is of concern because it is less than the LOC of 10. The dermal MOEs for induction range from 21 to 210 and most are of concern because they are less than the LOC of 100. The dermal MOEs for elicitation range from 0.001 to 0.01 and all are of concern because they are less than the LOC of 10.

Occupational handler inhalation exposures were assessed for MIT vapors emitted from MIT preserved paints using the Wall Paint Exposure Model (WPEM). The inhalation MOE of 0.5 is of concern because it is less than the LOC of 10.

Dermal and inhalation risks were assessed for machinists using Metal Working Fluids (MWF) treated with MIT. The dermal MOE of 46 for induction is of concern because it is less than the LOC of 100. The dermal MOE of 0.002 for elicitation is of concern because it is less than the LOC of 10. The inhalation MOE of 250 is not of concern.

Dermal and inhalation risks were assessed for workers using MIT/CMIT during the pressure treatment of wood. The dermal MOEs for induction and elicitation and the inhalation MOEs are not of concern.

Dermal and inhalation risks were assessed for workers using MIT/CMIT during the sapstain treatment of wood. The clean-up crew inhalation MOE of 0.75 is of concern for short and intermediate term exposures because is less than the LOC of 10. The remaining MOEs, which range from 16 to 26, are of concern only for long term exposures because they are less than LOC of 30. The cleanup crew dermal MOE of 48 for induction is of concern because it is less than the LOC of 100. The remaining induction MOEs are not of concern. The elicitation MOEs, which range from 0.004 to 0.06, are of concern because they are less than the LOC of 10.

Ecological Risk Summary

Based on the current use patterns for MIT/CMIT, no terrestrial exposures are expected. Several of the use patterns could result in aquatic exposure, however. Of these, the potential risks from the cooling water tower, pulp and paper mill, paints, and wood treatments are expected to result in the highest aquatic exposures and were assessed.

A screening-level risk assessment of MIT/CMIT used in cooling towers and pulp and paper mills was performed using the Exposure and Fate Assessment Screening Tool (E-FAST). Various scenarios were evaluated: (1) high-end (low stream-flow rate) and average (average stream flow rate) assessments, (2) moderate-sized and large-sized cooling water towers, and (3) high and low application rates in pulp and paper mills. The cooling water tower and paper mill uses resulted in exposures that exceed levels of concern for freshwater fish, aquatic invertebrates, and aquatic plants. The quantities of CMIT and MIT actually present in the environment from these uses are likely lower than those modeled due to the rapid degradation of MIT/CMIT (which is not accounted for in the modeling) and the lack of degradates of concern. However, because of the toxicity of MIT/CMIT to aquatic organisms and the number of days that the Concentrations of Concern (COCs) were exceeded, especially for the high-end scenarios with the highest application rates (*i.e.*, 20 ppm and 153 ppm for water cooling towers and paper mills, respectively), risks to fish, aquatic invertebrates and aquatic plants from the water cooling tower and paper mills uses cannot be precluded at this time and are assumed.

Additionally, an ecological risk assessment was performed for MIT/CMIT used in exterior paints and pressure treated wood used in docks. No risk to aquatic organisms were associated with these uses. The equivalent of 28 one-story houses or >2,500 docks could be located in the watershed of a single waterbody before risks would occur from the paint and wood treatment uses, respectively.

No estuarine/marine or benthic invertebrate risk assessment was conducted for MIT/CMIT because no data were submitted to the Agency or the aquatic exposure models used were not appropriate for the receptor group. Risks to estuarine/marine fish and invertebrates are assumed to be similar to freshwater taxa, based on the available ecotoxicity data. However, risks to benthic organisms are not expected because of low exposure potential (*e.g.*, MIT/CMIT is not expected to accumulate in sediment due to its rapid degradation and its relatively low log K_{ow}).

No quantitative risk assessment has been performed for terrestrial organisms (including pollinators). However, due to low potential for exposure, risks to terrestrial organisms (including pollinators) are not expected.

2.0 INTRODUCTION

2.1 Case Overview

The registration review docket for Case 3092 (MIT/CMIT) has been established at http://www.regulations.gov in docket number EPA-HQ-OPP-2013-0605.

A Reregistration Eligibility Decision (RED) for MIT/CMIT and was completed in October of 1998. By June 2014, the Agency had completed its registration review Preliminary Work Plan (PWP) for MIT/CMIT and the Final Work Plan (FWP) was finalized in December of the same year.

2.2 Chemical Ingredient Profile

Table 1 contains the chemical identification of MIT and CMIT which belong to the isothiazolinone chemical family. There are six pesticidal active ingredients in this chemical family: N-butyl-1,2-benzisothiazolin-3-one **(**BBIT), 1,2-benzisothiazolin-3-one (BIT), 2-n-octyl-4-isothiazolin-3-one (OIT), 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT), 2-methyl-4-isothiazoline-3-one (MIT) and 5-chloro-2-methyl-4-isothiazoline-3-one (CMIT). The Agency concluded that it was appropriate to bridge the chemicals into one group due to the similarity of their pesticidal, toxicological and environmental behavior characteristics (see Section 4.1.1; IT Hazard Characterization Chapter (EPA, 2020)).

The chemical identification and physical/chemical properties of MIT/CMIT are presented below in Tables 1 and 2.

Table 1. Chemical Identification of CMIT and MIT

Chemical Name	5-Chloro-2-methyl-3(2H)-isothiazolone (CMIT)	2-Methyl-3(2H)-isothiazolone (MIT)
Chemical Classification	Isothiazolinone	Isothiazolinone
PC Code	107103	107104
CAS Number	26172-55-4	2682-20-4
Molecular Formula	C_4H_4ClNOS	C_4H_5NOS
Molecular Weight	149.60	115.2

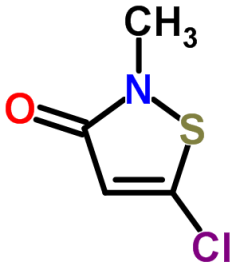
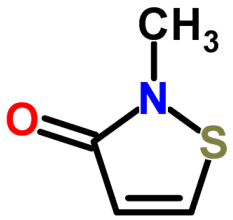
Chemical Name	5-Chloro-2-methyl-3(2H)-isothiazolone (CMIT)	2-Methyl-3(2H)-isothiazolone (MIT)
Molecular Structure		

Table 2. Chemical and Physical/ Environmental Fate Properties of MIT/CMIT

Guideline No.	**Parameter**	**Mixture of two AIs (3:1 ratio) CMIT = 70.1%, MIT = 26% (EPA Reg. No. 707-234)**	**MIT = 96% (EPA Reg. No. 707-255)**
830.7000	pH	2.1 at 25°C (5% solution in water)	2.58 (5% solution in water)
830.7050	UV/Visible Absorption	Stable to sunlight	Stable to sunlight
830.7200	Melting point	52-53°C	48.0-49.5°C
830.7220	Boiling point	N/A, this product is a semi solid at room temperature.	
830.7300	Density (g/ml)	1.42 at 25°C	1.35 at 25°C
830.7550	Octanol-water partition coefficient (Log K_{ow})	**CMIT**: 0.401 at 24°C in log Kow (PAI)	Log Kow=−0.486 at 24°C
830.7840	Solubility in water (grams/100 ml)	**CMIT:** ≥65.96 at 25°C **MIT:** ≥22.59 at 25°C	≥100
830.7950	Vapor pressure (mm Hg)	**CMIT**:$1.8x10^{-2}$ (PAI)	$6.2x10^{-2}$ at 25°C (PAI)
None	Henry's law constant (atm-m^3/mol)	**CMIT**: 5.37×10^{-9} at 25 °C [Calculated]	**MIT**: 4.16×10^{-8} at 25 °C [Calculated]

atm-m^3/mol=atmosphere cubic meter per mole; mmHg = millimeters of mercury

2.3 Use Patterns

2.3.1 Active Ingredient Registered Products and Uses

There are 140 EPA-registered products that contain MIT, MIT/CMIT mixtures or CMIT as an active ingredient (a.i.). There is one product (67071-112) that contains only CMIT and there are 19 products which contain only MIT. The remaining 120 products often contain MIT and CMIT in approximately a 3:1 ratio (CMIT: MIT) and are commonly referred as containing MIT/CMIT. Most of the products containing MIT, CMIT and MIT/CMIT are formulated as liquids in ready to use solutions, flowable concentrates or liquid soluble concentrates. Three products (464-8134, 464-8135 and 464-8137 are formulated as solid tablets. There is also a product (67071-38) listed as a wettable powder; however, this product is actually a liquid concentrate.

The application rates for the MIT, CMIT and MIT/CMIT registered end use products are included in Table 3. The application rates for MIT are based on products that only contain MIT. The application rates for MIT/CMIT are based on products that contain both MIT and CMIT. These application rates were calculated by adding the MIT percent a.i. to the CMIT percent a.i. to obtain a combined MIT/CMIT percent a.i. This combined MIT/CMIT percent a.i. was used along with the product application rates (*i.e.*, add 1 pound of product to 1000 gallons of cooling water) to calculate the application rate in terms of ppm a.i. The application rates for the product that contains only CMIT are within the range of the application rates for the MIT/CMIT products.

Table 3. MIT/CMIT Registered Uses and Application Rates

Use	Application Rate (ppm a.i.)	
	MIT	MIT/CMIT
Industrial Process and Water Systems		
Air conditioner/refrigeration condensate water systems	Not Registered	3.3 to 14
Air washer and industrial scrubbing water systems	3.3 to 153	1.4 to 38
Coal slurry systems	Not Registered	3.3 to 14
Commercial/industrial water-cooling systems	1.1 to 3.4	1.9 to 20
Evaporative condenser and heat exchanger water systems	Not Registered	3.3 to 14
Industrial auxiliary water systems	Not Registered	9.3 to 12
Industrial processing water and waste disposal systems	1.1 to 153	1.9 to 38
Oil recovery drilling muds/packer fluids	0.56 to 153	2.4 to 127
Pasteurizer/warmer/cannery cooling water systems	Not Registered	1.9 to 14
Pulp/paper mill water systems	153	11 to 97
Reverse osmosis water system	2.8 to 153	4.7 to 19
Secondary oil recovery injection water	1.1	2.0 to 45
Sewage systems	Not Registered	3.3 to 14
Wet-end additives/industrial processing chemicals	26 to 300	16 to 49
Material		
Adhesives, industrial	16 to 400	19 to 135
Coatings, industrial	Not Registered	12 to 233
Electronics Production Processing Solutions	153 to 255	19 to 440
Emulsions, resin/latex/polymer	125 to 400	20 to 135
Fuels/oil storage tank bottom water additive	18 to 34	1.0 to 33
Household cleaning products	112 to 400	15 to 135
Leather processing liquors	60	Not Registered
Metalworking cutting fluids	13 to 444	3.7 to 135
Paints and coatings	28 to 400	20 to 135
Paper/paper products	100 to 150	11 to 135
Pesticide formulations (as an inert ingredient)	Not Registered	35 (CMIT only)
Rubber products	Not Registered	55
Textiles/textile fibers/cordage	125 to 400	25 to 135
Wood Preservative		
Wood and wood products (pressure treatment)	Not Registered	13 to 63
Wood and wood products (spray and dip treatment)	Not Registered	15 to 54

2.3.2 Inert Ingredient Registered Products and Uses

As of 12/27/2019, there are 11 EPA registered pesticide products that contain MIT/CMIT (PC code 907106) as an inert ingredient.

2.3.3 Usage Information

MIT and CMIT are mentioned in the 2012 Kline report: "Specialty Biocides: Regional Market Analysis 2012- United States" published April 3, 2013 as a major use within adhesives, metalworking fluids, paints/coatings, synthetic latex polymers, cooling water, water treatment in paper production, and hygiene products. Estimated (2012) and forecasted (2017) consumption rates are in Tables 4 to 6.

Table 4. 2012 Kline Report: MIT/CMIT Uses

	2012 Estimated Consumption (Lb)	% of total market by volume	Forecasted Consumption for 2017 (Lb)
Adhesives and Sealants	136,000	4.8	146,600
Metalworking Fluids	57,000	0.7	65,000
Paints and Coatings	501,000	4.7	539,800
Synthetic Latex Polymers	345,000	22.6	381,200
Water Treatment- Cooling water	500,000	0.1	565,800
Water Treatment- Paper	80,000	<0.1	88,400
Hygiene- Household, Industrial, and Institutional (HI&I) Cleaning Products	5,000 (I&I only)	<0.1	5,600

Table 5. 2012 Kline Report: MIT Only Uses

	2012 Estimated Consumption (Lb)	% of total market by volume	Forecasted Consumption for 2017 (Lb)
Adhesives and Sealants	2,000	0.1	2,300
Metalworking Fluids	1,000	<0.1	1,000
Paints and Coatings	5,000	<0.1	5,600
Synthetic Latex Polymers	12,000	0.8	13,000

Table 6: 2012 Kline Report: BIT/MIT Uses

	2012 Estimated Consumption (Lb)	% of total market by volume	Forecasted Consumption for 2017 (Lb)
Synthetic Latex Polymers	10,000	0.7	11,400

In a study of the isothiazolinone content of residential interior wall paint (Goodier, 2018), 46 paints were purchased from home improvement stores and independent paint retailers in the Twin Cities area of Minnesota. In addition, a water-based paint that was advertised to be preservative free was purchased online. Forty-five of the paints were water based and two were oil based. The paints were analyzed for MIT (listed as MI), BIT, CMIT (listed as MCI), OIT and BBIT content using ultra high-performance liquid chromatography. All of the paints, including

the preservative free paint, contained at least one isothiazolinone and most of the paints contained MIT and BIT. Only the two oil-based paints contained CMIT and the paint that contained OIT also contained MIT and BIT. A summary of the isothiazolinone content is included in Table 7.

Table 7. Prevalence and Concentration of Isothiazolinones in Paint

Isothiazolinone	Number of Samples	Range (ppm)	Mean Content (ppm)	Standard Deviation
MIT (MI)	45	1.0 to 357.9	91.2	90.5
BIT	44	28.6 to 1110.7	170.6	179.2
cMIT (MCI)	2	8.2 to 13.1	10.6	3.4
OIT	1	43.2	N/A	N/A
BBIT	0	N/A	N/A	N/A

Source: Goodier, 2018.

2.4 Label Recommendations

For labels with registration numbers 39967-91 and 707-128, within the wood preservation directions, the maximum concentration of product presented (ppm) disagrees with the calculated concentration when applying the product (fl. oz) to the treatment solution.

3.0 HUMAN HEALTH RISK ASSESSMENT

3.1 Data Deficiencies

The toxicological databases for MIT and CMIT are considered complete for the purpose of this registration review case.

3.2 Tolerance Considerations

The Agency has not established exemptions from the requirement of a tolerance for MIT and CMIT**.**

3.3 Anticipated Exposure Pathways

Exposures from uses of MIT and CMIT occur via the oral, dermal and inhalation routes.

3.4 Hazard Characterization and Dose-Response Assessment

3.4.1 Summary of Toxicological Effects

The isothiazolinone biocides are reactive chemicals and as such, cause point of contact adverse effects such as irritation or corrosion of the skin and eyes, irritation of the respiratory tract, and irritation-type responses of the gastrointestinal tract. All the isothiazolinone biocides are Category I (corrosive) for eye and skin irritation except for BIT which is Category IV. All the isothiazolinones are known to cause allergic contact dermatitis (dermal sensitization).

In repeat dosing studies with the isothiazolinone biocides, evidence of irritation, such as lesions of the glandular stomach and skin, are observed as effects across the class of chemicals. Decreases in body weight across multiple species and emesis in dogs are also common adverse findings throughout the available toxicology studies for these chemicals. The effects of the isothiazolinone biocides are similar among members of the class, and include effects related to the irritant properties of the chemicals, such as hyperplasia/hyperkeratosis of the squamous mucosa of the forestomach from oral exposure; erythema and desquamation of the skin from dermal exposure; and inflammation/squamous metaplasia of the nasal cavity from inhalation exposure. The isothiazolinones are also positive dermal sensitizers. Isothiazolinones do not pose a mutagenic or carcinogenic concern based on the available data. Developmental and reproductive toxicities are also not observed with these chemicals. The available isothiazolinone databases indicate that they are not neurotoxic.

Although their toxicological effects are qualitatively similar, the isothiazolinone biocides differ in potency with no/lowest observed adverse effect levels (NOAELs/LOAELs) varying across the groups for these effects, the Agency concluded that it was appropriate to consider the toxicity databases of the chemicals as one group due to the similarity of the toxicity profiles, including the adverse effect of dermal sensitization. For risk assessment purposes, chemical-specific data are used when available. When chemical-specific data are not available, the most conservative endpoint for which there are data from other isothiazolinones is used. Refer to the IT Hazard Characterization Chapter for details (USEPA, 2020).

3.4.2 Evidence of Sensitivity/Susceptibility in the Developing or Young Animal

3.4.2.1 Safety Factor for Infants and Children (FQPA Safety Factor)

The 10X Food Quality Protection Act (FQPA) Safety Factor was not retained for MIT/CMIT as there were no evidence of increased quantitative or qualitative susceptibility was seen in rat and rabbit developmental toxicity and rat reproduction studies. All fetal and offspring effects were observed either in the presence of comparable maternal toxicity at the same dose or at doses higher than those that produced maternal toxicity.

3.4.3 Toxicity Endpoint and Point of Departure Selections

Toxicity endpoints and points of departure (POD) for dietary, residential and occupational exposure scenarios for antimicrobial uses of MIT/CMIT are summarized below.

Acute Dietary: For assessment of acute dietary risk from exposure to MIT/CMIT, the NOAEL of 79 mg/kg was selected as the POD from the acute oral toxicity study in the rat (MRID 00086092). At the LOAEL of 57 mg/kg/day, signs of intoxication were observed. An uncertainty factor of 10 was applied to this POD (3x interspecies extrapolation, 3x intraspecies variation) for calculation of the acute Population Adjusted Dose (aPAD).

Chronic Dietary: For assessment of chronic dietary risk from exposure to MIT/CMIT, the value of 2 mg/kg/day was selected as the POD from the 24-month drinking water chronic/oncogenic

study in rats for MIT/CMIT mixture (MRID 43140701). At the LOAEL value of 6.6/9.8 mg/kg/day (M/F), hyperplasia /hyperkeratosis of the squamous mucosa of the forestomach in both male and females was observed, as was necrosis of glandular mucosa of the stomach in females and edema/inflammation of the glandular stomach in females. An uncertainty factor of 10 was applied to this POD (3x interspecies extrapolation, 3x intraspecies variation) for calculation of the chronic Population Adjusted Dose (cPAD).

Short-/Intermediate-/Long-term dermal: The **MIT/CMIT Induction**: average *in vitro* EC3[1] = 0.49% (120 µg/cm^2) for MIT/CMIT is based on Model 4 from Hirota *et al*., 2015: DPRA + h-CLAT + KeratinoSens with an uncertainty factor of 100x was utilized to assess dermal exposure. Refer to the IT Hazard Characterization Chapter for details (EPA, 2020).

The induction POD selected for assessment of short, intermediate, and long-term dermal exposures for MIT/CMIT is based on the dermal sensitization induction threshold of 120 µg/cm^2 determined from the average EC3 value of 0.49% based on the Shiseido artificial neural network (ANN) Model (ANN D_hC_KS, "model 4" in Hirota *et al.*, 2015). ANN models are non-linear statistical models that combine multiple in vitro parameters covering various Key Events of the skin sensitization adverse outcome pathway (AOP) and predicts the local lymph node assay (LLNA) EC3 as an output. For a complete description of the use of these models for determination of the POD for MIT/CMIT and the other isothiazolinones, see NICEATM, 2020 and the IT Hazard Characterization Chapter for details (EPA, 2020). An uncertainty factor of 100x was applied to the POD.

Short-/Intermediate-/Long-term dermal: The **MIT Induction** The induction POD selected for assessment of short, intermediate, and long-term dermal exposures for MIT is based on the dermal sensitization induction threshold of 210 µg/cm^2 determined from the average EC3 value of 0.83% based on the Shiseido artificial neural network (ANN) Model (ANN D_hC_KS, "model 4" in Hirota *et al*., 2015).

The elicitation POD selected for assessment of short, intermediate, and long-term dermal exposures for MIT/CMIT are based on the Minimum Elicitation Threshold (MET) 18% of 0.0105 µg/cm^2 from a human Repeat Open Application Test (ROAT) study (Lundov *et al*., 2011) with an uncertainty factor of 10x (UF_H = 3x and UF_L= 3x). Refer to the IT Hazard Characterization Chapter for details (EPA, 2020).

Short-/Intermediate-/Long-term inhalation: There is a chemical specific inhalation study for MIT/CMIT. The NOAEC is 0.34 mg/m^3 and the LOAEC is 1.15 mg/m^3 based on the microscopic lesions in the nasal turbinates (rhinitis). The POD is further refined by calculating the Human Equivalent Concentration (HEC) from the LOAEC of 1.15 mg/m^3 using a Regional Dose Deposition Ration (RDDR) of 0.30 for extrathoracic effects. This RDDR was calculated using a Mass Median Aerodynamic Diameter (MMAD) of 1.1 microns, a Geometric Standard Deviation (GSD) of 2.0 and a study specific rat body weight of 400 grams based on the week 13 male and female average body weight. An uncertainty factor of 10x (UF_A =3x and UF_H=3) was applied to the short/intermediate -term exposures and an uncertainty factor of 30x (UF_A =3x,

[1] The EC3 is the effective concentration of a chemical (percent of chemical in vehicle) required to produce a 3-fold increase in the proliferation of lymph node cells compared to vehicle treated controls.

UF_H=3x and UF_D =3x) was applied for the long-term exposure. Refer to the IT Hazard Characterization Chapter for details (EPA, 2020).

Cancer: Carcinogenicity data are not available for MIT/CMIT but are not required based on the following: 1) available cancer studies for the isothiazolinone biocides are negative; 2) there is a lack of mutagenicity concern for the isothiazolinone biocides; 3) isothiazolinones are irritants following oral, dermal and inhalation exposures and produce similar effects following subchronic exposures; 4) the isothiazolones as a group have a known mode of action for antimicrobial activity, and; 5) irritation is the predominant effect and is the basis of the PODs and considered protective of any potential carcinogenic effects. CMIT/MIT was given a Group D classification by OPP Cancer Peer Review Committee.

Table 8. Toxicological Effects and Points of Departure for MIT and CMIT

Exposure Route	POD	LOC and UFs	Study and Effects
Acute Dietary (All populations, including infants, children and females 13 to 49)	NOAEL =79 mg/kg/day	aRfD = 7.9 mg/kg/day aPAD = aRfD/FQPA SF = 7.9 mg/kg/day FQPA SF= 1X UF= 10X	MIT/CMIT:Acute Oral – Rat (MRID 00086092) Death at 157 mg/kg/day (5), 313 mg/kg/day (10), and 625 mg/kg/day (10). **LOAEL = 157 mg/kg/day** based on signs of intoxication (lethargy, prostration, ataxia, dyspnea, severe irritation and hemorrhage were noticed in g.i).
Chronic Dietary (All Populations)	NOAEL =2 mg/kg/day	cRfD = 0.2 mg/kg/day cPAD = cRfD/FQPA SF = 0.2 mg/kg/day FQPA SF= 1X UF= 10X	MIT/CMIT: 24-month drinking water chronic/oncogenic study in rats -1994 (MRID 43140701) **LOAEL = 6.6/9.8 mg/kg/day (M/F)** based on hyperplasia /hyperkeratosis of the squamous mucosa of the forestomach in both M/F, necrosis of glandular mucosa of the stomach in females and edema/inflammation of the glandular stomach in the females.
Incidental Oral (Short- and Intermediate-Term)	NOAEL= 8.5 mg/kg/day	Residential LOC or MOE = 10 UF_A = 3 UF_H = 3	MIT/CMIT: Rat 2-gen reproductive study (MRID 44656101) **LOAELparental = 22.7/28 mg/kg/day** Based on increased incidence of histopathological lesions of the glandular and non-glandular stomach in the F0 and F1 male and female rats.
Dermal (Induction) MIT/CMIT All durations	EC3 = 0.49% (120 µg/cm^2)	Residential and Occupational LOC for MOE = 100 UF_A = 10 UF_H = 10	**Induction:** EC3 = 0.49% for MIT/CMIT based on Model 4 from Hirota *et al.*, 2015: DPRA + h-CLAT + KeratinoSens *in vitro* assays µg/cm^2 = [EC3 x 25µL x 10 µg/µL]/cm^2
Dermal (Induction) MIT Only All durations	EC3 = 0.83% (210 µg/cm^2)	Residential and Occupational LOC for MOE = 100 UF_A = 10 UF_H = 10	**Induction:** EC3 = 0.83% for MIT based on Model 4 from Hirota *et al.*, 2015: DPRA + h-CLAT + KeratinoSens *in vitro* assays µg/cm^2 = [EC3 x 25µL x 10 µg/µL]/cm^2

Exposure Route	POD	LOC and UFs	Study and Effects
Dermal (Elicitation) MIT/CMIT and MIT All durations	0.0105 µg/cm^2	Residential and Occupational LOC for MOE = 10 UF_A = 1 UF_H = 3 UF_L = 3	MIT/CMIT and MIT: **Elicitation**: 0.0105 µg/cm^2 = Minimum Elicitation Threshold (MET) = 18% (2/11) from Lundov human ROAT study
Inhalation (Short- and Intermediate-Term) **Inhalation** (Long-Term)	NOAEC = 0.34 mg/m^3 8 Hour HEC = 0.11 mg/m^3 24 Hour HEC = 0.038 mg/m^3	Residential and Occupational LOC for MOE = 10 UF_A= 3 UF_H = 3 Residential and Occupational LOC for MOE = 30 UF_A = 3 UF_H = 3 UF_D = 3	MIT/CMIT 90-day inhalation Study (MRID 00148418) **LOAEC = 1.15 mg/m^3**, based on microscopic lesions in the nasal turbinates (rhinitis)
Cancer (oral, dermal and inhalation)	Group D classification by OPP Cancer Peer Review Committee.		

FQPA Safety Factor =1 for all exposure routes. ST = Short term, IT = Intermediate term, LT = Long term
HEC = NOAEC * [6 hr animal / 8 or 24 hr human)]* RDDR (0.45 for ET effects)
UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). UF_L = LOAEL to NOAEL extrapolation, UF_D = Duration Adjustment, UF_L = LOAEL to NOAEL extrapolation, UF_D = Duration Adjustment. MOE = Margin of Exposure. LOC = Level of Concern. NOAEL= No Observable Adverse Effect Level. LOAEL= Lowest Observable Adverse Effect Level. Inhalation Uncertainty Factor lowered by a factor of 10 due to use of HEC. aRfD= acute reference dose, aPAD = acute population adjusted dose, cRfD= chronic reference dose, cPAD = chronic population adjusted dose.

3.5 Dietary Exposure and Risk Assessment

3.5.1 FFDCA Clearances

The Agency has not established exemptions from the requirement of a tolerance for MIT or CMIT as an active ingredient under the Federal Food, Drug, and Cosmetic Act (FFDCA) Section 408. However, there is an inert tolerance exemption for MIT/CMIT under 40 CFR Part 180.920. Details of this regulation are listed below in Table 9.

Table 9. Summary of EPA Tolerance Exemptions for CMIT (in combination with MIT)

40 CFR Section	Tolerance Exemption specifics	Use	Limit
180.920	**Inert ingredient used in pre-harvest**; CMIT (in combination with MIT) is exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredient in pesticide formulations applied to growing crops only:	Preservative	Not more than 0.0022% (22.5 ppm) in the formulation; 0.00022% (2.25 ppm) in the final solution applied to growing crops.

ppm = parts per million.

The Food and Drug Administration (FDA) has established several food additive regulations for indirect food uses of MIT/CMIT under FFDCA's section 409. Table 10 list regulation summaries. There is one Threshold of Regulation (TOR) Exemption for MIT and CMIT for components of pressure sensitive adhesives at 1% or less by weight; TOR No.2007-004[2].

Table 10. FDA's Clearances for indirect Food Additives for MIT and CMIT

21 CFR Section	FDA Clearances for Indirect Food Additives	Chemical CAS	Maximum Residue Level
175.105	**Adhesives and components of coating:** Preservative in the manufacturing of food contact adhesives	CMIT CAS No. 26172-55-4	No limit specified
175.300	**Resinous and polymeric coatings:** Coating applied as a continuous film or enamel over a metal substrate, or the coating is intended for repeated food-contact use and is applied to any suitable substrate as a continuous film or enamel that serves as a functional barrier between the food and the substrate.	CMIT (CAS No. 26172-55-4) and MIT (CAS No. 2682-20-4)	Ratio of 3 parts to 1 part, respectively, manufactured from methyl-3-mercaptopropionate and optionally containing magnesium nitrate at a concentration equivalent to the isothiazolone active ingredients (wt/wt). For use only as an antimicrobial agent in emulsion-based silicone coatings at a level not to exceed 50 mg/kg (based on isothiazolone active ingredient) in the coating formulations.
175.320	**Resinous and polymeric coatings for polyolefin films:** CMIT and MIT mixtures at a ratio of 3 parts to1 part, respectively, manufactured from methyl-3-mercaptopropionate and optionally containing magnesium nitrate at a concentration equivalent to the isothiazolone active ingredients (wt/wt).	CMIT (CAS No. 26172-55-4) and MIT (CAS No. 2682-20-4)	For use only as an antimicrobial agent in emulsion-based silicone coatings at a level not to exceed 50 mg/kg (based on isothiazolone active ingredient) in the coating formulation.
176.170	**Components of paper and paperboard:** For polymer latex emulsions in paper coatings; For finished coating formulations and for additives used in the production of paper and paperboard including fillers, binders, pigment slurries, and sizing solutions	CMIT (CAS No. 26172-55-4) and MIT (CAS No. 2682-20-4)	Polymer emulsion uses should not exceed 50 ppm (based on isothiazolone active ingredients) in coating formulations. For coating uses in paper and paperboard do not exceed 25 ppm (based on isothiazolone active ingredients) in the coating formulations and additives.

Several food contact notifications exist for MIT/CMIT mixtures and MIT alone. The notifications are dated back as early as 2001. Specification summaries are listed below in Table 11.

[2] Regulation specifics are found at https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=TOR&id=2007-004

Table 11. Food Contact Notifications for MIT and CMIT

FCN No.	Food Contact Substance	Intended Use	Specifications	Manufacture And Effective Date
1733	MIT (only) as a preservative in aqueous formulations, coatings, additive formulations and as a slimicide	In aqueous formulations of adhesives used in conditions defined in 21 CFR 175.105; In aqueous coating formulations to be used on paper under conditions defined in 21 CFR 176.170; In aqueous additive formulations (latex emulsions, fillers, binders, pigment slurries, and sizing solutions) used in paper and paperboard manufacturing to produce paper in compliance with 21 CFR 176.170 and 176.180; Slimicide in compliance with 21 CFR 176.170 and 176.180.	Adhesives at 150 ppm Paper coating formulations at 150 ppm; exception of latex coatings where max level is 250ppm Aqueous additive formulations for use in paper at 150 ppm slimicide for wet end of the paper process at 150 ppm in process water	LANXESS Corporation; July 7, 2017
1720[1]	A mixture of CMIT and MIT In a 3:1 ratio by weight, optionally stabilized with magnesium nitrate or sodium nitrate and optionally further stabilized with 0.15 % copper (II) nitrate.	Polymer latex solutions used in adhesives complying with 21 CFR 175.105; Preservative in resinous and polymeric coatings for polyolefin films complying with 21 CFR 175.320 In coatings and/or coating components complying with 21 CFR 175.300; and Polymer latex emulsions, coating formulations and additives for paper manufacture used for food packaging	For use at CIT and MIT levels: Levels specified in 21 CFR 175.105, stabilized with magnesium nitrate, sodium nitrate and up to 0.15 % copper(II) nitrate ~ to levels specified in 21 CFR 175.300 and 21 CFR 175.320, stabilized with magnesium nitrate, sodium nitrate and up to 0.15 % copper(II) nitrate as a preservative in resinous and polymeric coatings for polyolefin films and in coatings and/or coating components; ~ to levels specified in 21 CFR 176.170, stabilized with magnesium nitrate, sodium	Lonza, Inc.; Jan 5, 2017

FCN No.	Food Contact Substance	Intended Use	Specifications	Manufacture And Effective Date
			nitrate and up to 0.15 percent copper (II) nitrate as a biocide in polymer latex emulsions, coating formulations and additives for paper manufacture used for food packaging.	
1649	MIT (only)	as a preservative in coatings (resin and polymeric coatings for films)	Not to exceed 100 ppm on coatings on polyolefin film; emulsions of can-end cement not to exceed 150 ppm	LANXESS Corporation; Aug 27, 2016
1646	Mixtures of CMIT and MIT in a 3:1 ratio by weight, stabilized with magnesium nitrate and/or magnesium chloride, and further stabilized with 0.15% copper (II) nitrate	As an antimicrobial agent in polymer latex solutions used in adhesives complying with 21 CFR 175.105; as a preservative in resinous and polymeric coatings and/or coating components complying with 21 CFR 175.300; as a preservative in resinous and polymeric coatings for polyolefin films complying with 21 CFR 175.320; as an antimicrobial for polymer latex emulsions in paper coatings complying with 21 CFR 176.170; and as an antimicrobial for finished coating formulations and for additives used in the manufacture of paper and paperboard, including fillers, binders, pigment slurries, and sizing solutions, complying with 21 CFR 176.170; except for use in contact with infant formula and human milk.	For use at CMIT and MIT levels equivalent to those specified in the referenced regulations, further stabilized with up to 0.15% copper (II) nitrate. The mixture may contain magnesium nitrate up to 2.04% and/or magnesium chloride up to 0.9%. The FCS is not for use in contact with infant formula and human milk. Such uses were not included as part of the intended use of the substance in the FCN.	Troy Corporation; Aug 4, 2016
1633	A mixture of CMIT and MIT at a 3:1 ratio by weight, optionally stabilized with magnesium or sodium nitrate and optionally further stabilized with 0.15 percent copper +(II) nitrate.	The FCS is intended for use as: (1) an antimicrobial agent in polymer latex solutions used in adhesives complying with 21 CFR 175.105; (2) a preservative in resinous and polymeric coatings for polyolefin films complying with 21 CFR 175.320 and in coatings and/or coating components complying with 21 CFR 175.300; and (3) a biocide in polymer latex emulsions, coating formulations and additives	For use at CIT and MIT levels: (1) equivalent to levels specified in 21 CFR 175.105, stabilized with magnesium nitrate, sodium nitrate and up to 0.15 percent copper(II) nitrate as an antimicrobial agent in polymer latex solutions used in adhesives; (2) equivalent to levels specified in 21 CFR 175.300 and 21 CFR 175.320, stabilized with magnesium nitrate, sodium nitrate and up to 0.15 percent copper(II)	LANXESS Corporation; Mar 18, 2016

FCN No.	Food Contact Substance	Intended Use	Specifications	Manufacture And Effective Date
		for paper manufacture used for food packaging. Except for use in contact with infant formula and breast milk (see Limitations and Specifications).	nitrate as a preservative in resinous and polymeric coatings for polyolefin films and in coatings and/or coating components; (3) equivalent to levels specified in 21 CFR 176.170, stabilized with magnesium nitrate, sodium nitrate and up to 0.15 percent copper(II) nitrate as a biocide in polymer latex emulsions, coating formulations and additives for paper manufacture used for food packaging. The FCS is not for use in contact with infant formula and breast milk. Such use was not included as part of the intended use of the substance in the FCN.	
1515	Mixture of CMIT and MIT at a ratio of 3 parts to 1 part by weight, optionally stabilized with magnesium or sodium nitrate and further stabilized with 0.15% copper (II) nitrate.	The FCS is intended for use as a preservative in resinous and polymeric coatings for polyolefin films complying with 21 CFR 175.320 and in coatings and/or coating components complying with 21 CFR 175.300, except for use in contact with infant formula and breast milk (see Limitations/Specifications).	For use at CIT and MIT levels equivalent to those specified in 21 CFR 175.300 and 21 CFR 175.320, further stabilized with up to 0.15% copper (II) nitrate. The FCS is not for use in contact with infant formula and breast milk. Such use was not included as part of the intended use of the substance in the FCN.	Thor GmbH; Mar 6, 2015
1469	A mixture of CMIT and MIT at a ratio of 3 parts to 1 part by weight, optionally stabilized with magnesium or sodium nitrate at a 1 to 1 ratio (weight/weight) with the sum of isothiazolinones and further stabilized with 0.15 percent copper (II) nitrate.	As an antimicrobial agent in polymer latex solutions used in adhesives complying with 21 CFR 175.105, except for use in contact with infant formula and breast milk (see Limitations and Specifications).	For use at CIT and MIT levels equivalent to those specified in 21 CFR 175.105, further stabilized with up to 0.15 percent copper (II) nitrate. The FCS is not for use in contact with infant formula and breast milk. Such use was not included as part of the intended use of the substance in the FCN.	Thor GmbH; Oct 29, 2014
1396	Mixture of CMIT and MIT at a ratio of 3 parts to 1 part by weight, optionally stabilized with magnesium or sodium nitrate and copper (II) nitrate.	In polymer latex emulsions, coating formulations and additives for coating formulations used for food packaging, except for use in contact with infant formula and breast milk (see Limitations/Specifications).	For use at CIT and MIT levels equivalent to those specified in 21 CFR 176.170, further stabilized with up to 0.15% copper (II) nitrate. The safety of usage of the FCS in contact with infant formula and breast milk has not been evaluated.	Thor GmbH; Mar 25, 2014

FCN No.	Food Contact Substance	Intended Use	Specifications	Manufacture And Effective Date
1308	MIT (only)	Uncured liquid rubber latex used to manufacture repeat-use rubber gloves intended for use in contact with all types of food.	not to exceed 250 ppm in the latex emulsion.	Thor GmbH; Oct 2, 2013
999	Mixture of CMIT and MIT at a ratio of 9 parts to 1 by weight.	For use as an antimicrobial agent for finished coating formulations and for additives used in the manufacture of paper and paperboard including fillers, binders, pigment slurries, and sizing solutions.	The FCS will not be used in excess of 21 parts per million CIT/MIT (9:1) in the coating formulations and additives.	Thor GmbH; Nov 18, 2010
704	Mixture of CMIT and MIT at a ratio of 3 parts to 1 part by weight.	The FCS is intended for use as a preservative in resinous and polymeric coatings for polyolefin films complying with 21 CFR 175.320.	The FCS will be used at a level not to exceed 45 milligrams per kilogram (based on isothiazolone active ingredient) in the aqueous coating formulation. For use with aqueous, acidic, and low-alcoholic foods under Conditions of Use A through H and with fatty food under Conditions of Use C through G.	Thor GmbH, Germany; May 22, 2007
675	Mixture of 5-chloro-2-methyl-4-isothiazolin-3-one (CAS Reg. No. 26172-55-4) and 2-methyl-4-isothiazolin-3-one (CAS Reg. No. 2682-20-4) at a ratio of 3 parts to 1 part by weight.	The FCS is intended for use as a preservative in aqueous coatings formulated using components permitted for use in coatings and/or in coating components complying with 21 CFR 175.300.	The FCS will be used at a level not to exceed 45 milligrams per kilogram (based on isothiazolone active ingredient) in the aqueous coating formulation. For use with aqueous, acidic, and low-alcoholic foods under Conditions of Use A through H and with fatty food under Conditions of Use C through G, as described in Tables 1 and 2.	Thor GmbH, Germany; Feb 8, 2007

FCN No.	Food Contact Substance	Intended Use	Specifications	Manufacture And Effective Date
569	A mixture of 5-chloro-2-methyl-4-isothiazolin-3-one (CAS Reg. No. 26172-55-4) and 2-methyl-4-isothiazolin-3-one (CAS Reg. No. 2682-20-4) at a ratio of 3 parts to 1 part by weight. The mixture may contain magnesium or sodium nitrate at a 1 to 1 ratio (weight/weight) with the sum of the isothiazolinone ingredients.	As an antimicrobial agent in polymer latex solutions intended for use in adhesives complying with 21 CFR 175.105.	For use at temperatures not to exceed 120°F.	Thor GmbH; Mar 21, 2006
286	MIT (only)	Preservative for latex emulsions destined for use in latex gloves intended to contact all types of food.	At a level not to exceed 250 ppm in latex emulsion	Jan 16, 2003
131	MIT (only)	adhesives and components of adhesives used in accordance with 21 CFR 175.105. 2. as an antimicrobial agent for polymer latex emulsions in paper coatings complying with 21 CFR 176.170(b)	Not to exceed 250 ppm	Apr 17, 2001
111	MIT (only)	components of adhesives for food-contact articles. As an antimicrobial agent in coating formulations and in additives used in the manufacture of paper and paperboard intended for use in contact with all food types.	Not to exceed 150ppm	Feb 20, 2001

For a full description of each notification, refer to FDA's website (Content link functional as of March 6, 2020) https://www.accessdata.fda.gov/scripts/fdcc/?cat=foodingredpkg&type=basic&search= Begin the search by entering the appropriate CAS number (MIT; CAS 2682-20-4) or (CMIT; CAS 26172-55-4). Then select the notification of interest.

3.5.2 Food Exposure Profile

Indirect dietary exposure to MIT/CMIT mixtures are expected as both compounds are labeled to preserve various products such as surface cleaners and detergents used in households, commercial, and industrial sites. The active ingredients are also used as additives in pulp and papers systems to control slime and as a coating for paper and paperboard which includes food contact paper. The chemical pair is also widely used as an adhesive, glue or sealant which may have the potential for food contact.

3.5.3 Water Exposure Profile

Water exposure is expected to result from discharge in pulp and paper mill systems or cooling tower systems. More specifically, drinking water exposure may occur when pulp and paper mill effluent or cooling water tower blowdown water enter nearby water bodies. To evaluate exposures, the Agency uses the exposure and fate assessment screening model (E-FAST, 2014) to estimate concentrations from industrial sources. Refer to section 4.4.1 for further modeling details and Appendix B for model methodology. Table 11 provides the estimate drinking water concentrations (EDWCs) within drinking water from these sources. The 30Q5 concentration represents the lowest stream flow for 30 consecutive days over a 5-year period and is used to evaluate potential acute toxicity to humans via ingestion of drinking water. The harmonic mean flow concentration is used to evaluate potential chronic toxicity to humans via ingestion of drinking water.

Table 11. Drinking Water Exposure Estimates for MIT/CMIT Mixtures in Industrial Matrices

Use Site	Application Rate	30Q5 Concentration (Acute Exposure)	Harmonic Mean Concentration (Chronic Exposure)
Pulp and Paper Mills	11 ppm a.i.	0.47 µg/L	0.19 µg/L
Pulp and Paper Mills	153 ppm a.i.	6.47 µg/L	2.58 µg/L
Moderate Sized Water Cooling Towers[1]	1 ppm a.i.	0.57 µg/L	0.24 µg/L
Large-Sized Water Cooling Towers[2]	1 ppm a.i.	27.05 µg/L	11.63 µg/L
Moderate Sized Water Cooling Towers[1]	20 ppm a.i.	10.82 µg/L	4.65 µg/L
Large-Sized Water Cooling Towers[2]	20 ppm a.i.	540.70 µg/L	232.5 µg/L

1: Moderate-sized cooling water towers have a flow rate of 2,000 gal/minute
2: Large-sized cooling water towers have a flow rate of 100,000 gal/minute

Estimate water exposure concentrations at various application rates of a.i., listed in Table 12, were inputted into Dietary Exposure Evaluation Model with the Food Commodity Intake Database (DEEM-FCID)[3] Version 3.18. to determine acute and chronic drinking water risk and exposure. The software uses 2003-2008 food consumption data from the U.S. Department of Agriculture's (USDA's) National Health and Nutrition Examination Survey, What We Eat in America, (NHANES/WWEIA)[4]. Table 13 shows the exposure from the highest application rates of both industrial sources in addition to the highest exposed population subgroups. Assessment results show that acute and chronic exposures were negligible for paper mill discharged into nearby water. Moreover, cooling water risk and exposure estimates exist for all

[3] US EPA. 2014. Dietary Exposure Evaluation Model (DEEM) User's Guide. Version 3.18. User's Documentation Manual. https://www.epa.gov/sites/production/files/2015-09/documents/deem-user-guide-sep30-14.pdf
[4] Center for Disease Control and Prevention, National Center for Health Statistics. National Health and Nutrition Examination Survey. [Accessed March 25, 2020]; https://www.cdc.gov/nchs/nhanes/wweia.htm

population subgroups but are not of concern. The highest estimated risk if for all infants (<1-year-old) at 6% of the cPAD. Thus, drinking water risk and exposure estimates from cooling water towers are expected to co-occur with other use categories and has been added to the co-occurrence assessment section and found in Table 17. A complete DEEM analysis for both use patterns, their application rates and exposed subpopulations can be found in Appendix C.

Table 12. Drinking Water Exposure of MIT/CMIT Mixtures in Paper Mills and Cooling Water Towers

Scenario	Estimated drinking water concentration (ppm)	Exposure (mg/kg/day) and % PAD for total US population	Highly Exposed Subpopulation (mg/kg/day) and % a and cPADs	
Pulp and paper Mill systems at 153 ppm				
Acute	0.00647 ppm[a]	0.0003 (0.0%)	All Infants	0.0002 (0.0% aPAD)[c]
			Children 1-2	0.0004 (0.0% aPAD)[c]
Chronic	0.00258 ppm[b]	0.0000 (0.0%)	All Infants	0.0000 (0.0% cPAD)
			Children 1-2	0.0001 (0.0% cPAD)
Cooling Water Tower discharge at 20ppm - 100,000 gallon system				
Acute	0.5407 ppm	0.0295 (0.4%)	All Infants	0.0923 (1.2% aPAD)
			Children 1-2	0.0454 (0.6% aPAD)
Chronic	0.2325 ppm	0.0049 (2.4%)	All Infants	0.0126 (6.3%cPAD)
			Children 1-2	0.0070 (3.7%cPAD)

[a]Acute Concentration based on the 30Q5 Stream Flow Distribution. The 30Q5 is the lowest stream flow for 30 consecutive days over a 5-year period;
[b]Chronic concentration is based on Harmonic Mean Stream Flow Distribution;
[c]Values represent 95th percentile exposure

3.5.4 Dietary Risk Assessment for MIT/CMIT Mixtures and MIT Alone

To evaluate potential exposure to MIT and CMIT throughout various food use patterns, several dietary models were utilized within this assessment. To provide clarity of dietary models, each assessment table includes informative footnotes and default assumptions to explain how exposure results were obtained. Dietary model summaries are listed in corresponding dietary sections below. It should be noted that chemical exposures are assessed according to highest concentration rate with potential for food contact. This means that other registration labels or use sites may contain higher concentrations but aren't intended for food use. Those labels are typically accompanied by a "nonfood contact" or a similar food restriction statement. In absence of nonfood language, the Agency assumes that the labeled rate may potentially contact food items or food surfaces.

3.5.4.1 Residential Risk for MIT/ CMIT Mixtures and MIT only

The residential indirect dietary assessment utilizes a (Residential Tier 1A**)** model which considers antimicrobial products applied to hard surfaces in the home. This model is intended to estimate the dietary exposure of subpopulations from antimicrobial residues applied to or incorporated into residential food-contact surfaces. The model also uses average food consumption and average body weights from the National Health and Nutrition Examination

Survey and What We Eat in America (NHANES/WWEIA) information. In contrast to the commercial version, the residential model assumes food contact with a 2,000 cm^2 treated surface, and 100% of the chemical is available for transfer.

This assessment is being conducted for preservative uses of MIT/CMIT mixtures (Reg No. 5383-181) in household cleaning products. These are solutions which are sprayed directly on to surfaces or solutions incorporated into wetted wipes and applied to hard surfaces. The highest potential food contact rate for cleaning products was determined by the sum of both ai's. MIT (5.05% a.i.) and CMIT (0.15% a.i.) levels were adjusted to a final use concentration of 260 ppm, which is 252.5 ppm of MIT and 7.5 ppm of CMIT.

Acute- (Reg No. 5383-181)

In residential areas where MIT/CMIT mixtures are used to preserve surface cleaners at 260 ppm, dietary exposure and risk estimates are negligible (<1% aPAD) for all population subgroups. The highest estimated risk was for Children 1 to 2 years at 0.2% of the aPAD and exposure of 0.2 mg a.i/kg/day.

In residential areas where MIT only is used to preserve surface cleaners at levels of 400 ppm (Reg No. 67071-74) are not of the Agency's concern. Dietary exposure and risk estimates are negligible (<1% aPAD) for all population subgroups. The highest estimated risk was for Children 1 to 2 years at 0.4% of the aPAD with an exposure of 0.3 mg a.i/kg/day.

Chronic- (Reg No. 5383-181)

In residential areas where MIT/CMIT mixtures are used to preserve surface cleaners at 260 ppm, dietary exposure and risk estimates are below the Agency's level of concern (<100% cPAD) for all population subgroups. The highest estimated risk was for children 1-2 years old at 9% of the cPAD.

A second assessment was conducted for household products containing only MIT. The highest application rate for MIT household and commercial products is determined as 400ppm (Reg No. 67071-74). It should be noted that this level applies to household and commercial product use categories and is not applicable to other dietary sites.

In residential areas where MIT only is used to preserve surface cleaners at levels of 400 ppm (Reg No. 67071-74) are not of the Agency's concern (<100% cPAD) for all population subgroups. The highest estimated risk was for children 1-2 years old at 14% of the cPAD which is an exposure of 0.029 mg/kg/day.

Table 13. Residential Use of MIT/ CMIT mixtures in Surface Cleaners at 260 ppm and MIT only at 400 ppm

Population Group	Residue Value (mg ai)[1]	Dose prior to consumption adjustment	Consumption Ratio[2]	Exposure (Dose) (mg/kg/day)[3]	Risk Estimates % cPAD[4] (Food Only)[5]
CMIT and MIT uses in surface cleaners at 260ppm					
General U.S. Population	0.52	0.007	1.000	0.007	4
All Infants (<1-year-old)		0.068	0.196	0.013	7
Children 1-2 years old		0.041	0.453	**0.019**	**9**
Children 3-5 years old		0.028	0.496	0.014	7
Children 6-12 years old		0.014	0.629	0.009	4
Youth 13-19 years old		0.008	0.780	0.006	3
Adults 20-49 years old		0.006	1.051	0.007	3
Adults 50-99 years old		0.006	0.967	0.006	3
Females 13-49 years old		0.007	0.941	0.007	3
MIT only uses in surface cleaners at 400ppm					
General U.S. Population	1.60	0.011	1.000	0.011	6
All Infants (<1-year-old)		0.104	0.196	0.020	10
Children 1-2 years old		0.063	0.453	**0.029**	**14**
Children 3-5 years old		0.043	0.496	0.021	11
Children 6-12 years old		0.022	0.629	0.014	7
Youth 13-19 years old		0.012	0.780	0.009	5
Adults 20-49 years old		0.010	1.051	0.010	5
Adults 50-99 years old		0.010	0.967	0.010	5
Females 13-49 years old		0.011	0.941	0.010	5

[1] Residue Value (mg ai) = [ai concentration from the label (ppm) ÷ 1,000,000] x Residual Solution (1 mg product /cm^2) x surface area (2000 cm^2) x [fraction transferred (%)/100]

[2] The FDA assumption that a typical American's diet contacts 2000 cm^2 of treated surface per day is based on habits of the general U.S. population. Because different subpopulations consume various quantities of food, a consumption ratio (CR) is used in the residential hard surface sanitizer scenarios to account for this difference. CR (unitless) = Total food consumed by population subgroup (kg) ÷ Total food consumed by the general US Population (kg). For example, Children 1-2 years old's total food consumed is 1.77kg, while the general US population consumes 3.91kg. Therefore, the CR for Children 1-2 = 1.77kg / 3.91kg= 0.4526 or 0.453

[3] Exposure Dose (mg/kg/day) = Residue Value (mg ai) x Consumption Ratio ÷ BW (kg)

[4] %cPAD = [Exposure Dose (mg/kg/day) / cPAD (0.2 mg/kg/day)] * 100

[5]The most highly exposed subpopulation is in bold.

3.5.4.2 Commercial Risks for MIT/ CMIT Mixtures and MIT only

To assess dietary exposures in surface cleaners and solutions incorporated with MIT/CMIT the Commercial Tier1A model is used. Refer to the footnotes in Table 14 for model considerations and how exposures are calculated. This model is intended for antimicrobial products applied to hard non-porous surfaces and estimates the exposure of all subpopulations to chemical residues that will remain on surfaces and are available to transfer on food. Further, this conservative screening-level model is based on food consumption data from the US Department

of Agriculture's (USDA's) National Health and Nutrition Examination Survey and What We Eat in America (NHANES/WWEIA). It accounts for the average daily food consumption rates from the surveying data, assumes all food contacts a 4,000 cm^2 treated surface, and 100% of the chemical is available for transfer.

MIT/CMIT Mixtures of 260 ppm (MIT-252.5 ppm and CMIT-7.5 ppm)

Acute- (Reg No. 5383-181)

For commercial areas where MIT/CMIT are used to preserve household cleaners at 260 ppm, dietary exposure and risk estimates are not of the Agency's level of concern. Risk are negligible (<1% of the aPAD) for most population subgroups. The highest estimated risk was for children 1-2 years old at 0.037 mg ai/kg/day exposure and 0.5% which has been rounded to 1% of the aPAD.

Chronic- (Reg No. 5383-181)

For commercial areas where MIT/CMIT are used to preserve household cleaners or solutions (260 ppm) dietary exposure and risk estimates are below the Agency's level of concern (<100% cPAD) for all population subgroups. The highest estimated risk was for children 1-2 years old at 19% cPAD.

MIT only

MIT Acute- (Reg No. 67071-74)

In commercial areas where MIT alone is used to preserve household cleaners, dietary exposure and risk estimates are not of the Agency's level of concern for population subgroups. Risk are negligible (<1% of the aPAD) for most population subgroups. The highest estimated risk was for children 1-2 years old at 0.7 mg a.i./kg/day which has been rounded to 1% of the aPAD.

MIT Chronic- (Reg No. 67071-74)

For commercial areas where MIT/CMIT are used to preserve household cleaners or solutions (400 ppm) dietary exposure and risk estimates are below the Agency's level of concern (<100% cPAD) for all population subgroups. The highest estimated risk was for children 1-2 years old at 29% cPAD.

Table 14. Commercial Uses of MIT/CMIT Mixtures (260 ppm) and MIT (400 ppm)

Population Group	Residue Value (mg ai)[1]	Dose prior to consumption adjustment	Consumption Ratio[2]	Exposure (Dose) (mg/kg/day)[3]	Risk Estimates % cPAD (Food Only)[4]
CMIT and MIT uses in surface cleaners at 260 ppm					
General U.S. Population	1.04	0.015	1.000	0.015	7
All Infants (<1-year-old)		0.135	0.196	0.026	13

Population Group	Residue Value (mg ai)[1]	Dose prior to consumption adjustment	Consumption Ratio[2]	Exposure (Dose) (mg/kg/day)[3]	Risk Estimates % cPAD (Food Only)[4]
Children 1-2 years old		0.083	0.453	**0.037**	**19**
Children 3-5 years old		0.056	0.496	0.028	14
Children 6-12 years old		0.028	0.629	0.018	9
Youth 13-19 years old		0.015	0.780	0.012	6
Adults 20-49 years old		0.013	1.051	0.013	7
Adults 50-99 years old		0.013	0.967	0.012	6
Females 13-49 years old		0.014	0.941	0.013	7
MIT only uses in surface cleaners at 400ppm					
General U.S. Population		0.023	1.000	0.023	11
All Infants (<1-year-old)		0.208	0.196	0.041	20
Children 1-2 years old		0.127	0.453	**0.057**	**29**
Children 3-5 years old		0.086	0.496	0.042	21
Children 6-12 years old	1.60	0.043	0.629	0.027	14
Youth 13-19 years old		0.024	0.780	0.019	9
Adults 20-49 years old		0.020	1.051	0.021	10
Adults 50-99 years old		0.020	0.967	0.019	10
Females 13-49 years old		0.022	0.941	0.021	10

[1] Residue Value (mg ai) = [Active Ingredient Concentration from the label (ppm) ÷ 1,000,000] x Residual Solution (1 mg product /cm^2) x surface area (4000 cm^2) x [fraction transferred (%)/100]
[2] The FDA assumption that a typical American's diet contacts 4000 cm^2 of treated surface per day is based on habits of the general U.S. population. Because different subpopulations consume various quantities of food, a consumption ratio (CR) is used in the commercial hard surface sanitizer scenarios to account for this difference. CR (unitless) = Total food consumed by population subgroup (kg) ÷ Total food consumed by the general US Population (kg). For example, Children 1-2 years old's total food consumed is 1.77kg, while the general US population consumes 3.91kg. Therefore, the CR for Children 1-2 = 1.77kg / 3.91kg= 0.45269
[3] Exposure Dose (mg/kg/day) = Residue Value (mg ai) x Consumption Ratio ÷ BW (kg)
[4] %cPAD = [Exposure Dose (mg/kg/day) / cPAD (0.2 mg/kg/day)] * 100
[5]The most highly exposed subpopulation is in bold.

3.5.4.3 Pulp and Paper Risk for MIT/CMIT

To evaluate indirect dietary exposure via the food contact papermaking process where pesticide residues may migrate into food, a Slimicide Model is utilized. The screening level exposure is based on the use pattern (*i.e.*, slimicide added to the slurry, coating for finished paper) such that concentration inputs used in the calculator depends on where the pesticide is added during paper production. For example, a.i.'s that are used as slimicides are typically added early in the process to pulp and water mixtures while paper coating additives are applied to dried finished paper. Slimicide exposures are expected to be lower than paper coatings due to the entry point of additive a.i. in the paper making process. Thus, one model allows dietary exposure evaluations for both paper use sites. The assumptions and methodology are based on an FDA guidance for food contact surfaces.[5]

[5] (*Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations*, 2007). Link is functional as of 02/19/2020. For more information see https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-preparation-premarket-submissions-food-contact-substances-chemistry

Dietary risk and exposure for MIT/CMIT in pulp and paper water systems as a slimicide and paperboard are highly conservative in that the model assumes the following:

Assumptions based on FDA guidance for papermaking

- Slurry contains approximately 33% pulp and 67% slurry water;
- 100% of tehchemical migrates to from treated paper to food;
- Finished paper contains approximately 92% pulp and 8% water, and
- Body weights (kg) and total food consumed are derived from the NHANES/WWEIA 2003-2008 data.

Acute- slimicide uses at 0.195 lb./ton

For slimicide uses in pulp and paper slurry at 0.195 lb./ton, dietary exposure and risk estimates are below the Agency's level of concern (<100% aPAD) for population subgroups. Risk are negligible (<1% of the aPAD) for all population subgroups.

Chronic-slimicide uses at 0.195 lb./ton

For slimicide uses in pulp and paper slurry at 0.195 lb./ton, dietary exposure and risk estimates are below the Agency's level of concern (<100% cPAD) for all population subgroups. Risk are negligible (<1% of the cPAD) for all population subgroups.

Acute- paper and paperboard use as coatings

For paper coating uses at 110 ppm, dietary exposure and risk estimates are below the Agency's level of concern for population subgroups. Risk are negligible (<1 of the aPAD) for all population subgroups. The highest estimated risk was for children 1 to 2 years old, resulted in 0.014 mg ai/kg/bw/day dietary exposure which is 0.2% of the aPAD.

Chronic- paper and paperboard use as coatings

For paper coating uses at 110 ppm, dietary exposure and risk estimates are below the Agency's level of concern (<100% cPAD) for population subgroups. The highest estimated risk was for children 1 to 2 years old which is 7% of the cPAD.

Table 15. Coatings in Paper at 110 ppm

Population Group	DDD= Daily dietary dose (mg/kg/day)	Risk Estimates % cPAD[2,3]
General U.S. Population	0.006	3
All Infants (<1-year-old)	0.010	5
Children 1-2 years old	**0.014**	**7**
Children 3-5 years old	0.010	5
Children 6-12 years old	0.007	3
Youth 13-19 years old	0.005	2

Population Group	DDD= Daily dietary dose (mg/kg/day)	Risk Estimates % cPAD[2,3]
Adults 20-49 years old	0.005	3
Adults 50-99 years old	0.005	2
Females 13-49 years old	0.005	3

[1]Assumes a food mass to surface area ratio of 10 g food/in^2 paper (equivalent to 1.55 g food/cm^2). The Dietary Concentration (µg ai/g food) is calculated using the Slimicides Spreadsheet. Residue Value= Dietary concentration (µg ai/g food)* 1.55 g food/cm^2.
[2]%cPAD = [Exposure Dose (mg/kg/day) / cPAD (0.2 mg/kg/day)] * 100
[3]The most highly exposed population subgroup is in bold

3.5.5 Dietary Assessment for Adhesives and Detergents

Antimicrobials used to preserve adhesive or detergents formulations may result in the migration of the pesticide into the food that results in indirect dietary exposure. Below are some considerations when calculating the exposures for adhesives.

Exposure Calculations for Adhesives (based off of FDA guidance[6]):

- A daily dose (mg/kg/day) for the adhesive scenario calculated using the following formula

$$Daily\ Dietary\ Dose\left(\left.\frac{mg\ a.i.}{kg\ BW}\right/day\right) = \frac{EDI \times UCF}{BW}$$

- EDI = Estimated Daily intake
- BW = Body Weight (kg)
- UCF = Unit Conversion Factor (from mg to µg)

Where:

- DC= Concentration of Adhesive in food (µg a.i. /g food)
- TFC= Total food consumed (g of total food)
- Dietary concentration (DC) = 7 ppb (0.007 µg ai/g food). Assumes a maximum 7 ppb level of residues are likely to migrate from food packaging materials into food.

Acute -Adhesives

Risk and exposure estimates where MIT/CMIT mixtures are used to preserve adhesives and glues are negligible (<1% of the aPAD) for population subgroups. The highest label rate of 260 ppm.

[6] (*Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations*, 2007). Link is functional as of 02/19/2020. For more information see https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-preparation-premarket-submissions-food-contact-substances-chemistry

Chronic-Adhesive

Risk and exposure estimates to preserve adhesives and glues are negligible (<1% for the cPAD) for population subgroup at 260 ppm, the highest label rate. The highest estimated risk was for children 1 to 2 years old, resulted in 0.0010 mg ai/kg/bw/day dietary dose and 0.012% of the cPAD.

Dish detergents and dishwasher of MIT/CMIT at 260 ppm and MIT only at 400 ppm

When used to preserve dish detergents, dietary exposure and risk estimates are negligible (<1% a and c PADs) for all population subgroups.

3.5.6 Dietary Assessments for CMIT in combination with MIT as an Inert Ingredient in Agricultural Products

For the inert assessment, the Dietary Exposure Evaluation Model - Food Commodity Intake Database (DEEM-FCID^{TM}) software, version 3.18 was used to evaluate dietary exposure and potential risk involving antimicrobials used as inert ingredients. The version of DEEM uses 2003-2008 food consumption data from the USDA's NHANES/WWEIA.

CMIT (in combination with MIT) is formulated as an inert ingredient (not to exceed 0.0022% of the formulation) in agricultural pesticide products. See Table 9 for information about this tolerance exemption regulation.

For the inert chronic dietary assessment, the Dietary Exposure Evaluation Model software with the Food Commodity Intake Database (DEEM-FCID^{TM}, Version 3.18), and food consumption data from the U.S. Department of Agriculture's (USDA's) 2003-2008 National Health and Nutrition Examination Survey, What We Eat in America (NHANES/WWEIA) were used.

The resulting chronic dietary risk estimates for the general U.S. population and all population subgroups for CMIT in combination with MIT used as an inert are negligible (<1% of the cPAD) and not of concern, as shown below in Table 16. Also, there is no acute exposure concerns, as estimated risk was determined to be negligible for all population subgroups.

Table 16. Inert Use of CMIT (in combination with MIT) at Tolerance Exemption (0.0022% of Formulation)[1]

Population Group	Exposure (Dose) (mg/kg/day)	Risk Estimates % cPAD (Food Only)
General U.S. Population	0.000008	Negligible <1%
All Infants (<1-year-old)	0.000017	
Children 1-2 years old	**0.000031**	
Children 3-5 years old	0.000021	
Children 6-12 years old	0.000011	
Youth 13-19 years old	0.000006	
Adults 20-49 years old	0.000006	
Adults 50-99 years old	0.000007	
Females 13-49 years old	0.000006	

[1]The model uses the highest rates expected for inert on all commodities and incorporates adjustments for the tolerance exemption limitation of 0.0022%; 0.00022% for final growing crops.

3.6 Dietary Co-occurrence Risk Characterization

In order to conduct the dietary aggregate risk assessment, the co-occurrence of dietary sources of CMIT/MIT must be determine. As mentioned and assessed above, dietary exposure to MIT/CMIT mixtures occur from the following eight use sites: (1) Preservative of commercial cleaners, (2) preservative of household cleaning products; (3) adhesives and glues; (4) slimicide uses (5) paper coatings; (6) dish detergents considered in both commercial and residential settings (7) inerts and (8) potential drinking water exposure. MIT only exposure occur from three sites: (1) Commercial; (2) Residential; and (3) potential drinking water exposure from an industrial source.

The Agency has determined that for the purposes of this risk assessment, the assumption of concurrent exposure from all MIT/CMIT use sites is overly conservative, considering (1) the extensiveness of use sites, (2) compounding conservative assumptions contained within the models, and (3) the number of MIT/CMIT products on the market.

Based on the use pattern of products comprised of MIT/CMIT used in food areas, the Agency determined that it is highly unlikely for an individual food commodity to include residues from all areas treated with MIT/CMIT. This determination is further supported by the fact that all dietary models used in this assessment assume 100% residue transfer and account for the consumption of the same food items (*i.e.*, the commercial model utilizes average total food consumed from 4,000 cm^2 treated surfaces, while the residential model uses average consumed from 2,000 cm^2 treated surfaces). For example, if the identical amount of a.i. were applied to both commercial and residential surfaces, it is assumed that the larger surface retains more residues. In order not to overcount exposures in a conservative model, the commercial exposure is assumed to account for both exposure scenarios. A similar logic applies when evaluating risk and exposure in paper use scenarios. At identical use concentrations, slimicide exposure is expected to be lower than paper coatings due to the entry point of additive a.i. in the paper making process. Active ingredients used as a slimicide are typically added to a pulp and water mixture while paper coating additives are applied to dried finished paper.

Summation of exposure from all MIT/CMIT use sites in a co-occurrence assessment would result in compounding conservatisms. Therefore, the highest exposure from each use pattern or tolerance for residues was assessed and resulted in a combination of exposure from the following six use sites: Commercial areas, paper coatings, adhesives (included but has exceedingly low levels) , dish detergents (included but has exceedingly low levels), drinking water from an industrial source and inerts (included but has exceedingly low levels). The co-occurrence table below (Table 17) represents dietary assessments which result from multiple dietary sources, total likely co-occurring dietary exposure.

Acute Co-occurrence Summary for MIT and CMIT Mixtures

Acute co-exposure is not a concern. Most of the exposures are negligible (<1% of the aPAD) or around 1% of the aPAD for anticipated high exposure population subgroups. For this reason, a dietary co -occurrence summation table has not been added to this section. In commercial uses the highest estimated risk was for children 1-2 years old at 0.7% which has been rounded to 1% of the aPAD. For paper coating uses, the highest estimated risk was for children 1 to 2 years at 0.2% of the cPAD.

Acute and Chronic Summary for MIT only

MIT uses to preserve commercial, household cleaners and dish detergents at 400 ppm is not of concern. For surface cleaner uses, the highest estimated risk was for children 1-2 years old at 1% of the aPAD. Similar results were shown for dish detergents where exposures were negligible (<1% of the aPAD) for all exposed subgroups. Combined chronic exposures for commercial product cleaners and dish detergents were below 30% of the cPAD for the highest exposed population subgroup (children 1 to 2 years old). Therefore, MIT exposures as a single a.i. does not exceed co-occurring exposures of MIT/CMIT.

Chronic Co-occurrence Summary for MIT and CMIT Mixtures

Collective estimated risks of the highest exposed population subgroups are below 31% of the cPAD and therefore not of the Agency’s concern. The highest exposures were in commercial sites for preservation of consumer products and uses for paper coating, where there may be potential exposure to food. Risk estimates from adhesives, dish detergent and inert uses were determined to be insignificant (<1% of the cPAD) in which the outcome did not contribute to the overall risk estimates. Because, MIT uses and exposures as a single a.i. does not exceed co-occurring exposures of MIT/CMIT, MIT/MCIT risk estimates are determined as protective of MIT only uses; and therefore, there is no need to add MIT only exposures to the table below.

Table 17. Chronic Dietary Exposure Co-Occurrences MIT/CMIT Mixtures

Dietary Source	Dietary Exposure Co-Occurrence in mg/kg/day (% cPAD)			
	Highest Exposed[1] Subpopulation Children 1-2 yrs	**Infants (< 1 yr)**	**Females 13-49 yrs**	**General population**
Commercial[2]	**0.037 (19%)**	0.025 (13%)	0.013 (7%)	0.015 (7%)
Paper Coating	**0.014 (7%)**	0.010 (5%)	0.005 (3%)	0.006 (3%)
Adhesives[2]	Negligible <1%			
Detergents[2]	Negligible <1%			
Drinking water[4]	0.0070 (3.5%)	**0.0126 (6.3%)**	0.005 (2.4%)	0.005 (2.4%)
Inerts[5]	Negligible <1%			
Total Exposure	**0.058 (30%)**	0.048 (24%)	0.023 (12%)	0.026 (12%)

[1]The highest exposed population subgroups are bolded.
[2] Accounts for highest use rate for MIT/CMIT to preserve surfaces cleaners, adhesives and dish detergents.
[3] Highest application rate identified for paper coating with food contact potential.
[4] Drinking water exposures reflect large cooling towers systems at the highest labeled concentration allowed, 20ppm
[5] Inert tolerance exemption at 0.0022% of formulations.

3.7 Residential Handler Exposure/Risk Characterization

There is the potential for residential handler exposure when using paints and cleaners that are preserved with MIT/CMIT. These exposures are anticipated to be of a short to intermediate term duration because painting is conducted for a few days per year and cleaners are used intermittently.

The maximum application rate for the preservation of paints and floor cleaners is 400 ppm MIT, thus this rate is used to assess handler exposures. Although products that contain MIT/CMIT are also used to preserve paints and floor cleaners, these products are applied at a lower rate of 135 ppm.

3.7.1 Residential Handler Inhalation Exposure to MIT Aerosols

The MOEs for residential handler inhalation exposures to MIT aerosols were assessed as outlined in Table 18. The MOEs are greater than the LOC of 10 and are not of concern.

Table 18. Residential Handler Inhalation MOEs for MIT

Scenario	Application Rate[A]	Amount Product Applied per Day	Amount a.i. Handled[D,E] (lb/day)	Unit Exposure (mg/m^3/lb a.i.)	Inhalation Exposure[I] (mg/m^3)	MOE[J] (LOC = 10)
Airless Spray Application of Paint	400 ppm a.i. (MIT)	15 gallons[B]	0.060	0.124[F]	0.0074	15
Brush/Roller Application of Paint		2 gallons[B]	0.008	0.00097[G]	0.0000078	14,000
Trigger Spray and Wipe Application of Cleaners	400 ppm a.i. (MIT)	0.06 gallons[C]	0.00020	3.12[H]	0.00063	170
Mop Application of Cleaners		1 gallon	0.0033	0.0068[I]	0.000022	5,000

A. The application rates are the maximum rates from EPA Reg no. 67071-74 which contains 10% MIT.
B. Based on US EPA, 2012a.
C. Antimicrobial Exposure Joint Venture (AEJV) Survey Data (MRID 46799302).
D. Amount of a.i. Handled (lb/day) = Application Rate (ppm/1000000) x Amount Product Applied (gal) * Product Density (lb/gal)
E. Product density is 10 lbs/gal for paint and 8.35 lbs/gallon for cleaners.
F. AEATF II airless sprayer study (MRID 50879401).
G. AEATF II brush/roller study (MRID 50521701).
H. AEATF II Trigger Spray and Wipe Exposure Study (MRID 48375601).
I. AEATF II Mopping Exposure Study (MRIDs 48210201, 48231201, 48231901). Converted to an 8-hour TWA.
I. Inhalation Exposure (mg/m^3) = Amount a.i. Handled (lb/day) * Unit Exposure (mg/m^3/lb a.i.)
J. MOE = HEC (0.11 mg/m^3) / Inhalation Exposure (mg/m^3)

3.7.2 Residential Handler Dermal Exposure

The residential handler dermal exposures were calculated as a loading on the hands using the assumed hand surface area of 820 cm^2 from OPPTS Guideline 875.1200 (US EPA, 1996). The MOEs for these exposures were assessed as outlined in Table 19 using the induction POD of 210 µg/cm^2 that pertains to MIT and the elicitation POD of 0.0105 µg/cm^2 that pertains to MIT and MIT/CMIT. The induction MOEs range from 110 to 950 and are not of concern because they are greater than the LOC of 100. The elicitation MOEs range from 0.001 to 0.08 and are all of concern because they are less than the LOC of 10.

Table 19. Residential Handler Dermal MOEs for MIT

Scenario	Amount a.i. Handled[A] (lb/day)	Unit Exposure (mg/lb a.i.)	Dermal Exposure[F] (mg/day)	Dermal Loading[G] (µg/cm^2)	Induction MOE[H] (LOC = 100)	Elicitation MOE[I] (LOC = 10)
Airless Spray Application of Paint	0.060	105[B]	6.3	1.9	110	**0.005**
Brush/Roller Application of Paint	0.008	144[C]	1.2	1.1	190	**0.001**
Trigger Spray and Wipe Application of Cleaners	0.00020	1740[D]	0.35	0.21	1000	**0.05**
Mop Application of Cleaners	0.0033	82.1[E]	0.27	0.13	1600	**0.08**

A. Same values as used for calculating the residential handler inhalation MOEs in Table 18 above.
B. Short sleeve short pants value from the AEATF II airless sprayer study (MRID 50879401). Hand Exposure = 25%.
C. Short sleeve short pants value from the AEATF II brush/roller study (MRID 50521701). Hand exposure = 76%.
D. Trigger spray and wipe value from the AEATF II wipe study (MRID 48375601). Hand exposure = 50%.
E. Short sleeve, short pants value from AEATF II Mopping Exposure Study (MRIDs 48210201, 48231201, 48231901). Hands = 38%
F. Dermal Exposure (mg/day) = Amount a.i. Handled (lb/day) * Unit Exposure (mg/lb a.i.)
G. Dermal Loading = [Dermal Exposure (mg/day) * Hand Exposure (%/100) * 1000 µg/mg]/Hand Area (820 cm^2)
H. Induction MOE = POD (210 µg/cm^2 for MIT) / Dermal Loading (µg/cm^2)
I. Elicitation MOE = POD (0.0105 µg/cm^2) / Dermal Loading (µg/cm^2)

3.7.3 Residential Handler Inhalation Exposures to MIT Vapors from Preserved Paints

There are several labels that includes the use of MIT and MIT/CMIT as a preservative in paint. Both chemicals have relatively high vapor pressures at 25 ºC (0.062 mm Hg for MIT and 0.018 mm Hg for CMIT) thus there is the potential for exposures to MIT or CMIT vapors that volatizes out of paint. The application rate for MIT is 400 ppm and is greater than the application rate of 135 for MIT/CMIT and this in combination with the higher vapor pressure of MIT means that the exposures to MIT will be greater than the exposures to MIT/CMIT.

Inhalation exposures to the MIT in paint were assessed using the EPA's Wall Paint Exposure Model (WPEM). The exposure duration is assumed to be short term because painting is done on an episodic basis. WPEM was developed under a contract by Geomet Technologies for EPA OPPT to provide estimates of potential air concentrations and consumer/worker exposures to chemicals emitted from wall paint which is applied using a roller or a brush. WPEM uses mathematical models developed from small chamber data to estimate the emissions of chemicals from oil-based (alkyd) and latex wall paint. The emission data can then be combined with detailed use, workload and occupancy data (*e.g.*, amount of time spent in the painted room, *etc*.,) to estimate exposure. Specific input parameters include: the type of paint (latex or alkyd) being assessed, density of the paint (default values available), and the chemical weight fraction, molecular weight, and vapor pressure. Detailed information and the executable model can be downloaded from http://www.epa.gov/opptintr/exposure/docs/wpem.htm.

For this exposure assessment, the WPEM default scenario for the residential do it yourself painter (RESDIY) was used. This WPEM default scenario assumes that the residential painter is exposed to the chemical in paint when painting the bedroom of a house (Zone 1) and in adjacent rooms (Zone 2) after painting. This default scenario includes 3 hours of painting in Zone 1, 15 hours in Zone 2 and 6 hours outside of the house. The following chemical specific inputs and WPEM default assumptions were used in the model:

Chemical Specific Inputs

- The molecular weight of MIT is 115.2 grams/mole and the vapor pressure is 0.062 mm Hg at 25 ºC based on Table 2.
- The application rate is 400 ppm MIT based on EPA Reg. No. label 67071-74.
- MIT was selected because it is applied at a higher rate than MIT/CMIT and because it is more volatile.

WPEM Default Assumptions from the RESDIY Scenario

- The air exchange rate is 0.45 air changes per hour which is the median value from the Exposure Factors Handbook (US EPA, 1997).
- The painting is done in a house that has an internal volume of 15,583 ft^3 which is the mean value from the Exposure Factors Handbook (US EPA, 1997). This house has a wall loading ratio of 0.29 ft^2/ft^3.
- The walls of one bedroom are painted and the painted surface area is 452 ft^2. This bedroom has an internal volume of 1558 ft^3.
- One coat of paint which has a coverage of 400ft^2/gallon is applied.
- The paint is latex flat with a density of 4600 grams/gallon (10.1 pounds/gallon).
- The duration of painting is 3.42 hours and 1.13 gallons of paint are applied.

The WPEM model was set to run at one-minute intervals for 1 day (24 hours). To yield an average daily concentration that includes only the day of painting (for comparison to the HEC) the exposure frequency was set to 365 exposure events per year. Since a homeowner or do-it-yourself painter typically paints on an intermittent basis (*i.e.*, four times per year), only peak to short term exposures were assessed for comparison to non-cancer endpoints.

WPEM Output for the RESDIY Scenario

The output of the WPEM model run of the residential painter on the day of painting is shown in Figure 1. The MIT air concentrations increase rapidly as the paint is applied and reach a peak concentration of 193 µg/m^3 in the painted room (zone 1) as the paint application is completed.

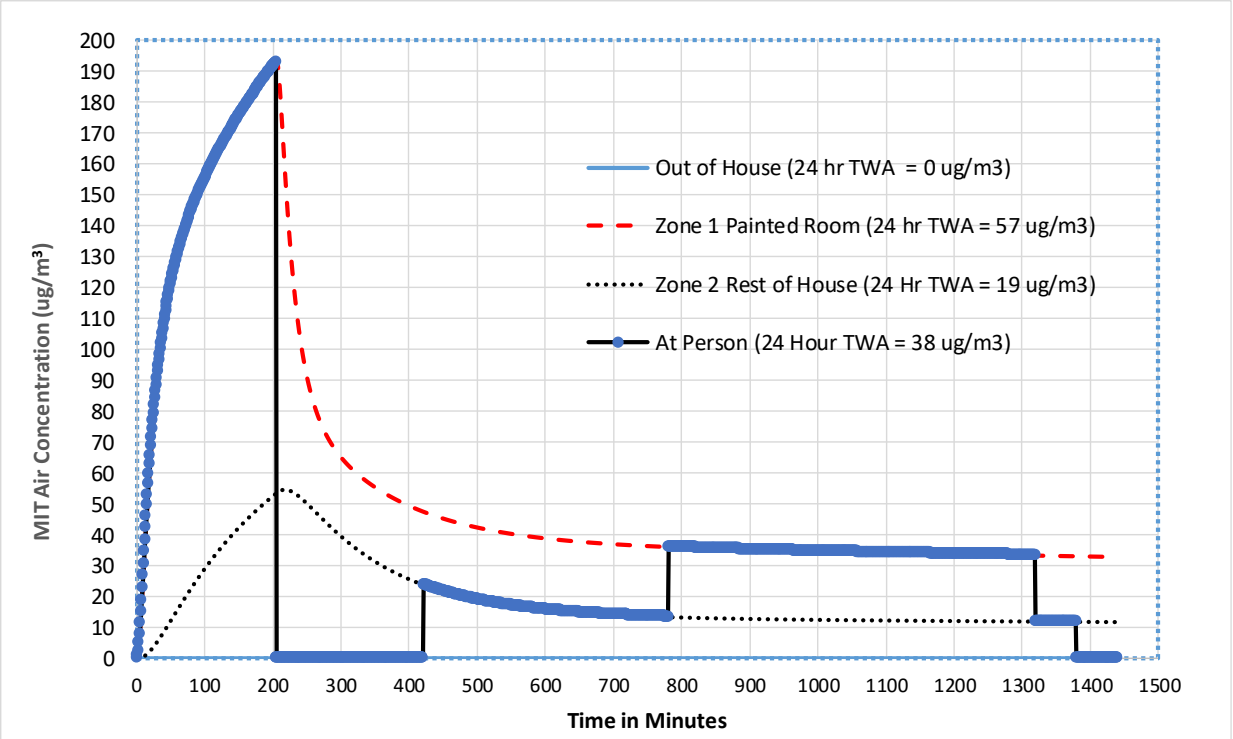


Figure 1 – Residential Painter MIT Air Concentrations on the Day of Painting (RESDIY)

Risk Summary

The results of the WPEM modeling run are compared to the 24-hour HEC as shown in Table 20. The MOE is 1.0 for the day of painting and is of concern because it is less the LOC of 10.

Table 20. MIT MOEs for Residential Painters

Days After Paint Application	Maximum Air Concentration in Zone 1 (µg/m^3)	24 hr Average in Zone 1 (µg/m^3)	24 hr Average in Zone 2 (µg/m^3)	24 hr TWA at Person (µg/m^3)	MOE[B] (LOC = 10)
0	193	57	19	38	**1.0**

A. Assuming the walls of one room are painted with one coat of paint as specified in the RESDIY scenario of WPEM.
B. MOE = 24 Hour HEC (0.038 mg/m^3) / 24 hr TWA MIT Air Concentration (µg/m^3) * 0.001 mg/µg

3.7.4 Residential Post Application Exposures to MIT Vapors from Preserved Paints

The post application exposures were assessed using the WPEM default scenario for the residential do it yourself painter (RESDIY). The same chemical specific and WPEM inputs were used as had been used for the handler assessment. The WPEM model was set to run at 15-minute intervals for 14 days to yield an MIT concentration profile for the painted room (zone 1) and the rest of the house (zone 2). The concentration profile is shown in Figure 2.

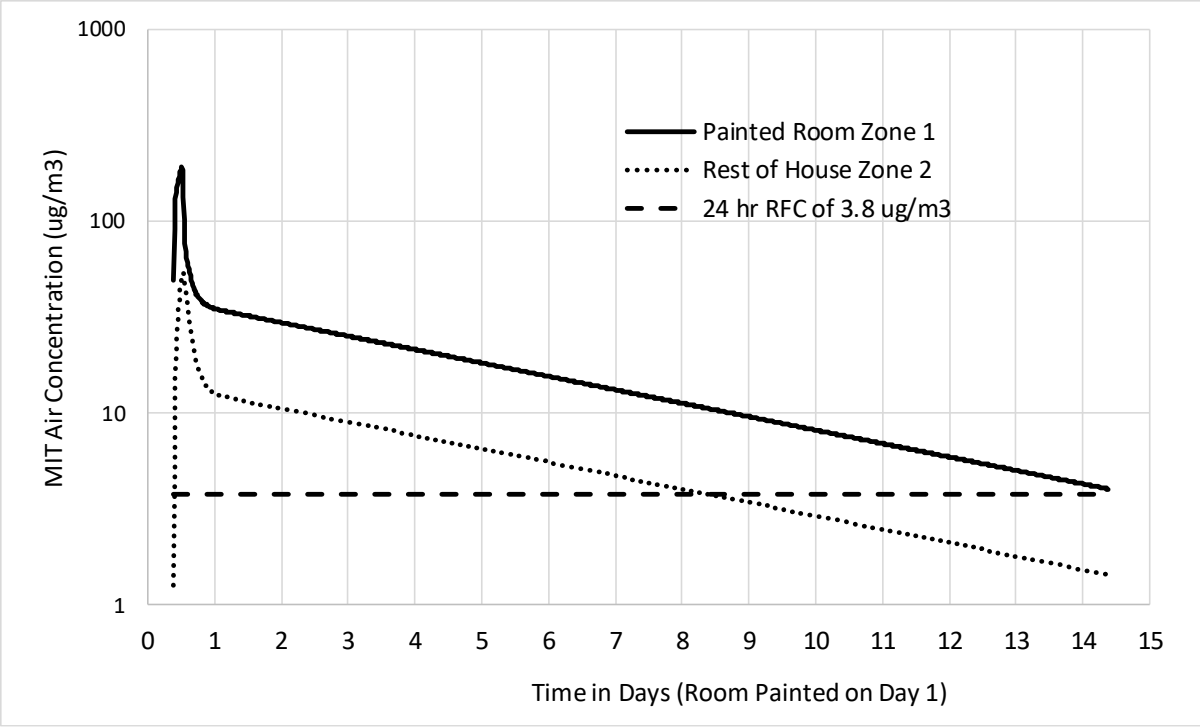


Figure 2 – Residential Painter MIT Air Concentrations for 14 Days after Painting

Risk Summary

The results of the WPEM post application modeling run are compared to the 24 hour HEC as shown in Table 21 by averaging the 24 hour average air concentrations for each day after painting based on the assumption that a person would spend 12 hours per day in zone 1 and 12 hours per day in zone 2. The MOE is 1.9 for 1st day after painting and is of concern because it is less than the LOC of 10. By the 12th day after painting, the air concentrations have decreased and the resultant MOE of 11 is not of concern.

Table 21. MIT MOEs for Post Application Exposures to Paint Vapors

Days After Paint Application	24 hr Average in Zone 1 (µg/m^3)	24 hr Average in Zone 2 (µg/m^3)	24 Hour TWA[B] (µg/m^3)	MOE[C] (LOC = 10)
1	30	11	20.5	**1.9**
7	10	3.5	6.6	**5.8**
12	5.1	1.8	3.5	**11**

A. Assuming the walls of one room are painted with one coat of paint as specified in the RESDIY scenario of WPEM.
B. Assuming 12 hours per day in Zone 1 and 12 hours per day in Zone 2
C. MOE = 24 Hour HEC (0.038 mg/m^3) / 24 hr TWA MIT Air Concentration (µg/m^3) * 0.001 mg/µg

MIT Paint Emissions Studies Reported in the Literature

There are two studies in the literature that include measurements of the amount of MIT emitted from paint.

In **Lundov (2014)**, MIT emissions were measured in laboratory chamber tests and in a field test at an apartment. In the laboratory test, 17 ml of paint containing a measured amount of MIT was applied to each side of a gypsum board according the paint manufacturers recommendations and two boards were placed in a chamber that has an internal volume of 51 liters (1.8 ft^3). The size of the boards is not reported, but if it assumed that the manufacturer specified a coverage of 400 ft^2 per gallon, which is typical for paint, then 17 ml of paint would cover 1.8 ft^2. Two coats of paint number 12, which contained 10 ppm MIT, was applied to one set of boards, and one coat of paint number 17 which contained 282 ppm MIT followed by one coat of paint number 18, which contained 19 ppm, was applied to the other set of boards.

The chambers were ventilated at a rate of 0.17 m^3/hour with air conditioned to 23 ºC and 50% relative humidity. This was intended to mimic the surface loading vs ventilation ratio for a model room (3.2 x 2.2 x 2.4 meters) where the walls and ceiling would be painted, and the room ventilated at 0.5 air changes per hour. Although the chamber had a ventilation rate of 3.3 air changes per hour, which is 6.6 times greater than the model room, the chamber loading of 7.2 ft^2 of painted surface (both sides of two boards at 1.8 ft^2 per side) per 1.8 ft^3 (4 ft^2/ft^3) was 6.6 times greater than the model room loading of 352 ft^2 of painted surface per 590 ft^2 (0.60 ft^2/ft^3). The two coats were applied with a 30-minute drying time between coats. The boards were placed in the chamber immediately after the second coat was applied.

In the apartment test, two coats of paint number 19, which contained 44 ppm MIT, were applied seven days before the air concentrations were measured. The ventilation rate was 0.8 air changes per hour.

The MIT concentrations for the boards painted with paints #17 (282 ppm MIT) and #18 (19 ppm MIT) reached a peak of 60 µg/m^3 during the first 24 hours and declined rapidly to approximately 5 µg/m^3 by day 4. The MIT concentration then remained at 5 µg/m^3 for five more days then declined at slower rate to 1 µg/m^3 by day 42. The MIT concentrations for the boards painted with paint #12 (10 ppm MIT), reached a peak of 1 µg/m^3 one day after painting and then slowly declined to 0.1 µg/m^3 by day 35. The MIT concentration in the apartment was approximately 40 µg/m^3 on the first day of measurement and then fluctuated around 3 µg/m^3 for the remaining 9 days.

The laboratory testing reported in Lundov (2014) provides some information about the emission of MIT from paints, but this information is limited because the application rates were much lower than the rate of 400 ppm which is allowed on the labels. Although one set of boards was painted with a paint that contained 282 ppm MIT, the second coat applied to the boards contained only contained 19 ppm MIT. The other set of boards was painted with paint containing 10 ppm MIT.

In **Nagorka (2014)**, MIT emissions were measured from building products including paints in test chambers and a test room. In the test chambers, 195 to 330 grams of paint containing a measured amount of MIT was applied to two glass plates with a face area of 0.64 m^2 which were placed in a chamber that has an internal volume of 1 m^3. The glass plates were approximately the area of the chamber floor. The density of the paint is not reported, but if it assumed to be 12 pounds per gallon, then the paint coverage ranged from 113 ft/gallon, when 330 grams were

applied to 192 ft/gallon when 195 grams were applied. The chambers were ventilated at a rate of 0.5 or 1.0 air changes per hour with air conditioned to 22 to 23 ^{o}C and 50% relative humidity.

In the test room, which was an unoccupied office that had at least one layer of old paint, 14.98 kg of paint was applied to an area of 46 m^2 in the room that had a volume of 50 m^3. This yielded a paint coverage of 180 ft^2 per gallon assuming a paint density of 12 lb./gallon. The windows were left open during painting and closed when painting was completed. The room temperature was 19 ^{o}C. The ventilation rate was not measured.

The MIT concentrations for the plates painted with wet room paint #10 (350 ppm MIT) in a chamber ventilated at 0.5 ACH reached a peak of 187 µg/m^3 by day 3 and then declined rapidly. The MIT concentrations for plates painted with wet room paint 8B (270 ppm MIT) in a chamber ventilated at 1.0 ACH, reached a peak of 158 µg/m^3 two days after painting. The MIT concentration in the test room painted with wet room paint #10 (350 ppm MIT) reached a maximum of 29 µg/m^3 on the 4th day after application and decreased by half three weeks later.

The chamber testing reported in Nagorka (2014) provides information about the emission of MIT from paints that is partially comparable to WPEM modeling results. The study was intended to measure emissions over a longer time period (30 to 35 days for the chambers and almost two years for the test room) than was assessed using WPEM. Because of this, the samples were collected over a period of 6 to 24 hours and the peak air concentrations that could have occurred within these periods were averaged out. A one-week emissions study with more samples taken during the first 24 hours would yield results that are more comparable to the WPEM assessment.

3.8 Residential Post Application Exposure/Risk Characterization

There is the potential for residential post-application exposure to textiles or household cleaning products preserved with MIT, MIT/CMIT or CMIT containing products. Because the MIT products are applied at the highest application rate, these exposures will be assessed for MIT. The Antimicrobial Exposure Assessment Task Force II (AEATF) has designed and is currently conducting residue removal/transfer studies for textiles and plastics. The residue studies are being designed to refine the 100% default assumption of residue transfer from the application rate from the uses in treated articles such as clothing/blankets/mattress pads, plastic toys, vinyl flooring, pool liners, etc. The AEATF II anticipates completing and submitting the study to the EPA in December 2021.

3.8.1 Residential Post Application Exposures from MIT Preserved Textiles

There is the potential for residential post-application incidental oral and dermal exposure to household items and clothing manufactured from textiles preserved with MIT. The exposure duration is anticipated to be short- to intermediate-term.

Incidental Oral Exposures to Textiles

Incidental oral exposures were calculated using the following equation:

Incidental Oral Exposure = Amount of MIT in Textile × Cloth Density × Surface Area Mouthed × Saliva Extraction Efficiency

Where:

- The application rate is 400 ppm MIT from EPA Reg no. 67071-74 which contains 10% MIT.
- The cloth density is 20 mg/cm^2 based on the density of cotton. This value is a standard assumption used in Office of Pesticide Programs risk assessments and was taken from the HERA Guidance Document Methodology (AISE/CEFIC, 2005).
- The surface area of fabric that is mouthed by a toddler per day is assumed to be 100 cm^2 (~16 in^2), which represents an estimate, for example, of the area of blanket or shirt sleeve.
- The saliva extraction efficiencies for mouthing fabric is 100%.
- The body weight of a child is 11.4 kg between 1 and <2 years (U.S. EPA, 2012a).

Incidental Oral MOE for Treated Textiles

The MOE for incidental oral exposure to MIT in textiles is summarized in Table 22. The MOE is 120 and is not concern because it is greater than the LOC of 10.

Table 22. Incidental Oral MOEs for Textiles Incorporating MIT

Application Rate (ppm)	Cloth Density (mg/cm^2)	MIT Surface Residue (mg/cm^2)	Surface Area Mouthed (cm^2/day)	Saliva Extraction Efficiency	Exposure[A] (mg/day)	Dose[B] (mg/kg/day)	MOE[C] (LOC = 10)
400	20	0.008	100	100%	0.8	0.070	120

A. Exposure = Surface Residues × Surface Area Mouthed × Saliva Extraction Efficiency
B. Dose = Exposure (mg/day) / Body Weight (11.4 kg)
C. MOE = POD (8.5 mg/kg/day) / Daily Dose (mg/kg/day)

Post Application Dermal Exposure from MIT Applied to Textiles

Dermal exposures were assessed as shown in Table 23. Since transferable residue data are not available for MIT treated textiles, the transfer factor was assumed to be 100% transfer. The induction MOE is 26 and is of concern because it is less than the LOC of 100. The induction MOE would be 100 if the transfer factor was 0.26 for 26 percent transfer. The elicitation MOE is 0.001 and is of concern because it is less than the LOC of 10. The elicitation MOE would be 10 if the transfer factor was 0.00013 for 0.013 percent transfer.

Table 23. Dermal MOEs for Textiles Incorporating MIT

Application Rate (ppm)	Cloth Density (mg/cm^2)	Surface Residue[A] (mg/cm^2)	Transfer Factor	Dermal Loading[B] ($\mu g/cm^2$)	Induction MOE[C] (LOC = 100)	Elicitation MOE[D] (LOC = 10)
400	20	0.008	1.0	8	**26**	**0.001**

A. Surface Residues (mg/cm^2) = Application Rate (ppm/1000000) * Cloth Density (mg/cm^2)
B. Dermal Loading ($\mu g/cm^2$) = Surface Residues (mg/cm^2) * Transfer Factor (1.0) * 1000 $\mu g/cm^2$
C. Induction MOE = [POD (210 $\mu g/cm^2$) / Dermal Loading ($\mu g/cm^2$)
D. Elicitation MOE = POD (0.0105 $\mu g/cm^2$) / Dermal Loading ($\mu g/cm^2$)

3.8.2 Residential Post Application Exposures from MIT Preserved Floor Cleaners

There is the potential for residential post-application incidental oral and dermal exposure to floors cleaned with MIT preserved floor cleaners. The maximum application rate for the preservation of floor cleaners is 400 ppm, thus this rate is used to assess post application exposures. Although products that contain MIT/CMIT are also used to preserve floor cleaners, these products are applied at a lower rate of 135 ppm.

Post Application Dermal Exposure from MIT in Floor Cleaners

Dermal exposures were assessed as shown in Table 24 by comparing the calculated dermal loading to the dermal PODs of 210 $\mu g/cm^2$ for MIT induction and 0.0105 $\mu g/cm^2$ for elicitation. The dermal MOE of 130 for induction is not of concern because it is greater than the LOC of 100. The dermal MOE of 0.007 for elicitation is of concern because it is less than the LOC of 10. The elicitation MOE would be 10 if the transfer factor was 0.00065 for 0.065 percent transfer.

Table 24. Dermal MOE for MIT in Floor Cleaners

Application Rate (ppm a.i.)	Application Rate[A] (g/gallon)	Cleaner Coverage (ft^2/gallon)	Surface Residue[B] (mg/cm^2)	Transfer Factor	Dermal Loading[C] ($\mu g/cm^2$)	Induction MOE[D] (LOC = 100)	Elicitation MOE (LOC = 10)
400	1.5	1000	0.0016	1.0	1.6	130	**0.007**

A Application rate (g/gallon) = Application rate (ppm a.i.) * Cleaning Solution Density (8.35 lb/gallon) * 454 g/lb
B. Surface Residue (SR) = Application Rate (g/gallon) * Coverage (1 gallon/1000 ft)* (1 ft^2/929 cm^2) * 1000 mg/g
C. Dermal Loading ($\mu g/cm^2$) = Surface Residue (mg/cm^2) * Transfer Factor * 1000 $\mu g/mg$
D. Induction MOE = POD (210 $\mu g/cm^2$ for MIT) / Dermal Loading ($\mu g/cm^2$)
E. Elicitation MOE = POD (0.0105 $\mu g/cm^2$) / Dermal Loading ($\mu g/cm^2$)

Post Application Incidental Oral Exposure from MIT in Floor Cleaners

Incidental oral exposures are assessed using the Post- Application Hand-to-Mouth Exposure Algorithm and assumptions. The exposure was calculated using the surface residue value of 0.0016 mg/cm^2 that was used for dermal exposures (Table 24) as the hand residue. The resulting incidental oral MOE of 400 in Table 25 is not of concern because it is greater than the LOC of 10.

Table 25. Incidental Oral MOE for MIT in Floor Cleaners

Surface Residue[A] (mg/cm^2)	Hand Residue[B] (mg/cm^2)	Fraction of Hand Mouthed (F_M)	Surface Area of Hand (SA_H)	Exposure Time (hours/day)	SHEDs Exponent Term[D]	Exposure[E] (mg/day)	Dose[F] (mg/kg/day)	Incidental Oral MOE[G] (LOC = 10)
0.0016	0.0016	0.13	150 cm^2	2.0	0.962	0.24	0.021	400

A. Based on the application rate of 400 ppm (see Table 24 above)
B. Hand Residue = Surface Residue (mg/cm^2) * Transfer Factor (1.0)
C. SHEDs Exponent Term = $[1 – (1\text{-}SE)^{FHtM/NR}]$, where SE = 0.48, FHtM = 20/hr and NR = 4/hr.
D. Exposure (mg/day) = HR (mg/cm^2) * F_M (0.13) * SA_H (150 cm^2) * ET (2 hrs) * NR (4/hr) * SHEDS Exponent Term
E. Dose (mg/kg/day) = Exposure (mg/day) / BW (11.4 kg child)
F. MOE = NOAEL (8.5 mg/kg/day) / Dose (mg/kg/day)

3.9 Aggregate Exposure/Risk Characterization

The Food Quality Protection Act amendments to the Federal Food, Drug and Cosmetic Act (FFDCA, section 408(b)(2)(A)(ii) require "*that there is reasonable certainty that no harm will result from aggregate exposure to pesticide chemical residue, including all anticipated dietary exposures and other exposures for which there are reliable information."* Aggregate exposure will typically include exposures from food, drinking water, residential uses of a pesticide, and other non-occupational sources of exposure. As established by the Food Quality Protection Act (FQPA), in order for a pesticide registration to continue, it must be shown "that there is reasonable certainty that no harm will result from aggregate exposure to pesticide chemical residue, including all anticipated dietary exposures and other exposures for which there are reliable information."

In performing aggregate exposure and risk assessments, EPA's Office of Pesticide Programs has published guidance outlining the necessary steps to perform such assessments (General Principles for Performing Aggregate Exposure and Risk Assessments, November 28, 2001. https://www.epa.gov/sites/production/files/2015-07/documents/aggregate.pdf). The basic framework for deciding whether to perform aggregate exposure and risk assessments are listed in this document, which include:

- Identification of toxicological endpoints for each exposure route and duration;
- Identification of potential exposures for each pathway (food, water, and/or residential);
- Reconciliation of durations and pathways of exposure with durations and pathways of health effects;
- Determination of which possible residential exposure scenarios are likely to occur together within a given time frame;
- Determination of magnitude and duration of exposure for all exposure combinations;
- Determination of the appropriate technique (deterministic or probabilistic) for exposure assessment; and
- Determination of the appropriate risk metric to estimate aggregate risk.

For MIT/CMIT, there are tolerance exemptions for the inerts under 40 CFR Part 180.920; additionally, there are material preservative uses that result in indirect food contact exposures. There is the potential for drinking water exposure as a result of MIT/CMIT discharges from pulp and paper mills and cooling towers as discussed in Section 3.5.3 There is also the potential for incidental oral, dermal, and inhalation exposure from the uses of MIT/CMIT as a material preservative in treated articles used by consumers. The residential exposure scenarios considered in the overall aggregate exposure are a result of MIT/CMIT's use as a material preservative in paints, cleaners, and impregnated clothing as described in Sections 3.7 and 3.8 above. Each of the three routes of exposure (oral, dermal, inhalation) are based on different toxicological endpoints as described in Table 8; thus, exposures across routes are not aggregated. The exposures to the treated paint and clothing trigger MOEs of concern for at least one of the three routes of exposure, and therefore, these exposures need to be mitigated and are not included in the aggregate (*i.e.,* risks for the individual uses fill the risk cup). Cleaners preserved with MIT/CMIT are considered in the aggregate since this use does not trigger MOEs of concern for the portion of the population not yet sensitized (*i.e*., using the induction POD). Note: For the portion of the population that has already been sensitized, the use of MIT/CMIT-treated cleaners would result in MOEs of concern (using the elicitation POD).

3.9.1 Acute Aggregate Risk

The acute assessment for MIT/CMIT is represented by the total dietary exposures which is considered minimal (<1% aPAD) for all populations as discussed above in Section 3.6.

3.9.2 Short- and Intermediate-Term Aggregate Risk

The inhalation route of exposure to be aggregated for the residential use of MIT-treated cleaners includes the co-occurrence of someone applying cleaners using a trigger spray & wipe plus mopping floors. Table 26 presents the aggregate inhalation MOE of 170 for the daily application of the cleaners which is not of concern (above the LOC of 10).

Table 26. Short- and Intermediate-term Inhalation Handler Aggregate Risks (Cleaners)

Exposure Scenario	Inhalation Exposure (mg/m^3)	Aggregate Inhalation Exposure[A] (mg/m^3)	Aggregate MOE[B] (LOC = 10)
Trigger Spray and Wipe Application of Cleaners	0.00063 (Table 18)	0.000652	170
Mop Application of Cleaners	0.000022 (Table 18)		

A. Aggregate Inhalation Exposure (mg/m^3) = Trigger Spray & Wipe + Mop Application.
B. MOE = ST/IT HEC 0.11 (mg/m^3) / dose (mg/kg/day); Uncertainty factor is 10x.

The dermal exposures are of concern for the induction of sensitization for paints and textiles and will need to be mitigated; leaving only the treated cleaners to be considered for the dermal aggregate. For those individuals already sensitized, the use of the cleaners also triggers risks of concern. Since the dermal endpoint is a localized effect, based on loading ($\mu g/cm^2$), and the dermal exposures for the applicator scenarios are based on hand residues, these residues would not be additive to the dermal exposure from clothing (*i.e*., residues to covered body areas

(µg/cm^2)) as these skin areas do not overlap, *i.e.*, no increase in the localized skin area loading of µg/cm^2.

The oral aggregate assessment considers the following exposure scenarios: total dietary and incidental ingestion from mouthing/sucking on MIT-treated clothing. Of these potential uses to consider for the oral aggregate, one of the other routes of exposure needs to be mitigated for the impregnated clothing use (*i.e.*, the incidental oral MOE by itself is not of concern, but the dermal route of exposure triggers an induction MOE of concern). Although it is atypical to conduct an aggregate assessment for a use that triggers risks of concern prior to mitigation, the oral aggregate is provided because the textile use is the only use contributing to the residential portion of the oral aggregate and there is the potential to refine the MOE of concern with residue transfer data to be submitted by the AEATF II. The aggregate oral MOE for children (1 to <2-year-old) illustrated in Table 27 is 66 which indicates the oral route is not the risk driver (above the LOC of 10).

Table 27. Short- and Intermediate-term Oral Aggregate Risk in Children for MIT/CMIT

Exposure Scenario	Dose (mg/kg/day)	Aggregate Dose[A] (mg/kg/day)	Aggregate MOE[B] (LOC = 10)
Total Dietary (children 1 to <2-year-old)	0.058 (Table 17)	0.128	66
Incidental oral from mouthing/sucking on clothing (children 1 to <2-year-old)	0.070 (Table 22)		

C. Aggregate Dose (mg/kg/day) = Total dietary + incidental oral toys + incidental oral laundered clothing.
D. MOE = ST/IT oral POD (mg/kg/day) / dose (mg/kg/day); Where ST/IT incidental oral POD = 8.5 mg/kg/day. Uncertainty factor is 10x.

3.9.3 Chronic Aggregate Risk

There are no chronic residential exposure scenarios to be added to the total dietary exposure. The chronic oral aggregate risk is not of concern with an MOE of 34 and LOC of 10 as shown in Table 17 above (based on the chronic oral NOAEL of 2 mg/kg/day / total dietary dose of 0.058 mg/kg/day).

3.10 Cumulative Exposure/Risk Characterization

In 2016, EPA's Office of Pesticide Programs released a guidance document entitled, *Pesticide Cumulative Risk Assessment: Framework for Screening Analysis*[7]. This document provides guidance on how to screen groups of pesticides for cumulative evaluation using a two-step approach beginning with the evaluation of available toxicological information and if necessary, followed by a risk-based screening approach. This framework supplements the existing guidance documents for establishing common mechanism groups (CMGs)[8] and conducting cumulative risk assessments (CRA)[9]. The Agency has utilized this framework for isothiazolinones and determined that although the isothiazolinones shares some chemical and/or

[7] [https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-cumulative-risk-assessment-framework]
[8] *Guidance for Identifying Pesticide Chemicals and Other Substances that have a Common Mechanism of Toxicity* (USEPA, 1999)
[9] *Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity* (USEPA, 2002)

toxicological characteristics (e.g., chemical structure or apical endpoint), the toxicological database does not support a testable hypothesis for a common mechanism of action. No further data are required to determine that no common mechanism of toxicity exists for the isothiazolinones and no further cumulative evaluation is necessary for the isothiazolinones (see USEPA, 2020).

3.11 Occupational Exposure/Risk Characterization

There is the potential for occupational handler exposure when MIT is used to preserve materials such as paints and plastics. There is also the potential for occupational handler exposure when using treated articles, such as paints, that are preserved with MIT.

Although products that contain MIT/CMIT, CMIT and MIT are used to preserve materials, the MIT products are applied at a higher rate than the MIT/CMIT or CMIT products.

3.11.1 Occupational Handler Exposures to MIT Aerosols

Occupational Handler Inhalation Exposures

The MOEs for occupational handler inhalation exposures to MIT aerosols were assessed as outlined in Table 28. The MOE of 4.4 for the airless spray application of paint is of concern because it is less than the LOC of 10. The other MOEs are not of concern.

Table 28. Occupational Handler Inhalation Exposures to MIT

Scenario	Application Rate[A]	Amount of Product Applied or Material Treated per Day[B]	Amount a.i. Handled (lb/day)[C]	Unit Exposure (mg/m^3/lb a.i.)	Inhalation Exposure[I] (mg/m^3)	MOE[J] (LOC = 10)
Open pour liquids for paint preservation	400 ppm a.i.	20,000 lbs of paint	8.0	0.00021[D]	0.0017	65
Airless Spray Application of Paint	400 ppm a.i.	500 lb of paint	0.20	0.124[E]	0.025	**4.4**
Brush/Roller Paint Application		50 lb of paint	0.020	0.00097[F]	0.000019	5800
Trigger Spray and Wipe Preserved Cleaners	400 ppm a.i.	0.26 gallons	0.00087	3.12[G]	0.0027	41
Mopping with Preserved Cleaners		45 gallons	0.15	0.0068[H]	0.0010	110

A. The application rates are the maximum rates from EPA Reg no. 67071-74 which contains MIT.
B. Standard assumptions used for occupational exposure assessments of AD chemicals.
C. Amount of a.i. Handled (lb/day) = Application Rate x Amount Product Applied or Treated.
D. Conventional pour unit exposure from AEATF II human exposure liquid pour study (MRID 48917401).
E. AEATF II airless sprayer study (MRID 50879401).
F. AEATF II brush/roller study (MRID 50521701).
G. AEATF II Trigger Spray and Wipe Exposure Study (MRID 48375601).
H. AEATF II Mopping Exposure Study (MRIDs 48210201, 48231201, 48231901). Converted to an 8-hour TWA.
I. Inhalation Exposure (mg/m^3) = Amount a.i. Handled (lb/day) * Unit Exposure (mg/m^3/lb a.i.)
J. MOE = HEC (0.11 mg/m^3) / Inhalation Exposure (mg/m^3)

Occupational Handler Dermal Exposures

The occupational handler dermal exposures were calculated as a loading on the hands using a hand surface area of 820 cm^2 from OPPTS Guideline 875.1200 (US EPA, 1996). The MOEs for these exposures were assessed using the induction POD of 210 µg/cm^2 that pertains to MIT and the elicitation POD of 0.0105 µg/cm^2 that pertains to MIT and MIT/CMIT as outlined in Table 29. The five induction MOEs range from 21 to 210 and four of these MOEs are of concern because they are less than LOC of 100. The elicitation MOEs range from 0.001 to 0.01 and are all of concern because they are less than the LOC of 10.

Table 29. Occupational Handler Dermal Exposures to MIT

Scenario	Amount a.i. Handled (lb/day)[A]	Unit Exposure (mg/lb a.i.)	Dermal Exposure[G] (mg/day)	Dermal Loading[H] (µg/cm^2)	Induction MOE[I] (LOC =100)	Elicitation MOE[J] (LOC = 10)
Open pour liquids for paint preservation	8.0	1.0[B]	8.0	9.7	**21**	**0.001**
Airless Spray Paint Application	0.20	43.6[C]	8.7	6.4	**33**	**0.002**
Brush/Roller Paint Application	0.020	115[D]	2.3	2.6	**81**	**0.004**
Trigger Spray and Wipe Preserved Cleaners	0.00087	1050[E]	0.91	1.0	210	**0.01**
Mopping with Preserved Cleaners	0.15	23.2[F]	3.5	4.0	**52**	**0.003**

A. From Table 28 above.
B. Conventional pour value from AEATF II human exposure liquid pour study (MRID 48917401) divided by 10X to account for the use of gloves. Hands = 99%.
C. Long sleeve long pants value from the AEATF II Airless Sprayer study (MRID 50879401). Hand exposure = 60%.
D. Long sleeve long pants value from the AEATF II brush/roller study (MRID 50521701). Hand exposure = 94%.
E. Long sleeve, long pants AEATF II Trigger Spray and Wipe Exposure Study (MRID 48375601). Hand Exposure = 92%
F. Long sleeve, long pants AEATF II Mopping Exposure Study (MRIDs 48210201, 48231201, 48231901). Hands = 93%
G. Dermal Exposure (mg/day) = Amount a.i. Handled (lb/day) * Unit Exposure (mg/m^3/lb a.i.)
H. Dermal Loading = [Dermal Exposure (mg/day) * Hand Exposure (%/100) * 1000 µg/mg] / Hand Area (820 cm^2)
I. Induction MOE = POD (210 µg/cm^2) / Dermal Loading (µg/cm^2)
J. Elicitation MOE = POD (0.0105 µg/cm^2) / Dermal Loading (µg/cm^2)

3.11.2 Occupational Handler Inhalation Exposures to MIT Vapors from Paint

There is the potential for occupational (*i.e.*, professional) painter inhalation exposure to MIT vapors from MIT preserved paints. Although painting is done by professional painters on a daily basis, the exposure duration for MIT is assumed to be short to intermediate term because it is highly unlikely that painters would be using MIT treated paint on a daily basis for more than six months at a time. The professional painter inhalation exposure to MIT vapors was assessed using the WPEM Model. The WPEM default scenario (RESPROF) for the professional painter was used and this scenario assumes that two professional painters paint an entire apartment in a workday. The following chemical specific inputs and WPEM default assumptions were used:

Chemical Specific Inputs

- The molecular weight of MIT is 115.2 grams/mole and the vapor pressure is 0.062 mm Hg at 25 ºC based on Table 2.
- The application rate is 400 ppm MIT based on EPA Reg No. 67071-74.

WPEM Default Assumptions from the RESPROF Scenario

- The air exchange rate is 0.45 air changes per hour which is the median value from the Exposure Factors Handbook (US EPA, 1997).
- The painting is done in an apartment that has an internal volume of 7,350 ft^3 which is the mean value from the Exposure Factors Handbook (US EPA, 1997).
- The walls are painted which have a surface area of 2131 ft^2.
- One coat of primer which has a coverage of 200 ft^2/gallon and one coat of paint which has a coverage of 400ft^2/gallon are applied.
- The paint is latex flat with a density of 4600 grams/gallon.
- Two professional painters are exposed while painting an entire apartment.
- The duration of painting is 9.4 hours based upon the labor production rate of 337.5 ft^2 per hour for painting with a roller at 400 ft^2/gallon.
- The amount of paint used is 10.66 gallons for the primer coat and 5.33 gallons for the finish coat.

WPEM Model Results for the RESPROF scenario

The WPEM model results for the RESPROF scenario are shown in Figure 3.

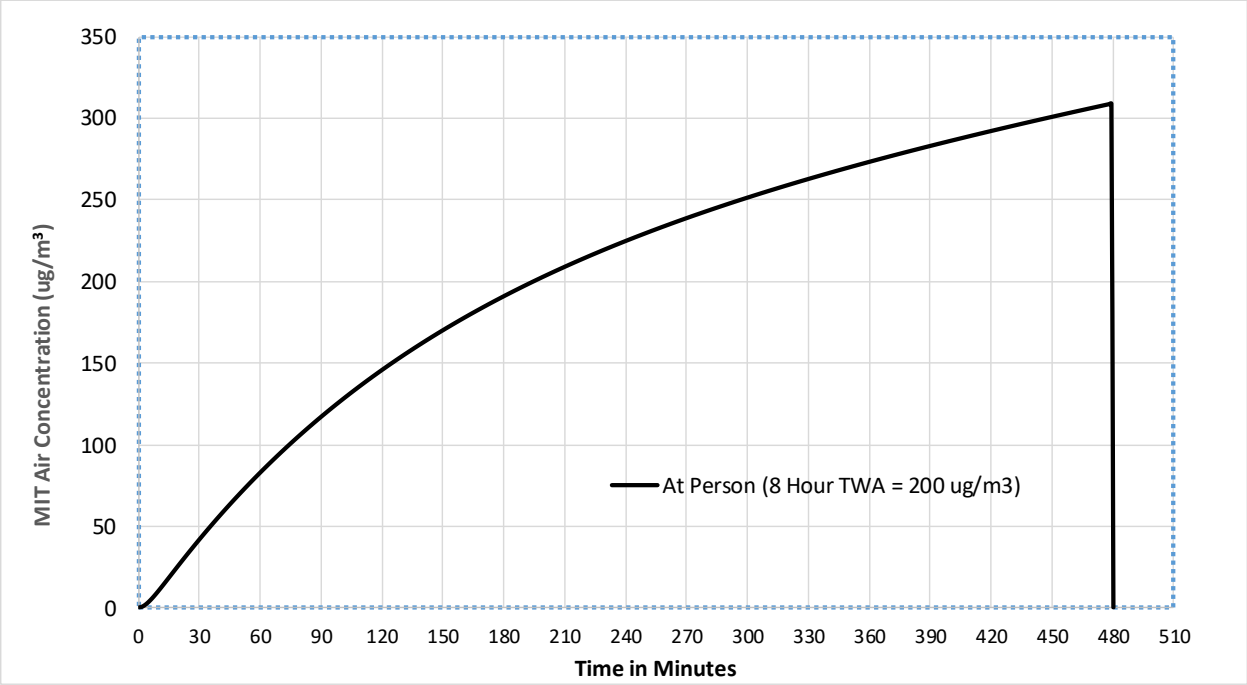


Figure 3 – Professional Painter MIT Air Concentrations (WPEM RESPROF Scenario)

Risk Summary

The results of the WPEM modeling run are compared to the 8-hour HEC as shown in Table 30. The MOE of 0.5 is of concern because it is less than the LOC of 10.

Table 30. Inhalation MOEs for Professional Painters Exposed to MIT Vapors

MIT Application Rate	Area Painted (ft^2)	Amount of Paint Applied (Gallons)	Ventilation Rate (Air changes/hr)	8 hr TWA at Person ($\mu g/m^3$)	MOE[C] (LOC = 10)
400 ppm	2131[A]	16[B]	0.45	200	**0.5**

A. Assuming the walls of an apartment are painted by two painters as specified in the RESPROF scenario of WPEM.
B. Assuming one primer coat at 200 ft^2/gal and one finish coat at 400 ft^2/gal as specified in the RESFROM scenario.
C. MOE = HEC (0.11 mg/m^3) / 8 hr TWA MIT Air Concentration ($\mu g/m^3$) * 0.001 mg/µg

3.11.3 Occupational Machinist Exposures to MIT in Metal Working Fluids (MWFs)

MIT, MIT/CMIT and CMIT are registered for use in metal working fluids (MWFs), therefore, there is the potential for machinists to be exposed when using treated MWFs. Both dermal and inhalation exposures are anticipated. Although products that contain MIT/CMIT, CMIT and MIT are used to preserve MWF, the MIT products are applied at a higher rate than the MIT/CMIT or CMIT products therefore the exposure assessment is based on MIT.

Inhalation Exposures

The inhalation MOE was calculated as outlined in Table 31. The MOE is not of concern because it is greater than the LOC of 10 for short/intermediate term exposure and the LOC of 30 for long term exposure.

Table 31. Inhalation MOE for Machinists Using MIT Treated MWF

Application Rate[A]	MWF Air Concentration (mg/m^3)	MIT Air Concentration[C] (mg/m^3)	Short/Intermediate Term MOE[D] (LOC = 10)	Long Term MOE[D] (LOC =30)
444 ppm	1.0[B]	0.00044	250	250

A. Noticeably fouled systems application rate from EPA Reg no. 67071-74. The maintenance rate is 222 ppm.
B. Average 8 hour TWA for oil mist (n=544) measured by OSHA (2000 to 2009) corrected for 25% volatilization loss.
C. MIT Air Concentration = Application Rate (ppm) * MWF Air Concentration (1.0 mg/m^3).
D. MOE = 8 Hour HEC (0.11 mg/m^3) / MIT Air Concentration (mg/m^3)

Dermal Exposures

The dermal exposure of machinists to MWFs treated with MIT were assessed by using the thin film approach for comparison to the POD which is expressed as the amount of a.i. per given area of skin. This approach using the following equation:

Dermal Loading ($\mu g/cm^2$) = WF (Application Rate/1,000,000) x Qu (mg/cm^2) x 1,000 µg/mg

The following assumptions were used in this assessment:

- WF. The weight fraction is based on the application rate.
- Qu. The quantity remaining on the skin is 10.3 mg/cm^2 based on the hand immersion with no wiping results for mineral oil reported in Cinalli (1992). This value is used to evaluate dermal irritation effects, because these effects can be localized.

- It is not feasible for machinists to wear chemical resistant gloves because they interfere with the fine motor skills needed to operated the metal working machines and measure the materials that being machined.

The dermal MOEs were calculated as outlined in Table 32. The induction MOE is 46 and is of concern because it is less than the LOC of 100. The elicitation MOE is 0.002 and is of concern because it is less than the LOC of 10.

Table 32. Dermal MOE for Machinists Using MIT-Treated Metal Working Fluids

Application Rate	Qu (mg/cm^2)	MIT Dermal Loading[C] (µg/cm^2)	Induction MOE[D] (LOC = 100)	Elicitation MOE (LOC = 10)
444 ppm[A]	10.3[B]	4.6	**46**	**0.002**

A. Noticeably fouled systems application rate from EPA Reg no. 67071-74. The maintenance rate is 222 ppm.
B. Standard value used by AD based on hand immersion and wiping experiments reported in Cinalli, 1992.
C. MIT Dermal Loading = Application Rate x MWF Thin Film Retention x 1000 µg/mg
D. Induction MOE = POD (210 µg/cm^2) / MIT Dermal Loading (µg/cm^2).
E. Elicitation MOE = POD (0.0105 µg/cm^2) / MIT Dermal Loading (µg/cm^2).

3.11.4 Pressure Treatment Worker Exposures to MIT/CMIT

There are several MIT/CMIT products that are used to pressure treat wood. Occupational handler exposures are anticipated to occur during these applications. These exposures are anticipated to be intermediate to long term in duration and they can occur via the dermal or inhalation routes. Because only the MIT/CMIT products are used to treat wood, these exposures will be assessed as MIT/CMIT rather than MIT.

Pressure Treatment Worker Inhalation Exposures

A summary of the inhalation MOEs for pressure treatment workers is included in Table 33. The MOEs are not of concern because they are greater than the LOC of 10.

Table 33. Pressure Treatment Workers Inhalation MOEs for MIT/CMIT

Job Function	Application Rate[A] (ppm a.i.)	Fraction a.i.[B]	Inhalation Unit Exposure[C] (µg/m^3/fraction a.i.)	Inhalation Exposure[D] (µg/m^3)	Inhalation MOE[E] (LOC = 10)
Treatment Operator Wood Handler	63	0.000063	3.0 11.6	0.00019 0.0022	580,000 50,000

A. Application rate based on EPA Reg No. 39967-91 which contains 10.75% CMIT and 3.6% MIT.
B. Fraction a.i. = Application Rate (ppm a.i.) / 1,000,000 ppm
C. Estimated Arithmetic Average (AMm) for the 8-hour TWA total inhalable fraction unit exposures from the AEATF II Pressure Treatment Exposure Study (MRID 49434501) for Sites ABDE.
D. Inhalation Exposure (mg/m^3) = Fraction a.i. * Inhalation Unit Exposure (mg/m^3/fraction a.i.) * 0.001 mg/µg
E. Inhalation MOE = HEC (0.11 mg/m^3) / Inhalation Exposure (µg/m^3) * 0.001 mg/µg

Pressure Treatment Worker Dermal Exposures

A summary of the dermal MOEs for pressure treatment workers is included in Table 34. The induction MOE 150 is not of concern because it is greater than the LOC of 100. The elicitation MOE of 26 is not of concern because it is greater than the LOC of 10.

Table 34. Pressure Treatment Workers Dermal MOEs for MIT/CMIT

Job Function	Application Rate[A] (ppm a.i.)	Fraction a.i.[B]	Dermal Unit Exposure[C] (mg/fraction a.i.)	Dermal Exposure[D] (µg/day^3)	Dermal Loading[E] (µg/cm^2)	Induction MOE[F] (LOC = 100)	Elicitation MOE[G] (LOC = 10)
Treatment Operator	63	0.000063	0.90	0.057	0.000069	1700000	150
Wood Handler			5.3	0.33	0.00040	300000	26

A. Application rate based on EPA Reg No. 39967-91 which contains 10.75% CMIT and 3.6% MIT.
B. Fraction a.i. = Application Rate (ppm a.i.) / 1,000,000 ppm
C. Estimated Arithmetic Average (AMm) from the AEATF II Pressure Treatment Exposure Study (MRID 49434501) for Sites ABDE. Hands = 99 percent.
D. Dermal Exposure (µg/day) = Fraction a.i. * Unit Exposure (mg/fraction a.i.) * 1000 µg/mg
E. Dermal Loading = [Dermal Exposure (µg/day) * Hand Exposure (%/100)] / Hand Area (820 cm^2)
F. Induction MOE = POD (120 µg/cm^2 for MIT/CMIT) / Dermal Loading (µg/cm^2)
G. Elicitation MOE = POD (0.0105 µg/cm^2) / Dermal Loading (µg/cm^2)

3.11.5 Sapstain Control Worker Exposures

There are several MIT/CMIT products that are used to spray or dip fresh cut lumber to prevent sapstain. Occupational handler exposures are anticipated to occur during these applications. These exposures are anticipated to be intermediate to long term in duration and they can occur via the dermal or inhalation routes. Because only the MIT/CMIT products are used for sapstain wood treatment, these exposures will be assessed as MIT/CMIT rather than MIT.

Sapstain Treatment Worker Inhalation Exposures

The inhalation MOEs for sapstain control worker inhalation exposures to CMIT/ MIT aerosols were assessed as outlined in Table 35. The MOE of 0.75 for the cleanup crew is of concern for short intermediate term exposures because is less than the LOC of 10. The remaining MOEs, which range from 16 to 26, are of concern for long term exposures because they are less than LOC of 30.

Table 35. Sapstain Control Worker Inhalation MOEs for MIT

Application Rate	Job Function	Unit Exposure[B] (mg/m^3/% a.i.)	Exposure[C] (mg/m^3)	Inhalation MOE[D] (LOC = 10 or 30)
0.054 percent MIT/CMIT in the treatment solution[A]	Dip Tank Operator	0.0052	0.00028	**16**
	Millwright	0.0031	0.00017	**26**
	Chemical Attendant	0.0043	0.00023	**20**
	Clean-up Crew	0.111	0.0060	**0.75**

A. Based on label 5383-141 which contains 14.6% a.i. and is applied at the rate of one gallon per 350 gallons of water.
B. Unit exposures are from the Sapstain Phase III study (MRID 455243-01).
C. Exposure (mg/m^3) = Application Rate (% a.i.) * Unit Exposure (mg/m^3/% a.i.)
D. Inhalation MOE = HEC (0.0045 mg/m^3) / Exposure (mg/m^3)

Sapstain Control Worker Dermal Exposures

The MOEs for sapstain control worker dermal exposures to MIT/CMIT were assessed as outlined in Table 36. The Induction MOE of 48 for the cleanup crew is of concern because it is less than the LOC of 100. The remaining Induction MOEs, which range from 150 to 670 are not of concern. The elicitation MOEs, which range from 0.004 to 0.06, are of concern because they are less than the LOC of 10.

Table 36. Sapstain Control Worker Dermal MOEs

Application Rate	Job Function	Unit Exposure[B] (mg/day/% a.i.)	Dermal Exposure[C] (mg/day)	Percent Hand Exposure[B]	Dermal Loading[D] (µg/cm²)	Induction MOE[E] (LOC = 100)	Elicitation MOE[F] (LOC =10)
0.054 percent MIT/CMIT[A]	Dip Tank Operator	2.99	0.16	91	0.18	670	**0.06**
	Millwright	7.10	0.38	51	0.24	500	**0.04**
	Chemical Attendant	17.1	0.92	71	0.80	150	**0.01**
	Clean-up Crew	72.4	3.91	52	2.48	**48**	**0.004**

A. Based on label 5383-141 which contains 14.6% a.i. and is applied at the rate of one gallon per 350 gallons of water.
B. Unit exposures are from the Sapstain Phase III study (MRID 455243-01). The workers were wearing chemical resistant gloves.
C. Dermal Exposure (mg/day) = Application Rate (% a.i.) * Unit Exposure (mg/day/% a.i.)
D. Dermal Loading (µg/cm²) = [Dermal Exposure (mg/day) * Hand Exposure (%/100) * 1000 µg/mg] / Hand Surface Area (820 cm²)
E. Induction MOE = POD (120 µg/cm²) / Dermal Loading (µg/cm²)
F. Elicitation MOE = POD (0.0105 µg/cm²) / Dermal Loading (µg/cm²)

3.12 Human Health Incidents

The incident Data System (IDS) was searched on Feb. 20, 2020, and there are three entries for major incidents reported for MIT and CMIT since 2013 (Incident number I026229). These entries are all related to a lawsuit against multiple plaintiffs including the manufacturer of MIT and CMIT. The suit alleges that several employees of an air compressor manufacturing company in Missouri developed respiratory disease and one employee died of breast cancer related to exposure to "coolant, biocide, hydraulic oil, bacteria, fungi, mold and mycobacteria at the air compressor plant." Also, there are 11 incidents which are categorized as moderate reporting irritation, rashes, blisters, burns, nausea and blurred vision.

There are multiple incidents of Allergic Contact Dermatitis reported in published literature and 39 comments were unofficial incident reports related to the use of MIT and CMIT primarily in personal care products, but also in cleaning products. See the Final Work Plan for a summary of these incidents reported as comments and the docket for the full text of the comments. www.regulations.gov Docket Number EPA-HQ-OPP-2013-0605.

4.0 ENVIRONMENTAL RISK ASSESSMENT

Based on the current use patterns for MIT/CMIT, no terrestrial exposures are expected. Several of the use patterns could result in aquatic exposures, however. Of these, the potential risks from the water cooling towers, pulp and paper mills, paints, and wood treatment uses can be quantified and are expected to result in the highest aquatic exposures.

4.1 Environmental Fate

Based on their chemical, physical, and environmental fate properties (Table 2), CMIT and MIT in the aquatic environment are expected to be soluble in water (225,900-669,600 mg/L) and to rapidly degrade to straight-chain nitrogenous carboxylic acids that eventually mineralize to carbon dioxide and formic acid with half-lives of 5.3-9.8 hours for CMIT and 9.1 hours for MIT. The low log K_{ow} values for CMIT and MIT (-0.486 to 0.40, respectively) indicate that bioconcentration in aquatic organisms and sorption to soil, sludge, and sediments are expected to be limited. CMIT and MIT present in aqueous media are not likely to partition into air based on the low Henry's Law constants (5.4×10^{-9} and 4.2×10^{-8} atm m^3 mol^{-1}), but based on their vapor pressures (10^{-2} mm Hg), these chemicals have the potential to volatilize from soil.

CMIT (parent compound, 5-chloro-2-methyl-4-isothiazolin-3-one) degrades by dechlorination to form MIT (dechlorinated CMIT, methyl-3(2H)-isothiazolone), which then loses a sulfur in the process of ring cleavage to form the straight-chain degradate, N-methyl malonamic acid (NMMA). NMMA is a low-molecular weight nitrogenous carboxylic acid compound that undergoes demethylation and loss of CO_2 (decarboxylation) by biotic processes to eventually form the terminal degradates, formic acid and CO_2. However, NMMA is stable to abiotic degradation (MRIDs 42086901, 43753203, -04 and -05).

4.1.1 Available Data

CMIT and MIT are both miscible in water (Table 2) and are expected to preferentially partition to the water phase of the aqueous environment rather than to sediment, air, and fish. For CMIT, the Freundlich Kads values, which predict partitioning to whole soil and sediment, ranged from 0.08-4.9 L/kg for five soils with a mean of 1.5 L/kg. The Koc values, which represent the potential for sorption to the organic carbon portion of soil and sediment, ranged from 30-310 L/kg in five soils. Based on these Koc values, CMIT is expected to be mobile to moderately mobile based on the FAO classification system.[10] Data on sorption of MIT to soil was not submitted, but MIT degraded with a half-life of 9.1 hours in an aquatic metabolism study which would prevent significant sorption to soil and sediment. As a result, data on CMIT is appropriate for bridging to MIT because of structural similarity. CMIT and MIT have Henry's Law constants of 10^{-9} to 10^{-8} atm m^3 mol^{-1}, indicating that partitioning to air from aqueous media is not a significant route of dissipation. CMIT is not likely to bioconcentrate in fish to a significant degree based on relatively low reported BCF values of 41-54X for two concentrations tested (MRID 44113102).

[10] http://www.fao.org/docrep/003/x2570e/x2570e06.htm

The degradation rate of CMIT and the extent of degradate formation and decline depends on the route of degradation (abiotic or biotic). Abiotic dissipation routes include hydrolysis and photodegradation in water. For parent CMIT, abiotic degradation only occurs at pH 9 with calculated times for 50% dissipation (DT_{50}) values of 14-20 days and DT_{90} values of 46-65 days for hydrolysis; the DT_{50} value was 6.6 days and calculated time for 90% dissipation (DT_{90}) value was 22 days for photodegradation in water. For MIT, the DT_{50} was 11 days and the DT_{90} was 37 days for photodegradation in water. For both hydrolysis and photodegradation, apparent accumulation of the degradate NMMA occurs because NMMA did not degrade abiotically.

CMIT degraded by biotic processes with DT_{50} values of 5.3-9.8 hours (aerobic and anaerobic aquatic metabolism) and MIT degraded with a DT_{50} of 9.1 hours (aerobic aquatic metabolism). The DT_{90} values were 18-33 hours for CMIT and 32 hours for MIT. In these aquatic metabolism studies, no major degradates ($\geq$10 % of applied parent compound) were detected. Based on these results, microbial degradation in the aquatic environment is expected to rapidly degrade CMIT and MIT.

CMIT and MIT are expected to leach from treated wood into water, but no leaching studies have been submitted to the Agency for the paint and textile uses. The registrant conducted a wood leaching study using Southern Yellow Pine and five water types, including separate treatments of distilled, deionized (DI) water, artificial seawater, and pH 5, 7, and 9 buffer solutions. For all treatments, approximately 55% of CMIT and MIT leached in the first 5 days and approximately 75-80% leached in 28 days.

Data on degradation of CMIT and MIT during wastewater treatment have not been submitted. Sorption to sludge is not likely based on the low log K_{ow} values of 0.40 for CMIT and -0.49 for MIT (Table 1). Based on the anaerobic and aerobic aquatic metabolism half-lives ranging from about 5.3 to 9.8 hours, rapid degradation of CMIT and MIT to NMMA is indicated. Consequently, any CMIT and/or MIT that persists long enough to reach a wastewater treatment plant from its point of discharge would not be expected to persist long enough to enter surface water in large amounts. The straight chain degradates, including NMMA, are transient degradates that are rapidly biodegraded and were not reported to be formed at significant quantities ($\geq$10 % of applied parent) in aerobic soil metabolism and aquatic metabolism studies listed in Table 37. In addition, these are not expected to enter surface water from WWTPs.

Table 37. MIT and CMIT Environmental Fate Studies

Test Guideline	Study result	Significant Degradation Products[11]	Reference (MRID) and Comments
Hydrolysis (835.2120)	CMIT stable at pH 5 and 7 14 days (DT_{50}) at pH 9 46 days (DT_{90}) at pH 9 MIT stable at pH 5, 7, and 9	Unidentified polar degradates adding up to 63% of applied parent[12]	43971801 Separate studies in same MRID for applied CMIT and MIT as parent products R^2=0.99, Chi sq.=0.60 (<15%) 25 °C

[11] Significant degradates formed at $\geq$10 % of applied parent compound.
[12] No degradates formed at $\geq$10 % of applied compound.

Test Guideline	Study result	Significant Degradation Products[11]	Reference (MRID) and Comments
	20 days (DT_{50}) 65 days (DT_{90})	N-methyl malonamic acid increased to 64.5% of parent by 42 days (end of study)	44700501 42681301 CMIT tested pH 9 25 ºC
	Stable at pH 5, 7, and 9	None[13]	42578401 24.1 ºC MIT
Photodegradation in water by sunlight (835.2210)	6.6 days (DT_{50}) 22 days (DT_{90})	N-methyl malonamic acid increased to 30-31 % of parent by 15 days (end of study) 5-chloro-3-methyl-4-thiozolin-2-one increased to 37.6% by 15 days	43753201 CMIT pH 7 at 24.8 ºC r^2=0.99, Chi sq=3.8 (<15%)
	11 days (DT_{50}) 37 days (DT_{90})	Mix of N-methyl malonamic acid, N-methylacetamide, and N-methyl oxamic acid increased to 37.5% of parent by 30 days (end of study) 3-methyl-4-thiazolin-2-one increased to 39.6% by 30 days	43753202 MIT r^2=0.87, Chi sq=16.7 (>15%)
Aerobic soil metabolism (835.4100)	5.3 hours (DT_{50}) 18 hours (DT_{90})	No major non-volatile degradates formed	42086901 25 ºC ~90 % degraded by 2 days r^2=0.93, Chi sq.=17.8 (>15%) CMIT
Anaerobic aquatic metabolism (835.4400)	5.3 hours (DT_{50}) 18 hours (DT_{90})	No major non-volatile degradates formed	43753203 25 ºC r^2=0.99, Chi sq.=3.9 (<15%) CMIT
Aerobic aquatic metabolism (835.4300)	9.8 hours (DT_{50}) 33 hours (DT_{90})	No major non-volatile degradates formed	43753204 r^2=0.9, Chi q.=23.1 (>15%) CMIT 25 ºC
	9.1 hours (DT_{50}) 32 hours (DT_{90})	N-methyl malonamic acid	43753205 r^2=0.99, Chi sq.=3.1 (<15%) MIT 25 ºC
Leaching-Adsorption-Desorption (unaged) 835.1230	Adsorption K_f values of 0.08-4.9 ml/g (mean of 1.5 ml/g) Koc values of 30-310 ml/g (mean of 136 ml/g)	No degradates measured	42086902 CMIT Sorption mostly related to organic carbon content (r^2=0.33) and pH [H^+] (r^2=0.24)
Bioconcentration in Fish (850.1730)	BCF of 41-54X 50% depuration of 0.64-1.6 day	None (not required)	44113102 CMIT

[13] Degradates cannot form if parent compound is stable.

Test Guideline	Study result	Significant Degradation Products[11]	Reference (MRID) and Comments
Leaching from treated wood (AWPA E11-12)	55% leached in first 5 days 84 % leached from treated Southern Yellow Pine in 28 days	No degradation at pH 5 and 7 but significant degradation occurred in the pH 9 solution. Degradate(s) not identified, but most likely N-methyl-malonamic acid (NMMA) based on hydrolysis study (MRIDs 44700501, 42681301)	43478401 Mixture of CMIT and MIT Kathon WT

4.1.2 Environmental Fate Data Gaps

There are no outstanding environmental fate data for CMIT or MIT.

4.1.3 Degradates of Potential Concern

There are no degradation products of potential concern for either CMIT or MIT because they form low molecular-weight, organic acids that metabolize to formic acid and CO_2, none of which contain an intact isothiazolinone ring.

4.1.4 Water Quality – Total Maximum Daily Load

Based on a March 3, 2020 search, CMIT and MIT are not identified as a cause of impairment for any water bodies listed as impaired under section 303(d) of the Clean Water Act.[14] In addition, no Total Maximum Daily Loads (TMDL) have been developed for CMIT.[15] More information on impaired water bodies and TMDLs can be found at EPA's website.[16]

4.1.5 Monitoring Data

The Agency is not aware of any surface or ground water monitoring data for CMIT, MIT, or its degradation products. No monitoring data for CMIT or MIT were found in a March 3, 2020 search of the USGS Water Quality Portal[17].

4.2 Selected Ecotoxicity Endpoints

The complete available ecotoxicity data set is provided in Appendix A. The most sensitive acute and chronic endpoints for each receptor group are presented in Table 38. These data characterize MIT/CMIT as being highly to very highly toxic to aquatic organisms and moderately toxic to birds on an acute exposure basis. No ecotoxicity data are available for MIT/CMIT degradates.

There is some uncertainty within the chronic freshwater fish endpoint. No acute to chronic ratio (ACR) could be performed because there was no acute endpoint for fathead minnows or chronic endpoint for rainbow trout. Since the rainbow trout is the most acutely sensitive fish species

[14] http://iaspub.epa.gov/tmdl_waters10/attains_nation_cy.cause_detail_303d?p_cause_group_id=885
[15] http://iaspub.epa.gov/tmdl_waters10/attains_nation.tmdl_pollutant_detail?p_pollutant_group_id=885&p_pollutant_group_name=PESTICIDES
[16] http://www.epa.gov/owow/tmdl/
[17] https://www.waterqualitydata.us/

tested, the fathead minnow chronic endpoint is within an order of magnitude of the LC_{50} of the rainbow trout, and the relative sensitivity of the two fish species is unknown, the chronic endpoint may be lower than presented.

Table 38. Selected Ecological Effects Endpoints for MIT/CMIT

Receptor Group	Surrogate Species	Exposure Scenario	Toxicity	MRID
Freshwater fish	Rainbow trout (*Oncorhynchus mykiss*)	Acute	LC_{50} = 0.07 mg ai/L	41963503
	Fathead Minnow (*Pimephales promelas*)	Chronic	NOEL= 0. 02 mg ai/L[1]	42012201
Freshwater invertebrates	Water flea (*Daphnia magna*)	Acute	EC_{50} = 0.18 mg ai/L	41718803
	Water flea (*Daphnia magna*)	Chronic	NOEL= 0. 10 mg ai/L	41963502
Estuarine/ marine fish	Sheepshead minnow (*Cyprinodon variegatus*)	Acute	LC_{50} = 0.36 mg ai/L	00042556
Estuarine/ marine invertebrates	Eastern oyster (*Crassostrea virginica*)	Acute	EC_{50} = 0.028 mg ai/L	00042558
Aquatic plants	Green Alga (*Selenastrum capricornutum*)	N/A	EC_{50} = 0.023 mg ai/L	43783201
Birds	Northern bobwhite (*Colinus virginianus*)	Acute	LD_{50} = 62.7 mg ai/kg-bw	41719501
	Mallard (*Anas platyrhynchos*)	Sub-acute	LC_{50} = 717 mg ai/kg diet	41719503
Honeybee	Honeybee (*Apis mellifera* L.)	Acute (contact)	48 hr LD_{50}= 3.9 µg AI/bee	51021501

1: No acute to chronic ratio (ACR) could be performed and the chronic endpoint may actually be lower than presented here.
N/A = Not applicable
LC_{50} = Median Lethal Concentration. A statistically derived concentration of a substance that can be expected to cause death in 50% of test animals. It is usually expressed as the weight of substance per weight or volume of water, air or feed, e.g., mg/L, mg/kg or ppm.
EC_{50} = Median Effective Concentration. A statistically derived concentration of a substance that can be expected to cause a 50% reduction in either algae growth, algae growth rate, or daphnid immobilization.
NOEC = No Observed Effect Concentration
LOEC = Lowest Observed Effect Concentration

4.3 Ecological Incident Data

There were no reported ecological incidents for MIT or CMIT in the Agency's Incident Data System (IDS) as of 2/5/2020.

4.4 Aquatic Exposure Modeling

Exposure to aquatic species has the potential to occur after MIT/CMIT use in various antimicrobial use sites. This risk assessment focuses on the risks from four major uses, which are: (1) water cooling towers with a use rate of 1-20 ppm and estimated use of 500,000 lb. in 2012 according to the Kline Report (Kline, 2012), (2) pulp and paper process water with a use rate of 11-153 ppm and a 2012 estimated use rate of 80,000 lbs., (3) paints, stains, and coatings

with a use rate of 20-400 ppm and an estimated use rate of 501,000 lbs., and (4) pressure treated wood in docks with a use rate of 63 ppm. Other MIT/CMIT uses have the potential for environmental exposure, but these use patterns were determined to result in the highest aquatic exposures and would be protective of other uses.

4.4.1 Uses with Effluent Going to Waste Water Treatment Plants

The MIT/CMIT water cooling tower and pulp and paper process water uses both involve effluent or blowdown entering WWTPs before environmental release. Therefore, the General Population and Ecological Exposure from Industrial Releases Module (herein called the Industrial Release module) of the Exposure and Fate Assessment Screening Tool (E-FAST) (US EPA, 2014) was used to perform an upper bound and average screening level estimate of exposure for aquatic organisms located downstream of treated industrial recirculating water systems and pulp and paper mills.

Since the product labels do not limit how MIT/CMIT is used in cooling towers or paper mills, several scenarios were modeled to characterize potential exposures. For cooling towers, the analysis was conducted using two sizes of towers (2,000 gallons/minute and 100,000 gallons/minute) at two application rates (1 and 20 ppm). For pulp and paper, the Agency estimated exposure from two use rates, the highest application rate (153 ppm) and a lower application rate (11 ppm) and assumed MIT/CMIT is used on the wet-end of the paper making process.

The Agency has conducted a high-end (low flow) and an average (average flow) analysis to determine the conditions under which there might be exposure and potential adverse risks to freshwater aquatic organisms. The high-end scenario is based on the 10^{th} percentile of the distribution of the ratio of 7Q10 stream flows to WWTP flows. The average case scenario is based on the median of the distribution of the ratio of 7Q10 stream flows to WWTP flows. The 7Q10 is the lowest 7 consecutive day stream flow over a 10-year period. For the high-end scenario, the ratio of stream flow to plant flow is relatively low since plant flows can contribute considerable volume to the flow of the stream and the resulting surface water concentrations can be relatively high. For the average case scenario, the ratio of stream flow to plant flow is more typical.

In this analysis, it was assumed that 0% of the MIT/CMIT that enters WWTPs would be removed during wastewater treatment due to a lack of data on the removability of the MIT/CMIT when it enters WWTPs. For more information on the E-FAST model assumptions and inputs, see Appendix B for details.

4.4.1.1 Concentrations of Concern (COCs)

The results of the E-FAST analysis are expressed as number of days of exceedance of concentrations of concern (COCs) for aquatic organisms. A COC is the aquatic concentration of active ingredient that if exceeded is expected to cause adverse effects to freshwater organisms. COCs are grouped into 3 categories: acute, chronic, and endangered/listed (the endangered/listed species COCs are provided in Appendix B; the non-listed species values are discussed below). The Agency uses the most sensitive ecotoxicology endpoints for surrogate species to assess risk

to each aquatic receptor group, such as freshwater fish, freshwater invertebrate, and aquatic plants. See Table B1 in Appendix B for calculations.

Although endpoints for MIT/CMIT are available for organisms that represent estuarine/marine fish and invertebrates, the Industrial Release module is appropriate only for estimating exposures in flowing water bodies (streams) and cannot be used to estimate potential exposures to aquatic organisms in estuarine/marine environments.

4.4.2 Cooling Tower Use

4.4.2.1 Cooling Tower Release Rate Calculations

The EPA's Office of Pollution Prevention and Toxics (OPPT) Chemical Engineering Branch's (CEB) generic scenario for recirculating cooling towers (USEPA, 1991) was used to estimate daily releases to surface water of MIT/CMIT in blow-down water in kilograms per site per day. Blow-down, also sometimes referred to as "Draw-off", is the portion of circulating water flow that is removed to reduce Total Dissolved Solids (TDS) and other impurities. Reducing TDS minimizes formation of scale, biological growth, and corrosion, which if unchecked can reduce the efficiency of cooling towers to remove heat from process water to the atmosphere. For complete calculations, see Appendix B.

Table 39 shows the environmental releases (kg/day/site) for MIT/CMIT in moderate and large size cooling towers based on the label information.

Table 39. Environmental releases (kg/site/day) of MIT/CMIT for Water Cooling Towers

Active ingredient concentration (ppm)[1]	Moderate size cooling tower (2,000 gallons/minute)[2]	Large size cooling tower (100,000 gallons/minute)[2]
1	0.07 kg AI/site/day	3.3 kg AI/site/day
20	1.3 kg AI/site/day	67 kg AI/site/day

1- From Table 3 in this document

2- The Environmental release (kg MIT/CMIT/site/day) is based on the quantity of MIT/CMIT within the blowdown. Environmental release = (0.6%) (ppm MIT/CMIT) (Recirculation rate) (5580 x 0.000001 min-kg/day-gal). Where 0.6% is the percentage of cooling tower water that is assumed to be released to surface water via blowdown, 5580 x 0.000001 min-kg/day-gal is a conversion factor, and the recirculation rate of the cooling water (gal/min) is either 2,000 or 100,000gal/min.

4.4.2.2 Cooling Tower Results

Tables 40 and 41 presents screening-level estimates of numbers of days of exceedance of COCs for freshwater organisms downstream of WWTPs receiving cooling tower blowdown assuming (1) all releases occur over the course of one year, (2) all water used in recirculating cooling towers is discharged to WWTPs, and (3) 0% of the MIT/CMIT that enters the WWTPs is removed during treatment. The environmental release (kg/site/day) is based on the initial doses of 1 to 20 ppm AI applied to the system as indicated on the label (Table 39). Surface water concentrations are based on the distribution of plant flows and stream flows. Model results are expressed as per days per year of exceedance of concentrations of concerns for aquatic organisms downstream of recirculating cooling towers. For detailed information on the features,

data, and methods on which the model in E-FAST version 2.0 are based, refer to the latest version of the documentation manual for E-FAST (US EPA, 2014).

Table 40 provides the number of days per year COCs are exceeded based on the average and high-end scenarios for moderate size cooling tower (2000 gallon/minute). Risks are of concern for all taxa when MIT/CMIT is used at the maximum application rate (20 ppm) with exceedances of the COCs 14 to 65 days per year when discharged into streams with average streamflow.

Risks are greatly reduced when the lower application rate of 1 ppm was used. No days of exceedances occurred for acute and chronic non-listed invertebrates when blowdown was released to average-flow streams. COCs were exceeded 1 day per year for non-listed invertebrates (acute), 5 days per year for acute exposure to non-listed fish, 15 days for chronic exposure to non-listed fish, 12 days for aquatic plants, and 0 days for chronic exposure to invertebrates when blowdown was released to streams with low-flow conditions. Exceedances of the COCs for listed species also occurred for all taxa and scenarios and the results are provided in Appendix B.

Table 40. Aquatic Risks for Moderate-Size Cooling Towers (2,000 gal/min)

Concentrations of concern (COC)	Application Rate: 1 ppm[1]		Application Rate: 20 ppm[2]	
	High-End	Average	High-End	Average
Non-Listed Freshwater Fish, Invertebrates, and Plants				
Acute Non-Listed Fish (COC=35 µg AI/L)[3]	5	1	219	42
Acute Non-Listed Invertebrate (COC= 90 µg AI/L)[4]	1	0	112	16
Chronic Non-Listed Fish (COC= 20 µg AI/L)[5]	15	2	276	65
Chronic Non-Listed Invertebrate (COC= 100 µg AI/L)[6]	0	0	101	14
Aquatic Plant (COC= 23 µg AI/L)[7]	12	1	263	59

The high-end scenario is based on the 10th percentile of the distribution of the ratio of 7Q10 stream flows to WWTP flows. The average case scenario is based on the median of the distribution of the ratio of 7Q10 stream flows to WWTP flows and is more typical.
1: 0.07 kg MIT/CMIT/site/day. Calculated in Table 39
2: 1.3 kg MIT/CMIT/site/day. Calculated in Table 39
3: Based on rainbow trout (*Oncorhynchus mykiss*) study with an LC50=70 µg AI/L. MRID 41963503.
4: Based on water flea (*Daphnia magna*) study with a 48 hr EC50= 180 µg AI/L. MRID 41718803
5: Based on Fathead Minnow (*Pimephales promelas*) study with a NOEL= 20 µg ai/L. MRID 42012201
6: Based on water flea (*Daphnia magna*) study with a NOEL=100 µg ai/L. MRID 41963502
7: Based on a Green Alga (*Selenastrum capricornutum*) study with an EC50= 23 µg ai/L. MRID 43783201

Table 41 outlines the average and high-end scenarios for a large-size cooling tower (100,000 gallon/minute). Risks are of concern for all taxa when MIT/CMIT is used at either at minimum (1 ppm) or maximum application rate (20 ppm). For the maximum application rate, exceedances of the COCs occurred 186 to 257 days per year when discharged into streams with average streamflow.

Risks are reduced when the lower application rate of 1 ppm was used, but still have exceedances for much of the year. Acute COCs were exceeded 42 days per year for non-listed invertebrates, 82 days per year for acute exposure to non-listed fish, and 104 days per year for aquatic plants when released to streams with average-flow. Chronic COCs were exceeded 38 days for invertebrates and 112 days for fish. Exceedances of the COCs for listed species also occurred for all taxa and scenarios and the results are outlined in Appendix B.

Table 41: Days Exceeding Concentrations of Concern for Large-Size Cooling Towers (100,000 gal/min)

Concentrations of concern (COC)	Application Rate: 1 ppm[1]		Application Rate: 20 ppm[2]	
	High-End	Average	High-End	Average
Non-Listed Freshwater Fish, Invertebrates, and Plants				
Acute Non-Listed Fish (COC=35 µg AI/L)[3]	306	82	365	234
Acute Non-Listed Invertebrate (COC= 90 µg AI/L)[4]	218	42	364	191
Chronic Non-Listed Fish (COC= 20 µg AI/L)[5]	337	112	365	257
Chronic Non-Listed Invertebrate (COC= 100 µg AI/L)[6]	206	38	363	186
Aquatic Plant (COC= 23 µg AI/L)[7]	330	104	365	251

The high-end scenario is based on the 10th percentile of the distribution of the ratio of 7Q10 stream flows to WWTP flows. The average case scenario is based on the median of the distribution of the ratio of 7Q10 stream flows to WWTP flows and is more typical.
1: 3.3 kg MIT/CMIT/site/day. Calculated in Table 39
2: 67 kg MIT/CMIT/site/day. Calculated in Table 39
3: Based on rainbow trout (*Oncorhynchus mykiss*) study with an LC50=70 µg AI/L. MRID 41963503.
4: Based on water flea (*Daphnia magna*) study with a 48 hr EC50= 180 µg AI/L. MRID 41718803
5: Based on Fathead Minnow (*Pimephales promelas*) study with a NOEL= 20 µg ai/L. MRID 42012201
6: Based on water flea (*Daphnia magna*) study with a NOEL= 100 µg ai/L. MRID 41963502
7: Based on a Green Alga (*Selenastrum capricornutum*) study with an EC50= 23 µg ai/L. MRID 43783201

4.4.2.3 Cooling Towers Uncertainties and Limitations

The cooling tower assessment presented above is a conservative, high-end, screening-level approach that uses many assumptions which may not be representative of real-world conditions in the environment. The major assumptions are:

- MIT/CMIT is only used in recirculating-cooling towers and not once-through systems
- 0% removal of MIT/CMIT during wastewater treatment was assumed in the absence of WWTP data.
- Fate and degradation of MIT/CMIT within the environment are not accounted for in these calculations.

4.4.3 Pulp and Paper Mill Use

4.4.3.1 Pulp and Paper Mill Release Rate Calculations

Retention Rate of the Chemical on the Paper

Depending on where MIT/CMIT may be applied within the paper/paperboard making process, the average retention rate on the paper or within the paper sludge can vary. Based on the MIT/CMIT label directions, it was determined that the retention rate of chemicals applied in the wet-end operations was the most appropriate. According to the 2009 OECD Paper Scenario's Emissions Table (Table 4.3, OECD 2009), the quantity of chemical from these sources going to an effluent treatment plant is approximately 10% (90% retention on paper and paper sludge).

Environmental Release Calculations

To determine the maximum amount of MIT/CMIT (kg/site/day) that could be used in a paper and paperboard mill, it is necessary to know the maximum amount of paper that can be produced. Based on industry expert opinion, it is assumed that 500 US tons of paper is produced per site per day in pulp and paper mills in a moderate sized paper mill. The total AI used per day (kg/site/day) was calculated based on this assumption and based on 90% absorption of chemical in pulp and paper. The following formula was used for calculation of kg/site/day. (See Appendix B for detailed calculations).

Total AI used per site per day (kg ai/site/day):

$$\frac{X\ mg\ AI}{1\ kg\ paper} x \frac{1{,}000\ kg\ paper}{1\ tonne\ paper} x \frac{1\ tonne\ paper}{1.10231\ US\ ton\ paper} x \frac{X\ US\ tons\ paper}{site-day} x \frac{1\ kg\ AI}{1x10^6\ mg\ AI}$$

= X kg AI/site/day Used

Al Released to Surface Water (kg ai/site/day):

$$Total\ AI\ Used\ x\ (1 - X\%\ retention\ in\ paper) x\ (1 - X\%\ removed\ in\ WWT)$$

= X kg AI/site/day released to the environment

Table 42 shows the environmental releases (kg/site/day) for MIT/CMIT in pulp and paper mills based on the label information.

Table 42. Environmental releases of MIT/CMIT based on the label information

PPM active ingredient[1]	Environmental release [2]
11	0.50 kg AI/site/day
153	6.9 kg AI/site/day

[1] From Table 3 of this document

[2] See calculations above for details

4.4.3.2 Pulp and Paper Mill Results

Tables 43 presents screening-level estimates of the numbers of days of exceedance of COCs for freshwater organisms downstream of WWTPs receiving effluent from pulp and paper mills assuming: (1) all releases occur over the course of one year, (2) all water used in pulp and paper mills is discharged to a WWTPs, and (3) 0% of MIT/CMIT that enters WWTPs is removed during wastewater treatment. The environmental release rates (kg/site/day) are based on 11 and 153 ppm active ingredient application rates (Table 42). Surface water concentrations are based on the distribution of plant flows and stream flows. Model results are expressed as per days per year of exceedance of concentrations of concerns for aquatic organisms downstream of a pulp and paper mill.

Table 43 shows the average and high-end scenarios for the 11 and 153 ppm MIT/CMIT use rates. Risks are of concern for all taxa when MIT/CMIT is used at 153 ppm within paper mills. There are exceedances of the COCs for non-listed species for 13 to 57 days per year when effluent is discharged to streams with average streamflow.

For pulp and paper mills using lower concentrations of MIT/CMIT (11 ppm) and releasing effluent into streams with low stream-flow rate, acute COCs are exceeded 9 day per year for non-listed freshwater fish, 1 day for non-listed freshwater invertebrates, and 20 days for aquatic plants. If these same mills release effluent into streams with average stream-flow rates, acute COCs are exceeded 1 day per year for non-listed freshwater fish, 0 days for invertebrates, and 2 days for aquatic plants. Exceedances of the COCs for listed species also occurred for all taxa and scenarios and the results are outlined in Appendix B.

Table 43. Days Exceeding Concentrations of Concern for Pulp and Paper Mills (Application Rates of 11 and 153 ppm)

Concentrations of concern (COC)	Application Rate: 11 ppm		Application Rate: 153 ppm	
	High-End	Average	High-End	Average
Non-Listed Freshwater Fish and Invertebrates				
Acute Non-Listed Fish (COC=35 µg AI/L)[3]	9	1	227	37
Acute Non-Listed Invertebrate (COC= 90 µg AI/L)[4]	1	0	110	15
Chronic Non-Listed Fish (COC= 20 µg AI/L)[5]	26	3	289	57
Chronic Non-Listed Invertebrate (COC= 100 µg AI/L)[6]	1	0	99	13
Aquatic Plant (COC= 23 µg AI/L)[7]	20	2	275	52

The high-end scenario is based on the 10th percentile of the distribution of the ratio of 7Q10 stream flows to WWTP flows. The average case scenario is based on the median of the distribution of the ratio of 7Q10 stream flows to WWTP flows and is more typical.
1: 0.50 kg MIT/CMIT/site/day. Calculated in Table 42
2: 6.9 kg MIT/CMIT/site/day. Calculated in Table 42
3: Based on rainbow trout (*Oncorhynchus mykiss*) study with an LC50=70 µg AI/L. MRID 41963503.
4: Based on water flea (*Daphnia magna*) study with a 48 hr EC50= 180 µg AI/L. MRID 41718803
5: Based on Fathead Minnow (*Pimephales promelas*) study with a NOEL = 20 µg ai/L. MRID 42012201

6: Based on water flea (*Daphnia magna*) study with a NOEL=100 µg ai/L. MRID 41963502
7: Based on a Green Alga (*Selenastrum capricornutum*) study with an EC50= 23 µg ai/L. MRID 43783201

4.4.3.3 Paper Mills Uncertainties and Limitations

The paper assessment presented above is a conservative, high-end, screening-level approach that uses many assumptions which, may not be representative of real-world conditions in the environment. The major assumptions used were: 90% of MIT/CMIT adheres to paper and paper sludge and 10% of MIT/CMIT is released during paper treatment and enter a WWTP (based on OECD 2009 paper scenarios emissions table), 0% removal of MIT/CMIT during wastewater treatment was assumed based on the lack of submitted WWTP studies, retention ponds were not used, dilution did not occur during treatment. Further, fate and degradation of MIT/CMIT within the environment was not accounted for in these calculations.

4.4.4 Paint and Stains Use

4.4.4.1 Exterior Paints and Stains Release Rate Calculations

Aquatic exposure has the potential to occur when MIT/CMIT leaches from painted/stained exterior surfaces during rain events and subsequently runs off into aquatic habitats. There are no leaching rate data available for use of MIT/CMIT in paints. Therefore, it was conservatively assumed that 100 percent of MIT/CMIT will leach into the environment. The maximum application rate used in paint is 400 ppm AI which corresponds to Reg No. 67071-74 (Table 3). The Agency has calculated the amount of surface area needed to result in risk using Equation 1:

Equation 1:

$$SA = \frac{EEC \cdot V}{AR \cdot WF \cdot PA \cdot D \cdot CF1 \cdot CF2 \cdot LR \cdot T}$$

Where:

SA= Surface Area of painted surface (ft^2)
EEC= Minimum Estimated Environmental Concentration (µg ai/L) that results in risks[18]
AR= Application Rate (0.003 gallons paint per square feet)[19]
WF= Weight Fraction of product in paint (0.4% product in paint)[20]
PA = Percent Active ingredient in product (10% AI)[21]
D = Density of formulation (1.38 g/ml)[22]
CF1= Conversion Factor one (1,000,000 µg/g)

[18] Endpoint * level of concern (LOC). *E.g.* the endpoint for freshwater fish is 70 µg/L and the LOC for non-listed fish is ≥0.5. Thus, the minimum EEC that results in risk is 70 µg/L * 0.5 = 35 µg/L
[19] According to paint manufacturers a gallon of paint may cover 250-400 ft^2 of wall in a single coat. Therefore, the average gallon covers 325 ft^2 which is equivalent to 0.003 gal paint/ft^2
[20] The Agency used Acticide SR 8213C (Reg No 67071-74) as the representative label. The label states that the final paint may contain 0.05-0.4% Acticide SR 8213C (0.05-0.4 lbs per 100 lbs formula)
[21] The Agency used Acticide SR 8213C (Reg No 67071-74) as the representative label. The label states that the percent MIT in the product is 10%.
[22] A gallon of paint weighs approximately 11.5 pounds, which converts to 1.38 g/mL. https://bhs.econ.census.gov/bhs/cfs/weightConversion.html

CF2= Conversion Factor two (3,795.4 ml/gallon)
LR = Leach Rate (100% AI/day)[23]
T = Time (1 day)
V = Volume of water in a waterbody (20,000,000 L)[24]

4.4.4.2 Acute Risk from Use in Paints, Stains, and Coatings

Due to the variability in house sizes, number, and distribution of houses in the United States, the maximum quantity of treated surface adjacent to a waterbody that would result in no acute risk to non-listed, non-target aquatic organisms (Table 44) was derived. Based on the most sensitive species (fish and aquatic plants), the Agency estimated that up to 64,000 ft^2 could be treated without exceeding a concentration that would result in a LOC exceedance. Concentrations for listed species are outlined in Appendix B.

Table 44. Maximum MIT/CMIT Treated (Painted) Surface Area Adjacent to a Waterbody That Results in No Acute Risk

Taxonomic Group	Study Type	Minimum EEC that Results in Risk (µg AI/L) [1]	Maximum Surface Area to Result in No Risk (ft^2) [2]
Freshwater Fish[3]	Acute	35 µg AI/L	110,000
Freshwater Invertebrate[4]	Acute	90 µg AI/L	290,000
Freshwater Fish[5]	Chronic	20 µg AI/L	64,000
Freshwater Invertebrate[6]	Chronic	100 µg AI/L	320,000
Aquatic Plant[7]	All	23 µg AI/L	73,000

1: Endpoint * level of concern (LOC). *E.g*. the endpoint for freshwater fish is 70 µg/L and the LOC for non-listed fish is ≥0.5. Thus, the minimum EEC that results in risk is 70 µg/L * 0.5 = 35 µg/L
2: See Equation 1 for details
3: Based on rainbow trout (*Oncorhynchus mykiss*) study with an LC50=70 µg AI/L. MRID 41963503.
4: Based on water flea (*Daphnia magna*) study with a 48 hr EC50= 180 µg AI/L. MRID 41718803
5: Based on Fathead Minnow (*Pimephales promelas*) study with a NOEL= 20 µg ai/L. MRID 42012201
6: Based on water flea (*Daphnia magna*) study with a NOEL=100 µg ai/L. MRID 41963502
7: Based on a Green Alga (*Selenastrum capricornutum*) study with an EC50= 23 µg ai/L. MRID 43783201

To characterize the quantity of houses that could make up this maximum surface area, the Agency has calculated the number of median-sized houses that result in the maximum surface area. These calculations are based on statistics within the 2015 Characteristics of New Housing document from the US Department of Commerce[25] which states that single-family houses built in the United States in 2015 contained a median square footage of 2,467 ft^2.

[23] No paint leach rate data are available for MIT/CMIT.
[24] The Agency's standard waterbody for pesticide ecological assessments is the 'farm pond' which is a 20,000,000 liter waterbody. This waterbody is used as a proxy for all non-flowing aquatic habitats.
[25] 2015 Characteristics of New Housing. US Department of Commerce and US Department of Housing and Urban Development. https://www.census.gov/construction/chars/pdf/c25ann2015.pdf

The Agency has calculated that 64,000 ft^2 of siding equates to approximately 24 one-story or 20 two-story single-family, median-sized houses that are painted with MIT/CMIT preserved products (Table 45).

Table 45. Maximum Number of Houses Next to a Waterbody that Result in No Risk from MIT/CMIT in Paints/Stains

Number of Stories[A]	Floor Plans With 2500 ft^2 of Space	Square Feet of Siding on the House[B]	# Houses Represented by 64,000 ft^2 of Siding[C]
1 Story	25ft x 100ft	2,625 ft^2	24
2 Stories	25ft x 50ft	3,125 ft^2	20

[A] The Agency has assumed that each story is 10 feet tall. Therefore, the exterior wall height is either 10 feet (one story) or 20 feet (two story) and the peak has a height of 10 ft.
[B] Square Feet of Siding = The surface area of the exterior walls including the peaks. (Length*Height)*2 + (Width*Height)*2 + (0.5*Base*10 ft)*2
[C] The number of houses has been rounded to two significant figures

4.4.4.3 Paint Calculations Uncertainties and Limitations

The exterior paint modeling presented above is a conservative, high-end, screening-level approach that uses many assumptions which, may not be representative of real-world conditions in the environment. The major assumptions are:

- All painted surfaces are impacted by rain equally and eaves and gutters do not protect the house's siding from rainfall.
- Houses are newly painted, and 100% leaching is assumed during a rain event.
- Every house next to a waterbody is painted with paint/stain preserved with MIT/CMIT.
- The application rate assumes that a gallon of paint covers 325 ft^2 (*i.e.,* 0.003 gallons paint per ft^2 /gal), whereas many paints manufacturers state that one gallon of paint may cover 250-400 ft^2 of wall in a single coat.
- All leachate goes into a waterbody with a volume of 20,000,000 liters via run-off and no degradation, sorption, or removal of the leachate occurs before entering the pond.
- The conversion of ft^2 to a specific number of houses assumes 100% of exterior surfaces are painted.

This risk assessment could be further refined with production data, paint leach data, and site specific weather conditions.

4.4.5 Pressure Treated Wood Use

4.4.5.1 Pressure Treated Wood Release Rate Calculations

Aquatic exposure has the potential to occur when MIT/CMIT leaches directly into aquatic areas from pressure treated wood used in docks. There are leaching rate data available for use of MIT/CMIT in wood preservatives which indicate 55% of the AI leaches in the first 5 days

(MRID 43478401). The maximum application rate used in pressure treated wood is 63 ppm AI in the wood preservative treatment solution (Table 3, Reg Nos. 39967-91 and 707-128).

Leachable Wood Volume of a Medium Sized Dock
Based on the OECD revised emission scenario document for wood preservatives a medium sized dock has the following dimensions and volume (OECD, 2013):

Length = 6 meters
Width = 1.2 meters
Thickness of the wood = 0.05 meter

Dock volume = 6 m x 1.2 m x 0.05 m = 0.36 m^3 = **12.7 ft^3**

Amount of Active Ingredient Applied to the Wood in a Dock
According to Table 3, MIT/CMIT can be used as a wood pressure treatment for forest products at concentrations up to 63 ppm AI in the wood preservative solution. Additionally, the American Wood protection Association (AWPA) recommends 0.8 pounds of solution per cubic feet of wood (SFA, 2010). The calculations below go through the amount of active ingredient (lbs.) applied to the medium sized dock.

Amount of AI applied per ft^3 of wood = 0.8 lb. treatment solution/ft^3 x 0.0063% AI = 0.0000504 lbs. AI/ft^3 wood preserved

Amount of AI within a dock= 12.7 ft^3 x 0.0000504 lbs. AI/ft^3 = 0.00064 lbs. AI = **290.3 mg AI**

4.4.5.2 Calculating Estimated Environmental Concentrations (EECs) for Wood

There are leaching rate data available for use of MIT/CMIT in wood preservatives which indicate 55% of the AI leaches in the first 5 days (MRID 43478401). Additionally, the Agency assumes that the dock is on a waterbody that contains 20,000,000 liters of water. Therefore, the estimated environmental concentration (EEC) of MIT/CMIT within the waterbody from use in wood preservatives is calculated using the following equation:

$$EEC = \frac{mg\ AI\ in\ the\ Dock\ X\ Leach\ Rate}{L\ Water\ in\ a\ Farm\ Pond} = \frac{290.3\ mg\ AI\ X\ 55\%}{20{,}000{,}000\ L} = \frac{159.7\ mg\ AI}{20{,}000{,}000\ L}$$

$$= 0.0000079\ mg/L = \mathbf{0.0079}\ \mu\boldsymbol{g/L}$$

4.4.5.3 Determining Risk from Wood Preservative Use

For the MIT/CMIT wood preservative assessment, the Agency uses the RQ method used by the Office of Pesticide Programs (OPP) to compare the estimates of acute exposure (EECs) to the acute ecotoxicity endpoint values for each receptor group being assessed. For fish and aquatic invertebrates, acute and chronic RQs are calculated as follows:

Acute RQ = acute EEC/LC_{50} (or EC_{50})

Chronic RQ = chronic EEC/NOAEC

The RQs were then compared to OPP's levels of concern (LOCs) to identify potential acute risks to each receptor group (Table 46). The development of the LOCs is discussed in detail in the Agency's Overview Document[26]. OPP's LOCs are tabulated below for listed[27] and non-listed species.

Table 46: Risk Presumptions and LOCs

Aquatic and Terrestrial Animals	**LOC**
Acute presumption of risk to listed aquatic species	RQ ≥ 0.05
Acute presumption of risk to listed terrestrial species	RQ ≥ 0.1
Acute presumption of risk to non-listed aquatic and terrestrial species	RQ ≥ 0.5
Chronic presumption of risk to listed and non-listed aquatic and terrestrial species	RQ ≥ 1.0
Risk Presumption for Aquatic/Semi-aquatic Plants	**LOC**
Presumption of risk to listed species	RQ ≥1
Presumption of risk to non-listed species	RQ ≥1

For MIT/CMIT leaching from a single medium sized pressure treated wood dock into freshwater ecosystems, there are no acute or chronic risks to non-listed freshwater fish and invertebrate. Additionally, there are no risks to aquatic vascular or non-vascular plants. It would require greater than 2,500 treated docks to exceed a LOC for the most sensitive aquatic taxon (Table 47).

Table 47. MIT/CMIT Risk Quotients for Wood Preservatives

Receptor Group	**EEC[1]**	**Toxicity**	**RQ**	**Number of Modeled Docks Needed to Exceed a LOC**
Acute Non-Listed Freshwater Fish[2]	0.0079 µg/L	LC_{50} = 70 µg AI/L	0.0001	4,400
Acute Non-Listed Freshwater Invertebrate[3]	0.0079 µg/L	EC_{50} = 180 µg AI/L	0.00004	11,000
Chronic Non-Listed Freshwater Fish[4]	0.0079 µg/L	NOEL= 20 µg AI/L	0.0004	2,500
Chronic Non-Listed Freshwater Invertebrate[5]	0.0079 µg/L	NOEL = 100 µg AI/L	0.00008	12,500
Aquatic Plant[6]	0.0079 µg/L	EC_{50} = 23 µg AI/L	0.0003	2,900

1: See equations above (this is per dock).
2: Based on rainbow trout (*Oncorhynchus mykiss*) study with an LC50=70 µg AI/L. MRID 41963503.
3: Based on water flea (*Daphnia magna*) study with a 48 hr EC50= 180 µg AI/L. MRID 41718803
4: Based on Fathead Minnow (*Pimephales promelas*) study with a NOEL= 20 µg ai/L. MRID 42012201
5: Based on water flea (*Daphnia magna*) study with a NOEL=100 µg ai/L. MRID 41963502
6: Based on a Green Alga (*Selenastrum capricornutum*) study with an EC50= 23 µg ai/L. MRID 43783201

[26] http://www.epa.gov/espp/consultation/ecorisk-overview.pdf

[27] A listed species is a species that has been designated as endangered or threatened by the U.S. Fish and Wildlife Service or the U.S. National Marine Fisheries Service.

4.4.5.4 Wood Preservative Calculations Uncertainties and Limitations

The wood preservative modeling presented above is a high-end, screening-level approach that uses many assumptions which, may not be representative of real-world conditions in the environment. The major assumptions are:

- The size and other specifications of the dock used for these calculations may not be representative of all docks which are built in water bodies.
- The dock is newly painted and 55% leaches in the first 5 days (MRID 43478401)
- Immediate dispersion throughout the waterbody is assumed
- Environmental conditions such as temperature, rainfall and pH, may affect the amount of leaching of the chemical from wood.
- The chemical and biological reactions which usually take place under regular environmental conditions are not considered in calculations for this report.
- All leachate goes into a waterbody with a volume of 20,000,000 liters and fate or degradation of MIT/CMIT within the pond is not accounted for in these calculations

4.5 Ecological Risk Characterization

Overall, risks to terrestrial taxa (including pollinators) are not expected from the currently registered uses of MIT/CMIT due to low exposure potential. Of the current uses, the cooling tower, paper mill, paint, and wood treatment uses are expected to result in the highest aquatic exposure. Of these uses, the paint and wood treatment use do not result in any risks of concern for aquatic organisms. The cooling tower and paper mill uses, however, do result in potential risks of concern for aquatic organisms based on screening-level modeling.

The treated water for the cooling tower and paper mill uses would pass through wastewater treatment plants (WWTPs) before reaching aquatic habitats. Data on the environmental fate in WWTPs has not been submitted, and therefore the Agency assumes that some environmental exposure may occur. Based on the chemical and physical properties of these compounds (Table 2), sorption to sludge is not likely to occur and any residues are expected to be in the water phase of WWTPs. If these aqueous residues leave in the effluent, they would be rapidly degraded with half-lives of 5.3-9.8 hours by metabolic processes and not be persistent. Soil residues are not likely because sludge sorption is expected to be minimal. Table 37 contains the environmental fate data showing half-lives of 5.3-9.8 hours for CMIT and MIT. The screening-level models used to estimate exposures from the water cooling and paper mill uses do not take removal through WWT or degradation into account. Therefore, the estimated exposures likely over-estimate the actual exposures found in the environment from these uses. However, the level of over-estimation cannot be quantified at this time.

4.5.1 Freshwater Fish and Invertebrates

The high-end, screening-level assessment of the cooling tower use, acute concentrations of concern (COCs) were exceeded for much of the year when MIT/CMIT products were used in large-sized cooling towers, released to low-flow streams, or used at the maximum application rate (20 ppm). Risks were reduced when the AI was used in moderate-sized cooling towers (2,000 gal/min), released to average-flow streams, and application rate was lower (1 ppm).

Similar trends were observed for the screening-level pulp and paper mill use. All scenarios had at least one day of exceedance for non-listed fish. For freshwater invertebrates, the only scenario that resulted in no days of exceedances of the COC was the 11 ppm application rate releasing to an average-flow stream. Potential risks to the receptor groups cannot be ruled out for any day in which COCs are exceeded.

Even though CMIT and MIT have the potential to reach freshwater environments via these pathways, the risk conclusions presented above are conservative as it is known that the chemicals degrade very rapidly via biotic processes. 90% of CMIT and MIT are expected to be removed from aquatic environments via degradation after 18-33 hours (CMIT) and 32 hours (MIT), however current screening-level exposure models are unable to take this into account at this time. Additionally, when CMIT and MIT undergo metabolic degradation in the environment, they form low molecular-weight organic acids (see Table 37) which are not expected to be persistent and are not of concern. Therefore, the quantities of CMIT and MIT present in the environment after WWT are likely lower than those assessed. However, because of the toxicity of MIT/CMIT to fish and aquatic invertebrates and the number of days that the COCs were exceeded, especially for the high-end scenarios with the highest application rates (*i.e.*, 20 ppm and 153 ppm for water cooling towers and paper mills, respectively), risks to fish and aquatic invertebrates from the water cooling tower and paper mills uses cannot be precluded and are assumed.

No risk to freshwater fish and invertebrates were associated with the MIT/CMIT paint or treated dock uses. Approximately 24 one-story houses, 20 two-story houses, or more than 2,500 docks could be located on a waterbody (20,000,000 L) before potential risks of concern would occur for any aquatic taxon assessed (freshwater fish (chronic)).

4.5.2 Estuarine/marine Fish and Invertebrates

No estuarine/marine assessment was performed for MIT/CMIT use in cooling towers or pulp and paper mill uses because E-FAST is only appropriate for estimating the magnitude of exposures in flowing waterbodies (streams) and therefore cannot be used to estimate potential exposures to aquatic organisms in estuarine/marine environments. However, based on the ecotoxicity endpoints, if MIT/CMIT were used in cooling towers or paper mills releasing blowdown and effluent into estuarine/marine environments of similar volume, the magnitude of risks to estuarine/marine organisms would be comparable to the freshwater organisms outlined above.

Additionally, no estuarine/marine assessment was performed for the paint or wood preservative uses of MIT/CMIT because the current calculations assume a waterbody containing freshwater. However, based on the ecotoxicity endpoints, if MIT/CMIT leaching from paints or wood were to enter a similarly sized estuarine/marine water body as assessed, the magnitude of risk to estuarine/marine organisms would be comparable to the risks to freshwater organisms outlined in this assessment and risk would not be expected.

4.5.3 Benthic invertebrates

No ecotoxicity data is available for benthic invertebrates however, MIT/CMIT is highly toxic to aquatic invertebrates residing in the water column. Nevertheless, the low log Kow of CMIT and MIT (-0.486 to 0.40) indicate that bioconcentration in aquatic organisms and sorption to soil and

sediments are not expected, indicating exposure and risks to benthic invertebrates are expected to be negligible.

4.5.4 Aquatic plants

Aquatic plants represent one of the most sensitive taxa of those tested (along with chronic exposure to freshwater fish) and the estimated exposures for the water cooling and paper mill uses exceeded the levels of concern for several days in all of the scenarios modeled. As discussed above, the actual exposures found in aquatic environments from these uses would likely be lower than modeled, risks to aquatic plants cannot be precluded and are assumed, especially for the high-end scenarios with the highest application rates (*i.e.*, 20 ppm and 153 ppm for cooling water towers and paper mills, respectively).

No risk to aquatic plants is associated with the MIT/CMIT paint or treated dock uses.

4.5.5 Terrestrial Species

No quantitative risk assessment has been performed for terrestrial organisms (including pollinators). However, due to low potential for exposure, risks to terrestrial organisms (including pollinators) are not expected.

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APPENDIX A: Ecotoxicity Profile

Ecological effects toxicity testing was performed using formulation intermediates (*i.e.* Kathon® 886F, 14.17%), or end-use formulations (*i.e.*, Kathon® WT, Kathon® OM, or Kathon® WT, 1.5%). Testing of the formulation intermediate and end-use products is sufficient to fulfill guideline data requirements where ecological effects testing of the technical grade is indicated, due to the inherent instability of the active ingredients at higher percentages. All products contained a combination of MIT and CMIT.

Aquatic Organisms

Acute Freshwater Fish and Invertebrates

Receptor Group	Species	Test material (% ai)	Toxicity MIT/CMIT	MRID
Fish	Rainbow trout (*Oncorhynchus mykiss*)	14.17[1]	96-h LC_{50} = 0.07 mg ai/L	41963503
		14.17[1]	96-h LC_{50} = 0.19 mg ai/L	41718802
	Bluegill (*Lepomis macrochirus*)	14.17[1]	96-h LC_{50} = 0.30 mg ai/L	41718801
Aquatic Invertebrates	Water flea (*Daphnia magna*)	14.17[1]	48-h EC_{50} = 0.18 mg ai/L	41718803
	Water flea (*Cerodaphnia* sp.)	1.5 [2]	48-h EC_{50} = 0.20 mg ai/L	42358701

1: The product tested was Kathon 886F (Reg No 707-130), which contains 10.4% CMIT and 3.7% MIT.
2: The product tested was Kathon WT 1.5 Percent Biocide (Reg No 707-133), which contains 1.11% CMIT and 0.39% MIT.

Chronic Freshwater Fish and Invertebrates

Receptor Group	Species	Test material (% ai)	Toxicity MIT/CMIT	MRID
Fish	Fathead Minnow (*Pimephales promelas*)	14.17[1]	NOEL= 0. 02 mg ai/L (mean measured)	42012201
Aquatic Invertebrates	Water flea (*Daphnia magna*)	14.17[1]	NOEL= 0. 10 mg ai/L (mean measured)	41963502

1: The product tested was Kathon 886 (Reg No 707-130), which had a purity of 14.17% AI

Saltwater Fish and Invertebrates

Receptor Group	Species	Test material (% ai)	Toxicity MIT/CMIT	MRID
Fish	Sheepshead minnow (*Cyprinodon variegatus*)	13.9[1]	96-h LC_{50} = 0.36 mg ai/L	00042556
Aquatic Invertebrates	Eastern oyster (*Crassostrea virginica*)	13.9[1]	48-Hr EC_{50} = 0.028 mg ai/L	00042558
	Marine copepod (*Acartia tonsa*)	11-35[2]	48-Hr LC_{50} = 0.05 mg product/L	42840301
	Pink shrimp (*Penaeus duorarum*)	13.9[1]	96-Hr LC_{50} = 2.3 mg ai/L	00042559

Receptor Group	Species	Test material (% ai)	Toxicity MIT/CMIT	MRID
	Fiddler Crab (*Uca pugilator*)	13.9[1]	96-Hr LC_{50} = 59.0 mg ai/L	00042557

1: The product tested was Kathon WT (Reg No 707-138), which contains 10.4% CMIT and 3.7% MIT. Study states % AIs as 13.9%.
2: The product tested was Kathon OM and was said to contain 11-35% active substances (10-30% CMIT, 1-5% MIT)

Aquatic Plants

Receptor Group	Species	Test material (% ai)	Toxicity MIT/CMIT	MRID
Aquatic plants	Green Alga (*Selenastrum capricornutum*)	13.84 [1]	(Number of cells) 96-hr EC_{50} = 23 µg ai/L 96-hr NOEC = 9.9 µg ai/L (Growth Rate) 96-hr EC_{50} = 36 µg ai/L 96-hr NOEC = 9.9 µg ai/L	43783201

1: The product tested was Kathon WT 14% (Reg No 707-138), which contains tested as having 13.84% active ingredient

Terrestrial Organisms

Birds

Receptor Group	Species	Test material (% ai)	Toxicity MIT/CMIT	MRID
Birds	Northern bobwhite (*Colinus virginianus*)	14.17[1]	LD_{50} = 62.7 mg/kg-bw/day	41719501
		14.17[1]	LC_{50} = 2200 mg ai/diet	41719502
	Mallard (*Anas platyrhynchos*)	14.17[1]	LC_{50} = 717 mg ai/diet	41719503

1: The product tested was Kathon 886, which contained 5-Chloro-2-methyl-4-isothiazolin-3-one calcium chloride with 2-methyl-4-isothiazolin-3-one calcium (PC 107105)

HoneyBee

Receptor Group	Species	Test Material (% ai)	Toxicity MIT/CMIT	MRID
HoneyBee	Honeybee (*Apis mellifera* L.)	14.68[1]	48 hr LD_{50}= 3.9 µg AI/bee (Contact)	51021501

1: The product tested was Preventol IT 14, which contains 14.68% w/w MIT/CMIT.

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APPENDIX B: Ecological Risk Estimation Methods

Risk Estimation Methods

Risk estimation integrates the results of the exposure and ecotoxicity data to evaluate the potential for the active ingredient and its transformation products to cause adverse effects to nontarget organisms. Depending on the uses being assessed, risk estimates are determined from calculations of acute and chronic risk quotients (RQs) or, for down-the-drain (DtD) assessments, from concentrations of concern (COCs).

EFAST Methodology

Within this assessment, the General Population and Ecological Exposure from Industrial Releases Module (herein called the Industrial Release module) of the Exposure and Fate Assessment Screening Tool (E-FAST) (US EPA, 2007) was used to perform an upper bound and average screening level estimate of the potential exposure for aquatic organisms located downstream of wastewater treatment plants processing water containing MIT/CMIT from recirculating cooling water systems and pulp and paper mills.

The results of this analysis are based on the probabilistic dilution model (PDM) option of the Industrial Release module of E-FAST. Stream flows are not single point estimates since streams have a highly variable seasonal flow pattern. PDM uses probability distributions as inputs and calculates the resulting probability distribution of the stream concentration. The model develops a distribution of stream dilution factors (SDFs) based on the ratio of stream flows to plant flows for wastewater treatment facilities. The PDM approach provides information on the number of days of exceedance of concentrations of concern (COCs) for freshwater aquatic organisms located downstream of wastewater treatment plants. Key factors that influence these results include: the wastewater treatment plant influent volume of MIT/CMIT, the percent of MIT/CMIT removed during wastewater treatment, and ratio of the distribution of stream flows to wastewater treatment plant flows.

The Agency has conducted a high end (low flow) and an average (average flow) analysis to determine the conditions under which there might be exposure and potential adverse risks to freshwater aquatic organisms. The high-end scenario is based on the 10^{th} percentile of the distribution of the ratio of 7Q10 stream flows to WWTP flows. The average case scenario is based on the median of the distribution of the ratio of 7Q10 stream flows to WWTP flows. The 7Q10 is the lowest 7 consecutive day stream flow over a 10-year period. For the high-end scenario, the ratio of stream flow to plant flow is relatively low since plant flows can contribute considerable volume to the flow of the stream and the resulting surface water concentrations can be relatively high. For the average case scenario, the ratio of stream flow to plant flow is more typical.

The results of the Industrial Release analysis are expressed as number of days of exceedance of COCs for aquatic organisms. COCs are grouped into 3 categories: acute, chronic, and endangered/listed. The Agency uses the most sensitive ecotoxicology endpoints for surrogate species to assess risk to each aquatic receptor group, such as freshwater fish, freshwater invertebrate, and aquatic plants. See Table B1 for calculations.

Although acute endpoints are available for organisms that represent estuarine/marine fish and invertebrates, the EFAST model is appropriate only for estimating magnitude of exposures in flowing water bodies (streams) and cannot be used to estimate potential exposures to aquatic organisms in estuarine/marine environments.

Table B1. Calculations of Concentrations of Concern (COCs)

Aquatic Receptor Group	Endpoint	Calculation
Acute risk to non-listed fish	LC_{50}	0.5 x LC_{50}
Acute risk to non-listed aquatic invertebrates	EC_{50}	0.5 x EC_{50}
Chronic risk to listed and non-listed fish	NOAEC	1 x NOAEC
Chronic risk to listed and non-listed aquatic invertebrates	NOAEC	1 x NOAEC
Acute risk to listed fish	LC_{50}	0.05 x LC_{50}
Acute risk to listed aquatic invertebrates	EC_{50}	0.05 x EC_{50}
Risk to non-listed aquatic plants	EC_{50}	1 x EC_{50}

Complete Calculations and Results for Cooling Water Use Site:

Estimated Environmental Releases Calculations

EPA/OPPT/CEB's generic scenario for recirculating cooling towers (US EPA, 1991) was used to estimate daily releases to surface water of MIT/CMIT in blow-down water in kilograms per site per day. Blow-down, also sometimes referred to as "Draw-off", is the portion of circulating water flow that is removed in order to maintain low enough levels of Total Dissolved Solids (TDS) and other impurities to adequately minimize formation of scale, biological growth, and corrosion, which if unchecked can reduce the efficiency of cooling towers to remove heat from process water to the atmosphere.

To estimate the rate of release of a chemical substance to surface water from blowdown (B), the following equation is used:

$$B = (0.6\%)\ (X_R)\ (R)\ (5580 \times 10^{-06}\ \text{min-kg/day-gal}) \qquad (1)$$

Where:

B = rate of release of chemical substance via blowdown water to surface water (kg/site/day);
X_R = concentration of antimicrobial pesticide in recirculating water (ppm); and
R = recirculation rate of cooling water (gallons per minute).

The conversion factor, 5580×10^{-06} min-kg/day-gal is derived from:

(1440 min/day) (3.875 kg/gal) (10^{-06} ppm) in which the blowdown water is assumed to have a specific gravity of 1.0; thus, 1liter weighs 1 kilogram, and the conversion of 3.78 liters per gallon is expressed in kilograms per gallon. The value of 0.6% is the percentage of cooling tower water that is assumed to be released to surface water via blowdown.

The following input values were used in this sample calculation:

- percentage of cooling tower that is assumed to be released to surface water by blowdown: 0.6% or 0.006

- concentration of MIT/CMIT in recirculating cooling tower water was based on information from the label
- recirculation rate – 2,000 gallons per minute (moderate size) and 100,000 gallons per minute (large size) were used in these calculations (EPA, 1991)
- a conversion factor of 1 x 10^{-6} based on a concentration of parts-per-million.

Table B2 show the environmental releases (kg/day/site) for MIT/CMIT in moderate and large size cooling towers based on the label information.

Table B2: Environmental releases (kg/site/day) of MIT/CMIT for use in PDM model based on registered use and application rate information

Active ingredient concentration (ppm)[1]	Environmental release (kg/site/day) for moderate size cooling tower (2000 gallons/minute)[2]	Environmental release (kg/site/day) for large size cooling tower (100,000 gallons/minute)[2]
1	0.07 kg AI/site/day	3.46 kg AI/site/day
20	1.38 kg AI/site/day	69 kg AI/site/day

1: From Table 3 in this document
2: The Environmental release (kg MIT/CMIT/site/day) is based on the quantity of MIT/CMIT within the blowdown. Environmental release = (0.6%) (ppm MIT/CMIT) (Recirculation rate) (5580 x 0.000001 min-kg/day-gal). Where 0.6% is the percentage of cooling tower water that is assumed to be released to surface water via blowdown, 5580 x 0.000001 min-kg/day-gal is a conversion factor, and the recirculation rate of the cooling water (gal/min) is either 2,000 or 100,000gal/min.

Release Sites Information:
In order to run EFAST, various inputs about the release sites must be determined and are as follows:

- Days per year of release; the default assumption is 365 days[28].
- Standard Industrial Classification (SIC) code analysis or facility analysis; the SIC code "POTWs industrial, includes POTWS which receive industrial discharge" was chosen because no specific facility was being analyzed.
- The number of use sites. The Agency estimated the exposure downstream from one site, as no data were available to determine how many use sites may be using MIT/CMIT.

Cooling Tower Results:
The results of the cooling water assessment are outlined in Tables B3 and B4. The results indicate that concentrations of concern (COCs) are exceeded for multiple days a year especially when MIT/CMIT is used in large-sized cooling towers, blow-down is introduced to low-flow streams, the maximum application rate (20 ppm) is applied, and when listed species are present. Potential risks to the receptor groups cannot be ruled out for any day in which COCs are exceeded.

[28] The Agency typically runs the EFAST model assuming operation for 360 days per year. For this assessment the Agency used 365 days instead of 360 day to show the high-end scenario. For example, when modeled for paper and paperboard mills at 360 days (Average case scenario), the number of exceedances for the COC=9.0 µg/L was 90 days which was 2 days less than 92 days for the 365 days run for the same COC.

Table B3: Aquatic Risks for MIT/CMIT Used in Moderate-size Cooling Towers (2,000 gal/min)

Concentrations of concern (COC)	Application Rate: 1 ppm[1]		Application Rate: 20 ppm[2]	
	High-End	Average	High-End	Average
Acute Freshwater Fish and Invertebrates				
Acute Non-Listed Fish (COC=35 µg AI/L)[3]	5	1	219	42
Acute Non-Listed Invertebrate (COC= 90 µg AI/L)[4]	1	0	112	16
Chronic Freshwater Fish and Invertebrates				
Chronic Fish (COC= 20 µg AI/L)[5]	15	2	276	65
Chronic Invertebrate (COC= 100 µg AI/L)[6]	0	0	101	14
Aquatic Plants				
Non-Listed Aquatic Plant (COC= 23 µg AI/L)[7]	12	1	263	59
Listed Species				
Listed Fish (COC= 3.5 µg AI/L)[3]	149	23	359	156
Listed Invertebrate (COC=9.0 µg AI/L)[4]	55	7	331	105
Listed Aquatic Plant (COC= 9.9 µg AI/L)[7]	48	6	329	99

The high-end scenario is based on the 10th percentile of the distribution of the ratio of 7Q10 stream flows to WWTP flows. The average case scenario is based on the median of the distribution of the ratio of 7Q10 stream flows to WWTP flows and is more typical.

1: 0.07 kg MIT/CMIT/site/day. Calculated in Table B2

2: 1.3 kg MIT/CMIT/site/day. Calculated in Table B2

3: Based on rainbow trout (*Oncorhynchus mykiss*) study with an LC50=70 µg AI/L. MRID 41963503.

4: Based on water flea (*Daphnia magna*) study with a 48 hr EC50= 180 µg AI/L. MRID 41718803

5: Based on Fathead Minnow (*Pimephales promelas*) study with a NOEL= 20 µg ai/L. MRID 42012201

6: Based on water flea (*Daphnia magna*) study with a NOEL= 100 µg ai/L. MRID 41963502

7: Based on a Green Alga (*Selenastrum capricornutum*) study with an EC50= 23 µg ai/L. NOEC= 9.9 µg ai/LMRID 43783201

Table B4: Aquatic Risks for MIT/CMIT Used in Large-Size Cooling Towers (100,000 gal/min)

Concentrations of concern (COC)	Application Rate: 1 ppm[1]		Application Rate: 20 ppm[2]	
	High-End	Average	High-End	Average
Non-Listed Freshwater Fish and Invertebrates				
Acute Non-Listed Fish (COC=35 µg AI/L)[3]	306	82	365	234
Acute Non-Listed Invertebrate (COC= 90 µg AI/L)[4]	218	42	364	191
Chronic Freshwater Fish and Invertebrates				
Chronic Fish (COC= 20 µg AI/L)[5]	337	112	365	257
Chronic Invertebrate (COC= 100 µg AI/L)[6]	206	38	363	186
Aquatic Plants				
Non-Listed Aquatic Plant (COC= 23 µg AI/L)[7]	330	104	365	251
Listed Freshwater Fish and Invertebrates				
Listed Fish (COC= 3.5 µg AI/L)[3]	365	203	365	312
Listed Invertebrate (COC=9.0 µg AI/L)[4]	358	155	365	285
Aquatic Plant (COC= 9.9 µg AI/L)[7]	357	150	365	282

The high-end scenario is based on the 10th percentile of the distribution of the ratio of 7Q10 stream flows to WWTP flows. The average case scenario is based on the median of the distribution of the ratio of 7Q10 stream flows to WWTP flows and is more typical.
1: 3.3 kg MIT/CMIT/site/day. Calculated in Table B2
2: 67 kg MIT/CMIT/site/day. Calculated in Table B2
3: Based on rainbow trout (*Oncorhynchus mykiss*) study with an LC50=70 µg AI/L. MRID 41963503.
4: Based on water flea (*Daphnia magna*) study with a 48 hr EC50= 180 µg AI/L. MRID 41718803.
5: Based on Fathead Minnow (*Pimephales promelas*) study with a NOEL= 20 µg ai/L. MRID 42012201
6: Based on water flea (*Daphnia magna*) study with a NOEL=100 µg ai/L. MRID 41963502
7: Based on a Green Alga (*Selenastrum capricornutum*) study with an EC50= 23 µg ai/L. NOEC= 9.9 µg ai/LMRID 43783201

Complete Calculations and Results for Pulp and Paper Mill Use Site:

Estimated Environmental Releases Calculations:

In order to determine the amount of AI released from the paper mill, the amount of AI used per day needed to be calculated. The calculation includes the assumptions that MIT/CMIT is being used in a moderately sized paper mill and therefore 500 US tons of paper is produced per site per day.

Total AI used per site per day (kg/site/day):

$$\frac{X\ mg\ AI}{1\ kg\ paper} x \frac{1{,}000\ kg\ paper}{1\ tonne\ paper} x \frac{1\ tonne\ paper}{1.10231\ US\ ton\ paper} x \frac{X\ US\ tons\ paper}{site-day} x \frac{1\ kg\ AI}{1x10^6\ mg\ AI}$$

= X kg AI/site/day

Total AI used at the maximum labeled rate per site per day (153 ppm):

$$\frac{153\ mg\ AI}{1\ kg\ paper} x \frac{1{,}000\ kg\ paper}{1\ tonne\ paper} x \frac{1\ tonne\ paper}{1.10231\ US\ ton\ paper} x \frac{500\ US\ tons\ paper}{site-day} x \frac{1\ kg\ AI}{1x10^6\ mg\ AI}$$

= 69.39 kg AI/site/day

Total AI used at the minimum labeled rate per site per day (11 ppm):

$$\frac{11\ mg\ AI}{1\ kg\ paper} x \frac{1{,}000\ kg\ paper}{1\ tonne\ paper} x \frac{1\ tonne\ paper}{1.10231\ US\ ton\ paper} x \frac{500\ US\ tons\ paper}{site-day} x \frac{1\ kg\ AI}{1x10^6\ mg\ AI}$$

= 4.98 kg AI/site/day

In order to estimate the total environmental release to surface water after use within the mill and wastewater treatment, the amount of AI retained within/on the paper and the amount of AI removed during WWT must be taken into account. The following calculation includes two base assumptions: (1) MIT/CMIT is applied during wet-end operations and its retention rate in paper is 90% (10% of MIT/CMIT applied ends up in the effluent water) (OECD, 2009) and (2) no degradation or removal occurs within the WWTP.

AI Released to Surface Water:

$$Total\ AI\ Used\ x\ (1 - X\%\ retention\ in\ paper)\ x\ (1 - X\%\ removed\ in\ WWT)$$

For the maximum labeled rate (153 ppm):

AI Released to Surface Water = 69.39 kg/site/day x (1-0.90) x (1 - 0.00) = **6.9 kg AI/site/day**

At the minimum labeled rate (11 ppm):

AI Released to Surface Water = 4.98 kg/site/day x (1-0.90) x (1 - 0.00) = **0.5 kg AI/site/day**

Release Sites Information:

In order to run EFAST, various inputs about the release sites must be determined and are as follows:

- Days per year of release; the assumption used was 365 days[29].

[29] The Agency typically runs the EFAST model assuming operation for 360 days per year. For this assessment the Agency used 365 days instead of 360 day to show the high-end scenario. For example, when modeled for paper and paperboard mills at 360

- Standard Industrial Classification (SIC) code analysis or facility analysis; the SIC code "Paper and Paperboard Mills" was chosen because no specific facility was being analyzed.
- The number of use sites. The Agency estimated the exposure downstream from one site, as no data were available to determine how many use sites may be using MIT/CMIT

Pulp and Paper Mill Exposure Results:

The results of the pulp and paper exposure modeling for both listed and non-listed aquatic species are outlined in Table B5. The results indicate that concentrations of concern (COCs) are exceeded for multiple days a year especially when the maximum application rate (20 ppm) is applied, when effluent is released to low-flow streams, and when listed species are present. Potential risks to the receptor groups cannot be ruled out for any day in which COCs are exceeded.

Table B5: Aquatic Risks for Pulp and Paper Mills (Application Rates of 11 and 153 ppm)

Concentrations of concern (COC)	Application Rate: 11 ppm[1]		Application Rate: 153 ppm[2]	
	High-End	Average	High-End	Average
Non-Listed Freshwater Fish and Invertebrates				
Acute Non-Listed Fish (COC=35 µg AI/L)[3]	9	1	227	37
Acute Non-Listed Invertebrate (COC= 90 µg AI/L)[4]	1	0	110	15
Chronic Freshwater Fish and Invertebrates				
Chronic Fish (COC= 20 µg AI/L)[5]	26	3	289	57
Chronic Invertebrate (COC= 100 µg AI/L)[6]	1	0	99	13
Aquatic Plants				
Non-Listed Aquatic Plant (COC= 23 µg AI/L)[7]	20	2	275	52
Listed Freshwater Fish and Invertebrates				
Acute Listed Fish (COC=3.5 µg AI/L)[3]	187	28	362	142
Acute Listed Invertebrate (COC= 9 µg AI/L)[4]	78	10	342	92
Listed Aquatic Plant (COC= 9.9 µg AI/L)[7]	69	8	338	87

The high-end scenario is based on the10th percentile of the distribution of the ratio of 7Q10 stream flows to WWTP flows. The average case scenario is based on the median of the distribution of the ratio of 7Q10 stream flows to WWTP flows and is more typical.

1: 0.5 kg MIT/CMIT/site/day. Calculated in above

2: 6.9 kg MIT/CMIT/site/day. Calculated in above

days (Average case scenario), the number of exceedances for the COC=9.0 µg/L was 90 days which was 2 days less than 92 days for the 365 days run for the same COC.

3: Based on rainbow trout (*Oncorhynchus mykiss*) study with an LC50=70 µg AI/L. MRID 41963503.
4: Based on water flea (*Daphnia magna*) study with a 48 hr EC50= 180 µg AI/L. MRID 41718803.
5: Based on Fathead Minnow (*Pimephales promelas*) study with a NOEL= 20 µg ai/L. MRID 42012201
6: Based on water flea (*Daphnia magna*) study with a NOEL=100 µg ai/L. MRID 41963502
7: Based on a Green Alga (*Selenastrum capricornutum*) study with an EC50= 23 µg ai/L. NOEC= 9.9 µg ai/LMRID 43783201

Complete Results for Paints/Coating Use Site:

The paints and coatings use site calculations were outlined within section 4.4 of this risk assessment. Table B6 outlines the maximum square footage of MIT/CMIT painted wood (at 400 ppm AI) that could be on a waterbody d without causing risk to listed and non-listed aquatic species.

Table B6: Maximum Treated (Painted) Surface Area for use of MIT/CMIT in paint Adjacent to a Waterbody That Results in No Acute Risk

Taxonomic Group	Study Type	Minimum EEC that Results in Risk (µg AI/L) [1]	Maximum Surface Area to Result in No Risk (ft^2) [2]
Non-Listed Species			
Freshwater Fish[3]	Acute	35 µg AI/L	110,000
Freshwater Invertebrates[4]	Acute	90 µg AI/L	290,000
Freshwater Fish[5]	Chronic	20 µg AI/L	64,000
Freshwater Invertebrates[6]	Chronic	100 µg AI/L	320,000
Aquatic Plants[7]	All	23 µg AI/L	73,000
Listed Species			
Listed fish[3]	Acute	3.5 µg AI/L	11,000
Listed Invertebrates[4]	Acute	9.0 µg AI/L	29,000
Listed Aquatic Plants[7]	All	9.9 µg AI/L	31,400

1: Endpoint * level of concern (LOC). *E.g*. the endpoint for freshwater fish is 70 µg/L and the LOC for non-listed fish is ≥0.5. Thus, the minimum EEC that results in risk is 70 µg/L * 0.5 = 35 µg/L
2: See equation in the body of the assessment for details
3: Based on rainbow trout (*Oncorhynchus mykiss*) study with an LC50=70 µg AI/L. MRID 41963503.
4: Based on water flea (*Daphnia magna*) study with a 48 hr EC50= 180 µg AI/L. MRID 41718803.
5: Based on Fathead Minnow (*Pimephales promelas*) study with a NOEL= 20 µg ai/L. MRID 42012201
6: Based on water flea (*Daphnia magna*) study with a NOEL=100 µg ai/L. MRID 41963502
7: Based on a Green Alga (*Selenastrum capricornutum*) study with an EC50= 23 µg ai/L. NOEC= 9.9 µg ai/LMRID 43783201

The Agency has calculated approximate number of one-story and two-story single-family, median sized houses with MIT/CMIT preserved products that could be on a waterbody without causing risk to listed and non-listed aquatic species (Table B7).

Table B7: Maximum Number of Houses Next to a Waterbody to Result in No Risk from MIT/CMIT in Paints/Stains

Number of Stories[A, B]	Square Feet of Siding on the House[B]	# Houses Represented by 64,000 ft^2 of Siding (Non-listed Fish [chronic])[D]	# Houses Represented by 73,000 ft^2 of Siding (Non-listed Plants)[D]	# Houses Represented by 11,000 ft^2 of Siding (Listed Fish)[D]	# Houses Represented by 31,400 ft^2 of Siding (Listed Plants)[D]
1 Story	2,625 ft^2	24	28	4	12
2 Stories	3,125 ft^2	20	23	3	10

A: The Agency has assumed that each story is 10 feet tall. Therefore, the exterior wall height is either 10 feet (one story) or 20 feet (two story) and the peak has a height of 10 ft.
B: Square Feet of Siding = The surface area of the exterior walls including the peaks. (Length*Height)*2 + (Width*Height)*2 + (0.5*Base*10 ft)*2
C: The representative 1-story house is 25ft x 100 ft which results in a square footage of 2,625ft^2; the 2-story house is 25ft x 50 ft which results in a square footage of 3,125ft^2
D: The number of houses has been rounded to two significant figures

REFERENCES

USEPA. 2007. Exposure and Fate Assessment Screening Tool (E-FAST). Version 2.0. Documentation Manual. http://www.epa.gov/opptintr/exposure/pubs/efastdl.htm

U.S. EPA. 1991. Chemical Engineering Branch Manual for the Preparation of Engineering Assessments. (OPPT, CEB) February 28, 1991. https://nepis.epa.gov/Exe/ZyPDF.cgi/P10000VS.PDF?Dockey=P10000VS.PDF

APPENDIX C: DEEM Drinking Water Analysis Results for MIT/CMIT Uses

The following definitions are from the FCID-WWEIA frequently asked questions (FAQs, no date) and help to explain the food codes selected within this assessment.

The FCID Commodity tab contains three choices of water available for analysis: Water, direct, tap; water, direct bottled; and water, indirect, all sources.

Direct water is water consumed from a tap or faucet ("water, direct, tap") or from bottled water ("water, direct, bottled"). For example, drinking fountain water, tap water from restaurants, and your kitchen sink (including filtered water like Brita) are all direct water sources. Bottled water includes those bought in stores (e.g., Evian) as well as water from a water cooler (e.g., Poland Spring in your office).

Indirect water is water added by a food preparer (individual or restaurant) to make beverages or foods. For example, water added to re-constitute frozen orange juice concentrate or to make tea, coffee, infant formula, soups, and pasta, would be considered in calculation of consumption of indirect water. For example, when (dry) pasta such as spaghetti is boiled, it absorbs a certain amount water, and this water is considered indirect water when consumed, with the amount based on the difference in water content between dry (uncooked) and cooked spaghetti.
It should be noted that each DEEM run considered both direct and indirect drinking water sources.

Pulp and paper Uses

50th percentile distribution of stream flow at 0.00647 ppm

Acute Pulp and paper mill discharge-135 ppm

```
US EPA                                                        Ver. 3.18, 03-08-d
DEEM-FCID ACUTE Analysis for MIT/CMIT                      NHANES 2003-2008 2-Day
Residue file: MIT.CMIT DW. 0.00647ppm.Acute.paper.r08
Adjustment factor #2 NOT used.
Analysis Date: 02-12-2020/16:06:25    Residue file dated: 02-12-2020/13:59:06
RAC/FF intake summed over 24 hours
Run Comment: "DW 0.00647 ppm; Discharge from pulp and paper mill sytems"
================================================================================

Summary calculations--per capita:

                  95th Percentile      99th Percentile     99.9th Percentile
                  Exposure   % aRfD    Exposure   % aRfD    Exposure   % aRfD
                 ---------- --------  ---------- --------  ---------- --------
Total US Population:
                  0.000266     0.00    0.000444     0.01    0.000721     0.01
All Infants:
                  0.000180     0.00    0.000373     0.00    0.001151     0.01
Children 1-2:
                  0.000411     0.01    0.000669     0.01    0.002017     0.03
Children 3-5:
                  0.000357     0.00    0.000567     0.01    0.001044     0.01
Children 6-12:
                  0.000269     0.00    0.000478     0.01    0.000833     0.01
Youth 13-19:
                  0.000259     0.00    0.000457     0.01    0.000653     0.01
Adults 20-49:
                  0.000276     0.00    0.000443     0.01    0.000683     0.01
Adults 50-99:
                  0.000218     0.00    0.000338     0.00    0.000538     0.01
Female 13-49:
                  0.000284     0.00    0.000453     0.01    0.000693     0.01
```

50th percentile distribution of stream flow harmonic mean at 0.00258 ppm

Chronic **Pulp and paper mill discharge -153 ppm**

```
US EPA                                                          Ver. 3.16, 03-08-d
DEEM-FCID Chronic analysis for MIT/CMIT                      NHANES 2003-2008 2-day
Residue file name: C:\Users\dmcshan\Documents\MIT.CMIT DW. 0.00258ppm.Chronic.paper.r08
                                                 Adjustment factor #2 NOT used.
Analysis Date 02-12-2020/14:56:01     Residue file dated: 02-12-2020/13:56:24
Reference dose (RfD, Chronic) = .2 mg/kg bw/day
COMMENT 1: DW 0.00258 ppm ; Discharge from pulp and paper mill systems
================================================================================
                    Total exposure by population subgroup
--------------------------------------------------------------------------------
```

Population Subgroup	Total Exposure mg/kg body wt/day	Total Exposure Percent of Rfd
Total US Population	0.000034	0.0%
Hispanic	0.000033	0.0%
Non-Hisp-White	0.000035	0.0%
Non-Hisp-Black	0.000030	0.0%
Non-Hisp-Other	0.000039	0.0%
Nursing Infants	0.000009	0.0%
Non-Nursing Infants	0.000018	0.0%
Female 13+ PREG	0.000040	0.0%
Children 1-6	0.000047	0.0%
Children 7-12	0.000033	0.0%
Male 13-19	0.000029	0.0%
Female 13-19/NP	0.000033	0.0%
Male 20+	0.000031	0.0%
Female 20+/NP	0.000037	0.0%
Seniors 55+	0.000029	0.0%
All Infants	0.000015	0.0%
Female 13-50	0.000038	0.0%
Children 1-2	0.000050	0.0%
Children 3-5	0.000046	0.0%
Children 6-12	0.000035	0.0%
Youth 13-19	0.000031	0.0%
Adults 20-49	0.000037	0.0%
Adults 50-99	0.000030	0.0%
Female 13-49	0.000038	0.0%

Cooling Water Tower Uses

50th percentile distribution of stream flow at 0.54070 ppm -30Q5

Acute **Cooling tower discharge- 100K gal 20ppm**

```
US EPA                                                        Ver. 3.18, 03-08-d
DEEM-FCID ACUTE Analysis for MIT/CMIT(20 PPM)              NHANES 2003-2008 2-Day
Residue file: MIT.CMIT DW. 0.54070ppm.Acute.100KCT.r08
Adjustment factor #2 NOT used.
Analysis Date: 02-20-2020/14:15:02    Residue file dated: 02-20-2020/14:12:21
RAC/FF intake summed over 24 hours
Run Comment: "DW 0.54070ppm; Cooling tower 100K gal "
================================================================================

Summary calculations--per capita:
```

	95th Percentile Exposure	% aRfD	99th Percentile Exposure	% aRfD	99.9th Percentile Exposure	% aRfD
Total US Population:	0.029483	0.37	0.048618	0.62	0.092356	1.17
All Infants:	0.092345	1.17	0.125080	1.58	0.183320	2.32
Children 1-2:	0.045463	0.58	0.068459	0.87	0.168595	2.13
Children 3-5:	0.036890	0.47	0.056499	0.72	0.090687	1.15
Children 6-12:	0.028187	0.36	0.046258	0.59	0.071298	0.90
Youth 13-19:	0.024553	0.31	0.040440	0.51	0.061058	0.77
Adults 20-49:	0.029013	0.37	0.043176	0.55	0.062813	0.80
Adults 50-99:	0.025844	0.33	0.039187	0.50	0.061770	0.78
Female 13-49:	0.029426	0.37	0.043271	0.55	0.060685	0.77

50th percentile distribution of stream flow harmonic mean at 0.23250 ppm

Chronic Cooling tower discharge- 100K gal at 20 ppm

Analysis Date 02-20-2020/13:23:18 Residue file dated: 02-20-2020/13:22:44
Reference dose (RfD, Chronic) = .2 mg/kg bw/day
COMMENT 1: DW 0.23250 ppm; Cooling tower system 100K gal

Total exposure by population subgroup

Population Subgroup	Total Exposure mg/kg body wt/day	Total Exposure Percent of Rfd
Total US Population	0.004868	2.4%
Hispanic	0.004648	2.3%
Non-Hisp-White	0.005007	2.5%
Non-Hisp-Black	0.004017	2.0%
Non-Hisp-Other	0.005611	2.8%
Nursing Infants	0.004404	2.2%
Non-Nursing Infants	0.016192	8.1%
Female 13+ PREG	0.004584	2.3%
Children 1-6	0.006235	3.1%
Children 7-12	0.004059	2.0%
Male 13-19	0.003347	1.7%
Female 13-19/NP	0.003750	1.9%
Male 20+	0.004535	2.3%
Female 20+/NP	0.005113	2.6%
Seniors 55+	0.004707	2.4%
All Infants	0.012553	6.3%
Female 13-50	0.004845	2.4%
Children 1-2	0.007023	3.5%
Children 3-5	0.005917	3.0%
Children 6-12	0.004267	2.1%
Youth 13-19	0.003550	1.8%
Adults 20-49	0.004857	2.4%
Adults 50-99	0.004802	2.4%
Female 13-49	0.004839	2.4%

APPENDIX D: IDEEM Analysis for MIT/CMIT Inert Uses

Acute

```
US EPA                                                          Ver. 3.18, 03-08-d
DEEM-FCID ACUTE Analysis for DEEM RESIDUE FILE FOR MIT/CMIT
                                                              NHANES 2003-2008 2-Day
Residue file: INERTS_57ACTIVE_100PPBH2OREV MIT-CMIT.R08
    Adjustment factor #2 used.
Analysis Date: 02-25-2020/13:26:59    Residue file dated: 02-25-2020/13:16:05
NOEL (Acute) =  79.000000 mg/kg body-wt/day
RAC/FF intake summed over 24 hours
Run Comment: "Inert 57 active ingredients + drinking water (100ppb) "
================================================================================

Summary calculations--per capita:

---   95th Percentile----   ---   99th Percentile----     ---99.9th Percentile----
 Exposure  % aRfD     MOE    Exposure  % aRfD     MOE    Exposure  % aRfD     MOE
---------- ------- -------- ---------- ------- -------- ---------- ------- --------
Total US Population:
  0.000025    0.00>1000000    0.000047    0.00>1000000    0.000091    0.00  872389
All Infants:
  0.000053    0.00>1000000    0.000082    0.00  968967    0.000138    0.00  570669
Children 1-2:
  0.000076    0.00>1000000    0.000111    0.00  713294    0.000179    0.00  442183
Children 3-5:
  0.000052    0.00>1000000    0.000076    0.00>1000000    0.000105    0.00  755443
Children 6-12:
  0.000030    0.00>1000000    0.000043    0.00>1000000    0.000069    0.00>1000000
Youth 13-19:
  0.000016    0.00>1000000    0.000025    0.00>1000000    0.000040    0.00>1000000
Adults 20-49:
  0.000017    0.00>1000000    0.000026    0.00>1000000    0.000039    0.00>1000000
Adults 50-99:
  0.000017    0.00>1000000    0.000026    0.00>1000000    0.000042    0.00>1000000
Female 13-49:
  0.000018    0.00>1000000    0.000026    0.00>1000000    0.000039    0.00>1000000
```

Chronic

```
US EPA                                                          Ver. 3.16, 03-08-d
DEEM-FCID Chronic analysis for DEEM RESIDUE FILE FOR MIT/CMIT
                                                          NHANES 2003-2008 2-day
Residue file name: C:\Users\dlieu\Desktop\INERTS_57ACTIVE_100PPBH2OREV MIT-CMIT.R08
                                                   Adjustment factor #2 used.
Analysis Date 02-25-2020/13:17:15     Residue file dated: 02-25-2020/13:16:05
Reference dose (RfD, Chronic) = .2 mg/kg bw/day
COMMENT 1: Inert 57 active ingredients + drinking water (100ppb)
================================================================================
                    Total exposure by population subgroup
--------------------------------------------------------------------------------
```

Population Subgroup	Total Exposure mg/kg body wt/day	Total Exposure Percent of Rfd
Total US Population	0.000008	0.0%
Hispanic	0.000009	0.0%
Non-Hisp-White	0.000008	0.0%
Non-Hisp-Black	0.000008	0.0%
Non-Hisp-Other	0.000010	0.0%
Nursing Infants	0.000011	0.0%
Non-Nursing Infants	0.000020	0.0%
Female 13+ PREG	0.000007	0.0%
Children 1-6	0.000024	0.0%
Children 7-12	0.000010	0.0%
Male 13-19	0.000006	0.0%
Female 13-19/NP	0.000006	0.0%
Male 20+	0.000006	0.0%
Female 20+/NP	0.000007	0.0%
Seniors 55+	0.000007	0.0%
All Infants	0.000017	0.0%
Female 13-50	0.000006	0.0%
Children 1-2	0.000031	0.0%
Children 3-5	0.000021	0.0%
Children 6-12	0.000011	0.0%
Youth 13-19	0.000006	0.0%
Adults 20-49	0.000006	0.0%
Adults 50-99	0.000007	0.0%
Female 13-49	0.000006	0.0%

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: **April 6, 2020**

SUBJECT: **Hazard Characterization of Isothiazolinones in Support of FIFRA Registration Review.**

PC Codes: 098951, 128101, 098901, 107104, 107103, 099901	DP Barcode: 456952
	Decision Number: 558144
Regulatory Action: Registration Review	Case No.: 5023, 3092, 3026, 5017, 2475
Risk Assessment Type: Hazard Assessment	CAS No.: 4299-07-4, 2634-33-5, 55965-84-9, 64359-81-5, 2682-20-4, 26530-20-1

TO: Stephen Savage, Chemical Review Manager
Rick Fehir, Ph.D., Team Lead
Rose Kyprianou, Branch Chief
Regulatory Management Branch II
Antimicrobial Division (7510P)

FROM: Timothy McMahon, Ph.D., Senior Toxicologist
Risk Assessment and Science Support Branch
Antimicrobial Division (7510P)

AND

Anna Lowit, Ph.D., Senior Science Advisor
Immediate Office
Office of Pesticide Programs

THROUGH: Melissa Panger, Ph.D., Branch Chief
Laura Parsons, Associate Branch Chief
Judy Facey, Ph.D., Human Health Risk Assessment Process Leader
Timothy Leighton, Senior Human Health Scientist
Risk Assessment and Science Support Branch
Antimicrobial Division (7510P)
Office of Pesticide Programs

This document provides the hazard characterization assessment for the individual isothiazolinones (BBIT, BIT, OIT, DCOIT, MIT and CMIT) risk assessments.

HAZARD CHARACTERIZATION OF ISOTHIAZOLINONES IN SUPPORT OF FIFRA REGISTRATION REVIEW

April 6, 2020

US Environmental Protection Agency, Office of Pesticide Programs, Antimicrobials Division

In Collaboration with the National Toxicology Program's Interagency Coordinating Committee for the Evaluation of Alternative Toxicological Methods

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Preface

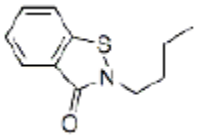
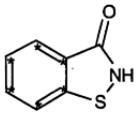
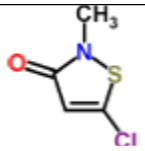
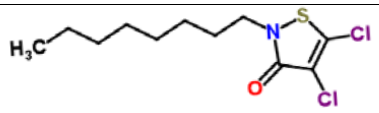
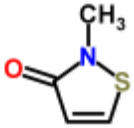
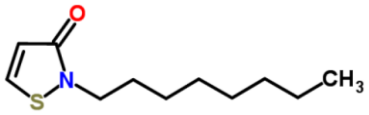
This chapter presents the human health hazard characterization for the isothiazolinone biocides, a class of chemicals registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as antimicrobial pesticides (biocides). The class consists of six pesticidal active ingredients in this chemical family: N-butyl-1,2-benzisothiazolin-3-one **(**BBIT), 1,2-benzisothiazolin-3-one (BIT), 2-n-octyl-4-isothiazolin-3-one (OIT), 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT), 2-methyl-4-isothiazoline-3-one (MIT) and 5-chloro-2-methyl-4-isothiazoline-3-one (CMIT). There are a wide variety of use patterns for this class of chemicals, including industrial process and water systems, wood preservatives, and materials preservatives in consumer goods such as paints, clothing, plastics, household cleaning products, laundry detergents, etc. Specific use patterns for these chemicals have been previously published in EPA dockets for the individual chemicals of this class listed in Table 1. It has been previously determined by the Office of Pesticide Programs (OPP) that the effects of the isothiazolinone biocides are similar among members of the class, and include effects related to the irritant properties of the chemicals, such as hyperplasia/hyperkeratosis of the squamous mucosa of the forestomach from oral exposure; erythema and desquamation of the skin from dermal exposure; and inflammation/squamous metaplasia of the nasal cavity from inhalation exposure. Isothiazolinones do not present a mutagenic or carcinogenic concern based on the available data. Developmental and reproductive toxicities are not observed with these chemicals. The available isothiazolinone databases indicate that they are not neurotoxic.

Among the adverse effects noted from exposure to isothiazolinones is the potential for skin sensitization. All the isothiazolinones are positive skin sensitizers. Use of isothiazolinones as materials preservatives presents a concern for skin sensitization potential, as the products containing these chemicals do not bear pesticide labels and therefore do not communicate potential skin sensitization hazard to consumers. Therefore, the Agency is using a quantitative approach to assess potential skin sensitization for the isothiazolinones by identifying induction and/or elicitation skin sensitization thresholds for each of the isothiazolinones. These threshold values are used to characterize risk from dermal exposure. This approach is consistent with the Agency's previous approaches for assessing skin sensitization potential for materials preservatives (EPA-HQ-OPP-2004-0099). The assessment for isothiazolinones uses these same principles and extends this approach through the use of *in vitro* and *in chemico* assays and neural network-based defined approaches (DAs) for quantitative assessment of dermal sensitization. The use of *in vitro* and *in chemico* assays and neural network-based defined approaches (DAs) is the first use of such information in regulatory risk assessment and is described in further detail in this chapter.

1. Introduction

The isothiazolinone biocides are a class of chemicals commonly formulated as antimicrobial pesticide products to be used as material preservatives to control of bacteria, fungi, and/or algae. These pesticide products can be used in/on countertops/utensils (food use), pulp and paper (food packaging), vinyl flooring, household cleaning products, laundry detergent, metalworking fluids, paint (in-can preservative and antifoulant paint for ship hulls), plastics, textiles/carpets and wood (pressure treatment). Table 1 provides the chemical names, docket numbers, CAS numbers, and structures of the isothiazolinone biocides evaluated in this document.

Table 1. Chemical names, CAS numbers, Structures, and Docket Numbers of the Isothiazolinone Biocide Class

Common Name	Chemical Name	CAS #	Structure
BBIT	1,2-benzisothiazolin-3-one, 2-butyl EPA-HQ-OPP-2015-0736	4299-07-4	
BIT	1,2-Benzisothiazolin-3-one EPA-HQ-OPP-2014-0159	2634-33-5	
CMIT/MIT	Mixture (structure provided for CMIT) EPA-HQ-OPP-2013-0605	55965-84-9	
DCOIT	4,5-Dichloro-2-octyl-3(2h)-isothiazolone EPA-HQ-OPP-2014-0403	64359-81-5	
MIT	2-Methyl-4-isothiazolin-3-one EPA-HQ-OPP-2013-0605	2682-20-4	
OIT	2-n-Octyl-4-isothiazolin-3-one EPA-HQ-OPP-2014-0160	26530-20-1	

For purposes of hazard identification, the isothiazolinone biocides are being evaluated together as they share structural and toxicological characteristics. In addition, as noted in the Final Workplans (FWPs) under registration review, the toxicological databases for some individual

isothiazolinone biocides are incomplete and bridging of toxicology data is being conducted for some risk assessment scenarios. As such, no additional toxicology studies are being required for the isothiazolinone biocides. The Agency concluded that bridging across the isothiazolinone biocides was appropriate, although chemical-specific data are preferred. The isothiazolinones are known irritants and dermal sensitizers, and dermal, oral and inhalation points of departure are needed for risk assessment purposes.

For characterization of skin sensitization hazard, the Agency initially reviewed repeat open application test (ROAT) studies conducted in humans for MIT and CMIT/MIT. These studies, which measured skin concentrations of MIT and CMIT/MIT that caused elicitation reactions (reactions in people already sensitized to these chemicals), were reviewed by the Human Studies Review Board (HSRB) from the perspectives of ethical and scientific conduct and the HSRB agreed with the Agency's conclusions on their use in risk assessment. However, these data could not be used to characterize skin sensitization potential of the other members of the isothiazolinone class. Therefore, data gaps exist for assessment of skin sensitization potential for some of the isothiazolinones.

The approach described in this chapter for quantitative assessment of skin sensitization for the

isothiazolinone chemicals uses data generated from *in vitro* and *in chemico* assays on each of the isothiazolinone chemicals. The results of these assays have been used to derive concentrations of the isothiazolinone chemicals that can cause induction of skin sensitization (concentrations that can cause sensitization in persons not previously exposed). The skin sensitization section of this hazard characterization was developed in collaboration with the National Toxicology Program's (NTP) Interagency Coordinating Committee for the Evaluation of Alternative Toxicological Methods (ICCVAM) and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

The risk assessment scenarios considered for the isothiazolinones are summarized in Table 2 below. The routes and durations of exposure are also summarized.

Table 2. Exposure Scenarios Relevant for the Risk Assessment of Isothiazolinones

Chemical	Exposure Scenario	Exposure Route				Duration		
		Diet, Indirect	IO	Dermal	IH	ST	IT	LT
BIT	Open Pour Liquid			x	x	x	x	
	Painting, Preserved Paints			x	x	x	x	
	MWF			x	x	x	x	x
	Mop/wipe/spray			x	x	x	x	
	Countertops, pulp & paper, DW, utensils	x				A		x
	Kids PA (textile, carpet, plastic)		x	x		x	x	
DCOIT	Open pour liquid			x	x	x	x	
	Painting, Preserved Paints			x	x	x	x	
	Painting, Antifoulant Paint			x	x	x	x	

Chemical	Exposure Scenario	Exposure Route				Duration		
		Diet, Indirect	IO	Dermal	IH	ST	IT	LT
	Wood treatment (PT[2])		x	x	x	x	x	x
	Countertops, DW	x				A		x
MIT/ CMIT	Open pour liquid			x	x	x	x	
	Painting, Preserved Paints			x	x	x	x	
	Mop/wipe/spray			x	x	x	x	
	MWF			x	x	x	x	x
	Wood treatment (PT)		x	x	x	x	x	x
	Countertops, pulp &paper, No DW	x				A		x
	Kids PA (floors, carpets, PT wood)		x	x		x	x	
	Postapplication (paint vapor)				x	x		
OIT	Open pour liquid			x	x	x	x	
	Painting, Preserved Paints			x	x	x	x	
	Wood treatments (PT and sap)			x	x	x	x	x
	MWF			x	x	x	x	x
	Mop/wipe/spray			x	x	x	x	
	Countertops, pulp &paper, DW, utensils	x				A		x
	Kids PA (carpet, flooring, textiles, plastics)		x	x		x	x	
BBIT	Liquid pour			x	x	x	x	
	Kids postapplication (floor coverings)		x	x		x	x	
	MWF			x	x	x	x	x

ST= short term; IT= intermediate term; LT=long term, IO = incidental oral, IH = inhalation; A= Acute; PT= pressure-treated; DW= drinking water

2. Toxicological Effects of Isothiazolinone Biocides

2.1 Overall Summary

In general, the isothiazolinone biocides are reactive chemicals and as such, cause point of contact adverse effects such as irritation or corrosion of the skin and eyes, irritation of the respiratory tract, and irritation-type responses of the gastrointestinal tract (Appendix A). All of the isothiazolinone biocides are Category I (corrosive) for eye irritation. Similarly, the isothiazolinone biocides are Category I (corrosive) for skin irritation with the exception of BIT which is Category IV. All the isothiazolinones are known to cause allergic contact dermatitis (dermal sensitization).

In repeat dosing studies with the isothiazolinone biocides, evidence of irritation, such as lesions of the glandular stomach and skin, are observed as effects across the class of chemicals. Decreases in body weight across multiple species and emesis in dogs are also common adverse findings throughout the available toxicology studies for these chemicals. Although their

toxicological effects are qualitatively consistent, the isothiazolinone biocides differ in potency with No Observed Adverse Effect Levels/Lowest Observed Adverse Effect Levels (NOAELs/LOAELs) varying across the group. For example, histopathological lesions of the stomach are observed from repeated oral dosing of CMIT/MIT at a dose of 22/28 mg/kg/day (MRID 44656101), whereas these same lesions are observed at an oral dose of 96-120 mg/kg/day for OIT (MRID 47815801). The effects of the isothiazolinone biocides are similar among members of the class, and include effects related to the irritant properties of the chemicals, such as hyperplasia/hyperkeratosis of the squamous mucosa of the forestomach from oral exposure; erythema and desquamation of the skin from dermal exposure; and inflammation/squamous metaplasia of the nasal cavity from inhalation exposure.

Isothiazolinones are also positive dermal sensitizers. Isothiazolinones do not present a mutagenic or carcinogenic concern. Based on examination of the data, carcinogenicity studies are not required because 1) available cancer studies for the isothiazolinone biocides are negative; 2) there is a lack of mutagenicity concern for the isothiazolinone biocides; 3) isothiazolinones are irritants following oral, dermal and inhalation exposures and produce similar effects following subchronic exposures; 4) the isothiazolinones as a group have a known mode of action for antimicrobial activity, and; 5) irritation is the predominant effect and is the basis of the PODs (see also HASPOC, 2007 and TXR 0056210). Developmental and reproductive toxicity is also not observed with these chemicals.

2.2 Dermal Route of Exposure: Skin Sensitization

Dermal contact is a major source of human exposure to the isothiazolinone biocides, and skin sensitization is known to occur from dermal exposure to these biocides (Cosmetic Ingredient Review (CIR), 2014; Basketter *et al*., 1999; Aerts *et al*., 2017). Some worker exposure scenarios for isothiazolinone biocides require personal protective equipment such as gloves and respiratory protection. However, isothiazolinone biocides are commonly used as materials preservatives in EPA-regulated products that do not bear pesticide labels and for which personal protective equipment (PPE) cannot be required (*i.e.*, treated articles). For one widely used member of the isothiazolinone biocide class (MIT), the use of this chemical as a materials preservative has resulted in increasing incidences of contact allergy reported to be associated with exposures to MIT (CIR, 2014). All the isothiazolinones are known to be positive dermal sensitizers. An approach to quantifying risk from exposure to products containing dermal sensitizing pesticide chemicals that do not bear labels was developed by EPA for assessment of risk from exposure to treated wood (USEPA, 2004). For the isothiazolinone biocides, EPA is also using a quantitative approach to assess the risk to isothiazolinone biocides for skin sensitization. For this approach, each registered member of the class has been tested using the Organization for Economic Co-operation and Development (OECD) *in vitro* and *in chemico* skin sensitization assays and most (except BBIT) have been tested using the local lymph node assay (LLNA) for purposes of quantitative estimation of skin sensitization potential.

2.2.1. Adverse Outcome Pathway

The adverse outcome pathway (AOP) for skin sensitization is described in the OECD document entitled "The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins" (OECD 2012a; OECD 2012b). The AOP for skin sensitization (Figure 1) is initiated by key event 1 (KE1), which is followed sequentially by three KEs with well-accepted biological significance: (KE2) keratinocyte activation, (KE3) dendritic cell activation, and (KE4) proliferation of antigen-specific T cells.

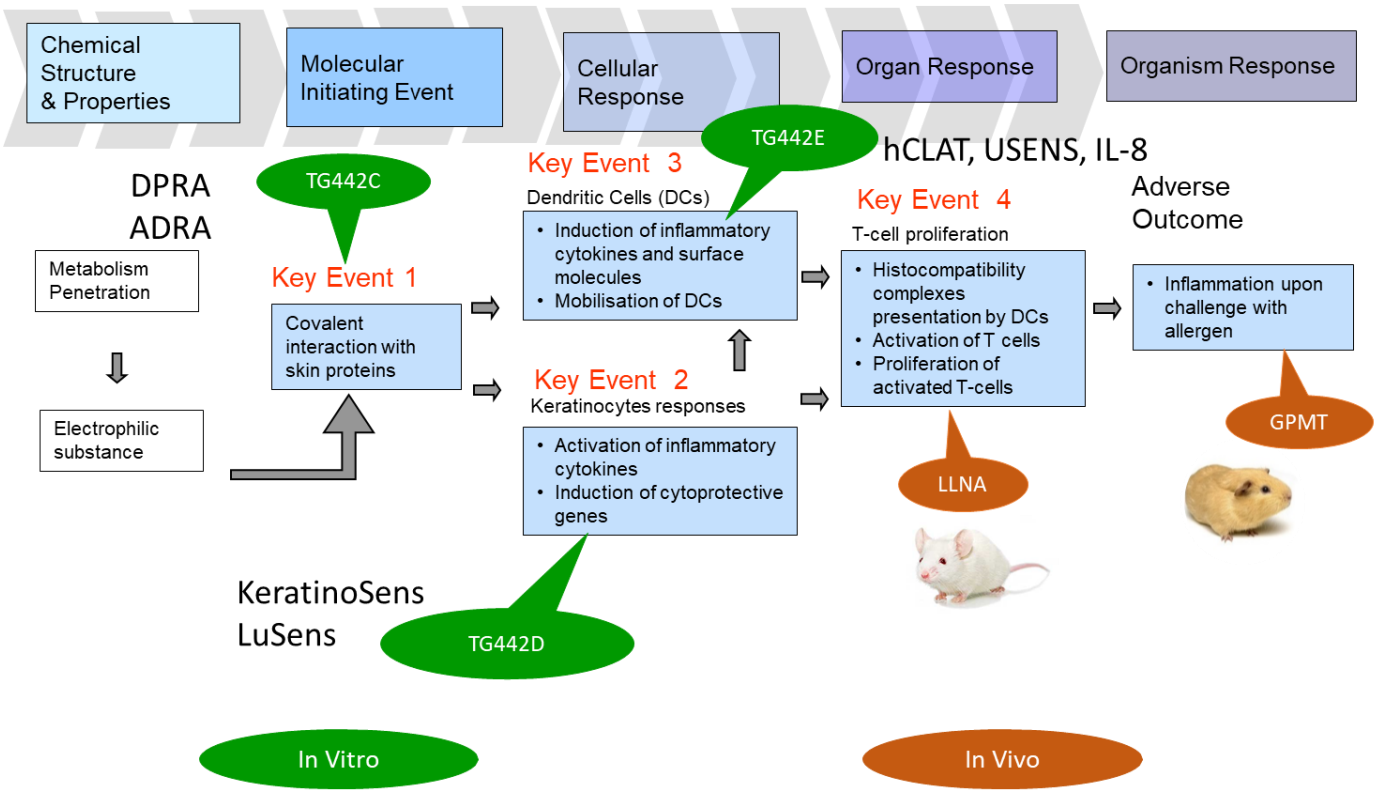


Figure 1. The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins (Adapted from Strickland et al. 2018)

2.2.2. Methods

Several non-animal methods with internationally recognized test guidelines adopted by OECD member countries assess the ability of chemicals to activate the first three KEs (OECD 2015a; OECD 2015b; OECD 2017). Examples of these methods are shown in Figure 1 and are detailed below. There are currently no validated non-animal methods that assess the ability of substances

to activate KE4, the proliferation of activated T cells. Burleson Research Technologies, Inc., the NTP contract laboratory for immunotoxicity testing, tested the six isothiazolinone compounds using direct peptide reactivity assay (DPRA), KeratinoSens, and human Cell Line Activation Test (h-CLAT). None of these methods are currently accepted as stand-alone replacements for the animal methods (OECD 2019; OECD 2018; OECD 2018b). Instead, information from these methods need to be integrated into a defined approach (DA).

- OECD TG 442C covers assays that assess the ability of a substance to form a hapten–protein complex, *i.e*., the molecular initiating event, KE1 (Figure 1) (OECD 2019). The direct peptide reactivity assay (DPRA) is an example of an *in chemico* test that maps to KE1. Average cysteine and lysine depletion > 6.38% indicate a sensitizer outcome. If the lysine peptide co-elutes with the test chemical, peptide reactivity can be assessed using cysteine depletion only. In that case, a sensitizer outcome is indicated when cysteine depletion is >13.89%. The measurement endpoints provided by the DPRA are: cysteine peptide depletion (Cys), lysine peptide depletion (Lys), average depletion of cysteine and lysine peptides (Avg.Lys.Cys). For this evaluation Avg.LysCys was used.
- OECD TG 442D covers assays that assess the ability of substances to activate cytokines and induce cytoprotective genes in keratinocytes, KE2 (OECD 2018). The KeratinoSens™ and the LuSens are ARE-Nrf2 luciferase test methods that map to KE2. The KeratinoSens test method was used for this risk assessment. A sensitizer outcome is indicated when luciferase induction is statistically significant and at least 1.5-fold higher than control values at a concentration with cell viability > 70%. The KeratinoSens assay provides the effective concentration at 1.5-fold luciferase induction (EC1.5), the effective concentration at 3-fold induction (EC3), the maximum induction (Imax) and the inhibitory concentration at 50% viability (IC50). The Imax was used for this evaluation.
- OECD TG 442E covers assays addressing activation of dendritic cells, KE3. The human Cell Line Activation Test (h-CLAT), Interleukin-8 Reporter Gene Assay (IL8-Luc) and Myeloid U937 Skin Sensitization Test (U-SENS™) all assess the ability of substances to activate and mobilize dendritic cells in the skin, KE3. (OECD 2018b). The h-CLAT was for used for this assessment. This test measures the induction of two cell surface markers, CD86 and CD54, which indicate dendritic cell activation. A cytotoxicity assay to determine 75% cell viability (CV75) is used to select the doses to be tested. The measurement endpoints for the h-CLAT include the effective concentration at 150% induction for the CD86 marker (EC150) and the effective concentration at 200% induction for the CD54 marker (EC200). A sensitizer outcome is indicated when CD86 expression is at least 150% or CD54 expression is at least 200% with cell viability > 50%. All the DAs applied here used the minimum induction threshold (MIT) from the CD86 and CD54 measurements. The MIT is the lower value of these two measurements.

Historically, skin sensitization testing has been accomplished using the murine local lymph node assay (LLNA), the guinea pig maximization test (GPMT), and the Buehler test (OECD, 1992; 2010a). Multiple, validated non-animal tests (EURL ECVAM Scientific Advisory Committee,

2016a; b; Joint Research Centre of the European Union, 2013; 2014; 2015) are available that are mechanistically associated with key events in the AOP as shown in the illustration above (Figure 1). However, at the present time, none of the internationally recognized test guidelines adopted by the OECD (OECD, 2015a; b; 2017a) are recommended as a stand-alone replacement for the animal tests. As such, international efforts have focused on the development of integrated strategies, referred to as defined approaches (DAs; OECD, 2016b), that use multiple testing (*in vitro* and *in chemico*) and non-testing (*in silico*) information sources.

The OECD makes a distinction between integrated approaches to testing and assessment (IATA) and DAs. As defined by the OECD, IATAs are defined as pragmatic, science-based approaches for chemical hazard or risk assessment that rely on an integrated analysis of existing information coupled with the generation of new information using testing strategies. IATAs follow an iterative approach to answer a defined question in a specific regulatory context, taking into account the acceptable level of uncertainty associated with the decision context (OECD 2017b). The overall assessment process within IATA is based on weight-of-evidence, which involves expert judgment in the weighing of the different pieces of information. Non-animal approaches based on a fixed set of information sources and fixed data interpretation procedure are designated as "defined approaches to testing and assessment" (OECD 2016a). The DA designation emphasizes that predictions generated by these approaches are rule-based and are not influenced by expert judgment. Moreover, the fixed nature of DAs are expected to facilitate their consideration under the OECD mutual acceptance of data (MAD), in contrast to IATAs which can be flexible and adaptable to particular regional requirements or regulatory statutes. The OECD has published two guidance documents (GD) (GD 255 and GD 256, OECD 2016a, b, c) on the harmonized reporting of DAs.

To promote regulatory acceptance of integrated non-animal approaches to skin sensitization assessment, the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) convened the International Cooperation on Alternative Test Methods (ICATM) Workshop on the International Regulatory Applicability and Acceptance of Alternative Non-animal Approaches to Skin Sensitization Assessment of Chemicals on October 4-5, 2016, in Ispra, Italy. The workshop was attended by 36 experts representing international regulatory authorities from 14 countries to facilitate a common understanding of the available non-animal methods (i.e., *in vitro*, *in chemico*, *in silico* and read-across) and their role within DAs. Workshop participants reviewed the performance of multiple non-animal integrated strategies for skin sensitization hazard assessment. Follow-up activities from the ICATM workshop have led to publications (Strickland *et al*., 2018; Casati *et al*. 2017, Daniel *et al*., 2018) and an OECD project to develop test guidelines for skin sensitization DAs which is being co-led the US, EU, and Canada. The work to development of OECD test guidelines is on-going.

Annex I of OECD GD 256 contains twelve case studies submitted by international stakeholders, covering various DAs and IATA for skin sensitization (OECD 2016c). The National Toxicology Program's (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) has worked collaboratively with Cosmetics Europe to evaluate these DAs for skin sensitization. The effort produced a database of highly curated animal and non-animal test data

for 128 chemicals (Hoffman *et al*., 2018) and a scientific evaluation of multiple DAs (Kleinstreuer *et al*., 2018). The evaluation was conducted in two phases and considered a variety of data interpretation procedures, ranging from simple (*e.g*., decision trees) to complex (*e.g*., machine learning algorithms). In the first phase, six qualitative evaluation categories were used: characteristics (*e.g*., purpose of the approach); input data (*e.g*., *in vitro*, *in chemico*, *in silico* and expert systems); prediction algorithm; mechanistic relevance with respect to the OECD AOP and the relevant key event(s); applicability domain; and practical aspects (*e.g*., relative cost and availability through contract research organizations, CROs). In the second phase, six of the twelve DAs were quantitatively assessed for their ability to predict skin sensitization. All six DAs evaluated for performance demonstrate comparable or superior performance to the LLNA (Kleinstreuer *et al*., 2018). Among these six was the artificial neural network (ANN) model developed by Shiseido (Hirota *et al*., 2015) which is unique in its ability to estimate LLNA EC3[1] values.

In April of 2018, EPA released a draft Science Policy to reduce animal use in testing through the use of two relatively simple DAs to identify potential skin sensitizers (EPA-HQ-OPP-2016-0093-0090). This draft Science Policy was developed in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). Under this policy, both OPP and OPPT accepted submissions for single chemicals (e.g., pesticide active ingredients or pesticide inert ingredients) that can be tested using the defined approach methods described in the policy, which included the DPRA, h-CLAT, and Keratinosens assays.

Since that time, EPA's has been collaborating with industry, NTP's Toxicology Branch, and NICEATM to use the isothiazolinone class of material preservatives as a case study for considering the regulatory relevance of the ANN-EC3 DA specifically for purposes of deriving quantitative points of departure. This work has resulted in the draft NTP report Application of Non-animal Test Methods and Defined Approaches to Skin Sensitization Assessment of Isothiazolinone Compounds (DHHS, 2019). Draft versions of this report were reviewed by the ICCVAM Skin Sensitization Expert Group (SSEG) and edited based on the comments from the ICCVAM experts. As described in detail below, EPA determined that the *in vitro* and *in chemico* studies provide information that is more reliable, reproducible and human-relevant than the LLNA. Therefore, EPA has used the results of the ANN-EC3 DA to derive EC3 values to extrapolate dermal risk for the currently registered isothiazolinones as part of registration review. This use of the *in vitro* and *in chemico* assays and the artificial neural network-based DA are the first use of such information in regulatory risk assessment.

[1] EC3: effective chemical concentration required for a stimulation index of 3 in proliferation of lymph node cells

2.2.3 Results

2.2.3.1 *In Vivo* Data

LLNA data were obtained from two major sources: a report submitted to EPA from Dow Chemical Company (Begolly, 2019; MRID 50790801) and from publicly available scientific literature. No LLNA studies were available for BBIT. The LLNA data were evaluated to determine a representative effective concentration from the Dow data alone and one from the NICEATM evaluation at a stimulation index (SI) of three (EC3), the threshold for a positive response, to represent the *in vivo* potency of each substance.

The Dow report included two to four studies for each of five substances, totaling 17 LLNA studies. Dow determined a representative EC3 for each substance by selecting the tests that were performed using acetone or acetone:olive oil as the solvent. Two EC3 values, 0.20% and 0.25% were calculated for OIT as there were two studies for this chemical.

The NICEATM approach used the 17 studies provided by Dow and 15 studies from the scientific literature to determine a representative EC3 for each substance. A total of 32 studies were available with three to 13 studies for each of the other five substances. One MIT test with EC3 = 1.9% from Gerberick *et al.* (2005) was excluded because it was the same test reported by Basketter *et al*. (2003); it had the same stimulation index values with erroneous test concentrations and EC3 value (Roberts 2013). The remaining individual LLNA tests were evaluated for inclusion in determining a single representative mean EC3 using the approach designed by the OECD Expert Group for Defined Approaches for Skin Sensitization. The NICEATM evaluation rejected 10 studies because they did not meet the criteria described in the NTP report (DHHS, 2019). Two to nine studies were then available for each of the five substances with LLNA studies. A representative EC3 for each substance was calculated by determining the mean EC3 for each substance as shown in Table 3 below.

Table 3. Representative LLNA EC3 Values

Chemical	Dow LLNA EC3 (%)[a]	NICEATM LLNA EC3 (%)[b]	Number of Studies for NICEATM LLNA EC3
DCOIT	0.004	0.008 (0-0.053)	2
CMIT/MIT	0.002	0.018 (0.0011-0.034)	9
OIT	0.2-0.25 (n=2)	0.361 (0.029-0.69)	4
MIT	0.863	1.154 (0-3.476)	3[b]
BIT	1.54	10.57 (0-23.36)	7

Chemical	Dow LLNA EC3 (%)[a]	NICEATM LLNA EC3 (%)[b]	Number of Studies for NICEATM LLNA EC3
BBIT	NA	NA	0

[a]Dow criteria for inclusion of the LLNA study for analysis included the following: studies that used the same vehicle [acetone:olive oil]; studies that used the same strain of mouse.

[b] NICEATM criteria for inclusion of the LLNA study in the analysis included the following: the test substance must be applied topically to both ears of the mice; lymphocyte proliferation must be measured in the lymph nodes draining the site of test substance application; lymphocyte proliferation must be measured during the induction phase of skin sensitization; a vehicle control must be included; either individual or pooled animal data were collected; concentrations tested and corresponding SI values are available. The numbers in parentheses represent the range of EC3 values (%) in the studies.

2.2.3.2 *In Vitro/In Silico*

The hazard classification results for each of the *in vitro* non-animal test methods (DPRA, KeratinoSens, h-CLAT) and for the *in silico* read-across were the same for each of the six IT compounds and are shown below in Table 4 **.** All six compounds were classified as sensitizers. With the exception of BBIT, which had no LLNA data, the hazard classification of the DAs was concordant with that of the LLNA. The potency classification of 1A (high frequency of occurrence in humans and/or a high potency in animals and can be presumed to potentially produce significant sensitization in humans**)** for all compounds was concordant across the DAs and with the LLNA data, except for the NICEATM LLNA for BIT, which yielded a 1B classification (low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals and can be presumed to potentially produce significant sensitization in humans**)**, and BBIT, which had no LLNA data.

Table 4. Potency Classification Prediction for Isothiazolinones

Chemical	Dow LLNA	NICEATM LLNA	DA: ANN D_hC[a] Potency	DA: ANN D_hC_KS[b] Potency
DCOIT	1A	1A	1A	1A
CMIT/MIT	1A	1A	1A	1A
OIT	1A	1A	1A	1A
MIT	1A	1A	1A	1A
BIT	1A	1B	1A	1A
BBIT	NA	NA	1A	1A

[a] Model 1 from Hirota et al. 2015: DPRA + h-CLAT

[b] Model 4 from Hirota et al. 2015: DPRA + h-CLAT + KeratinoSens

2.2.4 Defined Approaches

Per OECD Guidance Document 256 (OECD GD 256), "a defined approach consists of a fixed data interpretation procedure (DIP) (*e.g.*, statistical, mathematical models) applied to data (*e.g.*, *in silico* predictions, *in chemico*, *in vitro* data) generated with a defined set of information sources to derive a prediction. In contrast to the assessment process within Integrated Approaches to Testing and Assessment (IATA), that necessarily involves some degree of expert judgment, predictions generated with defined approaches are rule-based and can either be used on their own if they are deemed fit-for-purpose or considered together with other sources of information in the context of IATA." (OECD 2016a). A defined approach (DA) should contain the following: defined endpoint, defined purpose, description of the underlying rationale, description of the individual information sources used, description of how data from the individual information sources are processed, and consideration of the known uncertainties.

Annex I of OECD GD 256 contains twelve case studies submitted by international stakeholders, covering various DAs and IATA for skin sensitization (OECD 2016c). NICEATM and Cosmetics Europe collaborated to evaluate various technical and practical aspects, along with predictive performance, of these proposed alternative approaches (Hoffman *et al*., 2018; Kleinstreuer *et al*., 2018). The Shiseido artificial neural network (ANN) model was one of the DAs evaluated and determined to be robust for estimating EC3 values. The ANN-EC3 approaches are non-linear statistical models that combine multiple *in vitro* parameters covering various Key Events of the skin sensitization AOP and predicts the LLNA EC3 as an output. Two of the four Shiseido ANN models described in Hirota *et al*. (2015) were evaluated here and chosen based on availability of the input data and published performance of the models. The first model (ANN D hC, "model 1" in Hirota *et al*. 2015) used quantitative values from the DPRA (Avg.Lys.Cys) and the h-CLAT (MIT) to predict the EC3 value that would be produced in the LLNA. The second model (ANN D hC KS, "model 4" in Hirota *et al*. 2015) used the same structure with an additional value from the KeratinoSens (Imax) used as the third input. The ANN DAs were coded in R, and logistic activation functions were used for the hidden and output layers, 10,000 iterations were used for training, and learning rate, scaling functions, and momentum parameters were inferred from Hirota *et al*. 2015. For each IT compound, each model was run 100 times and mean EC3 prediction and 95% confidence intervals were calculated.

The quantitative EC3 predictions derived from the ANN DAs were similar to the LLNA EC3 values, with overlapping 95% confidence intervals (CI) in most cases, with the exception of CMIT/MIT, where the upper bound of the *in vivo* CI was 3.5-fold less than the lower bound of the *in silico* CI (for the ANN D_hC DA; Figure 2). There are several possible reasons for the CMIT/MIT result, including results that may be confounded by the testing of a mixture, possible unknown components present in addition to the active ingredients, and comparison to the *in vivo* test results that may have tested the CMIT/MIT mixture that had a different composition.

While the *in vivo* and *in silico* CIs for BIT did overlap, the average EC3 predictions derived from the DAs were closer to the *in vivo* estimate provided by Dow than that calculated by NICEATM

(as illustrated in Table 5 and Figure 2). The largest discrepancy between the two ANN DAs was seen for the CMIT/MIT mixture, with a 4-fold difference between the average EC3 predictions.

Table 5. Quantitative EC3 Prediction for Isothiazolinones (Extracted from Table 7 of the NTP/NICEATM Report)

Chemical	Dow LLNA EC3 (%)	NICEATM LLNA EC3 (%)[a]	DA: ANN D_hC[b] EC3 (%)[a]	DA: ANN D_hC_KS[c] EC3 (%)[a]
DCOIT	0.004	0.008 (0-0.053)	0.0566 (0.0555 – 0.0578)	0.023 (0.02 – 0.026)
CMIT/MIT	0.002	0.018 (0.0011-0.034)	0.121 (0.119 – 0.123)	0.492 (0.4 – 0.605)
OIT	0.2-0.25	0.361 (0.029-0.69)	0.0569 (0.0559 – 0.058)	0.015 (0.013 – 0.017)
MIT	0.863	1.154 (0-3.476)	1.775 (1.732 – 1.818)	0.826 (0.759 – 0.9)
BIT	1.54	10.57 (0-23.36)	0.934 (0.909 – 0.959)	0.341 (0.317 – 0.367)
BBIT	NA	NA	0.148 (0.146 – 0.151)	0.061 (0.055 - 0.068)

[a] Numbers in parentheses are the 95% confidence limits

[b] Model 1 from Hirota *et al*., 2015: DPRA + h-CLAT

[c] Model 4 from Hirota *et al*., 2015: DPRA + h-CLAT + KeratinoSens

Note: To convert the EC3 (%) into loading in units of ug/cm^2 = [EC3 x 25uL x 10 ug/uL]/cm^2

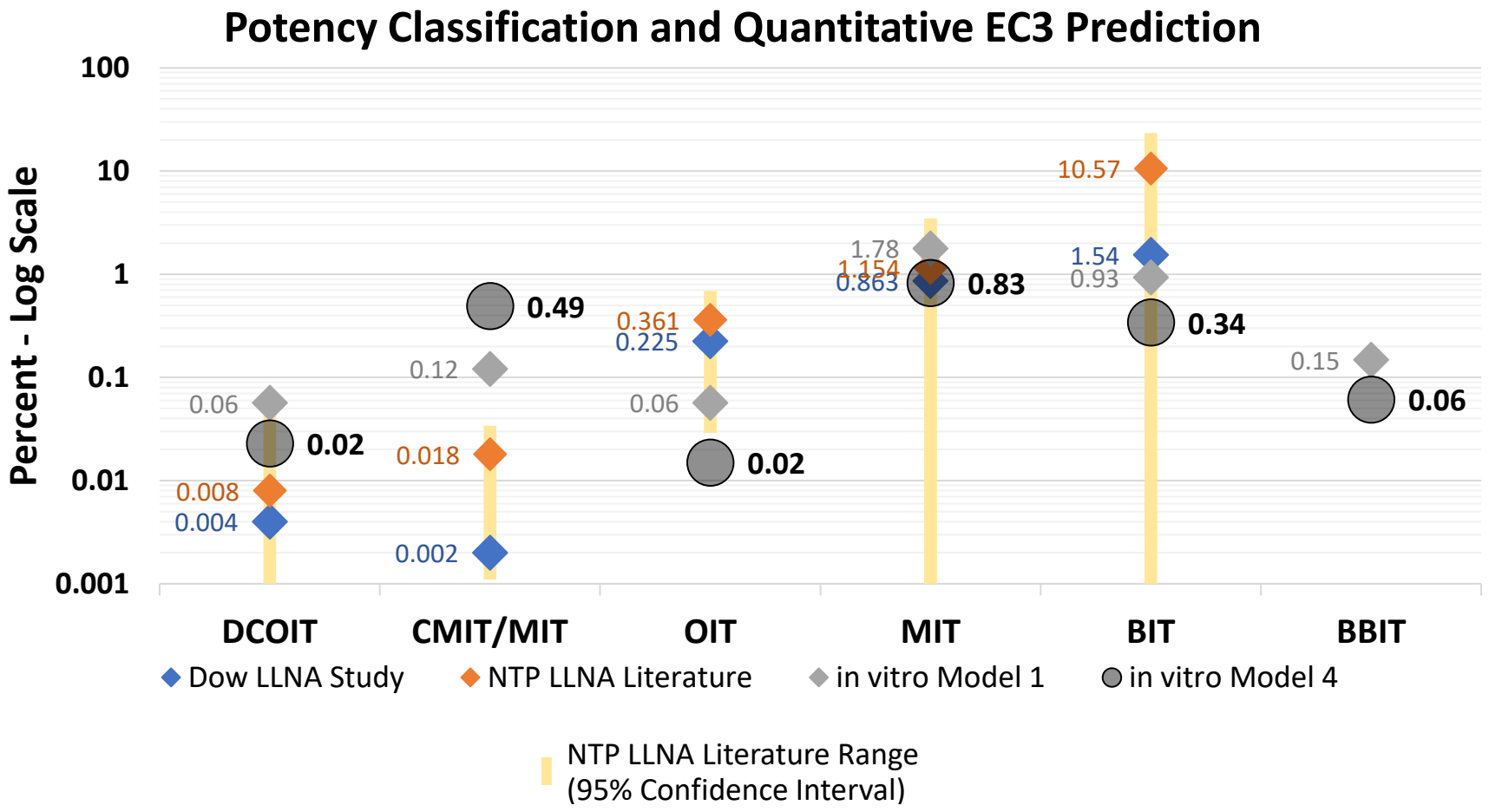


Figure 2. Comparisons of EC3 (%) Predictions and Overlays of the 95% Confidence Intervals for the Isothiazolinones

2.2.5 Evaluation of *In Vivo* & *In Vitro/In Chemico* Assays

All toxicity tests, animal and non-animal, have strengths and limitations. EPA considers these strengths and uncertainties when selecting the critical study(ies) for use in human health risk extrapolation. EPA has determined that the *in vitro* and *in chemico* studies, covering multiple key events in the skin sensitization AOP and with associated OECD guidelines, provide information that is more reliable and human relevant than the LLNA. Therefore, EPA is using the results of the ANN-EC3 DA to derive EC3 values to extrapolate dermal risk for the currently registered isothiazolinones as part of registration review.

The OECD *Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment (GD 34)*[2] states that "new test methods undergo validation to assure that they employ sound science and meet regulatory needs", "the validation process should be flexible and adaptable", and that performance must be "demonstrated using a series of reference chemicals" and "evaluated in relation to existing relevant toxicity data."

Based on these internationally accepted standards, there are two major components to establishing scientific confidence in new methods: *relevance* and *reliability*. OECD GD 34 defines relevance as encompassing the regulatory need, with full consideration of the usefulness of the alternative method(s) and associated limitations. As such, relevance incorporates *fit for purpose* and *utilization* as a contextual evaluation and application of the new approach methodologies (NAM) or integrated NAMs and may include a weight of evidence (WOE) analysis of their use, based on all available evidence, for making qualitative or quantitative predictions. The KeratinoSens and h-CLAT assays use human cells and human molecular targets that are anchored to key events in the AOP. The first cells which come into contact with compounds applied topically to the skin are the keratinocytes. The KeratinoSens assay includes a human HaCaT keratinocyte cell line containing a reporter construct with a single copy of the ARE-element of the human AKR1C2 gene (Emter *et al*., 2010). The Nrf2-Keap1-ARE regulatory pathway, corresponding to the second key event in the AOP, is induced by electrophilic chemicals and is considered one of the most relevant pathways for the identification of potential skin sensitizers (Natsch *et al*., 2015[3]). The h-CLAT is a cell-based assay that identifies skin sensitizers by examining changes in the expression of cell surface markers (CD54 and CD86) implicated in dendritic cell activation, the third key event of the skin sensitization AOP. Following exposure of the THP-1 human monocyte cell line to the test substance, expression levels of CD54 and CD86 are quantified by flow cytometry and compared to controls (Ashikaga *et al*., 2008[4]). Thus, the KeratinoSens and h-CLAT are considered more human relevant and mechanistically driven, compared to the LLNA which uses the mouse and models an apical outcome.

[2] http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2005)14&doclanguage=en
[3] https://www.ncbi.nlm.nih.gov/pubmed/25338925
[4] https://onlinelibrary.wiley.com/doi/full/10.1111/j.1600-0536.2011.01952.x

Reliability is defined in GD34 as the extent of reproducibility of results from a test within and among laboratories over time, when performed using the same standardized protocol. ICCVAM reviewed LLNA variability in a 1999 report (ICCVAM 1999), and several more recent reviews provide an evaluation of LLNA variability, specifically for use in assessing predictivity of NAMs and defined approaches (Dumont *et al*., 2016; Hoffmann 2015; Roberts *et al*., 2016; Kleinstreuer *et al*., 2018). Based on a dataset of LLNA studies collected by the European Commission's Joint Research Center, Dumont *et al*. (2016) analyzed LLNA reproducibility for three different classification schemes. They showed that for chemicals tested using the same solvent, approximately 10% had concordant negative studies, and for positive studies, 68% were concordant for hazard, 62% for GHS (3-potency classes), and 48% for ECETOC (5-potency classes). When including studies using different solvents, the reproducibility of the LLNA for the different classification endpoints dropped even further. Hoffmann *et al*. (2015) also evaluated the variability of LLNA for assigning substances to one of five potency classes: extreme, strong, moderate, weak, and non-sensitizer. Analyzing tests in the same vehicle, 75.6% yielded the same classification; 9.3% resulted in less severe classification, and 15.2% resulted in a more severe classification. Most recently, using the Cosmetics Europe database of 128 chemicals (Hoffman et al. 2018), the reproducibility of the LLNA for hazard classification was 78%, and 63-73% for potency prediction, depending on the summary statistic used for comparison (*e.g*., median, mean, etc.). In contrast, the between laboratory reproducibility was approximately 80%, 85%, and 80% for the DPRA, KeratinoSens and h-CLAT, respectively (OECD, 2019, 2018a, 2018b). Further, the predictive performance of the *in vitro* assays, both individually and when combined into DAs, when compared to the LLNA was equivalent to the ability of the LLNA to reproduce itself (Hoffmann *et al*., 2018, Kleinstreuer *et al*., 2018).

Specific to the isothiazolinone LLNA data, the confidence limits for the NICEATM analyzed studies are given in Table 5 and Figure 2 and show a wide range of plausible values from studies meeting defined inclusion criteria for most of the isothiazolinones. (Note, no LLNA data are currently available for BBIT). Moreover, the EC3 predicted from Model 4 from the ANN model is within the confidence limits of the LLNA data for three of five isothiazolinones with LLNA data. For CMIT/MIT, the ANN EC3 is higher than that for the NICEATM LLNA (*i.e*., less protective) and close to the lower bound LLNA value for OIT (0.015 vs 0.029).

Given all the available evidence, EPA has concluded that in the context of the OECD GD 34, that the NAM approach is more reliable and relevant for assessing dermal sensitization of the isothiazolinones and, thus, appropriate for extrapolating to dermal human health risk.

2.2.6 ROAT Studies

EPA reviewed Repeat Open Application Tests (ROAT) conducted for MIT and CMIT/MIT in human volunteers and presented these studies to the Human Studies Review Board (HSRB) for review of the studies from ethical and scientific perspectives. The HSRB indicated: "*The HSRB* [agreed with EPA's conclusion] *that when considered all together, the three studies described in*

Lundov et al., Yazar et al., and Zachariae et al., do provide a scientific weight of evidence in support of establishing a point of departure for the determination of an elicitation threshold for methylisothiazolinone (as potentially identified by the Lundov et al., study) for use in risk assessments."[5] Summaries of these studies are presented below.

In a ROAT study conducted by Lundov *et al*. (2011) MIT was examined for concentrations that elicited dermal sensitization in human volunteers using both a patch test protocol and a Repeat Open Application Test (ROAT). The study was performed in 11 individuals determined to be previously allergic to MIT and 14 control subjects without an allergy to MIT. In the first experiment, patch testing was performed using 12 decreasing doses of MIT (60, 30, 15, 8.82, 4.41, 2.94, 1.47, 0.441, 0.21, 0.147, 0.105 and 0.0105 µg MIT/cm^2). The purpose of the patch test study was two-fold: (1) to examine the influence of including phenoxyethanol in the MIT patch test on reactivity to MIT; and (2) to use a previously developed model equation to determine if patch test results could be used to predict responses in the ROAT. Patch tests were applied on the back and occluded for 2 days. Readings from day 3 and day 4 post-exposure were used in statistical calculations, using the scale of Johansen *et al*. (1997)

The ROAT study used the same participants as for the patch test. The conduct of the patch testing and the ROAT portion occurred concurrently. For the ROAT, study participants applied 3 different concentrations of MIT and a control solution in a 20 µl volume to a 3 x 3 cm area on the volar aspect of the forearm twice a day for up to 21 days. The intent was to mimic the use of a cosmetic cream applied daily. Concentrations used in the ROAT test were 0, 0.21, 0.105 and 0.0105 µg MIT/cm^2 per application.

Results of patch testing with MIT showed that all participants reacted to the 60, 30, 15, and 8.82 µg MIT/cm^2 concentrations. The lowest eliciting concentration in the patch test was 1.47 µg MIT/cm^2, where 6 participants (55%) showed reactions.

In the ROAT study, 9 of the 11 MIT-allergic test subjects completed the 21-day study duration. One test subject completed only 19 days of the protocol due to travel; one subject lost the test materials and missed 4 days of test material application (which days the applications were missed was not stated). Seven test subjects (64%) reacted to the highest dose of MIT (0.21 µg/cm^2). The same 7 test subjects also reacted to the middle dose of MIT (0.105 µg/cm^2). Reactions at the high and mid dose were statistically significant from the control. Two (18%) reacted to the lowest dose (0.0105 µg/cm^2); this was not statistically significantly different from the control. None of the participants reacted to the control solution, and none of the control subjects developed any reactions in the ROAT.

In comparing the frequency of reactions to the dose per application in the ROAT and the same dose used in the patch test, none of the participants developed a reaction to the patch test MIT doses of 0.21, 0.105 and 0.0105 µg/cm^2, but in the ROAT, as noted, reactions were noted from the repeated dermal administration of the same concentrations.

[5] https://www.epa.gov/sites/production/files/2017-05/documents/january_2017_hsrb_final_report.pdf

The Lowest Adverse Effect Level in this study from the ROAT is 0.0105 MIT µg/cm^2.

In a ROAT study conducted by Zachariae *et al*. (2006), a double blind, placebo-controlled dose-response ROAT was conducted in 25 subjects with confirmed allergy to CMIT/MIT by patch test results and 10 non-CMIT/MIT allergic control subjects. The first ROAT study exposed all test and control subjects to 0.025 µg/cm^2 (2 ppm) CMIT/MIT for 4 weeks. Subjects were then allowed a 4-week washout period. A second ROAT was then conducted on these same subjects using 0.094 µg/cm^2 (7.5 ppm) MCI/MI for 4 weeks.

In the first ROAT (0.025 µg/cm^2), 7 of the 25 test subjects showed a positive reaction with an average time to reaction of 16.5 days. Five weak, 2 moderate, and 0 strong reactions were observed. In the second ROAT (0.094 µg/cm^2), 14 of 25 test subjects showed a positive result with and average time to reaction of 12.1 days. Seven weak, 6 moderate, and 1 strong reaction were observed. The difference in the number of positive reactions to the 0.025 µg/cm^2 application of CMIT/MIT was stated by the investigators as being not significantly different than vehicle control, while the number of positive reactions at 0.094 µg/cm^2 was significantly different from vehicle control. The number of positive reactions in the ROAT at 0.094 µg/cm^2 was statistically different than the number of positive reactions at 0.025 µg/cm^2. All subjects reacting to the 0.025 µg/cm^2 concentration had either a similar or worse strength skin reaction at 0.094 µg/cm^2 CMIT/MIT. The control subjects had no reaction to either concentration during the ROATs. While it was not possible to establish an elicitation threshold for CMIT/MIT in this study, the data suggest that the LOAEL is in the area of 0.025 µg/cm^2. It would likely be somewhat lower, as this concentration showed a 28% response rate based on the reactions of the 25 test subjects.

The results of this study, where positive reactions were observed with CMIT/MIT at 0.025 and 0.094 µg/cm^2 (7 of 25 test subjects (28%) responding at 0.025 µg/cm^2 and 14/25 subjects (56%) responding at 0.094 µg/cm^2), are supported by the results of other studies on MIT and CMIT/MIT showing low elicitation threshold concentrations. In Yazar *et al*., (British Journal of Dermatology 173: 115-122 (2015); MRID 50035301), a positive reaction was observed in a ROAT study in 7/9 subjects (77%) to MIT at 0.24 µg/cm^2. In Lundov *et al*., an 18% response to MIT was reported at approximately 0.0105 µg/cm^2 in the ROAT portion of the study. These studies provide a weight of evidence to the results of Lundov *et al*., for supporting derivation of a point of departure for an elicitation threshold to MIT.

3. Dose-Response Assessment

Endpoints selected for oral (acute oral Table 6, chronic oral Table 7, and incidental oral Table 8), dermal (Table 9), and inhalation (Table 10) risk assessments for the isothiazolinone biocides are presented in the following summary tables. Discussion of the studies and application of uncertainty factors are found in the individual DRAs.

3.1 Oral Endpoint Selections

Selection of oral points of departure and derivation of endpoints is shown below in Tables 6, 7, and 8. These values were selected by the Office of Pesticide Programs' Toxicity Science Advisory Council in 2015 and are reproduced below. Note that the intra-species and inter-species uncertainty factors (UF_A and UF_H) are reduced from the default value of 10x to values of 3x, based on the irritation-type adverse effects observed in the toxicity database. The reduction of these values is consistent with the recommendations of the 2001 report from the National Resource Council (NRC, 2001), when evidence supports the finding of direct-acting irritation effects that are not influenced by systemic physiologic processes and the magnitude of response is not expected to differ when compared to systemic effects.

Table 6. Acute Dietary Endpoints Selected for the Isothiazolinone Biocides

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
BBIT	LOAEL = 2000 mg/kg/day	UF = 100x (UF_A = 3X, UF_H = 3X and $UF_{LOAEL \rightarrow NOAEL}$ = 10X) FQPA SF = 1	Acute (gavage) oral toxicity study – rat. (MRID 44364915) **LOAEL = 2000 mg/kg/day** based on clinical signs of toxicity were observed on Day 1 (piloerection, sides pinched in, upward curvature of spine, labored breathing, gasping, signs of salivation, breathing irregular, ↑ breathing depth & rate, prostrate, and tip toe gait) death of one female rat on day 3 at 2000 mg/kg/day.
BIT	100 mg/kg/day	UF = 10x (UF_A = 3x, UF_H = 3x) FQPA SF = 1	Acute oral (gavage) toxicity study – rat (MRID41022101/42858101) **LOAEL = 300 mg/kg** based on piloerection and upward curvature of the spine.
CMIT/MIT	79 mg/kg/day	UF = 10x (UF_A = 3x, UF_H = 3x) FQPA SF = 1	Acute Oral Toxicity Study (MRID 00086092) Formulation TRD 76-52 (13.2% a.i.) **LOAEL = 157 mg/kg** based on signs of intoxication (lethargy, prostration, ataxia, dyspnea, severe irritation and hemorrhage were noticed in g.i).

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
DCOIT	500 mg/kg/day	UF = 30x (UF_A = 3X, UF_H = 3X and $UF_{LOAEL \rightarrow NOAEL}$ = 3X) FQPA SF = 1	Acute Oral Toxicity in the rat (gavage) MRID 42977701 LOAEL= 500 mg/kg/day based on There was no mortality at 500 mg/kg. Diarrhea and mucus in stool were observed at 500 mg/kg.
OIT	LOAEL= 100 mg/kg/day	UF = 30x (UF_A = 3X, UF_H = 3X and $UF_{LOAEL \rightarrow NOAEL}$ = 3X) FQPA SF = 1	Acute oral toxicity study – rat (gavage) (MRID 00070456) **LOAEL = 100 mg/kg/day** based on diarrhea and unkempt fur. $UF_{LOAEL \rightarrow NOAEL}$ is reduced to 3X because no death involved at this dose for acute toxicity study.

Table 7. Chronic Dietary Endpoints Selected for the Isothiazolinone Biocides

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
BBIT	2 mg/kg/day	UF = 10x (UF_A = 3x, UF_H = 3x) FQPA SF = 1	24-month drinking water chronic/oncogenic study in rats for CMIT/MIT mixture -1994 (MRID 43140701) LOAEL = 6.6/9.8 mg/kg/day (M/F) based on hyperplasia/ hyperkeratosis of the squamous mucosa of the forestomach in both M/F, necrosis of glandular mucosa of the stomach in females and edema/ inflammation of the glandular stomach in females.
BIT	2 mg/kg/day	UF = 10x (UF_A = 3x, UF_H = 3x) FQPA SF = 1	24-month drinking water chronic/oncogenic study in rats for CMIT/MIT mixture -1994 (MRID 43140701) LOAEL = 6.6/9.8 mg/kg/day (M/F) based on hyperplasia/ hyperkeratosis of the squamous mucosa of the forestomach in both M/F, necrosis of

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
			glandular mucosa of the stomach in females and edema/ inflammation of the glandular stomach in females.
CMIT/MIT	2 mg/kg/day	UF = 10x (UF_A = 3x, UF_H = 3x) FQPA SF = 1	24-month drinking water chronic/oncogenic study in rats for CMIT/MIT mixture -1994 (MRID 43140701) LOAEL = 6.6/9.8 mg/kg/day (M/F) based on hyperplasia/ hyperkeratosis of the squamous mucosa of the forestomach in both M/F, necrosis of glandular mucosa of the stomach in females and edema/ inflammation of the glandular stomach in females.
DCOIT	30 mg/kg/day	UF = 100x (UF_A = 10X, UF_H = 10X) FQPA SF = 1	Two generation reproduction Toxicity Study in rats for DCOIT (MRID 45756501) LOAEL (reproductive P/F1) is 62-88 mg/kg/day [M] and 67-93 mg/kg/day [F], based on significantly delayed vaginal opening (35.1 days vs. 31.9 days in control) and preputial separation (46.2 days vs. 42.9 days in control) in F1 offspring. No effects on reproductive performance at any dose level.
OIT	2 mg/kg/day	UF = 10x (UF_A = 3x, UF_H = 3x) FQPA SF = 1	24-month drinking water chronic/oncogenic study in rats for CMIT/MIT mixture -1994 (MRID 43140701) **LOAEL = 6.6/9.8 mg/kg/day** (M/F) based on hyperplasia/ hyperkeratosis of the squamous mucosa of the forestomach in both M/F, necrosis of glandular mucosa of the stomach in females and edema/ inflammation of the glandular stomach in females.

Table 8. Incidental Oral Endpoints Selected for the Isothiazolinone Biocides

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
BBIT	49 mg/kg/day	UF = 100x (UF_A = 10x, UF_H = 10x) FQPA SF = 1	BBIT (99.4 ±1% a.i.) Two-generation reproduction toxicity (dietary) – rat) - 2007 (MRID 48261201) **$NOAEL_{parental\ toxicity}$ = 49 mg/kg/day** **$LOAEL_{parental\ toxicity}$ = 141 mg/kg/day** based on decreased body weights, body weight gains, and food consumption. **$NOAEL_{offspring\ toxicity}$ = 49 mg/kg/day** **$LOAEL_{offspring\ toxicity}$ = 141mg/kg/day,** based on decreased body weights, body weight gains, and spleen weight (F_2 pups only).
BIT	8.42 mg/kg/day	UF = 10x (UF_A = 3X, UF_H = 3X) FQPA SF = 1	BIT (84.2%) 90-day oral (gavage) –Wistar rats (MRID 46346201) **NOAEL=8.42 mg/kg/day a.i.** **LOAEL=25.26 mg/kg/day a.i.,** based on macroscopic and microscopic lesions in non-glandular and glandular regions of the stomach.
CMIT/MIT	8.5 mg/kg/day	UF = 10x (UF_A = 3X, UF_H = 3X) FQPA SF = 1	Rat 2-gen reproductive study (MRID 44656101) **NOAEL parental = 8.5 / 11.8 mg/kg/day** **LOAEL Parental = 22.7/28 mg/kg/day** Based on increased incidence of histopathological lesions of the glandular and non-glandular stomach in the F0 and F1 male and female rats.
DCOIT	30 mg/kg/day	UF = 100x (UF_A = 10x, UF_H = 10x) FQPA SF = 1	Rat 2-gen reproductive study (MRID 45756501) **NOAEL (reproductive P/F1) is 30-39[M] 33-41[F] mg/kg/day**

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
			LOAEL (reproductive P/F1) is 62-88 mg/kg/day [M] and 67-93 mg/kg/day [F], based on significantly delayed vaginal opening (35.1 days vs. 31.9 days in control) and preputial separation (46.2 days vs. 42.9 days in control) in F1 offspring.
OIT	43 mg/kg/day	UF = 10x (UF_A = 3X, UF_H = 3X) FQPA SF = 1	Rat 2-gen reproductive study (MRID 47815801) 0, 13-15, 43-51, 96-120 mg/kg/day dietary **NOAEL parental= 43-51 mg/kg/day** **LOAEL parental= 96-120 mg/kg/day** based on decreased body weight, hyperplasia/hyperkeratosis of the forestomach, decreased spleen weights, increased adrenal weight **NOAEL offspring= 43-51 mg/kg/day** **LOAEL offspring = 96-120 mg/kg/day** based on decreased body weight gain and decreased spleen weight.

3.2 Dermal Endpoint Selection

The resulting points of departure for dermal risk assessments of isothiazolinones from data provided through *in vitro* and artificial neural network (ANN) outputs are shown in Table 9. Endpoints for the isothiazolinones use dermal sensitization induction thresholds calculated from the *in vitro* and ANN outputs, while for CMIT/MIT, both induction and elicitation threshold values are available. The use of the elicitation threshold for CMIT/MIT from the human study results in a lower uncertainty factor (UF of 10) than the uncertainty factor applied to the induction threshold values for the other isothiazolinones (UF of 100). The elicitation study for CMIT/MIT is conducted in humans, so there is no need for an inter-species extrapolation factor; the intra-species variation factor of 10 is applied. The use of induction threshold values for the other members of the isothiazolinone class utilizes an uncertainty factor of 100. This factor includes the inter-species extrapolation factor of 10 (since the data are based on animal studies), and an intra-species factor of 10. The use of induction threshold values would be protective for

persons not yet exposed to isothiazolinone chemicals, while the use of elicitation threshold values is protective for those persons already sensitized to these chemicals. However, the exact quantitative relationship between the induction and elicitation threshold for any individual isothiazolinone chemical is not known. As an example, the induction threshold calculated for MIT (120 µg/cm^2) is considerably greater than the elicitation threshold calculated for MIT (0.0105 µg/cm^2). It is expected that elicitation thresholds for the other isothiazolinones will also be lower than the induction thresholds.

Table 9. Dermal Endpoints Selected for the Isothiazolinone Biocides

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
BBIT	**Induction:** Average *in vitro* EC3 = 0.061% (15.3 µg/cm^2); 95% Confidence Interval = 0.06 to 0.07%	UF = 100x (UF_A = 10x, UF_H = 10x)	Based on Model 4 from Hirota *et al.* 2015: DPRA + h-CLAT + KeratinoSens
BIT	**Induction:** Average *in vitro* EC3 = 0.34% (85 µg/cm^2) 95% Confidence Interval = 0.32 to 0.37%	UF = 100x (UF_A = 10X, UF_H = 10X)	Based on Model 4 from Hirota *et al*. 2015: DPRA + h-CLAT + KeratinoSens
CMIT/MIT	**Elicitation:** Minimum Elicitation Threshold (MET) for MIT of 0.0105 µg/cm^2 producing a response in 18% of tested individuals	UF = 10x (UF_A = 3X, UF_H = 3X)	Lundov *et al*., (2011): Methylisothiazolinone Contact Allergy and Dose–Response Relationships. Contact Dermatitis 64: 330-336. Human Repeat Open Application Test using doses of 0, 0.0105, 0.105, and 0.21 µg MIT/cm^2
	Induction CMIT/MIT EC3 = 0.49% (120 ug/cm^2)	Residential and Occupational LOC for MOE = 100 UFA = 10 UFH = 10	EC3 = 0.49% for CMIT/MIT based on Model 4 from Hirota *et al.*, 2015: DPRA + h-CLAT + KeratinoSens in vitro assays
	Induction MIT only EC3 = 0.83% (210 ug/cm^2)	Residential and Occupational LOC for MOE = 100 UFA = 10 UFH = 10	EC3 = 0.83% for MIT based on Model 4 from Hirota *et al*., 2015: DPRA + h-CLAT + KeratinoSens in vitro assays

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
DCOIT	**Induction:** Average *in vitro* EC3 = 0.023% (5.8 µg/cm^2); 95% Confidence Interval = 0.02 to 0.03%	UF = 100x (UF_A = 10x, UF_H = 10x) FQPA SF = 1	Based on Model 4 from Hirota *et al.* 2015: DPRA + h-CLAT + KeratinoSens
OIT	**Induction:** Average *in vitro* EC3 = 0.015% (3.75 µg/cm^2); 95% Confidence Interval = 0.01 to 0.02%	UF = 100x (UF_A = 10X, UF_H = 10X) FQPA SF = 1	Based on Model 4 from Hirota *et al.* 2015: DPRA + h-CLAT + KeratinoSens

Note: To convert the *in vitro* EC3 (%) into units of µg/cm^2 = [EC3 x 25µL x 10 µg/µL]/cm^2

3.3 Inhalation Endpoint Selection

Inhalation PODs and endpoints are presented in Table 10. The 4,5-Dicholoro-2-n-octyl-4-isothiazoln-2-one (DCOIT) 90-day inhalation toxicity study was used to bridge to BIT and BBIT as there were no chemical-specific inhalation toxicity data for these members of the isothiazolinone chemicals. The DCOIT study is appropriate as it is route-specific with the most conservative inhalation endpoint and covers the duration of exposure. The NOAEC is 0.02 mg/m^3 and the LOAEC is 0.63 mg/m^3 based on the histopathological alterations observed in the nose (min/mild subacute inflammation and transitional respiratory epithelial and goblet cell hyperplasia), larynx (chronic-active inflammation and hyperplasia of the squamous and cuboidal epithelium), and lungs (acute inflammation and goblet cell hyperplasia at high-dose). The POD is refined by calculating the Human Equivalent Concentration (HEC) from the LOAEC of 0.63 mg/m^3. An uncertainty factor of 10x (UF_A =3x and UF_H=3) is applied for short/intermediate - term exposures and an uncertainty factor of 30x (UF_A =3x, UF_H=3x and $UF_{Duration}$ =3x) is applied for long-term exposures. The UFs for short- and intermediate term inhalation exposures are reduced from the default 10x values based on the use of irritation-type effects as the point of departure, and the refinement of the POD to a Human Equivalent Concentration.

Table 10. Inhalation Endpoints Selected for the Isothiazolinone Biocides

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
BBIT	Bridged to the DCOIT 90-day inhalation toxicity study.		
BIT	Bridged to the DCOIT 90-day inhalation toxicity study.		
CMIT/MIT	NOAEC=0.34 mg/m^3 8-hr HEC = 0.11 mg/m^3 24-hr HEC=0.038 mg/m^3 HEC = NOAEC * [6 hr animal / 8 or 24 hr human)]* RDDR (0.45 for ET effects, BW= 400 grams, MMAD = 1.1 um, GSD = 1.9 to 2.0)	10X (for short/intermediate-term) (UF_A = 3x, UF_H = 3x) 30X (for long-term) (UF_A = 3x, UF_H = 3x, and $UF_{long\text{-}term}$ = 3x)	90-day inhalation Study for CMIT/MIT (MRID 00148418) **NOAEC = 0.34 mg/m3 (both M/F),** **LOAEC = 1 15 mg/m3**, based on microscopic: lesions in the nasal turbinates (rhinitis).
DCOIT	NOAEC=0.02 mg/m^3 HEC=0.0045 mg/m^3 HEC = NOAEC * (6-hour animal/8-hour human) * RDDR (0.30 for ET effects, BW= 420 grams, MMAD = 1.4 um, GSD = 4.6)	ST/IT LOC = 10 UF_A= 3 UF_H = 3 LT LOC = 30 UF_A = 3 UF_H = 3 UF_D = 3	90-Day inhalation study for DCOIT (MRID 43487501) **NOAEC = 0.02 mg/m^3**, **LOAEC = 0.63 mg/m^3**, based on the histopathological alterations observed in the nose (min/mild subacute inflammation and transitional respiratory epithelial and goblet cell hyperplasia), larynx (chronic-active inflammation and hyperplasia of the squamous and cuboidal epithelium), and lungs (acute inflammation and goblet cell hyperplasia at high-dose).
OIT	NOAEC=0.64 mg/m^3 HEC = 0.12 mg/m^3	10X (for short/intermediate-term) (UF_A = 3x, UF_H = 3x) 30X (for long-term)	90 Day Inhalation Toxicity - OIT (MRID 41544701) **NOAEC = 0.64 mg/m^3** **LOAEC = 6.3 mg/m^3** Based on rales (5-22/22 males and females, week 1-13), dyspnea (3/22 females week 4 and 3-9/22 females week 7-10),

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
	HEC = NOAEC * (6-hour animal/8-hour human) * RDDR (0.25 for ET effects, BW= 370 grams, MMAD = 1.4 um, GSD = 5.5)	(UF_A = 3x, UF_H = 3x, and $UF_{long\text{-}term}$ = 3x)	thriftless (3/22 males week 8 and 10 and 9-22/22 females week 7-10), red staining on dropping sheet (11/22 males week 2, 6/22 females week 8), decreases in body weight gain, organ weight changes (decreased liver, spleen, kidney and adrenal weight), gross pathology (fluid in uterus of 4/11) and pulmonary and nasal cavity pathology including effects that are in report as minimal to mild but have no severity list in report tables: secretory cell hyperplasia in nasal septum (7/11 females), squamous metaplasia in the lateral wall of the nasal cavity (3/11 male, 4/11 female), squamous metaplasia in the maxilloturbinate (4/11 female), acute inflammation of the nasal mucosa (4/11 male, 4/11 female), Eosinophilic intraepithelial droplets in the nasal cavity III (7/11 male, 10/11 female) and IV (8/11 female) . During recovery period rales present in 3/11 males and 3/11 females at week 14. By week 20 rales was no longer present in males and 1 female presented rales throughout recovery period.

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Appendix A. Toxicity Profiles for Isothiazolinones

Toxicity Profiles of the individual isothiazolinone chemicals are presented in Tables A1-A5.

Table A1. Toxicity Profile for 1,2-Benzisothiazolin-3-one (BIT)

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.3100 90-Day oral toxicity rodents	MRID 419104-01 (1990) 0, 200, 900, 4000 ppm M: 0, 15.3, 69.0, 322 mg/kg/day F: 0, 17.6, 78.3, 356 mg/kg/day **Acceptable**	NOAEL = 15.3 mg/kg/day (males), 78.3 mg/kg/day (females) LOAEL = 69 mg/kg/day (males) and 356 mg/kg/day (females) based on decreased body weights in both males and females and increased incidence of non-neoplastic lesions (forestomach hyperplasia) in females only.
870.3150 90-Day oral toxicity in nonrodents	MRID 42205701(1991) 0, 5, 20, 50 mg/kg/day (both male and female dogs) **Acceptable**	NOAEL = 5 mg/kg/day LOAEL = 20 mg/kg/day based on the incidence of emesis and clinical alterations at this dose.
870.3700a Prenatal developmental in rodents	MRID 409612-01 (original, 1988), 428581-04 (addendum, 1993) 0, 10, 40, 100 mg/kg/day (TEP) **Acceptable**	**Maternal** NOAEL = 40 mg/kg/day LOAEL = 100 mg/kg/day based on decreased body weight gain and decreased food consumption. **Developmental** NOAEL = 40 mg/kg/day LOAEL = 100 mg/kg/day based on decreased fetal body weight and increased incidence of delays in skeletal ossification.
870.3700a	MRID 43663008 (1994) 0, 10, 30, 90 mg/kg/day (100% a.i.)	**Maternal** NOAEL = 10 mg/kg/day

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
Prenatal developmental in rodents	**Acceptable**	LOAEL = 30 mg/kg/day based on increases in mortality, clinical signs of toxicity, and gross pathology findings. **Developmental** NOAEL = 30 mg/kg/day LOAEL = 90 mg/kg/day based on increases in litter and fetal incidence of extra skull bone ossification sites and unossified sternebra.
870.5265 *Salmonella typhimurium* reverse mutation assay	MRID 43584007 6.67-333 µg/plate -S9, 3.33-100 µg/plate +S9 **Acceptable**	Negative, with or without S9 activation.
870.5300 In vitro mammalian cell gene mutation test	MRID 43584009 0.0625-0.800 µg/mL -S9 0.075-0.600 µg/mL +S9 **Acceptable**	Weak positive under nonactivated conditions but only in presence of high toxicity (≤15% relative total growth). Negative under S9-activated conditions. Thus, no biologically significant increase in the mutation frequency noted at the thymidine kinase locus either with or without metabolic activation at any of the concentrations tested.
870.5300 In vitro mammalian cell gene mutation test	MRID 41022103 (original), 42858105 (addendum) 0.03-2.00 µg/mL -S9, 1-64 µg/ml +S9 **Core-acceptable**	Negative, with or without S9 activation.
870.5395 In vivo mammalian erythrocyte micronucleus test	MRID 43584008 225-900 mg/kg via oral gavage **Acceptable**	Negative
870.5395 In vivo mammalian erythrocyte micronucleus test	MRID 41022104 (original), 42858106 (addendum) M: 245, 392 mg/kg F: 331, 529 mg/kg	Negative. No significant increases in the frequency of micronucleated polychromatic erythrocytes noted in the bone marrow after any treatment time.

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
	Acceptable	
870.5550 Unscheduled DNA synthesis in mammalian cells in culture	MRID 41022105 (original), 42858107 (addendum) 10^{-8} to 10^{-4} M **Acceptable**	Negative

Table A2. Toxicity Profile for 1,2-Benzisothiazolin-3-one, 2-butyl (BBIT)

Guideline No./ Study Type	MRID No. (year)/ Classification/Doses	Results
870.3700 Developmental Toxicity in the Rat (gavage) BBIT, 95.5%	**MRID 44364920** Doses: 0, 30, 100, or 300 mg/kg/day on gestation days (GD) 7-16, inclusive **Acceptable**	Maternal NOAEL = 30 mg/kg/day Maternal LOAEL = 100 mg/kg/day based on ulcerated areas of the stomach. Developmental NOAEL > 300 mg/kg/day Developmental LOAEL > 300 mg/kg/day
870.3100 Subchronic Oral Toxicity in the Rat (dietary administration) BBIT, 95.5% a.i.	**MRID 44403001** M: 0, 3.1, 15.3, or 149.2 mg/kg/day F: 0, 3.4, 16.6, or 162.4 mg/kg/day **Acceptable**	NOAEL = males: 15.3 mg/kg/day; females: 16.6 mg/kg/day LOAEL =_ males: 149.2 mg/kg/day; females: 162.4 mg/kg/day, based on submucosal inflammation of the non-glandular stomach
870.3150 Subchronic Oral Toxicity in the non-	**MRID 48262204** Doses: 0, 25, 75 or 250 mg/kg/day (high dose reduced to 200 mg/kg/day on Day 10)	NOAEL = 25 mg/kg/day LOAEL = 75 mg/kg/day, based on treatment-related clinical findings in both sexes and dose-rlated decreases in albumin (males) and total protein concentration (females).

Guideline No./ Study Type	MRID No. (year)/ Classification/Doses	Results
rodent (Dog) – capsule administration BBIT, 99.4% a.i.	**Acceptable**	
2-generation Reproduction Toxicity in the Rat Dietary administration BBIT, 99.4% a.i.	**MRID 48261201** Doses: 0/0, 25/27, 49/56, and 141/157 mg/kg/day (M/F) **Acceptable**	Parental/systemic NOAEL = 49/56 mg/kg/day (M/F) Parental/systemic LOAEL = 141/157 mg/kg/day in males/females, respectively) based on decreased body weights and food consumption. Offspring toxicity NOAEL = 49/56 mg/kg/day Offspring toxicity LOAEL = 141/157 mg/kg/day, based on decreased body weights, body weight gains, and spleen weight (F2 pups only) Reproductive toxicity NOAEL $\geq$ 141/157 mg/kg/day Reproductive toxicity LOAEL > 141/157 mg/kg/day
870.5300 Bacterial Reverse Mutation Test mouse lymphoma L5178Y TK± cells BBIT 95.5% a.i.	**MRID 44364923** Cells tested in three independent assays with and witout metabolic (S9) activation. Concentrations ranging from 0.1-50 ug/l **Acceptable**	BBIT was tested up to cytotoxic concentrations. No evidence of induced mutant colonies over background
870.5395 Erythrocyte Micronucles Test in Mice BBIT, 95.5% a.i.	**MRID 44364924)** Oral gavage at at the maximum tolerated doses of 1250 mg/kg (males) or 2000 mg/kg (females). **Acceptable**	BBIT produced no significant increase in the frequency of bone marrow micronucleated polychromatic erythrocytes at either 24 or 48 hours after treatment
870.6200 Neurotoxicity Screening Battery (part of the oral subchronic toxicity study)	**MRID 44403001**	No treatment-related neurotoxic effects or effects on motor activity observed, in the Functional Observational Battery (FOB) and motor activity conducted.

Guideline No./ Study Type	MRID No. (year)/ Classification/Doses	Results
870.5375 Chromosomal aberrations in human lymphocytes BBIT, 95.5% a.i.	**MRID 44364922** Tested under non – activated and activated conditions at concentrations ranging from 1-25 ug/l **Acceptable**	BBIT was tested up to cytotoxic concentrations. Statistically significant increases in mean percentage of cells with aberrations (primarily breaks and fragments) were observed at 92 hours both in the presence and absence of S9-mix. BBIT was clastogenic to human lymphocytes *in vitro* as tested
870.5550 Unscheduled DNA Synthesis in Rat Hepatocytes BBIT, 95.5% a.i.	**MRID 44364925** Doses: 500 and 800 mg/kg in two or three male Alpk:AP SD rats per test group by oral gavage. **Acceptable**	mean net nuclear grain count was below zero for both doses at both treatment times indicating no induction of UDS as tested in this study

Table A3. Toxicity Profile for Octhilinone (OIT)

Guideline No./ Study Type	MRID No./ Classification/Doses	Results
Non-Guideline 14-day oral (rat)	**MRID 43935707** Fuchs, A. (1995) N-Octylisothiazolone (OIT) 96%: 14-Day Oral (Gavage) Dose Range-Finding Study in the Female Rat: Final Report: Lab Project Number: 1248-1154-050: 1154-050. Unpublished study prepared by Hazleton Deutschland GmbH. 108 p. Octhilinone administered orally at 0, 10, 60, or 120 mg/kg/day, 14 days 10 rats/sex/dose, 90 days	**Subchronic Toxicity:** **NOAEL not established.** **LOAEL = 10 mg/kg/day** based on clinical signs of salivation and gross pathology in the liver. Clinical observations and gross pathology findings indicated dose-related signs of toxicity in all treatment groups. Observations of salivation and abnormal position increased in incidence and frequency of occurrence with increasing dose level. Piloerection, soft feces, and excessive urination were also noted in the 120-mg/kg/day animals. Treatment-related gross pathology findings included a prominent lobular pattern in the liver (≥10 mg/kg/day), and whitish-colored and thickened fundus of the stomach (≥60 mg/kg/day). Gastric hemorrhages of the fundus were also noted in one 120-mg/kg/day rat. Body weight gain and food consumption were reduced at ≥60 mg/kg/day. However, body weight gain was not statistically different from controls due to large standard deviations, and food consumption was only significantly reduced at the Day 1-3 treatment interval. This study had numerous deficiencies in meeting criteria for a repeat dose oral toxicity study in rodents. However,

	Purity 96% Batch K1217 **Acceptable** Non-Guideline	because the purpose of this study was for dose selection for a rat developmental toxicity study and not for fulfillment of a specific guideline, the information contained in this study may be scientifically useful for examining toxic effects from short-term oral exposure Based on the results of this study, doses of 5, 30, or 60 mg/kg/day were suggested for the rat developmental toxicity study.
870.3100 90-day oral (rat)	**MRID 136524** Powers, M.; Kundzin, M.; Ferrell, J. (1970) Three-month Dietary Administration--Rats: RH-893 (Technical): Project No. 417-320. Final rept. (Unpublished study received Feb 9, 1971 under 707-100; prepared by Hazleton Laboratories, Inc., submitted by Rohm & Haas Co., Philadelphia, PA; CDL:004372-H) Octhilinone administered orally at 0, 100, 500 and 2000 ppm (equivalent to 0, 7.0, 34.6, or 139.9 mg/kg/day) 10 rats/sex/dose, 90 days Purity: Not reported Lot SW 70/0293 **Unacceptable** Not Upgradeable	**Subchronic Toxicity:** **NOAEL, LOAEL not determined**. There was no effect on survival, feed efficiency, urinalysis, or gross and microscopic pathology. Body weight, body weight gain, and feed consumption were low during the first week of treatment for high-dose males and females but were comparable to the respective control groups for the remainder of the study. The results for hematology, clinical chemistry, and organ weight were equivocal because of the high variability, low number (5 rats/sex/treatment group for clinical chemistry and hematology), inconsistency between the sexes, and lack of a dose response. The results, however, suggest a possible decrease in hemoglobin and RBCs in all female treatment groups, which was accompanied by an increase in serum carbon dioxide levels. Similar results were not observed in the males. There was a decrease in total WBC in the high-dose group at 13 weeks for both sexes and at 4 weeks in females. There also were some changes in the differential leukocyte counts with an increase in the percent of segmented neutrophils accompanied by a decrease in the percent of lymphocytes in all male (4 weeks only) and female (13 weeks only) treatment groups. All treated males and females also had increases in absolute and relative thyroid weight. The deficiencies in this study were numerous with several being sufficiently severe enough to compromise the overall integrity of the study. These deficiencies are mainly due to the age of the study, having been performed prior to the implementation of Good Laboratory Practices (GLP). These deficiencies include high variability in the data (which could be a result of the variability in the initial body weight or an artifact of the strain); the lack of any analyses on the dose formulation to indicate that homogeneity, stability, or adequate concentrations were achieved and maintained throughout the study; an inadequate number of animals (only 5 animals/sex/treatment) used for hematology, clinical chemistry, and histopathology examinations; and the lack of numerous measured parameters (e.g., neurological exams). **The deficiencies in this study are such that neither a LOAEL nor NOAEL could be determined.**
870.3150 90-day oral (dog)	**MRID 136525**	**Subchronic Toxicity:** **NOAEL, LOAEL not established.**

	Powers, M.; Ferrell, J. (1970) Three-month Dietary Administration-- Dogs: RH-893 (Technical): Project No. 417-334. Final rept. (Unpublished study received Feb 9, 1971 under 707-100; prepared by Hazleton Laboratories, Inc., submitted by Rohm & Haas Co., Philadelphia, PA; CDL:004372-I) Octhilinone administered orally at 0, 100, 500 and 2000 ppm 4 dogs/sex/dose, 90 days Purity: 100% Lot SW70/0293 **Unacceptable** Not Upgradeable	Data were highly variable, even at the initial measurement, making it difficult to determine treatment-related effects. The results indicate the following as possibly related to the administration of RH-893: reduced body weight gain in 500-ppm males and 2000-ppm males and females, with reduced food efficiency in these groups; slightly reduced hematocrit and hemoglobin and increased alkaline phosphatase in the high-dose groups; decreased total protein in high-dose males and all females, accompanied by some disruption in the protein electrophoresis; increased relative liver weight (high-dose males only); increased relative kidney weight (high-dose females only); and decreased relative testis weight with an increase in slight to moderate epididymitis in high-dose males. The major deficiency in this study was the complete lack of analyses on the dose formulation. Because there was high variability in the results, coupled with very little apparent affects, this becomes a great deficiency. The animals had such variability initially in their body weights and the majority of the other measured parameters that it cannot be determined if there is an inadequate homogeneity, stability, or concentration in the dose formulation or if there were just great variability in the animals. Given the great variability, the slight differences between the groups, and the lack of information on the dose formulations, it cannot be determined what effects are actually related to the treatment. Although it is possible that higher doses may have yielded more definitive results, this would again largely depend on the homogeneity, stability, and accuracy of the dose formulations. The deficiencies in this study are so great that neither a **LOAEL nor NOAEL can be assigned.** This 90-day oral toxicity study in the dog is UNACCEPTABLE/NOT UPGRADEABLE and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409). This study is unacceptable because homogeneity, stability, and concentration of the dose formulations were not measured. Additionally, the animals were not randomized and were possibly of different ages or older than 9 months prior to dosing. These and other numerous deficiencies discussed in this review make the study not Upgradeable.
Non-Guideline 14-day dermal (rat)	**MRID 43935705** Zuehlke, U. (1995) N-Octylisothiazolone (OIT) 96%: 14-Day Dermal Subacute Toxicity Study in the Rat: Final Report: Lab Project Number: 1247-1154-052: 1154-052. Unpublished study prepared by Hazleton Deutschland GmbH. 145 p.	**Dermal Irritation** **NOAEL = 10 mg/kg/day** **LOAEL = 100 mg/kg/day** based on dermal irritation in both males and females. There were no treatment-related effects in mortality, clinical signs, and food consumption. Dermal irritation was observed at ≥ 100 mg/kg/day in both males and females at Weeks 1 and 2. Slight to moderate erythema, edema, atonia, and desquamation were observed in 100 mg/k/kg/day-treated rats. The severity of dermal irritation increased to moderate to severe in the 1000 mg/kg/day-treated rats. Some 1000 mg/kg/day females also exhibited slight to moderate fissures at Week 1 (1/5) and Week 2 (3/5). In addition to these observations, scabbing was observed at ≥ 100 mg/kg/day at Weeks 1 and 2 and scabbing with exfoliation was observed at 1000 mg/kg/day in males and females at Week 2. There were no statistically

	Octhilinone applied to the skin at 0, 10, 100, 1000 mg/kg/day, 6 hours/day, for 14 days 5 rats/sex/dose Purity: 96% **Acceptable** Non-Guideline	significant findings for body weight and body weight gain data. Body weight gain, however, was reduced in 1000 mg/kg/day males and females at Week 2 and in overall weight gain. The lowered weight gain was greater in males than in females. Food consumption data did not provide an explanation for the reductions observed in body weight gain. There were no statistically significant effects in mean food consumption in treated rats throughout the study. Macroscopic findings reported at necropsy were generally insignificant. A dermal-related finding of subcutaneous redness in the neck and indurated skin in the treated area was observed in one 1000 mg/kg/day male.
870.3200 21-day dermal (rabbit)	**MRID 136526** Powers, M.; Kwapien, R. (1970) Three-week Dermal Application--Rabbits: RH-893-50%: Project No. 417-321. Final rept. (Unpublished study received Feb 9, 1971 under 707-100; prepared by Hazleton Laboratories, Inc., submitted by Rohm & Haas Co., Philadelphia, PA; CDL:004372-J) Rabbits (10sex/dose) 0, 1% or 10%, for 6 hours/day, 5 days/week, for 3 weeks. Half of the animals were abraded, half intact. Vehicle control: 1% Tween 80/distilled water solution Purity: 50% **Unacceptable** Not upgradeable	**Dermal irritation:** **NOAEL not established.** **LOAEL = 1%** based on dermal irritation (clinical findings and microscopic findings) in both males and females. **Systemic Toxicity:** **NOAEL not determined (males).** **NOAEL = 1% (females).** **LOAEL = 1% (males)** based on body weight gain. **LOAEL = 10% (females)** based on body weight gain. There were no treatment-related effects on mortality, appearance, and behavior. The 10% treatment affected dermal clinical findings, body weight, body weight gain, hematological parameters (leukocytes, segmented neutrophils, and lymphocytes) and histological findings of the skin (acanthosis, hyperkeratosis, dermatitis, pseudoepithelial hyperplasia, pustular epidermitis [males], ulcerations, folliculitis [males]) with the abraded animals displaying a slightly more severe skin lesions but with the small number of intact skin animals examined at necropsy comparisons were difficult. The 1% animals, displayed clinical signs of dermal effects, decreases in body weight gains (males with a greater effect noticed in males with abraded skin), and histological findings of the skin (acanthosis, leukocyte infiltration, hyperkeratosis, dermatitis, and pustular epidermitis) with the abraded animals displaying a slightly more severe skin lesions but with the small number of intact skin animals examined at necropsy comparisons were difficult. In addition, a 10% dose-male, had severe hepatic necrosis and inflammation and suppurative hepatitis; these changes cannot be fully attributed to dosage due to the low number of control animals for comparison but cannot be ruled at as incidental.
870.3250	**MRID 42007301**	**Dermal Toxicity:**

90-day dermal (rat)	Bernacki, H. and Hamilton, J (1991) Rh-893 HQ Technical: Three Month Dermal Toxicity Study in Rats. Rohm and Haas. (Pennsylvania) Rpt. #: 90R-31 Octhilinone administered dermally at 0, 2.97, 5.95 and 14.87 mg/kg/day (0, 0.3%, 0.6% and 1.5%) 10 rats/sex/dose, 5 days/week for 13 weeks Purity: 99.1% **Acceptable** Guideline	**NOAEL <2.97 (0.3%) mg/kg/day** **LOAEL =2.97 (0.3%) mg/kg/day,** based on skin irritation: hyperkeratosis, acanthosis, foci of necrosis, eschar formation, sebacious gland hyperplasia and chronic inflammation **Systetmic Toxicity:** **NOAEL = 5.95 (0.6%) mg/kg/day** **LOAEL = 14.87 (1.5%) mg/kg/day,** based on decreases in HGB, HCT, RBC, albumin, glucose and total protein in females and a decrease in body weight gain in males)
870.3250 90-day dermal (rat)	**MRID 43935706** Zuhlke, U. (1995) N-Octylisothiazolone (OIT) 94 +/- 3% 90-Day Dermal Subchronic Toxicity Study in the Rat. Corning Hazelton GMBH, (Germany). Study #: 1154-051. Octhilinone applied to the skin at 0, 5, 25 or 125 mg/kg/day for 6 hours/day for 90 days 10 Sprague-Dawley rats/sex/dose Purity: 94% **Supplemental**	Male and female rats in the 125 mg/kg treatment groups exhibited slight to moderate (mean score <=2.1 on a scale of 0 to 3) erythema, edema, atonia, desquamation, and fissures, and lesions described as squamous cell hyperplasia, sebaceous cell hyperplasia, folliculitis, dermatitis, and hemorrhages at the site of treatment. Body weight gains of male rats in the 125 mg/kg treatment group were 21% less than rats in the control group after 90 days of treatment; the depressed weights were first noted at 22 days. The body weight and body weight gains of female rats in the 125 mg/kg treatment group were unaffected. Male and female rats in the 25 mg/kg treatment groups were found to have minimal (mean scores <=1) erythema, edema, and atonia; no lesions were observed. No dermal irritation was observed in the 5 mg/kg treatment group during the study. Body weights of rats in the 5 and 25 mg/kg treatment groups were similar to the controls. For all treatment groups, food consumption was similar to the controls. There were no treatment-related differences in ophthalmology, hematology parameters, clinical blood chemistry, urine chemistry and appearance, organ weights, or macroscopic or microscopic organ morphology (with the exception of treated skin in the high dose rats) between rats in the treated and the control groups. No neoplastic tissue was observed. However, the investigator failed to microscopically examine samples of tissue from the treatment sites was not assessed. Therefore, the NOAEL/LOAEL cannot be determined.
Non-Guideline 10-day inhalation (rat)	**MRID 136527** Hiddemen, J.; Ferrell, J. (1971) Subacute Inhalation Study--Rats: RH-893-50%: Project No. 417-345.	**NOAEL and LOAEL could not be determined.** Animals in the 10% exposure group were found dead or sacrificed due to moribundity 9/10 males and 7/10 females died as early as Day 3. Clinical signs of toxicity related to treatment

	Final rept. (Unpublished study received Feb 9, 1971 under 707-100; prepared by Hazleton Laboratories, Inc., submitted by Rohm & Haas Co., Philadelphia, PA; CDL:004372-K) Whole-body inhalation exposure to 2 mg/L of 0, 1% or 10% solution, 1 hour per day, for 10 days 5 rats from each treatment group were sacrificed and necropsied at Day 12; rest of animals sacrificed and necropsied Day 26. 10 Rats/sex/exposure Vehicle: propylene glycol Purity: 50% **Unacceptable** Not upgradeable	were nose shuffling (1% and 10%), dried red material around the eyes (10%), gasping (10%), lacrimation (10%), red nasal exudate (10%), and coughing (10%); preening, hypoactivity, wet hair, excessive water consumption, and wheezing were exhibited in control and treated animals. Data were highly variable, making it difficult to determine treatment-related effects. Weight loss was observed in the 10%-RH-893-50% males and females; however, only 1 male rat and 3 female rats survived to Day 8 so the body weights on Days 8, 12, and 26 could not be statistically compared to controls. By the end of the recovery period the 10%-RH-893-50% group had gained weight; however, the surviving 10%-treated male displayed a weight gain of 69% of control, the 1% males a weight gain of 26% of control, 10%-treated female displayed a weight gain of 86% of control; the 1% females a weight gain of 91% of control. Blood was only taken on Day 26 from the surviving animals and not on Day 12, after exposure termination, it is difficult to determine treatment-related effects. Analysis of hematological data demonstrated a decrease in total white blood cells (10% males and females; 1% females), an increase in segmented neutrophils (1 and 10% males and females), and a decrease in lymphocytes (10% males and females; 1% males), which may indicate an irritation response. However, without data from Day 12 this is speculation. A significant decrease in glucose was observed in the high-dose male. Clinical pathology data does not correlate to this change; therefore, it could be an incidental change. Without data from Day 12 when the treatment was terminated it is impossible to make a conclusion as to the relevance of this finding. An increase in aspartate aminotransferase levels was observed in the 10% animals; however due to data variability and the fact that only 1 male and 3 females remained after treatment, these values were not statistically significant. These changes may have corresponded with gross pathology findings of mottled liver; however, without clinical chemistry data from Day 12 when the treatment was terminated it is impossible to make a conclusion as to the relevance of this finding. Because there was high variability in the results, coupled with very little data from the day treatment was terminated, this becomes a great deficiency. Microscopic treatment related effects were limited to the respiratory tract and included mild to moderate bronchiolitis in multiple 10% RH-893-50% rats. Additionally, four male rats from the 1%-RH-893-50% group exhibited severe lung lesions, which were consistent with chronic murine pneumonia that may have been exacerbated by inhalation treatment.
870.3465 90-day inhalation (rat)	**MRID 41544701** Hagan, JB, B. Kulwich and J. Fisher (1989) Skane M-8 HQ Microbicide 13-Week Inhalation Toxicity Study in Rats. Rohm and Haas. Rpt. #: 87R-013.	**NOAEL = 0.64 mg/m3** **LOAEL = 6.39 mg/m3,** based on decreased body weights, fluid in the uterus, and the pulmonary histopathological findings at the terminal sacrifice for males and females in the 6.39 mg/m3 group.

	Octhilinone administered via inhalation at 0.05, 0.64 and 6.39 mg/m3 11 Rats/sex/exposure Purity: 42% **Acceptable** Guideline	Clinical signs in the 6.39 mg/m3 exposure group included: rales [males and females: 5-22/22: weeks 1-13]; dyspnea [females: 3/22: wee 4; 3-9/22, weeks 7-10] thriftless [(males: 3/22: week 8 and 10) (females: 9-22/22; weeks 7-10)] Red staining of the drop sheet [(males: 11/22: week 2) and (females: 6/22: week 8)]. There were significantly decreases in body weights (3.3 to 8.8% for weeks 1, 7, and 13) and body weight gains (11.3 to 68.9% for weeks1, 7, and 13) in male and female rats in the 6.39 mg/m3 group.
870.3700a Developmental Toxicity – oral gavage (rat)	**MRID 41482508** Nemec, M. (1987) A Teratology Study in Rats with Skane M-8 HQ: Final Report: Lab Project Number: WIL-91003: 87RC-0009. Unpublished study prepared by WIL Research Laboratories, Inc. 269 p. Octhilinone administered orally to 3 groups of 25 female rats at 0, 1, 5 and 30 mg/kg from days 6-15 of gestation. 25 female rats/dose Purity: 43% Lot SW 86 6155 **Acceptable** Guideline	**Maternal Toxicity:** **NOAEL = 5 mg/kg/day** **LOAEL = 30 mg/kg/day,** based on death, salivation and decreased defecation, decreased body weight and body weight gain, decreased corrected body weight and body weight gain, and decreased food consumption. **Developmental Toxicity:** **NOAEL = 30 mg/kg/day** (highest dose tested) **LOAEL not established.** Maternal toxicity was observed at 30 mg/kg/day. Signs of toxicity at 30 mg/kg/day include: one death; salivation and decreased defecation; decreased body weight and body weight gain; decreased corrected body weight and body weight gain; and decreased food consumption. The investigator also concluded that there was a slight, treatment-related increase in resorptions/dam and post-implantation loss at 30 mg/kg/day. Upon further inspection, however, the values appear comparable to control values, as well as other treatment groups. Additionally, these changes did not reach statistical significance. Although the investigator concluded that maternal toxicity also was observed at 5 mg/kg/day, this determination does not seem reasonable. Changes in body weight, corrected body weight and body weight gain, and food consumption observed at the mid-dose and deemed treatment-related by the investigator were slight, and the values were not statistically different in comparison to the control values. The values actually appear to be comparable to control values, especially when standard deviations are taken into consideration. The findings at the mid-dose do not warrant being called a treatment-related effect. Developmental toxicity was not observed at any dose level. The investigator concluded that developmental toxicity was observed at 30 mg/kg/day due to an increased percentage of litters and fetuses with any type of malformation (external, visceral, or skeletal). Malformations occurred in only three fetuses from three litters at 30 mg/kg bw/day in comparison to one control fetus from one control litter. The difference was not statistically

		significant, and the apparently high percentage values (1.2% of fetuses and 17.6% of litters at 30 mg/kg/day; 0.4% of fetuses and 5.3% of litters in the control; and 0.3% of fetuses and 4.3% of litters in the historical control) are most likely an artifact of the very small number of fetuses and litters available for evaluation. In addition, the malformations observed are known to occur spontaneously in this strain of rats as demonstrated by the historical data.
870.3700a Developmental Toxicity – oral gavage (rat)	**MRID 43944401** Fuchs, A. (1995) N-Octylisothiazolone (OIT) 94 +/- 3% Oral (Gavage) Teratogenicity Study in the Rat. Hazelton GMBH, (Germany). Report #: 1272-1154-049. Octhilinone administered orally at 0, 5, 30 or 60 mg/kg/day from days 6-15 of gestation. 25 female Sprague-Dawley rats/dose Purity: 94 ±3% **Acceptable** Guideline	**Maternal Toxicity:** **NOAEL = 30 mg/kg/day** **LOAEL = 60 mg/kg/day, based on reduced body weight gains** **Developmental Toxicity** **NOAEL = 60 mg/kg/day (HDT)** **LOAEL not observed** Maternal toxicity was demonstrated by reduced mean body weight gains on days 6-9 of gestation (14%, compared to controls). No other treatment-related effects in mortality, clinical signs of toxicity, body weight, food consumption, or cesarean parameters were noted at any dose level.
870.3700a Developmental Toxicity – oral gavage (rat)	**MRID 46403** Powers, M.B. (1971) Final Report: Teratology Study--Rats: Project No. 417-349. (Unpublished study received May 25, 1971 under unknown admin. no.; prepared by Hazleton Laboratories, submitted by Rohm & Haas Co., Philadelphia, Pa.; CDL:107967-A) Octhilinone administered orally at 0, 200 and 1000 ppm from days 6-15 of gestation. 18 female rats/dose	**Maternal Toxicity:** **NOAEL = 1000 ppm (highest dose tested)** **LOAEL not established.** **Developmental Toxicity:** **NOAEL = 1000 ppm (highest dose tested)** **LOAEL not established.** There was no maternal toxicity observed at any dose level. It should be noted, however, that there may be treatment-related changes in maternal body weight. Mean maternal body weight values show a slight decrease in body weight at the high dose that is difficult to interpret due to the lack of individual data, standard deviations, and statistical analysis, as well as the low number of animals. **The maternal NOAEL is 1000 ppm.** There were no developmental effects attributed to oral exposure to RH-893 in the diet; therefore, **the developmental NOAEL is 1000 ppm.**

	Purity: 100% Lot SW 70-093 **Unacceptable** Not Upgradeable	This developmental toxicity study in the rat is **UNACCEPTABLE/NOT UPGRADEABLE** and does not satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats. Several major study reporting deficiencies including a lack of animal data would not allow this study to be upgraded.
870.3700a Developmental Toxicity – oral gavage (rat)	**MRID 43935707** Fuchs, A. (1995) N-Octylisothiazolone (OIT) 96%: 14-Day Oral (Gavage) Dose Range-Finding Study in the Female Rat: Final Report: Lab Project Number: 1248-1154-050: 1154-050. Unpublished study prepared by Hazleton Deutschland GmbH. 108 p. Octhilinone administered orally to 3 groups of 8 female rats at 0, 10, 60 and 120 mg/kg for 14 days 8 female rats/dose Purity: 96% **Acceptable** Non-Guideline	**Short-term toxicity:** **NOAEL not established.** **LOAEL = 10 mg/kg/day based on clinical signs of salivation and gross pathology in the liver.** Clinical observations and gross pathology findings indicated dose-related signs of toxicity in all treatment groups. Observations of salivation and abnormal position increased in incidence and frequency of occurrence with increasing dose level. Piloerection, soft feces, and excessive urination were also noted in the 120-mg/kg/day animals. Treatment-related gross pathology findings included a prominent lobular pattern in the liver (≥10 mg/kg/day), and whitish-colored and thickened fundus of the stomach (≥60 mg/kg/day). Gastric hemorrhages of the fundus were also noted in one 120-mg/kg/day rat. Body weight gain and food consumption were reduced at ≥60 mg/kg/day. However, body weight gain was not statistically different from controls due to large standard deviations, and food consumption was only significantly reduced at the Day 1-3 treatment interval. This study had numerous deficiencies in meeting criteria for a repeat dose oral toxicity study in rodents. However, because the purpose of this study was for dose selection for a rat developmental toxicity study and not for fulfillment of a specific guideline, the information contained in this study may be scientifically useful for examining toxic effects from short-term oral exposure Based on the results of this study, doses of 5, 30, or 60 mg/kg/day were suggested for the rat developmental toxicity study.
870.3700b Developmental Toxicity – oral gavage (rabbit)	**MRID 41482509** Solomon, H.; Lutz, M. (1987) Skane M-8 HQ Industrial Mildewcide: Oral (Gavage) Developmental Toxicity Study in Rabbits: Lab Project Number: 87R-019: 86P-504. Unpublished study prepared by Rohm and Haas Co. 178 p. Octhilinone administered orally to 5 groups of 19 female rats at 0, 5, 20 and 80 mg/kg from days 7-19 of gestation.	**Maternal Toxicity:** **NOAEL = 20 mg/kg/day** **LOAEL = 80 mg/kg/day,** based on clinical signs of toxicity, decreased body weight and body weight gains, and increased number of abortions. **Developmental Toxicity:** **NOAEL = 20 mg/kg/day** **LOAEL = 80 mg/kg/day** based on decreased male fetal body weight and total (male and female) fetal body weight. Maternal toxicity was observed at 80 mg/kg/day and included clinical signs of toxicity (anorexia and scant, soft, or no feces), decreased body weight and body weight gains, and increased number of abortions.

	19 female rabbits/dose Purity: 46.3% **Acceptable** Guideline	There was a significant treatment-related decrease in male fetal body weight and total (male and female) fetal body weight at 80 mg/kg/day. There were no other treatment-related signs of developmental toxicity.
870.3700b Developmental Toxicity – oral gavage (rabbit)	**MRIDs 58029 and 136528** Powers, M. (1970) Teratology Study: Rabbits: RH-893 (Technical): Project No. 417-346. Final rept. (Unpublished study received Feb 9, 1971 under 707-100; prepared by Hazleton Laboratories, Inc., submitted by Rohm & Haas Co., Philadelphia, PA; CDL: 004372-L) Octhilinone administered orally at 0, 6 and 60 mg/kg from days 6-18 of gestation. 45 female rabbits/dose Purity: 100% **Unacceptable** Not upgradeable	**Maternal toxicity:** **NOAEL not established** **LOAEL = 6 mg/kg/day** based on mortality, body weight loss, decreased food consumption, and gross pathology findings. **Developmental toxicity:** **NOAEL and LOAEL not determined.** The test material was toxic at both dose levels and resulted in high maternal mortality rates. Death occurred in 6 low-dose dams (3 designated for Caesarean section and 3 designated for normal hutching) and 10 high-dose dams (6 designated for Caesarean section and 4 designated for normal hutching). Changes in body weight and food consumption appear treatment-related with those animals dying prior to scheduled sacrifice exhibiting body weight losses and decreased food consumption. Animals that died prior to study termination also showed gross pathology findings, with the predominate findings being dark, red lungs or chest cavity anomalies. There were no apparent treatment-related changes in the number of implantation sites compared to the number corpora lutea. Additionally, there were no apparent treatment-related changes in the number and placement of implantation and resorption sites or in the number of live and dead fetuses/pups. The lack of a treatment-related response could be the result of too few animals available for comparison. Additional data are needed to fully assess these endpoints. **The maternal LOAEL is 6 mg/kg/day based on mortality, body weight loss, decreased food consumption, and gross pathology findings. A Maternal NOAEL was not achieved.** There were no apparent developmental effects. Fetal and pup body weight and length in treated groups appeared to be comparable with the control groups. Skeletal changes occurred more frequently in high-dose fetuses; however, these effects predominately occurred in one litter where one out of five fetuses died. There were only two high-dose litters to examine. Therefore, it is difficult to draw any meaningful conclusions from these data. **A developmental NOAEL/LOAEL could not be determined.**
870.4200b Oncogenicity – Oral gavage (mice)	**TRID 470103024** **MRIDs 00139417, 00139419 and 00139484**	There was no RH-893 treatment effect on mortality, but both positive controls (i.e., DEN and AAF) caused an increase in mortality. No clinical findings were attributed to chronic feeding of octhilinone. High-dose RH-893 mice generally had reduced body weights in the beginning

	Hennigar, G.R.; Larson, P.S. (1974) Eighteen-Month Study in Which RH-893 Is Being Added to the Diet of Mice: Monthly Reports|. (Unpublished study received Jun 4, 1975 under 5F1632; prepared by Medical Univ. of South Carolina, Dept. of Pathology and Medical College of Virginia, Health Sciences Center, Dept. of Pharmacology, submitted by Rohm & Haas Co., Philadelphia, Pa.; CDL: 094944-D) Octhilinone administered orally at 500 or 1000 ppm for 78 weeks 125 mice/sex/dose Purity: Not reported Lot # not reported **Unacceptable** Not upgradeable	of the study compared to the negative control group; however, by Week 3 in males and Week 10 in females the high-dose RH-893 treated mice had comparable or greater body weight and body weight gain than the control group. Body weight was depressed in the positive controls. There were no treatment-related effects on liver weight or microscopic pathology in RH-893 treated mice. Positive controls did exhibit an increase in lesions. The DEN animals were found with an increased incidence of carcinoma of the hepatocytes in 6-mg/kg/day males and females at weeks 26 and 30; the 4-mg/kg/day animals were not examined histopathologically. The DEN 6-mg/kg/day males and females were found with bronchitis or bronchiectasis. The AAF mice had increased incidence of hyperplasia of the bladder in the males, urinary bladder carcinoma in both sexes and hepatocellular carcinomas in both sexes. Under the conditions of this 18-month feeding carcinogenicity study, there was no apparent carcinogenic potential demonstrated for RH-893 in either male or female (C 57BL/6 x C3H/anf) F_1 mice exposed to 500 or 1000 ppm. However, a number of study deficiencies precluded selection of a LOAEL or NOAEL for this study. At the doses tested, there was no treatment-related increase in tumor incidence when compared to controls; however, only 48 animals at the high dose were examined histologically. Therefore, any carcinogenic response would be potentially under-reported. Dosing was not adequate for this study, based on a lack of toxicity at the highest dose. Even considering the data from the preliminary 7-week study used to determine appropriate doses for the carcinogenicity study, a dose of 1500 ppm or one between 1000 and 1500 ppm may have been a more appropriate high dose for this 18-month carcinogenicity study. In the preliminary 7-week study a single male administered 1500 ppm died, which is why this dose was neglected.
870.5100 Bacterial reverse mutation test	**MRID 43935708** Ballantyne, M. (1995) N-Octylisothiazolone (OIT) 94 +/- 3% Reverse Mutation in 5 Histidine-requiring strains of Salmonella typhimurium. Hazelton GMBH, (Germany). Study #: 1154/53. Octhilinone administered to S. typhimurium strains at concentrations of 0.064, 0.32, 1.6,	**Negative** There was no evidence of induced mutant colonies over background at any concentration in repeat experiments. N-octylisothiazolone (96%) was tested up to cytotoxic concentrations and the limit concentration, 5000 ug/plate. The positive controls induced the appropriate responses in the corresponding strains.

	1.875, 3.75, 7.5, 8, 15, 30, or 40 ug/plate (+/-S9) Strain: TA98, TA100, TA102, TA1535, and TA 1537 of S. typh. Purity: 96% **Acceptable** Guideline	
870.5300 In Vitro mammalian cell gene mutation test	**MRID 43471606** Pant, K.J. (1994) Test for Chemical Induction of Gene Mutation at the HGPRT Locus in Cultured Chinese Hamster Ovary (CHO) Cells with and Without Metabolic Activation. SITEK Research Labs, (Rockville, MD), Report #: 93RC-231. Octhilinone administered in two independent CHO in vitro gene mutation assays at the S9 activation doses of 0.5, 1.0, 2.5, 5.0, 10.0 or 25 ug/lm (initial) or 2.5, 5.0, 6.0, 8.0, 9.0, 10 or 15 ug/ml (confirmatory) S9 derived from adult male Sprague-Dawley rat liver Purity: 98.8% **Acceptable** Guideline	**Negative** There was no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. CHO cells responded in the expected manner to the nonactivated and S9activatted positive controls. Cytotoxicity was achieved at levels >= 0.5 ug/ml S9 and >=10 ug/ml +S9.
870.5300 In Vitro mammalian cell gene mutation test	**MRID 43471607** Kumaroo, P.V. (1994) Test for Chemical Induction of Chromosome Aberration in Cultured Chinese Hamster Ovary (CHO) Cells with and Without Metabolic Activation. SITEK Research Labs, (Rockville, MD), Report #: 93RC-233.	**Negative** Cytotoxicity, as indicated by reduced mitotic indices (50%), was seen at >=0.7 ug/ml -S9 and 8.0 ug/ml +S9; interference with cell-cycle kinetics was also observed at nonactivated doses as low as 0.05 ug/ml and at S9-activated levels >=4.0 ug/ml. Slight increase in the frequency of cells bearing structural aberrations, particularly complex aberrations, were noted in cultures harvested after the prolonged recovery from exposure to the highest nonactivated or S9-activated dose. Complex structural aberrations were also scored at the remaining doses

	Octhilinone administered in two independent CHO in vitro cytogenetic assays at the S9 activation concentrations of6.0, 7.0 or 8.0 ug/ml (both trials). S9 derived from 4- 6 week male Sprague-Dawley rat liver Purity: 98.8% **Acceptable** Guideline	+/-S9. However, the increase in aberrant cells never exceeded 3% of the examined population and never approached a level of statistical significance. The evidence is, therefore, not sufficient to conclude that RH-287, tested up to cytotoxic levels, induced a clastogenic response in the presence or absence of S9 activation.
870.5300 In Vitro mammalian cell gene mutation test	**MRID 43935709** Clements, J. (1995) N-Octylisothiazole (OIT) 94+/-3%: Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphoma L5178Y Cells using the Microtitre Fluctuation Ttechnique. Corning Hazelton (North Yorkshire) Report #: 1154-54. Octhilinone administered in the mutation assays as follows: Initial: 8 doses at .1563, .3125, .625, 1.25, 2.5, 5, 10, or 20 ug/ml (+/-S9) Repeat: 6 doses at 0.125, .25, .50, .75, 1, or 1.25 ug/ml (without activation) 7 doses at .125, .25, .50, 1, 1.5, 2, or 2.5 ug/ml (with S9 activation) Test Cells: Mouse lymphoma L5178Y cells. Purity: 96% **Acceptable**	**Negative** N-Octylisothiazolone was tested up to toxic concentrations and the limit of solubility. Mutation frequencies were determined for concentrations selected on the basis of relative survival. In the initial mutation assay, concentrations selected were 0.156, 0.313, or 0.625 ug/ml nonactivated, and 0.156, 0.313, 0.625, or 1.25 ug/ml activated. In a repeat assay, concentrations selected were 0.25, .0.5, .0.75, 1, or 1.25 ug/ml nonactivated and 0.5, 1, 1.5, 2, or 2.5 ug/ml activated. For these concentrations, percent relative survival ranged from 27.4 to 100.7 without activation and from 15.4 to 120.3 with activation in the initial assay; and from 11.2 to 82.9 without activation and from 14.6 to 64.7 with activation in the repeat assay. No statistically significant increase in mutant frequency was observed at any dose level tested in the absence of S9 activation. A linear trend was observed in the absence of S9 in both assays and in the presence of S9 in one assay. However, the linear trends did not reflect a true dose-relationship in all cases and were not considered to be biologically significant. Therefore, it was determined that the test material was not mutagenic under both nonactivated and activated conditions in this in vitro assay. In both the nonactivated and activated conditions, the positive controls induced the appropriate responses.

	Guideline	
870.5385 Mammalian bone marrow chomosomal aberration test	**MRID 43935710** Riley, S. (1995) N-Octylisothiazole (OIT) 94+/-3%: Induction of Micronuclei in the Bone Marrow of Treated Mice. Corning Hazelton (North Yorkshire) Report #: 1154-55. Octhilinone administered orally at 163, 325, or 650 mg/kg/day 5 CD-1 Mice/sex/dose Purity: 96% **Acceptable** Guideline	**Negative** There was no significant increase in the frequency of MPCEs in bone marrow after any OIT treatment time; therefore, the test article is considered negative in this micronucleus assay
870.5900 In Vitro sister chromatid exchange assay	**MRID 41177901** Murli, H (1989) Mutagenicity Test on DCDIC in an In Vitro Cytogenetic Assay Measuring Sister-Chromatid Exchange (SCE) Frequencies in Chinese Hamster Ovary (CHO) Cells. Hazelton Labs. Study #: (HLA) 10855-0-438. DCDIC (octhilinone) administered at 2000, 3000, 4000 or 5000 ug/ml for 2 hours Species: Chinese hamster (ovary) Purity: not stated **Acceptable** Guideline	**Positive** Test article induced significant dose related increases in SCE in activated (S9-supplemented) CHO cell cultures under pH-controlled conditions spanning the (nontoxic) dose range 3000-5000 ug/ml.
870.5915 In Vivo sister chromatid exchange assay	**MRID 43471608** Sames, JL and Elia, MC (1994) RH-287 Technical: Micronucleus Assay in CD-1 Mouse Bone Marrow Cells.	A slight dose—related increase in MPEs was observed in the males of the mid- and high-dose groups at the 24-hour sacrifice. The increase was significant ($p<0.05$) at 325 mg/kg. However, the findings are only suspect and do not provide sufficient evidence to classify RH-287 as clastogenic/aaneugenic in this test system. This issue can only be resolved by exposing the test animals to the MTD.

	Rohm and Haas. Study #: Rpt No. 93P-232. DCDIC (octhilinone) administered orally at 32.5, 162.5 or 325 mg/kg 5, 5 or 7 mice/sex/dose Purity: 98.8% **Unacceptable**	

Table A4. Toxicity Profile for DCOIT

Guideline No./ Study Type	MRID No./ Reference Information/Dosing/ Study Classification	Dosing and Animal Information	Results
Subchronic Toxicity			
Non-Guideline 28-day oral (rat)	**MRID 42214903** RH-287 administered orally at 0, 20, 100, or 500 mg/kg/day, 28 days 10 rats/sex/dose Purity 97.5% **Acceptable** Non-Guideline	--	**Subchronic Toxicity:** **NOAEL = 20 mg/kg** **LOAEL = 100 mg/kg/day,** based on increased water consumption, hematological/clinical chemistry changes, histopathological lesions in stomach and small intestine. This 28-day repeated dose oral toxicity study is classified as **ACCEPTABLE NONGUIDELINE**
870.3100 90-day oral (rat)	**MRID 43471603** RH-287 administered orally at 0, 100, 500,1000, and 4000 ppm (equivalent to 0, 6.2/7.2, 32.5/36.7, 60.7/74.7, 248.2/278.4 mg/kg/day) 10 rats/sex/dose, 90 days	--	NOAEL = 32.5 mg/kg/day (M)/ 36.7 mg/kg/day (F) **LOAEL = 60.7 mg/kg/day** (M)/74.7 mg/kg/day (F) Based on microscopic forestomach lesions, decreased triglyceride levels. .

Guideline No./ Study Type	MRID No./ Reference Information/Dosing/ Study Classification	Dosing and Animal Information	Results
	Purity: 98.8% **Acceptable**		
870.3150 90-day oral (dog)	**MRID 45747201** RH-287 administered orally at 0, 100, 300 and 1500 ppm (3.4/3.4, 10.2/10.1, 47.5/45.9 mg/kg/day) 4 dogs/sex/dose, 13 weeks Purity: 98.4% **Acceptable**	--	NOAEL = 10.2 mg/kg/day (M)/10.1 mg/kg/day (F) **LOAEL = 47.5 mg/kg/day** (M)/ 25.9 mg/kg/day (F) Based on decreased hematology and clinical chemistry parameters
Non-Guideline 90-day inhalation (rat)	**MRID 43487501** Nose-only inhalation exposure to 0.02, 0.63, 6.72 mg/m3 6 hours per day, 5 days per week. Purity: 32.6% 10 Rats/sex/exposure **Acceptable**	--	**NOAEC = 0.02 mg/m3** **LOAEC = 0.63 mg/m^3 (**HEC=0.0045 mg/m^3**),** based on histological alterations of the nose, larynx, and lungs. HEC = NOAEC * (6-hour animal/8 hour human) * RDDR (0.30 for ET effects, BW= 420 grams, MMAD = 1.4 um, GSD = 4.6)
Developmental Toxicity			
870.3700a	**MRID 43471604**	--	**Maternal Toxicity:** **NOAEL = 10mg/kg/day**

Guideline No./ Study Type	MRID No./ Reference Information/Dosing/ Study Classification	Dosing and Animal Information	Results
Developmental Toxicity – oral gavage (rat)	RH-287 administered orally to 3 groups of 25 female rats at 0, 1, 10, 30, and 100 mg/kg from days 6-15 of gestation. 25 female rats/dose Purity: 98.8% **Acceptable** Guideline		**LOAEL = 30 mg/kg/day,** based on decreased food consumption, scant feces, soft feces, or diarhhea. **Developmental toxicity** **NOAEL = 30 mg/kg/day** **Developmental toxicity LOAEL = 100,** based on increased incidence of wavy ribs (21 fetuses in 11 litters vs. 2 fetuses from 1 litter in control).
2-generation reproduction toxicity-rats	MRID 45756501 RH-287 administered in the diet to groups of 26 rats/sex/dose at dose levels of 0, 200, 800, or 3200 ppm (0, 16-20, 62-88, and 235 m/k/d (M) and 0, 18-21, 67-93, 259 m/k/d (F) Purity: 100.3% **Acceptable**	--	**$NOAEL_{Parental}$** = 33-39 mg/kg/day (M)/ 33-41 mg/kg/day (F). **$LOAEL_{Parental}$** = 62-88 mg/kg/day (M)/ 67-93 mg/kg/day (F) based on decreased body weight/weight gain. **$NOAEL_{Offspring}$** = 16-20 mg/kg/day (M)/ 18-21 mg/kg/day (F) **$LOAEL_{Offspring}$** =30-39 mg/kg/day (M)/ 33-41 mg/kg/day (F) based on decreased absolute and relative spleen and thymus weight
Carcinogenicity			
No data available			
Mutagenicity			
870.5100 Bacterial reverse mutation test	**MRID 43935708** Ballantyne, M. (1995) N-Octylisothiazolone (OIT) 94 +/- 3% Reverse Mutation in 5 Histidine-requiring strains of Salmonella typhimurium. Hazelton GMBH,	Octhilinone administered to S. typhimurium strains at concentrations of 0.064, 0.32, 1.6, 1.875, 3.75, 7.5, 8, 15, 30, or 40 ug/plate (+/-S9)	**Negative** There was no evidence of induced mutant colonies over background at any concentration in repeat experiments. N-octylisothiazolone (96%) was tested up to cytotoxic concentrations and the limit concentration, 5000 ug/plate. The positive controls induced the appropriate responses in the corresponding strains.

Guideline No./ Study Type	MRID No./ Reference Information/Dosing/ Study Classification	Dosing and Animal Information	Results
	(Germany). , Study #: 1154/53. **Acceptable** Guideline	Strain: TA98, TA100, TA102, TA1535, and TA 1537 of S. typh. Purity: 96%	
870.5300 In Vitro mammalian cell gene mutation test	**MRID 43471606** Pant, K.J. (1994) Test for Chemical Induction of Gene Mutation at the HGPRT Locus in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation. SITEK Research Labs, (Rockville, MD), Report #: 93RC-231. **Acceptable** Guideline	RH-287 administered in two independent CHO in vitro gene mutation assays at non-S9 activated doses of 0.005, 0.025, 0.05, 0.1, and 0.5 ug/ml (initial trial) and 0.025, 0.05, 0.1, 0.2, 0.4, 0.5, and 0.75 ug/ml (confirmatory) S9 activated doses of 0.5, 1.0, 2.5, 5.0, 10.0 or 25 ug/lm (initial) or 2.5, 5.0, 6.0, 8.0, 9.0, 10 or 15 ug/ml (confirmatory) S9 derived from adult male Sprague-Dawley rat liver Purity: 98.8%	**Negative** There was no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. CHO cells responded in the expected manner to the nonactivated and S9activatted positive controls. Cytotoxicity was achieved at levels >= 0.5 ug/ml -S9 and >=10 ug/ml +S9.
870.5375 In Vitro mammalian chromosome aberration test	**MRID 43471607** Kumaroo, P.V. (1994) Test for Chemical Induction of Chromosome Aberration in Cultured Chinese Hamster Ovary (CHO) Cells With and	RH-287 exposed to cell cultures at non-activated doses of 0.3, 0.6, 0r 0.7 ug/ml (initial trial) or 0.5,	**Negative** Cytotoxicity, as indicated by reduced mitotic indices (50%), was seen at >=0.7 ug/ml -S9 and 8.0 ug/ml +S9; interference with cell-cycle kinetics was also observed at nonactivated doses as low as 0.05 ug/ml and at S9-activated doses of 7 and 8 ug/ml. A slight increase in the frequency of cells bearing structural aberrations, particularly complex

Guideline No./ Study Type	MRID No./ Reference Information/Dosing/ Study Classification	Dosing and Animal Information	Results
	Without Metabolic Activation. SITEK Research Labs, (Rockville, MD), Report #: 93RC-233. **Acceptable** Guideline	0.6, or 0.7 ug/ml (confirmatory trial) S9 activated doses of 6, 7, or 8 ug/ml (both trials)	aberrations, were noted in cultures harvested after the prolonged recovery from exposure to the highest nonactivated or S9-activated dose. Complex structural aberrations were also scored at the remaining doses +/-S9. However, the increase in aberrant cells never exceeded 3% of the examined population and never approached a level of statistical significance. The evidence is, therefore, not sufficient to conclude that RH-287, tested up to cytotoxic levels, induced a clastogenic response in the presence or absence of S9 activation.
870.5915 In Vivo sister chromatid exchange assay	**MRID 43471608** Sames, JL and Elia, MC (1994) RH-287 Technical: Micronucleus Assay in CD-1 Mouse Bone Marrow Cells. Rohm and Haas. Study #: Rpt No. 93P-232. **Unacceptable**	RH-287 administered orally at 32.5, 162.5 or 325 mg/kg 5, 5 or 7 mice/sex/dose Purity: 98.8%	A slight dose—related increase in MPEs was observed in the males of the mid- and high-dose groups at the 24-hour sacrifice. The increase was significant ($p<0.05$) at 325 mg/kg. However, the findings are only suspect and do not provide sufficient evidence to classify RH-287 as clastogenic/aaneugenic in this test system. This issue can only be resolved by exposing the test animals to the MTD.

Table A-5. Toxicity Profile for CMIT/MIT

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
Subchronic Toxicity			
870.3100 90-day oral (rat)	**MRID 42810101** DiDonato, LJ and Hara, GPO. (1982) Kathon 886 NAR: Three month rat drinking water study and one generation reproduction	Kathon 886 administered orally in drinking water at 0, 25, 75 or 225 ppm (equivalent to 0, 2.4, 6.3, and 16.3	**Subchronic Toxicity:** **NOAEL = 75 ppm (6.3/ 10.8 mg/kg/day M/F)** **LOAEL = 225 ppm (16.3/24.7 mg/kg/day M/F)**, based on microscopic findings in the stomach on both sexes.

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
	study.: Project No. 417-320., submitted by Rohm & Haas Co., Philadelphia, Report No.: 81R-162 **Acceptable Guideline**	mg/kg/day (M) and 0, 4.1, 10.8, or 24.7 mg/kg/day) (F) 25 rats/sex/dose, 90 days Purity: 15.5%	There was a slight decrease (96% of control) in body weight in the high dose males during weeks 1 and 2 and decreases in body-weight gain in both sexes (males 82-89% and females 82-85% of control) at the high dose level during the first two weeks of the study. The mid- (90-91% of control) and low-dose (86-88% of control) females also displayed decreases in body weight gains compared to the controls during weeks 1 and 2, but there was no dose response. A dose-related decrease in food consumption was observed in males during weeks 1 through 3, which was statistically significant at all dose levels during weeks 1 and 3. Females displayed a dose-related decrease in food consumption during the first two weeks, which was statistically significant at the high dose during week 1 and at the mid- and high-dose levels during week 2. No adverse effects were observed on hepatic mixed function oxidase activity in either sex. Although differences in several parameters were observed (↓ cholesterol in females, ↑ SGOT in females, ↓ BUN, foci of erosion and focal blunting of the superficial epithelium of the glandular mucosa of the stomach in both sexes), there were no toxicologically significant effects observed in either sex. This 90-day oral toxicity study in the rat is **ACCEPTABL** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rodents.
870. 14-day dermal (rat)	**MRID 43834701** Zuhlke, U. (1994) Acticide 14, 14 Day Dermal Dose Range-Finding Study in the Rat. Hazelton, Report No: 1127-1154-001; Project No. 1154-001. Unpublished **Supplementary for MRID #: 43462005 90-Day Dermal Study**	Kathon 886 applied dermally at 0, 0.288, 2.88 and 28.8 mg/kg/day 5 rats/sex/dose Purity: 13.9%	**Systemic Toxicity** **NOAEL = 28.8 mg/kg/day (HDT)** **LOAEL = NA** **Dermal Toxicity** **NOAEL= 2.88 mg/kg/day** **LOAEL = 28.8 mg/kg/day**, based on skin alterations Dermal application of Acticide resulted in skin effects in both sexes at the high-dose level only. There were no adverse effects on survival, body-weight gain, food consumption, or gross lesions [except skin] at any dose in either sex.

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
870.3200 21-day dermal (rat)	**Open literature** Evaluation of the toxicity of kathon Biocide, (1984) Tox Dept. Rohm and Haas, pp59	0.1 % Kathon 886 aqueous solution applied topically at 0.1 and 5 mg/kg/day, 5 days/wk 10 rabbits/sex/dose Purity: technical	Moderate dermal irritation was observed in all treated animals. Therefore, it is concluded that RH-886 produced no evidence of systemic toxicity.
870.3250 90-dermal (rat)	**MRID 43462005** Zuhlke, U. (1994) Acticide 14, 90 Day Dermal Subchronic study in the rat. Hazelton, Report No: 1127-1154-002; Project No. 1154-002. Unpublished **Acceptable** Guideline	Kathon 886 applied dermally at 0, 0.75, 3.75 and 18.75 mg/kg/day for 13 weeks 10 rats/sex/dose Purity: 13.9%	**Systemic Toxicity:** **NOAEL = 18.75 mg/kg/day (HDT)** **Dermal Toxicity:** **NOAEL =** not determined **LOAEL =** not determined There were no adverse effects on survival, body weight gain, food consumption, hematology, clinical chemistry, urinalysis, ophthalmoscopy, gross and microscopic lesions [except skin] at any dose level in either sex.
870.3465 90-day inhalation (rat)	**MRID 00148418** Hagan, JB, and Baldwin, BC (1984) kathon 886 MMPA process 13-week inhalation toxicity study in rats. Rohm and Haas. Rpt. #: 82R-245 **Acceptable** Guideline	Kathon 886 administered via inhalation at 0.34, 1.15, or 2.64 mg/m3 16 Rats/sex/exposure Purity: 14.8%	**NOAEL = 0.34 mg/m3** **LOAEL = 1.15 m.g/m3,** based on microscopic lesions in the nasal turbinates (rhinitis). With the exception of decreased body weight gain, there were no toxicologically significant effects observed in either sex, but the corrosive properties of the test material imposed limitation on the dose levels tested for any duration. There were treatment-related deaths, and no effects were observed in the hematology, clinical chemistry, ophthalmoscopic, and gross pathology parameters monitored that could be attributed to treatment. Treatment-related lesions in the nasal turbinate were observed at the mid- and high-dose levels, which consisted of eosinophilic droplets in the anterior respiratory mucosa (2.64 mg/m3) and rhinitis in the lining of the anterior portion of the nasal cavity (1.15 and 2.64

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
			mg/m3). These are consistent with a normal physiological response to a respiratory irritant.
Developmental Toxicity			
870.3700a Developmental Toxicity – oral gavage (rat)	**MRID 00078831 (HED chapter)** Weatherholtz, W.M. et al. (1980) Kathon: Teratogenicity study in rats; Project No.: 417-399. Rohm and Hass Company. Report No. 80RC-081 (also in CAL EPA) **Classified as Unacceptable but upgradeable in CAL EPA document)**	Kathon 886 administered via gavage to 25 rats/group at 0, 10, 30 or 100 mg/kg from days 6-15 of gestation. 25 female rats/dose Purity: 14%	**Maternal Toxicity:** **NOAEL = 10mg/kg/day** **LOAEL = 30 mg/kg/day,** based on decreased body weight gains, with support from the dose-related increase in deaths. **Developmental Toxicity:** **NOAEL = 100 mg/kg/day** (highest dose tested) **LOAEL not established.** There were no adverse findings during the visceral or skeletal examinations of the fetuses that could be attributed to treatment. Kathon 886 was found not to be fetotoxic, embryotoxic, or teratogenic in rats.
870.3700a Developmental Toxicity – oral gavage (rat)	**MRID 43419615** Fuchs, A. (1994) Acticide 14 Oral (Gavage) Teratogenicity Study in the Rat; Project No.: 1154-003.; Report No. 1178-1154-003. Rohm and Hass Company. **Acceptable Guideline**	Kathon 886 administered orally to groups of 25 rabbits at 0, 28, 70 or 139 mg/kg from days 6-15 of gestation. 25 female rabbits/dose Purity: 13.9%	**Maternal Toxicity:** **NOAEL = 28 mg/kg/day** **LOAEL = 70 mg/kg/day,** based on clinical signs [gasping/wheezing respiration] **Developmental Toxicity:** **NOAEL = 139 mg/kg/day (HDT)** All mated females survived, but there was decreased body-weight gain at the high-dose level during days 6-9 of the dosing period [55% of control] and over the entire dosing period [80% of control] with a concomitant decrease [87-91% of control] in food consumption during the first and last third of the dosing period, a slight, non-statistically significant decrease [76% of control] in body-weight gain at the mid-dose level during days 6-9 of dosing; and treatment-related clinical signs [gasping/wheezing respiration] at the mid- and high-dose levels. There were no statistically significant differences in the number of corpora lutea/dam, implantations/dam, live fetuses/dam,

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
			resorptions/dam, dead fetuses/dam, or in pre- or post-implantation losses, litter weight, or fetal body weight (combined and per sex). There were comparable numbers of fetuses and litters with variations [external, visceral and skeletal] among the groups, and although the number of fetuses with malformations increased with increasing dose, there were more fetuses available for examination at all dose levels compared to the Control. Additionally, there was a slight, comparable increase in the number of fetuses [3] and litters with fetuses [3] with external/visceral malformations at the two highest dose levels compared to the low and control incidences [1/1], and the mean litter percent of malformations was slightly increased at all dose levels compared to the control [0.39, 0.98. 0.98 and 1.68 for the control, low-, mid-, and high-dose groups, respectively.
870.3700b Developmental Toxicity – oral gavage (rabbit)	**MRID 42311701** Thomas, TL; Solomon, HM and O'Hara, GP (1992) Kathon Biocide: Oral (gavage) developmental toxicity study in rabbits. Study prepared by Rohm and Haas Co. Report No. 91R-074 **Acceptable**	Kathon 886 administered orally to groups of 16 rabbits/group at .0.5, 2, 8 or 20 mg/kg from days 7-19 of gestation. 16 female rabbits/dose Purity: 13.4%	**Maternal Toxicity:** **NOAEL = 2 mg/kg/day** **LOAEL = 8 mg/kg/day** based decreased body weight gain, corrected body weight, food consumption, and scant/no feces and diarrhea. **Developmental Toxicity:** **NOAEL = 2 mg/kg/day** **LOAEL = 8 mg/kg/day** based on the slight increase in fetal alterations. Although there were no statistically significant differences in the incidence of any fetal alteration (external, visceral or skeletal malformations, variations, or retarded development), there was a tendency for these to occur to a greater degree at the 8 mg/kg/day dose level than in the control or other treatment groups.
Reproduction			
870.3800 Reproduction Toxicity – oral (rat)	**MRID 44656101** Robinson, P., L.P. Craig and T.L. Danberry. (1989) Kathon 886F Biocide: Two-Generation Reproductive Toxicity Study in Rats: Report Number: 96R-189:	Kathon 886F Biocide was administered to groups of 26 male and 26 female Cr1:CD BR rats at drinking water concentrations of 0, 30, 100, or 300 ppm for two generations.	**Parental Toxicity:** **NOAEL: 30 ppm (2.8, 4.4 mg/kg/day M/F)** **LOAEL: 100 ppm (8.5 (M) and 11.8 (F) mg/kg/day),** based on reduced water consumption and histopathological lesions in the stomach of F_0 and F_1 males and females**.** **Reproductive Toxicity:** **NOAEL: ≥300 ppm (≥22.7, ≥28.0 mg/kg/day M/F)**

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
	Unpublished study prepared by Rohm and Haas Co. **Acceptable** Guideline	26 rats/sex/dose Purity: 14.8%	**LOAEL: not determined** One litter was produced in each generation. Premating doses were 2.8, 8.5, and 22.7 mg/kg/day, respectively, for F_0 males and 4.4, 11.8, and 28.0 mg/kg/day, respectively, for F_0 females. Premating doses were 4.3, 13.4, and 35.7 mg/kg/day, respectively, for F_1 males and 5.5, 16.0, and 39.1 mg/kg/day, respectively, for F_1 females. F_1 adults were chosen from the F_1 pups and administered the same drinking water concentration as their parents. Animals were given treated or control water for at least 10 weeks before mating within the same dose group. All animals were continuously exposed to test material either in the drinking water or during gestation and lactation until sacrifice. Several intercurrent deaths of the F_0 males were considered incidental to treatment. No treatment-related clinical signs of toxicity were observed in males or females of either generation at any time during the study. Food consumption was not affected by treatment. Gross necropsy of the parental animals was unremarkable. Body weights and body weight gains of the low- and mid-dose F_0 males and females and of all treated F_1 males and females were not affected at any time during the study. Body weight gain of the F_0 high dose males and females were similar to the controls showing changes of <10%. Body weights of the high-dose F_0 males were significantly (95-96% of control; $p \leq 0.05$) less than the water control group, but not different from the salt control group, during weeks 2-4. Cumulative weight gains by the high-dose F_0 males were significantly (78-91% of controls; $p \leq 0.05$) less than both control groups through week 5 of the premating interval. Absolute body weights of the high-dose F_0 females were similar to both control groups throughout the premating interval. Body weight gains by the high-dose F_0 females were significantly (80-81% of controls; $p \leq 0.05$) less than both control groups during week 0-1. In the mid- and high-dose F_0 and F_1 males and females, water consumption was significantly ($p \leq 0.05$) less than one or both control

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
			groups throughout the premating interval. As compared to one or both control groups, water consumption by the mid- and high-dose groups was 70.5-91.9% and 65.5-73.8%, respectively, by F_0 males, 63.3-83.3% and 49.4-68.2%, respectively, by F_0 females, 74.4-91.7% and 63.5-79.7%, respectively, for F_1 males, and 62.7-82.3% and 47.5-70.2%, respectively, for F_0 females. Water consumption by the low-dose F_0 males and females and F_1 males was occasionally significantly ($p \leq 0.05$) less than one or both control groups. Low-dose F_1 females had significantly (71.6-89.4% of controls; $p \leq 0.05$) reduced water consumption throughout premating as compared to one or both control groups. Similar decreases in water consumption were observed for the F_0 and F_1 females during gestation and lactation and are considered a continuation of the systemic effects. Histopathological examination of the reproductive organs from the F_0 and F_1 adults was unremarkable. Treatment-related microscopic findings were observed in the stomachs of the high-dose parental animals of both generations: focal superficial erosions, edema and inflammation in the submucosa of the glandular and nonglandular areas (except F_1 females) and hyperplasia and hyperkeratosis of the limiting ridge of the nonglandular stomach occurred vs. the controls. The mid dose level animals of both generations showed an increase in hyperplasia/ hyperkeratosis compared to the controls while the F_1 females showed an increase in erosions. Other lesions, in both sexes and generations seen at the mid dose levels were comparable to the controls. No treatment-related effects were noted on maternal body weights, body weight gains, or food consumption during gestation and lactation. Mating fertility, gestation, and parturition indices, mean precoital interval, and mean gestation length were similar between the treated and control groups of both generations. Mean litter size, live births, and pup survival were also similar between the treated and control groups for both generations. No treatment-related clinical signs of toxicity were observed in the F_1 or F_2 pups during lactation.

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
			Mean pup body weights during lactation were similar between treated and control groups for both generations. Gross necropsy of the pups was unremarkable. No histological lesions in the stomach were observed in the pups.
Carcinogenicity			
870.4300 Chronic/Carcinogenicity (rat)	**MRID 43140701** (1994) Kathon Biocide: 24-Month drinking water chronic/oncogenicity study in rats., submitted by Rohm & Haas Co., Philadelphia, Pa.Report No. 90R-149. **Core-Minimum** Guideline	Kathon administered via drinking water at 30, 100 or 300 ppm (2.0/3.1, 6.6/9.8 or 17.2/25.7 mg/kg (M/F) for 24 months 90/80 (M/F) rats/dose Purity: 14.2%	**NOAEL: 30 ppm (2/3.1 mg/kg/day (M/F))** **LOAEL: 100 ppm (6.6/98 mg/kg/day (M/F)),** based on microscopic lesions (hyperplasia/hyperkeratosis in both sexes, necrosis of glandular mucosa in females) in the stomach. Decreased body weight [95-98% of control]/body weight gain [87% of control at week one, from 91-98% thereafter] were observed in the high-dose males, although statistical significance was not always attained. Females displayed an equivocal decrease in body weight throughout the study, with the mid- [93-96% of control] and high-dose [87-96% of control] groups showing comparable decrease that were not always dose-related. During the second year, body-weight gains of the high-dose females were significantly decreased [83-88% of control]. High dose males displayed a significant decrease (91-98% of control) in food consumption compared to the control groups throughout most of the study, and females at all dose levels displayed significant decreases in food intake but a dose response was not always evident. The hematology and clinical chemistry parameters monitored were comparable among the groups of both sexes, as were the organ weights and eyes. During the first 6 months of the study, the specific gravity of the urine was increased in both sexes, which may be attributed to the decrease in water consumption. With the exception of the stomach, none of the gross and non-neoplastic lesions observed could be attributed to treatment. AN increased incidence in hyperplasia and hyperkeratiosis of the squamous mucosa of the stomach was observed at the mid- and high-dose levels in both sexes, which correlated with the finding of prominence of the limiting ridge and/or thickened nonglandular mucosa of the forestomach on gross examination. There was no treatment-related increase in the incidence of any tumor in either sex.

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
Mutagenicity			
870.5265 S. Typhimurium reverse mutation assay	**MRID 43419616** Clare, CB (1994). Study to Determine the Ability of Acticide 14 to Induce Mutation in Five Histidine-requiring Strains of Salmonella typhimurium [Reverse Mutation Assay]; Hazelton Microtest, Harrogate, England; Report No. S15RETU **Acceptable Guideline**	Kathon 886 administered to S. typhimurium strains at nonactivated doses of 1.25-15 µg/plate (both trials) and S9-activated doses of 6.25-100 µg/plate (Trial I) or 10-80 µg/plate (Trial II) Strain: TA98, TA100, TA102, TA1535, and TA1537, or TA1538 of S. typh. Purity: 13.9%	**Positive** Severe cytotoxicity was seen at ≥50 µg/plate –S9 and ≥250 µg/plate +S9; cytotoxic effects were still apparent at ≥525 µg/plate-S9 and ≥100 µg/plate +S9. There was also a clear, reproducible and dose-related evidence that the nonactivated test material induced a powerful mutagenic response (14-16.5-fold increase in histidine revertants of TA100 at ≥15 µg/plate to a 1.6-fold increase in mutant colonies at 1 µg/plate). Mutagenic activity was also detected in strains TA1535, TA1537, TA1538, TA98 and TA102 in both nonactivated trials. Genotoxicity was also seen in the S9-activated conditions (4.4-fold increase in revertant colonies of strain TA100) was observed at 80 µg/plate. In general, reactivity was limited to this strain under S9-activated conditions.
870.5100 Typhimurium reverse mutation assay	**MRID 00078827/00096992** Scribner, HE; Melly, JG and Lohse, KL (1981). Kathon 886 MW: Microbial mutagen test. Rohm and Haas Company Report No. 81-R-96	Kathon 886 administered to S. typhimurium strains at concentrations of 0, 20, 50, 200, 500 and 1000 ug/plate with activation; and 5 to 500 ug/plate without activation Strain: TA98, TA100, TA102, TA1535, and TA1537 Purity: 97.5%	**Negative** The test compound did not cause a positive increase in the number of histidine revertants in any of the testerstrins either in the presence or absence of mammalian mincrosomal enzymes under test organisms.

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
870.5100 S. Typhimurium reverse mutation assay (MIT)	**MRID 44958404** Sames, JL and Streelman, DR (1999). Kordek 573T: Salmonella Typhimurium Gene Mutation Assay (Ames Test). Rohm and Haas Company Study No. 99R-062	Kordek 573T (97.5% a.i.) administered to S. typhimurium strains TA98, TA100, TA1535, TA1537, and TA102 at concentrations ranging from 5-1000 ug/plate (+/-S9). . Purity: 97.5% a.i.	Kordek 573T did not induce a mutagenic response in any of the Salmonella strains tested in the absence or presence of metabolic (S9) activation.
870.5275 Sex-linked recessive lethal test in Drosophila melanogaster	**MRID 00130751** Valencia, R. (1982). Drosophila sex-linked recessive lethal test on Kathon. Rohm and Hass, Report No. 82P-152.	Kathon 886 administered orally at 86 µg/ml and via injection at 258 µg/ml Purity: Not reported	**Negative**
870.5300 In vitro mammalian cell gene mutation test	**MRID 43419617** Clements, J. (1994) Study to Determine the Ability of Acticide 14 to Induce Mutations at the Thymidine Kinase (tk) Locus in Mouse Lymphoma L5178Y Cells Using a Fluctuation Assay; Hazelton Microtest, Harrogate, England; Report No. 2TKRETUC.001. Unpublished. **Acceptable Guideline**	Mice cells exposed in microsuspension to nonactivated doses of Acticide 14 ranging from 0.375-12 µg/mL and S9-activated levels of 0.1875-6 µg/mL (initial trial) and doses of 0.5-6 µg/mL +/- (confirmatory trial) Test cells: mouse lymphoma L5178Y cells Purity: 13.9%	**Positive** Cytotoxicity (i.e., ≤10% total viability) was seen at ≥5 µg/mL –S9 and at 6 µg/mL +S9. The positive controls induced the expected response in the target cells in both trials. Reproducible and dose-related increases in the mutation frequency (MF) were seen at 1-6 µg/mL –S9 (confirmatory trial) with fold increase in mutation ranging from 1.8 to 9.4, respectively (the MF of the solvent control was $145.17x10^6$). Although the high MF at 6 µg/mL was accompanied by severe cytotoxicity (0.5% survival), a MF of $742.91x10^6$ (5.1-fold increase) was calculated at 4 µg/mL with 18.7% survival. In the presence of S9 activation, dose-related mutagenesis was detected over a concentration range of 2-6 µg/mL in the confirmatory trial with MFs of $247.18\text{-}478.50x10^6$, respectively, versus a MF of 173.52 x10 of the solvent control. Under both conditions of testing and in both trials, Acticide 14 induced large and small mutant colonies. The overall findings provide compelling evidence that Acticide 14 is a mutagen in this in vitro test system.

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
870.5375 In vitro cytogenetics assay in cultured human lymphocytes	**MRID 43419618** Marshall, R. (1994) Study to Evaluate the Chromosome Damaging Potential of Acticide 14 by its Effects on Cultured Human Peripheral Blood Lymphocytes Using an In Vitro Cytogenetics Assay; Hazelton, Report No.2HLRETUC.001R. Unpublished. **Acceptable Guideline**	Human lymphocytes exposed to Acticide 14 at nonactivated doses of 1.394-5000 µg/mL (initial trial) or 33.55-250 µg/mL (confirmatory trial) or 70.61-250 µg/mL + S9 (initial and confirmatory trial). S9 fraction derived from Aroclor 1254 induced Sprague-Dawley male rat livers 1 male and 1 female human donor. Purity: 13.9%	**Positive** A marked reduction in the mitotic index was seen in cultures treated with ~ 160 µg/mL +/-S9. Findings with the positive controls confirmed the sensitivity of test system to detect clastogenesis. There was, no reproducible evidence that the nonactivated test material was clastogenic. However, S9-activated Acticide 14 at 132.9, 147.6 and 164 µg/mL (initial trial) and at 119.6 and 132.9 µg/mL (confirmatory trial) induced significant (generally $p \leq 0.001$) increases in the yield of cells with abnormal chromosome morphology. Only the low dose in the confirmatory trial (107.6 µg/mL) was negative. The test material induced both chromatid-and chromosome-type aberrations; however, chromatid-type damage predominated. Therefore, it can be concluded that Acticide 14 is active in this system.
870.5385 Mammalian bone marrow chomosomal aberration test	**MRID 43935710** Riley, S. (1995) N-Octylisothiazole (OIT) 94+/-3%: Induction of Micronuclei in the Bone Marrow of Treated Mice. Corning Hazelton (North Yorkshire) Report #: 1154-55.	Kathon 886 administered orally at 3, 15 or 30 mg/kg/day Mice/sex/dose Purity: Not reported	**Negative** Kathon 886 did not cause a significant increase in the frequency of structural chromosome aberrations in mouse bone marrow cells.
870.5395 Mammalian erythrocyte micronucleus test	**MRID43419619** McEnaney, S. (1994) N-Octylisothiazole (OIT) 94+/-3%: Induction of Micronuclei in the Bone Marrow of Treated Mice.	Acticide 14 administered via a single oral gavage doses of 7.5, 15 or 30 mg/kg once daily for 2 consecutive days	**Negative** The positive control induced the expected high yield of MPEs in males and females. No overt toxicity in the treated animals or cytotoxicity in the target organ was seen at any dose or sacrifice time. There was also no indication that the test material induced a

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
	Corning Hazelton (North Yorkshire) Report #: 1154-55. **Unacceptable**	5 CD-1 mice/sex/dose Purity: 13.9%	clastogenic or aneugenic effect in either sex at any dose or sacrifice time. However, the inability to demonstrate that the maximum tolerated dose (MTD) was achieved renders the study unacceptable. This is of particular concern since S9-activated Acticide 14 induced a clear clastogenic response in the in vitro human lymphocyte cytogenic assay (see MRID 43419618). Based on the above considerations, it was concluded that the findings of this in vivo study do not support a negative conclusion for the test material.
870.5550 Unscheduled DNA synthesis in mammalian cells in culture	**MRID 43419620** Ward, PJ (1994) Study to Evaluate the Potential of Acticide 14 to Induce Unscheduled DNA Synthesis in Rat Liver Using an In Vivo/In Vitro Procedure; Hazelton Microtest; report No. ILURETUC.001. Unpublished. **Acceptable Guideline**	Acticide 14 administered via oral gavage at doses of 19 or 60 mg/kg Groups of six male rats Purity: 13.9%	**Negative** Two deaths, attributable to treatment, occurred at 60 mg/kg; there was no evidence of a cytotoxic effect on the target organ. The results obtained with the positive controls confirmed the sensitivity of the test system to detect UDS. There was, however, no indication of a genotoxic responses at any dose or sacrifice time.
Metabolism			
870.7485 Metabolism and pharmacokinetics	**MRID 41101402** Udinsky, J.R. , T.B. Tran and C.B. Frederick (1988) Kathon Biocide: Comparison of ^{14}C-Metabolite Profiles Following Oral and Dermal Dosing in Male Rats; Rohm and Hass; report No. 86R-232. Unpublished.	**Oral dosing:** 14C-Kathon (RH-651 or RH-573) was administered via gavage at 6.25 mg/kg **Dermal dosing:** 14C-Kathon (RH-651 or RH-573) applied at a dose of 1.67 mg/kg Animal: **oral**; 6 male CD rats and **dermal;** 3 male CD rats	Oral dosing of Kathon radiolabeled in either isothiazolinone, RH-651 or RH-573, resulted in very rapid excretion of the dosed radioactivity in the urine (50 to 77%) and feces (23 to 54%) by 24 hr after dosing. By contrast, dermal dosing resulted in much slower elimination with most of the excreted radioactivity appearing in the urine (20 to 28%) of the applied dose) and very little in the feces (1 to 2%) by 48 hr after dosing. These results generally correspond to those previously reported for these compounds. The profile of urinary metabolites observed following either dermal or oral dosing of RH-651-labeled 14C-Kathon appears to be qualitatively similar; differences were observed regarding the relative amounts of specific metabolites. It may be concluded that metabolism of RH-651-labeled 14C-Kathon is similar following either dermal or oral dosing. Based on chromatography with

Guideline No./ Study Type	MRID No./ Reference Information/ Study Classification	Dosing and Animal Information	Results
		Purity: 98.1%	synthetic standards and chromatographic behavior (comparative retention times with or without an alkyl amine paired ion reagent) the urinary metabolites of RH-651-labeled Kathon are concluded to be small polar organic acids. None of the parent RH-651 was recovered in the urine unchanged. Since the rate of excretion for RH-651-labeled 14C-Kathon is much less following dermal dosing, the amount of each radiolebelled urinary metabolite excreted following dermal dosing is quite low. The profiles of urinary metabolites observed following either dermal or oral dosing of RH-573-labeled 14C-Kathon are similar; the dominant peak in both profiles (29.1-30.5 min) is an unknown metabolite. Quantitative differences in the relative amounts of specific metabolites of 14C-RH-573 labeled Kathon following oral and dermal dosing were observed. None of the parent RH-573 was recovered in the urine unchanged. The use of 3H-radiolabelled glutathione and either 14C-RH-651 or 14C-RH-573 provided no evidence for the formation of a covalently bound product containing both radioactive isotopes indicating that there was no conjugate formation.

Appendix B. Isothiazolinones: Cumulative Screening Analysis Memorandum

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: **April 3, 2020**

SUBJECT: **Isothiazolinones:** Screening Analysis of Toxicological Profiles to Consider Whether a Candidate Common Mechanism Group Can Be Established

PC Codes: 098951, 128101, 098901, 107104, 107103, 099901	DP Barcode: NA
	Reg Review Docket number: See below
Regulatory Action: Registration Review	Case No.: See below
Risk Assessment Type: NA	CAS No.: See below

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THROUGH: Melissa Panger, Ph.D., Branch Chief
Risk Assessment and Science Support Branch
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AND

Anna Lowit, Ph.D., Senior Science Advisor
Immediate Office
Office of Pesticide Programs

This document provides a screening analysis to determine whether a cumulative assessment for the isothiazolinones needs to be conducted.

In 2016, EPA's Office of Pesticide Programs released a guidance document entitled, *Pesticide Cumulative Risk Assessment: Framework for Screening Analysis Purpose* (https://www.regulations.gov/document?D=EPA-HQ-OPP-2015-0422-0019). This document provides guidance on how to screen groups of pesticides for cumulative evaluation using a two-step approach. This begins with the evaluation of available toxicological information and, if necessary, is followed by a risk-based screening approach. This framework supplements the existing guidance documents for establishing common mechanism groups (CMGs)[6] and conducting cumulative risk assessments (CRA)[7]. This CRA screening-level approach ultimately allows the Agency to address the FFDCA statutory requirements while efficiently using resources.

The Agency's screening of chemical groups involves a weight-of-evidence analysis of all relevant scientific information to determine whether a candidate CMG can be supported. Under the framework, the process for evaluating a group of pesticides for a potential common mechanism of toxicity begins with several considerations: chemical structural similarity, toxicological profile, and information on the mode of action/adverse outcome pathway (MOA/AOP). Shared chemical structure may be a good starting point for considering a group of chemicals; however, shared chemical structure is not solely sufficient as support for considering a candidate CMG. EPA also conducts an initial review of the experimental toxicology data to evaluate the extent to which common patterns of effects are observed. Data and knowledge of mammalian MOA/AOP provides the strongest information and is the foundation for establishing a CMG. Where mammalian MOA/AOP data are lacking, information on the pesticidal MOA may be a useful starting point for screening chemicals; however, consideration of the pesticidal MOA needs to be done with caution as some pesticidal MOAs may not be relevant to humans or have unknown relevance.

The Agency has utilized this framework for the isothiazolinone class of chemicals and determined that the toxicological database does not support a testable hypothesis for a common mechanism of action relevant to the exposures from residues of isothiazolinones in or on food. No further mechanistic data are required and no further cumulative evaluation is necessary for the isothiazolinones.

Table 11. Chemical names, PC Codes, CAS numbers, and CFR citations of the Isothiazolinones

Common Name	Chemical Name	PC Code	40 CFR	CAS #
BBIT	1,2-benzisothiazolin-3-one, 2-butyl	098951	NA	4299-07-4

[6] *Guidance for Identifying Pesticide Chemicals and Other Substances that have a Common Mechanism of Toxicity* (USEPA, 1999).

[7] *Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity* (USEPA, 2002).

Common Name	Chemical Name	PC Code	40 CFR	CAS #
BIT	1,2-Benzisothiazolin-3-one	098901	§180.920	2634-33-5
CMIT	5- chloro-2-methyl-4-isothiasolin-3-one	107103	§180.920	55965-84-9
DCOIT	4,5-Dichloro-2-octyl-3(2h)-isothiazolone	128101	NA	64359-81-5
MIT	2-Methyl-4-isothiazolin-3-one	107104	§180.920	2682-20-4
OIT	2-n-Octyl-4-isothiazolin-3-one	099901	NA	26530-20-1

The isothiazolinone class consists of six pesticidal active ingredients which are listed in Table 1 above. This group of chemicals was identified because the substances share chemical structural similarities (a thiazolone ring) and a common adverse effect (irritation of the gastrointestinal tract) from oral exposure and dermal skin sensitization. Each of these chemicals is registered for use in pesticide products in the United States. The isothiazolinone biocides are commonly formulated as antimicrobial pesticide products to be used as material preservatives to control bacteria, fungi, and/or algae. These pesticide products can be used in/on countertops/utensils (food use), pulp and paper (food packaging), vinyl flooring, household cleaning products, laundry detergent, metalworking fluids, paint (in-can preservative and antifoulant paint for ship hulls), plastics, textiles/carpets and wood (pressure treatment).

As materials preservatives, these compounds can contribute to indirect dietary exposures, when they are used to preserve paper, plastics and household cleaners. Some of the uses have Food and Drug Administration Food Contact Notifications (FDA FCNs), but there are no tolerances for the isothiazolinones. Three members of the class, BIT, CMIT and MIT, have tolerance exemptions from their use as inert ingredients in conventional pesticidal products.

Consistent with the framework, the toxicological knowledgebase for the isothiazolinones was evaluated in terms of information on MOA/AOP, chemical structural similarity, and toxicological profile to determine if the available data supports establishing a candidate CMG for this class of pesticides.

2.0 MOA/AOP

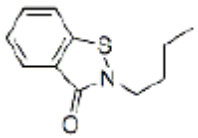
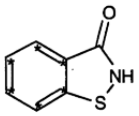
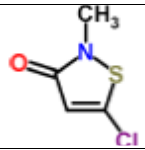
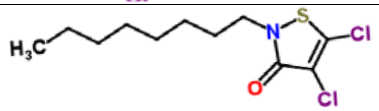
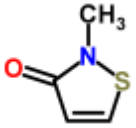
The isothiazolinone biocides are reactive chemicals. Depending on the route of exposure, point of contact adverse effects such as irritation or corrosion of the skin and eyes, irritation of the respiratory tract, and irritation-type responses of the gastrointestinal tract are observed. Although irritation and corrosion are relevant to humans, the irritation or corrosion effects of the isothiazolinone biocides are not related to a specific mode of action and are direct tissue effects based on the chemical reactivity of these chemicals.

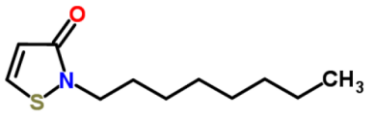
Dermal sensitization is also an adverse effect observed from dermal exposure to the isothiazolinone biocides. Dermal sensitization has a well-established adverse outcome pathway that has been discussed by the Organization for Economic Cooperation and Development (OECD Series on Testing and Development No. 168, 2012) and the International Program on Chemical Safety of the World Health Organization (IPCS, 2012). While a candidate grouping can be constructed for this effect of the isothiazolinone biocides based on chemical structural similarities and the commonality of the dermal sensitization adverse outcome, effects from dermal exposures cannot be cumulated with the non-specific toxic effects seen through oral exposure.

2.1 Chemical Structural Similarity

The isothiazolinone class of chemistry is characterized by the thiazolone ring which is broken during metabolism to form straight chain degradates such as N-methyl malonamic acid. Two of the compounds BIT and BBIT also have a benzene ring in addition to the thiazolone ring. The addition of the additional aromatic ring structure does not slow the breakage of the thiazolone ring, but the benzene ring is more persistent which results in straight carbon chains with a benzene ring attached. The ring structures are provided in Table 2 below. All isothiazolinones are water soluble, not hydrolysable or photo sensitive, but are susceptible to microbial degradation.

Table 2. Structures, and Registration Review Docket numbers of the Isothiazolinone Biocides

Common Name	Chemical Name	Structure
BBIT	EPA-HQ-OPP-2015-0736	
BIT	EPA-HQ-OPP-2014-0159	
CMIT/MIT	EPA-HQ-OPP-2013-0605	
DCOIT	EPA-HQ-OPP-2014-0403	
MIT	EPA-HQ-OPP-2013-0605	

Common Name	Chemical Name	Structure
OIT	EPA-HQ-OPP-2014-0160	

2.2 Toxicological Profile

The *Pesticide Cumulative Risk Assessment: Framework for Screening Analysis Purpose* document states that reviewers should conduct an initial review of the experimental toxicology data from that submitted for pesticide registration. These data are then used to evaluate the extent to which common patterns of effects are observed among the members of the potential class. The document also emphasizes that effects at lower doses, particularly those used in deriving points of departure (PODs) for single pesticide risk assessments, get more weight in the screening analysis. With these points in mind, the isothiazolinones were first screened to determine if there were common durations of exposure in which the effects observed for deriving the PODs could be compared for common effects.

In general, the isothiazolinone biocides are reactive chemicals and as such, cause point of contact adverse effects such as irritation or corrosion of the skin and eyes, irritation of the respiratory tract, and irritation-type responses of the gastrointestinal tract. All of the isothiazolinone biocides are Category I (corrosive) for eye irritation. Similarly, the isothiazolinone biocides are Category I (corrosive) for skin irritation with the exception of BIT which is Category IV. All the isothiazolinones are known to cause allergic contact dermatitis (dermal sensitization).

In repeat dosing studies with the isothiazolinone biocides, evidence of irritation, such as lesions of the glandular stomach and skin, are observed as effects across the class of chemicals. Decreases in body weight across multiple species and emesis in dogs are also common adverse findings throughout the available toxicology studies for these chemicals. Although their toxicological effects are qualitatively consistent, the isothiazolinone biocides differ in potency with No Observed Adverse Effect Levels/Lowest Observed Adverse Effect Levels (NOAELs/LOAELs) varying across the group. The effects of the isothiazolinone biocides are similar among members of the class, and include effects related to the irritant properties of the chemicals, such as hyperplasia/hyperkeratosis of the squamous mucosa of the forestomach from oral exposure; erythema and desquamation of the skin from dermal exposure; and inflammation/squamous metaplasia of the nasal cavity from inhalation exposure. These gastric irritation effects are considered non-specific toxic effects, which, as stated in EPA's cumulative risk guidance, do not support a candidate CMG, unless tied to a MOA/AOP or testable hypothesis related to a potential MOA/AOP, which is not the case for the isothiazolinones.

Also, as noted above, dermal sensitization is also an adverse effect observed from dermal exposure to the isothiazolinone biocides, and there is a well-established adverse outcome pathway. Despite the more specific nature of this toxic effect and the known MOA/AOP for this effect, EPA is not forming a candidate CMG based on this effect for the following reasons. The

FFDCA requires EPA to consider available information on cumulative risk from pesticides with a common mechanism of toxicity when determining whether a tolerance or exemption, which allows for residues of the pesticide in or on food, is safe. Because the toxic effects seen through the dermal exposure pathway differ from the effects seen through the oral pathway, the dermal exposures are not additive to the effects resulting from dietary exposure; therefore, they would not be aggregated in a cumulative risk assessment.

Toxic effects, points of departure, uncertainty factors and study references are provided in Tables 3-7 below..

3.0 Conclusions from CMG Screening Analysis and Options for Further CRA

The isothiazolinones share chemical structural similarities and a common adverse effect (irritation of the gastrointestinal tract) from oral exposure. This adverse effect is non-specific and does not support a testable hypothesis for a common mechanism group. Although the isothiazolinones share a common adverse effect (dermal sensitization), for which there is a known adverse outcome pathway relevant to humans, through the dermal route of exposure, this exposure cannot be cumulated with the non-specific toxic effects seen through oral exposure. Therefore, EPA is not conducting a cumulative risk assessment for the isothiazolinones.

Table 3. Acute Dietary Endpoints Selected for the Isothiazolinone Biocides

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
BBIT	LOAEL = 2000 mg/kg/day	UF = 100x (UF_A = 3X, UF_H = 3X and $UF_{LOAEL \rightarrow NOAEL}$ = 10X) FQPA SF = 1	Acute (gavage) oral toxicity study – rat. (MRID 44364915) LOAEL = 2000 mg/kg/day based on clinical signs of toxicity observed on Day 1 (piloerection, sides pinched in, upward curvature of spine, labored breathing, gasping, signs of salivation, breathing irregular, ↑ breathing depth & rate, prostrate, and tip toe gait) death of one female rat on day 3 at 2000 mg/kg/day.
BIT	100 mg/kg/day	UF = 10x (UF_A = 3x, UF_H = 3x) FQPA SF = 1	Acute oral (gavage) toxicity study – rat (MRID41022101/42858101) **LOAEL = 300 mg/kg/day** based on piloerection and upward curvature of the spine.
CMIT/MIT	79 mg/kg/day	UF = 10x (UF_A = 3x, UF_H = 3x) FQPA SF = 1	Acute Oral Toxicity Study (MRID 00086092) Formulation TRD 76-52 (13.2% a.i.) **LOAEL = 157 mg/kg/day** based on signs of intoxication (lethargy, prostration, ataxia, dyspnea, severe irritation and hemorrhage were noticed in g.i).
DCOIT	500 mg/kg/day	UF = 30x (UF_A = 3X, UF_H = 3X and $UF_{LOAEL \rightarrow NOAEL}$ = 3X) FQPA SF = 1	Acute Oral Toxicity in the rat (gavage) MRID 42977701 **LOAEL= 500 mg/kg/day** based on No mortality at 500 mg/kg/day. Diarrhea and mucus in stool were observed at 500 mg/kg/day.
OIT	LOAEL= 100 mg/kg/day	UF = 30x (UF_A = 3X, UF_H = 3X and $UF_{LOAEL \rightarrow NOAEL}$ = 3X) FQPA SF = 1	Acute oral toxicity study – rat (gavage) (MRID 00070456) **LOAEL = 100 mg/kg/day** based on diarrhea and unkempt fur

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
			$UF_{LOAEL \rightarrow NOAEL}$ is reduced to 3X because no death involved at this dose for acute toxicity study.

Table 4. Chronic Dietary Endpoints Selected for the Isothiazolinone Biocides

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
BBIT **BIT** **CMIT/MIT** **OIT**	2 mg/kg/day	UF = 10x (UF_A = 3x, UF_H = 3x) FQPA SF = 1	24-month drinking water chronic/oncogenic study in rats for CMIT/MIT mixture -1994 (MRID 43140701) **LOAEL = 6.6/9.8 mg/kg/day** (M/F) based on hyperplasia/ hyperkeratosis of the squamous mucosa of the forestomach in both M/F, necrosis of glandular mucosa of the stomach in females and edema/ inflammation of the glandular stomach in females.
DCOIT	30 mg/kg/day	UF = 100x (UF_A = 10X, UF_H = 10X) FQPA SF = 1	Two generation reproduction Toxicity Study in Rats (MRID 45756501) LOAEL (reproductive P/F1) is 62-88 mg/kg/day [M] and 67-93 mg/kg/day [F], based on significantly delayed vaginal opening (35.1 days vs. 31.9 days in control) and preputial separation (46.2 days vs. 42.9 days in control) in F1 offspring. No effects on reproductive performance at any dose level.

Table 5. Incidental Oral Endpoints Selected for the Isothiazolinone Biocides

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
BBIT	49 mg/kg/day	UF = 100x (UF_A = 10x, UF_H = 10x) FQPA SF = 1	BBIT (99.4 ±1% a.i.) Two-generation reproduction toxicity (dietary) – rat) - 2007 (MRID 48261201)

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
			$NOAEL_{parental\ toxicity}$ = 49 mg/kg/day **$LOAEL_{parental\ toxicity}$ = 141 mg/kg/day** based on decreased body weights, body weight gains, and food consumption. **$NOAEL_{offspring\ toxicity}$ = 49 mg/kg/day** **$LOAEL_{offspring\ toxicity}$ = 141mg/kg/day,** based on decreased body weights, body weight gains, and spleen weight (F_2 pups only).
BIT	8.42 mg/kg/day	UF = 10x (UF_A = 3X, UF_H = 3X) FQPA SF = 1	BIT (84.2%) 90-day oral (gavage) –Wistar rats (MRID 46346201) **NOAEL=8.42 mg/kg/day a.i.** **LOAEL=25.26 mg/kg/day a.i.,** based on macroscopic and microscopic lesions in non-glandular and glandular regions of the stomach.
CMIT/MIT	8.5 mg/kg/day	UF = 10x (UF_A = 3X, UF_H = 3X) FQPA SF = 1	Rat 2-gen reproductive study (MRID 44656101) **NOAEL parental = 8.5 / 11.8 mg/kg/day** **LOAEL Parental = 22.7/28 mg/kg/day** based on increased incidence of histopathological lesions of the glandular and non-glandular stomach in the F0 and F1 male and female rats.
DCOIT	30 mg/kg/day	UF = 100x (UF_A = 10x, UF_H = 10x) FQPA SF = 1	Rat 2-gen reproductive study (MRID 45756501) **NOAEL (reproductive P/F1) is 30-39[M] 33-41[F] mg/kg/day** **LOAEL (reproductive P/F1) is 62-88 mg/kg/day [M] and 67-93 mg/kg/day [F],** based on significantly delayed vaginal opening (35.1 days vs. 31.9 days in control) and preputial separation (46.2 days vs. 42.9 days in control) in F1 offspring.

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
OIT	43 mg/kg/day	UF = 10x (UF_A = 3X, UF_H = 3X) FQPA SF = 1	Rat 2-gen reproductive study (MRID 47815801) 0, 13-15, 43-51, 96-120 mg/kg/day dietary **NOAEL parental= 43-51 mg/kg/day** **LOAEL parental= 96-120 mg/kg/day** based on decreased body weight, hyperplasia/hyperkeratosis of the forestomach, decreased spleen weights, increased adrenal weight **NOAEL offspring= 43-51 mg/kg/day** **LOAEL offspring = 96-120 mg/kg/day** based on decreased body weight gain and decreased spleen weight.

3.2 Dermal Endpoint Selection

Table 6. Dermal Endpoints Selected for the Isothiazolinone Biocides

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study or Model
BBIT	**Induction:** Average *in vitro* EC3 = 0.061% (15.3 µg/cm^2); 95% Confidence Interval = 0.06 to 0.07%	UF = 100x (UF_A = 10x, UF_H = 10x)	Based on Model 4 from Hirota *et al.* 2015: DPRA + h-CLAT + KeratinoSens
BIT	**Induction:** Average *in vitro* EC3 = 0.34% (85 µg/cm^2) 95% Confidence Interval = 0.32 to 0.37%	UF = 100x (UF_A = 10X, UF_H = 10X)	Based on Model 4 from Hirota *et al*. 2015: DPRA + h-CLAT + KeratinoSens
	Elicitation: Minimum Elicitation Threshold (MET) for MIT of 0.0105 µg/cm^2 producing a response in 18% of tested individuals.	UF = 10x (UF_A = 3X, UF_H = 3X)	Lundov et al. (2011): Methylisothiazolinone Contact Allergy and Dose–Response Relationships. Contact Dermatitis 64: 330-336.

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study or Model
CMIT/MIT			Human Repeat Open Application Test using doses of 0, 0.0105, 0.105, and 0.21 µg MIT/cm^2
	Induction CMIT/MIT EC3 = 0.49% (120 ug/cm^2)	Residential and Occupational LOC for MOE = 100 UFA = 10 UFH = 10	EC3 = 0.49% for CMIT/MIT based on Model 4 from Hirota *et al*., 2015: DPRA + h-CLAT + KeratinoSens in vitro assays
	Induction MIT only EC3 = 0.83% (210 ug/cm^2)	Residential and Occupational LOC for MOE = 100 UFA = 10 UFH = 10	EC3 = 0.83% for MIT based on Model 4 from Hirota *et al.*, 2015: DPRA + h-CLAT + KeratinoSens in vitro assays
DCOIT	**Induction:** Average *in vitro* EC3 = 0.023% (5.8 µg/cm^2); 95% Confidence Interval = 0.02 to 0.03%	UF = 100x (UF_A = 10x, UF_H = 10x) FQPA SF = 1	Based on Model 4 from Hirota *et al.* 2015: DPRA + h-CLAT + KeratinoSens
OIT	**Induction:** Average *in vitro* EC3 = 0.015% (3.75 µg/cm^2); 95% Confidence Interval = 0.01 to 0.02%	UF = 100x (UF_A = 10X, UF_H = 10X) FQPA SF = 1	Based on Model 4 from Hirota *et al.* 2015: DPRA + h-CLAT + KeratinoSens

Note: To convert the *in vitro* EC3 (%) into units of µg/cm^2 = [EC3 x 25µL x 10 µg/µL]/cm^2

3.3 Inhalation Endpoint Selection

Table 7. Inhalation Endpoints Selected for the Isothiazolinone Biocides

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
BBIT	Bridged to the DCOIT 90-day inhalation toxicity study.		
BIT	Bridged to the DCOIT 90-day inhalation toxicity study.		
CMIT/MIT	NOAEC=0.34 mg/m^3 8-hr HEC = 0.11 mg/m^3 24-hr HEC=0.038 mg/m^3	10X (for short/intermediate-term) (UF_A = 3x, UF_H = 3x) 30X (for long-term)	90-day inhalation Study for CMIT/MIT (MRID 00148418) **NOAEC = 0.34 mg/m3 (both M/F),**

Chemical Name	Dose for Use in Risk Assessment	Target MOE, Uncertainty Factors, FQPA SF	Study and Toxicological Effects
	HEC = NOAEC * [6 hr animal / 8 or 24 hr human)]* RDDR (0.45 for ET effects, BW= 400 grams, MMAD = 1.1 um, GSD = 1.9 to 2.0)	(UF_A = 3x, UF_H = 3x, and $UF_{long\text{-}term}$ = 3x)	**LOAEC = 1 15 mg/m3**, based on microscopic: lesions in the nasal turbinates (rhinitis)
DCOIT	NOAEC=0.02 mg/m^3 HEC=0.0045 mg/m^3 HEC = NOAEC * (6-hour animal/8 hour human) * RDDR (0.30 for ET effects, BW= 420 grams, MMAD = 1.4 um, GSD = 4.6)	ST/IT LOC = 10 UF_A= 3 UF_H = 3 LT LOC = 30 UF_A = 3 UF_H = 3 UF_D = 3	90-Day inhalation study for DCOIT (MRID 43487501) **NOAEC = 0.02 mg/m^3**, **LOAEC = 0.63 mg/m^3**, based on the histopathological alterations observed in the nose (min/mild subacute inflammation and transitional respiratory epithelial and goblet cell hyperplasia), larynx (chronic-active inflammation and hyperplasia of the squamous and cuboidal epithelium), and lungs (acute inflammation and goblet cell hyperplasia at high-dose).
OIT	NOAEC=0.64 mg/m^3 HEC = 0.12 mg/m^3 HEC = NOAEC * (6-hour animal/8 hour human) * RDDR (0.25 for ET effects, BW= 370 grams, MMAD = 1.4 um, GSD = 5.5)	10X (for short/intermediate-term) (UF_A = 3x, UF_H = 3x) 30X (for long-term) (UF_A = 3x, UF_H = 3x, and $UF_{long\text{-}term}$ = 3x)	90 Day Inhalation Toxicity - OIT (MRID 41544701) **NOAEC = 0.64 mg/m^3** **LOAEC = 6.3 mg/m^3** Based on rales (5-22/22 males and females, week 1-13), dyspnea (3/22 females week 4 and 3-9/22 females week 7-10), For further details, please see the Hazard Characterization Chapter Appendices.

References

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