Harmonised classification and labeling proposal for N,N'-methylene bismorpholine (MBM) - Lubrizol comments for the public consultation

Executive Summary

MBM belongs to a category of biocidal actives known as formaldehyde-releasers (or formaldehyde-donors). These substances control microbial activity by the release of formaldehyde when diluted to their effective concentration. The different members of the formaldehyde-releasing biocides category exhibit different release characteristics and these are dependent on several factors including amongst others the type of chemical structure (N-formal or O-formal), the concentration of biocide, the dilution needed for hydrolysis and fluid pH. MBM contains one of the lowest levels of total ‘releasable’ formaldehyde per molecule (16% w/w) within the entire category of formaldehyde-releasers.

MBM has been on the market in the EU since the early 1990s and is used to control bacterial growth in water-based metalworking fluids and fuel at a maximum end use fluid concentration of 1500 ppm. MBM as manufactured is 98.5% by weight MBM with the remaining trace components comprising N-methylolmorpholine, morpholine, water and ‘free’ (unbound) residual formaldehyde that is present at less than 0.005% (50 ppm) by weight. MBM has low volatility (low vapour pressure and Henry’s Law Constant) and is considered to be stable in MWF concentrations and end use fluids with a half-life in terms of months.

Occupational measurements and measurement of the stability of MBM in end use fluids, which were not included in the Competent Authority Report that was submitted to the Biocidal Products Committee, are presented in this paper. These demonstrate that the MBM molecule is relatively stable in the form in which it is “reasonably expected to be used” (i.e. its intended use) and which would potentially result in the highest exposure of workers to the non-volatile MBM molecule via aerosolisation, with a half-life estimated to be 5-8 months in an end use metalworking fluid emulsion. This contrasts significantly with the hypothesis used to justify the proposed harmonised classification of MBM as a carcinogen, mutagen and sensitiser that sufficient formaldehyde would be released from the MBM molecule by contact with moisture from workers’ nasal mucosa or skin to cause an adverse toxicological event. Critically, it also demonstrates that all ‘bound’ formaldehyde is not released instantaneously upon contact with water in the end use fluid. Measurements of worker exposure to airborne formaldehyde and oil mist in a metalworking machining workshop utilising a fluid containing MBM demonstrate that the real-life exposure to either ‘released’ formaldehyde via volatilisation or MBM by aerosolisation will be negligible under conditions of normally expected use. This information, as well as consideration of published work suggesting that the nasal mucosa that is proposed to be responsible for the release of formaldehyde from MBM by hydrolysis upon contact following inhalation may also provide a partial barrier to direct contact with tissue means therefore that there will be insufficient exposure (bioavailability) to MBM by the inhalation route to give...
scientific credibility to the classification proposal based on total releasable formaldehyde; in summary, the data presented in this paper clearly demonstrates that inhalation exposure of workers to MBM is negligible and de minimis as supported by its physico-chemical properties, its intended reasonable use, its relative stability in an end use fluid, data from a UK Exposure Study, and based on arguments contained in an earlier risk assessment that used conservative models (e.g., for notification in Belgium where a product comprising 100% MBM is approved until 2024).

With regard to carcinogenicity in particular, there is no credible scientific evidence that MBM is a carcinogen. No carcinogenicity studies have been conducted with MBM and there is significant weight-of-evidence that MBM is not inherently a carcinogen. MBM is not genotoxic in vivo following oral administration indicating that MBM is not expected to be carcinogenic, at least by a primary genotoxic mechanism. Additionally, Quantitative Structure Activity Relationship (QSAR) analysis of the MBM molecular structure by the OECD methodology presents no alerts for carcinogenicity (or mutagenicity) and no histopathological findings such as hyperplasia or neoplastic lesions were observed in the 90 day oral gavage study with rats or in the oral prenatal developmental toxicity study on MBM. Finally, the final concentration of released formaldehyde in an end use fluid (both calculated and measured) is well below the regulatory threshold for classification of substances and mixtures as a carcinogen (i.e. << 0.1%, <<1000 ppm) and below the level (i.e. 2 ppm) previously recognised by RAC as resulting in no significant effects over the course of MBM’s intended use.

With the exception of skin irritation/corrosion hazard classification, the current harmonised classification proposal is entirely reliant on the assumption by the evaluating Competent Authority that there is rapid hydrolysis of MBM in contact with moisture to instantaneously release ‘bound’ formaldehyde, such that sufficient formaldehyde reaches relevant biological tissues to exert an adverse toxicological effect. The information presented in this paper demonstrate that this is a significant oversimplification of what happens when MBM (or another formaldehyde donor) is used in the workplace (i.e. in the form that it is placed on the market or can reasonably be expected to be used). While the RAC has previously considered hydrolysis by-products when assessing the hazard classification of other substances, it has done so in the context of specific acute inhalation hazard associated with its intended use (e.g. metal phosphides generating phosphine gas for use as a fumigant). The release characteristics demonstrated by MBM in aqueous metalworking fluid emulsions under in-use conditions means that a similar approach is not justified in this case, especially for the proposed classification as a carcinogen which relies on chronic exposure of workers’ nasopharyngeal epithelium to sufficient ‘released’ formaldehyde (i.e. at a supra-threshold level).

The current harmonized classification proposal for MBM based on releasable formaldehyde is therefore neither robust nor scientifically defensible; it does not reflect the intrinsic properties of the molecule, the supporting
experimental data, its reasonable use, weight of evidence, and is not therefore in accordance with the EU CLP Regulation.

I. Use and Hydrolytic Stability

MBM is an active substance used in biocidal products with intended uses in the preservation of fuels (0.04% w/w final concentration in fuels) or for the preservation of emulsifiable metal working fluids (0.15% w/w final concentration in end use metalworking fluid). Aerosolisation of metalworking fluid emulsions in high energy applications (e.g. milling, grinding, cutting etc.) presents the most opportunity for exposure of workers to the non-volatile MBM. In metalworking applications, MBM is added up to a 0.15% w/w (1,500 ppm) final concentration in the end use fluid and hydrolysis data presented in Table 1 shows that high dilution in water is required for notable hydrolysis to begin. It is therefore critical to recognise that experimental data demonstrates that there is no discernible hydrolysis of MBM and therefore negligible release of ‘bound’ formaldehyde in the form that MBM is manufactured and placed on the market. As manufactured it contains less than 0.005% w/w (50 ppm) formaldehyde and the available data conclusively demonstrate that the MBM molecule as placed on the market is very stable even in the presence of moisture. This situation holds until certain levels of dilution in water are achieved where the conversion (release) is 43 to 46%, with an upper limit of releasable formaldehyde of 16% of the original dose (see Table 1). Significantly, experimental data presented in this paper (Appendix 1) also shows that MBM is much more stable under conditions of normal use (i.e. in metalworking fluid concentrates and in end use metalworking fluid emulsions) than is predicted by the hydrolysis data presented in the dossier, with MBM half-life estimated to be 5-8 months (with 95% confidence) in end use metalworking fluid emulsions.

Although laboratory based hydrolysis test data with pure water demonstrates that MBM undergoes rapid hydrolysis at high dilution, the weight-of-evidence presented in this paper will demonstrate that it is misleading to extrapolate this finding to this substance for classification purposes. This is because “in use” formulations are not entirely aqueous but instead consist of complex metal working emulsions or micro dispersions in fuel. The rate of formaldehyde release from MBM at the effective dose in an end use metalworking fluid can be estimated indirectly to be in excess of several months. The data presented in Appendix 1 shows that MBM is relatively stable in an end-use metalworking fluid aqueous emulsion with an estimated half-life of between 5 and 8 months. This data clearly demonstrates that it is an oversimplification to suggest that MBM instantaneously releases sufficient ‘bound’ formaldehyde upon contact with water in the metalworking fluid aqueous emulsion, as suggested by the findings of the hydrolysis study cited in the harmonisation proposal. Industry experience with MBM also supports this observation since a normal fluid maintenance schedule requires the addition of fresh biocide only after a relatively long period of time to maintain fluid integrity. As formaldehyde is readily biodegradable in an aqueous medium (93% degradation based on CO₂ measurements within 28 days) and can be depleted through microbial metabolic pathways it is clearly deducible that if MBM formulated at the effective dose hydrolysed immediately
to release all ‘bound’ formaldehyde (which is what is suggested by the hydrolysis study presented in the dossier) then additional biocide would be required within a short space of time to maintain fluid integrity. The reason for the observation by end users that MBM is capable of providing longer-lasting antimicrobial activity in metalworking emulsions and other hydrocarbon-based solutions when used at the effective dose is not fully understood but it is demonstrable that under conditions of normal use total ‘bound’ formaldehyde is released only gradually into the end use fluid resulting in a very slow depletion of the biocide reserve (i.e. the ‘bound’ formaldehyde) rather than there being an immediate release of all available (‘bound’) formaldehyde upon contact of MBM with moisture, as suggested by the hydrolysis study presented in the dossier. Finally, the fact that the release of formaldehyde from the MBM molecule only occurs under certain in-fluid conditions that gradually develop over a period of time means that instantaneous release of total (bound) formaldehyde cannot be considered an intrinsic property of the MBM molecule (Onyekwelu et al 1981). This observation and logic is equally applicable to other substances belonging to the formaldehyde releaser category.

It follows therefore that any hazard classification based on an assumed immediate/instantaneous release of a sufficient amount of ‘bound’ or ‘releasable’ formaldehyde leading to the formation of sufficient ‘free’ formaldehyde at the nasal epithelium cell surface to cause adverse effects also cannot be considered an intrinsic property of the MBM molecule. With reference to sections 3.5.2.3.2 and 3.6.2.2.1 of the CLP Regulation classification of MBM as hazardous on this basis in the absence of any other conclusive evidence contradicts the concept of hazard classification as defined by CLP (CLP uses the term “hazard classification” to indicate that only the intrinsic hazardous properties of substances or mixtures are considered). The classification of MBM must instead be based solely on the level of residual formaldehyde present in the substance as placed on the market (which is <<0.1%).

**Table 1. Determination of ‘free’ formaldehyde in MBM as manufactured and at commercially-relevant dilutions**

<table>
<thead>
<tr>
<th>Concentration of MBM (w/w)</th>
<th>% water (aqueous solution)</th>
<th>Maximum releasable (bound) formaldehyde (by calculation)</th>
<th>% “free” formaldehyde detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% (as manufactured)</td>
<td>0%</td>
<td>16%</td>
<td>&lt; 0.005 % (&lt; 50 ppm)</td>
</tr>
<tr>
<td>50%</td>
<td>50%</td>
<td>8%</td>
<td>&lt;&lt; 0.10 % (&lt; 1000 ppm)¹</td>
</tr>
<tr>
<td>3%</td>
<td>97%</td>
<td>0.48%</td>
<td>0.03 % (300 ppm)</td>
</tr>
<tr>
<td>0.15%</td>
<td>99.85%</td>
<td>0.024%</td>
<td>0.012 % (120 ppm)</td>
</tr>
</tbody>
</table>

¹ This is an estimated value based on indirect measurement. No degradation of 50% MBM dilution in water by hydrolysis was observed. Confirmatory analysis of ‘free’ formaldehyde for 50% MBM dilution in water is underway and will be available at earliest within one month and latest by the relevant RAC meeting.
II. Exposure and Availability Assessment

Ordinarily exposure considerations have no part to play in the classification of a substance ("unless a chemical can be considered as not being biologically available"). This is because CLP defines hazard classification to be based on the inherent properties of the substance in question and not its fate characteristics. However, the proposal by the Austrian Competent Authority to classify MBM for carcinogenicity, mutagenicity and sensitisation by definition introduces an exposure element because there is an absolute requirement for complete and immediate hydrolysis of MBM in contact with biological tissues generating sufficient amount of released formaldehyde to have a site-specific localised effect, or even a systemic effect. As this is the sole basis for the proposed classification as Carcinogen Category 1B, Mutagen Category 2 and Skin Sensitisation the relevancy of exposure considerations becomes critical and must to be explored further to determine the appropriateness of the proposed classification.

UK Workplace Exposure Study for MBM in Metalworking Application

Lubrizol has generated workplace exposure data for a metalworking fluid emulsion containing MBM at the effective dose that shows the typical occupational exposure of workers to ‘free’ airborne formaldehyde and oil mist generated during the metalworking operation. The mist measurements can be considered to be representative of “reasonably expected use” and worst-case worker exposure to MBM via aerosolisation because the non-volatile MBM is dispersed within the metalworking emulsion. Additionally, the analytical method used to determine the concentration of airborne formaldehyde (including that present in the aerosol mist) is destructive and also converts any MBM present in the aerosol mist to formaldehyde. Thus, the total measured formaldehyde comprises formaldehyde released from the destruction of any airborne MBM present in the oil mist during analysis and background (‘free’) formaldehyde present in the worker atmosphere. The measured formaldehyde concentration shown in Table 2 therefore represents a worst-case scenario for a typical machining application. The full study report has been provided to this consultation separately and brief details of the exposure study are given below:

On-site occupational exposure data were collected over 3 days in a workshop machining cast steel automotive brake components using Computer Numerical Control (CNC) machines. These machines were semi-enclosed, meaning that they were open at the top and there was no mechanical exhaust. A total of 22 samples were taken: personal samples near the machine (operator position) and at various distances from the machine including 1-2 meters from the machine and at the sump/metalworking reservoir. Average representative sample collection
time was 362 minutes/day. The samples were collected by drawing air at a measured rate of approximately 1 litre per minute through a treated glass fibre filter held in a cassette. The filter was treated with dinitrophenylhydrazine (DNPH) and was analysed by HPLC at the end of the sampling period. Sampling results from aerosol mists generated during the machining process are shown below in Table 2. Airborne formaldehyde results ranged from 1 to 6 ppb regardless of where the sample was taken. The 95% confidence range of the data was 1.1 to 3.3 ppb as verified by the United States Environmental Protection Agency in its registration decision. An almost identical result was obtained in a study by Lillienberg published in 2008. It should be noted that MBM was not identified in the paper but later confirmed in a personal communication when the author was contacted. The data demonstrate that occupation exposures levels are several orders of magnitude below the lowest OEL and long-term local inhalation REACH DNEL for formaldehyde and the calculated DNEL for MBM (considered to be present in the oil mist).

Using these representative data, the average worker exposure to MBM and formaldehyde during typical machining operations (i.e. under conditions of reasonably expected use) was estimated. The measured level of atmospheric ‘free’ formaldehyde is significantly lower than accepted safe levels. Assuming MBM is present in the oil mist (185 µg/m3) at the typical effective end-use fluid concentration of 1500 ppm (0.15% w/w), the work place atmosphere would contain a theoretical maximum of 0.28 µg/m3 MBM. As each molecule of MBM contains a maximum of 16% releasable formaldehyde, the maximum amount of formaldehyde that can be released from MBM contained in the oil mist is estimated to be approximately 0.04 ppm. Thus, a worker can be maximally exposed to this extremely low formaldehyde concentration from potential contact with MBM as placed on the market or under conditions of normally expected use, following either inhalation or contact with exposed skin.

<table>
<thead>
<tr>
<th>Sampling Results</th>
<th>Formaldehyde</th>
<th>Oil Mist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average airborne = 1.97 ppb (0.00197 ppm)</td>
<td>185 µg/m3</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference Values</th>
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<tbody>
<tr>
<td>REACH DNEL (long-term, local, inhalation) = 0.3 ppm. Lowest OEL inhalation = 0.12 ppm (Netherlands, 2011; ‘safe’ level = 0.12 mg/m³ or 0.10 ppm established by DGUV measurement)</td>
<td>REACH DNEL (acute, local, dermal) for MBM is 1.06 mg/m³</td>
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</table>

Even when this amount is combined with the measured airborne formaldehyde levels in the workshop this value is several orders of magnitude lower than the lowest OEL for formaldehyde among all the EU Member States, and

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2 See Appendix 2 for derivation of maximum worker exposure to formaldehyde released from MBM under conditions of reasonably expected use.
the level considered to be ‘safe’ by DGUV for German workers. This value is also several orders of magnitude below the level that the RAC previously considered to represent the limit for ‘significant’ events associated with formaldehyde toxicity (i.e. 2 ppm or 2.46 mg/m³).

Additionally, it is well known that the human body naturally produces formaldehyde (O’Sullivan et al. 2004; Cloos et al. 2008; Hou and Yu 2010) and that formaldehyde detoxification by cellular enzymes (Friedenson 2011; MacAllister et al. 2011) results in a steady state balance between formaldehyde-generating and formaldehyde-disposing processes leading to normal blood formaldehyde concentrations of around 0.1 mM (Heck and Casanova 2004). One of the primary means of formaldehyde disposition is by exhalation meaning that there is clearly a level below which no adverse effects of formaldehyde can be detected. For instance, measurements of exhaled formaldehyde in a subset of the human population show significant levels of exhaled formaldehyde ranging from 1–10 µg/m³ (Kuhsch et. al. 2008) exclusive of contribution from MBM. These data are supported by further studies conducted by Moser et. al. (2005).

In further support of a limit for significant toxicological events, sub-chronic studies conducted in rhesus monkeys have shown that blood formaldehyde concentration was not measurably altered by exposure to airborne formaldehyde at 6 ppm (7.37 µg/m³) for 6 hr/day, 5 days/week for 4 weeks (Casanova-Schmitz et al. 1984). The apparent steady state of formaldehyde, even in the face of significant exposures, means that normal physiological processes can tolerate levels in excess of those considered safe by the authorities, and maximum levels of formaldehyde that can be generated by exposure to MBM under reasonably expected conditions of use. This is not surprising since the formaldehyde metabolic pathway describes several enzymatic processes that serve to ameliorate physiological formaldehyde concentrations (Tenga et. al 2001).

The above calculated maximum worker exposure concentration to formaldehyde (i.e. 0.04 ppm), which is based on real-life workplace exposure measurements, demonstrates that exposure to ‘released’ formaldehyde from MBM during its normally expected (intended) use in metalworking fluids (which is the worst-case exposure scenario) is therefore of a similar magnitude to the amount of formaldehyde typically present in human breath. The unavoidable conclusion that can be drawn from these measurements and calculations is that worker inhalation exposure to exogenous (anthropogenic) formaldehyde potentially formed by hydrolysis of MBM will be no greater than concentrations of measured endogenous (physiological) formaldehyde during exhalation. Consequently, exposure of formaldehyde to the nasal mucous membranes from either source is comparable during normally expected use of MBM, even under the worst-case exposure scenario. As exhaled endogenous formaldehyde palpably does not result in an increased incidence of adverse effects in humans including carcinogenicity and mutagenicity it is therefore demonstrably unjustifiable to classify MBM as a carcinogen and a
mutagen as a means of worker protection, even assuming classification based on a concept of total releasable formaldehyde is appropriate.

**Notification of MBM in Belgium**

In 2014 Lubrizol conducted an environmental and human health exposure assessment at the request of the Belgian authorities for the purposes of extending the notification of a biocidal product containing MBM in Belgium. Computer modelling of potential workplace exposures was conducted using Tier 1 ECETOC TRA modelling and Tier 2 assessments were performed using measured workplace exposure concentrations. Safe use was conclusively demonstrated with RCRs <<1 being achieved for all relevant exposure scenarios including the most dispersive uses in metalworking applications. In fact, RCRs <<1 were demonstrated even when using conservative models and worst case assumptions for both MBM and formaldehyde, which were shown to predict PECs >10 fold higher than actual measured levels from the UK machine shop study described above.

Several studies have been conducted to determine the extent to which formaldehyde is systemically available subsequent to inhalation. In a very convincing evaluation of the relative contribution of exogenous formaldehyde exposure to circulating levels in the blood of humans, rats and monkeys, formaldehyde concentrations in blood after inhalation exposure to formaldehyde were not elevated compared to physiological blood-levels of formaldehyde of about 0.1 mM (Casanova et al., 1988; Heck et al., 1982; Heck et al., 1985). This indicates that formaldehyde has a substantial and high “first-pass” effect such that systemic availability is extremely low. It is therefore apparent that local effects seem to play a more important role compared to systemic effects due to the reactivity of the compound and its rapid metabolism in the cells lining the skin (BFR 2006). Finally, in its review of USEPA’s revision of the IRIS assessment for formaldehyde, the National Research Council (NRC) concluded that “…the weight of evidence suggests that it is unlikely for formaldehyde to appear in the blood as an intact molecule, except perhaps after exposures at doses that are high enough to overwhelm the metabolic capability of the tissue at the site of entry.” (NRC 2011).

In addition to the numerous studies that support no delivery of inhaled formaldehyde to distant sites in the body, and combined with the fact that formaldehyde naturally occurs throughout the body and throughout the natural environment, it is also important to note that the nasal mucosa may also provide a partial barrier to ameliorate the adverse effects of potential formaldehyde exposure (see Priha et al. 1996). This is in contrast to the hypothesis contained in the CLH dossier that this mucosal layer just provides the source of moisture to release formaldehyde locally at the nasopharyngeal epithelium cell surface following inhalation of MBM. Mechanistically, albumin in the mucus that lines the human nasal lining forms an additional barrier to the systemic absorption of
formaldehyde (Bogdanffy et al. 1987; Casanova-Schmitz et al. 1984; Heck and Casanova (2004)) while the solubility of formaldehyde in mucus, and the ciliary movement and ingestion of mucus may account for the removal of as much as 42% of the inhaled dose in a murine model (Schlosser 1999). This observation adds to the weight-of-evidence that insufficient formaldehyde would be released following inhalation exposure to MBM to cause toxicologically-relevant adverse effects in the nasopharyngeal epithelium.

III. Carcinogenicity

Based on the data presented in the CLH dossier it cannot be safely concluded that MBM is inherently a carcinogen. Instead, the current classification proposal is based on the concept that MBM results in human exposure that liberates formaldehyde, which is the carcinogenic component. Since the classification proposal is dependent on exposure factors which govern the liberation of formaldehyde, it is therefore essential that such exposure factors are fully taken into account by RAC to assess the degree of potential exposure because they are patently integral to the classification discussion.

In accordance with EU CLP Regulation we strongly suggest that classification of MBM for carcinogenicity is inappropriate based on numerous lines of evidence presented below. Further, in view of the explanation of the hydrolytic stability of MBM in the form that it is placed on the market and the very slow rate of formaldehyde-release (as a proportion of total dosed MBM) during its use as intended i.e. in end use diluted metal working fluids, there is demonstrably no credible scientific justification for classifying MBM as a suspected carcinogen, either in terms of direct evidence or on a weight-of-evidence approach.

1) MBM as manufactured and in the form that it is placed on the market contains significantly less than 0.1% ‘free’ or ‘unbound’ formaldehyde as an impurity (measured ‘free’ formaldehyde was < 50 ppm).

2) CLP states that “carcinogenic potential can be inferred from in vivo and in vitro ...mutagenicity studies”. The higher tier in vivo studies demonstrate that MBM is not genotoxic by oral administration.

3) Using the decision logic for classification of substances for carcinogenicity (Guidance on the Application of CLP criteria section 3.6.2.6), when the substances do not have carcinogenicity data then classification as a carcinogen based on actual data is not possible.

3.6.2.6. Decision logic for classification of substances

The decision logic which follows is taken from the GHS Guidance. It is strongly recommended that the person responsible for classification, study the criteria for classification before and during use of the decision logic.

<table>
<thead>
<tr>
<th>Does the substance have carcinogenicity data?</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification not possible</td>
<td></td>
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</table>
CLP states that non testing data for the substance such as QSAR and Read Across predictions can be used when a substance has not been tested for carcinogenicity. In order to evaluate the potential for carcinogenicity according to CLP the OECD Toolbox version 3.2 was used to profile MBM. As shown below, based on QSAR predictions for carcinogenicity as well as read across predictions from chemicals with analogous structures having some experimental data MBM was confirmed to have a very low probability for carcinogenic potential. Thus, MBM should not be classified as a carcinogen based on model data.

Similarly, read-across from formaldehyde to MBM has been demonstrated in this paper to be scientifically unsound because there is no credible evidence to suggest repeated exposure of workers to MBM would release **sufficient** formaldehyde to cause tumours. On this basis, MBM itself cannot be considered to be inherently carcinogenic in accordance with the classification guidance.

4) The proposed classification of MBM for carcinogenicity relies solely on the carcinogenic effects of released formaldehyde and that a **sufficient** amount of formaldehyde is released at the nasopharyngeal cell surface to result in tumours at the site of contact. This is because numerous scientific articles and the previous RAC opinion for formaldehyde recognise that there is a concentration below which critical effects and carcinogenicity of formaldehyde have not been demonstrated (e.g., at 2 ppm; RAC 2012). The conclusion that the occurrence of tumours at higher levels is the result of chronic proliferative processes and that the genotoxicity of formaldehyde plays essentially no part in its carcinogenic potential is expertly summarized by Gelbke et al. The published literature also considers exposure to exogenous formaldehyde to be insignificant compared to exposure to endogenously formed formaldehyde, and that in the absence of irritation there are no long term toxicity issues arising from formaldehyde exposure. Finally, the literature confirms that there is essentially no risk to tissues other than those at the local site of contact.
The current proposal to classify MBM as a carcinogen relies entirely on the hypothesis that sufficient formaldehyde would be released rapidly in contact with biological media. This hypothesis, as noted by the proposal, is in “qualitative terms” supported by hydrolysis data generated from MBM/water solutions at very low dilutions. The experimental stability data (Appendix 1) and workshop exposure data (Table 2) presented in this paper actually demonstrate that quantitative application of this data for use in the read across is not appropriate. It should be noted that the RAC has previously concluded that the available data on low dose effects of formaldehyde suggest that the dose-related ‘key events’ seen below 2 ppm were considered to be non-significant (RAC 2012). Indeed, formaldehyde contact with biological tissue appears to require sufficient levels to trigger an irritant (cytotoxic) and/or cell proliferative response in the nasopharyngeal epithelium leading subsequently to cancers. An irritant/cytotoxic and/or cell proliferation response in the nasopharyngeal epithelium is believed to be a necessary precursor to the development of local tumours in the nasal epithelium (NRC 2011). Thus, being able to demonstrate this with MBM rather than formaldehyde, or at least put forward a credible argument that it occurs, should be a necessary pre-requisite for classifying MBM as a carcinogen.

The RAC opinion for formaldehyde (RAC 2012) and that of the US NRC (NRC 2011) also confirmed that there is no evidence or plausible mechanistic process for any systemic distribution and effect of formaldehyde distant to the site of exposure. As a consequence we consider that there are numerous flaws in the proposal to classify MBM as a carcinogen based on release of total (‘bound’) formaldehyde following contact with moisture in the nasopharyngeal epithelial mucus layer. Each flaw in the overall hypothesis can be addressed in turn:

1. Most crucially, there is a false assumption that hydrolysis of the MBM molecule occurs immediately upon contact with the nasopharyngeal epithelium and would release sufficient ‘bound’ formaldehyde leading to release of sufficient ‘free’ formaldehyde to cause an irritation/cell proliferation response. Stability data shows that concentrated MBM shows only very slow hydrolysis even when diluted to 50% in water (see Table 1 and Appendix 1). Furthermore, the demonstrated limited hydrolysis of formaldehyde (Priha 1995) and the protein-rich composition of nasopharyngeal mucus (111 proteins have been identified; Casado et al. 2005) suggests that rapid hydrolysis in a fully aqueous matrix in the respiratory system is unlikely. Further, as concentrated MBM is demonstrably corrosive to dermal skin it is reasonable to conclude that occupational exposure of the nasopharyngeal epithelium to neat MBM would result in the destruction of the epithelial cells rather than subtle cytotoxic effects or induction of cell proliferation that would act as the precursor to tumour formation. Similarly, inhalation exposure to low concentrations of MBM for example through aerosolisation of an end-use metalworking fluid containing MBM at the typical effective dose of 1500 ppm would be well below the calculated DNEL for local irritant effects.
2. It is an unrealistic assumption that the nasal epithelium of metalworkers will be exposed to sufficient MBM in the workplace.

MBM is non-volatile (calculated vapour pressure; 0.625 Pa at 25 °C or 0.443 Pa at 20 °C; Section 1.3, Table 9 of the dossier) and there is therefore no possibility of workers throughout the supply chain being repeatedly exposed to the neat substance by inhalation during handling and during any reasonably expected (intended) use due to the non-volatile property of the substance. Additionally, aerosolisation is not a credible route of inhalation exposure to neat MBM during handling by workers when formulating a mixture as insufficient energy would be generated during the formulation process to disperse an aerosol. There is however the possibility of inhalation exposure of metalworkers to dilute levels of MBM due to aerosolisation of an end-use fluid during high energy operations such as grinding, cutting or milling. However, actual workplace measurements show this to be practically irrelevant in terms of delivering sufficient MBM to the workers’ respiratory system. Furthermore, this route of exposure (via high energy aerosolisation) would not be appropriate for other approved uses of MBM (e.g. PT6).

3. It is an unrealistic assumption that workers’ nasopharyngeal epithelium will be exposed to supra-irritating levels of formaldehyde released from MBM on repeated occasions.

The preponderance of evidence accumulated through numerous studies and repeated analysis of the extensive body of toxicology data indicates that formaldehyde causes localized nasopharyngeal tumours following repeated inhalation exposure by chronic irritation and/or cellular proliferation of the nasopharyngeal epithelium. The recently finalised RAC opinion on the harmonised classification of formaldehyde also agreed that specific cellular mechanisms must occur for formaldehyde to cause nasopharyngeal cancer, and it follows that chronic exposure to sub-irritating levels of formaldehyde does not result in nasopharyngeal tumours (RAC 2012). The exposure data included in this paper clearly demonstrates that exposure of workers’ nasopharyngeal epithelium to supra-irritating levels would not happen under conditions of intended and reasonably expected use even in the worst-case occupational environment. As above, chronic irritation of the workforce respiratory system would be required to elicit adverse effects and such conditions would not be unnoticed or deemed acceptable in an industrial environment.

There is no indirect evidence that MBM is carcinogenic. In addition to there being no evidence of a genotoxic response in whole animals, we have followed ECHA’s own CLP guidance for carcinogenicity and critically assessed the other experimental data to seek evidence of pre-neoplastic changes to compensate for the absence of a carcinogenicity study on MBM. In the absence of any pre-neoplastic changes in these studies and in the absence of any genotoxic response in whole animals the weight-of-evidence suggests that MBM is not a carcinogen and therefore there is no scientific justification for its classification as such.
IV. Mutagenicity

In accordance with EU CLP (Regulation (EC) No. 1272/2008) classification of MBM is not required for genotoxicity based on the absence of genotoxicity in vivo. The mutagenic potential of MBM has been evaluated using a number of in vitro assays. MBM is weakly mutagenic in the presence of metabolic activation in Salmonella typhimurium strain TA100 and is positive with and without metabolic activation in the chromosome aberration assay with CHL cells and in the mouse lymphoma assay. In vivo studies, however, indicate that it is not genotoxic. MBM did not induce a significant increase in micronuclei in the in vivo mouse micronucleus assay and did not induce DNA synthesis in the liver from rats given orally administered doses up to 900 mg/kg. In accordance with the CLP guidance, the results from the in vivo assays on MBM in the form that it is placed on the market should be more heavily weighted as an indicator of the inherent genotoxic properties of MBM than the in vitro assays. Information presented elsewhere in this paper provide sufficient reasons why it is not scientifically credible to rely on data generated from experiments involving MBM at very low concentrations in an aqueous medium to define the inherent hazard character of this substance by consideration of the hydrolysis by-products.

Additionally, under CLP classification as a Mutagen is only required where there are demonstrated adverse effects on germ cells (i.e. inducing hereditable changes), or where hereditary effects can be predicted from effects on somatic cells. The hypothesis supporting the proposed classification of MBM as a mutagen, namely the hydrolytic release of sufficient ‘bound’ formaldehyde leading to ‘free’ formaldehyde at the site of contact means that the proposed classification is neither scientifically credible nor defensible. Numerous studies and RAC’s own previous opinion on formaldehyde accept that formaldehyde has no significant toxicological effect distant to the site of exposure (RAC 2012). The absence of a credible mechanism for systemic distribution supports the conclusion that a worker’s germ cells would never be exposed to sufficient formaldehyde released from MBM, and so the proposed classification of MBM as a mutagen is both disproportionate and not scientifically defensible.

V. Skin sensitisation

In accordance with EU CLP (Regulation (EC) No. 1272/2008), there is no credible, scientific justification for classifying MBM as a skin sensitisier based on the information presented in the dossier. The skin sensitization test (guinea pig maximization) was considered inconclusive by the evaluating Competent Authority because the 10% topical induction concentration did not cause any irritation. However, rejection of this scientifically-valid sensitisation study in favour of extrapolation to a hypothesis of instantaneous release of sufficient ‘bound’ formaldehyde to cause an adverse effect following dermal exposure is unjustified. As hapten formation and distribution to the lymph through systemic circulation is required to elicit the sensitisation effect, the conclusion that there is no credible mechanism that would facilitate translocation from the site of exposure to systemic circulation (NRC 2011) is supportive of no classification for sensitisation. Further, from the experimental perspective, the aforementioned assay was conducted using a non-aqueous vehicle to evaluate the sensitisation
profile of the MBM molecule to support a conclusion of intrinsic toxicity. Given the recognised confounding factor associated with the known sensitisation potential of formaldehyde and the anticipated high rate of hydrolysis of MBM at the low concentrations predicted to be needed during the experiment because of the known corrosive nature of MBM the conclusion that MBM is inherently sensitising is unfounded. The criticism of the study and the reason for the findings being considered equivocal by the evaluating Competent Authority was the lack of irritation seen during the induction phase of the main study at the dose levels selected. Using a higher intradermal and induction concentration than tested in the main study was not possible due to MBM’s inherent corrosivity and concerns for animal welfare (i.e. severe lesions (necrosis) were observed for several animals during the sighting assay). Despite the equivocal outcome of this study experimental data should not be superseded by the unproven hypothesis of an immediate release of sufficient ‘bound’ formaldehyde upon dermal contact and transfer across the skin barrier in sufficient amounts to cause a cellular reaction. Toxicokinetic measurements using radio-labelled material showed that the highest amount of radioactivity was retained in the stratum corneum with formaldehyde reacting with macromolecules mostly at the outer layers of the skin thus limiting further penetration and systemic distribution. From these experiments it must be inferred that insufficient released formaldehyde would penetrate into the epidermis to induce sensitisation following dermal exposure to MBM under normally expected use conditions. It follows therefore that there is no convincing evidence that dermal sensitisation is an intrinsic property of the MBM molecule. Instead classification considerations for sensitisation should be based on the amount of free (unbound) formaldehyde present when MBM is placed on the market rather than being based on a consideration of the amount of ‘releasable’ formaldehyde that may occur under uncertain experimental conditions. The stability study shown in Table 1 demonstrates that release of formaldehyde by hydrolysis is not expected to occur at higher concentrations even in the presence of moisture and the actual release kinetics of MBM in the form that it can reasonably be expected to be used do not support the hypothesis presented in this dossier to justify classification of MBM as a skin sensitiser.

VI. Administrative Argument
The intent of CLP is to provide a high level of protection of human health. This is achieved by classifying substances (and mixtures) for hazard classes and categories based on their intrinsic hazard as placed on the market or in the form that it can reasonably be expected to be used. Additionally, CLP states that classification should be achieved using a weight of evidence approach involving expert judgment, especially in those circumstances where criteria cannot be applied directly such as in the absence of relevant test data. CLP also recognises that for reasons of proportionality and workability there is a level below which identified impurities or by-products should not be considered in determining the hazard classification of substances and mixtures (i.e. 0.1% w/w for carcinogens). In other words, CLP’s authors recognised that to make the system workable (practical) for worker safety purposes a specific threshold should apply to all hazard categories, even for carcinogens. It is
therefore proportionate and reasonable that the same consideration should be applied to MBM for the hazard classes under consideration: in other words, MBM should be classified for carcinogenicity, mutagenicity and skin sensitisation based upon the amount of residual (i.e. unreacted or unbound) formaldehyde present in the substance as it is placed on the market or in the form in which it can be reasonably be expected to be used. Classification should not be based on an hypothesis that relies on the extrapolation of a single data point (i.e. hydrolysis) justifying an assumption that there is instantaneous release of sufficient ‘bound’ formaldehyde following hydrolysis of MBM upon contact with biological tissue.

References


Deutsche Gesetzliche Unfallversicherung (DGUV) Berufsgenossenschaft Holz und Metall Information sheet 029 (2014) Formaldehyde and -donors Risk assessment for users of cooling lubricants

Heinz-Peter Gelbke, Sibylle Gröters, Peter Morfeld (2014). Lowest adverse effects concentrations (LOAECs) for formaldehyde exposure. Regulatory Toxicology and Pharmacology. Volume 70, Issue 1, October 2014


Appendix 1 – Analytical study demonstrating stability of MBM in metalworking fluid emulsion

Analytical Report:
Assessment of the Stability of CONTRAM™ ST-1
N,N’-methylenebismorpholine
(CAS# 5625-90-1)
In Metalworking Fluid Emulsions
Appendix 1 (contd) – Analytical study demonstrating stability of MBM in metalworking fluid emulsion

1.0 ABSTRACT

Aqueous soluble oil metalworking fluids (oil in water macro emulsions) incorporate preservative compounds, e.g. CONTRAM™ ST-1 to inhibit microbial growth. A concentration of 1500 ppm is normally recommended in the diluted metalworking fluid. This chemistry employs formaldehyde in a bound form, released by varying mechanisms, in aqueous metalworking fluids.

Quantitative measurement of formaldehyde condensates in aqueous metalworking fluids is problematic. It can be very difficult to differentiate free formaldehyde from bound formaldehyde associated with formaldehyde condensates using standard formaldehyde analysis methods as these methods are destructive in nature. The analytical strategy used in this report is to isolate the free formaldehyde from its condensate by extraction of the metalworking fluid emulsion with chloroform. Free formaldehyde is not miscible in chloroform and remains in the aqueous phase. The chloroform phase is then analyzed by HPLC with normal phase separation of the N,N-methylenebismorpholine from N-hydroxymethylmorpholine, coupled with post column derivatization to enable detection of any formaldehyde containing substance. The environment of the post column reactor is severe enough to quantitatively decompose the formaldehyde condensate and release formaldehyde which is in turn derivatized. There are several examples of this approach in the literature (see 5.1). These examples employ aqueous reversed phase HPLC and aqueous post column derivatization chemistry. Some formaldehyde condensates commonly used in metalworking fluids however, readily decompose in water, particularly at low concentration, and cannot be measured in this manner. N,N-Methylenebismorpholine (CONTRAM® ST-1) is an example of this type of formaldehyde condensate.

The post column derivatization reaction is an adaptation of the Hantzsch reaction:

\[
\begin{align*}
2 \text{R}_1 \text{R}_2 \text{C}=\text{O} + \text{R}_2 \text{C}=\text{O} + \text{R}_3 \text{NH}_2 & \rightarrow \text{R}_1 \text{R}_2 \text{R}_3 \text{N} + 3 \text{H}_2\text{O} \\
\end{align*}
\]
The derivatizing reagent contains 2,4-pentanedione, acetic acid and ammonium acetate and is made in 1-butanol. The acidity of the derivatizing reagent and the temperature of the post column reactor is sufficient to decompose N,N-Methylenebismorpholine to quantitatively release formaldehyde. The product of the derivatization reaction with formaldehyde is 3,5-diacyl-1,4-dihydrolutidine which is chromophoric at 410 nm. A typical chromatogram for ST-1 is shown in 3.1. The sum of the 6 and 13 minute retention band areas at 410 nm divided by the sample weight is proportional to the amount of ST-1 present in the metalworking fluid. This approach is taken since pure standards of N,N'-methylenebismorpholine and N-hydroxymethylmorpholine are not available to establish individual

Appendix 1 (contd) – Analytical study demonstrating stability of MBM in metalworking fluid emulsion

response factors. A response (mAU*s/g) constructed in this manner can be used to monitor the decomposition of ST-1 with time in an aqueous metalworking fluid.

2.0 Experimental

2.1 Apparatus:

<table>
<thead>
<tr>
<th>Apparatus</th>
<th>Vendor</th>
<th>Description</th>
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<tbody>
<tr>
<td>HPLC</td>
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<tr>
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<td>Restek Ultra Cyano 2.1x150 mm</td>
</tr>
<tr>
<td>Temperature Controller</td>
<td>Waters</td>
<td>Waters, TCM II</td>
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<td>Static Mixer</td>
<td>ASI</td>
<td>ASI, binary, 25 µL cartridge</td>
</tr>
<tr>
<td>Post Column Reactor</td>
<td>ASI</td>
<td>ASI, 1.00 mL, stainless steel, 3000 p.s.i., 150°C</td>
</tr>
<tr>
<td>Reagent Pump</td>
<td>Dionex</td>
<td>Dionex, GP-40</td>
</tr>
</tbody>
</table>

2.2 Chromatography Conditions:

<table>
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<th>Description</th>
<th>Setting</th>
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</thead>
<tbody>
<tr>
<td>Injection</td>
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<tr>
<td>Flow</td>
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<tr>
<td>Detection</td>
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<td>Column Temperature</td>
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</table>
2.3 Post Column Reactor Conditions:

<table>
<thead>
<tr>
<th>Description</th>
<th>Setting</th>
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</thead>
<tbody>
<tr>
<td>Flow from Reagent Pump</td>
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<tr>
<td>Reactor Temperature</td>
<td>92 °C</td>
</tr>
</tbody>
</table>

Appendix 1 (contd) – Analytical study demonstrating stability of MBM in metalworking fluid emulsion

2.4 Reagents:

2.4.1 Color Reagent: 12.5 g of ammonium acetate is added to a 500 mL volumetric flask. Next, about 475 mL 1-butanol is added to the flask. 1500 µL glacial acetic acid is and 1650 µL 2,4-pentanediol are added to the flask. The volume of fluid in the flask is made to the mark with 1-butanol. The content of the flask are mixed using a magnetic stirrer until dissolution is complete. The solution is vacuum filtered through 0.45 um PTFE filter. The resulting solution should be clear and slightly yellow. Note: Two (2) PTFE filter pads are used.

2.4.2 [n-heptane | 2-propanol : 90 | 10 %v] Solution: 100 mL of 2-propanol is added to a 1.00 liter volumetric flask. Enough n-heptane is added to bring the level of the resulting solution to the mark. The resulting solution is thoroughly mixed.

2.4.3 10% NaCl Solution: 10 g of NaCl is added to 90 g of water. The resulting solution is mixed until dissolution is complete.

2.4.4 [2-propanol | water: 90 | 10 %v] Solution: 100 mL of water is added to a 1.00 liter volumetric flask. Enough 2-propanol is added to bring the level of the resulting solution to the mark. The resulting solution is thoroughly mixed.

2.5 Sample Preparation:

~2 g of metalworking fluid containing approximately 1500 mg/L of CONTRAM™ ST-1, weighed to .01 mg, is placed in a 10 mL vial, 4 mL chloroform is added to vial. 1000 uL of 10% NaCl aqueous solution is carefully added to vial. Contents of the vial are vigorously shaken for 10 to 15 seconds. Contents of the vial are allowed to stand for 10 minutes. A Tesla Coil can be used to improve the separation between the phases. ~2000 uL is carefully pipetted from the organic layer (bottom layer), filtered through 0.45 um PTFE filter. The resulting solution is assayed using the HPLC method below.

3.0 Results
3.1 A typical Chromatogram for CONTRAM™ ST-1 is shown below:

Appendix 1 (contd) – Analytical study demonstrating stability of MBM in metalworking fluid emulsion

3.1.1 The bands observed in the above chromatogram before 5 minutes are due to chloroform. Identical bands are seen in the chloroform blank. We believe the two bands seen in the chromatogram to be N,N'-methylenebismorpholine (6.1 minute retention) and N-hydroxymethylmorpholine (13.1 minute retention). See structures below:

3.2 Monitoring the concentration of N,N'-methylenebismorpholine and N-hydroxymethylmorpholine in the aqueous emulsion metalworking fluid versus time was conducted over several months using the procedures above. Samples were stored under static, ambient laboratory conditions (20-25 °C) throughout this time.

3.3 The chart below plots responses (mAU*s/g) from the composite analyses of three aqueous soluble oil metalworking fluids using the previously described normal phase HPLC with post column derivatization method versus time (days). Day zero for the three fluids each had a different starting day: 29 September 2005, 18 October 2005 and 2 November 2005. Each data point was obtained by a separate extraction from the metalworking fluid as in 2.5 (Sample preparation).
Appendix 1 (contd) – Analytical study demonstrating stability of MBM in metalworking fluid emulsion

3.4 The plotted data was regressed in Excel to obtain the regression equation below.

\[ \text{Response, (mAU*s/g)} = 313 - 1.08 \times \text{Time, (days)} \]

Assuming the intercept in the above regression equation (313 mAU*s/g) to be a good estimate for the Response at Time (0 days). Division of the intercept by 2 is an estimate of the Response at \( t_{1/2} \) for ST-1 (157 mAU*s/g). Substituting the Response value at \( t_{1/2} \) into the regression equation and solving for time to obtain an estimate of \( t_{1/2} \). The process was repeated using the 95% confidence levels for the intercept and slope obtained from Excel.

4.0 Conclusions and Discussion

An estimate of the half-life for 1500 mg/L of CONTRAM™ ST-1 in an aqueous soluble oil metalworking fluid was calculated to be between 5.13 and 8.68 months based on the 95% confidence levels as shown below:
The overall RSD is high. The RSD within analysis or day is acceptable. To compensate for the high RSD analyses were always replicated. However, enough data was collected to obtain a statistically significant regression equation. The high RSD is also reflected in the relatively large 95% confidence interval for the ST-1 half-life estimate.

These results also strongly suggest that CONTRAM® ST-1 is protected from rapid decomposition in emulsion type metalworking fluids by its likely residence in the oil micelle. Although this is somewhat speculative, it is a reasonable explanation of the experimental data obtained given that CONTRAM™ ST-1 rapidly decomposes in water at low concentration and has good solubility in many organic solvents.

### Appendix 1 (contd) – Analytical study demonstrating stability of MBM in metalworking fluid emulsion

#### 5.0 References

5.1 Literature references


5.1.2 Nash, T.  *Biochem. J.* 1953, 55, 416-421

5.1.3 Engelhardt, H. Klinkner R.  *Chromatographia*, 1985, 20, 559-565

5.2 All chromatography work was performed by J. Michael Burk, Lubrizol Corporation, Wickliffe, Ohio, USA during the time frame of November 2004 to April 2006.

Appendix 2 – derivation of maximum amount of worker exposure to formaldehyde released from MBM under conditions of reasonably expected use.

The estimated exposure to formaldehyde released from MBM under worst-case conditions of reasonably expected (intended) use (i.e. in a metalworking emulsion) in terms of ppm was calculated using the following equations and assumptions. The MBM concentration in the oil mist was not measured directly but it is reasonable to assume that the oil mist contained the same concentration of MBM as the metalworking fluid (i.e. 1500 ppm or 0.15% w/w) and that this concentration remained constant over the sampling period. The average oil mist concentration during the sampling period was 0.185 mg/m$^3$ (185 ũg/m$^3$). The maximum amount of formaldehyde contained in each molecule of MBM is 16% and it follows that the maximum amount of formaldehyde contained in the oil mist would be (185 ũg/m$^3$ x 0.15% x 16%) = 0.0444 ũg/m$^3$. This converts to 0.036 ppm (36 ppb) using the following equations and where gram molecular weight of formaldehyde is 30.03 g/mol.
The American Conference of Industrial Hygienists booklet "Threshold Limit Values (TLVs™) for Chemical Substances and Physical Agents and Biological Exposure Indices (BEIs™)" uses the conversion formulas:

\[
\text{TLV in mg/m}^3 = \frac{(\text{gram molecular weight of substance}) \times (\text{TLV in ppm})}{24.45}
\]

\[
\text{TLV in ppm} = \frac{24.45 \times (\text{TLV in mg/m}^3)}{(\text{gram molecular weight of substance})}
\]

These formulas are appropriate for converting between ppm and mg/m\(^3\) for gases when measurements are taken at 25 °C and the air pressure is 760 torr (= 1 atmosphere or 760 mm Hg). Adjustments are usually made for deviations in temperature and pressure at the workplace/site of measurement but for the purposes of this illustration standard temperature and pressure are considered to be adequate and realistic. The number 24.45 in the equations above is the volume (liters) of a mole (gram molecular weight) of a gas or vapour when the pressure is at 1 atmosphere (760 torr or 760 mm Hg) and at 25°C.