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Agreed

APPLICATION FOR AUTHORISATION: ESTABLISHING A REFERENCE DOSE RESPONSE RELATIONSHIP FOR CARCINOGENICITY OF TECHNICAL MDA

Background

At the 22nd meeting of the Committee for Risk Assessment (RAC) in September 2012, the ECHA Secretariat presented a proposal to set DNELs/DMELs and dose response relationships for substances prior to receiving applications for authorisation (AfAs). This was initially approved by RAC as a trial exercise. However, in early 2015, ECHA agreed to continue supporting the practise for Annex XIV substances, recognising its value to the Authorisation process and its efficiency[1].

The DNELs/DMELs and dose response relationships so derived will serve as a non-legally binding 'reference value'. They provide applicants with a clear signal as to how RAC is likely to evaluate these important elements of the risk assessment of AfA.

This initiative is intended to improve the efficiency of the AfA process as a whole by discussing and when possible publishing reference values such as DNEL's or dose response relationships in advance of applications, so providing greater consistency and better use of the legally defined periods of opinion-development in the RAC.

Requested action:

Following the Committee's agreement on the document, it will be published on the ECHA website.

Annex 1: Reference dose response relationship for carcinogenicity of Technical MDA

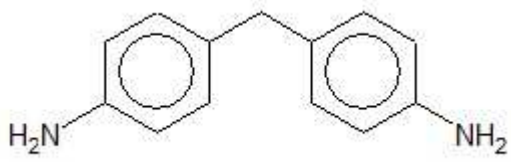
[1] At the Conference on "Lessons learnt on Applications for Authorisation" co-organised by ECHA and the European Commission that took place on 10-11 February 2015.

Annex 1 Reference dose response relationship for carcinogenicity of Technical MDA

Formaldehyde, oligomeric reaction products with aniline (also known as poly[(aminophenyl)methyl]aniline, polymeric MDA, PMDA or technical MDA; CAS RN: 25214-70-4; EC Number: 500-036-1) is a mixture, predominantly containing 4,4'- methylenedianiline (MDA), higher oligomers of MDA, and the isomers 2,4'-MDA and 2,2'-MDA (Table 3.1; OECD, 2002; ECHA, 2014; ECHA, 2011). **It is included in Annex XIV of REACH "List of substances subject to authorisation".**

Table.1 Composition of a typical standard product of technical MDA

Constituent	% w/w
4,4'-Methylenedianiline (MDA)	47 - <65
Higher oligomers of MDA (tri- and polynuclear amines)	~38.4 - <65
2,4'-MDA	<1.4 - ~10
2,2'-MDA	~0.2 – 3
Water	<1



Structure of 4,4'-MDA

Risk assessment of Technical MDA and 4,4'-MDA data

Very limited data are available specifically on technical MDA. In the REACH dossier for technical MDA, pure MDA is frequently used as a read-across substance in view of this lack of data (ECHA, 2014). In the Chemical Safety Reports (CSR) for this REACH dossier, several justifications are given for this read-across (Air Products (Chemicals) PLC, 2010; Air Products (Chemicals) PLC, 2013). The CSR states that since 4,4'-MDA is the main constituent of both pure MDA and technical MDA, the toxicological properties of the incompletely tested technical MDA can therefore be extrapolated from 4,4'-MDA based on a worst case consideration. The report also mentions the fact that studies have shown technical MDA to be of lower acute toxicity to experimental animals than 4,4'-MDA (data not reported here) and higher oligomers to be better tolerated in a chronic subcutaneous study compared to 4,4'-MDA. Therefore, in

light of this, information on pure MDA is also included in this review, and the form of MDA used for each study is specified where this information is available.

The main toxicity described for 4,4'-MDA is hepatotoxicity, carcinogenicity and sensitisation and this appears to be due, at least partly to the functional diamine two-ring structure in the 4,4'- position. This function still exists to some extent in the higher oligomers present in technical MDA.

There are two options that can be considered in assessing the risk of technical MDA.

Firstly, the amount of 4,4'-MDA in technical MDA (47-<65%; see Table 1) could be taken into consideration in a quantitative risk assessment of technical MDA. The higher oligomers, which form nearly all of the remainder of technical MDA, have a higher molecular weight and are likely to be less easily absorbed and taken up by cells. Therefore, they are likely to be less toxic than 4,4'-MDA from their toxicokinetics without taking into consideration toxicodynamic effects. There are a little data (outlined above) to show that technical MDA is less toxic than 4,4'-MDA. The option of taking only 4,4'-MDA toxicity into consideration and correcting for its presence in technical MDA would have an effect of about 2 or less on the risk estimates.

The second option is to base the risk estimates of technical MDA entirely on the toxicity of 4,4'-MDA. The first option considers that the higher oligomers are not toxic; however, they also possess the functional diamine two-ring structure in the 4,4'- position to some extent and are likely to possess similar toxicity to some extent. Therefore, consideration of the toxicity of only 4,4'-MDA as in the first option might lead to an underestimate of the toxicity of technical MDA. So, while assessment of the toxicity of 4,4'-MDA might be considered a pragmatic approach to the risk assessment of technical MDA as it has been the target of the toxicity studies, it is a precautionary approach to consider 4,4'-MDA as a surrogate, as other components of technical MDA are likely to have similar toxicity if less potent.

A further problem in assessing the toxicity of technical MDA according to the proportion of 4,4'-MDA toxicity is that the composition of the 4,4'-MDA and the higher oligomers varies and so any proportionality of toxic response would be difficult to ascertain unless the product was more strictly defined.

In conclusion, the second option using the toxicity of 4,4'-MDA for estimating the risk of technical MDA has been used in this risk estimate as the most precautionary and pragmatic approach.

Relevance of endpoints

For applicants applying for authorisation under Article 60(2) (adequate control route), in order to conclude whether the adequate control is demonstrated, only endpoints (i.e. properties of concern) for which the substance is included in Annex XIV need to be addressed in the hazard assessment[2]. However, information on other endpoints might be necessary for comparing the risks with the alternatives.

For applicants aiming at authorisation based on Article 60(4) (socio-economic analysis route) Article 62(4)(d) also applies and the socio-economic analysis (SEA) route will as a consequence focus on the risks that are related to the intrinsic properties specified in Annex XIV. The SEA should in turn consider the impacts related to such risks. In practice the applicant is expected to provide this information in their (Chemical Safety Report) CSR for which an update may be advisable. However, for an authorisation to be granted, the applicant

[2] Article 60(2) states "…an authorisation shall be granted if the risk to human health or the environment from the use of the substance arising from **intrinsic properties specified in Annex XIV** is adequately controlled."

should also demonstrate that there are no suitable alternatives. In this latter analysis it may be the case that other endpoints than those for which the substance was listed in 'Annex XIV' become relevant in order to demonstrate that no suitable alternative is available.

MDA was included on Annex XIV due to its carcinogenic properties. The reference dose response relationships proposed in the present document are only based on carcinogenicity arising from MDA exposure[3].

Carcinogenicity

Table 2 below provides an overview of expert assessments on the carcinogenic mode of action, the assumed carcinogenic mechanism and the low-dose extrapolation approaches that were used:

[3] Endpoints relevant to the authorisation are also discussed in section 5 of the document: "How RAC and SEAC intend to evaluate the applications" (common approach of RAC and SEAC in opinion development on applications for authorisation, agreed RAC-20/SEAC14, 24/03/2012). Link: http://echa.europa.eu/web/guest/applying-for-authorisation/additional-information

Table 2 Overview of the findings of Expert assessments on the carcinogenic mode of action of MDA

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
IARC (1986)	Not addressed	Not addressed	No data on humans available Sufficient evidence in experimental animals of carcinogenicity – the main target tissues were liver and thyroid sufficient evidence in short-term tests for genetic activity	not addressed
ATSDR (1998)	Non-genotoxic mechanism deemed likely for both liver and thyroid carcinogenicity	Not addressed	Oral: NTP chronic drinking water studies in F344 rats and B6C3F1 mice. Lamb *et al* (1986); NTP (1983) • Increased incidence of neoplastic nodules in the liver (M rats) • Malignant lymphoma and adenoma / carcinoma of the liver (F mice) Dermal: 104-week study in C3Hf/Bd mice. Holland *et al* (1987) • Increased incidence of hepatic tumours	**CELs** = 9 mg/kg bw (oral; M rats) 19 mg/kg bw (oral; F mice) 5.3 mg/kg bw (dermal; F mice)
Dybing *et al.*, (1997)	Not addressed	Non-threshold	NTP chronic drinking water studies in F344 rats and B6C3F1 mice	**T25** = 8.4 mg/kg bw/day value derived for MDA dihydrochloride (EU, 2001)

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
EU (2001) OECD (2002)	Genotoxic mechanism assumed	Non-threshold assumed linear dose response cannot be excluded	NTP chronic drinking water studies in F344 rats and B6C3F1 mice	**$T25_{MDA}$** = 6.2 mg/kg bw/day (based on T25 derived for MDA dihydrochloride by Dybing *et al.*) **WORKERS:** **inhalation:** **modified T25** **(human, inhalation, workplace time schedule)** = 12 mg/m^3 **dermal:** **modified T25** **(human, dermal, workplace time schedule** = >250 mg/person/day **CONSUMERS:** Exposure is not expected
Norway FSA (2006)	Genotoxic mechanism assumed, in the absence of evidence for chronic tissue-damaging (liver) and tissue-stimulating(thyroid) mechanisms of carcinogenicity	Non-threshold linear approach	Weisburger *et al* (1984); Lamb *et al* (1986), NTP (1983) most sensitive endpoint in chronic NTP drinking water studies: • Neoplastic hepatic nodules in male F344 rats	**BMDL10** = 2.33 mg/kg bw/day (4,4'-MDA dihydrochloride); reported to be 1.7 mg/kg bw/day (4,4'-MDA base) **T25** = 8.33 mg/kg bw/day (4,4'-MDA dihydrochloride); reported to be 6.1 mg/kg bw/day (4,4'-MDA base) **hT25** = 1.7 mg/kg bw/day (linear extrapolation based on T25) **hT100** = 6.8 mg/kg bw/day human cancer risk = (based on hT100) 2.3 x10^{-3}
NSF (2009)	May have both genotoxic and non-genotoxic modes of action, though the relative importance of the modes *in vivo* has not been elucidated	Thyroid: multiple models used • non-threshold, low-dose linearity (genotoxic	NTP chronic drinking water studies in F344 rats and B6C3F1 mice NTP (1983) • combined incidence of hepatocellular adenomas	**RSD** = 0.000024 mg/kg bw/day (derived for a cancer risk level of 10^{-5}) Based on a **BMDL10** = 0.67 mg/kg bw/day

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
		mechanism) • non-linear (non-genotoxic mechanism) Liver: non-threshold, linear	and carcinomas in female mice selected as the critical endpoint	
AGS (2010) cited in ECHA (2011)	Mechanism not clear; genotoxic or non-genotoxic mechanism could be assumed. Genotoxic mode of action assumed, in order to be conservative	Non-threshold linear approach	Weisburger *et al* (1984); Lamb *et al* (1986) most sensitive endpoint in chronic NTP drinking water studies: • neoplastic nodes / carcinomas in the liver of male F344 rats	**hT25** (point of departure) = 45.7 mg/m^3 Acceptance risk (4:10 000; inhalation) = 73 µg/m^3 Acceptance risk after 2013 at the latest 2018 (4:100 000; inhalation) = 7.3 µg/m^3 Modified acceptance risk (dermal) = 10 µg/kg bw/day
Air Products (Chemicals) PLC (2010); Air Products (Chemicals) PLC (2013)	Genotoxic and/or secondary mechanisms (e.g. thyroid stimulation following glucuronidation in the liver) can be postulated; genotoxic mechanism assumed in order to be conservative	linear approach	NTP chronic drinking water studies in F344 rats and B6C3F1 mice Weisburger *et al* (1984); Lamb *et al* (1986), NTP, 1983 • carcinogenic for both species, producing liver and thyroid tumours • most critical endpoint identified as neoplastic liver nodules in male rats • LOAEL of 9 mg/kg bw/day used for DMEL calculation	**Long-term exposure – systemic effects** **dermal:** **T25$_{oral, rat}$** = 9.375 mg/kg bw **T25$_{dermal, rat}$** = 18.75 mg/kg bw (assuming 100% oral bioavailability and 50% dermal bioavailability) AF = 12 500 Correction factor = 2.8 (to account for differences in worker and experimental exposure conditions) **DMEL** = 4.2 µg/kg bw **inhalation:**

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
				$T25_{inhalation, human}$ = 16.5 mg/m^3 AF = 3125 Correction factor = 2.8 (to account for differences in worker and experimental exposure conditions) **DMEL** = 14.8 µg/m^3

BMDL10: the lower confidence limit on the benchmark dose associated with a 10% response.
CEL: Cancer Effect Level – the lowest dose that produces significant increases in the incidence of cancer (or tumours) between the exposed population and the control.
DMEL: Derived Minimum Effect Level.
F: Female.
hT25: the equivalent dose corresponding to a 25% tumour incidence in humans (calculated from the T25).
M: Male.
RSD: Risk specific dose.
T25: the dose corresponding to a 25% tumour incidence.

Mechanism of action

In chronic studies the liver and thyroid appear to be the main target organs, with tumours observed in both rats and mice orally administered MDA (as its dihydrochloride) in drinking water. The mechanism for tumour formation is not completely understood but there are currently several proposed hypotheses.

Firstly, a genotoxic mechanism has been postulated for liver carcinogenicity, where a reactive metabolic intermediate of MDA binds to cell macromolecules, including DNA. The metabolism of MDA is reported to consist of both N-acetylation and N-hydroxylation. There is a large body of evidence for the N-acetylation of MDA, including the detection of N-acetylated metabolites in the urine of both experimental animals and occupationally exposed workers. N-acetylation is generally thought to represent a detoxification pathway, since the metabolites, N-acetyl-MDA and N,N'-diacetyl-MDA are reported to be non-mutagenic (Cocker *et al*., 1986; Tanaka *et al*., 1985). It is thought that MDA also undergoes N hydroxylation, where it is considered that the N-hydroxylation reaction can potentially lead to the formation of toxic intermediates (EU, 2001; ATSDR, 1998; Chen *et al*., 2008). In particular, many of the toxic properties of MDA have been attributed to N-hydroxy-MDA, which is reported to occur due to the enzymatic oxidation of MDA. In support of this mechanism, the results from genotoxicity studies with MDA indicate a weak genotoxic potential, particularly at high doses and with metabolic activation, and DNA- and haemoglobin-adducts have also been detected in both experimental animals and humans (EU, 2001). This genotoxic mechanism, involving DNA binding, is frequently assumed to have no threshold for tumour formation, although other processes involved, such as the formation of the intermediate, may have a threshold.

Additional evidence for the involvement of metabolic activation in the mechanism of MDA toxicity is provided by reports that the toxicity of MDA may be dependent on acetylator phenotype. Genetic polymorphisms have been identified, in both experimental animals and humans, in the gene that codes for N-acetyltransferase 2 (NAT2), an enzyme that catalyses the N-acetylation of aromatic amines. In an *in vivo* study in which strains of rat with fast and slow acetylator phenotypes were orally administered MDA, liver damage was reported to be more severe in fast acetylators compared to slow acetylators (Zhang *et al*., 2006; AGS, 2010). Although intuitively a fast acetylator would be associated with an increased detoxification capacity, for diamines (such as MDA) N-acetylation has been suggested to actually enhance oxidation of the second amine group, leading to the formation of the toxic metabolites and increasing the risk of toxicity in those with NAT2 fast acetylator phenotypes (Zhang *et al*., 2006; AGS, 2010). For other diamines, such as benzidine, the NAT2 slow acetylator phenotype has been associated with having a protective effect on bladder cancer in humans (Carreón *et al*., 2006).

A non-genotoxic mechanism has also been proposed, where tumour formation is due to chronic tissue damage (liver) or tissue stimulation (thyroid). Tumour initiation in the thyroid has been hypothesised to partially result from hyper-secretion of thyroid stimulating hormone (TSH). A decrease in T4 and T3 is thought to possibly trigger secretion of TSH, which can then induce thyroid hyperplasia (ATSDR, 1998). The formation of goitres with MDA treatment has also been suggested to support a non-genotoxic mechanism (Lamb *et al*., 1986; ATSDR, 1998).

The formation of the thyroid and liver tumours in two species (rats and mice), and in both males and females of each species in the chronic oral studies, could be interpreted as being more indicative of genotoxic action than a non-genotoxic mechanism, although it is not conclusive. Many risk assessments of MDA have been conducted, and the majority of these have assumed a genotoxic mechanism, taking a precautionary approach in light of the results from genotoxicity studies. ATSDR, however, concluded that a non-genotoxic mechanism, due to chronic tissue damage (liver) or tissue stimulation (thyroid), is most likely (ATSDR, 1998). In their risk assessment, NSF initially evaluated multiple models where these were based on a genotoxic mechanism for liver tumour formation, and both genotoxic and non-genotoxic mechanisms for thyroid tumour formation. They subsequently selected the model for formation

of liver tumours in female mice (genotoxic mechanism) as the most sensitive system and endpoint (NSF, 2009).

Genotoxicity
The information available indicates that all genotoxicity studies have been conducted with either pure MDA or MDA dihydrochloride, rather than with the technical product (OECD, 2002). However, since the various forms of MDA are structurally similar, the genotoxicity profiles are also likely to be similar.

Mixed results have been reported from both *in vitro* and *in vivo* genotoxicity studies with MDA. The US Agency for Toxic Substances and Disease Registry (ATSDR) concluded that the data show that, with few exceptions, MDA is genotoxic with metabolic activation. They attributed these genotoxic properties to the formation of a reactive metabolite formed by N-hydroxylation (ATSDR, 1998). The EU and OECD reported that high doses of MDA led to slight increases in micronuclei and DNA fragmentation *in vivo*, with weak or negative effects observed in other assays.

The weight of evidence in the genotoxicity studies suggests that it should be considered as a genotoxic chemical.

Animal studies
Several studies have shown the occurrence of bladder cancer in workers occupationally exposed to MDA (no information available on the form of MDA). This is consistent with the reported occurrence of low incidences of urinary bladder tumours in female rats in the 2-year carcinogenicity study, where these tumours were considered to possibly be related to MDA exposure since they are very rare in untreated animals. However, there are several difficulties in interpreting the results of these human studies, due to the limited quality of the data and potential confounding factors (such as exposure to other chemicals), and in their risk assessment report the EU stated that no clear conclusion could be drawn regarding carcinogenicity in humans (EU, 2001).

In 1986, the International Agency for Research on Cancer (IARC) classified MDA as Group 2B (possibly carcinogenic to humans) (IARC, 1986). This classification is on the basis that chronic studies in rats and mice showed MDA treatment via the oral route to be associated with thyroid and liver tumours, but there is a lack of clear evidence of carcinogenicity in humans.

It should be noted that no studies were located that specifically use technical MDA. The studies in experimental animals that are considered to be the most robust are those conducted by the US National Toxicology Program (NTP) using MDA dihydrochloride. Epidemiology studies in humans do not state to which form of MDA the study participants were exposed. The Annex XV Dossier for technical MDA states that the structurally similar compound, 4,4'-diaminodiphenylmethane (4,4'-MDA) has been identified as carcinogenic: Carc. 1B (H 350:"May cause cancer."). The dossier states that 4,4'-MDA is a major constituent of the UVCB substance formaldehyde, oligomeric reaction products with aniline (technical MDA) and therefore the classification for 4,4'-MDA applies also for this UVCB substance. In addition, the REACH registration dossier for technical MDA presents the chronic NTP study using MDA dihydrochloride for use as read across. Therefore, it seems reasonable to include data from these studies using other forms of MDA in this section.

The most critical studies for quantitative risk assessment are the oral, long-term, 2-year drinking water studies conducted on F344 rats and B6C3F1 mice as part of the NTP programme. MDA treatment led to both thyroid and liver tumours in these studies. However, the liver tumours are more likely to be caused by a genotoxic mechanism than the thyroid tumours, for which there are potentially plausible non-genotoxic mechanisms based on hormonal disruption due to liver damage. Therefore, authoritative evaluations of MDA have concentrated on the frequency of liver tumours detected in these studies with the combined neoplastic nodules and carcinoma in male rats being the most common endpoint for risk

assessment. The frequency of these hepatic nodules and carcinoma in MDA-treated male rats are the target genotoxicity for this review.

From the NTP long-term study on F344 rats administered MDA dihydrochloride in drinking water (NTP, 1983), the drinking water concentrations of 0, 150 and 300 mg/l, have been converted to total dose per body weight. The incidence of total liver tumours is outlined in Table 3. Of the total liver tumours, 12/50 are hepatic nodules and 1/50 hepatocellular carcinoma.

Table 3 Tumour incidence for total liver tumours in F344 rats (NTP, 1983)

Doses (mg/l)	0	150	300
Ingested Dose (mg/kg bw/day)	**0**	**9**	**16**
Total Tumours/animals	**1/50**	**13/50**	**25/50**
Incidence	**0.02**	**0.26**	**0.50**

Human studies

The CSR did not review epidemiological studies on MDA. The extended follow-up to the Epping Jaundice outbreak, which was due to the ingestion of contaminated bread, found no association between ingestion of MDA and mortality. There was also no evidence of an association with overall risk or bladder cancer in power generator workers potentially exposed to MDA (there was one bladder cancer in an unexposed sub-cohort). Of ten MDA-exposed workers who had developed jaundice, one developed bladder cancer. In two studies on workers occupationally exposed to epoxy resins, there was an excess of bladder cancers. Although there were a number of confounders and multiple chemicals present in these studies, MDA was implicated mainly due to structural similarity to other aromatic amines that cause bladder cancer (Cragle *et al*., 1992).

Reviews of these studies indicate that they are not suitable for quantitative risk assessment due to a lack of detailed exposure dose and time, and the potential for multiple chemical exposures. However, there is a some suggestion of an association with cancer, particularly bladder, and this, together with the experimental animal and genotoxicity data, would suggest that a non-threshold approach to risk assessment might be the most precautionary. Bladder cancers were not observed in MDA-treated animals except for a low incidence in female rats. However, different sites of tumours are often seen in animals and humans treated with the same chemicals, although obviously at very different doses.

Bioavailability

It is considered that the primary route of human exposure to MDA is dermal exposure, followed by inhalation exposure, during its manufacture and use as an intermediate in occupational settings.

Oral

No studies were located in which absorption via the oral route was specifically studied or quantified in humans or experimental animals. However, the oral bioavailability of MDA is expected to be high based on its water solubility and log Kow (NSF, 2009). A water solubility of 1.0-1.25 g/l at 20°C has been reported for 4,4'-MDA (NSF, 2009) and water solubilities of 0.36 g/l (20°C, pH 7.1-7.1, 1 g test substance/1 litre of water) and 1.22 g/l (20°C, pH 7.5-7.6,

10 g test substance/1 litre of water) have been reported for technical MDA (ECHA, 2011; ECHA, 2014). Partition coefficients (log Kow) of 1.59 (temperature not reported) and 2.5 (23°C) have been reported for 4,4'-MDA and technical MDA, respectively (NSF, 2009; ECHA, 2011).

Absorption in humans can also be inferred from the observation of adverse health effects in humans following accidental poisoning with MDA in the "Epping Jaundice" incident and in the many experimental animal studies in which MDA has been administered orally (ATSDR, 1998). In addition, in one study where rats were administered a single oral dose of MDA, MDA metabolites were detected in the urine, providing further evidence of initial absorption (Tanaka et al., 1985; ATSDR, 1998).

In the absence of any specific data on oral absorption, the physicochemical data suggest that oral bioavailability is expected to be high and there is evidence that it is absorbed in humans. Expert opinion suggests that oral absorption is likely to be higher than absorption through the skin for which there is evidence for 50% absorption. This being the case, oral absorption of 100% is used in the cancer risk estimates.

Dermal

In a patch test on the forearm of five male volunteers, approximately 28% of a dose of MDA in isopropanol was absorbed, where the original doses were reported to be 0.75-2.25 µmol and application was for 1 hour (Brunmark *et al*., 1995; ATSDR, 1998; EU, 2001).

Dermal absorption in humans can also be inferred from studies of workers exposed to MDA primarily by the dermal route (although in many case, exposure via inhalation may also occur). Adverse health effects have been reported in these exposed workers (ATSDR, 1998), and MDA and/or metabolites have been detected in the urine (quantitative data not available) (Cocker *et al*., 1986, 1994; ATSDR, 1998).

Application of MDA (17.7-40.6 µg/cm^2 in ethanol; form not stated) to unoccluded rat and human skin *in vitro* resulted in 6.1% and 13.0% absorption, respectively, after 72 hours. Higher absorption was observed under occluded conditions, with 13.3% and 33% absorption reported for rat and human skin, respectively (Hotchkiss *et al*., 1993; EU, 2001). This study suggests that absorption through human skin may be higher than through rat skin. However, another *in vitro* study found no significant difference between absorption through rat and human skin at three different doses (0.01, 0.1 and 1 mg per skin membrane of 0.32 cm2; MDA form not stated) (Kenyon *et al*., 2004).

Application of MDA (form not stated) to hairless mouse skin (0.9 cm^2) in vitro in an aqueous solution at a concentration of 1000 µg/cm^2 resulted in cumulative absorption of 240 µg. When methanol or acetone was used as the solvent, a solution of 600 µg/cm^2 resulted in cumulative absorptions of 80 and 35 µg, respectively (Hinz *et al*., 1991; EC, 2000).

In an *in vivo* study, topical administration of ^{14}C-MDA (4,4'-MDA) to male rats, guinea pigs and monkeys at doses of 2 or 20 mg/kg bw resulted in dose-dependent absorption in rats and guinea pigs, with evidence that the process was saturable (no data available on adsorption in monkeys). In both rats and guinea pigs, a lower percentage of the dose was absorbed following administration of the high dose. However, in rats the total amount absorbed (~0.225 mg/animal) was the same after both doses, but in guinea pigs twice as much material was absorbed following the higher dose (El-Hawari *et al*., 1986; EU, 2001). The highest absorption seen in Fischer rats was 53% (El-Hawari *et al.,* 1986).

There are a number of studies on dermal absorption giving a range of results. The highest absorption observed was just over 50% in rats, which is the value that will be used in the risk estimates.

Inhalation

No studies were located in which inhalation absorption was specifically studied or quantified in humans or experimental animals. However, absorption in humans can be inferred from the detection of MDA in the urine of workers exposed to MDA via inhalation (Cocker *et al*., 1994; Schütze *et al*., 1995; ATSDR, 1998). Similarly, in experimental animals inhalation absorption can be inferred from the detection of retinal lesions (attributed to the test compound) in guinea pigs following nose-only aerosol exposure to MDA intermittently for 2 weeks (Leong *et al.*, 1987; ATSDR, 1998).

In the absence of any specific data on absorption by inhalation, the REACH Guidance suggests a default value of 100% and this will be used in the risk estimates.

Carcinogenicity risk assessment

Oral

The T25 value for MDA has been derived by a number of authoritative bodies (see Table 2) using information from the NTP long-term study on F344 rats administered MDA dihydrochloride in drinking water (NTP, 1983).

- lowest dose with a significant increased frequency (C) of 9 mg MDA base/kg bw/day
- incidence at C, 13 tumours in 50 animals, 0.26
- control incidence, 1 tumour in 50 animals, 0.02

T25 is derived using the following calculation:

C x (Reference incidence 0.25)/(incidence at C – control incidence) x (1-control incidence)/1

This value is also corrected for a study duration of 103 weeks rather than the standard 104 weeks.

$$\mathbf{T25_{(oral,\ rat)}} = \mathbf{9 \times 0.25/(0.26–0.02) \times (1–0.02)/1 \times 103/104}$$

$$\mathbf{= 9.01\ mg/kg\ bw/day}$$

This calculation results in **$T25_{(oral,\ rat)}$ of 9.01 mg/kg bw/day** and this value is used as the PoD for the derivation of route-specific risk estimates for workers and the general population.

A number of other T25s have been derived giving slightly lower values (see Table 2) and mostly based on the value derived by Dybing *et al*. (1997) as an example in the original paper on T25, with some adjustment for the use of MDA dihydrochloride or MDA base. The origin of the data used in Dybing *et al.* (1997) for MDA was not attributed and is unclear.

An oral risk estimate is not set for workers as it is generally taken that this route of exposure is not relevant in the controlled occupational environment.

The following risk estimates have been derived using the following absorption: 100% for inhalation, 100% for oral absorption and 50% for dermal absorption based on published studies.

Workers

Workers inhalation risk estimate

Using the PoD as the $T25_{(oral,\ rat)}$ was corrected for inhalation exposure assuming 100% absorption and correcting for:

- rat oral intake (mg/kg bw/day) to rat inhalation (0.8 l/min/8h); 0.38 m^3/kg bw/8 h
- oral absorption rat/inhalation humans (100/100)
- activity driven difference for workers (standard respiratory volume for humans, 6.7/respiratory volume for workers, 10), the T25 value for human inhalation is as follows:

$$\mathbf{T25_{(Inhalation,\ human)} = 9.01 \times 1/0.38 \times 100/100 \times 6.7/10 = 15.9\ mg/m^3}$$

Correcting for workers' exposure:

- workers' exposure is 5 day/week, 48 weeks/year, 40 years in an average lifespan of 75 years
- Correction factor for workers' exposure of 7/5 x 52/48 x 75/40 = 2.8

$$\mathbf{T25_{(Inhalation,\ workers)} = 15.9 \times 2.8 = 44.5\ mg/m^3}$$

Workers dermal risk estimate

Taking the $T25_{(oral,\ rat)}$ and correcting for:

- dermal exposure of 50% and oral absorption of 100%
- allometric scaling of 4 from rats to humans

$$\mathbf{The\ T25_{(dermal,\ human)} = 9.01/(50/100)/4 = 4.5\ mg/kg\ bw/day}$$

Correcting for workers' exposure as above

$$\mathbf{Therefore\ T25_{(Dermal,\ worker)} = 2.25 \times 2.8 = 12.6\ mg/kg\ bw/day}$$

General population

Oral and inhalation risk estimate have been calculated for the general population.

General population Inhalation risk estimate

$T25_{(oral\ rat)}$ corrected for general population exposure according to the ECHA Chapter R8 guidance:

- allometric scaling for rats to humans, 4,
- human weight, 70 kg,
- human general population breathing, 20 m^3 per person
- 100% oral absorption to 100% absorption by inhalation.

$T25_{(inhalation,\ gen.\ pop.)}$ = 9.01/4 x 70/20 x 100/100 = 7.9 mg/m^3

General population oral risk estimate

$T25_{(oral,\ rat)}$ corrected to $T25_{(oral,\ human)}$ by allometric scaling from rats to humans, 4

$T25_{(oral,\ gen.\ pop.)}$ = 9.01/4 = 2.25 mg/kg bw/day

The cancer risk estimates are summarised in Table 2.

Table 4 Cancer risk estimates for Technical MDA

Route of exposure	Population	T25 Descriptor	Cancer risk for 1 unit amount
Oral	General population	$T25_{(oral,\ gen.\ pop.)}$ 2.25 mg/kg bw/day	**1.1 x 10^{-4} per µg/kg bw/day**
Inhalation	Workers	$T25_{(inhalation,\ worker)}$ 44.5 mg/m^3	**5.6 x 10^{-6} per µg/m^3**
	General population	$T25_{(inhalation,\ gen.\ pop.)}$ 7.9 mg/m^3]	**3.2 x 10^{-5} per µg/m^3**
Dermal	Workers	$T25_{(dermal,\ worker)}$ 12.6 mg/kg bw/day	**1.9 x 10^{-5}per µg/kg bw/day**

Assuming linearity of response the cancer risk for lifetime exposure to each unit amount of technical MDA will increase in proportion, e.g. for workers' exposure by inhalation.

1 µg/m^3	**5.6 x 10^{-6}**
2 µg/m^3	**1.1 x 10^{-5}**
5 µg/m^3	**2.8 x 10^{-5}**
10 µg/m^3	**5.6 x 10^{-5}**

Biomonitoring

The basic principle of this risk assessment is to compare the cancer risk estimate for a internal/systemic dose of the chemical in humans. Often this human exposure is, in itself, an estimate derived from secondary measurements such as air concentrations. A better practice where possible is to measure systemic dose by means of biomonitoring (e.g. via total MDA

concentration in urine). This systemic dose can then be compared with the corresponding cancer risk estimate.

Because of the low vapour pressure and good skin absorption, biomonitoring of MDA is the best way to assess the occupational exposure to MDA. Since the finalization of SCOEL recommendation on MDA one new paper on occupational MDA exposure has been published (Weiss *et al*., 2011). It clearly demonstrates the importance of skin exposure in fiber reinforced laminate technology industry and concludes that the exposure assessment of MDA should be carried out by biological monitoring rather than ambient air monitoring. Urine samples midweek or at the end of the week were recommended based on the observed delay in the excretion of MDA after dermal absorption (Weiss *et al*., 2011).

MDA is analyzed as described in SCOEL (2012) as a sum of free and conjugated 4,4'-diaminodiphenylmethane in urine. When exposure to MDA is through inhalation (as solid material or contaminated dust), peak MDA excretion in urine can be seen in post-shift urine samples whereas in the case of dermal exposure the peak excretion is delayed (Cocker *et al*., 1994, Brunmark *et al*., 1995a, Weiss *et al*., 2011). Therefore, the urine samples for MDA monitoring are recommended to be taken both as post-shift samples (especially when inhalation exposure is dominant) or next morning pre-shift samples (when there is likely to be significant dermal exposure).

Because of the importance of the skin absorption, correlations between air levels and urinary MDA levels have been generally poor, as stated in the SCOEL documentation. However, it has been shown that after a single experimental one hour dermal exposure, urinary excretion of MDA fits well to the first order one compartment model (Brunmark *et al*., 1995a). According to Brunmark *et al.* (1995a), although there was significant variation in excretion kinetics between individuals (2-26%, partly explained by individual acetylation status), the median excreted MDA in urine during 48 h was 16% of the absorbed dose (Brunmark *et al*., 1995a)[4]. The major part (~80-90%, estimated on the basis of figure 3, in Brunmark *et al*., 1995a) of the urinary MDA was eliminated within 24 h. Terminal half-time ($T_{1/2}$) in urine varied between 4.6-11 h.

The absorbed dose per day can be estimated from the urinary concentration of the chemical, if the proportion excreted in the urine is known (e.g. Angerer *et al*., 2011)

$$D = \frac{Css \times V24}{Fue \times BW} \qquad \text{(Formula 1)}$$

where D = absorbed dose (mg/kg body weight) per day, Css = average concentration in the urine, V24 =24-hour volume of urine excreted, Fue = proportion of dose excreted in urine, BW = body weight kg.

However, in practice, V24 and Css are not available when total 24-h urine is not collected. For V24, a default value of 1.7 litres can be used. The relationship between the MDA level in a single sample collected at a specified time (either post-shift or next morning pre-shift sample) and daily average level can be made assuming first-order elimination kinetics. According to this, the level of MDA after exposure is decreasing following the formula

$$Ct = Cp \times e^{-t \times kelim}$$

where Ct = concentration at time point t after the peak concentration; Cp = peak concentration, and K_{elim} = elimination rate constant, = $\ln 2/T_{1/2}$.

[4] The fate of the remaining 84% of the absorbed dose was unspecified. Urinary excretion is the main route in monkeys and rats, while faeces are the principal route of excretion in guinea pigs.

There are some uncertainties related to these calculations:

1) the half-time of MDA which seems to vary between individuals (partly because of the acetylation status)

2) time of the appearance of the peak concentration (C_{max}) after dermal exposure

After inhalation exposure peak concentration appears rapidly, but after dermal exposure it may appear significantly later. In practice, at workplaces, the exposure is usually mixed (i.e. includes both dermal and inhalation exposure).

In the estimations below the following, rather conservative, assumptions have been made based on the experimental work of Brunmark *et al*. (1995a) and supported by the findings of Weiss (2011) and Cocker (1994):

- C_{max} is delayed 6 h because of the slow dermal component
- $T_{1/2}$ is 11 h, the longest value measured
- F_{ue} is 16%

Applying these to first order elimination kinetics model it is estimated that in the steady state, the average C_{ss} concentration is ~70% of the urinary MDA concentration in post-shift sample and ~150% of urinary MDA concentration in next morning pre-shift sample.

Thus, if the level of urinary MDA concentration is 1 µg/l (the typical detection limit for MDA; SCOEL, 2012) in a post-shift specimen at the end of the working week, this corresponds to an internal dose of **0.11 µg/kg bw in post-shift sample**

Using formula 1 from above: D (Daily dose) = 0.7 x 1 µg/l x 1.7 l/(70 kg x 0.16)
= 0.11 µg/kg bw

Similarly, in next morning **pre-shift sample, 0.22 µg/kg bw**.

If air concentration of 1 µg/m^3 (corresponding to an absorbed dose of 0.14 µg/kg bw, if 100% absorption via inhalation (10 m^3/work shift) is assumed) corresponds to a cancer risk of 5.6 x 10^{-6} (derived from the risk estimates above).

Then, assuming linearity of cancer risk with absorbed dose, the urinary level of

1 µg/l in post-shift sample corresponds to a cancer risk of 0.44 x 10^{-5}

1 µg/l in next morning pre-shift sample corresponds to a cancer risk of 0.9 x 10^{-5}

10 µg/l in post-shift sample corresponds to a cancer risk of 0.44 x 10^{-4}

10 µg/l in next morning pre-shift sample corresponds cancer risk of 0.9 x 10^{-4}

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