Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

4,4'-methylenedimorpholine; [MBM]

EC Number: 227-062-3
CAS Number: 5625-90-1

CLH-O-0000001412-86-94/F

Adopted

4 December 2015

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4 December 2015

CLH-O-0000001412-86-94/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), the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonized classification and labelling (CLH) of:

Chemical name: **4,4'-methylenedimorpholine; [MBM]**

EC Number: **227-062-3**

CAS Number: **5625-90-1**

The proposal was submitted by **Austria** and received by RAC on **16 September 2014.**

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

PROCESS FOR ADOPTION OF THE OPINION

Austria 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 **9 December 2014**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **23 January 2015**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Agnes Schulte**

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 harmonized classification and labelling was adopted on **4 December 2015** by **a simple majority of all members present and having the right to vote**.

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	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	**No current Annex VI entry**										
Dossier submitters proposal	607-721-00-5	4,4'-methylenedimorpholine; [MBM]	227-062-3	5625-90-1	Carc.1B Muta. 2 Skin Corr. 1B Skin Sens. 1	H350 H341 H314 H317	GHS08 GHS07 GHS05 Dgr	H350 H341 H314 H317		Skin sens 1 C>1.2%	
RAC opinion	607-721-00-5	4,4'-methylenedimorpholine; [MBM]	227-062-3	5625-90-1	Carc. 1B Muta. 2 Acute Tox. 4 Acute Tox. 4 Acute Tox. 4 STOT RE 2 Skin Corr. 1B Skin Sens. 1	H350 H341 H332 H312 H302 H373 (gastrointestinal tract, respiratory tract) H314 H317	GHS08 GHS07 GHS05 Dgr	H350 H341 H332 H312 H302 H373 (gastrointestinal tract, respiratory tract) H314 H317	EUH071	-	-
Resulting Annex VI entry if agreed by COM	607-721-00-5	4,4'-methylenedimorpholine; [MBM]	227-062-3	5625-90-1	Carc. 1B Muta. 2 Acute Tox. 4 Acute Tox. 4 Acute Tox. 4 STOT RE 2 Skin Corr. 1B Skin Sens. 1	H350 H341 H332 H312 H302 H373 (gastrointestinal tract, respiratory tract) H314 H317	GHS08 GHS07 GHS05 Dgr	H350 H341 H332 H312 H302 H373 (gastrointestinal tract, respiratory tract) H314 H317	EUH071	-	-

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

On contact with biological tissues and media and with dilute aqueous media, 4-(morpholin-4-ylmethyl)morpholine (MBM) hydrolyses to formaldehyde and morpholine. It is assumed that the toxicity of MBM is related to the released formaldehyde. Where data on MBM were not available, data from the hydrolysis products was considered.

The maximum (calculated) 'releasable' formaldehyde per molecule of MBM is 16.7% w/w

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

A single oral OECD technical guideline (TG) 423 compliant study in rats was available. No dermal or inhalation studies were presented. In the oral study, the LD_{50} was between 500 and 2000 mg/kg/d. The DS indicated that the respiratory LC_{50} of formaldehyde and the dermal LD_{50} of formaldehyde may be applied to MBM for classification purposes, but in the end it was concluded by the DS that classification of corrosive substances for acute toxicity is redundant.

Comments received during public consultation

Two MSCA suggested that the classifications for acute toxicity are not covered by the classification on skin corrosion and proposed to refer to formaldehyde. Two MSCA suggested to consider classification as acute toxicity 4.
One MSCA suggested additional labelling with EUH071 and EUH 029.

Assessment and comparison with the classification criteria

Acute oral toxicity

Morpholine
The oral LD_{50} in rats varied between 1050 and 1900 mg/kg bw (limited documentation). In guinea pigs an oral LD_{50} of 900 mg/kg bw was observed. There is a minimum harmonised classification of morpholine as Acute Tox. 4* - H 302 (Harmful if swallowed).

Formaldehyde
Formaldehyde has a minimum classification in CLP, Annex VI for Acute oral toxicity, in Category 3* (H301 – Toxic if swallowed).

MBM
Regarding the OECD TG 423 study on MBM, the DS stated that there is evidence from pathology results in rats which died during the post exposure observation period that toxicity of the undiluted test substance after oral administration is due to local effects on the mucous membranes in the gastro-intestinal tract.

The study summary (cf. MBM_Doc IIIA6_1_1) stated that clinical signs at 500 mg/kg bw were seen in males only (hunched posture, lethargy, ataxia, decreased respiratory rate, and laboured respiration) and 1/3 males died (0/3 females). Clinical signs at 2000 mg/kg bw were hunched posture, lethargy, ataxia, ptosis, pilo-erection, prostration, decreased respiratory rate, noisy

respiration, tiptoe-gait. At 2000 mg/kg bw 3/3 females died (no males tested). Rats which died during the post exposure observation period revealed 'varying degree of mucosal lesions' in the gastro-intestinal tract (stomach and intestine). No effects were detected in survivors.
No effects were observed in rats receiving the diluted test substance (2%) at 200 mg/kg bw (cf. MBM – Doc III A6.1.1).

From the available acute oral study on MBM it remains unclear whether local effects on the gastro-intestinal tract were the cause of deaths as the general health status was severely affected and the information of 'varying degree of mucosal lesions' does not allow a conclusion on the cause of death. In addition, classification for corrosivity does not cover the classification for acute toxic effects. Lethalities (from all possible causal effects) in the relevant dose ranges given by the CLP Regulation have to be considered for classification on acute toxicity.

The observed acute toxicity is in line with the observed range of LD_{50} for morpholine which would support classification as Acute Tox. 4.

This conclusion is supported by the findings from a 14-day range-finding study on MBM (cf. Doc IIIA6_3_1). In this study 1 male rat died after dosing on day 2, 1 male at the end of day 2 and 2 females died on day 3. The application was terminated on day 4 in this dose group. Clinical symptoms at 1000 mg/kg bw/d were similar to those observed in the acute toxicity study (see above). Reddening and haemorrhages of gastric epithelium and limiting ridge, thinning of the non-glandular gastric epithelium, red intestinal content as well as gaseous distension of the GI-tract was reported at this dose. Hunched posture, noisy respiration and increased salivation was also seen at 250 mg/kg bw/d; no mortalities were seen at this dose and at 50 mg/kg bw/d.

Using data from formaldehyde would result in a corrected LD_{50} of 3840 mg/kg bw (based on the LD_{50} of 640 mg/kg bw for formaldehyde from a non-guideline rat study and following a correction by factor of 6 due to the maximum release of 16.7% formaldehyde from MBM). This alone would not justify classification for acute toxicity of MBM based on data from formaldehyde only. However it should be taken into account that formaldehyde is presently classified as Acute Tox. 3 indicating that lower LD_{50} in mice (LD_{50} 42 mg/kg bw) and guinea pigs (LD_{50} 260 mg/kg bw) from non-guideline studies had been taken into account when the decision on classification was taken.

Data from morpholine may also be considered; morpholine is classified as Acute Tox. 4.

In conclusion, an OECD TG 423 (acute toxic class method) study on MBM revealed an acute toxic estimate (ATE) value of $500 < LD_{50} < 2000$ (m&f combined) which is consistent with the acute mortalities seen at 1000 mg/kg bw/d in a 14-day range-finding study in rats. Thus, RAC agrees to classify as **Acute Tox. 4 - H302 (Harmful if swallowed)** according to CLP (oral ATE values for this category are from > 300 to $\leq$ 2000 mg/kg bw).

Acute inhalation toxicity

Morpholine
Symptoms described in an acute inhalation toxicity study were haemorrhage of the nose, mouth and eyes as well as spasm and tremor in rats; the LC_{50} in male and female rats was reported to be ca. 8 mg/L (but there was limited documentation available to RAC). Similar LC_{50} values for mice are available (6.9 mg/L (f) and 5.2 mg/L (m)). Based on these data the available LC_{50} values would not justify classification, however, there is a minimum harmonised classification for morpholine as Acute Tox. 4* - H 332 (Harmful if inhaled).

Formaldehyde
Formaldehyde is classified in CLP, Annex VI for Acute inhalation toxicity, in Category 3 (H331 – Toxic if inhaled).
There are acute inhalation studies (see Formaldehyde Core Document) suggesting that corrosive effects in the upper respiratory tract may contribute (possibly in addition to other effects) to lethality: Histopathological examination revealed excessive mucus secretion, mucociliary dysfunction, single cell necrosis, and discontinuous nasal epithelium with erythrocyte leakage following 4 h exposure of rats to formaldehyde gas at concentrations of 12 µg/L (Bhalla et al., 1991). Higher concentrations (0.6-1.7 mg/L) resulted in haemorrhage and oedema of the lung as

well as oedema in liver and kidneys and hepatocyte necrosis (Skog, 1950). The Formaldehyde Core Document indicates an LC_{50} of 0.6 mg/L (4 h).

MBM
Studies on acute inhalation toxicity were not available on MBM.
RAC considers using data from formaldehyde justified as MBM contains 16.7% releasable formaldehyde. As proposed by two MSCA during public consultation, classification should be considered based on the data leading to the classification of formaldehyde (in Cat. 3) and taking the maximum amount of releasable formaldehyde into account.
The possible contribution of morpholine (classified as Acute Tox. 4*) to acute inhalation toxicity of MBM is unknown.

Acute Tox. Cat 4 is considered justified based on the assumption that the acute inhalation toxicity of MBM would be totally dependent on 16.7% releasable formaldehyde. For MBM a corrected LC_{50} of about 3.6 mg/L (factor of 6 applied on a LC_{50} of 0.6 mg/L (4h) for formaldehyde) would result. For MBM mists, this is consistent with the criteria in the CLP Regulation for classification as Acute Tox. 4 (LC_{50} guidance values >1 and ≤5 mg/L). Thus, RAC agrees to classify MBM as **Acute Tox. 4 - H332 (Harmful if inhaled)**.

EUH071
The supplemental labelling with the hazard statement EUH071 – Corrosive to the respiratory tract - was proposed by one MSCA. If in addition to classification for inhalation toxicity, data are available that indicate that the mechanism of toxicity is corrosivity (CLP, Note 1 in Table 3.1.3), EUH071 could be assigned.

RAC notes that the CLP criteria for EUH071 are not clearly defined. EUH071 can also be applied to inhaled corrosive substances not tested for acute inhalation toxicity. According to CLP Annex II, Section 1.2.6 (which states '*For substances and mixtures in addition to classification for skin corrosivity, if no acute inhalation test data are available and which may be inhaled*') EUH071 may then be appropriate without a corresponding classification for acute inhalation toxicity.

In line with previous RAC recommendations (including those on other formaldehyde releasers) where EUH071 has been assigned in addition to the classification for acute inhalation toxicity and based on the corrosive properties of both hydrolysis products (formaldehyde and morpholine) which, in addition to other possible mechanisms, may have contributed to mortalities, **RAC agrees to assign EUH071 toMBM**.

EUH029
The labelling EUH029 - Contact with water liberates toxic gas - was suggested for consideration by one MSCA. CLP, Annex II, Section 1.2.1 defines substances and mixtures which in contact with water or damp air, evolve gases classified for acute toxicity in category 1, 2 or 3 in potentially dangerous amounts.

RAC emphasises that the liberation of toxic gas after contact with water will not be a major concern as sufficiently high amounts of toxic gas may not be produced immediately. Formaldehyde will also be generated and released without direct contact with water as aqueous conditions arise under normal room air conditions following contact with mucous membranes (of the eye, the respiratory tract and the upper gastrointestinal tract) and in contact with sweaty skin. **RAC agrees that EUH029 is not warranted**.

It is also noted that the CLP Regulation (Annex II, Section 1.2.1) provides for the additional labelling with EUH029 only for substances classified for acute toxicity in category 1,2 or 3 and not for Acute Tox. Cat.4 substances.

Acute dermal toxicity

Morpholine
Acute dermal toxicity studies in the rabbit revealed an LD_{50} of 500 mg/kg bw (non-guideline study, 1954). In Annex VI of the CLP Regulation, there is a harmonised minimum classification for acute toxicity for morpholine as Acute Tox. 4* - H312 (Harmful in contact with skin).

The data could be considered to support classification of morpholine as Acute Tox. 3 – H 311 (Toxic in contact with skin). The applicant presumed that death was caused by the corrosive effects of morpholine (cf. MBM_Doc III App. Morpholine). This conclusion was based on the observation that all of the 7 animals that received a repeated dermal dose of 900 mg/kg morpholine (at 33% in an aqueous solution) died before the 11^{th} dose. In this study the skin was reported to be necrotic, having a thickened oedematous area under the application site; the underlying organs showed inflammation and congestion (Shea, 1939, no further data documented in the MBM_Doc III App. Morpholine).
However, from the acute dermal data (with lack of data indicating local skin effects) and from the repeated dermal data (with lack of data on systemic effects that could have contributed to the deaths) no clear conclusion can be drawn on which effects caused the acute mortalities resulting in the LD_{50} dose of 500 mg/kg.
The corresponding concentration in the acute test on rabbits that received 500 mg/kg bw (4 h) was calculated to be 18.3% (based on the information from the repeated dose study) which is much lower than the testing of pure substances in testing on skin irritation/corrosion and make it more unlikely that corrosive effects were the only cause of the observed deaths in the study on rabbits.

Formaldehyde
Formaldehyde is classified in CLP, Annex VI for Acute dermal toxicity, in Category 3 (H311 – Toxic in contact with skin).

MBM
Studies on acute dermal toxicity were not available on MBM.
RAC considers that for MBM data for morpholine and formaldehyde can be used. Based on the dermal LD_{50} (4 h) for formaldehyde (270 mg/kg bw), which, corrected with a factor of 6 the corresponding LD_{50} for MBM is 1620 mg/kg bw, which is in the range > 1000 mg/kg and <2000 mg/kg for Category 4.

This category corresponds to the current harmonised (minimum) classification of morpholine as Acute Tox. 4*. Based on the dermal LD_{50} of 500 mg/kg bw for morpholine and taking no correction factor for the maximum releasable amount of morpholine into account (since in the presence of water 100 mg MBM will produce 93 mg morpholine), this value would, however, correspond to Acute Tox. 3 (guidance value for Category 3 > 200 and ≤1000 mg/kg). It may be noted that the available dermal studies on formaldehyde and on morpholine were conducted before 1981 and have limitations in comparison with currently available technical guidelines.

RAC agrees that based on the data from formaldehyde and the corrected acute dermal LD_{50} value of 1620 mg/kg bw (based on formaldehyde), and taking into account the harmonised classification of morpholine as Acute Tox. 4 and some uncertainties (based on the available summary information from a study published in 1954) from the acute dermal toxicity study on morpholine, MBM should be classified as **Acute Tox. 4 - H312 (Harmful in contact with skin)** according to CLP (dermal LD_{50} criteria in the CLP Regulation for this category are from 1000 to 2000 mg/kg bw).

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

Summary of the Dossier submitter's proposal

The DS has argued that there is no evidence for effects justifying STOT SE 1 or 2 and that STOT SE 3 - H335 is not appropriate as the substance is corrosive.

Comments received during public consultation

There was no comment that supported classification for STOT SE. One MSCA agreed that no classification for STOT SE3 is required and commented that respiratory irritation is covered by the classification as acutely toxic and/or corrosive.

Assessment and comparison with the classification criteria

Morpholine
For morpholine, there is no entry in Annex VI to the CLP Regulation for STOT SE.

Formaldehyde
For formaldehyde, there is no entry in Annex VI to the CLP Regulation for STOT SE; some notifiers have self-classified for STOT SE.

MBM
Based on the acute toxicity data on MBM there were no effects beyond those covered by the classifications on acute dermal and oral toxicity that would justify STOT SE 1 or 2.

There are no experimental/other data that justify an additional classification as STOT SE 3 (H335) for respiratory tract irritation, and the CLP Guidance 3.8.2.5, states as follows

> *'In general, a classification for corrosivity is considered to implicitly cover the potential to cause RTI and so the additional Category 3 is considered to be superfluous, although it can be assigned at the discretion of the classifier. The Category 3 classification would occur only when more severe effects in the respiratory system are not observed.'*

Based on the CLP criteria, STOT SE 3 should also be considered as covered by the classification as skin corrosive.

RAC agrees with the DS that **no classification on STOT SE is warranted**, and that the potential for respiratory tract irritation is covered by the classification of MBM as corrosive to the skin.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

One OECD TG 404 in rabbits was presented in the CLH report. The study indicated that the test substance is corrosive. The data make subcategorisation difficult but as the option with category 1 without subcategorisation is not currently in the legal text the DS proposed classification as Skin Corr. 1B.

The DS noted that the hydrolysis products of the substance, formaldehyde and morpholine, are also corrosive.

Comments received during public consultation

Two MSCA disagreed with the subcategorisation, however, they apparently were not aware that the the CLP Regulation required subcategorisation.

Assessment and comparison with the classification criteria

Morpholine
Morpholine is classified in Annex VI to the CLP Regulation, as Skin Corr. 1 B, H314 'Causes severe skin burns and eye damage'.

Formaldehyde
Formaldehyde is classified in Annex VI to the CLP Regulation, as Skin Corr. 1 B, H314 'Causes severe skin burns and eye damage'.

MBM
Corrosive effects of undiluted MBM were seen in all of 3 rabbits in an OECD TG 404 study at 24 h after a 4 h exposure. No information is available on effects at shorter exposure times as testing was conducted before 2002 i.e. before the relevant OECD TG was published.

Although testing data with exposure for 3 min and 1 h were not available, and it could not be demonstrated whether skin necrosis would have developed after shorter exposure time than after 4 h, RAC propose that data from the hydrolysis products formaldehyde and morpholine should be applied for MBM.
The observation that necrosis was noted after 24 h after the end of exposure to MBM does not exclude the possibility that necrosis could also occur during the post-exposure observation period after exposure for ≤ 1 h.

RAC took note of the difficulties identified by the DS and MSCA concerning on the selection of the appropriate a subcategory. As subcategorisation is required based on the CLP Regulation, RAC agrees to refer to the data for formaldehyde and morpholine which both are classified as Skin Corr. 1B – H 314 'Causes severe skin burns and eye damage'. The same classification is warranted for MBM**. RAC thus agrees that Skin Corr. 1B – H314 (Causes severe skin burns and eye damage) is warranted.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier submitter's proposal

No data were available, but the DS concluded that classification as skin corrosive also covers eye effects.

Comments received during public consultation

One MSCA proposed classification for serious eye damage (Eye Dam.1) but no labelling as explained in CLP Guidance, Chapter 3.3.2.4.

Assessment and comparison with the classification criteria

Morpholine
For morpholine the labelling 'H314 – Causes severe skin burns and eye damage' covers the potential for eye damage. There is no separate classification for eye damage in Annex VI of the CLP Regulation, but many notifiers have self-classified the substance as Eye Dam. 1.

Formaldehyde
There is no Annex VI entry on a separate classification for eye irritation/damage on formaldehyde, however the majority of notifiers have self-classified the substance as Eye Dam. 1.
The Formaldehyde Core Dossier summarises that although no guideline-conforming testing has been conducted, testing on dilutions (up to 15%) indicated severe irreversible eye damage that would justify the classification as Eye Dam. 1.

Due to specific concentration limits assigned to the existing Annex VI entry, mixtures containing formaldehyde at concentrations within the range 5% ≤ C < 25% are classified as Eye Irrit. 2; H319.

In humans, indications of eye irritation such as increased eye blink frequency and conjunctival redness were seen from gaseous concentrations of 600 µg/m³ (WHO 2010).

MBM

With regard to the comment from one MSCA, CLP Guidance is not clear with regards to a separate classification for corrosive effects on the eye.

CLP Guidance stipulates in Section 3.3.2.4:

> *A skin corrosive substance is considered to also cause serious eye damage which is indicated in the hazard statement for skin corrosion (H314: Causes severe skin burns and eye damage). Thus, in this case both classifications (Skin Corr. 1 and Eye Dam. 1) are required but the hazard statement H318 'Causes serious eye damage' is not indicated on the label because of redundancy (CLP Article 27).*

However, the first sentence of CLP Guidance, Section 3.3 recommends:

> *It should be noted that if a substance or mixture is classified as Skin corrosive Category 1 then serious damage to eyes is implicit and there is no need to proceed with classification for eye effects.*

In line with previous decisions where a separate classification on eye damage may be considered if separate studies on eye effects are available and had demonstrated irreversible eye damage, **no classification for irreversible eye effects is warranted for MBM**. Studies on eye irritation are not available for MBM. The classification of MBM as Skin Corr. 1B coupled with the labelling with H314 (Causes severe skin burns and eye damage) covers corrosive effects on the eyes.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

There was one inconclusive OECD TG 406 (GPMT) study summarised in the CLH report and no human data were available. The DS proposed either classification based on the calculated release of formaldehyde by the substance or no classification based on the amount of free formaldehyde. An SCL was calculated based on the SCL for formaldehyde and the fraction of the substance that could be released as formaldehyde.

Comments received during public consultation

Four MSCA supported the classification. However, the proposed SCL was questioned and the DS agreed in the response to MSCA comments that the SCL should be removed. Industry disputed the classification proposal claiming that there is no data on the substance supporting classification.

Assessment and comparison with the classification criteria

Morpholine
No sensitising effect was observed in a Buehler assay with invalid test design.

Formaldehyde
The existing classification of the hydrolysis product formaldehyde in Annex VI is Skin Sens. 1; H317 with a specific concentration limit of ≥0.2%.

MBM
A GPMT test (OECD TG 406) on MBM revealed 3/20 responders after challenge with 5%. MBM. A positive rate below 30% would normally not justify classification, however the test was judged as inconclusive as only one very low concentration (0.1%) was tested for intradermal induction. The study authors did not consider the effects as indication of hypersensitivity as the topical induction concentrations up to 10% MBM did not cause irritation. No indication of slight irritation (no erythema or oedema) appeared at topical (challenge) concentrations of 1% MBM in any of the 30 animals in the main study. This was in contrast to the observation that 1/6 animals at 1% and 5% MBM had grade 1 erythema in the preliminary studies. Irritant effects were seen in 5/6 animals at 10% in the pilot study, but this concentration was not tested in the main study as a challenge concentration.

The DS highlighted that the dermal absorption rate of MBM was estimated at 60-70%, which is similar to that of formaldehyde (40-65%).
RAC is of the view that there ispotential for formaldehyde to be produced at the skin surface after hydrolysis in contact with (sweaty) skin and this will then be absorbed, or formaldehyde may be produced following dermal absorption of MBM. Read across to formaldehyde (without subcategorisation) and classification of MBM as **Skin Sens. 1; H317 (May cause an allergic skin reaction) is proposed by RAC**.

The initial proposal of the DS to correct for the amount of releasable formaldehyde is also considered. A correction of the SCL of formaldehyde with a factor of 6 would result in a SCL of 1.2% for MBM. This is in the range of the general concentration limit and instead of calculating a theoretical SCL **RAC proposes** (in agreement with the DS) **to apply the generic concentration limit for a Category 1 sensitiser**.

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

Summary of the Dossier submitter's proposal

One OECD TG 408 and one 14-day non-guideline study were presented. In both studies there where effects in the fore-stomach and in the glandular stomach. The effects were seen at doses that could warrant classification as STOT RE 2, but the DS concluded that the effects were due to the corrosive action of the substance and thus suggested no classification.

Comments received during public consultation

Two MSCA suggested that classification for STOT RE is warranted.

Assessment and comparison with the classification criteria

Oral route
Morpholine

Repeated oral exposure to MOAS (morpholine oleic acid salt; 0.15%, 0.3%, 0.6%, 1.25% and 2.5% MOAS in drinking water daily *ad libitum*) in sub-chronic studies in mice (OECD TG 409) corresponded in males to 77, 154, 256, 436 and 795 mg/kg bw/d morpholine, respectively, and in females to 77, 128, 205, 410 and 667 mg/kg bw/d morpholine, respectively. Treatment resulted in lower body weight gain (at the highest dose), increased blood urea nitrogen and increased specific gravity of urine (≥0.6% MOAS), increased renal weight (≥1.25% MOAS), and cloudy swelling of proximal renal tubules (at the highest dose). The study authors concluded that MOAS produced a mild toxic nephrosis, while the applicant considered the kidney effects to have been related to decreased water consumption. A decrease in water consumption (that was not

reported in the study summary) may explain the higher specific gravidity, but is unlikely to be responsible for the increased blood urea nitrogen. This effect indicates damage to the renal tubuli.

Although there is evidence on kidney toxicity by MOAS, the effect levels were clearly above the guidance level for classification as STOT RE.

In a chronic drinking water study on MOAS with mice (96-weeks followed by a post-observation period of 8 weeks), a lower body weight gain was observed in females at ≥ 0.06% (128 mg/kg bw/d morpholine) and in males at 0.25% (385 mg/kg bw/d), however the influence of reduced water consumption is unclear. Morpholine concentrations of 0.25% (385 mg/kg bw/d) induced local forestomach effects and blood urea nitrogen increase in male mice.
Some evidence on kidney toxicity was confirmed, however the dose range is not relevant for classification. The examination at week 8 after the end of treatment limits the reliability of the study.

Formaldehyde
There is no harmonised classification on formaldehyde for STOT RE.
Lesions related to the irritancy in the stomach are - similar to MBM - the main effects after repeated oral administration of formaldehyde. However, available studies suggest that the lesions were seen at comparatively higher doses or occurred with lower severity grades.

After 12 months exposure to 300 mg/kg bw/d, forestomach squamous cell hyperplasia/hyperkeratosis, glandular hyperplasia and erosion/ulceration of the glandular stomach were seen (Tobe et al. 1989, formaldehyde core Doc III A6.3.1). No local effects in the gastrointestinal tract were observed in a 90-day study in rats receiving drinking water with formaldehyde up to concentrations of 1000 mg/L (150 mg/kg bw/d) (Johannsen et al., 1986). A 4-week oral study on rats (Til et al., 1988, formaldehyde core Doc III.A6.3.1) receiving 0, 5, 25 or 125 mg/kg bw/d with drinking water revealed at 125 mg/kg bw/d very slight to moderate hyperkeratosis of the forestomach (all animals) and very slight to moderate gastritis (3/10 males, 5/10 females) of the glandular stomach. A focal papillomatous hyperplasia was observed in one female. None of the available studies conducted were fully compliant with the relevant guidelines.

MBM
A 14-day range-finding test on MBM (cf. Doc IIIA6_3_1) revealed acute mortalities at 1000 mg/kg bw/d until day 3 of treatment that were considered for the assessment of acute toxicity. At 250 mg/kg bw/d no mortalities occurred and clinical findings were
hunched posture, noisy respiration and increased salivation. Other findings (also at the lower dose of 50 mg/kg bw/d) were increased neutrophils, thickening of the non-glandular stomach, pale kidneys and increased kidney weights in males and females. There were no data on histopathology (note:the kidney was also a target organ in subchronic studies on morpholine in mice).
After correction for the study duration the adverse effects at 50 mg/kg and 250 mg/kg (corresponding to 25 and 125 mg/kg bw/d for a 28-day test design) are supportive for classification as STOT RE 2.

In a subchronic gavage study consistent with OECD TG 408 in rats receiving 0, 5, 15, 50 or 250/150 mg/kg bw/d (cf. MBM-Doc III A6.4.1) hunched posture and noisy respiration were observed in some rats at 50 mg/kg; 1 male died at day 29. Physical condition was severely deteriorated in rats at 250 mg/kg, but reducing the dose to 150 mg/kg bw/d did not result in noticeable improvements. Six females and 4 males of this dose group died between day 13 and day 90. A decrease in body weight was noted in decedents prior to death. A significantly increased urine volume was noted in this group, while no effect was seen on water consumption.
From 50 mg/kg bw/d onwards lesions were detected mainly in the fore-stomach but also in the glandular stomach. Acanthosis, hyperkeratosis and inflammation in the forestomach occurred in males and females at ≥ 50 mg/kg bw/d; males seem to be less susceptible at 50 mg/kg bw than females. At 250/150 mg/kg bw/d ulceration of the stomach was observed in 6/6 surviving male rats and in 3/4 decedent males and 1/6 decedent female. A few males and females of the high dose group revealed also effects in the larynx (hyperkeratosis, hyperplasia and inflammation) and oesophagus (inflammation). This inflammation reaction was not considered by the authors to be

treatment related but there is some indication for such local effects. Minimal lymphoid atrophy of the thymus, mesenteric lymph nodes and spleen were observed in some females and males among the unscheduled deaths.
With a tendency for higher incidences with dose, inflammation, necrosis, fibrosis, ulceration and epithelial hyperplasia of the nasopharynx was observed in a number of animals from 15 mg/kg bw/d and above. Although such effects should normally not occur in a gavage study it was explained as being related to accidental application during dosing or related to the gavage dosing.
The observed delayed mortalities and lesions on the gastrointestinal tract (including larynx and oesophagus) at 50 mg/kg bw/d and above are considered to warrant classification (although this is a borderline case). The effects were most prominent at 250/150 mg/kg bw/d, which is above the guidance value of 100 mg/kg bw/d (see Table 3.9.2-a of the CLP Guidance), but started at 50 mg/kg bw/d (including one delayed mortality). No data are available on the dose range > 50 mg/kg and ≤ 100 mg/kg bw/d.

The biocide applicant considered the observed effects as related to exposure to the hydrolysis products formaldehyde and morpholine.

The CLP Guidance does not suggest that effects on the tissues along the administration routes resulting from repeated exposures are covered by classification for corrosion, while it gives some recommendation concerning Annex I 3.9.1.6, when STOT SE might be more appropriate than STOT RE:

> *Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. In such a case classification with STOT-SE only would be appropriate.*

In addition, CLP Guidance, Section 3.9.2.5.1 gives guidance on the doses, as follows:

> *If the dose is more than half an order of magnitude lower than that mediating the evident acute toxicity (corrosivity) then it could be considered to be a repeated-dose effect distinct from the acute toxicity.*

The dose at which the effects in the gastrointestinal tract occurred in the 90-day study was lower than the oral acute toxic doses (1/3 males and 0/3 females died at 500 mg/kg, 3/3 females died at 2000 mg/kg). Local effects in the stomach of varying degree were observed in the oral acute toxicity study (test substance was undiluted, no further information available on any dose-response relationship of the lesions). RAC, in line with comments during the public consultation from two MSCA does not agree with the view of the DS that the local irritant effects are mechanistically sufficiently addressed with the classification for corrosion and should not support the classification for STOT RE.

The delayed mortalities (day 13 – 90) and the toxic effects in the gastrointestinal tract are considered as chronic toxic effects that resulted from prolonged/repeated exposure to low concentrations/doses of MBM. The effects are considered as reflecting repeated exposure toxicity and not just acute toxicity. Because they occurred within the range of guidance values (CLP Guidance, Table 3.9.2-a, ≤100 mg/kg bw/d for an oral 90-day study) and the effective dose is considerably lower than the acutely toxic dose, it should be classified for STOT RE. Local effects in the gastrointestinal tract (such as chronic oesophagitis, gastritis) after repeated/prolonged exposure are toxicogically relevant as they impair not only the morphology and/or function of the locally targeted organ, but also bear the potential to impair adherent tissues/organs by transmural extension of the chronic inflammation (e.g. peritonitis, pleuritis) or to cause delayed mortalities (after ulceration into body cavities). Thus, RAC agrees to classify MBM as STOT RE 2, H 373 - May cause damage to (gastrointestinal tract) through prolonged or repeated exposure.

Dermal route

Morpholine
No guideline-conforming repeated dose study using the dermal route is available. Only limited information is available from a study published in 1939 describing repeated dermal exposure of

rabbits to morpholine diluted with 2 parts of water (33% solution) at a dose level of 900 mg/kg bw/d, which resulted in death of all 7 animals before the 11th dose (cf. MBM_Doc III App. Morpholine) . It is stated that the skin was necrotic, having a thickened oedematous area under the application site; the underlying organs showed inflammation and congestionIn contrast, only a thickening of the skin, but no relevant effects, were detected with morpholine (using sulphuric acid for neutralization) applied to 3 guinea pigs after 30 daily applications (Shea, 1939)..

With regards to systemic toxicity after repeated dermal exposure, no information is given from these early studies.

Formaldehyde
No valid dermal repeated dose study seems to be available (see core document on formaldehyde). There are several long-term studies with an unusual treatment regimen (twice weekly for 60 wks, thrice weekly for 26 wks, 2-3 weeks with documentation on the application frequency) on formaldehyde at concentrations of 0.1% to 10% that revealed mild to moderate irritation from concentrations of 0.5% onwards. Whether systemic effects were examined in these studies, is neither documented in the CLH report nor in the core document on formaldehyde.

MBM
No repeated dose study using the dermal route is available.

Taking the data from formaldehyde into account and the fact that reliable studies on MBM (and morpholine) are not available, the overall database is not sufficient to conclude on classification for STOT RE for this route.

Inhalation route

Morpholine
Irritation of the eyes and nasal cavity: nasal cavity with infiltrates, metaplasia and necrosis, but no systemic effects were observed in a 104 week study in rats exposed to 181 mg/m³ morpholine and in rats exposed to 900 mg/m³ for 7 or 13 weeks (MBM_Doc III App. Morpholine). Considering the exposure duration and the effect levels (in comparison to Table 3.9.2-a in CLP Guidance) no classification for morpholine is warranted.

Formaldehyde
Due to the lack of data on MBM, data on formaldehyde were assessed for STOT RE:

Classification on effects from repeated inhalation exposure may be considered if doses are much lower than those that induce acute irritant or corrosive effects.

As explained for the oral route, CLP Guidance does not say that effects on tissues along the administration routes resulting from repeated exposures are covered by classification for skin corrosion, while it gives some recommendation in Annex I 3.9.1.6, when STOT SE might be more appropriate than STOT RE:

> *Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. In such a case classification with STOT-SE only would be appropriate.*

In addition, Section 3.9.2.5.1 gives guidance on the relevant doses

> *Substances (or mixtures) classified as corrosive may cause severe toxicological effects following repeated exposure, especially in the lungs following inhalation exposure. In such cases, it has to be evaluated whether the severe effect is a reflection of true repeated exposure toxicity or whether it is in fact just acute toxicity (i.e. corrosivity). One way to distinguish between these possibilities is to consider the dose level which causes the toxicity. If the dose is more than half an order of magnitude lower than that mediating the*

evident acute toxicity (corrosivity) then it could be considered to be a repeated-dose effect distinct from the acute toxicity.

In short, if doses are considerably lower than those being acute toxic/irritant and these low doses induce serious health damage after repeated inhalation with accumulation/ exacerbation of repeated insult, classification for STOT RE should be considered.

For formaldehyde, the acute inhalation LC_{50} was reported to be 0.6 mg/L (600 mg/m³) by Nagorny et al. (1979) (see Formaldehyde Core Document II, Table 3-2). Taking the AEC of 0.12 mg/m³ from human data into account, the surrogate effect for repeated inhalation toxicity occurs at 5000-fold concentrations below the acute toxic dose, thus indicating that a classification for repeated inhalation effects is warranted.

There are no human data that examined chronic non-neoplastic lesions in the respiratory tract in humans under controlled exposure conditions. Instead, existing limit values were derived from surrogate data on sensory irritation effects on eyes, nose and throat as this effect is considered as the most sensitive adverse (non-neoplastic) effect. SCCS (2014) in their evaluation considered eye irritation as the most sensitive effect:

Eye irritation was revealed as most sensitive adverse endpoint. In susceptible individuals, slight discomfort due to eye irritation occurred at 0.25 ppm but dose-dependent increases in eye irritation were not observed below 1 ppm.Objective ratings for eye irritation (conjunctival redness and eye blinking frequency) have been investigated in healthy volunteers and a NOAEL of 0.5 ppm (without exposure peaks) and 0.3 ppm (with exposure peaks of 0.6 ppm) was established.

However data on sensory irritation can not be used to decide on classification for chronic toxic effects.

Repeated inhalation studies in animals reported dose-dependent non-neoplastic lesions in the nasal cavity that increased in severity and extent with exposure time and dose (for review see SCCS, 2014; BfR, 2006). Following inhalation exposure up to 24 months, squamous metaplasia was observed in rats at 6 ppm formaldehyde. Epithelial hypertrophy, hyperplasia and metaplasia, mixed inflammatory cell infiltrates and turbinate adhesions were seen at 10 ppm; in addition destruction of turbinate architecture occurred at 15 ppm (Monticello et al., 1996, cited from BfR, 2006). While lesions of the respiratory epithelium in the nasal cavity were not reported after 6 weeks exposure up to 2 ppm (Monticello et al., 1991; Formaldehyde Core document IIIA), inhalation exposure of ≥12 months to ≥2 ppm (2.456 mg/m³) formaldehyde caused purulent rhinitis, epithelial dysplasia and squamous metaplasia at level I of the nasal cavity (Kerns et al., 1983 a, b, cited from BfR, 2006). At higher concentrations than 2 ppm, lesions extended to more posterior parts of the nose (level I to III) and reached the trachea at 14.3 ppm. Monticello (1989, cited from RAC Opinion on formaldehyde) has demonstrated that inhalation of 6 ppm formaldehyde for 1 or 6 weeks induced loss of cilia, inflammatory response, epithelial hyperplasia and squamous metaplasia and increased cell proliferation in the nasal passages of rhesus monkeys. Like in rats, lesions in monkeys showed an anterior-posterior gradient and a duration-related increase in severity and extent of lesions, but these were more widespread than in rats. Inhalation of 3 ppm formaldehyde over 26 weeks induced squamous metaplasia and hyperplasia in the nasoturbinates in 6/6 Rhesus monkeys, but no effects were observed at 0.2 and 1 ppm (Rusch et al., 1983, see SCCS, 2014).
Taking 2 ppm formaldehyde as a robust LOAEC for chronic inflammatory and meta/hyperplastic lesions secondary to initial cytotoxicity in the nasal mucosa from repeated/prolonged inhalation and using the standard Haber's rule extrapolation from 12-month to 90-day exposure to compare with the guidance values, 2 ppm for 12 months corresponds to 8 ppm (9.824 mg/m³ = 0.01 mg/L) after 90 days. This is clearly below the guidance concentration for gases of 50 ppm and would justify a classification of formaldehyde as STOT RE 1.

MBM
No repeated dose study using the inhalation route is available.

The DS suggested read across to the hydrolysis product formaldehyde on which a local inhalative AEC of 0.12 mg/mg³ was based on human data on eye irritation.

Referring to the CLP Regulation Section 3.9.2.10.3, RAC agrees with the DS on that data from formaldehyde may be used, as data on repeated inhalation toxicity of MBM are lacking. However RAC does not agree that effects from repeated inhalation are covered by the classification for corrosion.

The absence of an entry on formaldehyde for STOT RE in CLP, Annex VI does not by itself justify non-classification for MBM.

It was noted in the CLH report that MBM contains about 16.7% releasable formaldehyde. Assuming that under prolonged inhalation exposure conditions MBM would continuously release the maximal releasable amount of MBM%, a factor of 6 should be applied to correct for the lower content of formaldehyde. As the human AEC was based on eye irritation, an acute receptor-mediated sensory irritation effect (without obvious cytotoxicity and infiltration of inflammatory cells) as surrogate for the lowest adverse effect in humans, animal data on repeated inhalation toxicity may be more appropriate to conclude on the classification for STOT RE.

For MBM, the LOAEC for repeated inhalation exposure is based on the LOAEC of 2 ppm for formaldehyde (2.456 mg/m³, derived from a rat 12-month study; Kerns et al., 1983 a,b) (corresponding to 8 ppm (9.824 mg/m³ = 0.01 mg/L)/90-day inhalation based on Haber's rule), corrected for the maximal amount of releasable formaldehyde (16.7%) from MBM with a factor of 6 and reveals a (corrected) concentration of 0.06 mg/L for MBM which is below the guidance value (for mists) for STOT RE 2 (≤0.2 mg/L). As inhalation exposure to the aerosol is expected to be the main concern for MBM, the guidance values for the gaseous form were not considered.

If the chronic toxicity occurred at the same dose level as the acute inhalation toxicity, chronic toxicity would be covered by the classification for acute toxicity. The inhalative LC_{50} was unknown for MBM as no acute inhalation study is available. As a substitute, information on the difference between the level of the inhalation LC_{50} and the LOAEC for chronic effects for formaldehyde was considered. The Formaldehyde Core Document indicates an LC_{50} of 0.6 mg/L (4 h) which is markedly higher than the LOAEC for chronic effects (2 ppm = 2.456 mg/m³). Thus the acute toxicity classification does not cover the classification for STOT RE.

Repeated inhalation exposure to MBM generates the hydrolysis products formaldehyde and morpholine. That morpholine may exert additive effects to those expected from formaldehyde may be expected (as repeated inhalation induced irritant effects to the respiratory tract), however its quantitative impact remains unknown.

Based on the data on formaldehyde (see above), RAC agrees to classify MBM with regards to target organ toxicity from repeated inhalation as **STOT RE 2**.

All routes/Overall classification on STOT RE
If classification for STOT RE is proposed based on data from several routes with different target organs, the final labelling should consider all the relevant target organs. RAC agrees that classification of MBM is warranted as **STOT RE 2, H373: (May cause damage to the respiratory tract and the gastrointestinal tract through prolonged or repeated exposure)**. No specific route should be indicated.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

The DS proposed to classify MBM as a Caterory 2 mutagen based on the existing classification of its hydrolysis product formaldehyde.
There are several mutagenicity studies *in vitro* and *in vivo* for MBM that were considered valid. Predominantly clastogenic effects were induced in cells of mammalian cell cultures with and

without metabolic activation; a bacterial gene mutation test was weakly positive only with metabolic activation. Regarding the *in vivo* testing, a negative micronucleus test and a negative UDS test were available.

The DS additionally provided information on negative results of *in vitro* mutagenicity tests and *in vivo* genotoxicity tests for the hydrolysis product morpholine.

The DS argued that due to the hydrolysis of MBM to fromaldehyde at contact to biological tissues, induction of local genotoxic effects is to be expected at site of first contact *in vivo*. Therefore the DS refered to the existing classification of formaldehyde as a Category 2 mutagen based on the induction of genotoxic effects *in vivo* on somatic cells at site of contact which are supported by positive results in numerous *in vitro* mutagenicity and genotoxicity tests.

Due to the mechanistic considerations of formaldehyde release from MBM the applicant proposes to classify the substance MBM as a Category 2 mutagen on the basis of its hydrolysis product formaldehyde.

Comments received during public consultation

Two Member States expressed their support for the proposed classification. One individual comment disagreed with the proposed classification as a Category 2 mutagen due to the lack of relevant mutagenicity data.

Assessment and comparison with the classification criteria

Formaldehyde
RAC agrees with the approach of the applicant to take into account the classification of formaldehyde as Category 2 mutagen for justification of the classification of MBM.

Formaldehyde that is released from MBM at contact with biological tissues is classified as a Category 2 mutagen based on the induction of genotoxic effects *in vivo* on somatic cells at the site of contact which are supported by positive results in numerous *in vitro* mutagenicity and genotoxicity tests. Although it seems likely that the amount of formaldehyde may vary depending on different uses, the inherent potential of MBM to release formaldehyde is a critical fact.

Testing of the *in vitro* mutagenicity of MBM shows that the observed positive effects are consistent with those known from formaldehyde alone. It is assumed that MBM has a low systemic availability *in vivo* due to its hydrolysis. Therefore it seems to be unlikely that genotoxic effects are induced at a site distant from first contact.

Information on the hydrolysis product formaldehyde was used to assess the mutagenic potential of MBM.

Morpholine
RAC takes note of the additional information by the applicant that no indication for *in vitro* mutagenicity and *in vivo* genotoxicity of the hydrolysis product morpholine has been detected in available *in vitro/in vivo* studies and no relevant structural alerts are present.

MBM
The evaluation of the mutagenicity data of MBM by the DS and RAC do are in agreement. RAC also comes to the conclusion that a proposal for classification of MBM as a Category 2 mutagen is justified.

In vitro data
The available bacterial gene mutation test is weakly positive with S9-mix (Lubrizol (MBM-Doc III A6.6.1), 2000).

A mouse lymphoma assay (Lubrizol (cf. MBM Doc III A6.6.3), 2001) is positive with and without S9-mix. At the analysis of the colony sizes, predominantly small colonies were found, which indicates clastogenic activity of MBM.
A chromosomal aberration test is positive in CHL cells with and without S9-mix (Lubrizol Corporation (cf. MBM Doc III A6.6.2), 2001).

In vivo data
An *in vivo* mouse bone morrow micronucleus is negative after single oral application up to the highest tested dose of 1000 mg/kg bw (Lubrizol (MBM Doc III A6.6.5), 2001). It was reported that the highest tested dose induces cytotoxic effects; necropsy data were not available.
An *in vivo* UDS test with rats is negative after single oral application of 300 and 900 mg/kg bw (Lubrizol (MBM Doc III A6.6.5), 2002). After the application of the highest tested dose clinical signs were observed; necropsy data were not available.

The quantity of test data for MBM is limited and the mutagenicity studies are not published. Thus, only the data given by the DS are available. These data allow neither a detailed test evaluation nor do they allow to conclude on whether a test performance is fully in accordance with the corresponding guideline. Despite these limitations, the following conclusion can be drawn: In bacteria as well as in soma cell cultures mutagenic effects are induced. The results of an *in vivo* micronucleus test as well as an *in vivo* UDS test are negative.

RAC considered that due to its reactivity, a low systemic availability is expected for MBM and therefore the induction of systemic genotoxic effects is unlikely. However, a local genotoxic effect produced by the hydrolysis product formaldehyde is expected and RAC consideres that use of data from formaldehyde, which is classified as a mutagen Cat. 2 based on its local genotoxic action, is justified. For information regarding the induction of local effects at the sites of contact after repeated oral dosing (gavage) or repeated inhalation of MBM see point '4.7. Repeated dose toxicity' of the CLH report.

Some RAC members expressed their view that the guidance relates only to classification of substances that caused germ cell mutations. This view is reflected in a minority position supported by three RAC members. RAC recognised that according to CLP Guidance, Section 3.5.1, classification is also warranted if there is evidence of only somatic cell genotoxicity that leads to classification in Category 2 if genotoxic substances are only acting locally.

RAC agrees with the proposal of the DS to classify MBM as a Mutagen, Category 2 H341 (Suspected of causing genetic defects) based on relevant data from'its hydrolysis product formaldehyde.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

No cancer bioassay or human data were available for the substance. The DS proposed classification for carcinogenicity based on data for formaldehyde.

Comments received during public consultation

Four MSCA supported the classification for carcinogenicity. Industry argued that classification is not warranted.

Assessment and comparison with the classification criteria

Morpholine
No carcinogenic effect was observed in a combined chronic toxicity and carcinogenicity drinking water study (reported as comparable to OECD TG 453, only two test doses) in mice on MOAS (morpholine oleic acid salt). Shibata et al.(1987a) (see MBM_Doc III App. Morpholine) exposed 50

male and 50 female B6C3F1 mice to MOAS for 96 weeks followed by a post-observation period of 8 weeks via the drinking water containing 0, 0.25% and 1.0% MOAS (equivalent to 0, 400 and 1500 mg/kg bw/d, respectively, in males and 0, 500 and 1500 mg/kg bw/d, respectively, in females; 0.25% and 1% MOAS corresponded to 0.06% and 0.24% morpholine). In high dose mice, reduced body weight gain, water intake, increased blood urea nitrogen (males only) and increased incidences of squamous hyperplasia of the forestomach) were observed. At the low dose, lower body weight gain was seen in female mice.

In a 104-week inhalation study in Sprague-Dawley rats (57-60 animals/sex/group), the animals were exposed to 0, 10, 50, 150 ppm morpholine vapour (0, 36, 181, 543 mg/m³, respectively), 6 h/d, 5 d/wk (Harbison et al, 1989, see App. Morpholine). Local irritation of eyes and nares, chromodacryorrhea and urine stains of the fur were observed at 150 ppm. Similar effects in a few mid dose males and females were reported in the study summary and it was noted that no details were given and the reported incidence was found questionable. The RMS added that the grossly observed effects exhibited as localized sores, bloody crust about the eyes, nose, face and body, localized necrosis (skin) and chromodacryorrhea and urine stains at the 181 and 543 mg/mm³ doses. A concentration-related increase in necrosis, infiltration of neutrophils and metaplasia of the nasal turbinates were observed at ≥50 mg/m³. No indication of systemic chronic toxicity or carcinogenicity was detected.

Formaldehyde
The hydrolysis product formaldehyde is classified in CLP, Annex VI for carcinogenicity, Cat 1B.

MBM
There are no long-term/carcinogenicity studies on MBM available.
Human data on MBM are not available (except a summary on a medical data letter report on medical screening results from three workers in the production area).

The non-submission of data was justified by a read across to formaldehyde and probable carcinogenic effects of MBM were considered by the applicant to be related to the hydrolysis product formaldehyde (Doc III A6.7 MBM non sub doc).

RAC considers read-across to the hydrolysis products of MBM justified.
Based on the study summary available (and the identifiable weaknesses of the studies) there was no indication of a tumour response for morpholine from long-term oral studies in mice and from long-term inhalation studies in rats. Carcinogenic potential cannot be totally excluded based on these studies; the squamous hyperplasia of the forestomach seen in oral long-term studies in mice may indicate potential for these to develop into tumours.

Although no concern for a carcinogenic effect has been identified from the available long-tem studies, the effects of morpholine at the site of contact seem to be similar to those of formaldehyde (cytotoxicity, inflammation, metaplasia, hyperplasia in the gastrointestinal tract and the respiratory tract). No information is given on a possible additive contribution to the carcinogenic potential that is derived from the hydrolysis product formaldehyde.

The DS considered that the equilibrium of MBM and formaldehyde shifts towards formaldehyde by dilution and by the reaction of formaldehyde with biological media. The formaldehyde release is – in qualitative terms- supported by the hydrolysis study, the intratracheal kinetic study, which indicated that formaldehyde is rapidly released, and by the effects at the site of contact observed after repeated oral and inhalation exposure.

The hydrolysis products formaldehyde and morpholine are unlikely to induce systemic genotoxicity or carcinogenicity as demonstrated by respective carcinogenicity studies and negative *in vivo* genotoxicity tests. It is therefore assumed for MBM that, similar to formaldehyde, systemically increased bioavailability and concern for systemic carcinogenic responses are not to be expected.

It is expected that MBM exerts similar effects as formaldehyde such as cytotoxicity, hyperplasia, metaplasia, tumours and local mutagenic effects at the sites of contact – i.e on the epithelium of

the respiratory tract, following prolonged inhalation, since formaldehyde is one of the hydrolysis products from MBM.

Formaldehyde is classified based on its carcinogenic potential at the sites of exposure, primarily on the nasopharyngeal tumours observed in man and rodents after prolonged inhalation[1].

CLP Guidance, Section 3.6.2.2.7 states

> '*A substance that has not been tested for carcinogenicity may in certain instances be classified in Category 1A, Category 1B or Category 2 based on tumour data from a structural analogue together with substantial support from consideration of other important factors such as formation of common significant metabolites, e.g. for benzidine congener dyes.*'

CLP Guidance (Section 1.4.3) explicitly foresees the read across of information from 'source' substances to predict the same hazard for another 'target' substance. For MBM, it is not about the similarity of source and target substance, but MBM should be classified as a carcinogen based on the release of the *identical* substance (formaldehyde) resulting from hydrolytic transformation of MBM.

Endpoints, on which data on MBM are available, show that the similiarity of effects at the site of contact support the use of data from formaldehyde as justified. Similar effects were noted e.g. for the oral repeated toxicity, with the observation that the toxicity may be more severe for MBM when comparing the dose levels or the severity of effects observed with formaldehyde. However uncertainties remain as to the lack of carcinogenicity studies on MBM with full guideline compliance and as to an additional (unknown) contribution of the other hydrolysis product morpholine to the effects by formaldehyde. The kidney was a target organ by repeated exposure to morpholine; the findings in the 14-day range-finding study of pale kidneys and increased kidney weight provide some hint of systemic effects of MBM exposure that may be attributable to absorbed morpholine.

From a quantitative aspect, the hydrolysis rate of MBM to formaldehyde may depend on several environmental factors (temperature, increases at lower pH, and at higher dilutions with aqueous media). Due to a rapid rate of hydrolysis it was not possible to detect MBM at the beginning of measurement and after 2.4 h for the tested pH-levels (37°C for pH 1.2, and at 50°C for pH 4, 7 and 9) (see MBM Doc III A7.1.1.1.1). MBM hydrolysed so quickly that the exact hydrolysis half-life could not be estimated (less than 2.4 h for 50°C). In the view of the RMS, a significantly lower hydrolysis half-life than 1 day at 25°C can be concluded but not quantified based on the present data. The rate may be assumed to be in the range of hours/ minutes.

However, water contact or dilution of MBM with aqueous solutions are not a necessary condition for exerting toxic effects of MBM. For the aerosol, aqueous conditions were given at contact sites (mucous membranes with oral & inhalation exposure, sweaty skin). The CLH report stated that the equilibrium of MBM shifts towards formaldehyde by dilution and by the reaction of formaldehyde with biological media.

In the public consultation several commenters disagreed with the classification of MBM based on data from formaldehyde and stated that MBM contains one of the lowest levels of total releasable formaldehyde per molecule (16.7%) (*in comparison to other formaldehyde releasers on the market*), less than 0.005% of free (unbound) formaldehyde and that MBM is relatively stable in end use fluids. The release of formaldehyde via volatilisation or MBM by aerosolisation was found negligible and the resulting exposure level at workplaces were not sufficient to cause tumours under conditions of normal use (*in the end products*). Overall, the probability of a carcinogenic

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1 http://echa.europa.eu/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling?search_criteria_name=Formaldehyde&search_criteria_ecnumber=200-001-8&search_criteria=Formaldehyde

potential of MBM was seen as negligible. RAC notes that the CLP Regulation states that classification is based on intrinsic hazards of a substance and does not take the exposure conditions, the exposure to mixtures containing the substance of concern or the anticipated risk level into account.

The option to classify MBM as carcinogen, in category 2, in order to account for uncertainties for substances such as this that are unstable, showing equilibrium behaviour and having variable half-lives depending on dilution, temperature and pH, as discussed as an option in the CLH report is not supported by RAC. By weighing the evidence from data from the specific substance (and hydrolysis product) that is known to have carcinogenic properties (formaldehyde), no reasons (such as uncertainty about structural similarity or qualititative differences in the mechanistic aspects) could be identified to justify a downgrading of the classification category. Hydrolysis tests demonstrated that formaldehyde is generated within short time periods.
These hydrolysis tests and the intratracheal instillation study support qualitatively that hydrolysis of MBM will occur in contact with aqueous biological media on mucous membranes. Inhalation exposure to aerosolic MBM is expected to result in hydrolysis at the site of contact and toxicologically significant concentrations of formaldehyde could be reached on the surface of the mucous membranes in the respiratory tract, eye or upper GI tract or skin. The inhalation exposure to gaseous formaldehyde that evaporated from MBM is assumed to contribute in (an unknown extent) in addition to the toxic/carcinogenic effect resulting from the direct impact of hydrolysis products at the contact site. Demonstrating that the volatility and the room concentrations of released gaseous formaldehyde will be rather low would not be sufficient to discount the hazardous potential that may result from the inhalation exposure to MBM aerosol.

As no data are available to demonstrate that a sufficiently high concentration of formaldehyde can not (meaning never) be reached, there is no evidence to justify a downgrading. This prerequisite for the evidence is in contrast to the opinion of some commenters who found that the classification is only justified if evidence from exposed workers demonstrates that sufficient formaldehyde will be released and have caused tumours.

Information on the hydrolysis product is used to assess the hazardous properties including the carcinogenic potential of MBM. More guidance is given in REACH, Annex XI, 1.5.2 that specifies that similarities to substantiate the read across may be based on common precursors or common breakdown products via physical or biological processes, which results in structurally similar chemicals.

RAC agrees with the proposal of the DS to classify MBM based on the released formaldehyde as a Carcinogen, Category 1B; H350 (May cause cancer).

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

No animal or human data were available on sexual function and fertility. There is, according to the DS, no evidence for such effects from the repeat dose studies.

There is one OECD TG 414 study in rabbits. No adverse effects on sexual function and fertility or development were seen. The DS proposed no classification.

Comments received during public consultation

One MSCA supported no classification for reproductive toxicity.

Assessment and comparison with the classification criteria

Fertility

Morpholine
According to the DS (and the applicant) no studies were available which are directly related to this endpoint (e.g. OECD TG 415, 416, or 422). In subchronic and chronic inhalation studies in rats the reproductive organs were investigated and no adverse effects were found. Consistent with this, in a long-term drinking water study in mice exposed to morpholine oleic acid salt no effects were also detected in the reproductive organs.

Formaldehyde
The Formaldehyde Core Document summarised repeated (14-day or 90-day) inhalation studies on rats which revealed testis atrophy, reduced sperm counts and motility and increased sperm abnormalities or reduced serum testosterone at doses which influenced food consumption and body weight gain. As no quantitative information on the reduction in food consumption and bw gain is reported, no conclusion can be drawn. Studies with intraperitoneal application confirmed adverse effects on sperm.

MBM
From the (14-day range-finding and) 90-day study on MBM there was no indication of effects on the reproductive organs in males and females.

There were no other specific studies on reproductive effects that assessed the sexual function and fertility effects of MBM.

In conclusion, no concern on fertility effects from the available repeated dose studies was identified. **RAC agrees that due to the lack of specific studies, no conclusion on effects on sexual function and fertility can be drawn and based on the currently available data classification for this endpoint is not warranted**.

Developmental toxicity

Morpholine
Data on teratogenicity in rats after oral exposure to morpholine oleic acid salt (MOAS) are available (pregnant rats gavaged with 0, 234, 468, and 936 mg/kg bw/d on gestation day 6-15). The LOAEL for MOAS for maternal toxicity was 234 mg/kg bw/d (NOAEL < 234 mg/kg bw/d) corresponding to a LOAEL for morpholine of 88 mg/kg bw/d. No developmental effects were detected in any treatment group. The information was taken from the tables and English language abstract of a study published in Japanese (Sakemi et al., 2000, see doc. App. Morpholine).

Formaldehyde
No teratogenic effects were observed in inhalation or oral developmental toxicity studies conducted according to OECD TG 414. Fetotoxic effects (lower bw and retardations) were observed at the high dose with maternal toxicity (bw loss) (see Formaldehyde Core Document).

MBM
In a gavage study in rabbits (conducted according to OECD TG 414; cf. MBM-Doc III A6.8.1) local effects (erosion and granular aspect of stomach) were found in the stomach of dams at ≥ 30 mg/kg bw/d on GD 6-18 (3% MBM in corn oil). Significant decreases in body weight gain and relative food consumption at 100 mg/kg bw/d (10% MBM in corn oil) are considered to be secondary to these local effects. No developmental effects were detected at the high dose level of 100 mg/kg bw/d.
The RMS evaluation revealed that the gravid uterus weight was significantly reduced at 100 mg/kg bw/d. This seems to have been caused by a combination of (non significant) reductions of empty uterus weight, reductions of fetal weight and increases in resorptions. The latter two were also without a clear dose-response relationship. Delayed ossification at several sites, but without dose-dependency, was also reported.

The summary (MBM Doc III A6.8.1) stated that there were some premature deaths in all groups due to mis-dosing; these deaths were not test-substance related (no further details available from the summary). White spots and some haemorrhages in the stomach of all treated dams and controls found at necropsy were found to be most probably due to the stomach tube itself. Due to the limited study quality it is difficult to decide on the MBM-related effects in the gastrointestinal tract.

Testing in a 2nd species was considered as scientifically unjustified because also no teratogenic effects are expected due to concentration dependent local effects.

From the developmental study available (in rabbits) no adverse developmental effects were identified, therefore **no classification with regards to this endpoint is proposed by RAC**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier submitter's proposal

4-(morpholin-4-ylmethyl)morpholine (MBM) hydrolyses in water to formaldehyde and morpholine. It is assumed that the toxicity of MBM is related to the formaldehyde release.

Degradation

The DS proposed to consider MBM as rapidly degradable. The basis for this proposal was that the OECD 301B test results show that MBM and its hydrolysis products are readily biodegradable (93% degradation based on CO_2 evolution within 28 days).

Aquatic Bioaccumulation

The DS proposed that MBM does not meet the CLP criteria for bioaccumulation. The basis for this proposal was an estimated log $P_{ow} \leq 0.3$ and no significant bioaccumulation potential is expected for MBM.

Acute Toxicity

The DS proposed to not classify MBM as acutely hazardous to the aquatic environment. The basis for this proposal was that the acute toxicity of N,N'-methylenebismorpholine to aquatic organisms was tested in several studies covering all three trophic levels (fish, daphnia, and algae). The test solutions were prepared some hours before the test organisms were introduced, ensuring complete hydrolysis of the test substance OS 157340 (=MBM, purity 98% w/w). In all acute tests the hydrolysis product morpholine was monitored, in contrast to formaldehyde, which was not analysed. The test organisms were therefore exclusively exposed to the hydrolysis products, instead of the parent substance. Monitoring revealed that the test substance (measured as morpholine) was stable over the test period. The nominally confirmed concentrations based on morpholine were used to derive the effect values for the test substance.

All available acute $L(E)C_{50}$ values for MBM for all three trophic levels were >1 mg/L. The lowest $L(E)C_{50}$ values available was for algae; a 96h-ErC_{50} = 9.5 mg/L (based on nominal conc. of morpholine ≈ 10 mg/L MBM (calc.) ≈ 1.6 mg/L formaldehyde) (calculated)

Chronic Toxicity

The DS proposed to not classify MBM as chronically hazardous to the aquatic environment. The basis for this proposal was that long-term NOECs were available for crustaceans and algae, which were both >1 mg/L. For algae a 72h-NOE_rC of 2.0 mg/L (based on a nominal concentration of

morpholine ≈ 2.1 mg MBM ≈ 0.36 mg formaldehyde) was derived. A study on chronic fish toxicity with N,N'-methylenebismorpholine as the test substance is not available.

Comments received during public consultation

One MSCA CA commented on the ENV part of the classification dossier and requested further evaluation of an algae study on formaldehyde, which is one hydrolysis degradation product of MBM. The algae study is only available as a literature publication without any raw data or concentration-response curves. Only the 72h-E_rC_{50} of 5.7 mg/L was published. Consequently, the literature publication does not allow the derivation of a NOE_rC, nor an E_rC_{10} or an E_rC_{20} and no assessment against the chronic classification criteria for formaldehyde.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS to assess MBM as being rapidly degradable.

Aquatic Bioaccumulation

RAC agrees with the DS that MBM does not fulfil the criteria on aquatic bioaccumulation.

Acute Toxicity

RAC agrees with the dossier submitter to not classify MBM as acutely hazardous to the aquatic environment.

Chronic Toxicity

RAC agrees with the dossier submitter to not classify MBM as chronically hazardous to the aquatic enviroment.

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).