**Committee for Risk Assessment**

**RAC**

Annex 2

# Response to comments document (RCOM)

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

# Dimethyl disulphide

**EC Number: 210-871-0**
**CAS Number: 624-92-0**

CLH-O-0000001412-86-218/F

**Adopted**

**8 June 2018**

**ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DIMETHYL DISULPHIDE**

## COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public 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 public 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 public 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.

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**Substance name: dimethyl disulphide**
**EC number: 210-871-0**
**CAS number: 624-92-0**
**Dossier submitter: Industry (ARKEMA France)**

### GENERAL COMMENTS

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 07.07.2017 | France | | MemberState | 1 |
| Comment received | | | | |
| Dimethyl disulphide (DMDS) is currently under assessment by France as Rapporteur Member State in view of its approval under PPP Regulation (EC) No 1107/2009. The dossier has been submitted by ARKEMA.<br>It should be noted that France may propose different NOAEC than those reported by the applicant in the CLH report. Nevertheless, the comments will be focused on classification proposal only.<br><br>In the frame of the ongoing approval process of the active substance DMDS according to Regulation (EC) No. 1107/2009, a study has been provided in order to identify the major metabolites of DMDS in air via photo-oxidation (Anonymous, 2002., CNRS/LCSR "Etude de l'oxydation atmosphérique du disulfure de diméthyle"). This study was conducted in Atmospheric Simulation chambers. Formaldehyde, methanesulfonic acid, and sulphur dioxide were identified as major metabolites of DMDS in air via photo-oxidation. The yields of the reactions implied in the formation of each metabolite (98% for formaldehyde, 88 % for sulphur dioxide and 35 % for methanesulfonic acid) were considered as the most relevant for further risk assessments. The corresponding formation fractions are 0.47 for SO2, 0.25 for formaldehyde and 0.28 for MSA.<br><br>Some toxicity studies reported in the CLH report have not been provided in the dossier submitted under PPP regulation. However, no impact on the classification proposal is expected. | | | | |

According to the monography of DMDS, the minimum purity of active substance is 98% and not ≥99.5% as indicated.

According to the monography of DMDS and 5-batch analysis study, the maximum specification limit of Methanethiol has been set at 0.05% (not 0.01% as indicated).

According to the monography of DMDS and 5-batch analysis study, the maximum specification limit of confidential impurity 1 has been set at 0.1% (not 0.15% as indicated).

Dossier Submitter's Response

The Dossier Submitter would like to thank France for these comments and submits respectfully the following response :

General comment :
DMDS is an ubiquitous substance with a distinctive garlic odour that is part of the naturally occurring global sulphur cycle. It is released to the atmosphere in emissions from soil, plants, soil and water micro-organisms, food, animal, biological wastes and from the oceans (Pr J. Auger, 2012, "Scientific Peer reviewed Open Litterature for the natural occurrence of DMDS", University François Rabelais[1]).

DMDS's natural occurrence in food is documented in onion, garlic, milk, cheese, meats, mushrooms (REACH CORAP Substance Evaluation Conclusion July 2017).

Uses of DMDS in Europe include the following:
- DMDS is listed on the positive list of food flavouring agents in Europe (FL N° 12.026 EFSA, approved by WHO ECFA under N° 564).
- DMDS is used in confined industrial applications as intermediate for chemical synthesis, as anti-coking agent and as catalyst activator (REACH CORAP Substance Evaluation Conclusion July 2017).

An application for the approval of DMDS as Plant Protection Product under PPP regulation (EC) N° 1107/2009 has been submitted to France as Rapporteur Member State who confirmed the completeness of the dossier in February 2013. Additional data requirements by the RMS have been addressed since and the Draft Assessment Report is expected to be finalised by the end of 2017.

On the atmospheric degradation of DMDS:
As other organic volatile chemicals (terpenes such as pinene, limonene, carotene, linalool, isoprenoids, C2-C4 alkane, alcohols such as methanol, aldehydes and esters, sulphides, etc..), gaseous DMDS undergoes atmospheric oxidation initiated by OH radicals in the presence of $NO_x$ ; formaldehyde is a common product of atmospheric oxidation for most of these biogenic VOCs and is itself rapidly photo oxydised in air to $CO_2$. The Master Chemical Mechanism from the University of Leeds lists about 140 formaldehyde precursors (MCM @ http://mcm.leeds.ac.uk/MCM/, (T. Aubert, 2015,"Bibliography on the formation of formaldehyde from plant residues decomposition in the soil and from atmospheric decomposition of biogenic volatile organic compounds"[2], and T.

[1] Unpublished report submitted under reference IIA 6/01 in the PPP dossier
[2] Unpublished report submitted under reference IIIA 7.3.3.2/04 in the PPP dossier

Aubert, 2017, "Bibliography on the emission of Biogenic Volatile Organic Compounds including formaldehyde"[3]).

Based on the fact that the physical state in which DMDS is placed on the European market and is expected to be used in industrial exposure scenarios is as a liquid, based on the absence of consideration of transient products of atmospheric degradation by OH radicals for organic volatile chemicals in existing classification decisions, based on the fact that formaldehyde is a product of atmospheric degradation and not a metabolite, the Dossier Submitter considered that the information on the atmospheric oxidation degradation of DMDS is not relevant for the classification proposal dossier.

The proposed agricultural use may result in limited emissions of gazeous DMDS to air in certains pedo-climatic conditions so the Dossier Submitter submitted the information on the atmospheric degradation of DMDS in the PPP dossier and considered this information in the risk assessment in the initial dossier. At the demand of the Rapporteur Member State, modelling of air concentrations of atmospheric degradation products was conducted and indicated non significant levels (order of magnitude 1 µg/m$^3$) (Sullivan *et al*. 2013 "Modelling of DMDS ecological exposures"[4]). At the demand of the Rapporteur Member State, the Dossier Submitter organised the analysis of background levels of formaldehyde in rural sites in Southern Europe over 3 years in absence of any agricultural use of DMDS ; these analyses indicated quantifiable and variable levels of formaldehyde in air and confirmed the existence of other sources of emission of formaldehyde in rural sites (F.Ferrari *et al.*, 2015, Poster at Aarhus Conference (Denmark) "Science for the Environment"; F.Ferrari *et al.*, 2016, Poster at Brighton Crop Protection Congress Conference (UK), F. Ferrari *et al.*, 2016, Poster at EFSA Conference (Italy) "Environmental risk assessment of pesticides"). As agreed with the Rapporteur Member State, the Dossier Submitter also conducted a comparative study investigating the levels of formaldehyde in 72 air samplers during 45 days on two fields, one treated with water and one treated with DMDS, in worst case climatic conditions favourable to emission of gaseous DMDS : the results indicated no quantifiable difference in the levels of background formaldehyde in the two fields (Faivre Y.,2015 "Statistical Analysis of the experimental results obtained in Study 27/2015" [5] and Minuto A. *et al*, 2015 "DMDS: Determination of atmospheric Concentrations of HCHO and occupational fumigators exposure during and after shank professional application under barrier film in open air in Italy in 2015"[6]). Based on these results, the Dossier Submitter concludes that the contribution from atmospheric degradatation of DMDS, on the risk assessment of the proposed agricultural use, is negligible.

<u>On the specifications of DMDS:</u>
Regarding the difference of DMDS purity and maximum levels of impurities between the classification dossier and the PPP dossier, this is linked to the existence of different qualities and different regulatory analytical requirements applicable for industrial chemicals (REACH) and for PPP. No impact on classification proposal is expected.

[3] Unpublished report submitted under reference IIIA 7.3.3.2/07 in the PPP dossier
[4] Unpublished Report submitted under reference IIA 10/31 in the PPP dossier
[5] Unpublished Report submitted under reference IIIA 7.3.3.2/07 in the PPP dossier
[6] Unpublished Report submitted under reference IIIA 7.3.3.2/06 in the PPP dossier

**ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DIMETHYL DISULPHIDE**

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| General comment:<br>Some unpublished references quoted in these comments were only submitted in the PPP dossier: the Dossier Submitter is willing to submit them separately to RAC if useful. |
| RAC's response |
| RAC notes that it is possible that additional information or a different interpretation of existing studies may result from the PPP application. It is unfortunate that the dossier was submitted before this evaluation was available. The Commission may wish to review the proposal once the PPP evaluation has been completed.<br><br>The atmospheric degradation of dimethyl disulfide is not directly relevant to classification based on aquatic data. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 27.06.2017 | Germany | | MemberState | 2 |
| Comment received | | | | |
| DMDS was noted as new pesticide active ingredient in 2013. FR as RMS intends to provide the DAR by end of October to EFSA. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank Germany for this comment and has no additional elements to submit. | | | | |
| RAC's response | | | | |
| Noted – see response to comment #1. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 03.07.2017 | Finland | | MemberState | 3 |
| Comment received | | | | |
| The actual comparison of results with the CLP criteria was missing several times in the sections "Comparison with the CLP criteria" (p. 20, 22, 24, 30, 35, 42, 45). Instead, only results were stated there, without comparison to the CLP criteria. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank Finland for this comment and would like to respond as follows:<br><br>We think that this comment is based on a previous version of the CLH report because in pages 24, 30, 35, 42 and 45, there are no sections related to "Comparison with the CLP criteria". The same comment of ECHA on an earlier version of the CLH report was addressed by the dossier sumitter in the version submitted to public comments. | | | | |
| RAC's response | | | | |
| Noted. | | | | |

## MUTAGENICITY

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 07.07.2017 | France | | MemberState | 4 |

Comment received

Based on the available studies, France considered that no conclusion can be drawn on the genotoxic potential of dimethyl disulphide (DMDS), and consequently that data are not conclusive to propose or not a classification for this endpoint.
Indeed, several of the currently available genotoxicity studies show limitations and/or positive/equivocal results (see details below). The in vitro chromosome aberration test gives a positive result, and biologically relevant effects are observed in the in vitro gene mutation assay in mammalian cells. In vivo, a rat bone marrow micronucleus test is negative but the bone marrow was not demonstrated to be exposed in this study. A mouse rat bone marrow micronucleus assay, showing increased MnPCE at the low dose, is also available but shows important limitations. Therefore, a combined in vivo micronucleus assay and in vivo alkaline comet assay of dimethyl disulphide was considered necessary to conclude on the genotoxicity of DMDS and is currently ongoing. Results should be provided to France as Rapporteur Member State for the assessment of DMDS under PPP regulation before the end of 2017.

Moreover, taking into account the results of the study provided in order to identify the major metabolites of DMDS in air via photo-oxidation (Anonymous, 2002., CNRS/LCSR "Etude de l'oxydation atmosphérique du disulfure de diméthyle") (see General comments), formaldehyde (25%), sulphur dioxide (47%) and methanesulfonic acid (28%) are shown to be major air metabolites of DMDS. As no in vivo ADME study is available, the possibility that DMDS degrades itself into the same metabolites in rats is unknown. Therefore, the ongoing in vivo genotoxicity study is a key point in order to conclude on the endpoint and classification, taking also into account the lack of a carcinogenicity study with DMDS. The study would also include the effects of such metabolites.

Page 26: In vitro mammalian cell gene mutation assay (Rutten 1990a):
Non dose-related increased mutant frequencies were observed in the absence and in the presence of metabolic activation system in this study. It should be noted that the absence of a dose-related effect in the mutagenic response may be due to the high volatility of DMDS, resulting in very low recoveries (about 50% of DMDS lost directly on incubation and an additional amount of 25% DMDS is lost during incubation). Therefore, considering this important limitation, it is considered that DMDS induced biologically significant response with and without metabolic activation in this study.

Page 27:
Two studies have not been provided in the in the dossier of DMDS submitted under PPP Regulation (Jones E, 1985 and Barfknecht TR, 1985). This has no impact on the proposed classification.

Page 28: In vitro mammalian chromosome aberration test (Vogel 1990):
In the absence of S9 mix, a significant increase in cells with aberrations was observed exclusively at cytotoxic concentration and may be due to interference with cytotoxicity. Nevertheless, the result is clearly positive in the presence of S-

9 mix as it was found that DMDS induced structural chromosomal aberrations in cultured human lymphocytes. The cytotoxicity is not considered too excessive and thus, the observed effect should be considered biologically relevant. Moreover, a statistically significant increase in the number of exchanges is noted in the presence of S9 mix at the highest tested dose. It is noteworthy to mention that this type of aberrations is rare on human lymphocytes due to their genetic stability.

Page 29: In vivo Micronucleus test on rat bone marrow (Weinberg 2007):
The test is considered negative but no proof of bone marrow exposure was provided (no relevant PCE/NCE decrease). Moreover, no plasma analysis was carried out and no in vivo ADME study is available so that it is not possible to predict bone marrow exposure following DMDS administration in rats. Therefore, the results of this study would not be considered as relevant.

Page 29-30: In vivo Micronucleus test on Mouse bone marrow (Willems MI 1989):
A statistically significant increase of MnPCE was observed in males at the low dose. This concern was not thoroughly explained due to several limitations of the study such as the high (cyto)toxicity at the high dose level (which may prevent a genotoxic response at this dose level), the use of two concentrations instead of at least three, a low number of males and no historical control data.

No carcinogenicity study on DMDS is available. As the genotoxicity endpoint is not fully assessed and no conclusion can be currently derived from the available data, the need for classification for carcinogenicity may be impacted.

Dossier Submitter's Response

The Dossier Submitter would like to thank France for these comments and addresses them respectfully with the following response :

General comments:
This analysis differs from the conclusions reached by EFSA : DMDS is used as a food flavouring agent (12.026) and is included in subgroup V (acyclic & cyclic disulphides). In the EFSA opinion on flavouring group evaluation, rev. 1 (EFSA journal 2011 ;9 :2459) have reviewed genetic toxicity data and whilst limited they have come to the conclusion that for 39 substances which included DMDS 12.026, the available genotoxicity did not precluded their evaluation through the procedure and approval for use as food flavouring agent.

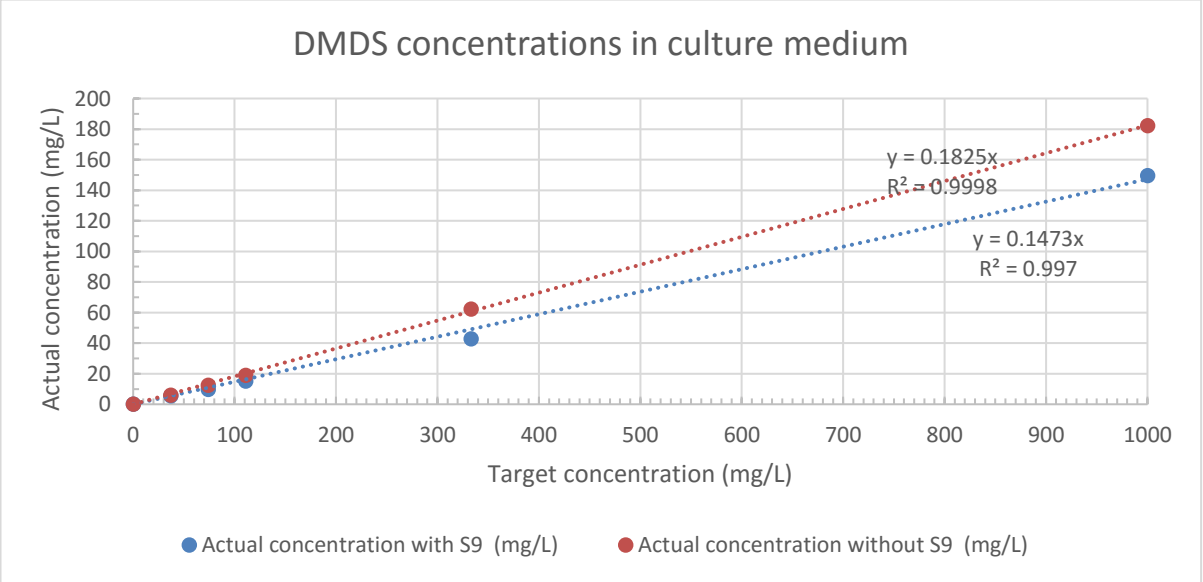
Formaldehyde is an aerial decomposition product of DMDS, formed by atmospheric oxidation under a mechanism of degradation by OH radicals common to volatile organic substances (including those emitted by plants and soils). Formaldehyde is not relevant for the toxicological evaluation of dimethyl disulphide. All data available on dimethyl disulphide and similar products (dipropyl disulphide) indicate metabolisation in animals in mercaptan, sulphide, sulphoxide or even sulphone but never in aldehyde. This information has been submitted in the REACH dossier and also the PPP dossier which was considered acceptable in the last written feedback from France RMS in 2015.

As indicated, a combined in vivo micronucleus assay and in vivo alkaline comet assay of dimethyl disulphide is currently ongoing under PPP regulation.

The objectives of this study were to assess the potential of vaporized DMDS to induce micronuclei in polychromatic erythrocytes (PCEs) in rat bone marrow and to cause DNA damage in rat liver, lung, and nasal tissue when administered via whole-body inhalation to Sprague Dawley rats for 6 hours per day for 3 consecutive days. The study was performed following the OECD Testing Guidelines 474 (29 July 2016) and 489 (29 July 2016). The report of the micronucleus assay is already available and DMDS was negative at the maximal tolerated concentration of 700 ppm. The RSS of this study (Randazzo, 2017) is displayed in Appendix 1. The comet assay in nasal, liver and lung tissues is just completed. DMDS was negative in liver and lung at the maximal tolerated concentration of 700 ppm and in nasal tissue at the maximal non cytotoxic concentration of 175 ppm.

Specific comments:
Page 26: In vitro mammalian cell gene mutation assay (Rutten 1990a):
This analysis differs from the conclusion reached in 2012 by the US Environmental Protection Agency (EPA) in the frame of the high production volume chemicals (HPV) program. Regarding the CHO/HGPRT assay, the agency concluded that ”a slight increase in mutant frequency was observed at several concentrations with metabolic activation, but the increase was not concentration-related and that DMDS was not mutagenic in this assay”. Finally, EPA concluded that “DMDS was not mutagenic in bacteria or mammalian cells in vitro. DMDS did not induce micronuclei in mice in vivo or unscheduled DNA synthesis in vitro or in vivo.”

The issue of this assay was the volatilisation of DMDS during the incubation of the cells. However, there was a clear dose-response ($R^2 > 0.99$) between the target concentrations and the actual concentrations of DMDS in the culture medium (see graph below). Therefore, the absence of a dose-related mutagenic effect could not be ascribed to the high volatility of DMDS, considering the actual concentrations which were sufficient to induce a significant cytotoxicity.




According to the OECD guideline no. 476 (29 July 2016) (§21), when determining the highest test chemical concentration, the concentration producing precipitation in the culture medium should be avoided and care should

be taken to assure that the precipitate does not interfere with the conduct of the test. At the target concentration of 1000 mg/L, DMDS droplets attached to the bottom of the tissue culture flask, indicating that this concentration was above of the solubility limit. In addition, the DMDS droplets could induce local concentrations far in excess of the target concentration.
According to the acceptability criteria of the guideline, the selection of top concentration must be consistent with the criteria described in §21. Therefore, the results obtained at the precipitating concentration of 1000 mg/L must be excluded of the interpretation of the assay. Excluding this concentration, there was no dose-related and reproducible increase of the mutant frequencies even at cytotoxic concentrations.

Page 27:
No comment.

Page 28: In vitro mammalian chromosome aberration test (Vogel 1990):
According to the actual version of the OCDE guideline no. 473 (29 July 2016), the highest concentration should aim to achieve reduction in mitotic index (MI) for primary cultures of lymphocytes to 45± 5% of the concurrent negative control.
At the concentrations of 300 µg/mL without S9 and 100 and 300 µg/mL with S9, the MI was reducted by 50% or more compared to the solvent control.
Therefore, according to the OECD criteria for selection of top concentration, the effects observed at these cytotoxic concentrations are not relevant to assess the clastogenic potential of dimethyl disulphide.

Page 29: In vivo Micronucleus test on rat bone marrow (Weinberg 2007):
Even if no relevant PCE/NCE decrease was observed in this study in the absence of plasma analysis and in vivo ADME study, it did not mean that the bone marrow was not exposed. In this study, the rats were exposed for 4 hours to the maximal tolerated concentration (MTC) of 825 mm. This MTC was selected on the basis of the acute inhalation toxicity study (Kirlpatrick, 2005) and was the non lethal concentration, the higher concentration of 1188 ppm being lethal. Clinical sign of hypoactivity during exposure at all concentrations and a slight body weight loss at 825 ppm post exposure were indicating of a systemic exposure. Therefore, once in the blood, there is a no reason to suspect that the DMDS and/or its metabolites would not reached the bone marrow. In addition, potential DMDS metabolites, like methyl mercaptan, dimethyl sulphide, dimethyl sulphoxide, dimethyl sulphone and methane sulphonic acid were tested in micronucleus assays in bone marrow and were all negative.

Page 29-30: In vivo Micronucleus test on Mouse bone marrow (Willems MI 1989):
The incidence of MPE's in males and females exposed to DMDS was quite variable and compared with male controls a statistically significant increase occurred in males exposed to 250 ppm DMDS. This increase was not considered to be of any biological significance as it was very small and was seen only at 250 ppm and not at 500 ppm. Moreover , the incidence of MPE's in the 250 ppm males (2.6 MPE/1000 PE) was fully comparable to that seen in female control animals (2.5 MPE/1000 PE). The incidences of micronucleated erythrocytes per 1000 NE and 1000 E in males and females exposed to 250 or 500 ppm were fully comparable with that in negative controls. In this study, it was concluded that

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| the results of the micronucleus test did not provide any indication of chromosomal damage and/or damage to the mitotic apparatus in bone marrow cells of mice exposed to DMDS.<br><br>The Dossier Submitter supports the conclusion 'conclusive but not sufficient for classification' based on the weight of evidence derived from the results of the existing studies and of the newly conducted Comet assay coupled with a Micronucleus test. |
| RAC's response |
| Thank you for pointing out several issues to be considered in the assessment of existing data and requiring further explanation. It made the justification more comprehensive. Nevertheless, in our view data on germ cell mutagenicity are conclusive but not sufficient for classification for germ cell mutagenicity. RAC also notes that carcinogenicity has not been under the scope of this CLH proposal, and furthermore, mutagenicity data alone is not sufficient to justify a classification for carcinogenicity. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 27.06.2017 | Germany | | MemberState | 5 |
| Comment received | | | | |
| Based on the presented summaries, the conclusion "conclusive but not sufficient for classification" is supported. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank Germany for these comments and would like to add that results of a new Comet assay coupled with a Micronucleus test are now available and support further this conclusion. | | | | |
| RAC's response | | | | |
| Agree. Thank you for comment. | | | | |

**TOXICITY TO REPRODUCTION**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 27.06.2017 | Germany | | MemberState | 6 |
| Comment received | | | | |
| Based on the presented summaries, the conclusion "conclusive but not sufficient for classification" is supported. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank Germany for this comment and has no additional response to submit. | | | | |
| RAC's response | | | | |
| Agree. Thank you for comment. | | | | |

**OTHER HAZARDS AND ENDPOINTS – Acute Toxicity**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 30.05.2017 | Germany | <confidential> | Company-Downstream user | 7 |

| Comment received |
|---|
| no data available |
| Dossier Submitter's Response |
| The Dossier Submitter would like to thank the Downstream user Company for this comment and has no additional response to submit. |
| RAC's response |
| Noted. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 07.07.2017 | France | | MemberState | 8 |
| Comment received | | | | |
| Acute oral toxicity (pages 14, 15):<br>The result of Shapiro R. study (1985a) should not be completely disregarded. Although the purity of the test material was not reported in the study report, the notifier informed FR as the RMS that the purity was at least 98%. The study was performed according to GLP and followed EPA guideline, considered equivalent to OECD 401. In this study different LD50 may be derived for males and females and males are more sensitive to DMDS than females. The LD50 are between 125-188 mg/kg bw/day for males and between 188 and 250 mg/kg bw/day for females. Unfortunately, in the Pelcot C (2010) study males were not tested. Overall, when the results of the other available acute toxicity studies by oral route are also considered (Pelcot C, 2010: LD50>300 mg/kg bw but only females are used while males are considered the most sensitive sex, and Lombard A, 1986 in which LD50 is between 290 and 500 mg/kg bw/day), a classification of DMDS with Acute Tox. 3 H301 is proposed.<br>Three studies (Gilotti AC 2006 and 2007, Shapiro R 1985b) were provided in the CLH report and not in the dossier of DMDS submitted under PPP Regulation. There is no impact on classification proposal.<br><br>Acute toxicity by inhalation (page 18):<br>The LC50 given in the CLH report is for males and females combined. However, taking into account LC50 obtained for males and females separately (more accurate method), males are more sensitive than females with an LC50 between 3.26 mg/L (847 ppm) and 4.57 mg/L (1188 ppm) compared to the LC50 of 5.36 mg/L (1391 ppm, with 95% confidence limits of 1167-1471ppm) for females. No impact on the classification Acute Tox.3 H331 that is supported. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank France for these comments.<br><br>Acute oral toxicity (pages 14, 15):<br>Dossier submitter hereby submits further explanation on why the conclusion was reached:<br>1. The conclusion of France is based on the study of Shapiro R. (1985a). DMDS tested in this study was produced in a plant in the USA. Even if the purity was at leat 98%, its specifications included a higher level of toxicological relevant impurity (methyl mercaptan) and do not correspond to the specifications of DMDS manufactured in France by the dossier submitter. This study was submitted for completeness only but should not be considered as a key study for establishing the classification of the substance; | | | | |

2. In a second reliable study (Lombard, 1986) conducted on DMDS as manufactured in France by the registrant (purity = 99.31%), the mortality was as followed:

| Dose (mg/kg) | Males | Females |
|---|---|---|
| 100 | 0/5 | 0/5 |
| 170 | 0/5 | 0/5 |
| 290 | 1/5 | 2/5 |
| 350 | 0/5 | 0/5 |
| 500 | 5/5 | 5/5 |

There is no apparent differential mortality between males and females at dose levels of 290 and 350 mg/kg bw. An approximate LD50 of 385 mg/kg was calculated for both sex combined.

3. As from the Lombard's study there was no difference in sensitivity between the sexes, only females rats were used in a third study (Pelcot, 2010) as recommended by the OCDE test guideline no. 423. Using a sample manufactured in France by the registrant (purity = 99.88%), no mortality was observed at the dose level of 300 mg/kg.

4. In order to address the requirements of the Chinese authorities for the registration of DMDS in China as a PPP, a new acute oral toxicity study in male and female rats (Yasso, 2015) was performed by the Arkema's subsidiary in USA on the technical DMDS (purity 99.89%). This study was performed following the Chinese guideline, which is comparable to the former OECD TG 401. LD50 and 95% confidence limits were 415 (207 to 833) mg/kg for male rats and 750 (362 to 1552) mg/kg for female rats. The RSS of this study is displayed in the Appendix 2.

The Dossier Submitter supports the conclusion 'Acute Tox. 4 (H302)' based on the weight of evidence derived from the results of the existing studies and of the recently conducted test.

Acute toxicity by inhalation (page 18):
According to the study report (Kirkpatrick, 2005a), a LC50 could not be determined for males due to inverted mortality in the 1188 and 1308 ppm groups. Therefore, the LC50 for males could be also considered to be between 4.57 mg/L (1188 ppm) and 6.35 mg/L (1650 ppm), which is not demonstrating a specific sensitivity of males.

| RAC's response |
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| Thank you for the comment. Noted. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 27.06.2017 | Germany | | MemberState | 9 |
| Comment received | | | | |
| Regarding the oral study by Lombart (1986), the mortality incidences in males and females should be reported separately and not only combining both sexes to allow the assessment of possible sex differences.<br><br>The oral study by Shapiro (1985) points to a classification with Acute Tox. 3 | | | | |

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| (LD50 of 190 mg/kg bw). This conclusion of Acute Tox. 3 (H301) would be in line with the results of the REACH substance evaluation.<br>Additionally, this would be supported by the studies by Lombard (1986) and Gilotti (2006 and 2007), which could not clearly describe the effects at the classification boundary of 300 mg/kg bw.<br><br>Based on the presented summaries the classification proposals with Acute Tox. 3 (H331) for the inhalation route and non-classification for the dermal route are supported. |
| Dossier Submitter's Response |
| The Dossier Submitter would like to thank Germany for these comments, similar to those made by France under comment 8: as a result, the response of the Dossier Submitter for comment 8 also addresses comment 9. |
| RAC's response |
| Thank you for comment. Noted. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 03.07.2017 | Finland | | MemberState | 10 |
| Comment received | | | | |
| FI CA supports the proposed classification of Acute Tox. 4; Harmful if swallowed for dimethyl disulphide.<br>FI CA supports the proposed classification of Acute Tox. 3; Toxic if inhaled for dimethyl disulphide. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank Finland for this comment and would like to add that the response of the Dossier Submitter for comment 8 provides additional information supporting Finland's comments. | | | | |
| RAC's response | | | | |
| Thank you for comment. Noted. | | | | |

**OTHER HAZARDS AND ENDPOINTS – Eye Hazard**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 07.07.2017 | France | | MemberState | 11 |
| Comment received | | | | |
| Serious eye damage/eye irritation (pages 20-22):<br>In Guillot study the scores for iritis and redness conjunctivae lead to a classification Eye Irrit. 2 H319. Nevertheless, effects were still observable until 72 hours which is the end of the study. Therefore the reversibility of the effects were not assessed and a classification with Eye Dam. 1 H318 would be applicable. However, in the second study available (Shapiro R 1985), reversibility of the effects was shown 7 days post treatment. Overall, the classification Eye Irrit. 2 H319 is supported. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank France for these comments and would like to share the results of a new eye irritation study in rabbits (Hall, 2015) that support the classification Eye Irrit.2 H319 by France. | | | | |

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| For the registration of DMDS in China as a PPP, an eye irritation study in rabbits (Hall, 2015) was performed by the Arkema's subsidiary in USA on the technical DMDS (purity 99.89%). This study was performed following the Chinese guideline, which is comparable to the OECD TG 405. Over 24, 48 and 72h, corneal opacity and iris scores were 0 for the 4 rabbits, conjunctival scores were 0.66 for rabbits 1 and 2, 1.0 for rabbit 3 and 0.33 for rabbit 4 and chemosis scores were 0.33 for rabbits 1 and 3 and 0 for rabbits 2 and 4. All effects reversed in 48-72 hours. The RSS of this study is displayed in the Appendix 3. |
| RAC's response |
| Thank you for comment.<br>In the acceptable study (Guillot JP 1985b), the effects meet CLP criteria for eye irritation category 2 (a mean score of ≥1 for iritis) were observed in tested animals. The reversibility of the effects were not assessed in this study, however reversibility of all lesions was demonstrated in a supporting studies (Shapiro, 1985e and Hall, 2015). |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 27.06.2017 | Germany | | MemberState | 12 |
| Comment received | | | | |
| Based on the presented summaries the classification proposal with Eye Irr. 2 (H319) is supported. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank Germany for this comment. | | | | |
| RAC's response | | | | |
| Agree. Thank you for comment. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 03.07.2017 | Finland | | MemberState | 13 |
| Comment received | | | | |
| FI CA supports the proposed classification of Eye Irrit. 2; Causes serious eye irritation for dimethyl disulphide. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank Finland for this comment. | | | | |
| RAC's response | | | | |
| Thank you for the comment. | | | | |

**OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 04.07.2017 | Sweden | | MemberState | 14 |
| Comment received | | | | |
| The dossier submitter has included and discussed 4 studies in the CLH-dossier – one in vivo LLNA and three in vitro tests addressing key events in the AOP for skin sensitisation (DPRA, LuSens, MUSST). The LLNA resulted in a SI>3 for 25% and 50% test concentrations and an SI>=3 for 2.5%. There was no dose-response relationship. Based on these results dimethyl disulphide fulfils the | | | | |

classification criteria for skin sensitisation (SI>3). Moreover, since concentrations of dimethyl disulphide below 2.5% were not tested in the LLNA and hence subcategory 1A cannot be excluded (EC3 = 2.5%), classification as Skin Sens. 1 seems appropriate. Further support for the skin sensitizing potential of dimethyl disulphide comes from the positive outcome in two out of three in vitro tests. However, in the CLH-report the DS has omitted one study available at ECHAs dissemination site – a Buehler assay from 1985, which was negative. Without a thorough assessment and discussion of the results from this assay we find it difficult to conclude on the skin sensitization potential of dimethyl disulphide. For increased readability, we also propose the DS to include more detail on the set-up of the LLNA (regarding for example the number of animals, vehicle, etc.) in Table 16 of the CLH-report. We would also appreciate a more in depth discussion about the fulfilment of the classification criteria – including an explanation on why Skin Sens. 1 is proposed (section 2.1 at page 3 of the CLH report) instead of subcategory 1B as suggested in section 10.7.3 at page 26 of the CLH-report.

Dossier Submitter's Response

The Dossier Submitter would like to thank Sweden for this comment and addresses them respectfully with the following response :

All details concerning the set-up of the LLNA study (Rokh, 2012) are provided in section 3.7 (p.84) of the annex I of the CLH report.
Skin Sens. Cat. 1 proposed in section 2.1 at page 3 of the CLH report was a mistake, it must be Cat. 1B.
The Buehler assay from 1985 was not reported in the CLH report because it was negative and therefore of limited value to for the sub-categorisation of the sensitising potential of dimethyl disulphide.

Another LLNA assay was performed with a plant protection formulation (named Atomal13) containing 93.1% of dimethyl disulphide (Watzinger, 2011). This study was not included in the CLH report but will be added in a next update (the RSS of this study is displayed in the Appendix 4). The results were as followed:

| Treatment | Concentration (%) | Irritation level | Stimulation Index (SI) |
|---|---|---|---|
| Atomal13 | 2.5 | non-irritant | 0.91 |
| Atomal13 | 5 | non-irritant | 1.07 |
| Atomal13 | 10 | non-irritant | 0.79 |
| Atomal13 | 25 | non-irritant | 3.46 |
| Atomal13 | 50 | non-irritant | 4.10 |
| HCA | 25 | - | 4.83 |

A significant lymphoproliferation (SI > 3) was noted at the concentrations of 25% and 50% with a dose relationship. In the absence of local irritation, the significant lymphoproliferative responses observed were attributed to delayed contact hypersensitivity. The EC3 value is equal to 22.41%.

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| Therefore, the SI>=3 at 2.5% of dimethyl disulphide in the Rokh's study (2012) is suspicious and not relevant for sub-categorisation. The SI observed at 25 and 50% in both studies are comparable and categorized 1B. |
| RAC's response |
| Thank you for the comment. Noted. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 07.07.2017 | France | | MemberState | 15 |
| Comment received | | | | |
| Skin sensitisation (pages 22-26):<br>In the LLNA, the SI reaches 3 at several concentrations. However, there is no clear dose-related effect and as the lowest concentration (2.5%) reached SI=3, lower concentration (≤2%) could have a SI=3 as well. Therefore, the classification Skin sens. 1 H317 is supported but it is considered that no sub-categorisation can be proposed. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank France for this comment similar to Comment number 14 : response provided to Comment number 14 is therefore also relevant to address comment number 15. | | | | |
| RAC's response | | | | |
| Thank you for comment. Noted. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 27.06.2017 | Germany | | MemberState | 16 |
| Comment received | | | | |
| Based on the presented summaries the Stimulation Index was > 3 under the conditions of described the LLNA. Additionally, 2 out of 3 in vitro assays were positive. Due to the different results of the two counting exercises in the LLNA, it is unclear, whether the EC3 is above or below 2 %. Therefore, a classification proposal with Skin Sens 1 or 1B (H317) should be considered. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank Germany for this comment similar to Comment number 14 : response provided to Comment number 14 is therefore also relevant with regards to comment number 16. | | | | |
| RAC's response | | | | |
| Thank you for the comment. Noted. | | | | |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 03.07.2017 | Finland | | MemberState | 17 |
| Comment received | | | | |
| FI CA supports the proposed classification of Skin Sens. 1 for dimethyl disulphide. However, the CLH report contains inconsistency related to the proposed classification category. In section 2.1 "Proposed classification and labelling according to the CLP criteria", table 6 the proposed classification for DMDS is Skin Sens. 1 (p. 7), whereas in section 10.7.3. "Conclusion on classification and labelling for skin sensitisation" (p. 30) it is stated that "DMDS | | | | |

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| was classified as skin sensitizer 1B (Hazard statement H317; May cause an allergic reaction)". Although the criteria for classification as Skin Sens. 1B is met (EC3 > 2%), the FI CA considers that enough data for subcategorization does not exist. While the lowest concentration in the test was 2,5% (with SI > 3), it cannot be exluded that also at concentration ≤ 2% the SI would be ≥ 3. |
| Dossier Submitter's Response |
| The Dossier Submitter would like to thank Finland for this comment similar to Comment number 14 : response provided to Comment number 14 is therefore also relevant with regards to comment number 17. |
| RAC's response |
| Thank you for comment. Noted. |

**OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 27.06.2017 | Germany | | MemberState | 18 |
| Comment received | | | | |
| Based on the presented summaries, the classification proposal with STOT-SE 3 (H335) is supported. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank Germany for this comment and has no additional response to submit. | | | | |
| RAC's response | | | | |
| Thanh you for the comment. Noted. | | | | |

**OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 07.07.2017 | France | | MemberState | 19 |
| Comment received | | | | |
| Specific target organ toxicity-repeated exposure 28-day dermal exposure study (pages 39, 40):<br>In the 28-day toxicity study by dermal route, severe skin irritation was observed 48 hours after treatment at the lowest dose (10.6 mg/kg bw/day) onwards. Based on the dose-related severity of the local effects (e.g. necrosis, encrustation…), a classification with STOT RE 1 H372 (skin) is proposed (concentration below 60 mg/kg bw/day for a 28-day study).<br><br>The effects not detailed in the CLH report, but available in the study report and assessed by France under PPP Regulation, are described below:<br><br>During week 1 the following dermal effects were observed in DMDS treated animals:<br>- low dose group (10.6 mg/kg bw/day): very slight, well-defined or moderate erythema, very slight or slight oedema, and ischemic necrosis<br>- mid dose group (106.3 mg/kg bw/day): well-defined, moderate or severe erythema, slight, moderate, or severe oedema, ischemic necrosis, and slight encrustation | | | | |

- high dose group (1063 mg/kg bw/day): well-defined, moderate, or severe erythema, moderate or severe oedema, ischemic necrosis, haemorrhages, and very slight, slight, or moderate encrustation.

During the second week of exposure, the treated skin of all test animals showed incrustation, which almost completely covered the treated skin area. The degree of erythema and oedema could only be scored on the small areas of the application site that did not show incrustation, or at the periphery. At the end of the second week, upon regeneration of the skin underneath, the crusts loosened. In addition, due to the inflexibility, the formed crusts cracked, especially in the animals of high dose group. Administration of DMDS to the newly formed skin underneath these cracks caused small wounds. During the third and fourth week of exposure, the severity of the encrustation in most animals of the three dose groups was such that scoring of erythema and oedema was no longer possible. After study day 16 (i.e. exposure day 13) it was decided to stop treatment of the animals of the high dose group, because of the high mortality that had occurred. Furthermore, it was decided not to record skin effects of this group on a daily basis. Instead, skin effects were recorded only on study day 21 (i.e exposure day 16) and on study day 28 or 29, just prior to autopsy in order to determine the reversibility or irreversibility of the lesions.

In the animals of the high dose group the severity of the skin effects had decreased during the recovery period. However, the 12 to 13-day recovery period was clearly not long enough to fully evaluate the reversibility or irreversibility of the dermal effects observed.

Histopathological examination identified treatment-related changes in the treated skin and the heart. The dermal lesions were seen in the treated skin of nearly all rabbits treated with topical DMDS. The changes mainly consisted of acanthosis, hyper- and/or parakeratosis, subcutaneous infiltrates of mononuclear cells and/or polymorphonuclear inflammatory cells, oedema and incidentally congestion. The severity of some of the lesions was slightly higher in the mid dose than in the low dose animals. In the high dose female rabbits that survived the experimental period, acanthosis and mononuclear cell infiltrate in the subcutis were somewhat more pronounced than in intercurrent deaths, whereas parakeratosis, subcutaneous oedema and polymorphonuclear inflammatory cell infiltrate were less pronounced. In the males of the high dose group the skin lesions of survivors and intercurrent deaths were comparable. The untreated skin of test and control animals did not reveal any abnormalities.

Dossier Submitter's Response

The Dossier Submitter would like to thank France for this comment and addresses them respectfully with the following response :

The France CA consider the skin irritation observed after repeated exposure of DMDS to rabbit skin relevant for STOT-RE 1 classification, however, according to the CLP regulation, section 3.9.1. "Definitions and general considerations", sub-section 3.9.1.1.:

" Target organ toxicity (repeated exposure) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included. However, other specific

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| toxic effects that are specifically addressed in sections 3.1 to 3.8 and 3.10 are not included here."<br>The skin irritation potential was specifically addressed according to section 3.2 of the CLP regulation by a classification skin irritant cat. 2 H315. Following the reasoning of the France CA, all substances classified skin irritant cat 2 or 1 should be classified STOT-RE 1 due to their potential to induce severe irritation after repeated exposure, which is clearly not the aim of the CLP regulation.<br>The dossier submitter concluded that classification STOT RE 1, H372 based on the severity of the skin irritation is not relevant. The risk phrase EUH066 - "Repeated exposure may cause skin dryness or cracking" would be more appropriate to cover the risk of skin irritation after repeated exposure.<br>The Dossier Submitter also would like to indicate that supported use and manufacturing exposure scenarios do not involve repeated dermal exposure to DMDS. |
| RAC's response |
| Thank you for comments. Noted.<br><br>RAC notes that exposure scenarios are not taken into consideration in classification. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 27.06.2017 | Germany | | MemberState | 20 |
| Comment received | | | | |
| Based on the presented summaries, the conclusion "conclusive but not sufficient for classification" is supported. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank Germany for this comment and has no additional response to submit. | | | | |
| RAC's response | | | | |
| Thank you. Noted. | | | | |

**OTHER HAZARDS AND ENDPOINTS – Aspiration Hazard**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 27.06.2017 | Germany | | MemberState | 21 |
| Comment received | | | | |
| According to the DS no toxicological data are available for this endpoint. However, the low kinematic viscosity would support a classification for Asp. Tox. 1. According to the criteria (section 3.10.2 of Annex I to the CLP regulation), classification into category 1 includes but is not limited to hydrocarbons. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank Germany for this comment.<br>According to CLP regulation a substance is classified in Category 1:<br>(a) based on reliable and good quality human evidence or<br>(b) if it is a hydrocarbon and has a kinematic viscosity of 20,5 $mm^2/s$ or less, measured at 40°C. | | | | |

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| There is no reported evidence of aspiration hazard in human for DMDS. Even if the kinematic viscosity of DMDS is lower than 20,5 mm²/s, DMDS is not an hydrocarbon.<br>Therefore, the Dossier Submitter concludes that the criteria for classification Asp.tox.1 are not met for DMDS. |
| RAC's response |
| Thank you for commenting.<br>Taking into account that there is no reported evidence of aspiration hazard in human for DMDS and DMDS is not an hydrocarbon the classification criteria for category 1 of aspiration toxicity for DMDS are not fulfilled. |

**OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment**

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 07.07.2017 | France | | MemberState | 22 |
| Comment received | | | | |
| The studies presented in the CLH report were also submitted in the frame of the ongoing approval process of the active substance DMDS according to Regulation (EC) No. 1107/2009. The assessment of this dossier is currently ongoing in France.<br><br>Regarding the degradation in water-sediment study (Allan J., 2011), the experimental device was not considered relevant since it was not closed throughout the experimentation. Considering that DMDS is a very volatile active substance (vapour pressure of 3000 Pa at 20°C), the choice of working with open recipients under fume hood with a constant air flow could be questioned since it induces a very important bias in the experimentation. Consequently, this study was not considered acceptable by France in the ongoing assessment under Regulation (EC) No. 1107/2009.<br><br>Regarding the ecotoxicological studies reported, three of them have not been provided in the dossier of DMDS submitted under Regulation (EC) 1107/2009: the acute toxicity test on Daphnia magna (Thiebaud H, 1996) and the toxicity tests on Pseudokirchnerella subcapitata (Scheerbaum 2007c and Thiebaud Lespagnol 2002). This is not expected to change the outcome of the proposed classification.<br><br>For the alga studies, the endpoints used for the risk assessment of DMDS are based on 72 hours exposure. For Anabaena flos-aquae (Minderhout et al., 2008b), the 72h EC50 and NOEC based on growth rate are 5.10 and 1.90 mg/L (mean measured), respectively. For Navicula pelliculosa (Minderhout et al., 2008c), the 72h EC50 and NOEC based on growth rate are 20.0 and 9.5 mg/L (mean measured), respectively. For Skeletonema costatum (Minderhout et al., 2008d), the 72h EC50 and NOEC based on growth rate are 3.6 and 2.6 mg/L (mean measured), respectively. This will not change the outcome of the proposed classification.<br><br>We agree that DMDS is considered not rapidly degradable and should be classified H400 (M = 1) and H410 (M = 10). | | | | |

| Dossier Submitter's Response |
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| The Dossier Submitter would like to thank France for this comment and to submit the following response.<br><br>Regarding the simulation degradation study (Allan J., 2011), it should first be noted that the OECD 308 test guideline clearly states in paragraph 5 that "The test should not be applied to chemicals which are highly volatile from water (e.g. **fumigants**, organic solvents) and thus cannot be kept in water and/or sediment under the experimental conditions of this test". This study was nevertheless requested for DMDS registration as a pesticide in the USA. Before conduction, the experimental design was agreed and validated by the US EPA. Since, the study was available at the time of REACh registration and pesticide dossier submission under Regulation EC 1107/2009, this study was also included in these dossiers. This study provides key information on DMDS environmental behaviour. DMDS was shown to dissipate rapidly from sediment and water compartments to the air compartment ($DT_{50}$ = 1 h). The dossier submitter considers this study valid and useful at least for risk assessment.<br>In the present case, because no degradation could be observed, the results of this study does not impact the environmental classification under regulation EC 1272/2008. DMDS is considered not rapidly biodegradable. The dossier submitter thanks France CA for its support on this classification. |
| RAC's response |
| RAC agrees that the water-sediment simulation study cannot be used to assess degradation potential in the context of the CLP Regulation, but recognises that it is relevant in terms of an assessment of overall environmental fate.<br><br>RAC notes the provision of additional information for some algal toxicity end points. This does not affect the classification. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 13.06.2017 | Netherlands | | MemberState | 23 |
| Comment received | | | | |
| "We thank you for submitting this CLH report. We note that the key study for the classification for Aquatic Chronic 1 is based on nominal concentrations (long-term invertebrate study with Daphnia magna). As in line with the CLP guidance, we however prefer measured concentrations above nominal concentrations for classification. However, we realize that measurements were not possible for the NOEC (= 0.0025 mg/L) as concentrations are below the limit of quantification (= 0.0139 mg/L). As the NOEC concentration is much lower compared to the other studies available we suggest to elaborate on this aspect, for instance by:<br><br>- considering stability of the substance;<br>- providing information on the measured concentration of the highest test concentration in the same test (i.e. 0.020 mg/L nominal concentration which is above the limit of quantification).<br><br>Such an elaboration will strengthen the CLH proposal. We also like to note that another long-term Daphnia magna toxicity study is available (Aquatic Japan MoE; OECD QSAR Toolbox v3.3; NOEC-21d = 0.089 mg/L) which supports that this species is most sensitive." | | | | |

| Dossier Submitter's Response |
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| The Dossier Submitter would like to thank Netherlands for this comment and would like to submit the following response.<br><br>The Dossier Submitter acknowledges the remark on the issue that may arise when relying on nominal concentrations rather than measured concentrations. Rebstock's study was performed under semi-static conditions with daily renewal. Concentrations were measured in new and old solutions on four occasions. They were reliably measured in the two highest treatments. Table 1 below is extracted from the Rebstock's study report. It shows that at 0.01 mg/L and 0.02 mg/L DMDS, there was in general less than 40% loss of the test substance, except on one occasion, but this may be related to an analytical or technical error.<br><br>The Dossier Submitter thanks the Netherlands for bringing to its attention the avaibility of an OECD 211 study report we were not previously aware of. The full study report was available and was analysed by the Dossier Submitter in August 2017. A robust study summary is provided in appendix 5. First, according to our conclusions, this study is valid as all validity criteria were met and we found no reason to question the scientific value of this study. In addition, this study was performed under GLP conditions. It is therefore considered Klimisch 1.<br>The test was performed under semi-static conditions, with daily renewal. Nominal concentrations were 0.1 ; 0.18 ; 0.32 ; 0.56 and 1 mg/L (plus a control). No solvent was used. Analytical quantification was possible and performed in all treatments on three occasions during the test. Measured concentrations showed that the nominal targets were reached in the fresh test solutions and that loss was higher than 20% but globally limited to 33% in the old solutions (except on one occasion for one treatment where 54% loss was observed). These losses are comparable to the losses observed in the Rebstock study. Concentrations used for statistical analysis and expression of the results were based on time weighted means (see appendix 5).<br>No dose-related parental mortality was observed. The mean number of living neonates per parent alive at the end of the test followed a dose-response curve. The NOEC for reproduction was 0.089 mg/L.<br>We propose to classify DMDS on the basis of the lowest NOEC result obtained in the two tests which support a classification aquatic chronic category 1, H410 M=10 for DMDS. |

Table 1. Measured Concentrations of Dimethyl Disulfide (DMDS) During the Chronic Static-Renewal Toxicity Test with *Daphnia magna*

| | Measured Concentration Expressed as mg DMDS/L (Percent of Nominal) | | | | | | |
|---|---|---|---|---|---|---|---|
| Sample Day | Control | Vehicle Control | Level 1 (0.0013) [a] | Level 2 (0.0025) [a] | Level 3 (0.0050) [a] | Level 4 (0.010) [a] | Level 5 (0.020) [a] |
| 0 [b] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | 0.00539 (108) [e] | 0.00896 (90) [f] | 0.0199 (100) |
| 1 [c] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | 0.00663 (66) [f] | 0.0120 (60) [f] |
| 6 [b] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | 0.00658 (132) [f] | 0.0113 (113) [f] | 0.0194 (97) |
| 7 [c] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | 0.0136 (68) [f] |
| 13 [b] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | 0.00429 (86) [e] | 0.00905 (91) [f] | 0.0211 (106) |
| 14 [c] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | 0.00828 (83) [f] | 0.0145 (73) |
| 20 [b] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | 0.0111(111) [f] | 0.0225 (113) |
| 21 [c] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | <MQL [d] | 0.00596 (60) [f] | 0.0132 (66) [f] |

[a] Nominal concentration as mg dimethyl disulfide/L.
[b] Measured concentration of DMDS from "fresh" test solutions.
[c] Measured concentration of DMDS from "spent" test solutions.
[d] MQL = 0.00584 mg a.i./L.
[e] Measured concentration is <MQL and below the validated range of the analytical method, but within 10% of the lowest standard response.
[f] Measured concentration is >MQL, but below the validated range of the analytical method.

RAC's response

The analytical data for the two highest treatments in the Rebstock (2011) study might suggest that losses did not exceed 60 % in the other treatments. However, the extent of losses in these treatments is unknown so no conclusion can be drawn.

The additional long-term *Daphnia* study supports the view that invertebrates are a sensitive trophic group, but does not affect the classification (the lowest nominal concentration in that study was five times higher than the highest nominal concentration in the Rebstock (2011) study).

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 03.07.2017 | United Kingdom | | MemberState | 24 |

Comment received

The key chronic ecotoxicity endpoint (Daphnia magna 21-NOEC of 0.0025 mg/l) is based on non-verified nominal concentrations.

It would be useful to present the analytical information for the highest exposure concentration (0.02 mg/l) where analysis was above the LoQ to illustrate typical losses in the test system.

In addition, it may be useful to consider losses in other ecotoxicity tests to estimate typical losses in the chronic Daphnia study and exposure concentrations.
We note that around 60% loss would need to have been observed to result in a lower M-factor.

Dossier Submitter's Response

The Dossier Submitter would like to thank United Kingdom for this comment, similar to comment number 23 : as a result, response provided to comment number 23 is also relevant for comment number 24.

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| In the Rebstock study, DMDS stability in test solutions was far over 40% at the two highest tested concentrations, for which analytical determination of the test substance was technically feasible. This suggests that there is no need for considering a lower M-factor. In addition, a reliable additional OECD 211 study shows that the NOEC based on nominal concentrations obtained in the Rebstock study is probably a worst case (see answer to comment number 23). Altogether, these results supports classification aquatic chronic I, H410 M=10, for DMDS. |
| RAC's response |
| See response to comment #23. |

| Date | Country | Organisation | Type of Organisation | Comment number |
|---|---|---|---|---|
| 27.06.2017 | Germany | | MemberState | 25 |
| Comment received | | | | |
| We support the proposal of classification for environmental hazards as Aquatic Acute 1 (H400), Aquatic Chronic 1 (H410), the Acute M-factor of 1 and Chronic M-factor of 10. | | | | |
| Dossier Submitter's Response | | | | |
| The Dossier Submitter would like to thank Germany for this comment and has no additional response to submit.<br>The dossier submitter thanks Germany CA for its support on this classification. | | | | |
| RAC's response | | | | |
| Noted. | | | | |

## CONFIDENTIAL APPENDICES

Appendix 1 : Randazzo 2017, OECD 474

Appendix 2 : Yasso 2015, OECD 401

Appendix 3 : Hall 2015, OECD 405

Appendix 4 : Watzinger 2011, OECD 429

Appendix 5 : Japanese Ministry of the Environment 1999, OECD 211