BIT CIG COMMENTS TO THE PUBLIC CONSULTATION ON THE CLH FOR 1,2-BENZISOTHIAZOL-3(2H)-ONE (BIT), CAS NUMBER: 2634-33-5

The BIT Common Interest Group (BIT CIG) consists of the 6 participants Nutrition & Biosciences (Switzerland) GmbH, Laboratorios Miret, S.A., Lanxess Deutschland GmbH, Lonza Ltd, Thor GmbH and Troy Chemical Company BV. These companies support BIT within the EU Biocidal Product Regulation (BPR) review programme.

The BIT CIG wishes to provide comments on the following proposals for classification that are open points under consideration within this CLH process.

1. Skin sensitisation
2. Acute and chronic toxicity to the environment
3. Acute inhalation toxicity
4. Skin Irritation
5. Ozone depletion

In providing our comments, we refer to the Regulation (EC) 1272/2008, on classification, labelling and packaging of substances and mixtures (CLP), Regulation (EU) No 2020/1182, the 15th amendment of the CLP for the purpose of adaptation to technical and scientific progress (15th ATP), and the most recent Guidance on the Application of the CLP Criteria Version 5.0, (CLP Guidance) released by ECHA in July 2017.

Summary

The BIT CIG confirms its agreement with the CLH proposal set out by the dossier submitter to update the current harmonised CLP classification to the following:

Acute Tox. 4, H302
Acute Tox. 2, H330
Eye Dam. 1, H318
Skin Sens. 1B, H317 [SCL c≥0.05%]
Aquatic Acute 1 H400 [M=1]
Aquatic Chronic H410 [M=1]

Furthermore, we confirm our agreement with the proposal that BIT does not meet the CLP criteria to classify as a skin irritant nor does it meet the criteria to classify as hazardous to the ozone layer.

1. Skin sensitisation

As previously stated the BIT CIG agrees with the dossier submitter proposal to classify BIT as a Skin Sensitiser, Cat. 1B with a specific concentration limit (SCL) for skin sensitisation at 500 ppm (C≥0.05%).

We believe the proposal from the dossier submitter is well supported but we have additional information that supports the proposal.

In order to facilitate the review of the additional information the comments detailed below are subdivided into the following sub-sections:

a. An assessment of the available animal data
b. Comment on the reference to BIT previously considered within the CLH dossier for MBIT
c. An assessment of cross-reactivity between isothiazolinones
d. A brief study overview and comments on the Aalto-Korte et al., 2006 and 2007 "BIT in glove studies"
e. Emission of BIT from paint and the likelihood of airborne-derived contact dermatitis

a. Comment on Skin sensitisation studies cited in the 1,2-benzisothiazol-3(2H)-one (BIT) CLH dossier (CLH Report issued February 2021)

In this document we would like to comment on the animal skin sensitisation studies which were cited in the BIT CLH Dossier.

An overview of all animal studies we refer to are presented in table 11 "Summary table of animal studies on skin sensitisation" on page 23 of the BIT CLH report.

Ten of the eleven listed independent studies have shown that BIT induces skin sensitisation. The two LLNAs reported in the dossier showed that BIT was a skin sensitiser at concentrations higher than 2%. One of them shows an EC3 < 2% (Skin Sens. 1A) and the other one shows an EC3 > 2% (Skin Sens. 1B). An LLNA mentioned in the TC C&L document (1991), which is not further described in the evaluation text, revealed that BIT was skin sensitiser at concentrations higher than 10%. Other three LLNAs on BIT can be found in the RAC opinion on MBIT (CLH-O-0000001412-86-209/F) with EC3 values > 2%. 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% (Skin Sens. 1B). The fifth GPMT even concluded that BIT did not meet the requirement to classify as a skin sensitiser.

Overall, only one study resulted in an EC3 value < 2%, leading to the classification of Skin Sens. 1A. However, the following should be considered: The study was conducted in 1991 prior to the adoption of OECD 429 and OPPTS guideline 870.2600, skin sensitisation: local lymph node assay, it is a non GLP study consisting of data collected for the purpose of method development. Consequently, it is assigned a reliability score of 2.

In summary, we have:

- 1 x GPMT study showing BIT is not a skin sensitiser
- 1x LLNA study showing BIT is a strong skin sensitiser (Skin Sens. Cat. 1A)
- 5x LLNAs and 4x GPMTs showing BIT is a moderate skin sensitiser (Skin Sens. Cat. 1B)

The majority of the studies show that BIT is a moderate skin sensitiser. Only one study, which does not fulfil the robust guideline criteria indicates that BIT is a strong sensitiser. It has to be acknowledged that there is also a study with reliability score of 1, showing that BIT is not a skin sensitiser at all. Therefore, these two studies with extremely contrasting results should be disregarded.

Thus, taking into account the result of the remaining nine studies, that can be considered valid, BIT must be classified as Skin Sens. 1B (H317: May cause an allergic skin reaction) according to the CLP 3.4.2.2.3.3. (Table 3.4.4.), because of EC3 > 2 % in five LLNA studies and ≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose in four guinea pig maximisation tests. This conclusion would also be in line with the weight of evidence approach that should be followed in accordance with the CLP Guidance (Chapter 3.4.2.2.3.7 Weight of evidence, Annex I, chapter 3.4.2.2.4).

The data available clearly demonstrate that BIT is a moderate skin sensitiser. Following the criteria laid down in the guidance on the application of the CLP criteria (version 5.0 chapter 3.4.2.5; table 3.9) a GCL (Generic Concentration Limit) of 1 % could be assigned.

In a HRIPT study a realistic no effect level for BIT was in the region of 500 ppm and 9% positive reactions were observed at 725 ppm (Basketter *et al*., 1999; SCCNFP on BIT, 2004, p.15). In this study, BIT (purity approx. 78%, but containing approx. 21.5% ethylene diamine as the free base) was used. Since ethylene diamine is a known skin sensitiser, it is not clear whether the positive reactions were caused by BIT or ethylene diamine. However, consideration of the available data based on the conservative assumption that the reactions seen were all attributed to BIT led to the conclusion that a realistic no effect level was in the region of 500 ppm (Basketter *et al*., 1999).

Therefore, based on the considerations made above, we do support the dossier submitter's proposal of keeping the current SCL of 500 ppm, as it is well supported by robust and guideline conforming toxicological data. The proposal is sufficiently conservative and already takes into account a safety margin.

References:

Basketter DA, Rodford R, Kimber I, Smith I, Wahlberg JE. Skin sensitization risk assessment: a comparative evaluation of 3 isothiazolinone biocides. Contact Dermatitis. 1999 Mar;40(3):150-4.

SCCNFP on BIT. The Scientific Committee on Cosmetic Products And Non-Food Products Intended For Consumers Opinion concerning Benzisothiazolinone COLIPA n° P 96. SCCNFP/0811/04

b. Comment on the referencing of the MBIT Opinion CLH-O-0000001412-86-209/F of 8 June 2018 issued by the Committee for Risk Assessment RAC proposing harmonised classification and labelling at EU level of 2-methyl-1,2-benzothiazol-3(2H)-one; [MBIT] EC Number: - CAS Number: 2527-66-4

Page 12 and 13: **Assessment and comparison with the classification criteria**

Page 12: …
"Furthermore, the EC3 values for MBIT were notably lower than those for BIT, which has a concentration limit of 0.05 % (500 ppm). This SCL may not be fully protective for BIT, either, since there are some reports suggesting BIT caused skin allergies from PVC gloves containing 20-30 ppm of BIT (Aalto-Korte *et al*., 2006; 2007). Also the Scientific Committee on Consumer Safety (SCCS, 2012) concluded in their opinion that BIT is known to be a sensitiser in man and has induced sensitisation at circa 20 ppm in gloves."

Page 13: …
"In summary, RAC is of the opinion that an SCL is necessary for MBIT. For deriving the limit, starting with the exposure level used in the HRIPT test (500 ppm) is considered justified. However, 20 % of the volunteers were reported to become sensitised at this dose level, and therefore RAC is of the opinion that the SCL should be considerably lower than that, clearly less than a tenth of 500 ppm. Furthermore, there are published reports suggesting that a closely related isothiazolinone, BIT, may induce skin sensitisation in humans at already levels of 20–30 ppm in gloves."

Comment:

In our view, a reference to BIT in the RAC opinion on MBIT was not appropriate.

The intrinsic hazard properties of BIT are very well and comprehensively assessed with robust guideline studies, as documented in multiple BIT dossiers available under the Biocidal Products Regulation (EU No. 528/2012). These studies were not considered or evaluated when drawing a conclusion on BIT in the MBIT RAC Opinion. Instead, BIT was only assessed on the basis of two literature references[1] *(reference to Aalto-Korte et al., 2006, 2007 (BIT in gloves)* which clearly have deficiencies. A detailed commentary on these two

[1]Aalto-Korte K, Alanko K, Henriks-Eckerman ML, Jolanki R. Antimicrobial allergy from polyvinyl chloride gloves. Arch Dermatol. 2006 Oct;142(10):1326-1330.

Aalto-Korte K, Ackermann L, Henriks-Eckerman ML, Välimaa J, Reinikka-Railo H, Leppänen E, Jolanki R. 1,2-benzisothiazolin-3-one in disposable polyvinyl chloride gloves for medical use. Contact Dermatitis. 2007 Dec;57(6):365-370.

references is attached to this submission [BIT CIG CLH comment 1d, page 12] Generally, it has to be acknowledged that there is only a very low number of allergic skin reactions towards glove material apparently caused by BIT reported in the literature. Importantly, these positive findings were limited to dental and healthcare workers with long history of dermatitis and permanent use of gloves in occupational settings leading to occlusive exposure conditions. Therefore, the limit value of 20-30 ppm indicated in those literature references is not considered as scientifically robust to be used for establishing a BIT skin sensitisation threshold.

Further, for MBIT 20% of the volunteers were sensitised at 500 ppm in the relevant HRIPT study and hence RAC justified a lower SCL for MBIT. By contrast, for BIT, a realistic no effect level was in the region of 500 ppm in the relevant HRIPT study and 9% positive reactions were observed at 725ppm (Basketter *et al*., 1999; SCCNFP on BIT, 2004, p.15). In this study, BIT (purity approx. 78%, but containing approx. 21.5% ethylene diamine as the free base) was used. Since ethylene diamine is a known skin sensitiser, it is not clear whether the positive reactions were caused by BIT or ethylene diamine. However, consideration of the available data based on the conservative assumption that the reactions seen were all attributed to BIT led to the conclusion that a realistic no effect level was in the region of 500 ppm (Basketter *et al*., 1999).

The family of isothiazolinones consists of different members, which have very different physico-chemical properties due to their different chemical structures. For each isothiazolinone, a comprehensive data package is available which also addresses all relevant eco-toxicological, environmental toxicological and mammalian toxicological endpoints. Based on this very extensive database, the conclusion can be drawn that the substances are different in their properties. Any conclusion drawn in the MBIT opinion regarding BIT should be disregarded. A comprehensive and scientifically sound data package on BIT that addresses relevant and important toxicological endpoints multiple times is available and should exclusively form the basis for the evaluation and assessment of BIT. No read-across should be used.

References:

Basketter DA, Rodford R, Kimber I, Smith I, Wahlberg JE. Skin sensitization risk assessment: a comparative evaluation of 3 isothiazolinone biocides. Contact Dermatitis. 1999 Mar;40(3):150-4.

SCCNFP on BIT. The Scientific Committee on Cosmetic Products And Non-Food Products Intended For Consumers Opinion concerning Benzisothiazolinone COLIPA n° P 96. SCCNFP/0811/04

c. BIT: An assessment of cross-reactivity

Summary

Benzisothizolinone (BIT) has been evaluated for cross-reactivity in humans, patients presenting to dermatological clinics, and experimental animals. The human data indicates that BIT does not cross-react with other tested isothiazolinones in patch test panels. The experimental data are inconclusive, since numerous methodological and reporting deficiencies obfuscate interpretation. Overall, it is appropriate to consider that reactions to BIT are independent to those of other isothiazolinones.

Human Data

The German Information Network of Departments of Dermatology (IVDK) assessed concomitant reactions to benzisothizolinone (BIT), methylisothiazolinone (MIT), and octylisothiazolinone (OIT) in patients presenting to its clinical network. Patients were tested with 0.05% aqueous MIT and 0.1% BIT in petrolatum. Of 38,762 patients tested for reactivity to MIT, 1771 responded positively, while of 8728 patients tested for reactivity to BIT, 141 responded positively. Less than 10% of MIT-sensitised patients also reacted to BIT. This is in contrast to more than 60%, which would be expected of well-known cross-reacting allergens and was attributed by the investigators to be more likely related to co-exposure rather than immunological cross-reactivity (Geier *et al*., 2015).

In another patch test series performed at the IVDK between 1990 to 1994, 928 patients were patch tested with methylchloroisothiazolinone C(M)IT/MIT (100 ppm aq.), BIT (0.1% pet.) and OIT (0.025% pet.). 36 patients (3.9%) showed an allergic reaction to C(M)IT /MIT, 12 (1.3%) to BIT and 6% (0.6%) to OIT. Only 2 patients reacted simultaneously to C(M)IT /MIT and BIT and 1 patient reacted to BIT and OIT, but not to C(M)IT /MIT (Geier et al. 1996).

Craig *et al*. (2017) conducted a patch test series with C(M)IT /MIT (0.02% aq.), MIT (0.2% aq.), OIT (0.1% pet.), and BIT (0.1% pet.) as part of a baseline series. Out of 1287 patients 118 (9.2%) showed a positive reaction to any isothiazolinone. Of these 118 patients only ten patients showed a positive reaction to BIT; in total 10 out of 1287 patients (0.8%) reacted to BIT. Eighty-one patients reacted positive to C(M)IT/MIT, 8 patients to OIT and 87 patients showed a positive reaction to MIT. Only 1 patient each reacted simultaneously to C(M)IT/MIT and BIT; C(M)IT/MIT, MIT and BIT; MIT, OIT and BIT or OIT and BIT, showing that positive reactions to BIT tended to occur in isolation.

In a much smaller cohort from the Finnish Institute of Occupational Health (FIOH), of 647 patients who were patch tested during the period 2012–2017, only nine responded to BIT (0.05% pet.), compared to 61 responding to MIT (0.03% or 0.2% aq.) and nineteen to OIT (0.1% pet.). Of these, two had

reactions to both OIT and MIT, while one reacted to MIT alone. The authors concluded, "Allergic reactions to OIT were strongly associated with extreme reactions to MIT, which suggests cross-sensitization. In contrast, BIT reactions were mostly independent" (Aalto-Korte & Suuronen, 2017). The FIOH had earlier reported that of 2264 patients tested during the period 1991–2005 with 0.05% or 0.1% BIT in petrolatum or alcohol, seventeen (0.75%) gave a positive reaction. A further three patients also reacted positively to BIT, making a total of twenty, but were not included in the analysis for logistical reasons. Four of these 20 patients reacted to the mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (C(M)IT /MIT) and 2 to OIT. BIT was not considered to cross react with C(M)IT /MIT or OIT and as per study authors, concomitant reactions to these isothiazolinones supported separate sensitization (Aalto-Korte *et al*., 2006). In another study (Aalto-Korte *et al*., 2007), BIT was patch tested at 0.05% in petrolatum in 5450 patients at Hospital of Helsinki University Central Hospital (HUCH). The study also reported data on 3 previously unpublished BIT allergic patients from Finnish Institute of Occupational Health (FIOH). Sixteen 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 (Kathon CG) or to OIT.

A non-systematic patch testing of 194 out of 335 C(M)IT/MIT-positive Belgian patients during 2010–2012 revealed that four reacted to BIT and eight reacted to both BIT and OIT. Direct exposure to BIT could be determined for only seven of the twelve BIT-positive patients (Aerts *et al*., 2014).

Animal Data

Cross-reactivity between MIT and BIT was evaluated using a "modified local lymph node assay", in which groups of mice were sensitised with MIT and then challenged at a later date with BIT. Although a reference to a validated method was not given, it broadly conformed to the enhanced LLNA by BrdU flow cytometry (eLLNA:BrdU-FC) test, which has not been validated by ICCVAM or OECD but is referenced in OECD Test Guideline 442B (OECD, 2010). There are, however, substantial methodological and reporting deficiencies that hinder the interpretation and applicability of this study (Schwensen *et al*., 2016).

General principles of skin sensitisation testing require that irritation—monitored by assessment of erythema, measurement of ear thickness, and ear punch weight—should be controlled to eliminate nonspecific responses (OECD, 2010). A pilot sensitisation/challenge study was conducted for MIT at concentrations of 0.13%, 0.4% (EC_3), and 1.2%, but there is no evidence that the authors assessed erythema or ear punch weight, and it appears that the two highest concentrations of MIT statistically-significantly increased skin thickness by more than 25%, which is the OECD/ICCVAM cut-off. The same

concentrations of MIT were used in the subsequent 'cross-reactivity' test with BIT. The interaction with BIT at its supposed EC_3 value (cited as 1.9%, see below[2]) was assessed three weeks after exposure to MIT, which will have lessened the initial irritation response, but the ear-thickening response to BIT challenge at the highest MIT sensitisation concentration still exceeded 25%. Therefore, it is clear that the highest, and possibly middle, sensitisation concentration of MIT should not have been used.

Nevertheless, even at the lowest concentration of MIT (0.13%), which was not excessive in terms of ear thickness (although, as noted above, reddening and ear punch weight were not assessed), there appeared to be a significant interaction with BIT. BrdU-labelled cells were sub-classified into three subtypes (CD4+, CD8+, CD19+), and the authors stated that "BIT induced the same concentration-dependent inflammatory response [as MIT] with proliferation of CD4+ and CD8+ T cells and CD19+ B cells in MIT-sensitised mice". However, only CD4+ cells were elevated at sensitisation concentrations of MIT that did not also induce excessive ear thickening, while the response in CD19+ cells appeared to be weighted by one extreme outlier in each of the lower MIT-sensitised groups. Furthermore, in this case an appropriate control was not conducted as no MIT-sensitised group was later challenged with the vehicle alone; this would have been the appropriate comparison control group, not the vehicle-sensitised/-challenged group. Therefore, the specificity of the finding to BIT is unclear, and it is impossible to state with any certainty whether there was a genuine cross-reactivity or wherever the response was a residual from the initial sensitisation to MIT.

Finally, the investigators conducted the test in duplicate with groups of four mice and combined the groups for statistical analysis. No justification is given for the duplication, nor was any statistical analysis presented to support the combination of groups from two independent experiments, rather than reporting the two experiments separately. Intergroup differences were determined by ANOVA followed by Tukey HSD, a multiple comparison test that compares all group mean values against all others, but only comparisons between treated groups and control were presented. For this, Dunnett's test for multiple comparisons against a common control would have been preferable. In addition, it is questionable whether parametric data analysis was appropriate for some cases where the data appear to be skewed.

[2] Schwensen *et al*. (2016) cited literature to support the EC3 used in their study, but none of their citations refer to BIT. The value of 1.9% that they cite is for MIT, based on a 19.7% solution uncorrected for active content (Roberts, 2013). The CLH dossier presents the following reported EC3 values: 28.8% (calculated); 1.5% (calculated); 2.3%; 10.2%; >10%; 4.8% and 32.4%.

Other information

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.

Conclusion

An experimental study in mice has been conducted to determine whether BIT can elicit an allergenic response following sensitisation with MIT. There are numerous methodological and reporting deficiencies that obfuscate the intended goal and call into question the conclusion of cross-reactivity between these two substances. Human patch testing of MIT- and BIT-sensitised patients indicates that patients that react to both isothiazolinones do so probably due to co-exposure, not cross-reactivity.

References

Aalto-Korte K., Alanko K., Henriks-Eckerman M.J., Jolanki R. (2006). Antimicrobial allergy from polyvinyl chloride gloves. *Archives of Dermatology* **142**:1326–1330.

Aalto-Korte K, Ackermann L, Henriks-Eckerman ML, Välimaa J, Reinikka-Railo H, Leppänen E, Jolanki R. 1,2-benzisothiazolin-3-one in disposable polyvinyl chloride gloves for medical use. *Contact Dermatitis.* 2007 Dec;57(6):365-370.

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

Aerts O., Baeck M., Constandt L., Dezfoulian B., Jacobs M.C., Kerre S., Lapeere H., Pierret L., Wouters K., Goossens A. (2014). The dramatic increase in the rate of methylisothiazolinone contact allergy in Belgium: a multicentre study. *Contact Dermatitis* **71**:41–48.

Ashby J., Basketter D.A., Paton D., Kimber I. (1995). Structure activity relationships in skin sensitization using the murine local lymph node assay. *Toxicology* **103**:177–194.

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

Geier J., Schnuch A. (1996). No cross-sensitization between MCI/MI, benzisothiazolinone and octylisothiazolinone. *Contact Dermatitis* **34**:148–9.

Geier J., Lessmann H., Schnuch A., Uter W. (2015). Concomitant reactivity to methylisothiazolinone, benzisothiazolinone, and octylisothiazolinone. International Network of Departments of Dermatology data, 2009–2013. *Contact Dermatitis* **72**:337–346.

OECD. (2010). OECD Guideline for the Testing of Chemicals 442B. Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA. Organisation for Economic Cooperation and Development, Paris.

Roberts D.W. (2013). Methylisothiazolinone is categorised as a strong sensitiser in the murine local lymph node assay. *Contact Dermatitis* **69**:261–262.

Schwensen J.F., Menné Bonefeld C., Zachariae C., Agerbeck C., Petersen T.H., Geisler C., Bollmann U.E., Bester K., Johansen J.D. (2017). Cross-reactivity between methylisothiazolinone, octylisothiazolinone and benzisothiazolinone using a modified local lymph node assay. *British Journal of Dermatology* **176**:176–183.

d. Brief study overview and comments on studies (Aalto-Korte et al., 2006 and 2007)

From January 1991 to September 2005, benzisothiazolinone (BIT) was patch tested on a total of 2264 patients at the Finnish Institute of Occupational Health (FIOH) at 0.05% or 0.1% BIT in petrolatum. The acetone, water and ethanol extracts of glove material was also patch tested and concentrations of BIT in gloves were analyzed by high-performance liquid chromatography with a UV detector at 230 nm (detection limit - 2 ppm). Twenty patients (0.88%) were positive to BIT. The reaction index for the test substance, 0.05% (petrolatum), in 1978 patients was −0.34. Out of total 8 patients in connection with both BIT allergy and PVC glove use: 2 patients tested positive; one patient had doubtful; 3 patients were negative to glove material. BIT concentration measured in PVC gloves ranged from 9-31ppm. A common feature of patients was a long history of hand dermatitis, and they had also been diagnosed with other types of hand dermatitis besides BIT contact allergy. Four of these 20 patients reacted to the mixture of (C(M)IT/MIT) and 2 to OIT. Despite the similarity in chemical structure, BIT was not considered to cross react with C(M)IT/MIT or OIT. As per the author's concomitant reactions to these isothiazolinones supports the idea of separate sensitization.

In another study (Aalto-Korte et al., 2007), BIT was patch tested at 0.05% in petrolatum in 5450 patients and glove material was tested 'as is' under Scanpore tape moistened with acetone, at a clinic of general dermatology, the Skin and Allergy Hospital of Helsinki University Central Hospital (HUCH). The concentrations of BIT in disposable PVC gloves were analyzed similar to the previous study. The study also reported data on 3 previously unpublished BIT allergic patients from the Finnish Institute of Occupational Health (FIOH). Sixteen patients (0.003%)[3] were positive to BIT. In the chemical analysis, BIT containing gloves were labelled from low-BIT (2–5 ppm) to high-BIT (≥20 ppm). The patch test results to the glove material: 3 positive; 4 doubtful; 4 negative patient reactions (out of 11 patients with BIT allergy and PVC glove use). As reported in the study, BIT positive patients were either dental or health care workers who use protective gloves most of their working day and might be exposed to other factors that irritate the skin such as frequent hand washing and the use of disinfectants. None of the 16 BIT allergic patients in HUCH or 3 patients in FIOH had patch test reactions to the C(M)IT/MIT (Kathon CG) or to OIT.

[3] % Positive incidence incorrectly calculated in the study report as 0.003%. It should be 0.29% (16/5450=0.00294*100%= 0.29%)

Both diagnostic patch studies indicate low incidence of positivity to BIT (0.88% and 0.29% in Aalto-Korte *et al*., 2006 and 2007 respectively). If both studies are taken together, the overall incidence is 0.47%, i.e. 20+16 out of 2264+5450, which also is considered very low. Further, in the 2006 study, a negative reaction index for the test preparation, 0.05% (petrolatum), was reported which characterizes doubtful and/or questionable patch reactions. Patch testing with glove material indicated very low positive incidence (2 of 8 and 3 of 11 patients who were positive to BIT and had used PVC gloves in Aalto-Korte *et al*., 2006 and 2007 respectively). Most of the patients have had a relatively long history of hand dermatitis/defective skin barrier because of irritation or pre-existing eczema and the occlusive effect of the gloves probably enhanced percutaneous penetration of BIT so they had become sensitized despite the low allergen concentration. Hence, considering the above factors (constant use of occlusive gloves, exposure to other irritants, long history of dermatitis and a defective skin barrier), the authors did not consider sensitization to BIT in gloves as the primary event. Based on very low positive findings to the glove material limited to dental and healthcare workers with previous skin conditions, the study is not considered scientifically robust to be used for establishing a BIT elicitation threshold. Further, as indicated by the study authors, BIT is not considered to cross react with C(M)IT/MIT or OIT.

References

Aalto-Korte K, Alanko K, Henriks-Eckerman ML, Jolanki R. Antimicrobial allergy from polyvinyl chloride gloves. *Arch Dermatol*. 2006 Oct;142(10):1326-1330.

Aalto-Korte K, Ackermann L, Henriks-Eckerman ML, Välimaa J, Reinikka-Railo H, Leppänen E, Jolanki R. 1,2-benzisothiazolin-3-one in disposable polyvinyl chloride gloves for medical use. *Contact Dermatitis*. 2007 Dec;57(6):365-370.

Reinikka-Railo H, Leppänen E, Jolanki R. 1,2-benzisothiazolin-3-one in disposable polyvinyl chloride gloves for medical use. *Contact Dermatitis*. 2007 Dec;57(6):365-370.

e. Emission of BIT from paint and the likelihood of aerogene contact dermatitis caused by BIT

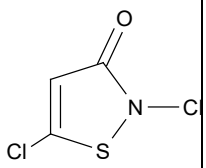
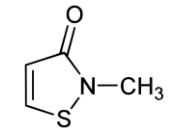
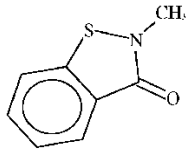
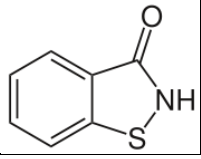
Emission of BIT from paint

Sensitisation to BIT is not very common in the general population compared to other isothiazolinones. As an example, for C(M)IT/MIT 3:1 and MIT, a large number of cases are reported in the cosmetics literature where an allergic reaction was triggered by direct skin contact from application of leave-on and rinse-off cosmetics. It seems important to note that BIT was never an approved active ingredient in the cosmetics sector.

Further, the low sensitisation potential of BIT, compared to the above mentioned isothiazolinones, needs to be recognised. In addition, the fact that BIT is much less volatile and therefore cannot react via the ambient air, e.g. in the case of paints containing BIT, via so-called aerogenic dermatitis, is also an important aspect. Once BIT preserved paints are applied to the wall, there is no further exposure to BIT via the inhalative route.

The volatility of a substance has no defined numerical value. However, it can be described by physico-chemical parameters such as the vapour pressure or boiling point. A high vapour pressure indicates a high volatility, and vice versa, while a high boiling point indicates a low volatility, and vice versa. The table below shows the vapour pressures and boiling points of the different isothiazolinones.

Table: Vapour pressure and boiling point of different isothiazolinones

Isothiazolinone	**CAS no.**	**Structural formula**	**Molecular weight [g/mol]**	**Vapour pressure [Pa] at 25°C**	**Boiling point [°C]**
Chloromethyl-Isothiazolinone (C(M)IT)[4]	26172-55-4		149.6	2.8	167 *)
Methyl-Isothiazolinone (MIT)[5]	2682-20-4		115.2	1.6	236 *)
Methylbenz-Isothiazolinone (MBIT)[6]	2527-66-4		165.2	0.043	285 *)
Benz-Isothiazolinone (BIT)[7]	2634-33-5		151.2	0.00037	328.7*)

*) decomposition

BIT with its benzyl group, clearly stands out from the other isothiazolinones when for example comparing vapour pressure and boiling point to each other. Out of the isothiazolinones listed, BIT has by far the lowest vapour pressure and the highest boiling point temperature and thus the lowest volatility. This is an important aspect when, for example, considering possible exposures of end users that could result from emissions into room air. Investigations according to the so-called test chamber method and also real measurements of freshly painted rooms with paints containing BIT show quite clearly that BIT adapts to the paint matrix and does therefore not cause any exposure in the room air at any time. Details of those measurements can be found in Appendix

[4] https://echa.europa.eu/documents/10162/b070e52b-52ea-435b-3a15-4b69078695d2 (europa.eu)

[5] http://dissemination.echa.europa.eu/Biocides/ActiveSubstances/1229-13/1229-13_Assessment_Report.pdf

[6] https://echa.europa.eu/documents/10162/5a180829-e031-777e-db83-477195663762 (europa.eu)

[7] https://echa.europa.eu/documents/10162/580644f7-c28b-f232-c560-206c24323469

1. Since we do not have any BIT emissions, the potential exposure via this route is consequently also close to zero and can be considered negligible.

Aerogene Contact Dermatitis

Although some literature references speculate about the possibility of airborne contact dermatitis, there is clearly no evidence to support this assumption. For example, a case report by Kaur-Knudsen (2012) speculates about airborne contact dermatitis due to BIT, although the patient clearly showed positive test results for other isothiazolinones (e.g. C(M)IT/MIT) as well. A recent literature search covering 2010-2021 and using the keywords "BIT", “airborne” and “contact dermatitis" clearly indicates that the aerogenic route of exposure is not relevant for causing an allergic skin reaction for BIT.

Conclusion

There are numerous case studies in the literature that report people becoming sensitised with isothiazolinone-preserved cosmetic products. Low concentrations of these isothiazolinones in the ambient air can then trigger an allergic reaction via the inhalation route, i.e. causing an aerogenic contact dermatitis.

BIT is neither used to preserve cosmetic products nor is it present in indoor air due to its low volatility. This clearly distinguishes BIT from the other isothiazolinones.

Literature-References

Aerts O et al: Contact allergy caused by isothiazolinone derivatives: an overview of non-cosmetic and unusual cosmetic sources, Eur J Dermatol., 2017 Apr 1;27(2):115-122.

Herman A et al: Isothiazolinone derivatives and allergic contact dermatitis: a review and update, J Eur Acad Dermatol Venereol,2019 Feb;33(2):267-276.

Kaur-Knudsen D et al: Systemic allergic dermatitis following airborne exposure to 1,2-benzisothiazolin-3-one Contact Dermatitis, 2012; 67, 306-320.

Tokunaga M et al: Occupational Airborne Contact Dermatitis by Isothiazolinones Contained in Wall Paint Products, Allergology International, 2013;62:395-397

Appendix 1

The studies referred to below are submitted separately as company-confidential data in the CLH commenting process.

Summary of the tests conducted:

Study 1, 2004:
Measurement of 1,2-Benzisothiazolin-3-one (BIT) emitted from water-based paints – Test Chamber Method

Emissions of BIT emitting from water-based paints was investigated according to the Test chamber method, EN 13419-1. The analytical evaluation of the air samples was conducted following ISO 1600-6. A commercially available water-based dispersion paint was preserved with 100 ppm BIT and applied on two different sample carrier materials, i.e. glass and gypsum board. The emission investigation covered 28 days in each case. The sample was permanently inside the test chamber under constant climatic conditions (temperature, rel. humidity, air changes). During the entire test duration of 28 days, the biocide agent BIT could be detected neither in the sample applied on glass nor in the paint applied on the gypsum board in the test chamber atmosphere.

Study 2, 2021a:

Study on indoor emissions from paints: Determination of the concentration of Benzisothiazolinone (BIT) in the room air after applying BIT-containing paint

The aim of the investigations was to obtain information on the emission behaviour of the active ingredient BIT, which is used as an in-can preservative in paints, after the application of a BIT-preserved paint in an office room. During sampling and measurements, the room remained unfurnished until sampling was completed. The paint sample contained 333 ppm BIT, which was confirmed by HPLC analysis. The paint was applied to wallpapered walls. A "zero value" was sampled in the room the day before the painting work started. Sample collecting started right after the painting was completed and continued for the commencing 4 weeks. At none of the sample collecting events BIT was detectable in the collected room air.

Study 3, 2021b:

Analysis of Benzisothiazolinone (BIT) Indoor Emissions – a Field Experiment

In this trial, a room of a company building was equipped with BIT preserved products. In particular, paints with BIT preservation, carpet backing and carpet adhesives with BIT treatment as well as ceiling and wall primers containing BIT were used. As a reference and for comparison, a room ("reference room") was used in which commercially available professional quality products were used that were proven to be free of BIT. Over a period of 8 months, room air measurements were regularly carried out under specified conditions.

No emissions for BIT were measured during the entire period.

2. Comment on proposal to classify 1,2-benzisothiazol-3(2H)-one (BIT) as very toxic to aquatic life with long lasting effects, CLH dossier (CLH Report issued February 2021, page 70-1)

The BIT CIG welcomes the opportunity to provide assistance and guidance on the CLH classification proposal for BIT. In providing our comments, we refer to the Regulation (EC) 1272/2008, on classification, labelling and packaging of substances and mixtures (CLP), and the most recent Guidance on the Application of the CLP Criteria Version 5.01, (CLP Guidance) released by ECHA in July 2017.

Specific Comment - Environmental Hazards

In the CLH Report the proposed classification and labelling for environmental hazards is Aquatic Acute 1 with an M-factor of 1, based on an ErC50 (24 h) of 0.108 mg BIT/L (geometric mean of four studies with *Pseudokirchneriella subcapitata*) and Aquatic Chronic 1 with an M-factor of 1, based on an ErC10 (24 h) of 0.026 mg BIT/L (geometric mean of four studies with *Pseudokirchneriella subcapitata*).

The BIT CIG agrees with this CLH classification proposal as it is in line with the relevant criteria laid out in tables 4.1.0 (b)(i) and 4.1.3 of Annex 1 of the CLP regulation and takes into account the specific mode of action of BIT as well.

Additional experimental data on the ecotoxicity of the BIT metabolites mentioned in Annex I of the CLH report (15.1) are not available among the BIT CIG.

3. Comment on proposal to classify 1,2-benzisothiazol-3(2H)-one (BIT) as a fatal if inhaled, CLH dossier (CLH Report issued February 2021, page 18)

The BIT CIG welcomes the opportunity to comment on the proposal by the CLH dossier submitter that BIT meets the criteria for classification as Acute Tox. 2 [H330] Fatal if inhaled.

The CLH dossier submitter details an inhalation study provided by one of the BIT participants in the BPR review programme however an additional study that had also been made available within the BPR review process has been omitted.

This additional BIT study provided as confidential data details an acute inhalation study (4hr) in rat to guideline OPPTS 870.1300. The study estimated that LC50 for males and females were 0.50 and 0.57 mg BIT/L of air respectively, while the combined LC50 was 0.50 mg BIT/L air.

Taking both of these studies and their endpoints into consideration it is appropriate to classify BIT as Acute Tox. 2 (H330: Fatal if inhaled) as in accordance with section 3.1.2.1 of the CLP regulation, (Table 3.1.1), Acute inhalation classification for Category 2 is applicable where $0.05 < LC50 \leq 0.5$ according to the toxicity test result.

We are therefore in agreement with the dossier submitter's conclusion on assigning this hazard classification.

We request that the assignment of an ATE = 0.25 mg/L is further investigated as the current conclusion does not appear consider the above referenced study as it is absent from the CLH proposal.

4. Comment on the proposal not to classify 1,2-benzisothiazol-3(2H)-one (BIT) as irritating or corrosive to skin, CLH dossier (CLH Report issued February 2021, pages 19-22)

The BIT CIG welcomes the opportunity to comment on the proposal by the CLH dossier submitter that BIT does not meet the criteria for classification as a skin irritant.

As already indicated by the CLH proposer several guideline studies have been provided by the BIT CIG within the BPR active substance review program. Within these studies, the highest erythema and oedema scores were 1.33 and 0.33, respectively (both recorded 24 hours after exposure). All effects were confirmed as reversible. Therefore, BIT does not meet criteria to be classified for hazard of skin irritation or skin corrosivity.

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

We are in agreement on the conclusion by the CLH dossier submitter that BIT does not meet the CLP criteria to be classified as a skin irritant and therefore its current classification as Skin Irrit. 2 (H315) should be removed.

5. Comment on proposal not to classify 1,2-benzisothiazol-3(2H)-one (BIT) as a hazardous to the ozone layer, CLH dossier (CLH Report issued February 2021, page 71)

The BIT CIG welcomes the opportunity to comment on the proposal by the CLH dossier submitter that BIT does not meet the criteria for classification as hazardous to the ozone layer.

As outlined by the CLH dossier submitter there is no specific study to determine the hazard of BIT to the ozone layer. However, there is information provided in the CAR of BIT which includes predictions of the half-life of BIT expected in the air.

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

BIT does not have Cl, Br or F atom substituents within its molecular structure. Nor given the short half-life of BIT expected in the air (i.e. 23 hours) and the consideration of its low vapour pressure (i.e. 1.1×10^{-4} 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 may only be found in potentially negligible amounts in the stratospheric ozone layer. Therefore it is expected that the ozone depletion potential of BIT is negligible, approaching zero.

According to Annex I to CLP 'a substance shall be classified as Hazardous to the Ozone Layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer. Patently, for BIT, this is not the case and we agree with the CLH dossier submitter that the appropriate conclusion is not to classify BIT as hazardous to the ozone layer.