Committee for Risk Assessment

RAC

Annex 1

Background document

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

1,2-benzisothiazol-3(2*H*)-one; 1,2-benzisothiazolin-3-one

EC Number: 220-120-9
CAS Number: 2634-33-5

CLH-O-0000007051-86-01/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 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

26 November 2021

CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name: 1,2-BENZISOTHIAZOL-3-(2H)-ONE (BIT)

EC Number: **220-120-9**

CAS Number: **2634-33-5**

Index Number: **613-088-00-6**

Contact details for dossier submitter:

Version number: 2.0 **Date: February 2021**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,2-BENZISOTHIAZOL-3(2*H*)-ONE; 1,2-BENZISOTHIAZOLIN-3-ONE

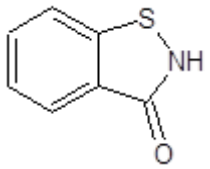
CONTENTS

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,2-BENZISOTHIAZOL-3(2*H*)-ONE; 1,2-BENZISOTHIAZOLIN-3-ONE

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1,2-benzisothiazol-3-(2*H*)-one 1,2-benzisothiazolin-3-one 2,3-dihydro-1,2-benzothiazol-3-one
Other names (usual name, trade name, abbreviation)	BIT
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	220-120-9
EC name (if available and appropriate)	-
CAS number (if available)	2634-33-5
Other identity code (if available)	-
Molecular formula	C_7H_5NOS
Structural formula	
SMILES notation (if available)	O=C1NSC2=C1C=CC=C2
Molecular weight or molecular weight range	151.19 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	*96.5% - 98.5%*

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3 (CLP)**	**Current self-classification and labelling (CLP)**
1,2-benzisothiazol-3-(2*H*)-one CAS: 2634-33-5	96.5% - 98.5%	H302, H315, H318, H317, H400	H302, H315, H318, H317, H400; GHS07, GHS05, GHS09; Dgr

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
-	-	-	-	-

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
-	-	-	-	-	-

2 PROPOSED HARMONISED CLASSIFICATION AND LABELING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: For substance with an existing entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	613-088-00-6	1,2-benzisothiazolin-3-one (BIT)	220-120-9	2634-33-5	Acute Tox. 4 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1	H302 H315 H318 H317 H400	GHS07 GSH05 GHS09 Dgr	H302 H315 H318 H317 H400		Skin Sens. 1; H317: C ≥ 0,05 %	
Dossier submitters proposal	613-088-00-6	1,2-benzisothiazol-3(2*H*)-one; 1,2-benzisothiazolin-3-one	220-120-9	2634-33-5	**Retain** Acute Tox. 4 Aquatic Acute 1 **Add** Acute Tox. 2 Aquatic Chronic 1 **Modify** Skin Sens. 1B **Remove** Skin Irrit. 2	**Retain** H302 H317 H400 **Add** H330 H410 **Remove** H315	**Retain** GHS09 **Add** GHS06 **Modify** GHS07	**Retain** H302 H317 **Add** H330 **Modify** H410 **Remove** H315		**Add** Oral: ATE = 454 mg/kg Inhalation: ATE = 0.25 mg/L M (acute)=1 M (chronic)=1 **Modify** Skin Sens. 1B; H317: C ≥ 0,05 %	
Resulting Annex VI entry if agreed by RAC and COM	613-088-00-6	1,2-benzisothiazolin-3-one (BIT)	220-120-9	2634-33-5	Acute Tox. 4 Acute Tox. 2 Eye Dam. 1 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H302 H330 H318 H317 H400 H410	GHS06 GHS05 GHS09 Dgr	H302 H330 H318 H317 H410		Oral: ATE = 454 mg/kg Inhalation: ATE = 0.25 mg/L Skin Sens. 1B; H317: C ≥ 0,05 % M=1 M=1	

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Harmonised classification proposed	Yes
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Harmonised classification proposed	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Harmonised classification proposed	Yes
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity	Hazard class not assessed in this dossier	No
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	Yes

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELING

The current entry in Annex VI of BIT does not include harmonised classification in category Acute Tox. (inhalation), however based on the evidence we propose the classification as H330 (Acute Tox. 2).

The current entry in Annex VI of BIT already includes harmonised classification in category Skin Irrit. 2, however data are conclusive but not sufficient for classification.

The current entry in Annex VI of BIT already includes harmonised classification in category Skin Sens. 1 (H317: C > 0.05%), however based on the evidence we proposed the classification as Skin Sens. 1B retaining SCL.

The current entry in Annex VI of BIT already includes harmonised classification in category Aquatic Acute 1, however it does not include harmonised classification of long-term aquatic hazard.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

Further detail on need of action at Community level:

Although this biocidal active substance has a current entry in Annex VI of CLP regulation, it is necessary to update the current human health and environmental hazards due to differences in acute toxicity, skin irritation, skin sensitization and aquatic chronic hazards, as well as, its ATEs and M-Factors with the current harmonised classification.

5 IDENTIFIED USES

BIT can be used with biocidal purposes as disinfectant or as preservative, such as these following uses:

1. For the preservation of manufactured products, other than foodstuffs or feeding stuffs, in cans, tanks or other closed containers by control of microbial deterioration to ensure their shelf life. Relevant applications include:
    - Washing and cleaning fluids (professional use), hygienic products (professional and nonprofessional use)
    - Detergents (professional and non-professional use)
    - Paints and coatings (professional and non-professional use)
    - Fluids used in paper, textile and leather production (professional use)
    - Lubricants (professional use)
    - Fuels
    - Glues and adhesives
2. In the metal industry, which can be divided into different working sectors, as follows:
    - Blast furnaces: production of steel
    - Iron foundry: moulding of steel into half or end products
    - Rolling mills: rolling of steel to half products to be used by the steel production industry
    - Metal forming: forcing of metal products in the shape of the end product
    - Metal cutting: creation of products by cutting away chips of the product
      Galvanic industry: application of protective metal coatings to metal products
3. For the preservation of fibres, leather, rubber, or polymers (e.g. vinyl membranes)
4. For the preservation for liquid cooling systems, (e.g. once-through cooling systems and recirculating cooling systems)
5. For controlling slimes (preservation) in mineral oil extraction and in papermills

BIT is also used with the following uses:

1. Scientific research and development

2. As a co-formulant in Plant Protection Products (e.g. spray applications and seed and granular applications by professionals or consumers)
3. As an ingredient of cosmetics and personal care products

6 DATA SOURCES

The following data sources were used by the Dossier Submitter (DS):

1. Draft final CAR of 1,2-benzisothiazolin-3-one (BIT) notified for different product types (PT 2, 6, 9, 11, 12 and 13) as existing substance, and for PT 10 as new substance according Art. 7, 8 and 9 of Regulation (EU) 528/2012.
2. Registration dossier of 1,2-benzisothiazolin-3-one (BIT) according to REACH Regulation
3. RAC opinion on MBIT CLH-O-0000001412-86-209/F
4. SCCS opinion on BIT COLIPA n° P96
5. US EPA RED for BIT
6. US EPA review draft risk assessment for BIT
7. TC C&L, 1993 and 1995

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Applicant	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	Solid powder Damp powder	CAR; Proyect 1274163 CAR; Proyect 1248276	
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	Fine, non free-flowing crystalline powder with a few large hard clumps Fine, non free-flowing crystalline powder that tends to form clumps, which are easily broken, with a few large hard clumps	CAR; Report No. 1606/0043 CAR; Report No. 1606/0043	At 22.0 ± 0.5°C At 22.0 ± 0.5°C
	Troy Chemical	Crystalline solid Solid powder	CAR; Report No. 9-94B02BIT CAR; Report No. 61524	
Melting/freezing point	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	157.1 ± 0.4°C	CAR; Project 1274163	

Property	Applicant	Value	Reference	Comment (e.g. measured or estimated)
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	157 ± 0.5 ºC	CAR; Report No. 1606/0043	
	Troy Chemical	159.5 - 160ºC	CAR; Report No. 9-94B02BIT	
Boiling point	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	328.7°C	CAR; Project No. 840980	BIT decomposed above 300°C
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	Not Applicable	CAR; Report No. 1606/0043	DSC Thermogram does not exhibit a boiling point below 340ºC
Relative density	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	1.483 at 20.0 °C	CAR; Study No. RS/01/025	
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	1.50 at 22.0 ± 0.5 ºC	CAR; Report No. 1606/0043	
	Troy Chemical	1.361 ± 0.02 g/mL at 20 ± 1 ºC	CAR; Report No. 9-94B02BIT	
Vapour pressure	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	6.3×10^{-5} Pa at 20 °C 1.4×10^{-4} Pa at 25 °C	CAR; Report No. 00036	
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	1.1×10^{-4} Pa at 20 °C 2.3×10^{-4} Pa at 25.0 °C	CAR; Report No. 1606/0043	

Property	Applicant	Value	Reference	Comment (e.g. measured or estimated)
	Troy Chemical	$1.5x10^{-4}$ Pa at 25 ± 1 ºC 3.02×10^{-3} Pa at 20 ºC 8.91×10^{-3} Pa at 25 ºC	CAR; Report No. 9-94B02BIT CAR; Report No. 3911	
Surface tension	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	The surface tension of an aqueous solution 1 g/L at 20°C was 72.6 mN/m.	CAR; Report No. B 013/2007	BIT is not a surfactant
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	71.5 mN/m (0.881 g/L solution) at 21.6 ± 0.5ºC	CAR; Report No. 1606/0043	
	Troy Chemical	71.5 mN/m (0.881 g/L solution) at 21.6 ± 0.5 ºC	CAR; Report No. 1606/0043	
Water solubility	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	Water solubility (in g/L) Nominal pH 4.8: 10ºC 0.727 20ºC 0.938 30ºC 1.196 Nominal pH 6.7: 20ºC 1.288 Nominal pH 9.1: 20ºC 1.651	CAR; Study No. RS/01/029	
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	The water solubility has been demonstrated to vary with pH and temperature (in g/L) Nominal pH 5: 10ºC 0.712 20ºC 0.976 30ºC 1.400 Nominal pH 7: 10ºC 0.860 20ºC 1.150 30ºC 1.590 Nominal pH 8: 10ºC 8.840 20ºC 9.330	CAR; Report No. 1606/0043	

Property	Applicant	Value	Reference	Comment (e.g. measured or estimated)
		30ºC 9.880		
	Troy Chemical	1.118 g/L at 20ºC	CAR; Report No. 9-94B02BIT	
Partition coefficient n-octanol/water	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	pH 5 at 20 °C $logP_{ow}$ of 0.99 pH 7 at 10, 20 and 30 °C $logP_{ow}$ of 0.63, 0.70 and 0.76, respectively pH 9 at 20 °C $logP_{ow}$ of -0.90	CAR; Study No. RS/01/021	
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	pH / T ºC / P_{ow} / $Log_{10}P_{ow}$ 5 / 10 / 30.0 / 1.48 5 / 30 / 26.8 / 1.43 7 / 10 / 19.8 / 1.30 7 / 20 / 15.4 / 1.19 7 / 30 / 17.2 / 1.24 9 / 10 / 1.67 / 0.224 9 / 30 / 1.07 / 2.78×10^{-2}	CAR; Report No. 1606/0043	
	Troy Chemical	$logP_{ow}$ 1.40 ± 0.06 at 21 ºC	CAR; Report No. 9-94B02BIT	
Flash point	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	Not required the test substance is a solid at ambient temperature with a melting point >50ºC	CAR	
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	Not required the test substance is a solid at ambient temperature with a melting point >50ºC	CAR	
	Troy Chemical	The flash-point is not required since BIT is a solid	CAR	
Flammability	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	Not highly flammable	CAR; Report No. B 053/2006	

Property	Applicant	Value	Reference	Comment (e.g. measured or estimated)
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	Not highly flammable	CAR; Report No. 1606/0043	
	Troy Chemical	Not highly flammable Not highly flammable	CAR; Report No. 1606/0043 CAR; Study No. 62630	
Explosive properties	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	Thermal Sensitivity: No Reaction Mechanical Sensitivity (shock): No Reaction Mechanical Sensitivity (friction): No Reaction TGAI has no explosive properties.	CAR; Report No. B 003/2007	
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	No explosive properties were observed	CAR; Report No. 1606/0043	
	Troy Chemical	The molecular structure of BIT indicates that the substance has no explosive properties. Therefore, a study is not required	CAR	
Self-ignition temperature	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	No self-ignition temperature below melting temperature.	CAR; Report No. B 004/2007	
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	No self-ignition temperature below the melting temperature	CAR; Report No. 1606/0043	
	Troy Chemical	No relative self-ignition temperature below its melting point No relative self-ignition temperature below its melting point	CAR; Report No. 1606/0043 CAR; Report No. 0044/1391	

Property	Applicant	Value	Reference	Comment (e.g. measured or estimated)
Oxidising properties	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	No oxidizing properties were observed	CAR; Report No. B 001/2007	
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	No oxidizing properties were observed	CAR; Report No. 1606/0043	
	Troy Chemical	The molecular structure of BIT indicates that the substance has no oxidising properties. Therefore, a study is not required	CAR	
Granulometry	-	-	-	-
Stability in organic solvents and identity of relevant degradation products	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	Active substance as manufactured does not contain any organic solvent.	CAR	
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	Active substance as manufactured does not contain any organic solvent.	CAR	
	Troy Chemical	Stable in deionised water, heptane, ethyl acetate and 1-octanol at ambient temperature for 24, 48 and 120 h	CAR; Report No. 9-94B02BIT	
Dissociation constant	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	pKa of 7.2 at 25 °C pKa of 7.5 ± 0.1 at 25 °C	CAR; Report No. SSL00801 CAR; Project 175	
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	pKa= 7.17	CAR; Report No. 1606/0043	

Property	Applicant	Value	Reference	Comment (e.g. measured or estimated)
	Troy Chemical	pKa = 7.04	CAR; Report No. 9-94B02BIT	
Viscosity	**Lonza (Arch UK Biocides Ltd)** **Thor GmbH**	Not required the test substance is a solid.	CAR	
	Specialty Electronic Materials Switzerland (Rohm and Haas) and Lanxess	Not required the test substance is a solid.	CAR	
	Troy Chemical	The viscosity is not required since BIT is a solid	CAR	

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Hazard class not assessed in this dossier.

8.2 Flammable gases (including chemically unstable gases)

Hazard class not assessed in this dossier.

8.3 Oxidising gases

Hazard class not assessed in this dossier.

8.4 Gases under pressure

Hazard class not assessed in this dossier.

8.5 Flammable liquids

Hazard class not assessed in this dossier.

8.6 Flammable solids

Hazard class not assessed in this dossier.

8.7 Self-reactive substances

Hazard class not assessed in this dossier.

8.8 Pyrophoric liquids

Hazard class not assessed in this dossier.

8.9 Pyrophoric solids

Hazard class not assessed in this dossier.

8.10 Self-heating substances

Hazard class not assessed in this dossier.

8.11 Substances which in contact with water emit flammable gases

Hazard class not assessed in this dossier.

8.12 Oxidising liquids

Hazard class not assessed in this dossier.

8.13 Oxidising solids

Hazard class not assessed in this dossier.

8.14 Organic peroxides

Hazard class not assessed in this dossier.

8.15 Corrosive to metals

Hazard class not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Hazard class not assessed in this dossier.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 8: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, n°/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD TG 425 US EPA OPPTS 870.1100	Rat Sprague-Dawley derived albino F 3 rats/group	89.8% a.i. Gavage	340 and 1078 mg BIT/kg bw	606 mg/kg	[redacted], 2007 IIIA6.1.1/01 ([redacted])

Method, guideline, deviations if any	**Species, strain, sex, n°/group**	**Test substance**	**Dose levels, duration of exposure**	**Value** LD_{50}	**Reference**
OECD TG 401	Rat Crl:CD®BR M+F 5 rats/sex/group	99.29% a.i. Gavage	M: 600, 1200 and 1500 mg BIT/kg bw F: 600, 900 and 1200 mg BIT/kg bw	M: 1246 mg/kg F: 944 mg/kg C: 1010 mg/kg	[redacted], 1993 AIII6.1.1/1 ([redacted])
OECD TG 401	Rat Wistar M+F 5 rats/sex/group	97.42% a.i. Gavage	438, 585, 680 and 877 mg BIT/kg bw	C: 582 mg/kg	[redacted], 2003a AIII6.1.1/2 ([redacted])
Comparable to OECD TG 401 and EC B.1	Rat Wistar-derived albino M+F 5 rats/sex/group	73.1% a.i. Gavage	100, 300, 500 and 900 mg PROXEL™/kg bw (PROXEL™ contains 73.1% BIT)	(Adjusted for 73.1% purity) M: 490 mg/kg F: 573 mg/kg C: 532 mg/kg	[redacted], 1988a IIIA6.1.1/1 ([redacted]) (REACH registration dossier)
OECD TG 401, EC B.1	Rat CD M (+ F at lowest dose) 5 rats/sex/group	Purity not specified Gavage	202, 320 and 506 mg BIT/kg bw	M: 454 mg/kg	[redacted], 1994a IIIA6.1.1/2 ([redacted]) (REACH registration dossier)

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Five independent studies of acute oral toxicity displayed a range of LD_{50} records between 454 and 1010 mg/kg bw. The most common clinical signs were hypoactivity, piloerection, lethargy, hunched posture, staggering gait and miosis. Gastrointestinal and pulmonary lesions were found very often in necropsies.

10.1.2 Comparison with the CLP criteria

Classified as Acute Tox. 4 (H302: Harmful if swallowed) because, according to the CLP 3.1.2.1. (Table 3.1.1), $300 < LD_{50} \leq 2000$ according to the toxicity test result. Taking into account the LD_{50}, ES-eCA proposes an ATE = 454 mg/kg.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Acute Tox. 4; H302: Harmful if swallowed; GHS07; Warning; Oral: ATE = 454 mg/kg.

10.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Table 9: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
OECD TG 403 (nose-only) US EPA OPPTS 870.1300	Rat Crl:CD(SD) M+F 5 rats/sex/group	89.8% a.i. Dust/mist MMAD = 2.5 + 2.75, 2.8 + 2.63, and 3.3 + 2.49 µm for the 0.088, 0.25 and 0.32 mg/L groups, respectively	0.088, 0.25 and 0.32 mg BIT/L 4 h	0.25 mg/L	[redacted], 2007 IIIA6.1.3/01 ([redacted])

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

There were no fatalities in animals exposed to 0.088 mg BIT/L air. Clinical observations for this group included rales, gasping, laboured respiration, partial closure of eyes, increased respiration, hypoactivity, and vocalization upon handling. The necropsy recorded pale kidneys in 1 male belonging to this group.

Four males and 1 female died during the exposure to 0.25 mg BIT/L of air. Clinical findings for this group included rales, gasping, laboured respiration, partial closure of eyes, and increased respiration. The abnormalities recorded during the necropsies included dark red discoloration of the mandibular lymph nodes of 3 females, an enlarged mandibular lymph node in 1 female and pale lung that were not fully collapsed in 1 male.

Only 1 male and 1 female survived to the exposure to 0.32 mg BIT/L of air (all males died during or immediately following exposure and all females died during the exposure). Rales, gasping, laboured respiration, and partial closure of eyes were recorded during clinical observations of the animals. The necropsy of 1 female detected dark red discoloration of the thyroid gland.

The estimated LC_{50} for males and females were 0.21 and 0.28 mg BIT/L of air respectively, while the combined LC_{50} was 0.25 mg BIT/L air.

10.3.2 Comparison with the CLP criteria

Classified as Acute Tox. 2 (H330: Fatal if inhaled) because, according to the CLP 3.1.2.1. (Table 3.1.1), $0.05 < LC_{50} \leq 0.5$ according to the toxicity test result. Taking into account the LC_{50}, ES-eCA proposes an ATE = 0.25 mg/L.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Acute Tox. 2; H330: Fatal if inhaled; GHS06; Danger; Inhalation: ATE = 0.25 mg/L

RAC evaluation of acute toxicity

ACUTE TOXICITY – ORAL ROUTE

Summary of the Dossier Submitter's proposal

1,2-benzisothiazolin-3-one (BIT) has a minimum classification for acute oral toxicity as Acute Tox. 4* (H302: Harmful if swallowed). The DS proposed classification as Acute Tox. 4 (H302: Harmful if swallowed) based on five independent studies which displayed a range of LD_{50} values between 454 and 1010 mg/kg bw (300 < LD_{50} ≤ 2000 mg/kg bw, corresponds to Category 4). The DS proposed an ATE of 454 mg/kg bw.

Comments received during consultation

Three Industry Stakeholders agreed with the classification, but one of them suggested that the ATE should be based on the Anonymous (2003a) study since this appears to be guideline compliant, with both male and female animals exposed to a highly pure test material and 4 dose groups were employed, enabling more accurate estimation of the LD_{50}. They therefore argued that the ATE for BIT should be 582 mg/kg.

Assessment and comparison with the classification criteria

***Table**: Summary of the acute oral toxicity studies with BIT.*

Method, guideline, deviations if any	**Species, strain, sex, nº/group**	**Test substance**	**Dose levels**	**LD_{50}** **Mortalities**	**Reference**
OECD TG 425 US EPA OPPTS 870.1100 GLP	Rat Sprague-Dawley derived albino F 3 rats/group	89.8% a.i. Gavage vehicle: 0.5% Carboxymethyl-cellulose in distilled water	340 and 1078 mg BIT/kg bw	606 mg/kg 340 mg/kg: 0/3 1078 mg/kg:3/3	Anonymous, 2007 IIIA6.1.1/01
OECD TG 401 GLP (self certified)	Rat Crl:CD®BR M+F 5 rats/sex/group	99.29% a.i. Gavage vehicle: distilled water	M: 600, 1200 and 1500 mg BIT/kg bw F: 600, 900 and 1200 mg BIT/kg bw	M: 1246 mg/kg F: 944 mg/kg C: 1010 mg/kg Dose (mg/kg bw) / Male / Female 600 / 0/5 / 0/5 900 / - / 2/5 1200 / 2/5 / 5/5 1500 / 5/5 / -	Anonymous 1993 AIII6.1.1/1

<table>
<tr>
<td>OECD TG 401

GLP</td>
<td>Rat

Wistar

M+F

5 rats/sex/group</td>
<td>97.42% a.i.

Gavage

vehicle: 0.5% solution of Carboxymethyl-cellulose</td>
<td>438, 585, 680 and 877 mg BIT/kg bw</td>
<td>C: 582 mg/kg
<table>
<tr><th>Dose (mg/kg bw)</th><th>Male</th><th>Female</th></tr>
<tr><td>438</td><td>0/5</td><td>3/5</td></tr>
<tr><td>585</td><td>2/5</td><td>2/5</td></tr>
<tr><td>680</td><td>3/5</td><td>4/5</td></tr>
<tr><td>877</td><td>5/5</td><td>5/5</td></tr>
<tr><td>1315</td><td>3/5</td><td>5/5</td></tr>
</table>
</td>
<td>Anonymous, 2003a

AIII6.1.1/2</td>
</tr>
<tr>
<td>Comparable to OECD TG 401 and EC B.1

GLP</td>
<td>Rat

Wistar-derived albino

M+F

5 rats/sex/group</td>
<td>73.1% a.i.

Gavage

vehicle: 0.5% aqueous polysorbate 80</td>
<td>100, 300, 500 and 900 mg PROXEL™/kg bw

(PROXEL™ contains 73.1% BIT)</td>
<td>(Adjusted for 73.1% purity)
M: 490 mg/kg
F: 573 mg/kg
C: 532 mg/kg
<table>
<tr><th>Dose (mg/kg bw)</th><th>Male</th><th>Female</th></tr>
<tr><td>73</td><td>0/5</td><td>0/5</td></tr>
<tr><td>219</td><td>0/5</td><td>0/5</td></tr>
<tr><td>366</td><td>0/5</td><td>1/5</td></tr>
<tr><td>658</td><td>5/5</td><td>3/5</td></tr>
</table>
</td>
<td>Anonymous, 1988a

IIIA6.1.1/1 (REACH registration dossier)</td>
</tr>
<tr>
<td>OECD TG 401, EC B.1

GLP</td>
<td>Rat

CD

M (+ F at lowest dose)

5 rats/sex/group</td>
<td>Purity not specified

Gavage

vehicle: 0.5% aqueous methylcellulose</td>
<td>202, 320 and 506 mg BIT/kg bw</td>
<td>M: 454 mg/kg
<table>
<tr><th>Dose (mg/kg bw)</th><th>Male</th><th>Female</th></tr>
<tr><td>202</td><td>0/5</td><td>0/5</td></tr>
<tr><td>320</td><td>1/5</td><td>-</td></tr>
<tr><td>506</td><td>3/5</td><td>-</td></tr>
</table>
</td>
<td>Anonymous, 1994a

IIIA6.1.1/2 (REACH registration dossier)</td>
</tr>
</table>

Five studies were summarised in the CHL dossier, all performed according to GLP, 1 conducted according to OECD TG 425, and 4 according to OECD TG 401 or comparable TG,. The LD_{50} values range from 454 mg/kg bw to 1010 mg/kg bw, which are all in the range ($300 < LD_{50} \leq 2000$ mg/kg bw) for Category 4.

The Anonymous (2007) study is not appropriate for establishing an ATE, as it used only 2 doses, and at the lower dose all 3 animals survived, while at the higher dose all died. The Anonymous (1993) study had LD_{50} values which are in a higher range than in the other studies (males: 1246 mg/kg bw, females: 944 mg/kg bw, combined: 1010 mg/kg bw). The remaining 3 studies had LD_{50} values in the same range. The Anonymous (2003a) study gave a combined LD_{50} of 582 mg/kg bw, the Anonymous (1988a) study gave LD_{50} values of 490 mg/kg bw (males), 573 mg/kg bw (females), and 532 mg/kg bw (combined), while the Anonymous 1994a study provided an LD_{50} value of 454 mg/kg bw for male rats.

From the 3 studies that used both sexes, two (Anon., 1993; Anon. 2003a) indicated that females are more sensitive, but the third study (Anon. 1988a) indicated the opposite, so no unequivocal conclusion can be drawn on one of the sexes being more sensitive than the other.

The DS proposed to use the lowest LD_{50} (454 mg/kg bw) for the ATE, derived from the Anonymous (1994a) study. Although the purity of the substance was not specified and it used only male rats, since the sex of the animals does not appear to affect the results, and the study was done according to the OECD TG 401 and under GLP, there was no reason to disregard it. RAC agreed to use this LD_{50} value, rounded to 450 mg/kg bw.

The LD_{50} values of all 5 studies are in the range for Category 4 ($300 < LD_{50} \leq 2000$ mg/kg bw), therefore RAC agrees that BIT should be classified as **Acute Tox. 4 (H302: Harmful if swallowed), with an ATE value of 450 mg/kg bw.**

ACUTE TOXICITY – INHALATION ROUTE

Summary of the Dossier Submitter's proposal

The CLH dossier summarised one 4-hour inhalation study, which is an OECD TG 403 compliant (nose-only) study performed according to GLP. The derived LC_{50} for combined sexes was 0.25 mg BIT/L. The DS proposed to classify BIT as Acute Tox. 2 (H330: Fatal if inhaled), with an ATE of 0.25 mg/L.

***Table**: Summary of the acute inhalation toxicity study with BIT.*

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**LC_{50}** **Mortalities**	**Reference**
OECD TG 403 (nose-only) US EPA OPPTS 870.1300 GLP	Rat Crl:CD(SD) M+F 5 rats/sex/group	89.8% a.i. Dust/mist MMAD = 2.5± 2.75, 2.8 ± 2.63, and 3.3 ± 2.49 µm for the 0.088, 0.25 and 0.32 mg/L groups, respectively	0.088, 0.25 and 0.32 mg BIT/L 4 h	M: 0.21 mg/L F: 0.28 mg/L C: 0.25 mg/L (95% CI: 0.21-0.30 mg/L) Dose (mg/L) / Male / Female 0.088 / 0/5 / 0/5 0.25 / 4/5 / 1/5 0.32 / 4/5 / 4/5	Anonymous, 2007 IIIA6.1.3/01

Comments received during consultation

Three Industry Stakeholders agreed with the proposed classification. Two of them drew attention to an additional inhalation study which had not been included in the CLH dossier. They asked that this study (Anonymous, 2012) be taken into account when determining an ATE.

***Table**: Summary of the additional acute inhalation toxicity study with BIT.*

Method, guideline, deviations if any	**Species, strain, sex, nº/group**	**Test substance**	**Dose levels**	**LD_{50}** **Mortalities**	**Reference**
US EPA OPPTS 870.1300 GLP	Rat Sprague-Dawley derived albino M+F 5 rats/sex/group	84-85% a.i. MMAD= 3.2, 3.6 and 3.5 for the 0.054, 0.55 and 2.21 mg BIT/L groups, respectively	0.054, 0.55 and 2.21 mg BIT/L 4 h nose-only	M: 0.5 mg/L (95% CI: 0.25-1.00) F: 0.57 mg/L (95% CI: 0.05-2.94) C: 0.5 mg/L (95% CI: 0.18-0.98) Dose (mg/L) / Male / Female 0.054 / 0/5 / 0/5 0.55 / 3/5 / 3/5 2.21 / 5/5 / 4/5	Anonymous, 2012

Assessment and comparison with the classification criteria

There are 2 acute inhalation toxicity studies, one included in the CLH dossier, and one submitted during the consultation. Both tests used 4-hour, nose-only exposures, and are guideline compliant, performed according to GLP. Both used 5 rats/sex/group and tested 3 dose levels.

The doses in the Anonymous (2007) study (Table "Summary of the acute inhalation toxicity study with BIT", above) were 0.088, 0.25 and 0.32 mg BIT/L, with a calculated LD_{50} for combined sexes of 0.25 mg/L (95 % CI: 0.21-0.30 mg/L). The LD_{50} for males is 0.21 mg/L, while the LD_{50} for females is 0.28 mg/L, so males appear to be the more sensitive sex (see also mortality data in the Table referred to above).

The Anonymous (2012) study (Table "Summary of the additional acute inhalation toxicity study with BIT", above) used doses of 0.054, 0.55 and 2.21 mg BIT/L, and calculated an LD_{50} of 0.5 mg/L (95 % CI: 0.18-0.98) for the combined sexes, 0.5 mg/L (95 % CI: 0.25-1.00) for males and 0.57 mg/L (95 % CI: 0.05-2.94) for females. This study also indicates, although to a lesser degree than in the other study, that males are the more sensitive sex.

Both studies give LD_{50} values which correspond to Category 2 ($0.05 < LC_{50} \leq 0.5$). RAC proposes to use the lowest LD_{50}, calculated for males in the Anonymous (2007) study to derive an ATE.

RAC proposes that BIT warrants the classification of **Acute Tox. 2 (H330: Fatal if inhaled), with an ATE of 0.21 mg/L (dusts and mists).**

10.4 Skin corrosion/irritation

Table 10: Summary table of animal studies on skin corrosion/irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, n°/group</th><th>Test substance</th><th>Dose levels duration of exposure</th><th>Results
-Observations and time point of onset
-Mean scores/animal
-Reversibility</th><th>Reference</th></tr>
<tr>
<td>OECD TG 404
US EPA OPPTS 870.2500</td>
<td>Rabbit
New Zealand albino
1 M + 2 F</td>
<td>89.8% a.i.
Semi-occlusive tape on the clipped intact skin of dorsal area of trunk</td>
<td>65% w/w BIT in distilled water; 450 mg, 0.5 mL
4 h</td>
<td>
<table>
<tr><th>Score (average animals)</th><th>Time</th><th>Erythema</th><th>Edema</th></tr>
<tr><td rowspan="4">Average Draize scores (0 to maximum 4)</td><td>1 h</td><td>0.3</td><td>0.0</td></tr>
<tr><td>24 h</td><td>0.0</td><td>0.0</td></tr>
<tr><td>48 h</td><td>0.0</td><td>0.0</td></tr>
<tr><td>72 h</td><td>0.0</td><td>0.0</td></tr>
<tr><td></td><td>7 days</td><td>Not applicable</td><td>Not applicable</td></tr>
<tr><td></td><td>14 days</td><td>Not applicable</td><td>Not applicable</td></tr>
<tr><td>Average score</td><td>24, 48, 72 h</td><td>0.0</td><td>0.0</td></tr>
<tr><td colspan="2">Reversibility*</td><td>C</td><td>Not applicable</td></tr>
<tr><td colspan="2">Average time for reversibility</td><td>1 h</td><td>Not applicable</td></tr>
<tr><td colspan="4">*C: completely reversible
NC: not completely reversible
N: not reversible</td></tr>
</table>
</td>
<td>[redacted], 2007
IIIA6.1.4.a/01
([redacted])</td>
</tr>
<tr>
<td>OECD TG 404
US EPA OPPTS 870.2500</td>
<td>Rabbit
New Zealand albino
3 M</td>
<td>98% a.i.
Semi-occlusive pad on 6 cm^2 of skin on the dorsal area of the trunk previously clipped</td>
<td>80% w/w BIT in distilled water; 500 mg
4 h</td>
<td>
<table>
<tr><th></th><th colspan="3">Erythema</th><th colspan="3">Oedema</th></tr>
<tr><th>Animal no.</th><th>5849 M</th><th>5850 M</th><th>5851 M</th><th>5849 M</th><th>5850 M</th><th>5851 M</th></tr>
<tr><td>After 24 h</td><td>1</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>After 48 h</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>After 72 h</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>
</td>
<td>[redacted], 2002c
IIIA6.1.4/1
([redacted])</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, n°/group</th><th>Test substance</th><th>Dose levels duration of exposure</th><th>Results
-Observations and time point of onset
-Mean scores/animal
-Reversibility</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td></td><td>
<table>
<tr><td>Mean score 24-72 h</td><td>0.22 (reversed at 48 h)</td><td>0.0</td></tr>
</table>
</td><td></td></tr>
<tr><td>OECD TG 404</td><td>Rabbit
New Zealand White
3 M</td><td>97.42% a.i.
Semi-occlusive pad on 6 cm^2 of skin on the dorsal area of the trunk previously clipped</td><td>500 mg BIT moistened with distilled water
4 h</td><td>
<table>
<tr><th></th><th colspan="3">Erythema</th><th colspan="3">Oedema</th></tr>
<tr><th>Animal no.</th><th>1</th><th>2</th><th>3</th><th>1</th><th>2</th><th>3</th></tr>
<tr><td>After 1 h</td><td>1</td><td>1</td><td>2</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>After 24 h</td><td>1</td><td>1</td><td>2</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>After 48 h</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>After 72 h</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Mean score 24-72 h</td><td colspan="3">0.55 (reversed at 72 h)</td><td colspan="3">0.0</td></tr>
</table>
</td><td>[redacted], 2003c
IIIA6.1.4.b/02
([redacted])</td></tr>
<tr><td>US EPA PAG 81-5</td><td>Rabbit
New Zealand White albino
6 M</td><td>74.3% a.i.
Semi-occlusive tape on the clipped left flank (test site area was 2.5 cm^2)</td><td>1 g BIT/mL in deionized water; 0.5 mL
4 h</td><td>Erythema (reversed at 72 h)
<table>
<tr><td>30-60 min</td><td>24 h</td><td>48 h</td><td>72 h</td><td>96 h</td></tr>
<tr><td>1.33</td><td>1.0</td><td>1.0</td><td>0.5</td><td>0.0*</td></tr>
</table>
Oedema (reversed at 72 h)
<table>
<tr><td>30-60 min</td><td>24 h</td><td>48 h</td><td>72 h</td><td>96 h</td></tr>
<tr><td>0.83</td><td>0.33</td><td>0.33</td><td>0.17</td><td>0.0*</td></tr>
</table>
* Average of 3 scores rather than 6 at 96h</td><td>[redacted], 1993
IIIA6.1.4/1
([redacted])
(REACH registration dossier)</td></tr>
<tr><td>Comparable to OECD TG 404</td><td>Rabbit
New Zealand White albino
3 M</td><td>Purity not specified
Semi-occlusive bondage to the closely-clipped dorsal</td><td>500 mg in 0.2 mL distilled water
4 h</td><td>
<table>
<tr><th rowspan="2">Animal</th><th rowspan="2">Type of response</th><th colspan="8">Score</th></tr>
<tr><th colspan="2">1 h</th><th colspan="2">24 h</th><th colspan="2">48 h</th><th colspan="2">72 h</th></tr>
<tr><th></th><th></th><th>T</th><th>C</th><th>T</th><th>C</th><th>T</th><th>C</th><th>T</th><th>C</th></tr>
<tr><td rowspan="2">1</td><td>E</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>O</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="2">2</td><td>E</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>O</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="2">3</td><td>E</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>O</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>
T = Test; C = Control; E = Erythema; O = Oedema</td><td>[redacted], 1993
(REACH registration dossier)</td></tr>
<tr><td>Not specified</td><td>Guinea pig
Strain not specified</td><td>Purity not specified
Application method not</td><td>1% in a non-specified vehicle
Time of exposure not</td><td>Strong irritation. No further details of the study were given.</td><td>[redacted], 1985
(TC C&L document)</td></tr>
</table>

Method, guideline, deviations if any	**Species, strain, sex, nº/group**	**Test substance**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
	nº/sex/group not specified	specified	specified		
Not specified	Guinea pig Strain not specified nº/sex/group not specified	Purity not specified Application method not specified	1% in a non-specified vehicle Time of exposure not specified	Strong irritation. No further details of the study were given.	[redacted], 1980 (TC C&L document)
Not specified	Human volunteers Sex not specified 25 volunteers	Purity not specified Applicantion method not specified	0.8%, 0.16% and 0.04% mg in water	At 0.8% and 0.16% some skin irritation. No further information about the test method is stated.	[redacted], 1992 (TC C&L document)

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Several independent studies have showed that BIT is not able to induce skin irritation. The highest erythema and oedema scores were 1.33 and 0.33, respectively (both recorded 24 hours after exposure). All effects were reversible. Thus, BIT does not meet criteria to be classified for hazard of skin irritation and corrosion.

10.4.2 Comparison with CLP criteria

BIT does not meet the EU criteria to be classified as skin irritant. According to CLP 3.2.2.1.2.1, average primary skin irritation score < 2.3.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Classification as Skin Irrit. 2 (H315) should be removed.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

According to the DS, several independent animal studies have showed that BIT does not induce skin irritation. The highest erythema and oedema scores were 1.33 and 0.33, respectively (both recorded 24 hours after exposure), and all effects were reversible. The DS concluded that BIT does not meet the criteria to be classified for skin irritation or corrosion, and accordingly the previous classification as Skin Irrit. 2 (H315) should be removed.

Comments received during consultation

There were four comments on this endpoint. Two Industry Stakeholders agreed with the removal of the classification for skin irritation.

One MSCA commented that five reliable guideline compliant studies showed results leading to non-classification of BIT for skin irritation/corrosion according to CLP. The MSCA pointed out that there was more human data on the skin irritating effect of BIT from experiments conducted to determine skin sensitising properties additional to that provided by the DS under this endpoint. Due to the higher level of documentation and standardisation and the higher susceptibility of rabbits/animals compared to humans, animal studies are preferred over human studies, therefore the MSCA supported non-classification of BIT for skin irritation.

One Industry Stakeholder disagreed with the removal of the classification as Skin Irritation Cat. 2 (H315). They argued that while several studies in animal models do indicate BIT is not an irritant, several studies listed in the skin sensitisation section of the dossier described irritation reactions in humans. In addition, false positive, irritant responses are observed in clinical patch testing. According to ECHA guidance on IR & CSA Section

R.7.2.4.2, existing human data can be used for a classification and labelling conclusion. Furthermore, according to the ECHA guidance on the application of the CLP criteria (section 3.2.2.6), human data indicating the substance is an irritant may be used to assign a Skin Irritation Cat. 2 classification.

Assessment and comparison with the classification criteria

There is an existing classification of BIT as Skin Irrit. 2, based on 2 guinea-pig studies where strong skin irritation was seen at 1 % BIT, and several human studies: irritation was seen in 8/10 (80 %) controls at 1.0 % BIT (Freeman, 1984), in patch tested patients, irritation was seen in 121/404 (30 %) at 1.0 % BIT and in 7/466 (1.5 %) at 0.5 % Proxel XL 0.1 % BIT (Andersen and Hamann, 1984). Also, in a patch test to determine the optimal patch test concentration in 25 healthy, non-dermatological volunteers, irritation was observed at concentrations above 0.04 % BIT (Damstra *et al.,* 1992).

***Table**: Summary of the studies on skin corrosion/irritation.*

<table>
<tr><th>Method, guideline, deviations if any</th><th>Purity, Dose levels</th><th>Results</th><th>Reference</th></tr>
<tr><td>OECD TG 404
US EPA OPPTS 870.2500
Rabbit
New Zealand albino
1 M + 2 F

Semi-occlusive
4 h

GLP</td><td>89.8 % a.i.
65 % w/w BIT in distilled water;
450 mg BIT, 0.5 mL</td><td>
<table>
<tr><th>Time</th><th>Erythema</th><th>Oedema</th></tr>
<tr><td>1 h</td><td>0.3</td><td>0.0</td></tr>
<tr><td>24 h</td><td>0.0</td><td>0.0</td></tr>
<tr><td>48 h</td><td>0.0</td><td>0.0</td></tr>
<tr><td>72 h</td><td>0.0</td><td>0.0</td></tr>
<tr><td>Average score (24- 72 h)</td><td>0.0</td><td>0.0</td></tr>
<tr><td>Reversibility</td><td>Complete</td><td></td></tr>
<tr><td>Average time for reversibility</td><td>1 h</td><td></td></tr>
</table>
</td><td>Anonymous, 2007
IIIA6.1.4.a/01</td></tr>
<tr><td>OECD TG 404
US EPA OPPTS 870.2500
Rabbit
New Zealand albino
3 M

Semi-occlusive
4 h

GLP (self certified)</td><td>98 % a.i.
80 % w/w BIT in distilled water;
500 mg</td><td>
<table>
<tr><th>Time</th><th>Erythema</th><th>Oedema</th></tr>
<tr><td>24 h</td><td>1,1,0</td><td>0,0,0</td></tr>
<tr><td>48 h</td><td>0,0,0</td><td>0,0,0</td></tr>
<tr><td>72 h</td><td>0,0,0</td><td>0,0,0</td></tr>
<tr><td>Mean score (24-72 h)</td><td>0.22 (reversed at 48 h)</td><td>0.0</td></tr>
</table>
</td><td>Anonymous, 2002c
IIIA6.1.4/1</td></tr>
<tr><td>OECD TG 404
Rabbit
New Zealand White
3 M

Semi-occlusive
4 h
GLP</td><td>97.42 % a.i.
500 mg BIT moistened with distilled water</td><td>
<table>
<tr><th>Time</th><th>Erythema</th><th>Oedema</th></tr>
<tr><td>1 h</td><td>1,1,2</td><td>0,0,0</td></tr>
<tr><td>24 h</td><td>1,1,2</td><td>0,0,0</td></tr>
<tr><td>48 h</td><td>0,0,1</td><td>0,0,0</td></tr>
<tr><td>72 h</td><td>0,0,0</td><td>0,0,0</td></tr>
<tr><td>Mean score (24-72 h)</td><td>0.55 (reversed at 72 h)</td><td>0.0</td></tr>
</table>
</td><td>Anonymous, 2003c
IIIA6.1.4.b/02</td></tr>
<tr><td>US EPA PAG 81-5
Rabbit
New Zealand White albino
6 M

Semi-occlusive
4 h

GLP</td><td>74.3 % a.i.
1 g test material/mL in deionized water;
0.5 mL</td><td>
<table>
<tr><th>Time</th><th>Erythema</th><th>Oedema</th></tr>
<tr><td>30-60 min</td><td>1.3</td><td>0.83</td></tr>
<tr><td>24 h</td><td>1.0</td><td>0.33</td></tr>
<tr><td>48 h</td><td>1.0</td><td>0.33</td></tr>
<tr><td>72 h</td><td>0.5</td><td>0.17</td></tr>
<tr><td>96 h</td><td>0.0</td><td>0.0</td></tr>
<tr><td>Average score (24-72 h)</td><td>0.83</td><td>0.28</td></tr>
</table>
</td><td>Robinson, 1993
IIIA6.1.4/1
(REACH registration dossier)</td></tr>
</table>

Comparable to OECD TG 404 Rabbit New Zealand White albino 3 M Semi-occlusive 4 h	Purity not specified 500 mg in 0.5 mL distilled water	<table><tr><th>Time</th><th>Erythema</th><th>Oedema</th></tr><tr><td>1 h</td><td>0.0</td><td>0.0</td></tr><tr><td>24 h</td><td>0.0</td><td>0.0</td></tr><tr><td>48 h</td><td>0.0</td><td>0.0</td></tr><tr><td>72 h</td><td>0.0</td><td>0.0</td></tr><tr><td>Average score (24-72 h)</td><td>0.0</td><td>0.0</td></tr></table>	Rees P.B., 1993 (REACH registration dossier)
Guinea pig Strain not specified nº/sex/group not specified Application method not specified	Purity not specified 1 % in a non-specified vehicle Time of exposure not specified	Strong irritation. No further details of the study were given.	Alomar *et al*., 1985 (Technical Committee on Classification & Labelling i.e. TC C&L document)
Guinea pig Strain not specified nº/sex/group not specified Application method not specified	Purity not specified 1 % in a non-specified vehicle Time of exposure not specified	Strong irritation. No further details of the study were given.	Cronin, 1980 (TC C&L document)
Patch test to determine optimal patch test concentration (4h? 48h?) 25 healthy, non-dermatological volunteers Sex not specified	Proxel BD, 33 % dilution of BIT in water 0.16 %, 0.08 %, and 0.04 % in water	400 ppm: no irritation 800 ppm: some skin irritation 1600 ppm: some skin irritation	Damstra *et al*., 1992 (TC C&L document)
Work place study Medical surveillance	Undiluted BIT.	Minor irritation. 1 worker experienced skin irritation on his arms and legs due to a small splash of chemical while transferring BIT from an intermediate container to a dilution tank.	Specialty Electronic Materials Switzerland manufacturing plant, 2003
HRIPT 111 volunteers (26 males and 85 females); 24 hour contact, semi-occlusive patches.	19.2 % a.i. (Proxel GxL) 0.05 % BIT (500 ppm) in Rhoplex AC-64 (sample A); 0.1 % BIT (1000 ppm) in Rhoplex AC-64 (sample B); undiluted Rhoplex AC-64 (sample C).	1000 ppm: irritation in 1/111 subject at challenge (Irritation in 3 subjects but two also showed irritation to vehicle (Rhoplex AC-64)). Three subjects (No. 46, 66 and 96) exhibited irritation during the challenge period and subject 46 also displayed mild erythema (grade 1) during induction and challenge applications to samples A and C. Another subject (96) displayed mild erythema (grade 1) 48-hour after challenge. Subject 66 displayed a papular response to samples B and C at challenge application only. Subject 66 participated in a rechallenge of samples B and C. There were no observable clinical reactions noted to the test samples at rechallenge. The overall response pattern for subject number 66 is consistent with clinical irritation. No sensitisation.	Anonymous, 1991 IIIA6.12.6/01
HRIPT (Preliminary irritancy screen) 10 healthy adult volunteers 3 applications over a nine day period	Purity not stated. 500, 750 and 1000 ppm (0.05, 0.075 and 0.1 %) in propylene glycol	500 ppm: slight irritation 750 ppm: more than slight irritation 1000 ppm: more than slight irritation	Anonymous, 1975
HRIPT (main study) 50 volunteers (21 males and 29 females); 24 hour	0.5 mL of 0.05 % BIT (500 ppm) in propylene	Induction: 42/50 volunteers: barely perceptible to slight erythema (associated with papule formation in 6 volunteers) 7/50: moderate erythema (accompanied by papule	Anonymous, 1975

contact, semi-occlusive patches.	glycol (induction and first challenge tests) or liquid paraffin (second challenge test) (64.45 µg BIT/cm^2 or 250 µg BIT/patch).	formation and/or oedema in 3 volunteers) Challenge: 27/45 following application barely perceptible to slight erythema on the original and /or alternate arm to a similar degree to that seen during the induction phase, generally ameliorated slightly by 72 hours. 9/45, on original arm, reaction was as great or greater than that seen previously (from faint erythema to vesicular formation with oedema) at 24 hours, + one atypical reaction. However, the vehicle propylene glycol also elicited dermal irritation which was greater than expected. Sensitisation in 5 volunteers (11 %) confirmed by rechallenge.	
Patch test (48h) Eczema patients BIT and Proxel XL included in the standard patch-test series at the Dept. Dermatology, Gentofte Hospital, Denmark. During 14 months, 404 (1 % BIT) and 466 (0.5 % Proxel XL) eczema patients were tested.	Purity of BIT not specified. 20 % a.i. (Proxel XL) 1 % BIT (10000 ppm) in alcohol 0.5 % Proxel XL (0.1 % BIT (1000 ppm)) in water.	10000 ppm: 121/404 (30 %) irritation 1/404 (0.25 %) sensitised 1000 ppm: 7/466 (1.5 %) irritation 1/466 (0.22 %) sensitised	Andersen and Hamann, 1984 (TC C&L document)
Patch test 10 controls	Purity of BIT not specified. 0.01, 0.1 and 1 % of BIT (100, 1000 and 10000 ppm).	10000 ppm: 8/10 (80 %) irritation	Freeman, 1984 (TC C&L document)

There are 8 studies included in the CLH dossier for this hazard class. Five of them were performed according to the OECD TG 404 (or comparable guideline), 4 of them according to GLP. The studies used 3 or 6 New Zealand White Rabbits, with the test substance mixed with/wetted by distilled water. 0.5 mL was applied to clipped skin in a semi-occlusive way for 4 hours. All 5 studies showed no or minimal erythema and oedema scores, which were fully reversible.

There are two other, older guinea pig studies (Alomar *et al.*, 1985, Cronin, 1980) mentioned in the CLH dossier, in which 1 % BIT caused strong irritation. As no further information is available (purity of the substance, vehicle, application method, number of animals, exposure duration) these studies could be deemed reliable and were not taken into consideration.

One human study (Damstra *et al*., 1992) was mentioned in the CLH dossier for this endpoint. There is very little information in the dossier about this study, but the original publication states: *"Proxel® BD (ICI), a 33 % dilution of BIT in water, was used for patch testing. Water was chosen as the vehicle [] To define the optimal patch test concentration, a range (0.016 %, 0.08 %* and 0.04 %) of BIT was patch tested in 25 healthy, non-dermatological volunteers. As some irritant reactions were observed at the higher 2 concentrations, 0.04 % aq. (400 ppm) was chosen as the optimal patch concentration*."

(*The CLH dossier and the TC C&L document mentions 0.8 %, but the original paper states 0.08 % BIT)

Other studies which were not discussed by the DS for irritancy, but were included in the section dealing with skin sensitisation, that are considered by RAC to be relevant for skin irritation are summarised below:

1 worker experienced skin irritation on his arms and legs due to a small splash of undiluted BIT (medical surveillance report, 2003).

In the Anonymous (1991) (IIIA6.12.6/01) HRIPT study, it is stated that out of 111 participants, 3 displayed irritation to BIT at 0.05 % or 0.1 % diluted in Rhoplex AC-64. In two of the subjects irritation occurred with undiluted Rhoplex AC-64 as well as the samples containing BIT at challenge. From the data it can be deduced that only one participant showed irritation to 0.1 % BIT (1000 ppm) with no reaction to Rhoplex AC-64.

The Anonymous (1975) study conducted a preliminary irritancy screen preceding a repeat insult patch test (not mentioned in the CLH dossier). BIT at concentrations of 500, 750 and 1000 ppm (0.05, 0.075 and 0.1 %) in propylene glycol was applied to the skin on three occasions in an attempt to identify a non-irritating dilution. The evaluation was made on ten, healthy, adult volunteers over a nine-day period. In this preliminary trial the concentration of BIT which could be applied without producing more than slight skin irritation was 500 ppm. In the main study, at 500 ppm BIT, during induction 42/50 (84 %) volunteers had barely perceptible to slight erythema (associated with papule formation in 6 volunteers) and 7/50 (14 %) showed moderate erythema (accompanied by papule formation and/or oedema in 3 volunteers). Following the challenge application (500 ppm), barely perceptible to slight erythema on the original and/or alternate arms was found in 27/45 (60 %) volunteers.

The Andersen and Hamann (1984) study on dermatitis patients showed irritant reactions in 7/466 (1.5 %) patients patch tested with 0.5 % aqueous Proxel XL (0.1 %= 1000 ppm BIT), and 121/404 (30 %) patients showed weak irritant reactions who were patch tested with 1 % (10000 ppm) BIT in alcohol.

In the Freeman (1984) study, 10000 ppm BIT was irritating in 8/10 control subjects.

The OECD TG 404 studies do not support classification of BIT for skin irritation. The highest score for erythema was 1.33 (Anonymous, 2003c) and the highest score for oedema was 0.33 (Robinson, 1993), at 24 hours. The highest average score for 24-72 hours was given by the Robinson *et al.*, 1993 study: 0.83 (erythema) and 0.28 (oedema). Both reactions were fully reversed by 96 hours, so the criteria for skin irritation "*Mean score of ≥ 2.3-≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions*" are not met.

The human studies on the other hand demonstrate that irritation of the skin does occur. In a patch test with BIT diluted in water, there was no irritation at 400 ppm, but some skin irritation occurred at 800 and 1600 ppm in 25 healthy volunteers (Damstra *et al*, 1992). In a preliminary irritancy screen test in 10 healthy volunteers (3 applications during 9 days), 500 ppm caused slight irritation, 750 and 1000 caused more than slight irritation. The main study thus used 500 ppm BIT for the HRIPT and registered slight erythema in 42/50 subjects (papule formation in 6 of them) and moderate erythema in 7/50 subjects (papule formation/oedema in 3 of them) at induction, and in 27/45 subjects slight

erythema was seen at challenge (Anonymous, 1975). Although the study report states that the vehicle (propylene glycol) also elicited dermal irritation greater than expected and some of the irritating effects may have been caused by propylene glycol, the preliminary irritancy test, using the same vehicle, showed a dose response for BIT. In dermatitis patients skin irritation was seen in 1.5 % of patients patch tested with aqueous Proxel XL (1000 ppm BIT) and weak irritant reactions were seen in 30 % of the patients patch tested with 10000 ppm BIT in alcohol (Andersen and Hamann, 1984). In a patch test of 10 controls with 10000 ppm BIT, 8/10 subjects showed irritation (Freeman, 1984).

The OECD TG 404 studies on rabbits used 4 hour applications, while the human studies used longer periods. The doses showing irritating effects in the human studies on the other hand, are up to 3 orders of magnitude lower (500-10000 ppm = 0.05-1.0 %), compared to the animal studies (78.4 % in the Anonymous (2002c) study and 37.2 % in the Robinson *et al* (1993) study).

The CLP Regulation does not contain clear criteria for classification for skin irritation based on human data, nevertheless such data are appropriate to be used for classification purposes. From human data, it can be concluded that starting at doses of 500 ppm some skin irritation can be observed, and with increasing doses both the severity of the irritant reaction and the incidence of cases increases. It is the opinion of RAC that human experience should be taken into account, and that negative data from animal studies should not negate positive results from human experience.

RAC therefore concludes that BIT warrants classification as **Skin Irrit. 2 (H315)**.

10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

10.6 Respiratory sensitisation

Hazard class not assessed in this dossier.

10.7 Skin sensitisation

Table 11: Summary table of animal studies on skin sensitisation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, n°/group</th><th>Test substance</th><th>Dose levels and duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>OECD TG 429</td><td>Mouse
CBA/Ca
5 F</td><td>89.8% a.i.
25 µL of dose solution was applied to the dorsum of each mouse ear.</td><td>3, 10 and 30% w/v BIT (30000, 100000 and 300000 ppm). Vehicle not specified
Once/day for 3 consecutive days</td><td>
<table>
<tr><th>Treatment</th><th>Dosage (%)</th><th>Group Mean (DPM)</th><th>Stimulation Index</th></tr>
<tr><td>Dimethyl-formamide</td><td>0</td><td>1387</td><td>1.0</td></tr>
<tr><td>BIT</td><td>3</td><td>2075</td><td>1.5</td></tr>
<tr><td>BIT</td><td>10</td><td>2135</td><td>1.5</td></tr>
<tr><td>BIT</td><td>30</td><td>4287</td><td>3.1</td></tr>
</table>
</td><td>[redacted], 2007
IIIA6.1.5/01 ([redacted])</td></tr>
<tr><td>LLNA (before OECD TG 429)</td><td>Mouse
CBA/J
5 F</td><td>19.2% a.i. (Proxel GxL)
25 µL of dose solution was applied to the dorsum of each mouse ear.</td><td>0.5, 1, 2.5, 5 and 10% w/v BIT (5000, 10000, 25000, 50000 and 100000 ppm). Vehicle not specified
Once/day for 4 consecutive days</td><td>
<table>
<tr><th>Treatment</th><th>Dose (ppm)</th><th>DPM (mean)</th><th>SI (test/ control ratio)</th><th>Results[1]</th></tr>
<tr><td>Acetone: olive oil</td><td>0</td><td>9035</td><td>1.0</td><td>-</td></tr>
<tr><td>BIT, 0.5%</td><td>5000</td><td>25119</td><td>2.78</td><td>-</td></tr>
<tr><td>BIT, 1.0%</td><td>10000</td><td>23834</td><td>2.64</td><td>-</td></tr>
<tr><td>BIT, 2.5%</td><td>25000</td><td>32910</td><td>3.64</td><td>+</td></tr>
<tr><td>BIT, 5.0%</td><td>50000</td><td>24609</td><td>2.72</td><td>-</td></tr>
<tr><td>BIT 10.0%</td><td>100000</td><td>30281</td><td>3.35</td><td>+</td></tr>
</table>
[1]Test/Control ratio ≥ 3.0 = positive result
DPM = Disintegrations Per Minute
</td><td>[redacted], 2007
IIIA6.1.5/02 ([redacted])</td></tr>
<tr><td>LLNA</td><td>Not stated.</td><td>Not stated.</td><td>Not stated.</td><td>EC_3 = 2.3% - Skin Sens. 1B</td><td>[redacted], 2005
(RAC opinion on MBIT)
(SCCS opinion on BIT)</td></tr>
<tr><td>LLNA</td><td>78% a.i. (+</td><td>Not stated.</td><td>Not stated.</td><td>EC_3 = 10.4% - Skin Sens. 1B</td><td>[redacted],</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, n°/group</th><th>Test substance</th><th>Dose levels and duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td>20% ethylene diamine)</td><td></td><td></td><td></td><td>1999
(RAC opinion on MBIT)
(SCCS opinion on BIT)</td></tr>
<tr><td>LLNA (guideline not specified) (conducted in duplicate)</td><td>Mouse
CBA/Ca
Sex not specified
4 mice/group</td><td>Purity not specified.
A non-specified volume of dose solution was applied to the dorsum of each mouse ear.</td><td>0, 3, 10, 30 and 50% w/v BIT (0, 30000, 100000, 300000 and 500000 ppm) in dimethyl formamide
Once/day for 3 consecutive days</td><td>A 3-fold increase in the mean incorporation of ^{3}HTdR/node was considered to be a reliable indicator of sensitization potential. BIT had no effect below 10%. At 10%, the increment was only significant in experiment 2. 50% gave a significant response in both tests. BIT was a skin sensitizer.
No further information about the test method is stated.</td><td>[redacted], 1991
(TC C&L document)</td></tr>
<tr><td>LLNA</td><td>Not stated.</td><td>Not stated.</td><td>Not stated.</td><td>EC_3 = 4.8% - Skin Sens. 1B
EC_3 = 32.4% - Skin Sens. 1B</td><td>[redacted], 1991
(RAC opinion on MBIT)</td></tr>
<tr><td>OECD TG 406</td><td>Guinea pig
Hartley
10M+10F (test)
5M+5F (control)</td><td>97.42% a.i.
1st day: three pairs of intradermal induction.
7th day: topical application (occlusive – 48 h).
21st day: challenge induction (occlusive – 24 h).</td><td>Intradermal induction (2.5% BIT (25000 ppm) in propylene glycol)
Topical induction (100 mg BIT moistened with 80% ethanol)
Adjuvant (50% v/v Complete Freund`s Adjuvant in distilled water)
Challenge induction (100 mg BIT moistened with acetone)</td><td><table><tr><th></th><th colspan="4">Animals with allergic reactions/ Animals in group</th></tr><tr><th></th><th>Sham control</th><th>Test group</th><th>Positive control</th><th>Sham positive control group</th></tr><tr><td>Scored 24h</td><td>0/10 (0%)</td><td>4/20 (20%)</td><td>9/20 (45%)</td><td>0/10 (0%)</td></tr><tr><td>Scored 48h</td><td>0/10 (0%)</td><td>2/20 (10%)</td><td>5/20 (25%)</td><td>0/10 (0%)</td></tr></table></td><td>[redacted], 2003e
IIIA6.1.5/2 ([redacted])</td></tr>
<tr><td>OECD TG 406
US EPA OPPTS</td><td>Guinea pig</td><td>98% a.i.
1st day: three pairs of</td><td>Intradermal induction (5% w/w BIT (50000 ppm) in</td><td><table><tr><th>Animals with allergic reactions/ Animals in group</th></tr></table></td><td>[redacted], 2002e</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, n°/group</th><th>Test substance</th><th>Dose levels and duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>870.2600</td><td>Hartley albino
10M+10F (test)
5M+5F (control)</td><td>intradermal induction.
7th day: topical application (occlusive – 48 h).
23rd day: challenge induction (occlusive – 24 h).</td><td>distilled water)
Topical induction (80% w/w BIT (800000 ppm) in distilled water)
Adjuvant (50% v/v Complete Freund`s Adjuvant in distilled water)
Challenge induction (80% w/w BIT (800000 ppm) in distilled water)</td><td><table><tr><th></th><th>Sham control</th><th>Test group</th><th>Positive control</th><th>Sham positive control group</th></tr><tr><td>Scored 24h</td><td>0/10 (0%)</td><td>6/20 (30%)</td><td>9/10 (90%)</td><td>0/5 (0%)</td></tr><tr><td>Scored 48h</td><td>0/10 (0%)</td><td>3/20 (15%)</td><td>7/10 (70%)</td><td>0/5 (0%)</td></tr></table></td><td>IIIA6.1.5/1 ([redacted])</td></tr>
<tr><td>OECD 406</td><td>Guinea pig
Dunkin-Hartley albino
10M+10F (test)
5M+5F (control)</td><td>Purity not specified.
1st day: three pairs of intradermal induction.
8th day: topical application (occlusive – 48 h).
22st day: challenge induction (occlusive – 24 h).</td><td>Intradermal induction (1 and 5 % w/v BIT (10000 and 50000 ppm) in purified water)
Topical induction (5% w/v BIT (50000 ppm) in purified water)
Adjuvant (not specified)
Challenge induction (0.5 and 3% w/v BIT (5000 and 30000 ppm) in purified water)</td><td><table><tr><th></th><th></th><th></th><th colspan="2">Incidence of significant responses</th><th></th></tr><tr><th>Group</th><th>Treatment</th><th>Animals</th><th>24 h</th><th>48 h</th><th>Total responders</th></tr><tr><td>Ctrl.</td><td rowspan="2">Purified water</td><td>10</td><td>0</td><td>0</td><td>0 (0%)</td></tr><tr><td>Test</td><td>20</td><td>1</td><td>2</td><td>2 (10%)</td></tr><tr><td>Ctrl.</td><td rowspan="2">3% w/v BIT</td><td>10</td><td>0</td><td>0</td><td>0 (0%)</td></tr><tr><td>Test</td><td>18</td><td>9</td><td>7</td><td>10 (56%)</td></tr><tr><td>Ctrl.</td><td rowspan="2">0.5% w/v BIT</td><td>10</td><td>0</td><td>0</td><td>0 (0%)</td></tr><tr><td>Test</td><td>18</td><td>3</td><td>3</td><td>4 (22%)</td></tr></table></td><td>[redacted], 1994
(REACH registration dossier)</td></tr>
<tr><td>Equivalent to US EPA PAG 81-6</td><td>Guinea pig
Alpk:Dunkin-Hartley albino
20 F (test)
10 F (neg. ctrl.)</td><td>Purity not specified.
1st day: three pairs of intradermal induction
7th day: topical application</td><td>Intradermal induction (0.01% w/v BIT (100 ppm) in 3% w/v DMF in corn oil)
Topical induction (30% w/v BIT (30000 ppm) in DMF)
Adjuvant (50% w/v Complete Freund`s</td><td><table><tr><th></th><th></th><th>Animals with allergic reactions/Animals in group</th></tr><tr><th></th><th>Dose (%)</th><th>Test group</th></tr><tr><td>Scored 24h</td><td>10</td><td>13/20 (65%)</td></tr><tr><td></td><td>3</td><td>2/20 (10%)</td></tr></table></td><td>[redacted], 1990
IIIA6.1.5/1 ([redacted])
(REACH registration dossier)</td></tr>
</table>

Method, guideline, deviations if any	Species, strain, sex, n°/group	Test substance	Dose levels and duration of exposure	Results	Reference
	20 F (pos. ctrl.)	(occlusive – 48 h) 21st day: challenge induction (occlusive – 24 h)	Adjuvant in 3% w/v DMF in corn oil) Challenge induction (3% and 10% w/v BIT (30000 and 100000 ppm) in DMF)	Scored 48h: 10 — 13/20 (65%); 3 — 1/20 (5%)	
EC B.6	Guinea pig Strain not specified Sex not specified 20 guinea pigs/group	30% a.i. (Proxel XL) 20% a.i. (Proxel HL)	Intradermal induction (5 % w/v Proxel (1 or 1.5% BIT (10000 or 15000 ppm)) in propilenglycol) Topical induction (25% w/v Proxel (5 or 7.5% BIT (50000 or 75000 ppm)) in petrolatum) Adjuvant (not specified) Challenge induction (1% w/v Proxel (0.2 or 0.3% BIT (2000 or 3000 ppm)) in purified water)	Proxel XL - 3/20 (15%) Proxel HL - 1/20 (5%) Weak to mild sensitizer No further information about the test method is stated.	[redacted], 1984 (TC C&L document)

Table 12: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Work place study Medical surveillance	Undiluted BIT.	Minor irritation.	1 worker experienced skin irritation on his arms and legs due to a small splash of chemical while transferring BIT from an intermediate container to a dilution tank.	Specialty Electronic Materials Switzerland manufacturing plant, 2003
HRIPT	78% a.i. (+ 20% ethylene diamine) 0.036 and 0.073% BIT (360 and 725 ppm) in	5 volunteers (9%) were sensitized by BIT using 0.073% BIT. None of them (0%) were sensitized by 0.036% BIT.	Information was drawn from a historic Zeneca database. 54 volunteers were exposed to 0.036% BIT and 58 volunteers to 0.073% BIT.	Basketter D.A. *et al.*, 1999 (RAC opinion on MBIT)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
	water.			(SCCS opinion on BIT)
	19.2% a.i. (Proxel GxL) 0.05% BIT (500 ppm) in Rhoplex AC-64 (sample A); 0.1% BIT (1000 ppm) in Rhoplex AC-64 (sample B); and undiluted Rhoplex AC-64 (sample C).	Irritation.	111 volunteers (26 males and 85 females); 24 hour contact, semi-occlusive patches. Three subjects (46, 66 and 96) exhibited irritation during the challenge period and subject 46 also displayed mild erythema (grade 1) during induction and challenge applications to samples A and C. Another subject (96) displayed mild erythema (grade 1) 48-hour after challenge. Subject 66 displayed a papular response to samples B and C at challenge application only. Subject 66 participated in a rechallenge of samples B and C. There were no observable clinical reactions noted to the test samples at rechallenge. The overall response pattern for subject number 66 is consistent with clinical irritation.	Plaza M.E. and Rheins L.A., 1991 IIIA6.12.6/01 (Specialty Electronic Materials Switzerland and Lanxess)
	Purity not stated. 0.5 mL of 0.05% BIT (500 ppm) in propylene glycol (induction and first challenge tests) or liquid paraffin (second challenge test) (64.45 µg BIT/cm^2 or 250 µg BIT/patch).	Mild to moderate or severe irritation. Sensitization in 5 volunteers (10%).	50 volunteers (21 males and 29 females); 24 hour contact, semi-occlusive patches. At challenge, mild irritation was seen in 27/45 volunteers, of a similar degree to that seen during the induction phase. In nine volunteers, the reaction was as great or greater than that seen previously. However, the vehicle propyleneglycol, also elicited dermal irritation which was greater than expected. In order to clarify the results obtained from the first challenge, a second challenge application was made to these nine volunteers, and in addition, six other volunteers also received the second challenge application because of atypical reactions produced by the other test compounds following the first challenge. Therefore fifteen volunteers were rechallenged using liquid paraffin as the vehicle. Marked reactions indicative of dermal sensitisation were observed in five individuals following the second challenge. BIT produced evidence of dermal sensitisation in five volunteers. There was also evidence of slight dermal irritation in most individuals. Therefore, BIT has the potential to cause	Davies R.E. *et al*., 1975 (TC C&L document)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			skin sensitisation in humans.	
Diagnostic patch test	Purity not specified. 0.05% BIT (500 ppm) in petrolatum.	20 patients (0.88%) showed a positive response to BIT. 7 of them (0.31%) seems to be sensitized by using gloves with 0.0006 to 0.002% BIT.	From January 1991 to September 2005, BIT was tested on a total of 2264 patients.	Aalto-Korte K. *et al.*, 2007 (RAC opinion on MBIT) (SCCS opinion on BIT)
	Purity not stated. 0.001% to 0.16% BIT (10 to 1600 ppm) (0.01 to 0.16 g/L).	Patch tests with the preservative 1,2-BIT used in the wallpaper paste showed positive reactions in concentrations down to 0.003% (0.03 g/L).	A 45-year-old paper-hanger presented a long-standing hand dermatitis which was resistant to conventional therapy. Avoidance of contact with this particular paste resulted in complete disappearance of the hand dermatitis in this individual.	Damstra R.J. *et al.*, 1992 (TC C&L document)
	Not specified.	4 patients (23%) contact allergy to BIT was found.	17 patients at occupational exposure risk sensitized to unknown BIT concentrations.	
	0.04% BIT (400 ppm) in a non-specified vehicle.	10 patients (1.8 %) showed positive patch test to BIT	556 consecutive dermatological patients without clear occupational risk. In 3 of these cases, contact allergy was related to domestic paper-hanging. Sensitization with unknown BIT concentrations.	
	33% a.i. (Proxel CRL) Purity of BIT not specified. 0.01, 0.03, 0.3 and 1% Proxel CRL (0.003, 0.01, 0.1 and 0.33% BIT (30, 100, 1000 and 3300 ppm)) and 0.05% BIT (500 ppm).	4 patients (50%) showed a positive reaction. 3 of them at 0.03% Proxel CRL (0.01% BIT) and 0.05% BIT and the other one at 0.3 and 1% Proxel CRL (0.1 and 0.33% BIT).	Occupational contact allergy to Proxel CRL were reported among 8 employees. No information about the concentrations inducing allergy is stated.	Díaz M. *et al*., 1992 (TC C&L document)
	1000 ppm or 0.1% Mergal K-10 (% BIT not stated) in petrolatum at 2 and 4 days.	Patch testing with the GEIDC standard series and all the components of the paints with which he was in contact gave positive reactions to Mergal K-10. The dermatitis cleared up on treatment with	A 52-year-old man had been working in a paint factory for 18 years. There was no previous history of atopy or skin disease. He started using Mergal K-10 as a biocide (% BIT not stated). Suddenly, he developed an itchy vesicular dermatitis on the dorsum of both hands and on the chest.	Sanz-Gallén P. *et al.*, 1992 (TC C&L document)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		topical corticosteroids.	The patient discontinued contact with BIT by changing his work station within the paint factory. After 6 months, he remained asymptomatic with no skin lesions.	
	Purity of BIT not specified. 0.05% BIT (500 ppm) in water	55 patients (5.6%) were positive to Katon CG (% BIT not stated). 1 patient (0.1%) cross-reacted to 0.05% BIT.	977 patients with a history of allergy to cosmetics were tested for allergy to a number of substances.	Ledieu G. *et al*., 1991 (TC C&L document)
	Purity not stated. 0.1% BIT (1000 ppm) in petrolatum.	14 patients (0.9%) showed positive reaction to BIT. However, this reaction could not be explained.	BIT was included in the routine patch testing panel in 6 Danish outpatient clinics. 1652 consecutive eczema patients were tested.	Andersen K.E. and Veien N.K., 1985 (TC C&L document)
	Purity not specified. 0.1% BIT (1000 ppm). Vehicle not stated.	48 patients (20.9%) had a positive allergic response.	230 patients with occupational dermatoses from cutting oils. No further information is stated.	Alomar A. *et al*., 1984 (TC C&L document)
	Purity of BIT not specified. 20% a.i. (Proxel XL) 1% BIT (10000 ppm) in alcohol and 0.5% Proxel XL (0.1% BIT (1000 ppm)) in water.	1 patient (0.25%) showed a positive reaction to BIT and 121 reactions (30%) were classified as irritations. 1 patient (0.22%) showed a positive reaction to Proxel XL and 7 reactions (1.5%) were classified as irritations.	BIT and Proxel XL were included in the standard patch-test series at the Dept. Dermatology, Gentofte Hospital, Denmark. In a period of 14 months, 404 (1% BIT) – 466 (0.5% Proxel XL) eczema patients were tested.	Andersen K.E. and Hamann K., 1984 (TC C&L document)
	33% a.i. (Proxel CRL) Purity of BIT not stated. 1% Proxel CRL (0.33% BIT (3300 ppm)) and 1% BIT (10000 ppm). Vehicle not specified	Positive reaction.	A case of contact dermatitis was reported on a person working in the rubber industry exposed to Proxel CRL.	Foussereau J. *et al*., 1984 (TC C&L document)
	0.13% a.i. (gum arabic) 19.2 a.i. (Proxel GxL)	The patient reacted to gum arabic, 0.1 % and 1% Proxel GxL and BIT.	A 24-year old printer showed hand dermatitis soon after change to a new company. At his job, he was handling gum arabic without gloves.	Freeman S., 1984 (TC C&L document)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
	Purity of BIT not stated. Gum Arabic (0.026% BIT (260 ppm)). 0.01, 0.1 and 1% of Proxel GxL (0.002, 0.02 0.19% BIT (20, 200 and 1920 ppm)). Vehicle not stated. 0.01, 0.1 and 1% of BIT (100, 1000 and 10000 ppm). Vehicle not stated.	1 control (10%) showed a strongly positive allergic reaction lasting for 2 weeks to 0.1% BIT. He had twice previously served as a control and had become sensitized by low concentrations of Proxel (probably 1%).	Patch testing was carried out on batches of 10 controls.	
	Purity not stated. 0.1% BIT (1000 ppm) in water.	4 patients (20%) had positive reactions.	20 metal workers with dermatitis of the hands, possibly due to exposure to cutting oils were patch tested. Proxel HL (30% BIT) was added to the cutting oils in a concentration of 0.1 to 0.3% (0.03% to 0.1% BIT). No further information is stated.	Alomar A., 1981 (TC C&L document)
	10% a.i. (Proxel XL2) 1% Proxel XL2 (0.1% BIT (1000 ppm)) and 0.1% BIT (1000 ppm) (vehicle not specified).	Positive patch tests were obtained.	A 27-year old man employer as a mouldmaker in the pottery industry presented a few months' history of an eczema on the backs and sides of his fingers. The mouldmaker dips a sponge into an open bucket of an oil-based emulsion; gloves were not used. For about one year, the factory had been using an oil containing Proxel XL2 (9-10% BIT) in a concentration of 1.6% Proxel XL2 (0.16% BIT).	Roberts D.L. *et al.*, 1981 (TC C&L document)
	Not specified.	Not specified.	2 other cases of allergy to BIT induced by working with oil containing Proxel XL2 in a non-specified concentration are referred.	
	10% a.i. (Proxel XL2) 1 and 5% Proxel XL2 (0.1 and 0.5% BIT (1000 and 5000 ppm)) in water.	Weak positive reaction in 3 persons (27%) with 5% Proxel XL2 and no reactions with 1% Proxel XL2.	Eleven controls.	
	Purity not specified. 0.01, 0.1 and 1% BIT	7/11 persons (63.6%) were sensitized to 1% BIT.	11 about 16 men working in the quality control laboratory of a chemicals firm developed dermatitis. The work involved	Slovak A.J.M., 1979

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
	(100, 1000 and 10000 ppm) in methanol.	4/7 were tested at 0.01 and 0.1% BIT. None of the 0/4 (0%) reacted to 0.01% BIT, 2/4 (50%) reacted to 0.1% BIT.	analysis of several chemicals including BIT.	(TC C&L document)
	Purity not stated. 5 and 10% BIT (50000 and 100000 ppm) in a non-specified vehicle.	Positive reaction.	A worker with occupational exposition to cutting fluids developed dermatitis.	Brown R., 1979 (TC C&L document)
	33% a.i. (Proxel CRL) Purity of BIT not stated. 0.1% Proxel CRL (0.033% BIT (330 ppm)) in water and 0.1% BIT (1000 ppm) in ethanol.	The 2 patients (100%) showed positive reaction to Proxel CRL and BIT. One of them at 0.1% BIT and the other one at 0.1 and 0.01% BIT.	2 male patients with hand eczema, both exposed to BIT through working with preserved plastic emulsions. There is no information about the concentration of BIT in plastic emulsions.	Pedersen N.B., 1976 (TC C&L document)

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Eight independent studies have showed that BIT induces skin sensitisation. The two LLNAs in the dossier showed that BIT was sensitizer at concentrations higher than 2%. One of them shows an EC_3 < 2% (Skin Sens. 1A) ([redacted], 2007) and the other one shows an EC_3 > 2% (Skin Sens. 1B) ([redacted], 2007). Other three LLNAs can be found in RAC opinion about MBIT with four EC_3 > 2%. Four of the five available GPMTs (OECD TG 406) showed that BIT was able to sensitize more than 30% of animals after challenges with intradermal doses higher than 1%. Thus, these eight studies showed that BIT must be classified as a Skin Sens. 1B (H317).

Taking into account information about human data, one HRIPT shows positive responses at 64.45 µg BIT/cm^2 and another one showed a negative response. The third one showed a positive response at 0.073% BIT. A great amount of diagnostic patch tests showed positive responses to BIT in contact dermatitis, so high exposure to this substance is to be expected, with a relatively low incidence. These data support the classification as Skin Sens. 1B.

Also, regarding human data, HRIPTs showed positive responses at 0.05% or 0.073% while diagnostic patch tests assess the exposure of several patients to a great concentration of BIT. The most recent of them (Aalto-Korte, 2007) shows that some patients may have been sensitized by wearing gloves with a BIT concentration between 0.0006% and 0.002% BIT. However, incidence is very low and they could have been sensitized before using the gloves. For these reasons, ES-eCA propose to retain the SCL ≥ 0.05%, although BIT has to be closely monitores.

10.7.2 Comparison with the CLP criteria

Classified as Skin Sens. 1B (H317: May cause an allergic skin reaction) because, according to the CLP 3.4.2.2.3.3. (Table 3.4.4.), EC_3 > 2 % in five LLNA studies and ≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose in four guinea pig maximisation tests.

Regarding the human data, according to CLP 3.4.2.2.2.2.b. incidence is relatively low to a relatively high exposure, so the classification as Skin Sens. 1B is supported.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Skin Sens. 1B; SCL ≥ 0.05%; H317: May cause an allergic skin reaction; GHS07; Warning.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

According to the DS, eight independent studies have shown that BIT induces skin sensitisation. The two LLNA studies in the dossier showed that BIT was a skin sensitiser at concentrations greater than 2 %. One of them showed an EC_3 < 2 % (pointing to Skin Sens. 1A) and the other one showed an EC_3 > 2 % (pointing to Skin Sens. 1B). Another three LLNA studies can be found in the RAC opinion for MBIT, with four EC_3 values > 2 %. According to the DS, four of the five available GPMTs (OECD TG 406) showed that BIT was able to sensitise more than 30 % of animals after challenges with intradermal doses higher than 1 %. Thus, the animal studies showed that BIT should be classified as Skin Sens. 1B (H317).

The DS summarised the human data: one HRIPT showed positive responses at 64.45 µg BIT/cm^2 and another one showed a negative response. The third one showed a positive response at 0.073 % BIT. A large number of diagnostic patch tests showed positive responses to BIT, especially in contact dermatitis patients, so high exposure to this substance is to be expected, with a relatively low incidence. These data support the classification as Skin Sens. 1B.

Also, regarding human data, HRIPTs showed positive responses at 0.05 % or 0.073 %, while diagnostic patch tests assessed the exposure of several patients to a high concentration of BIT. The most recent of them (Aalto-Korte, 2007) showed that some patients may have been sensitised by wearing gloves with a BIT concentration between 0.0006 % and 0.002 % BIT. However, the incidence was very low and they could have been sensitised prior to using the gloves. For these reasons, the DS proposed to retain the existing SCL at ≥ 0.05 %.

Comments received during consultation

There were comments from 3 MSCAs and 17 stakeholders.

An MSCA commented that the SCL has to be revised since the potential of cross-reactivity of BIT with other isothiazolinones has not been addressed in the CLH report. As the chemical structure of BIT is closely related to other isothiazolinones, especially MBIT, the cross reactivity has to be considered in setting the SCL.

The second MSCA proposed that BIT retain the harmonised classification as Skin Sens. 1; H317: C ≥ 0.05 %, as Skin Sens. 1A cannot be ruled out.

The third MSCA disagreed with the classification proposal, and proposed that Skin Sens 1A would be more appropriate. They argued that although the majority of animal studies performed support classification of BIT as Skin Sens. 1B (H317), classification of BIT should be based on the large amount of human data. The MSCA emphasised that human data on incidences in HRIPT and patch tests provided in the CLH-Report support classification with Skin Sens. 1A. According to the evaluation of these studies by the MSCA, skin sensitising effect was found with "relatively high frequency" in 16 studies and a "relatively low/moderate frequency" in 3 studies. The three studies that indicated a "relatively low/moderate frequency" effect are studies with unselected dermatitis patients (i.e. studies that are often particularly well standardised according to CLP Guidance chapter 3.4.2.2.3.1) and have large cohort sizes (404-2264 patients), so that a high relevance may be assumed. However, other studies with a large number of subjects (Aalto-Korte *et al.,* 2007, Damstra *et al.*, 1992 and Ledieu *et al.,* 1991) indicate a "relatively high frequency" of the sensitising effect of BIT. The MSCA also mentioned two relatively recent publications not included in the CLH report: a study by Geier *et al*. (2015), with a cohort size of 8728 dermatitis patients and a positive rate of 1.8% which indicates a "relatively high frequency", and the study by Madsen and Andersen (2016) indicating frequencies of occurrence ≥ 2 % in patients tested in dermatology offices/departments of dermatology in Denmark that also result in "high frequency". Another indication for a "high frequency" is the 191 published cases, which considerably exceeds the criterion for a "high frequency" according to table 3.2 of the CLP Guidance (> 100). In summary, the overall picture of the available human data on BIT points to a skin sensitising effect with "high frequency".

Isothiazolinones are usually used in very low concentrations and sensitisation by BIT has already been described for very low concentrations (Aalto-Korte *et al*., 2007: ≤ 0.002 % BIT; Alomar *et al*., 1984: 0.03-0.1 % BIT; Roberts *et al*., 1981: 0.16 % BIT; Freeman, 1984: probably 0.19 % BIT), so that the criterion of "relative low exposure" for the parameter "concentration/dose" of table 3.3 of the CLP Guidance is fulfilled. This conclusion is independent of whether one assumes low or high exposure for the parameters "Repeated exposure" and "number of exposures". According to table 3.4 of the CLP guidance, the combination of "high frequency" and "low exposure" leads to classification in subcategory 1A. The MSCA further reasoned that even assuming a "relatively high exposure" due to the ubiquitous use of isothiazolinones and the postulated cross-reactivity to other isothiazolinones, no conclusion for classification in subcategory 1B can be made based on human data due to the "relatively high frequency" determined. In that case the CLP Guidance specifies that classification in category 1 should be applied instead of category 1B, if category 1A cannot be excluded.

On the topic of setting the SCL, the MSCA stated that the available animal studies indicate a "moderate" skin sensitising potency for BIT, which may result in the assignment of a GCL of 1 %. However, if there is reliable information that the specific hazard is evident below the GCL, a lower SCL can be assigned. Such information for BIT is, on the one hand, the reports on sensitising effects even at very low concentrations (e.g. Aalto-Korte *et al*., 2007) that could lead to a classification as Skin Sens. 1A, and, on the other hand, the assumption of cross-reactivity to other isothiazolinones. The concern of cross-reactivity has already been used in the past by RAC to justify SCLs for other isothiazolinones. Therefore, the MSCA agrees that an SCL should be established, but before defining the relevant value, the concern for cross-reactivity should be evaluated.

The stakeholders all agreed with the classification proposal as Skin Sens 1B, and the proposed SCL of 500 ppm. Several pointed out that the results of the LLNA studies indicate that BIT, in contrast to the other isothiazolinones, is a moderate sensitiser (corresponding to category 1B at EC_3 values > 2 %), and the SCL should be assigned accordingly. As a moderate sensitiser, a GCL of 1 % could be assigned. Some of the stakeholders referred to the HRIPT study in which no reactions to BIT occurred at 360 ppm, while 9 % of volunteers reacted at 725 ppm, thus the HRIPT results show that the SCL can be set above 360 ppm and below 725 ppm, indicating that an SCL of 500 ppm may be appropriate. One of the stakeholders pointed out that in this HRIPT study the authors calculated that a realistic no effect level for BIT was in the region of 500 ppm. One stakeholder commented that in the case of BIT, setting the SCL at 0.05 % (i.e., 20× lower than the standard GCL for a moderate skin sensitiser) is expected to be conservative and protective of both workers, professionals and consumers who may use products containing BIT. They stated that already sensitised persons are protected by the hazard statement EUH208 (Contains <BIT>. May produce an allergic reaction) with a derived limit of 50 ppm. Several stakeholders commented that they are not aware that BIT has ever caused any induction of skin sensitisation from its presence in their products.

A stakeholder commented that the information provided by the dossier submitter, combined with the relatively few reports of allergic contact dermatitis in the open literature, would indicate that 2 points in the CLP regulation on human evidence for sub-category 1B apply in the case of BIT, namely "*diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high*

exposure" and "*other epidemiological evidence where there is a relatively low but substantial incidence of allergic contact dermatitis in relation to relatively high exposure*".

Several stakeholders commented on the 2 publications of Aalto-Korte *et al*. in which there were indications that BIT caused skin allergies from PVC gloves containing 20-30 ppm BIT. The study investigated contact allergy to plastic gloves, which is considered a rare phenomenon. All patients had displayed hand dermatitis for years, and the authors concluded that small amounts of BIT in the gloves may sensitise those who already have hand dermatitis. The stakeholders emphasised that a sensitisation threshold (i. e. the elicitation threshold for provoking an effect on the skin) for patients with existing hand dermatitis is not relevant for the setting of the SCL under CLP (SCL is set for induction of sensitisation). Furthermore, such human case studies cannot be validated, lack details, do not show a dose-response relationship and can hence only be considered "as supporting additional evidence". A lower SCL based on this publication was not supported. Several stakeholders asked that the classification and the SCL setting be based on data for BIT, and did not support an SCL for BIT of 15 ppm used for other isothiazolinones.

One stakeholder submitted historical HRIPTs covering nearly 1000 panellists, performed using consumer products containing BIT, to confirm the absence of skin sensitisation effects. All studies support the low risk of using BIT under conditions relevant for consumer exposure and further support the current and proposed SCL of 500 ppm for BIT.

One stakeholder submitted 3 studies in which it was shown that there is no release of BIT from paints, which can be explained by the low volatility of BIT compared to other isothiazolinones.

One stakeholder gave a detailed assessment of the cross-reactivity between isothiazolinones, citing several publications (Geier *et al*., 2015; Geier *et al*., 1996; Craig *et al*., 2017; Aalto-Korte & Suuronen, 2017 ; Aalto-Korte *et al*., 2006; Aalto-Korte *et al*., 2007; and Aerts *et al*., 2014) which indicate that BIT does not cross-react with other tested isothiazolinones in patch test panels, concluding that overall, it is appropriate to consider that reactions to BIT are independent to those of other isothiazolinones. They also cited a publication that indicates no cross-reactivity between BIT and CMIT: Ashby *et al*. (1995) evaluated a large number of chemicals in the LLNA in an attempt to identify structural alerts for positive reactions. They identified that the heterocyclic sulphur in BIT might form disulphide bonds with thiol sulphurs in proteins. C(M)IT, however, was identified as an electrophilic aromatic alkylating agent. The chemical reactivity of C(M)IT, and therefore its sensitisation potency and potential for cross-reactivity, does not apply to BIT.

Some other stakeholders also commented on human and animal data to evaluate the potential risk of BIT cross reactivity (e.g., elicitation in MIT-sensitised individuals following exposure to BIT). Human patch testing data of MIT- and BIT-sensitised patients were reviewed and indicated that the fraction of patients that reacts to both isothiazolinones is very small and driven mostly by individuals pre-sensitised to both substances, and not from cross-reactivity. They reviewed a publication evaluating the cross-reactivity between MIT and BIT using a "modified local lymph node assay". The publication indicated cross reactivity, but several stakeholders pointed to substantial methodological and reporting deficiencies that hinder the interpretation and applicability of this study.

The Information Network of Departments of Dermatology[1] (IVDK) submitted data they had obtained from their database. They had conducted a retrospective analysis of data from 29590 patients who were patch tested with BIT, sodium salt, 0.1 % in petrolatum (pet.) in the departments of dermatology, who were members of the IVDK, between 2000 and 2019. Benzisothiazolinone, sodium salt, 0.1 % in pet. was part of several special DKG patch test series and therefore mostly tested in a more or less aimed manner (which usually leads to higher reaction frequencies than patch testing in consecutive patients). Positive reactions to BIT were noted in 731 patients (2.47 % of 29590).

***Table**: IVDK, 2000-2019:patch test results with BIT 0.1% in pet.*

Reaction	n (patients)	% (patients)
Negative (neg.)	28,182	95.24
Doubtful (?)	506	1.71
Follicular (f)	54	0.18
+	599	2.02
++	109	0.37
+++	23	0.08
Irritant (ir.)	117	0.40
Total	29,590	100.00

There were 731 positive reactions (2.47%). The Reaction Index (RI) was +0.04, the Positivity Ratio (PR) was 82%.

Although BIT is a known skin irritant and the patch test concentration is rather high, the diagnostic discriminatory power is fairly good, characterised by a Reaction Index (RI) of + 0.04, and a Positivity Ratio (PR) of 82 % (table above). The fact that the proportion of BIT-positive patients was significantly higher among patients with an irritant reaction to the control patch test with Sodium Lauryl Sulphate (SLS) (3.5 %) than among those not reacting to SLS (1.9 %) indicates that individuals with "sensitive skin" (at the time and in the place of patch testing) react more easily to this patch test preparation. Hence, possibly some of the positive reactions to BIT might be attributable to its irritation properties, i.e. false-positive results. Reproducibility of positive patch test reactions to BIT 0.1 % in pet. is not satisfactory. In total, only 5 out of 14 positive test reactions (and only 3 out of 12 weak positive reactions) could be reproduced on a second occasion. This also points to a certain proportion of false-positive reactions, particularly among the weak positive reactions, although it should not be concluded that all (weak) positive patch test reactions to BIT 0.1% pet. are false-positives. But it should be considered that probably not every positive patch test reaction to BIT 0.1 % pet. truly indicates contact sensitisation. In other words: the data probably slightly overestimate the frequency of BIT sensitisation rather than under-diagnosing this effect.

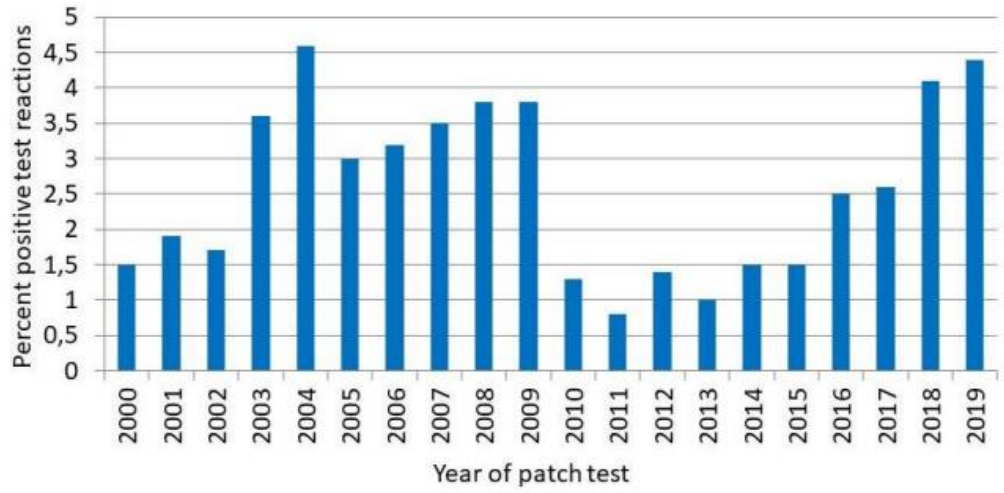
Overall sensitisation frequency in dermatitis patients patch tested was about 2.5 %. However, there was considerable variation in the reaction frequencies during the 20- year study period. From 2000 to 2002, sensitisation frequency was 1.7 % only, followed by 3.9 % from 2003 to 2009, 1.3 % from 2010 to 2015, 2.5 % from 2016 to 2017, and 4.3 %

[1] According to its website, IVDK membership is comprised of independent dermatological clinics but the organisation's activities are sponsored by industry.

from 2018 to 2019 (see the Figure below). IVDK stated that they have no complete conclusive explanation for these marked differences.

Painters and metalworkers handling metalworking fluids have a significantly increased risk of BIT sensitisation, probably due to BIT in paints and water-based metalworking fluids. Of note, cleaners (who are commonly exposed to BIT and MIT in cleaning agents) were not over-represented among those sensitised to BIT.

Figure: *Percentages of positive patch test reactions to BIT 0.1 % pet. during the years 2000 to 2019*



Concomitant sensitisation to BIT and other isothiazolinones may be acquired by co-exposure, in particular to BIT and MIT, which are often used in combination. As there are common chemical structures, immunological cross reactions between different isothiazolinones also seem possible. The table below gives an overview of concomitant reactions to BIT and other isothiazolinones tested.

Table. IVDK data on concomitant reactions to BIT 0.1 % in pet. and other isothiazolinones.

		Reaction to BIT 0.1% pet.		
		neg, ?, ir	pos.	total
MCI/MI	neg, ?, ir	26,169	551	26,720
0.01% aq.	pos.	1,110	116	1,226
2000-2019	total	27,279	667	27,946
MI	neg, ?, ir	16,912	306	17,218
0.05% aq.	pos.	964	77	1,041
2009-2019*	total	17,876	383	18,259
OIT	neg, ?, ir	17,468	462	17,930
0.025% pet.	pos.	127	18	145
2000-2019	total	17,595	480	18,075

*MI 0.05% aq. was not included in the DKG test series before 2009.

BIT and CMIT/MIT were patch tested in parallel in 27946 patients. Of these, 667 reacted positively to BIT, and 1226 to CMIT/MIT. One hundred and sixteen patients were positive

to both isothiazolinones, which is 17.4 % of the BIT-positive patients, and 9.5 % of those sensitised to CMIT/MIT.

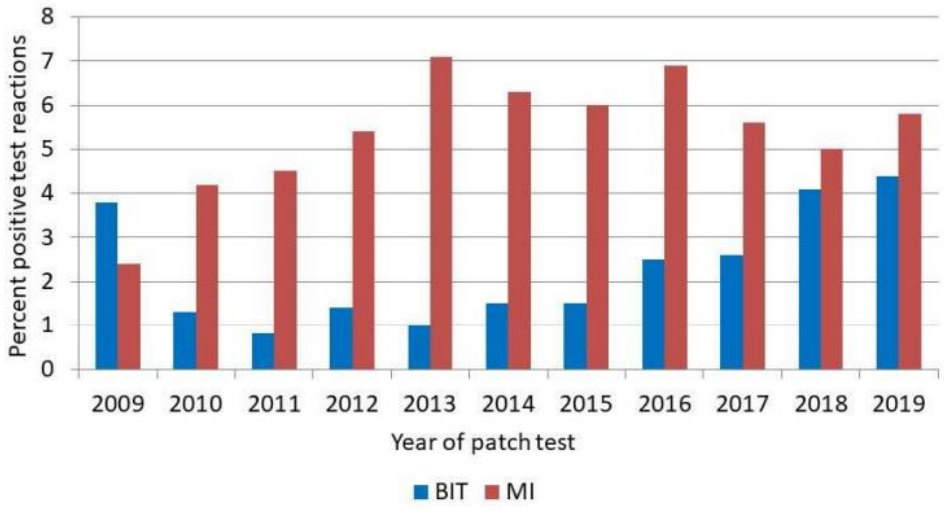
BIT and MIT were patch tested in parallel in 18259 patients. Of these, 383 reacted positively to BIT, and 1041 to MIT. Seventy-seven patients were positive to both isothiazolinones, which is 20.1 % of the BIT-positive patients, and 7.4 % of those sensitised to MIT.

BIT and OIT were patch tested in parallel in 18075 patients. Of these, 480 reacted positive to BIT, and 145 to OIT. Eighteen patients were positive to both isothiazolinones, which is 3.8 % of the BIT positive patients, and 12.4 % of those sensitised to OIT.

The analysis of concomitant reactions to BIT and other isothiazolinones (CMIT/MIT, MIT, OIT) clearly indicated that there was no relevant immunological cross-reactivity (table above), but a certain proportion (about 20 %) of BIT-sensitised individuals acquired sensitisation to MIT, probably by co-exposure. In contrast, only 7.4 % of those sensitised to MIT were also allergic to BIT. Co-exposure causing co-sensitisation occurs in industry and from products used in crafts, but not from cosmetics because BIT is prohibited for this field of application.

The Figure below illustrates the annual frequencies of sensitisation to MIT and BIT in patients tested with both MIT 0.05 % aq. and BIT 0.1 % in pet. The increase in MIT sensitisation due to cosmetics during the last decade was not accompanied by an increase in sensitisation to BIT. The complete lack of concordance of both curves underlines that there is no immunological cross-reactivity between BIT and MIT. In addition, it illustrates that the overwhelming mass of MIT sensitisation was acquired independently from BIT exposure.

Figure IVDK data on annual frequencies of sensitisation to MIT and BIT in patients tested with MIT 0.05 % aq. and BIT 0.1 % in pet.



Assessment and comparison with the classification criteria

BIT has an existing classification as Skin Sens 1, with an SCL of 0.05 %, based on data from occupational exposure, where people have been sensitised due to exposure to 1.0,

0.16, 0.1 and 0.03 % BIT (TC C&L document).

***Table**: Summary of animal studies on skin sensitisation.*

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, nº /group</th><th>Test substance</th><th>Dose levels</th><th>Results</th><th>Reference</th></tr>
<tr><td>LLNA

OECD TG 429

GLP</td><td>Mouse

CBA/Ca

5 F</td><td>89.8 % a.i.</td><td>3, 10 and 30 % w/v BIT

Vehicle DMF</td><td>EC_3=29 % - Skin Sens. 1B

<table><tr><th>Treatment</th><th>Dosage (%)</th><th>Group Mean (DPM)</th><th>SI</th></tr><tr><td>DMF</td><td>0</td><td>1387</td><td>1.0</td></tr><tr><td>BIT</td><td>3</td><td>2075</td><td>1.5</td></tr><tr><td>BIT</td><td>10</td><td>2135</td><td>1.5</td></tr><tr><td>BIT</td><td>30</td><td>4287</td><td>3.1</td></tr></table></td><td>Anonymous,

2007

IIIA6.1.5/01</td></tr>
<tr><td>LLNA (before OECD TG 429)

Deviation: Once/day for 4 consecutive days

GLP</td><td>Mouse

CBA/J

5 F</td><td>19.2 % a.i. in aqueous dipropylene glycol (Proxel GxL)</td><td>0.5, 1, 2.5, 5 and 10 % w/v BIT

Vehicle acetone:olive oil</td><td>EC_3 = 1.54 % - Skin Sens. 1A

<table><tr><th>Treatment</th><th>Dose (%)</th><th>DPM (mean)</th><th>SI</th></tr><tr><td>Acetone: olive oil</td><td>0</td><td>9035</td><td>1.0</td></tr><tr><td>BIT</td><td>0.5</td><td>25119</td><td>2.78</td></tr><tr><td>BIT</td><td>1.0</td><td>23834</td><td>2.64</td></tr><tr><td>BIT</td><td>2.5</td><td>32910</td><td>3.64</td></tr><tr><td>BIT</td><td>5.0</td><td>24609</td><td>2.72</td></tr><tr><td>BIT</td><td>10.0</td><td>30281</td><td>3.35</td></tr></table></td><td>Anonymous,

2007

IIIA6.1.5/02</td></tr>
<tr><td>LLNA

(guideline not specified) (conducted in duplicate)</td><td>Mouse

CBA/Ca

Sex not specified

4 /dose</td><td>100 %

Vehicle: DMF</td><td>0, 3, 10, 30 and 50% w/v BIT in DMF</td><td>EC_3 = 32.4 % - Skin Sens. 1B* (Experiment no.1.)

EC_3 = 4.8 % - Skin Sens. 1B* (Experiment no.2.)

Table 2. Induction of lymphocyte proliferation in draining lymph nodes following exposure to 1,2-benzisothiazolin-3-one (BIT)

<table><tr><th>Test chemical concentration (% w/v)</th><th>^{3}HTdr incorporation (mean cpm/node × 10^{-2})</th><th>Increment</th></tr><tr><td>Experiment no. 1</td><td></td><td></td></tr><tr><td>0</td><td>1.00</td><td>–</td></tr><tr><td>50</td><td>4.53</td><td>4.53</td></tr><tr><td>30</td><td>2.79</td><td>2.79</td></tr><tr><td>10</td><td>1.22</td><td>1.22</td></tr><tr><td>3</td><td>1.56</td><td>1.56</td></tr><tr><td>Experiment no. 2</td><td></td><td></td></tr><tr><td>0</td><td>1.26</td><td>–</td></tr><tr><td>50</td><td>6.26</td><td>4.97</td></tr><tr><td>30</td><td>5.61</td><td>4.45</td></tr><tr><td>10</td><td>4.84</td><td>3.84</td></tr><tr><td>3</td><td>3.43</td><td>2.72</td></tr></table></td><td>Botham *et al.*, 1991

*NICEATM LLNA Database</td></tr>
<tr><td>LLNA</td><td></td><td>„Proxel active”</td><td>10, 30 and 50 %

Vehicle DMF</td><td>EC_3 = 2.3% - Skin Sens. 1B

<table><tr><th>Dosage (%)</th><th>Stimulation Index</th></tr><tr><td>10</td><td>3.8</td></tr><tr><td>30</td><td>4.4</td></tr><tr><td>50</td><td>4.9</td></tr></table>
(Botham Experiment 2., but didn’t use the 3 % dose, and had to extrapolate as the lowest dose</td><td>Gerberick *et al.*, 2005</td></tr>
</table>

<table>
<tr><td></td><td></td><td></td><td></td><td>gave an SI greater than 3)</td><td></td></tr>
<tr><td>LLNA</td><td>Not stated.</td><td>BIT</td><td>Not stated.</td><td>EC_3 = 10.4 % - Skin Sens. 1B

Botham data using quadratic regression analysis</td><td>Basketter et al., 1999</td></tr>
<tr><td>GPMT

OECD TG 406

Range finding study

GLP</td><td>Guinea pig

Hartley

10M+10 F (test)

5M+5F (control)</td><td>97.42 % a.i.</td><td>Intradermal: 2.5 % BIT in propylene glycol

Topical: 100 mg BIT moistened with 80 % ethanol

Challenge: 100 mg BIT moistened with acetone</td><td>20 % responding at 2.5 % intradermal induction dose – no classification
<table><tr><td></td><td colspan="4">Animals with allergic reactions/ Animals in group</td></tr><tr><td></td><td>Sham control</td><td>Test group</td><td>Positive control</td><td>Sham positive control group</td></tr><tr><td>Scored 24h</td><td>0/10 (0%)</td><td>4/20 (20%)</td><td>9/20 (45%)</td><td>0/10 (0%)</td></tr><tr><td>Scored 48h</td><td>0/10 (0%)</td><td>2/20 (10%)</td><td>5/20 (25%)</td><td>0/10 (0%)</td></tr></table></td><td>Anonymous,

2003e

IIIA6.1.5/2</td></tr>
<tr><td>GPMT

OECD TG 406
US EPA OPPTS 870.2600

Range finding study

Deviation: challenge on day 23

GLP (self certified)</td><td>Guinea pig

Hartley albino

10M+10 F (test)

5M+5F (control)</td><td>98 % a.i.</td><td>Intradermal: 5 % w/w BIT in distilled water

Topical: 80 % w/w BIT in distilled water

Challenge: 80% w/w BIT in distilled water</td><td>30 % responding at 5 % intradermal induction dose - Skin Sens. 1B
<table><tr><td></td><td colspan="4">Animals with allergic reactions/ Animals in group</td></tr><tr><td></td><td>Sham control</td><td>Test group</td><td>Positive control</td><td>Sham positive control group</td></tr><tr><td>Scored 24h</td><td>0/10 (0 %)</td><td>6/20 (30%)</td><td>9/10 (90%)</td><td>0/5 (0 %)</td></tr><tr><td>Scored 48h</td><td>0/10 (0 %)</td><td>3/20 (15%)</td><td>7/10 (70%)</td><td>0/5 (0 %)</td></tr></table></td><td>Anonymous,

2002e

IIIA6.1.5/1</td></tr>
<tr><td>GPMT

OECD 406

No range finding study, no positive control

GLP</td><td>Guinea pig

Dunkin-Hartley albino

10M+10 F (test)

5M+5F (control)</td><td>Purity not specified.</td><td>Intradermal: 1 % in purified water and 5 % w/v BIT in FCA

Topical: 5 % w/v BIT in purified water

Challenge: 0.5 and 3 % w/v BIT in purified water</td><td>56 % responding at 5 % intradermal induction dose - Skin Sens. 1B
<table><tr><td>Group</td><td>Challenge</td><td>Score at 24 h</td><td>Score at 48 h</td><td>Total responders</td></tr><tr><td>Ctrl.</td><td rowspan="2">3 % BIT</td><td>0/10</td><td>0/10</td><td>0 (0%)</td></tr><tr><td>Test</td><td>9/18</td><td>7/18</td><td>10 (56%)</td></tr><tr><td>Ctrl.</td><td rowspan="2">0.5 % BIT</td><td>0/10</td><td>0/10</td><td>0 (0%)</td></tr><tr><td>Test</td><td>3/18</td><td>3/18</td><td>4 (22%)</td></tr><tr><td>Ctrl.</td><td rowspan="2">Purified water</td><td>0/10</td><td>0/10</td><td>0 (0%)</td></tr><tr><td>Test</td><td>1/18</td><td>2/18</td><td>2 (11%)</td></tr></table></td><td>Rees., 1994

(REACH registration dossier)</td></tr>
<tr><td>GPMT

Equivalent to US EPA PAG 81-6

Range finding study conducted</td><td>Guinea pig

Alpk:Dunkin-Hartley albino

20 F (test)

10 F</td><td>Purity not specified.

Test substance pre-dried technical grade active substance</td><td>Intradermal: 0.01 % w/v BIT in 3 % w/v DMF in corn oil Adjuvant (50 % w/v Complete Freund’s Adjuvant in 3 % w/v DMF in corn</td><td>35 % responding at 0.01 % intradermal induction dose - Skin Sens. 1A
<table><tr><td></td><td></td><td colspan="2">Animals with allergic reactions/Animals in group</td></tr><tr><td></td><td>Topical Dose (%)</td><td>Test group</td><td>Control group</td></tr><tr><td>Scored 24h</td><td>10</td><td>13/20 (65 %)</td><td>3/10 (30 %)</td></tr></table></td><td>Anonymous,

1990

IIIA6.1.5/1</td></tr>
</table>

<table>
<tr><td>GLP</td><td>(neg. ctrl.)

20 F (pos. ctrl.)</td><td></td><td>oil

Topical: 30 % w/v BIT in DMF

Challenge: 3% and 10% w/v BIT in DMF</td><td><table><tr><td></td><td>3</td><td>2/20 (10 %)</td><td>0/10 (0 %)</td></tr><tr><td rowspan="2">Scored 48h</td><td>10</td><td>13/20 (65 %)</td><td></td></tr><tr><td>3</td><td>1/20 (5 %)</td><td></td></tr></table>Net percentage response at 10 % challenge dose: 35 %,
Net percentage response at 3 % challenge dose: 10 %</td><td></td></tr>
<tr><td>GPMT

EC B.6

Range finding study conducted</td><td>Guinea pig

Strain not specified

Sex not specified

20 guinea pigs/group</td><td>20% a.i. in aqueous propylene glycol (Proxel XL)

30 % a.i. in morpholine di-and triethanol amine (Proxel HL)</td><td>Intradermal: 5 % w/v Proxel (1 or 1.5 % BIT in propylene glycol)

Topical: 25 % w/v Proxel (5 or 7.5 % BIT in petrolatum)

Challenge: 1 % w/v Proxel (0.2 or 0.3 % BIT in petrolatum)</td><td>15 % responding at 1 % intradermal induction dose – no classification
Proxel XL - 3/20 (15 %)

Proxel HL - 1/20 (5 %)</td><td>Andersen and Hamann, 1984

(TC C&L document)</td></tr>
</table>

Animal studies: LLNA

There are essentially 3 LLNA studies on BIT. One is the Anonymous (2007) (IIIA6.1.5/01) study, which was conducted according to the OECD TG 429, and performed under GLP. The doses were 3 %, 10 % and 30 % BIT in DMF, and the calculated EC_3 was 29 %.

The second study (Anonymous 2007, IIIA6.1.5/02) is an LLNA performed under GLP, but preceded the OECD TG 429, and used 4 consecutive days of application of the substance (instead of the 3 days indicated in the Guideline). The doses were 0.5 %, 1 %, 2.5 %, 5 % and 10 % BIT in a vehicle of acetone:olive oil. The stimulation indices were 2.78, 2.64, 3.64, 2.72 and 3.35, respectively, showing no dose response relationship. The calculated EC_3 was 1.54 %. This study has limited value because of the lack of dose response relationship.

The third study is the Botham (1991) study, which used 3 %, 10 %, 30 %, and 50 % BIT (purity 100 %) in DMF, and in which 2 experiments were conducted. The results can be seen in the Table above. In the first experiment, only the 50 % BIT solution elicited an SI ≥ 3, while in the second experiment this was achieved with 10 % BIT. The publication did not calculate an EC_3. The NICEATM LLNA Database on the other hand does give two EC_3 values based on the Botham publication: 32.4 % and 4.8 %.

Two other LLNA studies are mentioned in the CLH dossier, but from the original publications, it can be deduced that both rely on the data of the second experiment in the Botham (1991) study. The Gerberick *et al* (2005) study "Compilation of Historical Local Lymph Node Data for Evaluation of Skin Sensitisation Alternative Methods" gives BIT data for 10 %, 30 %, and 50 % BIT in DMF and gives SI values which are essentially the same as the Botham values, resulting in a calculated EC_3 of 2.3 %. The second study is the Basketter (1999) study which cites the BIT LLNA study performed by Botham, and using quadratic regression analysis, calculates an EC_3 of 10.4 % for BIT.

Of the 3 independent LLNA studies, the Anonymous (2007) (IIIA6.1.5/02) study calculated an EC_3 of 1.54 %, which would warrant classification as Skin Sens 1A (EC_3 value ≤ 2 %). Nevertheless, because there was no dose response relationship seen in this study, and there was a deviation from the guideline protocol (4 instead of 3 days application), which could have affected the results, this study is deemed unreliable for calculating an EC_3, and is not taken into account.

The Anonymous (2007) (IIIA6.1.5/01) study calculated an EC_3 of 29 %. All EC_3s calculated for the Botham (1991) study (2.3 %, 4.8 %, 10.4 % and 32.4 %) are greater than 2 %. Both studies therefore give EC_3 values that correspond to Skin Sens. 1B (EC_3 value > 2 %).

Animal studies: GPMT

There are 5 GPMTs included in the CLH dossier (Table above). The Anonymous (2003e) study was conducted according to OECD TG 406, under GLP, and included a range finding study. The chosen intradermal induction dose was 2.5 %. 20 % of the animals responded at the 2.5 % intradermal induction dose – which warrants no classification. The Anonymous (2002e) study was conducted according to OECD TG 406, under GLP (self-certified), and included a range finding study. The chosen intradermal induction dose was 5 % BIT, which elicited a response in 30 % of the animals (warranting Skin Sens 1B). The Rees (1994) study was conducted according to OECD TG 406, under GLP, but the purity of the substance was not stated, it did not include a range finding study, and had no positive control group. At an intradermal induction dose of 5 % BIT, 56 % of the animals responded (warranting Skin Sens. 1B). The Anonymous (1990) study protocol was equivalent to US EPA PAG 81-6, was conducted under GLP, and included a preliminary range finding study. At the chosen intradermal elicitation dose of 0.01 %, a 35 % response rate was found (warranting Skin Sens. 1A). The Andersen and Hamann (1984) study was conducted according to EC B.6, and included a range finding study. At an intradermal induction dose of 1 %, 15 % of the animals responded (warranting no classification).

The results of the GPMT studies varied widely, indicating Skin Sens. 1A (one study), Skin Sens 1B (two studies) and no classification (two studies). The study warranting Skin Sens. 1A used a pre-dried technical grade active substance, with the purity not specified, and is in contradiction with the rest of the studies which are consistent: 1 % intradermal induction resulted in 15 % animals responding, 2.5 % intradermal induction resulted in 20 % responding, 5 % intradermal induction resulted in 30 % and 56 % responding. The latter two, using the highest intradermal induction doses warrant classification as Skin Sens. 1B (≥ 30 % responding at > 1 % intradermal induction dose). Thus, the results of the GPMT studies point to a classification of Skin Sens. 1B.

Human information

***Table**: Summary of human studies on skin sensitisation.*

Type	Test substance	Observations	Relevant information about the study (as applicable)	Reference
HRIPT	78 % a.i. (+ 20 % ethylene diamine*) 0.036 %=360 ppm, 45 µg/cm^2 and 0.073 %=725 ppm, 90.6 µg/cm^2) in water. * also a sensitiser	5/58 volunteers (9 %) were sensitised by BIT using 725 ppm BIT (90.6 µg/cm^2). None (0/54: 0 %) were sensitised by 360 ppm BIT (45 µg/cm^2).	Information was drawn from a historic Zeneca database. 58 volunteers were exposed to 0.073 % BIT. 54 volunteers were exposed to 0.036% BIT	Basketter *et al.*, 1999 (RAC opinion on MBIT) (SCCS opinion on BIT)

HRIPT	19.2 % a.i. (Proxel GxL) 0.05% BIT (500 ppm, 27.8 µg/cm^2) in Rhoplex AC-64 (sample A); 0.1 % BIT (1000 ppm 55.6 µg/cm^2) in Rhoplex AC-64 (sample B); undiluted Rhoplex AC-64 (sample C).	No sensitisation (0/111) at rechallenge of 1 subject Irritation in 3 subjects, two also showed irritation to vehicle (Rhoplex AC-64).	111 volunteers (26 males and 85 females); 24 hour contact, semi-occlusive patches. For details, see Table "Summary of the studies on skin corrosion/irritation" in the Section "RAC evaluation of skin corrosion/irritation".	Anonymous, 1991 IIIA6.12.6/01
HRIPT	Purity not stated. 0.5 mL of 0.05 % BIT (500 ppm) in propylene glycol (induction and first challenge tests in main study, also second challenge in 10 subjects) or liquid paraffin (second challenge test main study) (64.45 µg/cm^2 or 250 µg BIT/patch).	Sensitisation in 5/45 volunteers (11 %) at 64.45 µg/cm^2. Mild to moderate or severe irritation.	50 volunteers (21 males and 29 females); 40 subjects (main study), 10 subjects (preliminary irritancy screen) 45 concluded the study. A second challenge application was made to 9 volunteers. Marked reactions indicative of dermal sensitisation were observed in five individuals following the second challenge.	Anonymous, 1975
Diagnostic patch test	Purity not specified. 0.05 % BIT (500 ppm) in petrolatum.	Allergic reaction in 16/5450 (0.3 %*) patients. *Incorrectly calculated in study and incorrectly given in CLH report as 0.003 %	From January 2000 to April 2006 BIT was tested on 5450 patients at Helsinki University Central Hospital (general dermatology).	Aalto-Korte *et al.*, 2007
Diagnostic patch test	Purity not stated. 0.1 % BIT (1000 ppm) in petrolatum.	14/1652 patients (0.9 %) showed positive reaction to BIT. 4 may have had excited skin syndrome 3 negative with 0.1 % BIT in alcohol→ possible false positive?	BIT was included in the routine patch testing panel in 6 Danish outpatient clinics. 1652 consecutive eczema patients were tested.	Andersen and Veien, 1985 (TC C&L document)
Diagnostic patch test	Purity of BIT not specified. 20 % a.i. (Proxel XL) 1 % BIT (10000 ppm) in alcohol and 0.5 % Proxel XL (0.1 % BIT (1000 ppm)) in water.	1/404 patient (0.25 %) reacted to 10000 ppm BIT and 121 reactions (30 %) were classified as irritation. 1/466 patient (0.22 %) reacted to Proxel XL (1000 ppm BIT) and 7 reactions (1.5 %) were classified as irritation.	BIT and Proxel XL were included in the standard patch-test series at the Dept. Dermatology, Gentofte Hospital, Denmark. In a period of 14 months, 404 (1 % BIT) – 466 (0.5 % Proxel XL) eczema patients were tested.	Andersen and Hamann, 1984 (TC C&L document)
Diagnostic patch test	33 % in water (Proxel BD) 0.04 % BIT (400 ppm) in water	10/556 patients (1.8 %) showed positive patch test to BIT	537 consecutive dermatological patients without clear occupational risk+19 patients positive for Kathon CG. In 3 of the BIT positive cases, contact allergy was related to domestic paper-hanging. Sensitisation occurred in response to unknown BIT concentrations.	Damstra *et al.*, 1992 (TC C&L document)
Diagnostic patch test	Purity not specified. 0.05 % BIT	20/2264 patients (0.88 %) showed a	From January 1991 to September 2005, BIT was	Aalto-Korte *et al.*, 2006, 2007

	(500 ppm) in petrolatum.	positive response to BIT. Some might have been sensitised by using gloves with 0.002 % BIT (20 ppm BIT).	tested on a total of 2264 patients at the Finnish Institute of Occupational Health (highly selected patients).	(RAC opinion on MBIT) (SCCS opinion on BIT)
Diagnostic patch test	Purity of BIT not specified. 0.05 % BIT (500 ppm) in water	1/977 patients (0.1 %) cross-reacted to 0.05 % BIT.	977 patients with a history of allergy to cosmetics were tested for allergy to a number of substances. 35 patients (3.6 %) were positive to Kathon CG (CMIT/MIT).	Ledieu *et al*., 1991 (TC C&L document)
Diagnostic patch test	0.1 % and 0.05 % in petrolatum	27/575 (4.7 %) patients had positive patch test reactions to BIT (0.05 % and/or 0.1 %)	Eczema patients from 2001 to 2015 tested at the Department of Dermatology, Odense University Hospital, Denmark, and in dermatology offices. 392 patients were tested with 0.05 % BIT and 183 with 0.1 % BIT. Aimed testing.	Madsen *et al*., 2015 (public consultation)
Diagnostic patch test	BIT 0.1 % (1000 ppm) in petrolatum	731/29590 (2.5 %) tested positive	29590 dermatitis patients tested between 2000-2019 at the IVDK. Mostly aimed testing.	IVDK data (public consultation)
Diagnostic patch test	BIT 0.1 % (1000 ppm) in petrolatum	141/8465 (1.6 %) gave positive reaction.	8465 dermatitis patients tested between 2009-2013 at the IVDK. Mostly aimed testing.	Geier *et al*. 2015 (public consultation)
Patch test/ Workplace	Purity not specified. 0.1 % BIT (1000 ppm). Vehicle water.	48/230 patients (20.9 %) had a positive allergic response.	230 patients with occupational dermatoses from cutting oils. Recommended concentration in cutting fluids 0.075 %, but often it is added in quantity, with no special control.	Alomar*et al*., 1985 (TC C&L document)
Patch test/ Workplace	Purity not stated. 0.1 % BIT (1000 ppm) in water.	4/20 patients (20 %) had positive reactions.	20 metal workers with dermatitis of the hands, possibly due to exposure to cutting oils were patch tested. Proxel HL (30 % BIT) was added to the cutting oils in a concentration of 0.1 to 0.3 % (0.03 %=300 ppm to 0.1 % =1000 BIT). No further information is stated.	Alomar*et al*., 1981 (TC C&L document)
Patch test/ Workplace	33 % in water (Proxel BD) 0.04 % BIT (400 ppm) in water	In 4/17 patients (23 %) contact allergy to BIT was found.	17 hand dermatitis patients at occupational exposure risk.	Damstra *et al.*, 1992 (TC C&L document)
Patch test/ Workplace	Purity not specified. 0.01, 0.1 and 1 % BIT (100, 1000 and 10000 ppm) in methanol.	7/11 persons (63.6 %) reacted to 1 % BIT. 4/7 were tested at 0.01 and 0.1 % BIT. None of the 4 (0 %) reacted to 0.01 % BIT, 2/4 (50 %) reacted to 0.1 % BIT.	11/16 men working in the quality control laboratory of a chemicals firm developed dermatitis. The work involved analysis of several chemicals including BIT.	Slovak, 1979 (TC C&L document)
Patch test/ Workplace	33 % a.i. (Proxel CRL) and BIT (purity not specified). 0.01, 0.03, 0.3 and 1 % Proxel CRL: 0.003 %=30 ppm, 0.01 %=100 ppm 0.1 %= 1000 ppm	4/8 patients (50 %) showed a positive reaction. 3 of them at 0.03 % Proxel CRL (0.01 % = 100 ppm BIT) and 0.05 % = 500 ppm BIT and one at 0.3 and 1 % Proxel CRL (1000 and 3300 ppm BIT). None reacted at	Occupational contact allergy to Proxel CRL was reported among 8 employees. No information about the concentrations inducing allergy is stated.	Dias *et al*., 1992 (TC C&L document)

	0.33 %=3300 ppm BIT and 0.05 % BIT (500 ppm).	30 ppm BIT		
Patch test/ Case study	1000 ppm or 0.1 % Mergal K-10 (% BIT not stated) in petrolatum at 2 and 4 days.	Patch testing with the GEIDC standard series and all the components of the paints with which he was in contact gave positive reactions to Mergal K-10. The dermatitis cleared up on treatment with topical corticosteroids.	A 52-year-old man had been working in a paint factory for 18 years. There was no previous history of atopy or skin disease. He started using Mergal K-10 as a biocide (% BIT not stated). Suddenly, he developed an itchy vesicular dermatitis on the dorsum of both hands and on the chest. The patient discontinued contact with BIT by changing his work station within the paint factory. After 6 months, he remained asymptomatic with no skin lesions.	Sanz-Gallén *et al.*, 1992 (TC C&L document)
Patch test/ Case study	Purity not stated. 0.001 % to 0.16 % BIT (10 to 1600 ppm)	Patch tests with the preservative 1,2-BIT used in the wallpaper paste showed positive reactions in concentrations down to 0.003 % (30 ppm).	A 45-year-old paper-hanger presented a long-standing hand dermatitis which was resistant to conventional therapy. Avoidance of contact with this particular paste resulted in complete disappearance of the hand dermatitis in this individual.	Damstra *et al.*, 1992 (TC C&L document)
Patch test/ Case study	33 % a.i. (Proxel CRL) Purity of BIT not stated. 1 % Proxel CRL (0.33 % BIT (3300 ppm)) and 1 % BIT (10000 ppm). Vehicle not specified	Positive reaction.	A case of contact dermatitis was reported on a person working in the rubber industry exposed to Proxel CRL.	Foussereau *et al.*, 1984 (TC C&L document)
Patch test/ Case study	0.13 % a.i. (gum arabic) 19.2 a.i. (Proxel GxL) Purity of BIT not stated. Gum Arabic (0.026 % BIT (260 ppm)). 0.01, 0.1 and 1 % of Proxel GxL (0.002, 0.02 0.19 % BIT (20, 200 and 1920 ppm)). Vehicle not stated. 0.01, 0.1 and 1 % of BIT (100, 1000 and 10000 ppm). Vehicle not stated.	The patient reacted to gum arabic, 0.1 % and 1% Proxel GxL and BIT at all doses. 1 control showed a strongly positive allergic reaction lasting for 2 weeks to 0.1 % BIT. He had twice previously served as a control and had become sensitised by low concentrations of Proxel (probably 0.1 or 1 %).	A 24-year old printer showed hand dermatitis soon after change to a new company. At his job, he was handling gum arabic (containing 0.13 % BIT) without gloves. Patch testing was carried out on batches of 10 controls.	Freeman, 1984 (TC C&L document)
Patch test/ Case study	10 % a.i. (Proxel XL2) 1 % Proxel XL2 (0.1 %=1000 ppm BIT) and 0.1 % BIT	Positive patch tests were obtained.	A 27-year old man employed as a mouldmaker in the pottery industry presented a few months' history of an eczema on the backs and sides of his fingers. The mouldmaker dips a sponge into an open	Roberts *et al.*, 1981 (TC C&L document)

	(1000 ppm) (vehicle not specified).		bucket of an oil-based emulsion; gloves were not used. For about one year, the factory had been using an oil containing Proxel XL2 (9-10 % BIT) in a concentration of 1.6 % Proxel XL2 (0.16 % =1600 ppm BIT).	
Patch test/ Case study	Not specified.	Not specified.	2 other cases of allergy to BIT induced by working with oil containing Proxel XL2 in a non-specified concentration are referred.	
Patch test	10 % a.i. (Proxel XL2) 1 and 5 % Proxel XL2 (0.1 and 0.5% BIT (1000 and 5000 ppm)) in water.	Weak positive reaction in 3 persons (27 %) with 5 % Proxel XL2 (5000 ppm BIT) and no reactions with 1 % Proxel XL2 (1000 ppm BIT).	Eleven controls. (?)	
Patch test/ Case study	Purity not stated. 5 and 10 % BIT (50000 and 100000 ppm) in a non-specified vehicle.	Positive reaction.	A worker with occupational exposure to cutting fluids developed dermatitis.	Brown, 1979 (TC C&L document)
Patch test/ Case study	33 % a.i. (Proxel CRL) Purity of BIT not stated. 0.1 % Proxel CRL (0.033 %=330 ppm BIT) in water and 0.1 % BIT (1000 ppm) in ethanol.	The 2 patients (100 %) showed positive reaction to Proxel CRL and BIT. One of them at 0.1 % BIT and the other one at 0.1 and 0.01 % BIT.	2 male patients with hand eczema, both exposed to BIT through working with preserved plastic emulsions. There is no information about the concentration of BIT in plastic emulsions.	Pedersen, 1976 (TC C&L document)

HRIPT studies

There are 3 HRIPT studies in the dataset for human studies.

In the first HRIPT study (Basketter *et al*., 1999), information had been obtained from a historic Zeneca database, which contained a group of 58 volunteers who had been exposed to 0.073 % (725 ppm) BIT, and another group of 54 volunteers who had been exposed to 0.036 % (360 ppm) BIT. The original test substance was 78 % BIT + 20 % ethylene diamine, the latter of which is also a skin sensitiser. The authors employed a conservative approach and assumed that all the reactions seen were due to BIT. They found that 5/58 volunteers (9 %) were sensitised by BIT using 725 ppm BIT (90.6 µg/cm^2), while none of the volunteers were sensitised by 360 ppm BIT (45 µg/cm^2).

In the second HRIPT study (Anonymous, 1991) involving 111 volunteers, no sensitisation occurred. The doses employed were 0.05 % BIT (500 ppm, 27.8 µg/cm^2) and 0.1 % BIT (1000 ppm 55.6 µg/cm^2) in Rhoplex AC-64.

In the third HRIPT study (Anonymous, 1975), 50 volunteers were enrolled: 40 volunteers in the main study, and 10 who also took part in the pre-screening study to assess the irritancy of BIT. In the preliminary irritancy screen, BIT at concentrations of 500, 750 and 1000 ppm (0.05, 0.075 and 0.1 %) in propylene glycol was applied to the skin on three occasions over a nine-day period. Thus the induction doses in these 10 volunteers was not 500 ppm during the beginning of the study, as patches of 750 and 1000 ppm were also applied. However, the results were pooled, and the conclusion was that 5/45 (11 %) were

sensitised (45 volunteers completed the study) with an induction dose of 0.05 % (500 ppm BIT, 64.45 µg/cm^2).

The 3 HRIPT tests are nevertheless quite consistent: there was no sensitisation with doses of 27.8 µg/cm^2 (Anonymous, 1991), 45 µg/cm^2 (Basketter *et al*., 1999) and 55.6 µg/cm^2 (Anonymous, 1991), while sensitisation occurred at somewhat higher doses than these, namely at 64.45 µg/cm^2 (Anonymous, 1975) and 90.6 µg/cm^2 (Basketter *et al*., 1999).

On the basis of the HRIPT studies, BIT warrants classification as **Skin Sens. 1A** (positive responses at ≤ 500 µg/cm^2 (HRIPT – induction threshold)).

Diagnostic patch tests

Six diagnostic patch tests were included in the CLH dossier, 3 of them on unselected, consecutive dermatitis patients.

Unselected dermatitis patients

From January 2000 to April 2006 BIT was tested on 5450 patients at Helsinki University Central Hospital. The dose used was 500 ppm, and an allergic reaction was found in 16/5450 (0.3 %) patients (Aalto-Korte *et al*., 2007).

BIT was included in the routine patch testing panel in 6 Danish outpatient clinics at a dose of 1000 ppm. 1652 consecutive eczema patients were tested. 14/1652 patients (0.9 %) showed positive reaction to BIT, although this may have been lower (7/1652, 0.42 %), as the authors stated that 4 may have had excited skin syndrome, and 3 were negative with 0.1 % BIT in alcohol, so these might have been false positives (Andersen and Veien, 1985).

BIT and Proxel XL were included in the standard patch-test series at the Dept. Dermatology, Gentofte Hospital, Denmark. In a period of 14 months, a group of 404 eczema patients were tested with 1 % BIT (10000 ppm) and a group of 466 was tested with 0.5 % Proxel XL (1000 ppm BIT). 1/404 patient (0.25 %) reacted to 10000 ppm BIT and 121 reactions (30 %) were classified as irritation. 1/466 patients (0.22 %) reacted to Proxel XL (1000 ppm BIT) and 7 reactions (1.5 %) were classified as irritation (Andersen and Hamann, 1984).

Selected patients/aimed testing

556 dermatological patients without clear occupational risk were patch tested with 400 ppm BIT. 537 were randomly selected, but 19 patients with reproducible positive patch test reaction to Kathon CG (C(M)IT/MIT) were included additionally. 10/556 patients (1.8 %) showed a positive patch test. The results are not distinguished between the two groups; therefore the study cannot be deemed to have been wholly conducted on unselected patients. In 3 of the positive cases, contact allergy was related to domestic paper-hanging (Damstra *et al.*, 1992).

From January 1991 to September 2005, BIT was tested (at 500 ppm) on a total of 2264 patients at the Finnish Institute of Occupational Health (highly selected patients). 20/2264 patients (0.88 %) showed a positive response to BIT (Aalto-Korte *et al*., 2006, 2007).

In the last diagnostic patch study included in the CLH dossier, 977 patients with a history of allergy to cosmetics were tested for allergy to a number of substances, including Kathon CG (containing CMIT/MIT) and BIT. 35 patients were positive to Kathon CG, while 1/977

patient (0.1 %) cross-reacted to 0.05 % (500 ppm) BIT (Ledieu *et al*., 1991).

During the public consultation 3 other publications were mentioned, all 3 on selected patients:

575 eczema patients were tested at the Department of Dermatology, Odense University Hospital, Denmark, and in dermatology offices between 2001 and 2015. 392 patients were tested with 0.05 % BIT (500 ppm) and 183 with 0.1 % (1000 ppm) BIT. 27/575 (4.7 %) patients had positive patch test reactions to BIT at either/or 0.05 % and 0.1 % (Madsen *et al*., 2015).

29590 dermatitis patients were tested between 2000-2019 at the Information Network of Departments of Dermatology (IVDK), which holds the world's largest contact allergy database including BIT patch test data of almost 30000 patients from more than 50 departments of dermatology in Germany, Switzerland, and Austria. Mostly aimed testing was carried out. 731/29590 (2.5 %) patients tested positive to 0.1 % (1000 ppm) BIT (IVDK data, submitted in the public consultation).

8465 dermatitis patients were tested between 2009-2013 at the IVDK. Mostly aimed testing was carried out. 141/8465 (1.6 %) patients gave a positive reaction to 0.1 % (1000 ppm) BIT (Geier *et al*., 2015).

Workplace studies/case studies

Five workplace studies were included in the CLH dossier. They ranged from 8 to 230 participants.

Two reported dermatitis in workers exposed to cutting oils (Alomar *et al*., 1984; Alomar *et al*., 1981). In one of them 48/230 (20.9 %) dermatitis patients were found to give a reaction to 1000 ppm BIT. The author stated that although at the time the recommended concentration of BIT in cutting fluids was 0.075 %, often it was added in quantity, with no special control, so higher concentrations were probable. In the second study it is stated that BIT was added to cutting fluids at 300-1000 ppm. In this latter study 4/20 (20 %) workers showed a positive reaction to 1000 ppm BIT.

In the Damstra *et al*. (1992) study, 4/17 (23 %) patients with occupational exposure risk showed contact allergy to BIT at 400 ppm.

In the Slovak (1979) study, 7/11 (63.6 %) laboratory personnel analysing chemicals including BIT reacted to 10000 ppm BIT in methanol. The test concentration - considering the irritating properties of BIT - seems too high.

In the study of Dias *et al*. (1992), 4/8 (50 %) employees reported to have contact allergy to Proxel CRL showed reaction to BIT at 1000 ppm.

There are 8 case studies dating from 1976-1992, with exposure concentrations mainly unknown, but in one the subject of the study handled gum arabic containing 1300 ppm BIT (Freeman, 1984), and in another the subject was exposed to an oil based emulsion containing 1600 ppm BIT (Roberts *et al*., 1981). Neither of the workers were using gloves.

Frequency of sensitisation

The frequency of sensitisation in diagnostic patch tests on unselected, consecutive patients is 0.3 % (Aalto-Korte *et al*., 2007), 0.9 % (Andersen and Veien 1985), 0.25 % and 0.22 % (Andersen and Hamann, 1984), all pointing to low/moderate frequency of skin sensitisation

(frequency < 1 %).

The frequency of sensitisation in selected dermatitis patients is 1.8 % (Damstra *et al.*, 1992), 0.88 % (Aalto-Korte *et al*., 2006), 0.1 % (Ledieu *et al*., 1991) and 1.6 % (Geier *et al*., 2015). These four studies point to low/moderate frequency of skin sensitisation (frequency < 2 %). However, there are two additional studies with higher percentages of frequency of sensitisation: the retrospective study by IVDK spanning 20 years in which 2.5 % of the dermatitis patients were found to be sensitised to BIT, and the Madsen study, in which the sensitisation rate was 4.7 %. These studies point to a relatively high frequency of skin sensitisation (≥ 2.0 %).

The data of Geier et. al (2015) was an analysis of the IVDK data during the period between 2009 and 2015. During this period, the frequency of sensitisation in 2009 was rather high, but in the rest of the period it was rather low, which explains why the Geier frequencies are lower than that in the whole IVDK dataset.

The Figure " Percentages of positive patch test reactions to BIT 0.1 % pet. during the years 2000 to 2019" (under the heading "Comments received during consultation", section on IVDK) demonstrates that the frequency of sensitisation changes over time. IVDK states, that upon analysis of the data, that there truly are changing sensitisation frequencies during the last 20 years, with a more or less constant increase from 2013 to 2019, although as there are no BIT exposure data, one cannot tell if this is due to a more widespread or more intense use of BIT. It is remarkable, that from a frequency of 1.0-1.5 % between 2010-2015, the rate of frequency increases to 2.5 % in the next two years, and then to above 4.0 % in the next two years (2018-2019).

The frequency of sensitisation in selected workers with known exposure or dermatitis is 20.9 % (Alomar *et al*., 1985), 20 % (Alomar *et al*., 1981), 23 % (Damstra *et al.*, 1992), 63.6 % (Slovak 1979) and 50 % (Dias *et al*., 1992), all pointing to high frequency of skin sensitisation (frequency ≥ 1 %).

To summarise, the studies with unselected dermatitis patients show low frequencies of sensitisation (frequency < 1 %), while some of the studies with selected patients show low (frequency < 2 %), others show high (≥ 2.0 %) frequencies of sensitisation. The studies with selected workers with known exposure or dermatitis show high frequencies of sensitisation (frequency ≥ 1 %). BIT is an antimicrobial agent that is used in industry as a preservative in water-based solutions, such as pastes, paints and cutting oils. Indeed, exposure to BIT seems to occur predominantly in occupational settings, so it is logical that aimed studies have higher frequencies of sensitisation. The largest study, with nearly 30000 patients in 3 countries (Germany, Switzerland, and Austria), shows that in recent years sensitisation in selected patients has risen to 4.4 %. Therefore, RAC considers that a relatively high frequency of sensitisation can be expected for BIT.

Exposure

The CLH report does not elaborate on exposure, but some literature can be found on the occurrence and concentration of BIT in various products. The publication by Flyvholm MA (2005) provides an overview on the occurrence of preservatives in registered chemical products. The data was obtained from the Danish Product Register Database (PROBAS) in January 2005 and January 2002. The data include products registered by January 2005, which have been active on the market within the past 5 years and computerised with information on chemical composition and product category. All products containing the

studied preservatives either directly or from raw materials were included. The product categories that had the most products listed for BIT content were paints/lacquers (253 products in 2002 and 1084 in 2005), printing inks (110 and 112 respectively), impregnating agents ((75 and 74) polishes (55 and 84) and cleaning agents (52 and 76). BIT did not appear in any toiletries or cosmetic products. The products which may be of concern to the general public are cleaning agents and paints/lacquers (home-decorating).

The publication by Nielsen H (1994) used the data from the same database (PROBAS), on products that were on the market as of September 1992. In addition to products/product categories, it also contains concentration data in 139 products. 46 % of the products contained less than 0.01 % (100 ppm) BIT, 30 % of the products contained from 0.01 % (100 ppm) to less than 0.1 % (1000 ppm) BIT, 24 % of the products contained above or equal to 0.1 % (1000 ppm) BIT. The typical concentration of BIT in cleaning agents (34 products) was < 0.0015 %, the typical concentration of BIT in polishes (38 products) was < 0.0025 %, and the typical concentration of BIT in preservatives was > 10 %.

There is little information on concentrations inducing sensitisation in the workplace/case studies, but 3 of them have concrete values. In the Alomar (1981) publication, 0.03-0.1 % (300-1000 ppm) is used in cutting oils. In the Freeman (1984) publication, a lithoprinter, working without gloves, was sensitised by handling gum arabic containing 0.13 % (1300 ppm) BIT. In the Roberts *et al.* (1981) publication a mouldmaker was exposed to an oil-based emulsion containing 0.16 % (1600 ppm) BIT.

The information above indicates relatively low exposure to BIT (concentrations < 1.0 %).

Conclusion

Although the animal data (both the LLNAs and GPMTs) would only warrant classification as Skin Sens 1B, there is a wealth of reliable human data available for BIT.

The Guidance on the Application of the CLP Criteria states that human evidence for sub-category 1A can include:
(a) positive responses at ≤ 500 µg/cm^2 (HRIPT, HMT –induction threshold);
(b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure;
(c) other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure

Both the first and the second points are fulfilled by BIT:

(a) In two independent HRIPT tests, sensitisation occurred at induction doses below 500 µg/cm^2, namely at 64.45 µg/cm^2 (Anonymous, 1975) and 90.6 µg/cm^2 (Basketter *et al*., 1999).
(b) Diagnostic patch test data indicated that there is a relatively high and substantial incidence of reactions (4.4 % in selected dermatitis patients), in relation to relatively low exposure (concentrations < 1.0 %).

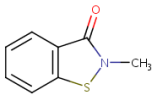
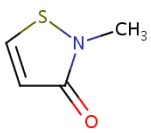
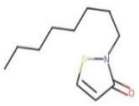
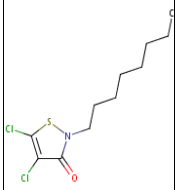
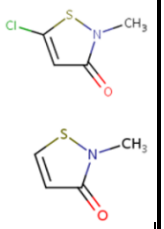
RAC therefore concludes that BIT warrants classification as **Skin Sens. 1A, H317: May cause an allergic skin reaction**.

Specific concentration limit

The results of the LLNA studies indicate that BIT is a moderate sensitiser (EC_3 values were >

2 %), and the same potency is indicated by the GPMTs (≥ 30 % responding at > 1 % intradermal induction dose). According to CLP, Annex I, Section 3.4.2.2.1.2, skin sensitisers classified in sub- category 1A are strong sensitisers, with a GCL of 0.1 %. Setting an SCL is possible when there is adequate and reliable scientific information available showing that the specific hazard is evident at below the GCL. Such data could be human data for which the exposures leading to sensitisation are defined. There is some evidence on concentrations inducing sensitisation in the workplace/case studies: in the Alomar (1981) publication, 0.03-0.1 % (300-1000 ppm) was used in cutting oils, in the Freeman (1984) publication, a lithoprinter, working without gloves, was sensitised by handling gum arabic containing 0.13 % (1300 ppm) BIT, and in the Roberts *et al*. (1981) publication a mouldmaker was exposed to an oil based emulsion containing 0.16 % (1600 ppm) BIT. There is an existing SCL for BIT of 0.05 %, and the DS proposed to retain it.

Table: *Comparison of skin sensitising properties of several isothiazolinones. Data taken from RAC opinions on MBIT (2018); MIT (2016); OIT (2018), DCOIT (2018) and CMIT/MIT (2016).*

	BIT (CAS 2634-33-5)	**MBIT (CAS 2527-66-4)**	**MIT (CAS 2682-20-4)**	**OIT (CAS 26530-20-1)**	**DCOIT (CAS: 64359-81-5)**	**CMIT/MIT (3:1) (CAS 55965-84-9)**
Chemical structure						
LLNA	EC_3 = 29 % EC_3 = 32.4 % EC_3 = 2.3 % EC_3 = 4.8 % EC_3 = 10.4 %	EC_3 = 1.04 % EC_3 = 0.69 %	EC_3 = 0.86 %	EC_3 = 0.46 % EC_3 = 0.66 % EC_3 = 0.24 %	EC_3 = 0.03 %	EC_3 = 0.003 % EC_3 = 0.007 %
Potency	strong	strong	strong to extreme	strong to extreme	extreme	extreme
Classification	Skin Sens. 1A (this opinion)	Skin Sens. 1A	Skin Sens. 1A	Skin Sens. 1A	Skin Sens. 1A	Skin Sens. 1A
HRIPT	5/58 (9 %) at 725 ppm aq. (90.6 µg/cm²), 0/54 (0 %) at 360 ppm aq (45 µg/cm²) 5/45 (11 %) volunteers at 500 ppm (64.5 µg/cm²) * 0/111 at (500 ppm, (27.8 µg/cm²) in Rhoplex AC-64	9/45 (20 %) volunteers at 500 ppm*	1/116 (0.9 %) volunteers at 400 ppm (20 µg/cm²) 1/210 (0.5 %) at 500 ppm (25 µg/cm²)	0/103 subjects at 50 ppm (0.005 %) (2.5 µg/cm²) 1/222 (0.45 %) subjects at 100 ppm (0.01 %) (5 µg/cm²)	4/34 (12 %) at 250 ppm (0.025 %) (12.5 µg/cm²) 14/34 (41 %) at 350 ppm (0.035 %)	–
SCL	0.036 % (this opinion)	0.0015 %	0.0015 %	0.0015 %		0.0015 %

*From same study (Anonymous 1975)

Of the isothiazolinones with a harmonised classification (see Table above), BIT is the least potent. In the LLNAs the EC_3 values for BIT are higher than 2 %, while for the other substances it is lower than 2 %. As a substance meeting the criteria for classifications as

Skin Sens. 1A, BIT is a strong sensitiser, while the other isothiazolinones range from strong to extreme sensitisers, which is reflected in their respective EC_3 values: EC_3 (BIT) > EC_3 (MBIT) ≈ EC_3 (MIT) ≈ EC_3 (OIT) > EC_3 (DCOIT) >> EC_3 (CMIT/MIT).

One of the factors that should be taken into consideration in the setting of an SCL for an isothiazolinone is that it might cross-react with other isothiasolinones. From their data on the concomitant reactions to BIT and other isothiazolinones (CMIT/MIT, MIT, OIT), IVDK concludes that their analysis clearly indicated that there is no relevant immunological cross-reactivity, but a certain proportion (about 20 %) of BIT-sensitised individuals acquired sensitisation to MIT, probably via co-exposure. On the other hand, only 7.4 % of those sensitised to MIT were also allergic to BIT. Co-exposure causing co-sensitisation occurs in industry and from products used in crafts, but not in cosmetics because BIT is prohibited for this field of application. Craig *et al.* (2017) conducted a patch test series with C(M)IT /MIT, MIT, OIT, and BIT. Out of 1287 patients 118 (9.2 %) showed a positive reaction to any isothiazolinone. Of these 118 patients only 10 showed a positive reaction to BIT. In their dataset, the majority of patients reacting to OIT reacted to other isothiazolinones, whereas positive reactions to BIT tended to occur in isolation. Conversely, few patients with MIT allergy reacted to OIT or BIT. As BIT is chemically less similar to MIT, co-exposure is more likely to explain co-reactivity. However, cross-reactivity could be considered between OIT and CMIT and MIT, which are chemically more similar. There are 3 publications by Aalto-Korte *et al.* investigating concomitant reactions of dermatitis patients to isothiazolinones. At the Finnish Institute of Occupational Health (FIOH), during the period 2012–2017, 647 consecutive patients were patch tested with OIT, BIT, and MIT. They found 61 (9.4 %) allergic reactions to MIT, 19 (2.9 %) reactions to OIT and 9 (1.4 %) reactions to BIT. Seventeen (89 %) of the OIT-positive patients had concomitant reactions to MIT, whereas only 3 (33 %) BIT-positive patients had allergic reactions to MIT. Allergic reactions to OIT were strongly associated with extreme reactions to MIT, which suggests cross-sensitisation. In contrast, BIT reactions were mostly independent (Aalto-Korte & Suuronen, 2017). In an earlier publication, the FIOH reported that of 2264 patients tested during the period 1991–2005, 20 gave a positive reaction to BIT. Four of these 20 patients reacted to C(M)IT/MIT and 2 to OIT. BIT was not considered to cross react with C(M)IT/MIT or OIT, concomitant reactions to these isothiazolinones supported independent sensitisation (Aalto-Korte *et al*., 2006). In another study, BIT was patch tested in 5450 patients at Helsinki University Central Hospital (HUCH). The study also reported data on 3 previously unpublished BIT allergic patients from FIOH. 16 patients were positive to BIT patch testing. None of the 16 BIT allergic patients in HUCH or 3 patients in FIOH had patch test reactions to the mixture of C(M)IT/MIT or to OIT (Aalto-Korte *et al.,* 2007). None of the studies suggest cross-sensitisation between BIT and other isothiazolinones; concomitant exposure remains the probable explanation for simultaneous reactions, therefore cross-reactivity to other isothiazolinones does not have to be taken into consideration when setting an SCL for BIT.

Another issue raised by the DS and during consultation of the CLH report, was that there is indication that BIT caused sensitisation from PVC gloves containing a very small concentration (20-30 ppm) of BIT. In the publications of Aalto-Korte *et al*, BIT was tested on 2264 patients at the FIOH. 20 patients had an allergic reaction to BIT, with 8 patients with BIT allergy and hand dermatitis in connection with PVC glove use. Some of them had used gloves that were shown to contain small amounts (≥ 20 ppm) of BIT. Nevertheless, the authors stated that "*a common feature of patients 1 to 8 was a long history of hand dermatitis (of at least 5 years' duration), and they had also been diagnosed with other*

types of hand dermatitis besides BIT contact allergy. Thus, sensitisation to BIT in the gloves was probably not the primary event. The occlusive effect of the PVC gloves on their eczematous skin might have enhanced the percutaneous penetration of BIT so that they had become sensitised despite the low allergen concentration" (Aalto-Korte, 2006). In the Aalto-Korte (2007) publication the authors stated that the sensitisation to BIT from PVC gloves seems to affect mostly dental personnel and that in addition to the constant use of occlusive gloves, dental workers are also exposed to other factors that irritate the skin such as frequent hand washing and the use of disinfectants. A defective skin barrier because of irritation or pre-existing eczema and the occlusive effect of the gloves probably enhance percutaneous penetration of allergens and increase the risk of sensitisation to glove allergens. Most of the patients have had a relatively long history of hand dermatitis, and it is possible that the sensitisation to BIT in the gloves requires pre-existing dermatitis, such as atopic dermatitis or irritant contact dermatitis. RAC is of the opinion that an SCL for BIT cannot be derived from studies of dermal patients who developed BIT allergy after a long history of dermatitis or a defective skin barrier, combined with exposure to other irritants and constant use of occlusive gloves.

The HRIPT studies on BIT indicated that an SCL of 360 ppm would be appropriate: in the most reliable study (Basketter *et al*., 1999), none of the volunteers were sensitised by 360 ppm BIT (45 µg/cm^2), while 5/58 volunteers (9 %) were sensitised by 725 ppm BIT (90.6 µg/cm^2). The original test substance was 78 % BIT + 20 % ethylene diamine, the latter of which is also a skin sensitiser. The authors employed a conservative approach and assumed that all the reactions seen were due to BIT. In a second HRIPT study (Anonymous, 1991) of 111 volunteers, no sensitisation occurred at doses of 0.05 % BIT (500 ppm, 27.8 µg/cm^2) and 0.1 % BIT (1000 ppm 55.6 µg/cm^2). In the third HRIPT study (Anonymous, 1975), 5/45 (11 %) volunteers were sensitised at an induction dose of 0.05 % (500 ppm BIT, 64.45 µg/cm^2), but this study is less reliable than the first because 10 of the volunteers received not only 500 ppm but also 750 and 1000 ppm at the first 3 induction applications.

RAC proposes to set an SCL of 0.036 (360 ppm) for BIT, on the basis of the Basketter *et al*. (1999) HRIPT study, in which none of the volunteers were sensitised by 360 ppm BIT (45 µg/cm^2), while 5/58 volunteers (9 %) were sensitised by BIT using 725 ppm BIT (90.6 µg/cm^2). As at 360 ppm no sensitisation occurred, this indicates it as an appropriate value for an SCL. This is supported by another HRIPT study, showing sensitisation in some volunteers at an induction dose of 0.05 % (500 ppm BIT, 64.45 µg/cm^2). Another reason to lower the existing SCL of 0.05 % (500 ppm) is the fact that there has been a rise in the frequency of BIT sensitisation in recent years, and therefore the current SCL does not seem to be sufficiently protective.

RAC noted that the widespread use of BIT and the rising frequency of sensitisation to the substance raises concerns, and therefore recommended that the SCL of 360 ppm should be reviewed at an appropriate time in the light of new data.

10.8 Germ cell mutagenicity

Hazard class not assessed in this dossier.

10.9 Carcinogenicity

Hazard class not assessed in this dossier.

10.10 Reproductive toxicity

Hazard class not assessed in this dossier.

10.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier.

10.13 Aspiration hazard

Hazard class not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

The following data sources were used by the Dossier Submitter (DS):

1. Draft final CAR (2020) of 1,2-benzisothiazolin-3-one (BIT) submitted. The assessment has been finalised according to the BPR criteria and procedures.
2. Registration dossier of 1,2-benzisothiazolin-3-one (BIT) according to REACH Regulation.

The DS has only included the studies rated as Reliability Index (R.I.) 1 or 2 according to the Guidance. Since the substance BIT has been assessed as biocidal a.s. by MS CA (Spain) and the proposed results have been already discussed and agreed in the BPC WG (ENV) with Experts from other MS CA, the following testing summaries include the last versions of accepted studies.

In the REACH Registration dossier most of the studies available are the same as included in the BPR Draft final CAR. Some of them were rated as R. I. 3 in the BPC WG (ENV) agreements and therefore they are not included hereafter. Only studies not available in the BPR Draft final CAR have been included in the present document, with the summaries reported by the corresponding Applicants. The results of these last studies are nevertheless considered in the conclusions reached by the DS for the proposal of harmonised C&L of BIT.

The rest of the studies, rated as R.I. 3 or 4, for transparency reasons, are included in Annex II (section 15.2).

11.1 Rapid degradability of organic substances

Table 13: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready biodegradation OECD TG 301B	0% degradation after 28 days	Not ready biodegradable BIT at 18 mg/L Activated sludge at 30 mg SS/L	Seyfrield, 2006 a CAR IIIA 7.1.1.2.1/3 IIIA7.1.1.2.1/01
Ready biodegradation OECD TG 301B	24% degradation after 28 days	Not ready biodegradable BIT at 0.313 mg/L Activated sludge at 90 mg SS/L	Burwood, 2007 CAR IIIA 7.1.1.2.1-04 III A7.1.1.2.1/02
Ready biodegradation OECD TG 301B	0% degradation after 28 days	Not ready biodegradable BIT at 0.83 mg/L and 2 mg/L Activated sludge	Hanstveit & Akdemir, 2002 CAR IIIA 7.1.1.2.1-1
Ready biodegradation OECD TG 301D	4.94% of ThOD after 28 days	Not ready biodegradable BIT at 2 mg/L Inocullum: river water, garden soil extract and septic tank supernatant	Patra, 2003 CAR IIIA 7.1.1.2.1-2
Ready biodegradation OECD TG 301C	<1% degradation after 63 days	Not ready biodegradable BIT at 1 mg/L Activated sludge at 30 mg SS/L	Brown *et al.*, 1994 CAR IIIA 7.1.1.2.1/1 REACH Dossier
Ready biodegradation OECD TG 301B	Maximum degradation 58.7% after 83 days (with 1.8 mg BIT/L and activated sludge with 30 mg SS/L)	Not ready biodegradable BIT at 1.8 and 9 mg/L Secondary effluent at 30 mg SS/L Activated sludge at 30, 300 and 2500 mg SS/L	Dempsey *et al.*, 1998 and Penwell and Roberts, 1999 CAR IIIA 7.1.1.2.1/2
Hydrolysis OECD TG 111	Loss of 3% at 50 °C and pH 4, 7 and 9	Hydrolytically stable Initial concentration: 10 mg/L	Graham, 2007 CAR IIIA7.1.1.1.1

Method	Results	Remarks	Reference
Hydrolysis OECD TG 111	Loss of 4.2, 2.9 and 1.3 % at pH 4, 7 and 9 and 50 °C after 5 d Half-life > 1 year at 25°C and pH 4, 7 and 9	Hydrolytically stable	Shrimali, 2003 CAR IIIA 7.1.1.1.1-2
Hydrolysis OECD TG 111	Loss of 2.4, 2.9 and 2.3% at pH 5, 7 and 9 and 50 °C after 7 d Half-lives not determined	Hydrolytically stable	Verhoef *et al.*, 2002 CAR IIIA 7.1.1.1.1/1
Hydrolysis EC Method C.7	Loss of 1.5, 0 and 2.36% at pH 4, 7 and 9 and 50 °C Half-life 219 and 145 d at 50 °C and pH 4 and 9	Hydrolytically stable	Greenwood, 2003 CAR IIIA 7.1.1.1.1/1 REACH Dossier
Inherent biodegradation OECD TG 302C	0% degradation after 28 d (31 mg BIT/L)	Not inherently degradable Inoculum, 100 mgSS/L	Seyfried, 2006 b CAR IIIA 7.1.1.2.2/1 IIIA 7.1.1.2.2
Inherent biodegradation OECD TG 302B	10% degradation after 28 d (0.04 mg BIT/L) 17% degradation after 28 d (0.4 mg BIT/L)	Not inherently degradable Inoculum, 500 mgSS/L	Gonsior *et al.*, 2008 CAR IIIA 7.1.1.2.2-2
Inherent biodegradation OECD TG 301B	40% degradation after 91 d (0.1 mg BIT/L) 52% degradation after 91 d (1 mg BIT/L)	Not inherently degradable Inoculum, 100 mgSS/L	Jenkins, 1999 CAR IIIA 7.1.1.2.2/1
Photochemical degradation: OECD Draft GD Direct and Indirect Photolysis (2000)	Half-lives under artificial light source: pH 5: 9 hours pH7: 0.7 hours pH9: 0.7 hours	Photolytically unstable	Graham and Troth 2007 CAR A7.1.1.1.2/01
Photochemical degradation: OECD Draft GD(97)21 Direct and Indirect Photolysis.	Half-life under artificial light source: pH 7: < 1 hour	Photolytically unstable	Ponte and Lopez 2007 CAR IIIA7.1.1.1.2-02
Photochemical degradation: OECD Draft GD Direct and Indirect Photolysis (2000)	Half-lives under artificial light source: pH=7 sterile water: <4.1 h Pond water (pH=8): <4..1 h	Photolytically unstable	Adam and Mégel 2009 CAR IIIA7.1.1.1.2
Photochemical degradation: OECD TG 316	Half-life not estimated due to rapid photodegradation	Photolytically unstable in natural pond water at pH 7	Gilbert, 2000 (REACH Registration dossier)
Aerobic degradation in estuarine water: OECD TG 309	Half-lives: 22.9 h at 12ºC (25.6 µg BIT/L) 29.8 h at 12ºC (105 µg BIT/L)	Primarily biodegradable in estuarine (brackish) water, yielding major 4 metabolites. After 9 d, <1% of AR was evolved CO_2.	Guo, 2008 CAR IIIA7.1.2.2.1
Aerobic degradation in sea water: OECD TG 309	Half-lives: 5.3 d at 12ºC (22 µg BIT/L) 12.2 d at 12ºC (105.2 µg BIT/L)	Primarily biodegradable in sea water, yielding major 3 metabolites. After 31 d, <0.1% (22 µg BIT/L) and 1.2% (105.2 µg BIT/L) of AR was evolved	Guo and Marbo, 2009 CAR IIIA7.1.1.2.3

Method	Results	Remarks	Reference
		CO_2.	
Aerobic degradation in sea water: OECD TG 306	Half-life was not estimated	Not ultimately degradable in sea water. Parent and metabolites were not measured. After 56 d, 0.9% (0.1 mg BIT/L) and ≤ 0.1% (1.0 mg BIT/L) of AR was evolved CO_2.	MacLean *et al.*, 2005 CAR IIIA7.1.1.2.3
Aerobic degradation in soil: OECD TG 307	Half-life: 5.6 h at 20°C (10.6 hours (0.44 d) at 12°C)	Sandy loam soil (UK) 40% AR was evolved $^{14}CO_2$ and 45% AR was NER at day 100	Graham, 2008 CAR IIIA7.2.1
Aerobic degradation in soil: OECD TG 307 US EPA OPPTS 835.4100	Half-lives: Bioactive soils: 0.01-0.27 d at 21ºC (0.02-0.54 d at 12ºC) Sterile soils: 0.4-0.6 d at 21ºC	Four soils: loam, sandy loam, silt loam and loamy sand (Germany) 40 - 56% AR was evolved $^{14}CO_2$ and 40-49% AR was NER at day 90	Piskorski, 2020 ; Mamouni *et al.*, 2020 CAR IIIA7.2.1/01

11.1.1 Ready biodegradability

Seyfried, 2006a

A test was performed following OECD TG 301B with BIT was dosed at 18 mg/L. The inoculum was activated sludge at a concentration of 30 mg suspended solids/L. Essentially no CO_2 above background was produced (0% degradation at day 28). Hence BIT was not ready biodegradable.

BIT at the concentration used to fulfill the requirements of test OECD 301B seemed to be toxic to the inoculum.

But in this study, in the toxicity control, containing both BIT as test item and the reference item sodium benzoate, no inhibitory effect on the biodegradation of the reference item was determined at the tested concentration of 18 mg/l. According to OECD 301 validity criteria, if in a toxicity test, containing both the test substance and a reference compound, less than 25% (based on total ThCO 2) occurred within 14 days, the test substance can be assumed to be inhibitory. In the toxixicty control of this study 301B, the degradation is 32.6% (CO2), so it cannot be considered to be inhibitory.

Burwood 2007

A study following OECD TG 301B was performed using ^{14}C-BIT at a concentration of 0.313 mg/L, which was not inhibitory for microrganisms. The inoculum was activated sludge at a concentration of 90 mg suspended solids/L. The pH was maintained at around 7.4. Initially there was a lag phase of 8 days but by day 11 of the test, 10% of the applied radioactivity (AR) was present as ^{14}CO2. From day 13 onwards, the rate of mineralization was slower reaching 20.1% AR on day 16 and 23.7% AR on day 28. Chromatographic examination of the solutions showed that on day 28 no BIT was present but two polar metabolites had been formed (at ca. 22% and 49% AR). These results indicate that the isothiazolone ring had been cleaved and extensively oxidized and that the benzene ring was being cleaved and oxidized. This indicates that primary degradation of BIT underwent fastly, resulting in the formation of the two polar metabolites; however ultimate degradation was limited to a maximum of ca. 24% at test end. Thus, BIT cannot be considered readily biodegradable.

The toxicity control (0.313 mg 12C-BIT/L plus 25.7 mg sodium benzoate/L) measured the mineralization of sodium benzoate in the presence of BIT. BIT at 0.313 mg/L did not suppress the microbial degradation and thus the mineralization of sodium benzoate. The test substance was tested at a non-biocidal concentration (0,313 mg/L). According to the study report, this low concentration is employed because the substance is suspected to be inhibitory to the test systems routinely employed to assess biodegradation.

An additional test was available in the CAR as a continuation of the principal study (Burwood 2007) and was prepared in an identical manner. This study identified the metabolites of BIT in a biological system and

assessed the primary degradation of the substance. On day 16 there was no parent subtance present (0.01% AR). The major metabolites isolated from the ready biodegradation flask were identified by LC-MS. 2-methylthiobenzamide was present at 61.47% of AR on day 16 and 2-methylsulfinyl-benzamide at 16.34% of AR. In addition, about 20% of the AR was present as $^{14}CO_2$ which indicates that cleavage of the benzene ring did occur.

Hanstveit and Akdemir 2002

In a study following OECD TG 301D the ready biodegradability of BIT was assessed at concentrations of 0.83 mg/L and 2.0 mg/L with activated sludge. The test substance clearly inhibited the inocullum both in the toxicity control and the test bottles. Therefore the biodegradability of BIT could not be established.

Patra 2003

In another study according to OECD TG 301D, the biodegradability of BIT was determined at a concentration of 2 mg/L in mineral medium inoculated with a mixture of river water, garden soil extract and supernatant of septic tank. During degradation of BIT the percentage of oxygen demand was 2.12%, 1.73%, 1.34%, and 7.79% (based on COD) and 1.35%, 1.10%, 0.85% and 4.94% (based on ThOD) on days 7, 14, 21 and 28, respectively. Therefore BIT was not readily biodegradable.

There is no toxicity control to provide evidence of inhibition to microorganisms.

Brown *et al.* 1994

This study assessed the ultimate biodegradability of BIT over 63 days following the OECD TG 301C modified to incorporate the analysis of BIT and metabolites. Samples of test medium with active sludge (30 mg suspended solids/L) were dosed with 1 mg/L of BIT, non-labelled and radiolabelled ([carbonyl-^{14}C]-BIT and [ring-^{14}C]-BIT).

BIT was neither readily nor ultimately biodegraded under the conditions of this test (i.e. $^{14}CO_2$ levels of $\leq$ 1% were measured for both the carbonyl and ring labelled BIT at test end). The analysis by HPLC showed that BIT was gradually disappeared while some metabolites appeared indicating that primary degradation occurred. At least four metabolites were detected by HPLC analysis, two less polar and two more polar than BIT, but they were not identified. The chromatographic data was interpreted with reference to chromatograms of known samples of BIT, saccharin and BIT-S-oxide. On this basis it appeared that saccharin may have been a metabolic product of BIT and BIT-S-oxide may have not been formed during the test (or it was outside the detection limit of the method).

The applicant has not reported biodegradation tests at BIT concentrations lower than 1 mg/L, and thus, it is not sure if inhibition of the bacterial activity is taking place. However, the applicants have reported other studies (see summary of reports above and below), where this concentration seemed to not affect the bacterial activity.

This was considered the key study in the CAR.

Dempsey *et al.*, 1998 and Penwell and Roberts, 1999

Two studies (Dempsey *et al.*, 1998 (ready biodegradation); Penwell and Roberts, 1999 (characterisation of metabolites)) were performed over 83 days at test concentrations of 1.8 and 9 mg BIT/L following a modification of the OECD TG 301B. Four inocula were tested: secondary effluent at 30 mg SS/L, activated sludge at 30 mg SS/L (typical of a ready biodegradation test), 300 mg SS/L (typical of an inherent biodegradation test) and 2500 mg SS/L (selected to simulate typical conditions at a sewage treatment works). Glucose was tested as a reference substance to demonstrate viability of the test inocula.

The extent of mineralisation of BIT was dependant on the inoculum and the concentration of BIT. At the highest concentration of inoculum (2500 mg/L) and 9.0 mg/L BIT, a lag period of 28 days was observed. At the end of the test, total mineralisation of 55.4% was achieved and 18.4% of the total radioactivity remained in the biomass. At 300 mg/L inoculum and 9.0 mg/L BIT, the lag period was 21 days. At the end of the test, total mineralisation was 51.1%, and 14.7% of the total radioactivity remained in the biomass. At 30 mg/L inoculum and 9.0 mg/L BIT, very low levels of mineralisation were observed (<10%), which were attributed to microbial toxicity of BIT at this concentration. However, with the 30 mg SS/L inoculum and 1.8 mg/L BIT, the lag period was 14 days. Up to 42.1% mineralisation was observed during the first 28 days in some test

vessels and at the end of the test (83 days) 58.7% mineralisation was observed, with 14.0% of the total radioactivity associated with the biomass. These data show that BIT, whilst not readily biodegradable, degrades when the sludge concentration is sufficient to counteract any biocidal effects of the test substance.

The metabolite profiles at the two concentrations of BIT were obviously different. At 1.8 mg BIT/L, BIT was rapidly degraded to more polar metabolites, which were then mineralised. Saccharin was not present and no firm characterisation of BIT S-oxide was possible. At 9 mg BIT/L, a lower number of metabolites were formed and saccharin was not present.Thus degradation run slower.

Conclusion on ready biodegradation

BIT is not readily biodegradable as demonstrated in all available tests since the substance did not pass the level of degradation in the required time window for readily biodegradable substances.

However, BIT underwent relatively fast primary degradation yielding several metabolites, which were different in the tests. Two metabolites were selected as relevant: 2-methylthiobenzamide and 2-methylsulfinyl-benzamide.

11.1.2 BOD_5/COD

There are not BOD_5 tests performed with BIT. Ready biodegradability tests and simulation studies regarding degradability in the aquatic and terrestrial environment are available.

11.1.3 Hydrolysis

Graham, 2007

The study was performed following the OECD TG 111 and EPA OPPTS 835.2110. Aqueous solutions, buffered to pH 4, 7 or 9 were dosed with ^{14}C-BIT at a concentration of ca. 10 mg/L and incubated in the dark at 50°C. At day 5 samples at pH 4, 7 and 9 showed that over 97% of the AR was recovered as BIT. Since BIT did not hydrolyze, the dissipation time was not determined. Therefore BIT was stable at the three pH values examined.

Shrimali, 2003

The hydrolysis of BIT was also examined in buffered solutions at pH 4, 7 and 9 and 50 °C with a test concentration of ca. 4 mg BIT/L. This study was conducted according to the OECD TG 111 with some deviations from the protocol, but not considered relevant.

The test solutions were analysed at 0 hours, 4 hours and 5 days. The hydrolysis of BIT was 0.74, 0.25 and 0.76 % after 4 hours of incubation and 4.22, 2.99 and 1.27 % after 5 days at pH 4, 7 and 9, respectively. The half-life of BIT was estimated to be > 1 year at 25 °C. Therefore, BIT is considered to be hydrolytically stable at the three pH values examined..

Verhoef et al, 2002

The hydrolysis of BIT was tested at pH 5, 7 and 9 and 50 °C. The study was conducted according to the OECD TG 111 with some deviations (i.e. a buffer solution at pH 5.0 was used instead of at pH 4.0; the study was carried out for 7 days instead of 5 days; etc.). However, these deviations were not considered to compromise the scientific validity of this study. The concentration of BIT used in this study was ca. 39 mg/L.

The concentration of BIT in the samples was determined at day 0, 3 and 7. The percent hydrolysis of BIT after 7 days was 2.4 %, 2.9 % and 2.3 % at pH 5, 7 and 9, respectively. The half-lives were not determined. It is concluded that the test substance is hydrolytically stable at the three pH values.

Greenwood, 2003

A 5-day hydrolysis study was performed at 50 °C and at pH 4, 7 and 9 according to EC Method C7. Solutions of BIT were prepared in buffer at a nominal concentration of 643 mg/L. After 5 days, the amount of BIT lost at pH 4 and 9 was determined as 1.56% and 2.36%, respectively. At pH 7 no loss was detected. The half-life for BIT at pH 4 and 9 was determined to be 219 and 145 days, respectively. Therefore the test compound is hydrolitically stable at pH 4, 7 and 9.

Conclusion on hydrolysis

Since the loss of BIT in water was <5% at pH 4, 7 and 9 and 50 °C in four studies, its half-life can be estimated as >1 year at 12 °C. Therefore BIT is hydrolytically stable at pH 4, 7 and 9.

11.1.4 Other convincing scientific evidence

Other convincing scientific evidence is not reported.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Field investigations and monitoring data are not reported.

11.1.4.2 Inherent and enhanced ready biodegradability tests

Seyfried 2006b

An inherent biodegradation test was performed following the OECD TG 302C during 28 days. BIT was tested at 31 mg/L. It was found not biodegradable under the test conditions, with a 0% degradation based on the $ThOD_{NH4}$ and $ThOD_{NO3}$. The suitability of the activated sludge (with 100 mg SS/L) was confirmed with the reference substance. BIT could not be assumed to be inhibitory on the activity of the sludge following the protocol criteria, because degradation of the reference substance in the toxicity control was higher than 25% (based on total ThOD) within 14 days. However, there was a decrease in biodegradation in toxicity control compared to procedure control which could indicate a certain inhibitory effect of BIT. This inhibitory effect could also explain the fact that BOD for BIT in the test media was lower than the normal range found for inoculum controls.

Gonsior *et al.*, 2008

The inherent biodegradability of BIT was also assessed in an activated sludge die-away study based on a modification of the OECD TG 302B in which a ^{14}C-labeled test compound was used. The study was set with two nominal concentrations of [^{14}C]-BIT (0.04 and 0.4 mg/L) added to activated sludge (with 500 mg SS/L) from an urban STP. 100% degradation was achieved within 3 and 24 hours for the 0.04 and 0.4 mg BIT/L samples respectively. Mineralization of [^{14}C]-BIT to $^{14}CO_2$ reached 10 and 17% of the initially AR after 28 days in reaction mixtures with 0.04 and 0.4 mg BIT/L, respectively. Degradation products were not identified.

According to the results of this study, BIT would fulfil the requirements to be classified as inherently biodegradable. However, in the abiotic control degradation reached 50% of intital concentration within 24h. In addition, the concentrations of BIT tested were lower than those expected in the environment with the biocidal uses. Hence, BIT cannot be considered inherently biodegradable at biocidal concentrations.

Jenkins 1999

This study was presented in the CAR of BIT as an inherent biodegradable test. The biodegradability of [^{14}C]-Nipacide® BIT (1,2-[benzene ring, [U-^{14}C]-benzisothiazolin) was assessed according to an adaptation of the CO_2 Evolution test (OECD TG 301B). [^{14}C]-Nipacide BIT was added at nominal concentrations of 0.1 or 1.0 mg/L to mineral salts medium inoculated with activated sludge (100 mg SS/L, not acclimated). The effects of BIT on the activity of the inoculum were assessed by comparing the degradation of the reference substance sodium benzoate in mixtures containing [^{14}C]-Nipacide BIT at 0.1 or 1.0 mg/L over a period of 91 days. Evolved CO_2 was determined throughout the test and the distribution of radioactivity was determined at the end of the test.

Sodium benzoate was degraded to 84% and 67% in the presence of [^{14}C]-Nipacide BIT at 0.1 mg/L and 1.0 mg/L, respectively. While it was 100% degraded without the presence of BIT. Therefore, BIT had an inhibitory effect on the inoculum (highest with increased BIT concentrations) but the inoculum had activity.

Mean cumulative $^{14}CO_2$ production by mixtures containing [^{14}C]-Nipacide BIT at 0.1 and 1.0 mg/L (with no sodium benzoate included) were equivalent to 40% and 52% of the AR by day 91, respectively. According to

the OECD TG 301B, a substance can be considered to show evidence of inherent primary biodegradation if the level of mineralisation is ≥20% or ultimate biodegradation if the level exceeds 70%. The high level of $^{14}CO_2$ production observed in this test confirmed that the benzene ring had been degraded; hence it was confirmed that BIT did show evidence of inherent primary biodegradation.

The authors argued in addition that total level of radioactivity liberated as $^{14}CO_2$ together with carbon present in the form of a non-extractable residue in the bacterial inoculum, ranged from 62% to 73% of the total AR after 91 days of exposure, suggesting that [^{14}C]-Nipacide BIT may have been ultimately degraded. However, according to OECD TG 301B, the pass levels are lower in the respirometric methods (i.e. >60%), since some of the carbon amount from the test chemical is incorporated into new cells. Thus, the DS believes it is not correct to sum up the incorporation of carbon into biomass with mineralization, since this has already been compensated for. Therefore, it is concluded that these data do not demonstrate that BIT was inherently biodegradable.

Conclusion on inherent biodegradation

According to the results of the first two available tests, it is concluded that BIT cannot be considered an inherently biodegradable substance. According to the third study, biodegradation of BIT was achieved when more favourable conditions were set in the tests (i.e. relatively high concentrations of microorganisms in the inoculum over a long time period) and this could be interpreted as inherent primary biodegradation, but not as inherent ultimate biodegradation.

Nevertheless it should be noted that the Guidance indicates that results of tests for inherent biodegradability or for enhanced ready biodegradability, because of the optimised conditions, should not be interpreted as evidence for rapid degradation of substances in the environment.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Aerobic aquatic degradation in water

Guo 2008

An aquatic aerobic degradation study determining the fate of BIT in estuarine surface water was performed following OECD TG 309. ^{14}C-BIT was added at 25.6 and 105 µg/L to estuarine water obtained from Pennsylvania, USA, and maintained in the dark at 20°C during 216 hours.

According to the study authors the half-life at 20°C was 30.8 hours at 25.6 ppb and 41.8 hours at 105 ppb. These values do not match, however, with table 11.1.4.3-2 on the quantification of parent and metabolites.

eCA recalculated BIT DT50 for both concentrations tested using FOCUS Kinetic Guidance. Results show that BIT degraded very fast in water. Degradation involves the cleavage of the isothiazolone ring yielding four major metabolites. The half-life for water dosed at 25.6 µg/L was 12.12 hours and at 105 µg/L, 15.7 hours at 20ºC. Degradation rates at 12ºC were 22.9 and 29.8 hours, respectively. Some metabolites were still increasing or remained constant by the end of the test, in particular for the lowest concentration.

In sterile surface water, little if any BIT had degraded after 120 hours, indicating that, in the dark with estuarine water, biodegradation is the primary route of dissipation. Less than 1% of the applied activity was present as $^{14}CO_2$ after 216 hours and there was no detectable volatile organics in the ethylene glycol trap.

Table 14: Aerobic degradation products of BIT in estuarine surface water

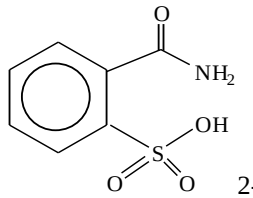
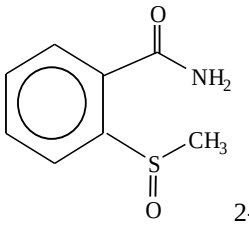
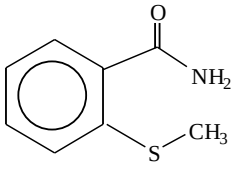
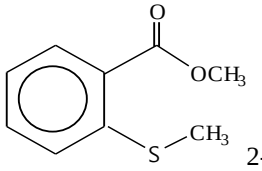
Structure	Maximum percent of AR during the test	
	25.6 µg/L	**105 µg/L**
 2-sulfobenzamide	30.0 %	27.5 %
 2-methylsulfinyl-benzamide	24.9 %	22.1 %
 2-methylthio-benzamide	48.3%	54.0 %
 2-methylthio-benzoic acid methyl ester	Not Detected	12.8 %

Table 15: Quantification of parent and metabolites

Sample time (h)	Percent of Applied ^{14}C-Activity (average of Sample duplicate samples)					
	BIT	M1 (2-Sulfobenzamide)	M2 (2-methylsulfinyl-benzamide)	M3 (2-methylthio-benzamide)	M4 (2-methylthio-benzoic acid methyl ester)	Mx[1]
25.6 ppb						
0	95.7	1.2	0.2	0.1	NS[2]	2.2
2	79.6	3	1.3	1.4	NS	0.8

6	67.2	8	3.7	2.2	NS	6.8
24	25.3	24.4	10.1	29.5	NS	6
48	7.1	24.2	11.8	48.3	NS	6.6
120	4	23.4	19.6	33.1	NS	6.6
216	0.6	30	24.9	33.7	NS	4.7
105 ppb						
0	107.7	1.4	NS	NS	NS	2.7
2	93.3	2.8	0	NS	NS	3.6
6	86.2	9.9	0	NS	NS	3.4
24	33.5	27.5	11.9	17.3	NS	6.7
48	7.5	10.7	22.1	52	NS	3.3
120	3.4	4.3	19.1	54	NS	5.2
216	3.7	2.3	20.7	43.8	12.8	4.7

1 Mx represents nonspecific radioactivity recovered from the TLC plate from areas other than those of M1, M2, M3, M4, and BIT. Given the large area represented by Mx, it is nonspecific and is comprised of multiple compounds.

2 NS = not significant or less than the liquid scintillation counter LOD.

Guo and Marbo, 2009

An aquatic aerobic simulation degradation study determining the fate of BIT in sea water was performed following OECD TG 309. ^{14}C-BIT was added at 22 and 105.2 µg/L to sea water obtained from Texas, USA, and maintained in the dark at 20°C. Test duration was 30 and 31 days for the lowest and highest concentrations tested, respectively.

The half-life for water dosed at 22 µg/L was 127.63 h (5.3 d) and at 105.2 µg/L, 292.06 h (12.2 d) at 12ºC. At the test temperature, 20ºC, degradation half-lives were 67.3 h and 154 h, respectively.

In sterile sea water, little if any BIT had degraded after 240 hours indicating that in the dark with sea water, biodegradation is the primary route of dissipation. The kinetic data suggest that at environmentally relevant concentrations, BIT will quickly biodegrade in sea water. Biodegradation involves the cleavage of the isothiazolone ring yielding 3 major metabolites (>10% of AR). The concentration of some metabolites was still increasing or remained constant by the end of the test (see table 11.1.4.3-3). The amount of $^{14}CO_2$ evolved was less than 0.1% for the lower dose and 1.2% for the higher dose after 744 hours. Therefore BIT was primarily but not ultimately biodegradable.

2-sulfobenzamide was previously detected in the photolysis and the ready biodegradation studies while 2-methylthiobenzamide and a minor metabolite were also detected in the ready biodegradation and estuarine water simulation studies.

Unlike the above metabolites, 2-(4-hydroxyphenylsulfanyl)-benzamide does not appear to be a universally relevant metabolite since it is the result of the particular water sample employed for the 105.2 ppb study. It

probably arises as a result of a halogenated phenol initiating a nucleophilic attack on the electrophilic BIT molecule. As reflected by it non-presence in the range finding and low dose experiments, the presence of this phenol contaminate in high dose water sample is probably an unusual occurrence.

Table 16: Quantitation of Parent and Metabolites

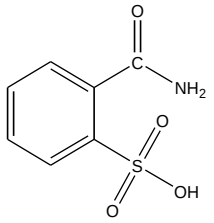
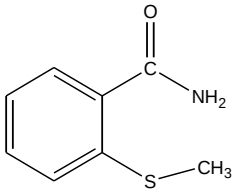
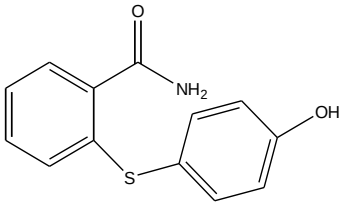
Sample time (hours)	Percent of Applied ^{14}C-Activity (average of duplicate samples)							
	BIT	M1 (2 - sulfobenzamide)	M2	M3 (2-methylsufinyl-benzamide)	M4 (2-methylthio-benzamide)	M5 (2-(4-hydroxyphenylsulfanyl)-benzamide)	M6	Mx[1]
22 ppb BIT								
0	86.9	1.7	NS[2]	NS	NS	NS		
2	83.3	5	NS	NS	NS	NS		
6	82.2	3.6	NS	NS	NS	NS		
24	72.2	6.6	NS	NS	NS	NS		
48	62	11.5	7.3	NS	NS	NS		
72	49.2	13	5.4	NS	NS	NS	1.9	
96	37.9	14.9	5.8	NS	14.5	NS	0.9	
144	17.7	11.7	3.5	0.6	29.1	NS	0.8	
720	18.1	8.8	9.2	NS	19.8	NS	NS	
105.5 ppb BIT								
0	85.9	3.5	NS	NS	NS	NS	NS	NS
10	88.3	3.2	NS	NS	NS	NS	NS	NS
24	89.8	4.8	NS	NS	NS	NS	NS	NS
48	84.7	4.2	NS	NS	3.1	NS	NS	NS
72	70.9	4.7	1.3	0.8	1.8	NS	9	2.7
96	77.2	5.3	1.4	1.4	1.8	NS	0.9	3.4
120	50.6	6.7	1.6	1	6.8	10.3	5	3
168	37.1	6	0.4	3.4	16.6	9	0.3	3.6

336	40.9	7.3	3.2	0.8	10.5	NS	0.4	4.5
774	2.8	13.2	5.3	4.8	11.9	16.3	4.3	4.6

[1] Mx = nonspecitic radioactivity recovered from the TLC plate areas other than the spots designated for M1, M2, M3, M4, M5, M6, and BIT. Significant Mx was not detected in the 22 ppb dosed samples.

[2] NS = less than LOD

Table 17: Aerobic aquatic degradation products of BIT in sea water.

Structure	Maximum percent of AR during the test	
	22 µg/L	105.2 µg/L
 2-sulfobenzamide	14.9 %	13.2 %
 2-methylthio-benzamide	29.1 %	16.6 %
 2-(4-hydroxyphenylsulfanyl)-benzamide	Not Detected	16.3 %

MacLean *et al.*, 2005

The biodegradation of radiolabeled BIT in natural sea water from Devon (UK), was assessed in a study following the OECD TG 306. Radioactivity from evolved $^{14}CO_2$ was measured to investigate the biodegradability of BIT at concentrations of 0.1 mg/L and 1.0 mg/L during 56 days. The test was performed under safelight to minimise the potential for abiotic, photolytically induced, degradation. OECD mineral nutrient solution was added to each vessel containing seawater. Temperature of test vessels was 17 ± 2°C and the pH ranged 5.5-7.1.

100% of the AR was recovered in the seawater for the BIT vessels. But specific analysis was not performed to determine whether the test material was still present as parent compound. A parallel test with a reference substance was run and it confirmed the validity of the study. The recovery of AR as evolved CO_2 from test vessels with 0.1 mg BIT/L was 0.9% and from the vessels with 1.0 mg BIT/L it was ≤ 0.1%. A negligible amount of CO_2 was trapped as bicarbonate in the seawater. In conclusion, BIT was not mineralized in seawater

at concentrations of 0.1 and 1.0 mg BIT/L. This study does not give an idea on the evolution of the parent compound along the tested period. It is therefore difficult to know whether BIT was primarily biodegraded at the tested conditions. Nevertheless the level of mineralisation obtained in this test was similar to Guo and Marbo, 2009.

It should be noted that studies following the OECD TG 306 do not provide information similar to ready biodegradability tests, since the inoculum is not added in addition to the micro-organisms already present in the seawater. Neither do the tests simulate the marine environment since nutrients are added and the concentration of test substance is very much higher than would be present in the sea.

Conclusion

According to the results of the first two degradation studies on estuarine (brackish water) and sea water, it is concluded that BIT can be rapidly degraded to four metabolites in estuarine water with a half-lives of 0.9-1.2 days at 12ºC; in sea water, degradation of BIT went slower, with half-lives of 5.3-12 days at 12ºC and producing three metabolites.

The results of these studies could suggest that BIT was primarily biodegradable in estuarine (brackish water) and sea water. However it was not ultimately biodegradable, since the level of mineralisation was very poor (≤ 1 % AR was evolved CO_2). Therefore BIT cannot be considered a rapid biodegradable substance.

Aerobic biodegradation in soil

Graham, 2008

An aerobic soil simulation metabolism study was performed following OECD TG 307. A sandy loam soil (pH 7.4) from the UK was dosed with 5 µg^{14}C-BIT /g_{dwt}. The soils were maintained in the dark and samples taken periodically over 100 days. BIT quickly biodegrades in soil with a half-life of 5.6 hours in soils incubated at 20°C (recalculated to 10.6 hours at 12°C). As demonstrated by the presence of significant percentage of evolved $^{14}CO_2$ (40.2% at day 100), metabolism was extensive and involved the cleavage of the isothiazolone and benzene rings. The degradation rate constant was estimated to be 0.1238 h^{-1} at 20°C (0.065 h^{-1} at 12°C).

About 45% of AR was non-solvent extractable bound residue at study termination. None of the bound residue was parent. There were two metabolite fractions present at greater than 10% AR and both of these fractions were transient and contained two major components. These four metabolites were subsequently identified by LC-MS. The structure, name and approximate percentage of AR of the degradation products were reported.

However the complete identification of the metabolites was not achieved and this undermines the validity of the results. Additionally, relatively high degradation was observed in the sterilized samples which would suggest that while degradation in the soil was primarily due to biodegradation, a portion may have been due to abiotic processes (probably the result of oxidation). Another possible explanation is that the sterilization of the soil was not efficient. In any case, it can be stated that in the non-sterilized soil, the degradation of the benzene ring was microbially mediated.

Piskorski, 2020; Mamouni *et al.*, 2020

In another study the the route and rate of degradation of radiolabeled BIT in soil was investigated according to the OECD TG 307 and the US EPA Guideline OPPTS 835.4100 in four standard fresh field soils from Germany with a wide range of soil properties (Soil I (loam), Soil II (sandy loam), Soil III (silt loam) and Soil IV (loamy sand)).

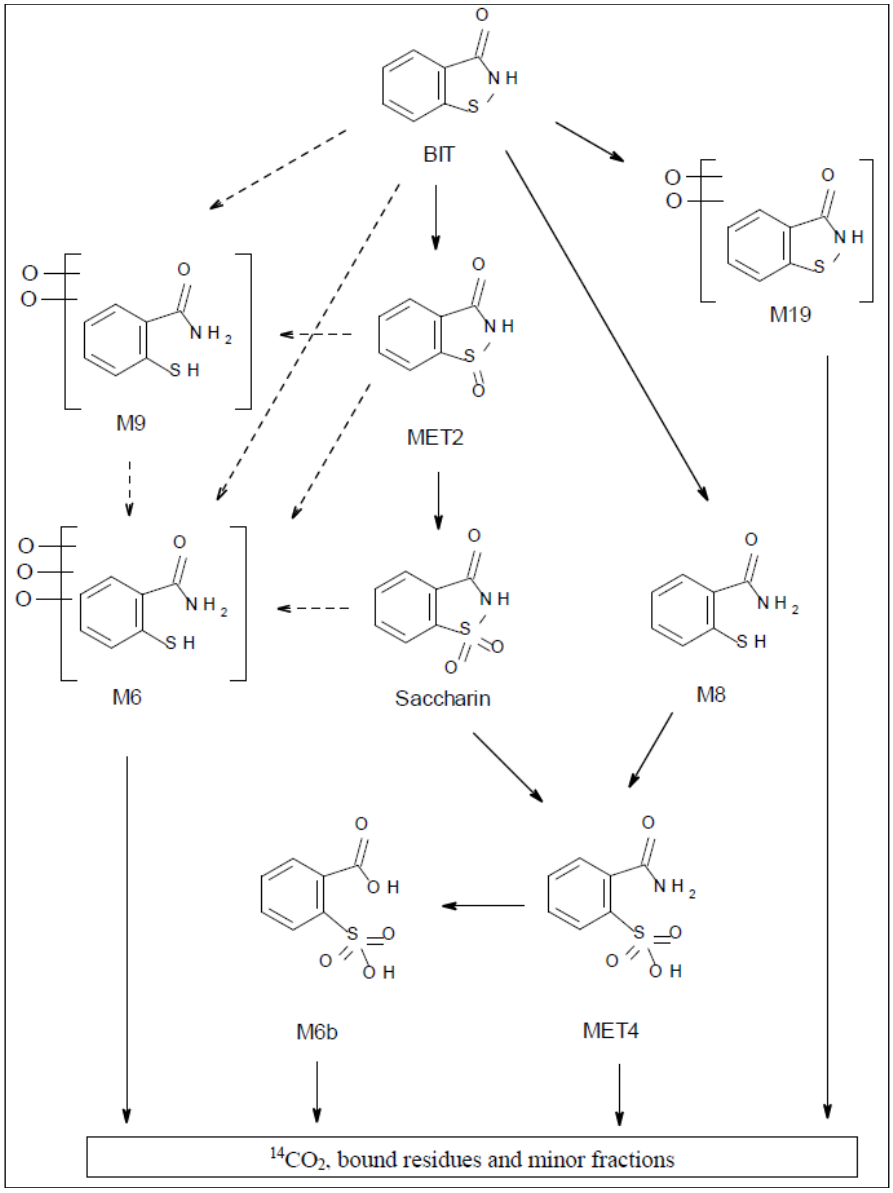
Following treatment with the test item at a rate of 0.5 mg BIT/kg_{dw} (375 g BIT/ha), soils were incubated at a temperature of 21°C and a soil moisture content of pF 2.0 for up to 120 days under aerobic conditions in the dark. Duplicate soil samples treated with the test item were extracted and analysed after 0, 0.04, 0.08, 0.17, 0.331, 1, 2.1, 4, 7, 14, 28, 56, 91 (Soils I-III only) and 120 (Soil IV only) days of incubation for bioactive soils. Corresponding duplicate sterile soil samples were extracted and analysed after 0, 1, 13, 28, 91 (Soils I-III only) and 120 (Soil IV only) days of incubation.

Each sample was submitted to exhaustive extraction (up to five times). Thereafter, the concentrated extracts were analysed for the test item and the degradation products by HPLC with co-chromatography. Selected extracts were additionally analysed by HPLC coupled with LC-MS in order to confirm the results and/or to identify relevant metabolites that did not match with the standards available. Total mean recovery of radioactivity during the incubation period accounted for 94.9-97.6% of AR for the four bioactive soils.

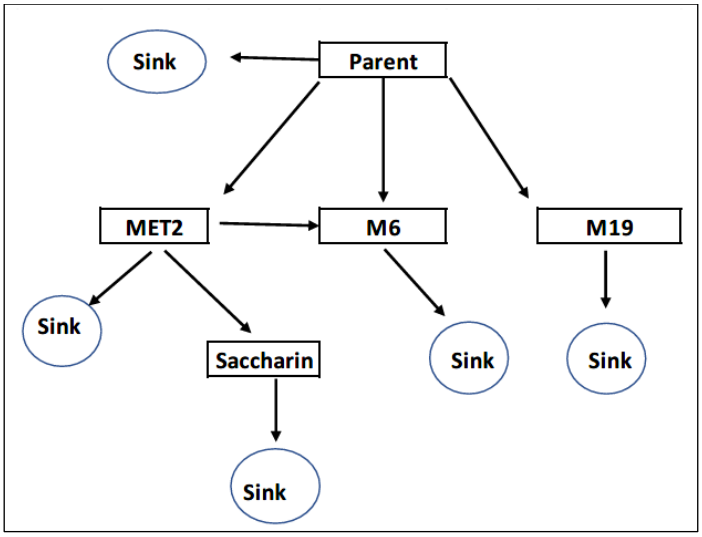
Mineralization of BIT was extensive in the bioactive soils, i.e. CO_2 released reached 47.9, 56.2, 46.1 and 39.9% of AR at the end of incubation in the four soils tested, respectively. In the sterile soils, the mineralization of BIT was negligible, i.e. evolved CO_2 ≤0.4% of AR in all soils tested. For the bioactive soils, the mean amount of non-extractable residues increased from 27.6, 10.3, 13 and 6.9% AR on 0 DAT to maximum levels of 52, 42.9, 44.6 and 45.6% AR on 56 DAT in the four soils. At the end of incubation, the amounts were 48.6, 39.9, 43.2 and 41.9% AR respectively for four soils tested.

In the bioactive soils the DT_{50} values of BIT were estimated to be 0.01-0.27 d (0.02-0.55 d at 12 ºC) and DT_{90} values were ≤0.90 d (<1.8 d at 12 ºC). In the sterile soils, the degradation was only slightly slower with DT_{50} values of 0.54-1.38 d and DT_{90} values of ≤4.6 d, showing the relevance of abiotic transformation.

Identified degradates include 1,2-benzisothiazolin-3-one-1-oxide (MET2) (24.9%), saccharin (M5) (8.9%), 2-sulphanyl benzamide (M8) (22%), 2-aminosulphinylbenzoic acid (M9) (21.4%), 2-sulphamoylbenzoic acid (M6) (46.6%), as well as 2-sulphobenzoic acid (M6b). Metabolite M19 did not exceed 5% in the non-sterile soils and reached the maximum of 4.9% AR. 2-Sulphanyl benzamide (M8) is a transient metabolite which is further rapidly degraded to M6. M9 also degraded fastly. Aditionally MET4 was found in sterile soils only. All metabolites but MET4 were found in bioactive and sterile soils. The proposed metabolic pathway is presented below.



The decline of BIT and its metabolites in the laboratory soil degradation study (Piskorski, 2020) was also modelled with CAKE 3.3 according to the recommendations of the FOCUS Kinetics Guidance (2014) (Mamouni *et al.*, 2020). To derive metabolites kinetics, a simplified metabolic pathway was derived from the metabolic pathway reported in the study.



Simplyfied proposed degradation pathway for BIT in four standard soils (Mamouni *et al.*, 2020).

The best fit for BIT was generally obtained by biphasic models, however, the slow phase was generally when over 90% degradation of the parent was reached. BIT was very rapidly degraded in all soils and similar and short DT_{50} values were derived regardless of model choice. The slow phase has no influence on the DT50 since this occurred once over 95% of BIT was degraded.

For modelling SFO showed acceptable fits except for soil IV where chi-square for SFO was above 15% and FOMC and DFOP are better options both showing similar results.[2] The modelling DT50 values ranged from 0.01 to 0.27 days (0.02 to 0.54 d at 12ºC). The optimum parent best fit kinetics was considered for the calculation of the metabolite kinetics. The metabolites generally showed acceptable to very good fits. Where value of $\chi 2$ were high, kinetics were calculated for the metabolite separately and showed similar values. The trigger DT_{50} values ranged from 0.01 to 0.25 days at 20.9ºC. The following table shows results obtained with CAKE only for parent.

2 If 10% of initial reached in study period calculate DT50 as FOMC DT90/3.32. FOMC provides a good value for modelling, chi-square = 6.32. DFOP would have better chi-square = 3.47 and a slightly better visual adjustment. Results of both models are similar.

Table 18: Degradation results for parent obtained with CAKE v.3.3.

Soil	Kinetic model for P/M	Mo	Parameter (K, K1, k2, g, tb, α, β)	χ2 %-error & visual fit	Prob>t	Lower CI	Upper CI	DT_{50} [days]	DT_{90} [days]
Soil I	**SFO (T & M)**	94.3	k=63.97	5.2 Very good	1.8E-09	56.5	71.5	0.01	0.04
	FOMC	94.3	α=1.192 β=0.004875	3.5 Very good	n.r. n.r.	0.48 -0.003	1.91 0.013	0.004/0.009** not reliable	0.029 not reliable
	DFOP	94.3	K1=70.9 K2=0.3004 g =0.9823	1.1 Very good	1.8E-09 0.27 n.r.	64.9 -0.80 0.97	76.97 1.4 0.99	nd not reliable	nd not reliable
Soil II	**SFO (M)**	93.8	k=32.12	9.9 Very good	1.4E-10	28.4	35.8	0.02	0.07
	FOMC (T)	94.1	α=1.545 β=0.02729	3.2 Very good	n.r. n.r.	1.09 0.014	2.0 0.04	0.02/0.03**	0.09
	DFOP	94.1	K1=45.44 K2=6.311 g =0.8532	4.3 Very good	1.9E-05 0.039 n.r.	30.96 -0.86 0.69	59.9 13.48 1.02	0.02/0.11* not reliable	0.09 not reliable
Soil III	**SFO (M)**	92.4	k=45.75	8.1 Very good	3.1E-09	40.07	51.44	0.02	0.05
	FOMC (T)	92.5	α=1.315 β=0.01197	3.6 Very good	n.r. n.r.	0.84 0.003	1.79 0.02	0.01/0.02**	0.06
	DFOP	92.5	K1=53.64 K2=1.344 g =0.9588	3.2 Very good	6.6E-09 0.06 n.r.	48.28 -0.48 0.94	59.0 3.17 0.98	0.01/0.52* not reliable	nd not reliable
Soil IV	SFO	84.5	k=6.67	17.3 Acceptable	1.5E-05	4.43	8.91	0.10	0.35
	FOMC	93.5	α=0.7476 β=0.04234	6.3 Very good	n.r. n.r.	0.51 0.02	0.98 0.07	0.06/0.27**	0.88
	DFOP (T&M)	94.2	K1=42.53 K2=2.731 g =0.4576	3.5 Very good	0.004 1.1E-04 n.r.	13.39 1.65 0.33	71.66 3.81 0.59	0.05/0.25*	0.02

* slow phase
** DT90/3.32
n.r. = not relevant.
nd = not determined
Bold: optimum fit / T= Trigger / M = Modelling
Prob >t: P value from the t-test (acceptability criteria $P \leq 0.05$)
CI: confidence interval (95%)

In addition, table 11.1.4.3-6 shows results obtain for parent when including metabolites in the iteration. The results obtained for the parent substance when including metabolites in the iterations were similar indicating a good adjustment of the models.

Table 19: Parent results when all metabolites are included in Cake iteration process.

Soil	Kinetic model for parent	Parameter (k, k1,k2, k3, g	Chi-square	T test	DT50	DT90
I	SFO	62.89	5.26	1.38E-29	0.01	0.004
II	FOMC	Alpha =1.452 Beta: 0.025	3.65	N/A	0.0157 0.0993/3.32 = 0.03	0.09
III	FOMC	Alpha: 1.308 Beta: 0.01178	3.56	N/A	0.00823 0.0567/3.32 = 0.017	0.06
IV	DFOP	K1: 41.23 K2: 2.5	3.64	8.93E-6 8.1E-10	Overall: 0.056 DT50(k1): 0.0168	0.656

					DT50(k2): 0.27	

It should be noted that in sterile soils the half-lives calculated were 0.37, 0.39, 0.37 and 0.6 days at 20.9ºC for Soil I, II, III and IV, respectively. The rapid dissipation of BIT in sterile soils was also shown in the first study (Graham, 2008). This indicates that the dissapearence of BIT is not related to ultimate biodegradation but to primary biodegradation.

MET2 metabolite, which was shown to be rapidly formed from the parent compound, was also very rapidly degraded in all soils with DT_{50} values ranging from 0.3 to 0.5 days. The rate of degradation of M6 metabolite (including M6b fraction and the transient metabolite M9) was much slower when compared to the parent compound. DT_{50} values ranging from 21.5 to 46.3 days were calculated (43.8-94 days at 12ºC). Saccharin (M5) and M19 metabolite were degraded with DT_{50} values ranging from 6.3 to 10.3 (12.6-20.6 d at 12 ºC) and from 2.0 to 23.2 days (4-46.4 d at 12ºC), respectively. Due to the rapid degradation and the lack of sufficient data points, no kinetics can be calculated for metabolites M8 and M9.

The required recovery of radioactivity (90-110% AR) was achieved for all samples with an exception of four replicates from bioactive soils from time points of 7, 14 and 28 DAT. For these four replicates, it can be assumed that the loss of radioactivity occurred in trapping of radiolabelled carbon dioxide, as might be noted from the lower levels of $_{14}CO_2$ found in these samples in comparison to corresponding other replicates, and intervals before and after.

Due to the rapid dissapereance of BIT, the number of data points before the DT_{50} occurs is limited in three of the soils; in fact only the initial value was measured. This adds uncertainty to the calculated half-lives for these three soils (soil I, II and III).

Conclusion

There are two valid studies available for BIT degradation in soil although the most complete and reliable is the study by Piskorski (2020) where four soil types were tested and a complete characterization of metabolites was done. Both studies show that BIT rapidly disappears from soils with half-lives values from 0.02 to 0.54 d at 12ºC.

Mineralization reached 40 - 56% depending on the soil type. The level of bound residues was 40-49% of AR at test ends. In both studies sterile soils showed the importance of abiotic degradation of BIT (half-lives 0.4-0.6 d at 21ºC). A number of metabolites were found in both studies which were rather similar. BIT transformed into 1,2-benzisothiazolin-3-one-1-oxide, saccharin, 2-sulphanyl benzamide (M8), 2-aminosulphinylbenzoic acid (M9), 2-sulphamoylbenzoic acid (M6), as well as 2-sulphobenzoic acid (M6b) and Metabolite M19.

The Guidance assumes that when a substance has been shown to be degraded rapidly in a soil simulation study (i.e. it is ultimately degraded within 28 days with a half-life < 16 days), it is most likely also rapidly degradable in the aquatic environment. However this is not the case for BIT as it was shown above in the estuarine and sea water simulation studies. In case of conflicting results of degradation simulation studies, the Guidance stipulates that simulation test data of surface water are preferred relative to aquatic sediment or soil simulation test data in relation to the evaluation of rapid degradability in the aquatic environment. Therefore on the basis of results of aquatic systems BIT cannot be considered a rapid biodegradable substance.

11.1.4.4 Photochemical degradation

Graham and Troth, 2007

An aqueous photolysis study was performed following the draft OECD Guideline on Direct and Indirect Photolysis in water (2000). Glass vessels containing nominal concentrations of 10 ppm ^{14}C-BIT in pH 5, 7 or 9 sterile buffer were continuously irradiated with a xenon lamp and samples were taken periodically over 30 days. The light source was adjusted so that the light intensity was approximately equivalent to 30 days of natural sunlight at latitude between 30°N - 50°N. The half-lives at pH 5, 7, and 9 were estimated to be 9 hours, 0.7 hours, and 0.7 hours, respectively.

Photodegradation of BIT involved the cleavage of the isothiazolone ring, hydroxylation of the benzene ring, and/or oxidation of the sulphur. A number of photodegradates were detected and some of them were identified by LC-MS. Evolution of CO_2 was less than 10% at all pH values after 30 days.

This study was presented also presented by other Applicant, but it was refered as Graham, 2007.

Ponte and Lopez, 2007

In another study the aqueous photolysis of BIT under artificial sunlight was examined according to the OECD Draft GD(97)21 Direct and Indirect Photolysis, EPA OPPTS 835.2210 and OECD TG 101.

In tier 1 experiments, the UV/Visible absorption spectra of BIT were examined at pH values of 4, 7 and 9. The maximum absorbance of BIT was below 290 nm, but there was some absorbance of the test substance at >290 nm. The absorbance of the test substance >290 nm was used to predict the half-life of photolysis of the test substance at 40° N latitude in summer, assuming a quantum yield of 1. The predicted half-life was calculated to be 0.0006 days at pH 7, indicating that direct photolysis is considered to be a significant process.

In Tier 2 experiments, BIT was exposed to artificial sunlight (Xenon arc lamp) during 3 days in pH 7 buffer at 25 °C (Phase 1). After 3 days of irradiation, the dark control samples contained >94% of the intital concentration of BIT. The light exposed samples contained <1% of the initial concentration of BIT after 3 days. The results indicate that hydrolysis was not a competing factor for photolysis in aqueous buffer.

In Phase 2 experiment, samples of BIT in pH 7 buffer were exposed to artificial sunlight over a period of 40 hours. The results showed that < 4% of the BIT initial concentration remained in the light exposed samples after 3 h of irradiation. However, the analysis of dark control samples revealed that BIT was stable in the dark samples. Based on these results, the photolysis half-life of BIT in pH 7 buffer aqueous solution was estimated to be < 1 hour under artificial light irradiation. Transformation products were neither detected nor identified.

Adam and Mégel, 2009

This study was performed according to the draft OECD Guideline on Direct and Indirect Photolysis in water (2000). The direct and indirect photochemical degradation of ^{14}C-BIT was investigated under simulated sunlight in sterilised buffer (pH 7) water and natural pond water (ca. pH 8). Samples of pH 7 buffer and pond water at 5 mg BIT/L were irradiated continuously during 15 days (corresponding to 32 days of midsummer sunlight at latitude 50°N and 94 days of spring sunlight at latitude 35°N -Tokyo, Japan-) at 25 °C. In dark controls, the test item remained stable in the buffer test solution, accounting for 101.1% of initial BIT amount on day 15.

^{14}C-BIT was rapidly photodegraded in the pH 7 solution and in natural pond water. Photodegradation involved the cleavage of the isothiazolone ring to form mainly CO_2 (31 and 22% of the AR in the irradiated buffer solution and pond water samples). The half-lives were estimated to be < 4.082 hours in both aqueous systems.

A number of radioactive fractions were detected, some accounted for >10% of AR. Only one common degradate was formed in both pond water and buffer solution, indicating essentially a different photolytic process in the two test systems. The major trasnformation products formed in the buffered solution were 2-sulfobenzoic acid, B1 (unknown) and benzamide; whereas in the pond water, detected products were M2 (unknown), 2-sulfobenzoic acid, 1,2-benzisothiazol-3(2H)-one-1,1-dioxide, M10 (unknown) and 2-carbamoylbenzenesulfonic acid. M2, 2-sulfobenzoic acid, M10 and B1 were very rapidly photolysed with half-lives of ≤1.2 days, whereas1,2-benzisothiazol-3(2H)-one-1,1-dioxide and benzamide degraded at a much slower rate (87-89 days).

Gilbert, 2000

A study was conducted to determine the phototransformation of the substance according to OECD TG 316. The ^{14}C-radiolabelled substance was instantly photo-degraded in a buffered solution at pH 7 and in natural pond water. Due to its very rapid rate of photodegradation, an accurate photolytic Suntest half-life could not be calculated. Photodegradation involved cleavage of the isothiazolone ring to form principally ^{14}CO2 and several major metabolites (2 -sulfobenzoic acid, 1,2 -benzisothiazol-3(2H)-one-1,1 -dioxide, benzamide (B3) and 2 -carbamoyl-benzenesulfonic acid), were very rapidly photolysed with Suntest half-lives of 1.2 days or less, whereas other metabolites degraded at a much slower rate (87-89 Suntest days).

This study comes from the REACH Registration dossier. The results are similar to the studies available in the CAR of BIT.

Conclusion on photolysis in water

BIT is very unstable under artificial sunlight in aquatic medium. BIT is very rapidly photolysed at all pH values. Considering the available studies, the photolysis half-life of BIT is estimated as <1 hour under artificial sunlight at pH 7 and 9. At pH 5, photolysis underwent slightly slower.

Photolytic degradation of BIT in water resulted in a number of degradation products at different amounts depending on the pH value of the medium. These products were further photolysed after several days.

It should noted that according to the Guidance, information on photochemical degradation is difficult to use for classification purposes since the actual degree of photochemical degradation in the aquatic environment depends on local conditions. Therefore the DS does not consider the results of photochemical degradation of BIT as indicative of rapidly degradability.

11.1.5 Conclusion on rapid degradability

According to classification scheme for environmental hazards (ECHA 2017), a substance is considered to be not rapidly degradable unless at least one of the following is fulfilled:

a. The substance is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability. The pass level of the test (70 % DOC removal or 60 % theoretical oxygen demand) must be achieved within 10 days from the onset of biodegradation, if it is possible to evaluate this according to the available test data (the ten-day window condition may be waived for complex multi-component substances and the pass level applied at 28 days, as discussed in point II.2.3 of Annex II to this document). If this is not possible, then the pass level should be evaluated within a 14 days time window if possible, or after the end of the test; or

b. The substance is demonstrated to be ultimately degraded in a surface water simulation test with a half-life of < 16 days (corresponding to a degradation of >70 % within 28 days); or

c. The substance is demonstrated to be primarily degraded biotically or abiotically e.g. via hydroysis, in the aquatic environment with a half-life <16 days (corresponding to a degradation of >70 % within 28 days), and it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

According to this scheme, data from biotic or abiotic degradation in aquatic system is preferable.

BIT is not readily biodegradable hence it does not fulfil first condition (a.).

BIT is not ultimately degraded in the aerobic aquatic degradation test (less than 1% of the applied activity was present as $^{14}CO_2$) thus it does not fulfil second condition (b.)

BIT is primarily degraded very fast in aerobic aquatic systems but it cannot be demonstrated that the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment as some metabolites might be toxic to aquatic organisms (see Annex I). Thus BIT does not fulfil the third condition (c.).

Therefore it can be concluded that BIT is a non-rapidly degradable substance.

11.2 Environmental transformation of metals or inorganic metals compounds

BIT is not a metal or an inorganic metal compound.

11.2.1 Summary of data/information on environmental transformation

Information on environmental transformation of metals and inorganic metal compounds is not relevant for the classification of BIT, since BIT is not a metal or an inorganic metal compound.

11.3 Environmental fate and other relevant information

Volatilisation

BIT has a low vapour pressure (62.76 x 10^{-4} Pa at 20ºC). The Henry's law constant of BIT is 1.45 x 10^{-5} - 7.4 x 10^{-6} Pa m^3 mol^{-1} at 20ºC. BIT is therefore not considered volatile and is not expected to partition from aqueous phases to air in significant quantities.

Adsorption / Desorption from soil and sediment

In the CAR of BIT there are three reliable studies available to show the adsorption/desorption potential from soil and sediment of BIT. The DS considers these studies should not be included in table 70, since these data are presented mainly for the sake of completeness.

Graham, 2007

Adsorption/desorption to four soils and one sediment was tested following OECD TG 106 and US EPA OPPTS 835.1220. The OC content of the soils ranged 0.8-4.8% and in the sediment it was 1.9%. The pH values ranged 5.1-8 in soils. The pH value of the sediment was 7.3. Gamma-irradiated samples were employed and checked for sterility. While determining the equilibration time it was found that BIT was degrading. This degradation was due to an abiotic process (oxidation) because the soils were sterile. It was necessary to use a short equilibration time (1 hr) to reduce the effect of degradation during the study, even though BIT did not come to a complete equilibrium.

The K_{oc} value of BIT in soils with acidic and alkalin pH values were a little lower than in the soil with neutral pH. The mean K_{oc} value of BIT in four soils was 114 L/Kg and in the sediment was 64 L/Kg. BIT can be considered as a highly mobile compound, because the K_{oc} values were low.

Schouten & Verhoef, 2005; De Vette & Jansen, 2002

Two adsorption/desorption studies were performed according to OECD TG 121 with both ionised and no-ionised forms in appropriate buffer solutions. The first test was performed under acidic conditions (pH 5.5) (Schouten & Verhoef 2005) and the adsorption was determined by HPLC with diode array detection. This resulted in an extrapolated log K_{oc} value of 0.73 (K_{oc} = 5.4 L/Kg) for BIT in acidic conditions. However, a major deviation in the procedure was found and the result is not reliable (R.I. 3).

The second study of the adsorption of BIT in neutral-pH conditions (pH 7), for which an important fraction of BIT molecules should be present in the ionised form (disassociated), considering the pKa value of 7.2. This test was a complementing study to the former study. A K_{oc} value of 128.8 L/Kg was obtained for BIT.

Wilkes, 1989

The adsorption/desorption properties of radiolabelled BIT on four different soil types (pH values 6-7.9) was assessed according to EPA Guideline 163.1. The equilibration uptake kinetics study did not ensure that the system was under equilibrated conditions, although this data can be used as a primary prediction of the sorption capacity of BIT to soil. The average adsorption coefficient value obtained was 399 L/kg, however the OC content of the soils was similar. No significant degradation of BIT for any of the soil types was found. The mean K_{oc} value obtained in the four soils tested is 399 L/Kg.

Greenwood, 2003

A study was conducted to determine the adsorption/desorption potential of the substance according to OECD TG 121. The adsorption/desorption coefficient on soil (log Koc) was estimated by an HPLC simulation procedure. The mean log Koc value for the test substance was 0.97, and was within a 95% confidence range of 0.76 to 1.19. The mean Koc value at 20 °C was 9.33 L/Kg.

This study comes from the REACH Registration dossier. No information is available to the DS on the number of soils tested and their related pH values. The result is much lower than in the other three studies.

As a conclusion, in the studies no clear pH dependency was observed for BIT, however realiable tests did not include a wide range of pH values. The geometric mean K_{OC} value is 196.87 L/kg for all tested soils in reliable studies of the CAR of BIT. For sediments, a K_{OC} value of 64 L/kg was available.

11.4 Bioaccumulation

Table 20: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Bioconcentration factor: Comparable procedure to OECD TG 305	Mean steady-state BCF was 6.95 L/Kg_{wwt} (whole fish) in *Lepomis macrochirus*	Not bioaccumulative Only additional information BCF not normalised to a lipid content of 5% Metabolites not monitored	Anonymous, 1973 CAR IIIA 7.4.2/1 REACH Dossier
Partition coefficient *n*-octanol/water: OECD TG 117 and EPA OPPTS 830.7570	pH 5 at 20°C logP_{ow} of 0.99. pH 7 at 10, 20 and 30°C logP_{ow} of 0.63, 0.70 and 0.76, respectively. pH 9 at 20°C logP_{ow} of -0.90	Not bioaccumulative	Seal, 2002 CAR IIIA 3.9
QSAR (Vieth *et al.* 1979) estimations	Estimated BCF = 0.78 L/Kg_{wwt} for freshwater fish Estimated BMF = 1	Not bioaccumulative	EUSES v. 2.0.3
QSAR estimations	Estimated BCF = 3.162 L/Kg_{wwt} for freshwater fish Estimated log Kow = 0.64	Not bioaccumulative	EPI Suite™ 4.11

11.4.1 Estimated bioaccumulation

The experimentally determined log Kow matches with KOWWIN (EPI Suite™ 4.11) predictions, where it estimates that BIT has a log Kow of 0.64 and a BCF of 3.162 L/Kg. Thus BIT is not expected to accumulate in aquatic organisms.

In EUSES v. 2.0.3 a QSAR developed by Vieth *et al.* 1979 is included to estimate BCF and BMF values based on the (experimental) log Kow. For BIT it was predicted that the BCF for fish is 0.78 L/Kg, which indicates very low potential for bioconcentration. In addition a Biomagnification Factor (BMF) of 1 for fish eating bird/predator is estimated.

In conclusion, the estimated values of the log Kow and BCF in fish show that BIT is not expected to be bioaccumulative in aquatic organisms. The estimated values match with the experimental values.

11.4.2 Measured partition coefficient and bioaccumulation test data

Seal, 2002

The experimental partition coefficient *n*-octanol/water (log Kow) of BIT was determined to be 0.70 at pH 7 and 20 °C, in a study according to OECD TG 117 and EPA OPPTS 830.7570.

Anonymous, 1973

A study on the bioconcentration of BIT in bluegill sunfish (*Lepomis macrochirus*) following a procedure similar to OECD TG 305 was available. Using a flow through system the fish were exposed to 0.01 mg/l or 0.1 mg/l BIT for 4 or 8 weeks (when including the depuration phase). The total residue in fish at both concentrations reached a maximum concentration within two to three weeks. Within seven days, the fish in the depuration phase exhibited a 38-54% decrease in total residue. At both concentrations, only trace amounts of total BIT residues were detected after four weeks in clean water. The mean steady-state Bioconcentration Factor (BCF) was 6.95 L/Kg_{wwt} for whole fish.

The weight of the fish throughout the duration of the study was not recorded and the lipid content of the fish was not determined; therefore the BCF was not normalised to a lipid content of 5%. The log Kow of BIT suggests that it will not bioaccumulate in the aquatic environment and the mean steady-state BCF (log BCF = 0.84) is comparable to the EPI Suite™ 4.11 estimated BCF value (Log BCF = 0.50).

This test is considered as additional information (R.I. 3) in this CLH report because the type and characteristics of illumination were not reported, although in REACH registration has a reliability of 2 (with restrictions). Particularly for BIT the photoperiod is very important due to the products of phototransformation generated at different pathways.

Experimental fish bioaccumulation studies are not available.

Conclusion

Since the estimated and experimental log Kow is < 4 and the BCF for fish is < 500 L/Kg, it can be concluded that BIT has not significant potential for bioaccumulation in the aquatic environment.

11.5 Acute aquatic hazard

Table 21: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
OECD TG 203; EPA OPPTS 850.1075	*Oncorhynchus mykiss*	BIT (TG, purity 89.8%)	96 h-LC_{50} = 1.9 mg/L	Flow-through, mean measured GLP	[redacted], 2006a CAR IIIA7.4.1.1.a/01
EPA OPPTS 850.1075	*Cyprinodon variegatus*	BIT (TG, purity 89.8%)	96 h-LC_{50} = 19 mg/L	Flow-through, mean measured GLP	[redacted], 2006c CAR IIIA7.4.1.1.b/01
OECD TG 203; EPA OPPTS 850.1075	*Oncorhynchus mykiss*	Nipacide®BIT (BIT purity 98.8%)	96 h-LC_{50} = 2.18 mg/L	Static, mean measured GLP	[redacted], 1995a CAR IIIA 7.4.1.1/01 REACH Dossier
Comparable to OECD TG 203	*Oncorhynchus mykiss*	PROXEL™ Press Paste (BIT TG, purity 76.9%)	96 h-LC_{50} = 1.23 mg/L	Continuous flow system, mean measured Non-GLP	[redacted], 1979 CAR IIIA 7.4.1.1/02
EPA-540/9-85-006	*Cyprinodon variegatus*	PROXELTM Press Paste (BIT TG, 76.1% purity)	96 h-LC_{50} = 9.47 mg/L	Static, mean measured GLP	[redacted], 1993 CAR IIIA.7.4.1.1/1b REACH Dossier
OECD TG 203	*Brachydanio rerio*	BIT (98% purity)	96 h-LC_{50} = 4.9 mg/L	Semi-static, nominal GLP	[redacted], 2002a CAR IIIA 7.4.1.1-2
OECD TG 203; EPA OPPTS 850.1075	*Oncorhynchus mykiss*	BIT (TG 97.42% purity)	96 h-LC_{50} = 1.49 mg/L	Semi-static, nominal GLP	[redacted], 2003 CAR IIIA 7.4.1.1-1

US EPA Subdiv. E, Sec. 72-1	*Oncorhynchus mykiss*	XBINX® (BIT purity 99.29%)	96 h-LC_{50} = 0.74 mg/L	Static, nominal GLP	[redacted], 1997a CAR IIIA 7.4.1.1-3
OECD TG 202; EPA OPPTS 850.1010	*Daphnia magna*	BIT (TG purity 89.8%)	48 h-EC_{50} = 3.7 mg/L	Flow-through, mean measured GLP	Palmer *et al.*, 2006b CAR IIIA7.4.1.2.a/01
EPA OPPTS 850.1035	*Americamysis bahia*	BIT (TG purity 89.8%)	96 h-EC_{50} = 1.9 mg/L	Flow-through, mean measured GLP	Palmer *et al.*, 2007a CAR III A7.4.1.2.b/01
OECD TG 202	*Daphnia magna*	Nipacide®BIT (BIT purity 98.8%)	48 h-EC_{50} = 2.9 mg/L	Static, mean measured GLP	Jenkins, 1995b CAR IIIA 7.4.1.2/1a REACH Dossier
EPA 72-3; SEP 600/9 78-010	*Mysidopsis bahia*	PROXELTM Press Paste (BIT TG, 76.1% purity)	96 h-EC_{50} = 0.99 mg/L	Static, mean measured GLP	Kent *et al.*, 1993 CAR IIIA 7.4.1.2/1b
OECD TG 202	*Daphnia magna*	BIT (98% purity)	48 h-EC_{50} = 4.0 mg/L	Static, nominal GLP	Hooftman *et al.*, 2002b CAR IIIA 7.4.1.2-2
US EPA Subdivision E, Section 72-2	*Daphnia magna*	XBINX® (BIT, 99.3% purity)	48 h-EC_{50} = 2.24 mg/L	Static, mean measured GLP	Terrell, 1997b CAR IIIA 7.4.1.2-3
OECD TG 201; EPA OPPTS 850.5400	*Pseudokirchneriella subcapitata*	BIT (TG 89.8% purity)	24 h-E_rC_{50} = 0.33 mg/L 48 h-E_rC_{50} = 0.8 mg/L 72 h-E_rC_{50} = 0.99 mg/L 96 h-E_rC_{50} = 1.31 mg/L	Static, mean measured GLP	Desjardins *et al.*, 2006b CAR IIIA7.4.1.3.a/01
OECD TG 201	*Pseudokirchneriella subcapitata*	PROXEL™ Press Paste (BIT TG, 73.3% purity)	24 h-E_rC_{50} = 0.08 mg/L 48 h-E_rC_{50} = 0.095 mg/L 72 h-E_rC_{50} = 0.087 mg/L	Static, mean measured GLP	Smyth *et al.*, 1994 CAR IIIA 7.4.1.3/1a REACH Dossier
Draft ISO Guideline "Marine Algal Growth Test"	*Phaeodactylum tricornutum*	PROXEL GXL (ca. 20% BIT)	24 h-E_rC_{50} = 0.21 mg/L 48 h-E_rC_{50} = 0.165 mg/L 72 h-E_rC_{50} = 0.177 mg/L	Static, mean measured GLP	Smyth and Brown, 1991 CAR IIIA 7.4.1.3/1b
OECD TG 201	*Pseudokirchneriella subcapitata*	BIT (TG 71.08% purity)	24 h-E_rC_{50} = 0.011 mg/L 48 h-E_rC_{50} = 0.017 mg/L 72 h-E_rC_{50} = 0.026 mg/L	Static, mean measured GLP	Kasthuri Raman, 2002 CAR IIIA 7.4.1.3-1
OECD TG 201	*Pseudokirchneriella subcapitata*	BIT (98% purity)	24 h-E_rC_{50} = 0.48 mg/L	Static, mean measured GLP	Oldersma *et al.*, 2002 CAR IIIA 7.4.1.3-2

			48 h-E_rC_{50} = 0.64 mg/L 72 h-E_rC_{50} = 0.67 mg/L		

11.5.1 Acute (short-term) toxicity to fish

[redacted], 2006a; [redacted], 2006c

The acute toxicity of BIT to fish was investigated in a freshwater (*O. mykiss*) and a marine (*C. variegatus*) species in two studies following OECD TG 203 and EPA OPPTS 850.1075. The tests were conducted in a flow-through system and the fish were exposed to BIT (TG AI, purity 89.8%). The 96-hour LC_{50} based on mean measured concentrations were 1.9 and 19 mg BIT/L respectively.

Binomial probability was used to calculate the 24 and 48-hour LC50 values and the probit method was used to calculate the 72 and 96-hour LC50 values.

The OECD validity criteria were fulfilled (Mortality of control animals <10%, Concentration of dissolved oxygen in all test vessels > 60% saturation, Concentration of test substance ≥80% of initial concentration during test). A 96 h dose response curve was obtained.

In first study (2006a), nominal concentrations (mg BIT/L) of 0.31, 0.63, 1.3, 2.5, 5.0 were used. One lethargic fish in the 1.2 (mean measured) mg BIT/L group and one fish lying on the bottom of the tank in the 2.3 (mean measured) mg BIT/L group. All other surviving fish appeared normal at test termination.

In the case of *C. variegatus,* nominal concentrations (mg BIT/L) of 1.9, 3.8, 7.5, 15 and 30 (this last was at the limit of water solubility as a white precipitate was observed). All water quality parameters were within acceptable limits during the test. All fish in the negative and solvent control groups and, based on mean measured concentrations, in the 1.8 and 3.5 mg BIT/L treatment groups appeared normal throughout the test. No mortality was observed in the 7.0 mg BIT/L group though the fish were lethargic at 48 hours. Percent mortality was 5 and 90% for the 14 and 24 mg BIT/L groups, respectively. Signs of toxicity observed in fish in the the 14 and 24 mg BIT/L groups during the test included lethargy, loss of equilibrium, erratic swimming, surfacing and.or lying on the bottom of the aquarium.

[redacted], 1995

In a third study, the acute toxicty to fish (*O. mykiss*) of Nipacide®BIT (organic purity of 99.8% of which 98.8% is BIT) was studied in a static test over 96 hours in accordance with OECD TG 203 (pH 7.7-8.2). The 96-hour LC_{50} was 2.18 mg BIT/L based on mean measured concentrations.

Nominal concentrations of 0.5, 1, 2, 4 and 8 mg/L were used. Overall mean measured levels of 0.442, 0.949, 1.86, 3.73 and 7.64 mg/L were obtained. Observations of the fish were made at least at 24-hour intervals.

The highest measured concentration at which no mortalities occurred and the lowest at which there was 100% mortality after 96 hours was 0.949 and 3.73 m g/L, respectively. Sub-lethal, treatment-related effects, which were seen at and above 1.86 mg/L, included darkened pigmentation, lethargic and nervous behaviour, hyperventilation and loss of coordination. At 7.64 mg/L, all of the fish were affected after exposure to the test material for four hours, and died within 24 hours. At 3.73 mg/L, all fish exhibited adverse symptoms after 48 hours and died within 96 hours. At 1.86 mg/L, effects were evident in some fish after 24 hours and were progressive during the test; after 96 hours, three fish had died at this level and five were severely affected.

Median lethal concentrations (LC50s) were estimated by the computer program of Stephan using the number of fish exposed and the number dead at each mean measured Nipacide BIT concentration. A mortality curve demonstrates the relationship between concentration and time for Rainbow Trout.

Validity criteria for acute fish test according to OECD Guideline 203 were all fulfilled.

[redacted], 1979

In another study performed to assess the acute toxicity of PROXEL™ Press Paste (a technical grade of BIT with purity of 76.9%) to *Oncorhynchus mykiss*, the study design was comparable to OECD TG 203 (pH was

7.3 to 7.8). It was a continuous flow system where twenty fish were used at each test concentrations. The nominal concentrations tested were 5.6, 4.2, 3.2, 1.8, 1.0 and 0.75 mg/L and at daily intervals analysis was performed to determine the actual concentration of Proxel Press Paste. The purity of the Proxel Press Paste is not reported.

Final effect concentrations are not calculated based on BIT concentrations for the present study. The RMS has performed an aproximation based on the typical percentages of BIT in PROXELTM Press Paste (an average value of 76.9 % in PROXELTM Press Paste has been used for the transformation of the effect concentrations):

24 hour LC50 = 3.5 mg/L "Proxel Press Paste"; equivalent to 2.69 mg/L BIT.

48 hour LC50 = 2.3 mg/L "Proxel Press Paste"; equivalent to 1.77 mg/L BIT.

72 hour LC50 = 1.7 mg/L "Proxel Press Paste"; equivalent to 1.31 mg/L BIT.

96 hour LC50 = 1.6 mg/L "Proxel Press Paste"; equivalent to 1.23 mg/L BIT.

The LC100 value was 2.84 mg/L at 72 hours; equivalent to 2.18 mg/L BIT.

Hence, the 96-hour LC_{50} was 1.23 mg BIT/L (1.6 mg PROXEL^{TM}Press Paste / L) based on measured concentrations.

[redacted], 1993

In addition for marine species, there is another study where marine fish (*C. variegatus*) were exposed to PROXEL^{TM} Press Paste (a technical grade of BIT with purity of 76.1%) under static conditions over 96 hours in accordance with EPA Standard Evaluation Procedure EPA-540/9-85-006 (pH 8.1). The mean measured concentrations of PROXEL^{TM} Press Paste used to determine the LC_{50} were derived using the mean of the 48 and 96 hours concentrations with the exception of the 32mg/L test solution where no 96-hour measurement was made (because treatment was terminated at 48 hours at 32 mg/L, since 100% mortality was observed) and therefore the 48-hour concentrations were used. The 96-hour LC_{50} was 9.5 mg BIT/L (12.5 mg/L of PROXEL^{TM} Press Paste). This is considered the key study for marine water fish, as it is clear that the sensitivity of marine fish is different from freshwater fish.

[redacted], 2002a

In this test the acute toxicity of BIT (98% purity) to *Brachydanio rerio* was determined in a 96-hour semi-static test. The test was conducted according to OECD TG 203. The concentrations tested were 0, 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L. The measured concentrations were 102-139% of the nominal concentrations; therefore, and since the actual concentrations were within 20% of the nominal concentrations, the test results were expressed as nominal concentrations. The LC_{50} value at 96 h was 4.9 mg/L.

The test met the validity criteria established in the recommended Guideline. There was no mortality in the control and the dissolved oxygen concentration was above 5.1 mg/L. The average percentage of the substance present during the test was > 80% of initial concentration.

[redacted], 2003

In this test the acute toxicity of BIT (97.42% purity) to *O. mykiss* was determined in a 96-hour semi-static test. The test was conducted according to OECD TG 203, E.C. method C.1 and EPA TG OPPTS 850.1075. Since the actual concentrations were within 80% of the nominal concentrations, the test results were expressed as nominal concentrations. Initial concentrations of test substance were 0.80, 1.30, 2.10, 3.40 and 5.51 mg BIT/L. The effect endpoints derived in the study report were for the wet material, the RMS has recalculated the values in order to obtain the corresponding values for BIT without water (purity of 71.08%). The test concentrations selected were not detectable by the analytical method, so a known detectable concentration of 10 mg/L was prepared to perform the active ingredient content and stability of the test solution. The nominal concentration was therefore 10 mg/L (purity 71.08 %) and the actual concentration was found to be 7.108 mg/L. After dosing, the measured concentration was 6.82 mg BIT/L, 96% of the actual concentration. After 24 hours, the measured concentration was 6.57 mg/L, 92% of the actual concentration.

The percent mortality observed at 96 h were 20, 50, 80 and 100 at the test concentrations of 1.30, 2.10, 3.40 and 5.51 mg BIT/L.

The 96-hour LC_{50} value was 1.49 mg/L. The protocol OECD 203 recommends a total length of test fish of 5cm (±1 cm), while individuals used for this study were among 7.17 and 7.63 cm. The length and weight of the fish are related to the sensitivity of animals towards the toxicant. Larger fish may be less sensitive. However the resulting values of the endpoints are similar to other tests with the same species thus the test results can be considered reliable.

Loss of equilibrium, sluggishness, swimming at surface, dark or light pigmentation, lying on bottom and rapid respiration were the clinical symptoms observed at concentrations above 0.80 mg/L. At 0.80 mg/L and the control concentrations tested, there were no adverse effects observed with respect to survival or condition.

[redacted], 1997

In this test the acute toxicity of XBINX® (BIT with purity 99.29%) to *O. mykiss* was determined in a 96-hour static test. The test was conducted according to US EPA Pesticide Assessment Guidelines Subdivision E 72-1.

The nominal concentrations tested were 0, 0.31, 0.65, 1.25, 2.5, 5.0 and 10.0 mg/L. The average percentage of the substance present during the test was >80% of initial concentrations, thus the endpoints were determined based on nominal concentrations.

As commented in the previous test, the size of test organisms employed in this test is different than the recommended in OECD TG 203. This document recommends a total length of test fish of 5 cm (±1 cm), while in this study, smaller individuals were used, with an average size of 2.9 cm. The length and weight of the fishes are related to their sensitivity towards the toxicant, but as the resulting values of the endpoints are similar to other tests with the same species, the test results can be considered reliable.

Mortality was observed at 24, 48, 72 and 96 hours. Complete mortality was observed in the 5.0 and 10.0 mg/L concentrations following 24 hours of exposure. Complete mortality was observed in the 2.5 mg/L concentration following 48 hours of exposure. No mortality was observed at the 0.31 mg/L concentration.

Some fish were observed to be quiescent, surfacing, gyrating, swimming upside down and/or ceasing to swim or have laboured respiration in the 0.31, 0.65, 1.25, 2.5 and 10.0 mg/L concentrations.

The LC_{50} value at 96 h was calculated to be 0.74 mg BIT/L based on mortality. Since the LC_{50} was the lowest reliable value, this assay was considered the key study for acute toxicity to fish in the risk assessment.

Conclusion on acute toxicity to fish

The 96 h-LC_{50} values obtained in the different studies with marine and freshwater species ranged 0.74 – 19 mg BIT/L. The most sensitive species was *Oncorhynchus mykiss*. Since there are five studies available with *O. mykiss*, which justify the use of the geometric mean as indicated in the Guidance, the mean 96 h-LC_{50} value for this species is 1.5 mg/L. This is considered the key acute endpoint for fish.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Palmer *et al.*, 2006b; Palmer *et al.*, 2007a

Acute effects of BIT to aquatic invertebrates were investigated both in freshwater and marine crustacean species. In the EPA guideline-conforming tests, water flea (*D. magna*) and shrimp (*A. bahia*) were exposed to various concentrations of technical BIT (purity: 89.8%).

D. magna: Nominal (mg BIT/L) concentrations used of 1.3, 2.5, 5.0, 10 and 20 correspond to mean measured of 1.1, 2.9, 5.1, 10 and 21 mg BIT/L.

The concentration response 48 h curve is reported for mortality/inmobility.

Analytical recoveries ranged from 83 to 115% of nominal concentrations on Day 0 and from 92 to 117% of nominal concentrations on Day 2. At test termination, all daphnids in the negative control and the solvent control appeared normal with no mortalities or immobile daphnids noted. Percent mortality/immobility at test termination in the 1.1, 2.9, 5.1, 10 and 21 mg BIT/L treatment groups was 5, 35, 60, 100 and 100%, respectively.

A. bahia: Nominal (mg BIT/L) of 0.31, 0.63, 1.3, 2.5, and 5.0 were used. The concentration response 96 h curve is reported for mortality. Percent mortality in the 2.4 and 5.0 mg BIT/L treatment groups was 80 and 100%, respectively. Surviving mysids in the 2.4 mg BIT/L group exhibited lethargy and erratic swimming behavior at test termination. The single mortalities in the 0.28 and 1.2 mg BIT/L groups were not considered to be treatment related.

The endpoints were based on mean measured concentrations. The EC_{50} values of freshwater water flea and marine mysid shrimp of 3.7 mg a.s./L (48-hour) and 1.9 mg a.s./L (96-hour), respectively, indicate a similar sensitivity of crustacean species from the different environments.

Jenkins, 1995b

The acute toxicity of Nipacide®BIT (98.8% BIT) to Daphnia magna was assessed under static conditions over a period of 48 hours in accordance with OECD TG 202. Analysis of the test solutions indicated that exposure concentrations of BIT were adequately achieved and maintained within 75 to 98 % respect to the initial. The light intensity during the study was not reported. This is especially relevant for BIT, for which a fast degradation via photolysis has been demonstrated. However, chemical analysis shows that BIT remained relatively constant during the test, and thus, it is assumed that photodegradation is not an issue in this case.

Groups of twenty *Daphnia* were exposed to Nipacide BIT at nominal concentrations of 0.625, 1.25, 2.5, 5 and 10 mg/L, selected following a rangefinding test. The concentrations of Nipacide BIT were measured in mid-vessel samples taken from each exposure level at the start and end of the test. Intended exposure concentrations of Nipacide BIT were achieved and adequately maintained, giving mean measured concentrations of 0.643, 1.51, 2.57, 5.55 and 11.0 mg/L.

All validity criteria for acute *Daphnia* immobilization test according to OECD Guideline 202 are fulfilled.

The 48-hour EC50 for immobilization, based on measured concentrations, was estimated to be 2.94 mg/L (equivalent to 2.90 mg a.s./L).

Kent *et al.*, 1993

The acute toxicity of PROXEL™Press Paste (76.1% BIT) to *Mysidopsis bahia* was assessed under static conditions in sea water (20 ‰ salinity) over 96 hours in accordance with EPA Guideline 72-3; SEP 600/9-78-010.

No mortality was seen in the controls over the duration of the study. Likewise no mortality was noted in measured concentrations up to and including 330 µg/L at any time period.

Analysis of the test solutions indicated that exposure concentrations were adequately maintained. The data reported are based on mean measured concentrations. The 96-hour EC50 for immobilization was 1.3 mg/L of PROXEL™Press Paste (equivalent to 0.99 mg/L of BIT).

Three other tests of acute toxicity to invertebrates were performed, the first two according OECD TG 202 and the third one according EPA Subdiv. E, Section 72-2.

The first test on *D. magna* under semi-static conditions resulted in a 48-hour EC_{50} of 0.43 mg BIT/L based on measured concentrations (Nair 2003; IIIA 7.4.1.2/01). However it was considered non-acceptable (R.I. 3) because the *Daphnia* were exposed to very low concentrations (ca. 0.2 to 2.0 mg a.s./L) during the test and the selected test concentrations were not detectable by the analytical method. Additionally the purity of BIT was not clearly defined in the lab report. Measurement of concentrations were only performed for the highest level of 10 mg a.s./L in a separate test in order to check the stability of the a.s. resulting in ca. 80% of the nominal at the test end. It is noted that the resulting EC_{50} is one other of magnitude below the other values obtained for the same species and following a similar procedure.

Hooftman *et al.*, 2002b

In this study the toxicity of BIT (98% purity) to *D. magna* was determined in a 48-hour static acute toxicity test. The initial concentrations of test substance were 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L. The test was conducted according to OECD TG 202 and EU Test Method C.2 with no deviations (the validity criteria are met). The average recovery of nominal concentrations during the test was 105%, thus the test results were expressed as nominal concentrations. The EC_{50} value at 48 h was 4.0 mg BIT/L, based on mobility (immobilisation data were given at 0, 24 and 48 hours are given. At 5.6 and 10 mg/L all animals were immobile).

Terrell, 1997b

A static study was available on acute toxicity of XBINX® (99.3% purity of BIT) to *D. magna* according to US EPA Pesticide Assessment Guidelines Subdivision E, Section 72-2. The nominal concentrations tested were 0, 0.31, 0.65, 1.25, 2.5, 5.0 and 10.0 mg/L. Mean analytical measurements of test substance concentrations ranged from 88.8 to 116.1% of the nominal, however the endpoints were estimated on the basis of measured concentrations.

During the test some organisms were observed quiescent in the 1.25 and 2.5 mg/L nominal concentrations. 10, 40, 100 and 100% mortality was observed in the 1.25, 2.5, 5.0 and 10.0 mg/L nominal concentrations, respectively, following 48 hours of exposure.

The test met the validity criteria established in the recommended Guideline. There was no immobility in the control and the dissolved oxygen level was above 60% of the initial oxygen. The average percentage of the substance present during the test was > 80% of initial concentration.

The 48-hour EC_{50} was determined to be 2.24 mg a.s./L based on mean measured concentrations.

Conclusion on acute toxicity to invertebrates

For freshwater species, the geomean of the four endpoint values towards *Daphnia magna* resulted in a 48 h-EC_{50} of 3.27 mg/L.

For marine invertebrates, the 96-hour EC_{50} of 0.99 mg/L with *Mysidopsis bahia* is the lowest value of the two tests with marine species.

The Guidance does not indicate that endpoints of marine or freshwater crustaceans predominate. Therefore the lowest endpoint of aquatic invertebrates is considered for classification purposes, i.e. the 96-hour EC_{50} of 0.99 mg/L with *Mysidopsis bahia*.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Please note that the following data from studies on toxicity to algae include reliable acute and chronic endpoints. During the risk assessment of BIT, after BPR WG ENV discussions all endpoints have been recalculated by the Applicants and the eCA (Spain). These new estimations are included below in the studies summaries and the table 11.5.3-1. The data summarised on table 21 include only the recalculated estimations of endpoints, since the DS considers that the proposal for harmonised C&L of BIT should be based on the accepted endpoints for the environmental risk assessment of BIT.

Desjardins *et al.*, 2006b

Acute and chronic effects of BIT to algae were investigated in the freshwater green alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) folowing EPA OPPTS 850.5400 and OECD TG 201. In this test, algae were exposed to various concentrations of technical BIT (purity 89.8%). Samples of test medium collected and analyzed for BIT concentrations resulted in recoveries that ranged from 105 to 106% of nominal concentrations on Day 0 and all <LOQ on Day 4. The test met the OECD TG 201 criteria.

The E_rC_{50} values estimated in the laboratory report were 0.3 (24h), 0.75 (48h) and 0.79 (72h) mg/L and the E_rC_{10} were 0.05 (24h), 0.2 (48h) and 0.33 (72h) mg/L.

However the initial measured concentrations, 0.019, 0.043, 0.095, 0.21, 0.47 and 1.1 mg BIT/L, were used by eCA for endpoints calculation, since 24h concentrations represent the most sensitive endpoint. The 24 h-E_rC_{50} of 0.33 mg BIT/L and 24 h-E_rC_{10} of 0.032 mg BIT/L were recalculated as indicated in 2015 ENV WG and explained below.

Smyth *et al.*, 1994

The toxicity of PROXEL™ Press Paste (purity 73.3% BIT) to green algae *P. subcapitata* (formerly *S. capricornutum*) was assessed over a period of 72 hours following the OECD TG 201. The analysis of the test solutions indicated that measured concentrations for almost all vessels at the end of the test (72 h) are <80% respect to the initial concentration. However, average concentrations remained above this percentage during the test and final concentrations were never <70 % respect to the 0 h concentrations.

The E_rC_{50} values estimated by the Applicant were 0.1 (24h), 0.1 (48h) and 0.11 (72h) mg/L and the E_rC_{10} were 0.02 (24h), 0.03 (48h) and 0.05 (72h) mg/L.

To estimate the relevant endpoints eCA has taken initial measured concentration (0.00185, 0.0062, 0.014, 0.027, 0.055, 0.110, 0.220 and 0.440 mg/l BIT) since the lowest endpoints occurs at 24h. After purity correction (from 73.3% of BIT in the press paste to express the endpoint as 100% a.s.), the E_rC_{50} of 0.08 mg BIT/L and the E_rC_{10} of 0.035 mg BIT/L were obtained.

Smyth and Brown, 1991

The toxicity of PROXEL GXL (ca. 20% BIT) to marine alga (*Phaeodactylum tricornutum*) was assessed over a period of 72 hours according to ISO Guideline "Marine Algal Growth Test" (4th working draft ISO/TC147/SC5/WGN120 (1988)). The mean measured PROXEL GXL concentrations ranged from 60 to 78%. After 72 h, measured PROXEL GXL concentrations were in all cases below the 80 % respect to the initial concentration.

The E_rC_{50} values estimated by the Applicant were 0.192 (24h), 0.208 (48h) and 0.262 (72h) mg/L and the E_rC_{10} were 0.098 (24h), 0.114 (48h) and 0.146 (72h) mg/L.

However, since the lowest endpoint does not occur at 24h, endpoints were recalculated assuming first order kinetics and applying to each concentration the constant k of degradation given that concentrations at the end of the test are known. Geomean between the estimated concentration at each time and initial measured concentration was then applied. Initial concentrations were 0.15, 0.35, 0.74, 1.60, 3.30 and 6.40 mg/L. Final endpoints were corrected to purity resulting in E_rC_{50} of 0.165 mg BIT/L and E_rC_{10} of 0.063 mg BIT/L at 48h and ErC50 of 0.21 mg BIT/L and ErC10 of 0.084 mg BIT/L at 24h.

Kasthuri Raman, 2002

A study was performed, in which the growth inhibition of BIT to *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) was determined in a 72-hour test comparing data obtained from each treatment group with that of the vehicle (acetone) control group. The test was conducted according to OECD TG 201 and EC Method C.3. The test concentrations selected were not detectable by the analytical method; therefore a known detectable concentration of 10 µg/mL was prepared to perform the active ingredient content analysis and to check the stability of the test solution. Despite the fact that the study deviates from validity criterion 2 of OECD TG 201 (the mean coefficient of variation section by section is 0.47 and 0.48 for the control and vehicle control respectively), it was considered valid for the following reasons:

- The biological section of the study can be considered good. Between control and vehicle control, each containing 6 replicates, there are not important differences in cell density values. The study is done under GLP.
- The study does not fulfill the second criterion by a 13%. Nevertheless when the study was done, the second criterion did not apply. Besides, there are other cases (e.g. MIT) where a study not fulfilling the second criterion has been accepted.
- Finally, despite there is no analytical verification at the concentrations tested, the study provides data that shows that concentrations of the test substance are maintained within the range of 20% of variation from nominal concentrations, making it possible to calculate endpoints based on nominal concentrations. From the 10 µg/mL test solution, BIT concentration was 7.108 µg/mL, and the detected concentrations of BIT at 0 and 72 h were 6.60 and 5.99 µg/mL, respectively.

  The E_rC_{50} values estimated by the Applicant were 0.017 (24h), 0.0281 (48h) and 0.045 (72h) mg/L and the E_rC_{10} were 0.05 (24h), 0.2 (48h) and 0.33 (72h) mg/L. The DS notes that the E_rC_{10} should be wrongly estimated, since these should be lower than the E_rC_{50} values. However the DS takes the re-calculated values (below) as relevant values for the harmonised C&L of BIT.

  The effects values were re-calculated by the eCA based on the content of 71.08% purity of BIT and nominal concentrations (0.0014, 0.0028, 0.0057, 0.0114, 0.0227, 0.0455 and 0.091 mg/L), resulting in 24 h-E_rC50 of 0.011 mg BIT/L and 24 h-E_rC10 of 0.0029 mg BIT/L at 24h.

  Oldersma *et al.* 2002

  In another study the toxicity of BIT (98% purity) to *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) was detemined in a 72h growth inhibition test according to OECD TG 201 and EC Method C.3, and in compliance with GLP. The test fulfils all validity criteria but was done with an initial cell density of 3000 cells/mL while de Guideline recommends a cell density range of 5000-10000 cells/mL.

  The E_rC_{50} values estimated by the Applicant were 0.192 (24h), 0.208 (48h) and 0.262 (72h) mg/L and the E_rC_{10} were 0.098 (24h), 0.114 (48h) and 0.146 (72h) mg/L.

  The nominal concentrations of BIT tested were 0, 0.03, 0.10, 0.33, 1.0 and 3.3 mg/L. The test included analytical determination of low, middle and high test substance concentrations at the start and the end of the test (0, 0.03, 0.33 and 3.3 mg/L). The measured concentration of the lowest nominal test concentration (0.03 mg/L) was not valid because it was near the detection limit. The measured concentration of the test concentration 0.33 mg/L was 97% of the nominal concentration at the start and 58% at the end of the study. The measured concentration of 3.3 mg/L was 97% of the nominal concentration at both the start and end of the study. Since there is not measured data for all concentrations eCA recalculated endpoints using nominal concentrations. The lowest endpoints are at 24 h, i.e. E_rC_{50} of 0.48 mg BIT/L and E_rC_{10} of 0.16 mg BIT/L.

  Effects assessment and re-calculation of endpoints for algae

  In algal toxicity tests (according to OECD TG 201), isothiazolinones typically dissipate during the exposure period to levels below their detection limit. This mode of action of the isothiazolinone biocides has been extensively researched. The biocidal effect is described as a two-step process involving rapid inhibition of growth and metabolism leading to a loss in viability of the cells. These effects occur within minutes at the enzymatic level and can result in loss of viability within hours of exposure. The mechanism of action of the isothiazolinones is, however, complex, as these molecules react with several specific enzymes, which are essential within critical metabolic pathways. According to this mechanism of action of BIT uptake through the cell wall and membrane of the algae occurs rapidly, within hours and facilitates the activity of the biocide. Concomitant with uptake and enzymatic inhibition, the isothiazolone ring is cleaved rendering the molecule inactive. This means that the inhibitory effect on algae is directly coupled with degradation of the molecule by the algae.

  The rapid mode of action of the isothiazolinone is apparent in certain algal studies. The growth curves indicate strong effect within the first 24h of exposure and a recovery of growth which is dependent on dosing concentrations. Based on this observation and information on the mode of action of the biocide, it has been suggested to estimate effects on algae after 24 hours of exposure based on initial measured concentrations in

place of the geometric mean measured concentration over the test duration (0-72 hours). This approach was accepted in the assessment of other isothiazolinones in the BPD/BPR Review Program (e.g. DCOIT and MIT). If the most sensitive time period is not 24 hours, the kinetics of BIT have to be considered. Thus, for each of the studies it has been assessed when the strongest effect occurs and the endpoint has been estimated accordingly.

The response in algal tests and other microbial growth tests is by nature a continuous response which takes the shape of a sigmoid curve. The Statistical Package R has been used to asses which model (i.e.: log-logistic, weibul, likewise) best fits to the data. eCA has estimated the endpoints fitting the curve taking into account negative growths and later calculating the 50% of the upper asymptote of the curve. NOEC values are estimated using Dunnett's test.

The applicants have also presented an analysis (after WG discussions) where endpoints evaluated were the 50% effect concentration for growth rate (E_rC_{50}) and the 10% effect concentration (E_rC_{10}). These were derived by generating a logistic sigmoid curve from 0% to 100% and applying a logistic model using a non-linear (weighted) regression using SAS version 9.2 (SAS Institute Cary North Carolina).

Since the variability of the growth of the algal controls at 24 hours leads to uncertainty in both the NOEC and the E_rC_{10} derivation, the E_rC_{10} is suggested as the key chronic endpoint as its determination is independent from the study design and is consistently derived for all the algal studies. Guidance recommends abandoning the concept of NOEC and replacing it with regression based point estimates ECx. For this reasons E_rC_{10} values are used for the risk assessment. The DS follows the same approach for the proposal on the harmonised C&L of BIT.

Table 22: Comparison of (reliable) estimated endpoints for algae at different time points based on mean measured concentrations and growth rate inhibition.

Study	Period	Applicants			eCA		
		E_rC_{50}	E_rC_{10}	NOEC	E_rC_{50}	E_rC_{10}	NOEC
Desjardins *et al*., 2006b (*P. subcapitata)*	0-24	0.3	0.05	-	0.33	0.032	0.04
	0-48	0.75	0.2	-	0.8	0.19	0.21
	0-72	0.79	0.33	-	0.99	0.24	0.47
	0-96	-	-	-	1.31	0.34	0.47
Smyth *et al.*, 1994 (*P. subcapitata*)	0-24	0.1	0.02	-	0.08	0.035	0.019
	0-48	0.1	0.03	-	0.095	0.043	0.019
	0-72	0.11	0.05	-	0.087	0.057	0.04
Kasthuri Raman , 2002 *(P. subcapitata)*	0-24	0.017	0.05	-	0.011	0.0029	0.0028
	0-48	0.0281	0.2	-	0.017	0.0032	0.0028
	0-72	0.0453	0.33	-	0.026	0.0044	0.0028
Smyth and Brown, 1991 (*P. tricornutum)*	0-24	0.192	0.098	-	0.21	0.084	0.12
	0-48	0.208	0.114	-	0.165	0.063	0.021

	0-72	0.262	0.146	-	0.177	0.081	0.022
Oldersma *et al.*, 2002 *(P. subcapitata)*	0-24	0.447	0.22	-	0.48	0.16	0.1
	0-48	0.543	0.306	-	0.64	0.3	0.03
	0-72	0.607	0.28	-	0.67	0.25	0.03

Conclusion on growth inhibition of BIT to algae

As it was mentioned above, it has been suggested to estimate effects on algae after 24 hours of exposure based on initial measured concentrations in place of the geometric mean measured concentration over the test duration (0-72 hours).

Results show that for most of the studies, 24h-ECx are the most sensitive endpoints with values lying within the same order of magnitude. No difference in sensitivity between freshwater and marine species is observed, thus values can be pooled. The lowest reliable 24h-E_rC_{50} value for algae is 0.011 mg BIT/L and the lowest 24h-E_rC10 is 0.0029 mg BIT/L (*P. subcapitata*).

Since there are four data points for the same species (*P. subcapitata*), the geometric mean has been used for the risk assessment and for the proposal on harmonised C&L, i.e. 24h-E_rC10 of 0.026 mg/L and 24h-E_rC_{50} of 0.108 mg/L.

The geomean obtained was based on four ErC50 (acute endpoint) and four ErC10 (chronic endpoint) at 24 h, two of them being based on initial measured concentrations (Desjardins and Smyth), as explained above, and two of them based on nominal concentrations (Kasthuri and Oldersma).

11.5.4 Acute (short-term) toxicity to other aquatic organisms

Thomas *et al.*, 2007a

Acute effects of BIT TG (purity of 89.8%) to the midge sediment dwelling aquatic invertebrates were investigated using the midge *Chironomus tentans*. In the guideline-compliant tests (EPA OPPTS 850.1735; ASTME 1706-96b), midge larvae were exposed to BIT in spiked sediment with acetone) under flow-through conditions for 10 days. BIT concentrations were measured at day 0 and day 10 in sediment, overlaying water and pore water. In the overlaying water measured BIT was not detectable except at day 0 in the highest level; in the sediment, they were the half of nominal at the beginning and at day 10, they strongly decreased; in pore water, they accounted for the majority of BIT.

The test showed no effects on survival over the 10 day test period; therefore EC_{50} was determined to be greater than the highest concentration tested (i.e. 100 mg a.s./kg_{dwt}). However according to the OECD TG 218 the effects concentrations expressed and based on dry weight, should be calculated preferably based on the measured sediment concetrations at the beginning of the test. Consequently the 10-day NOEC was 32.8 mg a. s./kg_{dwt} and the EC_{50} was determined to be >45.9 mg a. i. /kg_{dwt}.

Conclusion

The DS has included the results of this study for the sake of completeness, however it is not considered relevant for the harmonised C&L of BIT. The results were obtained in spiked sediment, while the sediment is not considered an environmental compartment of concern for BIT given the relatively low K_{OC} and low log K_{OW} of BIT. In addition sufficient data are available on aquatic organisms.

11.6 Long-term aquatic hazard

Table 23: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
OECD TG 210; EPA OPPTS 850.1400	*Pimephales promelas*	BIT (TG 89.8% purity)	33 d-NOEC 0.28 mg/L	Flow-through, mean measured GLP	[redacted], 2007b CAR IIIA7.4.3.2.a/01
Draft OECD TG 215	*Oncorhynchus mykiss*	PROXEL™ Press Paste (70% BIT)	28 d-NOEC 0.21 mg/L	Flow-through, mean measured GLP	[redacted], 2000a CAR IIIA 7.4.3.2/1
OECD TG 211; EPA OPPTS 850.1300	*Daphnia magna*	BIT (TG 89.8% purity)	21 d-NOEC 0.91 mg/L	Flow-through, mean measured GLP	Palmer *et al.*, 2007c CAR IIIA7.4.3.4.a/01
OECD TG 211	*Daphnia magna*	PROXEL™ Press Paste (70% BIT)	21 d-NOEC 1.2 mg/L	Semi-static, mean measured GLP	Penwell and Roberts, 2000b CAR IIIA7.4.3.4/1
OECD TG 201; EPA OPPTS 850.5400	*Pseudokirchneriella subcapitata*	BIT (TG, 89.8% purity)	24 h-E_rC_{10} = 0.032 mg/L 48 h-E_rC_{10} = 0.19 mg/L 72 h-E_rC_{10} = 0.24 mg/L	Static, mean measured GLP	Desjardins *et al.*, 2006b CAR IIIA 7.4.1.3.a/01
OECD TG 201	*Pseudokirchneriella subcapitata*	PROXEL™ Press Paste (BIT TG, 73.3% purity)	24 h-E_rC_{10} = 0.035 mg/L 48 h-E_rC_{10} = 0.043 mg/L 72 h-E_rC_{10} = 0.057 mg/L	Static, mean measured GLP	Smyth *et al.*, 1994 CAR IIIA 7.4.1.3/1a
Draft ISO Guideline "Marine Algal Growth Test"	*Phaeodactylum tricornutum*	PROXEL GXL (ca. 20% BIT)	24 h-E_rC_{10} = 0.084 mg/L 48 h-E_rC_{10} = 0.063 mg/L 72 h-E_rC_{10} = 0.081 mg/L	Static, mean measured GLP	Smyth and Brown, 1991 CAR IIIA 7.4.1.3/1b
OECD TG 201	*Pseudokirchneriella subcapitata*	BIT (TG 71.08% purity)	24 h-E_rC_{10} = 0.0029 mg/L 48 h-E_rC_{10} = 0.0032 mg/L 72 h-E_rC_{10} = 0.0044 mg/L	Static, mean measured GLP	Kasthuri Raman, 2002 CAR IIIA 7.4.1.3-1
OECD TG 201	*Pseudokirchneriella subcapitata*	BIT (98% purity)	24 h-E_rC_{10} = 0.16 mg/L 48 h-E_rC_{10} = 0.3 mg/L 72 h-E_rC_{10} = 0.25 mg/L	Static, mean measured GLP	Oldersma *et al.*, 2002 CAR IIIA 7.4.1.3-2

11.6.1 Chronic toxicity to fish

[redacted], 2007b

A chronic toxicity study on fish following OECD TG 210 and US EPA OPPTS 850.1400 was conducted in a flow-through system ([redacted], 2007b). Long-term effects of BIT (TG 89.8% purity) to fish was investigated in an early life-stage test, exposing *Pimephales promelas* to BIT for 33 days.

The nominal concentrations (mg BIT/L) used were 0.31, 0.63, 1.3, 2.5 and 5.0. The mean measured concentrations were 0.28, 0.59, 1.2, 2.4 and 4.8 mg BIT/L. Several fish in the 2.4 mg BIT/L group were surfacing between days 2 and 4 but the fish appeared normal from day 5 through test termination. Several fish in the 4.8 mg BIT/L group were weak, surfacing, swimming erratically or with morphological abnormalities such as crooked spines. Most of these 4.8 mg BIT/L weakened fish died prior to test termination. Length and weight were also reported.

The endpoints were based on mean measured concentrations. The assessed parameters were egg hatch, survival and growth. The most sensitive endpoint was growth. A 33-day NOEC of 0.28 mg a.s./L was determined based on growth-related effects.

[redacted], 2000a

The chronic effect of PROXEL™ Press Paste (70% BIT) on the growth rate of juvenile *Oncorhynchus mykiss* was assessed in a 28-day flow-through test according to Draft OECD TG 215. Test concentrations were measured throughout the test. Nominal concentrations of 0.10, 0.18, 0.32, 0.56 and 1.0 mg/L Proxel press paste are equivalent to 0.07, 0.13, 0.22, 0.39, and 0.7 mg/L 1,2-benzisothiazolin 3-one (BIT). The actual mean measured concentrations are 0.093, 0.16, 0.30, 0.61 and 0.87 mg/L Proxel press paste, equivalent to 0.07, 0.11, 0.21, 0.43, and 0.61 mg/L BIT.

For both the relative growth rate (RGR) and specific growth rate (SGR), the mean measured NOEC and the LOEC were 0.3 and 0.61 mg/L PROXEL™ Press Paste (equivalent to 0.21 mg/L and 0.43 mg/L BIT). For the condition indices the mean measured NOEC and LOEC values were 0.87 mg/L and >0.87 mg/L PROXEL™ Press Paste (equivalent to 0.69 mg/L and > 0.69 mg/L BIT, respectively). The mean measured NOEC and LOEC for food conversion efficiency were 0.3 and 0.61 mg/L PROXEL™ Press Paste (equivalent to 0.21 and 0.43 mg/L BIT, respectively). The overall 28-day NOEC and LOEC were 0.30 mg/L and 0.61 mg/L PROXEL™ Press paste which is equivalent to 0.21 and 0.43 mg/L BIT, respectively. The only symptom of toxicity noted in this study was a transitory reduction in rate of feeding at 0.61 and 0.87 mg/L between days 4 and 7. All fish in all concentrations were observed to be feeding eagerly by day 7 and continued to do so for the remainder of the study.

Conclusion on chronic toxicity to fish

Both studies were reliable and they showed similar sensitivity of the species tested. The lowest endpoint considered for chronic fish toxicity corresponds to 28 d-NOEC of 0.21 mg a.i./L in a test carried out with *O. mykiss*.

However according to the Guidance on the Application of the CLP Criteria only tests consistent with OECD TG 210 (Fish Early Life Stage), the fish life-cycle test (US EPA 850.1500), or equivalent can be used in the classification scheme. Technically, the OECD TG 210 is not a chronic toxicity test, but a sub-chronic test on sensitive life stages. However it is widely accepted as a predictor of chronic toxicity and is used as such for purposes of classification in the harmonised system.

Tests according to the OECD TG 215 are designed to assess the effects of prolonged exposure to chemicals on the growth of juvenile fish, but they do not include other typical long-term parameters such as hatching success, spawning success, and survival. The Guidance on I.R. and C.S.A. indicates that the juvenile growth test (OECD TG 215) can be accepted if there are well founded justifications indicating that growth inhibition is the most relevant effect in fish for the assessed substance. The study Palmer *et al.* 2007b demonstrates that the most sensitive endpoint was growth. Therefore the resulting 28d-NOEC of 0.21 mg a.i./L of the OECD TG 215 is proposed as key long-term endpoint for fish.

11.6.2 Chronic toxicity to aquatic invertebrates

Palmer *et al.*, 2007c

In a chronic *Daphnia magna* study according to OECD TG 211 and EPA OPPTS 850.1300, the reproductive efficiency and growth of adult water flea was studied after exposure to BIT TG (purity 89.8%). The test was conducted in a flow-through system with nominal concentrations 0.25, 0.50, 1.0, 2.0, and 4.0 mg a.s./L during 21 days. Measured concentrations were always > 80% of nominal.

Since no significant differences between the control groups were found for any parameter tested ($p > 0.05$) the control data were pooled for comparison with the BIT treatment groups. After 21 days survival in the negative and solvent control groups was 95% and 100%, respectively. The control data was pooled for comparisons with the BIT treatment groups. The first day of brood production in the negative control, solvent control and the BIT treatment groups was Day 8 of the test indicating there was no apparent delay in the onset of production at any BIT concentration tested.

Although the coefficient of variation for control fecundity (based on total number of living offspring per parent animal alive) and the plot of total number of living offspring per parent animal (for each replicate) alive at the end of the test vs concentration are missed in the report, this does not compromise the study reliability.

The 21-day NOEC was determined to be 0.91 mg a.s./L for reproduction related effects (number of offspring per reproductive day) and 1.91 mg a.s./L for growth related effects (length, dry weight) based on mean measured concentrations and the LOEC was 1.9 mg BIT/L.

Penwell and Roberts, 2000b

The chronic toxicity of BIT (PROXEL™ Press Paste, purity 70% BIT) to *Daphnia magna* was assessed in accordance with OECD TG 211 in a semi-static test during 21 days. Nominal concentrations tested were equivalent to 0.070, 0.13, 0.22, 0.39, 0.70, 1.2 and 3.2 mg BIT/L. Tested parameters were mortality, reproduction rate and length.

There were no mortalities observed in the dilution water control and at time weighted mean concentration of 0.066 and 0.12 mg/L PROXEL press paste. There were mortalities observed at time weighted mean concentrations of 0.23, 0.41, 0.85 and 1.7 mg/L however these were considered to be 'random events' and unrelated to any effect of the test substance.

Dunnett's T test was applied to the reproductive (from P0 *Daphnia* surviving to the end of the study) and length data and there was no significant difference ($P = 0.05$) in length of the P0 *Daphnia* or reduction in the number of offspring in any test concentration compared to the dilution water control.

The time-weighted mean measured concentrations ranged from 66 to 94% of nominal values. The NOEC for reproduction and growth was 1.2 mg BIT/L (1.7 mg/L PROXEL™ Press Paste) based on time-weighted mean measured concentrations. The overall NOEC was 1.2 mg BIT/L and the LOEC was 1.9 mg BIT/L.

Conclusion on chronic toxicity to invertebrates

Both studies were reliable and they showed similar results. Since only two values are available, the lowest value should be selected. The lowest long-term endpoint on *Daphnia magna* was the 21-day NOEC of 0.91 mg a.s./L.

11.6.3 Chronic toxicity to algae or other aquatic plants

As it has been explained above in section 11.5.3, the chronic toxicity studies to algae available to the DS are summarised in that section, since they come from the same studies as acute toxicity endpoints to algae. However the chronic toxicity endpoints to algae are repeated in table 74.

The lowest chronic toxicity endpoint is the 24 h-E_rC_{10} of 0.026 mg BIT/L, which is the geometric mean value of the 24 h-E_rC_{10} from the studies with the freshwater algae *Pseudokirchneriella subcapitata*.

Studies on chronic toxicity of BIT to other aquatic plants are not available.

11.6.4 Chronic toxicity to other aquatic organisms

Thomas *et al.*, 2007b

The objective of the this study was to determine the long-term effects of spiked sediment BIT TG (purity 89.8%) to *Chironomus riparius* during a 28-day exposure period under static conditions according to OECD TG 218. The organisms generally appeared normal and healthy throughout the study. The concentration in sediment, pore water and overlaying water decreased to <LOQ in the majority of concentrations. The 28-day EC_{50} value based on percent survival of midges and on the measured sediment concentrations at the beginning of the test was 32.79 mg a.s./kg, and the NOEC value was 11.7 mg a.s./kg.

Conclusion

The DS has included the results of this study for the sake of completeness, however it is not considered relevant for the harmonised C&L of BIT. The results were obtained in spiked sediment, while the sediment is not considered an environmental compartment of concern for BIT given the relatively low K_{OC} and low log K_{OW} of BIT. In addition sufficient data are available on aquatic organisms.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

The lowest acute endpoint value for fish is 96 h-LC_{50} of 1.5 mg/L (*Oncorhynchus mykiss*). The lowest acute endpoint of aquatic invertebrates is 96-hour EC_{50} of 0.99 mg/L with (*Mysidopsis bahia*). The lowest acute endpoint for algae is 24h-E_rC_{50} value of 0.108 mg BIT/L (*Pseudokirchneriella subcapitata*).

This endpoint for *P. subcapiptata* is the geomean obtained from four different studies with this species, and is based on four ErC50 at 24 h, two of them being based on initial measured concentrations (Desjardins and Smyth), as explained above, and two of them based on nominal concentrations (Kasthuri and Oldersma).

According to these data, algae are the most sensitive trophic level. For classification of a substance in relation to acute aquatic hazard, table 4.1.0 (a) of Annex I should be used. The acute endpoint selected has to be compared with the cut-off value. The 24 h-E_rC_{50} of 0.108 mg/L is $\leq$ 1 mg/L. Therefore BIT should be classified as Aquatic Acute 1. The corresponding Multiplying factor (M-factor) should be 1, since 0.1 < $EC_{50} \leq$ 1, according to Table 4.1.3 of Annex 1 of CLP.

The current entry in Annex VI of BIT already includes category Aquatic Acute 1. The DS proposes to keep the same hazard category and to add M-factor of 1.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Bioaccumulation potential

The experimental log K_{OW} is 0.70 at pH 7 and 20 °C and the estimated log K_{OW} is 0.6. The experimental BCF is 6.95 L/Kg_{wwt} (whole fish) and the estimated BCF is 3.162 L/Kg.

Since the estimated and experimental log Kow are < 4 and the BCF for fish is < 500 L/Kg, it can be concluded that BIT has not significant potential for bioaccumulation in the aquatic environment.

Degradation

BIT is hydrolytically stable at pH values 4, 7 and 9 and 50 °C.

BIT is very unstable under artificial sunlight in aquatic medium, the photolysis half-life of BIT is estimated as <1 hour under artificial sunlight at pH 7 and 9. At pH 5, photolysis underwent slightly slower. However the results of photochemical degradation of BIT are not considered as indicative of rapidly degradability in the field.

BIT is not readily biodegradable as demonstrated in four studies available, because the substance did not pass the level of degradation for readily biodegradable substances. However BIT underwent relatively fast primary degradation yielding several metabolites.

The results of two studies on inherent biodegradability concluded that BIT cannot be considered an inherently biodegradable substance. According to the third study, biodegradation of BIT was achieved when more favourable conditions were set in the tests. However the Guidance indicates that results of tests for inherent biodegradability, because of the optimised conditions, should not be interpreted as evidence for rapid degradation of substances in the environment.

According to the results of two simulation degradation studies of BIT in estuarine and sea water, it is concluded that BIT can be rapidly degraded to four metabolites in estuarine water with a half-lives of 0.9-1.2 days at 12ºC; in sea water, degradation of BIT went slower, with half-lives of 5.3-12 days at 12ºC and producing three metabolites. The results of these studies could suggest that BIT was primarily biodegradable in estuarine and sea water. However it was not ultimately biodegradable, since the level of mineralisation was very poor (degradation to $CO_2 \leq 1$ %). Furthermore, the degradation products of BIT might be toxic to aquatic organisms (see Annex I).

The results of two simulation degradation studies of BIT in soil showed that BIT rapidly disappears from soils with half-lives values from 0.02 to 0.54 d at 12ºC. Mineralization reached 40 - 56% depending on the soil type. The level of bound residues was 40-49% of AR at test ends. A number of metabolites were found in the studies as a result of degradation of BIT. In the studies sterile soils showed the importance of abiotic degradation of BIT (half-lives of 0.4-0.6 d at 21ºC).

The Guidance assumes that when a substance has been shown to be degraded rapidly in a soil simulation study (i.e. it is ultimately degraded within 28 days with a half-life < 16 days), it is most likely also rapidly degradable in the aquatic environment. However this is not the case for BIT as it was shown in the estuarine and sea water simulation testing. In case of conflicting results, the Guidance stipulates that simulation test data of surface water are preferred over aquatic sediment or soil simulation test data.

Therefore on the basis of results of aquatic systems, BIT cannot be considered a rapidly degradable substance for the following reasons (according to the classification scheme, see section 11.1.5):

- BIT is not readily biodegradable
- BIT is not ultimately degraded in the aerobic aquatic degradation test (less than 1% of the applied activity was present as $^{14}CO_2$)
- BIT is primarily degraded very fast in aerobic aquatic systems but it cannot be demonstrated that the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment as some metabolites might be toxic to aquatic organisms (see Annex I).

Conclusion on long-term aquatic hazard

There are adequate chronic toxicity data available for all three trophic levels (fish, crustacean, algae/aquatic plants).

The lowest long-term endpoint for fish (*Oncorhynchus mykiss*) is 28 d-NOEC of 0.21 mg/L. The lowest long-term endpoint for invertebrates was the 21-day NOEC of 0.91 mg a.s./L (*Daphnia magna*). The lowest chronic toxicity endpoint foe algae is the 24 h-E_rC_{10} of 0.026 mg BIT/L (*Pseudokirchneriella subcapitata*).

It can be concluded that the most sensitive aquatic organisms, on the basis of their chronic toxicity endpoints, are algae.

For the most sensitive species, there were four different studies, hence the geomean is used. The value obtained was based on four ErC10 at 24 h, two of them being based on initial measured concentrations (Desjardins and Smyth), as explained above, and two of them based on nominal concentrations (Kasthuri and Oldersma).

Since there are adequate chronic toxicity data available and the substance is non-rapidly degradable, the key chronic endpoint should be compared to the criteria of table 4.1.0 (b)(i) of Annex 1 of CLP. The 24 h-E_rC_{10} of 0.026 mg BIT/L is <0.1 mg/L. Thus BIT should be classified as Chronic 1.

The corresponding M-factor proposed should be 1, since 0.01 mg/L < $E_rC_{10} \leq$ 0.1 mg/L and the substance is non-rapidly degradable, according to Table 4.1.3 of Annex 1 of CLP.

The current entry in Annex VI of BIT does not include harmonised classification of long-term aquatic hazard. The DS proposes to classify BIT as Aquatic Chronic 1 with M-factor of 1.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

The DS proposes the following harmonised C&L for environmental hazards of BIT:

- Aquatic Acute 1 (H400), M = 1
- Aquatic Chronic 1 (H410), M = 1

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

1,2-benzisothiazol-3(2H)-one (BIT) is a biocidal active substance and is currently listed in Annex VI of the CLP Regulation (EC) 1272/2008 with a harmonised classification and labelling as Aquatic Acute 1 (H400).

The Dossier Submitter (DS) proposed to update the current environmental hazards since the substance has been assessed as a biocidal active substance by Spain and the proposed results have already been discussed and agreed in the BPC WG (ENV) with Ad hoc ENV Experts group from other MSCAs. Therefore, the DS proposed to retain Aquatic Acute 1, adding an M-factor of 1, and to add Aquatic Chronic 1 with an M-factor of 1.

Overall, the DS concluded that BIT is 'not rapidly degradable", has a low potential for bioaccumulation and proposed classification based on aquatic acute and chronic toxicity in the algae *Pseudokirchneriella subcapitata* as:

Aquatic Acute 1 (H400), M = 1, based on the 24-hour E_rC_{50} value of 0.1087 mg/L for *P. subcapitata* calculated as aquatic acute toxicity geometric mean value from four studies.

Aquatic Chronic 1 (H410), M = 1, based on the 24-hour E_rC_{10} value of 0.026 mg/L for *P. subcapitata* calculated as aquatic chronic toxicity geometric mean value from four studies.

Degradation

Various ready biodegradability tests were provided. Two studies following OECD TG 301B (Seyfried, 2006a and Burwood, 2007) shows 0 % and 23.8 % degradation after 28 days, respectively. Two studies according to OECD TG 301D study (Hanstveit and Akdemir, 2002 and Patra, 2003) shows 0 % and 4.94 % degradation after 28 days, respectively. One study following OECD TG 301C (Brown *et al*., 1994) shows < 1 % degradation after 63 days. One study following OECD TG 301B (Dempsey *et al*., 1998; Penwell and Roberts, 1999) shows 58.7 % degradation after 83 days.

Overall, the DS concluded that BIT is not readily biodegradable since the substance did not meet the pass level of > 70 % after 28 days. However, BIT underwent relatively fast primary degradation yielding several metabolites, which differed across the tests. Two metabolites were determined to be relevant: 2-methylthiobenzamide and 2-methylsulfinyl-benzamide.

Three studies on hydrolysis. following OECD TG 111 and one study following EC Method C.7, were provided. All studies indicated that BIT is hydrolytically stable at the three pH values (4, 7 and 9) at 50 °C. Therefore, the DS concluded that half-life of BIT can be estimated as > 1 year at 12 °C since the loss of BIT in water was < 5 % at pH 4, 7 and 9 and 50°C in all studies.

Three studies on inherent biodegradation were provided. Two of them (Seyfried, 2006b and Gonsior *et al*., 2008) indicate that BIT is not inherently degradable with 0-17 % degradation after 28d. According to the third study (Jenkins 1999), biodegradation of BIT with 40-52 % degradation after

91 days was achieved when more favourable conditions were set in the tests (i.e., relatively high concentrations of microorganisms in the inoculum over a long time period) and this could be interpreted as inherent primary biodegradation, but not as inherent ultimate biodegradation.

Four studies on photolysis in water were provided. All of them indicate that BIT is photolytically unstable at all pH values. Photolysis half-life of BIT was estimated as < 1 hour under artificial sunlight at pH 7 and 9. At pH 5, photolysis occurred slightly slower (9 hours). Photolytic degradation of BIT in water resulted in a number of degradation products at different amounts depending on the pH value of the medium. These products were further photolysed after several days. Therefore, the DS concluded that BIT is very unstable under artificial sunlight in aquatic medium and very rapidly photolysed at all pH values.

Three studies were provided on aerobic aquatic degradation in water (estuarine and sea water) of BIT. An aerobic degradation study in estuarine water (Guo, 2008) following OECD TG 309, indicated that BIT was primarily biodegradable in estuarine (brackish) water with half-lives of 22.9-29.8 h at 12 °C, yielding 4 major metabolites. An aerobic degradation study in sea water (Guo and Marbo, 2009) following OECD TG 309, indicated that BIT is primarily biodegradable in sea water with half-lives in the range 5.3-12.2 d at 12 °C, yielding major 3 metabolites. Another aerobic degradation study in sea water (MacLean *et al*., 2005) following OECD TG 306 showed that BIT was not ultimately degradable in sea water. Overall, the DS considered that the results suggest that BIT is primarily biodegradable in estuarine (brackish water) and sea water. However, it was not ultimately biodegradable, since the level of mineralisation was very poor (≤ 1 % AR was evolved as CO_2). Therefore, BIT was considered as not rapid biodegradable by the DS.

Two studies on aerobic degradation in soil of BIT following OECD TG 307 were provided. Both studies show that BIT rapidly disappears from soils with half-live values from 0.02 to 0.54 d at 12 ºC. Mineralisation reached 40-56 % depending on the soil type. The level of bound residues was 40-49 % of AR at test end. Although the CLP guidance assumes that when a substance has been shown to be degraded rapidly in a soil simulation study (i.e., it is ultimately degraded within 28 days with a half-life < 16 days), it is also likely to rapidly degrade in the aquatic environment. However, the DS considers that this is not the case for BIT as in case of conflicting results of degradation simulation studies, the CLP Guidance stipulates that simulation test data of surface water are preferred over aquatic sediment or soil simulation test data for the evaluation of rapid degradability in the aquatic environment.

Overall, due to the results summarised above, the DS concluded that the degradation information does not provide sufficient data to show that BIT is ultimately degraded to above 70 % within 28 days (equivalent to a half-life of less than 16 days) or is transformed to non-classifiable products. Therefore, BIT was considered by the DS to be not rapidly degradable according to the CLP criteria.

Aquatic Bioaccumulation

The available experimental mean steady-state BCF was 6.95 L/Kg_{wwt} for whole fish in *Lepomis macrochirus* (Anonymous, 1973). However, weight of the fish throughout the duration of the study was not recorded and the lipid content of the fish was not determined. Therefore, the BCF was not normalised to a lipid content of 5 %. Thus, the study was considered only as additional information by the DS. However, the log K_{OW} of BIT suggests that it will not bioaccumulate in the aquatic environment and the mean steady-state BCF (log BCF = 0.84) is comparable to the EPI Suite™ 4.11 estimated BCF value (Log BCF = 0.50).

The estimated BCF is 3.162 L/Kg (QSAR estimation, EPI Suite™ 4.11). The experimentally determined log K_{OW} is 0.70 at pH 7 and 20 °C (Seal, 2002). The estimated log K_{OW} is 0.64 (QSAR estimation, EPI Suite™ 4.11).

Overall based on the results summarised above, the DS concluded that BIT has a low potential for bioaccumulation.

Aquatic Toxicity

The aquatic toxicity test results from available acute and chronic studies (CAR and/or REACH dossier) for all trophic levels of BIT are summarised in the following table and sections. Only the valid acute and chronic studies on BIT which are relevant for hazard classification purposes are included in the following table and relevant endpoints from these studies are discussed in further detail below. The most sensitive trophic group for acute and chronic toxicity are algae (*P. subcapitata*). As there were large data sets (four or more values) available for the same species (*P. subcapitata*) and for the same endpoint (E_rC_{50} and E_rC_{10}), the geometric mean of toxicity values was applied by the DS to use as the representative toxicity value. Where test item was in a formulation (e.g., PROXEL PASTA) the recalculation of effect concentrations was applied based on the typical percentages of the BIT in formulation. During the risk assessment of BIT, after BPR WG ENV discussions all endpoints from the four studies on *P. subcapitata* were recalculated by the Applicants and the eCA (Spain), two of them based on initial measured concentrations (Desjardins et al., 2006b and Smyth *et al*., 1994), and two of them based on nominal concentrations (Kasthuri Ramen, 2002 and Oldersma *et al*., 2002).

Aquatic Acute toxicity

Test method	Test organism	Short-term result (endpoint) mg/L	Reference / Test item
Fish			
OECD TG 203; EPA OPPTS 850.1075 / GLP	*Oncorhynchus mykiss*	96h LC_{50} = 1.9 (mm)	Anonymous (2006a) (CAR)/BIT (89.8 %)
EPA OPPTS 850.1075/GLP	*Cyprinodon variegates*	96h LC_{50} = 19 (mm)	Anonymous (2006c) (CAR)/BIT (89.8 %)
OECD TG 203; EPA OPPTS 850.1075/GLP	*Oncorhynchus mykiss*	96h LC_{50} = 2.18 (mm)	Anonymous (1995a) (CAR, REACH Dossier)/Nipacide®BIT (BIT 98.8 %)
Comparable to OECD TG 203/non-GLP	*Oncorhynchus mykiss*	96h LC_{50} = 1.23 (mm)	Anonymous (1979) (CAR)/PROXEL™ Press Paste (BIT 76.9 %)
EPA-540/9-85-006/GLP	*Cyprinodon variegates*	96h LC_{50} = 9.47 (mm)	Anonymous (1993) (CAR, REACH Dossier)/PROXELTM Press Paste (BIT 76.1 %)
OECD TG 203/GLP	*Brachydanio rerio*	96h LC_{50} = 4.9 (nom)	Anonymous (2002a) (CAR)/BIT (98 %)
OECD TG 203; EPA OPPTS 850.1075/GLP	*Oncorhynchus mykiss*	96h LC_{50} = 1.49 (nom)	Anonymous (2003) (CAR)/BIT (97.42 %)
US EPA Subdiv. E, Sec. 72-1/GLP	*Oncorhynchus mykiss*	96h LC_{50} = 0.74 (nom)	Anonymous (1997a) (CAR)/XBINX® (BIT 99.29 %)
Aquatic invertebrates			
OECD TG 202; EPA OPPTS 850.1010/GLP	*Daphnia magna*	48h EC_{50} = 3.7 (mm)	Palmer *et al.* (2006b) (CAR)/BIT (89.8 %)
EPA OPPTS 850.1035 / GLP	*Americamysis bahia*	96h EC_{50} = 1.9 (mm)	Palmer *et al.* (2007a) (CAR)/BIT (89.8 %)
OECD TG 202/GLP	*Daphnia magna*	48h EC_{50} = 2.9 (mm)	Jenkins (1995b) (CAR, REACH Dossier) Nipacide®BIT (BIT 98.8 %)
EPA 72-3; SEP 600/9 78-010/GLP	*Mysidopsis bahia*	96h EC_{50} = 0.99 (mm)	Kent *et al.* (1993)

			(CAR)/PROXELTM Press Paste (BIT 76.1 %)
OECD TG 202/GLP	*Daphnia magna*	48h EC_{50} = 4.0 (nom)	Hooftman *et al.* (2002b) (CAR)/BIT (98 %)
US EPA Subdivision E, Section 72-2/GLP	*Daphnia magna*	48h EC_{50} = 2.24 (mm)	Terrell (1997b) (CAR)/XBINX® (BIT, 99.3 %)
Algae / other aquatic plants			
Draft ISO Guideline "Marine Algal Growth Test" / GLP	*Phaeodactylum tricornutum*	24h E_rC_{50} = 0.21 48h E_rC_{50} = 0.165 72h E_rC_{50} = 0.177 (mm)	Smyth and Brown (1991) (CAR)/PROXEL GXL (BIT 20 %)
OECD TG 201; EPA OPPTS 850.5400/GLP	*Pseudokirchneriella subcapitata*	**24h E_rC_{50} = 0.33** 48h E_rC_{50} = 0.8 72h E_rC_{50} = 0.99 96h E_rC_{50} = 1.31 (recalculated endpoints using initial measured con.)	Desjardins *et al.* (2006b) (CAR)/BIT (89.8 %)
OECD TG 201? GLP	*Pseudokirchneriella subcapitata*	**24h E_rC_{50} = 0.08** 48h E_rC_{50} = 0.095 72h E_rC_{50} = 0.087 (recalculated endpoints using initial measured con.)	Smyth *et al.* (1994) (CAR, REACH Dossier)/PROXEL™ Press Paste (BIT 73.3 %)
OECD TG 201/GLP	*Pseudokirchneriella subcapitata*	**24h E_rC_{50} = 0.011** 48h E_rC_{50} = 0.017 72h E_rC_{50} = 0.026 (recalculated endpoints using nom. con.)	Kasthuri Raman (2002) (CAR)/BIT (71.08 %)
OECD TG 201/GLP	*Pseudokirchneriella subcapitata*	**24h E_rC_{50} = 0.48** 48h E_rC_{50} = 0.64 72h E_rC_{50} = 0.67 (recalculated endpoints using nom. con.)	Oldersma *et al.* (2002) (CAR)/BIT (98 %)

mm: mean measured concentration, nom: nominal concentration

Several studies were submitted on the acute toxicity of BIT to freshwater fish and one for marine fish. The reported 96-hour LC_{50} values of BIT ranged from 0.74 to 19 mg/L. The lowest LC_{50} value of 0.74 mg/L to *O. mykiss* was determined in a 96-hour static test according to US EPA Pesticide Assessment Guidelines Subdivision E 72-1. The average percentage of the substance present during the test was > 80% of initial concentrations, so the endpoints were determined based on nominal concentrations.

Several studies were submitted on the acute toxicity of BIT to freshwater crustaceans and one marine crustacean species. For the freshwater species, all 48-hour EC_{50} values of BIT were in range from 1 to 10 mg/L. For the marine water species, the lowest 96-hour EC_{50} value of 0.99 mg/L for *Mysidopsis bahia* was assessed under static conditions in sea water in accordance with EPA Guideline 72-3; SEP 600/9 78-010. Analysis of the test solutions indicated that exposure concentrations were adequately maintained. The endpoint values are based on mean measured concentrations.

Regarding acute toxicity in algae, four studies with *P. subcapitata* following OECD TG 201 and one study with *Phaeodactylum tricornutum* following Draft ISO Guideline "Marine Algal Growth Test" were submitted. The DS considered that the growth curves indicate strong effects within the first 24-hours of exposure and a recovery of growth which is dependent on dosing concentrations. Based on this observation and information on the mode of action of isothiazolinones, the DS suggested to estimate effects on algae after 24 hours of exposure based on initial measured concentrations in place of the geometric mean measured concentration over the normal test duration (0-72 hours). Thus, for two

of the four studies (Desjardins *et al*., 2006b and Smyth *et al*., 1994) initial measured concentrations were used for endpoints calculation and for other two (Kasthuri Raman, 2002 and Oldersma *et al*., 2002) the nominal concentrations for endpoint calculations were used instead of the geometric mean measured concentration over the test duration (0-72 hours).

The reported 24-hour E_rC_{50} values from four studies were in a range from 0.011 to 0.48 mg/L for *P. subcapitata*.

The reported 48-hour E_rC_{50} values from four studies were in a range from 0.017 to 0.8 mg/L for *P. subcapitata*.

The reported 72-hour E_rC_{50} values from four studies were in a range from 0.026 to 0.99 mg/L for *P. subcapitata*.

The lowest acute endpoints (ErC_{50}) for algae (*P. subcapitata*) were observed at 24h. As there were four data points for the same species and the same endpoint following OECD TG 201, the geometric mean approach was taken by the DS to derive aquatic acute 24-hour ErC_{50} value. The geomean was obtained from four different studies based on four E_rC_{50}s at 24h, two of them being based on initial measured concentrations (Desjardins *et al*., 2006b and Smyth *et al*., 1994) and two of them based on nominal concentrations (Kasthuri Ramen, 2002 and Oldersma *et al*., 2002). This results in a 24-hour E_rC_{50} of 0.1087 mg/L for *P. subcapitata*, based on the geomean.

Overall, the DS proposed to classify BIT as Aquatic Acute in category 1 based on the 24-hour geometric mean E_rC_{50} for *P. subcapitata* of 0.1087 mg/L. As this acute toxicity value falls within the $0.1 < L(E)C_{50} \leq 1$ mg/L range, M-factor proposed by the DS is 1.

Aquatic Chronic toxicity

Test method	Test organism	Long-term result (endpoint) mg/L	Reference / Test item
Fish			
OECD TG 210; EPA OPPTS 850.1400/GLP	*Pimephales promelas*	33d NOEC 0.2 (mm)	Anonymous (2007b) (CAR)/BIT (89.8 %)
Draft OECD TG 215/GLP	*Oncorhynchus mykiss*	28d NOEC 0.21 (mm)	Anonymous (2000a) CAR/PROXEL™ Press Paste (BIT 70 %)
Aquatic invertebrates			
OECD TG 211; EPA OPPTS 850.1300/GLP	*Daphnia magna*	21d NOEC 0.91 (mm)	Palmer *et al.* (2007c) (CAR)/BIT (89.8 %)
OECD TG 211/GLP	*Daphnia magna*	21d NOEC 1.2 (mm)	Penwell and Roberts (2000b) (CAR)/PROXEL™ Press Paste (BIT 70 %)
Algae / other aquatic plants			
Draft ISO Guideline "Marine Algal Growth Test"/GLP	*Phaeodactylum tricornutum*	24h E_rC_{10} = 0.084 48h E_rC_{10} = 0.063 72h E_rC_{10} = 0.081 (mm)	Smyth and Brown (1991) (CAR)/PROXEL GXL (BIT 20 %)
OECD TG 201; EPA OPPTS 850.5400/GLP	*Pseudokirchneriella subcapitata*	**24h E_rC_{10} = 0.032** 48h E_rC_{10} = 0.19 72h E_rC_{10} = 0.24 (recalculated endpoints using initial measured conc.)	Desjardins *et al.* (2006b) (CAR)/BIT (89.8 %)
OECD TG 201/GLP	*Pseudokirchneriella subcapitata*	**24h E_rC_{10} = 0.035** 48h E_rC_{10} = 0.043 72h E_rC_{10} = 0.057 (recalculated endpoints	Smyth *et al.* (1994) (CAR)/PROXEL™ Press Paste (BIT 73.3 %)

		using initial measured conc.)	
OECD TG 201/GLP	*Pseudokirchneriella subcapitata*	**24h E_rC_{10} = 0.0029** 48h E_rC_{10} = 0.0032 72h E_rC_{10} = 0.0044 (recalculated endpoints using nom. conc.)	Kasthuri Raman (2002) (CAR) / BIT (71.08 %)
OECD TG 201/GLP	*Pseudokirchneriella subcapitata*	**24h E_rC_{10} = 0.16** 48h E_rC_{10} = 0.3 72h E_rC_{10} = 0.25 (recalculated endpoints using nom. conc.)	Oldersma *et al.* (2002) (CAR)/BIT (98 %)

mm: mean measured concentration, nom: nominal concentration

Two studies were submitted on the chronic toxicity of BIT to fish. The reported NOEC values of BIT were in a range from 0.21 to 0.28 mg/L, based on mean measured concentrations. Nevertheless, CLP prefers tests consistent with OECD TG 210, the fish life-cycle test, for classification as it includes all life stages. Tests according to the OECD TG 215 are designed to assess the effects of prolonged exposure to chemicals on the growth of juvenile fish, but they do not include other typical long-term parameters such as hatching success, spawning success and survival. However, the Information Requirement and Chemical Safety Assessment guidance indicates that the juvenile growth test (OECD TG 215) can be accepted if there are well founded justifications indicating that growth inhibition is the most relevant effect in fish for the assessed substance. The study demonstrates that the most sensitive endpoint is growth. Therefore, the resulting 28d NOEC of 0.21 mg/L derived using OECD TG 215 were proposed by the DS as a reliable and relevant long-term endpoint for fish.

Two studies were submitted on the chronic toxicity of BIT to invertebrates. The lowest reported chronic toxicity endpoint was a 21d NOEC of 0.91 mg/L based on mean measured concentration for reproduction related effects (number of offspring per reproductive day).

Regarding to chronic toxicity to algae, four studies with *P. subcapitata* according to OECD TG 201 and one study with *P. tricornutum* according to Draft ISO Guideline "Marine Algal Growth Test" were submitted. The reasoning of the DS to consider using a 24-hour E_rC_{10} was described in the acute aquatic toxicity section.

The reported 24-hour E_rC_{10} values from four studies were in a range from 0.0029 to 0.16 mg/L for *P. subcapitata*.

The reported 48-hour E_rC_{10} values from four studies were in a range from 0.0032 to 0.3 mg/L for *P. subcapitata*.

The reported 72-hour E_rC_{10} values from four studies were in range from 0.0044 to 0.25 mg/L for *P. subcapitata*.

The lowest chronic endpoints (E_rC_{10}) for algae (*P. subcapitata*) were observed at 24h. As there were four data points for the same species and the same endpoint following OECD TG 201, the geometric mean approach was taken by the DS to derive aquatic chronic 24-hour ErC_{10} value. A geomean was obtained from four different studies based on four E_rC_{10}s at 24h, two of them being based on initial measured concentrations (Desjardins *et al*., 2006b and Smyth *et al*., 1994) and two of them based on nominal concentrations (Kasthuri Ramen, 2002 and Oldersma *et al*., 2002). This results in a 24-hour E_rC_{10} of 0.0268 mg/L for *P. subcapitata* based on the geomean.

Overall, the DS proposed to classify BIT as Aquatic Chronic in category 1 based on the 24-hour geometric mean E_rC_{10} for *P. subcapitata* of 0.0268 mg/L. As the substance is considered not rapidly degradable and the chronic toxicity value falls within the $0.01 < NOEC \leq 0.1$ mg/L range, the M-factor proposed by the DS is 1.

Comments received during consultation

Two industrial companies, two MSCAs and one National Authority (NA) commented on the environmental part of the DS's proposals. Industrial comments were related to editorial changes and only requested clarification for some of the values used. The DS confirmed the requested values. Two MSCAs agreed with the proposed classification and suggested some editorial changes or clarifications to the proposal. The DS agreed with the editorial changes and provided the proposed clarifications for the MSCAs.

The NA agreed that based on the rapid MoA and loss of the test item, 24-hour algal endpoints based on initial measured or nominal concentrations are suitable for acute hazard classification. To support this approach, the NA asked for clarification on whether the OECD TG 201 validity criteria of control specific growth rate were met for each of the *P. subcapitata* 24-hour acute endpoints. In addition, the NA expressed doubt on PROXEL formulation suitability for hazard classification and asked about the impact of formulation ingredients and if the endpoints were reliable for hazard classification. The NA also agreed with the use of the geomean of the four *P. subcapitata* 24-hour E_rC_{50} values for acute classification if the endpoints using PROLEX will be considered as reliable for hazard classification.

The DS confirmed that in relation to PROXEL, the impurity profile of BIT does not imply any additional ecotoxicological hazards and such studies with PROXEL are considered valid for classification. The DS also confirmed that after discussions in the Biocides WG and an assessment of validity criteria for all tests available, the four studies with *P. subcapitata* were considered reliable.

For the aquatic chronic classification, the NA pointed out that 24 hours is not a suitable duration to assess long-term effects and prefer to use 72-hour endpoints in line with standardised hazard classifications. These 72-hour endpoints should be expressed as initial measured or nominal concentrations given that the test item is taken up by algae, so it is not available after the initial toxic effect. In addition, the NA considered that the *P. subcapitata* 72-hour endpoint from the study by Kasthuri Raman (2002) is not reliable for aquatic chronic classification because the OECD TG 201 validity criteria for control growth were not met over the 72-hour time period. Therefore, there are only three other *P. subcapitata* studies for BIT and they would not be applicable to calculate the geometric mean. Thus, the lowest 72-hour E_rC_{10} should drive the chronic classification instead. As the lowest chronic endpoint with *P. subcapitata* is 72-hour E_rC_{10} of 0.057 mg/L based on initial measured concentrations in the study Smyth *et al*. (1994), the NA asked for confirmation of the validity criteria for this study.

Regarding to study of the Smyth *et al*. (1994), the DS confirmed that all validity criteria are fulfilled:

- ✓ It fulfils exponential growth criteria.
- ✓ Mean coefficient of variation section by section = 0.119. It meets the criteria and does not exceed 35 %.
- ✓ Coefficient of variation of average specific growth rates for 72h = 0.031. It meets the criteria and does not exceeds 7 %.
- ✓ Initial cell density is 10400 cells/mL.
- ✓ Given reliability: 2

Regarding to the study of Kasthuri Raman (2002), the DS indicated:

- ✓ It fulfils exponential growth by more than a factor of 16.
- ✓ Mean coefficient of variation section by section is 0.47 and 0.48 for the control and vehicle control respectively. None of them meet the 35% criteria.
- ✓ Coefficient of variation of average specific growth rates for 72h = 0.021 meeting the 8 % criteria.
- ✓ Initial cell density is 12050 cells/mL. Guideline recommends a cell density equal to 10000 cells/mL.
- ✓ Reliability of the study: 2

In addition, the DS noted that the reasons why the study was evaluated as reliable after the BPC WG ENV and Adhoc ENV expert group follow up in 2015 by all Member States, were:

- ✓ The biological section of the study can be considered good. Between control and vehicle control, each containing 6 replicates, there are not important differences in cell density values. The study is done under GLP.
- ✓ The study does not fulfil the second criterion by a 13 %. Nevertheless, when the study was done, the second criterion did not apply. Besides, there are other cases where a study not fulfilling the second criterion was accepted. This is the case of MIT.
- ✓ Finally, despite there is no analytical verification at the concentrations tested, the study provides data that shows that concentrations of the test substance are maintained within 20 % of nominal concentrations making it possible to calculate endpoints based on nominal concentrations. Proper chemical analysis probably would have led to even lower test concentrations.

Regarding use of 24-hours vs 72-hours test values, the DS referred to the BPC WG (ENV) and to the Adhoc ENV group documents and discussions. The DS also pointed out that 72h endpoints based on initial measured concentration would not reflect the mode of action of the substance since it would allow for recovery and not consider the interaction between algal cell density and substance disappearance. Hence, use of 24h endpoints by the DS is justified. In addition, the DS noted that in other similar substances, such as MIT, the same approach was followed.

Assessment and comparison with the classification criteria

Mode of action of isothiazolinones

In algal toxicity tests (according to OECD TG 201), isothiazolinones typically dissipate during the exposure period to levels below the detection limit. This mode of action of isothiazolinones has been extensively researched. The biocidal effect is described as a two-step process involving rapid inhibition of growth and metabolism leading to a loss in viability of the cells. These effects occur within minutes at the enzymatic level and can result in loss of viability within hours of exposure. The MoA of isothiazolinones is, however, complex as these molecules react with several specific enzymes, which are essential within critical metabolic pathways. According to this MoA, uptake of BIT through the cell wall and membrane of the algae occurs rapidly, within hours and facilitates the activity of the biocide. Concomitant with uptake and enzymatic inhibition, the isothiazolinone ring is cleaved rendering the molecule inactive. This means that the inhibitory effect on algae is directly responsible for degradation of the molecule by the algae.

The rapid MoA of isothiazolinones is apparent in certain algal studies. The growth curves indicate strong effect within the first 24h of exposure and a recovery of growth which is dependent on dosing concentrations. Based on this observation and information on the mode of action of the biocide, it was suggested to estimate effects on algae after 24 hours of exposure based on initial measured concentrations. This approach was accepted in the assessment of other isothiazolinones in the BPD/BPR Review Program (e.g., DCOIT and MIT). If the most sensitive time period is not 24 hours, the kinetics of BIT has to be considered. Thus, for each of the studies it has to be assessed when the strongest effect occurs, and the endpoint has to be estimated accordingly.

Degradation

BIT is not demonstrated to be readily biodegradable in available 28-day tests for ready biodegradability. All available degradation tests indicate less than 25 % degradation after 28 days.

No hydrolysis of BIT is observed and the substance is stable in solutions at pH 4 to 9 at 50 ºC. the estimated half-lives for BIT were > 1 year at 12 °C.

BIT is very unstable under artificial sunlight in aquatic medium and very rapidly photolysed at all pH values. The half-life of BIT under artificial sunlight at pH 7 and 9 is estimated to be < 1 hour and at pH 5 it is 9 hours. Photolytic degradation of BIT in water resulted in a number of degradation products

in different amounts depending on the pH value of the medium. However, guidance on CLP criteria indicates that photochemical degradation is difficult to use for classification purposes since the actual degree of photochemical degradation in the aquatic environment depends on local conditions.

Aerobic degradation in estuarine and sea water indicates that BIT is primarily biodegradable in estuarine (brackish) and sea water with half-lives of 22.9 29.8 hours and 5.3–12.2 days at 12 °C, respectively, and formed 3–4 metabolites. However, BIT is not ultimately biodegradable since the level of mineralisation is very poor (≤ 1 % AR was evolved as CO_2). In addition, one more aerobic degradation study in sea water shows that BIT is not ultimately degradable. Therefore, BIT is primarily degraded very fast in aerobic aquatic systems, but it cannot be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

Aerobic degradation in soil indicates that BIT rapidly disappears from soils with half-live values from 0.02 to 0.54 days at 12 ºC. Mineralisation reached 40-56 % depending on the soil type. However, based on the CLP guidance, in case of conflicting results of degradation simulation studies, simulation test data of surface water are preferred to aquatic sediment or soil simulation test data in relation to the evaluation of rapid degradability in the aquatic environment.

Overall, due to the results summarised above, RAC considers that despite the ultimate photolysis in water and rapid aerobic degradation in soil BIT is not ultimately degraded to > 70 % within 28 days (equivalent to a half-life < 16 days), or rapidly transformed to non-classifiable products. Consequently, RAC agrees with the DS that BIT does not fulfil the CLP criteria for rapidly degradability.

Aquatic Bioaccumulation

The estimated BCF 3.162 L/Kg and the experimental mean steady-state BCF 6.95 L/kg_{wwt} for whole fish in *Lepomis macrochirus* is below the CLP trigger value of ≥ 500. The experimentally determined log K_{OW} 0.70 and the estimated log K_{OW} 0.64 are also below the CLP trigger value of ≥ 4. Although the experimental BCF of 6.95 L/kg_{wwt} was not normalised to a lipid content of 5 %, the log K_{OW} of BIT suggests that it will not bioaccumulate in the aquatic environment and the mean steady-state BCF is comparable to the estimated BCF value.

Therefore, RAC agrees with the DS that BIT is not bioaccumulative according to the CLP criteria.

Aquatic Toxicity

RAC notes that there are reliable acute and chronic aquatic toxicity data for all trophic levels. The most acutely and chronically sensitive trophic group is algae with *P. subcapitata* being the most sensitive species. RAC assumes that the test item PROXEL formulation is suitable for aquatic hazard classification as the constituent profile of BIT does not add any additional ecotoxicological hazards. However, RAC was not able to assess impurities/additivities of PROXEL formulation as this information was not provided.

Regarding the validation criteria of the available four studies with *P. subcapitata*, RAC would like to stress that the OECD TG 201 second validity criteria "*…the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35 %...*" was not met in the Kasthuri Raman (2002) study (please see "*Supplemental information*"). Nevertheless, RAC is aware that after discussions in the BPC WG (ENV) and Adhoc ENV expert group the validity criteria for available four studies with *P. subcapitata* were considered met. In addition, it should be noted that when the study was done, the second criterion did not apply. Additionally, there are cases (MIT) when studies not fulfilling the second criterion was accepted by RAC.

RAC has previously considered that initial measured concentrations are more appropriate for hazard classification purposes of isothiazolinones. The endpoints for *P. subcapitata* here were represented as a geomean of four different studies based on four ErC_{xx} at 24h, two of them being based on initial

measured concentrations (Desjardins *et al*., 2006b and Smyth *et al*., 1994) and two of them based on nominal concentrations (Kasthuri Ramen, 2002 and Oldersma *et al*., 2002). OECD TG 201 indicates that "*…if there is evidence that the concentration of the substance being tested has been satisfactorily maintained within ± 20 % of the nominal or measured initial concentration throughout the test, analysis of the results can be based on nominal or measured initial values…*".

Therefore, RAC considers that all four studies with the algae *P. subcapitata* are reliable and acceptable.

Therefore, RAC agrees that the geometric mean of toxicity values from four studies with *P. subcapitata* may be used as the representative toxicity value for this species as indicated in the CLP guidance.

ECHA guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b foresees the possibility to adopt a shorten test period (48h) with respect to the usual duration of 72h or 96h, still the 24h length for this test is not mentioned in the guidance.

The CLP Guidance indicates that acute aquatic toxicity is normally determined using a fish 96-hour LC_{50}, a crustacea species 48-hour EC_{50}, an algal species 72- or 96-hour EC_{50} and/or aquatic plants 7 days EC_{50}. However, the CLP Guidance indicates that there can be circumstances when a weight of evidence approach is appropriate. Chronic toxicity exposure durations can vary widely depending on the test endpoint measured and test species used.

OECD TG 201 allows use of shorter test periods if "*… the test which runs over a period of normally 72 hours, in spite of being a relatively brief test duration, effects over several generations can be assessed…. The test period may be shortened to at least 48 hours to maintain unlimited, exponential growth during the test as long as the minimum multiplication factor of 16 is reached.* "

RAC acknowledges that 72-hour endpoints in the case of BIT based on initial measured concentration would not reflect the MoA of the substance since it could allow for recovery, not taking into account the interaction between algal cell density and substance disappearance (please see Additional key elements). Although, RAC recognises that using 24-hour endpoints was considered by the BPC WG (ENV) and Adhoc ENV expert group, the DS does not clearly indicate that the validity criteria for relevant endpoints (minimum multiplication factor of 16 is reached at 24 and/or 48 hours) were met. However, the DS indicates that each endpoint of the studies was assessed in this regard when the strongest effect occurs, and the endpoint was estimated accordingly. Still, as the robust study summaries were not available to RAC and the DS does not provide the multiplication growth factor, RAC was not able to confirm that the validity criteria for the control performance (exponential control growth greater than a factor of 16) on all relevant endpoints was reached.

Nevertheless, RAC notes that using 48-hour or 72-hour endpoints for the geometric mean (based on initial measured concentrations from two studies (Desjardins *et al*., 2006b and Smyth *et al*., 1994) and on nominal concentrations from other two studies (Kasthuri Ramen, 2002 and Oldersma *et al*., 2002)) will result in the same classification outcome:

24-hour E_rC_{50} of 0.1087 mg/L (geomean)

48-hour E_rC_{50} of 0.1696 mg/L (geomean)

72-hour E_rC_{50} of 0.1968 mg/L (geomean)

24-hour E_rC_{10} of 0.0268 mg/L (geomean)

48-hour E_rC_{10} of 0.0529 mg/L (geomean)

72-hour E_rC_{10} of 0.0623 mg/L (geomean)

Overall, considering the MoA of isothiazolinones, the DS confirmations on tests criteria validation, indications that each endpoint of the studies was assessed in this regard when the strongest effect occurs, and that the endpoint was estimated accordingly as well assuming BPC WG (ENV) and Ad hoc ENV expert group opinions, RAC agrees that the use of 24-hours endpoint in case of BIT is

appropriate. Furthermore, the shorter test periods were used by RAC for previous assessments of isothiazolinones:

MIT (2-methylisothiazol-3(2H)-one CAS number: 2682-20-4): classification based on 24-hour E_rC_x values based on initial measured concentration (validity criteria of the control performance were met for the first 24h).

MBIT (2-methyl-1,2-benzothiazol-3(2H)-one; CAS number: 2527-66-4): classification based on 48-hour E_rC_x values based on initial measured concentrations (the validity criteria were met).

C(M)IT/MIT (Reaction mass 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H -isothiazol-3-one (3:1) CAS number: 55965-84-9): classification based on 48-hour E_rC_x values based on mean measured concentration (validity criteria fulfilled at 48h in the algal study).

DCOIT (4,5-dichloro-2-octyl-2H-isothiazol-3-one; CAS number: 64359-81-5): aquatic acute classification based on 24-hour E_rC_{50} based on initial measured concentrations (general validity criteria for the test are met including a growth rate higher than 0.92 per day at 24h). Aquatic chronic classification is based on 48-hour E_rC_{10} based on initial measured concentrations (instead of 24-hour because 48-hour endpoint is more relevant to assess the effect over several generations).

OIT (octhilinone (ISO); 2-octyl-2H-isothiazol-3-one; CAS Number: 26530-20-1): classification based on 48-hour E_rC_x value based on initial measured concentrations (validity criteria were met for 0-48 hours including exponential growth over this period).

Consequently, RAC agrees that the lowest acute endpoint for aquatic acute classification is the 24-hour E_rC_{50} geomean value for *P. subcapitata* of 0.1087 mg/L. The lowest chronic endpoint for aquatic chronic classification is the 24-hour E_rC_{10} geomean value for *P. subcapitata* of 0.0268 mg/L.

Conclusion on classification

BIT is considered as not rapidly degradable and does not fulfil the criteria for bioaccumulation. Based on the available and reliable information, RAC agrees with the DS that BIT warrants classification as:

Aquatic Acute 1 (H400) based on E_rC_{50} = 0.1087 mg/L for *P. subcapitata.* As this acute toxicity value falls within the 0.1 < L(E)C_{50} ≤ 1 mg/L range, **M = 1**.

Aquatic Chronic 1 (H410) based on E_rC_{10} = 0.0268 mg/L for *P. subcapitata*. As this chronic toxicity value falls within the 0.01 < NOEC ≤ 0.1 mg/L range, **M = 1.**

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Table 24: Summary table of data concerning hazardous properties of the substance for the ozone layer

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
TGD 2003	BIT	K_{OH} = 287.5 x 10^{-13} cm^3.$molecule^{-1}$.sec^{-1} K_{NO3} = 0.512 x 10^{-13} cm^3.$molecule^{-1}$.sec^{-1}	Half-life (OH): 10.3 hours Half-life (NO_3): 15.7 hours	Guo, 2007 CAR IIIA7.3.1
Atkinson calculation method. Aopwin v1.92	BIT	Photolysis rate constant (k^c_p): 16.9594 × 10^{-12} cm^3 $molecule^{-1}$ sec^{-1}	Half-life (OH): 0.946 days (22.7 h) (based on 24-hour days)	Ritter, 2006 McAteer, 2007 CAR IIIA 7.3.1

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

There are not studies for ozone layer hazard of BIT. However there is information provided in the CAR of BIT which includes predictions of the half-life of BIT expected in the air.

Effects on stratospheric ozone by a substance may be expected if the atmospheric lifetime is long enough to allow for transport to the stratosphere, and it contains one or more Cl, Br or F substituents.

BIT does not have Cl, Br or F substituents in its molecule. In addition, given the short half-life of BIT expected in the air (i.e. 23 hours), as a consequence of indirect photolytic reactions, and the low vapor pressure (i.e. 62.76 x 10^{-4} Pa at 20ºC) and Henry's Law Constant (1.45 x 10^{-5} - 7.4 x 10^{-6} Pa m^3 mol^{-1} at 20 ºC), the substance will be found in negligible amounts in the stratosphere. Thus it may be considered that the ozone depletion potential of BIT approaches zero.

12.1.2 Comparison with the CLP criteria

According to Annex I to CLP 'a substance shall be classified as Hazardous to the Ozone Layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer'.

The Guidance indicates that 'any substances having an Ozone Depleting Potential (ODP) greater or equal to the lowest ODP (i.e. 0.005) of the substances currently listed in Annex I to Regulation (EC) No 1005/2009 should be classified as hazardous to the ozone layer (category 1)'.

Since BIT will be found in negligible amounts in the stratosphere, it may be considered that the ozone depletion potential of BIT approaches zero.

Therefore BIT does not meet the CLP classification criteria and consequently it is not proposed to be classified as Hazardous to the Ozone Layer.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

The DS does not propose the classification and labelling of BIT for Hazardous to the Ozone Layer.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

The DS does not propose the classification and labelling of BIT for Hazardous to the Ozone Layer.

BIT does not have Cl, Br or F substituents in its molecule. In addition, given the short half-life of BIT expected in the air (i.e., 23 hours) as a consequence of indirect photolytic reactions, the low vapor pressure (i.e., 6.28 × 10^{-5} Pa at 20 ºC), and Henry's Law Constant (1.45 × 10^{-5}-7.4 × 10^{-6} Pa m^3 mol^{-1} at 20 ºC), the substance will be found in negligible amounts in the stratosphere. Thus, it may be considered that the ozone depletion potential of BIT approaches zero.

Comments received during consultation

One comment was received from a company-manufacturer which supported the DS's conclusion that no classification was warranted for hazards to the ozone layer.

Assessment and comparison with the classification criteria

A substance shall be classified as hazardous to the ozone layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

The BIT does not contain any moieties indicating Ozone Depleting Potential (currently listed in regulation EC No. 1005/2009) in its molecule.

BIT has a low vapour pressure (6.28×10^{-5} Pa at 20 ºC). The Henry's law constant of BIT is 1.45×10^{-5}-7.4×10^{-6} Pa m^3 mol^{-1} at 20 ºC. Thus, BIT is not volatile and does not partition from aqueous phases to air.

As a consequence of indirect photolytic reactions, the half-life of BIT expected in the air is short (i.e., 23 hours).

Therefore, RAC agree with DS that BIT does not meet the CLP classification criteria and consequently **does not warrant classification as Hazardous to the Ozone Layer**.

12.2 Endocrine disruption assessment

To evaluate a potential concern for endocrine diruption effects induced by BIT all available data were assessed for all levels of the OECD Conceptual Framework (Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009).

These data include experimental data generated by Lanxess Deutschland GmbH, Lonza Ltd, Specialty Electronic Materials Switzerland GmbH (former The Dow Chemical Company), Thor GmbH, Troy Chemical Company B.V. as well as public available information (from an updated literature research performed in Feb. 2019).

Toxicological and ecotoxicological studies have been performed with the active substance (BIT).

All relevant information available for BIT is showed below following the OECD Conceptual Framework levels:

LEVEL 1

Existing data and non-test information, covered, physical and chemical properties, toxicological data from standardized or not-standardized tests, and read-across, chemical categories, QSARs and another *in silico* predictions, and ADME model predictions.

Literature research

The applicant included a literature research which provided the following results:

An endocrine-related mechanistic study was identified for BIT from the published literature. These include:

- An *in vitro* study of human estrogen receptor (ER) binding and transactivation (Nakama *et al.*, 2007)
- An *in vitro* study of thyroid peroxidase (TPO) inhibition (Friedman *et al.*, 2016)

- An *in vitro* study of iodothyronine deiodinases (DIOs) inhibition (Olker *et al.*, 2019)

Additionally, the ES-CA performed a literature research following the strategy shown below:

Term "BIT" has been used as key word in a literature research, but not relevant information related to endocrine disruption has been found.

Additional literature research using different key words were developed in PubMed and Web of Science databases. After checking several searching strategies, the main wider search considered appropriate can be defined as follows:

- (hormone OR hormones OR hormonal OR endocrine OR disruptor OR disruption OR disruptors OR estrogenic OR androgenic OR thyroid) AND (1,2-benzisothiazol-3(2H)-one OR BIT OR benzo-[d]-isothiazol-2-one OR 1,2-benzisothiazolin-2-one).

  From the wider search strategy in PubMed database, 156 references, 0 have 1,2-benzisothiazol-3(2H)-one or BIT or benzo-[d]-isothiazol-2-one or 1,2-benzisothiazolin-2-one in the title. From the wider search strategy in Web of Science database, 3057 references, 0 have 1,2-benzisothiazol-3(2H)-one or BIT or benzo-[d]-isothiazol-2-one or 1,2-benzisothiazolin-2-one in the title.

A more restricted specific search was as follows:

- (((endocrine) AND ((disruptor or disruption or disruptors)))) AND (((endocrine) AND ((disruptor or disruption or disruptors)))) AND ((((BIT) OR 1,2-benzisothiazolin-2-one) OR 1,2-benzisothiazol-3-(2H)-one) OR 2634-33-5) OR (((((estrogenic) OR androgenic)) OR thyroid)) AND ((((BIT) OR 1,2-benzisothiazolin-2-one) OR 1,2-benzisothiazol-3-(2H)-one) OR 2634-33-5)

  In a manual review of the last strategy obtaining 259 articles in Web of Science database (0 issues in PubMed database), checking in title there are not studies with evaluation of effects potentially relevant to endocrine effects, including effects of androgenic, antiandrogenic, estrogenic, antiestrogenic, or thyroid. There are not related results.

Additionally, ES-CA performed a literature research using SIN and TEDX databases. 1,2-benzisothiazol-3(2H)-one was not included.

QSAR models

BIT is out of domain in most of the models included in the used databases, so no prediction is obtained for them. Models compatible with BIT give a negative prediction for AR or ER binding and activation and PXR binding (OECD Toolbox).

Endocrine Disruptome gives a medium probability (≈ 40%) of binding as antagonist to AR for BIT. The other human nuclear receptors analyzed give a negative prediction in binding and antagonist modality for ER and GR, and in binding modality only for AR, LXRα/β, MR, PPARα/β/δ/γ, PR and RXRα.

LEVEL 2

In vitro assays providing data about selected endocrine mechanism(s) and pathway(s).

Additionally, a revision of ToxCast and EDSP21 about BIT was carried out by the applicant and checked by the CA (Annexes III and IV).

BIT (CAS No. 2634-33-5) has several results in the US EPA Chemistry Dashboard (EDSP21 + ToxCast Dashboards); some of them were relevant endocrine endpoints addressed in the US EPA Chemistry Dashboard database: 26 estrogen assays, 16 androgen assays, 10 thyroid assays and 2 sterodiogenesis assays.

BIT has been tested in 18 of the 26 estrogen receptor EDSP21 assays that are informative regarding the estrogen modality. BIT was positive in four assays of antagonist activity at the human ER1 (one assay: over cytotoxic limit) and ER2 (one assay) and in two assays of complementation between ER2 (one assay: less than 50% efficacy; only one concentration above baseline; above cytotoxic limit) and ER1+ERE (one assay: less

than 50% efficacy; only highest concentration above baseline; over cytotoxic limit). CERAPP establishes BIT as inactive by agonist, antagonist and binding activities.

BIT has been tested in 11 of the 18 androgen receptor EDSP21 assays that are informative regarding the androgen modality. BIT was positive in three assays of antagonist activity at the human AR (three assays: over cytotoxic limit) and in two assays of complementation between AR+Src1 (two assays: above cytotoxic limit). COMPARA establishes BIT as inactive by agonist activity and active by antagonist and binding activities.

BIT has been tested in 1 of the 2 EDSP21 assays that are considered informative regarding the steroidogenesis modality. It was positive in the assay of aromatase inhibition assay (one assay: over cytotoxic limit).

BIT has been tested in 10 of the 10 EDSP21 assays that are considered informative regarding the thyroid hormone modality. BIT was positive in an assay of antagonist activity at the human TSHR (one assay: above cytotoxic limit), in two assays of antagonist activity at the human ThR and in two assays of Na^+-I^- symporter function (two assays: less than 50% efficacy; above cytotoxic limit).

LEVEL 3

In vivo assays providing data about selected endocrine mechanism(s) and pathway(s).

No information on such *in vivo* assays is available for BIT.

Table 24: Information on level three assays for endocrine disruption

Mammals	Information available in the current dossier
E, A modalities	
Uteroptrophic bioassay in rodents (OECD TG 440)	N
Hershberger bioassay in rats (OECD TG 441)	N
Non-mammals	
E, A, S modalities	
Fish short term reproduction assay (OECD TG 229)	N
21-dayfish assay (OECD TG 230)	N
T-modality	
Amphibians metamorphosis assay (OECD TG 231)	N

LEVEL 4

Table 25: *In vivo* assays providing data on adverse effects on endocrine relevant endpoints.

Mammals	Information available in the current dossier
T-modality	
Repeated dose 28-day study (OECD TG 407)	Y
Repeated dose 90-day study (OECD TG 408)	Y
Repeated dose 90-day study in non-rodents (OECD TG 409)	Y
Combined chronic toxicity and carcinogenicity studies (OECD TG 451-3)	N/A
Non-mammals	
T-modality	
Larval amphibian growth and development assay (LAGDA) (OECD TG 241)	N

In the repeated dose 28-day study (OECD TG 407), repeated dose 90-day studies (OECD TG 408) and in the repeated dose 90-day studies in non-rodents (OECD TG 409), in general terms, there are not significant dose-related or histopathological-supported changes so this study does not show endocrine related effects or adversity in any modality.

Competent Authority has determined that, based on available data from subchronic toxicity studies, genotoxicity assays, metabolism/disposition studies and structure-activity analyses, BIT and related isothiazolinones are unlikely to be carcinogenic . Therefore, conducting such studies is scientifically not justified and would not add any further weight to the overall analysis.

Although relevant studies to consider T-modality sufficiently investigated are missing for non-mammals, other optional studies are present.

In the fish early stage life toxicity test (OECD TG 210) adverse effects have been observed on survival, behaviour, hatching and growth at concentrations above the MATC; that could imply a systemic toxicity.

In the *Daphnia magna* reproduction test (OECD TG 211), despite the effects on survival, reproduction and growth, it is not possible to draw any conclusion from the assay due to the absence of the endpoint male production, which has not been measured.

In the chironomid toxicity test using spiked sediment (OECD TG 218) observed effects could be interpreted as a proof of some evidence for adverse *in vivo* effects, possibly but not necessarily caused by JH or EC (ant) agonists.

LEVEL 5

Table 26: *In vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism.

Mammals	Information available in the current dossier
EAST-modality	
Two-generation reproduction toxicity study (OECD TG 416)	Y
Non-mammals	
EAS-modality	
Medaka extended one-generation reproduction test (MEOGRT) (OECD TG 240)	N

In the two-generation reproduction toxicity study (OECD TG 416), in general terms, there are not significant dose- or develop-related and histopathology-supported changes so this study does not show endocrine related effects or adversity in EAS-modality. Changes in adrenal glands weight could be a systemic toxicity related effect, but in any case there are not coherence between studies or between sexes.

Conclusion

According to the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, EATS-mediated adversity and endocrine activity have not been sufficiently investigated. Additional information should be requested, but due to the Art. 90 BPR this is not possible.

However, despite that a clear conclusion cannot be reached about endocrine disruption properties, it is highly unlikely that BIT accomplishes such properties.

13 ADDITIONAL LABELLING

14 REFERENCES

Additional Appendix.

Additional references

Additional references not included in the CLH report

Aalto-Korte K., Suuronen K, Patterns of concomitant allergic reactions in patients suggest cross-sensitization between octylisothiazolinone and methylisothiazolinone. *Contact Dermatitis* 2017, 77:385–389.

Craig S, Urwin R, Latheef F, Wilkinson M, Patch test clinic experience of potential cross-reactivity of isothiazolinones. *Contact Dermatitis* 2017, 76:299–300.

Flyvholm MA Preservatives in registered chemical products. *Contact Dermatitis* 2005: 53: 27–32.

Geier J, Lessmann H, Schnuch A, Uter W. Concomitant reactivity to methylisothiazolinone, benzisothiazolinone, and octylisothiazolinone. International Network of Departments of Dermatology data, 2009-2013. *Contact Dermatitis* 2015; 72(5):337-9.

Madsen, J, Andersen, K Contact allergy to 1,2-benzisothiazolin-3-one. *Contact Dermatitis* 2016; 75(5): 324-6.

Nielsen H., Occupational exposure to isothiazolinones. A study based on a product register. *Contact Dermatitis* 1994 31:18-21.

15 ANNEXES

15.1 ANNEX I:

Ecotoxicity data of degradation products of BIT

BIT was primarily biodegraded in different screening and simulation degradation studies, yielding several minor and mayor metabolites. Experimental data on ecotoxicity of these metabolites is not available, however QSAR modelling data (EPI Suite™ 4.11) were estimated for mayor degradates. A summary of ecotox estimations is presented in the following table. According to these predictions, all metabolites are less or equally toxic to aquatic organisms than BIT.

Table 28: Major BIT degradates and their related QSAR modelling data regarding ecotoxicity.

	2-sulfobenzamide	2-methylthio-benzamide	2-methylsulfinyl-benzamide	2-methylthio-benzoic acid methyl ester
Measured in test	Surface water degradation Ready biodegradation	Surface water degradation Ready biodegradation	Surface water degradation	Surface water degradation
Maximum measured in degradation tests	30% (surface water)	61.5% (ready) 54% (surface water)	24.9%	12.8%

Fish 96-h LC50 (mg/L)	2.92E+06	4.97E+02	1.92E+04	1.36E+01
Daphnid 48-h EC50 (mg/L)	3.46E+07	1.04E+03	1.09E+05	2.71E+01
Green algae 96-h EC50 (mg/L)	3.02E+04	1.15E+01	2.81E+02	1.08E+01
Fish (NOEC/ EC_{10}) (mg/L)	2.33E+02	3.70E-01*	3.97E+00	9.40E-01
Daphnid (NOEC/ EC_{10}) (mg/L)	6.45E+04	2.33E+01	5.84E+02	1.64E+01
Green algae (NOEC/ EC_{10}) (mg/L)	3.22E+03	6.04E+00	5.87E+01	3.18E+00

*Prediction done with a small data set and low r^2 and therefore it is considered with caution.

15.2 ANNEX II:

Studies considered as supporting information, rated R.I. 3 or 4

Table 28. Supporting information. Studies rated R.I. 3/4

Method	Study/Species	Results	R.I.	Remarks	Reference
EPA 162-1	Aerobic biodegradation in soil	DT50 = 7.2 h (13.65 at 12ºC)	3	Only one soil and pH; Other defficiencies	Roberts, 1989; Powell, 1989. IIIA 7.2.1, Lonza and Thor
OECD TG 121	Adsorption coefficient	Koc = 5.4 L/Kg	3	Major deviation from OECD guideline	Schouten and Verhoef, 2005. Troy Chemical Company B.V./Dow (IIIA.7.1.3/2)
OECD TG 202	*Daphnia magna*	EC50 = 0.43 mg BIT/L (based on measured concentrations)	3	Low concentrations, below LOQ, purity not defined	Nair 2003; IIIA 7.4.1.2/01
OECD TG 201	*Skeletonema costatum*	24h-ErC50 = 0.030 mg BIT/L	3	Exponential growth criterion not fulfil; mean coefficient of variation growth rate > 35%.	Desjardins et al. 2006a; DOC IIIA7.4.1.3.b/01
-	*P. subcapitata*	-	4	No guideline, no endpoints calculated	Penwell, 1997; IIIA 7.4.1.3(3)
OECD TG 201	*Skeletonema costatum*	24h-ErC50 = 0.030 mg BIT/L	3	Below LOQ; mean coefficient of variation growth rate > 35%	Softcheck, K. 2008

- Aerobic biodegradation in soil (R.I. 3), Roberts, 1989; Powell, 1989. IIIA 7.2.1, Lonza and Thor: a new study should be conducted following OECD guidelines: use of different soils that vary in their pH values and use of one abiotic control or, if impracticable, performance of the aerobic biodegradation tests under dark conditions. In this study data are provided on day 0, 1 and 4. However BIT degrades in hours making measurements at 1 and 4 days not relevant. Besides, the laboratory report did not indicate whether the test was conducted in the dark, and an abiotic control was not included. These points undermine the validity of the test which will be considered as additional information.

- Adsorption coefficient, RI 3, Schouten and Verhoef, 2005. Troy Chemical Company B.V./Dow (IIIA.7.1.3/2). OECD 121. A major deviation in the procedure was found and the result is not reliable (OECD 121 says "For ionisable substances, two tests should be performed with both ionised and non-ionised forms in appropriate buffer solutions but only in cases where at least 10 % of the test compound will be dissociated within pH 5.5 to 7.5". The applicant states that the whole test was performed at a pH of 5.5. This is indeed an

environmental relevant parameter for soils, but the pKa of the test compound is of pKa 7.2. Thus, at the tested conditions, BIT molecules are mostly in their neutral form).

- Acute toxicity to invertebrates according OECD TG 202 (Nair 2003; IIIA 7.4.1.2/01), on *D. magna* under semi-static conditions, resulted in a 48-hour EC50 of 0.43 mg BIT/L based on measured concentrations. However it was considered non-acceptable (R.I. 3) because the Daphnia were exposed to very low concentrations (ca. 0.2 to 2.0 mg a.s./L) during the test and the selected test concentrations were not detectable by the analytical method. Additionally the purity of BIT was not clearly defined in the lab report. Measurement of concentrations were only performed for the highest level of 10 mg a.s./L in a separate test in order to check the stability of the a.s. resulting in ca. 80% of the nominal at the test end. It is noted that the resulting EC50 is one order of magnitude below the other values obtained for the same species and following a similar procedure.

- *Skeletonema costatum*, R.I. 3 (Desjardins et al. 2006a; DOC IIIA7.4.1.3.b/01). Chronic effects of BIT to algae were investigated in the marine diatom *Skeletonema costatum.* The study exposed this species during 96h to 0, 0.019, 0.038, 0.075, 0.15, 0.30 and 0.60 mg BIT/l. Analytical confirmation of test solution concentrations was performed at 0 h and 96 h, when the concentrations decreased below the LOQ. This test was considered invalid since exponential growth criterion was not fullfil and the mean coefficient of variation of the daily/section by section specific growth rate at 72 and 96h was greater than 35%.

- *P. subcapitata*, R.I. 4 (Penwell, 1997; IIIA 7.4.1.3(3)): following aerobic biodegradation by a modified OECD 301B procedure, the aquatic toxicity of the degradation products BIT was investigated on *P. subcapitata*. The analytical data showed 95% degradation of BIT in the 1 mg C/l vessel (highest concentration) which was subsequently tested for algal toxicity. The thawed samples were diluted with algal medium in the ratio 55:45 to provide test solutions with 0.055 mg/l of degraded BIT, based on the initial analysed concentration in the algal toxicity study. 30% inhibition was obtained at a concentration of 0.055 mg BIT/L, although it is not possible to know if this inhibition effect was only due to BIT or to its degradation products. The study concluded that degradation products did not significantly contribute to the toxicity of BIT to algae. The study was not conducted according to a standard procedure for metabolites and the results did not offer new information. The RMS considered this study as non-acceptable (Reliability 4).

- A study with *Skeletonema costatum* following OECD 201 was done by Softcheck, K. 2008. Nominal test concentrations were set at 0.010, 0.026, 0.064, 0.16, 0.40 and 1.0 mg a.i./L, plus a control. The measured concentrations of BIT in the test solutions at 0 hour approximated the desired nominal concentrations. The measured concentrations of BIT decreased at 96 hours and were below the limit of quantitation (LOQ) in the 0.010 mg a.i./L nominal treatment level. In this study the mean coefficient of variation section by section at 72h = 0.89 and at 96h =0.81 exceeding by far the 35% established in the OECD 201 criterion. When analysing the data it appears that it has both a high variability on the growth of the controls, variability in response for short duration and then either full inhibition or no effects when exposed to BIT. All these factors render this dataset not very suitable for the regression of the log-logistic model, as reflected by the Non convergence (NC) of either the effect concentration and/or the confidence intervals. For these reasons the study was considered not valid.