Committee for Risk Assessment

RAC

Annex 1

Background document

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

octhilinone (ISO); 2-octyl-2*H*-isothiazol-3-one; [OIT]

EC Number: 247-761-7
CAS Number: 26530-20-1

CLH-O-0000001412-86-255/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

30 November 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: octhilinone (ISO); 2-octyl-2*H*-isothiazol-3-one; [OIT]

EC Number: 247-761-7

CAS Number: 26530-20-1

Index Number: 613-112-00-5

Contact details for dossier submitter:

UK Competent Authority

Chemicals Regulation Division

Health and Safety Executive

United Kingdom

Version number: **2** **Date:** **January 2018**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***octhilinone (ISO); 2-octyl-2H-isothiazol-3-one; [OIT]***
EC number:	***247-761-7***
CAS number:	***26530-20-1***
Annex VI Index number:	***613-112-00-5***
Degree of purity:	***≥ 96 %***
Impurities:	***Confidential – Please refer to the technical dossier*** ***None that impact on the proposed classification***

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. 4* - H302: Harmful if swallowed Acute Tox. 3* - H311: Toxic in contact with skin Acute Tox. 3* - 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 (c ≥ 0.05 %) Aquatic Acute 1 - H400: Very toxic to aquatic life Aquatic Chronic 1 - H410: Very toxic to aquatic life with long lasting effects.
Current proposal for consideration by RAC	Acute Tox. 3 - H301: Toxic if swallowed Acute Tox. 3 - H311: Toxic in contact with skin Acute Tox. 2 - H330: Fatal if inhaled EUH071: Corrosive to the respiratory tract Skin Corr. 1B - H314: Causes severe skin burns and eye damage Eye Dam. 1 – H318 : Causes serious eye damage Skin Sens. 1A - H317: May cause an allergic skin reaction (C ≥ 0.005 %) Aquatic Acute 1 - H400: Very toxic to aquatic life Acute M factor = 100 Aquatic Chronic 1 - H410: Very toxic to aquatic life with long lasting effects Chronic M factor = 10

Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 3 - H301: Toxic if swallowed Acute Tox. 3 - H311: Toxic in contact with skin Acute Tox. 2 - H330: Fatal if inhaled EUH071: Corrosive to the respiratory tract Skin Corr. 1B - H314: Causes severe skin burns and eye damage Eye Dam.1 – H318 : Causes serious eye damage Skin Sens. 1A - H317: May cause an allergic skin reaction (C ≥ 0.005 %) Aquatic Acute 1 - H400: Very toxic to aquatic life Acute M factor = 100 Aquatic Chronic 1 - H410: Very toxic to aquatic life with long lasting effects Chronic M factor = 10

Proposed harmonised classification and labelling

Table 3: Proposed classification

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON OCTHILINONE (ISO); 2-OCTYL-2H-ISOTHIAZOL-3-ONE; [OIT]

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	Not classified	Not applicable	Not classified	Not considered in this proposal
2.2.	Flammable gases	Not classified	Not applicable	Not classified	Not considered in this proposal
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	Not considered in this proposal
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	Not considered in this proposal
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	Not considered in this proposal
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	Not considered in this proposal
2.7.	Flammable solids	Not classified	Not applicable	Not classified	Not considered in this proposal
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	Not considered in this proposal
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	Not considered in this proposal
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	Not considered in this proposal
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	Not considered in this proposal
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	Not considered in this proposal
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	Not considered in this proposal
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	Not considered in this proposal
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	Not considered in this proposal
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	Not considered in this proposal
3.1.	Acute toxicity - oral	**Acute Tox. 3 - H301: Toxic if swallowed**	Not applicable	**Acute Tox. 4*; H302: Toxic if swallowed**	-

	Acute toxicity - dermal	**Acute Tox. 3 - H311: Toxic in contact with skin**	Not applicable	**Acute Tox. 3*; H311: Toxic in contact with skin**	-
	Acute toxicity - inhalation	**Acute Tox. 2 - H330: Fatal if inhaled**	Not applicable	**Acute Tox. 3*; H331: Toxic if inhaled**	-
3.2.	Skin corrosion / irritation	**Skin Corr. 1B - H314: Causes severe skin burns and eye damage**	Not applicable	**Skin Corr. 1B - H314: Causes severe skin burns and eye damage**	-
3.3.	Serious eye damage / eye irritation	**Eye Dam. 1 – H318: Causes serious eye damage**	Not applicable	**Skin Corr. 1B - H314: Causes severe skin burns and eye damage**	-
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	**Skin Sens. 1A - H317: May cause an allergic skin reaction**	**C ≥ 0.005 %**	**Skin Sens. 1; H317: May cause an allergic skin reaction (C ≥ 0.05 %)**	
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	Not considered in this proposal
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	Not considered in this proposal
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	Not considered in this proposal
3.8.	Specific target organ toxicity – single exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	Not considered in this proposal
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	Not considered in this proposal
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1 - H400: Very toxic to aquatic life** **Aquatic Chronic 1 - H410: Very toxic to aquatic life with long lasting effects.**	**M = 100** **M = 10**	**Aquatic Acute 1 - H400: Very toxic to aquatic life** **Aquatic Chronic 1 - H410: Very toxic to aquatic life with long lasting effects.**	
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	Not considered in this proposal

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

Labelling:

Pictogram(s):
GHS05, GHS06, GHS09

Signal word:
Danger

Hazard statements:
H301 + H311: Toxic if swallowed or in contact with skin
H330: Fatal if inhaled
H314: Causes severe skin burns and eye damage
H317: May cause an allergic skin reaction
H410: Very toxic to aquatic life with long lasting effects

EUH071: Corrosive to the respiratory tract

Precautionary statements: Not included in Annex VI

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

OIT is a biocidal active substance. It was originally included in Annex I to Directive 67/548/EEC following a proposal submitted by the Danish Toxicology Centre In March 1993. The harmonised classification in table 3.1 of Annex VI of CLP (See section 2.3.1) was translated from the existing classification. This current proposal includes a reassessment of studies used at that time and also includes more recent studies carried out after the preparation of the original C&L document.

At the date of submission the substance is not registered under REACH.

2.2 Short summary of the scientific justification for the CLH proposal

OIT is a biocidal active substance with an existing entry in Annex VI of CLP (see section 2.3.1). As a result of the active substance review, it is proposed to update the existing entry in Annex VI as follows.

The substance is currently classified for acute toxicity via the oral dermal and inhalation routes. However, the existing classifications have been translated as minimum classification from the DSD equivalent. Given the available ATE's, this proposal seeks to confirm the acute dermal classification and to update the classification for acute oral and inhalation toxicity.

OIT is currently classified as Skin Corr 1B. This proposal seeks to confirm this and to add supplemental labelling EUH071: Corrosive to the respiratory tract given the nature of the effects observed.

OIT is currently classified as a skin sensitiser in Category 1, with a specific concentration limit of 0.05%. This proposal seeks to update this classification to Skin Sens Category 1A and propose a new SCL of 0.005% based on the available human data.

Finally, the substance is currently classified with Aquatic Acute 1: H400 and Aquatic Chronic 1: H410. This proposal seeks to confirm this classification and to add M factors of 100 (acute) and 10 (chronic).

2.3 Current harmonised classification and labelling

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

Classification		Labelling		Specific Concentration Limits
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Pictograms, Signal Word Code(s)**	
Acute Tox. 4*	H302	H302	GHS06	
Acute Tox. 3*	H311	H311	GHS09	
Acute Tox. 3*	H331	H331	GHS05	
Skin Corr. 1B	H314	H314	Dgr	
Skin Sens. 1	H317	H317		Skin Sens. 1: C ≥ 0.05 %
Aquatic Acute 1	H400			
Aquatic Chronic 1	H410	H410		

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling

There are a large number of self-classification entries listed in the classification and labelling inventory. Specifically, these show variations in the acute toxicity classification with oral toxicity varying between categories 3 (H301) and 4 (H302) and dermal and inhalation toxicity varying between categories 2 (H311 and H330 respectively) and 3 (H310 and H331 respectively). The addition of eye damage 1 (H318) is also included by a number of notifiers. In terms of specific concentration limits for skin sensitisation, many notifiers have given a concentration of $C \geq 0.05$ %.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

OIT is a biocidal active substance. In accordance with Article 36(2) of the CLP regulation, OIT should be subject to harmonised classification. As OIT already has a harmonised entry in Annex VI, this proposal seeks only to confirm or update the acute toxicity, skin corrosion and environmental classifications and to update the classification and specific concentration limit for skin sensitisation. Addition of the supplemental phrase EUH071 (corrosion to the respiratory tract) and the addition of M-factors should also be considered.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

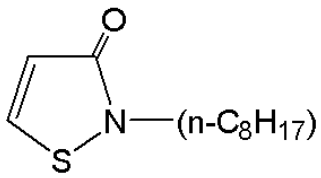
1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	247-761-7
EC name:	2-octyl-2H-isothiazol-3-one octhilinone (ISO)*
CAS number (EC inventory):	26530-20-1
CAS number:	26530-20-1
CAS name:	3(2*H*)-isothiazolone, 2-octyl-
IUPAC name:	2-octyl-1,2-thiazol-3(2*H*)-one
CLP Annex VI Index number:	613-112-00-5
Molecular formula:	$C_{11}H_{19}NOS$
Molecular weight range:	213.3 g/mol

* Note; the applicant advises that the common name octhilinon is not in common use and the substance should be referred to be the IUPAC name and abbreviation OIT.

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
OIT	≥ 96 %	≥ 96 %	< 99 %

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential			

There are a number of process impurities in this substance. These have been taken into consideration and are not considered to impact on the classification proposed in this dossier. Further information on the impurities is considered to be confidential but full details are provided in the IUCLID.

Two of the impurities have an existing entry in Annex VI of CLP. The classification of these substances has been taken into account and, given the concentrations at which they can be present and the information available on OIT, is not considered to impact on the proposed classification. Full information is provided in the IUCLID.

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

The test material is considered to be equivalent to that described above.

1.3 Physico-chemical properties

The physico-chemical properties of OIT are summarised below. Reference should be made to the UK Competent Authority Report – CAR – 2016 - Document IIIA – Section A3.

All studies were conducted to appropriate quality standards and were considered adequate during the peer review.

Table 8: 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	Yellow liquid	MSDS	Visual Inspection Purity: Not stated CAR (A3.3.1)
Melting/freezing point	21.4 °C	Tognucci A (2003)	EEC method A1 (DSC) Purity: 96.8 % CAR (A3.1.1)
Boiling point	No boiling point could be determined as decomposition started at 267 °C	Tognucci A (2002a)	EEC method A2 Purity: > 99 % CAR (A3.1.2)
Relative density	1.04	Seal K (2002a)	EEC method A3(pycnometer) Purity: > 99 % CAR (A3.1.3)
Vapour pressure	$3.1x10^{-3}$ Pa (20 °C) $6.1x10^{-3}$ Pa (25 °C)	Tognucci A (2002b)	EEC method A4 (gas saturation method) Purity: > 99 % CAR (A3.2)
Surface tension	35.97 mN/m at 20.1 °C surface active	Paulus J (2007c)	EEC method A5 (90 % saturation concentration) Purity: > 98 % CAR (A3.13)
Water solubility	pH 5: 456 mg/L at 10 °C 406 mg/L at 20 °C 394 mg/L at 30 °C pH 7: 451 mg/L at 10 °C 406 mg/L at 20 °C 395 mg/L at 30 °C pH 9: 483 mg/L at 10°C 433 mg/L at 20°C 448 mg/L at 30°C	Geffke T (2003a)	EEC method A6 (flask method) Purity: > 99 % CAR (A3.5)

Partition coefficient n-octanol/water	2.95, 2.92 and 2.93 at pH 7 and 10, 20 and 30 °C respectively 2.92, 2.92 and 2.94 at 20°C and pH 5, 7 and 9 respectively ----------	Seal K (2002b) ----------	EEC method A8 (HPLC) Purity: >99% CAR (A3.9) ----------
	2.5 (20°C) ----------	Tognucci A (1998) ----------	EEC method A8 (HPLC) Purity: 99.7% CAR (A3.9) ----------
	$LogP_{O/W}$ > 3.1	Kühne M (2010k)	Estimation (by calculation using the water and n-octanol solubilities at 20 °C) Purity: 97.6 % CAR (A3.9)
Flash point	156.5 °C	Paulus J (2007b)	EEC method A9 (pensky-Martens) Purity: 99.4 % CAR (A3.12)
Flammability	Based on the chemical structure of OIT and on experience in handling and use, it can be concluded that OIT is not flammable in contact with water and has no pyrophoric properties	-	CAR (A3.11)
Explosive properties	OIT contains no structural alerts with explosive properties.	-	CAR (A3.15)
Self-ignition temperature	Auto-ignition temperature of 330 °C	Paulus J (2007a)	EEC method A15 Purity: not specified CAR (A3.11)
Oxidising properties	OIT contains no structural alerts with oxidising properties.	-	CAR (A3.16)
Dissociation constant	5.2 to 6.0×10^{-4} mol/L in diluted aqueous solution	Werle H (1993a)	OECD method 112 (conductometric method) Purity: 90 % CAR (A3.6)
Viscosity	Dynamic viscosity: 78.95 mPa s (20 °C) Kinematic viscosity: 76.48 mm^2/s (20 °C) 29.02 mm^2/s (40 °C)	Rueb B (1995)	OECD method 114 (capillary method) Purity: > 95 % CAR (A3.14)

2 MANUFACTURE AND USES

2.1 Manufacture

The active substance, OIT, is manufactured within the EU.

2.2 Identified uses

OIT has a number of biocidal uses as a preservative, including an in-can preservative for non-food stuffs (product type 6) and a preservative for metalworking fluids (product type 13). It is currently under evaluation for use as a wood preservative (product type 8).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

3.1 Physico-chemical properties

No classification is proposed for physico-chemical properties (see Table 8). As the substance already has an existing entry in Annex VI of CLP this is not considered further in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

References are taken from the Competent Authority Report – CAR – 2016 - Doc IIA, Section 3 and DOC IIIA, Section A6.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Two guideline oral studies in Wistar rats are available (Anonymous (2007a), Anonymous (2008)), both carried out to GLP standards. Also available are three dermal absorption studies in Wistar rats (Anonymous (2006a), guideline, GLP), guinea pigs (Anonymous (1990a), non-guideline, non-GLP) and Sprague-Dawley rats (Anonymous (1991a), similar to guidelines, GLP).

The results of these studies showed that OIT is extensively (up to 70 %) and rapidly absorbed following either single or repeated exposure. Absorption by the dermal route is less extensive, with approximately 40 % of a non-irritant concentration being absorbed. Irritant concentrations were not tested.

Absorbed OIT is widely distributed throughout the body and metabolism is complete, both systemically and by the gastro-intestinal tract with cleavage of the sulphur-nitrogen bond to open up the isothiazolone ring.

Both biliary and urinary elimination were significant with almost complete elimination by 96 h. OIT and its metabolites were shown to have limited potential for bioaccumulation on repeated exposure.

4.1.2 Human information

There are no human data available.

4.1.3 Summary and discussion on toxicokinetics

Please refer to section 4.1.1.

4.2 Acute toxicity

This CLH proposal seeks to confirm and add to the classification and labelling proposal submitted by the Danish Toxicology Centre in March 1993 ("CLH Dossier 1993"), which was subsequently included in Annex I of DSD. This current proposal includes a reassessment of studies used at that time and also includes more recent studies carried out after the preparation of the original C&L document.

Table 9: Summary table of relevant acute toxicity studies

Acute Oral		
Method	**LD_{50}**	**Observations and remarks**
Rat, Charles River CD (10/sex/group) Test substance (OIT dilutions made in propylene glycol) 0, 300, 500, 800, 1200, 2000 mg/kg bw Equivalent to 0, 126, 210, 336, 504 and 840 mg OIT/kg bw (dilutions made in propylene glycol) OECD 401 GLP Test Material: OIT 42 – 46.7 % in propylene glycol Anonymous (1987)	Males: 318 mg **OIT**/kg* Females: 324 mg **OIT**/kg*	*Clinical signs and necropsy:* General signs of toxicity were noted. Redness of the stomach/intestinal mucosa and/or yellow/white-fluid filled stomachs or intestines in decedents. *CLH Dossier 1993 stated LD_{50} to be 760 mg/kg (males) and 767 mg/kg (females). However, these values were for the overall test material (a product containing 42-46.7% OIT) and were not amended to reflect the concentration of the active substance itself.
Rat, CF Nelson Albino (10/sex/group) *Females* Test substance (OIT dilutions made in propylene glycol): 0, 157, 313, 625 and 1250 mg/kg bw Equivalent to: 0, 71, 141, 281 and 563 mg **OIT**/kg bw *Males* Test substance (OIT dilutions made in propylene glycol): 0, 313, 625, 1250 and 2500 mg/kg bw Equivalent to: 0, 141, 281, 563 and 1125 mg **OIT**/kg bw Non-guideline Not GLP Test Material: OIT 42 – 46.7 % in propylene glycol Anonymous (1977) – original CLH report 1993	Males: 247 mg **OIT**/kg* Females: 292 mg **OIT**/kg*	*Clinical signs and necropsy:* insufficient data available. *CLH Dossier 1993 stated LD_{50} to be 550 mg/kg (males) and 650 mg/kg (females). However, these values were for the overall test material (a product containing 42-46.7% OIT) and were not amended to reflect the concentration of the active substance itself.

Rat, Sprague-Dawley Preliminary study: Test substance: 2500 mg/kg bw/day Equivalent to 1125 mg **OIT**/kg bw, 2/sex Main study: Test substance: 0, 200, 400, 1000 mg/kg bw/day Equivalent to 0, 90, 180, 450 mg **OIT**/kg bw, 5/sex/group (dilutions made in propylene glycol) EPA 81-1 (c.f. OECD 401) GLP Test Material: OIT 45 % in propylene glycol Anonymous (1991b)	125 mg **OIT**/kg	*Clinical signs and necropsy:* signs of general toxicity (no findings at gross necropsy).
Rat, Wistar (3/sex/200 and 500 mg/kg groups and 3 males/2000 mg/kg group) 0, 200, 500, 2000 mg/kg bw Vehicle: water OECD 423 GLP Test Material: OIT 96.4 % Anonymous (2002a)	500 – 2000 mg **OIT**/kg	*Clinical signs and necropsy:* Lesions in the lungs, mottling of the liver, bleeds to the stomach and white foci of the spleen and congestion of the kidneys.
Acute Inhalation		
Method	**LC_{50}**	**Observations and remarks**
Rats, Sprague Dawley (10/sex/group) Test substance (OIT in propylene glycol): 0, 0.125, 0.203, 0.490 and 1.437 mg/L Equivalent to 0, 0.058, 0.095, 0.229 and 0.671 mg **OIT**/L MMAD = 1.7 – 2.6 µm Nose only inhalation, aerosol (4 h) OECD 403 GLP Test Material: OIT 42 – 46.7 % in propylene glycol Anonymous (1986)	0.58 mg **OIT**/L*	*Clinical signs and necropsy:* Signs of sensory and upper airway irritation (dyspnoea, bradypnea, rales and gasping), central nervous system depression (ataxia, listlessness and prostration) secondary to respiratory distress, nasal mucosal irritation and reductions in body weight. Red and/or brown foci on lungs, brown areas on lungs and oedematous tongue *CLH Dossier 1993 stated LC_{50} to be 1.254 mg/L. However, these values were for the overall test material (a product containing 42-46.7% OIT) and were not amended to reflect the concentration of the active substance itself.
Rats, Sprague-Dawley (5/sex/group) Test substance: OIT 45% in propylene glycol 0, 0.256, 0.498 and 0.734 mg/L	0.27 mg **OIT**/L	*Clinical signs and necropsy: (decedents)* congestion of the lungs, gas-filled stomachs, some incidences of increase lung-body weight ratios.

Equivalent to 0, 0.115, 0.224, 0.330 mg **OIT**/L MMAD = 1.58 µm Whole-body, aerosol (4 h) OECD 403 GLP Test Material: OIT 45 % in propylene glycol Anonymous (1992)		

Acute Dermal		
Method	**LD_{50}**	**Observations and remarks**
Rabbits, Albino (5/males/group) Test substance: 0, 324, 646, 1293, 2585 and 5170 mg/kg bw (OIT dilutions made in propylene glycol) Equivalent to 146, 291, 582, 1163 and 2326 mg **OIT**/kg 24 h exposure (semi-occlusive) Pre-guidelines (c.f. OECD 402) Pre-GLP Test Material: OIT 42 – 46.7 % in propylene glycol Anonymous (2004a) (Study date 1977)	311 mg **OIT**/kg*	*Clinical signs and necropsy:* Signs of corrosion to the local area of skin. Irregular shaped spleens in surviving animals. *CLH Dossier 1993 stated LD_{50} to be 690 mg/kg. However, this value was for the overall test material (a product containing 42-46.7% OIT) and was not amended to reflect the concentration of the active substance itself.
Rats, Sprague-Dawley (5/sex) Test substance: OIT dilution made in propylene glycol, 2000 mg/kg bw Equivalent to 900 mg **OIT**/kg (dilution made in propylene glycol) 24 h exposure (occlusive) EPA Fifra 81-2 (c.f. OECD 402) GLP	> 900 mg **OIT**/kg	*Clinical signs and necropsy:* Severe damage to the local area of skin.

Test Material: OIT 45 % in propylene glycol Anonymous (1991c)		

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

There are three guideline studies and one non-guideline study available, investigating acute oral toxicity of OIT in rats. Two of these studies (Anonymous (1987) and Anonymous (1977)) were used to derive the harmonised classification of OIT in 1993 (Danish Toxicology Centre, March 1993) and the other two (Anonymous (2002a) and Anonymous (1991b)) provide additional information.

In the first study (Anonymous (1987)), male and female rats were treated by oral gavage with OIT (42-46.7 %) in propylene glycol at dose levels of 0, 126, 210, 336, 504 and 840 mg OIT/kg bw. Most animals treated with doses ≥ 210 mg/kg bw died on days 0-2. Clinical signs observed were CNS depression, distended stomachs, pale extremities, respiratory noise, cool-to-touch, lacrimation, scant droppings, diarrhoea, red-stained muzzle and stained anogenital area. Gross necropsy revealed the presence of redness of the stomach and intestinal mucosa and/or yellow or white fluid-filled stomach or intestines in the decedents.

The LD_{50} values reported were 318 mg OIT/kg bw (males) and 324 mg OIT/kg bw (females). The original proposal for classification and labelling reported these to be 760 and 767 mg/kg bw (males and females respectively), however it was later found that the values were not dose-corrected to the concentration of OIT itself; rather they had been calculated as though the test material had been 100% OIT instead of 42-46.7%.

Supporting evidence was provided by a second non-guideline study in rats (Anonymous (1977)) which reported an LD_{50} value of 247 mg OIT/kg bw (males) and 292 mg/kg (females). Similarly to the Anonymous, 1987 study, the LD_{50} values for this study were originally considered to be higher at 550 and 650 mg/kg bw for males and females respectively, however the concentrations of test material had also not been dose-corrected to that of OIT itself. The data provided for this study was considered insufficient to make a true assessment of its quality.

A third study was carried out in rats (Anonymous (1991b)). OIT (45 %) in propylene glycol was administered to male and females rats at dose levels of 0, 90, 180 and 450 mg OIT/kg bw. The result of the main study was an LD_{50} value of 125 mg OIT/kg bw for both males and females. Clinical signs included CNS depression, decreased respiratory rate, pallor of the extremities and piloerection. There was no abnormal findings during gross necropsy of the decedents/sacrificed.

A fourth, more recent, study (Anonymous (2002a)) investigated oral toxicity in rats after administration of OIT (96.4 %) in water at dose levels of 0, 200, 500 mg/kg bw in male and females and in males only at 2000 mg/kg bw. The LD_{50} was found to be greater than 500 mg/kg bw but less than 2000 mg/kg bw. Gross necropsy revealed lesions in the lungs, mottling of the liver, bleeds to the stomach, white foci of the spleen and congestion of the kidneys.

4.2.1.2 Acute toxicity: inhalation

Two guideline studies in rats are available for the assessment of acute inhalation toxicity following treatment with OIT. One of these studies (Anonymous (1986)) was used for the 1993 harmonised

classification proposal for acute inhalation toxicity (Danish Toxicology Centre, March 1993) and the second has been made available since then (Anonymous (1992)).

In the first study rats were exposed, nose-only, to an aerosol of OIT (42–46.7 %) in propylene glycol for 4 hours (Anonymous (1986)). Concentrations administered were 0.058, 0.095, 0.229 and 0.671 mg OIT/L (no control was mentioned in the Competent Authority Report). The resulting LC_{50} was 0.58 mg OIT/L. In the CLH Dossier 1993 this was reported to be 1.254 mg/L, however the value given was not dose-corrected to OIT. Clinical signs included signs of sensory and upper respiratory tract irritation (dyspnoea, bradypnoea, râles and gasping), CNS depression (ataxia, listlessness and prostration) which was considered secondary to respiratory distress and nasal mucosal irritation. Pathology of the decedents revealed red/brown foci and brown areas on the lungs and oedematous tongues.

A more recent inhalation study was performed in rats with OIT (45 %) in propylene glycol using whole-body exposure (Anonymous, 1992). Rats were exposed to 0 (air control), 0.115, 0.224 and 0.330 mg/L for a period of 4 h. The combined LC_{50} for males and females was determined to be 0.27 mg OIT/L, with most deaths occurring in the first 24 h period following exposure. Clinical signs included gasping, disturbed respiration (noisy and exaggerated), immobility and staining of the fur. Gross necropsy of the decedents revealed congestion of the lungs, gas-filled stomachs (thought to be caused by swallowing of air during attempts to breathe) and some incidences of increased lung weight (relative to body weight). There were no treatment-related abnormalities in surviving animals.

4.2.1.3 Acute toxicity: dermal

Two studies are available for acute dermal toxicity, one in rabbits and one in rats. Both studies used methods similar to that of OECD 402. The first study (Anonymous (2004a) – study date 1997) was carried out in 1977, pre-dating GLP, and was used for the 1993 harmonised classification proposal of OIT (Danish Toxicology Centre, March 1993). The second study, performed more recently, was carried out to GLP standards (Anonymous (1991c)).

In the first study (Anonymous (2004a)), OIT (42 – 46.7 %) in propylene glycol was applied to the shaved, intact skin of male albino rabbits at dose levels of 146, 291, 582, 1163 and 2326 mg OIT/kg bw for 24 h under semi-occlusive conditions (5 males/group). All animals treated with doses of ≥ 582 mg/kg bw died. Clinical signs were observed in animals treated with ≥ 291 mg/kg bw, these included lethargy, prostration, ataxia and partial paresis of hind limbs. During the experiment there were signs of corrosion to the local treatment area such as severe erythema and oedema, followed by eschar formation which preceded death. Necropsy of surviving animals at 291 mg/kg bw revealed irregular-shaped spleens; the significance of which was uncertain. The dermal LC_{50} was determined to be 311 mg OIT/kg bw. In the CLH Dossier 1993, this was given as 690 mg/kg bw, however this value was calculated as though the test material was 100% OIT, rather than being dose-corrected for OIT.

In the second dermal study (Anonymous (1991c)), a single dose of 900 mg/kg bw of OIT (45 %) in propylene glycol was applied to the shaven, intact skin of male and female rabbits (5/sex) for 24 h under occlusive conditions. There were no deaths, clinical signs of systemic toxicity or macroscopic abnormalities at necropsy. Oedema (slight to well-defined) was observed at application sites on day 2. Localised severe damage to the skin, associated with oedema (severe) and scabbing developed over the next few days. The magnitude of these responses prevented an assessment of the erythema and oedema; however there was evidence of skin healing on day 10 (post-application). Therefore, the dermal LC_{50} was considered to be > 900 mg/kg bw.

4.2.1.4 Acute toxicity: other routes

There are no acute toxicity data pertaining to other routes of administration to those already presented.

4.2.2 Human information

There is no relevant human information.

4.2.3 Summary and discussion of acute toxicity

Acute oral toxicity studies were performed in rats. The LD_{50}s ranged from 125 mg OIT/kg bw to > 500 - < 2000 mg OIT/kg bw and clinical signs included signs of general toxicity, CNS depression and distended stomachs. Gross necropsy revealed irritation/corrosion in the stomach or intestines and in some cases, lesions of the lungs and congestion of the kidneys.

OIT was tested for acute toxicity via the dermal route in two studies. In the first study (Anonymous (2004a) – study date 1977), the LD_{50} was determined to be 311 mg OIT/kg bw in rabbits. Clinical signs included lethargy, prostration, ataxia and partial paresis of the hind limbs. The second study in rats (Anonymous (1991c)) tested a single dose of 900 mg/kg, but there was no mortality. Severe skin corrosion at the application site was noted in both studies. Both the rat and rabbit are preferred species for evaluating acute toxicity via the dermal route and whilst, the earlier study was pre-GLP and pre-guideline, it has been considered to be of adequate quality for classification. As such, there do not appear to be any reasons to dismiss the results of the earlier study. Consequently, the LD_{50} of 311 mg OIT/kg bw is considered relevant for classification purposes.

Two acute inhalation toxicity studies were performed in rats, both following guidelines and carried out to GLP. The LC_{50} values were 0.58 mg OIT/L in a nose-only study (Anonymous (1986)) and 0.27 mg OIT/L in a whole-body study (Anonymous (1992)). Following inhalation exposure, clinical signs included dyspnoea, bradypnoea, râles and gasping. CNS depression, which was considered to be secondary to respiratory distress and nasal mucosa irritation were also noted. Pathology of decedents in the nose-only study revealed red/brown foci on the lungs and oedematous tongues. In the whole-body study, necropsy revealed congestion of the lungs, increased lung weight (relative to body weight) and gas-filled stomachs which was thought to be caused by animals swallowing air during attempts to breathe.

Whilst the possibility of additional exposure from the grooming of contaminated fur cannot be ruled out in the whole body study, it is not clear whether this accounts entirely for the discrepancy in the LC_{50} values identified in the two studies. In the whole body study, it is noted that necropsy revealed gas-filled stomach in the decedents; the study summary notes that this finding is often seen in rats that die as a result of respiratory distress and is due to swallowing air during attempts to breath. As such, it is proposed that the LC_{50} of 0.27 mg/L cannot be dismissed for classification purposes.

OIT is known to be corrosive to skin and eyes. In two separate skin irritation studies OIT was shown to cause necrosis in all animals tested (Section 4.4). Chemical burns and irreversible destruction of the dermal tissue occurred within one hour of removal of the dressings and effects were still apparent at the end of the treatment period. Given the nature of the clinical signs observed following inhalation exposure, it is likely that the mechanism of toxicity is, at least in part, due to corrosion of the respiratory tract.

4.2.4 Comparison with criteria

The lowest oral LD_{50} value in rats was found to be 125 mg OIT/kg bw for males and females. It is noted that this was from a study conducted on OIT formulated in propylene glycol and the LD50 in the study conducted on neat OIT was higher. However, it is considered that the results from the studies with OIT in propylene glycol cannot be dismissed and are therefore considered relevant for classification. The criteria for classification with acute oral toxicity category 3 are $50 < LD_{50} \leq 300$. Therefore, it is proposed that OIT should be classified with Acute Tox 3 via the oral route.

The lowest dermal LD_{50} value in rats was 311 mg OIT/kg bw. The criteria for classification with acute dermal toxicity category 3 are $200 < LD_{50} \leq 1000$ and so it is proposed that OIT should be classified with Acute Tox 3 via the dermal route.

The criteria for classification of aerosols with acute inhalation toxicity category 2 and category 3 in rats are $0.05 < LC_{50} \leq 0.5$ and $0.5 < LC_{50} \leq 1.0$ respectively. The lowest LC_{50} value was 0.27 mg OIT/L. Whilst it is noted that this was the result from a study that used whole body exposure and a higher LC50 value was obtained in a nose-only study, it is considered that the results of the whole-body study cannot be dismissed and are therefore considered relevant for the classification. As such, it is proposed that OIT should be classified with Acute Tox 2 via the inhalation route.

In addition to the classification for inhalation toxicity it is also proposed that the substance should be labelled as corrosive to the respiratory tract as there is evidence to suggest that the mechanism of toxicity is corrosivity. OIT is classified for skin corrosivity (please see section 4.4.1 and also clinical signs of respiratory irritation were observed in both acute inhalation studies. Therefore the supplementary phrase EUH071 – corrosive to the respiratory tract should be considered.

4.2.5 Conclusions on classification and labelling

Acute. Tox. 3 - H301: Toxic if swallowed
Acute. Tox. 2 - H330: Fatal if inhaled
Acute. Tox. 3 - H311: Toxic in contact with skin
EUH071: Corrosive to the respiratory tract

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) summarised eight acute toxicity studies on octhilinone (OIT) in the CLH report, covering oral (4 studies on rat), inhalation (2 studies on rat) and dermal (1 study on rabbit and 1 study on rat) routes of exposure. According to the DS, the existing harmonised classification of OIT was based on the LD_{50} or LC_{50} values derived in the oldest studies that were not dose-corrected for the dilution of OIT in propylene glycol (42 to 46.7 % w/w instead of 100 % w/w).

For **acute oral toxicity**, four studies were included in the CLH report. In the first study (Anonymous, 1987) according to OECD TG 401 and GLP, male and female Charles River

CD rats were treated by oral gavage with OIT (42-46.7 %) in propylene glycol at dose levels of 0, 126, 210, 336, 504 and 840 mg OIT/kg bw. Most animals treated with doses ≥ 210 mg/kg bw died on days 0-2. Clinical signs observed were central nervous system (CNS) depression, distended stomachs, pale extremities, respiratory noise, cool-to-touch, lacrimation, scant droppings, diarrhoea, red-stained muzzle and stained anogenital area. Gross necropsy revealed the presence of redness of the stomach and intestinal mucosa and/or yellow or white fluid-filled stomach or intestines in the decedents. The reported LD_{50} values were 318 mg OIT/kg bw (males) and 324 mg OIT/kg bw (females).

The second study (non-guideline, non-GLP) in CF Nelson Albino rats (Anonymous, 1977) reported an LD_{50} value of 247 mg OIT/kg bw (males) and 292 mg/kg (females). According to the DS, the study could not be assessed properly due to missing information.

A third study was carried out in Sprague-Dawley rats (Anonymous, 1991b) according to EPA 81-1 guideline (c.f. OECD TG 401) and under GLP. OIT (45 %) in propylene glycol was administered to male and females rats at dose levels of 0, 90, 180 and 450 mg OIT/kg bw. The result of the study was a combined LD_{50} value of 125 mg OIT/kg bw for males and females. Clinical signs included CNS depression, decreased respiratory rate, pallor of the extremities and piloerection. There were no abnormal findings during gross necropsy of the decedents/sacrificed.

A fourth study (Anonymous, 2002a) according to OECD TG 423 and GLP investigated oral toxicity in Wistar rats after administration of OIT (96.4 %) in water at dose levels of 0, 200, 500 mg/kg bw in male and females and in males only at 2 000 mg/kg bw. The LD_{50} was found to be greater than 500 mg/kg bw but less than 2 000 mg/kg bw. Gross necropsy revealed lesions in the lungs, mottling of the liver, bleeds to the stomach, white foci of the spleen and congestion of the kidneys.

The DS concluded that 125 mg OIT/kg bw was the relevant LD_{50} for both males and females and proposed to classify OIT as Acute Tox. 3; H301 (toxic if swallowed).

For **acute inhalation toxicity,** two guideline studies in Sprague-Dawley rats were assessed in the CLH report.

In the first study, rats were exposed, nose-only, to an aerosol (MMAD = 1.7-2.6 µm) of OIT (42-46.7 %) in propylene glycol for 4 hours (Anonymous, 1986) according to OECD TG 403 and GLP. Concentrations administered were 0, 0.058, 0.095, 0.229 and 0.671 mg OIT/L. The resulting combined LC_{50} for males and females was calculated to be 0.58 mg OIT/L. As for the oral studies, originally the LC_{50} value had not been dose-corrected for 100 % OIT and had been reported to be 1.254 mg/L. Clinical signs included signs of sensory and upper respiratory tract irritation (dyspnoea, bradypnoea, rales and gasping), CNS depression (ataxia, listlessness and prostration) which was considered secondary to respiratory distress and nasal mucosal irritation. Pathology of the decedents revealed red/brown foci and brown areas on the lungs and oedematous tongues.

The second study was performed in rats with an aerosol (MMAD = 1.58 µm) of OIT (45 %) in propylene glycol using whole-body exposure (Anonymous, 1992) according to OECD TG 403 and GLP. Rats were exposed to 0 (air control), 0.115, 0.224 and 0.330 mg/L for 4 h. The combined LC_{50} for males and females was determined to be 0.27 mg OIT/L, with

most deaths occurring during the first 24 h following exposure. Clinical signs included gasping, disturbed respiration (noisy and exaggerated), immobility and staining of the fur. Gross necropsy of the decedents revealed congestion of the lungs, gas-filled stomachs (thought to be caused by swallowing of air during attempts to breathe) and some incidences of increased lung weight (relative to body weight). There were no treatment-related abnormalities in surviving animals.

The DS argued that additional exposure from the grooming of contaminated fur could not be ruled out in the whole-body exposure study, but it was not clear whether this accounted entirely for inconsistent LC_{50} values obtained in the two studies. It was noted in the whole-body exposure study, that necropsy revealed gas-filled stomach in the decedents and that this finding was often seen in rats that died as a result of respiratory distress as they swallowed air during attempts to breath. The DS concluded that 0.27 mg/L was a relevant LC_{50} value and proposed to classify OIT as Acute Tox. 2; H330 (fatal if inhaled).

Since the mechanism of pulmonary toxicity was considered to be corrosivity (OIT has a harmonised classification as Skin Corr. 1, and also clinical signs of respiratory tract irritation were observed in both acute inhalation studies), the DS also proposed an additional labelling phrase EUH071 "corrosive to the respiratory tract".

For **acute dermal toxicity**, two studies were included in the CLH report. Both studies used methods similar to that of OECD TG 402. In the first study (Anonymous, 2004a– study date 1977), OIT (42-46.7 %) in propylene glycol was applied to the skin of male albino rabbits at dose levels of 146, 291, 582, 1 163 and 2 326 mg OIT/kg bw for 24 h under semi-occlusive conditions (5 males/group). All animals treated with doses of ≥ 582 mg/kg bw died. Clinical signs including lethargy, prostration, ataxia and partial paresis of hind limbs were observed in animals treated with ≥ 291 mg/kg bw. At the site of treatment, there were local signs of corrosion such as severe erythema and oedema followed by eschar formation, which preceded death. The dermal LC_{50} value was determined to be 311 mg OIT/kg bw. Previously, this had been given as 690 mg/kg bw, however this value had not been dose-corrected for pure OIT.

In the second dermal study (Anonymous (1991c), a single dose of 900 mg/kg bw of OIT (45 %) in propylene glycol was applied to the skin of male and female Sprague-Dawley rats (5/sex) for 24 h under occlusive conditions. There were no deaths, clinical signs of systemic toxicity or macroscopic abnormalities at necropsy. Oedema (slight to well-defined) was observed at application sites on day 2. Localised severe damage to the skin, associated with oedema (severe) and scabbing developed over the next few days. Therefore, the dermal LC_{50} was considered to be > 900 mg/kg bw.

The lowest dermal LD_{50} value in the studies was 311 mg OIT/kg bw. Therefore the DS proposed that OIT should be classified as Acute Tox. 3; H311 (toxic in contact with skin).

Comments received during public consultation

One MSCA supported the proposed classification for acute toxicity, but asked to determine harmonised ATE values.

Two Company-Manufacturers disagreed with the proposed Acute Tox. 3 classification for the oral route since the selected key study had been conducted on a formulated OIT product

(Anonymous 1991b) and not on the technical grade active substance. They were of the opinion that the study conducted on the technical grade material consisting of 96.4 % OIT (Anonymous 2002a) was more appropriate and justified the retention of the current harmonised classification as Acute Tox. 4 for OIT considering the LD_{50} of 500-2 000 mg OIT/kg bw of this study.

They also questioned the relevance of data obtained on an aerosol in view of the low vapour pressure of the substance and of the intended and reasonably expected use and conditions of handling of the substance. They did not agree that classification was warranted for acute inhalation toxicity, or with the supplementary labelling with EUH071 (corrosive to the respiratory tract). The Company-Manufacturers also objected to the chosen key study, and found the study via nose-only exposure to be more appropriate, as whole–body exposure might lead to exposure both orally (due to grooming) and dermally.

Both Company-Manufacturers supported the proposed classification for acute toxicity via the dermal route.

Assessment and comparison with the classification criteria

Oral route

The four oral rat studies gave a wide range of LD_{50} values. The studies with OIT in propylene glycol gave LD_{50} values of 318 mg OIT/kg bw (males) and 324 mg OIT/kg bw (females) in the first study; 247 mg OIT/kg bw (males) and 292 mg/kg (females) in the second study; and 125 mg OIT/kg bw in the third study. In the study carried out with OIT (96.4 %) in water the obtained LD_{50} was between 500-2 000 mg OIT/kg bw.

The lowest oral LD_{50} value in rats was 125 mg OIT/kg bw combined for both males and females. Although this value was obtained in a study conducted on OIT formulated in propylene glycol and the LD_{50} in the study conducted on neat OIT was higher, it is considered that the results of the studies on OIT formulated in propylene glycol cannot be dismissed and are considered relevant for classification. The criteria for classification with acute oral toxicity category 3 are $50 < LD_{50} \leq 300$. Therefore, RAC supports the DS's proposal to classify OIT as **Acute Tox. 3 via the oral route (H301)**. RAC also concludes that an **ATE value of 125 mg/kg bw** is warranted for OIT in a mixture.

Inhalation route

Two acute inhalation toxicity studies according to OECD TG 403 and GLP were performed in rats. In both studies rats were exposed to aerosols of OIT. The LC_{50} value was 0.58 mg OIT/L in the nose-only exposure study, which would warrant category 3 ($0.5 < LC_{50} \leq 1.0$), and 0.27 mg OIT/L in the whole-body exposure study, which would warrant category 2 ($0.05 < LC_{50} \leq 0.5$). Although additional exposure via grooming cannot be ruled out in the whole-body exposure study, it is not clear whether this accounts entirely for the discrepancy in the LC_{50} values. In the whole-body exposure study, necropsy revealed gas-filled stomachs in the decedents, and it was noted in the study summary that this finding was often seen in rats that died as a result of respiratory distress and was due to swallowing air during attempts to breathe. Therefore the results of the whole-body exposure study cannot be dismissed and are considered relevant for the classification. RAC supports the DS's proposal that the LC_{50} of 0.27 mg/L warrants classification as **Acute Tox. 2 via the**

inhalation route (H330). RAC also concludes that **an ATE value of 0.27 mg/L (dust and mist)** is warranted for OIT in a mixture.

Clinical signs observed during the acute inhalation studies were consistent with respiratory tract irritation/corrosion. These included dyspnoea, bradypnoea, rales and gasping. Pathology of decedents in the nose-only exposure study revealed red/brown foci on the lungs and oedematous tongues. In the whole-body exposure study, necropsy revealed congestion of the lungs, increased lung weight (relative to body weight) and gas-filled stomachs that could have been caused by swallowing air during attempts to breathe. Given the nature of the clinical signs observed following inhalation exposure, and that OIT is corrosive to skin and eyes, it is likely that at least one mechanism of toxicity is corrosion of the respiratory tract. Therefore RAC is of the opinion that an **additional labelling of OIT with EUH071 ("Corrosive to the respiratory tract") is warranted**.

Dermal route

Two studies were included in the CLH report. In the first study the dermal LC_{50} value was determined to be 311 mg OIT/kg bw in rabbits. In the second dermal study a single dose of 900 mg/kg was tested in Sprague-Dawley rats. As there were no mortalities, the dermal LC_{50} was considered to be > 900 mg/kg bw. The lowest dermal LD_{50} value in the studies was 311 mg OIT/kg bw. The criteria for classification with acute dermal toxicity category 3 are $200 < LD_{50} \leq 1\,000$. RAC agrees with the DS's proposal that OIT should be classified as **Acute Tox. 3 via the dermal route (H311)**. RAC also concludes that an **ATE of 311 mg/kg bw** is warranted for OIT in a mixture.

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

The effects observed following acute oral, dermal and inhalation exposure are outlined in section 4.2.1 of this report.

In acute inhalation toxicity studies in rats, clinical signs indicating respiratory irritation were observed. These were disturbed respiration, râles, gasping, dyspnoea, bradypnoea, nasal mucosal irritation. In the nose-only study (Anonymous (1986)) necropsy revealed red and/or brown foci on the lungs, brown areas on the lungs and oedematous tongues. Necropsies in the whole-body study (Anonymous (1992)) revealed gas-filled stomachs and congestion of the lungs. Also noted were increased lung-weight ratios in decedents.

Signs of respiratory tract irritation were also reported in repeated exposure inhalation studies in rats using OIT in propylene glycol. Lung to body weight ratio was increased and signs of upper airway and sensory irritation included râles, dyspnoea, bradypnoea and nasal discharge. Histopathology revealed focal squamous metaplasia of the respiratory epithelium, acute inflammation of the nasal mucosa, purulent exudates in the lumen of the nasal cavity, secretary cell hyperplasia and eosinophilic cell droplets.

Also available, as supporting information is a respiratory irritation study (Anonymous (1991d)). The results of this study were an upper airway RD_{50} (50 % decrease in respiratory rate) of 1.99 µg/L, determined for OIT (99.32 %) in mice. The upper airway test is a measure of sensory irritation and is commonly used for setting up workplace exposure limits, but not for classification purposes. As such, there is no accepted convention for interpreting RD_{50} values in terms of severity of respiratory tract irritant response.

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

OIT has been shown to be corrosive in skin irritancy studies (Section 4.4.1) and can be considered corrosive to the upper airway and respiratory tract, as indicated by the clinical signs and histopathology results in acute and repeated-dose toxicity studies.

4.3.2 Comparison with criteria

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. In a number of acute toxicity studies there were no indications of specific target organ toxicity relevant for classification with category 1 or 2.

Classification with STOT-SE category 3 is reserved for substances causing narcotic effects or respiratory tract irritation (RTI). There are currently no validated animal tests that specifically deal with RTI, however, useful information may be obtained from the single and repeated dose inhalation toxicity tests. Clinical signs of toxicity may include dyspnoea, rhinitis and histopathology (oedema, minimal inflammation, thickened mucous layer) which are *reversible*.

In the acute and short-term studies provided, OIT has been shown to cause significant irritation to the respiratory tract which was considered to have led to the *death* of the animals. The evidence from skin irritation and acute inhalation studies suggests that the mechanism of toxicity to the lungs was due to the corrosive nature of the substance. To this end, labelling with the supplementary phrase EUH071 is considered appropriate. In accordance with Section 3.8.2.2.1e of Annex I of CLP, classification with STOT-SE 3 should not occur if more severe organ effects including in the respiratory system are observed. Therefore, on the basis that it is proposed to label OIT as corrosive to the respiratory tract (EUH071), it should not be additionally classified with STOT-SE 3 (respiratory tract irritant).

4.3.3 Conclusions on classification and labelling

Not classified, conclusive but not sufficient for classification

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

Summary of the Dossier Submitter's proposal

The DS concluded that were no indications of specific target organ toxicity in the available studies warranting a classification as STOT SE 1 or 2. The DS did not either propose a classification for STOT SE 3, which is reserved for substances causing narcotic effects (H336) or respiratory tract irritation (RTI, H335). No specific comparison with the CLP criteria was performed for narcotic effects. As regards respiratory tract irritation, the DS reported reversible clinical signs of irritation (dyspnoea, rhinitis) and histopathology (focal squamous metaplasia of the respiratory epithelium, acute inflammation of the nasal mucosa). However, since OIT had a harmonised classification as corrosive to the skin, and as the DS proposed to classify OIT as Acute Tox. 2; H330 with an additional labelling as

corrosive to the respiratory tract (EUH071), the DS concluded that OIT should not be additionally classified with STOT SE 3 (respiratory tract irritant).

Comments received during public consultation

In relation to the inhalation route, two Company-Manufacturers questioned the relevance of data obtained on an aerosol in view of the low vapour pressure of the substance and of the intended and reasonably expected use and conditions of handling of the substance. They therefore agreed with the DS to not classify OIT for STOT SE 3 (transient respiratory tract irritation and narcotic effects). The DS responded that the low (lack of) potential for exposure to OIT during normal use does not prevent OIT to be classified based on its inherent hazards, using the information available and in accordance with the CLP Regulation.

Assessment and comparison with the classification criteria

According to the criteria, specific target organ toxicity (single exposure, STOT SE 1 and 2) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. With the exception of the skin and the lungs, no clear evidence of non-lethal effects on a specific target organ or tissues was observed in the oral, dermal and inhalation acute toxicity studies and therefore classification as STOT SE 1 or 2 is not warranted.

The hazard category STOT SE 3 covers transient respiratory tract irritation and narcotic effects. After inhalation, clinical signs of respiratory tract irritation (disturbed respiration, rales, gasping, dyspnoea, bradypnoea, nasal mucosal irritation) occurred, accompanied by histopathological findings in the lungs (red and/or brown foci on the lungs) at lethal doses. The supplementary label EUH071 and the classification as Acute Tox 2; H330 by inhalation cover the lung as a target organ after single exposure to OIT. RAC also notes that since no narcotic effects were reported at non-lethal doses, the classification as STOT SE 3; H336 is not warranted.

RAC agrees with the DS that **classification and labelling for STOT SE is not warranted**.

4.4 Irritation

4.4.1 Skin irritation

Table 10: Summary table of relevant skin irritation studies

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th></tr>
<tr><td>Rabbit, New Zealand White
6 Males

Vehicle: Propylene glycol

OECD 404
GLP

4 h Exposure (occlusive)

Test Material: OIT 42 – 46.7 % in propylene glycol

Anonymous (1984)</td><td>
<table>
<tr><td>Time (h)</td><td>Erythema</td><td>Oedema</td></tr>
<tr><td>1</td><td>4, 4, 4, 4, 4, 4</td><td>4, 4, 4, 4, 4, 4</td></tr>
<tr><td>24</td><td>4, 4, 4, 4, 4, 4</td><td>4, 4, 4, 4, 4, 4</td></tr>
<tr><td>48</td><td>4, 4, 4, 4, 4, 4</td><td>4, 4, 4, 4, 4, 4</td></tr>
<tr><td>72</td><td>4, 4, 4, 4, 4, 4</td><td>4, 4, 4, 4, 4, 4</td></tr>
<tr><td>168</td><td>4, 4, 4, 4, 4, 4</td><td>4, 4, 4, 3, 3, 3</td></tr>
<tr><td>Average (24 - 72)</td><td>4.0</td><td>4.0</td></tr>
<tr><td>Reversibility</td><td>N</td><td>N</td></tr>
</table>
</td><td>Corrosive</td></tr>
<tr><td>Rabbit, New Zealand White
6 Females

Vehicle: Propylene glycol

EPA 81-5 (c.f. OECD 404)
GLP

4 h Exposure (semi-occlusive)

Test Material: OIT 45 % in propylene glycol

Anonymous (1991e)</td><td>
<table>
<tr><td>Time (h)</td><td>Erythema</td><td>Oedema</td></tr>
<tr><td>0.5</td><td>2, 2, 2, 2, 2, 2</td><td>4, 4, 4, 4, 4, 4</td></tr>
<tr><td>24</td><td>4, 4, 4, 4, 4, 4</td><td>3, 2, 2, 2, 2, 2</td></tr>
<tr><td>48</td><td>4, 4, 4, 4, 4, 4</td><td>3, 3, 2, 2, 2, 2</td></tr>
<tr><td>72</td><td>4, 4, 4, 4, 4, 4</td><td>3, 3, 2, 2, 2, 2</td></tr>
<tr><td>168</td><td>4, 4, 4, 4, 4, 3</td><td>2, 2, 2, 2, 2, 2</td></tr>
<tr><td>336</td><td>4, 4, 4, 4, 4, 2</td><td>2, 2, 1, 1, 1, 1</td></tr>
<tr><td>Average (24 - 72)</td><td>4.0</td><td>2.3</td></tr>
<tr><td>Reversibility</td><td>N</td><td>N</td></tr>
</table>
</td><td>Corrosive</td></tr>
</table>

4.4.1.1 Non-human information

Two studies are available investigating skin irritation in rabbits. The first study (Anonymous (1984)) was used for the original harmonised classification in 1993 (Danish Toxicology Centre, March (1993)) and the second (Anonymous (1991e)) was made available after the submission. Both studies followed guidelines and were carried out according to GLP.

In the first study (Anonymous (1984)), OIT (45 – 50 %) in propylene glycol (0.5 mL) was applied to the shaven skin of six male rabbits for a period of 4 hours, under occlusive conditions. Animals were then observed for a period of 7 days post-exposure. Visible destruction of dermal tissue was observed in all animals and severe erythema and oedema was noted from 1 h. This was accompanied by eschar, blanching and effects persisted in all animals until the end of the study period.

In the second study (Anonymous (1991e)) OIT (45 %) in propylene glycol (0.5 mL) was applied to the shaven skin of six females. Exposure time was 4 hours, conditions were semi-occlusive and the observation period lasted for 14 days. Well-defined erythema with severe oedema was evident at all treatment sites from 30 minutes of removal of the dressing. Necrosis and chemical burns, with slight to moderate oedema had developed at all sites at 24 h. Reactions persisted up to day 14 in all but one animal where reactions improved slightly by day 8. This animal was observed to have desquamation

of the stratum corneum (sloughing) on days 7 and 8 and hyperkeratosis from day 9 to the end of the study period.

4.4.1.2 Human information

There is no relevant human information for this hazard class.

4.4.1.3 Summary and discussion of skin irritation

Two studies investigating the skin corrosion/irritation potential of OIT in rabbits are available. OIT was found to cause signs of necrosis in all animals studied. Chemical burns and visible destruction of the dermal tissue occurred within one hour of removal of the dressings and effects were still apparent at the end of the treatment period. As the severity of the erythema observed remained the same in all but one animal in each study until the end of the observation period, the effects were deemed irreversible.

4.4.1.4 Comparison with criteria

Each skin irritation study was conducted in six animals. A substance is considered to be corrosive when it produces destruction of skin tissue (visible necrosis through the epidermis and into the dermis) in at least one animal after exposure of the test substance for up to four hours. In both studies conducted, OIT caused irreversible necrosis of the dermal tissue, well-defined chemical burns and erythema and oedema that remained in all but one animal until the end of the study period. Therefore, OIT should be classified as corrosive. No data are available to inform on reactions at exposure times < 4 hours to allow for a direct assignment of a subcategory. However, in line with note 2 to table 1.1 in Annex VII of CLP, it is proposed to classify OIT in subcategory 1B.

4.4.1.5 Conclusions on classification and labelling

Skin Corr. 1B - H314: Causes severe skin burns and eye damage.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Two studies were included in the CLH report on skin irritation in rabbits. The first study (Anonymous, 1984) was considered by the TC C&L for the original harmonised classification in 1993. Both studies were in accordance with the OECD TG 404 and GLP (4-h application). OIT (45-50 %) in propylene glycol (0.5 mL) was tested in both studies.

In the first study (Anonymous, 1984), OIT (occlusive) produced visible destruction of dermal tissue in all animals and severe erythema and oedema was noted from 1 h onwards. Eschar and blanching persisted in all animals until the end of the study period (14 days).

In the second study (Anonymous, 1991e), OIT (semi-occlusive) produced a well-defined erythema with severe oedema at all treatment sites from 30 minutes of removal of the dressing. Necrosis and chemical burns, with slight to moderate oedema had developed at all sites at 24 h. Reactions persisted up to day 14 in all but one animal where reactions improved slightly by day 8. This animal had desquamation of the stratum corneum (sloughing) on days 7 and 8 and hyperkeratosis from day 9 to the end of the study period.

The DS concluded that OIT should be classified as corrosive. Although no data were available on reactions at exposure lengths < 4 hours to allow a direct assignment of the subcategory, the DS proposed to classify OIT in subcategory 1B in line with note 2 to table 1.1 in Annex VII of CLP.

Comments received during public consultation

One MSCA generally agreed with the DS's proposals for classification of OIT, but did not provide any specific comment for this endpoint. Two Company-Manufacturers agreed with the DS's classification proposal for skin and eye corrosion.

Assessment and comparison with the classification criteria

In two skin corrosion/irritation studies, both according to TG 404 and GLP, OIT was found to be corrosive to the skin causing irreversible necrosis of the dermal tissue, well-defined chemical burns, erythema and oedema until the end of the study. Exposure time was 4 hours in both studies, so there are no data on reactions at exposure lengths < 4 hours to allow a direct assignment of a subcategory. A substance tested for 4 hours only and where it is not possible to distinguish between Cat. 1C and Cat. 1B should not be subcategorised (Commission Regulation (EU) 2016/918).

Therefore RAC proposes classification of OIT as **Skin Corr. 1; H314 without sub-categorisation**.

4.4.2 Eye irritation

OIT was found to be corrosive in the dermal irritation studies, therefore, serious eye damage is considered implicit. However, the substances should not be labelled as such.

Eye Dam. 1 – H318: Causes serious eye irritation.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS stated that OIT was corrosive in the dermal studies, therefore, serious eye damage was considered implicit. The DS proposed to classify OIT as Eye Dam. 1; H318, based on the Skin Corr. 1; H314 classification, stating however, that the substances should not be labelled with H318 as the hazard statement H314 included the warning for eye damage.

Comments received during public consultation

One MSCA generally agreed with the DS's proposals for classification of OIT, but did not provide any specific comment for this endpoint. Two Company-Manufacturers agreed with the classification proposal for serious eye damage.

Assessment and comparison with the classification criteria

OIT is corrosive to the skin. As a consequence, and in accordance with the Technical Notes for Guidance on Data Requirements (chapter 2 section 6.1.4), OIT was not tested in rabbits for severe eye damage/irritation. OIT is classified as corrosive to skin and serious eye damage is thus implicit.

The Guidance on the application of the CLP Criteria (Version 5.0 – July 2017), section 3.3.2.4 states that a skin corrosive substance is also classified for serious eye damage which is indicated in the hazard statement for skin corrosion (H314: Causes severe skin burns and eye damage). However, although classification for both endpoints (Skin Corr. 1 and Eye Dam. 1) is required, the hazard statement H318 'Causes serious eye damage' is not indicated on the label because of redundancy (CLP Article 27).

Thus, RAC agrees with the DS that OIT should be classified as **Eye Dam. 1; H318**.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

Please see section 4.3 for a discussion on specific respiratory tract irritation following single exposure of OIT.

4.5 Corrosivity

Please see section 4.4 for the results, summary and discussion of corrosion to the skin caused by OIT.

Skin Corr. 1B - H314: Causes severe skin burns and eye damage.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 11: Summary table of relevant skin sensitisation studies

Method	Doses	No. sensitised/total no. or simulation index (SI)	Result
Local Lymph Node Assay in mice CBA/CaOlaHsd (4 females/group) OECD 429 GLP Vehicle: Acetone:olive oil (4: 1 v/v) Test Material: OIT 97.6 % Anonymous (2003)	0.25, 0.5, 4, 2.5 and 5 %	SI > 3 (for doses ≥ 0.5 %) EC3 = 0.46 % (w/v) Positive control: 25 % v/w HCA – SI > 3	***Positive***
Local Lymph Node Assay in mice CBA/J (5 females/group) OECD 429 GLP Vehicle: Acetone Test Material: OIT 99.8 % Anonymous (2004b)	0, 100.6, 320.4, 1036.6, 3062.1 and 11250 ppm	SI > 3 (11250 ppm) EC3 = 0.66 % (w/v) Positive control: 35 % HCA – SI: 2.75	***Positive***
Local Lymph Node Assay in mice CBA/J (6 females/group) Non-guideline (c.f. OECD 429) GLP Vehicle: acetone Test Material: OIT 99.32 % Anonymous (2006b)	100, 300, 1000, 3000 and 10000 ppm	SI > 3 (Doses ≥ 3000 ppm) EC3 = 0.24 % (w/v) No positive control	***Positive***

<table>
<tr>
<td>Buehler Test in guinea pigs

Hartley Albino (10/sex/test concentrations, 5/sex/controls)

OECD 406
GLP

Vehicle: ethanol aq. (induction), acetone (challenge)

Test material: OIT 48 % in propylene glycol

Anonymous (1983)</td>
<td>Induction:
25, 50, 100, 500, 750, 1200 and 2400 ppm (OIT)

Challenge:
100, 750 and 1200 ppm (OIT)</td>
<td>
<table>
<tr><td></td><td colspan="3">% Response (≥ grade 1 erythema)</td></tr>
<tr><td>Induction conc. OIT</td><td colspan="3">Challenge conc. OIT</td></tr>
<tr><td>%</td><td>0.01</td><td>0.075</td><td>0.12</td></tr>
<tr><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>0.0025</td><td>0</td><td>0</td><td>10</td></tr>
<tr><td>0.005</td><td>0</td><td>10</td><td>20</td></tr>
<tr><td>0.01</td><td>10</td><td>40</td><td>60</td></tr>
<tr><td>0.05</td><td>20</td><td>60</td><td>80</td></tr>
<tr><td>0.075</td><td>40</td><td>100</td><td>90</td></tr>
<tr><td>0.12</td><td>40</td><td>100</td><td>100</td></tr>
<tr><td>0.24</td><td>30</td><td>90</td><td>80</td></tr>
</table>
No positive control
</td>
<td>Positive</td>
</tr>
<tr>
<td>Maximisation Test in guinea pigs

Dunkin/Hartley (20/ females/ group)

Non-guideline (c.f. OECD 406)
GLP status unknown

Vehicle: Alembicol D

Test material: OIT 45 % in propylene glycol

Anonymous (1991f)</td>
<td>Induction:
Intradermal- 1 %

Topical - 2.5 %

Challenge:
0.5 and 1 %</td>
<td>20/20 animals sensitised following 1 % challenge</td>
<td>Positive</td>
</tr>
</table>

4.6.1.1 Non-human information

A number of skin sensitisation assays using OIT are available. These include three local lymph node assays (LLNA), one of which was performed to guidelines and GLP (Anonymous (2003)) and two others, both broadly following guidelines and to GLP, but with a number of methodological shortcomings (Anonymous (2004b)) and (Anonymous (2006b)). Also available are a Buehler test and a maximisation test in guinea pigs which broadly followed guidelines.

In the first LLNA carried out in mice (Anonymous (2003)) a pilot dose-ranging study was carried out using induction concentrations of up to 100 %. All mice receiving concentrations of 50 and 100 % of OIT died. One mouse per group receiving concentrations of 10 or 25 % suffered severe swelling of the local application site and/or moderate erythema. In the main study, induction concentrations of 0, 0.25, 0.5, 1, 2.5 and 5 % OIT in acetone: olive oil (4:1) were applied to the dorsal surface of both ears on three consecutive days. The SI value obtained at concentrations of 0.5 % and above was greater than three, indicating a positive result. The positive control responded appropriately and EC3 value for OIT was calculated as 0.46 %. Evidence of irritation in the form of erythema and oedema were observed at the site of application.

Two additional LLNAs in mice are available, both with methodological shortcomings. In the first study (Anonymous (2004b)), the results showed a clear sensitisation reaction with OIT with the stimulation index (SI) greater than three at the highest concentration of OIT tested (11250 ppm). The EC3 value was shown to be 0.66 %; however, the response of the positive control was unsatisfactorily low (SI < 3) indicating the test might not have worked appropriately. In the second study (Anonymous (2006b)), carried out following a similar protocol to OECD 420, OIT was shown to be positive for skin sensitisation, with an SI > 3 at concentrations of 3000 ppm and above and an EC3 value of 0.24 %. A positive control was not included in this study, thus compromising the validity of the results.

A Buehler study was carried out with OIT (48 % in propylene glycol) in guinea-pigs, following OECD 406 (Anonymous (1983)). In a deviation from the guideline, a range of induction (0, 25, 50, 100, 500, 750, 1200 and 2400 ppm) and challenge (100, 750 and 1200 ppm) concentrations were tested. A positive response was observed following induction of 50 ppm (0.005 %) whereby 20 % of animals responded to a challenge concentration of 1200 ppm. No positive control was used in this study and the vehicle used for induction [aqueous ethanol (80 %] differed from that used for challenge (acetone).

In a non-guideline guinea-pig maximisation test OIT (45 % in propylene glycol) was tested for skin sensitisation. A preliminary study investigated the intradermal and topical irritancy of a range of dilutions. A 1 % formulation in Alembicol D was found to be the maximum concentration that did not lead to skin irritation. Based on these results, intradermal injections of 1% and topical applications of 2.5 % were used for induction. For the topical challenge, concentrations of 0.5 and 1 % were used. In the main study, necrosis was observed following the intradermal injections at 1 % after the topical application of 2.5 %. Sensitisation reactions (slight to well-defined erythema, none to slight oedema) were observed in all animals following a challenge concentration of 1 %. Less severe or no reactions were observed after challenge with 0.5 % OIT.

Two further Buehler studies are available investigating the cross-potential of OIT (41.8 % in propylene glycol) with other biocidal products containing substances of a similar class. The results of these experiments are not deemed relevant to this assessment and so have not been included in this report.

4.6.1.2 Human information

There are four studies investigating the skin sensitisation potential of OIT in humans. These are summarised in the table below [taken from the UK Competent Authority Report (CAR) October 2013].

Table 12: Summary table of human skin sensitisation studies

Test	Results/Conclusion	Reference
21 day cumulative insult patch test	Confirmed sensitisation reactions occurred in 1/20 subjects induced/challenged with 500 ppm (0.05 %) OIT and in 5/20 volunteers with up to 1000 ppm (0.1 %) OIT	Emmet et al (1989)
Repeated insult patch test - An aqueous solution of OIT (50 ppm, 0.2 mL) was applied to an occluded patch measuring 2 x 2 cm. Dose was 0.0025 mg/cm2	No sensitisation reactions in 103 subjects induced and challenged with 50 ppm (0.005 %) OIT	Frank J (2000a)
Repeated insult patch test - An aqueous solution of OIT (100 ppm, 0.2 mL) was applied to an occluded patch measuring 2 x 2 cm. Dose was 0.005 mg/cm2	Confirmed sensitisation reaction in 1/222 subjects induced and challenged with 100 ppm (0.01 %) OIT	Frank J (2000b)
Repeated insult patch test - A solution of OIT in body lotion (100 ppm, 0.2 mL) was applied to an occluded patch measuring 2 x 2 cm. Dose was 0.005 mg/cm2	Sensitisation reactions in 3/222 subjects induced and challenged with 100 ppm (0.01 %) OIT in body lotion. Re-challenge was conducted in one of these subjects and sensitisation was confirmed.	Frank J (2001)

The skin sensitisation potential of OIT has been investigated in human volunteers across four challenge studies. These studies were conducted at the John Hopkins School of Hygiene and Public Health in six groups of adult volunteers (Emmet et al. (1988)). Various concentrations of OIT in petroleum and Tween-85 were applied to the paraspinal area of the back in Finn chambers. Approximately 24 hours after each of the daily 21 applications, the patch was removed and the skin left open to the air for 10 minutes to minimise maceration. Possible allergic reactions were noted. When the cumulative test was completed, any volunteer with suspected sensitisation reactions received a challenge patch test at a distant skin site. Challenge patches were left in place for 48 hours. Of the 9 volunteers with suspected sensitisation reactions, 6 were confirmed at challenge, as shown in the table below.

Table 13: Results of skin sensitisation in human volunteers (Emmet, 1988)

Group	Volunteers		OIT (ppm)	Tween-85 %	No. with suspected sensitisation reactions	No. with sensitisation response confirmed at challenge
	Males	Females				
1	3	2	100	2.5	0/5	
2	2	3	250	2.5	0/5	
3	4	1	500	2.5	0/5	
4	5	15	1000	2.5	8/20	5/8
5	4	15	250	0.625	0/19	
6	20 (unknown distribution)		500	0.625	1/20	1/1

In a separate study, OIT in water (50 ppm) was used in a series of repeat insult patch tests in 103 adult subjects (Frank (2000a)). The report states that this clinical investigation was conducted in a manner to comply with Title 212 of the code of US Federal investigations, Parts 50 (informed consent) and 56 (Institutional Review Board). Test material (0.2 mL) was applied by occluded patch to give a dose of 2.5 µg/cm^2 skin. In the induction phase, a fresh patch was applied to the same site 3 times per week for a period of 3 weeks. The patches were applied for 24 h and then removed for a further 24 h for an interim rest period. After a two week rest period following the ninth induction application, the subjects received a challenge application at an adjacent skin site. The challenge patch was removed after 24 hours and scored at 24, 48, 72 and 96 hours post application. No skin reactions were observed in either the induction of challenge phases.

In another similar repeat insult patch test study, 222 volunteers were treated with OIT in water (100 ppm) (Frank (2000b)). The test material (0.2 mL) was applied by occluded patch to give a dose of 5 µg/cm^2 skin. In an induction phase, a fresh patch was applied to the same site 3 times per week for 3 weeks. Each patch was removed 24 h after application for an interim rest period. After a two week rest period following the ninth induction application, the subjects received a challenge application at an adjacent skin site. The challenge patch was removed after 24 h and scored at 24 and 72 hours post application. A sensitisation reaction, confirmed by re-challenge, was observed in one volunteer.

A third repeat insult patch test was conducted in 207 volunteers (Frank (2001)). OIT in "body lotion" (composition not available) (100 ppm) was tested using the same induction and challenge application methods and schedule as described previously at a dose of 5 µg/cm^2 skin (Frank (2000)). Sensitisation reactions were reported at challenge in three volunteers. Re-challenge of one of these subjects confirmed sensitization. A second of these subjects was found to have participated previously in OIT patch testing.

4.6.1.3 Summary and discussion of skin sensitisation

There are five animal studies available, investigating the skin sensitisation potential of OIT in mice and guinea pigs. The most reliable study was a local lymph node assay in mice (Anonymous (2003)). In this study the stimulation index was found to be greater than 3 for OIT at doses ≥ 0.5 % and also for the positive control. An EC3 of 0.46 % (w/v) was derived. All of the other studies, whilst varying in the degree of reliability, provided supporting evidence that OIT is a strong sensitiser.

There is evidence from human volunteer studies that OIT diluted in either propylene glycol, water or body lotion can induce skin sensitisation. The lowest induction/challenge concentration resulting in skin sensitisation was 100 ppm (Frank (2000a)). Positive responses were observed at a dose of 5µg/cm^2 skin in two studies. An induction/challenge concentration of 50 ppm did not elicit

sensitisation reactions (Frank (2000b)). These human volunteer studies, consistent with the animal studies, provide evidence that OIT is a strong skin sensitiser.

4.6.1.4 Comparison with criteria

According to the CLP criteria, substances shall be classified as skin sensitiser category 1 when

a) a positive response in at least 30% of the animals is observed in an adjuvant type Guinea Pig test

b) a positive response in at least 15% of the animals is observed in a non-adjuvant Guinea Pig test

c) a stimulation index of three or more is observed in the LLNA.

These criteria were fulfilled in all of the corresponding studies with OIT and therefore classification as a skin sensitiser in Category 1 is appropriate.

In order to be further sub-categorised, the following evidence should be considered:

In animal studies, an EC3 value of ≤ 2 % obtained in a local lymph node assay would lead to sub-categorisation with 1A. In all three LLNAs available, the EC3 value was between 0.24 and 0.66 %. The most reliable study gave an EC3 value of 0.46 % (w/v); therefore classification with skin sensitisation Category 1A is appropriate. The results of these studies indicate that OIT is a strong sensitiser (EC3 of 0.2-2%)

Supporting evidence for classification in category 1A was provided by a Guinea Pig Maximisation Test (GPMT). In this study, 100 % of animals showed a response to a 1 % intradermal induction concentration. The criteria for sub-categorisation in 1A (≥ 60 % responding at > 0.1 % - ≤ 1 % intradermal induction dose) have been fulfilled. Based on the results of a GPMT, a substance is considered to be a strong sensitiser when ≥ 30-<60% of animals respond to an induction concentration of ≤ 0.1% or ≥60% animals respond to an induction concentration of >0.1-≤1.0%. In the GPMT, 100% of animals responded to an induction concentration of 1%, meeting the criteria for a strong sensitiser. However, a substance is considered to be an extreme sensitiser where ≥ 60% of the animals respond at an intradermal induction concentration of ≤ 0.1%. No information is available at lower induction concentrations to determine whether these criteria would be met.

A Buehler test is also available in which, 20 % of animals showed a response at a 0.005 % topical induction dose. The criteria for sub-categorisation with 1A for this assay (≥ 15 % responding at ≤ 0.2 % topical induction dose) have been fulfilled. Based on the results in a Buehler study, a substance is considered to be an extreme sensitiser where ≥ 60% of animals show a positive response to an induction concentration ≤ 0.2%. In the Buehler study, ≥60% of the animals showed a positive response to an induction concentration of ≥0.01%. Therefore, based on the Buehler study the substance would be considered an extreme sensitiser.

From these data, and in accordance with section 3.4.2.2.5 of the guidance document (ECHA (2015)) on the application of the CLP criteria, OIT can be regarded as a strong to extreme sensitiser.

In two human repeat insult patch tests, positive responses were observed at a dose of 5 µg/cm^2 skin in some volunteers. According to CLP, a positive response observed at ≤ 500 µg/cm2 provides evidence for classification in Category1A.

For a strong sensitiser, the recommended concentration limit in the guidance document is 0.1% (i.e., the same as the generic concentration limit GCL), whereas for an extreme sensitiser the recommended

concentration limit is 0.001%. At the moment a specific concentration limit (SCL) of 0.05% has been assigned to OIT in the existing harmonised entry. In one study, sensitisation reactions were confirmed in 5/20 volunteers induced with 0.1% OIT and 1/20 volunteers induced with 0.05%. In subsequent studies, sensitisation reactions were confirmed in 1/222 and 3/222 volunteers induced with 0.01% OIT. No reactions were observed at an induction concentration of 0.005%. In addition, topical inductions as low as 0.005% have been shown to cause positive skin sensitisation reactions in guinea pigs (Anonymous (1983)). Based on the information in humans and in the Buehler study, it is proposed to update the existing SCL to 0.005 %.

4.6.1.5 Conclusions on classification and labelling

Skin Sens 1A; H317 – May cause an allergic skin reaction

Specific concentration limit, C ≥ 0.005 %

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Non-human information

Five skin sensitisation studies were assessed in the CLH report; three local lymph node assays (LLNA), a Buehler test and a guinea pig maximisation test (GPMT).

The first LLNA (Anonymous, 2003) was performed according to the OECD TG 429 (originally adopted in 2002) and GLP. Induction concentrations of 0, 0.25, 0.5, 1, 2.5 and 5 % OIT in acetone: olive oil (4:1) were used. The SI value obtained at concentrations of 0.5 % and above was greater than three, indicating a positive result. The positive control responded appropriately and the EC3 value for OIT was calculated to be 0.46 %. Evidence of irritation in the form of erythema and oedema were observed at the site of application.

The second LLNA (Anonymous, 2004b) was conducted using induction concentrations of 0, 100.6, 320.4, 1 036.6, 3 062.1 and 11 250 ppm in acetone. According to the DS, it was conducted according to GLP but it had deficiencies as the response of the positive control was low (SI < 3). The results showed a clear sensitisation reaction with a SI > 3 at the highest OIT concentration tested (11 250 ppm). The EC3 value was 0.66 %.

The third LLNA (Anonymous, 2006b) was done under GLP, using induction concentrations of 100, 300, 1 000, 3 000 and 10 000 ppm OIT in acetone. The test also deviated from the OECD TG 429 as it lacked the positive control. OIT was shown to be positive for skin sensitisation, with an SI > 3 at concentrations of 3 000 ppm and above and an EC3 value of 0.24 %.

The Buehler test (Anonymous, 1983) was carried out with OIT (48 % in propylene glycol) following OECD TG 406. As a deviation from the guideline, a range of induction (0, 25, 50, 100, 500, 750, 1 200 and 2 400 ppm) and challenge (100, 750 and 1 200 ppm) concentrations were tested. The vehicle used for the induction was aqueous ethanol (80 %), and for the challenge it was acetone. 20 % of animals responded following induction at 50 ppm (0.005 %) and 60 % of the animals responded following induction at 100 ppm (0.01 %). No positive control was used in this study.

In the non-guideline GPMT (Anonymous, 1991f), OIT (45 % in propylene glycol) was tested. In the preliminary study a 1 % formulation in Alembicol D was found to be the maximum concentration that did not cause skin irritation. Intradermal injections of 1 % and topical applications of 2.5 % were tested for the induction, and concentrations of 0.5 and 1 % were tested for the topical challenge. Necrosis was observed following the intradermal injections at 1 % after the topical application of 2.5 %. Sensitisation reactions (slight to well-defined erythema and none to slight oedema) were observed in all animals following the challenge concentration of 1 %. Less severe or no reactions were observed after the challenge with 0.5 % OIT.

Human information

There were four studies (one 21-day cumulative insult patch test, and three human repeated insult patch tests, HRIPTs) summarised in the CLH report on the skin sensitisation potential of OIT in humans.

In the 21-day cumulative insult patch test six groups of adult volunteers were investigated (Emmet *et al*., 1988). Various concentrations of OIT in petroleum and Tween-85 were applied to the paraspinal area of the back in Finn chambers. Approximately 24 hours after each of the daily 21 applications, the patch was removed and the skin was left open to the air for 10 minutes to minimise maceration. Possible allergic reactions were noted. Any volunteer with suspected sensitisation reactions received a challenge patch test at a distant skin site. Challenge patches were left in place for 48 hours. Of the 9 volunteers with suspected sensitisation reactions, 6 were confirmed at challenge. Confirmed sensitisation reactions occurred in 1/20 subjects induced/challenged with 500 ppm (0.05 %) OIT and in 5/20 volunteers with 1 000 ppm (0.1 %) OIT.

In the first HRIPT (Frank, 2000a), OIT in Koralone™ 500 (50 ppm, 0.005 %, diluted with water) was tested in 103 adult subjects. Test material (0.2 mL) was applied by occluded patch to give a dose of 2.5 µg/cm^2 skin. In the induction phase, a fresh patch was applied to the same site 3 times per week for a period of 3 weeks. The patches were applied for 24 h and then removed for a further 24 h for an interim rest period. After a two-week rest period following the ninth induction application, the subjects received a challenge application at an adjacent skin site. The challenge patch was removed after 24 hours and scored at 24, 48, 72 and 96 hours post application. No skin reactions were observed in either the induction or challenge phases. RAC notes that the CLH report mentions OIT in water for the Frank 2000a and Frank 2000b studies, but according to the original study reports, Koralone™ 500 was used as the test material. The composition of Koralone™ 500 is confidential.

In the second HRIPT (Frank, 2000b), 222 volunteers were treated with OIT in Koralone™ 500 (100 ppm, 0.01 %, diluted with water) in the same manner as above. The test material (0.2 mL) was applied by occluded patch to give a dose of 5 µg/cm^2 skin. A sensitisation reaction, confirmed by re-challenge, was observed in one volunteer (1/222).

The third HRIPT (Frank, 2001) was done using the same method as described above, in 207 volunteers. OIT in "body lotion" (composition not available) was tested at a dose of 5 µg/cm^2 skin (equivalent to 100 ppm, 0.01 %). Sensitisation reactions were reported at challenge in three volunteers. Re-challenge was conducted in one of these three subjects and sensitisation was confirmed. A second subject was found to have participated previously in OIT patch testing and was therefore not eligible for the HRIPT.

The DS proposed to classify OIT as a Skin Sens. 1A on the basis of the most reliable LLNA study (Anonymous, 2003), in which the stimulation index was greater than 3 for OIT at doses

≥ 0.5 % (EC3 of 0.46 % (w/v)). According to the DS, the two human repeat insult patch tests showing positive responses at a dose of 5 µg/cm^2 skin in some volunteers also provided evidence for classification in Category 1A (a positive response observed at ≤ 500 µg/cm^2 warrants Category 1A).

The DS concluded that the LLNA results indicated that OIT was a strong sensitiser (EC3 of 0.2-2 %), that the GPMT results indicated the same, and that the Buehler test indicated OIT as an extreme sensitiser (≥ 60 % of the animals showed a positive response to an induction concentration of ≥ 0.01 %).

The DS proposed to lower the current SCL of 0.05 % assigned to OIT as in one human study, sensitisation reactions were confirmed in 1/20 volunteers induced with 0.05 %. In subsequent studies, sensitisation reactions were confirmed in 1/222 and 3/207 volunteers induced with 0.01 % OIT, while no reactions were observed at an induction concentration of 0.005 %. In addition, topical inductions as low as 0.005 % had been shown to cause positive skin sensitisation reactions in guinea pigs. Based on the information in humans and in the Buehler study, the DS proposed to update the existing SCL to 0.005 % (50 ppm).

Comments received during public consultation

All commenting parties (2 Company-Manufacturers, 2 MSCAs, 1 NGO) supported the proposed classification Skin Sens. 1A.

Two Company-Manufacturers agreed to Skin Sens. 1A based on the results of the 3 LLNAs conducted on the technical grade OIT material.

A trade association argued against the setting of an SCL and the socio-economic issues it would pose for the industry, also arguing that to be effective, usually a dosage of at least 250 ppm of OIT was needed, which was significantly above the proposed SCL of 50 ppm (0.005 %). Another trade association stressed the importance of OIT in industry, and argued against an SCL of 50 ppm, as it would mean that products containing OIT could not be sold to the public. A third industry/trade association noted the proposed SCL for OIT would significantly reduce the biocidal use of the substance. One downstream user argued that consumer paints containing the substance should be allowed to be sold if gloves were supplied with the product.

Two Company-Manufacturers in a jointly prepared document argue against setting an SCL of 0.005 % on the basis of the HRIPT results by Frank (2000a, 2000b and 2001), stating that in these studies, contrary to the CLH report, the test material was not aqueous OIT, but Koralone™ 500, as described in the original reports. Koralone™ 500 was a formulated product of OIT, and in accordance to Annex I CLP Section 3.4.3.1.1 'SCLs are set on the basis of testing of the substance and never on the basis of testing of a mixture containing the sensitising substance'. Thus, according to industry, the results obtained from the Frank studies should not be used for SCL setting. The joint paper also considered that HRIPT (the application of occlusion and exposure protocols) generally overestimated the hazard/potency associated with sensitisation. In their view, this was supported by the few reports of allergic contact dermatitis following exposure to OIT reported in the literature and was indicative that the current SCL of 0.05 % was protective.

One MSCA commented that lowering the SCL to 0.005 % needed further discussion, as the data represented a borderline case for a lower SCL limit, observing that no sensitised

individuals were observed at the exposure level of 50 ppm (0.005 %), recognising however that the Buehler test showed a positive response at a 0.005 % topical induction dose.

Another MSCA supported classification of OIT as Skin Sens. 1A with a strong to extreme potency as well as the proposal to reduce the current SCL. The MSCA cited recent publications (Schwensen *et al*., 2017; Aalto-Korte and Suuronen, 2017) which supported cross-reactivity between MIT (2-methyl-2*H*-isothiazol-3-one (CAS 2682-20-4) and OIT. The Aalto-Korte and Suuronen (2017) publication found that allergic reactions to OIT had become common during the MIT allergy epidemic. Their data showed that between 2012 and 2017, 2.9 % of 647 consecutively tested patients reacted to OIT (0.1 % OIT in petroleum) and that patients showing (extreme) reactions to MIT also reacted to OIT. Therefore it could not be excluded that patients previously sensitised to MIT would react to products containing OIT. The MSCA argued that the sensitising capacity of OIT was similar to or even stronger than that of MIT. Based on potency data from animal tests MIT was considered a "strong" and OIT a "strong to extreme" sensitizer. For a strong sensitizer a GCL of 0.1 % applied, but RAC had decided in 2016 to apply a SCL of 0.0015 % (15 ppm) for MIT due to cross reactivity to CMI (5-chloro-2-methyl-2*H*-isothiazol-3-one (CAS 26172-55-4) based on an Scientific Committee on Consumer Safety (SCCS) Opinion. The MSCA stressed that as the chemical structure of OIT was closely related to other thiazolinones (especially MIT) the cross-reactivity should be considered in SCL-setting to reduce the likelihood of OIT contributing to the rise in thiazolinone allergy.

The European Environmental and Contact Dermatitis Research Group (EECDRG) expressed a concern that the proposed SCL of 0.005 % was not low enough to protect workers and consumers from skin sensitisation. EECDRG cited two case reports that implied that concentrations lower than the proposed limit of 50 ppm may sensitise consumers: 1) An 89-year-old woman had wide-spread dermatitis on skin areas that corresponded contact areas of a new leather armchair. Clinical investigations revealed OIT contact allergy and a leather sample of the chair contained 28 ppm OIT following chemical analysis. The patient tested negative to MIT and BIT (Raison-Peyron *et al*., 2017). 2) In a series of 8 OIT-allergic patients from a Finnish occupational clinic, one was a sewing machine operator who had handled OIT-containing textiles and developed hand dermatitis. Four textile samples from the workplace contained 2, 10, 40 and 50 ppm OIT following chemical analysis (Aalto-Korte *et al*., 2007).

EECDRG additionally stressed that cross-reactivity between OIT and MIT should be taken into account, citing several publications: A recent Danish study indicated cross-reactivity between OIT, MIT and BIT in a modified local lymph node assay (Schwensen *et al*., 2017). Three clinical studies analysing patterns of concomitant reactions to OIT and MIT in patch-tested patients supported the cross-reactivity between OIT and MIT (Aerts *et al*., 2016; Craig *et al.*, 2017; Aalto-Korte and Suuronen, 2017). EECDRG also emphasised that MIT-sensitised patients need to be warned about the possibility to develop allergic contact dermatitis from OIT-containing products. Especially patients with extreme allergic patch test reactions to MIT were at risk (Aalto-Korte and Suuronen, 2017). As skin sensitization (delayed type cell-mediated contact allergy) was a life-long state, sensitised individuals continued living with the vulnerability to react not only to MIT but also to OIT. EECDRG proposed the same SCL as had been agreed for MIT, namely 0.0015 %, or if a SCL lower than 0.005 % could not be accepted, the limit for labelling of OIT in chemical products should be 0.00015 % to protect thousands of MIT-allergic individuals in the European population.

Assessment and comparison with the classification criteria

RAC agrees with the DS and all parties providing comments during the public consultation that OIT is a potent sensitiser. As shown in the following table, the animal studies on OIT provide results that meet the criteria for classification in sub-category 1A.

Animal test	Criteria for high potency (sub-category 1A)	OIT data	Conclusion
LLNA (Anonymous, 2003)	EC3 value ≤ 2 %	EC3 value = 0.46 %	The EC3 value meets the criteria for Cat. 1A
LLNA (Anonymous, 2004b)	EC3 value ≤ 2 %	EC3 value = 0.66 %.	The EC3 value meets the criteria for Cat. 1A
LLNA (Anonymous, 2006b)	EC3 value ≤ 2 %	EC3 value = 0.24 %	The EC3 value meets the criteria for Cat. 1A
Buehler test (Anonymous, 1983)	≥ 15 % responding at ≤0.2 % or ≥ 60 % responding at >0.2 % to ≤ 20 % topical induction concentration	20 % response at 0.005 % topical induction concentration	The response rate at a topical induction concentration of 0.005 % meets the criteria for Cat. 1A
GPMT (Anonymous, 1991f)	≥ 30 % responding at ≤ 0.1 % or ≥ 60 % responding at > 0.1 % to ≤ 1 % intradermal induction concentration	100 % response at 1 % intradermal induction concentration of OIT (observed at 1 % challenge concentration)	The response rate at an intradermal induction concentration of 1 % meets the criteria for Cat. 1A.

Human data also support the classification as in a 21-day cumulative insult patch test (Emmet *et al.*, 1988), confirmed sensitisation reactions occurred in 1/20 and 5/20 subjects induced/challenged with 500 ppm (0.05 %) and 1 000 ppm (0.1 %) OIT, respectively. Also two human repeat insult patch tests, in which positive responses were observed in some volunteers at 5 µg/cm^2 skin, provide evidence for classification in Category 1A (a positive response observed at ≤ 500 µg/cm^2 warrants Category 1A). More recent publications (Aalto-Korte and Suuronen, 2017) show that 2.9 % of 647 consecutively tested patients reacted to OIT (0.1 % OIT in petrolatum, 40 µg/cm^2) between 2012 and 2017.

Based on the LLNA (Anonymous, 2003), which is a reliable study without restrictions, and the other animal and human studies as supportive evidence, RAC agrees with the DS that **classification as Skin Sens. 1A; H317 (may cause allergic skin reactions)** is warranted.

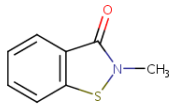
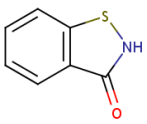
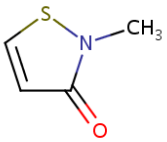
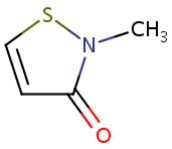
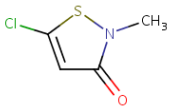
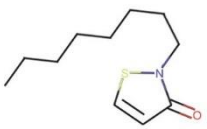
Specific Concentration Limit

The results of the LLNA studies indicate that OIT is a strong sensitiser (EC3 values were between 0.2 % and 2 %). In the GPMT 100 % of animals responded to an intradermal induction concentration of 1 %, meeting the criteria for a strong sensitiser (≥ 60 % animals responding

to an intradermal induction concentration of > 0.1 - ≤ 1.0 %). Lower induction concentrations have not been tested to determine whether the criteria for extreme sensitiser would be met. In the Buehler test, 60 % of animals showed a response at a 0.01 % topical induction dose of OIT, therefore the result of this study met the criteria for an extreme sensitiser (≥ 60 % of animals showing a positive response to a topical induction concentration ≤ 0.2 %). Based on the results of the available animal studies, in accordance with section 3.4.2.2.5 of the Guidance on the application of the CLP criteria (Version 5.0, July 2017), OIT can be regarded as a strong to extreme sensitiser.

Based on the LLNA results (see table comparing related thiazolinones), the sensitising capacity of OIT is similar to or even stronger than that of MIT. RAC proposed to apply an SCL of 0.0015 % (15 ppm) for MIT. The chemical structure of OIT is closely related to other thiazolinones, especially to MIT. Schwensen *et al*. (2017) demonstrated in a modified local lymph node assay that cross-reactivity occurs between related thiazolinones OIT (2-octyl-2*H*-isothiazol-3-one, CAS 26530-20-1, MIT (2-methyl-2*H*-isothiazol-3-one, CAS 2682-20-4) and BIT (1,2-benzisothiazol-3(2*H*)-one, CAS 2634-33-5). Cross-reactivity between MIT and OIT as well as between MIT and BIT has been demonstrated in humans in several publications (Aerts, 2017, Aalto-Korte and Suuronen, 2017, Amsler *et al*., 2017). Aalto-Korte and Suuronen (2017) found that allergic reactions to OIT have become common during the MIT allergy epidemic. Their data show that between 2012 and 2017 2.9 % of 647 consecutively tested patients reacted to OIT (0.1 % OIT in petroleum) and that patients showing (extreme) reactions to MIT also reacted to OIT. Therefore it cannot be excluded that patients previously sensitised to MIT will also react to products containing OIT.

Table: Comparison of skin sensitising properties of several thiazolinones. Data taken from RAC opinions on MBIT (2018); MIT (2016); CMIT/MIT (2016) and OIT (this opinion). For BIT the LLNA information was taken from the public NICEATM LLNA databank.

	MBIT (CAS 2527-66-4)	**BIT (CAS 2634-33-5)**	**MIT (CAS 2682-20-4)**	**CMIT/MIT (3:1) (CAS 55965-84-9)**	**OIT (CAS 26530-20-1)**
Chemical structure				 	
LLNA	EC3 = 1.04 % EC3 = 0.69 %	EC3 = 2.3 % EC3 = 32.4 % EC3 = 4.8 % EC3 = 10.4 %	EC3 = 0.86 %	EC3 = 0.003 % EC3 = 0.007 %	EC3 = 0.46 % EC3 = 0.66 % EC3 = 0.24 %
Classification	Skin Sens. 1A	Skin Sens. 1	Skin Sens. 1A	Skin Sens. 1A	Skin Sens. 1A (this opinion)
HRIPT	9/45 (20 %) volunteers showed dermal sensitization at 500 ppm	5/58 (9 %) at 725 ppm aq., 0/54 (0 %) at 360 ppm aq	1/116 (0.9 %) volunteers at 400 ppm and 1/210 (0.5 %) at 500 ppm	–	0/103 subjects at 50 ppm (0.005 %) 1/222 (0.45 %) subjects at 100 ppm (0.01 %)
SCL	0.0015 %	0.05 %	0.0015 %	0.0015 %	0.0015 % (this opinion)

There is an existing SCL for OIT of 0.05 % and the DS proposed a lower SCL of 0.005 %. The literature on OIT allergy in clinical patients is not very wide. Nevertheless, there are two case reports implying that concentrations lower than the proposed limit of 50 ppm may sensitise. In one case report, an 89-year-old woman had wide-spread dermatitis on skin areas that corresponded to contact areas of a new leather armchair. Clinical investigations revealed OIT contact allergy and a chemical analysis revealed that a leather sample of the chair contained 28 ppm OIT. The patient tested negative to MIT and BIT (Raison-Peyron *et al.*, 2017).

The second publication reports an OIT-allergic patient from a Finnish occupational clinic, who was a sewing machine operator who had handled OIT-containing textiles and developed hand dermatitis. A chemical analysis revealed that four textile samples from the workplace contained 2, 10, 40 and 50 ppm OIT (Aalto-Korte *et al.*, 2007).

Overall, taking into consideration that the animal tests indicate that OIT is a strong to extreme sensitiser, that there is cross-reactivity between OIT and MIT, and that there are case reports suggesting that concentrations lower than the proposed limit of 50 ppm may induce sensitisation, RAC is of the opinion that for OIT an SCL of 50 ppm is not low enough, and therefore proposes that **an SCL should be set at 15 ppm (0.0015 %).** This would also be in line with other thiazolinones.

4.6.2 Respiratory sensitisation

No data are available.

Conclusions on classification and labelling

Not classified – data lacking

4.7 Repeated dose toxicity

This hazard class is not considered in this assessment.

4.8 Germ cell mutagenicity (Mutagenicity)

This hazard class is not considered in this assessment.

4.9 Carcinogenicity

This hazard class is not considered in this assessment.

4.10 Toxicity for reproduction

This hazard class is not considered in this assessment.

4.11 Other effects

4.11.1.1 Neurotoxicity

This hazard class is not considered in this assessment.

4.11.1.2 Immunotoxicity

This hazard class is not considered in this assessment.

5 ENVIRONMENTAL HAZARD ASSESSMENT

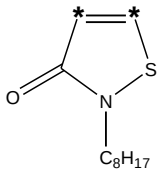
2-octyl-2H-isothiazol-3-one (OIT) is OIT has a number of biocidal uses as a preservative, including an in-can preservative for non-food stuffs (product type 6) and a preservative for metalworking fluids (product type 13). It is currently under evaluation for use as a wood preservative (product type 8). Available environmental fate and hazard studies have been considered under the Biocidal Products Regulation (EU) No. 528/2012 and summarised in the Competent Authority Report (CAR) 2016.

The key information pertinent to determining a classification is presented below.

All radiolabelled studies used ^{14}C-OIT with a purity of >95.8 % as shown in Figure 1.

Figure 1: Structure of OIT indicating positions of the ^{14}C labels.

^{14}C-OIT ^{14}C label was at the 4 and 5 position.



* site of ^{14}C label

[where ^{12}C-OIT also employed, this was added to the ^{14}C-material]

OIT has a quoted dissociation constant of 5.2 to 6.0 x 10^{-4} mol/l (Werle, 1993a) following OECD test guideline 112. This indicates pKa values in the range 3.2-3.3. Therefore, OIT is anticipated to dissociate and be ionised at environmentally relevant pH. Ecotoxicity studies were run at pH 5 or above reflecting environmental conditions where nearly all OIT would be in its ionised form

Where available, information on degradation products is included in Annex II.

5.1 Degradation

A summary of available valid information on the fate of OIT is presented in Table 14 below.

Table 14: Summary of relevant information on degradation of OIT

Method	Results	Remarks	Reference
Aquatic hydrolysis US EPA Guideline, Subsection N, Section 161-1 GLP, purity: 99.03 %	Stable at pH 4, 7 and 9 at 24 °C	Valid	Estigoy and Shepler (1992)
Aquatic hydrolysis US EPA Guideline, Subsection N, Section 161-1 GLP, purity: 97 %	Stable at pH 4, 7 and 9 at 25 °C	Valid	Lucas (1998)
Aquatic photolysis USE EPA Guideline, Subdivision N, Section 161-2 GLP, purity: 99 %	DT_{50} = 15.3 days at 37.4 °N (Richmond, California, USA)	Valid	Estigoy and Shepler (1995)
Aquatic photolysis USE EPA Guideline, Subdivision N, Section 161-2 GLP, purity: 98.4 %	Buffer solution DT_{50} = 3.7 days at 50°N (summer in central Europe) Pond water DT_{50} = 5.1 days at 50°N (summer in central Europe)	Valid	Adam (2007)
Ready biodegradation OECD Guideline 301D GLP, purity: 94.8 %	0 % degradation by day 28 Possible test item micro-organism inhibitory effect.	Valid	Noack (2002)
Freshwater aerobic mineralisation in surface water (simulation biodegradation), OECD Guideline 309, GLP, purity: 96.9 %	Mineralisation at study termination (day 29): 36.4 to 47.9 % AR	Valid	Mamouni (2007a)
Seawater aerobic mineralisation in surface water (simulation biodegradation), OECD Guideline 309, GLP, purity: 98.9 %	Mineralisation: max. 44.6 % AR, at day 17 study termination	Valid	Mamouni (2007b)

5.1.1 Stability

Aqueous hydrolysis

Two aqueous hydrolysis studies are available following GLP and US EPA Guideline subsection N, section 161.1.

Study 1 – Estigoy and Shepler (1992)
Using radio-labelled ^{14}C-OIT (10 mg/l), solutions at pH 5, 7 and 9 were incubated in the dark at 24 ± 0.3 °C for 32 days. No significant degradation was observed and analysis showed 100 % radioactivity as OIT at study termination. On this basis, the hydrolysis half-life was considered to be >1 year and OIT was considered hydrolytically stable.

Study 2 – Lucas (1998)
Using radio-labelled ^{14}C-OIT (6.25 mg/l), solutions at pH 5, 7 and 9 were incubated in the dark at 25 ± 1 °C for 30 days. No significant degradation was observed and analysis showed ≥97.5 %

radioactivity as OIT at study termination. On this basis, the hydrolysis half-life was considered to be >1 year and OIT was considered hydrolytically stable.

Aqueous photolysis

Two aqueous photolysis studies are available following GLP and US EPA Guideline subsection N, section 161.2.

Study 1 – Estigoy and Shepler (1995)
An aqueous photolysis study is available using ^{14}C radio-labelled OIT (10.0 mg/l). Test solutions were incubated at pH 7 for 718.5 hours (~30 days) at 24 °C ± 1°C under roof top sun in Richmond, California, USA (37.4°N, 122.26°W). The photolytic DT_{50} was 15.3 days.
The following degradants were observed:

- 2-(n-octyl)-4-thiazolin-2-one: 14.1 % AR at termination
- mixture of N-(n-octyl) malonamic acid (NNOMA) and oxamic acid: 12.5 % AR at termination
- N-(n-octyl)acetamide (NNOA): 11.2 % AR at termination
- sulfoxide of OIT: max. 10.1 % AR at 405 hours

Mineralisation accounted for 12.5 % AR based on carbon dioxide (CO_2) at study termination.

Study 2 – Adam (2007)
A second aqueous photolysis study used ^{14}C radio-labelled OIT (0.5 mg/l) in two systems: sterile buffer solutions at pH 7 and sterile natural pond water at pH 8. The irradiated pond water solutions were considered sterile throughout the study. Test solutions were incubated for 15 days at 25.1 ± 0.2 °C under constant irradiation (wavelengths below 290 nm filtered out). The 15 experimental days corresponded to 30.5 natural summer sunlight days at 50°N.

Test item, as Applied Radioactivity (AR), decreased from 97.9 % and 98 % to 1.1 % and 2.5 % in the buffer and pond solutions respectively.

The photolytic DT_{50} for the buffer solution system was 3.7 summer days at 50°N. The photolytic DT_{50} for the pond water system was 5.1 summer days at 50°N.

The following degradants were observed in the buffer solutions:

- N-(n-octyl)acetamide (NNOA): max. 23.3 % AR at day 4
- 2-(N-octyl) ethyl amine: 16.2 % AR at termination
- Unidentified degradant : 55.1 % AR at termination
- Mineralisation: 0.3 % AR CO_2 at termination

The following degradants were observed in the pond solutions:

- N-(n-octyl)acetamide (NNOA): max. 25.1 % AR at day 4
- Unidentified degradant: max. 11.3 % AR at day 4
- Unidentified degradant: max. 58.7 % AR at day 4
- Mineralisation: 7.9 % AR CO_2 at termination

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not available.

5.1.2.2 Screening tests

A ready biodegradation study following OECD Guideline 301D (Closed Bottle) (Noack (2002)) is available using OIT. Test solutions were prepared with 3 mg/l test item and 0.2 ml inoculum per 300 ml test vessel. Inoculum was sourced from a municipal waste water treatment plant treating predominantly domestic waste water. The reference control was considered valid with >60 % degradation after 5 days. The toxicity control (with 1.5 mg/l OIT and 5 mg/l sodium acetate) achieved 26 % degradation after 14 days and a maximum of 35 % degradation by day 21. While these values meet the validity criteria of 25 % degradation by day 14, the CAR evaluation considered the test item may have lead to a degree of microorganism inhibition. This is supported by an Activated Sludge Respiration Inhibition Test (ASRIT) (Ouellette (1995)) which determined an EC_{20} of 8.9 mg/l and 11 % inhibition observed at the lowest test concentration of 4 mg/l. A second ASRIT (Noack, 2001) also determined a similar EC_{20} of 7.3 mg/l.

No degradation of OIT was observed in test item solutions during the OECD 301D test. Overall, the substance is considered not readily biodegradable although this may be influenced by a partially inhibitory test concentration.

Ready biodegradation studies (Seyfried (2003a) and Seyfried (2003b)) are available for two degradants:
- N-(n-octyl) acetamide (NNOA)
- N-(n-octyl) malonamic acid (NNOMA)

Both studies followed OECD Guideline 301B (Modified Sturm test) and GLP. Both substances achieved over 60 % degradation meeting the 10-day window criterion. On this basis, both degradants are considered rapidly degradable.

5.1.2.3 Simulation tests

An aerobic sewage treatment simulation test following OECD guideline 303A is available (Fiebig (2002)) and was used with other data to consider the fate of OIT for the biocides assessment. However, results from tests simulating the conditions in a sewage treatment plant (STP) cannot be used for assessing the degradation in the aquatic environment for the purpose of classification. The main reasons for this are: microbial biomass in a STP is significantly different from the biomass in the environment; there is a considerably different composition of substrates; and that the presence of rapidly mineralised organic matter in waste water may facilitate degradation of the test substance by co-metabolism (ECHA (2015)).

A water–sediment simulation study is not available. Two aquatic biodegradation simulation studies are available using freshwater and seawater without a sediment phase.

<u>Study 1 – Mamouni (2007a)</u>

A freshwater aquatic biodegradation simulation study is available following OECD Guideline 309 and GLP. The study used ^{14}C-OIT and surface water from the River Rhine in Switzerland and did not include a sediment phase. While OIT is anticipated to adsorb to organic matter, the suspended solid concentration in the test water is not known. Test vessels containing 0.0103 and 0.1029 mg/l OIT were maintained for 29 days under aerobic conditions at 20 ± 2 °C. The test item was analysed by High Performance Liquid Chromatography (HPLC) and radioactivity confirmed by Liquid Scintillation Counting (LSC). Mean AR recoveries were 91.8 ± 5.6 % AR and 91.8 ± 5.6 % AR for low and high doses.

Levels of AR in water decreased from 96.7 and 102 % on day 0 to 41.8 and 52.5 % on day 29 in the low and high dose systems respectively. The decrease in water phase AR is considered due to primary biodegradation of OIT to degradants which were subsequently mineralised and some partitioning to dissolved organic solid matter. At study termination on day 29, levels of CO_2 as AR were 47.9 % in the low dose and 36.4 % in the high dose. No other volatile radioactivity was detected.

Measured concentrations of ^{14}C-OIT, degradants and CO_2 are presented in Tables 15 and 16 for low and high dose systems. Figures 2 to 5 show levels of OIT, degradants and CO_2 over time, and corresponding decrease in aquatic phase AR. It can be seen that ^{14}C-OIT concentrations rapidly decrease with an increase in degradant concentrations and subsequently CO_2 reflecting their mineralisation. This is presented in Figure 6. It is unclear if OIT bound to organic matter remains bound or is mobilised back into solution although it is noted that adsorbed AR peaks a few days after study initiation with a subsequent slight decline.

The study calculated DT_{50} values of 0.6 and 1.2 days for OIT. This is considered to represent primary degradation of OIT to degradants, adsorption to suspended organic matter and a degree of mineralisation. For the CAR, these values were converted 12 ^{o}C, resulting in DT_{50} values of 1.1 and 2.3 days.

Three degradants were detected (but not identified) above 10 % AR with maximums in each system as follows:

- M1 at 19 to 22.8 % AR;
- M5 at 14.7 to 15 % AR; and
- M6 at 9 to 10.5 % AR.

DT_{50} values were calculated at 12 ^{o}C as follows:

- M1 17.8 to 35.5 days;
- M5 19.3 to 30.9 days; and
- M6 8.3 to 22.9 days.

The study report states it was not possible to identify the above degradants M1, M5 and M6 as they contain multiple components. During the biocides process the UK Competent Authority concluded that there was insufficient evidence to support these degradants comprise multiple fractions. In addition, the assessment did not consider these degradants as transient given that their decline does not appear to be linked to the formation of minor degradants and that they are not present for a short enough time to be termed ‘transient’. It was acknowledged that these degradants do appear to ultimately undergo mineralisation.

Two additional degradants were observed at less than 10 % AR: M4 and M7. Due to the low concentrations, reliable DT_{50} values could not be calculated.

Table 15: Applied Radioactivity (% AR) in low dose (10 µg/l) system

ID	% AR at Incubation Time (days) [mean of 2 replicates]								
	0	**0.25**	**1**	**3**	**5**	**7**	**10**	**20**	**29**
OIT	96.7	75.5	26.6	5.6	0.9	*	*	*	*
M1	*	*	9.6	16.3	19.0	15.2	6.7	2.6	2.3
M2	*	*	*	2.5	3.2	1.6	1.2	1.6	1.8
M4	*	*	1.0	4.9	5.3	5.3	2.6	1.4	1.0
M5	*	*	7.6	13.8	15.0	6.8	6.2	6.0	5.2
M5A	*	*	*	*	2.7	1.5	1.3	1.3	1.5
M5B	*	*	*	0.7	2.2	1.5	1.2	0.4	*
M5C	*	*	*	*	1.3	1.9	0.8	*	*
M6	*	*	9.0	6.6	3.0	4.0	2.8	2.5	2.9
M7	*	*	1.9	5.0	6.4	7.3	3.5	2.1	1.4
M10	*	*	4.1	2.5	3.6	4.6	4.7	3.2	2.7
M11	*	*	*	*	0.5	1.2	0.3	*	*
M12	*	*	*	0.4	0.5	0.6	*	*	*
M13	*	*	*	*	0.5	*	*	*	*
M14	*	*	*	*	0.7	*	*	*	*
M15	*	*	3.4	*	*	*	*	*	*
Adsorbed AR to organic matter	na	18.1	31.6	28.5	17.6	18.2	17.8	14.2	22.7
$^{14}CO_2$ total	na	0.1	0.6	6.8	8.39	20.3	35.2	55.8	47.9
Other volatile	na	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

Notes:
* = not detected
na = not analysed

Table 16: Applied Radioactivity (% AR) in high dose (100 µg/l) system

ID	% AR at Incubation Time (days) [mean of 2 replicates]								
	0	**0.25**	**1**	**3**	**5**	**7**	**10**	**20**	**29**
OIT	102	93.4	75.1	8.1	3.0	1.7	*	*	*
M1	*	*	*	13.9	16.4	22.8	6.6	6.4	8.6
M2	*	*	*	1.0	2.0	1.0	1.1	1.0	1.6
M4	*	*	*	6.5	2.2	2.5	3.0	1.6	2.7
M5	*	*	*	1.35	13.3	14.7	4.1	2.3	5.2
M5A	*	*	*	*	4.9	3.1	1.7	1.8	1.2
M5B	*	*	*	*	1.5	0.9	0.7	0.7	*
M5C	*	*	*	*	1.3	1.2	0.3	0.5	1.1
M6	*	*	2.1	10.5	8.1	4.9	2.0	1.8	3.5
M7	*	*	*	1.2	3.8	5.7	1.9	0.8	1.7
M10	*	*	*	*	1.1	1.8	1.4	0.5	2.6
M11	*	*	*	0.9	*	*	0.6	*	*
M12	*	*	*	*	*	*	0.2	*	*
M15	*	*	*	*	1.4	*	*	*	*
Adsorbed AR to organic matter	na	5.6	20.2	29.7	22	15	15	31	24.4
$^{14}CO_2$ total	na	<0.1	<0.1	6.8	8.8	13.9	47.4	43.6	36.4
Other volatile	na	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

Notes:
* = not detected
na = not analysed

Figure 2: Levels of OIT, degradants and CO_2 (% AR) in the low dose (10 µg/l) system over the study period

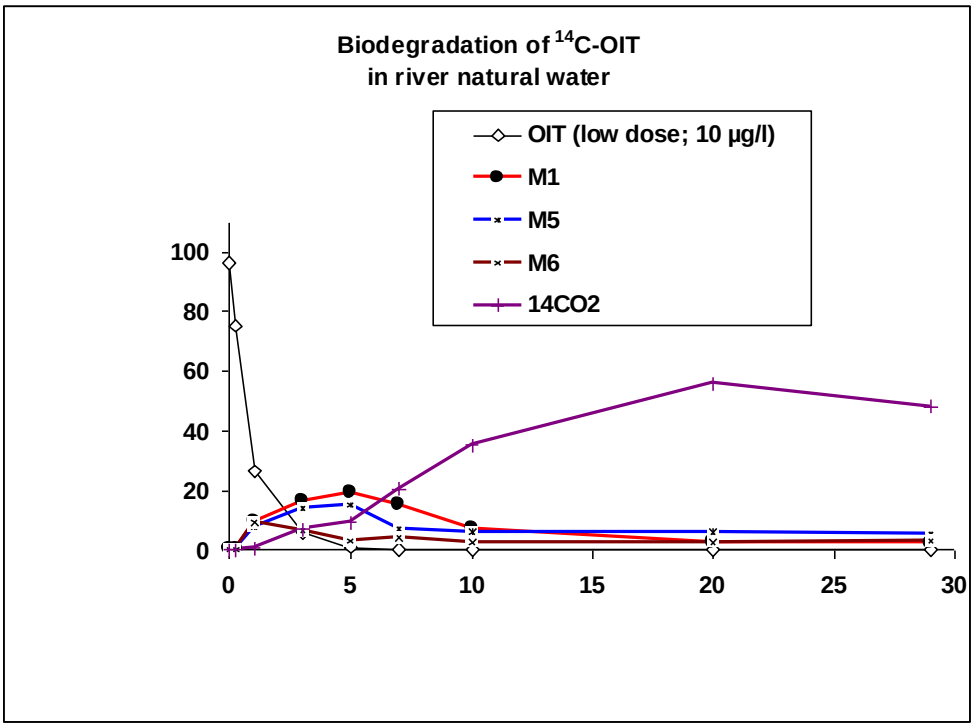


Figure 3: Levels of OIT, degradants and CO_2 (% AR) in the high dose (100 µg/l) system over the study period

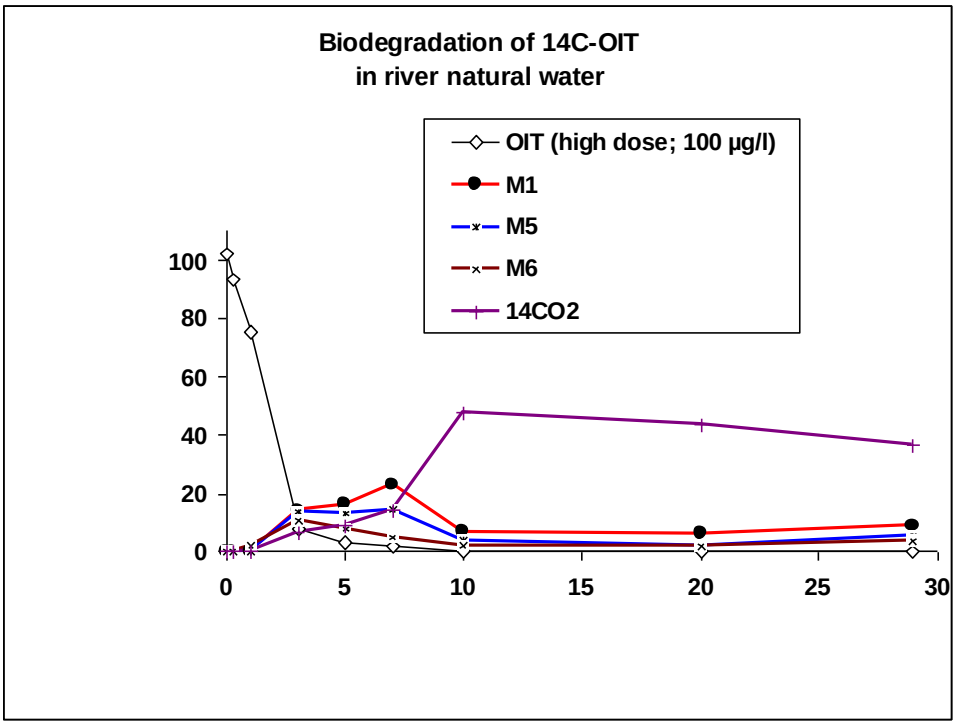


Figure 4: Applied Radioactivity (% AR) in the low dose (10 µg/l) system

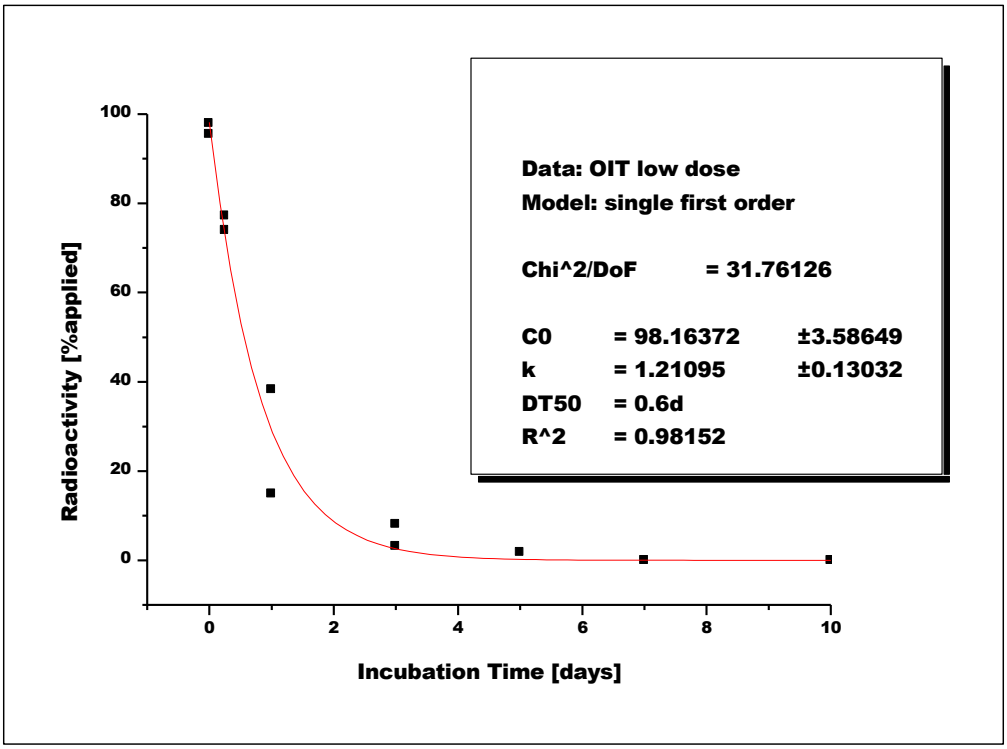


Figure 5: Applied Radioactivity (% AR) in the high dose (100 µg/l) system

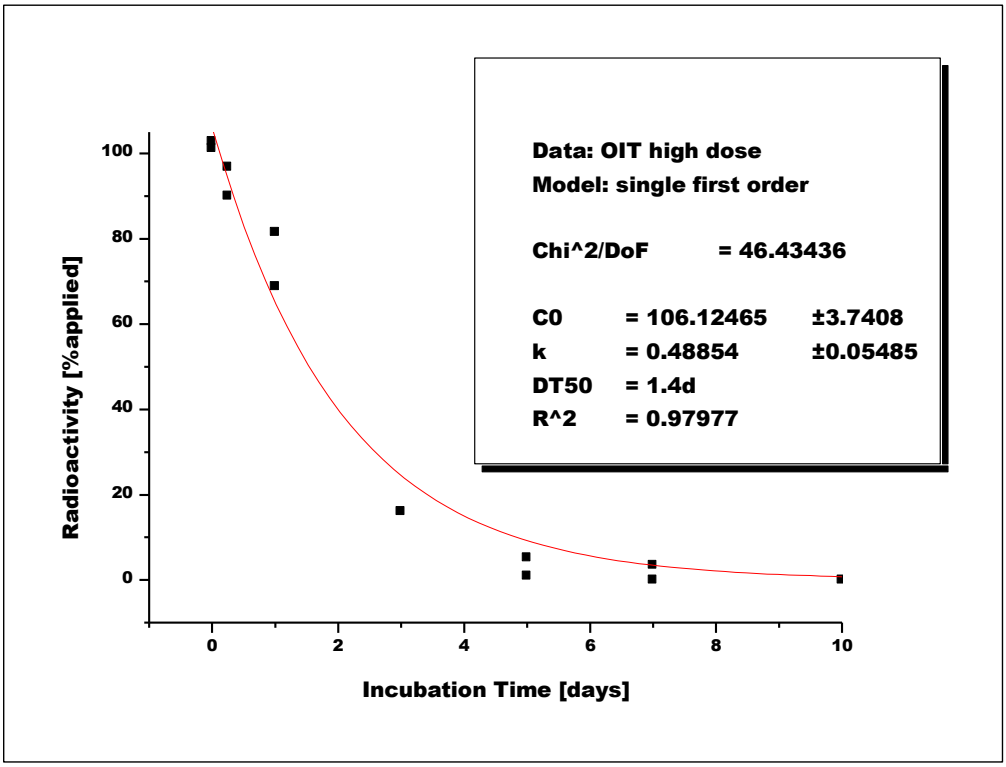
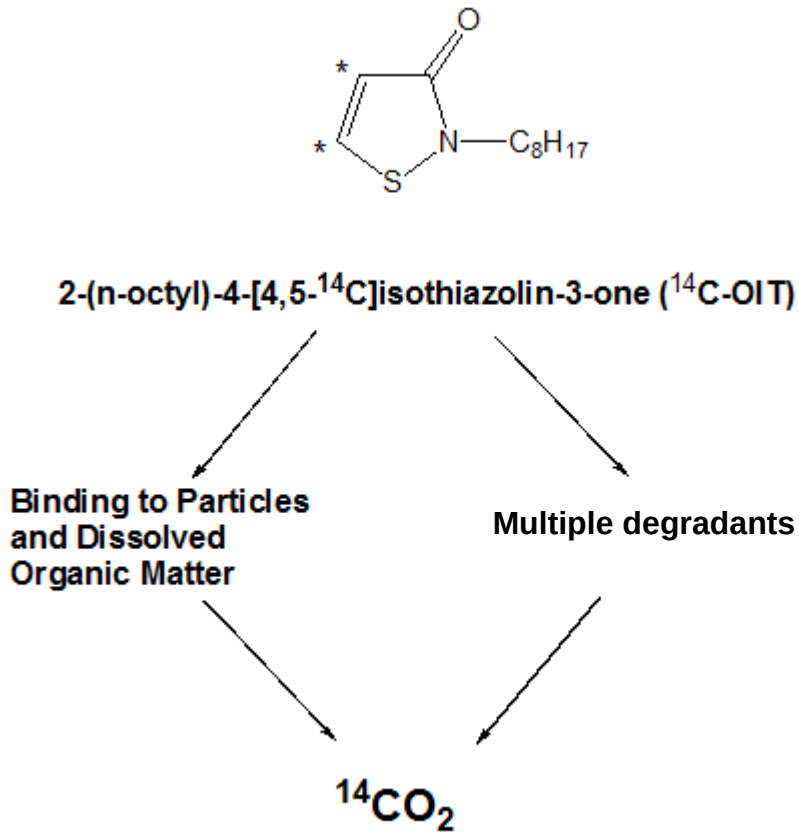


Figure 6: Proposed degradation pathway for ^{14}C-OIT in freshwater and seawater



Study 2 – Mamouni (2007b)

A seawater aquatic biodegradation simulation study is available following OECD Guideline 309 and GLP. The study used ^{14}C-OIT and surface water from St. Margaret's Bay, Kent, UK with unknown suspended solid or organic carbon concentrations. Seawater was filtered through a 0.2 mm sieve in the dark for 4 days at 4 ºC with aeration before use. A sediment phase was not included. Test vessels containing 0.0101 and 0.09986 mg/l OIT were maintained for 17 days under aerobic conditions at 20 ± 2 ºC. The test item was analysed by HPLC and radioactivity confirmed by LSC. Mean AR recoveries were 91.4 ± 7.6 % AR and 92.1 ±8.5 % AR for low and high doses.

Levels of AR in water decreased from 100.7 and 102.6 % on day 0 to 40.4 and 47 % on day 17 in the low and high dose systems respectively. The decrease in water phase AR is considered due to primary biodegradation of OIT to degradants which were subsequently mineralised and some partitioning to dissolved organic solid matter. This reflects the degradation pathways show in Figure 6 above. Levels of CO_2 peaked on day 17 at 44.6 % AR in the low dose system and at 36.8 % AR in the high dose system. No other volatile radioactivity was detected.

Measured concentrations of ^{14}C-OIT, degradants and CO_2 are presented in Tables 17 and 18 for low and high dose systems. Figures 7 to 10 show levels of OIT, degradants and CO_2 over time, and corresponding decrease in aquatic phase AR. It can be seen that ^{14}C-OIT concentrations rapidly decrease with an increase in degradant concentrations and subsequently CO_2 reflecting their mineralisation.

The level of AR in the dissolved organic matter fraction is higher in this study using seawater compared to the freshwater study, peaking at 49.4 % AR on day 7. This may be due to a higher organic matter fraction. The increase in adsorbed AR mirrored a slower rate of mineralisation from this point indicating less biodegradation in the presence of organic matter.

The study calculated DT_{50} values of 1.6 and 2.1 days for OIT reflecting primary degradation. For the CAR, these values were converted to 12 °C, resulting in DT_{50} values of 3 and 4 days.

Various degradants were observed at concentrations less than 10 % AR.

Table 17: Applied Radioactivity (% AR) in low dose (10 µg/l) system

ID	% AR at Incubation Time (days) [mean of 2 replicates]						
	0	**0.25**	**1**	**3**	**5**	**7**	**10**
OIT	96.2	88.8	19.9	2.7	3.7	na	na
M7	*	*	0.4	*	*	na	na
M5C	*	*	0.9	1.3	1.1	*	*
M1	*	*	0.3	1.1	1.4	*	*
M11	*	*	*	0.7	*	*	*
M12	*	*	*	1.4	*	*	*
M13	*	*	0.2	*	*	*	*
M15	*	*	0.3	*	*	*	*
M17	1.1	*	4.5	*	*	*	*
M20	*	*	0.3	1.0	*	*	*
M21	*	*	3.9	1.8	2.2	*	*
M22	*	*	0.9	0.9	1.7	*	*
M23	*	*	1.8	*	*	*	*
M24	*	*	0.6	0.7	*	*	*
Adsorbed AR to organic matter	3.4	9.5	46.3	60.9	50.2	68.5	40.4
$^{14}CO_2$ total	-	0.3	1.8	16	25.4	30.5	44.6
Other volatile	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

Notes:
* = not detected
na = not analysed

Table 18: Applied Radioactivity (% AR) in high dose (100 µg/l) system

ID	% AR at Incubation Time (days) [mean of 2 replicates]						
	0	**1**	**3**	**5**	**7**	**11**	**17**
OIT	99	95.5	41.4	15.1	3.1	2.2	0.7
M7	*	*	*	0.1	*	*	0.5
M10	*	*	2.4	0.4	*	*	0.1
M5B	*	*	*	*	0.5	0.5	0.1
M5C	*	*	0.4	0.6	0.9	*	*
M1	*	*	0.3	0.8	1.1	1.8	2.1
M11	*	*	*	0.4	0.9	0.8	0.1
M12	*	*	1.6	2.3	1.5	*	*
M13	*	*	0.6	0.3	*	*	*
M14	*	*	0.1	0.2	*	*	*
M15	*	*	*	0.1	*	*	*
M16	*	*	2.1	0.5	*	*	*
M17	*	*	*	1.7	*	*	*
M18	*	*	*	0.3	*	*	*
M19	*	*	3.3	0.4	*	*	*
M20	*	*	*	0.5	*	0.1	*
M21	*	*	9.2	5.4	4.6	2.6	0.4
M22	*	*	2.5	0.3	4	0.3	0.8
M23	*	*	3.6	3.3	2.6	6.4	3.4
M24	*	*	5.4	3.1	2.7	2.3	2.8
M25	*	*	0.9	3.2	1.1	2.2	1.4
M26	*	*	*	*	0.4	*	0.7
M27	*	*	*	*	*	*	*
Adsorbed AR to organic matter	3.6	6.2	17	40.8	49.4	38	34
$^{14}CO_2$ total	-	<0.1	0.7	16	17.2	22.1	36.8
Other volatile	na	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

Notes:
* = not detected
na = not analysed

Figure 7: Levels of OIT, degradants and CO_2 (% AR) in the low dose (10 µg/l) system over the study period

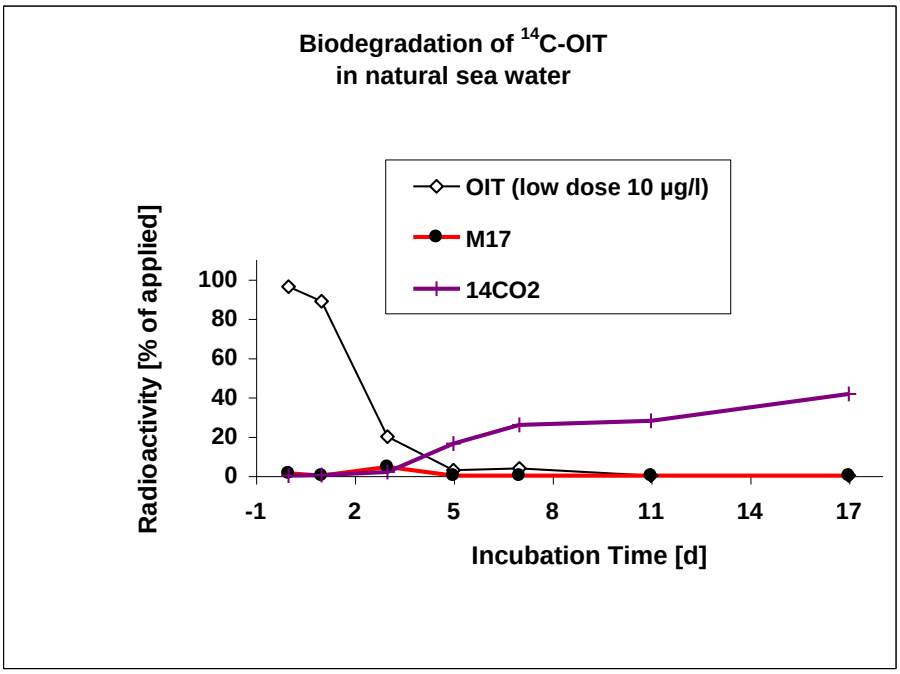


Figure 8: Levels of OIT, degradants and CO_2 (% AR) in the high dose (100 µg/l) system over the study period

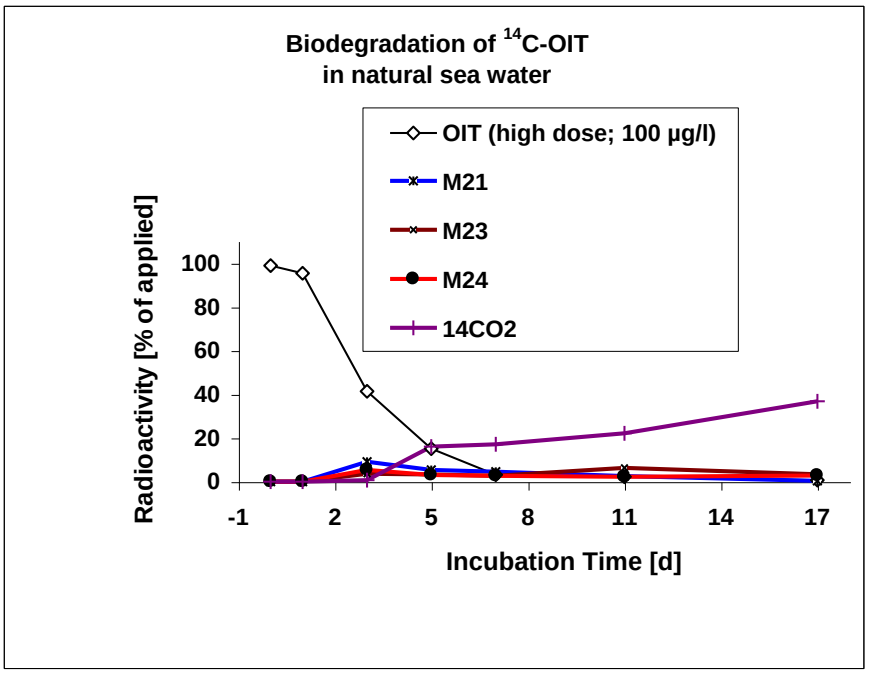


Figure 9: Applied Radioactivity (% AR) in the low dose (10 µg/l) system

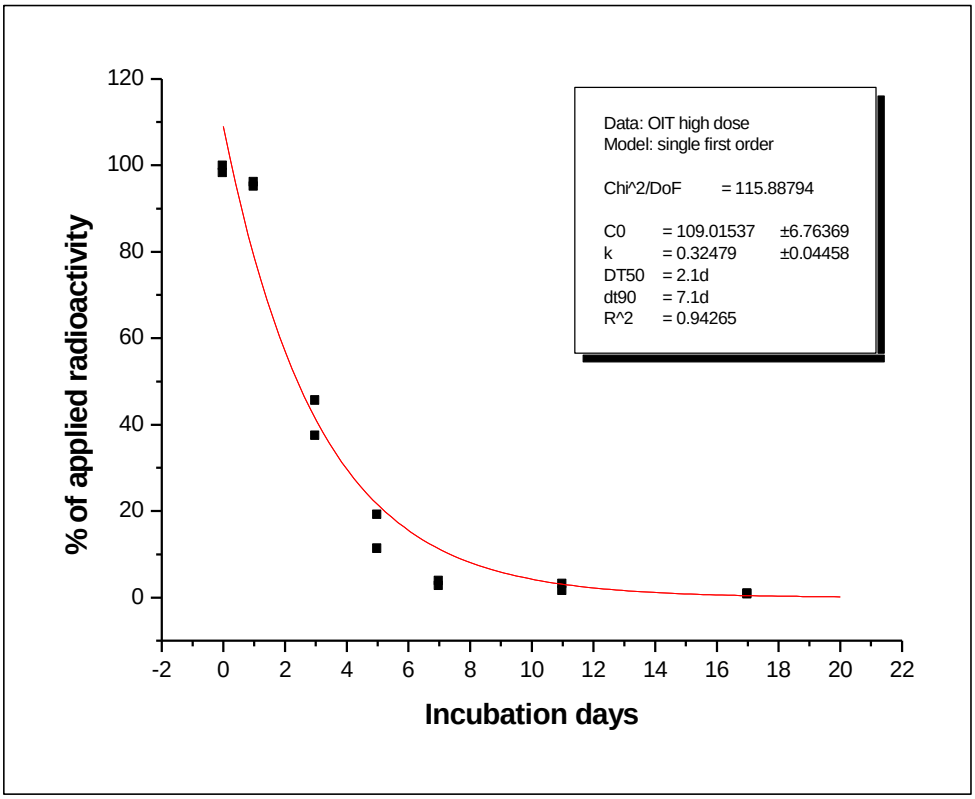
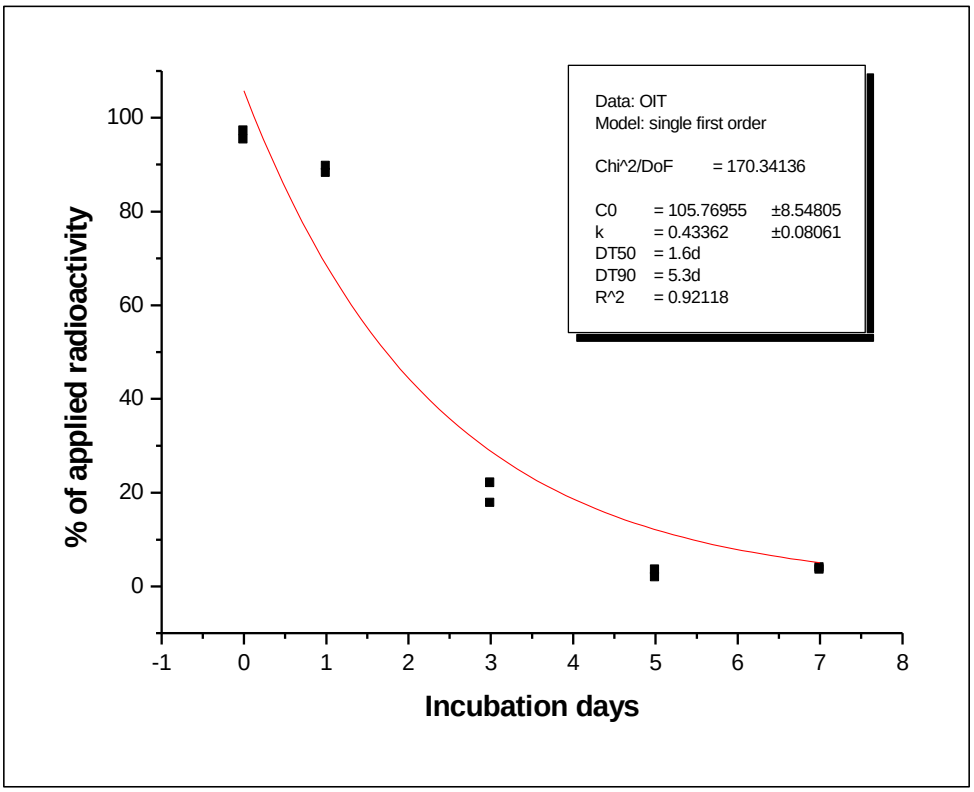


Figure 10: Applied Radioactivity (% AR) in the high dose (100 µg/l) system



5.1.3 Summary and discussion of degradation

OIT is considered hydrolytically stable.

OIT is susceptible to photodegradation. The experimental DT_{50} in sterile pure water was 3.7 days at 50°N in summer sunlight. The experimental DT_{50} in pond water was 5.1 days at 50°N in summer sunlight. The photodegradation DT_{50} values reflect degradation to degradants and mineralisation to CO_2. The maximum level of observed mineralisation was 12.5 % AR CO_2 by day 30. Of the observed photodegradants further information on the fate of two is available indicating that they are rapidly degradable.

It is noted that the actual degree of photodegradation in the aquatic environment depends on local conditions and seasons and is difficult to quantify. Given the available data, aquatic photolysis is not considered to meet the criteria for rapid degradation.

In a valid ready biodegradation study no degradation was observed and the substance was considered not rapidly biodegradable. However, based on degradation in the toxicity control, the test item may have limited microorganism activity to some extent.

Two aquatic biodegradation simulation studies are available using freshwater and seawater indicating that OIT undergoes significant microbial degradation. In both systems, OIT was observed to dissipate from the water column via transformation to various unidentified degradation products, adsorption to organic matter and mineralisation to CO_2. The maximum observed mineralisation was 58.5 % AR on day 20 in the freshwater system. Both studies did not include sediment although a significant proportion of AR was adsorbed to dissolved organic matter. In the seawater study this peaked at 49.4 % AR on day 7. This mirrored a slower rate of mineralisation from this point indicating less biodegradation in the presence of organic matter.

While fate data are available for the 2 key OIT degradants NNOA and NNOMA identified in the photolysis studies, the fate of all degradants is unclear e.g. those noted in simulation studies. In addition, full ecotoxicity data are not available for all degradants. This means the toxicity and classification of the wide range of identified and unidentified degradants is unknown.

Degradation half-lives can be used in defining rapid degradation provided that ultimate degradation of the substance, i.e. full mineralisation, is achieved. This equates to a half-life <16 days corresponding to a degradation of >70 % within 28 days. Primary biodegradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification. While significant degradation was observed in both aquatic simulation studies (primary degradation DT_{50} values between 1.1 and 4 days at 12 °C), ultimate degradation (as recorded by CO_2 reflecting mineralisation) did not reach >70 % within 28 days. Given the available ecotoxicity information for identified degradants (e.g. NNOMA ecotoxicity testing indicates that it would be classified for the environment – see Annex II) and the lack of ecotoxicity information for unidentified degradants, OIT degradants cannot be considered non-classifiable.

Overall, the degradation information does not provide sufficient data to show that OIT is ultimately degraded (mineralised) within 28 days (equivalent to a half-life < 16 days) or undergoes primary degradation to non-classifiable products with half-lives < 16 days. Consequently, OIT is considered not rapidly degradable for the purpose of classification and labelling.

This approach is consistent with other isothiazolone substances[1].

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Study 1 – Reynolds (2001)
Following OECD Test Guideline 106 and GLP, the study used ^{14}C and ^{12}C radiolabelled OIT with a purity of 95.8 % and 99.98 %. Three soils and one sediment from the USA were employed. Composition, pH and fraction organic matter was recorded. The K_{aoc} (adsorption coefficient based on organic carbon content) values ranged between 604 and 1297 ml/g. This equates to log K_{oc} values between 2.78 and 3.11. The geometric mean of the four results was 982 ml/g equating to log K_{oc} 2.99. This indicates a moderate propensity for distribution to sediments/sludge and a moderate mobility in soil.

Study 2 – Vökel (2007)
Following USA EPA OPPTS Guideline and GLP, the study used ^{14}C radiolabelled OIT with a purity of 99.2 %. The study used sterile domestic sewage sludge with varying concentrations of sludge. Adsorption was observed to be dependent on sludge concentration. The K_{foc} (Freundlich sorption constant based on organic carbon content) value for the sewage sludge was determined to be 6740 equating to log K_{oc} 3.8.

5.2.2 Volatilisation

Experimental data (Tognucci (2002)) indicate the vapour pressure for OIT is 3.1×10^{-3} Pa at 20 °C and 6.1×10^{-3} Pa at 25 °C following OECD Test Guideline 104. The values are based on extrapolation following linear regression considering experimental vapour pressure values at 50, 60 and 70 °C.

The Henry's Law Constant of 3.14×10^{-3} Pa m^3 mol^{-1} was calculated (Tognucci (2002)) using the extrapolated vapour pressure at 25 °C and an assumed water solubility of 414 mg/l at 20-30 °C. While this water solubility value is not a direct experimental value it is in broad agreement with experimental values (Geffke (2003a)) for the temperature range across environmentally relevant pHs.

Overall, OIT is unlikely to partition from the water phase to air.

5.2.3 Distribution modelling

Not relevant for classification and labelling.

[1] For example MIT [CAS: 2682-20-4] and C(M)IT/MIT [CAS: 55965-84-9] which were considered not rapidly degradable following RAC opinions dated 10 March 2016 were. Both substances were considered to undergo rapid primary degradation to identified and unidentified transformation products including one which was classifiable.

5.3 Aquatic Bioaccumulation

Table 19: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water (calculation from concentration in water and *n*-octanol)	Log K_{ow} >3.1 at 20 °C	Based on solubility in *n*-octanol and water at pH 5 and 7 at 20°C	Kühne (2010)
Experimental aquatic BCF OECD Guideline 305, GLP	OIT steady state whole fish BCF: 507 to 538 l/kg wet weight Depuration half-life DT_{50} whole fish: 1.97 to 1.53 days	Flow through, 14 days exposure, 14 days depuration Valid	Anonymous (2007b)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

OIT is considered surface active. Therefore, log K_{ow} studies determined using the HPLC method or the shake flask method are not considered reliable and not discussed further.

Kühne (2010) calculated the log K_{ow} based on the solubility of OIT in *n*-octanol (>524.8 g/l at 20°C) and measured water solubilities at different pH (pH 5, 7 and 9) and temperature (10 °C, 20 °C and 30 °C). As the solubility in water did not differ significantly, there was no significant effect on the log K_{ow} value which is considered to be >3.1.

5.3.1.2 Measured bioaccumulation data

An experimental aquatic BCF study for OIT (purity 99.2 %) is available following GLP and OECD Guideline 305 (Anonymous (2007b)). The study used ^{14}C-OIT, a flow-through system with Rainbow Trout (*Oncorhynchus mykiss*) and two exposure concentrations; 0.0001 and 0.00048 mg/l. The exposure period ran for 14 days followed by a 14 day depuration period. The pH values ranged from 7.8 to 8.2. Analysis of Total Radioactive Residues (TRR) by Liquid Scintillation Counting (LSC) indicated the parent compound (OIT) accounted for 97-99 % of radioactivity.

The study whole fish steady state BCFs were 507 ± 87 l/kg (high dose) to 538 ± 65 l/kg (low dose) wet weight. The depuration half-lives were 1.97 days for the low dose and 1.53 for the high dose.

BCFs were not growth corrected although given the uptake duration; any corrections are likely to have little impact on BCF values.

Lipid analysis of control fish on day 14 provided a whole fish lipid value of 31.4 mg/g equating to 3.14 %. Lipid normalising the BCFs to 5 % lipid content following OECD Test Guideline 305 dated October 2012, would increase BCFs to 843 to 886 l/kg wet weight.

5.3.2 Summary and discussion of aquatic bioaccumulation

The experimental log K_{ow} for OIT is considered >3.1 based on concentrations in pure water and *n*-octanol.

Experimental steady state whole fish BCFs were 507 to 538 l/kg wet weight. Lipid normalised (5 % lipid fraction) steady state whole fish BCFs were calculated as 843 to 886 l/kg.

Both experimental steady state BCFs and lipid normalised steady state BCFs are above the BCF trigger of ≥ 500 intended to identify substances with a potential to bioaccumulate.

5.4 Aquatic toxicity

A summary of available valid information on the aquatic toxicity of OIT is presented in Table 20. Where available, a summary of valid information for degradants is also included in Annex II, Table 1. Additional studies were noted during the Biocides Review. However these were considered non-key studies. They were assessed for this report but are not considered reliable for classification as they were not conducted to GLP or acceptable guidelines and did not include analytical verification. On this basis, further details have not been included.

Studies reviewed under the Biocidal Products Regulation (EU) No. 528/2012 and considered reliable are summarised in the CAR. Further details are presented for studies conducted on the active substance OIT but not for its degradants as these are relatively less toxic than the parent (see Annex II). These degradant data are not considered further in relation to the classification of OIT.

OIT is an isothiazolone biocide. Algae are the most sensitive trophic level. Isothiazolones are rapidly taken up by algae where they react with enzymes to disrupt metabolism and inhibit growth (Williams (2007)) as a toxic response. During this process cleavage of the isothiazole ring occurs and the parent substance is depleted. This mode of action in algae is rapid with uptake and enzyme effects in minutes affecting cell viability and resulting in cell death over hours. It also means that algal toxicity is dependent on initial substance concentration and algal cell density with greatest inhibition of algae resulting in slower degradation in test systems.

For some studies, results were presented as nominal or initial measured values during the Biocides Review. This approach was acceptable for the purpose of biocides risk assessment. In general for the purpose of classification, it is preferable to quote effect values based on measured concentration data. Where test substance losses >20 % are observed, endpoints are required to be reported on the basis of mean measured concentrations by most test guidelines. However, given the rapid uptake and depletion of OIT by algal cells results in significant losses over a short period of time, initial measured concentrations are considered to reflect representative concentrations which induce ecotoxic responses in algal studies. This is because mean measured concentrations would include a period of time when very little test item was available (generally below the analytical limit of detection) resulting in unrealistic calculated mean measured concentrations lower than those which induce the inhibition effect. It is considered that this non-standard approach is adequate to determine the appropriate aquatic classification and M-factors for OIT. Where available, reliable mean measured concentrations are included for comparison.

In the case of isothiazolones the rapid knock-down of algal cells with depletion of the substance by uptake and metabolism means that shorter duration endpoints may be appropriate for consideration of acute toxicity to algae. This is suitable as multiple generations are not required and up to 48 hours accounts for the period of time before algal populations recover when the test item is depleted from solution.

For the purpose of chronic classification, it is considered that the standard chronic time period of 72-96 hours for algal studies is appropriate to ensure multiple algal generations. It is noted that OECD Test Guideline 201 allows studies to be shortened to 48 hours if a 16 fold increase in cells is observed in controls indicating exponential growth and multiple generations. This is discussed for each study.

The environmental classification of similar isothiazolone substances (MIT, CAS: 2682-20-4; C(M)IT/MIT, CAS 55965-84-9) have been recently been harmonised following discussion at ECHA's Risk Assessment Committee (RAC 36 March 2016). It was agreed that algae are the most sensitive trophic level and given the mode of action, algal study results based on initial measured may be appropriate. In addition, for acute classification it was agreed a shorter duration algal endpoints may also be appropriate.

Table 20: Summary of relevant information on aquatic toxicity for OIT

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
Acute toxicity to fish US EPA FIFRA Guideline 72-1, GLP, purity: 98.5 %	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Flow-through	96 hours	LC_{50}	0.047 (mm)	Anonymous (1990b)
Acute toxicity to fish US EPA FIFRA Guideline 72-1, GLP, purity: 98.5 %	Bluegill Sunfish (*Lepomis macrochirus*)	Mortality	Flow-through	96 hours	LC_{50}	0.180 (mm)	Anonymous (1990c)
Acute toxicity to fish US EPA FIFRA Guideline 72-3, GLP, purity: 98.5 %	Sheepshead Minnow (*Cyprinodon variegatus*)	Mortality	Flow-through	96 hours	LC_{50}	0.160 (mm)	Anonymous (1990d)
Acute toxicity to fish US EPA OPPTS Guideline 850.1075, GLP, purity: 99.2 %	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	0.036 (mm)	Anonymous (1998)
Acute toxicity to fish US EPA OPPTS Guideline 72-1, GLP, purity: 96 %	Bluegill Sunfish (*Lepomis macrochirus*)	Mortality	Semi-static	96 hours	LC_{50}	0.16 (0 to 72h mm)	Anonymous (1995b)
Prolonged toxicity to fish, OECD Guideline 204, GLP, purity: 99.2 %	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality, weight and length	Static	21 days (extended from 14 days)	NOEC	0.022 (mm)	Anonymous (1999)
Fish Early Life-Stage (FELS) toxicity US EPA FIFRA Guideline 72-4, GLP, purity: 98.5 %	Fathead Minnow (*Pimephales promelas*)	Embryo survival and larval growth / survival	Flow-through	35 days	NOEC	0.0085 (mm) egg hatchability, growth and survival	Anonymous (1991g)
Daphnia sp Acute Immobilisation US EPA FIFRA Guideline 72-2, GLP, purity: 98.5 %	*Daphnia magna*	Acute immobilisation	Flow-through	48 hours	EC_{50}	0.32 (mm)	Mc Namara (1990)
Daphnia sp Acute Immobilisation US EPA FIFRA Guideline 72-2, GLP, purity: 99.2 %	*Daphnia magna*	Acute immobilisation	Semi-static	48 hours	EC_{50}	0.42 (n)	Noack (1998)
Daphnia sp Acute Immobilisation OECD Guideline 202 Part I, GLP, purity: 96 %	*Daphnia magna*	Acute immobilisation	Semi-static	48 hours	EC_{50}	0.1 (mm)	Wyness (1996)

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
Acute Toxicity US EPA FIFRA Guideline 72-3, GLP, purity: 98.5 %	Mysid Shrimp (*Mysidopsis bahia*)	Mortality	Flow-through	96 hours	LC_{50}	0.071 (mm)	Sousa (1990d)
Acute Toxicity US EPA FIFRA Guideline 72-3, GLP, purity: 98.5 %	Oyster (*Crassostrea virginica*)	Shell growth	Flow-through	96 hours	EC_{50}	0.013 (mm)	Dionne (1990)
Daphnia magna Reproduction US EPA FIFRA Guideline 72-4, GLP, purity: 98.5 %	*Daphnia magna*	Survival; reproduction; growth	Flow-through	21 days	NOEC	0.074 (mm)	Mc Namara (1991)
Daphnia magna Reproduction OECD Guideline 202, Part II, GLP, purity: 96 %	*Daphnia magna*	Survival; reproduction; growth	Semi-static	21 days	NOEC	0.003 (n)	Wyness (1996)
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 99.9 %	*Skeletonema costatum*	Cell multiplication inhibition	Static	24 hours	ErC_{50}	Not available	Seyfried (2007 and industry analysis 2016, 2017)
				48 hours	ErC_{50}	**0.00193 (im)**	
				72 hours	ErC_{50}	0.00161 (im)	
				96 hours	E_rC_{50}	0.00168 (im)	
				96 hours	E_rC_{50}	0.00029 (mm)	
				24 hours	ErC_{10}	Not available	
				48 hours	ErC_{10}	0.00118 (im)	
				72 hours	ErC_{10}	**0.00133 (im)**	
				96 hours	E_rC_{10}	**0.00138 (im)**	
				96 hours	E_rC_{10}	0.000264 (mm)	
				24 hours	NOErC	Not available	
				48 hours	NOErC	0.00068 (im)	
				72 hours	NOErC	0.00068 (im)	
				96 hours	NOErC	0.00038 (im)	
				96 hours	NOErC	0.000184 (mm)	
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 99.3 %	*Desmodesmus subspicatus**	Cell multiplication inhibition	Static	24 hours	ErC_{50}	>0.201 (im)	Scheerbaum (2000 and industry analysis 2016, 2017)
				48 hours	ErC_{50}	0.139 (im)	
				72 hours	ErC_{50}	0.0979 (im)	
				72 hours	E_rC_{50}	0.076 (mm)	
				24 hours	ErC_{10}	0.0208 (im)	
				48 hours	ErC_{10}	0.0208 (im)	
				72 hours	ErC_{10}	0.0239 (im)	
				72 hours	E_rC_{10}	0.0198 (mm)	
				24 hours	NOErC	0.0180 (im)	
				48 hours	NOErC	0.0180 (im)	
				72 hours	NOErC	0.0211 (im)	
				72 hours	NOErC	0.0156 (mm)	

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 99.2 %	*Pseudokirchneriella subcapitata***	Cell multiplication inhibition	Static	24 hours	ErC_{50}	Not available	Hoberg (1996 and industry analysis 2016, 2017) †
				48 hours	ErC_{50}	**0.0054 (im)**	
				72 hours	ErC_{50}	0.026 (im)	
				72 hours	E_rC_{50}	0.025 (mm)	
				24 hours	ErC_{10}	Not available	
				48 hours	ErC_{10}	0.0011 (im)	
				72 hours	ErC_{10}	**0.0059 (im)**	
				72 hours	E_rC_{10}	0.0068 (mm)	
				96 hours	ErC_{10}	0.0036 (im)	
				72 hours	NOErC	0.0011 (im)	
				72 hours	NOErC	0.00049 (mm)	
				96 hours	NOErC	0.031 (im)	
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity: 99.2 %	*Navicula pelliculosa*	Cell multiplication inhibition	Static	24 hours	ErC_{50}	0.00152 (im)	Porch et al (2011 and industry analysis 2016)
				48 hours	ErC_{50}	**0.00129 (im)**	
Lemna sp. Growth Inhibition Test OECD Guideline 221 (draft), GLP, purity: 99.9 %	*Lemna gibba*	Growth	Static	7 days	ErC_{50}	0.62 (mm)	Volz (2007)
					ErC_{10}	0.041 (mm)	
					NOErC	0.0087 (mm)	

Notes:
mm refers to mean measured concentration
n refers to nominal concentration
im refers to initial measured concentration
*formerly *Scenedesmus subspicatus*
**formerly *Selenastrum capricornutum*
Bold values indicate most sensitive acute and chronic endpoints used for hazard classification
† It is noted that mean measured endpoints are lower than initial measured endpoints which is unusual. This is due to a clearer distribution and dose response for the initial measured model. A reduced goodness of fit dose response model was observed using mean measured concentrations reflecting lower losses (less than 20%) for higher treatments and greater losses (60-80%) for lower treatments.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Valid static, acute toxicity to fish studies using OIT following GLP and guidelines similar to OECD Test Guideline 203 are discussed below.

Study 1 – Anonymous (1990b)
Using Rainbow Trout (*Oncorhynchus mykiss*) the nominal exposure range was 0.017, 0.028, 0.047, 0.078 and 0.13 mg a.s./l. Exposure solutions were prepared with the aid of the solvent triethylene glycol (TEG) at 0.094 ml/l and a solvent control was included. Study conditions were acceptable and validation criteria were met. Analytical concentrations by HPLC UV detector (HPLC-UV) were 86 to 101 % of nominal. The study 96-h LC_{50} was 0.047 mg a.s./l based on mean measured concentrations (95 % confidence interval 0.042 to 0.053 mg a.s./l).

Study 2 – Anonymous 1990c

Using Bluegill Sunfish (*Lepomis macrochirus*) the nominal exposure range was 0.065, 0.11, 0.180, 0.308 and 0.5 mg a.s./l. Exposure solutions were prepared with the aid of the solvent TEG at 0.092 ml/l and a solvent control was included. Study conditions were acceptable and validation criteria were met. Analytical concentrations by HPLC-UV were 0.069, 0.11, 0.16, 0.27 and 0.42 mg a.s./l. The study 96-h LC_{50} was 0.180 mg a.s./l based on mean measured concentrations with 95 % confidence interval 0.16 to 0.2 mg/l.

Study 3 – Anonymous 1990c

Using Sheepshead Minnow (*Cyprinodon variegatus*) the nominal exposure range was 0.052, 0.086, 0.14, 0.24 and 0.4 mg a.s./l. Exposure solutions were prepared with the aid of the solvent TEG at 0.094 ml/l and a solvent control was included. Study conditions were acceptable and validation criteria were met. Analytical concentrations by HPLC-UV were 0.04, 0.054, 0.11, 0.210 and 0.37 mg a.s./l. The study 96-h LC_{50} was 0.160 mg a.s./l based on mean measured concentrations with 95 % confidence interval 0.14 to 0.19 mg a.s./l.

Study 4 – Anonymous (1998)

Using Rainbow Trout (*Oncorhynchus mykiss*) the nominal exposure range was 0.01, 0.02, 0.4, 0.08 and 0.16 mg/l. Exposure solutions were prepared by ultrasonication. Study conditions were within the test guideline range and validation criteria were met. Analytical verification by HPLC-UV was 50 to 76 % of nominal concentrations at 0.0078, 0.0144, 0.0296, 0.0621 and 0.1416 mg/l. The study 96-h LC_{50} was 0.036 mg/l based on mean measured concentrations with 95 % confidence interval 0.003 to 0.039 mg/l.

Study 5 – Anonymous (1995b)

Using Bluegill Sunfish (*Lepomis macrochirus*) the nominal exposure range was 0.05, 0.07, 0.12, 0.19 and 0.3 mg/l. Exposure solutions were prepared by direct addition and study conditions followed the US EPA FIFRA OPPTS Guideline 72-1. The study employed a semi-static system with test media renewal at 24 hour intervals Analysis of fresh and expired media at 0, 24, 48 and 72 hours was conducted by HPLC-UV. The mean measured concentrations over this period were 0.04, 0.05, 0.07, 0.1 and 0.18 mg/l. The quoted study 96-h LC_{50} is 0.16 mg/l with 95 % confidence interval 0.13 to 0.26 mg/l based on 0 to 72 hour mean measured concentrations.

While analytical verification does not cover the whole 96 hour study period, it does reflect fresh and expired media renewals over the majority of the test period. In addition, the last 24 hour period is not anticipated to reflect a significant difference in test item losses than previous hour periods. On this basis, it is acceptable to include the study for the purpose of classification and labelling, noting the study is not the most sensitive endpoint for the fish trophic level.

Additional information – Anonymous (1995a)

Using Rainbow Trout (*Oncorhynchus mykiss*) the nominal exposure range was 0.06, 0.12, 0.25, 0.5 and 1.0 mg/l. Exposure solutions were prepared by direct addition and study conditions followed the US EPA FIFRA OPPTS Guideline 72-1. The study employed a semi-static system with test media renewal at 24 hour intervals. Limited analysis by HPLC-UV was conducted with only the lowest exposure concentrations of 0.06 mg/l nominal analysed at 0, 24, 48 and 72 hours (fresh and expired media) resulting in a mean measured concentration of 0.05 mg/l. At this treatment, 40 % mortality was observed at 96 hours. The remaining treatments only included analytical verification in fresh media at 0 hours and expired media at 24 hours. Over this period mean measured concentrations were 67 to 88 % nominal. The quoted study 96-h LC_{50} is 0.05 mg/l is based on the aforementioned limited measured concentrations.

Given the lack of analytical verification for the study period across the exposure range and the observed losses during the first 24 hours, the endpoints are not considered to be valid for classification and labelling. This is not considered to impact the classification proposal as adequate data for the endpoint and species is available.

Key acute fish trophic level endpoint:
Rainbow Trout are the most acutely sensitive fish species with LC_{50} values between 0.036 and 0.047 mg/l based on measured concentrations.

Additional information:
A prolonged toxicity to fish study following OECD Test Guideline 204 extended to 21 days is available (Anonymous (1999)). The study was extended to 21 days and included measurement of body weight and length. The study is not considered to assess long-term toxicity to fish as sensitive life stages such as eggs, larvae and juveniles were not exposed. No significant difference was observed between control and treatment fish for weight. The study 21-day NOEC was 0.022 mg/l based on mortality and mean measured concentrations. This endpoint is less sensitive than the 35-d NOEC from the (Anonymous (1991g)) study which is considered the current definitive value for the chronic fish toxicity endpoint.

5.4.1.2 Long-term toxicity to fish

A 35-day flow-through chronic toxicity to fish study (Anonymous 1991g) using OIT following GLP and US EPA FIFRA Guideline 72-4 is available. The study used Fathead Minnow (*Pimephales promelas*) and the following endpoints: time to hatch, hatching success, survival and growth (length and dry weight). General observations were also recorded. Study conditions were acceptable and validation criteria were met. The nominal exposure range was 0.0031, 0.0063, 0.013, 0.025 and 0.05 mg a.s./l. Exposure solutions were prepared with the aid of the solvent TEG at 0.0175 ml/l and a solvent control was included. Analytical verification was by HPLC-UV with measured values 65 to 87 % of nominal. Significant effects were determined by the William's Test. The most sensitive endpoints were egg hatchability, growth (length and wet weight) and survival where the 35-d NOEC for all these parameters was determined to be 0.0085 mg a.s./l based on mean measured concentrations.

Key chronic fish trophic level endpoint:
Based on current data, the key endpoint is the 35-day NOEC of 0.0085 mg a.s./l for Fathead Minnow based on measured concentrations.

It is noted that an acute toxicity to fish study using Fathead Minnow is not available. Therefore it is currently unclear if the available chronic fish test reflects the most sensitive fish species.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Study 1 – Mc Namara (1990)
A flow-through acute toxicity to *Daphnia magna* study using OIT is available following US EPA FIFRA 72-2 and GLP. The nominal exposure range was 0.130, 0.22, 0.36, 0.6 and 1.0 mg a.s./l. Exposure solutions were prepared with the aid of the solvent TEG at 0.061 ml/l and a solvent control

was included. Study conditions were acceptable and validation criteria were met. Analytical concentrations by HPLC-UV were 0.12, 0.21, 0.34, 0.59 and 1.0 mg a.s./l. The study 48-h LC_{50} was 0.32 mg a.s./l based on mean measured concentrations with 95 % confidence interval 0.21 to 0.59 mg a.s./l.

Study 2 – Noack (1998)

A semi-static acute toxicity to *Daphnia magna* study is available following GLP and US EPA OPPTS Guideline 850.1010. The nominal exposure range was 0.1, 0.18, 0.32, 0.58, 1.0 and 1.8 mg/l. Analytical measurement by HPLC-UV were 89 to 112 % of nominal for fresh media and 76 to 106 % of nominal for expired media. A greater than 20 % loss was observed in one replicate out of four samples at exposure concentrations 0.18 mg/l (78 % nominal) and 0.32 mg/l (76 % of nominal). The study is considered valid and reliable. Based on nominal concentrations, the 48-h EC_{50} was 0.42 mg/l with 95 % confidence interval 0.38 to 0.45 mg/l. While the >20 % loss of test item is noted in 2 samples, recalculating the EC_{50} based on measured data would not reduce the value below the most sensitive acute endpoints for algae.

Study 3 – Wyness (1996)

A static acute toxicity to *Daphnia magna* study is available following GLP and OECD Test Guideline 202 Part 1. The nominal exposure range was 0.065, 0.13, 0.25, 0.5, and 1.0 mg/l prepared by direct addition. Analytical measurement by HPLC-UV resulted in mean measured concentrations of 0.055, 0.096, 0.171, 0.356 and 0.742 mg/l. Using Probit analysis and log transformed data, the 48-h EC_{50} was 0.1 mg/l based on mean measured concentrations.

Study 4 – Dionne (1990)

A flow-through acute toxicity study to the marine oyster (*Crassostrea virginica*) is available using OIT. The study was run to GLP and followed US EPA FIFRA Guideline 72-3. The nominal exposure range was 0.0063, 0.013, 0.025, 0.05 and 0.1 mg a.s./l. Exposure solutions were prepared with the aid of the solvent TEG and a solvent control was included. Review under the Biocidal Products Regulation noted the solvent control contained 81 % of the maximum solvent concentration in exposure solutions but this is not considered to invalidate the study. Results were based on mean measured values: 0.003, 0.0062, 0.011, 0.024 and 0.057 mg a.s./l. The study is considered valid and reliable. Based on shell deposition, the study 96-h EC_{50} was 0.013 mg a.s./l with 95 % confidence interval 0.0064 to 0.025 mg a.s./l based on mean measured. A NOEC was not determined as effects were observed at all exposure concentrations.

Study 5 – Sousa (1990d)

A flow-through acute toxicity study to the marine mysid shrimp *Mysidopsis* [now *Americamysis*] *bahia* is available using OIT. The study was run to GLP and followed US EPA FIFRA Guideline 72-3. The nominal exposure range was 0.039, 0.065, 0.110, 0.180 and 0.3 mg a.s./l. Exposure solutions were prepared with the aid of the solvent TEG at 0.092 ml/l and a solvent control was included. Results were based on mean measured values: 0.034, 0.055, 0.086, 0.14 and 0.24 mg a.s./l. The study is considered valid and reliable. Based on the mortality, the study 96-h EC_{50} was 0.071 mg a.s./l with 95 % confidence interval 0.055 to 0.088 mg a.s./l based on mean measured concentrations. A NOEC was not determined due to ≥15 % mortality at all exposure concentrations.

Key acute invertebrate trophic level endpoint:

The marine oyster is the most acutely sensitive invertebrate species with an acute EC_{50} of 0.071 mg/l based on measured concentrations.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Study 1 – McNamara (1991)

A flow-through chronic toxicity to *Daphnia magna* study using OIT is available following GLP and US EPA FIFRA Guideline 72-4. The study assessed the following endpoints: survival, reproduction, length and weight. The study guideline validity criteria were met. The nominal exposure range was 0.0063, 0.013, 0.025, 0.05 and 0.1 mg a.s./l. Exposure solutions were prepared with the aid of the solvent TEG at 0.017 ml/l and a solvent control was included. Analytical verification was by HPLC-UV with measured values 51 to 80 % of nominal. Results were based on mean measured values: 0.0032, 0.0079, 0.017, 0.04 and 0.074 mg a.s./l.

No significant difference was observed between controls and solvent controls for length and survival. On this basis, exposure solutions were compared to pooled control and solvent control data. A significant difference was observed between controls and solvent controls for weight and reproduction. Therefore, exposure solutions were compared to the solvent control group only.

Significant effects were determined by the Dunnett's Test. No significant effects were observed in the four highest test concentrations for survival, reproduction, weight or length. Significant effects were observed at the lowest exposure concentration for length and reproduction (number of offspring). As no effects were observed at the higher concentrations, this was not considered to be in response to the test item. It was considered to be a result of smaller adult *Daphnia* randomly placed in lower concentration vessels.

The 21-day NOEC was considered to be 0.074 mg a.s./l (survival, reproduction, length and weight) based on mean measured concentrations.

Study 2 – Wyness (1996)

A semi-static chronic toxicity to *Daphnia magna* study using OIT is available following GLP and OECD Guideline 202 Part II. The study assessed the following endpoints: survival, reproduction and length. The nominal exposure range was 0.001, 0.003, 0.01, 0.03 and 0.1 mg/l. Exposure solutions were prepared by direct addition with analytical verification of the 0.1 and 0.03 mg/l treatments by HPLC-UV. The mean measured concentrations were 88 and 93 % of nominal. Validity criteria were met and the test is considered reliable. The 21-d NOEC for length was 0.01 mg/l based on nominal. The most sensitive endpoint was production of live juveniles with a 21-d NOEC of 0.003 mg/l based on nominal.

Additional information - Noack (1998b)

A semi-static chronic toxicity to *Daphnia magna* study using OIT is available following GLP and US EPA OPPTS Guideline 850.1300. The 21-day study assessed the following endpoints: survival, reproduction, length and weight. The nominal exposure range was 0.0016, 0.0032, 0.0064, 0.0126, 0.0256, 0.05, 0.1, 0.2 and 0.4 mg a.s./l. Validity criteria were met. Analytical measurement by HPLC-UV was conducted for the following exposure concentrations once a week: 0.0032, 0.0126, 0.05 and 0.2 mg a.s./l. Expired solutions were 39 to 58 % of nominal concentrations based on the quoted mean of selected exposure concentrations. Significant effects were observed at all exposure concentrations except the lowest of 0.0016 mg a.s./l. Therefore, the study 21-d NOEC for reproduction was quoted as 0.0016 mg a.s./l based on nominal.

Analytical verification is only available for some treatments (0.0032, 0.0128, 0.05, and 0.2 mg/l nominal) and not for the 0.0016 mg a.s./l treatment. It is noted that the quoted determination limits and detection limits are above the 0.0128 mg/l treatment level. This means it is not possible to validate the lower exposure concentrations. Given analytical losses were >20 % at higher exposure concentrations, therefore it is our opinion that it is not appropriate to present the NOEC based on nominal for classification purposes. Overall, as analytical concentrations cannot be verified and losses

were evident, the study is not used for the purpose of classification. However, it suggests that the true 21-d NOEC may be below 0.001 mg/l.

Key chronic invertebrate trophic level endpoint:
Based on available data, the most sensitive chronic endpoint is a 21-day NOEC of 0.003 mg/l for *Daphnia magna* based on verified nominal concentrations.

Acute ecotoxicity testing is available for the marine oyster and marine shrimp with short term NOECs of <0.003 mg/l and <0.034 mg/l respectively. It is unclear if such invertebrates would be more sensitive than *Daphnia magna* in chronic tests. Overall, it is not possible to rule out a chronic endpoint below the above acute NOECs for invertebrate species other than *Daphnia magna*.

5.4.3 Algae and aquatic plants

Algae:

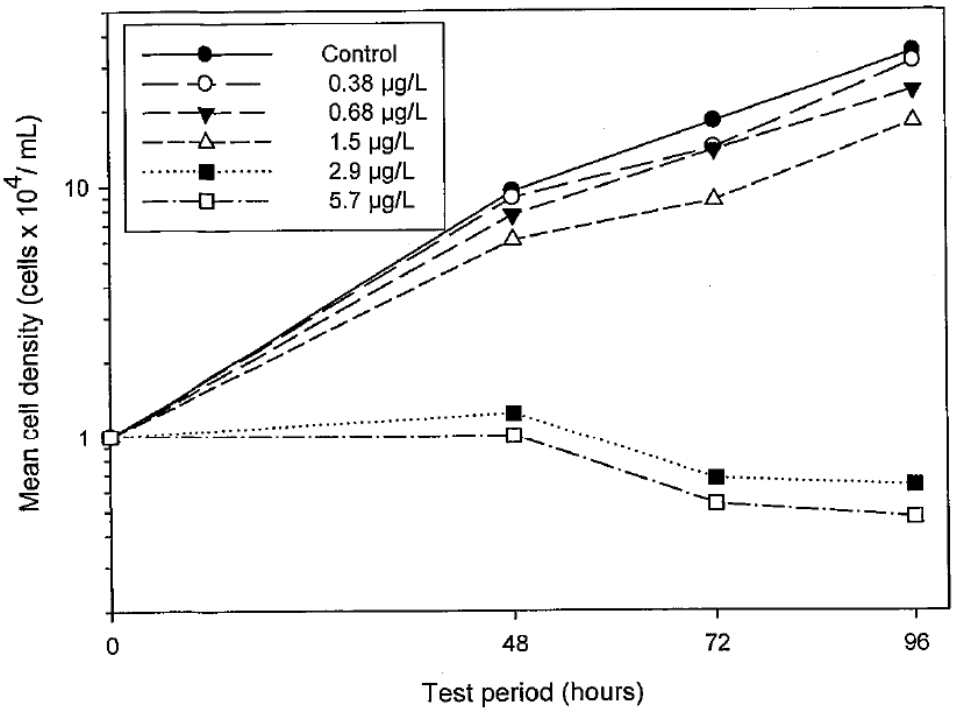
Study 1 – Seyfried (2007)
A static algal growth inhibition test using the marine diatom *Skeletonema costatum* is available following GLP. The study was initiated in early 2006 (before finalisation of the March 2006 OECD Test Guideline) and followed OECD Test Guideline 201 (1984). The study ran for 96 hours and observations are available for 48 and 72 hours. A 24 hour observation is not available. The nominal exposure range was 0.5, 1.0, 2.0, 4.0 and 8.0 µg/l. A 50 mg/l stock solution was prepared by intense stirring which was diluted to produce a 0.1 mg/l stock which was used to prepare exposure solutions with enriched sterile seawater.

The initial cell density was 10,000 algal cells/ml. A 16-fold growth increase in controls was observed at 72 and 96 hours indicating exponential growth over the study and meeting OECD Test Guideline 201 (1984) validity criteria. A 8.6 to 10.2 fold increase in algal cells was observed in controls at 48 hours which is below the 16-fold criteria indicating 48-hour endpoints are not suitable for chronic classification.

As a 24 hour observation was not made, it is not possible to fully consider additional control validity criteria in OECD Test Guideline 201 (2006). However, as an indication, the study has been considered as two section-by-section time periods of 0-48 hours and 48-96 hours noting coefficient of variant criteria included in OECD Test Guideline 201 (2006 and 2011) which suggest the controls are of sufficient validity over the study period.

The experimental growth curve dose response is shown below in Figure 11.

Figure 11: Growth curve dose response for *Skeletonema costatum* and initial 0 hour measured OIT concentrations.



Analysis by HPLC-UV was undertaken at 0 hours and 96 hours (Table 21). At 0 hours, initial measured values were 68 to 76 % of nominal values: 0.38, 0.68, 1.5, 2.9 and 5.7 µg/l. Stability samples were prepared with algae at all exposure concentrations and without algae at nominally 0.5, 2.0 and 83.0 µg/l. They were maintained following test conditions including a 14 hour light / 10 hour dark photoperiod. The stability samples were analysed after 96 hours. For all exposure concentrations containing algae, OIT was detected below the LOQ (0.1 µg/l). Samples without algae were 109 to 121 % of initial measured concentrations. This indicates losses were not due solely to photodegradation.

It is considered that losses in samples containing algae were due to adsorption to algal cells or incorporation. Given this, under the biocides review, the applicant considered the test item is bioavailable to the test organism and based study endpoints on initial mean measured concentrations.

During review of the study for the CLH report, it was noted that the First Amendment to the Study Plan stated analytical samples containing algae were not filtered before analysis due to an oversight and samples were directly extracted with organic solvent (100 ml ethylacetate). Given the mode of action for isothiazolone biocides, it is our understanding that the low recoveries of the test item in samples containing algae indicate the test item was taken up and degraded within the algal cells. Comments from industry support this reaction whereby OIT reacts with the biological cells resulting in cleavage of the isothiazolone ring and loss of the test item.

Table 21: Concentrations of OIT in exposure samples (µg/l)

Nominal concentration (µg/l)	0 hour measured concentration (µg/l)	96 hour measured concentration (µg/l)
0.5 with algae	0.38 (76 % nominal)	<LOQ
0.5 without algae		0.451 (90 % nominal)
1.0 with algae	0.675 (68 % nominal)	No value*
2.0 with algae	1.51 (75 % nominal)	<LOQ
2.0 without algae		1.65 (82 % nominal)
4.0 with algae	2.87 (72 % nominal)	<LOQ
8.0 with algae	5.74 (72 % nominal)	<LOQ
8.0 without algae		6.94 (87 % nominal)

NOTES:
LOQ (0.1 µg/l)
*value not available as sample quantification considered "disturbed by matrix" in the study report

Using Probit analysis, the study report 72-h E_rC_{50} was 1.5 µg/l (0.0015 mg/l) and the 96-h E_rC_{50} was 1.6 µg/l (0.0016 mg/l), both based on initial measured concentrations. The 95 % confidence intervals were not determined.

Using the Dunnett's test, the study report 72-h NOE_rC was 0.68 µg/l (0.00068 mg/l) and the 96-h NOE_rC 1.5 µg/l (0.0015 mg/l), based on initial measured concentrations.

Following discussion of other isothiazolones at the RAC, data for shorter duration E_rC_{50} endpoints were requested. The applicant for OIT provided recalculated E_rC_{50} and E_rC_{10} values using sigmoidal regression of the concentration-dose response relationship. Data are presented in Table 22 below.

As observations at 24 hours are not available, a 24-h E_rC_{50} is not available. Considering the growth curve in Figure 11, it is possible such a value may be lower than the available 48-hour E_rC_{50}.

Table 22: Effects data for different durations (recalculated using data from Seyfried, 2007) based on initial measured concentrations (µg/l)

Time	Endpoint	Value (µg/l) including 95 % CI
48-h	E_rC_{50}	1.93 (1.72 – 2.28 95% CI)
72-h	E_rC_{50}	1.61 (1.04 – 2.56 95% CI)
96-h	ErC50	1.68 (1.51 – 2.52 95% CI)
48-h	E_rC_{10}	1.18 (0.68 – 1.48 95% CI)
72-h	E_rC_{10}	1.33 (0.38 – 2.47 95% CI)
96-h	E_rC_{10}	1.38 (0.38 – 1.50 95% CI)
48-h	NOE_rC	0.68
72-h	NOE_rC	0.68
96-h	NOErC	0.38

Analytical verification was not undertaken at 48 or 72 hours. For comparison the 96-hour endpoints based on mean measured concentrations were as follows:

96-h E_rC_{50}: 0.290 µg/l (0.278 – 0.356 95% CI)

96-h E_rC_{10}: 0.264 µg/l (0.138 – 0.349 95% CI)

96-h NOE_rC: 0.184 µg/l

Based on initial measured concentrations, the 48-h E_rC_{50} of 1.93 µg/l (equivalent to 0.00193 mg/l) is considered appropriate for acute classification given the mode of action of isothiazolones. Overall, the 48-h E_rC_{50} is considered to be in the range 0.001 to ≤ 0.01 mg/l. It is noted that the slightly lower initial measured 72-h and 96-h E_rC_{50} values fall within the same range.

For the purpose of chronic classification, the 72-h E_rC_{10} of 1.33 µg/l based on initial measured concentrations (equivalent to 0.00133 mg/l) is considered appropriate. This is in the range 0.001 to ≤ 0.01 mg/l which is the same range as the 96-h ErC10 based on initial measured concentrations. While the NOE_rC value is lower, it is considered that the E_rC_{10} if available, should be used in preference as it is more statistically robust (ECHA, 2015). In addition, while a 48-h E_rC_{10} based on initial measured concentrations is available and slight lower, it is unlikely this time period represented multiple

generations given only an average 9.5 fold increase in control cultures over this period. On this basis, the 72-h endpoint is preferred for chronic classification.

While a 96-h E_rC_{10} based on mean measured concentrations is available, the value is not considered representative of a concentration which would induce such an ecotoxic response as it reflects a significant time period when the test item was depleted.

Study 2 – Scheerbaum (2000)

A static algal growth inhibition test is available using the freshwater algae *Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*) following GLP and OECD Test Guideline 201 (1984). Exposure concentrations were prepared by ultrasonication with the following nominal exposure range: 6.25, 12.5, 25, 50, 100 and 200 µg/l.

The initial cell density was 10,000 algal cells/ml. A16-fold growth increase in controls was observed at 48 and 72 hours indicating exponential growth over the study and meeting OECD Test Guideline 201 (1984) validity criteria.

It is also noted that study controls meet all coefficient of variance validation criteria in OECD Test Guideline 201 (2006 and 2011) at 48 and 72 hours indicating the controls were reliable over the study period.

Analytical verification by HPLC-UV was undertaken at the start and end of the test (0 and 72 hours). Initial measured concentrations without algae were 81 to 102 % of nominal. At 72 hours measured concentrations with algae were 32 to 63 % of nominal. Mean measured concentrations were: 3.6, 7.9, 15.6, 38.7, 72.6 and 153.7 µg/l. [2]

As per the study above following discussion of isothiazolones at the RAC, data for shorter duration E_rC_{50} endpoints were requested. The applicant provided recalculated E_rC_{50} and E_rC_{10} values using one-way ANOVA and Dunnettt's method. Data are presented in Table 23 below.

[2] For information, the study reported a 72-h E_rC_{50} of 84 µg/l (0.084 mg/l) based on mean measured concentrations with 95 % confidence interval 70 to 100 µg/l. Following statistical comparison of treatments with the controls using Dunnett's Test, the study reported a 72-h NOE_rC of 15.6 µg/l (0.0156 mg/l) based on mean measured concentrations.

Table 23: Effects data for different durations (recalculated using data from Scheerbaum (2000)) based on initial measured concentrations (µg/l)

Time	Endpoint	Value (µg/l) including 95 % CI
24-h	E_rC_{50}	>201
48-h	E_rC_{50}	139 (109 – 201 95 % CI)
72-h	E_rC_{50}	97.9 (80.2 – 121 95 % CI)
24-h	E_rC_{10}	20.8 (13.7 – 29.5 % CI)
48-h	E_rC_{10}	20.8 (15.4 – 27.3 % CI)
72-h	E_rC_{10}	23.9 (16.3 – 33.4 95 % CI)
24-h	NOE_rC	10.8
48-h	NOE_rC	10.8
72-h	NOE_rC	21.1

For comparison the 72-hour endpoints based on mean measured concentrations were as follows:

72-h E_rC_{50}: 76 µg/l (62.6 – 94.5 95% CI)

72-h E_rC_{10}: 19.8 µg/l (12.6 – 28.7 95% CI)

72-h NOE_rC: 15.6 µg/l

Based on initial measured concentrations, the 48-h E_rC_{50} of 139 µg/l (equivalent to 0.139 mg/l) is considered appropriate for acute classification given the mode of action of isothiazolones and in the range 0.1 to 1.0 mg/l). It is noted that the 72-h E_rC_{50} of 97.9 µg/l (equivalent to 0.0979 mg/l) is slightly lower but the time frame does not reflect the rapid model of action. In addition, given the test species is not the most sensitive, it is considered that the 48 hour value is preferred to be consistent with the treatment of data from other algal studies. The 72-hour EC_{50} is not more sensitive that acute endpoints from other studies.

For the purpose of chronic classification, the 72-h E_rC_{10} of 23.9 µg/l (equivalent to 0.0239 mg/l) is considered appropriate. This is in the range 0.01 to ≤ 0.1 mg/l. While the NOE_rC value is lower, it is considered that the E_rC_{10} if available, should be used in preference as it is more statistically robust (ECHA (2015)). The mean measured 72-hour E_rC_{10} value is also in the range 0.01 to 0.1 mg/l.

Study 3 – Hoberg (1996)

A static algal growth inhibition test using the freshwater algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) is available following GLP and OECD Test Guideline 201

(1984). The study was run for 120 hours with observations also made at 24, 48, 72 and 96 hours. The nominal exposure range was 0.0015, 0.003, 0.006, 0.013, 0.025, 0.05 and 0.1 mg a.s./l. The initial cell density was 3,000 cells/ml which is below the guideline recommended value of 10,000 cells/ml. Given the lower number of cells, it was not technically feasible to reliably determine cell counts at 24 hours which are considered as <10,000 cells/ml. In addition, examination of control growth rates indicate that 24 hour cell count results may be unreliable.

A16-fold growth increase in controls was observed at 72 and 96 hours indicating exponential growth over this time and meeting OECD Test Guideline 201 (1984) validity criteria.

As a reliable 24 hour observation is not made, it is not possible to fully consider additional control validity criteria in OECD Test Guideline 201 (2006). However, as an indication, the study has been considered as two section-by-section time periods of 0-48 hours and 48-96 hours noting coefficient of variation criteria included in OECD Test Guideline 201 (2006). This suggests the controls were sufficiently similar based on an acceptable coefficient of variation of average specific growth rates over the study period. The coefficient of variation for section-by-section specific growth rates indicates variation in section-by-section growth rates over the study period which could be due to the lower initial number of cells. Overall, the study controls are considered of sufficient quality over the study period considering the data limitations due to the lack of 24 hour observations.

Analytical verification was by HPLC-UV at 0, 72 and 120 hours with the Limit of Quantification (LOQ) at 0.0004 mg a.s./l. Initial mean measured concentrations were 0.0011, 0.0018, 0.0033, 0.0079, 0.016, 0.031 and 0.062 mg a.s./l. Table 24 presents measured concentrations.

Table 24: Concentrations of OIT in exposure samples (µg a.s/l)

Nominal concentration (µg a.s./l)	0 hour measured mean concentration (µg a.s./l)	72 hour mean measured concentration (µg a.s./l)	0-72 hour mean measured concentration (µg a.s./l)	120 hour mean measured concentration (µg a.s./l)
1.5	1.1	<LOQ	0.49	<LOQ
3.0	1.8	<LOQ	0.63	<LOQ
6.0	3.3	<LOQ	0.85	<LOQ
13	7.9	2.8	1.3	3.5
25	16	11	13	<1.5
50	31	24	27	<3.1
100	62	49	55	<6.2

NOTES:
LOQ (0.4 µg a.s./l)

The study only reported growth rate endpoints for 72 hours. The study report 72-h E_rC_{50} was calculated to be 0.031 mg a.s./l based on initial measured concentrations with 95 % confidence interval 0.018 to 0.045 mg a.s./l.

Reanalysis by industry reported a 72 hour E_rC_{50} of 0.026 mg a.s./l based on initial measured concentrations with 95 % confidence interval 0.021 to 0.032 mg a.s./l.

Based on 0-72 hour mean measured concentrations, the E_rC_{50} was 0.025 mg a.s./l with 95 % confidence interval 0.020 to 0.030 mg a.s./l.

A 24-h E_rC_{50} is not available due to the lack of observations at 24 hours. The 48-h E_rC_{50} based on initial measured concentrations was 0.0054 mg/l. Due to the lack of analytical verification at 48 hours, an endpoint based on mean measured concentrations is not available. Overall, the acute E_rC_{50} is considered to lie in the range 0.001 to 0.01 mg/l.

For chronic endpoints, E_rC_{10} data available for 0-72 hours and 0-96 hours based on initial measured concentrations are preferred to NOE_rCs since they are statistically more robust:
72-h E_rC_{10} 0.0059 mg/l
96-h E_rC_{10} 0.0036 mg/l

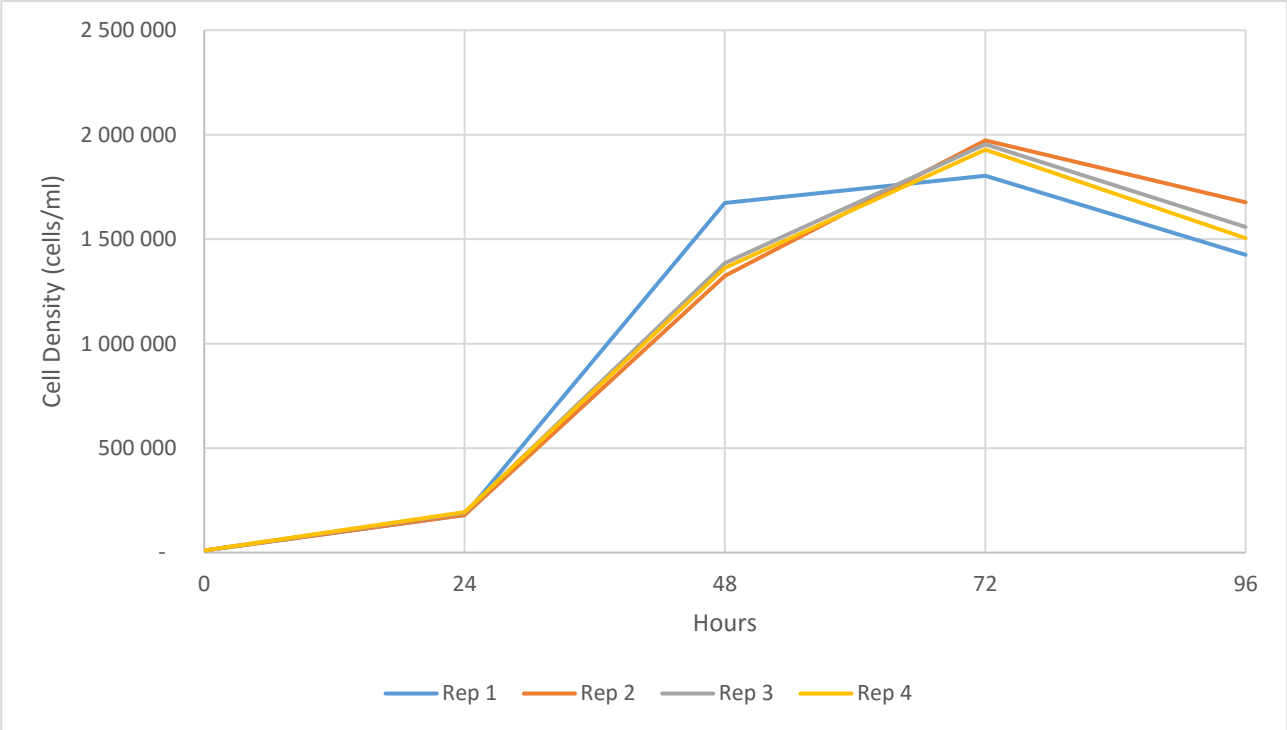
These values are in the range 0.001 to 0.01 mg/l. In addition the 96-h E_rC_{10} based on mean measured concentrations is 0.0068 mg/l and also within this range.

Study 4 – Porch *et al* (2011)
In 2016, Industry notified the UK CA of an algal growth inhibition study which was not considered during initial stages of the biocides review. It has now been reviewed for the purpose of hazard classification with details below.

A static algal growth inhibition test using the freshwater diatom *Navicula pelliculosa* is available following GLP and OECD Test Guideline 201 (1996). Exposure concentrations were prepared with added silica and selenium required for the test species with the following nominal exposure range: 0.16, 0.4, 1.0, 2.5 and 6.25 µg/l. The initial cell density was 10,000 cells/ml and the study ran for 96 hours.

A 16-fold growth increase in controls was observed at 72 and 96 hours indicating exponential growth meeting OECD Test Guideline 201 validity criteria. In addition the controls met the coefficient of variation of average specific growth rates (detailed in OECD Test Guideline 202, 2006 and 2011) over the study period indicating controls grew similarly over the study period. However, the coefficient of variation for section-by-section specific growth rates indicates significant variation in section-by-section growth rates over the study period with a low growth rate between 48 and 72 hours and negative growth rate between 72 and 96 hours. This indicates that while the controls appear valid for exponential growth (based on 16-fold control cell increase), from 48 hours exponential growth did not occur and the controls are not reliable. Figure 12 illustrates growth in the 4 controls over the study period. On this basis only endpoints up to 48 hours are considered reliable.

Figure 12: Growth in controls over the study period (Porch *et al*, 2011)



The 24-h and 48-h E_rC_{50} values are 0.00152 and 0.00129 mg/l, respectively, based on initial measured concentrations. These acute values are in the range 0.001 to 0.01 mg/l.

As data after 48 hours cannot be considered reliable, NOE_rC and E_rC_{10} endpoints are not available for consideration of chronic classification, although they would likely be lower than the 48-h E_rC_{50}.

Aquatic plants:

A static 7-day toxicity to *Lemna gibba* study (Volz (2007)) using OIT is available following GLP and draft OECD Test Guideline 221. Exposure solutions were prepared with the aid of the solvent dimethylformamide (DMF) (0.1 ml/l) and a solvent control was included. The nominal exposure range was 0.0032, 0.01, 0.032, 0.1, 0.32, 1.0, 3.2 and 10 mg/l. Validity criteria were met and the test is considered reliable. The study endpoints were frond number, frond yield, biomass, growth rate and dry weight. The growth rate endpoints were presented in the study report based on mean measured concentrations: 7-d E_rC_{50} 0.62 mg/l; 7-d E_rC_{10} 0.041 mg/l; and 7-d NOE_rC 0.0087 mg/l.

Key acute and chronic algae and aquatic plant trophic level endpoints:
Acute 24 hour endpoint data are only available for the *Navicula pelliculosa* study with an E_rC_{50} of 0.00152 mg/l based on initial measured concentrations. This is with the range 0.001-0.01 mg/l.

Acute 48-h E_rC_{50} data for three species (*P. subcapitata*, *N. pelliculosa* and *S. costatum*) lie within the range 0.001-0.01 mg/l based on initial measured concentrations. The lowest is 0.00129 mg/l for the freshwater diatom *Navicula pelliculosa*.

An additional species, *Desmodesmus subspicatus*, appears to be less acutely sensitive.

Chronic 72-h E_rC_{10} data for two species (*P. subcapitata,* and *S. costatum*) lie within the range 0.001-0.01 mg/l based on initial measured concentrations The marine species *Skeletonema costatum* is the most chronically sensitive with a 72-h E_rC_{10} of 0.00133 mg/l based on initial measured concentrations. Valid chronic data are not available for the most acutely sensitive species *Navicula pelliculosa* - it is possible a valid chronic endpoint for this species could be lower. The implications for this is considered below.

Additional information:
A summary of available testing with degradants is presented in Annex II. This includes a 72 hour algal growth inhibition study with *Skeletonema costatum* for the degradant NNOMA (Hahne, 2002c). The study 72-hour E_rC_{50} was 0.44 mg/l based on initial measured concentrations and the 72-hour NOE_rC was 0.064 mg/l based on initial measured concentrations. Analytical concentrations were verified within 20 % of initial measured concentrations.

As noted above, the OECD Test Guideline 201 (1984) states that for the test to be valid, the biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. At 72 hours a 3.7 fold increase was observed in control biomass compared to study initiation biomass. At 96 hours a factor of 19 was observed. The guideline states that the validity criterion may not be met when species that grow slower than those listed in Annex 2 are used. In this case, the test period should be extended to obtain at least a 16-fold growth in control cultures. The marine algal species used in this study is not a standard species for the guideline. Extending the study period to 96 hours allows for the exponential growth validity criteria to be met. The study 96-hour E_rC_{50} was 0.47 mg/l based on initial measured concentrations and the 96-hour NOE_rC was 0.13 mg/l based on initial measured concentrations. Analytical concentrations were verified within 20 % of initial measured concentrations.

5.4.4 Other aquatic organisms (including sediment)

No data

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

Degradation:

OIT is considered hydrolytically stable.

OIT is susceptible to photodegradation. The maximum level of observed mineralisation was 12.5 % AR CO_2 by day 30. It is noted that the actual degree of photodegradation in the aquatic environment depends on local conditions and seasons and is difficult to quantify. Given the available data, there is insufficient information to evaluate photodegradation in the European environment in terms of mineralisation or transformation to non-classifiable substances. Therefore aquatic photolysis is not considered to meet the criteria for rapid degradation.

In a ready biodegradation study, no biodegradation was observed although some microorganism inhibition is possible.

In aquatic biodegradation simulation studies using freshwater and seawater, OIT was observed to undergo degradation including some mineralisation. The maximum amount of mineralisation was 58.5 % AR within 20 days. Overall, OIT was not observed to mineralise sufficiently to meet the criteria for rapidly degradable based on 70 % ultimate aquatic degradation within 28 days. In addition,

classification of all main degradants cannot be excluded based on algal ecotoxicity data for the degradant NNOMA.

For the purpose of classification, OIT is considered not rapidly degradable.

Bioaccumulation:

The experimental log K_{ow} for OIT is >3.1 based on concentrations in pure water and *n*-octanol. Experimental steady state whole fish BCFs were 507 to 538 l/kg. Lipid normalised (5 % lipid fraction) whole fish BCFs were calculated as 843 to 886 l/kg.

Both experimental steady state BCFs and lipid normalised steady state BCFs are above the BCF trigger of ≥ 500 intended to identify substances with a potential to bioaccumulate.

Ecotoxicity:

Identified degradants are relatively less toxic than the parent (see Annex II) and not considered further for classification of OIT (except in relation to the above decision on rapid degradability).

OIT is an isothiazolone biocide. Algae are the most sensitive trophic level. Isothiazolones are rapidly taken up by algae where they react with enzymes to disrupt metabolism and inhibit growth. During this process the parent substance is depleted. This mode of action is rapid with uptake and enzyme effects in minutes affecting cell viability and resulting in cell death over hours.

This rapid knock-down of algal cells with depletion of the substance by uptake and metabolism means that shorter duration endpoints are appropriate for consideration of acute toxicity to algae. It is noted that given the comparison of 24 hour and 48 hour E_rC_{50} data, OIT appears exhibit slower algicidal effects than other isothiazolones. On this basis a 48 hour endpoint is considered relevant for acute classification. This is suitable as multiple generations are not required for acute algal endpoints and up to 48 hours accounts for the period of time before algal populations recover when the test item is depleted from solution.

For chronic classification, the standard chronic time period of 72-96 hours for algal studies is appropriate to ensure multiple algal generations.

Given OIT is rapidly taken up and depleted by algal cells resulting in significant losses over a short period of time, initial measured concentrations are considered to reflect representative concentrations in algal studies which induce ecotoxic responses in algal studies. This is because mean measured concentrations would include a period of time when very little test item was available (generally below the analytical limit of detection) resulting in unrealistic calculated mean measured concentrations lower than those which induce the inhibition effect. It is considered that this non-standard approach is adequate to determine the appropriate aquatic classification and M-factors for OIT.

Aquatic Acute Classification:

Aquatic acute toxicity data on OIT are available for fish, invertebrates, algae and aquatic plants.

Acute 48-h E_rC_{50} data for three algal species (*P. subcapitata, N. pelliculosa* and *S. costatum*) lie within the range 0.001 to 0.01 mg/l based on initial measured concentrations. There are no reliable mean measured 48 hour acute algal endpoints. It is noted that 72 hour endpoints are within the same classification range. One endpoint (96-hour E_rC_{50} 0.00029 mg/l for *S. costatum*) falls within the lower classification range 0.0001-0.001 mg/l – this value is mean measured and considered to be overly influenced by the long period of time when the test item was depleted and therefore not representative of the test item concentration inducing the ecotoxic effect. Overall, OIT should be classified as

Aquatic Acute 1 with an Acute M-factor of 100 based on acute endpoints in the range 0.001 to 0.01 mg/l for a not rapidly degradable substance.

Aquatic Chronic Classification:

Adequate chronic toxicity data on OIT are available for fish, invertebrates, algae and aquatic plants.

Chronic 72-h E_rC_{10} data for two algal species (*P. subcapitata* and *S. costatum*) lie within the range 0.001 to 0.01 mg/l based on initial measured concentrations. A 72-h E_rC_{10} for *P. subcapitata* is also in this range.

One endpoint (96-hour E_rC_{10} 0.000264 mg/l for *S. costatum*) falls within the lower classification range 0.0001-0.001 mg/l – this mean measured value considered to be overly influenced by the long period of time when the test item was depleted and therefore not representative of the test item concentration inducing the ecotoxic effect. In addition, a 72 hour endpoint is available for the species suitable for chronic classification.

Given that OIT is considered not rapidly degradable and has BCF data ≥ 500, OIT should be classified as Aquatic Chronic 1 with a Chronic M-factor of 10 based on chronic endpoints in the range 0.001 to 0.01 mg/l.

It is noted that chronic toxicity data for the most acutely sensitive species of fish (Rainbow trout) and other invertebrates (Oysters and Mysids) are not available. Considering the available acute ecotoxicity data and the surrogate classification approach for a not rapidly degradable substance, this would also result in Aquatic Chronic 1 and a chronic M-factor of 10.

While it is noted that acute ecotoxicity data is available for three species (*P. subcapitata*, *N. pelliculosa* and *S. costatum*) in the range 0.001-0.01 mg/l based on initial measured concentrations, chronic endpoints are not available for *N. pelliculosa* for which the lowest acute value was observed. Using the surrogate approach for *N. pelliculosa* for a not rapidly degradable substance would also result in Aquatic Chronic 1 but include a chronic M-factor of 100. Given the three acute endpoints are within the same classification range indicating similar ecotoxic responses and the valid chronic endpoints for *P. subcapitata* and *S. costatum* are within the same classification range, applying the surrogate approach is not appropriate considering:

i) the relative equal acute toxicity between species,
ii) there is no evidence that the algal species would exhibit differing chronic sensitivities, and
iii) due to the mode of action for algae and substance depletion, applying the acute endpoint might not be appropriate.

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

Aquatic Acute 1; H400: Very toxic to aquatic life
Acute M-factor = 100
Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects
Chronic M-factor = 10

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Octhilinone (OIT) is a biocide approved for use in wood preservatives with approvals for other types of use ongoing. It is currently listed in Annex VI of the CLP Regulation with Aquatic Acute 1 and Aquatic Chronic 1 classification without M-factors. As the current entry in Annex VI does not include M-factors, the DS proposed to add an M-factor of 100 to Aquatic Acute 1 classification based on initially measured algae 48-hour E_rC_{50} values in the range 0.001 to 0.01 mg/L. The proposed M-factor of 10 for Aquatic Chronic 1 classification was based on initially measured 72-hour E_rC_{50} values in the range 0.001 to 0.01 mg/L for algae. The substance was considered not rapidly degradable and having a potential to bioaccumulate. After the Public Consultation (PC) the DS changed the proposal for chronic toxicity. The new proposal was based on the algae initial measured 48-h E_rC_{10} of 0.000224 mg/L. The value is in the range 0.0001 to 0.001 mg/L giving an M-factor of 100 for a not rapidly degradable substance.

OIT is anticipated to dissociate and be ionised at environmentally relevant pH due to pK_a values in the range 3.2-3.3. Aquatic toxicity studies were run at pH 5 or above reflecting environmental conditions where nearly all OIT would be in its ionised form.

Degradation

There are two GLP hydrolysis studies available following US EPA Guideline, Subsection N, Section 161-1. Both studies were performed at pH 5, 7 and 9 and showed no significant degradation. On this basis, the hydrolysis half-life was considered to be > 1 year and OIT was considered hydrolytically stable.

Two aqueous photolysis studies were available following GLP and US EPA Guideline Subsection N, section 161.2. In the test using ^{14}C radio-labelled OIT (10.0 mg/L), the photolytic DT_{50} was 15.3 days. Mineralisation accounted for 12.5 % AR (Applied Radioactivity) based on carbon dioxide (CO_2) at study termination (~ 30 days). Degradants observed were 2-(n-octyl)-4-thiazolin-2-one, a mixture of N-(n-octyl) malonamic acid (NNOMA) and oxamic acid, N-(n-octyl)acetamide (NNOA), and sulfoxide of OIT at concentrations 14.1, 12.5 and 11.2 % of AR at study termination and max. 10.1 % AR at 405 hours, respectively. The second study used ^{14}C radio-labelled OIT (0.5 mg/L) in two systems: sterile buffer solutions at pH 7 and sterile natural pond water at pH 8. OIT, decreased from 97.9 % AR and 98 % AR to 1.1 % AR and 2.5 % AR in the buffer and pond solutions respectively. The photolytic DT_{50} for the buffer solution system was 3.7 summer days at 50°N. The photolytic DT_{50} for the pond water system was 5.1 summer days at 50°N. The degradant identified in both buffer and pond solutions was NNOA at max 23.3 % AR and 25.1 % AR, respectively. The degradant 2-(*N*-octyl) ethyl amine was identified in buffer solutions at test termination (16.2 % AR). In both systems, unidentified degradants were observed. Mineralisation at test termination was 0.3 % AR and 7.9 % of AR in buffer and pond solutions, respectively.

A ready biodegradation study following GLP and OECD TG 301D (Closed Bottle) was available. Test solutions were prepared with 3 mg/L test item and 0.2 mL inoculum per 300 mL test vessel. The toxicity control (with 1.5 mg/L OIT and 5 mg/L sodium acetate) achieved 26 % degradation after 14 days and a maximum of 35 % degradation by day 21. While these values

met the validity criteria of 25 % degradation by day 14, the CAR evaluation considered the test item may have led to a degree of microorganism inhibition. This was supported by an Activated Sludge Respiration Inhibition Test (ASRIT) which determined an EC_{20} of 8.9 mg/L and 11 % inhibition observed at the lowest test concentration of 4 mg/L. A second ASRIT (Noack, 2001) also determined a similar EC_{20} of 7.3 mg/L. No degradation of OIT was observed in test item solutions during the OECD TG 301D test. Overall, the substance was considered not readily biodegradable although this might be influenced by a partially inhibitory test concentration.

Two aquatic biodegradation simulation studies are available using freshwater and seawater. Study 1 is a freshwater aquatic biodegradation simulation study following OECD TG 309 and GLP. The study used ^{14}C-OIT and surface water from the River Rhine in Switzerland. While OIT is anticipated to adsorb to organic matter, the suspended solid concentration in the test water is not known. Test vessels containing 0.0103 and 0.1029 mg/L OIT were maintained for 29 days under aerobic conditions at 20 ± 2 °C. Mean AR recoveries were 91.8 ± 5.6 % AR and 91.8 ± 5.6 % AR for low and high doses. The levels of AR in water decreased from 96.7 and 102 % on day 0 to 41.8 and 52.5 % on day 29 in the low and high dose systems, respectively. DT_{50} values of 0.6 and 1.2 days were calculated for OIT. After conversion to 12 °C, DT_{50} values were 1.1 and 2.3 days. Three degradants over 10 % were detected but could not be identified (M1 at 19 to 22.8 % AR; M5 at 14.7 to 15 % AR; and M6 at 9 to 10.5 % AR). The DT_{50} values calculated at 12°C were, for M1: 17.8 to 35.5 days, for M5: 19.3 to 30.9 days, and for M6: 8.3 to 22.9 days. A seawater aquatic biodegradation simulation study is available following OECD TG 309 and GLP. The study used ^{14}C-OIT and surface water from St. Margaret's Bay, Kent, UK with unknown suspended solid or organic carbon concentrations. Test vessels containing 0.0101 and 0.09986 mg/L OIT were maintained for 17 days under aerobic conditions at 20 ± 2°C. Mean AR recoveries were 91.4 ± 7.6 % AR and 92.1 ± 8.5 % AR for low and high doses. Levels of AR in water decreased from 100.7 and 102.6 % on day 0 to 40.4 and 47 % on day 17 in the low and high dose systems, respectively. The study calculated DT_{50} values of 1.6 and 2.1 days for OIT, indicating rapid primary degradation. These values were converted to 12 °C, resulting in DT_{50} values of 3 and 4 days. Various degradants were observed at concentrations less than 10 % AR. A sediment phase was not included in either test. In both tests, the decrease in water phase AR is considered to be due to primary degradation of OIT to degradants which were subsequently mineralised and some partitioning to dissolved organic solid matter.

Ready biodegradation studies are available for two degradants NNOA and NNOMA. Both studies followed OECD TG 301B (Modified Sturm test) and GLP. Both substances achieved over 60 % degradation meeting the 10-day window criterion. On this basis, both degradants are considered rapidly degradable. Aquatic toxicity data is available for NNOMA with the lowest acute toxicity value being a 72-hour E_rC_{50} of 0.44 mg/L for algae. The lowest chronic toxicity value is a 72-hour NOE_rC of 0.064 mg/L for algae. Both values are based on initially measured concentrations at 0-hour. Consequently, NNOMA fulfils the classification as Aquatic Acute Category 1 and Aquatic Chronic Category 2.

Given the available aquatic toxicity information for NNOMA and the lack of aquatic toxicity information for unidentified degradants, OIT degradants cannot be considered non-classifiable.

Overall, the degradation information did not provide sufficient data to show that OIT was ultimately degraded (mineralised) within 28 days (equivalent to a half-life < 16 days) or

underwent primary degradation to non-classifiable products. Consequently, OIT was considered not rapidly degradable for the purpose of classification and labelling.

Bioaccumulation

OIT was considered surface active. Therefore, log K_{OW} studies determined using the HPLC method or the shake flask method were not considered reliable and not discussed further. Log K_{OW} based on the solubility of OIT in *n*-octanol (> 524.8 g/L at 20 °C) and measured water solubilities at different pHs (pH 5, 7 and 9) and temperatures (10 °C, 20 °C and 30 °C) was calculated. As the solubility in water did not differ significantly, there was no significant effect on the log K_{OW} value, which was considered to be > 3.1. An experimental aquatic BCF study for OIT was available following GLP and OECD TG 305. The study used ^{14}C-OIT, a flow-through system with Rainbow Trout (*Oncorhynchus mykiss*) and two exposure concentrations; 0.0001 and 0.00048 mg/L. The exposure period ran for 14 days followed by a 14-day depuration period. The pH values ranged from 7.8 to 8.2. The parent compound accounted for 97-99 % of radioactivity. The study whole fish steady state BCFs were 507 ± 87 L/kg (high dose) to 538 ± 65 L/kg (low dose) wet weight. BCFs were not growth corrected although given the uptake duration; any corrections were likely to have little impact on BCF values. Lipid normalising the BCFs to 5 % lipid content increased BCFs to 843 to 886 L/kg wet weight. The BCF values were greater than the CLP trigger value of 500 for potentially bioaccumulative substances.

Aquatic toxicity

A summary of available valid information on the aquatic toxicity of OIT is presented in the following Table. According to the data in the CLH report under Annex 2, the identified degradants NNOMA and NNOA are relatively less toxic than the parent and are not discussed further in relation to toxicity.

The DS explained that OIT is a thiazolinone biocide. Algae are the most sensitive trophic level. Thiazolinones are rapidly taken up by algae where they react with enzymes to disrupt metabolism and inhibit growth (Williams, 2007) as a toxic response. During this process, cleavage of the isothiazole ring occurs and the parent substance is depleted. This mode of action in algae is rapid with uptake and enzyme effects in minutes affecting cell viability and resulting in cell death over hours. It also means that algal toxicity is dependent on initial substance concentration and algal cell density with greatest inhibition of algae resulting in slower degradation in test systems. However, given the rapid uptake and depletion of OIT by algal cells results in significant losses over a short period of time, initial measured concentrations are considered to reflect representative concentrations which induce toxic responses in algal studies. This is because mean measured concentrations would include a period of time when very little test item was available (generally below the analytical limit of detection) resulting in unrealistic calculated mean measured concentrations lower than those which induce the inhibition effect.

In the case of thiazolinones, the rapid knock-down of algal cells with depletion of the substance by uptake and metabolism means that shorter duration endpoints may be appropriate for consideration of acute toxicity to algae. This is suitable as multiple generations are not required and up to 48 hours accounts for the period of time before algal populations recover when the test item is depleted from solution. For the purpose of chronic classification, it was considered that the standard chronic time period of 72-96 hours for algal studies was appropriate to ensure multiple algal generations. It was noted that OECD TG 201 allows studies to be

shortened to 48 hours if a 16-fold increase in cells is observed in controls indicating exponential growth and multiple generations. This was discussed for each study in the CLH Report.

***Table**: Summary of relevant information on aquatic toxicity for OIT*

Guideline/GLP	Species	Endpoint	Exposure		Results	
			Design	Duration	Endpoint	Toxicity mg/L
Fish						
Acute toxicity to fish US EPA FIFRA Guideline 72-1, GLP, purity: 98.5 % (1	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Flow-through	96-h	LC_{50}	0.047 (mm) 86-101 % of nom.
Acute toxicity to fish US EPA FIFRA Guideline 72-1, GLP, purity: 98.5 % (1	Bluegill Sunfish (*Lepomis macrochirus*)	Mortality	Flow-through	96-h	LC_{50}	0.180 (mm) 84-106 % of nom.
Acute toxicity to fish US EPA FIFRA Guideline 72-1, GLP, purity: 98.5 % (1	Sheepshead Minnow (*Cyprinodon variegatus*)	Mortality	Flow-through	96-h	LC_{50}	0.160 (mm) 63-92 % of nom.
Acute toxicity to fish US EPA OPPTS Guideline 850.1075, GLP, purity: 99.2 % (2	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96-h	LC_{50}	0.036 (mm) 50-76 % of nom.
Acute toxicity to fish US EPA FIFRA OPPTS Guideline 72-1, GLP, purity: 96 % (3	Bluegill Sunfish (*Lepomis macrochirus*)	Mortality	Semi-static (renewal at 24-h intervals)	96-h	LC_{50}	0.16 (0-72h mm) 53-80 % of nom.
Fish Early Life-Stage (FELS) toxicity US EPA FIFRA Guideline 72-4, GLP, purity: 98.5 % (1	Fathead Minnow (*Pimephales promelas*)	Embryo survival and larval growth/ survival	Flow-through	35 days	NOEC egg hatchability, growth and survival	0.0085 (mm) 65-87 % of nom.
Invertebrates						
Daphnia sp. Acute Immobilisation US EPA FIFRA Guideline 72-2, GLP, purity: 98.5 % (1	*Daphnia magna*	Acute immobilisation	Semi-static	48-h	EC_{50}	0.32 (mm) 92-100 % of nom.
Daphnia sp. Acute Immobilisation US EPA FIFRA Guideline 72-2, GLP, purity: 99.2 %	Daphnia magna	Acute immobilisation	Semi-static	48-h	EC_{50}	0.42 (n) Fresh media: 89-112 % of nom. Expired media: 76-

						106 % of nom.
Daphnia sp. Acute Immobilisation OECD Guideline 202 Part I, GLP, purity: 96 %	Daphnia magna	Acute immobilisation	Semi-static	48-h	EC_{50}	0.1 (mm) 68-85 % of nom
Acute Toxicity US EPA FIFRA Guideline 72-3, GLP, purity: 98.5 % (1	Mysid Shrimp (*Mysidopsis bahia*)	Mortality	Flow-through	96-h	LC_{50}	0.071 (mm) 78-87 % of nom.
Acute Toxicity US EPA FIFRA Guideline 72-3, GLP, purity: 98.5 % (1	Oyster (*Crassostrea virginica*)	Shell growth	Flow-through	96-h	EC_{50}	0.013 (mm) 44-67 % of nom.
Daphnia magna Reproduction US EPA FIFRA Guideline 72-4, GLP, purity: 98.5 % (1	*Daphnia magna*	Survival; reproduction; growth	Flow-through	21 days	NOEC	0.074 (mm) 51-80 % of nom.
Daphnia magna Reproduction OECD TG 202, Part II, GLP, purity: 96 % (3	*Daphnia magna*	Survival; reproduction; growth	Semi-static	21 days	NOEC production of live juveniles	0.003 (n) 88-93 % of nom.
Algae and aquatic plants						
Freshwater Algal Growth Inhibition OECD TG 201, GLP, purity: 99.9 % (4	*Skeletonema costatum*	Cell multiplication inhibition	Static	24-h (b 48-h (a 72-h 96-h 96-h 48-h (a 72-h 96-h 96-h 48-h (a 72-h 96-h 96-h	 E_rC_{50} E_rC_{50} E_rC_{50} E_rC_{50} E_rC_{10} E_rC_{10} E_rC_{10} E_rC_{10} NOE_rC NOE_rC NOE_rC NOE_rC	- 0.00193 (im) 0.00161 (im) 0.00168 (im) 0.00029 (mm) 0.00118 (im) 0.00133 (im) 0.00138 (im) 0.000264 mm 0.00068 (im) 0.00068 (im) 0.00038 (im) 0.000184 (mm)

						0-h: im 68-76 % of nom.
Freshwater Algal Growth Inhibition OECD TG 201, GLP, purity: 99.3 % (2	*Desmodesmus subspicatus**	Cell multiplication inhibition	Static	24-h 48-h 72-h 72-h 24-h 48-h 72-h 72-h 24-h 48-h 72-h 72-h	E_rC_{50} E_rC_{50} E_rC_{50} E_rC_{50} E_rC_{10} E_rC_{10} E_rC_{10} E_rC_{10} NOE_rC NOE_rC NOE_rC NOE_rC	>0.201 (im) 0.139 (im) 0.0979 (im) 0.076 (mm) 0.0208 (im) 0.0208 (im) 0.0239 (im) 0.0198 (mm) 0.0180 (im) 0.0180 (im) 0.0211 (im) 0.0156 (mm) 0 h: im 81-102 % on nom.
Freshwater Algal Growth Inhibition OECD TG 201, GLP, purity: 99.2 %	*Pseudokirchneriella subcapitata*	Cell multiplication inhibition	Static	24-h (c 48-h 72-h 72-h 48-h 72-h 72-h 96-h 72-h 72-h 96-h	 E_rC_{50} E_rC_{50} E_rC_{50} E_rC_{10} E_rC_{10} E_rC_{10} E_rC_{10} NOE_rC NOE_rC NOE_rC	 0.0054 (im) 0.026 (im) 0.025 (mm) 0.0011 (im) 0.0059 (im) 0.0068 (mm) 0.0036 (im) 0.0011 (im) 0.00049 (mm) 0.031 (im) im 55-73 % of nom.
Freshwater Algal Growth Inhibition OECD TG 201, GLP, purity: 99.2 %	*Navicula pelliculosa*	Cell multiplication inhibition	Static	24-h 48-h 48-h 48-h	E_rC_{50} E_rC_{50} NOE_rC (*	0.00152 (im) **0.00129 (im)**

					E_rC_{10} (*	0.000071 (im) **0.000224 (im)**
Lemna sp. Growth Inhibition Test OECD TG 221 (draft), GLP, purity: 99.9 %	*Lemna gibba*	Growth	Static	7 days	E_rC_{50} E_rC_{10} NOE_rC	0.62 (mm) 0.041 (mm) 0.0087 (mm)

(1 solvent triethylene glycol (TEG) used; (2 ultrasonication; (3 direct addition; (4 intense stirring; (5 solvent dimethylformamide (DMF) used

(a 8.6 to 10.2 fold increase in algal cells was observed in controls at 48 hours which is below the 16-fold criteria indicating 48-hour endpoints are not suitable for chronic classification. No reliable cell count at 24- hour.

(b observations not available

(c initial cell density was 3 000 cells/mL which is below the guideline recommended value of 10 000 cells/mL

(d It is noted that mean measured endpoints are lower than initial measured endpoints which is unusual. This is due to a clearer distribution and dose response for the initial measured model. A reduced goodness of fit dose response model was observed using mean measured concentrations reflecting lower losses (less than 20 %) for higher treatments and greater losses (60-80 %) for lower treatments.

(im) initial measured; (mm) mean measured; (n) nominal

Bold values indicate most sensitive acute and chronic endpoints used for hazard classification

(***** additional information in Public Consultation comments and Response to comments

Acute toxicity

There were five acute toxicity studies available on fish. *Oncorhynchus mykiss* is the most acutely sensitive fish species with LC_{50} values between 0.036 and 0.047 mg/L based on measured concentrations. There were five studies available on invertebrates, three for *Daphnia sp.*, one for *Mysidopsis bahia* and one for *Crassostrea virginica*. The marine oyster *Crassostrea virginica* was the most acutely sensitive invertebrate species with an acute EC_{50} of 0.013 mg/L based on measured concentrations.

Acute 24-hour endpoint data were only available for the *Navicula pelliculosa* study with an E_rC_{50} of 0.00152 mg/L based on initial measured concentrations. This was within the range 0.001-0.01 mg/L. A 16-fold growth increase in controls was observed at 72 and 96 hours indicating exponential growth meeting OECD TG 201 validity criteria. In addition, the controls met the coefficient of variation of average specific growth rates (detailed in OECD TG 201) over the study period indicating controls grew similarly over the study period. However, the coefficient of variation for section-by-section specific growth rates indicated significant variation in section-by-section growth rates over the study period with a low growth rate between 48 and 72 hours and negative growth rate between 72 and 96 hours. This indicates that while the controls appear valid for exponential growth (based on 16-fold control cell increase), from 48 hours exponential growth did not occur and the controls are not reliable. On this basis, only endpoints up to 48 hours are considered reliable. Acute 48-h E_rC_{50} data for three species (*S. costatum, P. subcapitata and N. pelliculosa*) lied within the range 0.001-0.01 mg/L based on initial measured concentrations. The lowest 48-hour E_rC_{50} was 0.00129 mg/L for the freshwater diatom *Navicula pelliculosa*. An additional species, *Desmodesmus subspicatus*, appears to be less acutely sensitive. There was also a 7-day

aquatic plant toxicity study on *Lemna gibba* available. The 7-day E_rC_{50} value was 0.62 mg/L based on mean measured concentrations.

Chronic toxicity

There was one chronic study available on fish. The key endpoint was the 35-day NOEC of 0.0085 mg/L for *Pimephales promelas* based on measured concentrations.

There were two chronic studies on *Daphnia magna*; the most sensitive chronic endpoint is a 21-day NOEC of 0.003 mg/L based on verified nominal concentrations.

Four freshwater Algal and one aquatic plant study were included in the dossier. For the aquatic plants, the 7-day E_rC_{10} value was 0.041 mg/L (*L. gibba*) based on mean measured concentrations. Chronic 72-h E_rC_{10} data for two species (*S. costatum* and *P. subcapitata*) lied within the range 0.001-0.01 mg/L based on initial measured concentrations. The marine species *Skeletonema costatum* was the most chronically sensitive with a 72-h E_rC_{10} of 0.00133 mg/L based on initial measured concentrations. Valid chronic data were not available for the most acutely sensitive species *Navicula pelliculosa* – it is possible a valid chronic endpoint for this species could be lower.

There were aquatic toxicity data available for the degradants NNOMA and NNOA. The highest toxicity was derived from a 72-hour algal growth inhibition study with *Skeletonema costatum* for the degradant NNOMA (OECD TG 201, GLP). The study 72-hour E_rC_{50} was 0.44 mg/L and the 72-hour NOE_rC was 0.064 mg/L based on initial measured concentrations. Analytical concentrations were verified within 20 % of initial measured concentrations. At 72 hours, only a 3.7-fold increase was observed in control biomass compared to study initiation. At 96 hours a factor of 19, fulfilling the validity criteria of the test guideline, was observed. The study 96-hour E_rC_{50} was 0.47 mg/L and the 96-hour NOE_rC was 0.13 mg/L based on initial measured concentrations.

Comments received during public consultation

Comments were received from four MSCAs and from the biocide applicants. Two MSCAs agreed with the proposed classification. Two MSCAs preferred effect concentrations expressed as mean measured concentrations rather than initial measured concentrations when determining the test result. One of the MSCAs wanted to use the 72-hour E_rC_{50} values instead of a shorter period. An MSCA agreed with the acute classification but wanted to use *Navicula pelliculosa* data from the draft final CAR not mentioned in the CLH Report for chronic classification. The use of the NOEC 0.071 µg/L would change the M-factor from 10 to 1 000. The two biocide applicants provided comments on the rapid degradability of OIT. They made available a new OECD TG 309 study and a metabolite identification study demonstrating, in their opinion, rapid degradability of OIT. Consequently, they proposed a chronic M-factor of 10.

Regarding the mean measured concentrations the DS explained that OIT is a thiazolinone with a specific mode of action in algae. OIT is taken up by algal cells and transformed so it no longer exists. This process occurs rapidly and induces algal toxicity. Taking a non-standard approach to use initial measured concentrations is considered appropriate in this special case. This approach has been used by RAC before with other thiazolinones.

The DS answered that the *Navicula pelliculosa* 48-hour NOEC of 0.071 µg/L was not used because of the uncertainty concerning the use of shorter duration chronic endpoints in hazard classification. Considering that RAC concluded before in two isothiazolinone opinions that

shorter duration chronic endpoints were relevant if validity criteria were met, the DS proposed to change the classification proposal and base the chronic classification on the 48-h E_rC_{10} of 0.000224 mg/L for *Navicula pelliculosa* resulting from the same study as the NOEC of 0.071 µg/L. The E_rC_{10} is based on statistical analysis and initial measured concentrations. OECD TG 201 (July 2011) validity criteria were met for 0-48 hours including exponential growth over this period. This value is in the M-factor range 0.0001 to 0.001 mg/L, which would result in a revised M-factor of 100 for a non-rapidly degradable substance.

In response to the biocide applicant's comments on rapid degradation, the DS explained that OIT undergoes rapid primary degradation in combination with some mineralisation. One of the degradation products NNOMA was observed during an aquatic photolysis study at 12.5 % AR as a mixture with oxamic acid. The aquatic toxicity and fate data for NNOMA indicate it would be classified as Aquatic Acute 1 and Aquatic Chronic 2. NNOMA was not confirmed in the two OECD TG 309 simulation studies, which were conducted in the dark indicating it may be formed under light conditions. The metabolite identification study identifies numerous degradants found in the former aerobic simulation studies in seawater and in river water in the dark. However, information on fate and aquatic toxicity data on the degradants are lacking. Consequently, it is not possible to evaluate if the degradants meet the classification criteria or not. The DS retained their conclusion that OIT is not rapidly degradable for classification purposes. The biocide applicants were of the opinion that the criteria for rapid degradability are fulfilled if not more than 30 % AR is left in the system after 28 days. However, CLP does not include such criteria or allow for such an interpretation. The identification and aquatic hazard classification of degradants is of importance when considering rapid degradation via primary degradation under CLP. The biocide applicants also proposed to consider non-extractable residue (NER) as irrelevant because it is not bioavailable. RAC agrees with the DS' conclusion that NER is under discussion in relation to persistency assessment and currently, unless there is data to the contrary, NER are not currently accounted for in the rate of removal.

Assessment and comparison with the classification criteria

Octhilinone was stable to hydrolysis, although it is susceptible to photodegradation. The experimental DT_{50} in sterile pure water was 3.7 days at 50°N in summer sunlight. The respective DT_{50} in pond water was 5.1 days. The photodegradation DT_{50} values reflect degradation to degradants and mineralisation to CO_2. No degradation of OIT was observed in an OECD TG 301D ready biodegradability test. Although this might have been influenced by a partially inhibitory test concentration, the substance is considered not readily biodegradable. In two aquatic biodegradation simulation studies using freshwater and seawater, the 12°C DT_{50} values were from 1.1 to 2.3 days and from 3 to 4 days, respectively. The decrease in water phase AR is considered due to primary biodegradation of OIT to degradants which were subsequently mineralised and some partitioning to dissolved organic solid matter. Three degradants over 10 % were detected but could not be identified in the freshwater study. In the seawater study, various degradants were observed at concentrations less than 10 % AR. Altogether, RAC does not agree to the biocide applicant's interpretation of the application of the CLP criteria. RAC agrees with the DS conclusion and reasoning on OIT being not rapidly degradable for classification purposes.

The Log K_{OW} based on the solubility of OIT in *n*-octanol and water at different pH and temperature was considered to be > 3.1. In the experimental aquatic BCF study with Rainbow Trout (*Oncorhynchus mykiss*), the whole fish steady state BCFs were 507 ± 87 L/kg (high dose) to 538 ± 65 L/kg (low dose) wet weight. Lipid normalising the BCFs to 5 % lipid content

increased BCFs to 843 to 886 L/kg wet weight. The BCF values are greater than the trigger value of 500 for potentially bioaccumulative substances. Consequently, RAC agrees with the DS that OIT has a potential to bioaccumulate.

There were acute data available for fish, invertebrates, algae and aquatic plants. RAC is of the opinion that the lowest acute aquatic toxicity value is a 48-hour E_rC_{50} of 0.00129 mg/L for the algae *Navicula pelliculosa*. In this study, the controls appeared valid for exponential growth (based on 16-fold control cell increase), but from 48 hours exponential growth did not occur and the controls were not reliable. On this basis only, endpoints up to 48 hours are considered reliable. The 48-hour endpoint is chosen because thiazolinones are rapidly taken up by algae where they react with enzymes to disrupt metabolism and inhibit growth. During this process, the parent substance is degraded and depleted from solution. This mode of action is rapid with uptake and enzyme effects in minutes affecting cell viability and resulting in cell death over hours. The 48-hour E_rC_{50} values from tests with *Skeletonema costatum* and *Pseudokirchneriella subcapitata* are in the same range as the 48-hour *Navicula pelliculosa* value. Given the mode of action, algal study results based on initial measured concentrations are appropriate. The value of 0.00129 mg/L fulfils the criteria for Aquatic Acute 1, i.e. < 1 mg/L. The value is in the range of $0.001 < L(E)C_{50} \leq 0.01$, giving an M-factor of **100**.

There were chronic data available for fish, invertebrates, algae and aquatic plants. RAC is of the opinion that the lowest value is a 48-h E_rC_{10} of 0.000224 mg/L for *Navicula pelliculosa*. OECD TG 201 (July 2011) validity criteria were met for 0-48 hours including exponential growth over this period. Therefore, the 48-h E_rC_{10} from the study is considered valid. Due to the specific mode of action of thiazolinones in algae, 48-hour test duration and use of initial measured concentrations are considered appropriate for chronic classification. In the other algae tests presented in Table xx, the initially measured E_rC_{10} values at 72 hours are already greater than at 48 hours reflecting the specific mode of action of OIT with the algae. The value of 0.000224 mg/L fulfils the criteria for Aquatic Chronic 1, i.e. ≤ 0.1 mg/L for a non-rapidly degradable substance. The value is in the range $0.0001 < NOEC \leq 0.001$, giving an M-factor of **100**.

Overall, RAC agrees with the DS proposal to classify octhilinone as **Aquatic Acute 1; H400 (M=100) and Aquatic Chronic 1; H410 (M=100)**.

6 OTHER INFORMATION

None available.

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8. ANNEXES

ANNEX II – Aquatic toxicity data for OIT degradants

Confidential Reference Annex

ANNEX II – Aquatic toxicity data for OIT degradants.

Table 1: Summary of relevant information on aquatic toxicity for OIT degradants

Degradant / Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/l)	
N-(n-octyl) malonamic acid (aka NNOMA)							
Acute toxicity to fish US EPA FIFRA Guideline 72-1, GLP, purity 96.9%	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	250 (mm)	Anonymous (1994)
Daphnia sp Acute Immobilisation OECD Guideline, 202, GLP, purity 99.72%	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	157 (mm)	Hahne, 2002a
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 99.7%	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	8.66 (initial 0h measured) 1.82 (initial 0h measured) (Note i)	Hahne, 2002b
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 99.72%	*Skeletonema costatum*	Cell multiplication inhibition	Static	72 hours	ErC_{50} NOErC	0.44 (initial 0h measured) 0.064 (initial 0h measured) Analytical verification of concentrations	Hahne, 2002c
N-(n-octyl)acetamide (aka NNOA)							
Acute toxicity to fish US EPA FIFRA Guideline 72-1, GLP, purity 97.06%	Rainbow Trout (*Oncorhynchus mykiss*)	Mortality	Static	96 hours	LC_{50}	25 (mm)	Anonymous (2002b)
Daphnia sp Acute Immobilisation OECD Guideline, 202, GLP, purity 97.06%	*Daphnia magna*	Acute immobilisation	Static	48 hours	EC_{50}	28 (mm)	Rhodes, 2002a
Freshwater Algal Growth Inhibition OECD Guideline 201, GLP, purity 97.06%	*Pseudokirchneriella subcapitata**	Cell multiplication inhibition	Static	96 hours	ErC_{50} NOErC	>3.15 mg/l (mm) 1.99 (mm) Note ii	Rhodes, 2002b

Notes:
mm refers to mean measured concentrations
n refers to nominal concentrations
*formerly *Selenastrum capricornutum*

Note i:
While losses greater than 20% of nominal were observed at some treatments, this was only in treatments below the study NOEC and ErC_{50}. Therefore, the MS CA feels it is acceptable to present study effects values based on nominal concentrations.

Note ii:
The nominal exposure range was 0.63, 1.3, 2.5, 5.0, 10 and 20 mg/l. At 0h this equated to measured concentrations 0.52, 1.3, 2.7, 5.9, 11 and 19 mg/l indicating the treatments were dosed near nominal values at study initiation.

Analysis at 72 hours was 0.407, 1.04, 1.47, 1.69, <MOQ, <MOQ mg/l (MOQ = 0.26 mg/l). This indicates significant losses over the 0-72 hour duration although the extreme losses in the highest treatments (10 and 20 mg/l nominal) are not explained in the study report.

The study NOE_rC was 2.5 mg/l nominal equating to 2.7 mg/l based on 0h measured data. The MS CA has calculated the geometric mean measured value for this treatment as 1.99 mg/l.

While noting the >20% loss in test concentrations, the MS CA feels the analytical data for the higher exposure concentrations (10 and 20 mg/l nominal) may not be reliable. Therefore, it is not possible to determine an E_rC_{50} based on measured data. At the 5 mg/l nominal exposure treatment, 4% growth inhibition was observed. This equate to 3.15 mg/l based on mean measured data. On this basis the ErC50 is considered > 3.15 mg/l based on mean measured concentrations.

References

Hahne, R. (2002a) Acute toxicity of N-(n-octyl) malonamic acid, technical, PMN to the water flea, *Daphnia magna*, under static test conditions, (metabolite); ABC Laboratories Study N° 47086, Rohm and Haas Report N° 01RC-0281 (May 22, 2002). Unpublished.

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Hahne, R. (2002c) Toxicity of N-(n-octyl) malonamic acid, technical, PMN to the saltwater diatom, *Skeletonema costatum* (metabolite); ABC Laboratories Study N° 47088, Rohm and Haas Report N° 01RC-0280 (March 14, 2002). Unpublished.

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Rhodes, J.E. (2002b) Toxicity of N-(n-octyl) acetamide to the unicellular green alga, *Selenastrum capricornutum* (metabolite); ABC Laboratories Study N° 47176, Rohm and Haas Report N° 01RC-0299 (April 1, 2002). Unpublished.