Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

2-methyl-2*H*-isothiazol-3-one hydrochloride; 2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride

EC Number: 247-499-3
CAS Number: 26172-54-3

CLH-O-0000007341-81-01/F

Adopted

14 September 2023

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ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-METHYL-2*H*-ISOTHIAZOL-3-ONE HYDROCHLORIDE; 2-METHYL-2,3-DIHYDRO-1,2-THIAZOL-3-ONE HYDROCHLORIDE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: 2-methyl-2*H*-isothiazol-3-one hydrochloride; 2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride
EC number: 247-499-3
CAS number: 26172-54-3
Dossier submitter: Slovenia

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2023	Germany		MemberState	1
Comment received				
We highly appreciate the clear and complete presentation of data by the Slovenian CA as well as usage of the RAAF to outline the proposed read across approach. Read Across: The read across proposal is supported for systemic endpoints given the physiological similarity of MIT-HCl (N-MIT-HCl) and MIT (N-MIT). It seems, that all experimental studies performed with MIT have been considered in the corresponding CLH-report from 2015 and the RAC-opinion from 2016. Thus, the classification of systemic effects can be based on the current entry of MIT in Annex VI. Of note, for some endpoints studies with Kathon were also considered (e.g., carcinogenicity). Kathon consists of CMIT and MIT in a 3:1 ratio. Thus, toxicity of the mixture is likely driven by CMIT, which was not included in the read across proposal as source substance. However, given the comparable toxicity of CMIT we agree that studies with Kathon may also be used for hazard assessment of MIT-HCl.				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.01.2023	Germany	Thor GmbH	Company-Manufacturer	2
Comment received				
Thor GmbH is a biocidal active substance manufacturer and a Review Programme participant for several active substances, among them 2-methyl-2H-isothiazol-3-one				

(MIT; CAS no. 2682-20-4). For MIT a harmonised classification is already available and it is an approved active substance for the PTs 11, 12 and 13.

N-MIT·HCl represents the hydrochloride form of MIT and is therefore only a special form of MIT. Under specific conditions e.g. neutral pH, like they occur in natural environments, N-MIT·HCl is available as MIT. Based on these fundamental chemical principles, the classification and labelling of N-MIT·HCl the same data basis should be used than for MIT. We are consequently of the opinion that the harmonised classification of N-MIT·HCl should be fully consistent with the harmonised classification of MIT.

Therefore, all available data on MIT should be included in the N-MIT·HCl CLH report and considered in the derivation of the harmonised classification. It seems that only a part of the MIT data has currently been included in this CLH report. However, there are two data sets available for MIT, one which is already partly included in the N-MIT·HCl CLH report (data owner Rohm and Haas according to CLH report) and another one from Thor which is at the moment not included at all in the CLH report. Both data sets are available at ECHA and we would like to refer to the MIT CLH report, the MIT RAC opinion (CLH-O-0000001412-86-105/F) and the MIT Assessment Reports published in connection with the active substance approval of MIT in PTs 11, 12 and 13 for an overview of the full data sets. For the sake of consistency it would be reasonable if all MIT data were included in the N-MIT·HCl CLH report.

Dossier Submitter's Response

Thank you for your comment.
The proposed classification for N-MIT·HCl is based on the data available for N-MIT·HCl, and as read-across to MIT, where no adequate data are available for N-MIT·HCl.

N-MIT·HCl in comparison to MIT is proposed to be more severely classified for skin corrosion based on an *in vitro* study with N-MIT·HCl (hazard class sub-categories Skin Corr. 1A and Skin Corr. 1B, respectively).

MIT is classified for acute inhalation and dermal toxicity endpoints. There is no N-MIT·HCl data for these endpoints. Read-across was not proposed for these endpoints, as the differences in pH between N-MIT and N-MIT·HCl (higher than 2.0 and less than 2.0, respectively) may contribute to the severity of local effects and hence also to the greater acute inhalation and dermal toxicity of N-MIT·HCl in comparison to MIT. It was agreed not to classify altogether for these hazard classes due to data lacking (data waived based on corrosivity).

Not all environmental data from the MIT RAC opinion are currently referred to. We agree that the MIT.HCl CLH report should refer to all environmental data used in the MIT RAC opinion. Otherwise, both the data sets that were used for MIT RAC opinion (CLH-O-0000001412-86-105/F) and the MIT Assessment Reports are included in the N-MIT·HCl CLH report. It can be seen from "Appendix V: Overall Reference List (Including Data Owner and Confidentiality Claim)" which data from Thor GmbH was included.

RAC's response

Noted and agreed. All the available MIT data have been considered in the opinion making.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2023	Germany		MemberState	3
Comment received				
No classification for carcinogenicity based on one oral and one dermal carcinogenicity study with CMIT/MIT is supported.				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Noted.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2023	Germany		MemberState	4
Comment received				
No classification for germ cell mutagenicity based on negative in vivo results obtained with the read-across substance MIT is supported. Most weight should be assigned to two negative MN tests with MIT, results of which are supported by toxicokinetic data showing that MIT can reach the target organ and thus presumably also the germ cells (see BD to RAC Opinion on MIT, 2016). Negative in vivo findings with the source substance MIT are further supported by negative results in vitro using the target substance MIT-HCl.				
Dossier Submitter's Response				
Thank you for your support and additional argumentation.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2023	Germany		MemberState	5
Comment received				
No classification based on multi-generation data for the source substance MIT is supported. Of note, some effects on sperm parameters were observed in a 90-d oral toxicity study in Wistar rats (which is described in the repeat dose toxicity section). However, since changes were within the HCD range and no such effects were seen in the 2-generation study we agree that these effects are not sufficient to trigger classification.				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Noted. Sperm effects in 90-d study added to the opinion.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2023	Germany		MemberState	6
Comment received				
We agree with the assessment of acute oral toxicity of MIT-HCl and with the proposed classification as Acute Tox. 3; H301 with an ATE of 175 mg/kg bw based on the acute oral				

toxicity study summarised in the report.
For comparison, MIT has also been tested for acute oral toxicity in rats in three studies. The LD50 values in females were 120 mg/kg bw (Crl:CD®BR), 183 mg/kg bw (Crl:CD®BR), and 247 mg MIT/kg bw (Wistar). A classification with Acute Tox. 3 (H301) was also derived for MIT by RAC (2016). Partly a different rat strain was tested, but it seems that the additional HCl included in MIT-HCl did not induce a substantially higher acute toxicity. The potency of both substances was comparable.

As for inhalation and dermal toxicity, it is agreed that the local potency of MIT-HCl might be higher than that of MIT. However, the oral LD50 values of MIT and MIT-HCl are comparable. Data from Kathon could also be considered, though the classification as Skin Corr. 1C at concentrations ≥ 0.6 % indicates a lower local potency. Kathon has a harmonised classification as Acute Tox. 2, H330 and Acute Tox. 2, H310.

Dossier Submitter's Response

Thank you for your comment and support regarding the acute oral toxicity classification. Regarding acute dermal and inhalation toxicity, the applicant proposed a read-across to MIT and the classification Acute Tox. 2, H330 and Acute Tox. 3, H331. Acute toxicity studies are conducted at higher concentrations than repeated toxicity studies; therefore, basic assumption that N-MIT·HCl dissolved in water is identical to MIT is considered not valid. N-MIT·HCl has a lower pH than MIT (below 2.0 and over 2.0, respectively). Based on the available data, it was concluded that the hazard classes (potency) could not be determined definitely, so no classification was proposed (data lacking due to data waived based on corrosivity).

RAC's response

Noted. Also RAC is of the opinion that it would be difficult to disregard the MIT data leading to classification, even if there are uncertainties related to the HCl component potentially increasing the acute toxicity. Further discussion on this is included in the opinion document.

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2023	Germany		MemberState	7

Comment received

MIT-HCl (98.7%) was tested in the three-dimensional human skin model EPIDERM (OECD 431) and the tissue viability was 5.6 % after 60 minutes and 22 % after 3 minutes exposure. The viability after 3 minutes was slightly below the border of < 25 % relevant for a sub-category 1A. It is noted by the DS, that the in vitro-test (OECD TG 431) results in over-classification in a proportion of cases and category 1A (H314) is proposed as worst-case.
For comparison, MIT (Category 1B, H314, GCL) has also been tested in the three-dimensional human skin model EPIDERM (OECD 431). A concentration of 51.5 % in water was not corrosive after 3 minutes exposure, but was corrosive after 60 minutes exposure as indicated by reduction of cell viability to 13.6 %. A higher local potency of MIT-HCl could be deduced, but a comparison is hampered by the difference in applied concentrations (MIT-HCl: 98.7 %, MIT: 51.5 %), MIT-HCl was more concentrated.
Comparing the potency of MIT and HCl it is noted, that HCl (as hydrochloric acid … %) is also classified as Skin Corr. 1B, but received an SCL (H314: C ≥ 25 %), which is higher than the GCL (H314: C ≥ 5%) allocated to MIT. Thus, the influence of the additional HCl might be limited.
Based on an EPIDERM study with MIT-HCl Skin Corr. 1A classification could be assigned ("optional" according to OECD TG 431). Considering the classifications for MIT and

CMIT/MIT (Skin Corr. 1B and Skin Corr. 1C for C ≥ 0.6 %, respectively), it could be argued that a classification of MIT-HCl as Skin Corr. 1 without sub-categorisation may be more appropriate.
Dossier Submitter's Response
Thank you for your comment. We agree that based on the presented argumentation, a classification of N-MIT·HCl as Skin Corr. 1 without sub-categorisation may be more appropriate.
RAC's response
Noted and agreed, further discussion is included in the opinion document.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2023	Germany		MemberState	8
Comment received				
Since MIT-HCl was established to be corrosive with a proposed classification as Skin Corr. 1(A), classification as Eye Dam. 1, H318 is implicit and thus supported. Corrosive substances are automatically considered to be severely damaging to the eye and are classified but not labelled for serious eye damage in addition to skin corrosion (Guidance: p. 302 and p. 304).				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
13.01.2023	Switzerland	<confidential>	Company-Downstream user	9
Comment received				
Considerations on the harmonized classification of 2-methyl-2H-isothiazol-3-one hydrochloride (Cas No. 26172-54-3) with a focus on the hazard endpoint of dermal sensitization and the proposed SCL of 0.0015% MIT.HCl is a registered biocide in the European Union, the harmonized classification proposed by Slovenia is based completely upon read-across to 2-methyl-2H-isothiazol-3-one (Cas No. 2682-20-4) referred to in the dossier as N-MIT. In 2016 the Risk Assessment Committee concluded that N-MIT should be classified as a skin sensitizer Cat. 1A with a defined SCL of 0.0015% (15ppm). This was based largely on clinical data indicating a high prevalence of allergic contact dermatitis associated primarily with MIT exposure in cosmetics with the SCL derived by read-across to CMIT/MIT given a) the absence of defined exposure concentrations in the clinical population upon which an appropriate SCL could be based and b) the consistent pattern exhibited between the two substances. The RAC opinion was subsequently adopted by the EU Commission and signed into law as part of the 13th ATP with legal implementation in May 2020. In addition to the RAC opinion, the SCCS developed numerous opinions on the safe use of MIT in cosmetics with particular focus on dermal sensitisation in 2013 and 2015, concluding that MIT could be used in rinse-off products only at concentrations of up to 15ppm and that further use in leave-on products could not be supported. The timings of these two legislative interventions is significant for the current discussions				

since following the SCCS opinion, but prior to the implementation of the 13th ATP, a significant decrease in allergic contact dermatitis was observed in numerous clinical centres in Europe (Herman et al 2018, Kreft et al. 2020, Uter et al. 2020, Havmose et al. 2021 & Uter et al. 2021). Together this information demonstrates that product/sector level intervention was highly effective in managing public health risk and that additional substance specific measures in the form of harmonized classification were overly restrictive (particularly so for those sectors of use where exposure can be adequately controlled), without necessarily being additionally protective.
In considering the appropriate SCL for N-MIT the RAC noted that limited information on exposure of individuals was available at the time for independent scrutiny and presumed that MIT was present in products at concentrations below 100ppm. Subsequent to those discussions, several papers have been published highlighting concentration of N-MIT in different products (e.g. Garcia-Hidalgo et al. 2017, Marrero-Aleman et al. 2019) indicating that in some products concentrations of up to 300ppm and above may be found. This is consistent with use rates mentioned for various product types in the Assessment Reports published as part of BPR approval.
In defining an SCL for a substance it is worth noting that according to section 3.4.2.2.5 of the Guidance on the Application of the CLP Criteria (ECHA 2017) 'SCLs are set on the basis of testing of the substance and never on the basis of testing of a mixture containing the sensitising substance (see CLP Annex I, 3.4.3.1.1). The setting of SCL is based on potency; potency is already considered for the subcategorisation defining generic concentration limits. SCLs are generally applied for the most potent skin sensitisers classified in 1A.' It should be noted that clinical data reports prevalence of allergic contact dermatitis arising as a result of exposure to complex products and mixtures, it should therefore only be used to determine whether a substance has the intrinsic ability to be a dermal sensitizer and not for derivation of labelling limits. In presuming exposure of individuals to N-MIT in products at concentrations below 100ppm, by default the approach to setting the SCL for N-MIT was contrary to what the current guidance states.

Choice of an appropriate SCL for MIT.HCl
In its recent opinion on BIT, the RAC compared the relative potencies of the various isothiazolones showing that BIT appears to be the weakest potency sensitizer of the group based upon animal data. In addition, the RAC concluded that an appropriate labelling limit for dermal sensitization should be 360ppm based on the HRIPT study conducted by Basketter et al (1999).
For N-MIT the same table shows a difference in potency between N-MIT and CMIT/MIT, (strong to extreme versus extreme) again based upon results from several animal models. It is noteworthy that in the original opinion for N-MIT, 4/5 animal studies (2xLLNA, 1xBeuhler and 1xGPMT) indicated that N-MIT was not an extreme sensitizer whilst only 1 GPMT assay was potentially indicative of this. In addition, the table presented indicates a significant difference in potency between N-MIT and CMIT/MIT as demonstrated by the results of the LLNA with reported EC3 values of 0.86% vs 0.003% respectively. Furthermore, a similar HRIPT study to that conducted using BIT has been performed in human volunteers using larger group numbers which indicated that 400ppm was a threshold for induction of sensitization, with exposure to 300ppm showing no dermal reactions. Taken together this information demonstrates that N-MIT and CMIT/MIT have a significant difference in potency and that identical labelling limits for dermal sensitisation are not warranted.
Based on the information provided in the dossier and the considerations described above, the current proposed SCL of 15ppm can be considered overly restrictive. Using the same approach as developed for BIT, i.e. consideration of potency and information available from controlled exposure studies using human volunteers, a more appropriate SCL of 100ppm can be defined. Whilst the HRIPT data indicate that up to 300ppm N-MIT may be

considered safe for use in controlled exposure situations, a comparison of the EC3 values from LLNA studies indicate that MIT is approximately 2 to 3 times more potent a sensitiser versus BIT (EC3 values of 0.86% versus lowest BIT value of 2.3%) as a result a comparable SCL between N-MIT and BIT would not be appropriate. A three-fold lower labelling limit for N-MIT of 100ppm in comparison to BIT therefore appears most justified along with a corresponding EUH208 labelling limit of 10ppm (0.001%).

References

Isothiazolinone derivatives and allergic contact dermatitis: a review and update. Herman A, Aerts O, de Montjoye L, Tromme I, Goossens A, Baeck M. J Eur Acad Dermatol Venereol. 2019 Feb;33(2):267-276.

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J Eur Acad Dermatol Venereol. 2020 Feb;34(2):333-339. .

The epidemic of contact allergy to methylisothiazolinone-An analysis of Danish consecutive patients patch tested between 2005 and 2019. Havmose M, Thyssen JP, Zachariae C, Menné T, Johansen JD.
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Patch test results with the European baseline series and additions thereof in the ESSCA network, 2015-2018. Uter W, Bauer A, Belloni Fortina A, Bircher AJ, Brans R, Buhl T, Cooper SM, Czarnecka-Operacz M, Dickel H, Dugonik A, Geier J, Giménez-Arnau AM, Gonçalo M, Johansen JD, Johnston GA, Mahler V, Rustemeyer T, Sanchez-Perez J, Schuttelaar MLA, Simon D, Spiewak R, Valiukevičienė S, Weisshaar E, White IR, Wilkinson M; ESSCA Working Group.
Contact Dermatitis. 2021 Feb;84(2):109-120.

Occurrence and concentrations of isothiazolinones in detergents and cosmetics in Switzerland. Garcia-Hidalgo E, Sottas V, von Goetz N, Hauri U, Bogdal C, Hungerbühler K.Contact Dermatitis. 2017 Feb;76(2):96-106.

Isothiazolinones in cleaning products: Analysis with liquid chromatography tandem mass spectrometry of samples from sensitized patients and market. Marrero-Alemán G, Borrego L, Antuña AG, Macías Montes A, Pérez Luzardo O.Contact Dermatitis. 2020 Feb;82(2):94-100.

Dossier Submitter's Response

Thank you for your comment. The proposal for classification of N-MIT·HCl is based on read-across to MIT; therefore, we support an SCL of 15 ppm. According to the RAC opinion for BIT, MIT has approximately the same potency based on EC_3 values as MBIT and OIT (EC_3 (BIT) > EC_3 (MBIT) ≈ EC_3 (MIT) ≈ EC_3 (OIT) > EC_3 (DCOIT) >> EC_3 (CMIT/MIT). OIT, MBIT, and MIT all have a harmonised SCL of 15 ppm.
We consider that the presented articles do not provide direct value that could be used to set SCL.

RAC's response
RAC agrees with the DS. In the case of MIT, setting of SCL was largely based on human evidence showing that MIT can cause sensitization at levels clearly below 100 ppm. The references given above support this; e.g. studies by Garcia-Hidalgo et al. 2017, Marrero-Aleman et al. 2019 show that in many cases the levels of MIT in the products were about 50 ppm. In addition, for example in the study by Garcia-Hidalgo et al., 2017, half of the products were collected from sensitized patients. The study by Uter et al., 2020 nicely show the decrease in the number of sensitization cases caused by MIT after the recognition of the hazards of MIT at low levels and implementation of the limit of 15 ppm for it. RAC therefore considers these studies supporting the limit of 15 ppm for MIT-HCl. The cited sentence in chapter 3.4.3.1.1. of annex I of REACH refers to animal testing data, not to the use of human evidence.

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2023	Germany		MemberState	10
Comment received				
The assessment is based on studies with MIT and it is noted, that four studies in guinea pigs (2 Magnusson-Kligman-tests, one Buehler-test, one open epicutaneous test) and one LLNA of the CLH report on MIT are missing. Read across to MIT is supported for skin sensitisation. Thus, Skin Sens. 1A, H317 with an SCL of 15 ppm applies.				
Dossier Submitter's Response				
Thank you for your comment and support. Studies considered missing are not listed in Table A.19 Summary table of animal studies on skin sensitisation, but they are mentioned in the text in Section A3.5.2 Comparison with the CLP criteria (reference to the RAC opinion for MIT).				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2023	Germany		MemberState	11
Comment received				
STOT SE 1/2: We support no classification for STOT SE Cat. 1 and 2 since no effects warranting classification in one of those categories were observed with the source nor the target substance. STOT SE 3 (RTI): Due to the presumably corrosive MoA, additional labelling with EUH071 is supported for respiratory tract effects observed with the source substance MIT (instead of STOT SE3 (RTI) classification). Furthermore, EUH071 labelling was also agreed for both MIT and CMIT/MIT. STOT SE 3(narcotic effects): Section A3.2.5.2 of the CLH report states: "There are no data from acute toxicity studies that indicate a narcotic effect (e.g. evidence of persistent lethargy, lack of coordination, loss of righting reflex, and ataxia) for N-MIT-HCl, or for N-MIT. The clinical signs reported in acute toxicity studies are consistent with a local irritant effect at the site of contact (and secondary effects) and do not indicate any systemic effect on the nervous system." According to CLP Regulation Annex I, 3.8.2.2.2 criteria STOT SE 3 classification for narcotic effects are symptoms observed in humans or "b) narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia." These effects should be transient in nature, otherwise STOT SE 1 or 2 may apply:				

"If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure."

Such effects were observed in several studies with the source and the target substances and are listed throughout the report but were not discussed as secondary to local toxicity in the respective sections (A3.2.1-A3.2.3). These effects were ataxia, hunched or prone posture, ptosis (acute oral toxicity studies with MIT-HCl and MIT), passiveness, lethargy, prostration, hypoactivity, incoordination, squatting, abnormal gait (inhalation toxicity studies with MIT). Passiveness and ataxia were also observed in the acute dermal toxicity study with MIT. Furthermore, hypoactivity was observed in one of the UDS studies with MIT and ataxia and passiveness in one MN study with MIT.
It may be argued that these effects were often seen at doses associated with mortality and may therefore not warrant classification as STOT SE 3, H336. However, a thorough discussion should be provided before dismissing effects consistently observed over several studies and routes of exposure as merely secondary to local toxicity.

Dossier Submitter's Response

Thank you for your comment. Clinical signs as lethargy/reduced responses/underactivity/ataxia, abnormal gait/hunched posture/prone gait, tremors, paddling movements, partially closed eye are all known and associated with stress/pain (OECD Series on Testing and Assessment No. 19. Guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation, 2000; https://your.yale.edu/policies-procedures/guides/4446-clinical-signs-pain-and-disease-laboratory-animals). N-MIT·HCl is a corrosive substance, and as already reported in the CLH report for N-MIT·HCl, the clinical signs reported in acute toxicity studies are consistent with a local irritant effect at the site of contact (and secondary effects) that cause pain and do not indicate any systemic effect on the nervous system. Macroscopic changes observed at (usually) high doses confirmed irritant/corrosive effects on tissues at the point of contact.

Acute oral toxicity study with N-MIT·HCl (Anonymous 10, 2018): Most clinical signs that can be potentially related to narcotic effects were observed immediately after dosing (550 mg/kg bw) or developed within one or two hours after dosing (550 mg/kg bw and 175 mg/kg bw). Along with ataxia, hunched or prone posture, ptosis also piloerection and dyspnoea were observed. Prone posture, hypothermia, ptosis, and tonic convulsion were observed immediately prior to death. There were no clinical signs observed at the dose level of 55 mg/kg bw. The signs lasted for up to four hours after dosing (middle dose) or until the animals were killed humanely (high dose). No macroscopic changes were noted at necropsy, except for pale lungs, a red and thick fundus region of the stomach, a gelatinous appearance of the mucosal surface of the fundus region of the stomach and a small caecum which were noted in the animal dosed at 550 mg/kg bw that was killed *in extremis* 6.5 hours after dosing. The onset of the clinical signs is directly related to dosing rather than a systemic effect. Hypoactivity and ataxia seen in some other gavage studies (one of the UDS studies with MIT and one MN study with MIT) are also considered to be responses to pain.

In the first acute dermal toxicity study with MIT (Anonymous 14, 1999), the clinical signs that can be related to pain and stress (red material around the muzzle, red material around the eyes, scant and/or no faeces, passiveness, laboured breathing, and ataxia) appeared together with the appearance of clinical signs on the skin (blanching, oedema, darkened areas, eschar, sloughing, scabbed areas, and desiccation) one day after dosing. Surviving rats recovered from signs that were probably related to pain and stress by day 5. Skin effects were observed continuing to Day 14. The effects were dose dependent. A

decrease in body weight gain was also observed. Reduced appetite can also be an indicator of pain (Castner and Moberg, Recognizing Pain and Distress in Laboratory Animals, ILAR Journal, 2000, 41(2), 62-71). In the second acute dermal toxicity study with MIT (Anonymous 23, 2000) only strong irritation of the skin was observed. No clinical signs of intoxication were observed. Body weight stasis was apparent in females. In three acute inhalation toxicity studies (Anonymous 11, 1995; Anonymous 12, 2001, Anonymous 13, 2002; Anonymous 31, 2000) several clinical signs were observed that could be related to narcotic effects. These signs were accompanied by other symptoms of respiratory tract irritation and pain (described in detail in the CLH report for N-MITHCl). Necropsies revealed irritating/corrosive effects on the respiratory tract, confirming the nature of the effects. To summarize, clinical signs such as ataxia, hunched or prone posture, ptosis (acute oral toxicity studies with MIT-HCl and MIT), passiveness, lethargy, prostration, hypoactivity, incoordination, squatting, abnormal gait (inhalation toxicity studies with MIT), passiveness and ataxia (acute dermal toxicity study with MIT), salivation and hypoactivity (one of the UDS studies with MIT), are behavioural signs attributed to severe pain and an inflammatory response at the point of contact (the nonglandular stomach in gavage studies, the skin in dermal studies and the nasal airway and its contiguous - ears, sinuses, eyes in inhalation studies). Therefore, the classification as STOT SE 3, H336 is not warranted.
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2023	Germany		MemberState	12
Comment received				
No classification for STOT RE due to lack of classifiable effects in repeat dose toxicity studies with the source substance MIT and CMIT/MIT is supported.				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2023	United Kingdom	Health and Safety Executive	National Authority	13
Comment received				
2-methyl-2H-isothiazol-3-one hydrochloride; 2-methyl-2,3-dihydro-1,2-thiazol-3-one hydrochloride (EC: 247-499-3; CAS: 26172-54-3). We agree that algae are the most acutely and chronically sensitive trophic group to N-MIT·HCl. This substance is an isothiazolone which as highlighted in the CLH report, has a two-step mechanism of action involving rapid inhibition (within minutes) of growth and metabolism, followed by an irreversible cell damage resulting in loss of viability (within hours). The isothiazolone ring of the substance is cleaved during this inhibition, resulting				

in rapid degradation/removal of the substance from the test system.

Given this mechanism of action, RAC have previously agreed with the use of 24- or 48-hour acute algal endpoints based on initial measured or nominal concentrations for the Aquatic Acute of other isothiazolones, MIT (CAS: 2682-20-4), OIT (CAS: 26530-20-1), MBIT (CAS: 2682-20-4), DCOIT (CAS: 64359-81-5) and BIT (CAS: 2634-33-5). RAC also agreed the Aquatic Chronic classifications based on initial measured concentrations. This approach recognises that endpoints based on initial measured or nominal concentrations are relevant for hazard classification of isothiazolinones because mean measured endpoints reflect a significant period of time when the substance was not available because it had been depleted and algal cells were recovering.

The proposed key aquatic acute and chronic toxicity endpoints used in the CLH report are the 72-hour ErC_{50} of 0.289 mg a.i./L and the 72-hour NOErC of 0.047 mg a.i./L from a study with Pseudokirchneriella subcapitata using N-MIT·HCl as the test item. Both endpoints are expressed as geometric mean measured concentrations. Given the position in previous RAC Opinions on isathiazolones, please can the DS provide acute and chronic endpoints for 24-, 48- and 72-hr exposure periods based on initial measured and nominal concentrations for this study. For Acute classification, we feel the key endpoint should mirror RAC's previous application of 24- or 48-hour endpoints depending on which is the lower. Considering that Chronic classification should reflect multiple generations in algae, please can the DS and RAC confirm which of these endpoints are the most suitable for the Aquatic Chronic classification of N-MIT·HCl by noting which of the 24/48/72 hour endpoints represent exponential growth and multiple generations.

We also note that read-across of ecotoxicity data for MIT (also called N-MIT) has been considered in the CLH proposal for N-MIT·HCl. Additional relevant ecotoxicity studies are available in the CLH report for MIT which should be included in the current CLH assessment. In particular, a Skeletonema costatum 24-hour ErC_{50} of 0.0695 mg a.i./L based on initial measured concentrations is available which has been used as the key endpoint for the harmonised classification of MIT as Aquatic Acute 1 (M=10). This indicates that S. costatum is more acutely sensitive than Pseudokirchneriella subcapitata to MIT. This endpoint could therefore lead to a higher acute M-factor for N-MIT·HCl.

For all read-across data, please can the DS provide the equivalent endpoints for N-MIT·HCl corrected for the difference in molar mass.

Dossier Submitter's Response

Thank you for your comment.
There is an acute and chronic endpoint available for the most sensitive thropic level, i.e. algae, from a study performed with N-MIT·HCl. For C&L intrinsic toxicity is leading and the study with N-MIT·HCl gets in our view precedence over read-across to MIT. In algal studies with MIT the first 24 hour is the most sensitive period for P. subcapitata and other species. In the study with N-MIT·HCl decline of the test substance is much slower compared to MIT and endpoints derived over 72 hours (ErC_{50} 0.290 mg/L) and 48 hours (0.249 mg/L) are clearly lower compared to 24-h endpoint (ErC_{50} 0.825 mg/L). This clearly indicates that there is a difference between environmental fate and intrinsic effects of N-MIT·HCl in a test with algae compared to a similar test with MIT. The use of 24-h endpoints based on initial measured concentrations derived from studies with MIT for classification of N-MIT·HCl is in our view not justified. The point of the algal study design and duration is that exponential growth of the population is occurring throughout the test duration. During the early study part (24, 48 hrs) there definitely should be exponential growth occurring, so multiple 'generations' are covered. Actually the only risk is that the

population grows too quickly so that by 72/96 hours exponential growth drops off. Therefore, Chronic classification could clearly be represented by 24-h and 48-h endpoints as in the case of Acute classification.

See above. We do not propose read-across to algal studies with MIT for the purpose of environmental classification. Degradation of the test substance was slower in the study with N-MIT·HCl compared to the study with MIT. We consider it therefore appropiate to derive endpoints based on geometric mean of the measured concentration at beginning and end of the test. 24-hour, 48-hour and 72-hour ErC_{50} values based on mean measured concentration are 0.825, 0.249 and 0.289 mg/L, respectively. 24-hour, 48-hour and 72-hour NOEC values are all equal to 0.047 mg/L. EC_{10} values are not available for 24-hour and 48-hour but from the data it is clear that the 24-h EC_{10} should be higher than 48-hour and 72-hour values. The lower EC_{50} after 48 hours (0.249 mg/L) of instead of the EC_{50} after 72 hours (0.289 mg/L) may be considered (see next comment) as this was also common practice for other isothiazolinone biocidal active substances noting that it does not make a difference for the overall conclusion. We refer to the CLH report for MIT from 2015 and thus implicitely to all studies referenced in this report. Read-across from data for MIT to conclude a higher acute sensitivity of S. costatum compared to P. subcapitata also for N-MIT·HCl does in our opinion not apply given the demonstrated difference between environmental fate and intrinsic effects of N-MIT·HCl in a test with algae compared to a similar test with MIT.

See above. We do not propose read-across to algal studies with MIT for the purpose of environmental classification. Equivalent endpoints for N-MIT·HCl corrected for the difference in molar mass should not be relevant as we do not propose read-across.

RAC's response

Thank you for your comment. RAC agree with the Comentor. The substance N-MIT·HCl is conjugated acid of the base N-MIT. At the same pH level the concentrations of both substances will be identical. No valid data that mode of action of N-MIT·HCl will be different from already confirmed mode of action of isothiazolones. From such point of view all aquatic toxicity results obtained for N-MIT has to be taken into account for the classification of N-MIT·HCl. In the new test from 2017 still the concentrations of test substance are maintained < 80 % of nominal. No valid data that substance degradation is slower. Most probably due to lower detections limit achieved (LC-MSMS is used for substance quantification) the concentrations are measured after 72 h. In addition, the initial cell density in the new test (2017) is twice lower and test results are not comparable with results from previous toxicity tests with *Pseudokierchneriella subcapitata*. RAC opinion is that still the most sensitive species is *Skeletonema costatum* and 24-hour ErC_{50} of 0.0695 mg a.i./L based on initial measured concentrations has to be used for aquatic acute classification of N-MIT·HCl (read across from the data obtained for N-MIT). In this way the classification for N-MIT·HCl will be in line with the classification of N-MIT – aquatic acute 1 with M-factor 10.

Date	Country	Organisation	Type of Organisation	Comment number
13.01.2023	Germany	Thor GmbH	Company-Manufacturer	14

Comment received

Thor GmbH is a biocidal active substance manufacturer and a Review Programme participant for several active substances, among them 2-methyl-2H-isothiazol-3-one (MIT; CAS no. 2682-20-4). For MIT a harmonised classification is already available and it is an approved active substance for the PTs 11, 12 and 13.

N-MIT·HCl represents the hydrochloride form of MIT and is therefore only a special form

of MIT. Under specific conditions e.g. neutral pH, like they occur in natural environments, N-MIT·HCl is available as MIT. Based on these fundamental chemical principles, the classification and labelling of N-MIT·HCl the same data basis should be used than for MIT. We are consequently of the opinion that the harmonised classification of N-MIT·HCl should be fully consistent with the harmonised classification of MIT.

Therefore, all available data on MIT should be included in the N-MIT·HCl CLH report and considered in the derivation of the harmonised classification. It seems that only a part of the MIT data has currently been included in this CLH report. However, there are two data sets available for MIT, one which is already partly included in the N-MIT·HCl CLH report (data owner Rohm and Haas according to CLH report) and another one from Thor which is at the moment not included at all in the CLH report. Both data sets are available at ECHA and we would like to refer to the MIT CLH report, the MIT RAC opinion (CLH-O-0000001412-86-105/F) and the MIT Assessment Reports published in connection with the active substance approval of MIT in PTs 11, 12 and 13 for an overview of the full data sets. For the sake of consistency it would be reasonable if all MIT data were included in the N-MIT·HCl CLH report.

When comparing the proposed classification of N-MIT·HCl with MIT, it is obvious that both substances are classified as Aquatic Acute Cat. 1 but they differ regarding the M-Factor acute which is proposed to be 1 for N-MIT·HCl and 10 for MIT. Based on the above and considering both MIT data sets, the M-Factor acute of N-MIT·HCl should be similar to MIT. Therefore, Thor does not agree with this the proposed M-Factor acute for N-MIT·HCl.

According to the CLH report the available data show that N-MIT·HCl can be classified as Aquatic Acute Cat. 1 with a M-Factor of 1. The relevant study for the derivation of this classification is an algae study with N-MIT·HCl from Roche with Pseudokirchneriella subcapitata (Anonymous, 2017b) with an EC_{50} of 0.289 mg/L. A second algae study is mentioned in this CLH report from Rohm and Haas (Anonymous, 1997) where the test item is MIT and the EC_{50} is 0.102 mg/L.

According to the CLH Report and the RAC Opinion on MIT of July 2015, the classification of MIT is based on an algae study with Skeletonema costatum with an EC_{50} of 0.0695 mg/L (A7.4.1.3.b/01 from Rohm and Haas) which is currently not included in the N-MIT·HCl CLH report. For the sake of consistency, we are of the opinion that this MIT study should be taken into account for the classification of N-MIT·HCl as well, because of the reasoning mentioned above. This would then result in a consistent harmonised classification of both substances of Aquatic Acute Cat. 1, M-Factor 10. In doing so, the harmonised classification of N-MIT·HCl would be suitable to specifically protect the environment against potential dangers of MIT which will be formed under natural conditions. The worst case classification M-Factor 10 would therefore be adequate here.

Additionally, we suggest that the timepoint with the lowest EC_{50} value from the algae study Anonymous, 2017b is used, i.e. the EC_{50} after 48 hours (0.249 mg/L) of instead of the EC_{50} after 72 hours (0.289 mg/L). This was so far also common practice for other isothiazolinone biocidal active substances.

Dossier Submitter's Response

Thank you for your comment.
There is an acute and chronic endpoint available for the most sensitive thropic level i.e. algae, from a study performed with N-MIT·HCl. For C&L intrinsic toxicity is leading and the study with N-MIT·HCl gets in our view precedence over read-across to MIT. In algal studies with MIT the first 24 hour is the most sensitive period for P. subcapitata and other species. In the study with N-MIT·HCl decline of the test substance is much slower

compared to MIT and endpoints derived over 72 hours (ErC_{50} 0.290 mg/L) and 48 hours (0.249 mg/L) are clearly lower compared to 24-h endpoint (ErC_{50} 0.825 mg/L). This clearly indicates that there is a difference between environmental fate and intrinsic effects of N-MIT·HCl in a test with algae compared to a similar test with MIT. Read-across using 24-h endpoints based on initial measured concentrations derived from studies with MIT for classification of N-MIT·HCl is in our view not justified. We agree that the lower EC_{50} after 48 hours (0.249 mg/L) of instead of the EC_{50} after 72 hours (0.289 mg/L) may be considered as this was also common practice for other isothiazolinone biocidal active substances noting that it does not make a difference for the overall conclusion.

We refer to the CLH report for MIT from 2015 and thus implicitely to all studies referenced in this report.

RAC's response

Thank you for your comment. RAC agree with the Commenter. The substance N-MIT·HCl is conjugated acid of the base N-MIT. At the same pH level the concentrations of both substances will be identical. No valid data that mode of action of N-MIT·HCl will be different from already confirmed mode of action of isothiazolones. From such point of view all aquatic toxicity results obtained for N-MIT has to be taken into account for the classification of N-MIT·HCl. In the new test from 2017 ((Anonymous, 2017b) still the concentrations of the test substance are maintained < 80 % of nominal. No valid data that substance degradation is slower. Most probably due to lower detections limit achieved (LC-MSMS is used for substance quantification) the concentrations are measured after 72 h. In addition, the initial cell density in the new test (Anonymous, 2017b) is twice lower and test results are not comparable with results from previous toxicity tests with *Pseudokierchneriella subcapitata* (initial cell density 10 000 cell/mL). RAC opinion is that still the most sensitive species is *Skeletonema costatum* and 24-hour ErC_{50} of 0.0695 mg a.i./L based on initial measured concentrations has to be used for aquatic acute classification of N-MIT·HCl (read across from the data obtained for N-MIT and assumption accepted by RAC committee in CLH report for N-MIT. In this way the classification for N-MIT·HCl will be in line with the already accepted classification of N-MIT – aquatic acute 1 with M-factor 10.

Date	Country	Organisation	Type of Organisation	Comment number
13.01.2023	France		MemberState	15

Comment received

The study containing the worst case algae acute endpoint from the MIT CLH report (24h EC50 = 0.0695) has not been taken into account. Considering the read across between MIT and MIT HCl, we think that the worst case data from the MIT data should be taken into account for the classification of MIT HCl. Therefore, we support, to maintain the M-factor of 10 for the aquatic acute classification, as it was already agreed for the harmonised classification of MIT. Hence, we do not agree with the M-Factor for the aquatic acute classification.

Dossier Submitter's Response

Thank you for your comment.
There is an acute and chronic endpoint available for the most sensitive thropic level i.e. algae, from a study performed with N-MIT·HCl. For C&L intrinsic toxicity is leading and the study with N-MIT·HCl gets in our view precedence over read-across to MIT. In algal studies with MIT the first 24 hour is the most sensitive period for P. subcapitata and other species. In the study with N-MIT·HCl decline of the test substance is much slower compared to MIT and endpoints derived over 72 hours (ErC_{50} 0.290 mg/L) and 48 hours (0.249 mg/L) are clearly lower compared to 24-h endpoint (ErC_{50} 0.825 mg/L). This

clearly indicates that there is a difference between environmental fate and intrinsic effects of N-MIT·HCl in a test with algae compared to a similar test with MIT. The use of 24-h endpoints based on initial measured concentrations derived from studies with MIT for classification of N-MIT·HCl is in our view not justified.
RAC's response
Thank you for your comment. RAC agree with the Commenter. The substance N-MIT·HCl is conjugated acid of the base N-MIT. At the same pH level the concentrations of both substances will be identical. No valid data that mode of action of N-MIT·HCl will be different from already confirmed mode of action of isothiazolones. From such point of view all aquatic toxicity results obtained for N-MIT has to be taken into account for the classification of N-MIT·HCl. In the new test from 2017 still the concentrations of test substance are maintained < 80 % of nominal. No valid data that substance degradation is slower. Most probably due to lower detections limit achieved (LC-MSMS is used for substance quantification) the concentrations are measured after 72 h. In addition, the initial cell density in the new test (Anonymous, 2017b) is twice lower and test results are not comparable with results from previous toxicity tests with *Pseudokierchneriella subcapitata*. RAC opinion is that still the most sensitive species is *Skeletonema costatum* and 24-hour ErC_{50} of 0.0695 mg a.i./L based on initial measured concentrations has to be used for aquatic acute classification of N-MIT·HCl (read across from the data obtained for N-MIT). In this way the classification for N-MIT·HCl will be in line with the classification of N-MIT – aquatic acute 1 with M-factor 10.