# Committee for Risk Assessment

# RAC

## Opinion

proposing harmonised classification and labelling
at EU level of

## mecetronium etilsulfate; N-ethyl-N,N-dimethylhexadecan-1-aminium ethyl sulfate; Mecetronium ethyl sulphate [MES]

## EC Number: 221-106-5
## CAS Number: 3006-10-8

CLH-O-0000001412-86-235/F

## Adopted

## 14 September 2018

14 September 2018

CLH-O-0000001412-86-235/F

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

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

**Chemical name:** **mecetronium etilsulfate; N-ethyl-N,N-dimethylhexadecan-1-aminium ethyl sulfate; Mecetronium ethyl sulphate [MES]**

**EC Number:** **221-106-5**

**CAS Number:** **3006-10-8**

The proposal was submitted by **Poland** and received by RAC on **5 May 2017.**

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

## PROCESS FOR ADOPTION OF THE OPINION

**Poland** has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **30 May 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **14 July 2017**.

## ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

Co-Rapporteur, appointed by RAC: **Michael Neumann**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **14 September 2018** by **consensus**.

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

| | Index No | International Chemical Identification | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors and ATE | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hazard Class and Category Code(s) | Hazard statement Code(s) | Pictogram, Signal Word Code(s) | Hazard statement Code(s) | Suppl. Hazard statement Code(s) | | |
| Current Annex VI entry | No current Annex VI entry | | | | | | | | | | |
| Dossier submitters proposal | TBD | mecetronium etilsulfate; N-ethyl-N,N-dimethylhexadecan-1-aminium ethyl sulfate; Mecetronium ethyl sulphate [MES] | 221-106-5 | 3006-10-8 | Acute Tox. 4<br>Acute Tox. 3<br>Skin Corr. 1C<br>Eye Dam. 1<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H302<br>H311<br>H314<br>H318<br>H400<br>H410 | GHS05<br>GHS06<br>GHS09<br><br>Dgr | H302<br>H311<br>H314<br>H410 | | M=100 (Acute)<br>M=10 (Chronic) | |
| RAC opinion | TBD | mecetronium etilsulfate; N-ethyl-N,N-dimethylhexadecan-1-aminium ethyl sulfate; Mecetronium ethyl sulphate [MES] | 221-106-5 | 3006-10-8 | Skin Corr. 1<br>Eye Dam. 1<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H314<br>H318<br>H400<br>H410 | GHS05<br>GHS09<br>Dgr | H314<br>H410 | EUH071 | M=100 (Acute)<br>M=1000 (Chronic) | |
| Resulting Annex VI entry if agreed by COM | TBD | mecetronium etilsulfate; N-ethyl-N,N-dimethylhexadecan-1-aminium ethyl sulfate; Mecetronium ethyl sulphate [MES] | 221-106-5 | 3006-10-8 | Skin Corr. 1<br>Eye Dam. 1<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H314<br>H318<br>H400<br>H410 | GHS05<br>GHS09<br>Dgr | H314<br>H410 | EUH071 | M=100<br>M=1000 | |

# GROUNDS FOR ADOPTION OF THE OPINION

## RAC general comment

N-ethyl-N,N-dimethylhexadecan-1-aminium ethyl sulphate (mecetronium ethyl sulphate) [MES] is not currently listed in Annex VI of the Regulation (EC) No 1272/2008. MES is a biocidal active substance according to Regulation (EU) No 528/2012 (BPR) and belongs to Product Type 1, (used for human hygiene purposes, applied on, or in contact with human skin or scalp for the primary purpose of disinfection). The active substance has not yet been approved under the BPR.

During the public consultation one Member State Competent Authority (MSCA) noted that the studies in the CLH report were not always reported in detail and no Annex I providing further information was available. The Dossier Submitter (DS) responded that all detailed information from the study reports was included in the dossier.

## RAC evaluation of physical hazards

### Summary of the Dossier Submitter's proposal

The DS proposed no classification for MES for physical hazards due for the following reasons:

- A test according to A.14 was not performed as the exothermic decomposition energy was less than 500 J/g with an onset of exothermic decomposition below 500 °C. Therefore MES was not considered to have explosive properties;
- In a preliminary test, MES was not ignited with a flame and therefore a main A.10 test was not performed. MES was not considered a highly flammable solid;
- MES was produced, handled and marketed as aqueous solution and pure MES was not considered a flammable solid;
- MES was not considered as self-reactive substance because heat of decomposition was determined to be below 300 J/g;
- MES was not considered a pyrophoric solid because the experience in manufacturing and handling showed that the substance does not spontaneously ignite when coming into contact with air at normal temperatures and because the NMR spectrum of MES powder after 24 hours of storage at 65 ºC showed no structural changes;
- MES was not considered as a self-heating substance because according to CLP substances with melting point lower than 160 ºC should not be considered as self-heating substances and an EU A.1 test showed that MES has a melting range from 87.6 to 111 ºC;
- MES was not considered a substance which in contact with water emit flammable gases because experience in production and handling showed that MES in contact with water did not emit flammable gases and according to CLP this justified no classification for this hazard class;
- From the structural formula and the composition of MES it was concluded that the substance did not show any oxidizing properties;
- There were no studies available to assess if MES should have been classified as corrosive to metals.

### Comments received during public consultation

No comments were received during the public consultation.

### Assessment and comparison with the classification criteria

According to CLP a substance shall not be classified as explosive if the organic substance contains chemical groups associated with explosive properties, but the exothermic decomposition energy is less than 500 J/g and the onset of exothermic decomposition is below 500 ºC. The results of test A.14 addressing explosive properties of MES confirmed these requirements and therefore the classification of MES as explosive is not warranted.

MES was reported as a non-flammable solid in an EU A.10 test and therefore the classification of MES as flammable solid is not warranted.

RAC also concurs with the reasons outlined by the DS for no classification of MES for the remaining physical properties.

In conclusion, RAC supports the DS's proposal for **no classification of MES for physical hazards**.

## HUMAN HEALTH HAZARD EVALUATION

## RAC evaluation of acute toxicity

### Summary of the Dossier Submitter's proposal

The DS proposed to classify MES as Acute Tox. 4; H302 (Harmful if swallowed) on the basis of an OECD TG 401 test in rats with 2000 mg/kg bw of a solution containing 30% of MES that caused 1/5 male mortality and 1/5 female mortality within 24 hours after the exposure. Based on this study, the DS concluded that the oral $LD_{50}$ was higher than 600 mg/kg bw ($LD_{50}$ for 30% solution was > 2000 mg/kg bw). In another study in accordance with OECD TG 401 in rats, no mortality was recorded with up to 2000 mg/kg bw of a solution containing 4% of MES.

The DS proposed to classify MES as Acute Tox. 3; H311 (Toxic in contact with skin) on the basis of an OECD TG 402 test in rats with 2000 mg/kg bw of a solution containing 30% of MES that caused no mortality. The DS concluded that the $LD_{50}$ of MES by the dermal route was higher than 600 mg/kg bw. In another study in accordance with OECD TG 402 in rats, a solution containing 4% MES did not cause mortalities up to a dose of 2000 mg/kg bw.

The DS proposed no classification of MES for acute inhalation toxicity since no toxicity studies via inhalation had been performed for the following reasons: 1) the physico-chemical properties of MES indicated that the active substance had no tendency to become airborne; 2) the exposure of professionals to MES during its production and formulation was limited to the dermal route; and, 3) the exposure of users to MES via the product containing 0.2% MES was limited to the dermal route.

### Comments received during public consultation

One MSCA considered that the proposal of classification as Acute Tox. 4; H302 and Acute Tox. 3; H311 were rather conservative, especially for the dermal route because no mortalities were reported at the tested dose, although the MSCA supported the proposed classification in the absence of additional experimental data with the pure substance. The same MSCA proposed a read-across from other quaternary ammonium compounds to assess the acute toxicity by the inhalation route. For the read-across, the MSCA supplied the following information (the original references were not available to RAC, but the information was published in an open review by The Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration):

- Wistar rats were exposed to an alkyl dimethyl ethyl benzyl ammonium compound at a concentration of 5.4 mg/L (the maximum attainable concentration) for one hour. All animals died at this concentration.
- A whole-body inhalation study on cetylpyridinium chloride with five rats per sex were exposed to air containing 0, 0.05, 0.07, 0.13 and 0.29 mg cetylpyridinium chloride dust/L for four hours (equal to 50, 70, 130 and 290 mg dust/m3). The particle size was less than 5 µm. The $LC_{50}$ was 0.09 mg/L (90 mg/m3) with upper and lower 95% confidence limits at 0.13 and 0.07 mg/L respectively. Deaths occurred in all treated groups (2/10, 1/10, 8/10 and 10/10, respectively). No deaths were seen among controls and all the deaths occurred within 4 days of exposure. Histopathological examination of the lungs and other major organs was not carried out and the author calculated that the total cetylpyrimidinium chloride exposure at the $LC_{50}$ level (0.09 mg/L) was about 4-8 mg/kg bw, and based upon this it was inferred that cetylpyrimidinium chloride could be more toxic by inhalation exposure than by oral or dermal exposure.
- A group of 196 farmers (with or without respiratory symptoms) was evaluated for the relationship between exposure to quaternary ammonium compounds (unspecified, exposure levels not given) and respiratory disorders by testing for lung function and bronchial responsiveness to histamine. After histamine provocation, statistically significant associations were found between the prevalence of mild bronchial responsiveness (including asthma-like symptoms) and the use of quaternary ammonium compounds as disinfectant. The association seemed even stronger in people without respiratory symptoms.

The DS responded that it did not consider the possibility of applying read-across for acute inhalation toxicity as the original study reports were not available and because in any case the generation of an aerosol of MES would be rather difficult due to the physico-chemical properties of MES (the physico-chemical properties indicated that the active substance had no tendency to become airborne). Regarding the observation about the conservativeness of the proposed dermal classification the DS reminded that the tests of acute dermal toxicity were not conducted with pure MES, but the corresponding dose levels were extrapolated from the diluted substance. The proposed classification was considered justifiable by the DS for precautionary reasons.

Another MSCA did not support the proposed classification for acute dermal toxicity, because it was based on a study in which none of the animals died and where only local skin effects were observed.

Also IND argued that the proposed classification for acute dermal toxicity was inappropriate and that Category 4 at most should be considered based on the following arguments.

- The only way to demonstrate the correct classification for acute dermal toxicity of MES would be by performing a new assay testing the substance up to 2000 mg/kg, which was unjustified on the basis of bioethical considerations since the substance was corrosive.
- The product was not manufactured in anhydrous form and the concentration of MES was 29% (the maximum available concentration).
- The local effects after dermal application of MES were covered by the classification as skin corrosive and an additional acute dermal toxicity study would not provide any additional scientific information.
- The available acute dermal toxicity test with the highest dose of pure MES showed only irritating effects on the skin without any mortality and without any other systemic effects.
- All available information including acute toxicity studies and studies with repeated dose application showed local effects at the site of contact, whereas systemic effects or relevant clinical signs related to systemic effects of toxicity were not shown.
- It was theoretically possible to extrapolate dermal acute toxicity from studies using oral route of exposure and considering 100% oral absorption and 3% dermal absorption

(which is deduced from an ADME study with $^{14}$C-labelled MES). Extrapolating from the $LD_{50}$ via the oral route, the $LD_{50}$ by dermal route would be higher than 20000 mg/kg bw (Dermal $LD_{50}$ = oral $LD_{50}$/dermal absorption = >600/0.03) and therefore the classification by dermal route was not considered warranted.

- Four cases of biocidal active substances (amines, N-C10-16-alkyltrimethylenedi-, reaction products with chloroacetic acid, poly(hexamethylenebicyanoguanide-hexamethylenediamine, peroxyacetic acid and iodine) hydrochloride, for which classification as acute Tox. 2 or Acute Tox. 3 could be derived from the available data, classification as Acute Tox. 4 was proposed for all four cases.

The DS considered Cat. 3 for acute dermal toxicity justifiable for precautionary reasons.

## Assessment and comparison with the classification criteria

The tables below summarise the available acute toxicity studies by oral and dermal routes, respectively. No studies of acute toxicity by inhalation route were available in the CLH report.

***Table**: Summary of the animal studies on acute oral toxicity studies with MES*

| Study | Dose level | Results | Reference |
|---|---|---|---|
| OECD TG 401<br><br>GLP<br><br>Limit test Oral<br><br>Gavage<br><br>Rat Wistars<br><br>5 animals/sex<br><br>Solution 30% MES<br><br>Post exposure period: 14 days | 2000 mg/kg bw | Clinical signs: Within 24 h after application 1/5 males and 1/5 females died. The main clinical signs observed up to day 4 (females more affected than males) were poor general condition, decreased respiratory rate, abnormal gait, squatting position and sunken flanks (there were no effects on day 5-14).<br><br>Body weight gain was normal in males but in females a slight decrease in body weight was observed on day 7 compared with day 0 (156 versus 159 g). There was an increase in body weight on day 14 (176 g).<br><br>$LD_{50}$ of the tested preparation for males and females was greater than 2000 mg/kg bw.<br><br>$LD_{50}$ of MES was greater than 600 mg/kg bw. | <confidential> (1992) |
| OECD TG 401<br><br>GLP<br><br>Limit test<br><br>Rat Wistars<br><br>Gavage<br><br>5 animals/sex<br><br>Solution of 4% MES<br><br>Post exposure period: 14 days | 2000 mg/kg bw | No mortality.<br><br>Clinical signs: piloerection in 10/10 rats, in 2 females slightly reduced activity, squatting position and decreased respiratory rate 2-6 h after application, later observations revealed no effects.<br><br>$LD_{50}$ of the tested preparation for males and females was greater than 2000 mg/kg bw.<br><br>$LD_{50}$ of MES was greater than 80 mg/kg bw. | <confidential> (1992a) |

The CLH report contains a summary of epidemiological studies on the general population reporting that the overall incidence of suspected reactions after exposure to biocidal product

containing 0.2% MES was 0.00018%. However no more information about the route of exposure and the type and severity of the adverse effects was reported.

***Table**: Summary of the animal studies on acute dermal toxicity studies with MES*

| Study | Dose level | Results | Reference |
|---|---|---|---|
| OECD TG 402<br><br>GLP<br><br>Limit test<br><br>5 animals/sex<br><br>Solution of 4% MES | 2000 mg/kg bw | No mortalities.<br><br>Mainly in the female rats erythema and oedema were observed up to 13 days in 2 females.<br><br>In a few females formation of fissures and degreasing of the treated skin.<br><br>$LD_{50}$ of the tested preparation for males and females was greater than 2000 mg/kg bw.<br><br>$LD_{50}$ of MES was greater than 80 mg/kg bw. | <confidential> (1992b) |
| OECD TG 402<br><br>GLP<br><br>Limit test<br><br>5 animals/sex<br><br>Solution 30% MES | 2000 mg/kg bw | No mortalities.<br><br>Moderate to severe erythema and very slight oedema were observed up to day 12 followed by a decline of these skin reactions up to the end of observation period.<br><br>Degreasing, induration, partial desquamation and formation of fissures.<br><br>$LD_{50}$ of the tested preparation for males and females was greater than 2000 mg/kg bw.<br><br>$LD_{50}$ of MES was greater than 600 mg/kg bw. | <confidential> (1992c) |

The key study for acute oral toxicity yielded an $LD_{20}$ of 600 mg MES/kg bw. Therefore, the $LD_{50}$ is higher than 600 mg/kg bw. The range for classification for a category 4 for acute oral toxicity is 300 mg/kg bw < $LD_{50}$ ≤ 2000 mg/kg bw. RAC notes that, despite the plausibility that $LD_{50}$ would be lower than 2000 mg/kg bw/day, the data is inconclusive for classification and for setting an ATE value.

The key study for acute dermal toxicity demonstrated that a dose of MES of 600 mg/kg bw was unable to cause mortalities and therefore the $LD_{50}$ is greater than 600 mg/kg bw. The range for classification in category 3 for acute dermal toxicity is 200 mg/kg bw < $LD_{50}$ ≤ 1000 mg/kg bw; while that for category 4 is 1000 mg/kg bw < $LD_{50}$ ≤ 2000 mg/kg bw. **RAC notes that it is extremely unlikely that the dermal $LD_{50}$ of MES would be lower than 1000 mg/kg bw because 60% of this dose caused no mortalities**, and therefore RAC does not support the DS's proposal for classifying MES in category 3. Industry has presented arguments in favour of category 4 or no classification on the basis of a route-to-route extrapolation and suggested that the dermal $LD_{50}$ was likely to be significantly higher than 2000 mg/kg bw. However, RAC notes that this extrapolation presents several uncertainties as it encompasses an assumption of an oral absorption of 100%. However, according to the CLH report the quaternary ammonium compounds are poorly absorbed from the gastrointestinal tract and the influence of first-pass metabolism in the stomach/intestines and liver should also be taken into consideration in the extrapolation from oral data according to the Guidance on the Application of the CLP Criteria (July 2017). RAC concludes that it is not possible to propose a classification for acute dermal toxicity due to lack of robust and conclusive information.

No acute toxicity studies via inhalation were presented in the CLH report. The DS argued that due to the physical properties of the substance it had a very low tendency to form aerosols and therefore the amount of substance potentially inhaled would be always very low. One MSCA

presented data suggesting that other quaternary ammonium compounds might require classification. However, RAC notes that there is not enough information available to justify the application of read-across from other quaternary ammonium compounds. Therefore, due to the absence of robust information, RAC proposes no classification of MES for acute toxicity via inhalation.

According to the CLP Annex II Section 1.2.6, in cases where no acute inhalation study is available for a corrosive substance, and such substances may be inhaled, the substance shall be supplementarily labelled with *EUH071: Corrosive to the respiratory tract*. Industry highlighted arguments in favour of no labelling MES with EUH071 based on its use as a biocide where the exposure was argued to be limited to the dermal route. RAC notes that classification is not based on risk assessment and concludes that EUH071 is warranted because there is no available acute inhalation toxicity study, the substance is corrosive and it could be inhaled in certain circumstances.

In summary, RAC concludes that **no classification of MES for acute toxicity is justified due to the absence of, or inconclusive data** by all three routes.

However, RAC concludes that **labelling of MES with a supplemental hazard statement code "EUH071: corrosive to the respiratory tract" is warranted**.

## RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

### Summary of the Dossier Submitter's proposal

DS proposed no classification of MES for STOT SE on the basis of the following:

- There was no evidence of specific target organ toxicity in animals after single exposure to MES.
- The overall incidence of suspected MES-induced effects in humans exposed to a preparation containing 0.2% MES was around 0.00018%. The reported effects included skin and ocular irritation, suspected allergy, respiratory tract irritation, diarrhoea and burns.

### Comments received during public consultation

No comments were received during public consultation.

### Assessment and comparison with the classification criteria

RAC notes that the acute oral and dermal toxicity studies in animals did not reveal any evidence of toxicity to a specific organ that are not specifically addressed under other hazard classes, which is a requirement for classification for STOT SE. RAC also notes that the epidemiological data base is not sufficiently robust to justify a classification for STOT SE, because the only adverse effects relevant for STOT SE 3 included only two cases of respiratory tract irritation in the general population in contact with a preparation containing 0.2% MES between January 2000 and August 2005 (the frequency of <0.0000001%).

In conclusion, RAC agrees with the DS that there is **no evidence to warrant classification of MES for STOT SE**.

## RAC evaluation of skin corrosion/irritation

### Summary of the Dossier Submitter's proposal

The DS proposed the classification of MES as skin corrosive in category 1C. In a study performed according to the OECD TG 404, 4% MES applied to White New Zealand rabbits during 4 hours caused severe skin reactions of varying degree and duration in all animals throughout most of the observation period. Specifically, from days 9-10 onwards, all animals showed skin fissures and one animal showed leathery skin until the end of observation period (18 days). The DS also considered that undiluted MES would be corrosive. In another study performed according to the OECD TG 404, 0.2% MES caused no erythema, oedema or any other effects on skin.

### Comments received during public consultation

One MSCA supported the classification as Skin Corr. 1C, but asked the DS to provide more clarity on the observed effects leading to the proposed classification (i.e., irreversibility, scars, fissures, etc.). The MSCA also requested the DS to clarify how the very limited human data was considered in the classification proposal.

Another MSCA commented that a substance is corrosive to skin when it produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, and that the CLH report did not contain information on observed necrosis. According to the MSCA, only local irritation effects were reported in both dermal irritation/corrosion study and dermal acute toxicity studies and therefore the criteria for Skin Corr. 1C was not met.

The DS responded that due to the irreversibility of skin damage within 14 days, the classification of MES as Skin Corr. 1C was justified.

### Assessment and comparison with the classification criteria

The table below summarises the available skin corrosion/irritation studies. The CLH report also contains information about an epidemiological study where 41 cases of skin irritation (representing a relative frequency of 0.0000006% from all hygienic hand disinfections) were reported in connection with a mixture containing 0.2% MES between January 2000 and August 2005.

***Table**: Summary of the animal studies on skin corrosion/irritation studies with MES*

<table>
<tr><th>Study</th><th>Dose level</th><th>Results</th><th>Reference</th></tr>
<tr><td>OECD TG 404<br><br>3 White New Zealand rabbits</td><td>0.5 ml of 0.2% MES</td><td>No erythema, oedema or any other effects on skin were observed at 30-60 min, 24 h, 48 h, 72 h after patch removal (Draize scores = 0)</td><td><confidential> (1992d)</td></tr>
<tr><td>OECD TG 404<br><br>3 White New Zealand rabbits</td><td>0.5 ml of 4% MES</td><td>Skin irritation in rabbits after dermal exposure to 4% MES.<br><table><tr><th>Score (average of 3 animals investigated)</th><th>Time</th><th>Erythema</th><th>Oedema</th></tr><tr><td rowspan="3">Average score Draize scores (0 to maximum 4)</td><td>0.5-1 h</td><td>1</td><td>0</td></tr><tr><td>24 h</td><td>2.0</td><td>2.3</td></tr><tr><td>48 h</td><td>2.0</td><td>2.3</td></tr></table></td><td><confidential> (1993)</td></tr>
</table>

| | | | | | |
|---|---|---|---|---|---|
| | | | 72 h | 2.3 | 2.3 |
| | | Average score | 24h, 48h, 72h | 2.1 | 2.3 |
| | | Other times | 4 d | 2.3 | 2.0 |
| | | | 5 d | 2.3 | 2.0 |
| | | | 6 d | 2.0 | 2.0 |
| | | | 7 d | 2.0 | 2.0 |
| | | | 8 d | 2.0 | 2.0 |
| | | | 9 d | 2.0** | 2.0 |
| | | | 10 d | 1.3*** | 1.0 # |
| | | | 11 d | 1.3*** | 1.0 # |
| | | | 12 d | 1.0*** | 0.6 # |
| | | | 13 d | 1.0*** | 0.6 # |
| | | | 14d | 0.6*** | 0.3 # |
| | | | 15 d | 0.6*** | 0.3 # |
| | | | 16 d (n=2) | 0.5** | 0.5 # |
| | | | 17 d (n=2) | 0.5** | 0.5 # |
| | | | 18 d (n=2) | 0.5** | 0.5 # |
| | | | reversibility: | n.c. | n.c. |
| | | n c: not completely reversible; *: formation of skin fissures (* in one rabbit, ** in 2 rabbits, *** in 3 rabbits); #: leathery skin in one animal | | | |

The CLP Criteria for skin corrosion consist of irreversible destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least one tested animal after exposure ≤ 4 h. RAC notes that 4% MES caused irreversible erythema and oedema of an average score of at least 2 according to the Draize scale in all three animals from 24 h to day 9, and afterwards lower Draize scores but also skin fissures in all animals and leathery skin in one animal until the end of the observation period (day 18). RAC also notes that the total destruction of skin as such was not reported in the CLH report, although it is remarkable that a preparation containing only 4% MES caused such skin responses and therefore much more severe irreversible effects would be expected for pure MES. Therefore, RAC concludes that the results observed in the study performed in accordance with the OECD TG 404 testing 4% MES trigger classification of MES for Skin Corrosivity in category 1.

The DS proposed the sub-categorisation of MES within category 1C because the skin fissures, considered as corrosive effects, were observed 9-10 days after a 4-hour exposure therefore meeting the CLP criteria for a sub-category 1C. However, RAC highlights the fact that the test substance contained only 4% MES and therefore more concentrated MES is likely to trigger its corrosive effects earlier than the 4% solution, and therefore a more severe sub-category cannot be excluded. This conclusion is also supported by the results of the acute dermal toxicity studies with a preparation of 30% MES in which degreasing, induration, partial desquamation and formation of fissures were observed. However, the results of these studies do not either allow a sub-categorization. Therefore, the available animal data is not sufficient for sub-categorisation.

RAC also notes that the available human data shows results in general population exposed to a biocidal product containing 0.2% MES, a concentration 20 times lower than the concentration used in animal studies, without any other information regarding a potential exposure to other co-formulants with skin irritation properties in the biocidal product. These human data also shows a very low frequency (0.0000006%) of irritation cases. RAC concludes that the available human data is too vague for setting a classification on the skin irritant properties of MES.

RAC concludes that **classification of MES as skin corrosive category 1; H314 (Causes severe skin burns and eye damage) is warranted**.

## RAC evaluation of serious eye damage/irritation

### Summary of the Dossier Submitter's proposal

The CLH report summarised a study with a preparation containing 0.2% MES that caused reversible redness, chemosis and discharge of the conjunctiva in rabbits. The DS also presented the human data on the general population obtained from the biocidal product containing 0.2% of MES with an incidence of 0.0000003% ocular irritation. The DS concluded that 0.2% MES was irritant to the rabbit eye, but there was no data on pure MES. However, according to CLP 3.3.2.3." Skin corrosive substances shall be considered as leading to serious damage to the eyes as well (Category 1)" and according to the CLP Guidance "Testing for eye irritation would not be carried out on substances known or predicted to be corrosive to skin. Such 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". Therefore, taking into account these considerations the DS proposed the classification of MES as Eye Dam. 1; H318 (Causes serious eye damage).

### Comments received during public consultation

The proposed classification was supported by one MSCA.

### Assessment and comparison with the classification criteria

The table below summarises the available eye corrosion/irritation study with animals. The CLH report also contains information about an epidemiological study where 24 cases of ocular irritation (representing a relative frequency of 0.0000003% per all hygienic hand disinfections) were reported in connection with a mixture containing 0.2% MES between January 2000 and August 2005. RAC concludes that this human data is inconclusive because of extremely low frequency of incidents (0.0000003%) in the general population exposed to a biocidal product containing a very low concentration of MES (0.2%) and without information on other potentially corrosive co-formulants present in the biocidal product.

***Table**: Summary of the animal study on eye corrosion/irritation with MES*

| Study | Dose level | Results | Reference |
|---|---|---|---|
| No guideline study but comparable to OECD TG 405 with acceptable restrictions:<br>i) no wash out after 24 h;<br>ii) limited data on test animals and clinical signs<br><br>8 White New Zealand rabbits | 0.1 ml of 0.2% MES | Mean score 2 for conjunctival redness (effects lasted 24 h)<br><br>Redness, chemosis and discharge of the conjunctiva reached score 2 (completely reversible after 4 days) | BODE Chemie (1978) |

The CLP criteria for severe eye damage (category 1) consist of irreversible effects on the cornea, iris or conjunctiva in at least one animal and/or of a positive response of corneal opacity equal or higher than 3 and or iritis higher than 1.5 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material in at least 2 of 3 animals. No records

for individual data were provided in the study summarised in the table above and therefore no classification can be set on the basis of such study. However, RAC notes that according to the data contained in the table above, a mixture containing 0.2% MES was irritant to the rabbit eye, and therefore much more severe effects would have been expected for pure MES. RAC also notes that according to the CLP, substances known to be corrosive to skin and classified as such (as is the case of MES) are automatically considered to be severely damaging to the eye. Therefore RAC supports the DS's proposal for classification of MES for serious eye damage category 1.

RAC also notes, in line with the DS, that according to the Guidance on the Application of the CLP Criteria (July, 2017), if a substance is classified as Skin corrosion Category 1, then serious eye damage is implicit as reflected in the hazard statement for Skin corrosion; H14 (Causes severe skin burns and eye damage). Thus, the corrosive substance is also classified for Eye Dam. 1; H318, but the corresponding hazard statement (H318) is not indicated on the label to avoid redundancy.

In conclusion, RAC concludes that the **classification of MES as serious eye damage category 1; H318** (without hazard statement on the label) is warranted.

## RAC evaluation of skin sensitisation

### Summary of the Dossier Submitter's proposal

The DS proposed no classification of MES for skin sensitisation on the basis of a study performed in accordance with an OECD TG 406, where a preparation of 30% MES did not cause sensitisation in any of the treated Guinea pigs. The DS also summarised briefly some human data and reported that a mixture containing 0.2% MES was unable to sensitise any of the 55 exposed volunteers and that in the general population there were only 43 cases of suspected allergy to a mixture containing 0.2% MES (0.0000006% of population exposed to all hygienic hand disinfections) reported in the period between January 2000 and August 2005.

### Comments received during public consultation

One MSCA expressed concern regarding the validity of the skin sensitisation test for the following reasons: i) it was unclear whether the 1.5% MES preparation was used for intradermal induction, topical induction or topical challenge; ii) there was no explanation on how the doses were selected and whether they were high enough as described in the OECD TG; and, iii) no information regarding the positive controls were provided.

The DS responded that the following conditions were used for induction: i) 0.1 mL of 0.5% preparation in water or FCA/water (containing 0.15% active component) was used for the intradermal injection; ii) 10% preparation in water solution (containing 3% active component) for 48 hours was used for occlusive topical induction. The DS also clarified that after this induction, 30% MES was unable to sensitise any animal and referred the requesting MSCA to the IUCLID for additional information.

### Assessment and comparison with the classification criteria

The table below summarises the available skin sensitisation study with animals. In addition to the animal study the following human data were also available: i) the relative frequency per all hygienic hand disinfections of suspected allergy in connection with a mixture containing 0.2% MES was 0.0000003% (24 cases) in the period between January 2000 and August 2005; and ii)

none of the 55 individuals voluntarily exposed to 0.2% MES for 24 hours under occlusive conditions experienced sensitising reactions. RAC notes that this study is not suitable for human sensitisation assessment since there were no induction phase according to the protocol provided in the CLH dossier.

***Table**: Summary of the animal study on skin sensitisation with MES*

| Study | Dose level | Results | Reference |
|---|---|---|---|
| OECD TG 406<br><br>GLP<br><br>Guinea pig<br><br>10 animals/sex/group | <u>Induction:</u><br>Day 0 (intradermal): 0.1 ml/injection site of 0.5% tested preparation (0.15% MES) in water or in Freunds Complete Adjuvant/water (it caused slight/no specific findings in preliminary experiments)<br><br>Day 7: Occlusive patch of 10% of the tested preparation (3% MES) during 48 hours (it caused slight erythema and no oedema in preliminary experiments)<br><br><u>Challenge:</u><br>Day 21 (dermal): Occlusive patch of 5% of the tested preparation (1.5% MES) during 24 hours (it caused no erythema and no oedema in preliminary experiments) | Positive control: 2,4-dinitrobenzene and benzocaine; worked as expected from the historical control data.<br><br><u>24 hours after challenge:</u><br>No skin reactions were detected in any of the treated or control animals.<br><u>48 hours after challenge:</u><br>1 treated male and 2 control females scored 1 for erythema.<br><br>16/20 test animals and 15/20 control animals showed scale formation on the treated skin.<br><br>**Conclusion:** 1.5% MES was not skin sensitiser. | <confidential> (1992e) |

A preparation of 1.5% MES was unable to sensitise animals in a test performed in accordance with the OECD TG 406. RAC concludes that none of the conditions requested for warranting classification have been met using a challenge dose of 1.5% MES and the data base on humans is not robust enough for supporting classification. Therefore, RAC concludes in agreement with the DS that **no classification is warranted for MES for skin sensitisation**.

## RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

### Summary of the Dossier Submitter's proposal

The DS proposed no classification of MES for STOT RE on the basis of the following studies: i) a 4-week oral (gavage) dose range-finding study in rat reporting NOAEL and LOAEL of 50 and 150 mg/kg bw/day, respectively; and, ii) a 90-day oral (gavage) study in rats showing NOAEL and LOAEL of 45 and 90 mg/kg bw/day, respectively. The DS concluded that the observed effects in these studies did not have the potential to produce significant toxicity in humans.

### Comments received during public consultation

No comments were received during public consultation.

### Assessment and comparison with the classification criteria

The table below summarises the available repeated dose toxicity studies with animals.

***Table:*** *Summary table for repeated dose toxicity studies in animals with MES*

| Method | Results | Reference |
|---|---|---|
| 4-weeks<br><br>GLP<br><br>Sprague-Dawley rats<br><br>Daily exposure: gavage<br><br>5 animals/sex/group<br><br>0, 50, 150, 450 mg MES/kg bw/day (in the high dose group dosing was discontinued on day 5) | <u>450 mg/kg bw/day</u><br>Clinical signs: Piloerection and reduced motility on day 4 in 1 male and 1 female; 1 female showed reduced motility, ataxia and ptosis on day 5. All animals were in a poor condition on day 5.<br>Mortality: 1 male and 2 females were found dead in the morning of day 5.<br>Body weight gain: Not reported.<br>Food consumption: Slight decrease (12%) in males (not statistically significant).<br>Gross pathology: No treatment-related effects during the macroscopic post mortem examination in surviving rats or in rats that died during the exposure.<br><br><u>150 mg/kg bw/day</u><br>No clinical signs.<br>Mortality: 1 female.<br>Body weight gain: Slight decrease (3-9%) only in males (but not statistically significant).<br>No changes in food consumption.<br>Gross pathology: No treatment-related effects during the macroscopic post mortem examination in surviving rats. In the female rat that died during the treatment a haemorrhagic, distended and empty gastro-intestinal tract was reported.<br><br><u>50 mg/kg bw/day</u><br>No clinical signs.<br>No mortalities.<br>No changes in body weight gain.<br>No changes in food consumption.<br>Gross pathology: No treatment-related effects during the macroscopic post mortem examination in surviving rats.<br><br>**<u>DS's conclusion:</u>**<br>**LOAEL: 150 mg/kg bw/day**<br>**NOAEL: 50 mg/kg bw/day** | <confidential> (2001) |
| 90 days<br><br>OECD TG 408<br><br>GLP<br><br>Sprague-Dawley rats<br><br>Daily exposure: gavage<br><br>10 animals/sex/group<br><br>0, 15, 45, 135/90 mg MES/kg bw/day (135 mg/kg bw/day until day 73, from day 74 onwards due to mortality 90 mg/kg bw/day) | <u>135/95 mg/kg bw/day</u><br>Clinical signs: irreversible piloerection on day 29 (4/10 males and 3/10 females) and day 30 (all rats).<br>Mortality: 3 male and 7 female (between days 34 and 73). On day 74 the dose was reduced to 90 mg/kg bw/day and no further mortality was observed in the high dose group.<br>Body weight gain: decrease from week 1 to termination (11-20% below control value) in males. A slight and not statistically significant decrease was observed in females from week 6 onwards (1-12% below control value).<br>Food consumption and compound intake: statistically significantly reduced at week 1 (not at week 2-13) in males. A transient decrease in food intake was seen in females at week 1-8 (statistically significant at week 1, 2, 6, and 7).<br>No changes in organ weights.<br>Haematology: Indicative of inflammatory responses in both males and females: increased leucocytes and a shift to the left in differential blood cell counts.<br>Clinical chemistry: In both males and females: i) ALAT (alanine aminotransferase) values are slightly above the historical control range presented by Charles River (no historical control data available from the performing facility); and, ii) aP (alkaline phosphatase) values are within the historical control range presented by Charles | <confidential> (2002) |

| | | |
|---|---|---|
| | River (no historical control data available from the performing facility).<br><br><u>45 mg/kg bw/day</u><br>Clinical signs: long-lasting piloerection starting on day 59 (20/20 rats on day 60).<br>No mortalities.<br>No changes in body weight gain.<br>No changes in food consumption.<br>No changes in organ weights.<br>No haematological changes.<br>Clinical chemistry: In both males and females: i) ALAT values are slightly above the historical control range presented by Charles River (no historical control data available from the performing facility); and, ii) aP values are within the historical control range presented by Charles River (no historical control data available from the performing facility).<br><br><u>15 mg/kg bw/day</u><br>No clinical signs.<br>No mortalities.<br>No changes in body weight gain.<br>No changes in food consumption.<br>No changes in organ weights.<br>No haematological changes.<br>No changes in clinical chemistry.<br><br>**<u>DS's conclusion:</u>**<br>**LOAEL: 90 mg/kg bw/day**<br>**NOAEL: 45 mg/kg bw/day** | |

In the 28-day repeated dose toxicity study mortalities and severe clinical signs were reported at 450 mg/kg bw/day. However, this dose is well above the guidance value range for classification as STOT RE category 2 (30 < C ≤ 300 mg/kg bw/day for a 28-day study). In the same study at 150 mg/kg bw/day (a dose that is within the guidance value range for category 2), a slight decrease in body weight gain as compared to controls (maximum 9%) and one death were reported. The gross pathology examination of the dead female showed haemorrhagic, distended and empty gastro-intestinal tract, which are effects not reported in cases of dead animals at 450 mg/kg bw/day, and therefore RAC considers this fatality as incidental and not relevant for classification. Furthermore, according to the CLP criteria small changes in bodyweight gain do not indicate significant toxicity and therefore should not be considered for classification. Therefore, RAC concludes that the effects observed in the 28-day repeated toxicity study, that are within the guidance value range for classification, do not warrant classification for STOT RE.

In the 90-day repeated dose toxicity study clinical signs, mortalities, reduction in body weight gain and food consumption and changes in haematological and clinical chemistry parameters were reported at the highest dose (135 mg/kg bw/day during the first 73 days and 90 mg/kg bw/day during the remaining 17 days). The initial dose is not within the guidance value range for classification, but the second one is within that range. However, no fatalities were reported after reduction of the dose to 90 mg/kg bw/day. RAC notes that 90 mg/kg bw/day is a dose within the guidance value range for STOT-RE 2. However, it is not known whether the administration of this dose during 90 days instead of only 17 days would have caused mortalities or not. Therefore, the information at this respect is inconclusive and does not give sufficient evidence to support a classification on the basis of the reported mortalities in this 90-day study. In this study, the mid-dose was 45 mg/kg bw/day (a dose that is also within the guidance value range for STOT RE 2) causing long-lasting piloerection and similar changes in clinical chemistry parameters as reported for the highest dose. These changes are considered to be mild, slightly above or within the historical control data of other facility, and no dose-response seems to be

noted between 45 and 135/90 mg/kg bw/day doses suggesting that these changes might be incidental and therefore not enough robust for justifying a classification.

RAC concludes that the effects observed in the 90-day repeated toxicity study are within the guidance value range for classification but do not warrant classification for STOT RE.

In addition to studies summarised in the table above, RAC notes that some mortalities were also reported in the 1-generation toxicity study and in the developmental toxicity study. However, in these two studies the mortalities were attributed to local toxicity in the contact point due to corrosivity of the substance. Indeed, dose-dependent incidences of stomach lesions consisting of acanthosis/hyperplasia, hyperkeratosis, edema, acute inflammation, erosion and ulceration were reported in the 1-generation toxicity study. Similar gastrointestinal effects were also reported in the developmental toxicity study. RAC concludes that mortalities reported in these reproductive studies do not warrant STOT RE classification since the mortalities are attributed to local corrosive effects rather than to systemic toxicity.

In conclusion, RAC agrees with the DS, that **no classification is warranted for STOT RE**.

## RAC evaluation of germ cell mutagenicity

### Summary of the Dossier Submitter's proposal

DS proposed no classification of MES for germ cell mutagenicity considering the results of the following tests:

- Two independent negative bacterial reverse mutation tests conducted according to OECD TG 471;
- One negative *in vitro* mammalian chromosome aberration test with 2 independent experiments and according to OECD TG 473;
- One *in vitro* mammalian gene mutation assay (OECD TG 473) with two independent experiments where one yielded a negative result while the other yielded positive result;
- One positive *in vitro* mammalian gene mutation assay in another independent test also according to OECD TG 476;
- One negative *in vitro* unscheduled DNA synthesis test (OECD TG 472) in mammalian cells with clear negative result without metabolic activation and one negative result with metabolic activation but without a robust positive control;
- A negative *in vivo* micronucleus test (OECD TG 474) performed with methodological deficiencies that allowed to consider this test as non-reliable and non-valid.

### Comments received during public consultation

One MSCA commented that the mutagenicity potential of MES was not conclusive because of contradictory results in mammalian cell gene mutation assays, positive and equivocal results with other quaternary ammonium compounds, a dose-response relationship (not statistically significant increase) in the incidence of micronuclei in females but no clastogenic effects in males, and because of a quantitative structural alert on the ethyl sulphate structure of the MES, and that classification was not possible without any specific additional information. The DS agreed with the comment.

### Assessment and comparison with the classification criteria

The table below summarises the results of the available mutagenicity and genotoxicity tests.

***Table**: Summary table of relevant in vitro and in vivo mutagenicity studies with MES*

| Method | Test system | Tested concentrations | Results | Remarks | Reference |
|---|---|---|---|---|---|
| OECD 471<br><br>GLP<br><br>Bacterial reverse mutation test | S. typhimurium: TA 1535, TA 1537, TA 98, TA 100<br><br>Metabolic activation: S9 mix from livers of Wistar rats which received i.p. 500 mg/kg bw Aroclor 1254 5 days before preparation | Main study: 0.16, 0.8, 4.0, 20 and 100 µg/plate.<br><br>Positive control without metabolic activation 10 µg/plate sodium azide in TA100 and TA1535, 50 µg/plate 9-aminoacridine in TA1537, 10 µg/plate 4- nitro-1,2-phenylene diamine in TA98.<br><br>Positive control with metabolic activation 3 µg/plate 2-aminoanthracene for all strains. | **MES did not induce gene mutations in bacteria.**<br><br>Reduced number of revertants/plate at concentration of 100 µg/plate in case of TA1535, TA1537 indicated cytotoxic effect. | Preliminary toxicity study (only TA100): 10, 32, 100, 320, 1000, 3200, 10000 µg/plate<br><br>The undiluted test substance contained 30% active component and all concentrations used were based on this content of active component. | BODE Chemie (1992f) |
| Comparable to OECD 471<br><br>GLP<br><br>Bacterial reverse mutation test | S. typhimurium: TA 1538, TA 1535, TA 1537, TA 98, TA 100<br><br>Metabolic activation: S9 mix from livers of male Wistar rats which received i.p. 500 mg/kg bw Aroclor 1254 5 days before preparation or after induction with phenobarbital for 7 days. | Concentrations: 20, 100, 500, 2500 µg/plate.<br><br>Positive control: For all strains the same substance was used with and without metabolic activation: TA98 and TA1538: 50 µg/plate dichlorobenzidine; TA100: 50 µg/plate methylcholathrene; TA1535 200 µg/plate cyclophosphamide; TA1537: 100 µg/plate aminoacridine. | **MES did not induce gene mutations in bacteria.** | | BODE Chemie (1981) |
| OECD 473<br><br>GLP<br><br>In vitro mammalian chromosome aberration test | Chinese Hamster Ovary (CHO) cells<br><br>Metabolic activation system: rat liver S9 mix | <u>Main study 1:</u> without metabolic activation 0, 1.5, 3, 6, 9, 12 µg/ml and with metabolic activation 0, 2.5, 5, 10, 20, 30, 40 µg/ml<br><br><u>Main study 2:</u> | **MES induced no clastogenic activity** (neither with metabolic activation nor without metabolic activation) at any dose level **in both** | Given concentrations are related to the 30% solution of MES<br><br>Preliminary study: 0, 0.78-50 µg/ml without metabolic | BODE Chemie (1994) |

| | | | | | |
|---|---|---|---|---|---|
| | | without metabolic activation 0, 2.5, 5, 7.5, 10 µg/ml or 0, 7.5, 10; with metabolic action 0, 5, 15, 25, 30 µg/ml or 0, 25.5, 30<br><br>Positive control; 0.2 µg/ml colcemide | **independent studies.**<br><br>Positive and negative control worked within the historical control data of the performing facility. | activation and 0, 3.12-100 µg/ml with metabolic activation (mitotic index determined after 18 or 28 h incubation). | |
| OECD 476<br><br>GLP<br><br>Mammalian cell gene mutation assay | Mouse lymphoma L5178Y TK+/- cells<br><br>Metabolic activation system: S9 mix from rat liver<br><br>Positive control: With metabolic activation 3 µg/ml benzo(a)pyrene; without metabolic activation 25 µg/ml methylmethanesulfonate | <u>1st assay:</u><br>Without metabolic activation: 0, 0.63, 1.25, 2.5, 5 µg/ml; with metabolic activation: 0, 3.13, 6.25, 12.5, 25.0 µg/ml<br><br><u>2nd assay:</u><br>Without metabolic activation: 0, 2.5, 5, 7.5, 10, 15 µg/ml; with metabolic activation: 0, 7.5, 10, 20, 30, 40 µg/ml | <u>1st assay:</u><br><br>**Positive** (statistically significant increases in the mutant frequency)<br><br><u>2nd assay:</u><br><br>**Negative** (no statistically significant increases in the mutant frequency)<br><br>In both cases positive and negative control worked within the historical control data of the performing facility. | | BODE Chemie (1994a) |
| OECD 476<br><br>GLP<br><br>Mammalian cell gene mutation assay | Mouse lymphoma L5178Y/ TK+/- cells | Without metabolic activation: 0, 0.63, 1.25, 2.5, 3.75, 5.00, 7.50 µg/mL<br><br>With S9-mix: 0, 2.5, 5.0, 10, 15, 20 µg/mL.<br><br>Positive control: with metabolic activation: 2.5 µg/mL cyclophosphamide monohydrate;<br><br>Positive control without metabolic activation: 7.5 µg/mL methyl | For both with and without metabolic activation:<br><br>**Negative** (MES did not induce statistically significant increases in the mutant frequencies).<br><br>Positive and negative control worked within the historical control data of the performing facility. | MES, both in the absence and presence of S9-mix, induced marked concentration-dependent cytotoxicity.<br><br>Plating efficiency and relative survival were not altered significantly by MES treatment (except 3.75 µg/mL without and 5.0 µg/mL with S9-mix). | BODE Chemie (2008) |

| | | | | | |
|---|---|---|---|---|---|
| | | methanesulfonate. | All MES-treated cultures exhibited mutant frequencies within the normal range for negative controls. | | |
| Comparable to OECD 482<br><br>GLP<br><br>Unscheduled DNA synthesis (UDS) in mammalian cells *in vitro* | HeLa S3 cells (human cell line)<br><br>Metabolic activation: Rat liver S9 mix from Wistar rats that received a single i.p. injection of 500 mg/kg bw Aroclor 1254 in corn oil | Concentrations: 0, 0.2, 0.02, 0.002, 0.0002 µg/ml<br><br>Positive control with metabolic activation: 10 µM NQO (no further specification, presumably 4-nitroquinoline-N-oxide)<br><br>Positive control without metabolic activation; 50 µM DCB (no further specification) | <u>Without metabolic activation:</u><br><br>No increase in radioactivity indicating **no UDS** and/or cytotoxic effects.<br><br>Valid positive control<br><br><u>With metabolic activation:</u><br><br>No increase in radioactivity indicating **no UDS** and/or cytotoxic effects.<br><br>Only a weak effect in the positive control limiting the reliability of the results (no statistical evaluation). | Cytotoxicity: Yes, reduced radioactivity at the high dose levels indicates cytotoxicity | BODE Chemie (1981a) |
| OECD 474<br><br>GLP<br><br>*In vivo* micronucleus test | Crl:NMRI BR mouse<br><br>5 males + 5 females/group/sampling time<br><br>Gavage administration<br><br>Single oral application<br><br>Dose: 0, 18.7, 56, 187 mg/kg bw plus positive control (all 24 h); 187 mg/kg bw (48 h) | Dose: 0, 18.7, 56, 187 mg/kg bw plus positive control (all 24 h); 187 mg/kg bw (48 h) | **MES did not induce a significant increase in the number of micronuclei** at a dose level up to 187 mg/kg bw. | 187 mg/kg bw did not reach the dose level recommended in the OECD TG 474 and did not induce cytotoxicity.<br><br>The study is not valid due to significant methodological deficiencies. | <confidential> (1994b) |

RAC notes that the database is not totally conclusive for establishing a classification. According to the CLP criteria, positive results in somatic cell mutagenicity tests *in vivo*, in mammals; or other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays are needed for classification in Category 2. Also substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens. None of these requirements were met with the available database because the only available *in vivo* result was negative (although of questionable validity due to methodological deficiencies) and the *in vitro* results were mainly negative as the only positive result in the mammalian cell gene mutation assay (the first assay in DE Chemie (1994a)) could not be confirmed in the other two equal independent assays (the second assay in DE Chemie (1994a) and BODE Chemie (2008)), in the unscheduled DNA synthesis assay (BODE Chemie (1981a), in the *in vitro* mammalian chromosome aberration test (BODE Chemie (1994)), or in the bacterial reverse mutation tests (BODE Chemie (1992f) and BODE Chemie (1981)). Therefore, RAC agrees with the DS's proposal that **no classification of MES is warranted for germ cell mutagenicity**.

## RAC evaluation of reproductive toxicity

### Summary of the Dossier Submitter's proposal

The DS proposed no classification of MES for reproductive toxicity on the basis of the results of the following studies:

- A one-generation reproductive toxicity study (OECD TG 415) in rats receiving 0, 10, 40 and 110 mg/kg bw/day of MES via oral gavage. At 110 mg MES/kg bw/day, a statistically significant decrease in the mean number of implantation sites (11.9 vs. 13.8 in controls, viability index (89.1% vs 97.7% in controls), mean body weight in pups on lactation days 7, 14 and 21 as compared to controls (-10%, -14% and 11%, respectively) and an increase in the postnatal loss (24 vs. 6 in controls) on days 0-4 co-occurring with 17% maternal mortality (4/24), clinical signs (salivation and rales during single or multiple days of the treatment period) and irritative and degenerative lesions in the forestomach;
- A teratogenicity study in rabbit (OECD TG 414) receiving 0, 4, 12, 30 and 40 mg/kg bw/day of MES via oral gavage. According to the DS there were no developmental effects of biological relevance or not secondary to local effects on the GI tract of dams at ≤ 40 mg/kg bw/day.

The DS concluded that there were no adverse effects on sexual function and fertility or on development at doses below those inducing severe maternal toxicity, and therefore classification was not warranted.

### Comments received during public consultation

No comments were received during public consultation.

### Assessment and comparison with the classification criteria

#### *One-Generation Reproductive Toxicity Study*

The one-generation toxicity study was performed according to OECD TG 415 and GLP. Wistar rats (96 males and 96 females; 24 males and 24 females per dose group) were exposed by oral

gavage to MES once daily. The dose levels for the F0-generation were 0 (control), 10, 40 and 110 mg/kg bw/day. F0 male animals were exposed to the test substance for a 70-day pre-pairing period, during the pairing period and for a 45-day after-pairing period until one day before the scheduled sacrifice, in total for 120 days. F0 females received the test substance during a 70-day pre-paring period and also during the pairing, gestation and lactation periods until one day before the scheduled necropsy, in total for maximally 120 days. Due to the occurrence of maternal toxicological effects, 10 females in groups 1 (vehicle control) and 4 (110 mg/kg bw/day) were further tested for reversibility of the effects. Therefore, these females were given a 4-week treatment-free period (recovery period) and they were mated again with untreated males. All females were allowed to give birth and rear their pups until day 4 post-partum. The dams were sacrificed on day 5 post-partum, ie. one day after the pups.

All animals were subjected to twice daily clinical observations. Daily body weight and food consumption were measured over the treatment period. The regularity and duration of the oestrus cycle were examined. At necropsy, macroscopic observations and organ weights were recorded. A histopathological examination was performed on all reproduction organs and tissues. Reproduction parameters, breeding data and pup development were assessed.

The table below summarises the parental toxicity in the one-generation reproductive toxicity study. Mortalities, clinical signs and irritative and degenerative lesions in the forestomach were reported at the highest dose (110 mg/kg bw/day). In the mid dose (40 mg/kg bw/day) clinical signs and histopathological alterations in stomach were also reported, although with a lower incidence.

***Table:*** *Parental toxicity in the one-generation reproductive toxicity study with MES*
*None of the effects were reported in the control group and in the group dosed with the lowest dose of 10 mg/kg bw/day.*

| **110 mg/kg bw/day** | **40 mg/kg bw/day** |
|---|---|
| 4 mortalities (females)<br><br><u>Body weight gain:</u><br>Pre-pairing period: 83 (males) and 90% (females) of control<br>Gestation period: 76% of control<br><br><u>Clinical observations:</u><br>Salivation: 13 males and 9 females<br>Rales: 15 males and 13 females<br><br><u>Histopathologic alterations:</u><br>Acanthosis/Hyperplasia: 18 males and 5 females<br>Hyperkeratosis: 20 males and 6 females<br>Oedema: 5 males and 4 females<br>Inflammation, acute: 4 males and 3 females<br>Erosion: 2 females<br>Ulceration: 1 female | 0 mortalities<br><br><u>Clinical observations:</u><br>Salivation: 2 males and 7 females<br>Rales: 3 males and 4 females<br><br><u>Histopathologic alterations:</u><br>Acanthosis/Hyperplasia: 3 males and 1 females<br><br>Hyperkeratosis: 2 males<br>Oedema: 3 males<br>Inflammation, acute: 1 male and 1 female<br>Erosion: 1 male<br>Ulceration: 1 male |

<u>Adverse effects on sexual function and fertility</u>

Adverse effects on sexual function and fertility were restricted to the highest tested dose. The number of implantation sites was decreased as compared to controls (11.9 vs. 13.8 in controls). However, as the maternal mortality was 17%, the maternal toxicity is considered excessive and the data at this dose level is not considered for further evaluation.

<u>Adverse effects on development</u>

The adverse effects on development were restricted to the highest tested dose. The total number of pups lost during the first 4 days was 24 compared to 6 dead pups in the control group. Among

the total number of pups lost, one total litter of 9 pups was found cannibalised on day 1 post-partum. One other litter was found dead with 8 pups. In another litter, five pups were found dead on day 2 post-partum (no milk in the stomach). This was considered to be a result of the moribund condition of the dams. Accordingly, the pup viability index was decreased (89.1% vs 97.7% in controls). From day 7 post-partum onwards, body weight development was statistically significantly reduced. All these findings were considered to be substance-related. However, as the maternal mortality was 17%, the maternal toxicity is considered excessive and the data at this dose level is not considered for further evaluation.

***Table:*** *Reproductive parameters in the One Generation Reproductive Toxicity Study with MES.*
**= Statistically different of control for p<0.05.*
***=Statistically significantly different from control p<0.01*

| | **Dose level (mg/kg bw/day)** | | | |
|---|---|---|---|---|
| | **0** | **10** | **40** | **110** |
| Number of implantation sites | 13.8 | 13.0 | 13.8 | **11.9*** |
| Living pups at first litter check (%males/females) | 41/59 | 50/50* | 45/55 | **53/47*** |
| Post-natal loss (days 0-4) | 6 | 10 | 4 | **24**** |
| Viability index (%) | 97.7 | 96.6 | 98.5 | **89.1**** |
| Pup mean body weight (day 7) | 14.3 | 14.3 | 14.2 | **12.9**** |
| Pup mean body weight (day 14) | 30.2 | 29.9 | 29.7 | **26.1**** |
| Pup mean body weight (day 21) | 47.3 | 47.7 | 46.8 | **41.9**** |

### *Teratogenicity study in the rabbit*

The teratogenicity study in rabbits was performed according to OECD TG 414 and GLP. Himalayan rabbits were dosed by gavage with the following doses: 0 mg/kg bw/day (20 animals); 4 mg/kg bw/day (20 animals), 12 mg/kg bw/day (20 animals), 30 mg/kg bw/day (16 animals), and 40 mg/kg bw/day (10 animals).

The table below summarises the maternal toxicity reported at the two highest doses. At 4 mg/kg bw/day, no test substance-related clinical effects were reported for mortality, body weight or body weight change or food consumption relative to the control group. At 12 mg/kg bw/day no effects were detected on body weight or body weight change or food consumption; 2 out of 24 dams were not pregnant (the incidence is within the normal range) and 1 out of 21 dams spontaneously aborted on gestation day 26.

***Table:*** *Maternal toxicity in the teratogenicity study in rabbits with MES.*
*No treatment-related effects were reported at 12 and 4 mg/kg bw/day*

| **30 mg/kg bw/day** | **40 mg/kg bw/day** |
|---|---|
| • 2 out of 24 dams (8%) died prematurely on gestation day 22 or 28 (both with diarrhoea and lesions of the stomach).<br>• 5 further dams were sacrificed after abortion between gestation day 23 and 27 (evaluation of individual test results revealedgastro-intestinal effects of the test substance in all 5 rabbits with abortions: 1 out of these 5 dams has stomach lesions; diarrhoea, partly haemorrhagic in 3 rabbits, and the last one showed minimal or no discharge of faeces).<br>• One dam was without viable foetuses (haemorrhagic diarrhoea observed).<br>• Obvious decrease (p<0.01) body weight gain (the numerical data was not provided)<br>• Reduced absolute and relative food intake (p<0.01) (the numerical data was not provided).<br>• Histological findings in stomach: | • 8 out of 24 dams (33%) died prematurely on GD 18 or 27 (all of them had stomach lesions, 6/8 liquid content in the intestine and 6/8 diarrhoea).<br>• Mydriasis in all dams from the 1st application onwards (starting 20-60 minutes after treatment and lasting 2-6 h).<br>• 4 further dams were sacrificed after abortion between gestation days 25 and 27 (3 out of these 4 dams had stomach lesions combined with liquid content in the intestine, the 4th had only a brownish liquid in the intestine; diarrhoea in 1/4 rabbits, 2/4 dams revealed minimal or no discharge of faeces).<br>• Decreased (p<0.01) body weight gain (the numerical data was not provided).<br>• Reduced absolute and relative food intake (p<0.01) (the numerical data was not provided). |

| | |
|---|---|
| Several/multiple haemorrhagic foci: 1<br>Multiple ulcers: 0<br>Mucosal detachment: 2<br>Whitish layer: 1<br>• Histological findings in intestine:<br>Liquid brownish content: 6<br>Aerated: 8<br>• Histological findings in spleen:<br>Reduced size: 0<br>• Histological findings in liver:<br>Pale: 0 | • Histological findings in stomach:<br>Several/multiple haemorrhagic foci: 12<br>Multiple ulcers: 2<br>Mucosal detachment: 0<br>Whitish layer: 0<br>• Histological findings in intestine:<br>Liquid brownish content: 12<br>Aerated: 1<br>• Histological findings in spleen:<br>Reduced size: 4<br>• Histological findings in liver:<br>Pale: 2 |

Adverse effects on sexual function and fertility

The table below summarises the main findings on sexual function and fertility in the rabbit teratogenicity study with MES.

***Table:*** *Effects on sexual function and fertility in the rabbit teratogenicity study with MES*

| Finding | Control (n=21) | 4 mg/kg bw (n=20) | 12 mg/kg bw, (n=20) | 30 mg/kg bw, (n=17) | 40 mg/kg bw, (n=10) |
|---|---|---|---|---|---|
| Corpora lutea total | 177 | 164 | 161 | 127 | 75 |
| Corpora lutea per dam | 8.4 | 8.2 | 8.1 | 7.5 | 7.5 |
| Total implantation sites | 162 | 145 | 153 | 116 | 63 |
| Implantation sites/dam | 7.7 | 7.3 | 7.7 | 6.8 | 6.3 |
| Mean pre-implantation loss (%) | 9.3 | 12.1 | 5.7 | 8.1 | 18.4 |

As the high dose caused excessive maternal mortality (33%), RAC did not further evaluate the effects observed at this dose. Maternal toxicity was considered to be excessive also at 30 mg/kg bw/day. RAC considers that there were no treatment-related effects on corpora lutea, implantation sites, or pre-implantation loss below the top dose.

Adverse effects on development

The table below summarises the main developmental effects in the rabbit teratogenicity study with MES.

***Table:*** *Developmental effects in the rabbit teratogenicity study with MES*

| Finding | Control (n=21) | 4 mg/kg bw (n=20) | 12 mg/kg bw (n=20) | 30 mg/kg bw(n=17) | 40 mg/kg bw (n=10) |
|---|---|---|---|---|---|
| Total resorptions | 18 | 5** | 2** | 8 | 3 |
| Resorptions/dam | 0.9 | 0.3 | 0.1 | 0.5 | 0.3 |
| Total early resorptions | 17 | 4** | 1** | 7 | 3 |
| Early resorptions/dam | 0.8 | 0.2 | 0.1 | 0.4 | 0.3 |
| Total late resorptions | 1 | 1 | 1 | 1 | 0 |
| Late resorptions/dam | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 |
| Mean% post-implantation loss | 10.0 | 4.4 | 1.3 | 8.7 | 3.8 |
| Total live foetuses | 144 | 140 | 151 | 108 | 60 |
| Live foetuses per dam | 7.2 | 7.0 | 7.6 | 6.8 | 6.0 |
| Total dead foetuses at laparatomy | 0 | 0 | 0 | 0 | 0 |
| Placental weight (m&f, litter mean) | 5.01±0.89 | 5.31±0.64 | 5.00±0.73 | 4.70±0.64# | 5.43±1.17 |
| Foetal weight (m, litter mean) | 38.9±4.8 | 39.5±3.3 | 38.9±4.1 | 34.9±5.9*# | 38.5±5.5 |

| | | | | | |
|---|---|---|---|---|---|
| Foetal weight (f, litter mean) | 38.1±4.1 | 39.4±3.9 | 38.9±3.4 | 33.4±6.3**# | 37.1±3.4 |
| Subdural haemorrhages of meninx (fetal incidence) | 3 (4.2%, n=72) | 8 (11.4%, n=70) | 3 (4.0%, n=75) | 11** (20.8%, n=53) | 1 (3.3%, n=30) |
| Subdural haemorrhages of meninx (litter incidence) | 3 (15.0%) | 6 (30.0%) | 2 (10.0%) | 9* (56.3%) | 1 (10.0%) |
| External malformation & variation | No test substance related findings | | | | |
| Skeletal variations & retardations | No test substance related findings | | | | |
| Visceral examination | No test substance related findings | | | | |
| Soft tissue of the head | No test substance related findings | | | | |
| *: p≤0.05; **: p≤0.01; #: incidental decrease, biologically not relevant, within the historical control range | | | | | |

According to CLP 3.7.2.4.4, maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation. As the high dose caused excessive maternal mortality (33%), this dose level was not considered for further evaluation by RAC. Maternal toxicity was considered to be excessive also at 30 mg/kg bw/day. RAC notes however that no test substance-related developmental effects were reported in the study. The observed effects in foetal weight at 30 mg/kg bw/day are within the historical control data (and no effect in foetal weight is observed at the top dose). Foetal and litter incidence of subdural haemorrhages in the meninx were elevated at 30 mg/kg bw/day. However, no corresponding effect was found at 40 mg/kg bw/day. Furthermore, this variation is also noted in the control and low dose group and might be due to methodological shortcomings during dissection. For these reasons the subdural haemorrhages are considered to be incidental. Altogether, no classification is warranted for the effects listed in the table above.

***Abortions and the co-occurring maternal toxicity***

1, 5 and 4 abortions were recorded at 12, 30 ad 40 mg/kg bw/day, respectively. The abortion at the lowest dose was considered spontaneous according to the study report. As the high dose caused excessive maternal mortality (33% due to stomach lesions (8/8); liquid content in the intestine and diarrhoea (6/8)), RAC does not further evaluate the abortions observed at this dose. Also, abortions at 30 mg/kg bw/day do not warrant classification because they are considered to be secondary non-specific consequences of maternal toxicity since all dams that suffered from the abortions showed gastrointestinal effects (stomach lesions (1/5), partly haemorrhagic diarrhoea (3/5) and minimal or no discharge of faeces (1/5)). The critical role of severe maternal toxicity on abortions is supported by the fact that these gastrointestinal effects in non-aborting animals caused 2/24 fatalities (both with diarrhoea and lesions of the stomach) in dams dosed with 30 mg/kg bw/day. The gastrointestinal damage reported in animals exposed at 30 and 40 mg/kg bw/day included several/multiple haemorrhagic foci in the stomach (13 animals in total), liquid brownish content in the intestine (18 animals in total) and aerated intestine (9 animals in total); together with lower but still significant incidences of multiple ulcers and mucosal detachment in the stomach and reduced spleen size.

***Comparison with the CLP criteria***

RAC notes that the adverse effects on reproduction in the one-generation reproductive toxicity study in rats were reported only at the top dose of 110 mg/kg bw/day, which also caused severe parental toxicity (17% of maternal mortality, salivation in 48% of animals and rale in 58% of animals in addition to histopathological evidence of severe degenerative lesions in the forestomach). RAC considers this maternal systemic toxicity as excessive, and therefore the

adverse effects are not further considered for classification for adverse effects on sexual function and fertility, on development or on or via lactation.

RAC concludes that there were no treatment-related effects on sexual function and fertility or development in the developmental toxicity study in rabbits below the doses causing excessive maternal mortality/severe maternal toxicity. Therefore, no classification of MES for adverse effects on sexual function and fertility or on development is warranted.

In conclusion, RAC supports the DS's proposal for **no classification of MES for reproductive toxicity**.

# ENVIRONMENTAL HAZARD EVALUATION

## RAC evaluation of aquatic hazards (acute and chronic)

### Summary of the Dossier Submitter's proposal

MES is a biocidal active substance and is currently under active substance review for approval as Product Type 1 (PT1; Human hygiene) under BPR for uses including that as a surgical disinfectant.

MES has an ionic structure, strong polarity and surface-active properties. There is currently no entry in Annex VI to CLP for the substance. However, in the ECHA C&L inventory, some notifiers self-classified the substance as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410.

#### *Degradation*

The DS proposed to consider MES as rapidly degradable for classification purposes. The basis for this proposal is a weight of evidence approach (as clarified in the DS´s response in the RCOM table following PC), giving more weight to recent studies in which MES under specific test conditions and under several test modifications fulfils the criteria to be considered readily biodegradable. No simulation studies are available which deal with the rate and route of degradation in aquatic systems (incl. sewage treatment plants) and with degradation in soil. No tests are available on hydrolysis and MES was assessed by the DS not to hydrolyse as it is marketed as a stable, 29% aqueous solution. No tests are available on photolysis. However, as MES has no chromophore and does not show any UV absorption above 290 nm, MES was assessed by the DS to not be a candidate for photolysis.

#### *Adsorption/desorption*

The DS concludes that MES has a strong adsorption potential, is immobile in soils and will strongly adsorb onto sewage sludge. The basis for this assessment are the measured adsorption coefficients by BODE Chemie (2002a, 2008d and 2008e).

#### *Inhibition to microbial activity (aquatic)*

The DS concludes that MES is toxic to bacteria. The basis for this assessment is an OECD TG 209 test by BODE Chemie (2002) which results in an $EC_{50}$ = 22 mg/L.

#### *Aquatic Bioaccumulation*

The DS proposed to not consider MES as being bioaccumulative in the aquatic environment for classification purposes. The basis for this proposal is an estimated log $K_{ow}$ of – 0.39 derived from the individual solubilities in water and n-octanol. This was in order to avoid the strong surface-active properties of the test substance, which typically sits on the interphase in a two-phase

(OECD TG 107/117) system or forms micelles. The study on solubility in n-octanol was performed according to CIPAC MT 181 (Collaborative International Pesticides Council) and the solubility was found to be 168-202 g/L. The study on solubility in water was performed according to OECD TG 105 with a determined solubility of 500-1000 g/L.

As a worst-case scenario, the highest solubility value of n-octanol and the lowest value of water were used for further calculation.

$K_{ow}$ estimated as 202 g/L /500 g/L = 0.404. From this the log $K_{ow}$ value is calculated log $K_{ow}$ = -0.39.

The CMC (critical micelle concentration) is not considered and there is no measured log $K_{ow}$ or measured bioaccumulation data available.

***Acute Aquatic Toxicity***

The DS proposed to classify MES as Aquatic Acute 1, H400 with an M-factor of 100. The basis for this proposal was that for all three trophic levels acute test data are available and that the lowest $L(E)C_{50}$ value is:

- 72 h $E_rC_{50}$ of 0.0039 mg a.i./L (time-weighted average concentration;
  derived from the nominal 72 h $E_rC_{50}$ of 0.054 mg/L

obtained from an OECD TG 201 Growth Inhibition Test on algae (BODE Chemie (2000)) and adjusted by BODE Chemie (2010).

***Chronic Aquatic Toxicity***

The DS proposed to classify MES as Aquatic Chronic 1, H410 with an M-factor of 10. The basis for this proposal is that for all three trophic levels chronic test data are available and that the lowest $NOEC/EC_{10}$ is:

- 72 h $NOE_rC$ of 0.00014 mg a.i./L (time-weighted average concentration;
  equal to the 72 h NOEC of 0.011 mg/L (nominal)) for algae

obtained from an OECD TG 201 Growth Inhibition test on algae (BODE Chemie (2000)). Available NOEC values for fish:

- 35 d NOEC 0.000555 mg a.i./L (adverse effect on survival, mean measured concentration))

and daphnia:

- 21 d NOEC of 0.00019 mg/L (time-weighted average concentration)

are in the same range. In addition, MES was proposed to be rapidly degradable and to have a low potential for bioaccumulation for classification purposes.

## Comments received during public consultation

Three MSCAs commented on the proposal for environmental classification, with two MSCAs agreeing with the proposed environmental classification. All three asked for more detailed information or further data.

For the assessment of the results on the available tests on ready biodegradability, it was questioned if the adaptations and modifications using silica gel and humic acid are relevant for assessing biodegradation for the purpose of classification and how environmentally relevant they are for classification purposes. The DS responded that adding silica gel to biodegradation testing balances the effects of toxicity towards microorganisms with non-availability to microorganisms.

However, the question of whether modifications are environmentally relevant for the purpose of classification was not answered in the DS's response in the RCOM table and is also not addressed in the weight of evidence approach applied by the DS.

To understand the test validity in relation to IC (Inorganic Carbon) concentrations and to ensure that the measured degradation solely reflects the test item, details of IC concentrations over time and mass balances were requested by MSCAs but not provided by the DS.

Concerning the acute aquatic toxicity test with *Daphnia magna*, it was requested for the BODE Chemie (2000) (A7.4.1.2 as referred to in the DS´s response in the RCOM table) study, that the $LC_{50}$ values should be corrected by time-weighted averages as it was for the second test with daphnia, the test with fish and the test with algae. However, the DS responded that for this test there was no kinetic study available simulating test conditions according to OECD TG 202 and that the endpoints from this study had to be based on nominal concentrations.

Further information was requested on the kinetic study (this study provided fate assays for MES following OECD TGs 201, 203, and 211) and provided in the response by the DS for OECD TG 211 only. Time-weighted average values were also used for one acute test on fish (A7.4.1.1 as referred to in the DS´s response in the RCOM table), on daphnia (A7.4.1.2_02 as referred to in the DS´s response in the RCOM table) and for the test on algae (A7.4.1.3 as referred to in the DS´s response in the RCOM table). In the kinetic study, it was confirmed that analytical measurements revealed that the MES concentrations are not stable during the test period. The extreme high adsorption potential of MES affected the test concentrations. From other aquatic toxicity studies, it is known that MES is difficult to recover due to its high potential for clustering and adsorption, resulting in an irregular distribution in the test vessels.

Concerning the test on algae, the DS confirmed that the validity criterion of cell concentration in control cultures was met.

## Assessment and comparison with the classification criteria

### *Degradation*

RAC has reassessed the available information on the tests on ready biodegradability and performed a weight of evidence approach.

### *Derive effect levels from ready test systems*

In their supplementary comment after PC, industry has submitted an effect level for each test system. RAC concludes that it is scientifically unjustified to derive effect levels from OECD TG 301 and OECD TG 310 test systems and that, consequently, it is not possible to derive a dose-response relation between these test systems.

### *Effect of potential to adsorb*

Furthermore, BODE Chemie (1999) found the percentage elimination of 33% in the abiotic control is in the same range as the elimination in the vessels with test substance. Thus, the observed elimination can be attributed to an abiotic removal, probably adsorption. Also, for BODE Chemie (2008), the duration of the lag phase (in test item and toxicity control assays) was affected by availability of MES to microorganisms. These findings are consistent with the high adsorption potential of MES.

RAC concludes that the potential of MES to adsorb may also influence the test result. However, adsorption is only a problem in tests measuring removal of DOC (see OECD TG 301). To investigate the influence of adsorption compared to the influence of toxicity, it would have been

necessary to include a toxicity control in those experiments with silica gel. It is unclear to RAC why this has not happened.

***Modification with silica gel***

RAC is of the opinion that the use of silica gel does not necessarily invalidate a test for the purpose of classification. Although this method is not specifically mentioned in OECD TG 301, silica gel (e.g. amorphic silicon dioxide) has also been used in the EU ring-test for OECD TG 310. ECHA Guidance R.7b states that reduction in the toxicity in the ready biodegradability tests may be achieved by the introduction of carriers allowing slower-release of the test substance during the test period. Silica gel is a preferred modification of ready biodegradability tests for improving the bioavailability of poorly water-soluble substances. In UBA (2017), it is concluded that the approach using silica gel matrices represents the first option for substances of low bioavailability. Silica gel is documented in several peer-reviewed publications (Handley *el al*., 2002; Painter *et al*., 2003, van Ginkel *et al*., 2008, Kowalczyk *et al*., 2015). Provided all other conditions in the ready biodegradability tests are fulfilled, such modified tests are regarded as ready biodegradability tests and the results may be used for the purpose of classification.

RAC concludes that the two test systems BODE Chemie (2011b) and BODE Chemie (2011c) are invalid because they do not fulfil the validity criteria of the OECD TG and not for the reason that silica gel was added.

The following Table shows all available test systems and presents the test results, their validity and the basis for the weight of evidence as assessed by RAC.

***Table***:

| Reference | TG | Batch | Degradation result | pass level fulfilled | Weight of evidence | validity fulfilled | Evaluation by DS |
|---|---|---|---|---|---|---|---|
| BODE Chemie (1995) | OECD TG 301D | A | < 5% at day 29 | no | not readily biodegradable | yes | reliable with restriction |
| | | B | < 5% at day 29 | no | not readily biodegradable | yes | reliable with restriction |
| BODE Chemie (1999) | OECD TG 301A | A | only abiotic removal, due to adsorption | no | not readily biodegradable | yes | reliable with restriction |
| BODE Chemie (2008) | OECD TG 301F | A | no degradation | no | not readily biodegradable | yes | key study |
| | | B | lag phase of 19 days; 42.5 % of ThOD at day 28 | no | not readily biodegradable | yes | key study |
| | | C | lag phase of 6 days 49.8 % of ThOD at day 28 | no | not readily biodegradable | yes | key study |
| | | D | lag phase of 6 days 60.2 % of ThOD at day 28 | no, 10-d window failed | not readily biodegradable | yes | key study |
| BODE Chemie (2011a) | OECD TG 310 | A | no degradation (-10.3%) | no | not readily biodegradable | No highest mean TIC produced in the blank flasks was 3.7 mg/L in 28 days | not valid and not reliable |
| | | B | no degradation (-4.88%) | no | not readily biodegradable | No highest mean TIC produced in the blank flasks was 3.7 mg/L in 28 days | not valid and not reliable |
| BODE Chemie (2011b) | OECD TG 310 | A | 65% at day 28 (at day 14 100% and at day 21 117 %) | yes | at day 14 readily biodegradable | No IC content in the inoculum blanks was 24.1 mg IC/L at test end | not valid and not reliable |
| | | B | 37% at day 28 (at day 14 70% and at day 21 80%) | yes | at day 14 readily biodegradable | No IC content in the inoculum blanks was 24.1 mg IC/L at test end | not valid and not reliable |
| BODE Chemie (2011c) | OECD TG 310 | A | 100 % at day 14; no lag phase | yes | readily biodegradable | No mean amount of TIC present in the blank controls at the end of the test is 3.66 C/L; Reference substance was not used | not valid and not reliable |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | B | 87 % at day 14; lag phase 7 d | yes | readily biodegradable | No difference of replicate values > 20%; mean amount of TIC present in the blank controls at the end of the test is 6.54 C/L; Reference substance was not used | not valid and not reliable |
| | | C | 76 % at day 14; lag phase 7 d | yes | readily biodegradable | No difference of replicate values > 20%; mean amount of TIC present in the blank controls at the end of the test is 9.80 C/L; Reference substance was not used | not valid and not reliable |
| BODE Chemie (2013a) | OECD TG 301 B | A | 48 % at day 28 | no | not readily biodegradable | yes | reliable with restriction |
| | | B | 97 % at day 28 | yes | readily biodegradable | yes, but not reliable for classification | reliable with restriction |

***Read-across and QSAR***

MES belongs to the group of quaternary ammonium compounds (QACs), a group of cationic surfactants, which are structurally similar with respect to the embedded quaternary nitrogen and at least one long carbon chain. The DS stressed that read-across is possible between the chemical members of this group. However, RAC concludes that a more detailed description of the structural analogues and their similarity with regards to other substances' properties would be needed in the CLH report to allow a fully valid read-across. Moreover, it seems difficult to apply a sufficiently high weight to the read-across approach and QSAR models to change the conclusion reached from valid and reliable tests on ready biodegradability.

***Conclusion on rapid degradability***

RAC in its evaluation assessed and considered all available information including available test results, information on read-across, QSARs, as well as input from industry.

In contrast to the DS, RAC concludes that there are no fully reliable or suitable data clearly showing ready biodegradability of MES.

Conversely, RAC notes that there are valid, reliable studies available showing that even with modifications to test systems (such as the addition of silica gel), MES is not readily biodegradable.

RAC bases the weight of evidence on valid and reliable studies (1) without modification and adjustment and (2) with modification by adding silica gel. RAC concludes that in these test systems, the degradation in the toxicity control fulfils the validity criteria of the OECD test guideline and inhibition did not influence the outcome of these test systems.

RAC notes that no simulation studies are available for MES. MES does not hydrolyse and is not accessible for photolysis. Based on the evidence presented, RAC in contrast to the DS and concludes that for the purposes of classification MES should be considered not rapidly degradable.

***Aquatic bioaccumulation***

No experimental data on the bioconcentration potential of MES are available. To assess this hazard , the DS applied a weight of evidence approach based on an estimated log $K_{ow}$ of -0.39, experimental BCF studies within the training data set of EPISUITE of seven QAC DODMAC, a read-across to an experimental uptake and depuration study with analogue substances by Versteeg and Shorter (1992) and toxicokinetic studies in rats. It should be noted that this is more of a qualitative comparison of the sparse available data than any structured attempt at read-across to other similar chemicals.

Estimated partition coefficient

The DS explains that the experimental determination of the partition coefficient of MES by OECD TGs 107 and 117 is technically not feasible. Thus, the log $K_{ow}$ was estimated from the individual solubilities in water and n-octanol. A very high water solubility of >500 g/L together with an n-octanol solubility of 202 g/L was used to calculate a log $K_{ow}$ of -0.39. However, the value of >500g/L does not account for the likely formation of micelles and adsorption to test vessel. As such, it does not represent the solubility of the substance in a manner that is suitable for the determination of Log $k_{ow}$ or aquatic toxicity testing.

The following QSAR predictions, conducted by ECHA to determine the water solubility, indicate much lower values:

- ACD/Labs (Release 2016.2): 0.0014 g/L
- EPISUITE WaterNT: 0.000047 g/L

Using the same calculation method as the DS, the estimated log $K_{ow}$ would be in the range of 3.15 to 6.63.

However, these value are uncertain because the substance has more instances of the aliphatic carbon $–CH_2-$ than covered by the training set and the $N^+$ fragment has a coefficient of zero. Consequently, RAC cannot evaluate the reliability of these QSAR predictions. They point towards a much lower water solubility than given in the CLH dossier. An overestimation of the water solubility would result in an unreliable and rough underestimation of the partitioning coefficient. The measurement of the CMC (critical micelle concentration) of MES is not available to RAC.

RAC concludes that the estimated log $K_{ow}$ of -0.39 is most likely an underestimation and that due to MES being an ionised surfactant, log $K_{ow}$ data in general is not suitable for concluding on bioaccumulation. However, the available models do suggest that MES may have a lower water solubility than the value provided by the DS and consequently, the log $K_{ow}$ could be higher.

***Conclusion on bioaccumulation***

In the absence of a direct measured log $K_{ow}$ value or a measured BCF value for MES, the conclusion for bioaccumulation must be based on the weight of the small amount of available evidence. The log $K_{ow}$ value is not appropriate for surface-active substances. The estimated log $K_{ow}$ of -0.39 is likely to be an underestimation and based on modelled water solubility (above), the log $K_{ow}$ could be higher. Information on the CMC (critical micelle concentration) of MES would have assisted in assessing the water solubility for testing purposes but this information is not available to RAC. Bioaccumulation in mammals appears unlikely.

Although some information on BCF of QACs indicate low bioaccumulation a high BCF of 1962 L/kg in fathead minnow was reported for a QAC comprising C 16/18 alkyl chains in a non-guideline test with short uptake and depuration phases. Finally, the calculated Klipw value of 6.58 for the MES cation could further indicate a potential for bioaccumulation but the model is not validated for regulatory use. RAC concludes a potential BCF value for MES would be under the 1962 L/kg reported for similar structures but that the value for MES is likely to be above 500 L/kg. Furthermore, the log $K_{ow}$ values provided by the log Klipw model indicate lipophilicity that could lead to a concern for bioaccumulation, although whether it avoids the problem with surface active materials is unclear. Overall, the potential of MES to bioaccumulate with a BCF above the criteria for the purpose of classification (BCF ≥ 500 L/kg) can currently not be excluded. Therefore, based on the evidence presented, RAC in contrast to the DS, considers MES to have a potential for bioaccumulation for the purpose of classification and labelling.

***Aquatic Toxicity***

Available studies on aquatic toxicity

Over all, seven aquatic toxicity studies were available to RAC. For acute toxicity, one fish study, two invertebrate studies, and one algae study were available (Table). For Long-term toxicity, One fish study, two invertebrate studies, and one algae study (as above) were available (Table).

RAC notes that as MES is highly adsorbing and therefore analytical confirmation of test concentrations is important as nominal concentrations significantly underestimate actual toxicity. However, MES concentrations measured in the studies are not available for all acute (see Table) and chronic tests (see Table). For the acute fish test (A7.4.1.1), the first acute daphnia study (A7.4.1.2/01), and the algae study (A.7.4.1.3), the analytical methods were not sensitive enough to measure MES, due to the rapid disappearance of MES within the test systems. Therefore, no direct MES measurements from within the studies were available. In order to provide data on test substance concentrations in the test systems, a retrospective kinetic study was conducted by Industry and used to theoretically recalculate the nominal endpoints of the original studies lacking measured test substance data, by calculating theoretical TWA MES concentrations. For tests with measured MES data, measurements within the studies were used to express each test concentration as either Time Weighted Average (TWA) test concentrations or as mean measured test concentrations.

***Table:***

| Acute aquatic hazard test results: | | **nominal** | **measured TWA** | **theoretical TWA** |
|---|---|---|---|---|
| **BODE Chemie (1992) A7.4.1.1** | OECD TG 203<br>Fish Acute toxicity test | 96 h $LC_{50}$<br>0.06 mg/L | - | 96 h $LC_{50}$ < 0.048 mg/L is not valid and not reliable |
| **BODE Chemie (2000) A7.4.1.2/01** | OECD TG 202<br>*Daphnia* sp. Acute Immobilisation test | 48 h $EC_{50}$<br>0.019 mg/L | - | 48 h $EC_{50}$ between<br>0.0042 to 0.0091 mg/L |
| **BODE Chemie (2010) A7.4.1.2/02** | OECD TG 202<br>*Daphnia* sp. Acute Immobilisation test | not available | 48 h $EC_{50}$<br>0.015 mg/L | - |
| **BODE Chemie (2000) A7.4.1.3** | OECD TG 201<br>Algae, Growth Inhibition Test | 72 h $E_rC_{50}$<br>0.054 mg/L | - | 72 h $E_rC_{50}$<br>< 0.0039 mg/L |

***Table:***

| Long-term aquatic hazard test results: | | **nominal** | **measured TWA / mean** | **theoretical TWA** |
|---|---|---|---|---|
| **BODE Chemie (2012) see A7.4.3.2** | OECD TG 210<br>Fish Early-life Stage Toxicity Test | not available | 35 d NOEC: 0.000555 mg/L | - |
| **BODE Chemie (2008) see A7.4.3.4** | OECD TG 211<br>*Daphnia magna* Reproduction Test | not available | 21 d $EC_{10}$ 0.00006 mg/L<br>21 d NOEC 0.00042 mg/L | 21 d $EC_{10}$ not available<br>21 d NOEC < 0.00019 mg/L |
| **Simon (2018)** | OECD TG 11<br>*Daphnia magna* Reproduction Test | not available | 21 d $EC_{10}$ not calculated<br>21 d NOEC > 0.00127 mg/L | - |
| **BODE Chemie (2000) see A7.4.1.3** | OECD TG 201<br>Algae Growth Inhibition Test | 72 h NOEC<br>0.011 mg/L | not available | 72 h NOEC < 0.00014 mg/L |

The retrospective kinetic approach was not applied to the second acute Daphnia study (A7.4.1.2/02). However, for reasons unknown, this approach was applied to the chronic daphnia study (A7.4.3.4) in addition to the available measured values and TWA values from the original study report.

The kinetic study (BODE Chemie (2010)) study presents three assays intended to simulate test conditions for the OECD TGs 201, 203, and 211 studies as follows:

| | |
|---|---|
| OECD TG 211 | ○ static conditions<br>○ 24 h<br>○ Purified drinking water without any test organism or biological material<br>○ 60 mL glass beakers with 50 mL of test solution<br>○ light/dark cycle of 16/8 hours<br>○ test temperature was 20.0 ± 2 °C |
| OECD TG 201 | ○ static conditions<br>○ 72 h<br>○ Purified drinking water with sterilised modified synthetic OECD medium without any test organisms or biological material<br>○ 250 mL conical glass flasks with 100 mL test medium<br>○ The vessels and caps were sterilised prior to use (autoclaving or heating). The aqueous phase was filtered by using a 0.22 µm filter without pre-filtration under sterile conditions to sterilise the media by filtration.<br>○ continuously illuminated with a light intensity adjusted between 60- 120 µE/m²s (4440 – 8880 lux) close to the surface of the liquid<br>○ test temperature was 20.0 ± 2 °C |
| OECD TG 203 | ○ static conditions<br>○ 96 h<br>○ Purified drinking water without any test organism or biological material<br>○ 12 L glass basin with 10 L test medium<br>○ light/dark cycle of 12/12 hours<br>○ test temperature was 20.0 ± 2 °C |

RAC notes that the loss of MES was monitored in the absence of the test organisms (fish, Daphnia and algae) and in the absence of food or any other biological material. It must be assumed that the presence of such material would have changed the results (*e.g.* due to adsorption). Consequently, RAC notes that the approach by BODE Chemie (2010) to correct the nominal values from the original studies represents the best case and needs to be assessed critically for the purpose of classification.

The results were very different for each guideline assay (Table). The two extremes were the 72 h OECD TG 201 assay which showed the expected result, an extremely rapid loss of MES within a few hours following a two-compartment degradation, and the 96 h OECD TG 203 assay, which showed a slow dissipation following a single first order model. The latter is based on a higher concentration in the test, which may explain a percentile lower adsorption but may not explain the different kinetic result with a 900-fold higher $DT_{50}$.

***Table***:

| | initial measured in % of nominal | aged test solutions in % of nominal | TWA concentration in % of initial measured | kinetic | Dissemination |
|---|---|---|---|---|---|
| OECD TG 211 | 115 to 270 % | after 24 h:<br>28 to 54 % | 24.2 to 35.9 % | two-compartment | $DisT_{50}$: 0.5 – 1.6 h<br>$DisT_{95}$: 78.9 – 115.5 h |
| OECD TG 201 | 99 and 107 % | after 72 h:<br>2.1 and 5.2 % | 1 % and 5 % | two-compartment | $DisT_{50}$: 0.1 and 0.2 h<br>$DisT_{95}$: 0.3 and 71.5 h |

| | | | | | |
|---|---|---|---|---|---|
| OECD TG 203 | 108 % | after 96 h: 66 % | 78 % | Single First Order | $DisT_{50}$: 90.5 h<br>$DisT_{95}$: 391.1 h |

RAC concludes that overall the results of the kinetic study BODE Chemie (2010) are of limited relevance to "correct" the nominal *in vivo* concentration data and likely represent a best-case scenario. The 96 h assay seems to be an outlier and should not be used. However, the assays following OECD TGs 201 and 211 are considered to provide a more reasonable estimate of substance concentrations than nominals. This is due to the rapid loss of substance from the test system causing nominal concentrations to underestimate toxicity. However, RAC also notes that this kinetic methodology would also underestimate toxicity to some degree due to further substance loss that would likely occur in the presence of test organisms or other biological material. Despite this, the corrected algal data provides the most stringent outcome and will be used for classification.

***Acute aquatic toxicity***

OECD TG 203 Fish Acute toxicity test

BODE Chemie (1992) (A7.4.1.1) did not confirm the test concentrations by analytical methods. The 96 h static test with *Leuciscus idus melanotus* did not have renewed test solution. BODE Chemie (2010) conducted a kinetic study to adjust the nominal endpoint to a TWA endpoint. Evaluating the results, RAC notes the extremely high measured concentrations and the large fluctuation that seems unreliable and thus incomparable to the original study.

***Table:***

| | **0 h** | **1 h** | **2 h** | **4 h** | **8 h** | **24 h** | **48 h** | **72 h** | **96 h** |
|---|---|---|---|---|---|---|---|---|---|
| Measured (mg/L) | 0.0649 | 0.102 | 0.0566 | 0.078 | 0.0986 | 0.0663 | 0.0747 | 0.0363 | 0.0397 |
| % of nominal 0.06 mg a.i./L | 108 | 170 | 94 | 130 | 164 | 111 | 125 | 61 | 66 |

RAC concludes that the OECD TG 203 Fish Acute toxicity test by BODE Chemie (1992) is not reliable because MES is highly adsorptive and analytical confirmation of test concentrations is missing. Consequently, the results may significantly underestimate actual toxicity.

RAC concludes that the calculated theoretical TWA 96 h $LC_{50}$ of < 0.048 mg/L (derived using BODE Chemie 2010) is not valid and not reliable for the purpose of classification since it clearly reflects best-case assumptions.

OECD TG 202 Daphnia magna Acute Immobilisation test

There are two tests available. The BODE Chemie (2010) study (A7.4.1.2/02) monitored the test substance MES at 0, 1, 2, 4, 8, 24 and 48 hours and calculated a TWA for each test concentration. RAC concludes that the resulting 48 h $EC_{50}$ of 0.015 mg/L is valid and reliable and can be used for the purpose of classification.

In the first study by BODE Chemie (2000) (A7.4.1.2/01), the monitoring of the test substance was not successful because of analytical problems, thus no TWA of test concentrations is available. The nominal results significantly underestimate the actual toxicity. RAC corrected in a worst-case approach the nominal endpoint of BODE Chemie (2000) (A7.4.1.2/01) by the correction factor from BODE Chemie (2010) of 22.3 to 47.9% (Table). This resulted in an estimated 48 h $EC_{50}$ between 0.0042 to 0.0091 mg/L ).

***Table:***

| | | Adjustment of nominal within the original study: | Endpoint recalculated by BODE Chemie (2010): | nominal mg a.i./L and test duration | reduction by factor (%) based on nominal |
|---|---|---|---|---|---|
| A7.4.1.2/02 BODE Chemie (2010) | OECD TG 202 Daphnia sp. Acute Immobilisation test | yes, TWA measured test concentrations | no | 0.003; 48 h | 22.3 |
| | | | | 0.006; 48 h | 25.8 |
| | | | | 0.012; 48 h | 43.0 |
| | | | | 0.024; 48 h | 40.5 |
| | | | | 0.048; 48 h | 47.9 |

OECD TG 201 Algae, Growth Inhibition Test

BODE Chemie (2000) (A7.4.1.3) did not confirm the test concentrations by analytical methods. In the 72 h static test with *Desmodesmus subspicatus*, the test solution was not renewed. BODE Chemie (2010) conducted a kinetic study to adjust the nominal endpoint to a TWA endpoint. In evaluating the results, RAC notes the extreme fast decrease in the first ca. 1 h and a slower decrease in the subsequent hours for the higher concentration but no further decrease in the lower test concentration. The measured values seems to be consistent and comprehensible.

***Table:***

| | **0 h** | **1 h** | **2 h** | **4 h** | **8 h** | **24 h** | **48 h** | **72 h** |
|---|---|---|---|---|---|---|---|---|
| Measured (mg/L) | 0.0107 | 0.00011 | 0.00010 | 0.00010 | <LOQ | <LOQ | 0.00015 | 0.00022 |
| % of nominal 0.01 mg a.i./L | 107 | 1.1 | 1.0 | 1.0 | n.a | n.a | 1.5 | 2.2 |
| Measured (mg/L) | 0.0396 | 0.00474 | 0.0438 | 0.003690 | 0.00284 | 0.00263 | 0.00243 | 0.00204 |
| % of nominal 0.04 mg a.i./L | 99.0 | 11.9 | 11.0 | 9.2 | 7.1 | 6.6 | 6.1 | 5.1 |

LOQ (Limit of quantification) = 0.0001 mg/L

RAC concludes that the results from OECD TG 201 Algae, Growth Inhibition Test by BODE Chemie (2000) is not reliable because MES is highly adsorptive, analytical confirmation of test concentrations is missing and consequently the results may significantly underestimate actual toxicity. The nominal 72 h $ErC_{50}$ 0.054 mg/L and the 72 h NOEC 0.011 mg/L are not valid for the purpose of classification. The results calculated using the correction factor from BODE Chemie (2010) are derived from the two nominal test concentrations of 0.01 mg a.i./L and 0.04 mg a.i./L and result in a theoretical TWA 72 h $ErC_{50}$ of <0.0039 mg/L and 72 h NOEC of <0.00014 mg/L.

Since these values were measured without test organisms, they represent a best case. It must be assumed that measurements during an experiment with algae might have resulted in lower $ErC_{50}$ and $ErC_{10}$/NOEC values. As the corrected TWA values represent the highest toxicity demonstrated under acute testing, RAC agrees to use the corrected algal data for the purpose of classification.

***Long-term aquatic toxicity***

OECD TG 210 Fish Early-life Stage Toxicity Test

BODE Chemie (2012) (7.4.3.2) performed with *Danio rerio* (zebrafish) an Early Life Stage Toxicity Test under flow through conditions. Test substance concentration in all test vessels was assessed

by chemical analysis using liquid chromatography and tandem mass spectrometry detection (LC-MS/MS). The LOQ was determined to be 0.20 µg/L.

***Table :*** *Results of analytical measurements of test substance concentration for the entire test duration (LOQ 0.2 µg MES/L)*

| **Nominal concentration [µg/L]** | **Mean measured concentration** | |
|---|---|---|
| | **[µg/L]** | **[%]** |
| 0.400 | 0.154 | 39 |
| 1.00 | 0.404 | 40 |
| 2.50 | 0.555 | 22 |
| 6.25 | 2.95 | 47 |
| 15.6 | 10.8 | 69 |

The concentration of MES could not be maintained throughout the test within ± 20% of mean measured values. All effect data were based on these mean measured concentrations.

RAC concludes that the resulting NOEC of 0.000555 mg MES/L (adverse effect on survival, mean measured concentration) and an LOEC of 0.00295 mg MES/L (adverse effect on survival, mean measured concentration) is valid and reliable and can be used for the purpose of classification.

<u>OECD TG 211 Daphnia magna Reproduction Test</u>

There are two tests available. BODE Chemie (2008) (A7.4.3.4) measured the stock solution for the concentration of the test item. Depending on the results, the individual test solutions were prepared by dilution with dilution water, sampled for chemical analysis and distributed to the test beakers. The stock solution was freshly prepared daily.

During the test duration, the test solutions with nominal concentrations of 0.30, 0.81, 2.19, 5.93, and 16.00 µg a.i./L were analysed at three times (once a week) right after preparation. The same test solutions of the nominal concentrations 0.81, 2.19, 5.93, and 16.00 µg a.i./L were analysed also after 24h of aging with algae. The solution with a nominal concentration of 0.30 µg a.i./L was neglected due to the results of the pre-tests (values < LOQ). All samples were centrifuged before measurement to pellet the algae.

The recovery rate after centrifugation was below the recovery rate in media aged without algae. The ratio between the two recovery rates was calculated. The analytical values were recalculated regarding the mean leakage due to algae centrifugation found in the pre-tests since test item bound on the algae is available for the daphnids.

Due to the strong decrease of concentration during aging, the available time-weighted mean was used as the relevant concentration for biological effects. To consider the bioavailable concentration, it was calculated based on the measured values of the fresh test solutions and the recalculated values of the aged test solutions. Values below the LOQ were set 0.1 µg a.i./L (= 1/2 LOQ) for calculation. The calculation was done in accordance with the equation given in OECD TG 211.

**Table** : Concentrations of the active substance (a.i. = active substance; LOQ = Limit of quantification (0.2 µg/L))

| **Nominal conc.** | **0.30 µg a.i./L** | **0.81 µg a.i./L** | **2.19 µg a.i./L** | **5.93 µg a.i./L** | **16.00 µg a.i./L** |
|---|---|---|---|---|---|
| Mean measured initial conc. | 0.59 (± 0.18) | 1.00 (± 0.24) | 2.43 (± 0.03) | 6.52 (± 0.66) | 16.03 (± 0.75) |
| % of nominal | 195.1 | 122.8 | 111.0 | 109.9 | 100.2 |
| Mean recalculated aged conc. | < LOQ | 0.04 (± 0.06) | 0.79 (± 0.25) | 2.68 (± 0.58) | 7.54 (± 2.67) |
| % of nominal | - | 5.0 | 36.0 | 45.2 | 47.2 |
| Time weighted mean conc. | 0.42 (± 0.07) | 0.58 (± 0.08) | 1.45 (± 0.17) | 4.31 (± 0.58) | 11.18 (± 1.66) |
| % of nominal | 140.9 | 71.0 | 66.2 | 72.7 | 69.9 |

The mean measured concentrations of MES in the freshly prepared test solution (initial concentrations once a week) were between 100 % and 195 % of nominal concentrations. During the time interval until renewal of the test solution, a.s. concentrations decreased considerably to 5 – 47% of nominal at the four highest concentrations (0.81 - 16.00 µg/L nominal). At 0.30 µg/L nominal concentration, no measurements were performed at all. The average time-weighted means of mean measured initial and recalculated mean measured aged concentrations (considering the mean leakage due to algae centrifugation) at test solution renewal were 0.42, 0.58, 1.45, 4.31, and 11.18 µg/L, corresponding with 141, 71, 66, 73, and 70 % of the nominal concentrations.

Concentration related mortality of the adults was observed. The $EC_{10}$ and $EC_{50}$ were estimated at 0.06 and 0.43 µg a.i./L available time-weighted mean (TWM), respectively. The NOEC (mortality) was found to be 0.42 µg a.i./L available TWM.

***Table :*** *Effect summary of the original study report based on concentrations calculated from geometric mean measured concentrations*

| **Concentration** | **Parental survival** | **Growth (length on day 21)** | **Age at 1st brood** | **Cumulative offspring per female** | **Intrinsic rate of increase** |
|---|---|---|---|---|---|
| $EC_{50}$ (95% CL) | 0.43 (0.16 – 1.17) | n.d. (n.d.) | n.d. (n.d.) | n.d. (n.d.) | n.d. (n.d.) |
| $EC_{10}$ (95% CL) | 0.06 (0.01 – 0.64) | n.d. (n.d.) | n.d. (n.d.) | n.d. (n.d.) | n.d. (n.d.) |
| NOEC | 0.42 µg/L | ≥ 11.18 µg/L | ≥ 11.18 µg/L | ≥ 4.31 µg/L | n.d. |

RAC notes that that some of the initial measured concentrations significantly exceeding 100 % of nominal at the start may be caused by analytical challenges related to a poorly soluble substance that forms micelles.

RAC notes, that the total number of living offspring produced per parent animal alive at the end of the test as a test parameter is not reliable, because of high or total mortality of parent animal. RAC further notes that the $EC_{50}$ value is nearly identical with the NOEC value and gives the $EC_{10}$ value more weight for the purpose of classification.

However, RAC recognises some uncertainty concerning the reliability of the $EC_{10}$ due to there being no measured test concentrations close to the $EC_{10}$ and that the dose-response regression was not properly fitted. Given the potential uncertainties surrounding the $EC_{10}$, RAC discussed if it would be more appropriate to discount the $EC_{10}$, resulting in reverting the available NOECs. In

this case, the chronic classification would be based on the fish NOEC, which is an order of magnitude higher than the $EC_{10}$ for invertebrates but is supported by values for invertebrates and algae which are of lower quality albeit in the same range ($0.0001 < NOEC//EC_{10} \leq 0.001$) (Table). However, and despite any shortcomings with the $EC_{10}$ and the regression that produced it, these were not sufficient to discount its use for classification. The NOECs (based on either mean measured or TWAs) may underestimate toxicity and the $EC_{10}$ may represent a more realistic situation.

Overall, RAC concludes that for the OECD TG 211 *Daphnia magna* reproduction test by BODE Chemie (2008) the $EC_{10}$ value of 0.00006 mg/L is of sufficient reliability for classification and provides a realistic toxicity value.

***Conclusion on Acute Aquatic Toxicity***

RAC agrees with the proposal of the DS to base the acute classification of MES on the 72 h $E_rC_{50}$ of 0.0039 mg a.i./L (theoretical time-weighted average concentration obtained from a OECD TG 201 Growth Inhibition Test on algae (BODE Chemie (2000) and retrospectively adjusted by BODE Chemie (2010)).

RAC notes that for fish the calculated theoretical TWA 96 h $LC_{50}$ of 0.048 mg/L from the OECD TG 203 Fish Acute toxicity test by BODE Chemie (1992) is not valid or reliable for the purpose of classification since it clearly represents a best-case value.

For Daphnia the lowest reliable result is taken from a worst case estimation and results in an estimated 48 h $EC_{50}$ between 0.0042 and 0.0091 mg/L for BODE Chemie (2000) (A7.4.1.2), which is in the same range as the acute algae study and supports the acute classification.

RAC agrees with the DS to classify MES as **Aquatic Acute 1, H400** with an **M-factor of 100**.

***Conclusion on Chronic Aquatic Toxicity***

In contrast to the DS's proposal to classify MES based on an algal NOEC of 0.00014 mg/L, RAC concludes to base the chronic classification of MES on the 21 d $EC_{10}$ 0.00006 mg/L (measured time-weighted average concentration) on *Daphnia magna* obtained from the OECD TG 211 *Daphnia magna* reproduction test by BODE Chemie (2008). This $EC_{10}$ is considered reliable for classification and its preferred use is consistent with the Guidance on the application of the CLP criteria.

Based on the conclusion that MES is not rapidly degradable and has a potential to bioaccumulate, RAC concludes to classify MES as **Aquatic Chronic 1, H410** with an **M-factor of 1000**.

## Additional references


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## ANNEXES:

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

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