Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Reaction mass of:
5-chloro-2-methyl-4-isothiazolin-3-one
[EC no. 247-500-7] and 2-methyl-2*H*-isothiazol-3-one
[EC no. 220-239-6] (3:1);
Reaction mass of:
5-chloro-2-methyl-4-isothiazolin-3-one
[EC no. 247-500-7] and
2-methyl-4-isothiazolin-3-one [EC no. 220-239-6] (3:1)

EC number: -
CAS number: 55965-84-9

CLH-O-0000001412-86-106/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

10 March 2016

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Reaction mass 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1) ; C(M)IT/MIT

EC Number: **no EC number for the mixture**

CAS Number: **55965-84-9 for the mixture**

Index Number: **613-167-00-5**

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Version number: **2** **Date:** **15/04/2015**

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Reaction mass 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1) ; C(M)IT/MIT
CAS number:	55965-84-9 for the mixture C(M)IT/MIT (3:1)
Annex VI Index number:	613-167-00-5
Degree of purity:	Min 57.9% for C(M)IT/MIT in dry weight (for the lower source)
Impurities:	Confidential data

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Acute Tox 3*/H301: Toxic if swallowed Acute Tox 3*/H311: Toxic in contact with skin Acute Tox3*/H331 : Toxic if inhaled Skin Corr 1B/H314: Causes severe skin burns and eye damage Skin Sens 1/H317: May cause an allergic skin reaction Aquatic Acute 1/H400: Very toxic to aquatic life Aquatic chronic 1/H410 Very toxic to aquatic life with long lasting effects.

	Skin Corr. 1B; H314: C ≥ 0.6% Skin Irrit. 2; H315: 0.06% ≤ C < 0.6% Eye Irrit. 2; H319: 0.06% ≤ C < 0.6% Skin Sens. 1; H317: C ≥ 0.0015%
Current proposal for consideration by RAC	Acute Tox.3/H301: Toxic if swallowed Acute Tox.2/H330: Fatal if inhaled Acute Tox.2/H310: Fatal in contact with skin Skin Corr 1C/H314: Causes severe skin burns and eye damage Skin Sens 1A/H317: May cause an allergic skin reaction Skin Corr. 1C; H314: C ≥ 0.5% Skin Sens. 1A; H317: C ≥ 0.0015% Aquatic Acute 1/H400: Very toxic to aquatic life (M-factor = 100) Aquatic chronic 1/H410 Very toxic to aquatic life with long lasting (M-factor = 100)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox.2/H330: Fatal if inhaled Acute Tox.2/H310: Fatal in contact with skin Acute Tox 3/H301: Toxic if swallowed Skin Corr 1C/H314: Causes severe skin burns and eye damage: C ≥ 0.5% Skin Sens 1A/H317: May cause an allergic skin reaction C ≥ 0.0015% ; Skin Irrit. 2; H315: 0.06% ≤ C < 0.6% Eye Irrit. 2; H319: 0.06% ≤ C < 0.6% Aquatic Acute 1/H400: Very toxic to aquatic life (M-factor = 100) Aquatic chronic 1/H410 Very toxic to aquatic life with long lasting (M-factor = 100)

***** Minimum classification

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	No classification			conclusive but not sufficient for classification
2.2.	Flammable gases	No classification			Not relevant
2.3.	Flammable aerosols	No classification			Not relevant
2.4.	Oxidising gases	No classification			Not relevant
2.5.	Gases under pressure	No classification			Not relevant
2.6.	Flammable liquids	No classification			conclusive but not sufficient for classification
2.7.	Flammable solids	No classification			conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	No classification			conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	No classification			conclusive but not sufficient for classification
2.10.	Pyrophoric solids	No classification			conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	No classification			conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	No classification			conclusive but not sufficient for classification
2.13.	Oxidising liquids	No classification			conclusive but not sufficient for classification
2.14.	Oxidising solids	No classification			conclusive but not sufficient for classification
2.15.	Organic peroxides	No classification			conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	No classification			conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox 3/H301	-	Acute Tox 3*/H301	
	Acute toxicity - dermal	Acute Tox.2/H310	-	Acute Tox 3*/H311	

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
	Acute toxicity - inhalation	Acute Tox.2/H330	-	Acute Tox3*/H331	
3.2.	Skin corrosion / irritation	Skin Corr 1C/H314	Skin Corr. 1C; H314: C ≥ 0.6% Skin Irrit. 2; H315: 0.06% ≤ C < 0.6% Eye Irrit. 2; H319: 0.06% ≤ C < 0.6%	Skin Corr 1B/H314	
3.3.	Serious eye damage / eye irritation	No classification	-	No classification	Covered by classification Skin Corr 1C.
3.4.	Respiratory sensitisation	No classification	-	No classification	Data lacking
3.4.	Skin sensitisation	Skin Sens 1A/H317: C ≥ 0.0015%	Skin Sens. 1A; H317: C ≥ 0.0015%	Skin Sens 1/H317	
3.5.	Germ cell mutagenicity	Not considered	-	No classification	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not considered	-	No classification	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not considered	-	No classification	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not considered	-	No classification	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not considered	-	No classification	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not considered	-	No classification	Conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1/H400: Very toxic to aquatic life Aquatic chronic 1/H410 Very	Acute M-factor = 100) Chronic M-factor = 100	Aquatic Acute 1/H400: Very toxic to aquatic life Aquatic chronic 1/H410 Very toxic to aquatic life with long lasting effects.	

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
		toxic to aquatic life with long lasting			
5.1.	Hazardous to the ozone layer				

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Danger
Hazard statements: H400, H410, H317, H319, H314, H315, H311, H301, H331

Proposed notes assigned to an entry:

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

C(M)IT/MIT has previously been discussed in TC C&L in 1999-2001. It appears that numerous discussions have taken place regarding limits to use for sensitisation.

2.2 Short summary of the scientific justification for the CLH proposal

The data presented below justify the modifications proposed of the existing entry.

2.3 Current harmonised classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

The classification of C(M)IT/MIT is harmonised in Annex VI of CLP under the index number *613-167-00-5* as follows:

Classification		**Labelling**	**Specific Concentration limits, M-Factors**
Hazard Class and Category Code(s)	Hazard Stat. Code(s)	Picto, Signal Word Code(s)	
Acute Tox. 3 *	H301	GHS06 GHS09 GHS05 Dgr	Skin Irrit. 2; H315: 0.06% ≤ C < 0.6% Eye Irrit. 2; H319: 0.06% ≤ C < 0.6% Skin Sens. 1; H317: C ≥ 0.0015% Skin Corr. 1B; H314: C ≥ 0.6%
Acute Tox. 3 *	H311		
Skin Corr. 1B	H314		
Skin Sens. 1	H317		
Acute Tox. 3 *	H331		
Aquatic Acute 1	H400		
Aquatic Chronic 1	H410		
Signal Words		**Pictograms**	
Danger		 Skull and crossbones  Environment  Corrosion  Environmen	

2.4 Current self-classification and labelling based on the CLP Regulation criteria

There are 33 aggregated notifications grouping 1459 notifiers that apply the following self-classification:

Hazard Class and Category Code(s)	Hazard Statement	Nb of notifiers applying the Hazard Class/ code/ statement
Acute Tox. 2	H310	115
Acute Tox. 2	H330	149
Acute Tox. 3	H331	1305
Acute Tox. 3	H301	1454
Acute Tox. 3	H311	1454
Aquatic Acute 1	H400	1453
Aquatic Chronic 1	H410	1431
Aquatic Chronic 4	H413	18
Eye Dam. 1	H318	607
Eye Irrit. 2	H319	1
Skin Corr. 1B	H314	1454
Skin Sens. 1	H317	1454
STOT SE 3	H335 (Respiratory sys...)	50
Met. Corr. 1	H290	1

RAC general comment

This substance is a reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one in the ratio 3:1 (C(M)IT/MIT). It is manufactured as a technical concentrate and produced in a solution with solvents and stabilisers. The majority of toxicity studies summarised in the CLH report have been performed on a specific aqueous formulation which contains around 14% of the 3:1 reaction mass. The SCSS opinion on C(M)IT/MIT (SCCS/1238/09) noted that the biocide is produced by an integrated production process (reaction mass), resulting in an approximate total of 14% active ingredients, 16% magnesium nitrate, 10% magnesium chloride and 62% water. However, the Biocidal Product Committee also mentioned that the theoretical (calculated) dry weight specification-minimum purity of C(M)IT-MIT (3:1) at 579 g/kg i.e. 60% w/w (BPC, 2015). Some manufacturers also report active ingredients of 14% w/w minimum on their website. Finally, Industry could not confirm whether it was possible to produce the substance at > 14%. In view of this uncertainty, RAC queried whether the concentration of 14% should be specified in the Annex VI entry for this substance. In order not to restrict the entry in Annex VI of CLP to a

concentration of 14% while considering that more diluted forms may be available to workers or professionals, no specification of the maximum concentration of the active ingredient C(M)IT/MIT is proposed. This approach is also in line with all entries of active substances in CLP. This reaction mass will be referred in the opinion as "C(M)IT/MIT".

Germ cell mutagenicity, carcinogenicity, reproductive toxicity and aspiration toxicity were not considered in this dossier and hence, RAC has not evaluated these hazard classes.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

C(M)IT/MIT is an active Biocide substance in the meaning of Regulation EC 528/2012. In accordance with Article 36(2) of the CLP Regulation, C(M)IT/MIT shall be subjected to harmonized classification and labeling for all endpoints.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

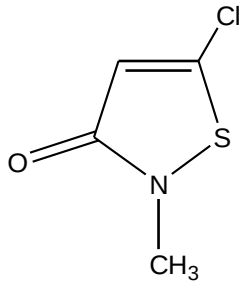
1.1 Name and other identifiers of the substance

Table 5: Substance identity

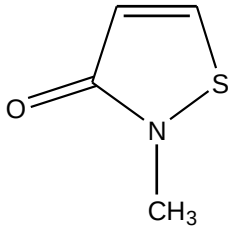
CAS number:	55965-84-9 for the mixture C(M)IT/MIT (3:1)
CAS name:	3(2H)-isothiazolone, 5-chloro-2-methyl- mixt. with 2-methyl-4-isothiazolone
IUPAC name:	Reaction mass 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)
CLP Annex VI Index number:	613-167-00-5
Molecular formula:	C_4H_4ClNOS for C(M)IT C_4H_5NOS for MIT
Molecular weight range:	149.6 g/mol for C(M)IT 115.2 g/mol for MIT

Structural formula:

C(M)IT



MIT



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Reaction mass 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)Reaction mass 5-chloro-2-methylisothiazol-3(2H)-one and 2-methylisothiazol-3(2H)-one (3:1) *55965-84-9*		Min 57.9% in dry weight (TC)	Mixture of C(M)IT/MIT (3:1) The active substance is manufactured as a TK. It is in a solution with solvents and stabilizers. Different solvents and stabilizers exist.
5-chloro-2-methylisothiazol-3(2H)-one *26172-55-4*		Min 45.7% in dry weight	
2-methylisothiazol-3(2H)-one *2682-20-4*		Min 12.2% in dry weight	

TC: technical material, pure C(M)IT/MIT with its impurities whose composition is theoretically calculated based on the composition of the solution

TK: technical concentrate, solution with the substance in solvents with stabilizers in order to have a stabilized product

See the confidential annex for further information.

Current Annex VI entry:

The following harmonised classification applies:

According to table 3.2	According to table 3.1		
T; R23/24/25 C; R34 R43 N; R50-53 C ≥ 0,6 % C; R34 0,06 % ≤ C < 0,6 % Xi; R36/38 C ≥ 0,0015 % R43	Acute Tox. 3 * Acute Tox. 3 * Skin Corr. 1B Skin Sens. 1 Acute Tox. 3 * Aquatic Acute 1 Aquatic Chronic 1	H301 H311 H314 H317 H331 H400 H410	Skin Corr. 1B; H314: C ≥ 0.6% Skin Irrit. 2; H315: 0.06% ≤ C < 0.6% Eye Irrit. 2; H319: 0.06% ≤ C < 0.6% Skin Sens. 1; H317: C ≥ 0.0015%

Table 7: Impurities (non-confidential information)

See the confidential annex for further information

Table 8: Additives (non-confidential information)

Additive	Function	Typical C.	Conc. range	Remarks: Self Classification
Magnesium nitrate 10377-60-3			Max 21.78%	Acute Tox. 4 H302 Ox. Liq. 1 H271 Ox. Liq. 3 H272 Ox. Sol. 1 H271 Ox. Sol. 2 H272 Ox. Sol. 3 H272 Skin Irrit. 2 H315 STOT SE 3 H335(Respiratory Skin Irrit. 2 H315 STOT SE 3 H335(Respiratory
Magnesium chloride 7786-30-3			Max 10.9%	Eye Irrit. 2 H319 Met. Corr. 1 H290 Skin Irrit. 2 H315 Skin Sens. 1 H317 STOT SE 2 H371(unknown) STOT SE 3 H335(respiratory tra...)

1.2.1 Composition of test material

C(M)IT/MIT (3:1) is very reactive with some substances and should be stabilized in the product. That's why C(M)IT/MIT is produced in a continuous process directly at the product stage. Therefore the active substance is manufactured as a TK, in a solution with solvents and stabilizers. Different solvents and stabilizers exist. Most of the (eco)toxicological studies and all the physico-chemical properties have been performed with a solution of C(M)IT/MIT (3:1) at 14% in water with magnesium salts which is the product mostly on the market.

C(M)IT/MIT (3:1) has been isolated just before being formulated only to be tested for physico-chemical properties. The purity is 95-99%. It is named "pure CMIT/MIT (3:1)" in paragraphs 1.3 and 3. It is different from the TC (see paragraph 1.2 under table 6 for the definition) which is theoretically calculated based on the composition of the product.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	<u>Pure C(M)IT/MIT (3:1):</u> Solid pale yellow at yellow, weakly sweet and pungent at 20-25°C <u>Solution at 14% with magnesium salts:</u> Clear liquid colourless to pale yellow with a mild odour at 20°C	Petigara R.B. (2003), Rohm and Haas Company Petigara R.B. (2001), Rohm and Haas Company MSDS Acticide 14	Visual observation Purity :95-99% Visual observation
Melting/freezing point	<u>Pure C(M)IT/MIT (3:1):</u> Onset at 22.2°C with peak at 35.1°C <u>Pure C(M)IT:</u> Onset at 51.3°C with peak at 54.9°C Onset at 46.6°C and peak at 48.9°C <u>Pure MIT:</u> 46.7-48.3°C Onset at 44.2°C and peak at 47.7°C <u>Solution at 14% with magnesium salts:</u> Less than -25°C at atmospheric pressure -23°C	Petigara R.B. (2003), Rohm and Haas Company Petigara R.B. (2003), Rohm and Haas Company Hoffmann (2000) Betteley, J.; Petigara, R. (2001), Rohm and Haas Company Hoffmann (2000) Petigara R.B. (2001), Rohm and Haas Company Lander (2007)	Measured Purity : 95-99%, EC A1, DSC Measured Purity :99.86% Purified, Measured Purity : 99.7% About 100% Measured EC A1 DSC
Boiling point	<u>Pure C(M)IT/MIT (3:1):</u> No boiling point, decomposition from 97.3°C <u>Pure C(M)IT:</u> No boiling point, decomposition from 167°C	Petigara R.B. (2003), Rohm and Haas Company Tognucci (2002) Tognucci (2002)	Measured Purity : 95-99%, EC A2, DSC Measured Purity : >98%, DSC Measured

	Pure MIT (>99%): No boiling point, decomposition from 236°C Solution at 14% with magnesium salts: 100.1°C 106.5°C	 Petigara R.B. (2001), Rohm and Haas Company Massmann and Werle (1992)	Purity : >99%, DSC Measured Simplified dynamic method Siwoloboff method
Relative density	Pure C(M)IT/MIT (3:1): 1.396 at 38°C molten phase 1.420 at 25°C solid phase Pure C(M)IT: 1.6 at 20.8°C Pure MIT: 1.39 at 20°C Solution at 14% with magnesium salts: 1.296 at 25°C 1.256 at 20°C	Petigara R.B. (2003), Rohm and Haas Company Broughton, H.S. (1992) Rohm and Haas Company Tognucci (1992) Tognucci (1992) Petigara R.B. (2001), Rohm and Haas Company Massmann and Werle (1992)	Measured Purity : 95-99%, pycnometer Measured Purity :>98%, gas comparison pycnometer Measured Purity : >99%, gas comparison pycnometer Measured Pycnometer Pycnometer
Vapour pressure	Pure C(M)IT/MIT (3:1): 2.2Pa at 20°C and 3.8Pa at 25°C Pure C(M)IT: 0.9Pa at 20°C and 1.3 at 25°C 1.6Pa at 20°C and 2.8Pa at 25°C Pure MIT: 2.1Pa at 33°C, 0.4 at 20°C and 0.7Pa at 25°C	Petigara R.B. (2003), Rohm and Haas Company Betteley, J.; Petigara, R. (2001), Rohm and Haas Company Badt-Tognucci (2007) Betteley, J.;	Measured Purity : 95-99%, Knudsen effusion method Measured Purity : 99.86%, Knudsen effusion method Purity : 98.4%, gas saturation method Measured Purity : 99.7%, vapour

	0.99Pa at 20°C and 1.6Pa at 25°C Solution at 14% with magnesium salts: 2080Pa at 20°C and 2726Pa at 25°C 20.8hPa	Petigara, R. (2001), Rohm and Haas Company Weissenfeld (2006) Petigara R.B. (2001), Rohm and Haas Company Werle (1994)	pressure balance Purity : 98.5%, gas saturation method Measured Static method
Henry's law constant	Pure C(M)IT/MIT (3:1): < 10-4 Pa.m3/mol Pure C(M)IT: <$4.26x10^{-4}$ $Pa.m^3$/mol at 20°C and <$7.07x10^{-4}$ $Pa.m^3$/mol at 25°C Pure MIT: <$2.72x10^{-5}$ $Pa.m^3$/mol at 20°C and <$4.39x10^{-5}$ $Pa.m^3$/mol at 25°C	Petigara R.B. (2003), Rohm and Haas Company Badt-Tognucci (2007) Weissenfeld (2006)	Calculated Purity : 95-99% Calculated Purity : 98.4% Calculated Purity : 98.5%
Surface tension	Pure C(M)IT/MIT (3:1): 72.3mN/m at 20°C Solution at 14% with magnesium salts: 73.0mN/m at 19.5°C 72.6mN/m	Petigara R.B. (2003), Rohm and Haas Company Petigara R.B. (2001), Rohm and Haas Company Lander (2007)	Measured Purity : >99%, EC A5 Measured EC A5
Water solubility	Pure C(M)IT/MIT (3:1): >3000g/L Pure C(M)IT: 1g/mL Pure MIT: 4g/mL Solution at 14% with magnesium salts: Not relevant for aqueous solution	Petigara R.B. (2003), Rohm and Haas Company Tognucci (2002)	Measured Purity : 95-99%, shake flask method Measured Shake Flask Method Measured Shake Flask Method

Partition coefficient n-octanol/water	Pure C(M)IT: 0.401 at 24°C Pure MIT: -0.486 at 24°C Solution at 14% with magnesium salts: C(M)IT: 0.75 MIT: -0.71	Petigara R.B. (2003), Rohm and Haas Company Bates ML, 1993	Measured Purity : 97.8% Purity : 98.1% Measured HPLC
Flash point	Pure C(M)IT/MIT (3:1): No flash point up to 110°C Solution at 14% with magnesium salts: No ignition up to 110°C	Petigara R.B. (2003), Rohm and Haas Company Lander (2007)	Measured Purity : 95-99%, closed cup method Measured EC A9
Flammability	Pure C(M)IT/MIT (3:1): Not highly flammable Solution at 14% with magnesium salts: Not highly flammable	Petigara R.B. (2003), Rohm and Haas Company Schied (2003)	Measured Purity : 95-99%, EC A10 Theoretical statement
Explosive properties	Pure C(M)IT/MIT (3:1): Not explosive Solution at 14% with magnesium salts: Not explosive	Petigara R.B. (2003), Rohm and Haas Company Hanstveit (2007)	Measured Purity : 95-99%, fall hammer test and Koenen steel tube test Theoretical statement
Self-ignition temperature	Pure C(M)IT/MIT (3:1): The auto-ignition temperature was found to be 395ºC at atmospheric pressure (99.7 kPa)	Petigara R.B. (2003), Rohm and Haas Company	Measured Purity : 95-99%, EC A15
Oxidising properties	Pure C(M)IT/MIT (3:1): Not oxidising Pure C(M)IT: Not oxidising Pure MIT: Not oxidising Solution at 14% with magnesium salts: Not oxidising	- Hanstveit (2007) Hanstveit (2007) Hanstveit (2007)	Theoretical statement

Granulometry	Not relevant	-	-
Stability in organic solvents and identity of relevant degradation products	Not applicable	-	-
Dissociation constant	Considered as not relevant since CMIT and MIT are covalent molecules that do not dissociate into ionic species. With respect to the molecular structures of CIT and MIT the chemical represents weak bases. Estimated pKb (QSAR): CIT>15, MIT>13. The weak acid properties of CIT and MIT sample in water in the course of the studies are probably due to acidic impurities from the preparation process.	- Werle (1995) Werle (1997) Werle (1997) Verhaar (2007)	-
Viscosity	Pure C(M)IT/MIT (3:1): Not required, solid Solution at 14% with magnesium salts: 11.4mPa.s at 25.7°C 8.4mPa.s at 44.6°C Dynamic viscosity: 4.8 mPa.s at 20°C, Kinematic viscosity: 3.8 mm²/s at 20°C 2.3 mm²/s at 40°C	- Petigara R.B. (2001), Rohm and Haas Company Werle (1993)	Measured rotational viscometer Capillary viscometer
pH	Solution at 14% with magnesium salts: Solution at 1% of the test material, at 20°C pH =3.43 At 1% of the test material, pH=2.5-3.0 Acidity due to hydrogen chloride in solution and acetic acid	Bates (2003) Rohm and Haas Hanstveit, Verhaar (2007)	Measured CIPAC MT 75 Measured In house method

Acidity/Alcalinity	0.342% as H_2SO_4 in the 14% solution Or 2.41% as H_2SO_4 in the active ingredients The acidity is due to acidic impurities, not to the active ingredients	Bates (2003) Rohm and Haas	Measured CIPAC MT 31

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

C(M)IT/MIT 14% is used as a biocidal product.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference

EC A9 Closed cup method	No flash point up to 110°C	Pure C(M)IT/MIT (3:1) 95-99%	Petigara R.B. (2003), Rohm and Haas Company
EC A9	No ignition up to 110°C	Solution at 14% with magnesium salts	Lander (2007)
EC A10	The test substance melted to form a red-brown liquid that only ignited in the presence of the test flame. Not highly flammable	Pure C(M)IT/MIT (3:1) 95-99%	Petigara R.B. (2003), Rohm and Haas Company
EC A15	The auto-ignition temperature was found to be 395ºC at atmospheric pressure (99.7 kPa)	Pure C(M)IT/MIT (3:1) 95-99%	Petigara R.B. (2003), Rohm and Haas Company
Theoretical statement	Not highly flammable	Solution at 14% with magnesium salts	Schied (2003)
EC A14 Fall hammer test and Koenen steel tube test	Not explosive	Pure C(M)IT/MIT (3:1) 95-99%	Petigara R.B. (2003), Rohm and Haas Company
Theoretical statement	Not explosive	Solution at 14% with magnesium salts	Hanstveit (2007)
Theoretical statement	Not oxidising There are no functional groups present, in either of the two component materials, which are capable of being significantly oxidising.	Pure C(M)IT/MIT (3:1)	-
Theoretical statement	Not oxidising	Pure C(M)IT	Hanstveit (2007)
Theoretical statement	Not oxidising	Pure MIT	Hanstveit (2007)
Theoretical statement	Not oxidising	Solution at 14% with magnesium salts	Hanstveit (2007)

3.1 Explosive property

A test with the method EC A.14 has been performed and shows that pure C(M)IT/MIT (3:1) is not explosive.

The solution of C(M)IT/MIT contains 60% of water and no constituents with explosive properties therefore it is considered that the test would give a negative result.

3.2 Inflammability

A test with the method EC A.9 has been performed and shows that pure C(M)IT/MIT (3:1) has no flash point up to 110°C. The same test gives the same result for the solution at 14%.

Moreover a test with the method EC A.10 has been performed and shows that pure C(M)IT/MIT (3:1) is not highly flammable.

The auto-ignition temperature of pure C(M)IT/MIT (3:1) was found to be 395°C at atmospheric pressure.

3.3 Oxidizing potential

There are no functional groups present, in either of the two component materials, which are capable of being significantly oxidising. Based on the chemical composition, it is considered that the test would give a negative result.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) did not propose classification for physical hazards. The data on physico-chemical properties did not indicate any concerns and as such, C(M)IT/MIT does not meet the criteria for classification. According to the assessment of the DS, C(M)IT/MIT was not explosive in a standard study (EC method A.14) and a test using EC method A.10 showed that C(M)IT/MIT was not highly flammable. Examination of the chemical structure indicated that C(M)IT/MIT would not have any oxidising properties, therefore C(M)IT/MIT does not meet the criteria for classification as an oxidising substance.

Comments received during public consultation

No comments were received regarding this endpoint.

Assessment and comparison with the classification criteria

RAC is in agreement with the DS that **classification is not required for physico-chemical hazards**.

4 HUMAN HEALTH HAZARD ASSESSMENT

C(M)IT/MIT is a mixture. It is normally supplied as an aqueous solution of 14% C(M)IT/MIT (Kathon^{TM}886F or ACTICIDE 14). According to the definition of a "substance" under REACh, the proposed entry is referring to the "pure" C(M)IT/MIT with a purity expressed in dry weight also referred as active ingredient (a.i) in the document.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Not considered in this dossier.

4.2 Acute toxicity

Table 4.2-1 Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Acute toxicity study (No guideline available in 1977) Oral route	$LD_{50\ males}$ = 457 mg Kathon^{TM}886/kg bw $LD_{50\ males}$ = 64 mg a.i/kg bw	Charles River CD males rats n = 10/group Gavage Test substance: aqueous solutions of C(M)IT/MIT 14% and 13.3%	Craig, 1993
Acute toxicity study GLP, OECD 401 EPA 81-1 Oral route	$LD_{50\ combined}$ = 472 mg ACTICIDE 14/kg bw $LD_{50\ combined}$ = 66 mg a.i/kg bw	Sprague Dawley male and female rats n = 5/sex/group Gavage Test substance: aqueous solution of C(M)IT/MIT 14%	Mercier, 1994
Acute toxicity study GLP, OECD 403 / US EPA 81-3 Inhalation route	$LC_{50\ combined}$ = 2.36 mg Kathon^{TM}886F / L_{air}/4h $LC_{50\ combined}$ = 0.33 mg a.i / L_{air}/4h	Crl:CD®BR male and female rats n = 6/sex/group Nose-only Test substance: aqueous solution of C(M)IT/MIT 14%	Wanner, 1991
Acute toxicity study GLP, OECD 403 Inhalation route	$LC_{50\ combined}$ = 1.23 mg ACTICIDE 14 /L_{air}/4h $LC_{50\ combined}$ = 0.171 mg a.i / L_{air}/4h	Sprague-Dawley male and female rats n = 5/sex/group Nose-only Test substance: aqueous solution of C(M)IT/MIT 14%	Jackson, 1997
Acute toxicity study GLP, OECD 402/US EPA 81-2 Dermal route	$LD_{50\ combined}$ > 10008 mg ACTICIDE 14/kg bw $LD_{50\ combined}$ > 141 mg a.i/kg bw Total mortality: 30%	Sprague-Dawley male and female rats n = 5/sex/group Test substance: aqueous solution of C(M)IT/MIT 14%	Mercier, 1994

Acute toxicity study (No guideline available in 1976) Dermal route	$LD_{50\ combined}$ = 660 mg Kathon™886/kg bw $LD_{50\ combined}$ = 87.12 mg a.i/kg bw	Albino male rabbits n = 5/sex/group Test substance: aqueous solution of C(M)IT/MIT 14%	Craig, 1993

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Two acute oral toxicity studies provide relevant information to evaluate the acute toxicity of C(M)IT/MIT by oral route. One of these studies was performed according the OECD guideline 401.

Charles River CD male rats (10/group) were exposed by gavage to concentrations of 221, 313, 442, 625 and 883 mg /kg of Kathon™ 886F (corresponding to 14% C(M)IT/MIT in aqueous solution for Lot 76/0445 and 13.3% for Lot 0098) (**Craig, 1993**)(1). The test article was administered as a single dose, followed by clinical observations at 0 and 6h after dosing and daily thereafter for 14 days.

Clinical signs

Clinical signs were observed in all dose levels of this study.

In Lot 76/0445, salivation, lethargy, ptosis, piloerection, lacrimation, ataxia, prostration, nasal discharge and diarrhea were observed. In Lot 0098, the same clinical signs were observed except salivation.

Necropsy

Necropsy of the decedents and survivors revealed gross changes in all dose levels.

In decedent animals of Lot 76/0445, reddening and irritation of the stomach and intestines and sloughing of the stomach mucosa were observed. In survivors, scar tissue of the stomach and reddening of the intestines were noted.

In decedent animals of Lot 0098, reddened intestines, reddening and edema of the stomach, sloughing of the stomach mucosa and gas distention of the intestines were reported. In survivors, scar tissue of the stomach and reddening of the stomach and intestines were observed.

Mortality is summarized in the table below:

Table 4.2-2: Table for Acute Oral Toxicity in Rats

Dose (mg Kathon™ 886F/kg)	Number of dead / number of investigated		Time of death (range)	
	Lot 76/0445	Lot 0098	Lot 76/0445	Lot 0098
221	1/10	0/10	day 6	no deaths
313	0/10	0/10	no deaths	no deaths
442	4/10	2/10	4 at 0-6 h	2 at 0-6 h
625	9/10	6/10	9 at 0-6 h	4 at 0-6 h; 1 at 24h ; 1 at 8-14 days
883	no animals dosed	10/10	no animals	8 at 0-6 h 2 at 24h

On the basis of this study, the acute oral LD_{50} in male rats is determined to be 457 mg Kathon™ 886F/kg (corresponding to 64 mg a.i./kg).

In another study, Sprague Dawley rats (5/sex/group) were exposed by gavage to concentrations of 0, 365, 504 and 718 mg/kg of ACTICIDE 17 (14% C(M)IT/MIT in aqueous solution) (**Mercier, 1994**)[(2)]. The test article was administered as a single dose and followed by a clinical observation period of 14 days.

Clinical signs

Mortality occured from 1 hour after administration of the test article to day 2:

Prostration and subdued behaviour were observed from 15 minutes to 4 hours after administration of the test article in the treated groups.

Necropsy

Most animals dying during the observation period showed stomach distended by a greenish liquid, congested mucosa of glandular stomach. Thymus, lungs or intestines were slightly or markedly congested in some animals.

No macroscopically detectable abnormality was noted in animals euthanatized on study termination (Day 15).

Mortality is summarized in the table below:

Table 4.2-3: Table for oral toxicity ACTICIDE 14 to rats

Dose [mg ACTICIDE 14/kg]	Number of dead / number of investigated	Duration of clinical signs	Time of death (Days after dosing)
0	0 / 10		
365	Male: 1/5 Female: 0/5	1 – 4 h	1 after 4 hours at day 1
504	Male: 3/5 Female: 2/5	1 – 4 h	Male: 1 after 4 h at day 1, 2 at day 2 Female: 1 after 1 h at day 1, 1 at day 2
718	Male: 5/5 Female: 5/5	1 – 4 h	Male: 2 after 1 h and 1 after 2 h at day 1, 2 at day 2 Female: 3 after 1 h and 2 after 2 h at day 1

On the basis of this study, the combined male and female LD_{50} is determined to be 472 mg ACTICIDE 14/kg (corresponding to 66 mg a.i./kg).

4.2.1.2 Acute toxicity: inhalation

Two acute inhalation toxicity studies provide relevant information to evaluate the acute toxicity of C(M)IT/MIT by respiratory route. These studies were performed according the OECD guideline 403.

Crl:CD®BR rats (6/sex/group) were exposed snout-only to atmospheres containing respirable particles of C(M)IT/MIT (prepared from a solution of Kathon^{TM}886F; the particle size distribution gave a MMD of 2.7 ± 0.9 µm and a mean respirable fraction (< 7µm) of 57 ± 9%) at concentrations of 0.19, 0.32, 0.5, 1.26, 2.24 and 3.02 mg Kathon^{TM}886F/L (14 % C(M)IT/MIT in aqueous solution) (corresponding to 0.027, 0.045, 0.07, 0.176, 0.314 and 0.422 mg a.i/L or group 1, 2, 3, 4, 5 and 6) for 4 hours (**Wanner, 1991**)[(3)]. Clinical observations were reported during exposure period and twice per day for 14 days after exposure.

Clinical signs

Death occurred during exposure period in rats exposed to Kathon^{TM}886F at 0.32, 1.26, 2.24 and 3.02 mg/L.

Signs of respiratory irritation, including gasping, rales, hyperpnea, dyspnea and vocalization, were seen in some animals in all groups immediately post-exposure. The number of animals showing these signs and the severity of the respiratory irritation correlated with the concentration of the test material to which the animals were exposed in the report. The signs of respiratory irritation disappeared in all surviving animals, taking from two to twelve days. Small red droplets were seen on the drop sheets in groups 3, 4, 5, and 6 (corresponding to 0.5, 1.26, 2.24 and 3.02 mg/L). This sign disappeared in all surviving animals taking from 6 to 12 days. This was judged to be expired nasal exudates and the result of nasal irritation due to

exposure of the test substance. Other treatment related signs, including scant feces, thriftlessness and black or crusty material on the muzzle were also seen in several of the groups. The crusty material on the muzzle was judged to be the result of direct contact with the test material. These and all other signs disappeared in all surviving animals by Day 12.

Necropsy

Animals in groups 4, 5 and 6 (corresponding to 1.26, 2.24 and 3.02 mg/L) showed stomachs and/or intestines filled with gas which correlated with the concentration of the test material to which the animals were exposed, the greater response was seen in animals exposed to the greater concentration of the test material. This was judged to be the result of swallowing air in an attempt to breathe. No other treatment-related necropsy observations were seen in any animal.

Mortality is summarised in the table below.

Table 4.2-4: Acute Toxicity Inhalation LC_{50} Rats

Dose (mg/L)	**Number of dead / number of investigated**	**Time of death (range)**
Group 1 0.19	0/12	No deaths
Group 2 0.32	1/12	1 died within 3 h after removal from chamber
Group 3 0.50	0/12	No deaths
Group 4 1.26	3/12	1 died within 3 h after removal from chamber, 2 died within 24 h
Group 5 2.24	4/12	3 died within 3 h after removal from chamber, 1 died within 24 h
Group 6 3.02	9/12	8 died within 3 h after removal from chamber, 1 died within 24 h

On the basis of this study, the combined male and female LC_{50} was determined to be 2.36 mg Kathon^{TM}886F/L air, corresponding to 0.33 mg a.i/L/4h.

Sprague-Dawley rats (5/sex/group) were exposed snout-only to atmospheres containing respirable particles of C(M)IT/MIT (prepared from a solution of ACTICIDE 14 (14% C(M)IT/MIT in aqueous solution); the particle size distribution gave a MMD of 2.1 – 3.2 µm and a mean respirable fraction (< 7µm) of 85 – 95.7%) at concentrations of 0.344, 0.366, 0.443, 1.16, 1.79 and 2.75 mg ACTICIDE 14/L (corresponding to 0.048, 0.051, 0.062, 0.16, 0.25 and 0.39 mg a.i/L or group 2, 3, 4, 5, 6 and 7) for 4 hours (**Jackson, 1997**)[(5)].

Clinical observations were reported during exposure period and twice per day for 14 days after exposure.

Clinical signs

Death occurred during exposure period in rats exposed to ACTICIDE 14 at 0.366, 1.16 mg/l, 1.79 mg/l or 2.75 mg/L.

Signs in all test groups during exposure were exaggerated respiratory movements indicative of an effect on the respiratory tract, and soiling of the fur with excreta. The soiling was attributed to the method of restraint. Gasping or exaggerated respiratory movements were apparent in all surviving tests rats following exposure. Other signs were seen following exposure to ACTICIDE 14 included lethargy, staining of the body fur and whole body tremors.
Some of the signs persisted for several days following exposure but all rats that survived exposure to ACTICIDE 14 were normal in appearance and behaviour within 5 days of exposure.

Examination of the mortality data for ACTICIDE 14 indicates that the mortality for female rats of Group 3 (ACTICIDE 14; 0.366 mg/L; 0.051 mg a.i/L) was not consistent with mortality seen in other groups exposed to ACTICIDE 14. Exposure of females at higher levels produced lower mortality and repeat exposures at 0.344 mg/L (0.048 mg/L) or 0.443 mg/L (0.062 mg/L) did not confirm the high mortality seen for female rats of Group 3. Furthermore, examination of the bodyweight data indicated that some female rats in Group 3 were losing weight at the days just before exposure. The mortality data for Group 3 had therefore been excluded from the LC_{50} calculations.

Necropsy

Congested lungs were seen in most decedent rats from all exposure groups.
External macroscopic findings for decedent rats included wet fur and/or brown staining around the snout and jaws and matted fur.
The lung to bodyweight ratio of decedent rats was generally higher than that of control rats.

Mortality is summarized in the table below.

Table 4.2-5: Table of inhalation toxicity

Dose a.i. [mg/L]	**Nb of dead animals/ Nb of animals with toxic signs/ nb of investigated animals**	**Time of death (no. per day)**	**Observations**
MALES			
Group 1: Air control	0/0/5	---	---
Group 2: 0.048	0/5/5	---	During exposition and post-exposure: – fur soiled with excreta – gasping – exaggerated respiratory movement
Group 3 :0.051	1/5/5	Day 1 during post exposure	
Group 4: 0.062	0/5/5	---	
Group 5: 0.16	2/5/5	3rd hour of exposure, Day 1	
Group 6: 0.25	3/5/5	3rd hour of exposure, Day 1, Day 2 during post exposure	
Group 7: 0.39	5/5/5	2nd hour of exposure - Day 2	

Dose a.i. [mg/L]	**Number of dead animals/ Number of animals with toxic signs/ number of investigated animals**	**Time of death (no. per day)**	**Observations**
FEMALES			
Group 1 Air control	0/0/5	---	---
Group 2 0.048	0/5/5	---	During exposition and post-exposure: – fur soiled with excreta – gasping – exaggerated respiratory movement
Group 3 0.051	4/5/5	Day 1 during post exposure	
Group 4 0.062	1/5/5	Day 1 during post exposure	
Group 5 0.16	2/5/5	3rd hour of exposure, Day 1	
Group 6 0.25	1/5/5	Day 1during post exposure	
Group 7 0.39	5/5/5	3nd hour of exposure - Day 2	

On the basis of this study, the male LC_{50} was determined to be 1.21 mg ACTICIDE 14/L air (corresponding to 0.169 mg a.i/L) and females LC_{50} was determined to be 1.38 mg ACTICIDE 14/L air, corresponding to 0.193 mg a.i/L/4h. The combined LC_{50} was determined to be 1.23 mg ACTICIDE 14/L air, corresponding to 0.171 mg a.i/L/4h.

4.2.1.3 Acute toxicity: dermal

Two acute dermal toxicity studies provide relevant information to evaluate the acute toxicity of C(M)IT/MIT by dermal route. One of these study was performed according the OAEC guideline 402. No guideline was available at the time the second study was conducted (1976).

SD rats (5/sex/group) were exposed by dermal route to 0.8 mL of ACTICIDE 14 (corresponding to 14% C(M)IT/MIT in aqueous solution) for 24h (**Mercier, 1994**)[(6)]. The test article was applied once only at the dose level of 1008 mg/kg (i.e. 141 mg a.i/kg) followed by a post exposure period of 14 days.

Clinical signs

Three animals showed subdued behaviour on day 2: Two males died on day 2 and one female on day 3.

Eight animals on day 2 and seven animals on day 3 showed a moderate oedema (less than 1mm thick). The seven surviving animals showed a slight oedema on day 4 and superficial eschars from day 5 to 15.

Necropsy

Three animals which died during the observation period showed an oedema of the subcutaneous tissue and one showed marked congested lungs. No macroscopically detectable abnormality was noted in animals euthanized on study termination.

A total mortality of 30% was calculated at the tested dose level, no LD_{50} was determined.

Mortality is summarized in the table below:

Table 4.2-6 : Table of dermal toxicity

Dose [mg/kg]	Number of dead / number of investigated	Time of death (range)
1008	2/5 males 1/5 female	Day 2 Day 3

On the basis of this study, the combined male and female LD50 is higher than 1 008 mg ACTICIDE 14/kg (corr. to 141 mg a.i/kg).

In another study, albino rabbit male (5/group) were exposed by dermal route to undiluted KathonTM886 (14% C(M)IT/MIT in aqueous solution) at doses of 313, 625, 1250 and 2500 mg/kg bw/d for 24h followed by a post exposure period of 14 days (**Craig, 1993**)[(7)].

Clinical signs

Clinical signs were observed in dose levels up to and including 1250 mg/kg. However, rabbits in the 2500 mg/kg dose group appeared to have died prior to the recording of the first clinical observations. Clinical signs observed included: lethargy, prostration, ataxia, dilation of pupils, hypothermia, slow respiration and poor food consumption.

Necropsy

Necropsy of the decedents and survivors revealed only subcutaneous tissue damage at the application site.

Skin irritation consisted of severe erythema and edema followed by eschar formation.

Mortality is summarized in the table below:

Dose (mg Kathon^{TM}886/ kg)	**Number of dead /number of investigated**	**Time of death (range)**
313	0/5	no deaths
625	2/5	2 at 24 h
1250	5/5	4 at 24 h; 1 at 48 h
2500	5/5	5 at 24 h

On the basis of this study, LD_{50} was determined to be 660 mg Kathon^{TM} 886/kg (with 95% confidence limits of 370 and 1210 mg/kg). This corresponds to LD_{50} = 87.12 mg a.i./kg.

4.2.1.4 Acute toxicity: other routes

Not considered in this dossier.

4.2.2 Human information

No data.

4.2.3 Summary and discussion of acute toxicity

C(M)IT/MIT is toxic/highly toxic by the oral, dermal and inhalation routes.

After acute oral, inhalation or dermal exposure, it induces effects in relation with its corrosive properties.

The acute oral LD_{50} of C(M)IT/MIT in rats ranges from 457 to 472 mg/kg (corresponding to 64 and 66 mg a.i./kg).

The 4-hr nose-only acute inhalation LC_{50} of C(M)IT/MIT in rats ranges from 1.21 to 2.36 mg/L air (corr. to 0.169 to 0.33 mg a.i./L air). The effects observed are consistent with the clinical signs of respiratory irritation.

The acute dermal LD_{50} of C(M)IT/MIT in rats is higher than 1 008 mg ACTICIDE 14/kg bw/d (corr. to 141 mg a.i/kg). In rabbits, the LD_{50} was determined to be 660 mg Kathon^{TM} 886/kg (corr. to LD_{50} = 87.12 mg a.i./kg).

4.2.4 Comparison with criteria

For C(M)IT/MIT, the acute oral LD_{50} ranges from 64 to 66 mg a.i./kg. These values lie within the range (50-300 mg/kg) for classification as Acute Tox.3 (H301: Toxic if swallowed) under regulation (EC) 1272/2008.

The LC_{50} in rats ranges from 0.169 to 0.33 mg a.i./L air. These values lie within the range (0.05-0.5 mg/L) for classification as Acute Tox.2 (H330: Fatal if inhaled) under regulation (EC) 1272/2008.

The acute dermal LD_{50} is equal to 87.12 mg a.i./kg. This value lies within the range (50-200 mg/kg) for classification as Acute Tox 2 (H310: Fatal by contact with skin) under regulation (EC) 1272/2008.

4.2.5 Conclusions on classification and labelling

Based on the results of the acute oral, dermal and inhalation toxicity studies, a classification **Acute Tox.3-- H301**; **Acute Tox.2-H330 and Acute Tox.2-H310** is proposed for C(M)IT/MIT.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed to remove the existing minimum classification for acute toxicity of C(M)IT/MIT by the oral route (Acute Tox. 3*; H301). In the two acute oral toxicity studies available (of which one was performed according the OECD Test Guideline (TG) 401), the oral LD_{50} in rats were 457 and 472 mg/kg bw, corresponding to 64 and 66 mg a.i./kg bw, respectively. These values lie within the range 50-300 mg/kg for classification as Acute Toxicity 3 (H301: Toxic if swallowed) under CLP.

Following inhalation exposure (nose-only, 4 h), the LC_{50} of C(M)IT/MIT in rats ranged from 0.169-0.33 mg/L air and the effects observed were consistent with respiratory irritation. These values lie within the range of 0.05 – 0.5 mg/L for classification with Acute Toxicity 2 (H330: Fatal if inhaled).

After dermal exposure, the LD_{50} of C(M)IT/MIT in rats was 141 mg/kg bw. In rabbits, the LD_{50} was determined to be 87.12 mg/kg bw. These values both lie within the range of 50-200 mg/kg bw and so meet the classification criteria for Acute Toxicity 2 (H310: Fatal by contact with skin).

The DS concluded that C(M)IT/MIT is highly toxic by the dermal and inhalation routes, inducing effects consistent with its corrosive properties.

Comments received during public consultation

Two industry stakeholders and one MS agreed with the proposed category 2 classification for acute dermal toxicity. The same MS also agreed with the proposal for acute inhalation toxicity category 2.

One of the stakeholder organisation agreed with acute oral toxicity category 3 but questioned the appropriateness of the proposed classification for acute inhalation toxicity. The low vapour pressure of C(M)IT/ MIT required generation of an aerosol. Such an atmosphere is unlikely to be generated under foreseeable conditions. Furthermore, the observed effects were considered to be primarily due

to the irritating/ corrosive nature of the test material.

The other industry stakeholders agreed with the proposed classification for acute inhalation toxicity (category 2) but did question the relevance of data obtained by means of an aerosol in view of the low vapour pressure of the substance.

In response, the DS explained that these exposure-related issues are not considered for classification. The proposal is based on the relevant criteria, which are based on the inherent hazardous properties of substance concerned.

One MS suggested applying the phrase EUH071 (Corrosive to the respiratory tract), making the case that this could be justified by the corrosive nature of C(M)IT to skin and the evidence from acute studies of toxicity via inhalation. The DS replied that EUH071 could be envisaged based on the classification for acute inhalation toxicity and the corrosivity of the substance.

Assessment and comparison with the classification criteria

Oral

Two acute oral toxicity studies in SD rats provide were considered acceptable and reliable although one study was conducted prior to OECD TG 404 and GLP. In both studies, diluted products containing 13.3 to 14% C(M)IT/MIT in aqueous solutions were administered as a single dose, followed by a clinical observation period of 14 days. Both studies provided very similar LD_{50} and the clinical signs were consistent with the corrosive properties of C(M)IT/MIT (see below). No gender difference was apparent. The oral LD_{50} in rats ranged between 64 (males only) and 66 (males and females combined) mg C(M)IT/MIT kg bw.

In conclusion, RAC agrees with the DS to remove the minimum classification for C(M)IT/MIT since oral LD_{50} values in rats lie within the range (50-300 mg/kg bw) for classification as Acute Tox. 3 (H301: Toxic if swallowed) under CLP.

Dermal

Two dermal studies are available, one in rats and one in rabbits.

The study in rats was carried out according to OECD TG 402 and GLP. In this study, male and female rats were exposed to an aqueous solution of C(M)IT/MIT (14%) i.e. a dose level of 141 mg C(M)IT/MIT/kg bw for 24 h. At this single dose level there was 30% mortality and so the LD_{50} was determined to be > 141 mg/kg bw.

A study in rabbits was also available, carried out in 1976 prior to OECD guidelines and GLP. In this study male rabbits were exposed to an aqueous solution of C(M)IT/MIT (14%) at doses of 313, 625, 1250 and 2500 mg test material/kg bw for 24 h. The LD_{50} was determined to be 87.12 mg C(M)IT/MIT/kg bw. This finding is in accordance with the criteria for classification with acute dermal

toxicity category 2 (50 < LD_{50} ≤ 200 mg/kg bw).

Inhalation

Two acute toxicity experiments via the inhalation route are available. Both of these studies were carried out according to OECD TG 403 and in compliance to GLP.

In both studies, male and female rats were exposed to an aerosol generated from an aqueous solution of C(M)IT/MIT (14%), nose-only, for 4 h. In the first study, the mean respirable fraction (< 7 µm) was 57 ± 9% and the combined LC_{50} for males and females was 0.33 mg/L C(M)IT/MIT. In the second study, the mean respirable fraction (< 7 µm) was 85-95.7% and the combined LC_{50} for males and females was 0.171 mg/L C(M)IT/MIT. In both studies, particle size in the test atmosphere achieved the recommended aerodynamic diameter standard of 1-4 µm.

The LC_{50} values of 0.33 and 0.171 mg/L are both within the range (0.05 < LC_{50} ≤ 0.5 mg/L) given in the criteria for classification in Acute Inhalation Toxicity Category 2 for dusts and mists.

An industry stakeholder queried the validity of using data from studies that involved the generation of an aerosol of C(M)IT/MIT claiming that such exposure conditions would not be generated under foreseen conditions. However, RAC agrees with the response of the DS that the classification should be based on the inherent properties of C(M)IT/MIT and therefore the data should be taken into account.

One MS and the DS considered that EUH071 ("corrosive to the respiratory tract") may also be applicable to C(M)IT/MIT. According to Annex II of the CLP Regulation, EUH071 "shall be assigned for substances and mixtures in addition to classification for inhalation toxicity, if data are available that indicate that the mechanism of toxicity is corrosivity". The findings in the rat acute inhalation studies indicate that the lethality observed is likely to have been due to severe local irritation or corrosion: gasping, rales, hyperpnea, dyspnoea and exaggerated respiratory movements observed immediately after exposure, also congested lungs and gas-filled stomachs and/or intestines at necropsy. This latter effect was deemed to be the result of swallowing of air in an attempt to breathe. The clinical signs disappeared in all surviving animals, taking at most from 6 to 12 days to resolve.

Given that C(M)IT/MIT is corrosive to the skin and eyes (see below), RAC considers the most likely explanation for the observed inhalation toxicity is its corrosive nature. On this basis, although the DS and those who responded during the public consultation did not consider the potential for other mechanisms of toxicity, RAC concludes using expert judgement that EUH071 should be applied.

In conclusion, RAC agrees with the DS that classification of C(M)IT/MIT is

warranted as follows:

- **Acute Tox. 3, H301: Toxic if swallowed;**
- **Acute Toxicity 2, H330: Fatal if inhaled;**
- **Acute Toxicity 2, H310: Fatal in contact with skin.**

In addition, RAC is of the opinion that the additional labelling phrase **EUH071: Corrosive to the respiratory tract** is justified.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not considered in this dossier.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

Specific target organ toxicity following a single exposure was not considered in this dossier.

Comments received during public consultation

No comments were received regarding this endpoint.

Assessment and comparison with the classification criteria

RAC noted the available information from acute toxicity studies. Clinical signs observed during the studies following inhalation exposure of C(M)IT/MIT were consistent with respiratory irritation. These included, gasping, rales, hyperpnea, dyspnoea and exaggerated respiratory movements. Necropsy revealed congested lungs and gas-filled stomachs and/or intestines. This latter effect was deemed to be the result of swallowing of air in an attempt to breathe.

Although the data suggest that C(M)IT/MIT is a respiratory irritant, the effects are accounted for by the classification for acute inhalation toxicity and the application of the EUH071 phrase. **Therefore RAC does not propose an additional classification for STOT SE**.

4.4 Irritation

Not considered in this dossier.

4.5 Corrosivity

Table 4.5-1: Summary table of relevant dermal irritation studies

Method	Results	Remarks	Reference
Acute dermal irritation or corrosion study OECD 404	Severely irritant. One animal presented a clear edema and a slight eschar. Erythema: Score: 2.5 (mean of 24, 48 and 72 h) Edema Score: 2.1 (mean of 24, 48 and 72 h)	New Zeland White Rabbit n = 3/group (male) Reversibility on day 11 for erythema, and on day 8 for edema Test substance: aqueous solution of C(M)IT/MIT 14%	Roubier, 1986
Acute dermal irritation or corrosion study OECD 404	Moderately irritating at 0.25% a.i. Severely irritating at 0.5% a.i. Corrosive at 0.75% and 1% a.i. Erythema (4h exposure) Mean score value at 24, 48 and 72h: 2.1 (0.25 % a.i.), 2.5 (0.50 % a.i.), 3.1 (0.75 % a.i.), 3.2 (1.0 % a.i.) Edema (4h exposure) Mean value at 24, 48, 72h: 2.5 (0.25 % a.i.), 3.3 (0.50 % a.i.), 3.1 (0.75 % a.i.), 3.7 (1.0 % a.i.)	New Zeland White Rabbit n = 3/group (male) Reversibility: - at 0.25 % ai 7-14 days after application - at 0.5 % ai 14-21 days after application - No at 0.75 and 1.0 % ai Test substance: aqueous solution of C(M)IT/MIT 1.5%	Morrisson, 1985
Acute dermal irritation or corrosion study OECD 404	Corrosive (irreversible burnt appearance) Erythema (4h exposure) Mean score value at 24, 48 and 72h: 4 Edema (4h exposure): Mean score value at 24, 48 and 72h: 3.7	New Zeland White Rabbit n = 1 (male) due to severe reaction No reversibility Test substance: aqueous solution of C(M)IT/MIT 14%	Mercier, 1994

Three dermal studies provide relevant information to evaluate corrosion of C(M)IT/MIT by dermal route. These studies were performed according the OECD guideline 404.

Six NZW rabbits were exposed *via* dermal route to 0.5 mL of C(M)IT/MIT (14% in water) as delivered for 1 hour (3 rabbits) and 4 hours (3 rabbits) (**Roubier, 1986**)[8]. Animals were examined 60 minutes after removal of gauze and then once daily for 12 days. Body weights were recorded prior to study initiation, on day 1 of the study and weekly during the study. No post-mortem examinations were conducted.

A severe edema (score = 4) was observed in five animals and one animal had a moderate edema (score = 3) one hour after patch removal. This edema was raised more than 2 mm and extended beyond the area of exposure. By day 3, this irritation reversed such that only 3 animals had a slight edema. There was total recovery after 8 days. One animal had a well-defined erythema with slight eschar formations. A reversal was observed after 72 h with total recovery after 11 days.

C(M)IT/MIT is a severe irritant.

The study results are as follow:

Table 4.5-2: Table for skin irritation study – 1 h exposure

score (average animals investigated)	**time**	**Erythema**	**Edema**
average score Draize scores (0 to maximum 4)	60 min	2.7	4.0
	24 h	2.3	2.3
	48 h	2.0	1.3
	72 h	0.7	0.3
other times	96 h (4 days)	0.7	0.3
	120 h (5 days)	0.7	0.3
	144 h (6 days)	0.7	0.3
	168 h (7 days)	1.0	0.3
	192 h (8 days)	1.0	0.3
	216 h (9 days)	0.3	0.0
	240 h (10 days)	0.3	0.0
	264 h (11 days)	0.0	0.0
reversibility: *		c	c
average time for reversibility		after 240 h	after 192 h
*c : completely reversible; n c : not completely reversible; n : not reversible.			

Table 4.5-3: Table for skin irritation study – 4 h exposure

score (average animals investigated)	**time**	**Erythema**	**Edema**
average score Draize scores (0 to maximum 4)	60 min	3.7	3.7
	24 h	3.0	3.7
	48 h	2.7	2.0
	72 h	1.7	0.7
other times	96 h (4 days)	1.7	0.7
	120 h (5 days)	2.0	0.7
	144 h (6 days)	2.0	0.7
	168 h (7 days)	2.0	0.7
	192 h (8 days)	1.7	0.0
	216 h (9 days)	1.7	0.0
	240 h (10 days)	0.7	0.0
	264 h (11 days)	0.3	0.0
reversibility: *		c	c
average time for reversibility		after 264 h	after 168 h
*c : completely reversible; n c : not completely reversible; n : not reversible			

Three male New Zealand White rabbits were exposed *via* dermal route to concentrations of C(M)IT/MIT of 0.25%, 0.5%, 0.75% and 1.0% a.i for 4 hours. Animals were examined during a post exposure period of 21 days (**Morrisson, 1985**)[(9)].

Erythema were observed at 72h with a mean score value of 1.3 at 0.25%, 3.0 at 0.5%, 3.3 at 0.75% and 3.0 at 1.0%. For edema, a mean score value of 1.7 at 0.25%, 2.3 at 0.5%, 2.7 at 0.75% and 3.3 at 1.0% were observed at 72h.

Based on the results, C(M)IT/MIT is moderately irritating to the skin of rabbits at a concentration of 0.25 % a.i., severely irritating to the skin of rabbits at a concentration of 0.50 % a.i. and 0.75 % a.i. and corrosive to the skin of rabbits at a concentration of 0.75% and 1.0 % a.i. Indeed, no reversibility is observed at 14 days post-treatment.

The study results were as follow:

Table 4.5-4: Table for Acute Dermal Irritation – 0.25% active ingredient

Score (average animals investigated)	Time	Erythema	Edema
Average score Draize scores (0 to maximum 4)	60 min	3.3	4.0
	24 h	2.7	3.7
	48 h	2.3	2.0
	72 h	1.3	1.7
	7 days	1.3	0.7
	14 days	0.0	0.3
	21 days	0.0	0.0
72 h mean irritation	72 h	3.0	3.0
Reversibility: *		C	C
Average time for reversibility		7-14 days	7-14 days
*c : completely reversible; n c : not completely reversible; n : not reversible			

Table 4.5-5: Table for Acute Dermal Irritation – 0.50% active ingredient

Score (average animals investigated)	Time	Erythema	Edema
Average score Draize scores (0 to maximum 4)	60 min	4.0	4.0
	24 h	2.3	4.0
	48 h	2.3	3.7
	72 h	3.0	2.3
	7 days	2.7	1.3
	14 days	1.3	0.0
	21 days	0.0	0.0
72 h mean irritation	72 h	5.3	5.3
Reversibility: *		C	C
Average time for reversibility		14-21 days	14-21 days
* c : completely reversible; n c : not completely reversible; n : not reversible.			

Table 4.5-6: Table for Acute Dermal Irritation – 0.75% active ingredient

Score (average animals investigated)	Time	Erythema	Edema
Average score Draize scores (0 to maximum 4)	60 min	2.7	4.0
	24 h	2.3	4.0
	48 h	3.3	2.7
	72 h	3.3	2.7
	7 days	3.3	3.0
	14 days	2.3	2.0
72 h mean irritation	72 h	6.0	6.0
Reversibility: *		n	n
Average time for reversibility		---	---
* c : completely reversible; n c : not completely reversible; n : not reversible.			

Table 4.5-7: Table for Acute Dermal Irritation – 1.0% active ingredient

Score (average animals investigated)	Time	Erythema	Edema
Average score Draize scores (0 to maximum 4)	60 min	4.0	4.0
	24 h	3.3	4.0
	48 h	3.3	3.7
	72 h	3.0	3.3
	7 days	4.0	2.7
	14 days	2.3	0.3
	21 days	1.7	0.0
72 h mean irritation	72 h	corrosive	corrosive
Reversibility: *		N	N
Average time for reversibility		---	---
* c : completely reversible; n c : not completely reversible; n : not reversible.			

NZW rabbit was exposed *via* dermal route to 0.5 mL of C(M)IT/MIT (14% in water) as supplied for 4 hours. The cutaneous examinations were performed, for erythema and edema, after removal of the bandage. Besides, these readings were continued on day 7 and 14 (**Mercier, 1994**)[10].

Severe skin reactions were observed with a mean score value of 4 at 72 h for erythema and 3.7 for edema. However, only one animal was tested.

Based on these results, C(M)IT/MIT is corrosive to skin.

The study results are as follow:

Table 4.5-8: Table for skin irritation study – mean scores for one rabbit

Assessment	**Time**	**Erythema**	**Edema**
Draize scores (0 to maximum 4)		**0-4**	**0-4**
		Average score (one rabbit)	
	1 hour	3	4
	24 hours	4	4
	48 hours	4	4
	72 hours	4	3
Average score		4	4
Reversibility*:		N	C
Time for reversibility to occur		NA	Day 7
*c : completely reversible; n c : not completely reversible; n : not reversible, NA: not applicable			

4.5.1 Summary and discussion of corrosivity

C(M)IT/MIT is corrosive to skin from a concentration of 0.75% a.i. After dermal exposure, it induces irreversible skin reactions in rabbits.

4.5.2 Comparison with criteria

For C(M)IT/MIT, irreversible burnt are observed in animals after a 4-hour exposure period. No irreversible skin damage was observed in rabbits after a one-hour exposure period from 0.75% a.i. These results are consistent with the criteria for classification as Skin Corr. 1C (H314: Causes severe skin burns and eye damage) under regulation (EC) 1272/2008 with a specific concentration limit (SCL) at 0.5% a.i.

4.5.3 Conclusions on classification and labelling

Based on the results of the dermal irritation studies, a classification **Skin Corr. 1C, -H314 with a specific concentration limit: C > 0.5%, Skin Corr. 1C-H314** is proposed for C(M)IT/MIT.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Several dermal studies provided relevant information to evaluate irritation/corrosion of C(M)IT/MIT by the dermal route. These studies were all performed according to OECD TG 404. The DS reported that C(M)IT/MIT was found to be corrosive to skin from a concentration of 0.75% in New-Zealand White (NZW) rabbits after 4h exposure and were found to be irreversible. No irreversible skin damage was observed in the study after a 1h exposure period. According to the DS, these results are consistent with criteria for classification with Skin Corr. 1C (H314: Causes severe burns and eye damage) with a proposed revised specific concentration limit (SCL) of C ≥ 0.5%.

Comments received during public consultation

One MS agreed with the proposed classification of Skin Corr. 1C with an SCL of C ≥ 0.5%. Another MSCA also agreed with classification for skin corrosion with an SCL of ≥ 0.5%, but questioned whether subcategorisation was appropriate because this was based on the absence of corrosivity in a 1 hour study with a low concentration. The DS responded that the proposed classification in category 1C was based on available data.

Additionally, the second MS requested adaptation of the SCL for skin irritation since the existing SCL (0.6 to 0.06%) is not in line with the proposed SCL of 0.5% for corrosion. One stakeholder organisation suggested an SCL of 0.06% ≤ C < 0.5% w/w for Skin Irrit. 2; H315 after taking into consideration the SCL of C ≥ 0.5% w/w applicable to the hazard class Skin Corr. 1C; H314.

A second industry stakeholder agreed that Skin Corr. 1C is appropriate but proposed retaining the existing SCL agreed under DSD (C ≥ 0.6%) on the basis that full reversibility of effects is observed 14-21 days after application of 0.5% dilution. This organisation also considered that the existing SCL for Skin Irrit. 2 (0.06% ≤ C < 0.6%) is adequate and should be maintained.

Another stakeholder also agreed with the proposal for categorisation of C(M)IT/MIT as Skin Corr. 1C, but since the data demonstrated that the corrosive effects of the substance were observed at 0.75% and above, the organisation proposed an SCL of 0.75% for corrosivity. Additionally, this organisation did not consider additional classification for dermal and eye irritation to be warranted. The stakeholder made reference to section 3.2.2.6 of the Guidance on the Application of the CLP Criteria, which states that substances shall be labelled as

corrosive or irritating and not both.

In response to the public comments, the DS explained that the proposed lower SCL of 0.5% was retained in order to address the severity of the effects of corrosion and the available data and to update Skin Irrit. 2 (H315) with SCLs as $0.06 \leq C < 0.5\%$.

As presented in Table 9 of the CLH report, a solution at 1% of the test material at 20°C has a pH of 3.4. This low pH is indicative of C(M)IT/MIT's potential to cause skin effects, which provides further support for classifying C(M)IT/MIT as corrosive.

Assessment and comparison with the classification criteria

C(M)IT/MIT currently has a harmonised classification of Skin Corr. 1B, with a specific concentration limit (SCL) of 0.6%. RAC was advised that this was translated from the classification agreed by the Commission Working Group on the Classification and Labelling of Dangerous Substances. In addition to several animal studies, that group had also been provided with data from human case studies to show that C(M)IT/MIT (14%) is corrosive to human skin.

No human data were presented in the CLH report. However, the results of three dermal irritation studies in rabbits were presented by the DS. Two studies showed evidence of corrosivity of C(M)IT/MIT to rabbit skin whereas another seemed to show severe irritation, not skin corrosion. One of the 2 studies showing the corrosive potential of C(M)IT/MIT also provided valuable information about potency that is of relevance for the setting of a specific concentration limit for this endpoint.

In a study conducted in 1994, one NZW rabbit was exposed via the dermal route to 0.5 mL of C(M)IT/MIT (14% in water) for 4 hours. No further animals were used in this study because of the severe irreversible burn produced. Erythema was observed with a mean score value at 24, 48 and 72h of 4. There was no reversibility of erythema by day 14. Oedema, which reversed by day 7, was observed, with a mean score value of 3.7. Given the severity of the lesions observed and the irreversibility of the erythema, the test substance is considered corrosive to skin. Since the damage occurred following a 4 hour exposure, these data would support categorisation of C(M)IT/MIT in at least Skin Corr. Cat. 1C. The study did not investigate whether shorter exposure times would also produce a corrosive effect and so do not inform on the applicability of a more severe sub-categorisation.

In a study conducted in 1985, three male NZW rabbits were exposed via the dermal route to C(M)IT/MIT at concentrations of 0.25%, 0.5%, 0.75% and 1.0% for 4 hours. The mean score values at 24, 48 and 72h are presented in the table below.

Concentration	Erythema	Oedema
0.25%	2.1	2.5
Average time for reversibility	7-14 days	7-14 days
0.5%	2.5	3.3
Average time for reversibility	14-21 days	14-21 days
0.75%	3.1	3.1
Average time for reversibility	no reversibility 14 days post-treatment	no reversibility 14 days post-treatment
1.0%	3.2	3.7
Average time for reversibility	no reversibility 14 days post-treatment	no reversibility 14 days post-treatment

According to the DS, this study indicates that C(M)IT/MIT is corrosive due to the severity and irreversible damage induced by exposure to the test substance at 0.75% and 1%. At concentrations of 0.25% and 0.5%, C(M)IT/MIT produced a skin irritant effect. However, the DS did not report the observations in full, but given the existing classification of C(M)IT for corrosivity, RAC has no reason to doubt this assessment. The study did not investigate whether shorter exposure times would also produce a corrosive effect, therefore it also does not inform on the possibility of a more severe sub-categorisation than Skin Corr. 1C.

A further dermal irritation study, in which six NZW rabbits (3/group) were exposed to an aqueous solution of C(M)IT/MIT (14%) for 1 hour and 4 hours was conducted in 1986. One animal presented a well-defined erythema and a slight eschar. The mean erythema Draize score at 24, 48 and 72 hours was 2.5. A reversal of erythema was observed at 72h, with total recovery after 11 days. Oedema was severe (scored the maximum Draize score of 4) in 5 animals, whilst the other animal had moderate oedema (score = 3) one hour after patch removal. The oedema extended beyond the area of exposure. By day 3, there was evidence that the irritation had reversed (only 3 animals had a slight oedema). The mean Draize score for oedema was 2.1 (mean of 24, 48 and 72 hours) and total recovery was observed after 8 days. This study produced similar results to the 1994 study but the effects observed were not as severe and according to the CLP criteria, the results indicate severe skin irritation rather than skin corrosion. No argument was provided in the CLH report to explain why exposure to the test substance in this study elicited a less severe reaction than in the other two studies.

The data from the 1994 and 1985 studies show that C(M)IT/MIT induced severe and irreversible damage to the skin of rabbits following exposure to the substance for 4 hours. According to Section 3.2.2.6.2 of Annex I of CLP, Skin Corrosion subcategory 1C is appropriate where such responses occur after exposures between 1 hour and 4 hours and observations of up to 14 days. The basis for the current harmonised classification in sub-category 1B is unclear. No studies were conducted with shorter exposure periods, so a definitive conclusion about the applicability of a higher classification in subcategory 1A or 1B cannot be reached. However, as the effects seen in the third rabbit skin irritation study matched the

criteria for classification of C(M)IT/MIT as an irritant and not a corrosive substance, a higher sub-categorisation would seem inappropriate.

Therefore, RAC agrees with the proposal to classify C(M)IT/MIT in category 1C for skin corrosion.

The existing harmonised entry for C(M)IT/MIT includes specific concentration limits of 0.6% for skin corrosion and 0.06% for skin irritation. These are considerably lower than the general limits of 5% and 1% for these hazard classes, respectively. Following comments made during the public consultation, the DS confirmed that their proposal was to reduce the limit for skin corrosion classification to 0.5% and maintain the limit for skin irritation at 0.06%. However, the CLH report does not provide an assessment of the data on the specific concentration limit previously reviewed by the Commission Working Group; in the one reliable rabbit study, a 0.5% solution of C(M)IT/MIT was only irritating to skin, not corrosive. In addition, no data have been provided to indicate that the specific concentration limit for skin irritation should be amended.

In conclusion, RAC agrees with the DS that classification as **Skin Corr. 1C** is warranted for C(M)IT/MIT. However, regarding SCLs for this hazard class, RAC proposes **no change to the existing SCL in Annex VI** of CLP:

- Skin Corr. 1C; H314: Causes severe skin burns and eye damage; C ≥ 0.6%
- Skin Irrit. 2; H315: Causes skin irritation; 0.06 ≤ C < 0.6%

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Eye corrosion/irritation was not considered in this dossier. However, classification with skin corrosion means it is implicit that the substance will also cause serious damage to the eyes.

Comments received during public consultation

One stakeholder organisation agreed with the proposal to classify C(M)IT/MIT as Skin Corr. 1C; H314, but considered that C(M)IT/MIT does not warrant additional classification for eye irritation.

One MS requested adaptation of the proposed SCL for eye irritation. A second stakeholder organisation suggested SCLs of 0.06% ≤ C < 0.5% w/w for Eye Irrit. 2. In response, the DS stated that their intention was to propose SCLs of 0.06 ≤ C < 0.5% w/w for Eye Irrit. 2; H319, as 0.5% is the SCL proposed for

classification as corrosive to skin.

Assessment and comparison with the classification criteria

As discussed above for skin corrosion/irritation, RAC considers that the existing harmonised specific concentration limits for corrosivity and irritancy should be maintained, as the DS did not provide any clear evidence to justify changing them.

In conclusion, the existing SCLs in Annex VI of CLP are maintained as Eye Irrit. 2; H319: Causes serious eye irritation; 0.06 ≤ C < 0.6%.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 4.6-1 Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Open epicutaneous test (No official guidelines)	Sensitizing 6/8 induced and challenged at. 5% (7200 ppm a.i) 6/8 induced and challenged at 2.5% (3600 ppm a.i.)	Dunkin Hartley female guinea pigs n = 8/group Induction: 0.1 mL at 0.021%, 0.04%, 0.08%, 0.25%, 2.5% and 5% (corresponding to 30, 58, 115, 360, 3600 and 7200 ppm a.i.) Challenge: 0.025 mL at 0.021%, 0.04%, 0.08%, 0.25%, 2.5% and 5% (corresponding to 30, 58, 115, 360, 3600 and 7200 ppm a.i.) Test substance: aqueous solution of C(M)IT/MIT 14%	Wiemann, 2001
Buehler test (9 induction doses) GLP	Sensitizing After induction at 0.1% a.i (1 000ppm a.i), 4/5, 3/5, 3/15 and 0/20 individuals showed erythema response at 0.1, 0.05, 0.02 and 0.005% a.i (1000, 500, 200 and 50 ppm ai) challenge concentration, respectively; After induction at 0.05% a.i (500 ppm a.i), 10/10, 3/10 and 0/10 individuals with erythema response at 0.2, 0.05 and 0.01% a.i.(2000, 500 and 100 ppm ai) challenge concentration, respectively; After induction at 0.01% a.i (100 ppm a.i), 9/15 and 1/15 individuals with erythema response at 0.2 and 0.01% a.i (2000 and 100 ppm ai) challenge concentration respectively.	Dunkin Hartley male and female guinea pigs 5-10/sex/group Induction 0.1%; 0.05%; 0.01%; 0.005% and 0.0025% a.i (corr. to 1000, 500, 100, 50 or 25 ppm ai) Challenge: 0.2%; 0.1%; 0.05%; 0.025%; 0.02%; 0.01%; 0.005%; 0.0025% and 0.002% a.i (corr. to 2000, 1000, 500, 250, 200, 100, 50, 25 or 20 ppm a.i.) Test substance: aqueous solution of C(M)IT/MIT 14%	Chan, 1982
Maximization test (Magnusson and Klingman)	Not sensitizing Challenge: 0/19	Dunkin Hartley female guinea pigs	Parno, 2000

GLP, OECD 406	Re-challenge: 3/19 at 0.02% ai. at 24 h only (corr to 200 ppm a.i.)	20/group: C(M)IT/MIT groups dosed with 30 or 50 ppm a.i (groups 1 and 2), 10/group: irritation control and positive control (groups 3 and 4), 5/group: irritation control for positive control (group 5), 4/group: irritation control (group 6). <u>Induction</u> 30 and 50 ppm a.i. (or 0.003 and 0.005 % a.i) <u>Challenge</u> 30 and 50 ppm a.i. (or 0.003 and 0.005 % a.i) <u>Re-challenge</u> 50, 100 and 200 ppm a.i. (or 0.005, 0.01 and 0.02% a.i) Test substance: aqueous solution of C(M)IT/MIT 14%	
Maximization test (Magnusson and Klingman) GLP, OECD 406	Sensitising at 0.0036% a.i. (or 36 ppm a.i.) re-challenge <u>Challenge</u> 3/10 control 10/10 at 1.42% a.i. 10/10 at 1.07% a.i. 5/10 at 0.71% a.i. 3/10 at 0.355% a.i. <u>After re-challenge</u> 0/10 control 4/10 at 0.00355% a.i. 0/10 at 0.000355%	Dunkin Hartley male and female guinea pigs n = 10 /group <u>Induction</u> Intradermal treatment: 0.71% a.i. Dermal induction exposure: 3.55% a.i. <u>Challenge</u> 1.42, 1.07, 0.71, 0.355% a.i. (or 14 200; 10 700; 7 100 and 3 550 ppm a.i) <u>Rechallenge</u> 0.00355, 0.000355% a.i. (or 36 and 3.6 ppm a.i) Test substance: aqueous solution of C(M)IT/MIT 14%	Stahl, 2000
LLNA	Sensitising at all concentrations	CBA/J female mice	House,

(OECD 429)		n = 5/group Induction 0, 30, 50, 70, 90, 360, 1000 ppm a.i. or 0.003; 0.005; 0.007; 0.009; 0.036 and 0.1% a.i) Test substance: aqueous solution of C(M)IT/MIT 14%	2000a
LLNA (OECD 429)	Sensitising ≥ 70 ppm a.i. (or 0.007% a.i)	CBA/J female mice n = 5/group Induction 0, 30, 50, 70, 90, 360, 1000 ppm a.i. (or 0.003; 0.005; 0.007; 0.009; 0.036 and 0.1% a.i) Test substance: aqueous solution of C(M)IT/MIT 14%	House, 2000b

4.6.1.1 Non-human information

Several studies provide relevant information to evaluate the sensitizing potential of C(M)IT/MIT by dermal route.

Female guinea pigs (8/group) were exposed topically to doses of 0.1 mL of 30, 58, 115, 360, 3600 and 7200 ppm a.i. diluted in ethanol/aqua bidest, corresponding to the induction phase of the open epicutaneous test (**Wiemann, 2001**)[(11)]. Five doses of the test substance were administered to animals during 4 consecutive weeks. During the challenge phase, animals were exposed at 0.025 mL of test substance at similar concentrations. The first challenge phase occurred 3 days after the 20th induction, the second, 14 days after the first challenge. After the 6 h exposure period the sites were washed with water.

During the induction phase the 5 % and 2.5 % test substance preparation caused discrete or patchy erythema to intense erythema, swelling, scaling to severe scaling and eczematoid skin change in animals of test groups 3 and 4 (5% and 2.5% ppm a.i respectively). In the 0.25% group, discrete or patchy erythema and scaling was observed. All other test group animals, 0.021% up to 0.08%, did not show any signs of skin irritation. Ethanol/aqua bidest applied as a vehicle control to control groups 1 and 2 did not cause any skin reactions.

During the first challenge, the treatment substance induced discrete or patchy erythema to intense erythema and swelling in animals of test groups 3-5 (5% and 0.25%). Test groups 6-8 (0.08% to 0.021%) and control groups 1 and 2 did not show any skin reactions.

The test substance induced discrete or patchy to intense erythema, swelling and scaling and severe scaling in animals of the test groups 3-7 (5% to 0.04%), during the second challenge. The animals of test group 8 and control groups 1 and 2 did not show any skin reactions.

Six out of eight animals were induced and challenged at 2.5% and 5% (corresponding to 3600 ppm a.i and 7200 ppm a.i). The test substance is considered to be a skin sensitizer that demonstrated a dose response relationship under the conditions of this test. All animals that showed inflammatory response at the first challenge responded also after the second challenge.

Results of skin sensitization test are summarized below:

Table 4.6-2: Table of skin sensitization results

First challenge		**Number of animals with signs of allergic reactions at 24, 48 and/or 72 hours after the challenge application / number of animals in group**						
Group	**Induction**	**5 %**	**2.5 %**	**0.25 %**	**0.08 %**	**0.04 %**	**0.021 %**	**vehicle**
Control group 1	**Vehicle**	0/8	0/8	-	0/8	-	0/8	-
Control group 2	**Vehicle**	-	-	-	-	-	-	0/8
Test group 3	**5 %**	6/8	-	-	-	-	-	-
Test group 4	**2.5 %**	-	6/8	-	-	-	-	-
Test group 5	**0.25 %**	-	-	1/8	-	-	-	-
Test group 6	**0.08 %**	-	-	-	0/8	-	-	-
Test group 7	**0.04 %**	-	-	-	-	0/8	-	-
Test group 8	**0.021 %**	-	-	-	-	-	0/8	-

Second challenge		**Number of animals with signs of allergic reactions at 24, 48 and/or 72 hours after the challenge application / number of animals in group**						
Group	**Induction**	**5 %**	**2.5 %**	**0.25 %**	**0.08 %**	**0.04 %**	**0.021 %**	
Control group 1	**Vehicle**	0/8	0/8	-	0/8	-	0/8	
Control group 2	**Vehicle**	0/8	0/8	-	0/8	-	0/8	
Test group 3	**5 %**	7/8	7/8	5/8	1/8	-	-	
Test group 4	**2.5 %**	8/8	8/8	7/8	3/8	-	-	
Test group 5	**0.25 %**	6/8	5/8	3/8	1/8	-	-	
Test group 6	**0.08 %**	2/8	2/8	2/8	1/8	-	-	
Test group 7	**0.04 %**	2/8	1/8	0/8	-	0/8	-	
Test group 8	**0.021 %**	0/8	0/8	0/8	-	-	0/8	

Hartley male and female guinea pigs (5-10/sex/group) were topically exposed to 9 induction doses, 0.4 mL each, of C(M)IT/MIT (prepared from a solution of Kathon™ 886) for three 6 hour periods per week for three consecutive weeks (**Chan, 1982**)[(12)]. Concentrations of test substance used for induction are 0.1, 0.05, 0.01, 0.005 and 0.0025 % a.i (corresponding to 1000, 500, 10, 50 and 25 ppm a.i).

After a 2-week rest period following the last induction application or 1-week rest period following the last challenge application, the guinea pigs in the treated and control groups were challenged or re-challenged with various dilutions of Kathon™ 886. Concentrations used for challenge are 0.2, 0.1, 0.05, 0.025, 0.02, 0.01, 0.005, 0.0025 and 0.0020% a.i. (corresponding to 2000, 1000, 500, 250, 200, 100, 50, 25 or 20 ppm a.i.). After the 6 hour exposure period, the patch was discarded and the exposure sites were washed with a water soaked paper towel and dried.

At 0.1% a.i (1000 ppm a.i.) induction, the incidence of erythema response was 4/5, 3/5, 3/15 and 0/20 individuals at 0.1, 0.05, 0.02 and 0.005 % a.i (1000, 500, 200 and 50 ppm a.i.) challenge concentration, respectively.
At 0.05% a.i (500 ppm a.i.) induction, the incidence of erythema response was 10/10, 3/10 and 0/10 individuals at 0.2, 0.05 and 0.01% a.i (2000, 500 and 100 ppm a.i.) challenge concentration, respectively.
At 0.01% a.i (100 ppm a.i.) induction, the incidence of erythema response was 9/15, and 1/15 individuals at 0.2 and 0.01 % a.i (2000 and 100 ppm a.i.) challenge concentration, respectively.
At 0.005% a.i (50 ppm a.i.) induction, the incidence of erythema response was 2/15, 1/15, 0/15 and 0/15 individuals at 0.2, 0.02, 0.01 and 0.005 % a.i (2000, 200, 100 and 50 ppm a.i.) challenge concentration, respectively.
At 0.0025% a.i (25 ppm a.i. induction), the incidence of erythema response was 1/20, 0/20, 0/20 and 0/20 individuals at 0.2, 0.02, 0.01 and 0.0025 % a.i (2000, 200, 100 and 25 ppm a.i.) challenge concentration, respectively.

No erythema reaction was observed in the non-induced individuals challenged at 0.2, 0.02, 0.005 and 0.0025% a.i (2000, 200, 50 or 25 ppm a.i.).

Under the conditions of this study, Kathon™ 886 is considered a skin sensitizer.

The results of skins sensitization test are summarized below:

Table 4.6-3: Table of skin sensitization results

	Incidence of erythema response/number of animals in group									
Induction **Treatment**	Induction concentration (ppm a.i.) in water	2000 ppm a.i. #	1000 ppm a.i. #	500 ppm a.i. #	250 ppm a.i. #	200 ppm a.i. #	100 ppm a.i. #	50 ppm a.i. #	25 ppm a.i. #	20 ppm a.i. #
Phase I										
non-induced (challenge control)	0	--	--	--	--	0/10	--	0/10	--	--
non-induced (re-challenge control)	0	--	--	--	--	--	--	0/10	--	--
Kathon™ 886	1000	--	4/5	3/5	--	3/15	--	0/20	--	--
Phase II										
non-induced	0	0/10	--	0/10	--	--	--	--	--	--
Kathon™ 886	500	10/10	--	3/10	--	--	0/10	--	--	--
Kathon™ 886	100	9/15	--	--	--	--	1/15	--	--	--
Phase III										
non-induced	0	0/10	--	--	--	--	--	0/10	0/10	--
Kathon™ 886	50	2/15	--	--	--	1/15	0/15	0/15	--	--
Kathon™ 886	25	1/20	--	--	--	0/20	0/20		0/20	--
non-induced	0	0/20	--	0/10	--	0/10	--	0/30	0/10	--
Kathon™ 886	2000	20/20 (2/2)[a]	2/2 [a]	1/2 [a]	1/2 [a]	2/10 [a]	--	--	--	0/10
Kathon™ 886	1000	--	4/5	3/5	--	3/15	--	0/20	--	--
Kathon™ 886	500	10/10	--	3/10	--	--	0/10	--	--	--
Kathon™ 886	100	9/15	--	--	--	--	1/15	--	--	--
Kathon™ 886	50	2/15	--	--	--	1/15	0/15	0/15	--	--
Kathon™ 886	25	1/20	--	--	--	0/20	0/20	--	0/20	--

 # = challenge concentration; [a] Re-challenged guinea pigs; Non-induced = challenge or re-challenge controls; -- = not applicable

Incidence of erythema was calculated. Incidence = number of animals with erythema of grade 1 or greater at either 24 or 48 h divided by the number of animals challenged.

The third assay (Magnusson & Kligman) was intended to evaluate the potential of sensitization of C(M)IT/MIT at concentrations relevant for human exposure (**Parno, 2000**)[(13)]. Hartley female guinea pigs received six intradermal injections (0.1 mL) of C(M)IT/MIT at 30 ppm and 50 ppm, followed, one week later, by one 24h topical doses, for induction phase. Two weeks after the topical induction application, topical challenge applications are realized at concentration of 30 and 50 ppm a.i. A re-challenge was realized with concentrations of 50, 100 and 200 ppm a.i.

On Day 10, one animal in Group 1 died and one animal with a prolapsed rectum in Group 2 was euthanized. Gross necropsies indicated that neither animal's death/condition was related to treatment with 30 or 50 ppm C(M)IT/MIT, respectively.

After challenge:

- Group 1 (induction 30 ppm): 1/19 animals exhibited a dermal reaction, grade 1 at 24 hours only to the challenge application of 30 ppm C(M)IT/MIT;
- Group 2 (induction 50 ppm): 0/19, no reaction (50 ppm C(M)IT/MIT);

After re-challenge:

- Group 1: 1/19 animals exhibited a dermal reaction, to the re-challenge application of 50 ppm, 1/19 at 100 ppm, 1/19 at 200 ppm and 1/19 responded to both 100 and 200 ppm C(M)IT/MIT; all responses were grade 1 at 24 h only;
- Group 2: 0/19, no reaction (50 or 100 ppm C(M)IT/MIT); 3/19 animals exhibited a dermal reaction to 200 ppm C(M)IT/MIT; 1/19 animals responded to sterile saline; all responses were grade 1 at 24 h only;

Results of the skin sensitization study are as follow:

Table 4.6-4: Table of skin sensitization results

Induction dose [ppm a.i.]	Challenge dose[ppm a.i.]		Re-challenge dose [ppm a.i.]		
	30	50	50	100	200
0	0/10	0/10	0/4	0/4	0/4
30	1/19	--	1/19	2/19	2/19
50	--	0/19	0/19	0/19	3/19

Number of animals with signs of allergic reactions / number of animals in group.

C(M)IT/MIT is not a sensitizer under the conditions of this study, since the incidence of erythema was less than 30 %.

In the fourth study, dunkin Hartley male and female guinea pigs (10 per group) were treated with intradermal injection at concentration of 0.71% a.i. (Magnusson & Kligman

assay), one week later the test material was applied dermally on the same site (**Stahl, 2000**)[14]. The animals were challenged by dermal exposure two weeks later with concentration of 1.42%, 1.07%, 0.71 and 0.355% a.i.

After the challenge with test item (ACTICIDE® 14) the following reaction were found:

In dose group I. (1.42% a.i for challenge) positive response was seen in ten animals out of ten in the test group. Intense erythema, edema and necrosis, in two animals, moderate erythema were observed on the treated skin surface, 24 hours after the challenge treatment. The mean of the scores were 2.80 and 2.90 according to the 24th and 48th-hour results.

In dose group II. (1.07% a.i for challenge) positive response was seen in ten animals out of ten in the test group. In eight animals intense erythema, edema and necrosis, in two animals moderate erythema were observed on the treated skin surface. The mean of the scores was 2.8 according to the 24th and 48th-hour results.

In dose group III. (0.71% a.i for challenge) in five animals were observed intense erythema, edema and necrosis. In four cases moderate, and in one animal discrete erythema was found on the treated skin surface, 24 hours after the challenge treatment. The mean of the scores were 2.4 and 2.5 according to the 24th and 48th-hour results.

In dose group IV. (0.36% a.i for challenge) in three animals intense erythema, edema and necrosis were observed. In two cases moderate, and in five animals discrete erythema was found on the treated skin surface, 24 hours after the challenge treatment. The mean of the scores were 1.8 and 1.9 according to the 24th and 48th-hour results.

One week after the challenge a re-challenge treatment was performed with animals of dose groups III. and IV.

In dose group III (in concentration of 0.025%) positive response was seen in four animals out of ten in the test group. The mean of the scores were 0.6 and 0.70 according to the 24th and 48th-hour results. The dermal scores represented discrete and moderate erythema developed on the skin of sensitized guinea pigs.

In dose group IV (in concentration of 0.0025%) positive response was not observed on the animals. The mean of the scores was 0.00 according to the 24th and 48th-hour results.

Positive response was observed in 40% of the test animals after re-challenge with the test item in concentration of 0.025% (dose group III). In animals of dose group IV (in concentration 0.0025%) and in the new control group positive response could not be found. C(M)IT/MIT is classified as a skin sensitizer under the test conditions (lowest sensitisation dose: 36 ppm active ingredient; highest tested non sensitisation dose: 3.6 ppm active ingredient).

Results of skin sensitization study are as follow:

Table 4.6-5: Table of skin sensitization results

Inductions	**GPMT**		**Observations/Remarks**
	Day of treatment	**Application**	**(Give information on irritation effects)**
Intradermal	0	3.55% a.i. w/wo FCA	Slight to moderate erythema and slight edema
Topical	7	0.71% a.i.	Local irritation

Control group[a]	21	1.42 % a.i	Discrete and moderate erythema. Mean of the scores: 24 hours: 0.50 (3/10) 48 hours: 0.50 (3/10)
Challenge	21	0.36 % a.i	Slight to moderate erythema and slight edema 24 hours: 1.80 (10/10) 48 hours: 1.90 (10/10)
Challenge	21	0.71 % a.i	Moderate to intense erythema, edema, necrosis. Mean of the scores: 24 hours: 2.40 (10/10) 48 hours: 2.50 (10/10)
Challenge	21	1.07 % a/i	Intense erythema, edema, necrosis. Mean of the scores: 24 hours: 2.80 (10/10) 48 hours: 2.80 (10/10)
Challenge	21	1.42 % a.i	Intense erythema, edema, necrosis. Mean of the scores: 24 hours: 2.80 (10/10) 48 hours: 2.90 (10/10)
Rechallenge	28	36ppm a.i.	Discrete and moderate erythema. Mean of the scores: 24 hours: 0.60 (4/10) 48 hours: 0.70 (4/10)
Rechallenge	28	3.6ppm a.i	24 hours: 0 (0/10) 48 hours: 0 (0/10)
New control group	28	36ppm a.i.	24 hours: 0 (0/5) 48 hours: 0 (0/5)

a: group treated with the vehicle during the induction phase and 1.42 % a.i substance for the challenge. So as we are on concentrations have a significant skin reaction to the corrosive properties of the substance is observed.

The fifth study performed was a murine local lymph node assay (LLNA) conducted on CBA/J female mice in order to evaluate the sensitizing potential of C(M)IT/MIT (**House, 2000a**)[(15)]. A dose of 25µL of the test solution was applied to the dorsal aspect of each mouse ear (5 animals per group). The test system concentrations used for the induction phase are 0, 30, 50, 70, 90, 360 and 1000 ppm a.i (or 0, 0.003%, 0.005%, 0.009%, 0.036% and 0.1% a.i).

After the 3 days of test substance application, the animals were rested for 2 days. On Day 6, the animals were given an intravenous injection of ^{3}H-thymidine into a tail vein equivalent to a total dose of 20 µCi ^{3}H-thymidine/mouse. Approximately 5 hours after the ^{3}H-thymidine injection, the animals were sacrificed and the auricular lymph nodes were removed intact. All samples were analyzed for radioactivity in a liquid scintillation counter. The samples were counted and the results were recorded as disintegrations per minute (dpm). No test material-related clinical observations were noted. No remarkable changes in body weights were noted during the course of the study.

A Stimulation Index (SI) was calculated for each induction concentration. All concentrations evaluated produced a SI greater than or equal to 3. The results of the study (OECD 429) indicate that the test material C(M)IT/MIT, exhibits a statistically significant, generally dose-related potential to induce contact hypersensitivity in mice.

Under the conditions of this study, C(M)IT/MIT was a sensitizer at concentrations greater than 30 ppm a.i..

Results of the skin sensitization study are as follow:

Table 4.6-6: Table of LLNA results

Treatment	**Measured dose (ppm a.i)**	**DPM (mean)**	**SI (Test/control Ratio)**	**Results [1]**
Untreated control	0 ppm	160	--	--
Acetone/olive oil (4:1 v/v)	0 ppm	225	1.0	negative
C(M)IT/MIT	30 ppm	776 *	3.4	positive
CMIT/MIT	50 ppm	1047 *	4.7	positive
C(M)IT/MIT	70 ppm	953 *	4.2	positive
C(M)IT/MIT	90 ppm	1507 *	6.7	positive
C(M)IT/MIT	360 ppm	4612 *	20.5	positive
C(M)IT/MIT	1000 ppm	10241 **	45.5	positive
Hexylcinnamaldehyde	20 %	1985 *	8.8	positive

[1]Test/control Ratio of 3.0 or greater represents a positive result.
* Statistically significant difference compared to the vehicle control group ($p<0.05$).
** Statistically significant difference compared to the vehicle control group ($p<0.01$).
-- Not applicable.
DPM = disintegrations per minute.
SI = stimulation index.

Finally, another LLNA was conducted on CBA/J female mice with the same conditions that the study presented above (same author, same induction concentrations…) (**House, 2000b**)[(16)]. No test material-related clinical observations were noted. No remarkable changes in body weights were noted during the course of the study.

In this study, a SI higher than 3 was observed for induction concentration from 70 ppm a.i up to 1000ppm a.i, leading to positive results for sensitizing properties. The results of the study (OECD429) indicate that the test material C(M)IT/MIT , exhibits a statistically significant, generally dose-related potential to induce contact hypersensitivity in mice.

Under the conditions of this study, C(M)IT/MIT was a sensitizer at concentrations ≥ 70 ppm a.i.

Results of the skin sensitization study are as follow:

Table 4.6-7: Table of LLNA results

Treatment	Measured dose (ppm a.i)	DPM (mean)	SI (Test/control Ratio)	Results [1]
Untreated control	0 ppm	6795	--	--
Acetone/olive oil (4:1 v/v)	0 ppm	8952	1.0	negative
C(M)IT/MIT	30 ppm	13807	1.5	negative
C(M)IT/MIT	50 ppm	17386 *	1.9	negative
C(M)IT/MIT	70 ppm	30204 *	3.4	**positive**
C(M)IT/MIT	90 ppm	29212 *	3.3	**positive**
C(M)IT/MIT	360 ppm	60330 *	6.7	**positive**
C(M)IT/MIT	1000 ppm	69146 *	7.7	**positive**
Hexylcinnamaldehyde	20 %	22528 *	2.5	**positive**

[1]Test/control Ratio of 3.0 or greater represents a positive result.
* Statistically significant difference compared to the vehicle control group ($p<0.05$).
-- Not applicable
DPM = disintegrations per minute
SI = stimulation index

4.6.1.2 Human information

In the dermatological literature, innumerable reports identified C(M)IT/MIT as a skin sensitizer.
At two time points the data was reviewed, in 1992 by the Cosmetic Ingredient Review Panel and in 1999 by Fewings and Menné. Both reports have been widely cited and were part of the main data considered during the Meeting of the Commission Working Group on the Classification and Labeling of Dangerous Substances of January 19-21, 2000[(17)]. This meeting took place to confirm the agreement of March 1999 to classify C(M)IT/MIT especially with R43 (May cause sensitization by skin contact) and a specific concentration limit: C ≥ 0.0015% (or 15 ppm); R43. During this meeting, the specific concentration limit of 0.0015% was challenged by industry, willing to increase the threshold to 0.003%.

Many discussion took place on the fact that the concentration limit of 15 ppm for classification and labeling of C(M)IT/MIT as a skin sensitizer is based on a large number of publications (animal data, patch-test results, epidemiological studies, case reports…). Moreover, in the safety assessment by the Cosmetic Ingredient Review Expert Panel[(18)] it was concluded from human repeat insult patch test that the lowest concentration of C(M)IT/MIT in a cosmetic formulation producing sensitization is 7.5 ppm. Indeed, the new RIPT sensitization test data included in the report and the new non clinical test data on formulation available at this time led the Expert Panel to the conclusion that C(M)IT/MIT may be safely used in "rinse-off" products at a concentration not exceeding 15 ppm and in "leave-on" cosmetic products at concentration not to exceed 7.5 ppm. However, raw data leading to these thresholds are not available.

In their update of the risk assessment for C(M)IT/MIT with focus on "rinse-off" product realized in 1999, **Fewings and Menné**[(19)] reported that "under normal use conditions

(i.e, concentration of C(M)IT/MIT < 15 ppm) the risk of primary sensitization from the use of "rinse-off" products is negligible, and elicitation of allergic contact dermatitis in C(M)IT/MIT-sensitized people rare, after exposure to "rinse-off" products preserved with C(M)IT/MIT". This report also referred to the permissible level of 15 ppm of C(M)IT/MIT in cosmetic products in the EU for "rinse-off" products and "leave-on" products (Appendix V of the Regulation (EC) 12231/2009). The same limit concentrations are recommended in the USA and Hungary, for "rinse-off" product and "leave-on" products. In Japan, a concentration of 15 mm of C(M)IT/MIT is approved for use in "rinse-off" products but no approval has been sought for use in "leave-on" products.

In 2012, **Mose *et al.***[20], identified the most common allergens associated with the occupational contact dermatitis observed in painters. Indeed, painters represent the occupational group that most commonly experienced occupational contact dermatitis. In this study, authors analyzed all the available data registered by the Danish Contact Dermatitis Group from 2001 to 2010. Three different isothiazolinones, including C(M)IT/MIT, were identified as the most frequent sensitizers among the tested allergens. Painters have an increased risk of developing hand eczema when they are exposed to paints containing C(M)IT/MIT.

C(M)IT/MIT is widely used since 1980s as a preservative in paints, glue, toiletries and many other products. Recently, different products, including paints, containing exclusively MIT (without C(M)IT) are used leading to various pattern of exposure and sensitization. In 2006, **Thyssen *et al.***[21] described in their article a factory outbreak of allergic contact dermatitis. Four patients of 14 persons working at paint manufacturer developed dermatitis mainly following the introduction of MIT as additives. Patch test series containing various preservatives were realized on these patients. The tested preservatives were: methylchloroisothiazolinone/ methylisothiazolinone (C(M)IT/MIT), methylisothiazolinone (MIT), benzisothiazolinone (BIT) and octylisothiazolinone (OIT) in aqueous solution. The results showed positive reaction for MIT and C(M)IT/MIT, with stronger reaction for MIT indicating a primary sensitization to MIT.

The potential pattern of cross-reactivity between different isothiazolinones has been investigated by **Isaksson *et al.***[22] in 2014. Patients reacting to C(M)IT/MIT and/or MIT were additionally patch tested with several isothiazolinones in serial dilutions. In order to determine the primary sensitizer (C(M)IT or MIT), the following isothiazolinones were tested: C(M)IT/MIT, C(M)IT, MIT, OIT and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT). This study was conducted on nearly 3 years. According to the results, the authors described three groups of reactors. For one group, no reaction was observed with MIT; for another group, reaction with both C(M)IT and MIT was described but higher patch test reactivity to C(M)IT was reported (with concentrations at 37.5 and 150 ppm). For the last group, reaction with both C(M)IT and MIT was noted with similar reactivity but reacted more often to OIT and DCOIT. Cross-reactivities between isothiazolinones are therefore possible but some differences exist considering the primary sensitizer that is involved.
In this article, authors concluded that in patients reacting to both C(M)IT/MIT and MIT, two patterns can be drawn. First, C(M)IT is considered the primary sensitizer when high patch test reactivity to C(M)IT is observed with cross-reactivity to MIT. In that case, no cross-reactivity is expected with OIT. Second, MIT is considered the primary sensitizer when high patch test reactivity to MIT is observed with cross-reactivity to C(M)IT. However, in that case, cross-reactivity to OIT is also expected.

4.6.1.3 Summary and discussion of skin sensitisation

C(M)IT/MIT is a potent skin sensitizer. After dermal exposure, it induces skin sensitization effects in animals (guinea pigs and mice) and humans.
According to the results obtained in the LLNA studies in mice, C(M)IT/MIT is sensitising at concentration ≥ 30 ppm a.i (or 0.003% a.i).
Several dilutions of C(M)IT/MIT have been tested to ascertain an appropriate diagnostic patch test concentration to include in patch test series (**Maibach, 1985**)[(23)]. The dilution of 100 ppm a.i induces low skin irritancy and is high enough to detect most cases of sensitization. It has been included in the European baseline patch test series since 1988. However, Sweden and some centres in Spain, in the United Kingdom and in Ireland used 200 ppm a.i in their baseline series (**Bruze *et al*., 2014**)[(24)]. Considering the results of this multicentre study, 200 ppm a.i could be considered the optimal patch test concentration for C(M)IT/MIT since it is demonstrated that it diagnosed significantly more contact allergy than a concentration of 100 ppm a.i without inducing more adverse reactions.

Information leading to ascertain the most optimal concentration to detect cases of sensitization exists but no new information is available to challenge the classification threshold value of 0.0015% a.i (15 ppm a.i) set during the Commission Working Group on the Classification and Labeling of Dangerous Substances in 2000 in order to avoid the induction of skin sensitization during exposure with product containing C(M)IT/MIT. The most relevant data leading to a modification of this threshold value have already been reviewed during this meeting. A concentration of C(M)IT/MIT in product not exceeding 15 ppm do not lead to a risk of primary sensitization and elicitation is not expected at this concentration. The reasoning to retain 15 ppm instead of 7.5 ppm is unknown and cannot be challenged in this dossier.
However, a new concern is arising with the use of products containing other isothizolinones, especially MIT (without C(M)IT) since a few years leading to various pattern of exposure and sensitization. An increase in the incidence of sensitization due to the use of isothiazolinones could be expected considering the potential pattern of cross-reactivity observed with these substances.

4.6.1.4 Comparison with criteria

According to the results obtained in the LLNA studies on C(M)IT/MIT, the lowest Estimated Concentration that will induce a stimulation index (SI) of 3 after topical application (EC_3 value), is 30 ppm a.i (or 0.003% a.i.). This value is below the threshold value of 2% for classification as Skin Sens. 1A (H317: May cause an allergic skin reaction) under regulation (EC) 1272/2008.

4.6.1.5 Conclusions on classification and labelling

Based on the results of the sensitization effect studies, a classification **Skin Sens. 1A, H317. The specific concentration limit: C < 0.0015%, Skin Sens. 1A-H317** is maintained due to unavailable data to challenge this value.

RAC evaluation of skin sensitisation
Summary of the Dossier Submitter's proposal
The DS considers C(M)IT/MIT to be a potent skin sensitiser. After dermal

exposure, it induced skin sensitisation effects in animals (guinea pigs and mice) and humans.

According to the results obtained in the LLNA studies in mice conducted according to OECD 429 (House, 2000a, 2000b), C(M)IT/MIT is sensitising at concentrations ≥ 30 ppm (or ≥ 0.003%) (see also the Table below).

Since 1988 the substance has been included in the European baseline patch test series and as a result, human historical data is available and summarised in the CLH report . A multicenter study within the European Environmental and Contact Dermatitis Research Group has been conducted (Bruze *et al*., 2014). Several dilutions of C(M)IT/MIT were tested to ascertain an appropriate diagnostic patch test concentration to include in a patch test series. dilution concentration of 100 ppm induced low skin irritancy and was high enough to detect most cases of sensitisation. It has been included in the European baseline patch test series since 1988. However, Sweden and some centres in Spain, in the United Kingdom and in Ireland used 200 ppm in their baseline series. Considering the results of this multicentre study, 200 ppm could be considered the optimal patch test concentration for C(M)IT/MIT since it has been demonstrated that it diagnosed significantly more contact allergy cases than a concentration of 100 ppm without inducing more adverse reactions.

Overall, the patch test data provides information establishing the optimal concentration to confirm cases of sensitisation but no new information is available to challenge the classification threshold value of 0.0015% (15 ppm) recommended by the Commission Working Group on the Classification and Labelling of Dangerous Substances in 2000 in order to avoid the induction of skin sensitisation during exposure with products containing C(M)IT/MIT.

According to the results obtained in the LLNA studies on C(M)IT/MIT, the lowest Estimated Concentration that will induce a stimulation index (SI) of 3 after topical application (EC_3 value), is 30 ppm (or 0.003%). This value is below the threshold value of 2% for classification as Skin Sens. 1A (H317: May cause an allergic skin reaction) under CLP. Overall, based on the results of the animal studies and knowledge of historical human data, the Dosser submitter proposed a classification of Skin Sens. 1A; H317. As no data were available to challenge the current specific concentration limit for this hazard class, the DS proposed that the existing SCL in Annex VI of CLP of C ≥ 0.0015%, Skin Sens. 1A (H317) should be retained.

Comments received during public consultation

Three MSCA and two industry stakeholders agreed with classification of C(M)IT/MIT as skin sensitiser Cat. 1A and retaining the specific concentration limit of 0.0015%.

One industry stakeholder reminded the DS that as a consequence of classifying

C(M)IT/MIT as a skin sensitiser, the EUH208 phrase ('*Contains [name of sensitising substance]. May produce an allergic reaction'*) will be required on all products containing C(M)IT/MIT above 1.5 ppm (0.00015%).

Assessment and comparison with the classification criteria

There is a large body of literature describing clinical studies and case reports in humans indicating that C(M)IT/MIT is a skin sensitiser. The data were reviewed in detail when C(M)IT/MIT was assessed by the Commission Working Group on the Classification and Labelling of Dangerous Substances during the period 1998-2000. The data contributed to the classification of C(M)IT/MIT as a skin sensitiser and to the the specific concentration limit of 0.0015%.

The following table shows the results of the animal studies presented by the DS to illustrate that C(M)IT/MIT is a potent sensitiser.

Test (date)	**Result**	**Observations and Conclusions**
LLNA (2000a) Measured doses: 0, 30, 50, 70, 90, 360, 1000ppm (0, 0.003, 0.005, 0.007, 0.009, 0.036 and 0.1% respectively)	Positive	OECD TG 429 **SI ≥ 3 for all concentrations** SI = 1.0, 3.4, 4.7, 4.2, 6.7, 20.5, 45.5 at 0, 30, 50, 70, 90, 360 and 1000 ppm respectively. **EC3 value of ≤ 2** EC3 = 0.003% Skin Sens. Cat. **1A**
LLNA (2000b) Measured doses: 0, 30, 50, 70, 90, 360, 1000ppm (0, 0.003, 0.005, 0.007, 0.009, 0.036 and 0.1% respectively)	Positive	OECD TG 429 **SI ≥ 3 from 70ppm** SI = 1.0, 1.5, 1.9, 3.4, 3.3, 6.7, 7.7 at 0, 30, 50, 70, 90, 360 and 1000 ppm respectively **EC3 value of ≤ 2** EC3 = 0.007% The data appear to show a positive result and support categorisation of C(M)IT/MIT as Skin Sens. Cat. **1A** However, the positive control did not give an SI ≥ 3, therefore the data cannot be assessed reliably.
GPMT (2000a)	Negative	OECD TG 416, GLP Very low induction concentrations used, 0.003% and 0.005% no classification

GPMT (2000b) <u>Induction</u> Intradermal treatment: 0.71% Dermal induction treatment: 3.55% a.i. <u>Challenge</u> 1.42, 1.07, 0.71, 0.355% a.i. (or 14200, 10700, 7100 and 3550 ppm a.i.) <u>Re-challenge</u> 0.00355, 0.000355% a.i. (or 36 and 3.6ppm a.i.)	Positive	OECD TG 416, GLP Following intradermal induction with 0.71%, the test material was applied dermally on the same site one week later. Two weeks later, the animals were challenged. The results are as follows. <u>Challenge</u> 3/10 control 10/10 at 1.42% a.i. (Dose group I) 10/10 at 1.07% a.i. (Dose group II) 5/10 at 0.71% a.i. (Dose group III) 3/10 at 0.355% a.i. (Dose group IV) Intense skin reactions and necrosis were observed following challenge. One week after challenge, animals in dose groups III and IV were re-challenged. <u>Re-challenge</u> 0/10 control 4/10 at 0.00355% a.i. (Dose group III) 0/10 at 0.000355% a.i. (Dose group IV) The result of this study appears to be positive since a positive response was observed in 40% of the test animals after re-challenge. However, it would be inappropriate to use this result to define potency given the corrosivity observed under the test conditions.
Buehler (1982)	Positive	GLP 9/15 animals responded to an induction concentration of 0.01%. Skin Sens. Cat. **1A**
Open Epicutaneous Test (2001)	Positive	Non-standard study and so sub-categorisation is not possible Skin Sens. Cat. **1**

According to Table 3.4.2 in Annex I of CLP, *substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.* Substances meeting this criteria fall into subcategory 1A. Some of the studies summarised above give potency data, which indicate that Skin Sens. 1A would be appropriate for C(M)IT/MIT.

In the CLH report, the human data have been summarised but not in sufficient detail to allow a totally independent assessment of potency. There are no data in

the CLH report or in the comments received during PC to justify an alternative specific concentration limit to that already listed in the harmonised classification of C(M)IT/MIT. Significantly, no studies showing that levels < 15 ppm can lead to sensitisation have been cited. Consequently, with reference to the agreement reached previously, RAC is in agreement with the DS that there are no grounds to recommend a change to the existing specific concentration limit of 0.0015% for skin sensitisation.

As noted during the public consultation, in accordance with Annex II of the CLP Regulation, labelling phrase EUH 208 phrase (*Contains [name of sensitising substance]. May produce an allergic reaction*) will be required on all products containing C(M)IT/MIT above 1.5 ppm (0.00015%).

In conclusion, RAC agrees with the DS that_C(M)IT/MIT warrants a classification as **Skin Sens. 1A; H317: May cause an allergic skin reaction**. RAC is of the opinion that the existing SCL in Annex VI of CLP of C ≥ 0.0015% should be retained.

4.6.2 Respiratory sensitisation

Information from scientific literature is summarized here for information only in relation to the discussion on skin sensitization.

In 2003, **Basketter *et al***.[25] compared the relative potency of four biocides using both LLNA and cytokine profiling to determine the induction capacity of these biocides for skin and/or respiratory allergy. The tested biocides were: formaldehyde, glutaraldehyde, C(M)IT/MIT and MIT. The authors used LLNA as a primary screen for allergenicity, and then they examined the cytokine profile of each substance to identify whether it also may be a respiratory allergen. Skin and respiratory allergens result in characteristic cytokine profiles: enhanced expression of cytokines associated with activation of T helper 1 (Th1) subset, including interferon-γ (IFN-γ) and interleukin 12 (IL-12) for skin allergen; activation of T helper 2 (Th2) subset, including enhanced expression of interleukin 4, 5, 10 and 13 (IL-4, IL-5, IL-10 and IL-13) for respiratory allergen.
In the two tested vehicle (Acetone Olive Oil (AOO) and Propylene Glycol (PG)), C(M)IT/MIT presented the lowest EC_3 values (the lowest Estimated Concentration that will induce a stimulation index (SI) of 3 after topical application) with 0.0082% in AOO and 0.063% in PG meaning that C(M)IT/MIT is the most potent skin allergen. Concerning the cytokine profile, C(M)IT/MIT induced production of low IL-10, IL-13, IL-5 and IL-4, and only low levels of IFN-γ in this experiment leading to the conclusion that C(M)IT/MIT presents a Th1-type response consistent with its skin sensitization properties. However, it has no significant potency to induce sensitization of the respiratory tract.

4.6.2.1 Summary and discussion of respiratory sensitisation

Based on these data from scientific literature, C(M)IT/MIT does not warrant classification for respiratory sensitisation. However, due to lack of robust data, no conclusion can be drawn concerning the respiratory sensitisation properties of C(M)IT/MIT.

4.6.2.2 Comparison with criteria

Due to lack of robust data, no conclusion can be drawn concerning the respiratory sensitisation properties of C(M)IT/MIT. Therefore, comparison with the classification criteria is not relevant.

4.6.2.3 Conclusions on classification and labelling

Due to lack of robust data, no conclusion can be drawn concerning the respiratory sensitisation properties of C(M)IT/MIT. Therefore, no classification is proposed.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS had originally included data about interleukins in order to present the available information on the allergenicity of C(M)IT/MIT more generally. In response to the comments received during public consultation, the DS clarified that the intention was not to make a proposal for classification for respiratory sensitisation.

Since no data have been collected specifically addressing respiratory sensitisation, the DS considered that no conclusion could be drawn on either the endpoint or the availability of data.

Comments received during public consultation

One MSCA provided a summary of a further study (see additional key elements).

One industry stakeholder considered that the scientific literature data are conclusive but not sufficient for classification.

A second industry stakeholder organisation commented that C(M)IT/MIT has been used for several decades in a multitude of industrial and consumer applications and in that time not a single case of clinically confirmed respiratory allergy has been described in the literature.

Additional key elements

One MSCA mentioned that an additional pulmonary hypersensitivity study (Rohm and Haas Report No. 94RC-096 (1995)) was included in the latest SCSS opinion on C(M)IT/MIT (SCCS/1238/09). It concluded that: "Under the conditions of this study, C(M)IT/MIT induction at 4.8 mg/m^3 did not result in an immediate or delayed pulmonary hypersensitivity response in guinea pigs when subsequently challenged with an aerosol of the test substance at 0.17, 0.35 and 0.72 mg/m^3 nor did C(M)IT/MIT produce respiratory sensitisation."

Assessment and comparison with the classification criteria

Since there are no available data from studies specifically investigating the potential of C(M)IT/MIT to induce respiratory sensitisation, it is not possible to draw a firm conclusion on this endpoint.

RAC concludes that **no classification** is justified for this hazard class.

4.7 Repeated dose toxicity

Not considered in this dossier.

4.8 Germ cell mutagenicity (Mutagenicity)

The studies for mutagenicity in the rat were conducted with the test substance C(M)IT/MIT and do not modify the current classification. Therefore, they are not presented in this dossier.

4.9 Carcinogenicity

The studies for carcinogenicity in the rat were conducted with the test substance C(M)IT/MIT and do not modify the current classification. Therefore, they are not presented in this dossier.

4.10 Toxicity for reproduction

The studies for the toxicity for reproduction in the rat were conducted with the test substance C(M)IT/MIT and do not modify the current classification. Therefore, they are not presented in this dossier.

4.11 Other effects

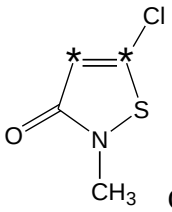
Not considered in this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

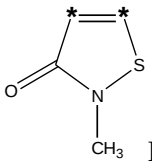
In the framework of the Biocidal Products Directive, two registrants (Dow and Thor) have provided complete data for the environmental section. These data have been gathered and compared, allowing to present below a comprehensive profile of environmental fate and aquatic ecotoxicity of C(M)IT/MIT.

5.1 Degradation

For studies of both applicants, when radiolabelled, C(M)IT and MIT were labelled on 4th and 5th carbon as described below:



C(M)IT



MIT

* site of ^{14}C label

Table 5.1-1: Summary of relevant information on degradation

Method	Results	Remarks	Reference / Owner

BIOTIC DEGRADATION			
Ready biodegradation OECD 301-B (CO_2 Evolution Test) EC method C.4-C Carried out on C(M)IT only	0.3 mg/L Day 28 – 38.8% 0.1 mg/L Day 28 – 55.3% 0.03 mg/L Day 28 – 62.0%	Inoculum is an activated sludge Wastewater treatment plant treating primarily domestic wastewater Not readily biodegradable	Bashir (1998 a) / Dow
Ready biodegradation OECD 301-B (CO_2 Evolution Test) EC method C.4-C Carried out on MIT only	0.1 mg/L Day 28 – 54.1% 0.03 mg/L Day 28 – 55.8% 0.01 mg/L Day 28 – 47.6%	Inoculum is an activated sludge Wastewater treatment plant treating primarily domestic wastewater Not readily biodegradable	Bashir (1998 b) / Dow
Ready biodegradation OECD 301-D (Closed Bottle) EC method C.4-E Carried out on C(M)IT /MIT	Day 7 - 76% Day 28 – 99%	Carried out with 4.2 mg C(M)IT/L and 1.4 MIT/L. Inoculum is an activated sludge from STP receiving both domestic wastewater and chemical waste. Therefore adaptation of the microorganism is not excluded Not readily biodegradable	Noack 2002 / Thor
Simulation test : Degradation in Two Water/Sediment Systems OECD 308 Carried out on C(M)IT only	**DT50 (days)** **1 Silt loam** Total system 0.38 (20°C) 0.72 (12°C)* calculated over the first 3 days, which has been considered as acceptable as more than 90% of dissipation was reached at 3 days **2 Loamy sand** Total system 1.3 (20°C) 2.47 (12°C)* **DT90 (days)** **1 Silt loam** Total system 1.3 **2 Loamy sand** Total system 4.2	Initial TS concentration : 1 mg/L In the silt loam system, calculated over the first 3 days, which has been considered as acceptable as more than 90% of dissipation was reached at 3 days In the loamy sand system, DT50 has been calculated over the first 7 days, which is considered as acceptable as more than 90% of dissipation was reached at 7 days	Schuck 2002a / Dow
Simulation test : Degradation in Two Water/Sediment Systems OECD 308 Carried out on C(M)IT only	**DT50 (days)** **1 Sand** Total system 2.04 (20°C) 3.86 (12°C)* **2 Sandy loam** Total system 1.86 (20°C) 3.53 (12°C)* **DT90 (days)** **1 Sand** Total system 6.78 **2 Sandy loam** Total system 6.17	Initial TS concentration : 0.5 mg/L	Noorlos 2007 a / Thor
Simulation test :	**DT50 (days)**	Initial TS concentration: 1	Schuck

Degradation in Two Water/Sediment Systems OECD 308 Carried out on MIT only	**1 Silt loam** Total system 0.46 (20°C) 0.87 (12°C)* **2 Sandy loam** Total system 1.4 (20°C) 2.7 (12°C)* **DT90 (days)** **1 Silt loam** Total system 1.5 **2 Sandy loam** Total system 3.3	mg/L In the silt loam system, calculated over the first 2 days, which has been considered as acceptable as more than 90% of dissipation was reached at 2 days In the sandy loam system, DT50 has been calculated over the first 7 days, which is considered as acceptable as more than 90% of dissipation was reached at 7 days	2002b / Dow
Simulation test : Degradation in Two Water/Sediment Systems OECD 308 Carried out on MIT only	**DT50 (days)** **1 Sand** Total system 1.28 (20°C) 2.43 (12°C)* **2 Loam** Total system 2.2 (20°C) 4.17 (12°C)* **DT90 (days)** **1 Sand** Total system 4.26 **2 Loam** Total system 7.31	Initial TS concentration: 0.5 mg/L	Noorlos 2007 b / Thor
Simulation test : Aerobic aquatic metabolism OECD 309 Carried out on C(M)IT only	Natural estuarine water **DT50 (days)** 22 µg/L 0.81 (20°C) 1.49 (12°C)* 115 µg/L 3.17 (20°C) 5.82 (12°C)*	Initial TS concentration :22 and 115 µg/L At 100 µg/L, Hockey-Stock model has been used to derive DT50, because of a lag phase**.	Guo et al., 2007a / Dow
Simulation test : Aerobic aquatic metabolism OECD 309 Carried out on C(M)IT only	Marine water **DT50 (days)** 10 µg/L 1.8 (20°C) 3.4 (12°C)* 4.3 (9°C)*** 100 µg/L 17.3 (20°C) 32.8 (12°C)* 41.7 (9°C)***	Initial TS concentration : 10 and 100 µg/L	Oteyza, 2008a / Dow
Simulation test : Aerobic aquatic metabolism OECD 309 Carried out on C(M)IT only	Marine water **DT50 (days)** >2 - <7 (15°C) >2.8 - <8.9 (12°C)* >3.2 - <11.3 (9°C)***	Initial TS concentration : 2 and 20 µg/L but no result was reported for the 2 µg/l tested concentration	Hamwijk and Cremers 2007a / Thor
Simulation test : Aerobic aquatic metabolism OECD 309 Carried out on MIT only	Natural estuarine water **DT50 (days)** 22 µg/L 1.38 (20°C) 2.63 (12°C)* 112 µg/L 1.24 (20°C) 2.35 (12°C)*	Initial TS concentration : 22 and 112 µg/L	Guo et al., 2007b / Dow

Simulation test : Aerobic aquatic metabolism OECD 309 Carried out on MIT only	Marine water **DT50 (days)** 10 µg/L 3.3 (20°C) 6.3 (12°C)* 8.0 (9°C)*** 100 µg/L 12.3 (20°C) 23.3 (12°C)* 29.7 (9°C)***	Initial TS concentration : 10 and 100 µg/L At 10 µg/L, FOMC model has been used to derive DT50**	Oteyza, 2008b / Dow
Simulation test : Aerobic aquatic metabolism OECD 309 Carried out on MIT only	Marine water **DT50 (days)** 3.6 (15°C) 4.6 (12°C)* 5.7 (9°C)***	Initial TS concentration : 1.5 and 87.5 µg/L but no result was reported for the 1.5 µg/l tested concentration	Hamwijk and Cremers 2007b / Thor
Simulation test Aerobic sewage treatment OECD 303 Carried out on C(M)IT only	**DT50 (days)** 0.27	Initial TS concentration: 100 µg/L Activated sludge from an aeration tank of a domestic STP	Daniel and Roberts, 2007 / Dow
Simulation test Aerobic sewage treatment OECD 303 Carried out on MIT only	**DT50 (days)** 0.03-0.04	Initial TS concentration: 100 µg/L Activated sludge from an aeration tank of a domestic STP	Oteyza et al., 2007 / Dow
Simulation test Aerobic sewage treatment OECD 303	**Degradation degree** CMIT >95% MIT > 80%	Initial TS concentration: 100 µg/L Activated sludge from a domestic STP	Fiebig, 2002 / Thor
Simulation test Aerobic degradation in soil OECD 307 Carried out on C(M)IT only	**DT50 (days)** **Silt loam** 0.11 (0.21 at 12°C*)	Initial TS concentration : 1 mg/kg DFOP model has been used to derive DT50**	Guo and Eisenschmid, 2006 / Dow
Simulation test Aerobic degradation in soil OECD 307 Carried out on C(M)IT only	**DT50 (days)** **Sandy loam** 0.22 (0.63 at 12°C*)	Initial TS concentration : 1 mg/kg DFOP model has been used to derive DT50**	Wang, 1991 / Dow
Simulation test Aerobic degradation in soil OECD 307 Carried out on MIT only	**DT50 (days)** **Silt loam** 0.27 (0.51 at 12°C*)	Initial TS concentration : 1 mg/kg	Guo, 2006 / Dow
Simulation test Aerobic degradation in soil OECD 307 Carried out on MIT only	**DT50 (days)** **Sandy loam** <0.08 (0.15 at 12°C*)	Initial TS concentration : 0.5 mg/kg	Oldersema and Salmon, 2007 / Thor
ABIOTIC DEGRADATION			
Hydrolysis	**DT50 at 25°C** pH 5 and 7 : not applicable / stable	Initial TS concentration : 11 mg/L	Jalali-

OECD 111 Carried out on C(M)IT only	pH 9 : DT_{50}= 22days **DT50 at 12°C** pH 5 and 7 : not applicable / stable pH 9 : DT_{50}= 62.2 days		Araghi and Shepler, 1993 / Dow
Hydrolysis OECD 111 Carried out on C(M)IT only	**DT50 at 25°C** pH 5 and 7 : not tested pH 9 : DT_{50}= 16.9days **DT50 at 12°C** pH 5 and 7 : not tested pH 9 : DT_{50}= 47.8 days	Initial TS concentration : 1.1 mg/L	Mazza, 1998 / Dow
Hydrolysis OECD 111 Carried out on MIT only	pH 5, 7and 9 : not applicable / stable	Initial TS concentration : 10-13 mg/L	Marx et al., 1992 / Dow
Hydrolysis OECD 111 Carried out on C(M)IT/MIT	**C(M)IT** **DT50 at 20°C** pH 4 and 7 : not applicable / stable pH 9 : DT_{50}= 63.6 **DT50 at 12°C** pH 5 and 7 : not applicable / stable pH 9 : DT_{50}= 120.6 days **MIT** pH 5, 7and 9 : not applicable / stable	Initial TS concentration : 20 mg C(M)IT/L and 8 mg MIT/L	Geffke, 2002a / Thor
Photolysis in water US EPA 161-2 Carried out on C(M)IT only	**DT50 (days)** 6.6	Initial TS concentration : 10 mg/L	Concha et al., 1994 / Dow
Photolysis in water US EPA 161-2 Carried out on C(M)IT only	**DT50 (days)** 6.3	Initial TS concentration : 2 mg/L	Purser, 1998 / Thor
Photolysis in water US EPA 161-2 Carried out on MIT only	**DT50 (days)** 11.1	Initial TS concentration : 11 mg/L	Shepler, 1995 / Dow
Photolysis in water US EPA 161-2 Carried out on MIT only	**DT50 (days)** 18.2	Initial TS concentration : 2 mg/L	Purser, 1998 / Thor

* recalculated value to reflect an average EU outdoor temperature
** See Generic guidance for estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration, 2011
*** recalculated value to reflect an average EU marine water temperature

5.1.1. Stability

5.1.1.1 Hydrolysis

Dow provided separate hydrolysis studies which are performed following OECD guidelines 111 and U.S. EPA guidelines 40 CFR § 158 Subdivision N §161-1 for C(M)IT (Jalali-Araghi

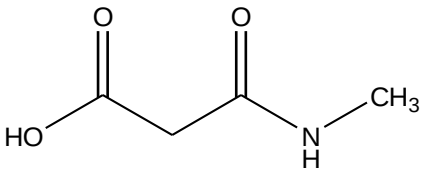
and Shepler, 1993; Mazza, 1998, Dow) and MIT (Marx et al., 1992, Dow). Thor provided a hydrolysis test on the biocidal product (C(M)IT/MIT 3:1, 14%); C(M)IT and MIT were dosed separately during the test performed following OECD guideline 111 (Geffke, 2002a, Thor).

For both applicants, aqueous solutions, buffered to pH 4, 5, 7 or 9 and stored at 25 °C (Dow) or 20°C and 30°C (Thor), are dosed with ^{14}C-C(M)IT and ^{14}C-MIT. MIT is stable at all pH. C(M)IT is stable at pH 5 and 7 while at pH 9 the half-lives normalised at 12°C are 62.24 and 47.81 days and 66.7 (test performed at 20°C) and 120.6 days (test performed at 30°C). In the environmental conditions (12 °C, pH7), C(M)IT and MIT are considered as stable.

Metabolites identification

Dow

Hydrolysis of C(M)IT involves cleavage of the isothiazolone ring leading to several transformation products: the major hydrolysis product is N-methyl malonamic acid (C(M)IT study, Mazza, 1998, 36.6% of applied radioactivity):



Thor

Several transformation products are formed due to hydrolysis, all more polar than the parent compound. However, only one minor hydrolytic transformation product could be identified in the hydrolysis test (carbone disulfide CS2) whereas identification of other transformation metabolites were not achieved. In general it is assumed that in pure aqueous solutions hydrolysis occurs by cleavage of the S-N bond, which is also occurring in presence of strong nucleophiles (which is the reason to minimize impurities in the C(M)IT/MIT solution (Paulus, 2005a,b, SSCNFP, 2003)). Also in a second study with radiolabelled test material (Lucas, 1996, Thor) identification of transformation products was unsuccessful due to technical difficulties associated with the chromatographic analysis of the metabolites formed and the lack of reference standards.

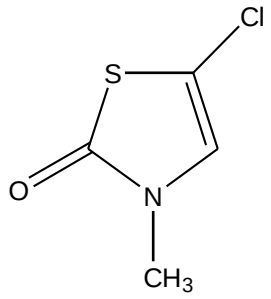
Considering that hydrolysis was observed only for C(M)IT and only at pH 9, ant that even at pH 9 the hydrolysis rate is still lower than the photolysis or biodegradation in water/sediment system rates, hydrolysis is considered of minor importance for the risk assessment. For this reason, it was accepted that the identity of metabolite is not further investigated.

5.1.1.2 Photolysis

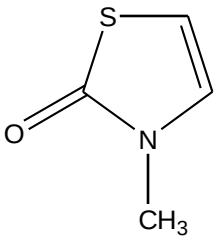
In the Dow studies, Glass tubes containing nominal 10 mg/L ^{14}C-C(M)IT or ^{14}C-MIT in a sterile pH 7 phosphate buffer are exposed to natural sunlight and samples taken periodically over 15 days for C(M)IT and over 30 days for MIT. Parent is quantitated by HPLC and the half-life in the presence of sunlight is 6.6 days and 11.1 days for C(M)IT and MIT respectively, when for dark control, 146.2 and 425 days respectively.

Metabolism involves cleavage of the isothiazolone ring. In addition to CO_2, the following photodegradates are identified and quantified above 10%: 5-chloro-3-methyl-4-thiazolin-2-

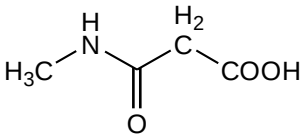
one (C(M)IT study by Dow, 38% of applied radioactivity at 15 d), 3-methyl-4-thiazolin-2-one (MIT study by Dow,, 40% of applied radioactivity at 30 d) and N-methyl malonamic acid (C(M)IT and MIT studies, 30.4 of applied radioactivity at 15 d and 37.5% of applied radioactivity at 30 d).



5-chloro-3-methyl-4-thiazolin-2-one



3-methyl-4-thiazolin-2-one



N-methyl malonamic acid

In the Thor study, optical dilute solutions of radiolabelled MIT and C(M)IT were tested separately, in flow through glass test systems with quartz lids, incubated in a Suntest apparatus. The irradiation was produced by a Xenon lamp. It was assumed that the behaviour of the two substances separately will be similar when tested as mixture. Identification of relevant metabolites was attempted by LC/MS.

[^{14}C]-C(M)IT in aqueous buffer solution of pH 7 degraded when irradiated. Test duration was 13.9 d (sunlight equivalent). The half-life, extrapolated to natural sunlight, was 6.3 days. Two relevant transformation products were formed: UNK 2 (max 35%, 9.8 d), and UNK 3 (max 17%, 9.8 d). UNK 2 is less polar that C(M)IT and is assumed to be a rearrangement of C(M)IT. UNK 3 is more polar than C(M)IT. Approximately 10.8% of the applied radioactivity was recovered from the gas traps, of which 6.21 % can be related to $^{14}CO_2$ and almost 4% is trapped in the polyurethane foam. C(M)IT was stable in the dark control at pH 7, but not at pH 9. This is consistent with the hydrolysis study.

[^{14}C]-MIT in aqueous buffer solution of pH 7 degraded when irradiated. Test duration was 23.5d (sunlight equivalent). The half-life, extrapolated to natural sunlight under the chosen conditions, was 18.2 days. Three relevant transformation products were formed: UNK 8 (max 27%, 25.3 d), UNK 4 (max 11%, 25.3 d) and UNK 10 (max 16%, 25.3 d). UNK 8 is slightly more apolar than MIT, the other compounds are assumed to be more polar. Less than 4% of the applied radioactivity was recovered from the gas traps. MIT was stable in the dark controls.

Three studies (Hamwijk, 2007 a, b, c) were performed in addition to the earlier photolysis test report (Purser, 1998) in order to elucidate the identity of three transformation products of MIT and two of C(M)IT formed by photolytic degradation. All studies were considered to be reliable, however, none of them resulted in a successful identification. Photolysis rates are higher than biodegradation rate in marine water, and photolysis degradates should be investigated for intended uses wich induce releases in marine water. However no use with direct release in marine water is intended in the Thor dossier and because of fast degradation in surface water, no indirect release in marine water is expected. Additionally, biodegradation

rate in estuarine water and water sediment studies are higher than those of photolysis and, further investigations were not performed.

In the two first studies, no reference products were used. In study by Hamwijk (2007 a, C(M)IT), it was shown that after an irradiation of 1 day, photolysis take place and the major metabolite formed is less polar than C(M)IT and has a molecular mass identical to that of C(M)IT. In study by Hamwijk (2007 b, MIT), it was shown that after an irradiation of 1 day, photolysis take place and the major metabolite formed is less polar than MIT and has a molecular mass identical to that of MIT. In the third study by Hamwijk (2007 c, C(M)IT, MIT) the following available polar reference compounds (of the proposed metabolites/ degradation products from Figure 5.1.3-1 and Figure 5.1.3-2) were analysed:

- Urea (MW = 60.1 g.mo/1),
- Malonic acid (MW = 104.1 g.mol/1),
- Malonamic acid (MW = 103.1 g.mol/1),
- Methylmalonic acid (MW = 118.1 g.mol/1)
- and Ethylene glycol (MW = 62.1 g.mol/1).

These were confirmed not to be the metabolites in the test samples. The test duration was 7 days.

For C(M)IT:

Three major degradation products were detected:

- One transient degradation product: DegC2 (max. 13.75%, 1d), less polar than C(M)IT, and which is an isomer of C(M)IT. The estimated DT_{50} of DegC2 is < 1 day. This is consistent with the findings of study by Hamwijk(2007 a). Moreover, in study by Purser (1998), one metabolite, less polar than the parent was found.
- Two stable polar degradation products: DegC1 (max. ≈17%, 7d) and DegC5 (max. ≈ 13%, 7d) were detected but not identified and there is no information on their degradability. The applicant indicates that extensive LC-MS work that has been conducted until now did not lead to any structural elucidation of Deg C1 and Deg C5. It is likely that these substances have a very low molecular mass and are therefore not detectable with MS-techniques.
- The other degradation products of C(M)IT were more polar than C(M)IT and did not occur in relevant amounts.

In this study, a DT_{50} < 1 day was estimated for C(M)IT.

For MIT

- The major degradation product (Deg M1, max. ≈ 40%, 1 d) that was less polar than the parent gave a protonated molecular ion with a mass trace of m/z 116, which is identical to the molecular mass of MIT. It was demonstrated that Deg M1 is different from the reference compound 2-methoxy-1,3-thiazole. Deg M1 was photolytically unstable. With Modelmanager a DT_{50} value of 4 days was calculated for DegM1.This is consistent with the finding of study by Hamwijk (2007 b) (major metabolite after 1 days, less polar, same mass), and in a lesser extent with those from study Pursur (1998), since the main metabolite is also less polar than MIT, formed in the same amount, but appear more slowly.
- The other degradation products of MIT were more polar than MIT and did not occur in relevant amounts. This is consistent with study by Purser (1998) where polar metabolites become major metabolites only after a period of time > 7 days.

In this study, a DT_{50} = 0.4 day was estimated for MIT.

Conclusion on photodegradation studies performed by Thor:

Degradation rate:

Degradation rates in the studies available are inconsistent and range from < 1 day to DT_{50} = 6.3 d for C(M)IT and DT_{50} = 18.2 d for MIT. The later would be the most relevant for risk assessment purpose. In studies where DT_{50} < 1 day were observed, the degradation product formed this first day was an isomer of the parent compound, which was found to be less polar than C(M)IT or MIT. Less polar isomeric structures of C(M)IT and MIT were also found in study by Purser (1998), but more slowly.

Photodegradation products:

Based on the available information, and common knowledge on the processes that might occur with structures such as of MIT and C(M)IT, the applicant postulated that the less polar compound with similar molecular weight as MIT or C(M)IT is a structure formed due to ring opening or the ether that is formed due to keto-enol tautomerization of C(M)IT and MIT, such as presented in Figure 5.1.1-1 for MIT.

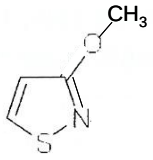


Figure 5.1.1-1: Methyl ether of isothiazo-3-one.

This latter hypothesis seems more sensible, since the opening of the ring is more likely to form a more polar compound. Unfortunately, compounds such as methyl malonamic acid, which would directly result from the opening of the ring were not tested.

Other photolysis products, more polar than the parent compounds were not identified.

The applicant indicated that technical difficulties would prevent further identification of the photolysis products.. Additionally:

- the less polar isomeric structures to C(M)IT and MIT were rapidly observed (< 1 day) in the systems and are likely to have been formed during the test on algae which is performed with light conditions and which is deemed relevant for PNEC derivation. However, the extent in which they were formed cannot be firmly defined, since results varied from a photolysis study to another.
- the relevance of the identity of the more polar photolytic transformation products, which are formed latter on, is considered limited, because the phototransformation rate is slower than the biodegradation rate in fresh water where release are expected according to uses intended in the Thor dossier,. These are small compounds and the applicant suggested that transformation products of these active substances, formed after the opening of the ring are not persistent and can be considered as not dangerous for the environment. This remark is supported by literature see Figure 5.1.3-1 and Figure 5.1.3-2) which proposed routes of degradation for C(M)IT. It is assumed that the degradation path for MIT will be similar.

Conclusion on photolysis in water

The worst case photolysis half lives are 6.6 days for C(M)IT and 18.2 days for MIT. Three photolytic transformation products were identified in the Dow dossier. Despite several tentatives, the identification of the transformation products in the Thor dossier was unsuccessfull. However, photolysis rate are slower than those of biodegradation in water and, further investigation were therefore not asked to the applicant. Additionnally, transformation products identified in the Dow dossier were in good accordance with the litterature information dealing with C(M)IT metabolism provided in the Thor dossier.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

C(M)IT/ MIT

In the Dow dossier Bashir, 1998 a and b), the ready biodegradation of the active substance was studied in separate tests for C(M)IT and MIT at three concentrations (0.3; 0.1 and 0.03 mg/L for C(M)IT and 0.1; 0.03 and 0.01 mg/L for MIT). C(M)IT can be considered to be readily biodegradable with a failure of the 10-day window: C(M)IT does not biodegrade from 10 % to 60 % of the applied dose within 10 days. However, at a concentration of C(M)IT that demonstrated only a small inhibition of the microbial population (0.03 mg/L ^{14}C-C(M)IT) the average percent biodegradation of two replicates after 28 days is 62.0%. No toxicity control has been carried out in this study. At the end of the study, similar microbial population were counted in the control (9.5 10^{10} CFU L^{-1}) and the 0.03 mg/L item (8.5 10^{10} CFU L^{-1}), whereas lower microbial density were reported for the highest tested concentrations (1.2-1.7 10^{10} CFU L^{-1}), indicating that some toxicity occurred.

MIT rapidly biodegrades up to 48-56%, but based on current guidelines, it cannot be classified as ready biodegradable since it does not biodegrade to 60% and does not satisfy the 10-day window requirement. The three tested concentrations are moderately toxic to the inoculum, showing a 50% reduction of the microbial activity. The RMS considers that the test is an acceptable worst case. As for the C(M)IT study above, no toxicity control was carried out but the microbial population in the item exposed to MIT (5.4-7.0 10^{10} CFU L^{-1}) was below than in the control (1.3 10^{11} CFU L^{-1}), indicating some toxicity.

In the Thor dossier, C(M)IT and MIT were also studied in the biocidal product (C(M)IT/MIT 3:1, 14%, Noack, 2002a). The biocidal product was readily biodegradable, when tested in an OECD 301D ready biodegradability test method. High degradation rate (93% within 14 days) were observed in a toxicity test carried out with 20 mg C(M)IT/MIT /L, indicating that the tested concentration was not toxic to the used inoculum.

These results are in contradiction with the studies by Dow, despite a higher tested concentration of substance and a lower initial inoculums concentration in the Thor study. However, the activated sludge in the Thor study comes from a STP receiving both domestic wastewater and chemical waste the inoculum and it can therefore not been excluded that the inoculum of this study was adapted to C(M)IT MIT. Therefore, C(M)IT/MIT has to be considered as not readily biodegradable. Nevertheless, simulation studies below show that C(M)IT/MIT is rapidly biodegradable, with similar degradation rate in the STP than default values from the TGD. Moreover, the mechanisms of action of C(M)IT/MIT support a fast

degradation, as it involved a rapid biding to protein thiols resulting of a cleavage of the ring through disulfide structure.

Because of the initial difference in the readily biodegradation results in the two dossiers, different approach has been developed by the two applicants. Relevant simulation studies have been provided by Dow with complete identification and quantification of relevant metabolites. Several simulation tests have also been provided by Thor and the resulting endpoint from the relevant study could be used in the risk assessment.

Relevant metabolites

The ready biodegradation of metabolites has only been investigated in the Dow dossier (Seyfried, 2003 a, b, c). Indeed, in Thor dossier, as metabolites have not been identified, no study on the degradation of metabolites has been performed.

Ready biodegradation tests have also been performed on N-methyl malonamic acid (NMMA, major metabolites identified in the hydrolysis, pholoysis and aerobic biodegradation in estuarine water study) and on malonamic acid (MA) or N-methyl acetamide (NMA). Although N -methyl acetamide (NMA) and malonamic acid (MA) are never been found above 10% of the applied radioactivity in any of the simulation tests, based on analogy with other compounds from the isothiazolinone family, these two substances are believed to be key in the metabolic pathway of the biodegradation of C(M)IT and MIT.

The studies on metabolites are performed following OECD Guideline 301B. An inoculum processed from activated sludge obtained from a wastewater treatment plant is dosed at 36 mg/L, 43 mg/L and 30.2 mg/L of N-methyl malonamic acid (NMMA), malonamic acid (MA) or N-methyl acetamide (NMA), respectively. Biodegradation is measured by the excess production of CO_2 in flask containing the test material versus controls.

All the three metabolites tested are ready biodegradable with the extent of biodegradation after 28 days reaching 78.2-94.7%.

5.1.2.3 Simulation tests

Biodegradation in water/sediment systems

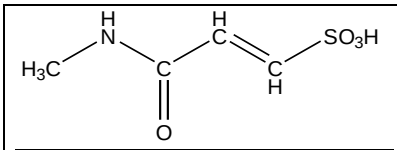
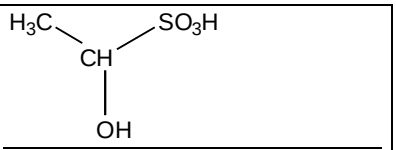
C(M)IT

The test provided by Dow (Schuck, 2002a) was conducted according to OECD guideline 308 with one water/sediment systems with low organic mater content (Cedar Hill) and one with high organic matter content (Alms-house) (A7.1.2.2.2a/01, Dow). The test substance concentration (radiolabelled material) was 1 mg/L. C(M)IT and metabolites in aqueous phase and in sediment extracts were also analyzed by liquid scintillation counting (LSC) and HPLC with sampling at 9 time-points within 30 days. The pre-incubation period was 10-11 days.

C(M)IT rapidly dissipates in fresh water/sediment microcosms with a dissipation (primary degradation) half-life (whole system) varying from 0.72 to 2.47 days at 12°C.

In both water/sediment systems, the ^{14}C-activity detected in KOH traps increases with time and reaches a maximum of 16.3% and 17.9% of the applied activity at the end of the study in the Alsmhouse and Cedar Hill systems respectively. The mineralization rate based on the production of $^{14}CO2$ is 202 days in the Almshouse water/sediment system and 161 days in the Cedar Hill water/sediment system.

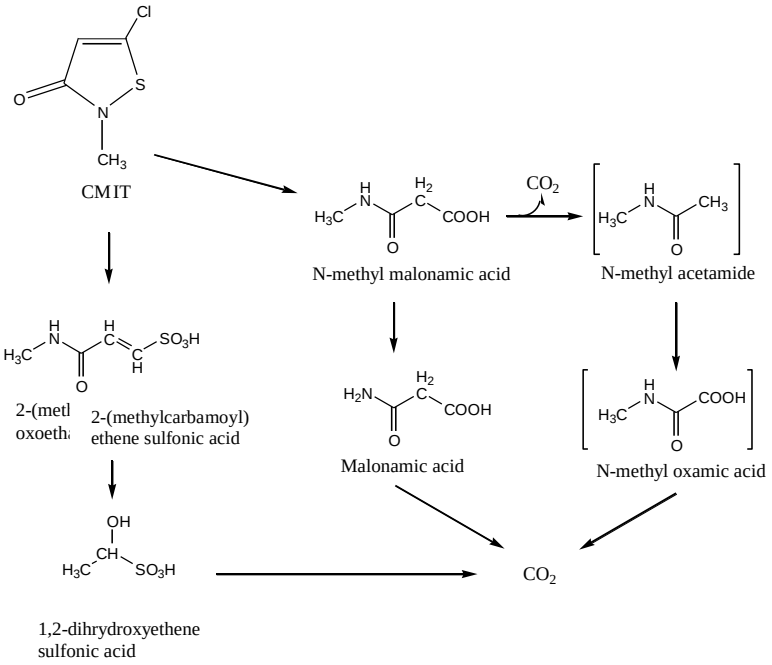
The degradation rates of the metabolites are not calculated because of the percent of applied for each detected metabolites is less than 1%, except for three of them. The maximum is detected of 9% for one metabolite in the aqueous phase in the Cedar Hill water/sediment system (9.9% in the whole system). These three metabolites, although they are transient, have been identified by LC-MS as 2-(methylcarbamoyl)ethene sulfonic acid and 2-hydroxyethane sulfonic acid and the metabolite found at 9% was identified as malonamic acid.

	
2-(methylcarbamoyl) ethene sulfonic acid	2-hydroxyethane sulfonic acid

Note: These two metabolites (2-(methylcarbamoyl)ethene sulfonic acid and 2-hydroxyethane sulfonic acid) are reported as sulfinic acids in the report. However, sulfinic acids are rare and not structurally favoured. Based on subsequent evaluation the metabolites are probably the pictured sulfonic acid metabolite. Associated with the initial fraction containing 2-hydroxyethane sulfonic acid is a second compound with the same mass weight as the 2-(methylcarbamoyl) ethene sulfonic acid

Metabolism pathway (see Figure 5.1.2-1) involves cleavage of the isothiazolone ring and subsequent oxidation to metabolites such as N-(n-methyl) malonamic acid, N-(n-methyl) acetamide, and N-methyl oxamic acid. N-methyl malonamic acid, malonamic acid, and N-methyl acetamide have been shown to be readily biodegradable.

Figure 5.1.2-1: Metabolic pathway of C(M)IT in water/sediment system



The range of non-extractable residues was 45.4-69.5 % of the applied ^{14}C-activity with 60.4 % at study termination (30 days) and 34.6-44.4 % with 42.2 percentage at study termination (30 days) for the Almhouse and Cedar Hill water/sediment systems, respectively. The % that was acid extractable was low (<8% of the non-extractable activity by day 30). By day 30, 43 and 33 % of the non-extracatable activity was extractable with NaOH for the Almhouse and Cedar Hill water:sediment systems, respectively. Of the NaOH extractable activity, 23 and 41 % was present in the fulvic acid fraction for the Almhouse and Cedar Hill water:sediment systems, respectively (9.9 and 13.5 % of the non-extractable activity).The largest fraction of activity, comprising about 40-55 % of the non-extractable activity, remained in the unextractable humin fraction.

The test provided by Thor (Noorloos, 2007 a) was conducted with two water/sediment systems (Goorven ('GV') with low %OC (organic carbon) and Schoonrewoerdsewiel ('SW') with high % OC according to OECD guideline 308, in an intermitted flow-through test design (aeration twice a day for 30 minutes) (A7.1.2.2.2-01, Thor). The test substance concentration (radiolabelled material) was 0.5 mg/L, reached by dosing a stock solution that contained acetonitrile as co-solvent, to the aqueous phase. C(M)IT and metabolites in aqueous phase and in sediment extracts were analyzed by liquid scintillation counting (LSC) and HPLC with radioactivity detection at 7 time-points within 58 days. The total duration of the incubation was extended to 100 days for measurement of the gas traps only. The pre-incubation period was 29 days.

For both substances the mass balance was not within the required range of 90-110% a.r. for all of the sediments. This was expected, because preliminary work showed the same trend, and no solution could be found for the loss of radioactivity. Possible explanations are that a volatile organic compound was formed that was not trapped in the gas traps or that the bound residue analysis underestimates the actual amount of radioactivity (see also the soil degradation part). Applicant's version could be not acceptable because the mass balance was not within the required range 90-110% for the two sediments. However DT_{50} of C(M)IT and MIT were determined to be max. 2.04 days and 2.2 days at 20°C, respectively. In this period, the range 90-110% was maintained. Therefore these studies can be considered to be reliable with restrictions.

C(M)IT is rapidly dissipated in water/sediment systems with an estimated DT_{50} of 3.45-3.72 d in the water layer and 3.53-3.86 d in the total system at 12°C (water layer and sediment extracts, first order model kinetics). Within a few days, most of the radioactivity had been transported into the sediment phase. After 3 days, it seemed that a plateau level has been reached for the bound residue, which content was between 35 and 44% a.r.: bound residues ranged from 17.0 to 43.9% of applied activity by day 1 and 58 respectively in the 'SW' water:system and from 17.8 to 51.4% by day 1 and 31.5 in the 'GV' water:sediment system. The mineralization rate is much slower: after 58 days 23% ('SW') or 26.2% ('GV') of the applied radioactivity (a.r.) was measured as $^{14}CO_2$ in the gas traps. The maximum extractable radioactivity was 13 (SW) to 18 (GV) % a.r., respectively in the two systems. The concentration in the water layer decreased to 4.0 (GV)/ 6.7 (SV) % a.r., respectively within 58 days of incubation. Only in the SW sediment (high %OC) two significant metabolites are formed: a polar degradation product (degradation product M1, 10.1% of applied activity by day 6, 4.6 by day 58) and a degradation product of polarity similar to C(M)IT (degradation product M2, 13.6% of applied activity by day 13, 3.0% by day 58). Their identities were not elucidated, despite efforts with LC/MS analysis.

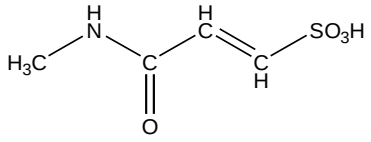
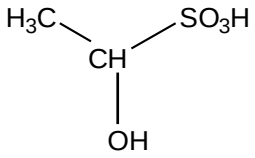
MIT

The test provided by Dow (Schuck, 2002b) was conducted according to OECD guideline 308 with one water/sediment systems with low organic matter content (Cedar Hill) and one with high organic matter content (Almshouse). The test substance concentration (radiolabelled material) was 1 mg/L. MIT and metabolites in aqueous phase and in sediment extracts were also analyzed by liquid scintillation counting (LSC) and HPLC with sampling at 9 time-points within 30 days. The pre-incubation period was 7 days.

MIT rapidly dissipates in fresh water/sediment microcosm with a dissipation (primary degradation) half-life at 12°C varying from 0.87 to 2.7 days.

In both water/sediment systems, ^{14}C-activity detected in KOH traps increases with time and reached a maximum of 27.9% and 19.7% of the applied activity at the end of the study in the Alsmhouse and Cedar Hill systems respectively. The mineralization rates are 150 days in the Almshouse water/sediment and 99 days in theCedar Hill water/sediment.

The degradation rates are not calculated because of the percent of applied for each detected metabolites is less than 1%, except for two of them. The maximum of the major metabolite in the Almshouse system is detected of 23.5% (0.9% at day 30) and (3.3% at day 30) the maximum of the major metabolite in the Cedar Hill system is detected of 20.5% on day 2. These two metabolites have been identified by LC-MS as 2-(methylcarbamoyl)ethene sulfonic acid and 2-hydroxyethane sulfonic acid.

	
2-(methylcarbamoyl) ethene sulfonic acid	2-hydroxyethane sulfonic acid

Note: These are reported as sulfinic acids in the report. However, sulfinic acids are rare and not structurally favoured. Based on subsequent evaluation the metabolites are probably the pictured sulfonic acid metabolite.

The range of non-extractable residues was 7.5-60.2 % of the applied ^{14}C-activity with 57.7 % at study termination (30 days) and 4.4-62.6 % with 61.5 % at study termination (30 days) for the Almhouse and Cedar Hill water:sediment systems, respectively. The % that was acid extractable was low (7.5-10.4 % of the non-extractable activity by day 30). By day 30, 42.8 and 35.6 % of the non-extracatable activity was extractable with NaOH for the Almhouse and Cedar Hill water:sediment systems, respectively. Of the NaOH extractable activity, 26.7 and 29.6 % was present in the fulvic acid fraction by day 30 for the Almhouse and Cedar Hill water:sediment systems, respectively (11.4 and 10.5 % of the non-extractable activity).An important fraction of activity, comprising about 43-48 % of the non-extractable activity by day 30, remained in the unextractable humin fraction.

The test provided by Thor (Noorloos, 2007 b) was conducted similarly to the test with C(M)IT, with water/sediment systems from the same source (Goorven ('GV') with low %OC (organic carbon) and Schoonrewoerdsewiel ('SW') with high % OC). The pre-incubation period was 19 ('GV') to 45 ('SW') days, the incubation time 39 ('GV') to 58 ('SW') days. Pre-incubation time was over the OECD recommendation for the 'SW' system however as high biodegradation rate were still observed, the test was considered as reliable.

MIT degraded rapidly in water/sediment systems with an estimated DT_{50} of 2.37-4 d (average 3.21 d) in the water layer and 2.43-4.17 d in the total system at 12°C (water layer and sediment extracts, first order model kinetics). The mineralization rate was slower: after ca. 100 days 24-42 % of the applied radioactivity was detected as $^{14}CO_2$. In both water/sediment system (high and low %OC) the bound residue content reached a plateau level of approximately 50% a.r. after 17 days of incubation: bound residues ranged from 13 to 47% of applied activity by day 1 and 58 respectively in the 'SV' water:system and from 16 to 42% by

day 1 and 39 in the 'GW' water:sediment system. One relevant, polar metabolite M1 was formed in both water/sediment systems. The maximum percentage was observed at 4 days in the 'GV' water sediment system (48.5%) and the percentage was lower (11.4%) at the end of the experiment (38 days). The maximum percentage was observed at 8 days in the 'SW' water sediment system (39.6%) and this metabolite was not detected at 58 days. The identity is not known. M1 might actually be two compounds with close retention times. The biomass content in the low %OC sediment ('GV') was too low at the end of the incubation, which might have influenced the results of the study. However, the percentage mineralization was higher in this 'GV' system then in the 'SW' system, and therefore it was assumed that the biomass was sufficiently viable.

The applicant proposed two relevant publications on the proposed degradation pathway of C(M)IT (see Figure 5.1.3-1 and Figure 5.1.3-2). These studies demonstrate that most of the metabolites were assumed to be small polar compounds and in most cases they were rapidly biodegraded. The applicant proposed to assume that the degradation path for MIT would be similar.

Additionally, a water/sediment study with one sediment (low %OC) and radiolabelled C(M)IT/MIT that was carried out at 10°C with an appropriate mass balance showed a similar behaviour for the two substances and no formation of organic volatiles (Lucas 1996, Thor). The radioactivity in the surface water reached 20.3% and 30.8% of the applied radioactivity after 100 days of incubation for C(M)IT and MIT respectively. The non-extractable radioactivity in the sediment increased reaching maximum values of 53% of applied radioactivity after incubation of C(M)IT for 58 days and 36.4% of applied radioactivity after incubation of MIT for 100 days. Increasing amounts of polar unknown metabolites were formed in the surface water, reaching maximum mean concentrations of 17.4% of the applied radioactivity after 14 days of incubation with C(M)IT and 41.1% after 7 days of incubation with MIT. Radioactivity in sediment extracts was split into four groups of unknown metabolites. Differences exist between C(M)IT and MIT sediments. In the C(M)IT sediments the main part of radioactivity was located in an unknown group of metabolites that eluted between 11 and 15 minutes, reaching a maximum of 6.9% of applied radioactivity. In the MIT sediments the main part of the radioactivity was located in a polar group of unknown metabolites that eluted after HPLC solvent (Rt 6 and 8 minutes), reaching a maximum of 8.2% of applied radioactivity.

Half life from the Thor dossier are higer than those from the Dow dossier and have been chosen for the risk assessment as worst case. Therefore the degradation half life at 12°C in whole system for C(M)IT is 3.86 days and the degradation half life in whole system for MIT is 4.17 days. However, we can note that half life from the Dow dossier are in the same range than those from the Thor dossier.

Biodegradation in only water microcosm systems

In the Thor dossier, the biodegradability of C(M)IT/MIT in marine water was initially determined according to OECD Guideline 306 (Hamwijk and Oldersma, 2005). However, in this test, the test substance at the concentration of 14.5 mg/L was considered to be inhibitory to bacteria and the Biochemical Oxygen Demand (BOD) of the test substance never reached a positive value. Alternative test procedures with lower concentrations of test substance and using radiolabelled test substance were needed to determine biodegradability. For that purpose marine surface water simulation tests were carried out with C(M)IT and MIT separately. These tests were performed according to the OECD Guideline 309.

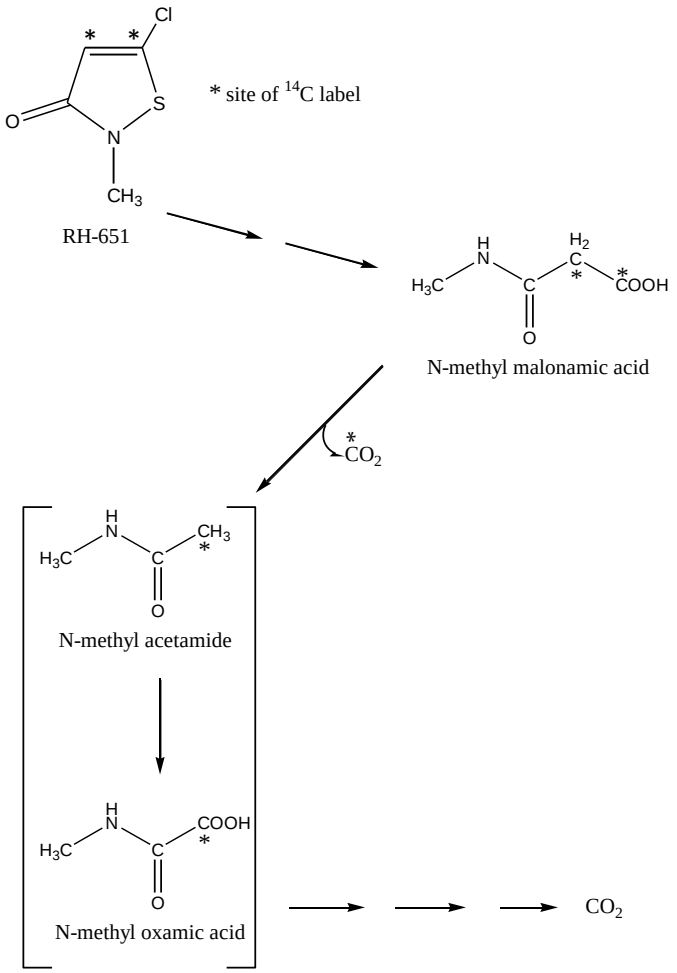
In the Dow dossier, estuarine water and marine surface water simulation tests were carried out with C(M)IT and MIT separately according to the OECD Guideline 309.

C(M)IT

Salinity in the estuarine water was 0.28-0.40 mmhos/cm (0.18-0.26 ‰) and microbial density was from $3.5\ 10^3$ - $1.7\ 10^4$ CFU/mL (Guo et al., 2007a). The duration of the tests was 5 days for the 22 µg/L concentration (8 sampling points) and 12 days for the 115 µg/L concentration (9 sampling points). C(M)IT rapidly dissipates in estuarine water: the dissipation (primary degradation) half-life of C(M)IT calculated at 12°C is 5.82 days for the highest tested concentration. Indeed, the test is conducted at two concentrations (22 and 115 µg/L), the higher seems to exhibit a toxicity for microorganisms, degradation rate being lower than the the degradation rate (1.49 d) derived at the lower concentration. However, in the absence of toxicity controls, it is not possible to check if the tested concentrations are toxic or not. Although not mandatory in OECD 309, toxicity control for this kind of biocidal substances would have been useful. Results are accepted, considering that if any toxicity would have occurred, then the degradation rate should be considered as a worst case. Additionally, since the dissipation of C(M)IT is well correlated with the formation of metabolites and because of the low adsorption capacity of C(M)IT on the solid phase, dissipation of C(M)IT could be assimilated to primary degradation.

Metabolism involves cleavage of the isothiazolone ring, leading to the formation of N-methyl malonamic acid and other polar compounds which have not been identifed (assumed to be N-methyl acetamide and N-methyl oxamic acid). The presence of $^{14}CO_2$ (27.7% after 5 days) indicates that these alkyl metabolites are undergoing additional oxidation which results in the evolution of $^{14}CO_2$. N-methyl malonamic acid comprises a maximum of 37.3% of the applied activity at 22 µg/L after 48 hours (17.1% at the end of the test) and a maximum of 78% at 115 µg/L after 7 days (68.0% at the end of the test). The proposed metabolic pathway of C(M)IT in surface is depicted in

Figure 5.1.2-2: Proposed metabolic pathway of C(M)IT in surface water



In the test provided by Dow, salinity in the marine water was 35.3 ‰ and microbial density was 1 10^3 CFU/mL (Oteyza, 2008a). The duration of the tests was 56 days with 9 sampling points. C(M)IT dissipates slower in marine water than in estuarine water: the dissipation (primary degradation) half-life of C(M)IT calculated at 12°C is 32.8 days at 100 µg C(M)IT/L and 3.4 d at 10 µg C(M)IT /L. The test is conducted at two concentrations (10 and 100 µg/L), and as for the biodegradation test in estuarine water, the higher concentration seems to exhibit a toxicity for microorganisms, even if the toxicity could not be confirmed in the absence of toxicity control. Therefore the DT50 determined in the highest concentration (41.7 days at 9°C) has to be considered as a worst case and the DT50 determined at the lowest concentration (4.3 days at 9°C) could be used for the risk assessment when the predicted concentrations is in the same range than this low experimental concentration (10 µg/L). Only one metabolite was detected at greater than 10% and it was characterized as N-methyl malonamic acid. Metabolism involved cleavage of the isothiazolone ring, leading to the formation of N-methyl malonamic acid and other polar compounds. N-methyl malonamic acid has been shown to be ready biodegradable (see 5.1.2.2 screening test).

In the test provided by Thor, salinity in the marine water was about 31 ‰ and information dealing with the microbial density was not provided (Hamwijk and Cremers, 2007a). The duration of the tests was 28 days with 5 sampling points. Data do not allow to accurately determine DT_{50} of C(M)IT. C(M)IT was rapidly biodegraded in marine water with an

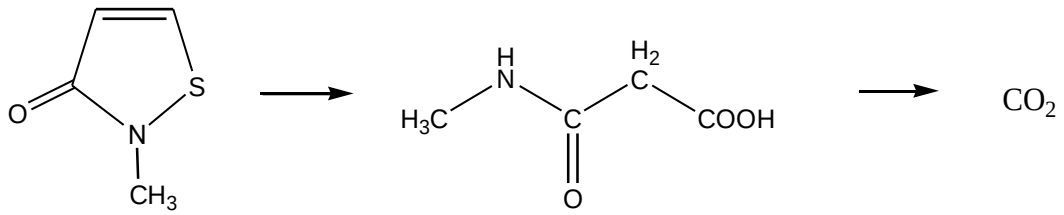
estimated DT_{50} comprised between 3.2 and 11.3 days at 9°C. The test substance did not inhibit the microbial activity at 20.0 µg/L and results were not available for the 2.0 µg/L tested concentration.

The mineralization after 28 d was 29.4% CO2 at 2µg/L, and 38.5% at 20 µg/L. Three metabolite regions with several peaks were found > 10%. They consisted of multiple metabolites and were not further evaluated as the concentration of each single metabolite was <10% of the applied radioactivity (a.r.). These metabolites are more polar than C(M)IT. One individual metabolite was detected on some sampling point but seemed transient and remained under 10%. Apart from the fact that the recovery in the microbial activity controls was slightly below 90% a.r. at the end of the test, the conditions of validity of the guideline were met.

MIT

Salinity in the estuarine water was 0.78-0.84 mmhos/cm (0.50-0.54‰) and microbial density was from 2.9 10^4 to 2.1 10^5 CFU/mL (Guo et al., 2007b). The duration of the tests was 6 days (7-8 sampling points). MIT rapidly dissipates in estuarine water: the dissipation (primary degradation) half-life of MIT is 2.63 days. The low adsorption property of MIT allows to state that the dissipation mostly results from primary degradation. Metabolism involves cleavage of the isothiazolone ring, leading to the formation of N-methyl malonamic acid and other polar compounds. The applicant stated that presence of $^{14}CO_2$ (0.3% after 2 days) indicates that these alkyl metabolites are undergoing additional oxidation, which results in the evolution of $^{14}CO_2$. Although this oxidation is likely to occur in view of the other data available, this mineralization rate is too low to draw any conclusion. N-methyl malonamic acid comprises a maximum of 27% of the applied activity at 22 µg/L after 72 hours (24.8% at the end of experiment) and a maximum of 33% at 112 µg/L after 6 days (end of the experiment).

Figure 5.1.2-3: Proposed metabolic pathway of MIT in surface water



MIT N-methyl malonamic acid

In the test provided by Dow, salinity in the marine water was 35.3 ‰ and microbial density was 1 10^3 CFU/mL (Oteyza, 2008b). The duration of the tests was 56 days with 9 sampling points. Dissipation in marine water is slower than in estuarine water: half –life of MIT calculated at 12°C is 23.3 d at 100 µg MIT/L (SFO model) and 6.3 d at 10 µg MIT/L (FOMC model). As for C(M)IT, toxicity should have occur at the highest tested concentration, even if no toxicity control has been carried out to support this observation.

The test has been conducted at two concentrations (10 and 100 µg/L). The major metabolite had similar chromatographic behavior to the N-methyl malonamic acid standard. N-methyl malonamic acid has been shown to be ready biodegradable.

In the Thor study, the ring was uniformally labelled. Salinity in the marine water was 30.9-31 ‰ and the microbial density was assumed to be $1\ 10^4$ CFU/mL (Hamwijk and Cremers, 2007b). The duration of the tests was 56 days with 6 sampling points. Despite tested at two concentrations, results were only available for the highest concentration (87.5 µg/L). MIT was rapidly biodegraded in marine water DT_{50} = 3.6 d at 15°C. This corresponds to a DT_{50} = 5.7 d at 9°C. Degradation rate was only determined from the higest concentration experiment, because of the non reliability observed at the lowest concentration.

After 56 days, approximately 95% primary biodegradation of MIT was determined. Approximately 30% was recovered as $^{14}CO_2$ within that period. Apart from the parent substance MIT, HPLC-RAD (HPLC with radiochemical detection) analyses revealed seven unidentified metabolites (met-1 – met-7) and three metabolite regions with several peaks (reg-1 – reg-3). Metabolite Met-1 was the only relevant metabolite, with a concentration increasing to a maximum value of 18.76% a.r after 7 days (replicates: 18.76% and 3.82%) and subsequently decreasing to a value of 2.52% a.r. after 56 days. The first order degradation rate of met-1 (obtained from a simultaneous fit with data of the parent) was 0.180 d^{-1} (DT_{50} = 3.8 d, DT_{90} = 12.8 d) at 20°C. This corresponds to a DT_{50} = 9.16 d at 9°C (k = 0.44 d^{-1}).

The applicant proposed two relevant publications on the proposed degradation pathway of C(M)IT and MIT (see Figure 5.1.3-1and Figure 5.1.3-2). These studies demonstrate that most of the metabolites were assumed to be small polar compounds. In most cases they were rapidly biodegraded.

Some degradation, which was assumed to be abiotic of nature, was observed in the dark controls. Met-1 was found with a max. of about 4% after 7 d. Apart from the high recovery at the lower test substance concentration (which was not used to determine the degradation rate), the conditions of validity of the guideline were met.

No biodegradation test in fresh water was provided by both registrants and only a biodegradation test in marine water was provided by Thor. Thus, estuarine water provided by Dow was considered as a realistic worst case for biodegradation in freshwater. Indeed, the biodegradation in estuarine water, with a lower salinity than marine water, was faster than the biodegradation in marine water and probably slightly slower than in fresh water. The lower biodegradation rate in marine water compared to the estuarine water could also results from the lower density of microorganisms in marine water, even if the difference remains low in the available degradation studies. For marine water, only a range of DT_{50} is derived for the degradation of C(M)IT in the Thor dossier, these data are only considered as supportive. For MIT, the Dow values have been selected as worst cases. Two DT_{50} values have to be considered depending of the predicted concentration because of the suspected toxicity that occurred at the highest tested concentration.

Biodegradation in sewage treatment plant

In the Dow dossier, C(M)IT and MIT were tested separately following the test guideline OECD 303A, Simulation Test-Aerobic Sewage Treatment: Activated Sludge Units (Daniel and Roberts, 2007; Oteyza et al., 2007). The test unit consists of two main vessels: an aeration vessel and a settling vessel. Activated sewage is pumped into the aeration vessel at a rate of 12 L/day and 300 mL/day of the mixed liquor in the aeration vessel is transferred to a waste

sludge flask. The hydraulic retention time in the aeration vessel is 6 hours and the sludge retention time, 10 days. The contents of the aeration vessel are transferred into a settling vessel where the sludge solids are allowed to settle and the supernatant transferred to a refrigerated effluent container. A pump transfers settled sludge solids back into the aeration vessel.

C(M)IT

The unit is allowed to equilibrate for 27 days prior to dosing with ^{14}C-C(M)IT. The dosing solution is transferred into the aeration vessel via a syringe pump. The dosing solution concentration is 100 ppm and the delivery rate is 12 mL/day. The resulting concentration of ^{14}C-C(M)IT in the mixing vessel is 100 ppb. The system is dosed for 33 days.

The half-life of total applied radioactivity (parent and metabolites) in the sewage treatment system studied is determined using two scenarios; steady state direct dissipation where the half-life is 0.27 day and mineralization where the half-life is 0.36 day.

In the sewage treatment plant simulation system dosed with ^{14}C-C(M)IT, 39.3%, 27.0% and 22.5% of the applied activity is detected in the aqueous fractions, the solid fractions, and the volatiles, respectively. Extraction of the sludge with methanol released only 27% of the radioactivity. Further extraction tentatives including ultrasonication and other solvent allowed recovering 39% of the radioactivity. Analysis of extracts indicated that the major metabolite(s) were chromatographically polar in nature.

No parent is detected in the effluent or sludge. No other metabolites appear to be present at greater than 10% of the applied activity. These results indicate that the isothiazolone ring has been cleaved and that there is extensive oxidation of the resulting alkyl metabolites.

MIT

The unit is allowed to equilibrate for 20 days prior to dosing with ^{14}C-MIT. The dosing solution is transferred into the mixing vessel via a syringe pump. The dosing solution concentration is 100 ppm and the delivery rate is 12 mL/day. The resulting concentration of ^{14}C-MIT in the mixing vessel is 100 ppb. A steady state is obtained after 27 days of dosing and is maintained for 51 days.

In this simulation system, 63.7%, 25.8% and less than 2% of the applied activity is detected in the aqueous fractions, the solid fractions, and the volatiles, respectively. The effluent comprises a majority of the applied radioactivity with 60.6% in the aqueous portion and 18.2% in the solids.

The half-life of MIT in the sewage treatment system studied is determined using two scenarios; steady state direct dissipation where the half-life of MIT is about 0.03 day when considering that no MIT remained in solids and 0.04 day when assuming that the radioactivity in solids is MIT; mineralization where the half-life is 1.69 days..

Parent comprises 12.2% of the applied activity in the aqueous phase of the effluent. N-methyl malonamic acid, N-methyl acetamide, and malonamic acid are also present but each at less than 10% of the applied activity.

The mass fraction of parent MIT adsorbed to sludge was undetermined but in a first approach, it was suggested that all the ^{14}C-activity associated with the sludge (6.64%) was considered as parent, as a worst case.

Two studies were provided by Thor. The first study was carried out on C(M)IT/ MIT without radiolabelling. The elimination and the primary and/or ultimate biodegradation of ACTICIDE®14 was determined in a continuous activated sludge tests according to OECD 303 A (Fiebig, 2002) that simulates the aeration tank of a sewage treatment plant. The test design included specific analysis of C(M)IT and MIT. This test showed that, when tested at a concentration of 15 mg ACTICIDE®14/L (1.55 mg C(M)IT/L and 0.6 mg MIT/L), C(M)IT and MIT were removed for >95% or >80% respectively.

It is difficult however to conclude if the substance was actually biodegraded or removed from the system. There is no information on the formation of carbon dioxide, or any other metabolite. Besides, it is indicated that it was confirmed in preliminary tests that C(M)IT and MIT did not adsorb to the sludge. It is not specified how it was done, and to which extent C(M)IT/MIT does not adsorb. However the low adsorption properties of C(M)IT and MIT is supported by the adsorption/desorption from soil and sediment test (see 5.2.1).

Another study conducted with MIT only (A7.1.2.1.1-02), seems to indicate that the removal observed in the previous study may correspond to a more complex behaviour than mineralization only. In this test, the percentage of $^{14}CO_2$ formed after 0.5h of incubation was 7 % of the total applied radioactivity (TAR). The mineralization process slowed down after this first phase. After 7 days of incubation the amount of $^{14}CO_2$ was approximately 18% of the initial radioactivity applied. Because of this two-phase degradation pattern, DT_{50} and DT_{90} were calculated using a multi-compartment model. The bound residues content increased to approximately 53 %TAR after 24h which again appeared to be a ‘plateau’ level. Five metabolites were found, but none was identified. The main metabolite (M3), slightly less polar than the parent, reached about 9% after 4-6 hours and starts decreasing afterwards. The amount of bound residues detected in this study is not in accordance with the result of the adsorption/desorption studies (see 5.2.1). Indeed those studies demonstrate that C(M)IT and MIT are highly mobile in soil. Consequently, it is possible that C(M)IT and MIT rapidly form metabolites less mobile than parent compound, which are bound to the substrates. The low adsorption property of MIT could indicate that the degradation was the main process that occurred during the experiment, and that a real half life degradation was measured. However, no similar data has been provided for C(M)IT and it can not be stated that the half live value obtained for MIT was the worst case value. Additionally, this study is a static test with only a single addition of radioactive-labeled test substance and not a continuously operated test system according to OECD 303A with a hydraulic retention time and a sludge age comparable to full-scale STPs as the previous studies. Thus, this experiment was only considered as supportive data.

Aerobic degradation in soil

C(M)IT

Two studies were provided by Dow. In C(M)IT rapidly dissipates in soil following a biphasic kinetic. Following discussions at the Working Group II 2014, DT50 values have been checked and re-calculated when required, according to FOCUS recommendations leading to $DT_{50,\ 12°C}$ = 0.21 days. (Guo and Eisenschmid, 2006).

Metabolism involves cleavage of the isothiazolone ring with the ultimate metabolite being CO_2. CO_2 increased from 0.2% of applied activity at Hour 2 to 75% on Day 100.

Two metabolites are being present at greater than 10% of applied activity: the first one at a maximum of 30.2% on day 2 (around 6% at day 30 and 100) and the second one at a maximum of 18.4% at 22 hours (9% at day 5, 0.5 at day 100). They are identified by LC-MS as 2-(methylcarbamoyl)-1-oxoethane sulfinic acid and 2-methylisothiazolin-3,5-dione.

The fraction of applied ^{14}C-activity that becomes incorporated into the bound residues increased from 0.2 % to 38.5 % after 30 days of incubation and then decreased to 13.1 % after 100 days of incubation. After 5 days (29.6% of the applied activity in the bound residues), acid hydrolysis extracted over 18% of the applied activity. NaOH extraction showed that most of the remaining activity (10.4% of the applied activity) and was mostly associated with the fulvic acid fraction (8.2% of the applied activity). The humic acid and humin fractions contained only small quantity of activity (less of 4% of the applied activity after 5 days).

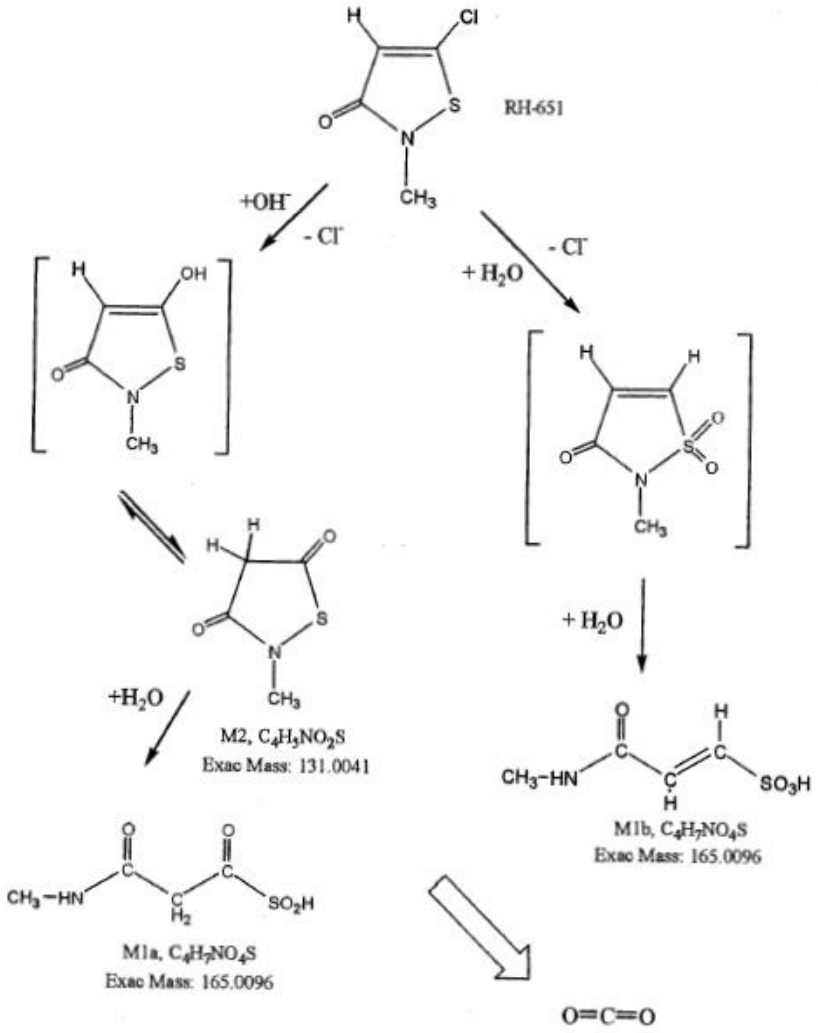
Similar trend (biphasic kinetic and dissipation rate) were observed in the older second study (Wang, 1991). Calculations according to FOCUS recommendations leading to $DT_{50,\ 12°C}$ = 0.63 days. These half life appear as worst case compared to the value derived from the previous study however, fit was statistically not acceptable.

CO_2 was the only metabolite detected and identified that was greater than 10% of the applied radioactivity and reached 27% at 64 days.

All detected metabolites were at or near the void volume and cochromatographed with the acids, malonic acid, malonamic acid, N-methyl malonamic acid, and N-methyl oxamic acid. While definitive identification of the metabolites could not be achieved, they can supposed to be a mixture of these 4 acids.

The fraction of applied ^{14}C-activity that becomes incorporated into the bound residues increased from 1.62 % to 76.49 % after 48 hours of incubation and then decreased to 58.70% after 64 days of incubation. No acid extraction has been performed before the NaOH extraction. At 24h, 58.8% of the applied activity was extracted by NaOH. The fulvic acid fraction was 48.8% of the non extractable activity (28.9% of the applied activity), while the acid insoluble fraction, the humic acid fraction, comprised about 29.8 % of the NaOH extractable activity (17.51% of the applied activity). The base insoluble fraction (humin) comprised about 21.3% of the non extractable activity (12.51% of the applied activity).

Figure 5.1.2-4: Metabolic pathway of C(M)IT in soil, based on identification of the metabolites in the study by Dow


RH-651
+OH⁻
- Cl⁻
- Cl⁻
+ H2O
+ H2O
+H2O
M2, C4H5NO2S
Exac Mass: 131.0041
M1b, C4H7NO4S
Exac Mass: 165.0096
M1a, C4H7NO4S
Exac Mass: 165.0096
O=C=O

The test proposed by Thor (Oldersma and Salmon, 2007a) is considered as not reliable because the % TAR was not maintained in the good range during the the test even for the first hours of the test. Therefore the following results are considered as indicative only. The results indicated that a rapid mineralization occurred during the first hours after dosing after which the process slowed down. A maximum percentage of $^{14}CO_2$ formation of approximately 15% of the applied radioactivity (% a.r.) was measured after one day, but thereafter the trapped amount of $^{14}CO_2$ decreased to 6% at the end of the test, probably due to leakage of the system. The amount of extractable radioactivity decreased from approximately 42% a.r. at the first sampling point to 12.5 % a.r. after 28 days of incubation. During the incubation, the bound residue content increased from approximately 40% a.r. at the start of the test (=0.5h) to approximately 49% a.r. at the end of the incubation (28 days). Again there is initially a fast process after which the process slows down. Apparently, C(M)IT or, more likely, its metabolites have a high affinity for the soil phase. This can be explained by the mode of action of C(M)IT as described by Paulus (2005 a and b). The reaction with SH-compounds results in cleavage of the N-S bond and the resulting compound can easily react with –SH containing humic / humin acids in soil. The adsorption test with C(M)IT in sterilized soil indicated that C(M)IT itself was only slightly adsorbed to soil in a batch equilibrium test

The HPLC analysis of the methanol/formic acid extract showed a fast, almost rapid degradation of C(M)IT into several more polar metabolites, of which one, metabolite 'M1' occurred in percentages >5% at least two time points, but it was not >10% of the applied radioactivity. This unknown metabolite M1 was subsequently degraded as well. Due to the rapid degradation of C(M)IT, the data could not be fitted reliably with standard curve fitting programmes. The DT_{50} for C(M)IT in soil was empirically estimated to be < 2h. Besides metabolite M1 it is possible that a volatile organic metabolite was formed that was not trapped in the gas trap series, because the recovery of radioactivity decreased in time without a known cause.

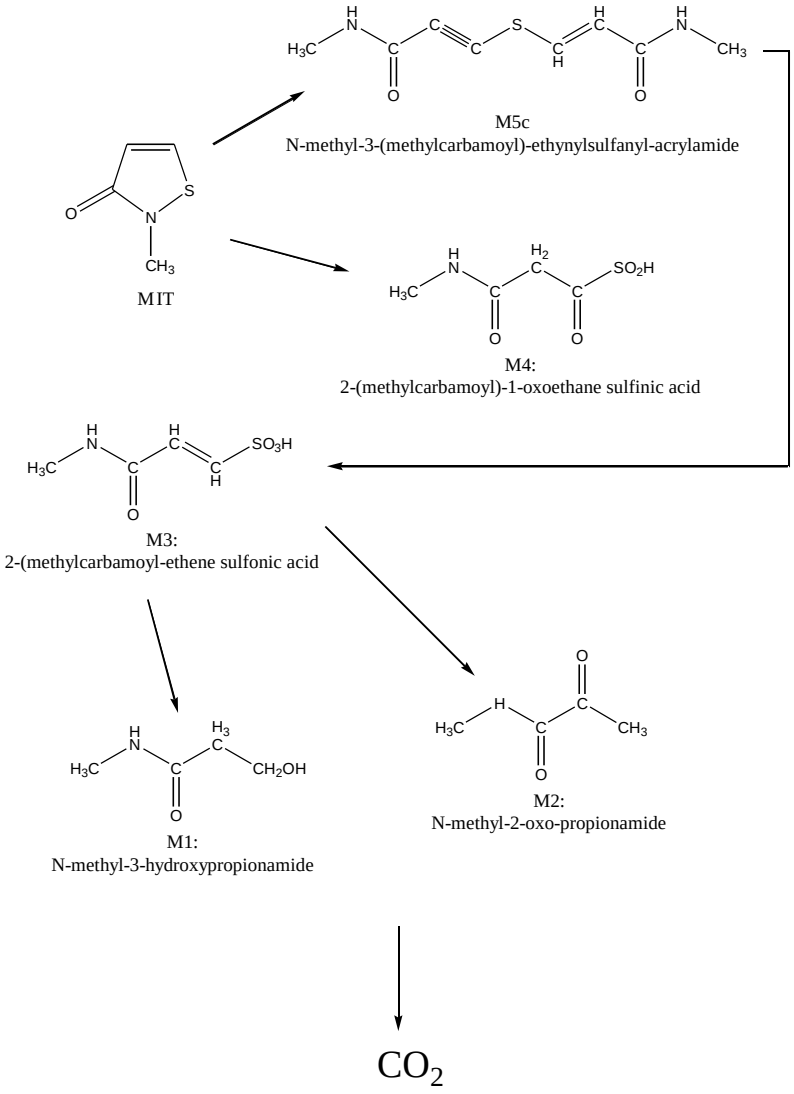
MIT

One study was provided by Dow (Guo, 2006). As C(M)IT, MIT rapidly dissipates in soil following kinetic dissipation with 2 phases: one of them is first order until 48 days, and the second is no longer first order. The $DT_{50,\ 12°C}$ value is 0.51 day.

CO_2 increased from 2% of the applied activity at 2 hours to 47% at day 100. Four metabolites are being present at or greater than 10% of applied activity: the first one (M3) at a maximum of 29% at 22 hours, the second one (M4) at a maximum of 21.4% at 22 hours, the third one (M1) at a maximum of 11.2% at day 5 and the fourth one (M2) at a maximum of 10% at 22 hours. They are identified by LC-MS: M3=2-(methylcarbamoyl)-ethene sulfonic acid, M4=2-(methylcarbamoyl)-1-oxo-ethane sulfinic acid (= isomere of M3), M1 = N-methyl-3-hydroxypropionamide and M2=N-methyl-2-oxo-propionamide and each of them may ultimately mineralize and produce CO_2.Identified metabolites are transient decreasing to less than 10% by Day 10 and less than 5% by day 30 and 100.

The fraction of applied ^{14}C-activity that becomes incorporated into the bound residues increased from 6.2 % to 39.7 % after 30 days of incubation and 38.8 % after 100 days of incubation. Acid hydrolysis extracted over 23.5 % of the applied activity after 30 days. NaOH extracted 9.2% of the applied activity and most of the NaOH extracted activity was associated with the fulvic acid fraction (6.5% pf the applied activity). The humin fraction contained 7.4 % of the applied activity after 30 days of incubation.

Figure 5.1.2-5: Metabolic pathway of MIT in soil



The test conducted by Thor seems to confirm the rapid dissipation of MIT (Oldersma and Salmon, 2007b). As for CIT, the test proposed by Thor should have been considered as not reliable because the % TAR was not maintained in the good range during the the test. However MIT is rapidly degraded with a DT_{50} <0.08d. During this period, % TAR was maintained in the range 90-110%. Therefore this study has been considered as acceptable with restrictions. The results indicated that a fast initial mineralization occurred during the first hours after dosing, after which the process slowed down. A maximum percentage of $^{14}CO_2$ formation of approximately 25% a.r. after 51 days of incubation was measured. The amount of extractable radioactivity from the soil decreased from approximately 57 % a.r. at the first sampling-point to less than 10 % a.r. after 51 days of incubation. During the incubation, the bound residue content increased from approximately 33% a.r. at t=2h to approximately 52% a.r. at the end of the incubation with a maximum at 55% at 28 days. This is similar to the results for C(M)IT and probably due to similar reactions.

The HPLC analysis of the methanol/formic acid extract showed a fast, almost complete degradation of MIT into at least one more polar metabolite 'M1'. This metabolite M1 is subsequently degraded as well and remained below 10% of the applied activity.. Two minor metabolites, less polar than the parent compound, were observed but not in relevant concentrations.

5.1.3 Summary and discussion of degradation

In the Dow dossier, C(M)IT is classified ready biodegradable failing 10-d window and MIT is classified as not ready biodegradable according to the criteria of the test, although significant biodegradation occurred. In the Thor dossier, threshold values of ready biodegradable substance are obtained. Nevertheless, as the test has been carried out with an activated sludge receiving both domestic wastewater and chemical, adaptation of the inoculum can not be excluded and C(M)IT/MIT is therefore considered as not ready biodegradable.

C(M)IT and MIT rapidly dissipate in the aquatic environment. Abiotically, C(M)IT and MIT have moderate hydrolytic (47.8 – 120.6 days at 12°C for C(M)IT at pH 9) and photolytic half-lives (6.6 days for C(M)IT and 18.2 days for MIT). Nonetheless, in fresh water and in STP, the biotic degradation of C(M)IT and MIT appears as the major metabolic pathway with half-lives of C(M)IT and MIT in simulation tests below than 6 days compared to abiotic degradation which is much less rapid than biodegradation. In the water sediment studies in the Thor dossier, similar half life are observed for the whole system and the water compartment, which is consistent with low adsorption capacities of C(M)IT and MIT (see 5.2.1). In marine water, half lifes are higher (until 41.7 days at 9°C). In soil, a fast degradation (2d) was observed.

Conclusion on metabolites –Dow

Metabolism involves cleavage of the isothiazolone ring, leading to the formation of metabolites, which are shown more polar than the parent compounds. The presence of $^{14}CO_2$ indicates that these metabolites are undergoing additional oxidation, which results in the evolution of $^{14}CO_2$.

Nine degradation products of C(M)IT/MIT are identified in the degradation tests:

- N-methyl malonamic acid (NMMA),

- N-methyl acetamide,
- Malonamic acid,
- 2-(methylcarbamoyl)-ethene sulfonic acid,
- 2-hydroxyethane sulfonic acid,
- 2-(methylcarbamoyl)-1-oxo-ethane sulfinic acid,
- 2-methyl-isothiazoline-3,5-dione,
- N-methyl-3-hydroxypropionamide,
- N-methyl-2-oxo-propionamide.

The metabolites found at >10% are reported in the table below in different environmental compartments:

Table 5.1-2: Metabolites identified in the degradation tests in the environmental compartment.

Environmental compartment	Metabolites of C(M)IT and MIT	Amount of metabolite (percentage)
Water	NMMA	Aquatic (estuarine) degradation (C(M)IT) 37.3% at day 2(22 µg/L) to 78% at day 7 (115 µg/L) Aquatic (estuarine) degradation (MIT) 27% at day 3 (22 µg/L) to 33% at day 6 (112 µg/L)
	Malonamic acid	*Water/sediment degradation (C(M)IT)* 9% at day 3 (Cedar Hill system) and 2% at day 3 (Almshouse system) in aqueous phase
	2-(methylcarbamoyl)-ethene sulfonic acid[1]	*Identification by LC-MS of the two compounds both associated:* *Water/sediment degradation (MIT)* 19.9% at day 2, 3.2% at day 30 (Cedar Hill system) and 22.3% at day 1.3, 0.5% at day 30 (Almshouse system) in aqueous phase
	2-hydroxyethane sulfonic acid	
Soil	2-(methylcarbamoyl)-ethene sulfonic acid[1]	Aerobic degradation in soil (MIT) 29% at day 1 1, 3.1% at day 30
	2-(methylcarbamoyl)-	Aerobic degradation in soil (C(M)IT)

Environmental compartment	Metabolites of C(M)IT and MIT	Amount of metabolite (percentage)
	1-oxo-ethane sulfonic acid[1]	30.2% at day 2, 6% at day 30 Aerobic degradation in soil (MIT) 21.4% at day 1, 2.7% at day 30
	2-methyl-isothiazoline-3,5-dione	*Aerobic degradation in soil (C(M)IT)* 18.4% at day 1, 9% at day 5
	N-methyl-2-oxo-propionamide	Aerobic degradation in soil (MIT) 10% at day 1, 1.1% at day 100
	N-methyl-3-hydroxypropionamide	Aerobic degradation in soil (MIT) 11.2% at day 5, 4.7% at day 100

[1] 2-(methylcarbamoyl)-ethene sulfonic acid and 2-(methylcarbamoyl)-1-oxo-ethane sulfonic acid are isomer.

One of the major metabolite, N-methyl malonamic acid (NMMA) and two other metabolites resulting from ring cleavage identified in simulation tests (N-(n-methyl) acetamide (NMA, sewage treatment plant study, MIT) and malonamic acid (MA sewage treatment plant test, MIT, and aerobic water sediment study, C(M)IT) are ready biodegradable and thus they will not be persistent in the aqueous phase, in the sediments or in the soil. The other metabolites will probably also expected to be quickly biodegraded in the environment, based on QSARs calculations (see section 4.3).

It can therefore be concluded that in the environment, C(M)IT/MIT is quickly biodegraded in degradation products, which are either been shown to be readily biodegradable or predicted to be rapidly biodegradable and shown to be transient in the simulation studies where they have been detected. Aquatic and soil metabolic pathways have been presented above.

Conclusion on metabolites –Thor

Metabolites were formed in all biodegradation tests, also at relevant concentration levels (> 10% of the applied radioactivity). Most of the metabolites were assumed to be small polar compounds, but they could not be identified due to those characteristics. In most cases the metabolites were also rapidly biodegraded.

The applicant proposed to use routes of degradation for C(M)IT found in literature, see Figure 5.1.3-1 and Figure 5.1.3-2.

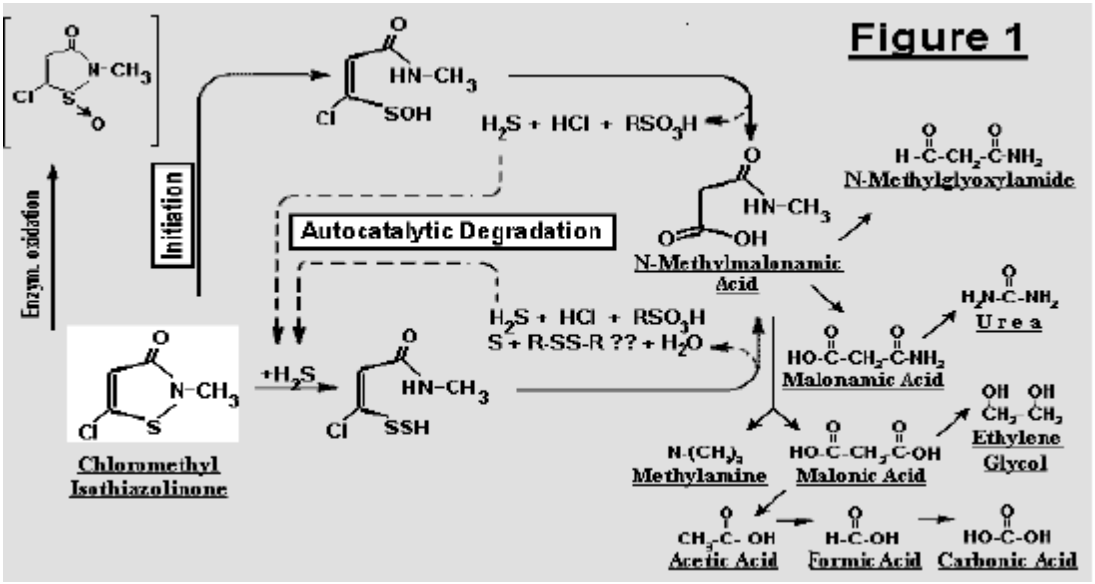


Figure 5.1.3-1: Proposed degradation pathway of C(M)IT taken from SCCNFP/0670/03(SCCNFP, 2003)

The first scheme, exposed by SCCNFP (2003) is for the major part similar to the proposed route of degradation by Krzeminski (Krzeminski *et al*, 1975a,b). This proposal was based on extensive experimental work, using various analytical methods for the identification of the metabolites:

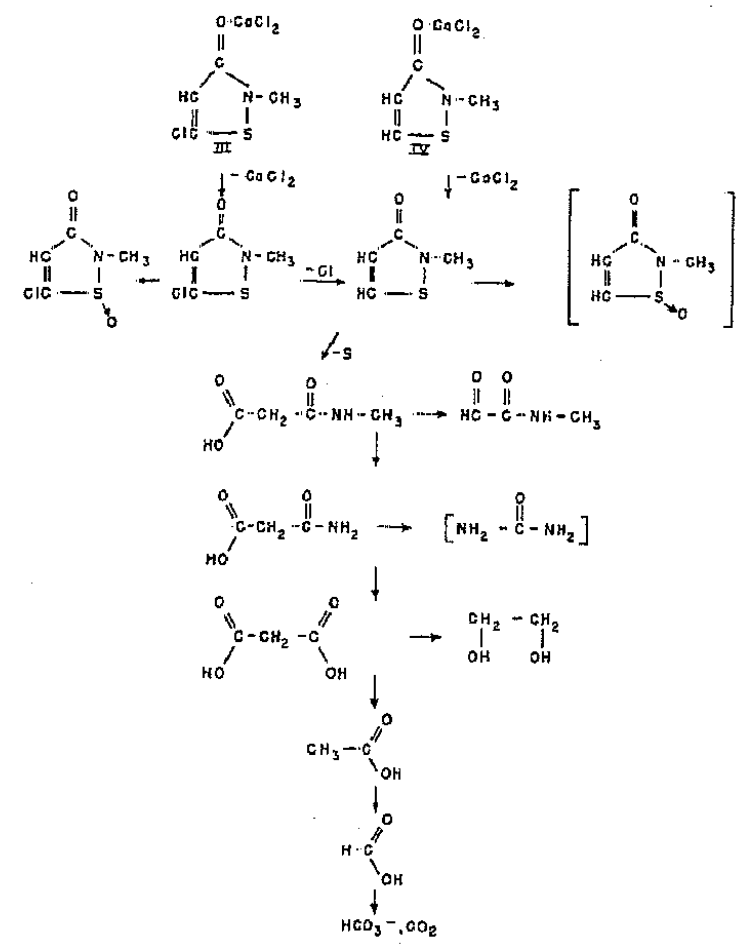


Figure 5.1.3-2: Proposed pathway for biodegradation of C(M)IT in the environment (Krzemski, 1975a,b)

The applicant proposed to assume that the degradation path for MIT would be similar. These proposals for the route of degradation confirm the formation of small polar metabolites. Most of the compounds are naturally occurring, and will not cause any harm to the environment. They are expected to be not bioaccumulating. It is very well possible that the observed metabolites in the degradation tests correspond with the proposed compounds.

The registrant proposed pathways for degradation of C(M)IT in the environment. The routes of degradation of MIT will be similar to C(M)IT. The disappearance of the two compounds is rapid with formation of small polar metabolites not harm to the environment (rapidly degraded, not bioaccumulable, not toxic). The principal degradative route involved the dissociation from $CaCl_2$, ring opening, loss of S and Cl and led to N-methylmalonamic acid. The degradation then proceeded through malonamic, malonic, acetic and formic acids to CO_2.

Mineralization and non-extractable residues:

In the Dow dossier, C(M)IT is classified ready biodegradable failing 10-d window and MIT is classified as not ready biodegradable according to the criteria of the test, although significant biodegradation occurred. In simulation tests of this dossier, the mineralization half-lives of both substances in aerobic fresh water/sediment microcosm are less than 372 days, and in anaerobic fresh water:sediment microcosm was 786 days. In a STP simulation test, the mineralization half-lives of both substances are less than 14 days.

In a soil aerobic test, the mineralization half-lives of both substances are less than 3 months (175 days maximum for MIT). In all study but one, the not extractable residues in amounts do not reached 70% of the initial dose. In a study by Dow. bound residues reached 76.5% oft he applied activity after 48h, but the amount of bound residues decreased to 57.8% after 64 days.

In the Thor dossier, C(M)IT/MIT is considered as not ready biodegradable. Mineralization rates have not been determined in the water sediment study, however less than 27% of C(M)IT has been mineralized after 53 days and less than 42% of MIT has been mineralized after 100 days. Mineralization rate was only partially investigated in the STP simulation test; in a simulation test with MIT, CO_2 amounts to 7% of total applied acitivity after 0.5h, and the mineralization process decrease after this first phase. In soil, the test degradation has not been considered as reliable because of the low recovery of total applied activity. A maximum of 15% of CO_2 after the first day and 25% of CO2 after 51 days were measured for C(M)IT and MIT respectively. The not extractable residues in amounts do never reached 70% of the initial dose.

Significant amount of non extractable residues were observed in the water sediment, STP and soil degradation studies . According to the low adsorption properties of C(M)IT and MIT, it is not expected that bound residues contained significant amounts of active substance. As for the leaching study, it is assumed that biodegradation lead to the formation of ring-cleaved metabolites that formed tight association with sludge, sediment or soil matrix.

Table 5.1-3: Non extractable residues in water sediment, STP and soil studies

Study	Non extractable residues, % of initial applied radioactivity			
	Dow		Thor	
	C(M)IT	MIT	C(M)IT	MIT
Sediment in aerobic water sediment	Max 44.4-69.5% 42.2 and 60.4% at the end of the test	Max 60.2-62.6% 57.7 and 62.6% at the end of the test	Max 43.9 – 51.4% 35.5 and 43.9% at the end of the test	Max 42.0-53.7% 42.0 and 47.5% at the end of the test
Solids in STP test (including waste sludge solids, effluent solids and aeration vessel solids)	19.7%	<25.9%*	n.a.	Max 52.8% 38.8% at the end of the test
Soil	Max 38.5%-76.5% 13.1 - 58.7% at the end of the test	Max 39.7% 38.8% at the end of the test	Max 52.6% 49.3% at the end of the test	Max 52.8% 51.8% at the end of the test

*no extraction was carried out as solids in this study

n.a. not available

5.2 Environmental distribution

Table 5.2-1: Results of the adsorption/desorption from soil and sediment studies on C(M)IT and MIT

Method	**Soil Class**	**Tested substance**	**Percent AS Adsorbed**	**K_a**	**K_{aoc}**	**K_d**	**K_{doc}**	**Reference / Owner**
US EPA 835-1110	Return activated sludge	C(M)IT	3-33.90	29.75-34.94	79.9-107.1*			Swales, 2002a / Dow
US EPA N163-1	Sandy loam	C(M)IT	9.1-67.0	1.48	91.71*	1.89	116.71*	Wang,1991 / Dow
	Silt loam	C(M)IT	4.1-16.5	0.42	30.00*	0.55	39.23*	
	Sand	C(M)IT	5.9-33.8	0.73	105.38*	0.56	80.83*	
	Clay loam	C(M)IT	0.9-3.3	0.08	143.46*	0.05	88.65*	
	Sandy loam sediment	C(M)IT	25.2-87.0	4.86	310.38*	6.6	421.49*	
US EPA 835-1110	Return activated sludge	MIT	2.58-51.12	20.11-56.82	54.1-152.7*			Swales, 2002b / Dow
OECD 121		C(M)IT	Not applicable		11.75	nr	nr	Geffke, 2002b / Thor
		MIT			<< 5.6			
OECD 106	Sediment 1: Valleikanaal	C(M)IT	< 25 in all test soils and sediments	0.76	45	nr	nr	Salmon and Cremers / Thor
	Sediment 2: Kromme Rijn			0.39	42	nr	nr	
	Sand			0.93	42	nr	nr	
	Loamy sand			0.6	26	nr	nr	
	Sandy loam			0.7	69	nr	nr	
	Sandy clay			0.94	49	nr	nr	
	Loam			0.97	44	nr	nr	

Method	Soil Class	Tested substance	Percent AS Adsorbed	K_a	K_{aoc}	K_d	K_{doc}	Reference / Owner
US EPA N163-1	Sandy loam	MIT	10.5	0.1	7.7*	0.67	ND	Gillings, 2006 / Dow
	Clay loam	MIT	24.7	0.27	6.9*	0.80	ND	
	Silty clay loam	MIT	16	0.14	6.7*	0.91	ND	
	Sand	MIT	1.9	0.03	10*	0.74	ND	
	Loam	MIT	46	1.07	6.4*	0.96	ND	

* Estimated values from the percent organic carbon of the sample: Koc = (Ka *100)/percent organic carbon

5.2.1 Adsorption/Desorption

C(M)IT

Two studies were provided by Dow. When tested in an activated sludge adsorption test, the Freundlich sorption constant of C(M)IT (K_f) is 55.6. The low value for the Freundlich sorption constant and the estimated Ka_{oc} ranged from 79.9-107.1 indicate that C(M)IT is not extensively sorbed to activated sludge and likely to remain predominantly in the aqueous phase for the typical concentrations of sludge expected in a waste treatment plant. When tested in a soil adsorption test, C(M)IT is weakly adsorbed to the examined soils and sediment (Ka_{oc} = 30-310, arithmetic mean Ka_{oc} = 136.2) and does desorb considerably (Kd_{oc} = 39-421). This indicates that C(M)IT if present would not be extensively adsorbed to soil.

In the Thor dossier, the adsorption/desorption characteristics of C(M)IT / MIT were determined in test using the HPLC estimation method following OCDE Guideline 121. For C(M)IT, the Koc value is 11.75 L/kg. A batch equilibrium test according to OECD guideline106 was carried out with two sediments and five soils. The batch equilibrium test confirmed the adsorption percentages and distribution coefficient for C(M)IT (Ka_{oc} = 26-69 , arithmetic mean Ka_{oc} = 45) that were measured with the HPLC method. It was therefore concluded that C(M)IT itself have a high affinity for the aqueous phase and can be considered (highly) mobile in soil and sediment.

MIT

Two studies were provided by Dow. When tested in an activated sludge adsorption test, the Freundlich sorption constant of MIT (K_f) is 6.12. The low value for the Freundlich sorption constant and the estimated Ka_{oc} ranged from 54.1 to 152.7 indicate that MIT is not extensively sorbed to activated sludge and likely to remain predominantly in the aqueous phase for the typical concentrations of sludge expected in a waste treatment plant. When tested in a soil adsorption test, MIT is adsorbed weakly to the examined soils and sediment (Ka_{oc} = 6.4-10, arithmetic mean Ka_{oc} = 7.5). MIT is considered highly mobile

In the Thor dossier, the adsorption/desorption characteristics of MIT were determined in test using the HPLC estimation method following OCDE Guideline 121.The only reliable result is that the K_{OC} value is much smaller than 5.6 L/kg. This value was extrapolated because no reference item with a shorter retention time than that of MIT was available. The reason is that MIT was not retained by the column's stationary phase and basically eluted with the solvent front. It was therefore concluded that MIT have a high affinity for the aqueous phase and can be considered (highly) mobile in soil and sediment.

5.2.2 Volatilisation

Due to their low vapour pressure, C(M)IT and MIT are very unlikely to be present in air. C(M)IT photodegrades quickly with half-life of 16.4 hours (Guo, 2003) and 17.5 hours (Hanstveit, 2006). MIT photodegrades quickly with half-life of 16.6 hours (Guo, 2003) and 14.3 hours (Hanstveit, 2006) and the half-lives of its metabolites range from 18.6 to 24.4 hours (Guo, 2003). Due to very low production and usage volume, the effect from C(M)IT, MIT and its potential photodegradation products towards global warming is minimal. Therefore, C(M)IT, MIT and its photodegradation metabolites impose no effect to global warming.

5.2.3 Distribution modelling

Not performed.

5.3 Aquatic Bioaccumulation

Table 5.3-1: Summary of relevant information on aquatic bioaccumulation

Guideline	Results	Remarks	Reference / Owner
OECD 305 (Static) C(M)IT	BCF = 54 and 41 at 0.01 and 0.12 mg a.i./L respectively	Log Kow ≤ 0.401 Initial concentration: 0.01 and 0.12 mg a.i./L Depuration Time (DT_{50}): 1.6 and 0.64 days at 0.01 and 0.12 mg a.i./L respectively	Madsen and Stuerman, 1996 / Dow
EPIWIN C(M)IT	3.16	Log Kow = 0.63 to 0.71	Verhaar, 2007 /Thor
EPIWIN MIT	3.16	Log Kow = -0.26 to -0.34	

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

MIT and C(M)IT are highly water soluble substances and have a low affinity for non-polar solvents and phases. The experimentally determined octanol/water partition coefficients (K_{ow})[1] for MIT and C (M)IT are, given as log (K_{ow}), -0.48 to -0.26 for MIT, and 0.63 to 0.71 for C(M)IT. EPIWIN estimates of the BCF are 3.16 for both MIT and C(M)IT. As such it can be stated that the active substances MIT and C(M)IT, do not

[1] Note that Pow and Kow are two different symbols for the same entity, the octanol/water partition coefficient.

possess any bioconcentration potential. There is no need to perform and submit a bioconcentration study to corroborate this, as the Kow and estimated BCF values are sufficiently far away from the cut-off values for classification and labelling (log Kow≥ 3 or BCF ≥ 100).

5.3.1.2 Measured bioaccumulation data

Nonentheless, a bioconcentration study has been conducted on C(M)IT in fish (Bluegill sunfish) by Dow. The provided study fulfils the requirement for C(M)IT fish bioaccumulation. The Bioconcentration Factor (BCF) of total ^{14}C residues, ≤ 54, is very low; well below regulatory thresholds. Additionally ^{14}C-residues depurate very rapidly. These results are expected given the low log Kow value and the high water solubility of C(M)IT. At environmentally relevant concentrations, the bioaccumulation of parent compound will be significantly less than the toxicity.

5.3.2 Summary and discussion of aquatic bioaccumulation

The potential of bioaccumulation or biomagnification of C(M)IT and MIT is negligible

5.4 Aquatic toxicity

The acute and chronic toxicity studies were conducted with the notified substance C(M)IT/MIT but with different mixture:

- ACTICIDE 14: 14 % C(M)IT/MIT (Thor) or
- Acticide PT: 2.1% C(M)IT/MIT (Thor) or
- Kathon^{TM}886F: 14% C(M)IT/MIT (Dow) or
- C(M)IT/MIT formulations (Kathon^{TM}886 all magnesium formulations which is considered as equivalent to the technical grade Kathon^{TM}886F).

When no more information are provided, test were carried out with C(M)IT/MIT 14%. However, the results are all presented on a C(M)IT/MIT active ingredient (a.i.) basis.

Table 5.4-1: Summary of relevant information on aquatic toxicity

Method			**Results (mg a.i./L)**	**Remarks**	**Reference / Owner**
Secondary consumers	Acute toxicity to **fish**	(*Oncorhynchus mykiss* - 96h LC50) US EPA FIFRA 72-1	LC_{50} = 0.19 (mmc)	F	Ward and Boeri, 1990a / Dow RI: 1
		(*Lepomis macrochirus* - 96h LC50) US EPA FIFRA 72-1	LC_{50} = 0.28 (mmc)	F	Ward and Boeri, 1990b / Dow RI: 1
		(*Oncorhynchus mykiss* - 96h LC50) OECD 203	LC_{50} = 0.22 (nc)	S	Wyness, 1994a / Thor RI: 2

		(*Oncorhynchus mykiss* – 14d LC50) OECD 204	LC_{50} = 0.07 (mmc)	F	Ward and Boeri, 1991a / Dow RI: 1
	Chronic toxicity to **fish**	(*Pimephales promelas* - 36d NOEC) US EPA FIFRA 72-4	NOEC = 0.02 (mmc) based on weight NOEC = 0.12 (mmc) based on percent survival at hatch, time to hatch, mortality of embryos, mortality of larvae and juveniles and total length	F	Ward and Boeri, 1991b / Dow RI: 1
		Oncorhynchus mykis- 28d NOEC) OECD 215	NOEC = 0.098 (nc) based on weight	SS	Scheerbaum , 1999 / Thor RI: 2
	Acute toxicity to **saltwater fish**	(*Cyprinodon variegatus* – 96h LC50) American Society for Testing and Materials Committee E-35 on Pesticides, 1980	LC_{50} = 0.30 (nc)	S	Heitmuller et al., 1980 / Dow RI: 2
		(*Cyprinodon variegatus* 96h LC50) USEPA FIFRA 72-4	LC_{50} = 0.48 (nc)	F	Boeri, 1998 / Thor RI: 1
Primary consumers	Acute toxicity to **freshwater invertebrates**	*(Daphnia magna* – 48h EC50) USEPA FIFRA 72-2	EC_{50} = 0.16 (mmc)	F	Ward and Boeri, 1990c / Dow RI: 1
		(Daphnia magna – 48h EC50) OECD 202	EC_{50} = 0.10 [a] (nc)	S	Mattock 1996 / Thor RI: 1
	Chronic toxicity to **freshwater invertebrates**	*(Daphnia magna* – 21d NOEC) US EPA 72-4	NOEC= 0.10 (mmc)	F	Ward and Boeri, 1991c / Dow RI: 1
		(Daphnia magna – 21d NOEC) OECD Guideline 202 Part II	NOEC = 0.0036[a] (mmc)	SS	Mattock 1996 /Thor RI: 2
	Acute toxicity to **saltwater invertebrates**	*(Americamysis bahia* – 96h LC_{50}) US EPA OPPTS 850.1035	LC_{50} = 0.282 (mmc)	F	Palmer et al., 2002 / Dow RI: 1
		(Mysidopsis bahia – 96h LC50) USEPA FIFRA 72-3	LC_{50} = 0.33 (nc)	F	Boeri 1998 b / Thor RI: 1
		(Acartia tonsa – 96h LC50) ISO TC 147/SC 5/WG 2: and PARCOM Ring Test Protocol	LC_{50} = 0.007 (nc)	S	Weideborg 1995 / Dow RI: 2

		(Crassostrea virginica – 96h EC_{50}) EPA, FIFRA 72-3 (b)850.1350	EC_{50} = 0.041 (nc) (shell deposition)	F	Boeri et al. 1998/ Thor RI: 1
Primary producers	Toxicity to **freshwater algae and aquatic plants**	*(Selenastrum capricornutum* – 24h) OECD 201, ISO 8692, US EPA FIFRA 122-2	NOErC = 4.995 10^{-3} Initial measured concentration (LOQ/2)	S	Boeri et al., 1995a / Dow RI: 2
		(Pseudokirchneriella subcapitata – 72 h) OECD 201/ EPA OPPTS 850.5400	ErC_{50} = 53.5 10^{-3} (mmc) NOErC = 1.16 10^{-3} (mmc)	S	Scheerbaum , 2008 / Thor RI: 1
	Toxicity to **saltwater algae**	*(Skeletonema costatum*- 48h) OECD 201, US EPA OPPTS 850.5400	ErC_{50} = 5.2 10^{-3} (mmc) NOErC = 0.49 10^{-3} (mmc)	S	Palmer et al., 2009 / Dow RI: 1

S: Static; SS: Semi-static; F: Flow-through;
R1/R2 : reliability of the study
mmc: mean measured concentration; nc = nominal concentrations
[a] test was carried out with C(M)IT/ MIT 2.1% instead of 14% in all others ecotoxicity tests

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Results from an acute (96-hour) flow-through and a static toxicity tests with rainbow trout (*Oncorhynchus mykiss*) and from a flow-through toxicity test with Bluegill sunfish (*Lepomis macrochirus*) indicate that C(M)IT/MIT is very toxic to freshwater fish: 96-hour Trout LC_{50} (mean measured concentrations) 0.19 mg a.i./L (Ward and Boeri, 1990a); 96-hour Bluegill LC_{50} (mean measured concentrations)= 0.28 mg a.i./L (Ward and Boeri, 1990b); 96-hour Trout LC_{50} (nominal) = 0.22 mg a.i./L (Wyness, 1994a). The result of the last study is expressed in nominal concentration as measured concentrations are ranged from 80% to 120% of the nominal values.

Results from an acute (96-hour) flow-through and a static toxicity tests with sheepshead minnow (*Cyprinodon variegatus*) indicate that C(M)IT/MIT is very toxic to saltwater (marine/estuarine) fish 96-hour LC_{50} = 0.30 mg a.i./L (Heitmuller et al., 1980); 96-hour LC_{50} = 0.48 mg a.i./L (Boeri, 1998). Results for this study are not based on analytically confirmed test concentrations.

Results from a 14-day prolonged toxicity test with rainbow trout (*Oncorhynchus mykiss*) indicate that the NOEC in freshwater fish is 0.05 mg a.i./L and the LC50 is 0.09 mg a.i./L (Ward and Boeri, 1991a).

5.4.1.2 Long-term toxicity to fish

In an early life stage toxicity test (36 days) with the fathead minnow (*Pimephales promelas*), the NOEC is 0.02 mg a.i./L based on weight and 0.12 mg a.i./L based on percent survival at hatch, time to hatch, mortality of embryos, mortality of larvae and juveniles and total length (Ward and Boeri, 1991b). In a 28-day juvenile growth test with Rainbow trout, (*Oncorhynchus mykiss),* the NOEC is 0.098 mg ai/L (Scheerbaum, 1999).

Results for the studies from the Dow dossier were based on analytically confirmed test concentrations whereas results for the study with rainbow trout from the Thor dossier were based on nominal concentrations.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

C(M)IT/MIT is very toxic to freshwater invertebrate, *Daphnia magna*, based on results from an acute flow-through and a static studies indicating a 48-hour EC_{50} (mean measured concentrations) = 0.16 mg a.i./L (Ward and Boeri, 1990c), and a 48h-EC50 (nominal) = 0.10 mg ai/L (Mattock 1996).

C(M)IT/MIT is very toxic to saltwater invertebrate, Mysid shrimp (*Americamysis bahia*), based on results from an acute flow-through study indicating a 96h-LC_{50} (mean measured concentrations) = 0.282 mg a.i./L (Palmer et al., 2002) and a 96h-LC50 (nominal) = 0.33 mg ai/L (Boeri 1998 b). Results from static acute toxicity tests with a marine copepod (*Acartia tonsa*) indicate a 48h-EC_{50} of 0.007 mg a.i./L (Weideborg 1995). Results for these studies are based on nominal test concentrations. Results from a flow through acute toxicity test with *Crassostrea virginica* indicate a 96h-LC_{50} (nominal) of 0.041 mg ai/L (Boeri et al. 1998).

5.4.2.2 Long-term toxicity to aquatic invertebrates

Results from a flow-through chronic toxicity test with *Daphnia magna* indicate a 21-day NOEC of 0.10 mg a.i./L) for survival, reproduction and length, a LOEC of 0.18 mg a.i./L for survival of first generation daphnids (Ward and Boeri, 1991c). Results from a semi-static chronic toxicity test with *Daphnia magna* indicate a 21-day NOEC of 0.0036 mg a.i./L for survival and reproduction (Mattock 1996). Results for both chronic toxicity studies are based on mean measured test concentrations.

5.4.3 Algae and aquatic plants

Both applicants have provided studies on fresh water and marine species. Other isothiazolinones have been assessed in the framework of the biocide product regulation and evaluation of the present algae studies take into account of the consultation and technical meeting/ working group discussion. At first, as other isothiazolinones, C(M)IT/MIT is expected to rapidly react with algal cells and the initial algal density has a large influence on the outcomes of the test. Therefore initial cells density of each study has carefully been checked and only studies carried out with the initial density recommended in the guideline have been considered as reliable. Additionally, endpoints of each study have been daily assessed to determine the most sensitive period on which endpoints should be chosen. At last depending of the relevant period, endpoints are expressed as initial measured concentrations or as geometric mean of measured concentrations.

Dow provided three growth inhibition tests on algae. The tests are carried out with test concentrations ranging from 4.9 to 320 µg ai/L for the test on *Selenastrum capricornutum* (Boeri et al., 1995a), from 0.35 to 22 µg ai/L for the test on the marine algae *Skeletonema costatum* (Boeri et al., 1995b) and from 0.82 to 27 µg/ ai/L for the second test on marine algae (Palmer et al., 2009).

Despite validity criteria were met, , the test on *Selenastrum capricornutum* was considered reliable with restriction due to the low sensitivity of the analytical method: analytical data indicate that the test substance cannot be quantified by the end of the study. Moreover, analyses of the five highest tested concentrations (20, 39, 78, 160, 320 µg ai/L) at the test initiation indicate that the nominal and measured concentrations are in close agreement but the two lowest concentrations (4.9 and 9.9 µg a.i./L) have not be proven to be really achieved at least at T0 in the test media. Statistics indicate that endpoints are the lowest after the first 24 hours of exposure. To have the same approach as for other isothiazolinones, the selected endpoint should have been derived as a function of initial measured concentration. Nevertheless, analytical data indicate that endpoints concentrations are below the limit of quantification at the beginning of the test and LOQ/2 (4.955 µg ai/L) has therefore been chosen as NOEC.

The first test on marine algae (Boeri et al., 1995b) was considered unreliable (reliability index of 3): analytical data indicate that the test substance cannot be quantified by the end of the study for all test concentrations except for the highest one (22 µg/L). Therefore, endpoints based on the nominal concentrations are considered as not reliable.

The second test on *Skeletonema costatum* (Palmer et al., 2009) asked for the applicant with strong analytical measurements was considered by RMS as reliable without restriction even if all validity criteria were met. Six concentrations were tested (initial measured concentration: 0.82, 1.6, 3.4, 6.6, 13.5, 27 µg ai/L). The concentrations in the test media were measured at every time (0, 24, 48, 72 and 96 hours) and the analysis showed that levels of the active substance rapidly declined. Even at 48h, the concentrations decreased to a value below the limit of quantification (LOQ) in all test concentrations except the two highest concentrations which had measured concentrations that were 38% and 7 % of nominal, respectively 12 and 24 µg a.i./L. Statistics indicate that endpoints are the lowest after the first 48 hours of exposure. Because of the fast dissipation of the active substance and as measured concentrations are available, it appears reasonable to choose endpoints based on measured concentrations, leading to NOE_rC 48h = 0.49 µg a.i.

Thor initially provided growth inhibition tests on algae: the toxicity of Acticide® 14 to the fresh water algal species *Pseudokirchneriella subcapitata* and the marine algae species *Skeletonema costatum* (Wyness, 1994 d) was investigated according to EPA Guideline Subdiv. J, Series 122. Parameters investigated were growth rate and biomass increase. In the first study, the growth factor in the control was lower than the growth factor required in the OECD Guideline. According to OECD Guideline 201 increase in cell counts in the control should be at least a factor 16 within 3 days for the freshwater algae. This validity criterion was not met for the *Pseudokirchneriella subcapitata* test. Moreover the initial inoculum was 10^3 cells/mL instead of 10^4 cells/mL. In the second study (marine species), no measure of concentration of C(M)IT / MIT had been carried out in and the test was considered as not reliable.

A new study was carried out according the OECD Guideline 201 (Scheerbaum, 2008). Parameters investigated were biomass, growth rate and yield over a period of 72 and 96 hours. The test was conducted under static conditions. All validity criteria were met. Nine concentrations (0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28 mg a.i./L) were tested with three replicates for each concentration and six replicates for control. The measured initial concentrations well met the nominal concentrations of C(M)IT and MIT. At the end of the study C(M)IT and MIT could be detected only in the 2 and 3 higher test concentrations. After 96h algae were transferred from the nominal concentrations of 0.64-1.28 mg a.i./l and control to fresh untreated medium and allowed to grow for further 5 days under test conditions. The test item effect was observed to be reversible at these test concentrations. Statistics indicate that the lowest NOEC and EC50 are derived at 72 hours, which is therefore considered as the most sensitive period of this study. Because the fast dissipation of the active substance at the range concentrations of the NOEC, and as measured concentrations are available, it appears reasonable to choose endpoints based on mean measured concentrations. Therefore NOE_rC 72h = 1.16 µg a.i./L.

5.4.4 Other aquatic organisms (including sediment)

Table 5.4-2: Toxicity of C(M)IT/ MIT to freshwater sediment-dwelling invertebrates

Method		Results (mg a.i./kg dry sediment)	Remarks	Reference
Toxicity to sediment dwelling organisms	(*Chironomus riparius* - 28d) OECD 218 "	NOEC = 7.03 (mmc)	28 day development rate	*Aufderheide*, 2006 / Dow RI = 1
	(*Lumbriculus variegatus* - 28d) Draft OECD Sediment-water *Lumbriculus* Toxicity test using Spiked Sediment Guideline, September 2006	0.37 < EC_{50}< 0.46 (mmc) NOEC = 0.27 (mmc)	28 day survival	Thomas et al, 2007 / Dow RI = 2
	(*Hyalella azteca* - 28d) US EPA OPPTS 850.1735, ATSM E 1706-00	1.83 < EC_{50}< 6.34 (mmc) NOEC = 1.11 (mmc)	28 day survival	Thomas, 2008 / Dow RI = 2

mmc: mean measured concentration;
R1/R2 : reliability of the study

In the Dow dossier, chronic toxicity studies are carried out on freshwater midge *Chironomus riparius*, on endobenthic oligochaete *Lumbriculus variegatus* and on freshwater amphipod *Hyalella azteca.* The results of the three tests on *Chironomus riparius*, *Lumbriculus variegatus* and *Hyalella azteca* are based on geometric mean of measured concentration on sediment samples. When analytical method is not sensitive enough to quantify the test substance by the end of the study (e.g. tests on *Lumbriculus variegatus* and *Hyalella azteca),* the concentration has been taken as half of the limit of quantification of the analytical method.. As results from the most sensitive species are provided only at the end of the test (28 days), the relevant endpoint based on nominal concentration is the NOEC (28d, survival, nominal) = 2.0 mg a.i./kg dry weight. This value based on measured concentration is considered as the relevant endpoint: NOEC (28d, survival, initial) = 0.27 mg a.i./kg dry weight.

Only one test on sediment dwelling organisms was presented by Thor. A test on the development of *Chironomus riparius* in a water-sediment system was carried out according to BBA-guideline proposal. This study cannot be accepted because the concentrations of C(M)IT and MIT are not detectable. The mean measured concentrations of MIT on day 0 were > 80% for all concentration levels of the aqueous phase. The recoveries for C(M)IT on day 0 were > 80% for concentration levels from 10-40 mg/l. Below 10 mg/l the recoveries were 0-68%. With progress of the study the recoveries of the aqueous phase showed a decreasing tendency for both active ingredients after 7 days and after 28 days none of them was detectable. No explanation is available.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Regarding all available toxicity data, algae are the most sensitive species for acute and chronic effects. These results are used to classify the active substance C(M)IT/MIT.

Considering that the 48h-EC50 = 5.2 µg/L value was obtained for *Skeletonema costatum* is lower than 1 mg/L, C(M)IT/MIT meets the criteria for classification as **Aquatic Acute 1** for environmental hazard according to CLP criteria. This value is extracted from a recent study dated on 2009, for which FR-MSCA

considers sufficient information available to be considered. As this value is within the range of 0.001-0.01 mg/L, an **M-factor of 100** is allocated.

The ready biodegradation studies provided by Dow show that C(M)IT is readily biodegradable but failing the 10-day window and MIT is not readily biodegradable, although a significant biodegradation occurred (around 50% at 28 days). Several concentrations of C(M)IT or MIT were tested indicating that even with low concentrations some toxicity occurred. In the study provided by Thor, the threshold for ready biodegradation is reached, however, the origin of the inoculum does not allow to confirm that the inoculum was not adapted. The fast biodegradability of the active substance is supported by the sewage treatment plant simulation study, showing a complete degradation of CMIT and a remaining fraction of MIT in the effluent similar to the fraction determined through Simple Treat Model for a ready biodegradable substance. Nevertheless, according to Guidance on the Application of the CLP Criteria (version 4.0, November 2013) results from such tests cannot be used for the classification as the microbial biomass in STP in significantly different from the biomass in the environment.

An active substance can be considered to be rapidly degradable if ultimate biodegradation reached 70% after 28 days, corresponding to a half life below 16 days. These thresholds were not achieved in the available aquatic simulation tests (Table 5.5-1). According to the section 4.1.2.9.3 from the Annex I of the Regulation (EC) No 1272/2008, "primary biodegradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment". The threshold for rapid degradation was obtained for the primary biodegradation in the estuarine water studies and in the water sediment studies. In the estuarine studies, relevant metabolites have been shown to be readily biodegradable and not toxic in acute aquatic tests (see in Annex Additional information on C(M)IT/MIT degradation products). The results of the water sediment study could be used for the classification because of the low adsorption properties of the active substance. However, in the Thor studies, relevant metabolites were detected but not identified. In the Dow studies, no relevant metabolite was detected for C(M)IT and the two identified relevant metabolites of MIT were considered as not toxic according to QSAR predictions. But whatever the Registrant and the tested substance, large amounts of bound residues were observed in each of the provided water sediment study. Moreover, the worst cases half life for primary biodegradation in marine water were over 16 days. At last, QSAR predictions indicate that C(M)IT and MIT are not expected to be readily biodegradable. Therefore in a weight of evidence approach, C(M)IT/ MIT should be considered as not rapidly degradable.

Table 5.5-1 : Results of aquatic degradation simulation studies

Compartment	Substance	Applicant	Primary degradation	Formed CO_2	Relevant metabolite	Toxicity of relevant metabolite
Estuarine	C(M)IT	Dow	$DT_{50,12°C} \leq 5.82$ d	<28% after 5 d	n-malonamic acid	Ready biodegradable and not toxic for aquatic species
	MIT	Dow	$DT_{50,12°C} \leq 2.63$ d	<1%	N-methyl malonamic acid	Ready biodegradable and not toxic for aquatic species
Marine	C(M)IT	Worst case	$DT_{50,9°C}$ >16d			
	MIT	Worst case	$DT_{50,9°C}$ >16d			
Water sediment	C(M)IT	Dow	$DT_{50,12°C} \leq 2.47$ d (whole system)	<20% at 30 d	No but large amount of bound residues	
		Thor	$DT_{50,12°C} \leq 3.86$ d (whole system)	<27% at 58 d	Two not identified metabolites in sediment + large amount of bound residues	
	MIT	Dow	$DT_{50,12°C} \leq 2.7$ d (système entier)	<28% at 30 d	2-(methylcarbamoyl) ethene sulfonic acid and 2-hydroxyethane sulfonic acid + large amount of bound residues	Not toxic for aquatic species according to QSAR predictions
		Thor	$DT_{50,12°C} \leq 2.47$ d (système entier)	<43% at 100 d	One not identified metabolite + large amount of bound residues	

Considering that C(M)IT/MIT is not rapidly degradable and that the 48d-NOEC = 0.49 µg/L value obtained for *Skeletonema costatum* is lower than 0.1 mg/L, C(M)IT/MIT meets the criteria for classification as **Aquatic Chronic 1** for environmental hazard according to CLP criteria. As the value is within the range of 0.0001-0.001 mg/L, an **M-factor of 100** is allocated.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

According to CLP Regulation criteria:

Classification:
Aquatic Acute 1; H400
Aquatic Chronic 1; H410
Acute M-factor: 100
Chronic M-factor: 100

Labelling:

Pictogram: 
Signal word: Warning
Hazard statements: H410: Very toxic to aquatic life with long lasting effects

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Reaction mass 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1); (C(M)IT/MIT) is currently listed in Annex VI to CLP (Regulation (EC) 1272/2008) as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410. The DS proposed to retain the existing harmonised classification but to add separate acute and chronic M-factors of 100 to both hazard classes.
In the framework of the Biocidal Products Regulation, two applicants have provided data for the environmental section, which have been gathered and compared in the submitted CLH report.

Degradation

Summary

The available hydrolysis studies showed that MIT and C(M)IT do have moderate hydrolytic half-lives. While MIT was stable at all pHs, C(M)IT was stable at pH 5 and 7, whereas at pH 9 the half-lives were 47.8 – 120.6 days at 12°C. Regarding photodegradation in water, half-lives were 6.6 days for C(M)IT and 18.2 days for MIT. A ready biodegradation study conducted with C(M)IT/MIT following OECD TG 301D was not considered valid by the DS due to the application of adapted inoculum. However, available ready biodegradation studies (OECD TG 301B) on the constituents showed that C(M)IT and MIT are not readily biodegradable. No reliable surface water simulation test for C(M)IT/MIT was available, however, MIT and C(M)IT were tested separately in marine and estuarine water (OECD TG 309). The worst case primary degradation DT_{50}s of C(M)IT and MIT in marine water at 9 °C were 41.7 and 29.7 days, respectively. In addition, simulation studies in estuarine water and in water/sediment (OECD TG 308) are available on C(M)IT and MIT showing that primary degradation half-lives were < 16 days, however, not all relevant metabolites were identified. Further details on the available degradation studies can be found in the background document to the opinion (Annex 1).

Taking into consideration the available information the DS conluded that based on a weight of evidence approach, C(M)IT/MIT cannot be considered as rapidly degradable for classification purposes.

Bioaccumulation

In the CLH report experimental log Kow values (determined at 24 °C) of 0.63 to 0.71 for C(M)IT and of -0.48 to -0.26 for MIT are reported, indicating no potential for bioaccumulation.

The DS furthermore reported a BCF value of 3.162, estimated by QSAR for C(M)IT, MIT and

metabolites, and an experimental BCF value for MIT (2.32) and for C(M)IT (in the range 11-51) was reported without further reference to the studies.

Aquatic toxicity

Four acute and two chronic aquatic toxicity tests to freshwater fish are available; two other acute tests to saltwater fish are also available. All tests were carried out with C(M)IT/MIT 14% and standard guidelines were followed.

Two acute and two chronic aquatic toxicity tests to freshwater invertebrates are available, four other acute tests to saltwater invertebrates are also available. All tests were carried out with C(M)IT/MIT 14%, and standard guidelines were followed.

Three toxicity tests, two on freshwater algae and one on marine water diatom are available from studies with C(M)IT/MIT, following standard guidelines.

In the following table a summary of the relevant information on aquatic toxicity studies is reported.

Table: Summary of relevant information on aquatic toxicity.

Method	Test organism	Conditions	Endpoint	Toxicity values in mg a.s./L	Reference
Short-term toxicity to fish					
US EPA FIFRA 72-1 Freshwater	*Oncorhynchus mykiss*	Flow-through mm	96-h LC_{50}	0.19	Ward and Boeri, 1990a/ Dow
US EPA FIFRA 72-1 Freshwater	*Lepomis macrochirus*	Flow-through mm	96-h LC_{50}	0.28	Ward and Boeri, 1990b/ Dow
OECD TG 203 Freshwater	*Oncorhynchus mykiss*	Static nom	96-h LC_{50}	0.22	Wyness, 1994a/ Thor
OECD TG 204 Freshwater	*Oncorhynchus mykiss*	Flow-through mm	14-d LC_{50}	0.09	Ward and Boeri, 1991a/ Dow
American Society for Testing Materials Committee E-35 on Pesticides, 1980 Marine water	*Cyprinodon variegatus*	Static nom	96-h LC_{50}	0.30	Heitmuller *et al*., 1980/ Dow
US EPA FIFRA 72-4 Marine water	*Cyprinodon variegatus*	Flow-through nom	96-h LC_{50}	0.48	Boeri, 1998/ Thor
Long-term toxicity to fish					
US EPA FIFRA 72-4 Freshwater	*Pimephales promelas*	Flow-through mm	36-d NOEC (based on weight) 36-d NOEC (based on	0.02 0.12	Ward and Boeri, 1991b/ Dow

			percent survival at hatch, mortality of embryos, mortality of larvae and juveniles and total length)		
OECD TG 215 Freshwater	*Oncorhynchus mykiss*	Semi-Static nom	28-d NOEC (based on weight)	0.098	Scheerbaum, 1999/ Thor
Short-term toxicity to aquatic invertebrates					
US EPA 72-2 Freshwater	*Daphnia magna*	Flow-through mm	48-h EC_{50}	0.16	Ward and Boeri, 1990c/ Dow
OECD TG 202 Freshwater	*Daphnia magna*	Static nom	48-h EC_{50}	0.10[a]	Mattock, 1996/ Thor
US EPA OPPTS 850.1035 Saltwater	*Americamysis bahia*	Flow-through mm	96-h LC_{50}	0.282	Palmer *et al.*, 2002/ Dow
USEPA 72-3 Saltwater	*Mysidopsis bahia*	Flow-through nom	96-h LC_{50}	0.33	Boeri, 1998b/ Thor
ISO TC 147/SC 5/WG 2: and PARCOM Ring Test Protocol Saltwater	*Acartia tonsa*	Static nom	96-h LC_{50}	0.007	Weideborg, 1995/ Dow
EPA 72-3 (b)850.1350 Saltwater	*Crassostrea virginica*	Flow-through nom	96-h EC_{50}	0.041 (based on shell deposition)	Boeri *et al.*, 1998/ Thor
Long-term toxicity to aquatic invertebrates					
US EPA 72-4	*Daphnia magna*	Flow-through mm	21-d NOEC	0.10	Ward and Boeri, 1991c/ Dow
OECD TG 202 Part II	*Daphnia magna*	Semi-Static mm	21-d NOEC	0.0036[a]	Mattock, 1996/ Thor
Toxicity to algae					
OECD TG 201 ISO 8692 US EPA FIFRA 122-2 Freshwater	*Pseudokirchneriella subcapitata (Selenastrum capricornutum)*	24h Static imc (LOQ/2)	NOE_rC	$4.995\ 10^{-3}$	Boeri *et al.*, 1995a/ Dow RI: 2
OECD TG 201 US EPA OPPTS 850.5400 Freshwater	*Pseudokirchneriella subcapitata*	72h Static mm	E_rC_{50} NOE_rC	$53.5\ 10^{-3}$ $0.49\ 10^{-3}$	Scheerbaum, 2008/ Thor RI: 1
OECD TG 201 US EPA OPPTS 850.5400 Saltwater	***Skeletonema costatum***	48h Static mm	**E_rC_{50}** **NOE_rC**	**$5.2\ 10^{-3}$** **$0.49\ 10^{-3}$**	Palmer *et al.*, 2009/ Dow RI: 1

mm – mean measured concentration
imc – initial measured concentration
nom – nominal concentration
a) test was carried out with C(M)IT/ MIT 2.1% instead of 14% in all others ecotoxicity tests
Key endpoints used in acute and long-term hazard classification are highlighted in bold.

All toxicity tests indicate that the substance is very toxic to fish. Almost all toxicity tests with invertebrates, both freshwater and saltwater, indicate that C(M)IT/MIT is also very toxic to this trophic level.
Algae is the most sensitive taxonomic group for this substance. Due to the peculiar mode of action of C(M)IT/MIT, linked to the algal concentration, initial cells density of each study has been carefully checked and endpoints have been daily assessed to determine the most sensitive period. Depending of the relevant period, endpoints are expressed as initial measured concentrations or as geometric mean of measured concentrations.

The key study on the aquatic algae *Skeletonema costatum* showed a rapid decline of the active substance concentration, due to its mode of action. The degradation of C(M)IT/MIT depends on algal concentration, because the substance determines an inhibitory effect on the enzymes of the algae, which will result in degradation of C(M)IT/MIT. At higher test concentrations, growth of algae is inhibited which in turn slows down the degradation of the substance by algae. In order to correctly assess the concentration of the substance in the test media, analytical measurements were performed every 24h. Since statistics indicate that the period of major sensibility of the algae relay within the first two days, the endpoints were determined as mean measured concentrations at 48h.

Moreover, three major metabolites of C(M)IT/MIT were identified: NMMA, NMA and MA. Acute toxicity data of these metabolites are available for all three trophic levels (Table below).

Table: Summary of relevant information on aquatic toxicity of C(M)IT/MIT metabolites (NMMA, NMA and MA).

Method	Test organism	Conditions/Metabolite	Endpoint	Toxicity values in mg a.s./L	Reference
Short-term toxicity to fish					
OECD TG 203, US EPA OPPTS 850.1075, US EPA 797.1400, US EPA 72-1, and EC Council Directive 91/414/EC Freshwater	*Oncorhynchus mykiss*	Static nom N-methyl malonamic acid (NMMA)	96-h LC_{50}	>1000	Madsen, 2002a/ Dow

OECD TG 203, US EPA OPPTS 850.1075, US EPA 797.1400, US EPA 72-1, and EC Council Directive 91/414/EC Freshwater	*Oncorhynchus mykiss*	Static mm N-methyl acetamide (NMA)	96-h LC_{50}	>694	Rhodes, 2002a/ Dow
OECD TG 203, US EPA OPPTS 850.1075, US EPA 797.1400, US EPA 72-1, and EC Council Directive 91/414/EC Freshwater	*Oncorhynchus mykiss*	Static nom malonamic acid (MA)	96-h LC_{50}	>1000	Madsen, 2002b/ Dow
Short-term toxicity to aquatic invertebrates					
OECD TG 202, US EPA OPPTS 850.1010, US EPA 797.1300, US EPA 72-2 Freshwater	*Daphnia magna*	Static mm N-methyl acetamide (NMA)	48-h EC_{50}	>>863	Madsen, 2002c/ Dow
OECD TG 202, US EPA OPPTS 850.1010, US EPA 797.1300, US EPA 72-2 Freshwater	*Daphnia magna*	Static mm N-methyl malonamic acid (NMMA)	48-h EC_{50}	>>986	Rhodes, 2002b/ Dow
OECD TG 202, US EPA OPPTS 850.1010, US EPA 797.1300, US EPA 72-2 Freshwater	*Daphnia magna*	Static nom malonamic acid (MA)	48-h EC_{50}	> 1000	Madsen, 2002d/ Dow
Toxicity to algae					
OECD TG 201 US EPA OPPTS 850.5400	*Pseudokirchneriella subcapitata*	96h-Static imc N-methyl malonamic acid (NMMA)	96-h E_rC_{50} 96-h NOE_rC	128 36	Madsen, 2002e/ Dow RI:1
OECD TG 201 US EPA OPPTS 850.5400	*Pseudokirchneriella subcapitata*	72h-Static imc N-methyil acetamide (NMA)	72-h E_rC_{50} 72-h NOE_rC	5.8 0.51	Rhodes, 2002c/ Dow RI:2
OECD TG 201 US EPA	*Pseudokirchneriella subcapitata*	96h-Static imc Malonamic acid (MA)	96-h E_rC_{50} 96-h	> 1080 519	Madsen, 2002f/ Dow

OPPTS 850.5400 US EPA TSCA 797.1050 US EPA FIFRA 122-2 and 123-2 EC Council Directive 67/548/EEC			NOE_rC		RI:1
mm – mean measured concentration nom – nominal concentration imc – initial measured concentration					

Short-term toxicity tests to fish and aquatic invertebrates indicate that all three metabolites are practically non-toxic for these trophic level. All three metabolites are less toxic to freshwater algae than the parent C(M)IT/MIT. However, an algae NOEC value of 0.51 mg/L for NMA shows that this metabolite is toxic to algae.

Comments received during the public consultation

Two MSCAs and three companies commented on the proposed environmental classification.

For one MSCA it was not clear if the validity criteria of the exponential growth in controls of the key algae study was fulfilled and if a minimum multiplication factor of 16 was reached after 48h of the test period. The DS stated in his response to comments that the above conditions were met.

All three commenting companies disagreed with the proposed environmental classification, particularly with the M-factor of 100 for the long-term aquatic hazard classification. They considered CMIT/MIT and their metabolites being rapidly degradable. The DS confirmed that for several simulation degradation studies, the DT_{50}s for primary degradation were below 16 days and the metabolites have been shown to be readily biodegradable. Nevertheless, in marine water, a DT_{50} for primary degradation of >16 days was observed for the highest tested concentration (100 µg/L) and C(M)IT/MIT cannot therefore be considered rapidly degradable. Moreover, MIT was considered not rapidly degradable, since not all metabolites formed at >10% have been successfully identified. Therefore, it has not been convincingly demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

One company proposed to use the 24h measured CMIT/MIT concentrations to derive the toxicity endpoints based on the specific mode of action of CMIT/MIT in bacteria, fungi and algal cells and its rapid time course and disagreed with the use of the 48h toxicity endpoints. The DS agreed with the analysis of the effects on algae but highlighted that the validity criteria mentioned in the comment were not clear. In addition, the DS questioned whether endpoints at 24h can be considered as chronic endpoints.

Another company expressed doubts about the long-term hazard classification based on the

algal endpoint because of the substance rapid dissipation from the test media. Moreover, the company did not consider it feasible to use shortened exposure times in algae tests and proposed a weight of evidence approach using another ecotoxicity study on the marine copepod *Acartia tonsa* (Weideborg, 1995). The DS noted that since the validity criteria were fulfilled at 48h in the algal study, there is no need to provide more information on the validity of the study carried out with *A. tonsa*.

The last company did not agree on a classification based on 48h E_rC_{50}/NOE_rC values. Following that consideration, it was proposed to classify C(M)IT/MIT using 72h E_rC_{50} value, which will lead to an acute M-factor of 10. The DS replied that in the study with the marine algae a multiplication factor of 55 was reported after 48h for cell density in the controls which supported study conditions generating exponential growth. The endpoints derived at 48h were therefore considered relevant for the acute and long-term hazard classifications.

Additional key elements

The ecotoxicity studies used by the DS in order to classify the substance for acute and chronic aquatic toxicity are based on 48h algal tests. The OECD TG 201 and the ECHA guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b foresee the possibility to adopt a shorten test period (48h) with respect to the usual duration of 72h or 96h, provided that the validity criteria for the control performance (exponential control growth greater than a factor of 16) are fulfilled. Since the test reports a multiplication factor of 55 for cell density after 48h, the data are considered reliable. Moreover, due to the peculiar behaviour of the substance in the presence of algae, the substance probably shows the strongest adverse effects in the first 48 hours.

Assessment and comparison with the classification criteria

Degradation

Regarding hydrolysis, MIT is stable at all pH, C(M)IT was stable at pH 5 and 7 while at pH 9 the half-lives were 47.8 – 120.6 days at 12°C.

Regarding photodegradation in water, half-lives were 6.6 days for C(M)IT and 18.2 days for MIT.

In a ready biodegradation study on C(M)IT/MIT the threshold was reached. Nevertheless, as the test was carried out with an activated sludge receiving both domestic wastewater and chemical waste, adaptation of the inoculum cannot be excluded and C(M)IT/MIT this study was therefore considered not valid for classification purposes. The ready biodegradation studies on the constituents of the substance show that C(M)IT and MIT are not readily biodegradable.

No reliable surface water simulation tests for C(M)IT/MIT are available. However, simulation studies in marine and estuarine water were performed on each of the constituents separately. The worst case primary degradation DT_{50}s of C(M)IT and MIT in marine water at 9°C are 41.7 and 29.7 days, respectively, therefore the single constituents of the substance were demonstrated to be not primarily degraded with half-lives of < 16 days. Furthermore, even if the primary degradation half-lives in the estuarine water studies and in the water sediment

studies on the constituents were < 16 days, some relevant metabolites were not identified. In addition, the transformation product NMA is considered classifiable as Aquatic Chronic 3, based on an algae NOEC value of 0.51 mg/L and rapid degradability. In light of this information, C(M)IT and MIT are separately considered as not rapidly degradable.

Based on the above weight of evidence, C(M)IT/MIT is considered not to be rapidly degradable for classification purposes.

Bioaccumulation

The experimental log Kow at 24 °C of MIT is -0.486 and log Kow of C(M)IT is 0.401, these values are more than four orders of magnitude lower than the trigger value (> 4) in the CLP Regulation.

Aquatic toxicity

Acute aquatic toxicity data are available for all three trophic levels. The most acutely sensitive trophic group is algae with a 48h E_rC_{50} value for *Skeletonema costatum* of 0.0052 mg/L. This acute endpoint is in the range of $0.001 < L(E)C_{50} \leq 0.01$ mg/L.

Chronic aquatic toxicity data are available for all three trophic levels. The most acutely sensitive trophic group is algae with a 48h NOE_rC value for *Skeletonema costatum* of 0.00049 mg/L. This chronic endpoint is in the range of $0.0001 < NOEC \leq 0.001$ mg/L.

Conclusion on the classification

C(M)IT/MIT is considered not rapidly degradable and does not fulfil the criteria for bioaccumulation potential. The lowest acute aquatic toxicity value falls in the range $0.001 < L(E)C_{50} \leq 0.01$ mg/L and the lowest chronic aquatic toxicity value lies in the toxicity range of $0.0001 < NOEC \leq 0.001$ mg/L.

RAC concluded that C(M)IT/MIT fulfils the CLP criteria for classification as **Aquatic Acute 1; H400** with an **M-factor of 100** and **Aquatic Chronic 1; H410** with an **M-factor of 100.**

6 ANNEX: ADDITIONAL INFORMATION ON C(M)IT/MIT DEGRADATION PRODUCTS

The major metabolites of C(M)IT/MIT have been identified and described in section 5.1.

These are the following biodegradation products:

- N-methyl malonamic acid (NMMA);
- 2-hydroxyethane sulfonic acid;
- 2-(methylcarbamoyl)-ethene sulfonic acid;
- 2-(methylcarbamoyl)-1-oxo-ethane sulfinic acid;
- 2-methyl-isothiazoline-3,5-dione;

It is currently assumed that 2-(methylcarbamoyl)-ethene sulfonic acid and 2-(methylcarbamoyl)-1-oxo-ethane sulfinic acid are probably in fact isomers.

Among the major metabolites of C(M)IT/MIT identified in simulation tests one of them has been tested in ecotoxicological tests: N-(methyl) malonamic acid (NMMA).

Two other ring-cleaved degradation products: N-methyl acetamide (NMA) and malonamic acid (MA) are also tested in aquatic ecotoxicological tests, although they are not found above 10% of the applied radioactivity in any simulation tests.

The results of these ecotoxicological tests (fish, daphnids, algae) are summarized in section 6.1 below.

For the other metabolites, which are not tested, the potential ecotoxicity is evaluated by QSARs. Results of the QSARs evaluations are summarized in section 6.2 below

6.1 C(M)IT/MIT metabolites aquatic toxicity: measured values

Rainbow trout, *Daphnia magna* and algal acute toxicity data are available for NMMA (N-methyl malonamic acid), NMA (N-methyl acetamide) and MA (malonamic acid). The results of the tests are summarized below (Table 6.1-1, Table 6.1-2 and Table 6.1-3).

NMMA is practically non-toxic to the rainbow trout (96-hour LC_{50} >1000 mg a.i./L; Madsen, 2002 a), practically non-toxic to *Daphnia magna* (48-hour LC_{50} >986 mg a.i./L; Rhodes, 2002 b), and slightly toxic to *Selenastrum* (96-hour E_rC_{50} = 128 mg a.i./L; Madsen, 2002 e).

NMA is practically non-toxic to the rainbow trout (96-hour LC_{50} >694 mg a.i./L; Rhodes, 2002 a), practically non-toxic to *Daphnia magna* (48-hour LC_{50} >863 mg a.i./L; Madsen, 2002 c), and moderately toxic to *Selenastrum* (72-hour E_rC_{50} 5.8 mg a.i./L; Rhodes, 2002 c).

MA is practically non-toxic to the rainbow trout (96-hour LC_{50} >1000 mg a.i./L; Madsen, 2002 b), practically non-toxic to *Daphnia magna* (48-hour LC_{50} >1000 mg a.i./L; study Madsen, 2002 d), and practically non-toxic to *Selenastrum* (96-hour E_rC_{50} >1080 mg a.i./L; study Madsen, 2002 f).

The three metabolites tested are therefore considered as less toxic than parent molecules.

Table 6.1-1 : Acute toxicity of NMMA, NMA and MA to fish

Method	Results mg/L	Remarks	Reference / Owner

Oncorhyn-chus mykiss – 96h OECD 203, US EPA OPPTS 850.1075, US EPA 797.1400, US EPA 72-1, and EC Council Directive 91/414/EC	LC_{50}>1000 (nc)	S Test substance : = N-methyl malonamic acid	Madsen, 2002 a/ Dow RI: 1
Oncorhyn-chus mykiss – 96h OECD 203, US EPA OPPTS 850.1075, US EPA 797.1400, US EPA 72-1, and EC Council Directive 91/414/EC	LC_{50}>694 (mmc)	S Test substance = N-methyl acetamide RI: 1	Rhodes, 2002 a / Dow RI: 1
Oncorhyn-chus mykiss – 96h OECD 203, US EPA OPPTS 850.1075, US EPA 797.1400, US EPA 72-1, and EC Council Directive 91/414/EC	LC_{50}>1000 (nc)	S Test substance = malonamic acid RI: 1	Madsen, 2002 b/ Dow RI: 1

S: Static; SS: Semi-static; F: Flow-through;
R1/R2 : reliability of the study
mmc: mean measured concentration; nc = nominal concentrations

Table 6.1-2 Acute toxicity of NMMA, NMA and MA to aquatic invertebrates

Method	Results mg/L	Remarks	Reference / Owner
Daphnia magna -48h OECD 202, US EPA OPPTS 850.1010, US EPA 797.1300, US EPA 72-2, and EC Council Directive 91/414/EC	EC_{50}>>863 (mmc)	S Test substance = N-methyl acetamide	Madsen, 2002 c/ Dow RI: 2
Daphnia magna -48h OECD 202, US EPA OPPTS 850.1010, US EPA 797.1300, US EPA 72-2, and EC Council Directive 91/414/EC	EC_{50}>>986 (mmc)	S Test substance : = N-methyl malonamic acid	Rhodes, 2002 b / Dow RI: 1
Daphnia magna -48h OECD 202, US EPA OPPTS 850.1010, US EPA 797.1300, US EPA 72-2, and EC Council Directive 91/414/EC	EC_{50}>1000 (nc)	S Test substance = malonamic acid	Madsen, 2002 d/ Dow RI: 1

S: Static; SS: Semi-static; F: Flow-through;
R1/R2 : reliability of the study
mmc: mean measured concentration; nc = nominal concentrations

Table 6.1-3 Acute toxicity of NMMA, NMA and MA to algae

Method	Results mg/L	Remarks	Reference / Owner

Selenastrum Capricornutum 96h OECD 201, US EPA OPPTS 850.5400	E_rC_{50} = 128 (nc) NOEC = 36 (nc)	S Test substance : = N-methyl malonamic acid	Madsen, 2002 e/ Dow RI:1
Selenastrum Capricornutum 72h OECD 201, US EPA OPPTS 850.5400	E_rC_{50} = 5.8 (nc) NOEC = 0.51 (nc)	S Test substance = N-methyl acetamide	Rhodes, 2002 c / Dow RI:2
Selenastrum Capricornutum 96h OECD Guideline 201, US EPA OPPTS 850.5400, US EPA TSCA 797.1050, US EPA FIFRA 122-2 and 123-2, EC Council Directive 67/548/EEC	E_rC_{50} > 1080 (mmc) NOEC = 519 (mmc)	S Test substance = malonamic acid	Madsen, 2002 f/ Dow RI:1

S: Static; SS: Semi-static; F: Flow-through;
R1/R2 : reliability of the study
mmc: mean measured concentration; nc = nominal concentrations

6.1.1 C(M)IT/MIT metabolites aquatic toxicity: calculated values (QSARs)

6.1.1.1 QSARs for key metabolites: NMMA, NMA and MA

Ready biodegradability tests and acute toxicity tests on the three trophic levels (fish, invertebrates and algae) are available for the three key metabolites (NMMA, NMA and MA) and have been summarized in sections 4.1.1.2.2 and 4.3.1 above. Quantitative Structure Activity Relationship (QSAR) modelling is employed to derive specific environmental fate parameters including water solubility, biodegradability, vapour pressure, Log Kow and Koc (as possible). Additionally, ecotoxicology parameters including fish 96-hour LC50 values, invertebrates 48-hour LC50s and algal 96-hour ErC_{50}s are also estimated. The USEPA's EPI Suite v 4.00 and ECOSAR models[2] are used for the QSAR analysis.

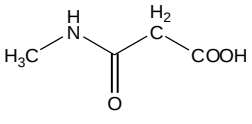
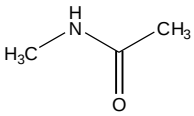
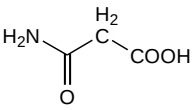
QSAR estimates for environmental fate and ecotoxicological parameters for the C(M)IT/MIT metabolites (NMMA, NMA and MA) are compared with measured data. The data presented in the table below illustrate that the measured and estimated ecotoxicity data for NMMA, NMA and MA are comparable within the accepted bounds of QSAR analyses; these 3 metabolites are several orders of magnitude less toxic than parent. Additionally, the results of the biodegradability studies for NMMA, NMA and MA agree with the QSAR estimates for biodegradability, i.e., that the compounds are readily biodegradable.

For NMMA, NMA and MA, the predicted and measured ecotoxicity endpoints for fish, aquatic invertebrates and algae are all indicate of very low toxicity to these organisms. While the actual estimated and measured L(E)C50 values are not statistically correlated, it is important to note that in the measured data, the toxicity endpoint is expressed as greater than the highest dose tested in virtually all cases, with two exceptions, that of the algal response to N-(methyl) malonamic and N-methyl acetamide. In that instance the measured 96-

[2] Meylan, W.M. and P.H. Howard. (1999a), User's Guide for EPIWIN, EPI suite: EPI-Estimation programs interface for Microsoft Windows. Syracuse Research Corporation, North Syracuse, NY. 33 pp. Software at http://www.syrres.com/esc/est_soft.htm.

hour E_rC50 value equals 128 mg/L and 5.8 mg/L respectively, a concentration orders of magnitude greater than that measured for the parent C(M)IT and/or MIT molecules.

Table 6.1-4:: QSAR Estimated Values for C(M)IT/MIT Metabolites

			
Chemical Name	N-methyl malonamic acid	N-methyl acetamide	malonamic acid
SMILES Notation	CNC(CC(O)=O)=O	CNC(C)=O	NC(CC(O)=O)=O
Environmental compartment(s)	Water	Water	Water
Molecular Weight	117.11	73.10	103.08
Vapor Pressure (Pa at 25°C)	0.01866	32.26	0.0329
Water Solubility(at 25°C, mg·L^{-1})	1E+06	1E+06	1E+06
K_H (at 25°C, Pa·m^3·mol^{-1})	1.85E-08	2.49E-03	8.43E-09
Log K_{OW}	-1.4419	-0.6962	-1.9078
Koc (L/kg)	1	3.5	1
BCF	3.162	3.162	3.162
Ready Biodegradability **Primary Biodegradation** **Ultimate Biodegradation**	Yes Hours-Days Days-Weeks (Yes)	Yes Days Weeks (Yes)	Yes Hours-Days Days-Weeks (Yes)
Fish 96-Hr LC50 (mg·L^{-1})	Amides Acid 7.95E+04 Neutral Organic 7.38E+04 (> 1000)	Amides 1.28E+03 Neutral Organic 1.08E+04 (> 694)	Amides 1.64E+05 Neutral Organic 1.61E+05 (> 1000)
Daphnid 48-Hr LC50 (mg·L^{-1})	Amides Acid 1.36E+04 Neutral Organic2.81E+04 > 986)	Amides 283.4 Neutral Organic 4.49E+03 (> 863)	Amides 2.39E+04 Neutral Organic 5.79E+04 (> 1000)
Green Algae 96-Hr EC50 (mg·L^{-1})	Amides Acid 23.41 Neutral Organic 2.95E+03 (E_rC50: 128)	Amides 0.91 Neutral Organic 639.6 (E_rC50: > 5.8)	Amides Acid 27.8 Neutral Organic 5.02E+03 (E_rC50:>1031.81)
Terrestrial organism(s)	No available QSAR	No available QSAR	No available QSAR

Values in parentheses reflect experimental data

ECOSAR -Amides : Fish n = 12 and R^2 = 0.9275. Daphnids n = 9 and R^2 = 0.7848. Green algae n = 3
ECOSAR -Neutral organic: Fish n = 388 and R^2 = 0.8753. Daphnids n = 152 and R^2 = 0.0.7712. Green algae n = 62 and R^2 = 0.0.5956

6.1.2 QSAR for 2-methyl-isothiazoline-3,5-dione, 2-(methylcarbamoyl)-1-oxo-ethane sulfinic acid, 2-(methylcarbamoyl)- ethene sulfonic acid and 2-hydroxyethane sulfonic acid

Initial QSAR studies are conducted to validate the QSAR model accuracy with regard to the isothiazolone chemistry class. QSAR estimates for environmental fate and ecotoxicologic parameters for 5-chloro-2-methyl-4-isothiazolin-3-one (C(M)IT) and 2-methyl-4-isothiazolin-3-one (MIT) are compared against measured data.

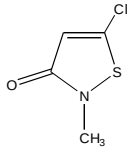
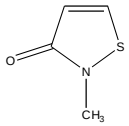
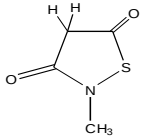
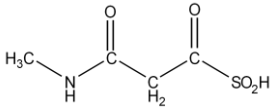
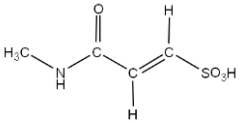
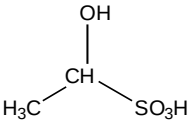
The estimated ecotoxicity values for C(M)IT and MIT are comparable to the measured toxicity endpoints in fish, aquatic invertebrates and algae. The QSAR is therefore considered predictive of the aquatic ecotoxicity of the isothiazolone chemistry class in general and especially for 2-methyl-isothiazoline-3,5-dione.

For these metabolites, 2-(methylcarbamoyl)-1-oxo-ethane sulfinic acid, 2-(methylcarbamoyl)- ethene sulfonic acid, 2-methyl-isothiazoline-3,5-dione and 2-hydroxyethane sulfonic acid, the QSARs indicate that the metabolites will not persist with half-lives ranging from “days” to “days-weeks”. The probability of rapid biodegradation indicated by the model is high, indicative of a significant potential for rapid degradation in the environment. The estimated degradation rates are considered somewhat conservative. For both C(M)IT and MIT QSAR estimates for primary degradation are days – weeks when measured DT_{50} values in water sediment and soil systems for both C(M)IT and MIT are generally in the hours range thereby supporting the indication that the QSAR estimates are indeed conservative. Likewise then it can be inferred that the degradation rates of the metabolites are similarly conservative and that the potential for persistence of the metabolites is negligible. This is confirmed by the transient nature of these metabolites, as observed in simulation tests.

The calculated L(E)C50 for the other 4 major metabolites which are not tested in ecotoxicological tests, indicate a low potential for toxic effects to aquatic organisms. The calculated L(E)C50 for this substances are orders of magnitude lower that the L(E)C50 calculated (and measured) for parent compounds.

For all the metabolites, the estimated Log Kow values are clearly indicative of a negligible potential for bioaccumulation. The estimated BCF values support the conclusion of a negligible potential for bioaccumulation

Table 6.1-5: QSAR Estimated Values for C(M)IT/MIT Metabolites

						
Chemical Name	5-Chloro-2-methyl-4-isothiazolin-3-one	2-Methyl-4-isothiazolin-3-one	2-methyl-isothiazoline-3,5-dione	2-(methylcarbamoyl)-1-oxo-ethane sulfinic acid	2-(methylcarbamoyl)-ethene sulfonic acid	2-hydroxyethane sulfonic acid
SMILES Notation	O=C1C=C(Cl)SN1C	O=C1C=CSN1C	O=C(N(C)S1)C([H])([H])C1=O	CNC(=O)CC(=O)(S(=O)=O)	CN([H])C(=O)\C([H])=C([H])\S(=O)(O)=O	CC(O)S(=O)(=O)(O)
Environmental compartment	Water Soil	Water Soil	Soil (18.4% at day 1, 9% at day 5)	Soil (C(M)IT degradation study: 30.2% at day 1, 6% at day 30; MIT degradation study: 21.4% at day 1, 2.7% at day 30)	Water (6.5% at day 3, 2.8% at day 30) Soil (29% at day 1, 3% at day 30)	Water (6.5% at day 3, 2.8% at day 30)
Molecular Weight	149.60	115.15	131.15	165.16	165.16	126.13
Vapor Pressure (Pa at 25ºC)	0.7199 (1.30)	4.133 (0.73)	0.171	1.44E-03	4.9E-06	0.0122
Water Solubility(at 25ºC, mg·L^{-1})	3.226E+05 (> 2000)	9.5876E+05 (>1000 g L^{-1})	1E+06	1E+06	1E+06	1E+06
K_H (at 25ºC, Pa·m^3·mol^{-1})	3.61E-03	5.02E-03	2.26E-04	5.57E-08	7.51E-10	6.19E-08
Log K_{OW}	-0.34 (0.401)	-0.8323 (-0.486)	-1.2329	-3.31	-3.63	-3.43
Koc	19.38 (30 – 310)	12.08 (K_f in sludge: 6.12)	1	10	1	1
BCF	3.162 (11 – 51)	3.162 (2.32)	3.162	3.162	3.162	3.162
Ready Biodegradability ***Primary Biodegradation*** ***Ultimate Biodegradation***	No Days-Weeks Weeks-Months (Not ready biodegradable although some biodegradation occurs)	No Days - Weeks Weeks (Biodegradable, failing the 10-day window)	No Days -Weeks Weeks	No Days-Weeks Weeks	No Days Weeks	No Days Days-Weeks

Fish 96-Hr LC50 (mg·L^{-1})	Isothiazolones 2.76 Amides 1.37E+03. Neutral Organic 1.10E+04 (0.09-0.28)	Isothiazolones 3.79 Amides 2.58E+03. Neutral Organic 2.21E+04 (–0.09-0.28)	Amides 6.09E+03 Neutral organic 5.50E+04	Amides 3.34E+05 Neutral organic 3.96E+06	Acrylamides acid 5.63+04 Amides Acid 6.08E+06 Neutral organic 7.45E+06	Neutral Organic acid 5.82E+07
Daphnid 48-Hr LC50 (mg·L^{-1})	Isothiazolones 3.49 Amides 342.87 Neutral Organic 4.79E+03 (0.10-0.16)	Isothiazolones 4.67 Amides 545.74. Neutral Organic 9.06E+03 0.10-0.16)	Amides 1.22E+03 Neutral organic 2.15E+04	Amides 3.01E+04 Neutral organic 1.19E+04	Acrylamides acid 7.47E+04 Amides Acid 4.89E+05 Neutral organic 2.18E+06	Neutral Organic acid 1.47E+07
Green Algae 96-Hr EC50 (mg·L^{-1})	Isothiazolones 0.37 Amides 1.48 Neutral Organic 790.11 (E_rC50: 10.7-53.5 µg/L)	Isothiazolones 0.326 Amides 1.56. Neutral Organic 1.22E+03 (E_rC50: 10.7-53.5µg/L)	Amides 2.29 Neutral organic 2.46E+03 (E_rC50:>1080)	Amides 10.9 Neutral organic 5.50E+04	Amides Acid 134.75 Neutral organic 9.30E+04	Neutral Organic acid 6.60E+05
Terrestrial organism(s) Earthworm 14d LC50	No available QSAR		No available QSAR	No available QSAR	No available QSAR	Neutral Organic acid 8.02E+04

Values in parentheses reflect measured data for C(M)IT/ MIT.
ECOSAR -Isothiazolones Fish n = 4 and R^2 = 0.8634. Daphnids n = 2. Green algae n = 2
ECOSAR -Amides : Fish n = 12 and R^2 = 0.9275. Daphnids n = 9 and R^2 = 0.7848. Green algae n = 3
ECOSAR -Neutral organic: Fish n = 388 and R^2 = 0.8753. Daphnids n = 152 and R^2 = 0.0.7712. Green algae n = 62 and R^2 = 0.0.5956
ECOSAR -Acrylamids Fish n = 8 and R^2 = 0.983. Daphnids n = 3 and R^2 = 0.9932.

6.1.3 Conclusion on C(M)IT/MIT degradation products

According to the TGD reduced lipophilicity may be one indication that the metabolites are less harmful than the parent compound. Preliminary information on toxicity are obtained with the help of measured Kow values and QSAR predictions for postulated and identified metabolites.

Based on actual tests or calculations, the major metabolites of C(M)IT/MIT are expected to be of low toxicity to aquatic organisms and by several orders of magnitude less toxic than parent compounds. Additionally, the major metabolites are anticipated to be quickly biodegraded in the environment and will not likely bioaccumulate. Based on this lack of persistence, low potential for bioaccumulation and the low toxicity, it is concluded that the potential for adverse environmental effects in response to exposures to the C(M)IT/MIT metabolites is considered negligible..

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