Committee for Risk Assessment

RAC

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

Adopted

26 November 2021

26 November 2021

CLH-O-0000007051-86-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

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

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

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

The proposal was submitted by **Spain** and received by RAC on **11 February 2021.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Spain has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **15 March 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **14 May 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Anna Biró**

Co-Rapporteur, appointed by RAC: **Žilvinas Užomeckas**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **26 November 2021** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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 GHS05 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** Eye Dam. 1 Aquatic Acute 1 **Add** Acute Tox. 2 Aquatic Chronic 1 **Modify** Acute Tox. 4 Skin Sens. 1B **Remove** Skin Irrit. 2	**Retain** H302 H318 H400 **Add** H330 H410 **Modify** H317 **Remove** H315	**Retain** GHS05 GHS09 Dgr **Add** GHS06 **Remove** GHS07	**Retain** H302 H318 **Add** H330 **Modify** H317 H410 **Remove** H315		**Add** oral: ATE = 454 mg/kg bw inhalation: ATE = 0.25 mg/L (dusts or mists) M = 1 M = 1 **Modify** Skin Sens. 1B; H317: C ≥ 0.05 %	
RAC opinion	613-088-00-6	1,2-benzisothiazol-3(2*H*)-one; 1,2-benzisothiazolin-3-one	220-120-9	2634-33-5	**Retain** Skin Irrit. 2 Eye Dam. 1 Aquatic Acute 1 **Add** Acute Tox. 2 Aquatic Chronic 1 **Modify** Acute Tox. 4 Skin Sens. 1A	**Retain** H302 H315 H318 H400 **Add** H330 H410 **Modify** H317	**Retain** GHS05 GHS09 Dgr **Add** GHS06 **Remove** GHS07	**Retain** H302 H318 H315 **Add** H330 **Modify** H317 H410		**Add** oral: ATE = 450 mg/kg bw inhalation: ATE = 0.21 mg/L (dusts or mists) M = 1 M = 1 **Modify** Skin Sens. 1A; H317: C ≥ 0.036 %	
Resulting Annex VI entry if agreed by COM	613-088-00-6	1,2-benzisothiazol-3(2*H*)-one; 1,2-benzisothiazolin-3-one	220-120-9	2634-33-5	Acute Tox. 2 Acute Tox. 4 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H330 H302 H315 H318 H317 H400 H410	GHS06 GHS05 GHS09 Dgr	H330 H302 H315 H318 H317 H410		oral: ATE = 450 mg/kg bw inhalation: ATE = 0.21 mg/L (dusts or mists) Skin Sens. 1A; H317: C ≥ 0.036 % M = 1 M = 1	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

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 ($300 < LD_{50} \leq 2000$ 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, n°/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
OECD TG 401 GLP	Rat Wistar	97.42 % a.i. Gavage vehicle: 0.5 %	438, 585, 680 and 877 mg BIT/kg bw	C: 582 mg/kg	Anonymous, 2003a AIII6.1.1/2

Method, guideline, deviations if any	Species, strain, sex, n°/group	Test substance	Dose levels	LD_{50} Mortalities	Reference
	M+F 5 rats/sex/group	solution of Carboxymethyl-cellulose		Dose (mg/kg bw) / Male / Female 438 / 0/5 / 3/5 585 / 2/5 / 2/5 680 / 3/5 / 4/5 877 / 5/5 / 5/5 1315 / 3/5 / 5/5	
Comparable to OECD TG 401 and EC B.1 GLP	Rat Wistar-derived albino M+F 5 rats/sex/group	73.1 % a.i. Gavage vehicle: 0.5 % aqueous polysorbate 80	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 Dose (mg/kg bw) / Male / Female 73 / 0/5 / 0/5 219 / 0/5 / 0/5 366 / 0/5 / 1/5 658 / 5/5 / 3/5	Anonymous, 1988a IIIA6.1.1/1 (REACH registration dossier)
OECD TG 401, EC B.1 GLP	Rat CD M (+ F at lowest dose) 5 rats/sex/group	Purity not specified Gavage vehicle: 0.5 % aqueous methylcellulose	202, 320 and 506 mg BIT/kg bw	M: 454 mg/kg Dose (mg/kg bw) / Male / Female 202 / 0/5 / 0/5 320 / 1/5 / - 506 / 3/5 / -	Anonymous, 1994a IIIA6.1.1/2 (REACH registration dossier)

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, n°/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).**

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 Irrit. 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 Irrit. 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 where 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>
<tr><td>Comparable to OECD TG 404 Rabbit
New Zealand White albino
3 M
Semi-occlusive
4 h</td><td>Purity not specified
500 mg in 0.5 mL distilled water</td><td><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></td><td>Rees P.B., 1993
(REACH registration dossier)</td></tr>
<tr><td>Guinea pig
Strain not specified
nº/sex/group not specified
Application method not specified</td><td>Purity not specified
1 % in a non-specified vehicle
Time of exposure not specified</td><td>Strong irritation. No further details of the study were given.</td><td>Alomar *et al*., 1985
(Technical Committee on Classification & Labelling i.e. TC C&L document)</td></tr>
</table>

Method, guideline, deviations if any	Purity, Dose levels	Results	Reference
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 contact, semi-occlusive patches.	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).	Induction: 42/50 volunteers: barely perceptible to slight erythema (associated with papule formation in 6 volunteers) 7/50: moderate erythema (accompanied by papule 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.	Anonymous, 1975

Method, guideline, deviations if any	Purity, Dose levels	Results	Reference
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)**.

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

Comments were received from 3 MSCAs and 17 stakeholders.

One MSCA commented that the SCL has to be revised since the potential for 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, at which sensitisation by BIT has already been described (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 consists of, 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 EC3 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 29,590).

***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 %.

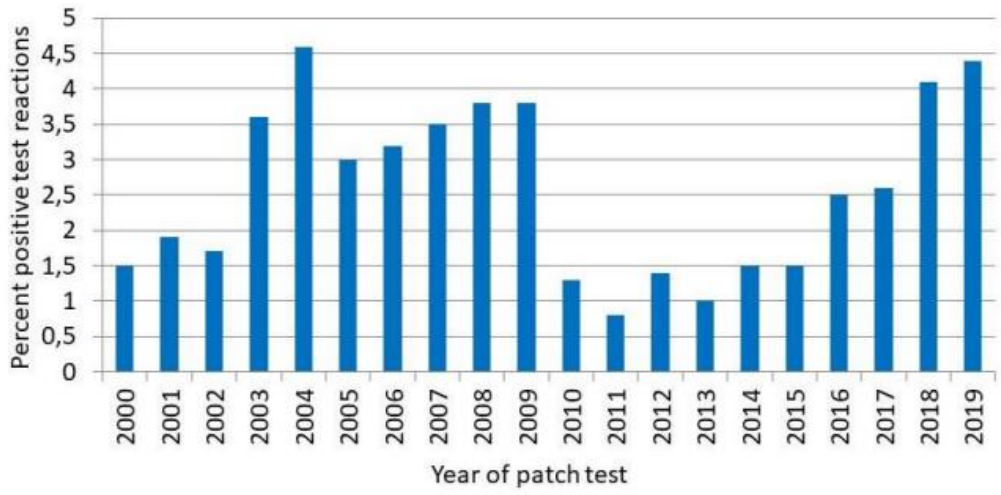
Although BIT is a known skin irritant and the patch test concentration is rather high, the diagnostic discriminatory power is fairly good, characterised by a Reaction Index (RI) of + 0.04, and a Positivity Ratio (PR) of 82 % (table above). The fact that the proportion of BIT-positive patients was significantly 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 % from 2018 to 2019 (see the Figure below). IVDK stated that they have no complete conclusive explanation for these marked differences.

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

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.

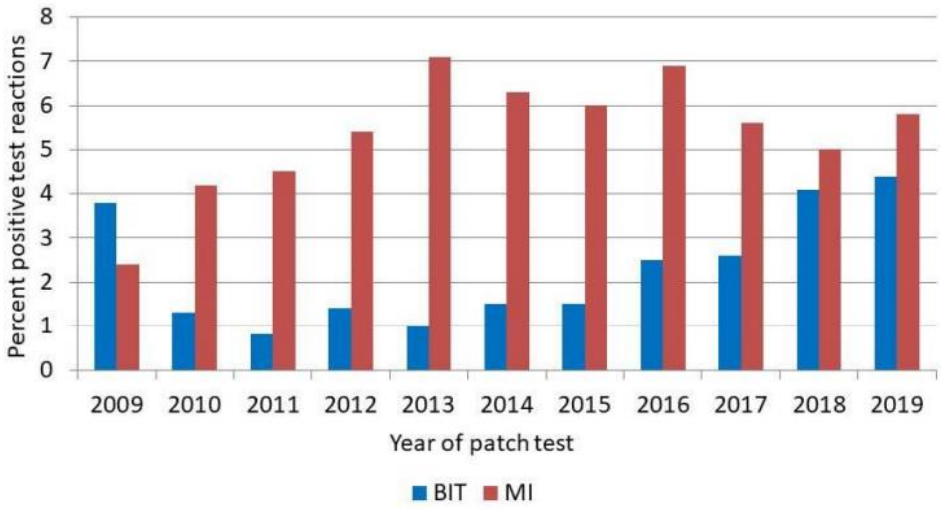
BIT and MIT were patch tested in parallel in 18259 patients. Of these, 383 reacted positively to BIT, and 1041 to MIT. Seventy-seven patients were positive to both isothiazolinones, which is 20.1 % of the BIT-positive patients, and 7.4 % of those sensitised to MIT.

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>EC₃=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>EC3=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>EC₃ = 32.4 % - Skin Sens. 1B* (Experiment no.1.)

EC₃ = 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>EC₃ = 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 gave an SI greater than 3)</td><td>Gerberick et al., 2005</td></tr>
<tr><td>LLNA</td><td>Not stated.</td><td>BIT</td><td>Not stated.</td><td>EC₃ = 10.4 % - Skin Sens. 1B

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

<table>
<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><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>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><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>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><th>Group</th><th>Challenge</th><th>Score at 24 h</th><th>Score at 48 h</th><th>Total responders</th></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

GLP</td>
<td>Guinea pig

Alpk:Dunkin-Hartley albino

20 F (test)

10 F (neg. ctrl.)

20 F (pos. ctrl.)</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 oil

Topical:
30 % w/v BIT in DMF

Challenge:
3 % and 10 % w/v BIT in DMF</td>
<td>35 % responding at 0.01 % intradermal induction dose - Skin Sens. 1A
<table>
<tr><th></th><th></th><th colspan="2">Animals with allergic reactions/Animals in group</th></tr>
<tr><th></th><th>Topical Dose (%)</th><th>Test group</th><th>Control group</th></tr>
<tr><td rowspan="2">Scored 24h</td><td>10</td><td>13/20 (65 %)</td><td>3/10 (30 %)</td></tr>
<tr><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>Anonymous,

1990

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

GPMT EC B.6 Range finding study conducted	Guinea pig Strain not specified Sex not specified 20 guinea pigs/group	20 % a.i. in aqueous propylene glycol (Proxel XL) 30 % a.i. in morpholine di-and triethanol amine (Proxel HL)	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)	15 % responding at 1 % intradermal induction dose – no classification Proxel XL - 3/20 (15 %) Proxel HL - 1/20 (5 %)	Andersen and Hamann, 1984 (TC C&L document)

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	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	Anonymous, 1975

Type	Test substance	Observations	Relevant information about the study (as applicable)	Reference
	10 subjects) or liquid paraffin (second challenge test main study) (64.45 µg/cm^2 or 250 µg BIT/patch).		observed in five individuals following the second challenge.	
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 (500 ppm) in petrolatum.	20/2264 patients (0.88 %) showed a positive response to BIT. Some might have been sensitised by using gloves with 0.002 % BIT (20 ppm BIT).	From January 1991 to September 2005, BIT was tested on a total of 2264 patients at the Finnish Institute of Occupational Health (highly selected patients).	Aalto-Korte *et al*., 2006, 2007 (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)

Type	Test substance	Observations	Relevant information about the study (as applicable)	Reference
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 0.33 %=3300 ppm BIT and 0.05 % BIT (500 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 3300ppm BIT). None reacted at 30 ppm BIT	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)
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	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)

Type	Test substance	Observations	Relevant information about the study (as applicable)	Reference
	(0.33 % BIT (3300 ppm)) and 1 % BIT (10000 ppm). Vehicle not specified			
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 (1000 ppm) (vehicle not specified).	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 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).	Roberts *et al.*, 1981 (TC C&L document)
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 55.6µg/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 %).

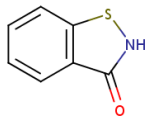
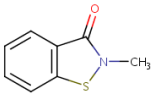
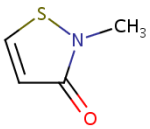
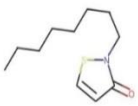
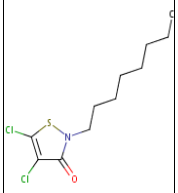
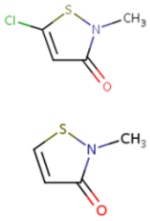
RAC therefore concludes that BIT warrants classification as **Skin Sens. 1A, H317: May cause an allergic skin reaction**.

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 GPMT studies (≥ 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/cm^2), 0/54 (0 %) at 360 ppm aq (45 µg/cm^2) 5/45 (11 %) volunteers at 500 ppm (64.5 µg/cm^2)* 0/111 at (500 ppm, (27.8 µg/cm^2) in Rhoplex AC-64	9/45 (20 %) volunteers at 500 ppm*	1/116 (0.9 %) volunteers at 400 ppm (20 µg/cm^2) 1/210 (0.5 %) at 500 ppm (25 µg/cm^2)	0/103 subjects at 50 ppm (0.005 %) (2.5 µg/cm^2) 1/222 (0.45 %) subjects at 100 ppm (0.01 %) (5 µg/cm^2)	4/34 (12 %) at 250ppm (0.025 %) (12.5 µg/cm^2) 14/34 (41 %) at 350ppm (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 an 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 Finnish Institute of Occupational Hygiene (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 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.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

1,2-benzisothiazol-3(2H)-one (BIT) 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.

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 (E_rC_{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 using initial measured conc.)	Smyth *et al.* (1994) (CAR)/PROXEL™ Press Paste (BIT 73.3 %)
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 (ErC_{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 ErC_{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 72 h = 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 72 h = 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 Ad hoc ENV group documents and discussions. The DS also pointed out that 72 h 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. The 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. BIT reacts with several specific enzymes, which are essential within critical metabolic pathways. 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 mode of action 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 °C. 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 24 h). 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 $0.1 < L(E)C_{50} \leq 1$ 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 $0.01 < NOEC \leq 0.1$ mg/L range, **M = 1**

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 °C), and Henry's Law Constant (1.45×10^{-5}-7.4×10^{-6} Pa m^3 mol^{-1} at 20 °C), 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**.

Additional references

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ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).