National Centre for Environmental Toxicology

SR 20 Support for the assessment of remaining risks related to the use of MOCA, MDA and EDC, and DNEL setting for reprotoxic properties of Diglyme

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Formaldehyde, oligomeric reaction products with aniline (technical MDA), 2,2'-Dichloro-4,4'-methylenedianiline (MOCA), 1,2-dichloroethane and bis(2-methoxyethyl)ether (diglyme) have been recommended for inclusion in Annex XIV authorisation list. Chemical companies can then apply for authorisation of a substance for a specific use, which is then assessed by ECHA's scientific committees which may pass on opinions to the Commission to add their decision making. Applications for authorisation are expected in 2015.

To provide greater consistency amongst applications, Derived No Effect Levels (DNELs) and dose response curves for the substances subject to authorisation are determined by the ECHA Risk Assessment Committee (RAC) before applications are received. This will also aid Industry when preparing applications, and for the RAC when evaluating them.

To aid this process in assessing the remaining cancer risks and setting relevant DNELs for relevant exposure routes, this report reviews the toxicokinetics, bioavailability and toxicological information for these chemicals. The report also includes the cancer risk estimates for technical MDA, MOCA and 1,2-dichloroethane, and the DNELs for diglyme agreed by the RAC, together with the calculations which underlie the derivations. For technical MDA and MOCA, concentrations are also suggested to be used in conjunction with biomonitoring.

The assistance and expertise of members of the ECHA RAC is gratefully acknowledged.

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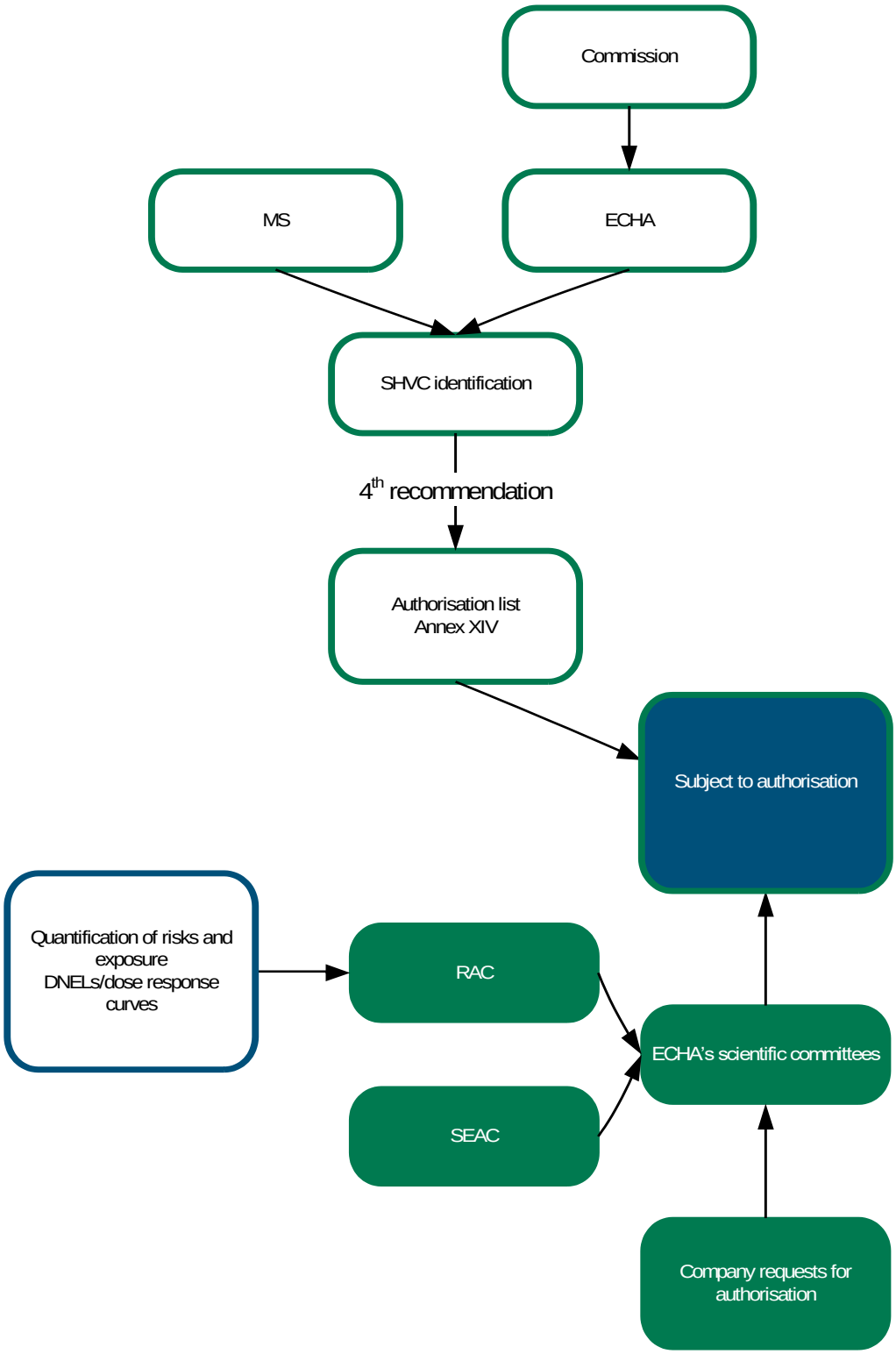
1. Introduction

Under REACH, chemicals may be subject to authorisation, whereby the manufacture of substances that are deemed to pose a threat of excessive harm are limited to individual authorisations for specific uses, whereby the use will not pose a risk due to minimal exposure, or the socio-economic benefits of using the substance outweigh the risks and there are no suitable alternatives. Substances are subject to authorisation via a specific process. Chemical companies can then apply for authorisation of a substance for a specific use, which is then assessed by ECHA's scientific committees which may pass on opinions to the Commission to aid their decision making. This process is outlined in Figure 1.1 below.

Figure 1.1 Authorisation process



The ECHA secretariat have proposed an efficient authorisation process that aims to provide greater consistency amongst applications and better use of the legally defined period of opinion forming in committees. As part of this process, the Derived No Effect Levels (DNELs) and dose response curves for the substances subject to authorisation are to be determined by the Risk Assessment Committee (RAC) before applications are received. This will also aid Industry when preparing applications, and for the RAC when evaluating them.

2,2'-Dichloro-4,4'-methylenedianiline (MOCA), formaldehyde, oligomeric reaction products with aniline (technical MDA), 1,2-dichloroethane and bis(2-methoxyethyl)ether (diglyme) are on the 4th recommendation for inclusion in the Annex XIV authorisation list, and applications for authorisation are expected in 2015.

Table 1.1 outlines the reasons for inclusion. According to ECHA's proposal above, ECHA and its committees, RAC and the Committee for Socio-Economic Analysis (SEAC) require information that will enable them to quantitatively assess the remaining risks on relevant exposure routes for applications for authorisation addressing MOCA, MDA and 1,2-dichloroethane and to set DNELs for diglyme on its toxicity for reproduction, by relevant exposure routes.

This report describes how WRc has assisted ECHA RAC by providing toxicological information on these chemicals and, after discussions the RAC has agreed risk estimates and DNELs. Specifically to:

- Assess the remaining cancer risks related to the use of technical MDA (EC Number: 500-036-1), MOCA (EC Number: 202-918-9) and 1,2-dichloroethane (EC Number: 203-458-1). This includes the review of registration dossiers and the relevant scientific literature related to the carcinogenicity of MOCA, MDA and 1,2-dichloroethane, and in particular previous risk assessments of international or national bodies, seek information related to its mechanism of action, and prepare relevant dose response relationships or other quantitative risk estimates for the substances' carcinogenicity.

- Set relevant DNELs or other risk estimates for the toxicity for reproduction from diglyme's use, by relevant exposure routes. This task includes the review of the registration dossiers and the relevant scientific literature related to the toxicity for reproduction of diglyme, and in particular previous risk assessments of international or national bodies, seek information related to its mode of action, and set DNELs (or prepare other relevant quantitative estimations) for the relevant exposure routes.

These cancer risk estimates and DNELs have been assessed by the RAC and the agreed values are listed in this report together with the calculations which underlie the derivations.

Table 1.1 Reasons for recommendations for inclusion on Annex XIV

Substance	EC Number	Proposed by	Volume of production	Number of sites	Potential for worker exposure	Decision number	Classification
Technical MDA	202-918-9	ECHA	Relatively high - high	High	High	ED/77/2011	Carc 1B
MOCA	500-036-1	ECHA	High	High	High	ED/77/2011	Carc 1B
1,2-Dichloroethane	203-458-1	ECHA	High	Medium	Significant	ED/77/2011	Carc 1B
Diglyme	203-924-4	ECHA	Very high	High	High	ED/77/2011	Repro 1B

Recommendation of the European Chemicals Agency of 17 January 2013 for the inclusion of substances into Annex XIV to REACH (List of substances subject to authorisation).

2. General Methodology

The work programme flow for the carcinogens, formaldehyde, oligomeric reaction products with aniline (technical MDA), 2,2'-Dichloro-4,4'-methylenedianiline (MOCA), and 1,2-dichloroethane is shown in Figure 2.1 and for the reproductive toxin, bis(2-methoxyethyl)ether (diglyme) in Figure 2.2.

Figure 2.1 Carcinogenicity work flow

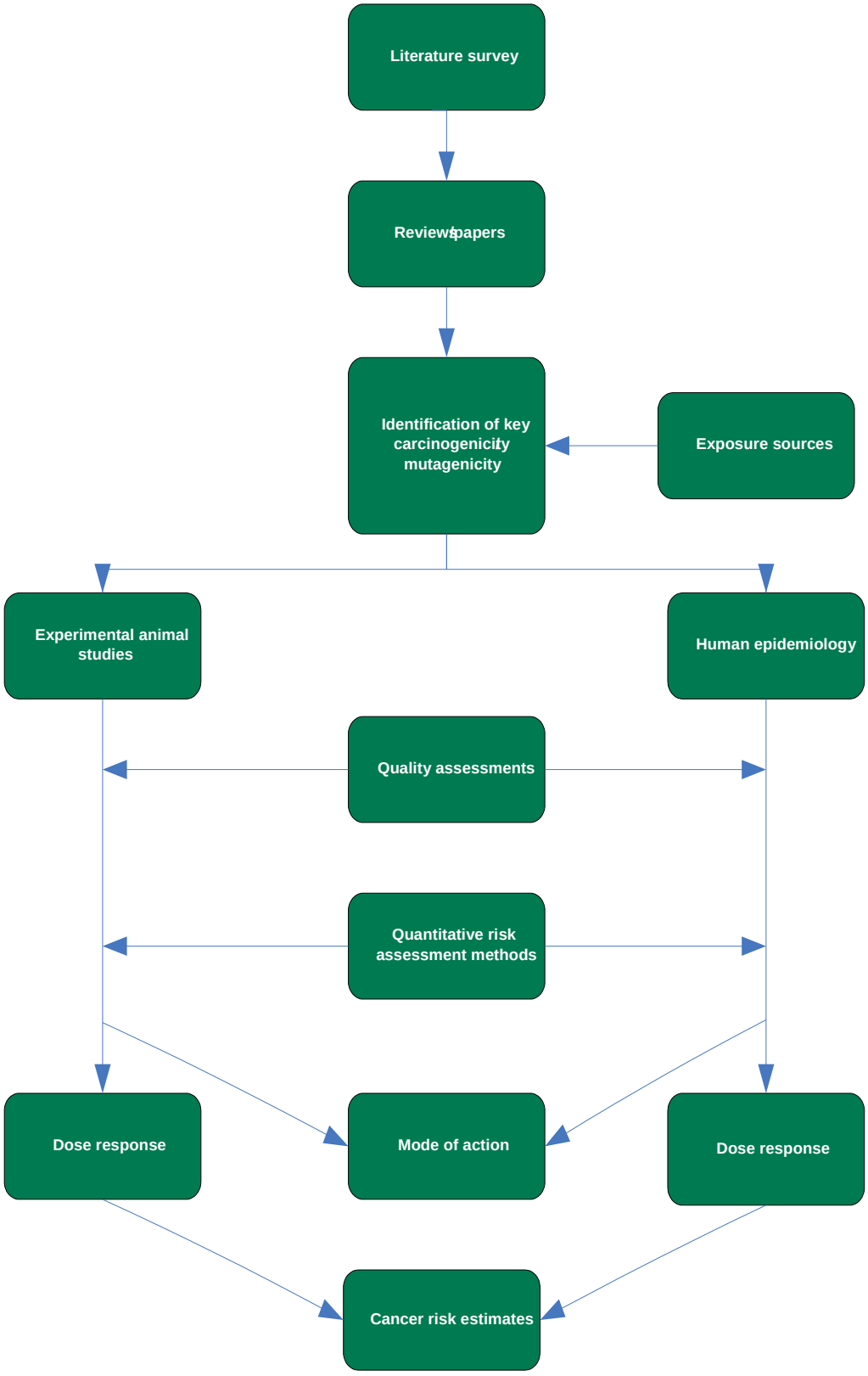


Figure 2.2 Reproductive toxicity work flow

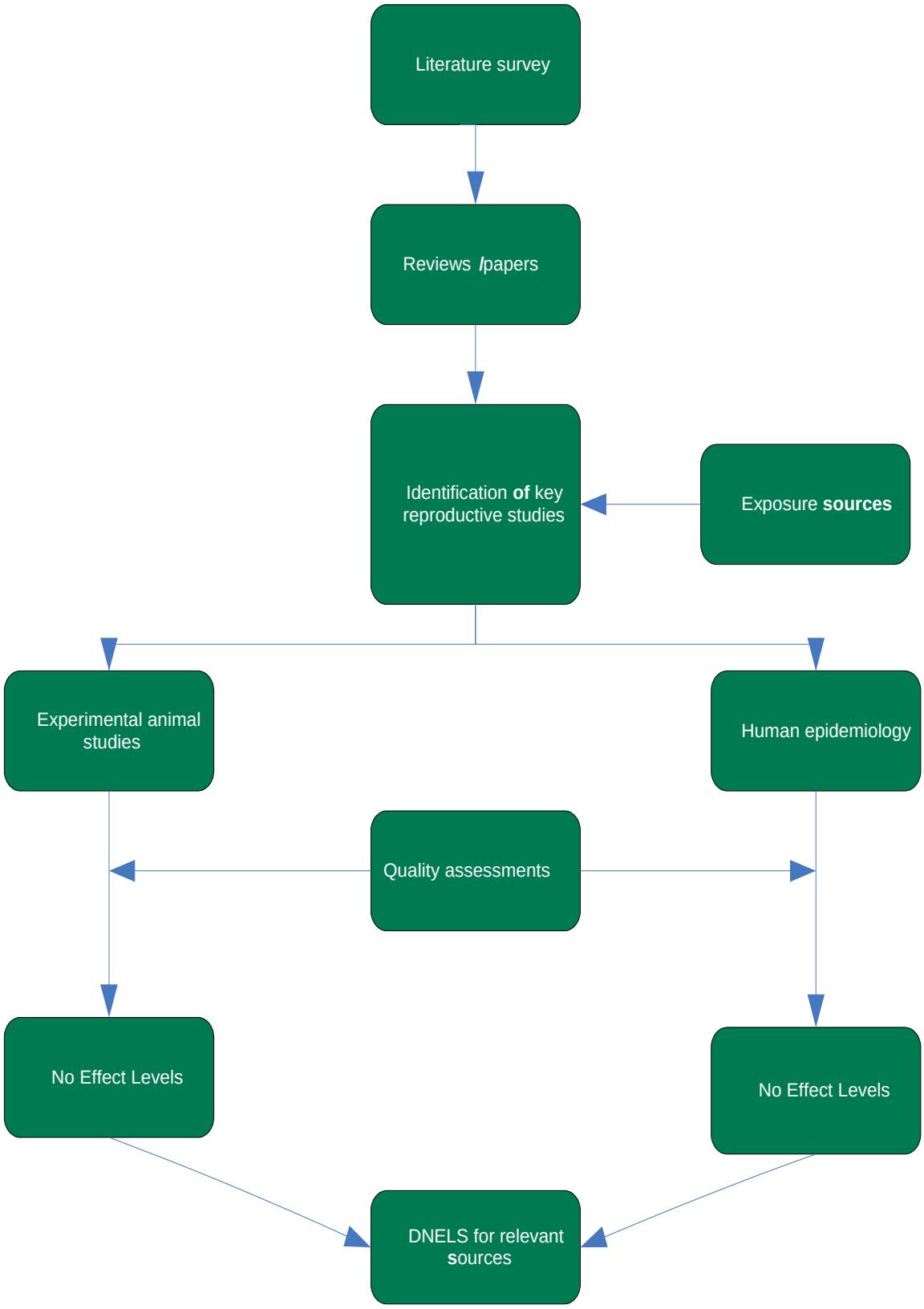


Table 2.1 summarises the nature of the information that has been collated on each of the specific substances during the data searching exercise.

Table 2.1 Information collated on each of the specific substance types

Type of information	Details
Carcinogenicity	Animal and epidemiology studies for MOCA, MDA and EDC
Reproductive toxicity	Animal and epidemiology studies for diglyme
Toxicokinetics	Toxicokinetic data for the substances will be sought to determine bioavailability
Toxicological data relevant to mechanisms of actions	For example, specialist studies on mode of action, chronic repeat dose studies where relevant
Any other relevant information	As relevant
Uncertainties	Identifying data gaps for key information Identifying data which is considered unreliable

For the purposes of this project relevant data sources have been divided into three groups:

- ***Regulatory sources*** – reports prepared by regulatory bodies in Member States, the European Union (including the ESIS and REACH Registration databases), government agencies in Australia, Canada, Japan, and the United States and international bodies such as the International Agency for Research on Cancer (IARC), International Standards Organisation (ISO), the Organisation for Economic Cooperation and Development (OECD) and the World Health Organization (WHO).

- ***Public Domain sources*** – information identified by literature searching of sources such as PubMed, Science Direct and Web of Science using defined search strings for each of the substance types).

- ***Commercial sources*** - The CSRs from the chemical registrants were also consulted.

The key experimental animal and epidemiological studies were identified and the study data obtained as close to the original primary sources as possible (e.g. for experimental animal studies, the original reports from the contract laboratories were obtained if possible).

Data sources for MDA, a similar substance to technical MDA were also sought, as in some instances data are present for both substances within the same review.

2.1 Quality assessment

An initial review of the collated information for coverage, reliability and consistency was carried out which identified the presence of data gaps and identify areas of uncertainty in the available data.

For each substance there were multiple data sources. Therefore, it was necessary to review each dataset for its reliability and to consider how consistent the information is in comparison with that from other sources. The experimental animal studies were assessed for quality and rated according to Klimisch coding (including accordance to OECD guidelines and GLP). Such a quality assessment of the data is vital in ensuring that outputs are robust and to provide confidence in any subsequent analysis. The Klimisch Criteria[1] has been used to assess the reliability of the chemical composition data which will comprise four categories:

1 - Reliable without restrictions, which relates to data generated in studies where a validated and standardised method has been used with no deviation from the defined procedure.

2 - Reliable with restrictions, which relates to data generated in studies where either a validated and standardised method with minor observations from the procedure or a non-standardised method with appropriate quality controls has been used.

3 - Unreliable which relates to data generated in studies where the method used is not considered reliable.

4 - Not assignable which relates to data generated in studies where there is insufficient information on the method used and how it was applied.

The definitions of each Klimisch code have been broken down into separate criteria. The criteria are; guideline compliance (including significant deviations that would need to be incorporated into risk/hazard assessment), Good Laboratory Practice (GLP) status, acceptability of the study with regards to its scientific quality, documentation of the study and detail provided.

Summaries were be made of each key study together with information on the dose response and other details essential for the risk assessment process. All of this information is available so that the underlying data behind all stages of the risk assessment is clearly identifiable. Klimisch classification is not appropriate for data which may be collated from human clinical and/or epidemiological studies based on cancer in humans and so an appropriate measure of quality will be developed for human studies based on a qualitative assessment.

[1] Klimisch, H-J, Andreae, M. and Tillmann, U. (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*, 25, 1–5.

Areas of uncertainty were also identified, i.e. the type of data which is required but for which there is currently an absence of reliable information or available data that is not considered sufficiently reliable to be used as part of regulatory assessments.

The following were major factors considered in the assessment of epidemiological studies:

- Populations – ideally two populations should only differ by exposure to the chemical in question. However, this is seldom possible and bias may be present in the selection of different cases and populations as well as end-point measurement.

- Exposure – This is important for disease with long-term aetiology such as cancer, and information about the level, duration, route and frequency of exposure of one population against another must be accurately assessed. The exact exposure regime is often not known and surrogate measures such as job description may be used. Any use of exposure or biological markers in a study may be useful.

- Confounders – In long-term studies many other factors may be important such as age itself, socioeconomic status, smoking and diet. Multiple exposure to chemicals over a long period may also be difficult to control for.

- Effects – the power of the study must be sufficient to observe an effect, for example, the rarity of the tumour type and the latency of the cancer.

- Statistical considerations – appropriate statistical methods must be applied to ascertain whether any particular association observed between exposure and effect could be expected by chance. Appropriate statistical correction for confounders and bias is also important.

2.2 Key studies

The key studies for the carcinogenic studies were appropriate experimental animal or human epidemiological studies meeting the criteria in terms of relevance and quality outlined above.

2.3 Dose response curves

2.3.1 Carcinogenicity

Quantitative Risk Assessment (QRA)

A number of authoritative bodies (e.g. WHO IPCS, US EPA, German Committee on Hazardous Substances[2]) have suggested methods for cancer risk estimates based on a number of risk assessment methodologies. These authoritative bodies and others have also prepared relevant dose-response curves for quantitative risk assessment from the key experimental animal studies which have been summarised in the literature survey. These have been collated and further studies (particularly human studies) have also been assessed to ascertain whether there is additional robust data upon which to base quantitative risk assessment. The suggested methods are outlined in the next section.

Experimental animal studies

Most of these quantitative methods are based on the derivation of benchmark dose response curves based on a Point of Departure (PoD) concentration, which is at the lower end of the observed results without the requirement for extrapolation to a lower dose. There are a number of descriptors available for this PoD to estimate the cancer potency reflected by the daily dose (expressed as mg/kg body weight) giving a tumour incidence upon lifetime exposure.

These methods necessarily require a definition of a dose needed to induce tumours and a number of approaches have been described. Two examples are: 1) the dose (TD50) required in order the remain tumour-less at the end of a standard life span, and 2) the lowest dose able to induce a statistically significant increase in tumour incidence of x percent (TDx).

The method favoured by the ECHA guidance[3] and others[4] is T25 which is the daily dose (in mg/kg body weight) inducing a tumour incidence of 25% upon lifetime exposure. This is based on an assumption of a linear dose response at all concentrations (including above the experimental doses) excluding the zero dose. The T25 method will be explored for the

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[2] Committee on Hazardous Substances (Ausschuss fur Gefahrstoffe – AGS) (2008) Guide for the quantification of cancer risk figures after exposure to carcinogenic hazardous substances for establishing limit values for the workplace. Germany.

[3] ECHA guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisation of dose (concentration)-response for human health. 2012.

[4] SCHER/SCENIHR/SCCP (2009) Risk assessment methodologies and approaches for genotoxic and carcinogenic substances. Scientific Committee on Health and Environmental Risks/Scientific Committee on Consumer Products/Scientific Committee on Emerging and Newly Identified Health Risks.

experimental animal data on the three carcinogenic chemicals as it has a number of advantages as a primary approach, namely:

- The 25% increase in tumour incidence should be within the range of the experimental data.
- Once the significant increase is derived, the computer modelling is not complex for estimating values from the dose response curves.
- Use of tumour incidence at lower doses reduces problems of mortality in higher dose groups.

Expert assessment also needed to be applied to the experimental data as there are a number of assumptions in this (and any other model system used):

- A linear relationship is assumed.
- The site, species, strain and gender of the tumour activity needs to be considered. For example, multiple routes of exposure, multiple tumour sites, tumours present in multiple species and strains and in both sexes, as well as latency of lesions, metastases, dose-related increases, etc.
- Genotoxicity of the chemical is assumed, or, for example, non-genotoxic mechanisms such as cell proliferation may not be linear and evidence for the presence of a threshold may be supported.
- Mechanism relevant to humans, as certain mechanisms such as liver tumours in rats due to peroxisome proliferation and renal tumours due to α-2μ-globulin neuropathy have been shown not to be relevant in humans.

The T25 or slope (potency) factor (mg/kg body weight/day) can also be expressed as a unit risk for oral and inhalation based on estimated risk of standard exposure to a unit amount of the chemical. This is often expressed as 1 in 10^4, 1 in 10^5 or 1 in 10^6.

As well as T25 method, which is mainly used for potency estimation, BMDL10 has also been used in the assessment of these chemicals by a number of toxicological bodies. BMDL10 is defined as the lower 95% confidence dose of a Benchmark Dose representing a 10% tumour response upon lifetime exposure. BMDL10 is used for the assessment of risk posed by food ingredients by EFSA. In the case of linear or close to linear dose response relationships, the results of the two procedures (T25 and BMDL10) are virtually identical.

There is still debate over the use of all these models and whether they accurately simulate the cancer process in humans and so expert opinion must also play a role in the risk assessment process. For example, the UK Committee on Carcinogenicity (2003)[5] indicated that models

[5] Guidance on a Strategy for the Risk Assessment of Chemical Carcinogens, Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (2003).

may give an impression of precision which cannot be justified by the assumptions and approximation upon which they are based.

Human studies

All the human studies considered to be acceptable based on the quality criteria have been assessed whether they yield positive or negative results, or even suggest protective effects against cancer. Conclusions about the overall evidence for carcinogenicity from human studies have been made and any uncertainties about any associations (such as potential bias and confounders) stated. The quality of the study and the value of the data in the assessment of cancer risk to humans have been made by expert judgement based on the strength of exposure information (and whether it is the only chemical exposure present), the quality of the cancer frequency data and any other potential bias and confounders.

Where necessary, the assessment of the causality of any experimental animal or human studies has made use of the nine Bradford-Hill criteria:

- Consistency of the observed association
- Strength of the observed association
- Specificity of the observed association
- Temporal relationship of the observed association
- Exposure response relationship
- Biological plausibility
- Coherence
- Experimental evidence
- Analogy

Included in the review of the carcinogenic (and reproductive) assessment of these chemicals are a number of other key factors:

- The term “mode of action” is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. A “key event” is an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically-based marker for such an element. Mode of action is contrasted with “mechanism of action”, which implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action.

- The toxicokinetic and bioavailability processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the defined mode of action, but any effects of toxicokinetics have been included in the review.

Genotoxicity is important in the assessing the mechanism of action of these chemicals. Cancer studies over the last 20 years have identified mutational events in genes involved in many key stages in the development of cancer, including cell proliferation, the inhibition of apoptosis and receptor activation/inactivation. Therefore, evidence of direction reaction with DNA is important but there may have also been indirect effects on gene expression and epigenetic mechanisms such as methylation. Other modes of action, such as cytotoxicity with reparative cell proliferation and immune suppression, may indicate potential non-genotoxic modes of action. Any measurement of biomarkers may also yield evidence into modes of actions.

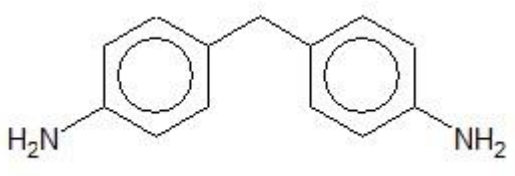
For each of the chemicals the evidence for mechanisms of action were summarised and a hypothesis derived. The strengths and weaknesses of such a hypothesis together with its relevance to humans will be outlined based on the Bradford-Hill criteria outlined above.

2.3.2 Reproductive Toxicity

Derived No Effect Levels (DNELs)

DNELs will be derived according to the ECHA guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisation of dose (concentration)-response for human health. 2012. This will be performed using a step-wise approach outlined in Figure 2.2 previously.

3. 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 reported to be a mixture, predominantly containing 4,4'-methylenedianiline (MDA), higher oligomers of MDA, and the isomers 2,4'-MDA and 2,2'-MDA.

PMDA is synthesized by reaction of formaldehyde and aniline in the presence of hydrochloricacid. All processes produce polymeric MDA (PMDA), which consists of mixtures of isomers and oligomers of MDA. 98% of PMDA is used as a precursor to methylene diphenyldiisocyanate (MDI). Other uses of PMDA are: as a hardener for epoxy resins in adhesives, in the production of rolls with composite cover, production of chemically resistant pipes, production of moulds, in the production of high performance polymers and as a starting point for the synthesis of 4,4'-methylenebis(cyclohexaneamine).

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

Very limited data are available specifically on technical MDA (see Table 3.1). In the REACH dossier for technical MDA, pure MDA (see Table 3.2) 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 3.1) 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. 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 assessment as the most precautionary and pragmatic approach.

Table 3.1 Composition of a typical standard product of technical MDA (OECD, 2002; ECHA, 2011; ECHA, 2014)

Constituent	% w/w	CAS Number	EC Number
4,4'-Methylenedianiline (MDA)	47 - <65	101-77-9	202-974-4
Higher oligomers of MDA (tri- and polynuclear amines)	~38.4 - <65	-	-
2,4'-MDA	<1.4 - ~10	1208-52-2	214-900-8
2,2'-MDA	~0.2 – 3	6582-52-1	229-512-4
Water	<1	7732-18-5	231-791-2
Aniline	<0.1	62-53-3	200-539-3

Table 3.2 Composition of pure MDA

Constituent	% w/w
4,4'-MDA	≥98
2,4'-MDA	≤2
2,2'-MDA	≤2
4-Amino-4'-methylaminodiphenyl methane:	trace
Aniline	trace

MDA has previously been assessed by the US Agency for Toxic Substances and Disease Registry (ATSDR), the European Union (EU), the International Agency for Research on Cancer (IARC), NSF International, the Organisation for Economic Cooperation and Development (OECD) and the US National Toxicology Program (NTP).

3.2 Toxicokinetics

The predominant routes of human exposure to MDA are likely to be dermal, followed by inhalation, during its manufacture and use as an intermediate (ATSDR, 1998; NSF, 2009).

3.2.1 Absorption

There is evidence that MDA is absorbed following oral, dermal and inhalation exposure in both experimental animals and humans (EU, 2001), where in humans the rate of absorption via inhalation may be faster than dermal absorption (ATSDR, 1998).

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*., 1986a, 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 cm^2; 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).

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.

3.2.2 Distribution

Oral

No studies were located which quantified the distribution of MDA to specific tissues following oral exposure in humans or experimental animals. However, the reported cases of liver toxicity resulting from accidental poisoning with MDA in the "Epping Jaundice" incident imply that MDA is distributed to the liver in humans. Based on the locations of adverse effects in experimental animal studies, it can be inferred that MDA (or its metabolites) can be distributed to the liver, kidneys and thyroid following oral exposure (ATSDR, 1998).

Dermal

In a patch test on the forearm of five male volunteers, plasma MDA concentrations reached a peak approximately 3-4 hours after the start of exposure, with a subsequent decline (Brunmark *et al.*, 1995; ATSDR, 1998). No quantitative information was located on distribution of MDA to specific tissues in humans. However, it is likely to distribute to the liver, based on case reports of liver toxicity following dermal exposure of workers (ATSDR, 1998).

In an *in vivo* study, following topical administration of ^{14}C-MDA (4,4'-MDA) at a dose of 2 mg/kg bw, 2% and 1% of the radioactivity was recovered in the tissues of male rats and guinea pigs, respectively, after 96-hour exposure (El-Hawari *et al.*, 1986; EU, 2001). In rats, after exposure for 6 and 24 hours, the highest amounts of radioactivity were detected in the gastrointestinal tract (3.8% and 3%, respectively) and the liver (2% and 1.2%, respectively). After 96 hours, 0.5% of the radioactivity was detected in each tissue, respectively. Overall (on a per gram basis), the tissues with the highest reported radioactivity were the liver, adrenals and kidneys. Preferential accumulation of MDA or its metabolites was not observed in any organ other than the liver (El-Hawari *et al.*, 1986; ATSDR, 1998). In guinea pigs, the highest amounts of radioactivity were again detected in the gastrointestinal tract (1.5% and 2.8%, respectively) and the liver (0.4% and 0.5%, respectively), with 0.5% of the radioactivity detected in each tissue, respectively, after 96 hours. Overall, the organs with the highest reported radioactivity were the adrenal glands, and preferential accumulation of MDA or its metabolites was not observed in any organ (El-Hawari *et al.*, 1986; ATSDR, 1998).

Inhalation

No studies were located which quantified the distribution of MDA to specific tissues following inhalation exposure in humans or experimental animals. However, the detection of retinal lesions in guinea pigs following nose-only aerosol exposure to MDA intermittently for 2 weeks (Leong *et al.*, 1987) implies that MDA or its metabolites can be distributed to the eye following inhalation exposure (ATSDR, 1998).

Other Routes

In an *in vivo* study, ^{14}C-MDA was administered to four male rats and two rabbits (one "slow acetylator" and one "fast acetylator") as a single intraperitoneal dose of 30 or 50 mg/kg bw, respectively. It was reported that the residual radioactivity localised in the liver, kidney, spleen and thyroid after both 24 and 96 hours (Morgott, 1984; EU, 2001).

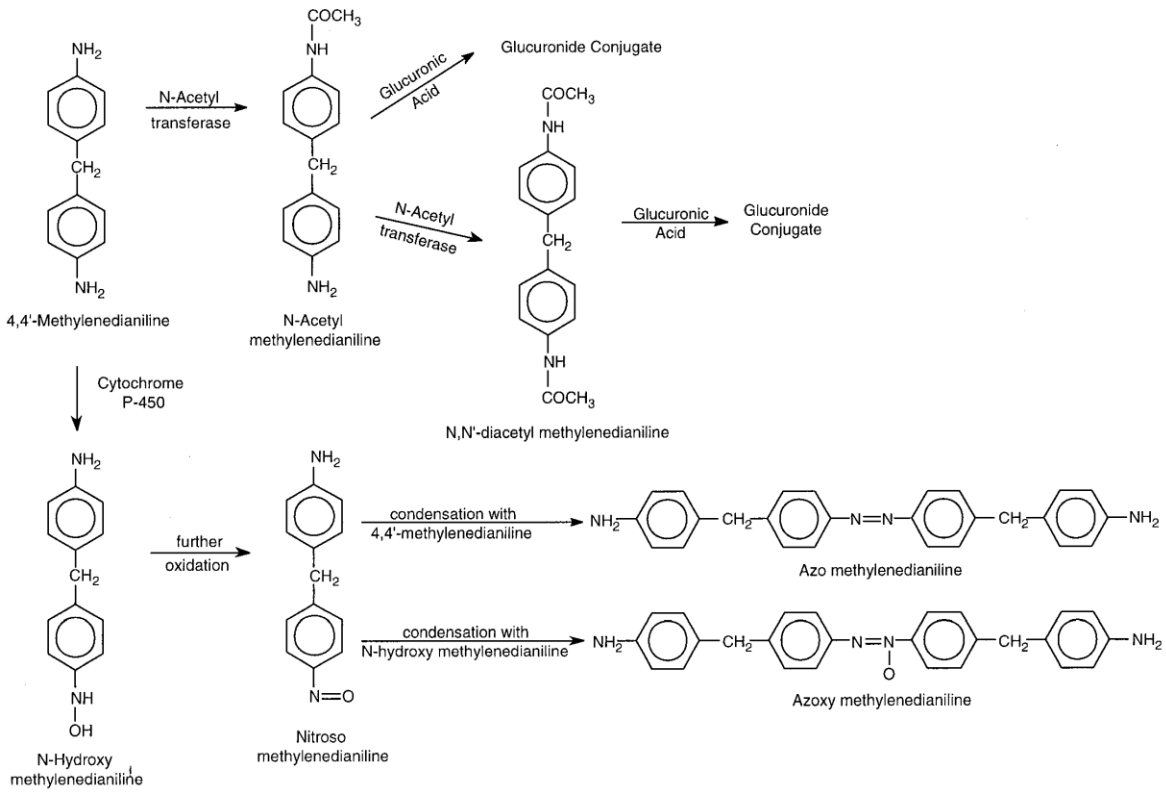
In another *in vivo* study, ^{14}C-MDA (4,4'-MDA) was administered to rats and guinea pigs as a single intraperitoneal dose of 2 mg/kg bw and sacrifices were conducted at 6, 24 and 96 hours after dosing. In rats, the liver had the highest concentration at all times points (on a per gram basis), followed by the lungs (6 and 24 hours) or the spleen (96 hours), and preferential accumulation of MDA or its metabolites was not observed in any organ other than the liver. In guinea pigs, the liver had the highest concentration of radioactivity when measured as a percentage of the applied dose, but on a per gram basis the highest concentrations were

detected in the spleen, followed by the liver, with suggestion of preferential absorption (El-Hawari *et al.*, 1986; ATSDR, 1998).

3.2.3 Metabolism

Although the pathways are not fully understood, the metabolism of MDA is reported to consist of *N*-acetylation as well as oxidation and conjugation to glucuronides and sulphates. *N*-acetylated metabolites have been detected in the urine of both occupationally exposed workers and experimental animals, and it is generally thought that *N*-acetylation of MDA represents a detoxification pathway. It is also assumed that MDA also undergoes *N*-hydroxylation, followed by further oxidation and conjugation, 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). There is currently less evidence for this *N*-hydroxylation pathway, because of the difficulty of detecting the initial *N*-hydroxylamine compounds due to their instability in aqueous solutions. However, data from an *in vitro* study (Kajbaf *et al.*, 1992) and from more recent *in vivo* studies (Chen *et al.*, 2008) increasingly indicate its likely importance in the metabolism of MDA, in addition to assumptions made from knowledge of the metabolism of structurally similar compounds. A simplified scheme of the proposed metabolic pathways of importance for MDA is shown in Figure 3.1 (ATSDR, 1998).

Figure 3.1 Metabolism of MDA



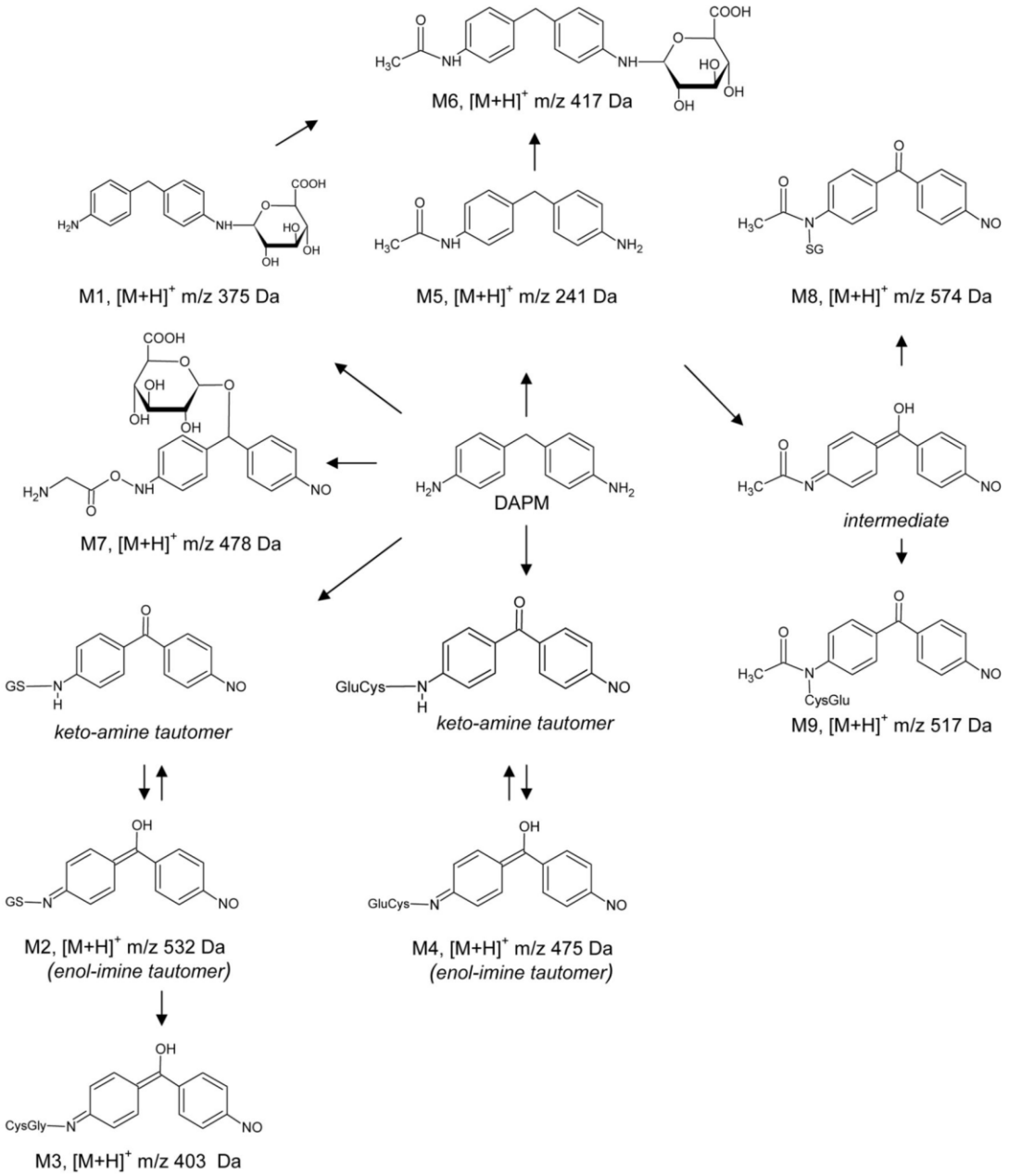
Oral

No studies were located which investigated the metabolism of MDA in humans following oral exposure.

In an *in vivo* study, Sprague-Dawley rats were administered MDA in gum arabic as a single dose of 50 mg/kg bw via oral gavage. *N*-acetyl-MDA was reported to be the major metabolite, although minor amounts of *N*,*N*-diacetyl-MDA and free MDA were also detected in the urine (no further information available) (Tanaka *et al.*, 1985; ATSDR, 1998; EU, 2001).

In another *in vivo* study, 16 male and 18 female Sprague-Dawley rats were administered ^{14}C-MDA via oral gavage at doses of 0, 25 or 50 mg/kg bw and the biliary metabolites were profiled. Several metabolites were detected, where nine of these were characterised using a range of analytical techniques. These nine metabolites included *N*-acetyl-MDA, various glutathione conjugates and a glycine conjugate. The most “prominent” metabolite excreted in the bile (the largest peak on the HPLC profile), assigned as “M2”, was identified as a nitroso-glutathione metabolite. The authors mentioned that in several similar metabolic pathways, the first step is metabolism to a nitroso intermediate, followed by addition of a glutathione to form a glutathione conjugated *N*-hydroxylamine, which is then rapidly reduced to a glutathione-conjugated imine due to the instability of the hydroxylamine in aqueous solutions. They therefore suggest that the metabolite “M2” may have been derived from an initial *N*-hydroxylamine. Another metabolite was assigned as “M7” and identified as a glycine conjugate. This was proposed to represent a bioactivation pathway, since amino acid conjugates of other *N*-hydroxylamines have been shown to induce DNA adduct formation. Overall, the data indicated that MDA is transformed via phase I and II metabolism, including hydroxylation, oxidation, acetylation, glucuronidation and glutathione conjugation, and a metabolic reaction scheme was proposed as shown in Figure 3.2 (Chen *et al.*, 2008).

Figure 3.2 Proposed metabolism of MDA (DAPM) based on analytical profiling



In addition, this same study (Chen *et al.*, 2008) investigated sex differences in the metabolism of MDA, since previous studies by the authors had indicated increased sensitivity to liver toxicity in females compared to males (thresholds of 25 and 50 mg/kg bw in females and males, respectively). The number of metabolites did not vary between sexes; however, differences were observed in the concentrations excreted. Overall, females were reported to excrete significantly less of the conjugated metabolites than males; higher concentrations of acetylated and glucuronyl metabolites were detected in the bile of males compared to females. The authors suggested that therefore the sex differences in sensitivity may be due to deficiencies in conjugation pathways in females, since the levels of several glutathione-S-transferase enzymes are reported to be 2-3 fold higher in males compared to females. They

concluded that conjugation pathways, including acetylation, glucuronidation and glutathione conjugation may therefore be important detoxification pathways in MDA metabolism (Chen *et al.,* 2008).

In an *in vivo* study, female F344 rats ("fast acetylator"), WKY rats ("slow acetylator") or F344/WKY hybrid rats were administered 4,4'-MDA in corn oil via oral gavage as a single dose of 37.5 mg/kg bw. These rat strains have different genetic polymorphisms in the gene that codes for *N*-acetyltransferase 2 (*NAT2*), an enzyme that catalyses the N-acetylation of aromatic amines. N-acetylation of MDA to *N*-acetyl-MDA was reported to be significantly (2-3 fold) higher in rapid acetylators (F344 rats) compared to slow acetylators (WKY rats). Liver damage, as evidenced by increased plasma alanine aminotransferase (ALT) levels, bile duct necrosis, inflammation, haemorrhage and portal expansion, was reported to be more severe in fast acetylators (F344 rats) compared to slow acetylators (WKY rats) (Zhang *et al.*, 2006; AGS, 2010).

Dermal

N-acetyl-MDA has been identified in the urine of workers occupationally exposed to MDA, where the most probably route of exposure was reported to be dermal (quantitative information not reported) (Cocker *et al.*, 1986a). *N*,*N*-diacetyl-MDA has also been detected in the urine of occupationally exposed workers, but at lower concentrations than for *N*-acetyl-MDA. In this study, 116 post-shift urine samples from 63 exposed workers were analysed, and the relative concentrations (arithmetic means) of MDA and its metabolites detected were: total MDA > *N*-acetyl-MDA (>50% of total MDA) > MDA (<15%) > *N*,*N*'-diacetyl-MDA (<3%). However, it was reported that the individual *N*-acetyl-MDA / total MDA ratios varied widely (Robert *et al.*, 1995).

No studies were located which investigated the metabolism of MDA in experimental animals following dermal exposure.

Inhalation

MDA and its metabolite, *N*-acetyl-MDA were detected in the urine of workers occupationally exposed to low levels of MDA (where the route was assumed to be inhalation) at concentrations of 0.013-2.76 nmol/l and 0.045-23.4 nmol/l, respectively. Out of 33 workers included in the study, only four were found not to have *N*-acetyl-MDA in their urine (Schütze *et al.*, 1995; ATSDR, 1998).

No studies were located which investigated the metabolism of MDA in experimental animals following inhalation exposure.

Other Routes

In an *in vivo* study, ^{14}C-MDA was administered to four male rats and two rabbits (one "slow acetylator" and one "fast acetylator") as a single intraperitoneal dose of 30 or 50 mg/kg bw, respectively. In the rats, more than 17 metabolites were identified in the urine, where these primarily consisted of acetylated compounds (*N*-acetyl-MDA, *N*,*N*-diacetyl-MDA, *N*,*N*-diacetyl-3-hydroxy-MDA, *N*-acetyl-4,4'-diaminobenzophenone and *N*,*N*-diacetyl-4,4'-diamino-benzhydrol). Of the initial dose, 69.6% of the radioactivity was detected in the urine (60.4% as free MDA, 3.8% as *N*-glucuronides, 1.7% as *O*-glucuronides, 1.2% as *O*-sulphates and 2.5% as acid labile). In the "slow acetylator" rabbit, 84.9% of the radioactivity was detected in the urine (69.8% as free MDA, 13.9% as *N*-glucuronides, 0.2% as *O*-glucuronides, 0.1% as *O*-sulphates and 0.9% as acid labile) and in the "fast acetylator" 81.1% of the radioactivity was detected in the urine (50.1% as free MDA, 25.8% as *N*-glucuronides, 2.4% as *O*-glucuronides, 0.3% as *O*-sulphates and 2.5% as acid labile). The metabolites *N*-acetyl-MDA and *N*,*N*-diacetyl-4,4'-diaminobenzhydrol were detected in the urine of the "fast acetylator", and a metabolite tentatively identified as *N*-acetyl-4-amino-4'-hydroxydiphenylmethane was identified in both rabbits (Morgott, 1984; EU, 2001; EC, 2000).

In an *in vitro* study in rabbit liver microsomes, the three metabolites detected were azo-MDA, azoxy-MDA and nitroso-MDA, where the authors suggested that the latter could have formed due to a non-enzymatic reaction (Kajbaf *et al.*, 1992; ATSDR, 1998; EU, 2001). *N*-hydroxylamine was not detected in this study. However, it is considered likely that the three metabolites detected were formed due to further oxidation of the hydroxylamine compound, which is therefore assumed to have been present initially (ATSDR, 1998; EU, 2001).

3.2.4 Excretion

Limited data are available on the excretion of MDA following certain routes of exposure. However, the data available indicate that urine is most likely to be the primary route of excretion in rats and monkeys, whereas excretion via faeces is also important in guinea pigs.

MDA has been detected in the urine of workers occupationally exposed to MDA via the dermal and inhalation routes. It was reported 4,4'-MDA was detected in 14.9% (>200 µg/l) and 0.09% (>20 µg/l) of urine samples from 27 workers producing 4,4'-MDA in the years 1970 and 1980, respectively (route of exposure not stated) (Vaudaine et al., 1982; IARC, 1986). In humans, excretion of MDA and its metabolites is reported to occur faster when exposure is via the inhalation route compared to the dermal route (quantitative information not available) (Cocker *et al.*, 1994; EU, 2001).

Oral

No studies were located which investigated the excretion of MDA in humans following oral exposure.

In an *in vivo* study, free MDA and MDA metabolites were detected in the urine of Sprague-Dawley rats who were administered MDA (form not stated) in gum arabic as a single dose of 50 mg/kg bw via oral gavage (quantitative information not available) (Tanaka *et al.*, 1985; ATSDR, 1998; EU, 2001).

Dermal

In several studies, 4,4'-MDA has been detected in the urine of workers exposed to MDA where the most probably route of exposure was reported to be dermal (Robert *et al.*, 1995; Cocker *et al.*, 1986a; Dalene *et al.*, 1995; Brunmark *et al.*, 1995). In one of these studies, over 300 samples were analysed and in those where 4,4'-MDA was detected, concentrations ranged from 6-175 nmol/mmol creatinine (average 26 nmol/mmol creatinine) (Cocker *et al.*, 1986a).

In another study, the excretion of 4,4'-MDA was investigated in seven male workers from a site where MDA was used as a curing agent for an epoxy resin and exposure was considered to be predominantly via the dermal route. The excretion rate in urine was reported to range between 0 and 90 µmol/hour, where the excretion rate was lower at the weekend than during the weekday (study was conducted over four workdays and one weekend). The cumulative excretion was reported as 0.04 to 1.2 µmol/day and 0.005-0.51 µmol/day during workdays and the weekend, respectively (Dalene *et al.*, 1995; ATSDR, 1998).

In a patch test on the forearm of five male volunteers, MDA was applied for a period of 1 hour at doses of 0.75-2.25 µmol. Excretion in urine was reported to represent 16% of the dose absorbed, where excretion peaked at 6-11 hours following exposure and was almost complete within 24 hours. Half-lives of 9.2-19 hours and 4.6-11 hours were reported for plasma and urine concentrations, respectively. It was reported that urinary excretion was proportional to the dose and that slow acetylation seemed to be associated with a short elimination half-life in the urine (Brunmark *et al.*, 1995; ATSDR, 1998; EU, 2001). MDA excretion is reported to be slower in workers with higher exposures, with reported half-lives of 48 hours (Smith *et al.*, 1990; EU, 2001). In this study, dermal absorption was suggested to be the rate limiting step (EU, 2001).

In an *in vivo* study, ^{14}C-MDA (4,4'-MDA) was topically administered to male rats, guinea pigs and monkeys at a dose of 2 mg/kg bw. In rats, radioactivity detected in the urine and faeces, respectively, was reported to be 2.5% and 0.04% after 6 hours, 20% and 2.3% after 24 hours, and 43% and 10% after 96 hours, indicating that the main route of excretion in rats is via the urine. Similarly, the main route of excretion in monkeys appeared to be the urine, with 18.8% and 1.9% of the radioactivity detected in the urine and faeces, respectively, following a 24-hour exposure and subsequent measurement of cumulative excretion over a 168-hour period. In contrast, both urine and faeces were indicated as important routes of excretion following dermal exposure in guinea pigs, with radioactivity detected in the urine and faeces, respectively, reported to be 0.35% and 0.1% after 6 hours, 7.8% and 5.7% after 24 hours, and 10.5% and 17.6% after 96 hours (El-Hawari *et al.*, 1986; EU, 2001; ATSDR, 1998).

Inhalation

MDA was detected in the urine of 33 workers occupationally exposed to low levels of MDA (mostly below the detection limit of 20 µg 4,4'-MDA/m^3; exposure was assumed to be via inhalation) at concentrations of 0.013-2.76 nmol/l (Schütze *et al.*, 1995; ATSDR, 1998). In another study, MDA was detected at a concentration of c100 nmol/mmol creatinine in the urine of workers exposed through inhalation of solid material of contaminated dust (Cocker *et al.*, 1994; ATSDR, 1998).

No studies were located which investigated the excretion of MDA in experimental animals following inhalation exposure.

Other Routes

In an *in vivo* study, ^{14}C-MDA was administered to four male rats and two rabbits (one "slow acetylator" and one "fast acetylator") as a single intraperitoneal dose of 30 or 50 mg/kg bw, respectively. This study was reviewed by the EU in their risk assessment report (EU, 2001). The original study report is unavailable and therefore the apparent reporting inconsistencies cannot be investigated further. In one section of the EU report, the radioactivity detected in the urine (as a percentage of the total) was reported to be 69.6%, 84.9% and 81.1% in the rats, "slow acetylator" and "fast acetylator", respectively, indicating that urine is an important route of excretion in all species. However, in another section of the report, it is stated that in rats the radioactivity detected in the faeces and urine represented 55.8% and 35.0% of the total, respectively, indicating that faeces was the main route of excretion following intraperitoneal administration. In this second section, it was reported that both rabbit phenotypes excreted approximately 80% of the radioactivity in the urine, but that total recovery of radioactivity was about 10% less in the rats and slow acetylator compared to the fast acetylator, where the difference was attributed to greater faecal excretion by the fast acetylator rabbit (Morgott, 1984; EU, 2001).

In an *in vivo* study, ^{14}C-MDA (4,4'-MDA) was administered to rats, guinea pigs and monkeys as a single intravenous dose of 2 mg/kg bw. In rats, radioactivity detected in the urine and faeces, respectively, was reported to be 55% and 0.3% after 6 hours, 67.4% and 21.8% after 24 hours, and 67% and 31% after 96 hours, indicating that the main route of excretion in rats is via the urine. Similarly, the main route of excretion appeared to be urine in monkeys, with 79% and 6.5% of the radioactivity detected in the urine and faeces, respectively, within the first 48 hours. In contrast, the faeces were indicated as the primary route of excretion in guinea pigs, with 34% and 51% of the radioactivity detected in the urine and faeces, respectively, after 48 hours (El-Hawari *et al.*, 1986; EU, 2001; ATSDR, 1998).

3.2.5 Summary

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

There is evidence that MDA is absorbed following dermal, inhalation and oral exposure in both experimental animals and humans, where in humans the rate of absorption via inhalation may be faster than dermal absorption. Data from *in vitro* studies are inconsistent on whether or not dermal absorption is higher in humans than in rats, and in experimental animals, dermal absorption has been reported to be a dose-dependent and saturable process.

Limited data are available on the distribution of MDA. However, studies using radiolabelled MDA in experimental animals have identified the liver, adrenal glands and kidneys as potential distribution sites following dermal exposure, and the liver, kidneys, spleen and thyroid following intraperitoneal exposure. However, there was no evidence of preferential accumulation of MDA or its metabolites in any organ other than the liver.

Although the pathways are not fully understood, the metabolism of MDA is reported to consist of *N*-acetylation as well as oxidation and conjugation to glucuronides and sulphates. N-acetylated metabolites, such as N-acetyl-MDA and N,N-diacetyl-MDA, have been detected in the urine of both occupationally exposed workers and experimental animals, and it is generally thought that N-acetylation of MDA represents a detoxification pathway. It is also thought that MDA also undergoes N-hydroxylation, followed by further oxidation and conjugation, where it is considered that the N-hydroxylation reaction can potentially lead to the formation of toxic intermediates.

Limited data are available on the excretion of MDA following certain routes of exposure; however, the available studies have shown MDA to be rapidly excreted following exposure of humans or experimental animals. Studies in occupationally exposed workers indicate that MDA excretion is dose- and route-dependent, being slower following exposure to higher concentrations and faster when exposure is via the inhalation route compared to the dermal route. Excretion of MDA appears to also be dependent on acetylator phenotype, where this has been suggested to account for the wide variation in excretion in humans. Studies in experimental animals indicate that the primary route of excretion may differ between species, where it is most likely that urine is the primary route of excretion in rats and monkeys, whereas excretion via faeces is also important in guinea pigs.

3.2.6 Bioavailability

There is evidence that MDA is absorbed following oral, dermal and inhalation exposure in both experimental animals and humans, and that there is no preferential accumulation of MDA or its metabolites in any organ, other than the liver in certain cases. In experimental animals, dermal absorption has been reported to be a dose-dependent and saturable process. In humans, it has been reported that the rate of absorption via inhalation may be faster than dermal absorption (quantitative information not available). Similarly, MDA excretion is reported to be dose- and route-dependent in humans, being slower following exposure to higher concentrations and faster when exposure is via the inhalation route compared to the dermal route. However, no quantitative data are available on absorption via

the oral route in humans or experimental animals, and therefore no conclusions can be made regarding the relative absorption via this route compared to different routes of exposure.

The majority of the data on the toxicokinetics of MDA in humans comes from studies in occupationally exposed workers, with only limited quantitative data reported. Therefore, it is difficult to differentiate between the toxicokinetics in animals and humans. The results from *in vitro* studies using human and rat skin are inconsistent, with one study suggesting that dermal absorption is 2-2.5 times higher in humans compared to rats and another study suggesting that there is no significant difference. Studies in experimental animals indicate that the primary route of excretion may differ between species, where it is most likely that urine is the primary route of excretion in rats and monkeys, whereas excretion via faeces is also important in guinea pigs. In humans, MDA has been detected in the urine of occupationally exposed workers, but no data are available on the relative contributions of the different routes of excretion. However, a large variation in MDA excretion has been reported in human studies, where this has been attributed to differences in acetylator phenotype, where slow acetylation has been associated with a short elimination half-life in the urine and with decreased liver toxicity compared to fast acetylation. One oral study in experimental animals suggested that females may be more susceptible to liver toxicity due to deficiencies in the metabolism of MDA. However, no information is available on whether there is a sex difference in MDA toxicokinetics in humans. Therefore, in the absence of robust data indicating otherwise, bioavailability for humans via the oral, dermal and inhalation routes of absorption is assumed to be the same as for animals. The variation in MDA toxicokinetics between human individuals should be accounted for by the assessment factors applied in the final assessment.

3.3 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 (Table 3.3 and Table 3.4). ATSDR concluded that the data show that, with few exceptions, MDA is genotoxic with metabolic activation. They also reported that the results from the *in vivo* DNA binding study suggested that MDA was a genotoxic compound. 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. However, due to insufficient evidence, they classified MDA as a category 3 mutagen (substance which causes concern for man owing to possible mutagenic effects; under the Dangerous Substances Directive) rather than a category 2 mutagen (substances which should be regarded as if they are mutagenic to man; under the Dangerous Substances Directive) (EU, 2001).

Table 3.3 Results of *in vitro* genotoxicity studies with MDA

Test	Metabolic Activation	Assay details	Result	Ref.
Prokaryotic organisms:				
Bacterial mutation test in *Salmonella typhimurium* strains TA98 and TA100	without	not reported	negative	Zeiger *et al.*, 1988; cited in EU, 2001
Bacterial mutation test in *Salmonella typhimurium* strains TA98 and TA100	with	3-333 µg/plate, or ≥30 µg/plate	positive a	
Bacterial mutation test in *Salmonella typhimurium* strains TA1535 and TA1537	with and without	0-150 µg/plate	negative	Darby *et al.*, 1978; cited in EC, 2000
Bacterial mutation test in *Salmonella typhimurium* strains TA1538	with and without	not reported	equivocal	Darby *et al.*, 1978; cited in EC, 2000
Bacterial mutation test in *Salmonella typhimurium* strains TA98, TA100, TA1537 and TA1538	without	3.5-3000 µg/plate	negative	BASF AG, 1977; cited in EC, 2000 and ECHA, 2014
Bacterial mutation test in *Salmonella typhimurium* strains TA98, TA100, TA1537 and TA1538	with	3.5-3000 µg/plate	negative (TA1537), positive (all other strains)	
Eukaryotic organisms:				
Fungi:				
Yeast gene mutation assay in *Saccharomyces cerevisiae* D4	with and without	1000 µg/plate	negative	Brusick, 1975; cited in EC, 2000
Mammalian:				
Chromosome aberration assay in Chinese Hamster Ovary (CHO) cells	without	up to 800 µg/ml, 2-hour exposure	weak positive b	Gulati *et al.*, 1989; cited in EU, 2001 and ECHA, 2014
Chromosome aberration assay in CHO cells	with	500-1000 µg/ml, 2-hour exposure	positive	

Test	Metabolic Activation	Assay details	Result	Ref.
Mouse lymphoma assay	without	not reported	weak positive c	McGregor *et al.*, 1988; cited in EU, 2001 and ECHA, 2014
Sister chromatid exchange assay	with and without	160-1600 µg/ml (with), 16-160 µg/ml (without)	weak positive	Gulati *et al.*, 1989; cited in EU, 2001
Unscheduled DNA synthesis in primary rat hepatocytes	not reported	various	equivocal d	Mori *et al.*, 1988; Shaddock *et al.*, 1989; cited in EU, 2001
DNA damage and repair in Chinese hamster V79 cells	with	0.1-3 mM (approximately 19.8-594 µg/ml)	positive e	Swenberg, 1981; cited in EC, 2000 and IARC, 1986
DNA fragmentation in rat and human hepatocytes and thyreocytes (Comet assay)	not reported	10-180 µM (approximately 1.98-35.7 µg/ml), 4 and 20 hours	positive	Martelli *et al.*, 2002
DNA fragmentation and micronucleus formation in primary cultures of rat and human kidney cells	not reported	0.056, 0.1 or 0.18 mM	negative	Robbiano *et al.*, 1999
DNA fragmentation in human kidney and urinary bladder cells (Comet assay)	not reported	10-180 µM (approximately 1.98-35.7 µg/ml), 4 and 20 hours	negative	Martelli *et al.*, 2002
DNA adducts in human skin	not reported	0.1 mg	positive	Kenyon *et al.*, 2004
Chromosome aberrations and sister chromatid exchanges in human leucocytes	with and without	not reported	negative	Ho *et al.*, 1979; Ho *et al.*,

Test	Metabolic Activation	Assay details	Result	Ref.
				1984; cited in EC, 2000
Chromosome aberrations in human lymphocytes	not reported	not reported	negative	Nunziata, 1985; cited in EC, 2000

a: Dose dependent.

b: Negative at concentrations up to 600 µg/ml; positive in one experiment at 800 µg/ml. No information available on cytotoxicity, so it may be that the clastogenic effects observed here are limited to high doses with cytotoxic effects.

c: Weak positive responses observed only in two out of three experiments at the top doses of 500 or 700 µg/ml.

d: Different responses were reported in three experiments: negative (up to 100 µg/ml; Sprague-Dawley rats), dose-dependent positive (1-100 µmol, approximately 0.198-19.8 µg/ml; 1000 µmol/l reported to be cytotoxic; male ACI/N rats) and weak positive (25-100 µg/ml).

e: DNA damage reported at the doses of 1 and 3 mM, where the dose of 3 mM was cytotoxic.

Table 3.4 Results of *in vivo* genotoxicity studies with MDA

Test	Route of administration	Dose (mg/kg bw)	Result	Ref.
Micronucleus test in bone marrow of male B6C3F1 mice	intraperitoneal (IP)	9.3, 18.5 or 37; 3 daily doses	weak positive a	Shelby *et al.*, 1993; cited in EU, 2001
Micronucleus test in peripheral blood of CD-1 mice	IP	various	weak positive b	Morita *et al.*, 1997; cited in EU, 2001
Micronucleus formation and DNA damage in the kidneys of male Sprague-Dawley albino rats (3/dosing regimen)	oral	Single dose of 415 mg/kg bw or three doses of 277 mg/kg bw/day (MDA >97% pure)	negative	Robbiano *et al.*, 1999
Unscheduled DNA synthesis in the liver of male Fischer-344 rats	oral	20, 80 or 350	negative	Mirsalis *et al.*, 1989; cited in EU, 2001 and IARC, 1986
Unscheduled DNA synthesis in liver of B6C3F1 mice	oral	50, 200, 500 or 1000	negative	Mirsalis *et al.*, 1989; cited in EU, 2001 and

Test	Route of administration	Dose (mg/kg bw)	Result	Ref.
				IARC, 1986
DNA strand breaks in liver of Sprague-Dawley rats	IP	74, single dose	positive	Parodi *et al.*, 1981; cited in EU, 2001
DNA binding assay in male Wistar rats (liver)	IP	5.6 or 116.5 µmol/kg bw (reported to be 1.1 and 23.1)	low binding capacity	Schütze *et al.*, 1996; cited in EU, 2001
Sister chromatid exchanges in bone marrow of male Swiss mice	IP	9 or 18	weak positive c	Parodi *et al.*, 1983; cited in EU, 2001
Sister chromatid exchanges in bone marrow of Balb/c mice	not reported	1-35	positive	Gorecka-Turska *et al.*, 1986; cited in EC, 2000

a: Not dose-dependent.

b: Three experiment: 1) doses of 28-112 mg/kg bw, single treatment, weak but dose-dependent increase; 2) 28-140 mg/kg bw, single treatment, marginal increase; 3) 22.8-90 mg/kg bw, two daily doses, negative.

c: Low reliability due to methodology insufficiencies such as the small number of animals included in the study.

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

3.4 Carcinogenicity

3.4.1 Human Epidemiological Studies

Information on individual studies is located in Appendix A1. The CSRs 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 subcohort). 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 which 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 exposure. However, there is a some suggestion of an association with cancer, particularly bladder, and this, together with the experimental animal 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.

3.4.2 Experimental Animal Studies

Initiation-Promotion Studies

Information on individual studies is located in Appendix A2.1.

Chronic Studies

Detailed information on individual studies is located in Appendices A2.2 and A2.3. Below in Table 3.5 is a summary of all the available chronic studies of MDA.

Table 3.5 Overview of the subchronic and chronic studies of MDA

Reference	Study (species, strain, sex, number of animals, duration and route of exposure)	Dose	Findings
Subchronic			
Griswold *et al.*, 1968	• Sprague Dawley rats F • 20 in MDA treatment group, 40 positive controls, 140 negative controls • administration every three days for 30 days and 9 months observation • oral exposure via gavage	• total MDA dose of 300 mg/rat (no further details available); MDA dihydrochloride • positive controls:	• mammary lesions observed in all groups (5/132 in negative controls, 29/29 in positive controls, 1/14 in MDA treated group)
Schoental 1968	• rats (strain not specified) M/F • 8/sex/group • <8 months and observation until death	• four or five doses of 20 mg/rat over the study period • 4,4'-MDA (purity unspecified)	• one hepatoma and a haemangioma-like tumour of the kidney (one M, after 18 months) • an adenocarcinoma of the uterus (one F, after 24 months)

Reference	Study (species, strain, sex, number of animals, duration and route of exposure)	Dose	Findings
	• oral administration via gavage		• varying degrees of liver fibrosis and inflammation (most animals; no further details available)
Chronic			
NTP 1983	• F344/N rats M/F • 50/sex/dose • 2-year exposure • oral exposure via drinking water	• 0, 150 or 300 mg/l M: 0, 9 and 16 mg/kg bw/day F: 0, 10 and 19 mg/kg bw/day • MDA dihydrochloride (purity 98.6%)	• significant increase in incidence of thyroid follicular cell carcinoma (high dose, M) and thyroid follicular cell adenomas (high dose, F) • significant increase in incidence of neoplastic nodules of the liver in both dose groups of M • low incidences of neoplastic lesions in other organ systems ○ bile duct, one high dose M; ○ urinary bladder, both doses F; ○ ovary, both doses F
NTP 1983	• B6C3F1 mice M/F • 50/sex/dose • 2-year exposure • oral exposure via drinking water	• 0, 150 or 300 mg/l M: 0, 25 and 57 mg/kg bw/day F: 0, 19 and 43 mg/kg bw/day • MDA dihydrochloride (purity 98.6%)	• increase in incidence of thyroid follicular cell adenoma in M & F (only significant at the high dose) • significant increase in combined incidence of thyroid follicular cell adenoma and carcinoma (high dose, F) • increase in incidence of hepatocellular carcinoma (significant in both dose groups of M and high dose F) • increase in incidence of hepatocellular adenomas (only high dose, F) • M: significant positive trend in adrenal phaeochromocytomas • F: significant positive trend in malignant lymphomas (significant at both doses) and alveolar/bronchiolar adenomas (significant only

Reference	Study (species, strain, sex, number of animals, duration and route of exposure)	Dose	Findings
			at high dose)
Deichmann *et al.,* 1978	• Beagle dogs F • 5 dogs administered "highly purified" 4,4'-MDA and 4 dogs administered "crude" 4,4'-MDA • treatment three times per week for 4.5-7 years • oral exposure; test substance dissolved in corn oil and placed in gelatinous capsules	• Total doses equivalent to: ○ 5.0-6.26 g/kg bw ("pure" 4,4'-MDA) ○ 4.0-6.25 g/kg bw ("crude" 4,4'-MDA; containing 50% 4,4'-MDA, 50% higher molecular weight analogues)	• no urinary or liver tumours observed • one tumour of the uterine horn and one in the spleen (not examined microscopically) • changes observed in the liver, kidneys and spleen
Steinhoff and Grundmann, 1970	• Wistar rats M/F • 25/sex/dose • treatment every 1-3 weeks for various durations (705 days for 4,4'-MDA) and observation for total animal lifespan • subcutaneous injection	• 30-50 mg/kg bw (total administered dose 1410 mg/kg bw) • 4,4'-MDA (purity unspecified) • additional groups were treated with 2,4'-MDA, 3,4-amine, 8-amine or the vehicles saline or groundnut oil:ethyl alcohol (9:1)	• 25/50 animals in the MDA treatment group developed malignant tumours (total malignant tumours: 33, total benign tumours: 29) compared to 13/50 control animals (total malignant tumours: 16, total benign tumours: 15) • equivalent numbers in the other treatment groups: ○ 15/50 animals (2,4'-MDA) ○ 27/50 (3,4-amine; benign tumours: 16) ○ 12/50 (8-amine; benign tumours: 18)
Holland *et al.,* 1978	• C3Hf/Bd mice M/F • no information on number of animals • treatment three times per week for 24 months • dermal exposure; application to clipped skin	• 0, 5.3, 10.7 or 21.3 mg/kg bw/day • 4,4'-MDA (in ethanol) • Benzo[a]pyrene used as a positive control	• no tumours observed at the site of application • dose-dependent increase in incidence of hepatic tumours in F (no information on statistical significance)

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 3.6. Of the total liver tumours, 12/50 are hepatic nodules and 1/50 hepatocellular carcinoma.

Table 3.6 Tumour incidence for total liver tumours in MDA hydrochloride-treated 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

3.5 Evaluations

Table 3.7 summarises the expert carcinogenic assessments of MDA including any derived threshold doses.

Table 3.7 Overview of the carcinogenic assessments of MDA

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
IARC (1986)	not addressed	not addressed	no data on humans were 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	**CELs** = 9 mg/kg bw (oral; M rats) 19 mg/kg bw (oral; F mice) 5.3 mg/kg bw (dermal; F mice)

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
			incidence of hepatic tumours	
Dybling *et al.,* (1997)	not addressed	not addressed	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)
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 Dybling *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}

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
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 mechanism) • non-linear (non-genotoxic mechanism) Liver: non-threshold, linear	NTP chronic drinking water studies in F344 rats and B6C3F1 mice NTP (1983) • combined incidence of hepatocellular adenomas and carcinomas in female mice selected as the critical endpoint	**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
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:** **T25**$_{inhalation, human}$ = 16.5 mg/m^3 AF = 3125 Correction factor = 2.8

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
				(to account for differences in worker and experimental exposure conditions) **DMEL** = 14.8 µg/m^3

BMDL10: 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: Equivalent dose corresponding to a 25% tumour incidence in humans (calculated from the T25).
M: Male.
RSD: Risk specific dose.
T25: Dose corresponding to a 25% tumour incidence.

3.6 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*., 1986b; Tanaka *et al.*, 1985). It is also 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. There is currently less evidence for the *N*-hydroxylation of MDA than for its acetylation, because of the difficulty of detecting the initial *N*-hydroxylamine compounds due to their instability in aqueous solutions. However, data from an *in vitro* study (Kajbaf *et al.*, 1992) and from more recent *in vivo* studies (Chen *et al.*, 2008) increasingly indicate the likely importance of this route in the metabolism of MDA, in addition to assumptions made from knowledge of the metabolism of structurally similar compounds. In support of this mechanism, the results from genotoxicity studies with MDA indicate a 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. The authors of an MDA risk assessment conducted in 2005 assumed a threshold for tumour formation, suggesting that the toxicity observed at high doses of MDA in experimental animal studies and in incidents of acute human poisoning may not be relevant for low dose exposure. This was on the basis that the process of metabolic activation, which is thought to be required for MDA toxicity, is in competition with detoxification processes which may become saturated at high doses (Lewandowski *et al.*, 2005).

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). Mixed results have been reported from both *in vitro* and *in vivo* genotoxicity studies with MDA (section 3.3), and an *in vivo* DNA binding study, where MDA was administered to male Wistar rats via the intraperitoneal route, demonstrated only weak binding capacity in the liver (Schütze et al., 1996). Tumour initiation in the thyroid has been hypothesised to partially result from hyper-secretion of thyroid stimulating hormone (TSH). In a tumour promotion study, a slight, but not significant, decrease was observed in serum concentrations of thyroxine (T4) and triiodothyronine (T3) in animals treated with MDA (Hiasa *et al.*, 1984). A decrease in T4 and T3 is thought to possibly trigger secretion of TSH, which can then induce thyroid hyperplasia (ATSDR, 1998). In this study, MDA treatment promoted the development of thyroid tumours, and the authors thought that the hypersecretion of TSH may have contributed to tumour formation in the initiated cells (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 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 (section 3.5), and the majority of these

have assumed a genotoxic mechanism, taking a precautionary approach in light of the results from genotoxicity studies. The 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).

3.7 Carcinogenicity risk assessment

3.7.1 Critical studies

The most critical studies for quantitative risk assessment are the oral, long-term, 2-year drinking water studies conducted on F-344 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 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.

3.7.2 Dose Response

The aim of this project is to identify information that can be used to quantify risk for relevant exposure routes. The review of the genotoxicity and carcinogenicity data leads to the conclusion that there is a potential for a genotoxic mode of action with metabolic activation and that exposure to technical MDA can give rise to tumours in experimental animals, and can presume to have carcinogenic potential in humans. Therefore the quantitative risks for technical MDA are based on a carcinogenic potential.

Review of the epidemiological studies on human occupational exposure to technical and other forms of MDA do not reveal any data that would be useful in identifying any quantitative risk for humans. Therefore the dose response curves are based on relevant, robust studies in experimental animals. There are very limited data on technical MDA but there are a number of toxicological studies on MDA and its salts and it is considered that this information is relevant to the risk assessment of technical MDA.

The value commonly used globally including Europe as a Point of Departure (PoD) for risk assessment is T25 which is the daily dose (in mg/kg body weight) inducing a tumour incidence of 25% upon lifetime exposure. This is based on an assumption of a linear dose response at all concentrations (including above the experimental doses) excluding the zero

dose. The derivation of a T25 for MDA (and relevant to technical MDA) will be the PoD for this risk assessment.

3.7.3 Derivation of T25

Oral

A T25 value for MDA has been derived by a number of authoritative bodies (see Table 3.6, 3.7) using information from the NTP long-term study on F344 rats administered MDA dihydrochloride in drinking water (NTP, 1983). Taking:

- 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.

$$\mathbf{T25_{(Oral,\ Rat)} = 9 \times 0.25/(0.26-0.02) \times (1-0.02)/1 \times 103/104}$$

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

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 3.7) 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.

$$\mathbf{T25_{(Inhalation,\ Gen.\ pop.)} = 9.01/4 \times 70/20 \times 100/100 = 7.9\ mg/m^3}$$

General population oral risk estimate

$T25_{(Oral,\ Rat)}$ corrected to $T25_{(Oral,\ Human)}$ by allometric scaling from rats to humans, 4

$$\mathbf{T25_{(Oral,\ Gen.\ pop.)} = 9.01/4 = 2.25\ mg/kg\ bw/day}$$

The cancer risk estimates are summarised in Table 3.8.

Table 3.8 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	**1.1×10^{-4} per µg/kg bw/day**
Inhalation	Workers	$T25_{(Inhalation,\ Worker)}$ 44.5 mg/m^3	**5.6×10^{-6} per µg/m^3**
	General population	$T25_{(Inhalation,\ Gen.\ pop.)}$ 7.9 mg/m^3]	**3.2×10^{-5} per µg/m^3**
Dermal	Workers	$T25_{(Dermal,\ Worker)}$ 12.6 mg/kg bw/day	**1.9×10^{-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}

3.8 Biomonitoring

The basic principle of this risk assessment is to compare the cancer risk estimate for an 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)[6]. 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)

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

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, = $\ln 2/T_{1/2}$.

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).

[6] 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.

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 an air concentration of 1 µg/m^3 (corresponding to an absorbed dose of 0.14 µg/kg bw, and 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}**

3.9 References

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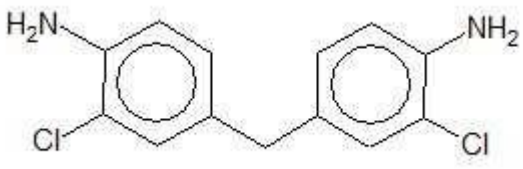
4. MOCA

2,2'-Dichloro-4,4'-methylenedianiline (MOCA) CAS RN: 101-14-4; EC Number: 202-918-9)



MOCA is used primarily to procuce polyurethane articles. Polyurethanes are produced by the reaction of a liquid isocyanate with a blend of liquid polyols, catalysts and other additives. MOCAis used as an additive in the polyol blend with the purpose to give the resulting polymer specific properties. Depending on the function MOCA has within the polymer, four uses can be differentiated: curing agent, cross-linker, chain extender and pre-polymer. The only further registered use is as laboratory chemical.

4.1 Toxicokinetics

The most probable route of occupational exposure is dermal from contact with contaminated surfaces, followed by inhalation and oral pathways (Cocker *et al*., 1990; Ichikawa *et al*., 1990).

4.1.1 Absorption

Oral

No studies were located on the absorption of MOCA in humans following oral exposure.

The results from rats administered a single oral dose of radiolabelled MOCA via oral gavage suggest that MOCA is partially absorbed following oral exposure. 16.5% MOCA was excreted in urine within 72 hours, 13.7% was retained in the tissue, while approximately 60% remained unabsorbed in faeces (Groth *et al*., 1984).

Dermal

Occupational workers can be exposed to MOCA during its manufacturing process, which can either exist as a liquid emulsion, solid pellets with dust, or as solid pellets without dust (IARC, 2012). NIOSH 1986 reported the concentrations of MOCA in the urine of exposed workers over a period of 22 months and identified the levels of MOCA from 5.3 to 43.8 µg/l. A detailed review of the data identified that the highest MOCA concentrations in urine detected were in workers in direct daily contact with MOCA i.e. mixers and moulders.

One study indirectly evaluated the absorption of MOCA in five male factory workers over a 5-day period. MOCA air concentrations were monitored for each worker over 6-7 hours every other day and urinary MOCA concentrations were obtained over the 5 days. MOCA air concentrations ranged from 0.0002 to 0.0089 mg/m^3. The concentration of MOCA detected in urine was greater than the reported air concentrations, identifying that another potential route of MOCA exposure is dermal (Ichikawa *et al*., 1990).

The differences in absorption rates of radiolabelled MOCA (^{14}C-MOCA) in Beagle dogs following either dermal or intravenous exposures were reported for 24 hours following MOCA administration. Only 2.4% MOCA was reported to be absorbed via dermal administration (Manis *et al*., 1984). Groth *et al*. (1984) reported 11.5-21.9% of MOCA absorption in Sprague-Dawley rats following 72 hours of dermal application to the skin.

The absorption and penetration of radiolabelled MOCA through 7 x 7 mm area of fresh human neonatal foreskin organ cultures was reported over a four-hour period. One hour following dermal application, 46% of the radiolabelled MOCA was reported on the skin, 0.5% was detected on the underlying membrane, while the remaining 53.5% radiolabelled MOCA was unabsorbed. Four hours after the initial radiolabelled MOCA, 61% was detected in the skin, 26% was detected on the underlying membrane and 12% remained unabsorbed. The authors suggested that MOCA was readily absorbed without being metabolised (Chin *et al*., 1983).

Inhalation

No additional studies were located on the direct measurement of MOCA absorption in humans or experimental animals via inhalation exposure.

Summary

Therefore for the cancer risk estimations, the following absorption values were used:

Oral absorption – no human data and partially absorbed in rats; therefore, an oral absorption of 50% is assumed and when extrapolating from oral to inhalation toxicity a correcting factor of 2 is used according to the REACH Guidance.

Dermal absorption – There are no *in vivo* dermal absorption data in humans, in one study in rats dermal absorption of 11.5-21.9% is observed and human tissue culture study suggests even higher absorption; 50% default value for dermal absorption is used according to the REACH Guidance.

Inhalation absorption - No studies located – 100% default value according to the REACH Guidance.

4.1.2 Distribution

Oral

No studies were located on the distribution of MOCA in humans following oral exposure.

In an oral study, male Sprague-Dawley rats were administered a single dose of 28 µmol/kg of radiolabelled ^{14}C-MOCA. The highest concentration of radiolabelled MOCA was in the liver after 24 hours of oral exposure. MOCA was reported as being distributed in the kidney, lung, spleen, bladder, testes, brain and lymphocytes in decreasing concentrations (Cheever *et al*., 1991). Similar results were reported in female Wistar rats. The distribution of radiolabelled MOCA 24 hours following oral administration (oral gavage) in the female rats was the liver, intestine, lung, kidney, blood, stomach, spleen and uterus in decreasing the concentrations (Sabbioni and Neumann, 1990). Cheever *et al*., 1991 reported that following 28 consecutive oral doses of ^{14}C-MOCA, the liver was the main tissue in which MOCA and its metabolites accumulated and the concentration of MOCA was 100-fold greater from the initial oral dose. Another study identified the liver as the main target organ in female rats following oral administration of 10 mg/kg ^{14}C-MOCA; however, the study was limited due to its use of only two female rats (Farmer *et al*., 1981).

An additional oral study in rats reported 64-87% removal of radiolabelled MOCA in urine or faeces, 48 hours after exposure. The study identifies that MOCA is rapidly metabolised and excreted within the first 24 hours of oral administration (Morton *et al*., 1988).

Dermal

The data suggest that MOCA is rapidly removed from the human body following dermal exposure. Nine hours after an occupational worker was exposed to 11.34 l of molten MOCA in an accidental spill, 1.7 mg/l of MOCA was detected in the urine of the exposed worker. The authors reported that MOCA was distributed via the circulatory system to the kidneys following dermal exposure (Osorio *et al*., 1990).

In another study, Beagle dogs were dermally administered 0.4 mg/cm^2 of radiolabelled MOCA. Control dogs were intravenously injected with radiolabelled MOCA to achieve 100% absorption to compare the distribution of MOCA following dermal exposure. No radioactivity was detected in blood or plasma 24 hours following MOCA administration. The results from the study identified that the circulatory system distributes MOCA to the liver, kidneys, fat, and lungs (Manis *et al*., 1984).

Inhalation

No additional studies were located on the distribution of MOCA in humans or experimental animals via inhalation exposure.

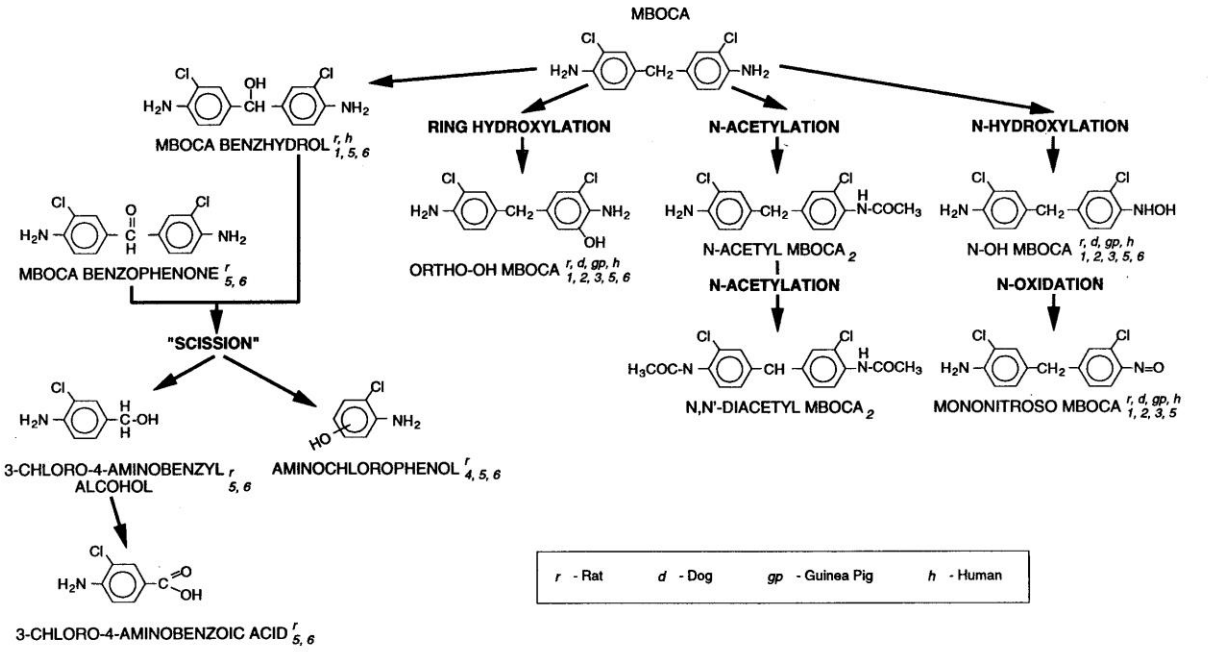
4.1.3 Metabolism

Oral

No studies were located on the metabolism of MOCA in humans following oral exposure.

Several metabolism pathways have been reported in experimental animals following oral exposure to MOCA, these include *N*-acetylation, *N*-hydroxylation, *N*-oxidation and ring hydroxylation (Cheever *et al*., 1991). *N*-acetylation is an important human metabolic pathway for other aromatic amines; however, data suggest that *N*-acetylation of MOCA is not a major pathway, due to only the minor detection of acetyl-MOCA-type metabolites (IARC, 2010). Figure 4.1 (ATSDR, 1994) identifies the main metabolic pathways of MOCA in humans and experimental animals following oral exposure.

Figure 4.1 Metabolism of MOCA



* Derived from Butler et al. 1989 (1); Cheever et al. 1991 (2); Chen et al. 1989 (3); Farmer et al. 1981 (4); Kuslikis et al. 1991 (5); Morton et al. 1988 (6).

Microsomal P450 enzymes are reported to be involved in the *N*-hydroxylation and *N*-oxidation of MOCA. CYP3A4 and CYP2A6 are identified as major and minor enzymes, respectively in the *N*-oxidation of MOCA (Yun *et al*., 1992). Human liver microsomes and purified rat liver cytochrome P450 monooxygenases were incubated with ^{14}C-MOCA in separate *in vitro* studies confirming the activation of MOCA by liver microsomal P450 enzymes (Butler *et al*., 1989).

Under acidic conditions, (pH 5 or lower (Kadlubar *et al*., 1977)) MOCA is activated via *N*-hydroxylation metabolic pathway in the liver and transported to the bladder (Swaminathan *et al*., 1996), which is reported to be the initial pathway to MOCA reacting with DNA to form MOCA-DNA adducts and consequently resulting in carcinogenicity (IARC, 2012). The two main hydroxylation metabolisms of MOCA in experimental animals include *N*-hydroxylation in guinea pigs and *O*-hydroxylation in dogs, while hydroxylation of MOCA in rats is a minor metabolic pathway (Chen *et al*., 1989). Morton *et al*., 1988 reported a higher rate of hydroxylation metabolic pathways in rat microsomal enzymes when compared with human microsomes.

Under less acidic conditions the *N*-hydroxylation of MOCA is followed by conjugation with either acetate (King, 1974; Shinohara *et al*., 1986), sulphate (King, 1974) or glucuronate (Kadlubar *et al*., 1977). The glucuronate-MOCA conjugates are reported to be relatively stable and are eliminated in the urine of experimental animals (Kadlubar *et al*., 1977).

Dermal

Cocker *et al*. (1988) collected urine samples from occupational workers dermally exposed to MOCA, to identify potential MOCA metabolites. 10 of the 23 urine samples contained small amounts of *N*-acetyl MOCA. Ducos *et al*. (1985) also identified *N*-acetyl MOCA as a metabolite but present in less than 10% of sampled urine. Further evaluation of urine, identified MOCA glucuronide as another potential MOCA metabolite (Cocker *et al*., 1990).

Dermally administered radiolabelled MOCA was reported to quickly metabolise in Beagle dogs (Manis *et al*., 1984) and rats (Groth *et al*., 1984) and only small amounts of unmetabolised MOCA were present in the urine samples. 1.3% of the dermal MOCA dose was present in the urine of the Beagle dogs 24 hours after administration. 0.005% of the detected radiolabelled MOCA dose was unmetabolised (Manis *et al*., 1984). 72 hours following dermal administration of radiolabelled MOCA to rats, Groth *et al*. 1984 reported that 2.54% of the dermal application to rats was detected in urine and of that 0.008% was unmetabolised MOCA.

Inhalation

In an occupational worker exposed to molten MOCA in an accidental spill, 35% of the MOCA metabolites excreted in urine were conjugates (Osorio *et al*., 1990). No additional information on the specific conjugates was provided.

No studies were located on the metabolism of MOCA in experimental animals via inhalation exposure.

4.1.4 Excretion

Oral

No additional studies were located on the excretion of MOCA in humans following oral exposure.

48 hours following a single oral dose of 10 mg/kg of ^{14}C-MOCA administered to female LAC:Porton rats, 60% of the radioactivity was excreted in faeces and the liver retained the most radioactivity at 2% (Farmer *et al*., 1981). Ducos and Gaudin (1983) reported a maximum excretion of MOCA 24 hours after male Sprague-Dawley rats were exposed to a single oral dose of 50 mg/kg/day. Biological half-lives of MOCA in rat liver and blood were 4.4 and 16.7 days, respectively, after a single oral dose of 75 mg/kg of ^{14}C-MOCA in rats (Cheever *et al*., 1988).

An additional oral study in rats reported 64-87% removal of radiolabelled MOCA in urine or faeces, 48 hours after exposure. The study identifies that MOCA is rapidly metabolised and excreted within the first 24 hours of oral administration (Morton *et al*., 1988).

Dermal

Nine hours following an accidental spill, exposing an occupational worker to 11.34 l of molten MOCA, 1.7 mg/l of MOCA was detected in the urine. A biological half-life of MOCA was estimated at 23 hours, after evaluating urine samples of the worker two weeks following the incident (Osorio *et al*., 1990). From the evaluation of urine samples, 94% of the initial MOCA dose was calculated to be excreted within 4 days (Osorio *et al*., 1990).

Hosein and Van Roosmalen (1978) reported an accidental spill of hot, liquid MOCA which sprayed on an occupational worker's face and entered the worker's mouth. 18 hours following the incident a maximum of 3.6 mg/l MOCA concentration was detected in the worker's urine. Three weeks post-MOCA exposure, only a trace amount of MOCA was detected in urine.

Groth *et al*. (1984) reported that 72 hours following a single dermal dose of 2.5 mg MOCA or ^{14}C-MOCA administered to male Sprague-Dawley rats, 2.54% of the initial MOCA dose was excreted as ^{14}C.

Inhalation

Urine samples of 34 occupational workers exposed to MOCA in the air at approximately <3 µg/m^3 were analysed. Concentrations of MOCA in workers involved in mixing had the highest level detected in urine. MOCA concentrations >5 µg/l were detected in all 10 mixers and half of the mixers had levels of MOCA >50 µg/l in their urine. Urine samples of moulders (intermediate MOCA exposure) reported concentrations of <5 to 50 µg/l of MOCA. No MOCA was detected in urine in the low exposure group, trimmers and supervisors (NIOSH, 1986). One study reported little difference between pre- and post-shift (48 hours after exposure)

urine concentrations in five male workers who were exposed to MOCA for between 3 and 27 years (Ichikawa *et al*., 1990).

A study over a period of 5 years was conducted involving 12 to 15 occupational workers in the manufacture of polyurethane elastomers, which uses MOCA in the process. During July to September 1978, the average level of MOCA in urine was 50 µmol/mol creatinine and by February 1980 the level had decreased to 5 nmol MOCA/mmol creatinine. The authors reported a reduction in MOCA exposure in workers due to an improvement in the ventilation system, the use of protective and dry cleaning of clothing and an improvement in hygiene in the factory (Thomas and Wilson, 1984).

An occupational study of 122 workers in 19 different works (1 MOCA manufacturer and 18 factory users) was conducted to identify the levels of MOCA in urine at the end of a shift. The highest levels were detected in urine of 12 workers who manufactured MOCA. The highest concentration reported was 600 µg/l (450 µg/g relative to creatinine), which decreased to an average of 62 µg/g of creatinine following modifications to the manufacturing process to reduce the workers' exposure to MOCA. The levels of MOCA in the urine samples varied between the remaining plants. Ducos and Gaudin (1983) concluded that the level of MOCA in urine depended on several factors, which included the frequency of MOCA use, the quantity of MOCA, the form of MOCA (i.e. granules, solid or solution), the methods of manufacturing and general work practices. The authors also reported that the predominant metabolite was *N*-acetyl MOCA at low concentration and in one worker, *N*,*N'*-diacetyl MOCA was also identified (Ducos and Gaudin, 1983).

No additional studies were located on the excretion of MOCA in experimental animals following inhalation exposure.

4.1.5 Summary

Data from occupational studies have identified that the most likely routes of exposure to MOCA are from contact with contaminated surfaces i.e. dermal, followed by inhalation and oral pathways. The results from studies suggest that MOCA is rapidly absorbed and excreted following acute dermal/inhalation exposure (48 hours) to low concentrations of MOCA. Studies have reported a slower excretion of higher concentrations of MOCA. No human absorption data were available following the oral exposure of MOCA. The liver was determined in experimental animals as the main target tissue following acute exposure. Radiolabelled MOCA in experimental animals also identified the kidneys, lungs, spleen, bladder, brain and lymphocytes as other potential sites of distribution. A similar distribution following oral and dermal exposure to MOCA was reported in rats and dogs, respectively. The metabolism of MOCA includes several different pathways including *N*-hydroxylation and *N*-oxidation to generate chemicals with the potential to form MOCA-DNA adducts.

4.1.6 Bioavailability

Urine samples from workers following dermal and inhalation exposure suggest that MOCA is rapidly absorbed and excreted (ATSDR, 1994).

4.2 Genotoxicity

IARC (2010, 2012) reported strong evidence of the carcinogenicity of MOCA via a genotoxic mechanism of action. The data suggest that the genotoxic mechanism includes metabolic activation of MOCA to form adducts with DNA, resulting in the induction of mutagenic and clastogenic effects in humans.

Table 4.1 and Table 4.2 summarise the available *in vitro* and *in vivo* genotoxic data for MOCA. The data suggest that MOCA is mutagenic in several strains of *Salmonella typhimurium* tested with metabolic activation in the Ames assay. The genotoxic assays conducted in several strains of *Saccharomyces cerevisiae* indicate that MOCA is not genotoxic in these test systems. MOCA induced chromosomal aberrations (including single DNA strand breaks) either with or without metabolic activation and unscheduled DNA synthesis without activation. The mouse lymphoma assay identified both positive and negative results with and without metabolic activation, respectively. The available *in vivo* data strongly suggest that MOCA is genotoxic in experimental animals following dermal, inhalation and oral exposure. MOCA induced DNA adduct formation in two species of rat following oral, dermal and intraperitoneal injection and mutations in the sex-linked recessive lethal assay conducted in *Drosophila melanogaster*. MOCA also induced micronuclei in B6C3F1 mice via intraperitoneal injection; however, it did not induce micronuclei via the same exposure route in CD-1 mice.

The weight of evidence from the genotoxicity data, particularly the *in vivo* studies, indicates that it should be considered a genotoxic agent.

Table 4.1 *In Vitro* Genotoxicity of MOCA

Species (test system)	End-point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium TA98, TA100 and TA1538 (histidine reversion)	gene mutation	positive	negative	Cocker *et al*., 1985; Dunkel *et al*., 1984; Brooks and Dean, 1981; Simmon and Shepherd, 1981
Salmonella typhimurium TA1535, TA1537	gene mutation	positive	no data	Cocker *et al*., 1985; Skopek *et al*., 1981; Trueman, 1981

Species (test system)	End-point	Results		Reference
		With activation	Without activation	
and TM677				
Salmonella typhimurium TA1535 and TA1537	gene mutation	negative	negative	Chemtura Belgium N.V., 2014; Limburge Urethane Casting N.V., 2010
Salmonella typhimurium mix of TA7001-TA7006	gene mutation	positive	no data	Chemtura Belgium N.V., 2014; Limburge Urethane Casting N.V., 2010
Escherichia coli WP2 uvrA, WP85 (tryptophan reversion)	gene mutation	positive	negative	Gatehouse, 1981; Venitt and Crofton Sleigh, 1981
Escherichia coli WP2 uvrA	gene mutation	negative	negative	Chemtura Belgium N.V., 2014; Limburge Urethane Casting N.V., 2010
Escherichia coli 58-161 envA (lambda lysogen)	phage lambda induction (SOS induction)	positive	no data	Thomson, 1981
Escherichia coli P3478, W3110, WP67 and CM871	differential killing	negative	positive	Rosenkranz *et al*., 1981; Tweats, 1981;
Escherichia coli JC2921, JC5519	differential killing	positive	no data	Ichinotsubo *et al*., 1981
Bacillus subtilis rec^-	differential killing	positive	positive	Kada, 1981
Eukaryotic organisms:				
Fungi:				
Saccharomyces cerevisiae XV185-14C (auxotroph reversion)	gene mutation	negative	negative	Mehta and Von Borstel, 1981
Saccharomyces cerevisiae XII	mitotic recombination	negative	negative	Kassinova *et al*., 1981
Saccharomyces cerevisiae D4	mitotic gene conversion	negative	negative	Jagannath *et al*., 1981
Saccharomyces cerevisiae JD1	mitotic gene conversion	positive	positive	Sharp and Parry, 1981
Saccharomyces	mitotic	positive	positive	Parry and Sharp,

Species (test system)	End-point	Results		Reference
		With activation	Without activation	
cerevisiae D6	aneuploidy			1981
Mammalian:				
Chinese hamster ovary cells	chromosomal aberrations	negative	negative	Galloway *et al*., 1985
Mammalian cultured lung-derived fibroblasts (species not reported)	chromosomal aberrations	positive	no data	Chemtura Belgium N.V., 2014; Limburge Urethane Casting N.V., 2010
Chinese hamster ovary cells	sister chromatid exchange	inconclusive	inconclusive	Galloway *et al*., 1985
Chinese hamster ovary cells	sister chromatid exchange	negative	negative	Perry and Thomson, 1981
HeLa cells	unscheduled DNA synthesis	positive	negative	Martin and McDermid, 1981
Rat primary hepatocytes	unscheduled DNA synthesis	no data	positive	McQueen *et al*., 1981
Mouse primary hepatocytes	unscheduled DNA synthesis	no data	positive	McQueen *et al*., 1981
Hamster primary hepatocytes	unscheduled DNA synthesis	no data	positive	McQueen *et al*., 1981
Rabbit primary hepatocytes	unscheduled DNA synthesis	no data	positive	McQueen and Williams, 1987
Primary hamster embryo cells	single strand DNA breaks	no data	positive	Casto, 1983
Human male embryonic lung cells	single strand DNA breaks	no data	positive	Casto, 1983
Mouse lymphoma cells (L5178Y TK +/-)	forward gene mutation	positive	negative	Caspary *et al*., 1988; Myhr and Caspary, 1988
RLV-infected rat embryo ($2FR_450$)	transformation (attachment independence)	no data	positive	Traul *et al*., 1981
Balb/3T3 mouse cells	transformation (attachment independence)	no data	positive	Dunkel *et al*., 1981
Baby hamster kidney cells (BHK21 C13)	transformation (attachment independence)	positive	positive	Daniel and Dehnel, 1981

Species (test system)	End-point	Results		Reference
		With activation	Without activation	
Baby hamster kidney cells (BHK21)	transformation (attachment independence)	positive	no data	Styles, 1981
C3H2K cells/MLV	viral integration enhancement	no data	negative	Yoshikura and Matsushima, 1981
Human bladder explant culture	DNA adduct formation	no data	positive	Stoner *et al.*, 1988
Dog bladder explant culture	DNA adduct formation	no data	positive	Stoner *et al.*, 1988

Table 4.2 *In Vivo* Genotoxicity of MOCA

Species (test system)	End-point	Results	Reference
Mouse CD1 (intraperitoneal)	micronucleus formation	negative	Tsuchimoto and Matter, 1981
Mouse B6C3F1 (intraperitoneal)	micronucleus formation	positive	Salamone *et al*., 1981
Drosophila melanogaster (oral, dermal)	wing spot test	positive	Kugler-Stegmeier *et al*., 1989
Drosophila melanogaster (oral, dermal)	sex-linked recessive lethal mutation	positive	Vogel *et al*., 1981
Drosophila melanogaster (inhalation, occupational)	sex-linked recessive lethal mutation	positive	Dormer *et al*., 1983
Rat (Sprague-Dawley; oral)	DNA adduct formation	positive	Cheever *et al*., 1990; Kugler-Stegmeier *et al*., 1989
Rat (Sprague-Dawley; dermal)	DNA adduct formation	positive	Cheever *et al*., 1990
Rat (Wistar-derived strain; intraperitoneal)	DNA adduct formation	positive	Silk *et al*., 1989

4.3 Carcinogenicity

Under Annex VI, part 3 of Regulation (EC) No 1272/2008 MOCA is classified as a carcinogen 1B, H350 chemical, which suggests that MOCA “may cause cancer” (EC, 2011).

4.3.1 Human Epidemiological Studies

Information on individual studies is located in Appendix B1. Four epidemiological studies were located and these mainly concentrated on the possible increased incidence of bladder cancer (Ward et al., 1990; Chen et al., 2005; Mason et al., 1990; Dost et al., 2009). This was based on the known properties of other similar amine compounds such as benzidine and naphthylamine. There were US, Taiwan and UK studies following workers exposed to MOCA and monitoring urine samples. There were low levels of bladder cancers and abnormalities in

cells in urine detected in these studies but the lack of appropriate controls and exposure to a number of other potentially carcinogenic chemicals and other confounders means that there is no convincing evidence of a causal association between MOCA exposure and bladder cancer. IARC (2010, 2012) reported that “no adequate epidemiology studies were available to the Working Group to evaluate an association between MOCA and bladder cancer risk”.

4.3.2 Experimental Animal Studies

Initiation-promotion Studies

Information on individual studies is located in Appendix B2.1.

Chronic Studies

Detailed summaries of individual chronic studies are located in Appendix B2.2. Table 4.3 summarises all the available chronic carcinogenicity studies of MOCA.

Table 4.3 Overview of the chronic carcinogenicity studies of MOCA

Reference	Study (species, strain, sex, number of animals, duration and route of exposure)	Dose	Findings
Russfield *et al*. 1975	• HaM/ICR mice M/F • 25/sex/dose • 18 months exposure and 6 months observation • oral exposure via diet	• 0, 130 or 260 mg/kg bw/day MOCA hydrochloride salt (purity 97%)	• significant increase in incidence of hepatomas in both dose groups of F
Grundmann and Steinhoff 1970	• Wistar rats M/F • 25/sex/dose and 50/sex controls • 500 days and observation period (lifetime) • oral exposure via protein-deficient diet	• 0 or 54 mg/kg bw/day MOCA (purity unspecified)	• significant increase in hepatomas and lung tumours in M & F • high mortality rate in M & F
Russfield *et al*., 1975	• Charles River CD-1 rats M • 25/dose • 18 months exposure and 6 months observation • oral exposure via standard protein diet	• 0, 25 or 50 mg/kg bw/day MOCA hydrochloride salt (purity 97%)	• no significant increase in tumours

Reference	Study (species, strain, sex, number of animals, duration and route of exposure)	Dose	Findings
Stula *et al*., 1975	• Charles River CD rats M/F • 50/sex/dose • 2 years • oral exposure via a standard-protein diet (23% protein) • 6/dose sacrificed for a one-year interim evaluation	• 0 or 50 mg/kg bw/day MOCA (purity ~95%)	• significant increase of lung adenomatosis (pre-neoplastic lesion) and lung adenomatosis in M & F
Stula *et al*., 1975	• Charles River CD rats M/F • 25/sex/dose • 16 months • oral exposure via a low-protein diet (7%) • 6/dose sacrificed for a one-year interim evaluation	• 0 or 50 mg/kg bw/day MOCA (purity ~95%)	• significant increase of lung adenocarcinomas and lung adenomatosis in M & F • significant increase in hepatocellular carcinomas and hepatocellular adenomas in M • significant increase in mammary gland adenocarcinomas in F
Kommineni *et al*., 1979	• Charles River CD rats M • 100 rats (control and low-dose group), 75 rats (mid-dose group) and 50 rats (high-dose group) • 18 months exposure and 6 months on diet and 32 weeks observation • **Group A**: ○ protein-adequate diet (27%) • **Group B**: ○ a protein-deficient diet (8%)	• **Group A**: ○ 0, 25, 50 or 100 mg/kg bw/day MOCA (industrial grade) • **Group B**: ○ 0, 12.5, 25 and 50 mg/kg bw/day MOCA	• significant increase in lung adenocarcinomas, all lung tumours, mammary gland adenocarcinomas, Zymbal gland carcinomas, hepatocellular carcinomas and haemangiosarcomas

Reference	Study (species, strain, sex, number of animals, duration and route of exposure)	Dose	Findings
Stula *et al*., 1978	• Beagle dogs F • 6/dose • 3 days/week for 6 weeks, then 5 days/week for 9 years • oral exposure	• 100 mg MOCA (~90% purity) in a gelatine capsule (average 10 mg/kg bw/day)	• Urinary bladder transitional cell carcinomas were reported in 4/5 (80%) of the treated female dogs The other treated dog died early, not related to treatment).
Steinhoff and Grundmann 1969	• Wistar rats M/F • 17/sex/dose and 25/sex controls • 88 weeks exposure and 23 weeks observation (lifetime) • subcutaneous injection	• 0, 500 or 1000 mg/kg bw MOCA (94% purity)	• significant increase in hepatocellular carcinomas and lung cancers

F: Female.

M: Male.

IARC classified MOCA as Group 2B (possibly carcinogenic to humans) because, while there is strong evidence of carcinogenicity in animals, there was no convincing evidence in humans (IARC, 2010, 2012).

There have been a number of carcinogenicity studies with MOCA although they are all rather old and conducted before the modern guidelines and GLP were implemented. These are outlined in Table 4.3. Although these studies in rats suffer from a limited range of doses and exposure times, and some experienced high mortality rates, they consistently show an increased incidence of lung and liver tumours.

There is an oral long-term (up to 9 years) study in Beagle dogs with a single dose in which bladder tumours were observed in 4 out of 5 surviving treated dogs. This result, together with the epidemiological studies, indicates weak evidence that bladder cancer may be associated with MOCA exposure (Stula *et al*., 1978). Due to the limited number of animals the study is not however, suitable for risk assessment. The most-complete dose-response study, although with high mortality, is that of Kommineni *et al.* (1979) in which rats with an adequate protein diet (a further treated group had inadequate protein) were treated orally. The use of T25 in the cancer risk estimates using lower dose tumour incidences counters this higher mortality in the study. This study was used for risk assessment in the Chemical Safety Reports (CSRs: Chemtura, 2014; Limburge Urethane Casting N.V., 2010).

In the Dutch DECOS (2000) assessment of the long-term carcinogenicity studies, the Kommineni *et al*. (1979) study had an incidence of tumours of 3.5 $x10^{-2}$/mg kg bw/day, close to the highest incidence of 3.7 $x10^{-2}$/mg/kg bw/day (Grundmann and Steinhoff, 1970). This assessment, while giving some indication of the comparative sensitivity of the carcinogenicity studies, uses different methodologies to those REACH Guidance methods used in this risk assessment and so the tumour frequencies are not suitable.

The frequency of combined lung tumours observed in the Charles River CD rat oral long-term study of Kommineni *et al.* (1979) will be used in this review to derive lifetime cancer risk estimates. In the part of the study to be used, male rats were exposed to industrial grade MOCA (unspecified purity) in protein-sufficient diets (27% protein; a further group had a protein-restricted diet, 8% protein) at 0, 250, 500 and 1000 ppm for 18 months following by a 6-month recovery period. This corresponded to a received dose of 0, 12.5, 25 and 50 mg/kg bw/day estimated by assuming that a rat consumes 5% of its body weight per day (US EPA, 2006). These doses were expanded to continuous lifetime exposure by multiplying by 18/24 months to give a corrected dose (US EPA, 2006). Tumours were detected in the lung, mammary gland, Zymbal gland and liver. Combined lung tumours (adenomas, epidermoid carcinomas and adenocarcinomas) gave the most complete dose response data, and lung tumours are the most frequently observed tumours seen in the experimental animal long-term studies. The tumour incidence is shown in Table 4.4 below.

Table 4.4 Lung tumour incidence in MOCA-treated male rats (Kommineni *et al.*, 1979)

Dietary Dose (ppm)	0	250	500	1000
Dose/animal (mg/kg bw/day	0	12.5	25	50
Corrected Dose (mg/kg bw/day)	0	9.4	18.8	37.5
Total Tumours/animals	1/100	23/100	28/75	35/50
Incidence	0.01	0.23	0.37	0.70

4.4 Evaluations

Table 4.5 summarises the expert carcinogenic assessments of MOCA including any derived threshold doses.

Table 4.5 Overview of the carcinogenic assessments of MOCA

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
IARC (2010)	genotoxic mechanism: • metabolic activation to *N*-hydroxy MOCA	not addressed	inadequate evidence in humans of carcinogenicity sufficient evidence in experimental animals of carcinogenicity – the main target tissues: • liver and lungs in rats • urinary bladder in dogs	not addressed
IARC (2012)	genotoxic mechanism: metabolic activation *N*-oxidation in the liver *O*-acetylation in the bladder	not addressed	inadequate evidence in humans of the carcinogenicity of MOCA sufficient evidence in experimental animals of the carcinogenicity of MOCA	not addressed
ATSDR (1994)	not reported	not addressed	reported to be a suspected bladder carcinogen considered a probable human carcinogen	not addressed
Chemtura Belgium N.V., 2014; Limburge Urethane Casting N.V., 2010	genotoxic mechanism	non-threshold	lung, mammary, zymbal gland and liver tumours detected in an 18-month study in male rats (Kommineni *et al*., 1979)	**WORKERS:** **dermal:** BMDL10 = 178 mg/kg bw/day AF = 40 000 DMEL = 4.45×10^{-3} mg/kg bw/day **inhalation:** BMCL10 = 7.76 mg/m^3 SF = 10 000 DMEL = 7.76×10^{-4} mg/m^3

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
Chemtura Belgium N.V., 2014; Limburge Urethane Casting N.V., 2010	genotoxic mechanism	non-threshold	lung, mammary, zymbal gland and liver tumours detected in an 18-month study in male rats (Kommineni *et al*., 1979)	**GENERAL POPULATION:** **dermal:** BMDL10 = 178 mg/kg bw/day SF = 40 000 DMEL = 4.45 x 10^{-3} mg/kg bw/day **inhalation:** BMCL10 = 3.07 mg/m^3 SF = 10 000 DMEL = 3.07 x 10^{-4} mg/m^3 **oral:** BMDL10 = 4.44 mg/kg bw/day SF = 40 000 DMEL = 1.11 x 10^{-4} mg/kg bw/day
DECOS, 2000	Genotoxic mechanism	Non-threshold	DECOS assessed all the studies for additional lifetime cancer risk associated with occupational exposure. Different methodology using malignant tumours to calculate incidence/mg/kg bw/day.	Results varied from 2.2 x 10^{-3} to highest incidence 3.7 x 10^{-2}/ mg/kg bw/day (Grundmann and Steinhoff, 1970) corresponds to additional lifetime cancer risk of 4 x 10^{-5} for 40 y exposure to 0.02 mg/m^3

BMCL10: Lower 95% confidence limit of a benchmark concentration representing a 10% tumour response following lifetime exposure.

BMDL10: Lower 95% confidence limit of a benchmark dose representing a 10% tumour response following lifetime exposure.

DMEL: Derived Minimum Effect Level.

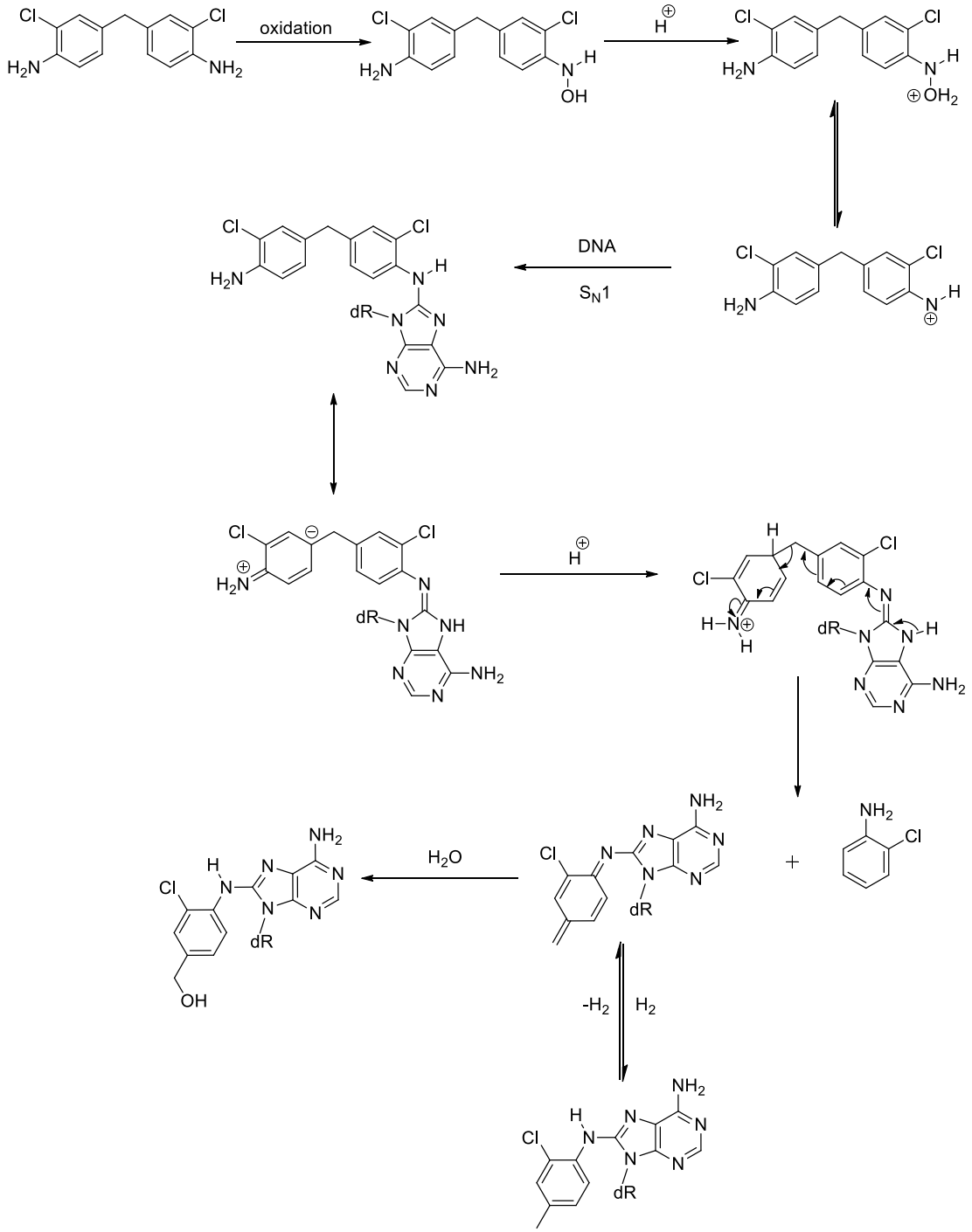
SF: Safety Factor (Assessment Factor).

4.5 Mechanism of Action

The precise mechanism of action for carcinogenicity of MOCA is not fully understood; however, MOCA has the potential to form adducts with DNA. It is reported that arylamines such as MOCA undergo a series of metabolic process, generating metabolites which can react with DNA to form adducts (ATSDR, 1994; IARC, 2012). Initially cytochrome P450 enzymes catalyse the *N*-oxidation of MOCA to *N*-hydroxy-MOCA. Following further

metabolism, the conjugated *N*-hydroxy-MOCA metabolite is hydrolysed under acidic conditions to form the reactive electrophilic aryl nitrenium ions (IARC, 2012; Swaminathan *et al*., 1996). The high acidity of urine in humans is suggested as a potential explanation for the incidence of urinary bladder tumours detected in occupational workers exposed to MOCA (Kadlubar *et al*., 1977). The reactive nitrenium ion was identified as reacting primarily with C8-deoxyadenosine in rats via a S_N1 nucleophilic substitution reaction mechanism as seen in Figure 4. (Beland and Kadlubar, 1990; IARC, 2010; IARC, 2012; Swaminathan *et al*., 1996). Following tautomerisation of the metabolite-DNA adduct and cleavage of the methylene bridge, the major adduct is identified as *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol, while the minor adduct is reported as *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorotoluene (IARC, 2010). These MOCA-DNA adducts have been reported in urothelial cells of an exposed worker; liver, kidney, lung and bladder of rat and dog *in vivo* studies (IARC, 2012; Swaminathan *et al*., 1996).

Figure 4.6 Reaction mechanism of MOCA with DNA



As well as forming MOCA-DNA adducts, data suggest that MOCA can also react and generate adducts with haemoglobin and serum albumin. MOCA-haemoglobin adducts have been detected in exposed workers in the polyurethane elastomer industry (Cheever *et al.*, 1988, 1990, 1991; Vaughan and Kenyon, 1996).

4.6 Carcinogenicity risk estimates

4.6.1 Critical studies

Dose response

The aim of this project is to identify information that can be used to quantify risk for relevant exposure routes. The review of the genotoxicity and carcinogenicity data leads to the conclusion that there is a potential for a genotoxic mode of action and that exposure to MOCA can give rise to tumours in experimental animals, and can presume to have carcinogenic potential in humans. Therefore the quantitative risks for MOCA are based on a carcinogenic potential. Review of the epidemiological studies on human occupational exposure to MOCA does not reveal any data that would be useful in identifying any quantitative risk for humans. Therefore the dose response curves are based on the most relevant, robust studies in experimental animals.

The value commonly used globally including Europe as a Point of Departure (PoD) for risk assessment is T25 which is the daily dose (in mg/kg body weight) inducing a tumour incidence of 25% upon lifetime exposure. This is based on an assumption of a linear dose response at all concentrations (including above the experimental doses) excluding the zero dose. The derivation of a T25 for MOCA will be the PoD for this risk assessment.

4.6.2 T25 Derivation

The T25 value for MOCA has been derived using information from a long-term study on Charles River CD rats administered MOCA in the diet with adequate protein (Group A) and using the frequency of all lung tumours (adenoma, epidermoid carcinoma and adenocarcinoma) (Table 4.4; Kommineni *et al*., 1979).

- lowest dose with a significantly increased frequency (C) of 9.4 mg/kg bw/day
- Incidence at C, 0.23
- Control incidence, 0.01

T25 is derived using the following calculation:

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

The lowest $T25_{(Oral, Rat)}$ = 9.4 x 0.25/0.23-0.01 x 1-0.01/1

= 10.6 mg/kg bw/day.

Therefore **$T25_{(Oral, Rat)}$ = 10.6 mg/kg bw/day**.

This value is used as the PoD for the derivation of route-specific risk estimates for workers and the general population.

Workers inhalation risk estimate

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/8 h);0.384 m^3/kg bw/8 h
- oral absorption rat/inhalation humans (50/100)
- activity driven difference for workers (standard respiratory volume for humans, 6.7/respiratory volume in light work for workers,10 m^3)

$$\mathbf{T25_{(Inhalation,\ Human)} = 10.6 \times 1/0.384 \times 6.7/10 \times 50/100 = 9.25\ mg/m^3}$$

Correcting for worker 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

$$\mathbf{T25_{(Inhalation,\ Workers)} = 9.25.\ mg/m^3 \times 2.8\ correction\ factor = 25.9\ mg/m^3}$$

Workers dermal risk estimate

Taking the $T25_{(Oral,\ Rat)}$ and correcting for

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

$$\mathbf{T25_{(Dermal,\ Human)} = 10.6/(50/50)/4 = 2.65\ mg/kg\ bw/day}$$

Correcting for workers' exposure as above:

$$\mathbf{T25_{(Dermal,\ Workers)} = 2.65 \times 2.8 = 7.4\ mg/kg\ bw/day}$$

General population inhalation risk estimate

$T25_{(Oral,\ Rat)}$ 10.6 mg/kg bw/day corrected for general population inhalation exposure:

- allometric scaling from rats to humans, 4
- human weight 70 kg
- human general population breathing 20 m^3 per person
- default oral absorption (50%) to inhalation absorption (100%).

$$\mathbf{T25_{(Inhalation,\ Gen\ pop)} = 10.6/4 \times 70/20 \times 50/100 = 4.6\ mg/m^3}$$

General population oral risk estimate

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

$$\mathbf{T25_{(oral,\ general\ pop)} = 10.6/4 = 2.65\ mg/kg\ bw/day}$$

A summary of the cancer risk estimates is shown in Table 4.7.

Table 4.7 Cancer risk estimates for MOCA

Route of exposure	Population	T25 Descriptor	Cancer risk for 1 unit amount
Oral	General population	$T25_{(Oral,\ General\ pop)}$ 2.65 mg/kg bw/day	9.43 x 10^{-5} per µg/kg bw/day
Inhalation	Workers	$T25_{(Inhalation,\ Workers)}$ 25.9 mg/m^3	9.65 x 10^{-6} per µg/m^3
	General population	$T25_{(Inhalation\ General\ pop)}$ 4.6 mg/m^3	5.43 x 10^{-5} per µg/m^3
Dermal	Workers	$T25_{(Dermal,\ Human)}$ 7.4 mg/kg bw/day	3.38 x 10^{-5} per µg/kg bw/day

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

1 µg/m^3	9.65 x 10^{-6}
2 µg/m^3	1.93 x 10^{-5}
5 µg/m^3	4.83 x 10^{-5}
10 µg/m^3	9.65 x 10^{-5}

4.7 Biomonitoring approach

An additional approach for assessing the exposure and risk of MOCA is the biomonitoring of occupationally exposed workers. This approach has been summarised by SCOEL particularly in the 2013 Annex to its recommendations on MOCA (SCOEL, 2010/2013).

There have been a number of studies measuring MOCA in urine. MOCA is excreted as 'free' MOCA but also as metabolites, glucuronide-MOCA and acetyl-MOCA. Commonly used methods have been developed to measure total MOCA (free and conjugated MOCA) expressed in µmol/l or µmol/mol creatinine (to correct for urinary creatinine excretion). Detection limits vary between 3.7-5 nmol/l (1-1.5 µg/l), corresponding approximately to 0.35-0.5 µmol/mol creatinine (SCOEL, 2010/2013). In workers not exposed to MOCA, urinary levels are below the detection limits of these modern analytical techniques.

Since MOCA is a genotoxic, non-threshold carcinogen, SCOEL has not set any biological limit value for MOCA, but has derived a Biological Guidance Value which typically represents the 95th percentile of the biomarker levels in occupationally non-exposed populations. In the case of MOCA, this is below the detection limit, and so any concentrations detected suggest occupational exposure.

There are no reliable measured data on correlations between urinary MOCA levels and MOCA air concentrations, so it is not possible to directly calculate urinary levels which correspond to occupational exposure, e.g. 1 or 10 µg/m^3.

In SCOEL (2010/2013), an open one-compartment model to calculate the daily dose corresponding to urinary MOCA level of 5 µmol/mol creatinine in the Friday afternoon (end of shift) sample is described. For a substance following first order elimination kinetics the decrease in urinary level follows 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, = $\ln 2/T_{1/2}$.

Assuming that the half-time of MOCA is 23 hours and the steady state is reached after one-week exposure, an average urinary concentration of MOCA at steady state is 2.6 µmol/mol creatinine when the concentration in the Friday afternoon sample is 5 µmol/mol creatinine.

Urinary excretion of 5 µmol/mol creatinine in the Friday afternoon can then be calculated to using the formula:

$$D = C_{ss} \times Cr_{24h} \times M/BW \times F_{ue}$$

where D = daily dose (µg), Css = average concentration in the urine, Cr_{24h} = average daily excretion of creatinine for a 50-year old man of 70 kg (12 mmol), Fue = proportion of dose excreted in urine (50% in the case of MOCA).

2.6 µmol/mol creatinine × 0.012 mol × 267.17 g/mol /0.5 = 17 µg

SCOEL then used unit cancer risk estimates derived by DECOS (2000) to calculate cancer risk for different urinary MOCA levels. These risk estimates were derived using a different method from that in the REACH Guidance. It should be noted, that SCOEL gave these risk estimates for information only, and did not set any limit value based on these calculations.

The risk estimates derived above using the REACH Guidance can be used to calculate the risk level for different urinary MOCA levels.

Since 1 µg/m^3 exposure (which corresponds to a daily dose of 10 µg in occupational exposure) represents a cancer risk of 9.65 x 10^{-6}.

5 µmol/mol creatinine in a Friday afternoon sample (corresponding to a daily dose of 17 µg) corresponds to a risk of 16.4 x 10^{-6}.

0.5 µmol/mol creatinine (detection limit of current analytical techniques) corresponds to cancer risk of 1.64 x 10^{-6}.

While these calculations to estimate daily dose are not precise and include some assumptions, biomonitoring is currently the best method to estimate the total exposure to MOCA in occupational settings. Therefore when biomonitoring data are available, these can be used to estimate cancer risks for occupational exposure.

4.8 References

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5. 1,2-Dichloroethane

1,2-dichloroethane (CAS RN: 107-06-2; EC: 203-458-1) is included in Annex XIV of REACH "List of substances subject to authorisation".

Over 95% of EDC in 2009 was consumed in the production of vinyl chloride monomer for polyvinyl chloride production. Other very small uses for EDC include chlorinated extraction and cleaning solvents, manufacture of ethyleneamines and vinylidene chloride).

5.1 Toxicokinetics

Studies suggest that 1,2-dichloroethane is rapidly and well absorbed by all routes of exposure in experimental animals. It is also noted that when 1,2-dichloroethane is administered orally and dermally with water as the vehicle, absorption, distribution, metabolism and excretion rates were all increased (Withey et al., 1983; Morgan *et al*., 1991).

5.1.1 Absorption

Oral

No studies examining the absorption of 1,2-dichloroethane in humans following oral ingestion were located. Case studies of people who exhibited toxic effects following accidental or intentional ingestion intimate that is rapidly absorbed into the circulatory system. 1,2-Dichloroethane is lipophilic and therefore it is expected that it will mainly be absorbed via passive diffusion across the mucosal membranes of the gastrointestinal tract (ATSDR, 2001).

Absorption following ingestion is rapid and complete in rats (Reitz *et al.,* 1980, 1982). The pharmacokinetics of 1,2,-dichloroethane are dose-dependent. Following oral ingestion of 1,2-dichloroethane dissolved in corn oil at doses of 25, 50 or 150 mg/kg bw, peak blood levels in Osborne-Mendel rats of 13–67 mg/l occurred within 10-15 minutes of administration. A positive linear correlation between blood plasma levels and dose was steeper up to doses of 50 mg/kg bw and the steepness of the correlation decreased up to 150 mg/kg bw (Reitz *et al.,* 1982). The authors suggested this may have been due to saturation of gastrointestinal absorption (Reitz *et al.,* 1982., Spreafico *et al.,* 1980).

In another study, Sprague-Dawley rats were administered 1,2-dichloroethane in corn oil via gavage at doses of 25, 50 or 150 mg/kg bw. Following administration, peak blood levels were

achieved in 30-60 minutes. One half of the lowest dose was absorbed in 3.3 minutes and one half of the highest dose was absorbed in 6.4 minutes (Spreafico *et al.,*1980).

In the same study, plasma elimination time (t½) increased from 25 minutes to 57 minutes over the dose range (25-150 mg/kg bw), while the area under the curve (AUC) increased 16-fold with a six-fold increase in dose. The maximum blood plasma concentration (C_{max}) is proportional to the dose up to 150 mg/kg bw (Spreafico *et al.,* 1980). No difference was observed between the kinetics of 1,2-dichloroethane if administered as a single oral dose of 50 mg/kg bw or single daily administrations of 50 mg/kg bw/day. Absorption via the gastrointestinal tract was more rapid if 1,2-dichloroethane was administered in an aqueous solution compared to corn oil (Withey *et al.,* 1983).

Dermal

Studies in experimental animals have shown that 1,2-dichloroethane is rapidly absorbed through the skin. Male rats were exposed to 2 ml of 1,2-dichloroethane via shaved skin and covered by a patch. After 24 hours, 1.08 ml had been absorbed and blood levels of 1,2-dichloroethane were 135 µg/l. Absorption exceeded distribution and excretion. The experiment was repeated using a 1,2-dichlorethane in aqueous solution and blood plasma levels peaked at 0.35-1.4 µg/ml, 1-2 hours following exposure, and then reduced to control levels after 24 hours. This suggests that 1,2-dichloroethane in aqueous solution is rapidly and completely absorbed allowing for rapid elimination from the body within the 24 hour time period (Morgan *et al*., 1991).

1,2,-dichloroethane is rapidly adsorbed through the skin in mice, rats and guinea pigs (Tsuruta, 1975, 1977). Absorption studies in rats exposed to 1,2-dichloroethane in aqueous solution applied to the skin showed peak blood levels correlating to the dose applied (Jakobson *et al*., 1982; IARC, 1999). Blood levels of 1,2-dichloroethane in guinea pigs increased rapidly (up to 7 mg/l) during the first 30 minutes following covered application of 1.0 ml of undiluted compound to the skin. The blood plasma levels of 1,2-dichloroethane then decreased until 1 hour after application when it increased again rapidly to a maximum of 17 mg/l (Jakobson *et al*., 1982).

The conclusion in experimental animals from a number of studies is that dermal absorption is rapid and complete. However, in contrast, a study on occluded human skin *in vitro* reported a low level of absorption of approximately 1.5% (Ward, 1992). The available study details indicate that the absorption rate for 1,2-dichloroethane in rat and human skin was measured over a period of 8 hours. However, data were only shown for 0.25 hour and then the rate/hour stated. This suggested that for undiluted 1,2-dichloroethane only 1.5% was absorbed in an hour; the study report stated that absorption had virtually ceased in an hour. It is unclear why the results of this study appear to contradict the findings from the earlier *in vivo* studies.

A recent study by Gajjar and Kasting (2014) investigated absorption of several volatile organic compounds (VOCs), including 1,2-dichloroethane in human skin *in vitro,* in a system that was

designed to allow the evaporation of the product at the surface of the skin. Absorption of 1,2-dichloroethane in this model was 0.2%. The authors noted that evaporation is likely to be a significant factor when considering 1,2-dichloroethane absorption via the skin and concluded that this figure might under-predict the absorption of these VOCs (except ethanol) related to their ability to disrupt or solubilise skin lipids.

The REACH Guidance in Chapter R7.12 on Toxicokinetics, indicates that physicochemical factors may also be considered. The guidance suggests that a default of 100% can be assumed unless the LogP (octanol-water partition coefficient; the lipophilicity of a chemical) is outside the range -1 to +4 and the molecular weight is high (over 500; larger molecules are not easily absorbed), when absorption of 10% can be considered. The LogP for 1,2-dichloroethane is 1.48 which favours dermal absorption especially when water solubility is high, and 1,2-dichloroethane is highly water soluble (8690 mg/l), and molecular weight is low at 98.97.

In spite of the *in vitro* findings, it therefore seems that the potential for 1,2-dichloroethane absorption is high and this has been observed *in vivo*. The extent of uptake in practice appears to depend not only on the rate at which transfer of 1,2-dichloroethane occurs across the skin, but importantly also on the degree of occlusion and ambient air current. The rate of transfer may be influenced by co-exposure to other substances: there is evidence for example that absorption was higher when water was used as a vehicle for delivery.

Therefore, in this cancer risk estimate, a default value of 50% dermal absorption is used. Other absorption values could be considered if there was convincing evidence that absorption was different in the application being reviewed.

Inhalation

1,2-Dichloroethane is rapidly absorbed through the lungs of humans and experimental animals upon inhalation exposure (ATSDR, 2001). In two studies looking at the occurrence of 1,2-dichloroethane in the breast milk of lactating women it was found that 1,2-dichloroethane inhaled during occupational exposure accumulates in breast milk (Urusova, 1953, US EPA, 1980). Urusova (1953) reported that nursing women exposed to 15.6 ppm of 1,2-dichloroethane in the workplace air accumulated 1,2-dichloroethane in the breast milk and the concentration gradually increased over time with the peak concentration found 1 hour after finishing work. A fatal case of 1,2-dichloroethane poisoning has been reported in which a man was exposed to 1,2-dichloroethane vapours for 30 minutes in an enclosed space indicating that it is readily absorbed through the lungs. Adverse effects in this case were not seen until 20 hours post-exposure and so the authors proposed that the formation of active metabolites were important in the induction of toxicity (Nouchi *et al.,* 1984, ATSDR, 2001).

Inhalation by experimental animals showed rapid adsorption. In rats, blood plasma concentrations of 1,2-dichloroethane peaked and remained constant at 8-10 µg/ml within 1-2 hours of continuous inhalation exposure of 600 mg/m^3 (150 ppm) for 6 hours (Reitz *et al.,*

1982, IARC, 1999). In a similar study, rats were exposed to 1,2-dichloroethane by inhalation at concentrations of 50 or 250 ppm. Rats exposed to 50 ppm 1,2-dichloroethane achieved steady state blood plasma levels in 1-2 hours after the start of exposure, whereas the rats in the top dose group did not achieve a steady state blood plasma level until 3 hours after the start of exposure (Spreafico *et al.,* 1980). These studies imply that the absorption of 1,2-dichloroethane increases from the start of exposure until an equilibrium is reached and that increasing the concentration increases the time it takes to achieve this equilibrium.

Summary

Experimental studies indicate rapid and high absorption and so 100% absorption is appropriate for oral and inhalation exposure (also no difference between routes of exposure is recommended in REACH Guidance when extrapolating from inhalation to oral routes). For dermal absorption, a default value of 50% is considered generally appropriate, given the potential for evaporation of 1,2-dichloroethane to compete with dermal flux. Convincing justification must be given to deviate from this default rate.

5.1.2 Distribution

Oral

Absorbed 1,2-dichloroethane was found widely distributed in the tissues of humans who had died following acute oral poisonings. Concentrations of 1,2-dichloroethane in these subjects ranged from 1-50 mg/kg in the spleen, and 100-1000 mg/kg in the stomach. Levels in the liver and kidneys were approximately 10 times those in the stomach (Luzinikov *et al*., 1985).

1,2-dichloroethane is distributed widely throughout the body of experimental animals exposed via inhalation or ingestion with the highest concentrations found in blood, kidneys, liver, brain and spleen (WHO,1998).

^{14}C-labelled 1,2-dichloroethane was administered to rats either as an oral single dose of 150 mg/kg bw or by inhalation over 6 hours at concentrations of 600 mg/m^3. Both routes demonstrated similar distribution patterns throughout the body although concentrations of residual radioactivity were 1.5 to 2 times higher following oral administration as opposed to inhalation. There was also a higher concentration of 1,2-dichloroethane in the forestomach when they were administered the compound orally when compared to administration by inhalation. The distribution pattern of macromolecular binding was similar 4 hours following oral administration or inhalation. Oral exposure produced lower levels of macromolecular binding but higher levels of DNA alkylation than inhalation (Reitz *et al*. 1982, IARC 1999).

In an oral study rats were administered single doses of 1,2-dichloroethane in corn oil at doses of 25, 50, or 150 mg/kg bw. Peak concentrations in the liver were reached 10 minutes following oral ingestion. 1,2-Dichloroethane accumulated most rapidly in the liver but the highest concentrations were found in the adipose tissues. Peak levels of 1,2-dichloroethane in the adipose tissues, 45-60 minutes after administration, were 3.9-8.3 times greater than in

blood, whereas peak levels in the liver 10 minutes after administration were 1.3 to 2.2 times greater than in the blood (Speafico *et al*., 1980).

Dermal

1,2-dichloroethane has been detected in the breast milk of women exposed occupationally via inhalation or dermal absorption, which suggests rapid distribution (Urusova, 1953). No studies on the distribution of 1,2-dichloroethane in humans and experimental animals following dermal exposure have been located. However, as it appears tissue distribution is not route dependent for inhalation and oral exposure and 1,2-dichloroethane is easily absorbed through the skin it is probable that distribution would be similar to oral and inhalation exposure (ATSDR, 2001).

Inhalation

1,2-dichloroethane has been detected in the breast milk of women exposed occupationally via inhalation or dermal absorption which suggests rapid distribution (Urusova, 1953).

Following inhalation of 200 or 1000 mg/m^3 of 1,2-dichloroethane for 6 hours, distribution of the compound to adipose tissue was observed although it was also found in the blood, liver, kidney, brain and spleen and levels in these tissues were dose-dependent. Within 2-3 hours of exposure, steady state levels were achieved, although the steady state concentration in the blood was 20 to 30 times greater in the high dose groups than in the low dose groups. Concentrations of 1,2-dichloroethane in the liver and lung were less than those in the blood (Spreafico *et al.,* 1980).

Pregnant rats were exposed to 1,2-dichloroethane via inhalation at concentrations of 150-2000 ppm for 5 hours. The concentration of 1,2-dichloroethane in the maternal blood and the foetuses increased linearly with the exposure concentration (Withy and Karpinkski, 1985).

1,2-dichloroethane was detected in the foetal tissue of rats on day 17 of gestation, following maternal exposure via inhalation of 1,2-dichloroethane at concentrations of 612-8000 mg/m^3 (153 to 2000 ppm) for 5 hours (Withey and Karpinkski, 1985).

Other routes of exposure

It has been reported that ^{14}C-1,2-dichloroethane binds to DNA in the liver, kidneys, lungs and stomach of mice in greater concentrations than in rats, 22 hours after intraperitoneal administration equivalent to 8.7 µmol/kg bw (Reitz *et al*., 1982).

Whole body autoradiography performed on mice one minute to four days following a single intravenous dose of ^{14}C-1,2-dichloroethane of 0.73 mg/kg bw, showed the highest levels of radioactivity to be predominantly present in the nasal olfactory mucosa and the tracheo-

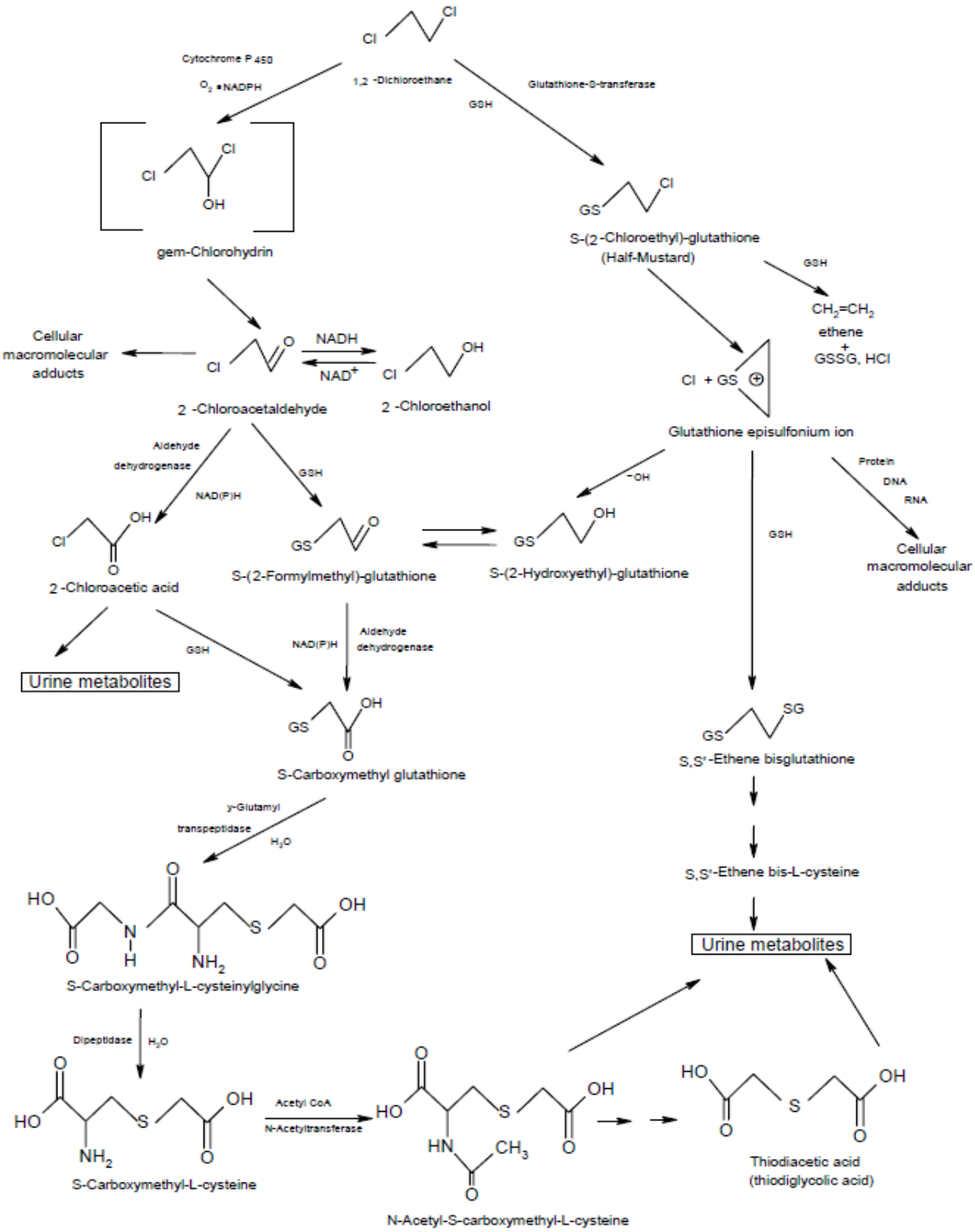
bronchial epithelium. Low levels of metabolites were also present the epithelium of the upper alimentary tract, vagina and eyelid and in the liver and kidney (Brittebo *et al*., 1989).

In vitro studies with tissues from mice found that reactive products from 1,2-dichloroethane were irreversibly bound to the nasal mucosa, lung, and liver but not the oesophagus, forestomach or vagina. Binding occurred to a greater extent in the nasal mucosa and the lung compared to the liver. The epithelium of the respiratory tract may be a potential target for the toxic effects of 1,2-dichloroethane due to in-situ metabolism and reactive ingredients (Brittebo *et al*., 1989).

5.1.3 Metabolism

1,2-Dichloroethane is metabolised by two principal pathways; it is catalysed by cytochrome P450 and the glutathione S-transferase. Cytochrome P450 enzymes catalyse oxidative transformation of 1,2-dichloroethane to 1-chloroacetaldehyde, 2-chloroacetic acid and 2-chloroethanol (Guengerich *et al*., 1980), which are conjugated enzymatically and non-enzymatically with glutathione (GSH). 1,2-dichloroethane is also directly conjugated with GSH to form S-(2-chloroethyl)glutathione, which is a sulphur half mustard (Schasteen and Reed, 1983, Foureman and Reed, 1987) (half mustard gas is similar to mustard gas used in chemical warfare). A non-enzymatic reaction of S-(2-chloroethyl)glutathione results in a putative alkylating agent (episulfonium ion) which may in turn react with water to form S-(2-hydroxyethyl)glutathione, with thiols such as GSH to form ethene bis-glutathione, or with DNA to form adducts. S-(2-chloroethyl)glutathione which forms DNA adducts, the relation products are considered non-toxic and undergo further metabolism (IARC, 1999).

Figure 5.1 Possible metabolic pathways for 1,2-dichloroethane (taken from ATSDR, 2001)



The metabolism of 1,2-dichloroethane is reported to be saturated in rats at levels of exposure resulting in blood concentrations of 5 to 10 mg/l, based on a non-linear relationship between levels in blood and administered doses or concentrations. Also the amount of metabolites was approximately double in rats administered 1,2-dichloroethane via gavage when compared to rats administered 1,2-dichloroethane via inhalation (Reitz *et al*., 1982, Spreafico *et al*., 1980).

The metabolic pathway is linear at low doses but as doses increase the P450 enzymes become saturated and the amount of glutathione conjugate produced rises. The GSH pathway is then saturated at very high doses and the glutathione conjugate produced declines. It is thought that the toxicity of 1,2-dichloroethane occurs when the metabolic processes are saturated allowing higher concentrations of 1,2-dichloroethane to circulate through the body and conjugate with glutathione instead of being detoxified and eliminated (Reitz *et al.,* 1982, D'Souza *et al.,* 1987).

Oral

The metabolism of 1,2-dichloroethane in rats administered a single oral dose of 150 mg/kg bw. The exact metabolic pathways were not characterised but observed depression of hepatic nonprotein sulfhydryl groups indicate that glutathione may play a major role in the metabolism of 1,2-dichloroethane. It appears that saturation of the biotransformation enzymes occurred at this dose as since only 60% of the administered dose was recovered as urinary metabolites and 29% of the administered dose was expired unchanged in air. It is reported that 70% of a single oral dose was transformed into metabolites of which 85% appeared in urine. The metabolism of 1,2-dichlorethane appears to be saturated or limited to blood plasma concentrations of 5-10 µg/ml (Reitz *et al*., 1982).

It has been reported that following a single oral dose of 1,2-dichloroethane at 150 mg/kg bw, mice showed greater metabolism and excretion than rats (100 and 85%, respectively) (Mitoma *et al.,* 1985).

The saturable pathways for oral administration and inhalation are similar; however, oral exposure by gavage appears to result in saturation at lower administered doses than inhalation (ATSDR, 2001).

Dermal

No data were available for metabolism following dermal exposure.

Inhalation

Following administration of 1,2-dichloroethane via inhalation over 6 hours at concentrations of 600 mg/m^3, 91% of the administered dose was transformed into metabolites of which 85% appeared in urine. The exact metabolic pathways were not characterised but observed

depression of hepatic non-protein sulfhydryl groups indicate that glutathione may play a major role in the metabolism of 1,2-dichloroethane (Reitz *et al*. 1982).

It is reported that metabolism occurs when blood levels of 1,2-chloroethane reach 5-10 µg/l or inhalation exposure is 150-250 ppm and when exposure exceeds these levels toxic effects become more apparent (Spreafico *et al.,*1980 and Reitz *et al.,* 1982). In one study it was reported that most of the toxicity observed at inhalation exposure concentrations of 250 ppm decreased when the concentration was reduced to 150 ppm and no treatment related effects were seen at 50 ppm (Maltoni, *et al.,* 1980).

5.1.4 Excretion

Unmetabolised 1,2-dichloroethane is excreted in expired air whereas 1,2-dichloroethane metabolites are excreted via the urine (WHO, 1998). Unchanged 1,2-dichloroethane was detected in the exhaled breath of women following dermal and inhalation occupational exposure. Airborne concentrations were 0.252 mg/m^3 (0.063 ppm) and the amount of 1,2-dichloroethane in exhaled breath was greater directly following exposure but decreased over time (Urusova, 1953).

Male and female rats (number not reported) were administered 1,2-dichloroethane by whole body inhalation at concentrations of 50 ppm (200 mg/cm^3) and within 10-14 days of cessation of administration by inhalation, male and female rats (number not reported) were administered 150 mg/kg ^{14}C-radiolabelled 1,2-dichloroethane by gavage. Analysis of the urine showed that 42.5 and 33.9% of the gavage administered dose was excreted in urine, and 27.3 and 40.3% was excreted as the unchanged compound in the breath. A small amount was detected in the faeces and as $^{14}CO_2$ in breath. During the same study some rats were administered disulfiram via their diet at the same time as 1,2-dichloroethane by inhalation. In these rats the amount of unchanged 1,2-dichloroethane (following administration of 150 mg/kg 1,2-dichloroethane via gavage) was higher in breath than non-disulfiram rats at 57.6 and 57.7%, but lower in urine at 27.6 and 24.9%. Levels of unchanged 1,2-dichloroethane in blood were higher in the rats exposed to 1,2-dichloroethane and disulfiram than those exposed to 1,2-dichloroethane alone (Cheever *et al.,* 1990). Rats exposed to 1,2-dichloroethane via inhalation at concentrations of 150 ppm excreted 84% of the absorbed dose as urinary metabolites, 2% was excreted as unchanged compound in the faeces and 7% was expired as CO_2 (Reitz *et al.,*1980). The elimination of metabolites was similar is rats and mice in the 48 hours following a single oral dose of ^{14}C-radiolaballed 1,2-dichloroethane of 100 or 150 mg/kg bw for rats and mice respectively. In rats, 8.2 and 69.5% of the radiolabelled dose was recovered as CO2 and in urine respectively and in mice 18 and 82% as CO2 or in urine respectively. Overall 96 and 110% of the administered dose was recovered in rats and mice respectively (Mitoma *et al.,* 1985).

There was no significant difference in the route of excretion of non-volatile metabolites in rats administered 1,2-dichloroethane by inhalation at concentrations of 600 mg/cm^3 (150 ppm) for 6 hours or administered 150 mg/kg by gavage. The major urinary metabolites following both

administration routes were thioadetic acid (67-68%) and thioadetic acid sulphoxide (26-29%) (Reitz *et al.,* 1982).

Elimination of 1,2-dichloroethane from blood is rapid regardless of whether it is administered orally or by inhalation. When rats were exposed via inhalation to concentrations of 50 or 250 ppm the elimination was monophasic with elimination half-times of 12.7 and 22 minutes at 50 and 250 ppm respectively. Only small amounts of 1,2-dichloroethane were detected in tissues such as the liver, kidney, lung, spleen, forestomach, stomach and carcass within 48 hours of exposure when administered orally or by inhalation (Speafico *et al.,*1982, Reitz *et al.,*1980).

In a repeat dose study, rats were administered increasing single doses of 1,2-dichloroethane from 0.25-8.08 mmol/kg bw via gavage in mineral oil. The percentage of metabolites in the urine decreased with increasing doses it is though that this may be due to saturation of the metabolism pathways and not due to kidney damage (Payan *et al.*, 1993).

1,2-dichloroethane is not thought to bioaccumulate in tissues even though it is eliminated more slowly from adipose tissue than from blood or other tissues such as the lung or liver (Speafico *et al*., 1982 Cheever *et al.,*1990). In rats, only 71 and 75% of an administered dose of 1,2-dichloroethane (150 mg/kg bw) via gavage directly following a 2-year study in which they were administered 1,2-dichloroethane via inhalation (200 mg/m^3 or 50 ppm for 6 hours a day five days a week). The authors speculated that the remainder may have been stored in the body fat (Cheever *et al.,* 1990).

5.1.5 Bioavailability

1,2-Dichloroethane is rapidly and completely absorbed by all routes of exposure in experimental animals. There is little difference between the rate at which it is absorbed between routes; however, it is noted that large bolus doses of 1,2-dichloroethane administered orally result in a more rapid saturation of the metabolic pathways at a higher peak blood level than similar doses administered via inhalation (Reitz et al., 1982, Spreafico et al., 1980). As the mechanism for toxicity is linked to the metabolites produced, this intimates that administrating 1,2-dichloroethane orally may have more of a toxic effect than a similar dose administered via inhalation. It is also noted that when 1,2-dichloroethane is administered orally and dermally with water as the vehicle, absorption, distribution, metabolism and excretion rates were all increased (Withey *et al.,* 1983).

There are limited data on the absorption of 1,2-dichloroethane in humans and therefore, it is not possible to compare it with absorption rates in experimental animals. Epidemiological studies have found that occupational inhalation and dermal absorption of 1,2-dichloroethane occurs and results in distribution to milk in lactating women (Urusova, 1953, US EPA, 1980). There is also a report of a fatality due to 1,2-dichloroethane poisoning following inhalation (Nouchi *et al.,* 1984). A comparative study also found that the rate of absorption across rat and human skin was similar (Ward 1992). It has been assumed in all evaluations assessed

that the rates of absorption in humans and experimental animals are comparable for all routes.

5.2 Genotoxicity

Available genotoxicity data for 1,2-dichloroethane have been summarised in Table 5.1 (*in vitro* data) and Table 5.2 (*in vivo* data).

Covalent binding studies with isolated calf thymus DNA have shown that 1,2-dichloroethane can form adducts in the presence of a metabolic activation system. The genotoxicity data indicate that 1,2-dichloroethane is mutagenic in most strains of *Salmonella typhimurium* tested with and without metabolic activation. In the TA1535 strain, for example, mutagenic activity was dependent on the addition of metabolic activation or specifically, glutathione S-transferase. (IARC, 1999).

Although some negative results have been reported in the literature, in mammalian cell assays *in vitro*, 1,2-dichloroethane has been found clearly to induce gene mutations, micronuclei and unscheduled DNA synthesis. (IARC, 1999).

The conclusion is that 1,2-dichloroethane is genotoxic *in vitro*.

1,2-dichloroethane induces DNA strand breaks in mouse liver after intraperitoneal injections and oral exposure, but not by inhalation. DNA strand breaks in rat liver were also induced following administration of 1,2-dichloroethane by gavage. 1,2-dichloroethane was found to bind to DNA, RNA and proteins in mice and rats *in vitro* and *in vivo* (IARC, 1999). A study looking at aneuploidy in human cell lines showed that 1,2-dichloroethane increased the frequency of non-staining kinetochore micronuclei (which is indicative of aneuploidy) (IARC, 1999).

A variety of different studies have been conducted *in vivo*, but a clear picture of the mutagenic potential of 1,2-dichloroethane is lacking. As reviewed by IARC (1999), 1,2-dichloroethane has been found to bind to DNA, RNA and proteins in a variety of tissues in mice and rats *in vivo.*

However, 1,2-dichloroethane treatment produced negative results in three *in vivo* micronucleus studies (IARC, 1999; US ATSDR, 2001). In one of these studies, there was no micronucleus induction seen in the peripheral blood of a transgenic strain of male and female mice administered 100 to 300 mg/kg 1,2-dichloroethane by oral gavage for 14 or 41 weeks. The positive control substances, benzene and 2-acetylaminofluorene, gave positive results, but diethylnitrosamine also gave a negative result. In another study, 30-hour intra-peritoneal treatment of NMRI mice with approximately 45-400 mg/kg bw 1,2-dichloroethane given twice, with bone marrow sampling 6 h after the second dose (several authoritative reviews have suggested that the time between treatment and sampling may be too long), also gave a negative result. The third study, a mouse bone marrow micronucleus test, was less well

reported but also gave a negative result. Additionally, a negative result was reported with 1,2-dichloroethane in a non-regulatory lacZ transgenic mouse mutation assay (Hachiya and Motohashi, 2000).

1,2-dichloroethane was also negative in a dominant lethal assay conducted as part of a reproduction study using 1% Emulphor EL-620 to give 5, 15, 50 mg/kg bw/day. Little weight is placed on this study as several authoritative reviews have indicated that there was incomplete documentation and the conclusion was unclear.

In contrast to these negative studies, reports of 1,2-dichloroethane genotoxicity *in vivo* can be found in a variety of other non-regulatory studies reported in the literature. In a multi-substance trial, Sasaki *et al* (1998) observed a positive Comet response in a variety of tissues of mice sacrificed 3 or 24 h after treatment. A bone marrow Sister Chromatid Exchange (SCE) assay in Swiss male mice given 1,2-dichloroethane (0.5, 1, 2, 4, 8, 16 mg/kg bw by intraperitoneal injection), with a sampling time of 24 hours, also gave a positive result. Inhalational exposure to 1000 ppm 1,2-dichloroethane for 4 hours produced irreversible DNA damage in mice as evidenced by single-stranded breaks in hepatocytes. However, this result should be viewed with caution especially as the genetic damage was seen at a concentration that produced mortality in 80–100% of treated mice within 24 hours. DNA single strand breaks have been seen in mice after single intraperitoneal injections of 45-360 mg/kg 1,2-dichloroethane.

In summary, there have been many genotoxicity studies reported, both regulatory and more experimental in nature. These genotoxicity studies yield a mix of results including a number of negative studies. However, in general, the bacterial mutation assays and tests in human and other mammalian cells *in vitro*, such as hprt, Unscheduled DNA Synthesis (UDS) and micronucleus were positive as were the non-regulatory assays involving DNA binding. *In vivo* tests in experimental animals were more mixed with negative micronucleus and dominant lethal assays but a positive SCE assay. Again there are a number of positive DNA binding assays. It is not possible to make a definitive conclusion about the *in vivo* mutagenic potential of 1,2-dichloroethane from the available data, but overall the possibility of a mutagenic hazard (at least in somatic cells) cannot be excluded.

Together with the toxicokinetic information, these findings from the available genotoxicity studies, indicate that on balance, 1,2-dichloroethane should be considered as a genotoxic chemical.

Table 5.1 Genotoxicity of 1,2-dichloroethane *in vitro*

Test	Metabolic Activation	Assay details (ug/ml)	Result	Ref.
Prokaryotic organisms				
SOS chromotest	with and without	-	Negative	Quillardet *et al.,* 1985
Differential toxicity (Spot test) *Escherichia coli pol* A	without	12 000	weakly positive	Brem *et al.,* 1974
Reverse mutation assay in *Salmonella typhimurium* TA100, TA1535, TA1537, TA1538, TA98	with an without	1782	negative	King *et al.,* 1979
Forward or reverse mutation assauy, *Escherichia coli* K12	with and without	990	negative	
Reverse mutation assay in *Salmonella typhimurium,* TA100,	with and without	3120	positive	Barber *et al.,* 1981
Reverse mutation assay in *Salmonella typhimurium,* TA1535,	with and without	1574	positive	
Reverse mutation assay in *Salmonella typhimurium,* TA98	with and without	11475	negative	
Reverse mutation assay in *Salmonella typhimurium,* TA100, TA98	with and without	60 000	negative	Principe *et al.,* 1981
Reverse mutation assay in *Salmonella typhimurium,* TA1535	with	60 000	weakly positive	
	without	60 000	negative	
Reverse mutation assay (spot test) in *Salmonella typhimurium,* TA1537, TA1538	with and without	60 000	negative	
Forward mutation assay in *Streptomyces coelicolor*	without	60 000	negative	
Forward mutation assay in *Aspergillis ridulans*	without	300 000	negative	
Reverse mutation assay in *Salmonella typhimurium,* TA100	with and without	3960	negative	Van Bladeren *et al.,* 1981
Reverse mutation assay in *Salmonella typhimurium,* TA100, TA1535, TA98	with and without	not reported	positive	Milman, *et al.,* 1988.
Reverse mutation assay in *Salmonella typhimurium,* TA1537,	with and without	not reported	negative	

Test	Metabolic Activation	Assay details (ug/ml)	Result	Ref.
Reverse mutation assay in *Salmonella typhimurium,* TA100	without	20 (µg/ml air)	weakly positive[a]	Simula *et al.,* 1993
Reverse mutation assay in *Salmonella typhimurium,* TA1530, TA1535,	without	495	weakly positive	Brem *et al.,* 1974
Reverse mutation assay in *Salmonella typhimurium,* TA1538	without	495	negative	
Reverse mutation assay in *Salmonella typhimurium,* TA1535	with and without	740	positive	Rannug and Ramel, 1977
Reverse mutation assay in *Salmonella typhimurium,* TA1535	with	990	positive	Cheh *et al.,* 1980
Reverse mutation assay in *Salmonella typhimurium,* TA1535	without	990	negative	
Reverse mutation assay in *Salmonella typhimurium,* TA1535	with	990	positive	Guengerich *et al.,* 1980
Reverse mutation assay in *Salmonella typhimurium,* TA1535	without	990	negative	
Reverse mutation assay in *Salmonella typhimurium,* TA1535	with	1250	positive	Moriya *et al.,* 1983
Reverse mutation assay in *Salmonella typhimurium,* TA1535	without	1250	negative	
Gene expression assay in *Salmonella typhimurium,* TA1535 + SOS/*umuC"lacZ, umuC*	without	50	positive	Oda *et al.,* 1996
Reverse mutation assay in *Salmonella typhimurium,* TA1535 + GST (NM 5004) + SOS/*umuC"lacZ*	without	1	positive	
Genetic crossing-over assay in *Aspergillis ridulans*	without	1.54% (in air)	negative	Crebelli *et al.,* 1988
Aneuploidy assay in *Aspergillis ridulans*	without	2500	positive	
Forward mutation assay in *Salmonella typhimurium* BA13	with	74	positive	Roldán-Arjona *et al.,* 1991
Forward mutation assay in *Salmonella typhimurium* BA13	without		negative	
Mammalian				
DNA repair assay in mouse hepatocytes	without	not reported	positive	Milman *et al.,* 1988
Unscheduled DNA synthesis in rat hepatocytes	without	not reported	positive	

Test	Metabolic Activation	Assay details (ug/ml)	Result	Ref.
Cell transformation in BALB/c-3T3 mouse cells	without	not reported	negative	
Gene mutation *hprt* locus assay in Chinese Hamster Ovary (CHO) cells	with and without	99	positive	Tan and Hsie., 1981
Gene mutation *hprt* locus assay in Chinese Hamster Ovary (CHO) cells	without	8 (µg/ml in air)	positive	Zamora *et al.,* 1983
Aneuploidy kinetochore staining in AHH-1 cells (CYP1A1 native)	without	495	negative	Doherty *et al.,* 1996
Aneuploidy kinetochore staining in MCL-5 cells (cDNAs for CYP1A2, 2A6, 3A4, 2E1 and epoxide hydrolase	without	495	weakly positive	
Aneuploidy kinetochore staining in h2E cells (cDNA for CYP2E1)	without	495	negative	
Micronucleus test in AHH-1 cells (CYP1A1 native)	without	198	positive	
Micronucleus test in MCL-5 cells (cDNA for CYP1A2, 2A6, 3A4, 2E1 and epoxide hydrolase)	without	198	positive	
Micronucleus test in h2E1 cells (cDNA for CYP2E1)	without	198	positive	
Cell transformation in BALB/c-3T3 mouse cells	without	50	negative	Tu *et al.,* 1985
Cell transformation in SA7/Syrian hamster embryo cells	without	1.3% in air	positive	Hatch *et al.,* 1983
Gene mutation in human EUE cells	without	99	positive	Ferreri *et al.*, 1983
Gene mutation in human lymphoblastoid cell line AHH-1	without	100	positive	Crespi *et al.,* 1985
Gene mutation in human lymphoblastoid cell line TK6	without	500	positive	
Covalent binding to DNA in calf thymus	with	99	positive	Guengerich *et al.,* 1980
Covalent binding to DNA in calf thymus	without	99	negative	
Covalent binding to DNA in calf thymus	with	3.6	positive	Arfellini *et al.,* 1984
Covalent binding to DNA in calf thymus	without	3.6	negative	
Covalent binding to DNA	with	6	positive	Colacci

Test	Metabolic Activation	Assay details (ug/ml)	Result	Ref.
Covalent binding to DNA	without	6	negative	*et al.,* 1985
Covalent binding to DNA in mouse hepatocytes	without	103 µg	positive	Banerjee, 1988
Unscheduled DNA synthesis in human peripheral lymphocytes	with	not reported	positive	Perocco and Prodi, 1981
Unscheduled DNA synthesis in human peripheral lymphocytes	without	not reported	negative	
Micronucleus test in human peripheral lymphocytes	with	not reported	negative	Tafazoli *et al.,* 1998
Micronucleus test in human peripheral lymphocytes	without	not reported	positive	
DNA damage assay in human peripheral lymphocytes	with	not reported	negative	
DNA damage assay in human peripheral lymphocytes	without	not reported	positive	

LED = Lowest Effective Dose. HID = Highest Ineffective Dose.

[a] Strains transfected with plasmids expressing α-class glutathione *S*-transferase (GST) were more sensitive than those expressing π-class GSTs or the control TA100 strain.

Table 5.2 Genotoxicity of 1,2-dichloroethane *in vivo*

Test	Route of administration	Dose (mg/kg bw)	Result	Ref.
Host mediated assay, *Escherichia coli* K12 in female NMRI mouse hosts	interperitoneal injection (single dose)	198	negative	King *et al*., 1979
Micronucleus test in NMRI mouse bone marrow	interperitoneal injection (dosed twice)	396	negative	
Micronucleus test in mice	not reported	not reported	negative	Sasaki *et al.,* 1994
DNA single-strand breaks assay in B6C3F_1 mouse liver	interperitoneal injection (single dose)	198	positive	Storer and Conolly, 1983
DNA single-strand breaks assay in B6C3F_1 mouse liver	oral (single dose)	100	positive	Storer *et al.,* 1984
DNA single-strand breaks assay in B6C3F_1 mouse liver	interperitoneal injection (single dose)	150	positive	

Test	Route of administration	Dose (mg/kg bw)	Result	Ref.
DNA single-strand breaks assay in B6C3F$_1$ mouse liver	inhalation for 4 hours	500 ppm	negative	
DNA damage in CD rats liver cells	oral (dosed twice)	134	positive	Kitchen and Brown, 1994
Mouse spot test in female C57BL/6J Hans mice	interperitoneal injection (single dose)	300	inconclusive	Gocke *et al.,* 1983
Sister chromatid exchange assay in male Swiss albino mouse bone marrow	interperitoneal injection (single dose)	1	positive	Giri and Que Hee, 1988
Micronucleus test in Eμ-PIM-1 transgenic mouse peripheral blood	oral for 41 weeks	300 mg/kg bw/day	negative	Armstrong and Galloway, 1993
Reverse mutation assay in bile of CBA mice, *Salmonella typhimurium* TA1535	interperitoneal injection (single dose)	80	positive	Rannug *et al.,* 1979
Dominant lethal test in ICR Swiss mice	oral for 7 days	50 mg/kg bw/day	negative	Lane *et al.,*1982
Covalent binding to DNA in rat liver, spleen, kidney and stomach	oral (single dose)	150	positive	Reitz *et al.,* 1982
Covalent binding to DNA in Wistar rat liver, spleen, kidney and stomach	interperitoneal injection (single dose)	0.86	positive	Arfellini *et al.,* 1984
Covalent binding to DNA in BALB/c mouse liver, kidney, lung and stomach	interperitoneal injection (single dose)	0.86	positive	
Covalent binding to RNA and proteins in Wistar rat liver, lung, kidney and stomach	interperitoneal injection (single dose)	0.86	positive	
Covalent binding to RNA and proteins in BALB/c mouse liver, kidney, lung and stomach	interperitoneal injection (single dose)	0.86	positive	
Covalent binding to DNA in Sprague-Dawley rat hepatocytes	interperitoneal injection (single dose)	150	positive	Inskeep *et al.,* 1986

Test	Route of administration	Dose (mg/kg bw)	Result	Ref.
Covalent binding to DNA in Sprague-Dawley rat liver	interperitoneal injection (single dose)	1.38	positive	Banerjee, 1988
Covalent binding to DNA in $B6C3F_1$ mouse liver	interperitoneal injection (single dose)	1.38	positive	
Covalent binding to DNA in Sprague-Dawley rat liver	oral (single dose)	150	positive	Cheever *et al.,*1990
Covalent binding to DNA in Fischer 344 rat lung	inhalation for 4 hours	34	positive	Baertsch *et al.,* 1990
Covalent binding to DNA in mouse, liver, kidney, lung and stomach	not reported	not reported	positive	Prodi *et al.,* 1986
Covalent binding to DNA in rat, liver, kidney, lung and stomach	not reported	not reported	positive	
Covalent binding to DNA in mouse, forestomach and kidney,	not reported	not reported	positive	Hellman and Brandt, 1986

Further considerations

A GLP-compliant *in vivo* Comet assay (Communication to ECHA, September 2014) has been conducted by the Dow Chemical Company, in accordance with the recently published OECD 489 guideline. This focused on the rat mammary gland as a possible target for 1,2-dichloroethane-related genotoxicity in an attempt to characterise the mode of action for the formation of 1,2-dichloroethane-induced mammary tumours in female F344/DuCrl rats. Rats were exposed to 0 or 200 ppm 1,2-dichloroethane by inhalation for 28 days. The positive control treatment in this study was a single dose of N-nitroso-N-methylurea (MNU) administered by gavage 3 hours before necropsy. A further group of rats were administered diethyl maleate by intra-peritoneal injection 2 hours prior to necropsy to investigate the effect of glutathione depletion in mammary and liver tissue.

Tissues were collected and processed within 2-6 hours of the final exposure period. Inhalation exposure to 1,2-dichloroethane for 4 weeks (28-31 exposures) had no effect on body weights, clinical observations, serum prolactin levels, mammary epithelial cell proliferation (measured by Ki-67)/numeric density or mammary gland morphology or histopathology. There was no evidence of a genotoxic response in isolated mammary epithelial cells measured by the Comet assay. There was an increase in Comet parameters in mammary tissue from positive control animals treated with MNU indicating DNA damage. 1,2-dichloroethane treatment also had no effect on oxidised or reduced glutathione levels in mammary tissue, but reduced levels

in liver were observed. Endogenous S-[2-(N^7-guanyl)ethyl]glutathione, the predominant adduct formed following 1,2-dichloroethane exposure, was detectable in mammary and liver tissue with the levels being approximately 54% higher in the liver.

The authors concluded that exposure to 200 ppm 1,2-dichloroethane (approximately 20% higher than the concentration reported to induce mammary tumours in long-term studies) in this sub-acute study had no effects on serum prolactin levels, oxidised and reduced glutathione levels, cell proliferation or DNA damage in mammary tissue. They suggested that this study does not support a genotoxic/mutagenic mode of action for the formation of 1,2-dichloroethane-induced mammary tumours.

The *in vivo* Comet assay is in principle applicable to any tissue from which analysable single cell/nuclei suspensions can be derived. Although performance of this assay was claimed to be in accordance with the new OECD test guideline, the results are regarded with some caution. Importantly, it is unclear whether the test had been optimised for assessing genetic damage in the mammary gland; the performing laboratory has not yet demonstrated its proficiency by building a historical database to establish positive and negative control ranges and distributions for this tissue.

Against this, there is clear evidence of genotoxicity in a number of assays and tumour formation in a number of different tissues leading to the conclusion that a genotoxic mode of action may be involved in the formation of tumours induced by 1,2-dichloroethane.

5.3 Carcinogenicity

5.3.1 Human epidemiological studies

All collated epidemiology studies are summarised in Appendix C1. The main deficiencies of the small number of human epidemiological studies is that the exposure levels of 1,2-dichloroethane are unknown and there are usually multiple chemicals present. The small study of Benson and Teta (1993) on chlorhydrin production workers indicated an increase in cancer; pancreatic and lymphopoietic cancers in particular. Although other chemicals were present, the authors implicated 1,2-dichloroethane in the increase in pancreatic cancer although there was no firm evidence. There are further studies on petrochemical workers, drinking water exposure, proximity to landfill sites and a spillage of 1,2-dichloroethane, but there are insufficient data on exposure to associate 1,2-dichloroethane with any human cancer. This being the case, there are no human data for use in the quantitative risk assessment of 1,2-dichloroethane and no robust evidence to implicate it as a human carcinogen.

5.3.2 Experimental animal studies

All collated epidemiology studies are in Appendix C2. Table 5.3 presents a summary of the studies evaluated as part of this assessment.

Table 5.3 Overview of the carcinogenicity studies of 1,2-dichloroethane

Reference	Study (species, strain, sex, number of animals, duration and route of exposure)	Dose	Findings
Maltoni *et al.,* (1980)	• Swiss mice • 90/sex/dose • Inhalation • 7 hours/day, 5 days/week for 78 weeks	0, 5, 10, 50, 150/250 ppm	No specific tumours or changes in the incidence of tumours were noted.
Maltoni et al., (1980)	• Sprague-Dawley rats • 90/sex/dose • Inhalation • 7 hours/day, 5 days/week for 78 weeks	0, 5, 10, 50, 150/250 ppm	Fibromas and fibroadenomas in female mammary glands. Significant differences in tumour incidence at the 5, 50 and 150/250 ppm dose groups compared to controls. Significant differences were also seen between control groups.
Cheever *et al.,* (1990)	• Sprague-Dawley rats • 50/sex/dose • Inhalation • 7 hours/day, 5 days/week for 2-years	0 or 50 ppm	Increased incidence of intrahepatic bile duct cholangiomas, intrahepatic bile duct cysts, liver neoplastic nodules and interstitial-cell tumours in the testis of males, mammary gland adenocarcinomas in females.
Nagano *et al.,* (1998)	• BDF1 mice • 50/sex/dose • Inhalation • 6 hours/day, 5 days/week for 2 years	0, 10, 30, or 90 ppm	Liver haemangiosarcomas in males and hepatocellular adenoma, bronchiolar/alveolar adenoma carcinoma, mammary gland adenocarcinoma and endometrial stromal polyps in females.
Nagano *et al.,* (1998)	• Sprague-Dawley rats • 50/sex/dose • Inhalation • 6 hours/day, 5 days/week for 2 years	0, 10, 40, or 160 ppm	Mammary fibroadenomas and subcutis fibromas in both sexes, peritoneal mesothelioma in males, and mammary adenomas and adenocarcinomas in females.
Nagano *et al.,* (2006)	• F344/DuCrj (SPF) rats • 50/sex/dose • Inhalation • 6 hours/day, 5 days/week for 2 years	0, 10, 40, or 160 ppm	Subcutis fibromas, mammary gland fibroadenoma and adenomas in both sexes, peritoneum mesothelioma in males, and mammary gland adenocarcinomas in females.

Reference	Study (species, strain, sex, number of animals, duration and route of exposure)	Dose	Findings
Nagano *et al.,* (2006)	• Crj:BDF1 (SPF) mice • 50/sex/dose • Inhalation • 6 hours/day, 5 days/week for 2 years	0, 10, 30, or 90 ppm	Liver haemangiosarcoma in males, lung bronchiolar/alveolar adenomas and carcinomas, uterus endometrial stromal polyps, and liver hepatocellular adenoma and carcinoma in females, and lymph node malignant lymphoma.
Van Duuren *et al.,* (1979)	• Ha ICR Swiss mice • 30/female/dose • Dermal • 3 times per week for 440-594.	0 (0.1 ml acetone), 126 mg/animal in 0.2ml acetone.	Benign lung papillomas.
Van Duuren et al., (1979)	• Ha:ICR Swiss mice • 30/female/dose • Dermal • 429-576 days	Single dose of 126 mg 1,2-dichloroethane/animal followed by 5 µg/animal phorbol myristyl acetate for life. Controls administered phorbol myristyl acetate alone.	No significant differences in occurrence of skin tumours between treated and control groups.
Klaunig *et al.,* (1986)	• B6C3F1 mice • 25/sex/dose • Oral, drinking water • 56 weeks	Administered *N*-nitrosadiethylamine (NDEA) for four weeks. Then given 1,2-dichloroethane in drinking water at 0, 835, or 2500 mg/l for 52 weeks.	No significant differences in either tumour incidence or number of tumours per mouse.
Milman *et al.,* (1988)	• Osborne-Mendel Rats • 10/sex/dose • Oral, gavage • 8 weeks	0 (vehicle), 0 (positive control) and 30 mg/kg bw.	No significant increase in γ-GT-positive-loci in rat liver cells.
Milman *et al.,* (1988)	• Osborne-Mendel Rats • 10/sex/dose • Oral, gavage • 5 days a week for 7 weeks	0 (vehicle), 0 (positive control) and 100 mg/kg bw.	No significant increase in γ-GT-positive-loci in rat liver cells.
NCI (1978)	• Osborne-Mendel Rats • 50/sex/dose • Oral, gavage • 5 days a week for 7 weeks	0 (untreated), 0 (vehicle), 47 or 95 mg/kg bw/day	Squamous-cell carcinomas of the forestomach haemangiosarcomas, and subcutaneous fibromas in males and adenocarcinomas in the mammary gland in females.

Reference	Study (species, strain, sex, number of animals, duration and route of exposure)	Dose	Findings
NCI (1978)	• B6C3F1 mice • 50/sex/dose • Oral, gavage • 5 days a week for 7 weeks	0 (untreated), 0 (vehicle), 47 or 95 mg/kg bw/day.	Mammary gland adenocarcinomas in females, hepatocellular carcinomas in males, endometrial stromal polyps, alveolar/bronchiolar adenomas in both sexes

There have been a number of long-term carcinogenicity studies on 1,2-dichloroethane by the oral and inhalation routes in rats and mice. In rats, the main tumours consistently seen in inhalation studies were in the mammary gland, together with some liver neoplasms. In the oral studies conducted by the National Cancer Institute (NCI, 1978), haemangiosarcomas, forestomach and mammary tumours were observed. In the mouse, the tumours detected were more varied with lung and reproductive tumours as well as liver and mammary tumours. There were also two studies where no increases in tumour incidence were seen. The US Environmental Protection Agency (EPA) in their Integrated Risk Information System (IRIS) assessment used the incidence of haemangiosarcomas in the NCI oral rat study as their point of departure and used extrapolation to derive an inhalation unit risk, although this review (and others, see Table 5.3) was conducted before the later Nagano study was published. The early oral NCI study had only two dose levels, lasted for 78 weeks rather than the usual 104 weeks and had very high mortality even in the control groups. It was not considered suitable for quantitative risk estimation.

The most consistent and sensitive results were the development of mammary tumours in rats. Inhalation (together with dermal) was considered the most likely exposure route in humans and the most complete dose-response study was by Nagano *et al.* (2006) with an endpoint of a combined tumour incidence of adenomas, fibroadenomas and adenocarcinomas of the mammary gland (see Table 5.4). The results of this study were used by registrants of 1,2-dichloroethane to derive inhalation and dermal DMELs. RAC agreed that this is the most suitable study to use in deriving cancer risk estimates.

The relevance of the mammary tumours to human risk assessment of 1,2-dichloroethane needs to be considered. The genotoxicity studies suggest that direct action of the metabolites of 1,2-dichloroethane on DNA is the primary reaction that could potentially lead to cancer with little evidence of other indirect mechanisms such as oxidative stress and reactive hyperplasia, although the presence of forestomach tumours in some studies may indicate irritancy. The available epidemiological studies are insufficient to reach any conclusions on the carcinogenicity of 1,2-dichloroethane and do not provide any useful information on tissue sensitivity. Tumours in experimental animals often differ from humans in the site of carcinogenicity; for example, the human bladder appears a possible site for tumours caused by aniline-derived compounds but this target is rare in experimental animal studies with these compounds. Therefore, the choice of mammary tumours for this risk assessment is based on

genotoxic potential and the best dose-response rather than its relevance to a specific human cancer.

Table 5.4 Total mammary tumour incidence in 1,2-dichloroethane-treated female F344/DuCrj (SPF) rats (Nagano *et al*, 2006)

Dose (ppm)	0	10	40	160
Approximate Dose (mg/m^3)	0	41	164	658
Total tumours/animals	8/50	8/50	11/50	25/50
Incidence (%)	16	16	22	50

5.4 Evaluations

Table 5.5 presents a summary of the evaluations and assessments that were located for 1,2-dichloroethane.

Table 5.5 Overview of the findings of assessments on the carcinogenic mode of action of 1,2-dichloroethane

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
ATSDR (2001)	Mutagenic forming DNA adducts	Threshold	Classified as a probable human carcinogen. Cheever *et al.,* 1990 • rats • inhalation • liver histopathology NTP (1991) • rats and mice • drinking water or gavage • kidney effects	MRL inhalation = 0.6 ppm MRL Oral= 0.2 mg/kg bw/day
ECHA (2011)	Not reported	-	Classification according to part 3 of Annex VI, Table 3.1 (list of harmonised classification and labelling of hazardous substances of Regulation (EC) No 1272/2008 = Carc. 1B.	-
Health Canada (1994)	Metabolites are genotoxic	Threshold	Classified as probable human carcinogen. NCI (1978); • rats • gavage • carcinomas in the stomach, haemangiosarcomas, subcutaneous fibromas, adenocarcinomas or fibroadenomas in the mammary gland. • mice • gavage • alveolar/bronchiolar adenomas, hepatocellular carcinomas, mammary gland adenocarcinomas and endometrial stromal polyps.	Doses associated with a 5% increase in tumour incidence $TD_{0.05}$s = 6.2–34 mg/kg bw
IARC (1999)	Not specified		Group 2B possible carcinogen to humans.	
OECD (2002)	Genotoxic	Not specified	Classified as a suspected human carcinogen.	Not specified

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
US EPA (2014)	Not specified	Non-threshold	Classified as probable human carcinogen. NCI (1978) • rats • gavage • carcinomas in the stomach, haemangiosarcomas, subcutaneous fibromas, adenocarcinomas or fibroadenomas in the mammary gland. • mice • gavage • alveolar/bronchiolar adenomas, hepatocellular carcinomas, mammary gland adenocarcinomas and endometrial stromal polyps Van Duuren *et al.,* (1979) • ICR/Ha Swiss mice • dermal • benign lung papillomas	Oral slope factor = $9x10^{-2}$ mg/kg/day Drinking water unit risk = $2.6x10^{-6}$
WHO (1998)	Metabolites are genotoxic	Not specified	Classified as probable human carcinogen. NCI (1978); • rats • gavage • carcinomas in the stomach, haemangiosarcomas, subcutaneous fibromas, adenocarcinomas or fibroadenomas in the mammary gland. • mice • gavage • alveolar/brochiolar adenomas, hepatocellular carcinomas, mammary gland adenocarcinomas and endometrial stromal polyps.	Not specified

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
			Induction of common and rare tumours, production of intermediate that alkylates DNA and positive results of genotoxicity *in vitro* and *in vivo*.	
WHO (1998)	Metabolites are genotoxic	Threshold	Classified as probable human carcinogen. NCI (1978); • rats • gavage • carcinomas in the stomach, haemangiosarcomas, subcutaneous fibromas, adenocarcinomas or fibroadenomas in the mammary gland. • mice • gavage • alveolar/bronchiolar adenomas, hepatocellular carcinomas, mammary gland adenocarcinomas and endometrial stromal polyps	Doses associated with a 5% increase in tumour incidence $TD_{0.05}$s = 6.2–34 mg/kg bw Guidance values: Air = 3.6-20 mg/m^3 or 0.36-2 mg/m^3 Ingestion = 1.2-6.8 mg/kg bw or 0.12-0.68 mg/kg bw.
WHO (2003)	Not specified	Threshold	Group 2B possible carcinogen to humans. NCI (1978) • male mice • gavage, 78 weeks • haemangiosarcomas	Linearised multistage model of upper-bound excess cancer risks = 300, 30 and 3 µg/l (10^{-4}, 10^{-5}, 10^{-6}, respectively). Guideline for Drinking Water Quality = 30 µg/l.
Arkema France (2012), BASF SE (2012), Instituto Suizo para el Formento de la Seguridad España S. L. U (2012), Solvay-Electrolyse-	Genotoxic	Threshold	Nagano *et al.,* 2006 • female rat • inhalation • mammary tumours	BMD10 = 42 ppm T25 = 101 ppm **Workers** Inhalation DMEL (lifetime cancer risk 10^{-5}) from BMD10 = 0.00429 ppm (17.6 µg/m^3) DMEL (lifetime cancer risk 10^{-5}) from T25 = 0.00404 ppm (16.6 µg/m^3)

Expert evaluation	Primary mechanism	Threshold/non-threshold approach	Studies	Threshold dose
France S.A.S. RUE DE CLICHY 25 75009 Paris France (2014), Solvic S.A. 310 rue de Ransbeek 1120 Brussels Belgium (2014)				However, they consider an inhalation DMEL of 0.004 ppm as conservative and proposed using a lifetime cancer risk of 4 x 10^{-3} or 1.6 ppm (6.6 µg/m^3) for a number of reasons. Dermal DMEL (lifetime cancer risk 10^{-5}) = 0.156 mg/kg bw/day Sponsors propose a Dermal DMEL for the same reasons (lifetime cancer risk 4 x 10^{-3}) = 62.4 mg/kg bw/day **General population** Inhalation DMEL (lifetime cancer risk 10^{-5}) based on BMD10 = 0.00075 ppm (3.1 µg/m^3) General population inhalation DMEL (lifetime cancer risk 10^{-5}) based on T25 = 0.00071 ppm (2.9 µg/m^3) Sponsors use 2.9 µg/m^3 in risk assessment
Dow Deutschland Anlangengesellschaft mbH (2014)	Not reported	Note reported	Not reported	**Workers** Inhalation DMEL (2014) = 6.6 µg/m^3 Dermal = 62.4 mg/kg bw/day **General population** Inhalation DMEL = 2.9 µg/m^3

5.5 Mechanism of action

Evidence suggests that the toxicity and carcinogenicity of 1,2-dichlorethane is dependent on its being metabolised to active, potentially genotoxic intermediates (US ATSDR, 2001), through two principal pathways.

In the first pathway, 1,2-dichloroethane is catalysed by cytochrome P450 and glutathione *S*-transferase. Cytochrome P450 enzymes catalyse oxidative transformation of 1,2-dichloroethane to 1-chloroacetaldehyde, 2-chloroacetic acid and 2-chloroethanol (Guengerich *et al.,* 1980), which are conjugated enzymatically and non-enzymatically with glutathione (GSH). This pathway can yield 2-haloacetaldehydes which readily bind to protein and non-protein thiols. There is evidence to suggest that some DNA damage may be induced via the P450 pathway *in vitro* (Banerjee *et al*., 1980; Guengerich *et al*., 1980; Lin *et al*., 1985), but studies have concluded that production of the 2-haloethanols and 2-haloacetaldehydes from 1,2-dichloroethane is inconsistent with a major role in DNA damage (Guengerich *et al*., 1981, Koga *et al*., 1986).

In the second pathway, 1,2-dichloroethane is directly conjugated with GSH to form *S*-(2-chloroethyl)glutathione, which is a sulphur half mustard (Schasteen and Reed, 1983, Foureman and Reed, 1987) (half mustard gas is similar to mustard gas used in chemical warfare). A non-enzymatic reaction of *S*-(2-chloroethyl)glutathione results in a putative alkylating agent (episulfonium ion) which can in turn react with water to form *S*-(2-hydroxyethyl)glutathione, react with thiols such as GSH to form ethene bis-glutathione, or with DNA to form adducts. With the exception of *S*-(2-chloroethyl)glutathione which forms DNA adducts, the reaction products are considered non-toxic and undergo further metabolism (IARC, 1999).

The available evidence suggests that conjugation with GSH may be the main route for DNA damage (Guengerich *et al*., 1980; Rannug, 1980; Guengerich *et al*., 1981; Van Bladeren *et al*., 1981; Sundheimer *et al*., 1982; Crespi *et al.,* 1985; Storer and Conolly, 1985; Inskeep *et al*., 1986; Koga *et al*., 1986; Cheever *et al*., 1990). The mutation frequency of 1,2-dichloroethane in human cell lines has been correlated with glutathione-S-transferase activity. In the AHH-1 human cell line, which has higher glutathione-S-transferase activity, mutation frequency was 25 times higher than the TK6 cell line (Crespi *et al*., 1985).

Furthermore, findings in studies using B6C3F1 mice are consistent with the hypothesis that reduction in GSH levels is associated with a reduction in DNA damage as the GSH metabolic pathway is associated with the formation of DNA adducts via the formation of S-(2-chloroethyl)glutathione. Male B6C3F mice were pretreated with piperonyl butoxide which inhibits P450 activity, and were then administered 1,2-dichloroethane and examined for the extent of hepatic DNA damage 4 hours later. Hepatic DNA damage, as measured by alkali-labile lesions, was potentiated by piperonyl butoxide. Treatment of mice with doses of 2-chloroethanol failed to produce DNA damage (Storer and Conelly, 1985). Evidence also suggests that the putative episulfonium ion, resulting from the formation of

S-(2-chloroethyl)glutathione, is a major intermediate in the formation of DNA adducts, via reaction with guanine to form S-[2-(N^7-guanyl)ethyl]glutathione (Inskeep *et al*. 1986).

No alternative, non-genotoxic mechanisms of action have been proposed in the literature.

5.6 Critical studies

There have been a number of long-term carcinogenicity studies on 1,2-dichloroethane by the oral and inhalation routes in rats and mice. In rats, the main tumours consistently seen in inhalation studies were in the mammary gland, together with some liver neoplasms. In the National Cancer Institute (NCI) oral studies, tumours were observed in the forestomach, haemangiosarcomas and mammary tumours. In the mouse, the tumours detected were more varied with lung and reproductive tumours as well as liver and mammary tumours. There were also two studies where no increases in tumour incidence were seen.

The most consistent and sensitive results were the development of mammary tumours in rats. Inhalation (together with dermal) was considered the most likely exposure route in humans and the most complete dose-response study was by Nagano *et al*. (2006) with an endpoint of a combined tumour incidence of adenomas, fibroadenomas and adenocarcinomas of the mammary gland. This study was used by the chemical sponsors in their CSRs to derive the inhalation DMEL and then the dermal DMEL by extrapolation.

US EPA in their IRIS assessment used the incidence of haemangiosarcomas in the NCI oral rat study as their point of departure and used extrapolation to derive an inhalation unit risk.

The relevance of the mammary tumours to human risk assessment of 1,2-dichloroethane needs to be considered. The genotoxicity studies suggest that direct action of the metabolites of 1,2-dichloroethane on DNA is the primary reaction that could potentially lead to cancer with little evidence of other indirect mechanisms such as oxidative stress and reactive hyperplasia, although the presence of forestomach tumours may indicate irritancy. The epidemiological studies are insufficient to determine a possible target site for tumours in humans and tumours in experimental animals often differ from humans in the site of carcinogenicity; for example, the human bladder appears a possible site for tumours caused by aniline-derived compounds but this target is rare in experimental animal studies with these compounds. Therefore, the choice of mammary tumours for this risk assessment is based rather on genotoxic potential and the best dose-response rather than its relevance to human cancer.

5.7 Dose Response

The aim of this project is to identify information that can be used to quantify risk for relevant exposure routes. The review of the genotoxicity and carcinogenicity data leads to the conclusion that there is a potential for a genotoxic mode of action with metabolic activation and that exposure to 1,2-dichloroethane can give rise to tumours in experimental animals, and can presume to have carcinogenic potential in humans. Therefore the quantitative risks for

1,2-dichloroethane are based on a carcinogenic potential, although there are no robust evidence that it is a human carcinogen. Review of the epidemiological studies on human occupational exposure to 1,2-dichloroethane does not reveal any data that would be useful in identifying any quantitative risk for humans. Therefore the dose response curves are based on relevant, robust studies in experimental animals.

The value commonly used globally including Europe as a Point of Departure (PoD) for risk assessment is T25 which is the daily dose (in mg/kg body weight) inducing a tumour incidence of 25% upon lifetime exposure. This is based on an assumption of a linear dose response at all concentrations (including above the experimental doses) excluding the zero dose. The derivation of a T25 for 1,2-dichloroethane will be the PoD for this risk assessment.

Based on the likely exposure routes for 1,2-dichloroethane, the T25 value has been derived for inhalation and the subsequent human risk extrapolated for dermal exposure.

5.8 Carcinogenicity risk assessment

A T25 for carcinogenicity in laboratory animals was derived from a 2-year inhalation study in F344/DuCrj (SPF) rats using the combined frequency of mammary tumours; adenomas, fibroadenomas and adenocarcinomas, reported (Table 5.4 : Nagano *et al*., 2006). This study is the most recent long-term study with three dose levels, giving a linear response that is sufficient for taking a non-threshold linear approach and derivation of a T25 value. The key information provided in this study is as follows:

- Lowest dose with a significantly increased frequency (C) of 160 ppm (658 mg/m^3)
- Incidence at C, 25 tumours in 50 animals, 0.50
- Control incidence, 8 tumours in 50 animals, 0.16
- Net increase in frequency above concurrent control of 0.34
- Exposures were made 6 hours per day, 5 days per week for 2 years (standard lifetime period)

The $T25_{(inhalation,\ rat)}$ from this study for a period of 6 hours/day for 5 days/week lifetime exposure is derived using the following equation:

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

$$\mathbf{T25_{(Inhalation,\ Rat)} = 160 \times (0.25)/(0.50 - 0.16) \times (1-0.16)/1}$$

$$\mathbf{= 98.8\ ppm\ (approximately\ 406\ mg/m^3)}$$

These values were then used in the registration CSRs to derive DMELs for long-term inhalation and dermal exposure (systemic effects) for workers and an inhalation DMEL for the general population.

Workers

Workers inhalation risk estimate

The $T25_{(Inhalation,\ Rat)}$ of 98.8 ppm applies for lifetime exposure, 6 hours/day, 5 days/week. A T25 for workers' inhalation exposure was calculated using the following:

- Light activity for workers is assumed during an exposure time of 8 h/day, 5 days/week, 48 weeks/year for 40 years out of a lifetime of 75 years.
- Activity driven difference for workers (standard respiratory volume for humans, 6.7/respiratory volume for workers, 10).

$$\mathbf{T25_{(Inhalation,\ Workers)} = 98.8 \times 6/8 \times 5/5 \times 52/48 \times 75/40 \times 6.7/10}$$
$$\mathbf{= 100.8\ ppm\ (414.4\ mg/m^3)}$$

Workers dermal risk estimate

The $T25_{(Inhalation,\ Workers)}$ of 100.8 ppm (414.5 mg/m^3) can be converted to workers dermal exposure using the following assumptions:

- Workers breathe in 10 m^3 of air per day
- $T25_{(Inhalation,\ Workers)}$ is 414.4 mg/m^3
- Adult human body weight 70 kg
- 50% dermal absorption was assumed compared to 100% following inhalation exposure

$T25_{(Dermal,\ Workers)}$ (uncorrected for dermal absorption) = 414.4 x 10/70kg = 59.2 mg/kg bw/day

For 50% dermal absorption: 59.2 x 100/50 = 118.4 mg/kg bw/day

General population

General population inhalation risk estimate

The lifetime $T25_{(Inhalation,\ Rat)}$ of 98.8 ppm can be converted into a lifetime $T25_{(Inhalation,\ Gen.\ pop.)}$, by correction for exposure period (24-hour exposure not 6 hours), exposure frequency (7 days/week not 5 days/week),

$T25_{(Inhalation,\ Gen\ pop)}$ = 98.8 x 6/24 x 5/7

= 17.6 ppm (72.5 mg/m^3)

General population dermal risk estimate

The $T25_{(Inhalation,\ Gen.pop.)}$ of 17.6 ppm (72.5 mg/m^3) can be converted to general population dermal exposure using the following assumptions:

- General population breathe in 20 m^3 of air per day
- T25 $_{(Inhalation,\ Gen.pop.)}$ is 72.5 mg/m^3
- Adult human body weight 70 kg
- 50% dermal absorption was assumed compared to 100% following inhalation exposure

T25 $_{(Dermal,\ Gen.pop)}$ (uncorrected for dermal absorption) = 72.5 x 20/70 = 20.7 mg/kg bw/day

For 50% dermal absorption: 20.7 x 100/50 = 41.4 mg/kg bw/day

General population oral risk estimate

The $T25_{(Inhalation,\ Gen.pop.)}$ of 17.6 ppm (72.5 mg/m^3) can be converted to general population oral exposure using the following assumptions:

- General population breathe in 20 m^3 of air per day
- T25 $_{(Inhalation,\ Gen.pop.)}$ is 72.5 mg/m^3
- adult human body weight 70 kg
- 100% absorption by the oral route compared to 100% following inhalation exposure

T25 $_{(Oral,\ Gen.pop)}$ = 72.5 x 20/70 x 100/100

= 20.7 mg/kg bw/day

The cancer risk estimates are summarised in Table 5.6.

Table 5.6 Cancer risk estimates for 1,2-dichloroethane

Route of exposure	Population	T25 Descriptor	Cancer risk for 1 unit amount
Oral	General population	$T25_{(Oral, Human)}$ 20.7 mg/kg bw/day	**1.2×10^{-5} per µg/kg bw/day**
Inhalation	Workers	$T25_{(Inhalation, Human)}$ 100.8 ppm (414.4 mg/m³)	**6.0×10^{-7} per µg/m³**
	General population	$T25_{(Inhalation, Human)}$ 17.6 ppm (72.5 mg/m³)	**3.45×10^{-6} per µg/m³**
Dermal	Workers	$T25_{(Dermal, Human)}$ 118.4 mg/kg bw/day	**2.1×10^{-6} per µg/kg bw/day**
	General population	$T25_{(Dermal, Human)}$ 41.4 mg/kg bw/day	**6×10^{-6} per µg/kg bw/day**

Assuming linearity of response the cancer risk for lifetime exposure to each unit amount of technical 1,2-dichloroethane will increase in proportion, e.g. for workers' exposure by inhalation.

1 µg/m³	6.0×10^{-7}
2 µg/m³	1.2×10^{-6}
10 µg/m³	6.0×10^{-6}
100 µg/m³	6.0×10^{-5}
1000 µg/m³	6.0×10^{-4}

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6. Diglyme

Bis(2-methoxyethyl) ether (Diglyme) CAS RN: 111-96-9; EC Number: 203-924-4

Diglyme is a solvent used in the manufacture of protective coatings. It is a member of the class of glycol ethers. DNELs have been derived for reproductive toxicity.

Where necessary, a conversion of 5.579 has been used to convert from ppm to mg/m³ (ECETOC, 2005).

6.1 Toxicokinetics

Dermal and inhalation exposure routes have been identified as the most important exposure routes for workers and the general population.

6.1.1 Absorption

Oral

Diglyme is rapidly and completely absorbed in the rat and mouse following oral administration. Within 96 hours, approximately 86-90% of administered dose is excreted (GBK, 2010).

Diglyme has a low molecular weight, logPow and solvating properties (ECHA, 2011).

Dermal

Diglyme will also be absorbed following skin exposure. Following application of diglyme to dermatomised human skin *in vitro*, absorption occurred following a lag time of 36 ± 3 minutes. The flux during steady state permeation was 0.952 ± 0.340 mg/cm²/hour.

Inhalation

Diglyme is also absorbed following exposure via inhalation.

6.1.2 Distribution

Glycol ethers are readily distributed throughout the body and eliminated via urine. Significant accumulation does not occur (ECHA, 2011).

6.1.3 Metabolism

Two initial metabolic oxidations occur during the metabolism of diglyme. Both involve Cytochrome P-450. The first pathway metabolises diglyme via oxidative dealkylation of an

interior ether bond to formally provide two molecules of 2-methoxyethanol. 2-Methoxyethanol is converted via oxidation, by way of the aldehyde, to 2-methoxyacetic acid.

2-Methoxyacetic acid has been associated with testicular toxicity in male experimental animals and development of the conceptus in pregnant female animals. In rats, most 2-methoxyacetic acid is excreted via the urine; however, some is conjugated with glycine to produce N-methoxyacetyl glycine.

The second pathway involves oxidative demethylation of diglyme, by unspecified cytochrome P450 isozymes to give 2-(2-methoxyethoxy)ethanol which is converted by oxidation of the aldehyde to 2-(2-methoxyethoxy)acetic acid. This molecule can be further metabolised via oxidative demethylation to give the alcohol 2-hydroxyethoxyacetic acid. This will then be oxidised to diglycolic acid and excreted.

The human pathway for metabolism is similar to that in experimental animals, with human and rat microsomal preparations producing qualitatively and quantitatively similar oxidative metabolic products. Human liver microsomes may be more efficient than rat liver microsomes at cleaving diglyme into 2-methoxyethanol.

The pathway of diglyme metabolism is dependent on where the oxidative attack occurs on the diglyme molecule, which is dependent on the relative quantities of cytochrome P450 isozymes that are present. The pathways of metabolism do not cross over.

The metabolite, 2-methoxyacetic acid may accumulate in animals and humans, with a human half-life of 77.1 hours.

6.1.4 Excretion

Oral

Diglyme is excreted via the urine, and is almost complete within 96 hours of dosing (GBK, 2010). No further information is available.

Dermal

No information is available.

Inhalation

No information is available.

6.1.5 Bioavailability

Orally, diglyme is rapidly and completely absorbed and 100% bioavailability will be taken for this route of exposure. Diglyme is also absorbed by inhalation (as

demonstrated in several inhalation toxicity studies showing systemic effects) and via the dermal route, although there are no quantitative data available. In the absence of specific data, 100% absorption via the inhalation and dermal route will be assumed. There are no data to differentiate toxicokinetics in humans and animals, therefore bioavailability for humans via the three routes of absorption is assumed to be the same as for animals.

6.2 Reproductive toxicity

6.2.1 Human Epidemiological Studies

Information on individual studies is located in Appendix D1.

Four studies have been located, which report findings in cohorts of semiconductor workers or shipyard painters. Workers in all of the studies were exposed to ethylene glycols, but not specifically diglyme itself. Exposure to ethylene glycols was not quantitatively measured in any of the studies. Increases in spontaneous abortions and decreases in the ability to conceive were observed in women working in the semiconductor industry. Adverse effects on sperm were observed in male shipyard workers. There are various deficiencies in each of the studies. The lack of information on diglyme itself and the inadequacy of the exposure quantification indicate that sufficient information is not available from epidemiological studies to provide an assessment of the effects diglyme may cause, and therefore safe levels of exposure for this substance.

6.2.2 Experimental Animal Studies

Information on individual studies is located in Appendix D2.

An oral fertility study has been completed in male rats, three inhalation studies have been completed in rats, and one inhalation study has been completed in mice.

One developmental toxicity study has been completed in rabbits via gavage, three have been completed in mice via gavage, and one study has been completed in rats via inhalation.

6.2.3 Reproductive toxicity

Table 6.1 Reproductive toxicity study summary for diglyme

Species	Route of exposure	Dose/concentration	Observations	NOAEL	Converted internal NOAEL	Reference	Reliability
Rat (Crl:CD) male 20/group	Inhalation 6 h/day 5 days/week for 2 weeks 84 days post exposure	0, 110, 370, 1100 ppm (approximately 0, 614, 2064, 6137 mg/m³)	370 and 1100 ppm: decrease in absolute weight of testis, epididymides, seminal vesicles and prostate. 1100 ppm: Decreased relative weight of testis. Testicular atrophy. Effects reversible at 84 days except 1100 ppm	LOAEC 110 ppm (approx. 614 mg/m³)	LOAEL 212 mg/kg bw/day (5 days/week)	DuPont (1988b), Valentine (1999)	1
Rat (Crl:CD) male 20/group	Inhalation 6h/day 5days/week for 2 weeks 14 days post exposure	0, 3, 10, 30, 100 ppm (approximately 0, 17, 56, 167, 558 mg/m³)	Minimal top mild lesions below 100 ppm (degenerative germ cells in epididymal; tubules, spermatic granuloma in epididymis, prostatitis. Not clear if lesions occurred in same or different animals. 100 ppm: decreased mean bodyweight, mild testicular atrophy. The NOAEL is that stated by the authors taking into consideration historical data (not shown) and	30 ppm (approx. 167 mg/m³)	58 mg/kg bw/day (5 days/week)	DuPont (1989)	2

Species	Route of exposure	Dose/concentration	Observations	NOAEL	Converted internal NOAEL	Reference	Reliability
			repeated in the CICAD (WHO, 2002)				
Rat (CD) male 10/group	Inhalation 7h/day 5 days	0, 250, 1000 ppm (approximately 0, 1395, 5579 mg/m³)	1000 ppm: Reduced bodyweight in males. Decreased pregnancy frequency. Preimplantation losses. Recovery complete in week 10.	NOAEC of 250 ppm (1395 mg/m³)	564 mg/kg bw/day (5 days)	McGregor *et al*. (1983)	3
Mice (B6C3F1)	Inhalation 7h/day 4 days	0, 250, 1000 ppm (approximately 0, 1395, 5579 mg/m³)	Reduced bodyweight gain in both groups. 4 mice at top dose died on day 4. 1000 ppm: morphologically altered sperm.	LOAEC of 250 ppm (1395 mg/m³)	LOAEL 774 mg/kg bw/day 4 days	McGregor *et al*. (1981)	3
Rats (Sprague-Dawley) Male 5/group	Oral 20 days	684 mg/kg bw 8 week recovery	Primary and secondary spermatocyte degeneration, spermatidic giant cells, reduced testis to bodyweight ration from day 12 until the end of study, testicular LDH-X activity decreased by day 18.	Effects at 684 mg/kg bw	-	Cheever *et al.* (1985, 1989)	3

Doses in mg/m³ calculated by a conversion factor of 5.579 from ppm. Rat breathing rate 0.24 l/minute. Rat bodyweight 0.25 kg (Guidance for Human Health Risk Assessment, Volume III, Part B, 2013). Mouse breathing rate 0.0.04 l/minute. Mouse bodyweight 0.0.3 kg (ECHA guidance, 2012)

6.2.4 Developmental toxicity

Table 6.2 Developmental toxicity study summary

Species	Route of exposure	Dose/concentration	Observations	Maternal NOAEL	Foetal NOAEL	Converted internal dose	Reference	Reliability
Rabbits (New Zealand) Female 15-25/group	Gavage Day 6-19	0, 25, 50, 100, 175 mg/kg bw/day	50 mg/kg bw/day: dams: decreased weight gain, increased adversely affected implants per litter. 100 mg/kg bw/day: decreased gravid uterine weight, increased prenantal mortality (resorptions), increased malformations 175 mg/kg bw/day: Decreased faecal output, increased mortality. Maternal toxicity only.	100 mg/kg bw/day 25 mg/kg bw/day	25 mg/kg bw/day 50 mg/kg bw/day	-	NTP (1987), WHO (2002) Schwertz *et al.*(1992)	2
Rats (CD) female 25-26/group	Inhalation 6h/day days 7-16	0, 25, 100, 400 ppm (approximately 0, 139, 558, 2232 mg/m³)	25 ppm: decreased foetal weight, variations 100 ppm: dams: increased relative liver weight, Foetus: structural malformations 400 ppm: dams: decreased food consumption, decreased bodyweight gain, total resorption	25 ppm (approx. 139 mg/m³)	LOAEC 25 ppm (approx. 139 mg/m³)	48 mg/kg bw/day	Dupont (1988a) Driscoll (1998)	1

Species	Route of exposure	Dose/concentration	Observations	Maternal NOAEL	Foetal NOAEL	Converted internal dose	Reference	Reliability
Mice (CD-1) 20-24 /group	Gavage day 6-15	0, 62.5, 125, 250, 500 mg/kg bw/day	125 mg/kg bw/day: decreased foetal weight 250 mg/kg bw/day: dams: decreased weight gain, increased late foetal death, increased malformations 500 mg/kg bw/day: dams: decreased bodyweight gain, increased resorptions	500 mg/kg bw/day	62.5 mg/kg bw/day	-	NTP (1985), Price *et al. (*1987)	1
Mice (CD-1) Not provided	Gavage day 11	0, 537 mg/kg bw	Only examination for gross external malformations and foetal bodyweight. 537 mg/kg bw/day: increased malformations	Effects at 537 mg/kg bw	Effects at 537 mg/kg bw	-	Hardin and Eisenmann (1986, 1987)	3
Mice (CD-1) 49/group	Gavage day 6-13	0, 3000 mg/kg bw/day	Reproductive screening according to Chernoff and Kavlock, no systematic examination for malformations. 3000 mg/kg bw/day. Dams: increased mortality, no viable litters	Effects at lowest dose	Effects at lowest dose	-	Schuler *et al.*(1984), Plasterer *et al.* (1985), Hardin and Eisenmann (1987)	3

Doses in mg/m³ calculated by a conversion factor of 5.579 from ppm. Rat breathing rate 0.24l/minute. Rat bodyweight 0.25 kg (ECHA guidance, 2012)

6.3 Mechanism of Action

Two initial metabolic oxidations occur during the metabolism of diglyme. Both involve cytochrome P450. The first pathway metabolises diglyme via oxidative dealkylation of an interior ether bond to formally provide two molecules of 2-methoxyethanol. 2-Methoxyethanol is converted via oxidation, by way of the aldehyde, to 2-methoxyacetic acid.

2-Methoxyacetic acid has been associated with testicular toxicity in male experimental animals and development of the conceptus in pregnant female animals. In rats, most 2-methoxyacetic acid is excreted via the urine, however some is conjugated with glycine to produce N-methoxyacetyl glycine.

The second pathway involves oxidative demethylation of diglyme, by unspecified cytochrome P450 isozymes to give 2-(2-methoxyethoxy)ethanol which is converted by oxidation by the aldehyde to 2-(2-methoxyethoxy)acetic acid. This molecule can be further metabolised via oxidative demethylation to give the alcohol 2-hydroxyethoxyacetic acid. This will then be oxidised to diglycolic acid and excreted.

The human pathway for metabolism is similar to that in experimental animals, with human and rat microsomal preparations producing qualitatively and quantitatively similar oxidative metabolic products. Human liver microsomes may be more efficient than rat liver microsomes at cleaving diglyme into 2-methoxyethanol.

The pathway of diglyme metabolism is dependent on where the oxidative attack occurs on the diglyme molecule, which is dependent on the relative quantities of cytochrome P-450 isozymes that are present. The pathways of metabolism do not cross over.

The metabolite, 2-methoxyacetic acid may accumulate in animals and humans, with a human half-life of 77.1 hours.

These data indicate that the developmental effects manifested following administration of diglyme occur after a threshold dose has been reached.

6.4 Critical studies

Multiple studies have been conducted via both the inhalation and oral routes to determine the reproductive toxicity of diglyme. The fertility studies have focused on dysfunctions in the male reproductive system (testicular toxicity) and foetal malformations were identified in the developmental studies. An oral fertility study has been completed in male rats, three inhalation studies have been completed in rats, and one inhalation study has been completed in mice. One developmental toxicity study has been completed in rabbits via gavage, three have been completed in mice via gavage, and one study has been completed in rats via inhalation. The studies have been summarised in Tables 6.1 and 6.2 above. The key points to their use in the setting of DNELs are outlined below.

DuPont/Valentine (DuPont, 1988b; Valentine *et al.,* 1999) exposed rats via inhalation for 6 hours/day, 5 days/week for only 2 weeks at concentrations of 0, 110, 370 or 1100 ppm (approximately 0, 614, 2064 and 6137 mg/m³). A decrease in the absolute weight of the testis, epididymides, seminal vesicles and prostate occurred at the mid and top dose, and decreased relative weight of the testis and testicular atrophy occurred at the top dose. At the lowest dose, spermatocytes in pachytene and meiotic division at spermatogenic stages XII-XIV were mainly affected. A LOAEC of 110 ppm (approximately 614 mg/m³) was identified from this study. Only a LOAEC was derived from this study, and the duration of exposure was extremely short.

A rat inhalation study was conducted with concentrations of 0, 3, 10, 30, 100 ppm (approximately 0, 17, 56, 167, 558 mg/m³) where minimal to mild lesions (degenerative germ cells in epididymal tubules, spermatic granuloma in epididymis, prostatitis) were described below 100 ppm (DuPont, 1989). It is not clear whether these slight lesions occurred in the same or different animals. A NOAEL of 30 ppm (approx. 167 mg/m³) was stated by the authors taking into consideration historical data (not shown) and this conclusion is repeated in the CICAD document (WHO, 2002).

WHO (2002) and the ECHA Annex XV dossier (2011) have evaluated diglyme and identified an NTP oral gavage study in rabbits administered 0, 25, 50, 100, 175 mg/kg bw/day as the key developmental toxicity study (NTP, 1987). This study was well reported and conducted to OECD standards and GLP. However, different interpretations of the maternal and foetal NOAELs exist for this study. A paper by Schwertz *et al.* (1992) identifies 50 mg/kg bw/day as the foetal NOAEL; however, a significant decrease in the number of implants per litter occurred at this dose, corresponding to the NOAEL identified by the NTP of 25 mg/kg bw/day.

A rat developmental toxicity study investigated effects via inhalatory administration of 0, 25, 100, 400 ppm (approximately 0, 139, 558, 2232 mg/m³) (DuPont 1988a, Driscoll *et al*., 1998). This study did not identify a NOAEC for foetal effects as a decrease in foetal weight and an increase in variations were observed at the lowest concentration tested. Converting the LOAEC identified in this study of 25 ppm (approximately 139 mg/m³) into an internal dose results in a LOAEL of approximately 48 mg/kg bw/day (based on rat breathing rate 0.24 l/minute and rat bodyweight 0.25 kg).

Mice were gavaged on days 6-15 of gestation at doses of 0, 62.5, 125, 250 or 500 mg/kg bw/day in a study by NTP (1985). Decreased foetal weight was observed at 125 mg/kg bw/day. Dams displayed decreased weight gain at 250 mg/kg bw/day and there was increased late foetal death and increased malformation. At the top dose there was decreased bodyweight gain in dams and increased resorptions. A foetal NOAEL of 62.5 mg/kg bw/day was identified from this study.

No data from dermal studies are available.

6.5 DNELs for exposure routes

Comparison of no effect levels with available exposure estimations.

Table 6.3 Previous DNELs and exposure estimates for diglyme

Long term systemic	DNELs Annex XV	DNELs registration	Guidance values (WHO 2002)	Exposure estimates (WHO 2002)	Exposure (registration dossier)
Worker dermal	0.8 mg/kg bw/day	2.08 mg/kg bw/day	-	-	*
Worker inhalation	11.6 mg/m³	26.8 mg/m³	0.6 mg/m³	36 mg/m³ Production 3 mg/m³ Semiconductor industry 31 mg/m³ Painting op	*
Worker oral	-	-	0.25 mg/kg bw/day	-	-
General population dermal	0.4 mg/kg bw/day	1.04 mg/kg bw/day	-	-	-
General population inhalation	2.8 mg/m³	6.7 mg/m³	0.6 mg/m³	-	-
General population oral		1.04 mg/kg bw/day	0.25 mg/kg bw/day	-	-

*Confidential

6.5.1 Important exposure routes

For completeness, DNELs have been derived fior all routes of exposure for both workers and the general population. Dermal and inhalation exposure routes have been identified as the most important exposure routes for workers and the general population. Consumers must not use diglyme and use by consumers is advised against (GBK, 2010), so any exposure would be oral or inhalation in the environment.

6.6 Derived No Effect Levels

For the inhalation route, DNELs have therefore been calculated based on the DuPont 1988a/Driscoll 1998 study (inhalation rat, developmental effects) and on the DuPont 1989 study (inhalation rat, testicular effects).

For the oral route, DNELs have been calculated based on the NTP 1987 and 1985 studies (oral rabbit and mouse, respectively, developmental effects).

The United Kingdom Interdepartmental Group on Health Risks from Chemicals document entitled 'Guidelines on route-to-route extrapolation of toxicity data when assessing health risks of chemicals' (IGHRC, 2006) states that oral to dermal extrapolation is common for industrial chemical and pesticide exposure. This document assumes that dermal bioavailability is less than oral (i.e. less substance will be absorbed via the skin due to its barrier properties), therefore, using the oral data is precautionary, providing that the skin is not compromised by the substance being a severe irritant and causing increased absorption through a more permeable barrier. Diglyme is not considered to be irritant, therefore, equivalent bioavailability can be assumed for oral and dermal exposure to provide a precautionary DNEL. The approach used in the IGHRC document is that used in the ECHA REACH Guidance and is widely referenced.

For the purposes of comparison, extrapolation was also made from the inhalation study of DuPont (1989) for both oral and dermal exposure using 100% bioavailability for both.

In the absence of substance-specific information, default assessment (uncertainty) factors (for inter- and intraspecies variation, exposure duration extrapolation etc.) as prescribed in the ECHA guidance (ECHA, 2008) are used.

RAC agreed that an assessment factor of 4 for testicular effects would be applied for extrapolation from sub-acute testicular toxicity studies to chronic effects. RAC considered that the assessment factor of 6 stated in the REACH Guidance was not appropriate due to two factors: firstly, the relatively short duration of the spermatogenesis process, and secondly, that bioaccumulation of diglyme had not been reported. This assessment factor was not required for developmental studies.

Inhalation exposure

Workers/General population

DuPont 1989 study

Rat
End-point: testicular toxicity
Exposure regime: 6 h/day; 5 days/week; 2 weeks

NOAEC = 167 mg/m^3
Assuming 100% bioavailability

NOAEC CORRECTION		
	Workers	General population
Exposure regime	6/8	(6/24) X (5/7)
Breathing rate	6.7/10	-
CORRECTED NOAEC (mg/m^3)	83.9	29.8

ASSESSMENT FACTORS		
	Workers	General population
Interspecies allometric scaling	-	-
Interspecies, remaining differences	2.5	2.5
Intraspecies	5	10
Subacute to chronic	4	4

Thus:
DNEL workers = 1.68 mg/m^3
DNEL general population = 0.30 mg/m^3

DuPont 1988a study

Rat
End-point: development
Exposure regime: 6 h/day; 10 days
LOAEC = 139 mg/m^3
Assuming 100% bioavailability

LOAEC CORRECTION		
	Workers	General population
Exposure regime	(6/8) x (7/5)	6/24
Breathing rate	6.7/10	-
CORRECTED LOAEC (mg/m^3)	34.8	97.8

ASSESSMENT FACTORS		
	Workers	General population
Interspecies allometric scaling	-	-
Interspecies, remaining differences	2.5	2.5
LOAEC to NOAEC	3	3
Intraspecies	5	10
Subacute to chronic	-	-

Thus:
DNEL workers = 2.61 mg/m^3
DNEL general population = 0.46 mg/m^3

DuPont 1988b study

Rat
End-point: testicular toxicity
Exposure regime: 6 h/day; 5 days/week, 2 weeks
LOAEC = 614 mg/m^3
Assuming 100% bioavailability

LOAEC CORRECTION		
	Workers	General population
Exposure regime	6/8	(6/24) X (5/7)
Breathing rate	6.7/10	-
CORRECTED LOAEC (mg/m^3)	308	110

ASSESSMENT FACTORS		
	Workers	General population
Interspecies allometric scaling	-	-
Interspecies, remaining differences	2.5	2.5
LOAEC to NOAEC	3	3
Intraspecies	5	10
Subacute to chronic	4	4

Thus:
DNEL workers = 2.1 mg/m^3
DNEL general population = 0.37 mg/m^3

The DNELs derived from the all three inhalation studies (two fertility and 1 developmental) are quite similar, which suggests that in the rat the DNELs for fertility and development are rather comparable.

Oral exposure

Workers/General population

NTP 1985

Mice
End-point: development
Exposure regime: 10 days
LOAEC = 62.5 mg/kg bw/day
Assuming 100% bioavailability

LOAEC CORRECTION		
	Workers	General population
Exposure regime	7/5	-
CORRECTED LOAEC (mg/kg bw/day)	87.5	62.5

ASSESSMENT FACTORS		
	Workers	General population
Interspecies allometric scaling	7	7
Interspecies, remaining differences	2.5	2.5
LOAEC to NOAEC	3	3
Intraspecies	5	10

Thus:
DNEL workers = 1.0 mg/kg bw/day
DNEL general population = 0.36 mg/kg bw/day

NTP 1987

Rabbit
End-point: development
Exposure regime: 14 days
LOAEC = 25 mg/kg bw/day
Assuming 100% bioavailability

LOAEC CORRECTION		
	Workers	General population
Exposure regime	7/5	-
CORRECTED LOAEC (mg/kg bw/day)	35	25

ASSESSMENT FACTORS		
	Workers	General population
Interspecies allometric scaling	2.4	2.4
Interspecies, remaining differences	2.5	2.5
LOAEC to NOAEC	3	3
Intraspecies	5	10

Thus:
DNEL workers = 1.2 mg/kg bw/day
DNEL general population = 0.42 mg/kg bw/day

By using an additional correction factor of 70 kg bw/person/20 m^3/day (for general population) and 70 kg bw/person/10 m^3/working day, these same assessment and correction factors can be used for deriving inhalation DNELs from these two oral studies. The results of such a derivation are shown below:

	DNEL (mg/m^3)	
	Workers	**General population**
NTP 1985	7.0	1.3
NTP 1987	8.2	1.5

It is noteworthy that these DNELs are around 4 times higher than the corresponding values derived using inhalation studies, which suggests that either mice and rabbit species might be

more resistant to diglyme or that bioavailability for the oral route is lower than for the inhalation route.

Therefore, and in absence of additional evidences, RAC agreed that all route-specific DNELs (oral, dermal and inhalation) are based on the most sensitive rat inhalation study (DuPont 1989).

Thus, the derivation for **oral DNELs** are:

DuPont 1989 study

Rat
End-point: testicular toxicity
Exposure regime: 6 h/day; 5 days/week; 2 weeks
NOAEC = 167 mg/m^3
Assuming 100% bioavailability
Assuming respiratory rate of 0.8 l/min/kg

Corrected NOAEC for workers = 167 mg/m^3 x 6 hours x 60 min/hour x 0.0008 m^3/min/kg = 48 mg/kg bw/day

An additional factor of 5/7 is needed for correct NOAEC in general population:

Corrected NOAEC for general population = 167 mg/m^3 x 6 hours x 60 min/hour x 0.0008 m^3/min/kg x 5/7= 34.2 mg/kg bw/day

ASSESSMENT FACTORS		
	Workers	General population
Interspecies allometric scaling	4	4
Interspecies, remaining differences	2.5	2.5
Intraspecies	5	10
Subacute to chronic	4	4

Thus:
DNEL workers = 0.24 mg/kg bw/day
DNEL general population = 0.09 mg/kg bw/day

Dermal exposure

Workers/General population

Assuming again 100% dermal bioavailability the DNELs derived for the dermal route would be the same as those derived for the oral route: DNEL workers, 0.24 mg/kg bw/day; DNEL general population, 0.09 mg/kg bw/day.

Summary

The RAC agreed that the critical study for setting DNELs for inhalation, oral and dermal routes of diglyme exposure is that of DuPont (1989). The final DNELs set are shown in Table 6.4.

Table 6.4 Final DNELs for diglyme

Point of departure for DNEL derivation by all routes for Diglyme (DuPont, 1989)		
Rat 2-week Inhalation NOAEC in mg/m³ (testicular toxicity)	**167**	
Dosing regime	6h/d, 5d/wk, 2wk	
Inhalation absorption percentage	100%	
Derivation of Reference DNELs		
	WORKERS	**GENERAL POPULATION**
Assessment Factors		
Interspecies, Allometric scaling	-	-
Interspecies, remaining differences	2.5	2.5
Intraspecies	5	10
Subacute to chronic	4	4
Hours/day	8	24
Days/week	5	7
INHALATION		
Absorption percentage	100%	100%
Correction for exposure regime	6/8	6/24 x 5/7
Breathing rate for workers light activity vs rest	6.7/10	
NOAEC (corrected)	83.9	29.8
Reference DNELs INHALATION in mg/m³	**1.68**	**0.30**

DERMAL		
Absorption percentage	100%	100%
Correction for exposure regime	6/8	6/8 x 5/7
Standard respiratory volume in m^3/kg bw/day	0.384	0.384
NOAEL (corrected)	48.1	34.4
Reference DNELs DERMAL in mg/kg/day	**0.24**	**0.09**
ORAL		
Absorption percentage	100%	100%
Correction for exposure regime	6/8	6/8 x 5/7
Standard respiratory volume in m^3/kg bw/day	0.384	0.384
NOAEL (corrected)	48.1	34.4
Reference DNELs ORAL in mg/kg/day	**0.24**	**0.09**

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Appendix A Technical MDA

A1 Epidemiology Studies

Reference 1.1

Reference
Hall, A.J., Harrington, J.M. and Waterhouse, J.A. (1992) The Epping jaundice outbreak: a 24 year follow up. J Epidemiol Community Health, 46, 327-328. Nichols, L. (2004) The Epping Jaundice outbreak: mortality after 38 years of follow-up. Int Arch Occup Environ Health 77, 592-594.
Study Design/Details
Several follow-up investigations have been conducted after the “Epping Jaundice”, a notable episode of MDA poisoning in 1965 in which 84 patients (28 males and 56 females) from the Epping district of Essex, England suffered from liver toxicity following consumption of wholemeal bread baked with flour contaminated by MDA. It was reported that in this incident a plastic jar had been spilled into the van transporting the flour, where the substance spilled was a hardener for epoxy resin consisting of 4,4’-MDA dissolved in butyrolactone, and that consumption of the contaminated wholemeal bread was the only established connection between the affected individuals (Kopelman *et al.*, 1966). Following the incident, it was estimated that the bread contained 0.26% MDA (when the moisture content of the bread sample was 11.5%) (Kopelman *et al.*, 1966), and from analysis of the bread samples, the estimated oral dose of MDA was reported to be 3 mg/kg bw (EU, 2001). Of 88 cases originally attributed to the contaminated bread, four were subsequently excluded from the analysis on the basis of these cases being questionable either in origin or diagnosis, leaving a total cohort of 84 (Nichols, 2004) The follow-up studies examined the health status (including mortality and cancer incidence) of these 84 patients at 24 years (Hall *et al.*, 1992) and 38 years (Nichols, 2004) after the original incident. In the 24-year follow-up study, causes of death were identified in those individuals that could be traced and who were no longer alive. Observed: expected ratios were calculated using the life table method and adjusted for the age distribution of the original population. Confidence intervals were not reported but one-sided Poisson distribution tests were calculated to indicate significance. In the 38-year follow-up, mortality was compared with expected values based on national rates for the period 1965-2002, and cancer registration data were analysed for the period 1971-2002.
Study Findings
After 24 years (Hall *et al.*, 1992), of the 68 patients who could be traced (81%), there were 18 deaths. Of these deaths, there was one case of carcinoma of the biliary tract. Observed/expected ratios for deaths from all causes were: 0.32 (males; one-sided Poisson: 0.999), 0.80 (females; one-sided Poisson: 0.813) and 0.56 (both sexes; one-sided Poisson: 0.997). Observed/expected ratios for deaths from cancers were: 0.53 (males; 2 cases; one-sided Poisson: 0.889), 0.78 (females; 3 cases; one-sided Poisson: 0.736) and 0.66 (both sexes; 5 cases; one-sided Poisson: 0.874). Authors commented

that since the numbers in the study are small, little significance can be obtained and the follow-up study provides no clear link between current health status and MDA poisoning. However, despite the lack of clear causal link, they nevertheless considered the cancer of the biliary tract to be of interest, since this cancer is very rare in humans.

In this study (Hall *et al.*, 1992), the individuals identified as still being alive (50 individuals) were either supplied with a questionnaire or were interviewed. They were asked about cigarette, coffee and alcohol consumption, as well as diet, health status and occupation, in order to identify potential confounding factors. The response rate was 58% (18 females and 11 males). Of those who responded, there were eight current smokers (27.5%), most (75-80%) drank tea and/or coffee, two were vegetarians, eight were following special diets and ten (45%) had some form of allergy (mostly asthma or hay fever). Eleven had some notable pathology (not more than one disorder), where this included one individual with each of gall stones, Crohn's disease and stomach ulcer. One individual indicated having hepatitis B prior to 1965 and four others indicated having episodes of jaundice (possibly related to hepatitis A) since the incident. The authors also speculated that those who ate that particular wholemeal bread in the 1960s may have been particularly health conscious or belonged to particular professional classes and may therefore have had a lower than expected mortality.

After 38 years (Nichols, 2004), there were 37 deaths among the cohort. Mortality from all causes was close to expectation among females (25 deaths; standardised mortality ratio (SMR): 82; 95% confidence interval (CI): 53-122), and below expectation among males (25 deaths; SMR: 45; 95% CI: 23-78; P<0.01). There were 8 deaths from malignant tumours (SMR: 60; 95% CI: 26-118), one from cancer of the gall bladder (SMR: 1113; 95% CI: 28-6260). There were no deaths from cancer of the liver or cancer of the urinary bladder. The author concluded that there was no evidence of any obvious association between ingestion of MDA and effects on mortality.

Study Deficiencies, Questions and Additional Comments

It seems highly likely that the exposure to the epoxy hardener was the cause of the majority of the original episodes of jaundice, although individuals may have been exposed to the solvent butyrolactone in addition to 4,4'-MDA. The exact exposure was not clear, although estimates were made by analysis of the bread samples following the incident. Some attempt was made by Hall *et al.*, (1992) to identify potential confounding factors. However, the response rate (58%) in the individuals who were alive and the lack of information on the individuals who died make it difficult to determine which (if any) additional factor(s) may be of importance.

These follow-up studies have only limited power to detect effects due to the small size of the cohort and the inability to trace a part of the original cohort. Both reports are very short, and therefore contain only limited detail of the follow-up studies. Authors of both follow-up studies comment that the relevance of the findings to occupationally exposed workers is unclear, as occupational exposure is normally at a lower dose for a longer period of time, and the exposure route is usually different.

Reference 1.2

Reference
Seldén, A., Berg, P., Jakobsson, R. and de Laval, J. (1992) Methylene dianiline: assessment of exposure and cancer morbidity in power generator workers. *Int Arch Occup Environ Health*, 63, 403-408.
Study Design/Details
In a Swedish retrospective study, the health of power generator workers was investigated, where these workers had been potentially exposed to MDA (form not reported), in a curing agent of an epoxy system, between the years 1963 and 1968. Company records were used to identify a cohort of coil winders who were employed before 1968, where these workers were supposed to have been the most heavily exposed to the epoxy system. No measurements were available on exposure to MDA. However, it was reported that the company consumption of the MDA-based curing agent increased gradually from its introduction in 1963 to approximately 1500 kg/year in the late 1960s, and then decreased to approximately 500 kg/year in the early 1980s. The formula of the curing agent was reported to be constant over the study period, and to consist of 35% MDA, 15% dibutyl phthalate (solvent) and 50% other condensation products of aniline and formaldehyde. The epoxy system was a 2:1 mixture of the resin and the curing agent. The total cohort included 595 workers (550 males and 45 females). No information was available on individual exposure to MDA, but the workers were retrospectively classified into three categories according to the probability of their exposure to the epoxy system: 1) exposed (males: n=192; 2 792 person-years), 2) possibly exposed (males: n=237; 2 988 person-years) and 3) unexposed (males: n=121; 1516 person-years). The mean age for males in 1985, was 42.9 ± 11.6 years (n=482). Very few details are given in the report on the female workers, other than the fact that between them they provided 710 person-years of observation. Cancer incidence in the cohort was matched with the national cancer register for the period 1964-1985. Urine samples, for the analysis of urinary MDA metabolites, were provided by eight male coil winders on two consecutive days.
Study Findings
The levels of MDA measured in the air during the work performed by the eight coil winders were very low, where the maximum reported level was 0.4 µg/m^3 (reported to be 0.05% of the suggested USA threshold limit value of 800 µg/m^3 for MDA). Despite this, the urine of these exposed workers was found to contain high levels of MDA metabolites (>400 µg/l in several samples). Therefore, although recorded incidences of skin contact and acetylator phenotype were reported not to correlate with the excretion profiles, the authors attributed the high levels of urinary MDA metabolites to percutaneous absorption. Authors noted that in this situation MDA was used as a solute, but that the opportunity for both dermal and inhalation exposure were likely to be greater with MDA in a solid form, as in the study conducted by Liss *et al.* (1983). A standardised cancer incidence ratio (SIR) of 0.52 (95% confidence interval (CI): 0.16-1.21) was reported for overall cancer risk among males (n=550) based on five observed cases. Of these five cases, two were observed in the possibly exposed subcohort (SIR: 0.62; 95% CI: 0.07-2.25) and three

were observed in the unexposed subcohort (SIR: 0.85; 95% CI: 0.17-2.65). No cancer cases were reported in the definitely exposed subcohort, over the observation period, compared to 2.89 expected cases.

One male urinary bladder cancer case was reported (SIR: 4.29; 95% CI: 0.10-23.90), but that was in the unexposed subcohort. An overall SIR of 1.67 (95% CI: 0.04-9.31) was reported for bladder cancer risk. Two cancer cases (not urinary bladder) were identified among females (n=45), compared to 2.7 expected cases (no further details reported).

It was concluded that there was no statistically significant evidence of an increased association with either overall risk or bladder cancer risk compared to the total population. However, the authors stated that due to the limitations of the study, no definitive conclusions could be made.

Study Deficiencies, Questions and Additional Comments

The authors report limitations regarding the size of the cohort, the young age of the cohort (who had therefore not reached cancer-prone age) and the short follow-up period which may not have allowed for cancer latency. In addition, information was lost on some of the original study participants due to emigration from Sweden, and workers were also exposed to other chemicals in addition to MDA (no information available on other potential confounding factors, such as smoking status). Only limited information was available on the females included in the study and the exposures were not fully defined.

Reference 1.3

Reference

Liss, G.M. and Giurguis, S.S. (1994) Follow-up of a group of workers intoxicated with 4,4'-methylenedianiline. *Am J Ind Med*., 26, 117-124.

Study Design/Details

Between 1967 and 1976 eleven male workers from a factory in Ontario developed acute jaundice (four in 1967, one in 1974, one in 1975 and five in 1976). Ten of these workers were subsequently followed until the end of 1991 and cancer incidence was investigated (one could not be traced). These workers were reported to be exposed to MDA (form not reported), due to its use as an epoxy hardener, by inhalation at a concentration of 0.04-3.11 mg/m^3 (geometric mean 0.40 mg/m^3) for 7 days - 2.5 months between the years 1967 and 1976. The process of manufacturing the epoxy concrete surfacing material, using MDA as a hardener, was reported to involve receiving the MDA as a powder in drums.

Study Findings

Bladder cancer was diagnosed in one out of the ten workers, 23 years after exposure to MDA (expected number based on provincial incidence rates: 0.64 for all cancers, 0.05 for bladder cancer; Standardised Incidence Ratio (SIR) for bladder cancer: 19.29, 95% confidence intervals (CI): 0.5-107, P=0.051). Between them, the workers were reported to provide 184 person-years of follow-up.

Due to the latency of aromatic amine-induced cancers, the authors concluded that this provided further

evidence of the carcinogenicity of MDA in humans, but that the findings should be interpreted with caution given the study limitations. The authors also commented that MDA has structural similarity to known human bladder carcinogens.

Study Deficiencies, Questions and Additional Comments

The product was only made intermittently, and workers were exposed for short times, but the doses to which they were exposed were reported (by the authors) to be large and intense. The study included only a very small numbers of workers. No information was available on smoking status or on co-exposures, and there is potential bias due to a known health concern.

Reference 1.4

Reference

Liss, G.M. and Chrostek, W. (1983) NIOSH Health Hazard Evaluation Report, HETA, 82-146-1388, Boeing Vertol Company.

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Seldén, A., Berg, P., Jakobsson, R. and de Laval, J. (1992) Methylene dianiline: assessment of exposure and cancer morbidity in power generator workers. *Int Arch Occup Environ Health*, 63, 403-408.

Liss, G.M. and Giurguis, S.S. (1994) Follow-up of a group of workers intoxicated with 4,4'-methylenedianiline. *Am J Ind Med*., 26, 117-124.

Study Design/Details

In a study conducted by the US National Institute for Occupational Safety and Health (NIOSH), the health of helicopter pattern and blade workers at Boeing Vertol Company (SIC-3728) in Philadelphia, Pennsylvania, was investigated in 1982-1983, due to the concerns of workers (approximately 4600) regarding skin problems and cancer risk. The workers were exposed to epoxy resins, curing agents and various other chemicals via inhalation for at least one month. Exposures were measured by collecting air samples, medical interviews were conducted with 20 exposed and 20 unexposed workers and skin examinations conducted, and a proportional cancer mortality ratio analysis was conducted for 179 white male workers who died between 1968 and 1980, where 46 of these had malignant tumours.

Study Findings

Concentrations of MDA were reported to range up to Hygienists Threshold Limit Value of 0.8 mg/m^3. Other chemicals detected, at concentrations below Occupational Safety and Health Administration (OSHA) standards, were butyl-glycidyl-ether (BGE), dust, methyl-isobutyl-ketone (MBK) and toluene.

The incidence of skin problems, such as redness, itching and cracking, was reported to be twice as high in exposed compared to unexposed workers.

Statistically significant excesses ($P < 0.05$) were observed for cancer of the intestine

(observed/expected: 7/3.1), and lymphosarcoma and reticulosarcoma (3/0.87). Three mortalities from bladder cancer were reported in the exposed individuals, compared to the expected 0.9 cases, which was also reported to represent a significantly elevated excess (no further details available). Only the excess of bladder cancer remained statistically significant in a proportional cancer mortality analysis. In unexposed individuals, no excess of bladder cancer was observed (1/0.89). Additionally, it was reported that two other current or former employees (still living) had a history of bladder cancer. The authors concluded that this high incidence of bladder cancer may be due to the exposure to MDA, but recommended further investigation of this association.

Study Deficiencies, Questions and Additional Comments

The original study report could not be obtained from NIOSH, and therefore only limited study details were located from the study abstract and citations in related studies. The workers were exposed to a number of chemicals in addition to MDA, and therefore this co-exposure may be a confounding factor; it is not clear whether this was taken into account in the analysis. From the limited details obtained, the levels of exposure are not clear and it is not clear when exposure occurred. There is potential for bias due to a known health concern.

Reference 1.5

Reference

Cragle, D.L., Wells, S.M. and Tankersley, G. (1992) An occupational morbidity study of a population potentially exposed to epoxy resins, hardeners, and solvents. *Appl Occup Environ Hyg.* 7, 826-834.

Study Design/Details

A morbidity study was conducted in workers from the gas centrifuge process at Oak Ridge Gaseous Diffusion Plant, USA, who were involved in a process which had the potential for exposure to epoxy resins and solvents. It was reported that the workers may have been exposed to a number of other chemicals in addition to MDA, where these included m-phenylenediamine, bis(2,3-epoxycyclopentylether) and diglycidyl ether of bisphenol A (the substances in the resin systems), and the solvents trichloroethylene and methylene chloride.

The study cohort consisted of 263 workers considered to have worked closest to the process for the longest amount of time and a comparison group of 271 workers who did not work in the process. The exposed workers had worked a maximum of approximately 5200 days and a minimum of 500 days in a job with responsibilities that would very likely provide routing exposure to industrial types and quantities of toxic materials. Comparison workers were matched to the exposed workers according to date of birth, race, sex, date of hire and presence at the plant on the date that the exposed worker began working in the centrifuge process (referred to as the “centrifuge date”).

This process was in operation at the plant from 1963-1985. Person-years of observation were calculated from the date of entry into the study until the midpoint of the year of first occurrence of a disease, or until the end of 1988 or the date of death (whichever was earlier) if a person did not have a disease.

Prevalence ratios were calculated in order to determine any underlying disease patterns, and

incidence ratios were calculated to determine the rate of development of a disease in the exposed workers compared with the comparison workers.

Study Findings

Numerous potential confounding factors were examined in exposed compared with comparison workers. No significant difference was observed in a range of factors including: religious background, marital status, number of times married, military service, branch of military, current employment status, smoking (smoking status, packs smoked per day, age at start of smoking, number of years smoked, number of years smoking filtered cigarettes, manner of inhalation, number of hours exposed to cigarette smoke of others, whether parents smoked, pipe smoking, cigar smoking, tobacco chewing, snuff dipping) beer, wine or liquor consumption, or change in drinking habits in the last ten years. Exposed workers were reported as more likely to have work experience in the chemical industry (2 times), mining experience (3.5 times) and experience in the construction industry (1.5 times), as well as more experience working with benzene (2.4 times), chromium (1.7 times), radiation (13 times), trichloroethylene (2 times), phenol (22 times) and methylene chloride (3 times).

No cancers were identified in either the process or comparison workers prior to the “centrifuge date” (the date when the exposed workers first entered the centrifuge process), and therefore no cancer prevalence ratios were calculated.

One cancer of the kidney was identified in a process worker (compared with 0 cases in the comparison workers). Two liver cancers were identified, one in a process worker and one in a comparison worker (prevalence ratio: 1.03; 95% confidence interval: 0.06-16.43), where this was not significant (P=0.99 (log rank test); P=0.67 (Wilcoxon test)). However, the most significant finding of the study was reported to be the identification of five bladder cancers among the process workers (compared with none in the comparison workers). Since no cases were reported in the comparison workers, in order to calculate an incidence ratio for bladder cancer the number of cases that would have been expected in the comparison group were estimated based on incidence data for the area of Atlanta, USA, the closest geographic area to the plant where incidence data was available. The calculated incidence ratio was 7.80 (95% confidence intervals 1.12-68.14). This was reported to be statistically significant, although no P values were reported.

The five workers with bladder cancer were subsequently interviewed, and none were found to have ever worked closely (routinely or hands-on) with the epoxy resin materials during their employment. All five workers were also reported to have been smokers during their lives (one was a current smoker, four had stopped). Several factors were reported to be significantly related to bladder cancer in this study, including work in the chemical industry prior to joining this particular plant and diabetes. The only significant difference between these five workers and the other workers in the study was reported to be the potential for exposure to trichloroethylene. However, the authors concluded that it was not possible to identify one specific agent or job duty as the causative factor for the bladder cancers, and further follow-up observation of the workers was recommended.

Study Deficiencies, Questions and Additional Comments

The matching process of comparison workers and examination of potential confounding factors appeared to be very comprehensive in this study.

However, the exposure of workers was not fully quantified, and workers were categorised into groups based purely on their likelihood of exposure to the chemicals in question. Workers in the centrifuge process were also exposed to multiple chemicals in addition to MDA, and those workers in whom

bladder cancers were identified had not worked closely with MDA. These confounding factors make it difficult to determine the cause of the increased incidence of bladder cancers.

A2 Experimental Animal Studies

A2.1 Initiation-Promotion Studies

Reference 1.6

Reference
Fukushima, S., Hirose, M., Hagiwara, A., Hasegawa, R. and Ito, N. (1981) Inhibitory effect of 4,4´-diaminodiphenylmethane on liver, kidney and bladder carcinogenesis in rats ingesting N-ethyl-N-hydroxyethylnitrosamine or N-butyl-N-(4-hydroxybutyl) nitrosamine. *Carcinogenesis*, 2, 1033-1037. Cited in: IARC (1986). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 39: 4,4'-Methylenedianiline and its Dihydrochloride. International Agency for Research on Cancer, Lyon. and: ATSDR (1998). Toxicological Profile for Methylenedianiline. Agency for Toxic Substances and Disease Registry, Public Health Service, US Department of Health and Human Services.
Study Design/Details
In a study to investigate the effects of MDA on “post-initiation” stage carcinogenicity, male F344 rats were administered the initiator N-ethyl-N-hydroxyethyl-nitrosamine as 0.1% in their drinking water for 2 weeks, followed by 4,4’-MDA as 0.1% in their diet (reported to be 100 mg/kg bw/day) for a further 32 weeks. In experiment 2, male F344 rats were administered 0.01% N-butyl-N-(4-hydroxybutyl)nitrosamine for 4 weeks, followed by 0.1% 4,4’-MDA (reported to be 88 mg/kg bw/day) for a further 34 weeks, via their drinking water.
Study Findings
In experiment 1, reduced incidence of hepatocellular carcinoma as well as neoplastic nodules and renal cell tumours of the kidney were observed in rats treated with MDA compared to controls In experiment 2, MDA treatment was reported to inhibit the induction of papillomas in the bladder. Authors stated that these results indicate that MDA administration in the “post-initiation” stage inhibited liver, kidney and bladder carcinogenesis in rats.
Study Deficiencies, Questions and Additional Comments
IARC noted the short duration of the study. No information was available on the number of animals included in the study, and only one sex was used.
Klimisch Rating

4 (not assignable)

Reference 1.7

Reference
Masui, T., Tsuda, H., Inoue, K., Ogiso, T. and Ito, N. (1986) Inhibitory effects of ethoxyquin, 4,4´-diaminodiphenylmethane and acetaminophen on rat hepatocarcinogenesis. *Jpn J Cancer Res*, 77, 231-237. Cited in: ATSDR (1998). Toxicological Profile for Methylenedianiline. Agency for Toxic Substances and Disease Registry, Public Health Service, US Department of Health and Human Services.
Study Design/Details
In an initiation-promotion study, male F344 rats were administered a single intraperitoneal injection of the initiator diethylnitrosamine at a dose of 200 mg/kg bw, followed by a basal diet containing 2-acetylaminofluorene at 0.02% from week 2 to week 8. From week 12 – 36, rats were administered 0.1% 4,4'-MDA via the diet (reported to be 100 mg/kg bw/day). Animals were sacrificed at week 40.
Study Findings
In the animals treated with MDA, significantly reduced incidence, number and area per unit liver area of hepatocellular carcinoma was observed. Authors stated that these results indicate that MDA exerted an inhibitory effect on the development of hepatocellular carcinoma.
Study Deficiencies, Questions and Additional Comments
No information was available on the number of animals included in the study or on the controls, and only one sex was used.
Klimisch Rating
4 (unassignable)

Reference 1.8

Reference
Hiasa, Y., Kitahori, Y., Enoki, N., Konishi, N. and Shimoyama, T. (1984) 4,4´-Diaminodiphenylmethane: promoting effect on the development of thyroid tumors in rats treated with N-bis(2-hydroxypropyl)nitrosamine. J Natl Cancer Inst, 72, 471-476. Cited in: IARC (1986). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 39: 4,4'-Methylenedianiline and its Dihydrochloride. International Agency for Research on Cancer, Lyon. and: ATSDR (1998). Toxicological Profile for Methylenedianiline. Agency for Toxic Substances and Disease Registry, Public Health Service, US Department of Health and Human Services.
Study Design/Details
In a tumour promotion study, male inbred W rats (21/group) were administered a single intraperitoneal injection of N-bis(2-hydroxypropyl)-nitrosamine (DHPN) at a dose of 2800 mg/kg bw, followed by a diet either with or without 1000 mg 4,4'-MDA/kg (reported to be equivalent to 84 mg/kg bw/day) for 19 weeks. Further groups were maintained either on the MDA diet without the initial injection, or were administered saline at 0.5 ml/100 g bw (negative controls) and maintained on a basal diet.
Study Findings
No deaths were observed in any of the treatment groups. A significantly increased incidence of thyroid tumours was observed in the group treated with DHPN and MDA (19/21) compared to those treated only with DHPN (6/21). No thyroid tumours were observed in rats treated only with MDA. A slight, but not significant, decrease was observed in serum concentrations of thyroxine (T4) and triiodothyronine (T3) in animals treated with only MDA (but not in those treated with DHPN followed by MDA). Kidney tumours were observed in 38% (8/21) and 28% (6/21) of rats treated with DHPN and MDA and those treated with only MDA, respectively. No kidney or lung tumours were observed in rats treated with only MDA or in control rats.
Study Deficiencies, Questions and Additional Comments
IARC noted the small number of animals, of only one sex, included in the study, and the short study duration.
Klimisch Rating
3 (unreliable)

A2.2 Subchronic Studies

Reference 1.9

Reference
NTP (1983). Carcinogenesis Studies of 4,4'-Methylenedianiline Dihydrochloride in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program, US Department of Health and Human Services. Also reported in: Lamb, J.C., Huff, J.E., Haseman, J.K., Murthy, A.S. and Lilja, H. (1986) Carcinogenesis studies of 4,4'-methylenedianiline dihydrochloride given in drinking water to F344/N rats and B6C3F1 mice. Journal of Toxicology and Environmental Health, 18, 325-337. Cited in: EU (2001). European Union Risk Assessment Report for 4,4'-Methylenedianiline.
Study Design/Details
In a 90-day study, F344/N rats (10/sex/dose) were administered MDA (as the dihydrochloride) orally in the drinking water at doses of 0, 50, 100, 200, 400 or 800 mg/l (reported to be 0, 3.8, 7.1, 13.2, 25.7 and 38.7 mg/kg bw/day in males and 0, 3.7, 7.5, 12.7, 20.4 and 44.4 mg/kg bw/day in females, respectively).
Study Findings
Reduced final mean bodyweight was observed in males at the top dose and females at the top two doses, and reduced water consumption was observed in both sexes at the top three doses. Yellowing of the pelt around the urogenital orifice was observed at the top dose (8/10 males and 9/10 females). Dose-dependent bile duct hyperplasia was observed in both sexes at the top two doses (all animals of both sexes at the top dose, and 4/10 males and 3/10 females at the second highest dose). Adenomatous goitre was also observed at the top two doses (8/9 males and 10/10 females at the top dose, and 3/10 males and 1/10 females at the second highest dose). Thyroid follicular hyperplasia was observed in some animals (5/10 males and 7/10 females) at the second highest dose and in one male at the top dose. At the top dose, pituitary basophil hypertrophy was observed in all males and in 5/9 females. A NOAEL of 100 mg/l (reported to be 7.1 and 7.5 mg/kg bw/day in males and females, respectively) was identified (EU, 2001).
Study Deficiencies, Questions and Additional Comments
No information is available regarding compliance to test guidelines. However, although this subchronic used only a limited number of animals (as it was predominantly used to select the doses for the chronic study), it appears to have been conducted according to robust scientific principles.
Klimisch Rating
2 (reliable with restrictions)

Reference 1.10

Reference
NTP (1983). Carcinogenesis Studies of 4,4'-Methylenedianiline Dihydrochloride in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program, US Department of Health and Human Services. Cited in: EU (2001). European Union Risk Assessment Report for 4,4'-Methylenedianiline.
Study Design/Details
In a 90-day study, B6C3F1 mice (10/sex/dose) were administered MDA (as the dihydrochloride) orally in the drinking water at doses of 0, 25, 50, 100, 200 or 400 mg/l (reported to be 0, 2.5, 5.7, 11.4, 26.5 and 54.9 mg/kg bw/day in males and 0, 3.5, 7.6, 14.4, 25.9 and 52 mg/kg bw/day in females, respectively).
Study Findings
Reduced mean bodyweight was observed in males at the top two doses and in females at the top dose. At the top dose, bile duct hyperplasia was observed in 5/10 males and 4/10 females. Adenomatous goitres (less severe than in rats) were observed in one male and one female at the top dose. A NOAEL of 100 mg/l (reported to be 11.4 and 14.4 mg/kg bw/day in males and females, respectively) was identified (EU, 2001).
Study Deficiencies, Questions and Additional Comments
No information is available regarding compliance to test guidelines. However, although this subchronic used only a limited number of animals (as it was predominantly used to select the doses for the chronic study), it appears to have been conducted according to robust scientific principles.
Klimisch Rating
2 (reliable with restrictions)

Reference 1.11

Reference
Ciba-Geigy (1982) 3 Month Toxicity Study in Rats (Drinking Water); TK 10504. GU Project No. 791743; GU2 Toxicology (25.06.1982). Cited in: EU (2001). European Union Risk Assessment Report for 4,4'-Methylenedianiline.

Study Design/Details
In a 90-day study, Tif:Ralf (SPF) rats (80/sex/dose) were administered MDA (>99%) orally in the drinking water at doses of 0, 80, 400 or 800 mg/l (reported to be 0, 7.5, 23 and 31 mg/kg bw/day in males and 0, 8, 22 and 32 mg/kg bw/day in females, respectively).
Study Findings
At the top two doses, various changes in haematological parameters (including anaemia) and in clinical chemistry (including elevated alkaline phosphatase, aspartate aminotransferase and urea) were observed in both sexes. In addition, reduced bodyweight was also observed in both sexes at the top two doses. Various non-neoplastic lesions were observed in the liver and thyroid of both sexes at the top two doses, although these were most severe at the top dose and only the liver lesions persisted after a 4-week recovery period. At the low dose, no liver lesions were observed but there was evidence of slight thyroid stimulation in both sexes (2/20 males and 2/20 females). Kidney mineralisation was observed at all doses in males (1 male at the low dose, all males at the mid-dose and 21/30 males at the top dose). A LOAEL of 80 mg/l (reported to be 7.5 and 8 mg/kg bw/day in males and females, respectively) was identified based on thyroid lesions (EU, 2001).
Study Deficiencies, Questions and Additional Comments
No information is available on compliance with GLP or test guidelines. However, the EU considered this study to be valid (although the validity was stated to be restricted due to missing ophthalmology examination).
Klimisch Rating
2 (reliable with restrictions)

Reference 1.12

Reference
Griswold, D.P., Jr, Casey, A.E., Weisburger, E.K. and Weisburger, J.H. (1968) The carcinogenicity of multiple intragastric doses of aromatic and heterocyclic nitro or amino derivatives in young female Sprague-Dawley rats. *Cancer Res*, 28, 924-933. Cited in: IARC (1986). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 39: 4,4'-Methylenedianiline and its Dihydrochloride. International Agency for Research on Cancer, Lyon.
Study Design/Details
Twenty female Sprague-Dawley rats were administered MDA (as the dihydrochloride) via oral gavage every three days for 30 days, followed by an observation period of 9 months. The total dose administered was reported to be 300 mg/rat (no further details available). Additional rats were used as

negative controls (140 rats) or positive controls (40 rats; a single dose of 7,12-dimethylbenz[a]anthracene (DMBA) at a dose of 18 mg).
Study Findings
At the end of the observation period, survival was reported to be 14/20, 127/140 and 19/40 in the MDA-treated, negative control and positive control groups, respectively. Mammary lesions were observed in all groups, where incidence was reported as 5/132 in the negative control group (three carcinomas, one fibroadenoma, five hyperplasias), 29/29 in the positive control group (75 carcinomas, ten fibroadenomas, five hyperplasias) and 1/14 in the MDA-treated group (one hyperplasia).
Study Deficiencies, Questions and Additional Comments
IARC noted that the study was of limited duration, only tested a small number of animals of one sex, and was principally aimed at examining use of the mammary gland in female Sprague-Dawley rats as a tool for identifying chemical carcinogens. There was also only one MDA treatment group, and the dosing regimen is not completely clear.
Klimisch Rating
3 (unreliable)

Reference 1.13

Reference
Schoental, R. (1968) Carcinogenic and chronic effects of 4,4’-diaminodiphenylmethane, an epoxyresin hardener. *Nature*, 219, 1162-1163. Cited in: IARC (1986). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 39: 4,4'-Methylenedianiline and its Dihydrochloride. International Agency for Research on Cancer, Lyon.
Study Design/Details
Rats (8/group/sex; strain not specified) were administered 4,4’-MDA via oral gavage as four or five doses of 20 mg/rat over a period of less than eight months, and rats were observed until death.
Study Findings
After 18 months, one hepatoma and a haemangioma-like tumour of the kidney was observed in a male rat. After 24 months, an adenocarcinoma of the uterus was observed in one female. Most animals were reported to have varying degrees of liver fibrosis and inflammation.

Study Deficiencies, Questions and Additional Comments
IARC noted that this was a pilot study, which is why only a small number of animals was used and limited experimental detail was reported.
Klimisch Rating
3 (unreliable)

A2.3 Chronic Studies

Reference 1.14

Reference
NTP (1983). Carcinogenesis Studies of 4,4'-Methylenedianiline Dihydrochloride in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program, US Department of Health and Human Services. Cited in: EU (2001). European Union Risk Assessment Report for 4,4'-Methylenedianiline and: ECHA (2014) REACH Registration Dossier for Poly[(aminophenyl)methyl]aniline.
Study Design/Details
In a 2-year study conducted by the US National Toxicology Program (NTP), F344 rats (50/sex/dose/species) were administered MDA (as the dihydrochloride; 98.6% pure) orally in the drinking water at doses of 0, 150 or 300 mg/l (reported to be 0, 9 and 16 mg/kg bw in males, and 0, 10 and 19 mg/kg bw in females, respectively). The European Union stated that although a number of other carcinogenicity studies have been conducted, these other studies were not performed well or well documented, and so this was the only study that they considered in their risk assessment of MDA (EU, 2001).
Study Findings
Reduced mean bodyweights were observed in females at the high dose. Increased follicular cysts (incidence of 1/49, 2/47 and 3/47 in males, 0/47, 3/47 and 7/48 in females, in the control, low dose and high dose groups, respectively) and follicular cell hyperplasia (1/49, 2/47 and 3/47 in males, 1/47, 3/47 and 8/48 in females, in the control, low dose and high dose groups, respectively) were observed in the thyroid of both sexes at both doses. A statistically significant increase in the incidence of thyroid follicular cell carcinoma was observed in males at the high dose (7/48 cases compared to none in the control or at the low dose) and a statistically significantly increased incidence of thyroid follicular cell adenomas was observed in females at the high dose (17/48 cases compared to 0/47 in the control and 2/47 at the low dose). Non-neoplastic liver lesions, including unspecified dilatation (males), fatty metamorphosis (both sexes)

and focal cellular change (both sexes), were observed at both doses. A statistically significantly increased incidence of neoplastic nodules of the liver was observed in males at both doses (incidence of 1/50, 13/50 and 25/50 in the control, low dose and high dose groups, respectively).

An increased incidence of mineralisation of the kidney was observed in males at the high dose (incidence of 9/50, 10/50 and 19/50 in the control, low and high dose groups, respectively).

Low incidences of neoplastic lesions were also observed in other organ systems, such as the bile duct (one male, high dose), urinary bladder (females, both doses; 2/50 and 1/50 in the low and high dose groups, respectively) and ovary (females, both doses; 3/50 and 2/50 in the low and high dose groups, respectively). Study authors noted that these tumours had previously been undiagnosed or observed only at very low incidence in historical control data from the NTP Bioassay program.

A LOAEL of 150 mg/l (reported to be 9 and 10 mg/kg bw/day in male and female rats, respectively) was identified for non-carcinogenic endpoints based on non-neoplastic lesions of the liver (EU, 2001).

Study Deficiencies, Questions and Additional Comments

No information is available in the original study report regarding compliance to GLP or test guidelines. However, the study appears to have been conducted according to robust scientific principles and was considered in the EU risk assessment of MDA. In the REACH registration dossier, where this study is used as read-across, it is stated that the method is equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies), with the deviation that only two doses have been tested.

Klimisch Rating

2 (reliable with restrictions)

Reference 1.15

Reference

NTP (1983). Carcinogenesis Studies of 4,4'-Methylenedianiline Dihydrochloride in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program, US Department of Health and Human Services.

Cited in: EU (2001). European Union Risk Assessment Report for 4,4'-Methylenedianiline

and: ECHA (2014) REACH Registration Dossier for Poly[(aminophenyl)methyl]aniline.

Study Design/Details

In a 2-year study conducted by the US National Toxicology Program (NTP), B6C3F1 mice (50/sex/dose/species) were administered MDA (as the dihydrochloride; 98.6% pure) orally in the drinking water at doses of 0, 150 or 300 mg/l (reported to be 0, 25 and 57 mg/kg bw in males and 0, 19 and 43 mg/kg bw in females, respectively).

The European Union stated that although a number of other carcinogenicity studies have been conducted, these other studies were not performed well or well documented, and so this was the only

study that they considered in their risk assessment of MDA (EU, 2001).

Study Findings

At the top dose, reduced mean bodyweights (both sexes) and reduced survival (males) were observed.

Thyroid follicular cell hyperplasia was observed in males at both doses (3 and 18 cases at the low and high dose, respectively) and in females at the high dose only (23/50). Thyroid follicular cell adenoma was observed in both sexes (3/49 and 16/49 in males, and 1/47 and 13/50 in females, at the low and high doses, respectively, compared to no cases in the controls), where this increased incidence was only statistically significant at the high dose. Two cases of thyroid follicular cell carcinoma were also observed in females at the high dose, and at this dose the combined incidence of adenoma and carcinoma was also statistically significant.

Liver degeneration was observed in a high proportion of males at both doses (40/50 and 30/50 at the low and high doses, respectively) and in 7/50 females at the high dose. A statistically significant positive trend was observed for hepatocellular carcinoma in both sexes. Incidence of hepatocellular carcinoma was 10/49, 33/50 and 29/50 in males, and 1/50, 6/50 and 11/50 in females, in the control, low dose and high dose groups respectively; the increased incidence was statistically significant in males at both doses and females only at the high dose. An increased incidence of hepatocellular adenoma was observed in females (3/50, 9/50 and 12/50 cases in the control, low dose and high dose groups, respectively), where this increase was only statistically significant at the high dose.

Increased tumour incidences were also observed in several other tissues. A statistically significant positive trend was observed in adrenal phaeochromocytomas in males (2/48, 12/49 and 14/49 cases in the control, low dose and high doses groups, respectively), where this increased incidence was also statistically significant in pairwise comparisons with the controls at both doses. In females, statistically significant positive trends were observed in malignant lymphomas (13/50, 28/50 and 29/50 cases in control, low dose and high dose groups, respectively; significant at both doses) and in alveolar/bronchiolar adenomas (1/50, 2/50 and 6/49 cases in control, low dose and high dose groups, respectively; significant only at the high dose).

Non-neoplastic lesions of the kidney were observed in both sexes, where these included increased incidences of nephropathy (both doses) and renal papillary mineralisation (high dose).

A LOAEL of 150 mg/l (reported to be 25 and 19 mg/kg bw/day in male and female mice, respectively) was identified for non-carcinogenic endpoints based on non-neoplastic lesions of the liver (EU, 2001).

Study Deficiencies, Questions and Additional Comments

No information is available in the original study report regarding compliance to GLP or test guidelines. However, the study appears to have been conducted according to robust scientific principles and was considered in the EU risk assessment of MDA. In the REACH registration dossier, where this study is used as read-across, it is stated that the method is equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies), with the deviation that only two doses have been tested.

Klimisch Rating

2 (reliable with restrictions)

Reference 1.16

Reference
Deichmann, W.B., MacDonald, W.E., Coplan, M., Woods, F. and Blum, E. (1978) Di-(4-aminophenyl)-methane (MDA): 4-7 year dog feeding study. *Toxicology*, 11, 185-188. Cited in: IARC (1986). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 39: 4,4'-Methylenedianiline and its Dihydrochloride. International Agency for Research on Cancer, Lyon. and: ATSDR (1998). Toxicological Profile for Methylenedianiline. Agency for Toxic Substances and Disease Registry, Public Health Service, US Department of Health and Human Services. and: Norwegian Scientific Committee for Food Safety (2006) Risk assessment of health hazards from 4,4'-methylene dianiline (4,4'-MDA) migrated from polyamide cooking utensils.
Study Design/Details
Female beagle dogs were orally administered MDA at a dose of 70 mg of either as "highly purified" 4,4'-MDA (5 dogs) or as "crude" 4,4'-MDA (50% 4,4'-MDA, 50% higher molecular weight analogues; 4 dogs) three times per week for 4.5 – 7 years, where the test substance was dissolved in corn oil and placed in gelatinous capsules. Total doses were reported to be equivalent to 5.0-6.26 g pure 4,4'-MDA/kg bw and 4.0-6.25 g crude 4,4'-MDA /kg bw.
Study Findings
At the end of the study period, there was one survivor in the group administered pure MDA, and two survivors in the group administered crude MDA. No urinary or liver tumours were observed. One tumour of the uterine horn and one in the spleen were observed, but these were not examined microscopically. Moderate to severe gross and micropathological changes were observed in the liver, and less severe changes were observed in the kidneys and spleen.
Study Deficiencies, Questions and Additional Comments
This study has several limitations. IARC noted the small number of animals included in the study and "the limited duration of the study" (presumably when compared to total lifespan). Additionally, ATSDR noted the lack of control group, and the fact that a range of doses was not tested. There is very limited description of the study results.
Klimisch Rating
3 (unreliable)

Reference 1.17

Reference

Steinhoff, D. and Grundmann, E. (1970) Cancinogenic activity of 4,4-diaminodiphenylmethane and 2, 4-diaminodiphenylmethane. Naturwissenschaften 57, 247-248.

Unspecified study report (1969) 1969-10-31.

Cited in: ECHA (2014) REACH Registration Dossier for Poly[(aminophenyl)methyl]aniline.

Study Design/Details

Wistar rats (25/sex/group) were administered test material by subcutaneous injection at intervals of 1-3 weeks. The animals were approximately 100 days of age at the study initiation, and it was reported they were given the highest possible, yet compatible, single doses of the test substance. Study design is summarised in the table below:

Compound	Dose (mg/kg bw)	Number of days administered	Total dose administered (mg/kg bw)
Control group – saline	5 ml/kg bw	460	170 ml/kg bw
Control group - groundnut oil:ethyl alcohol (9:1)	5 ml/kg bw	410	140 ml/kg bw
4,4'-MDA, a	30-50	705	1410
2,4'-MDA, a	200-400	410	7300
3,4-Amine (fraction of 3- and 4- ring amine), a,c	400	410	11 200
8-Amine (fraction containing up to ~8-ring amine), b,c	400-700	410	15 800

a: Vehicle used was described as physiol. saline.

b: Vehicle used was groundnut oil:ethyl alcohol (9:1).

c: Poly aromatic compounds.

All animals were observed for their total lifespan and tumour incidence recorded.

Study Findings

For each group, the average lifespan of the animals was reported, along with the numbers of malignant and benign tumours. No information was available on the types of tumours identified. Results are summarised in the table below:

Compound	Average lifespan	Animals with malignant tumours (%)	Total malignant tumours	Total benign tumours
Control group – saline	1007	13/50 (26)	16	15
Control group - groundnut oil:ethyl alcohol (9:1)	935	22/50 (44)	25	29
4,4'-MDA	1015	25/50 (50)	33	29
2,4'-MDA	865	15/50 (30)	-	17
3,4-Amine (fraction of 3- and 4- ring amine)	943	27/50 (54)	-	16
8-Amine (fraction containing up to ~8-ring amine)	1013	12/50 (24)	-	18

Study Deficiencies, Questions and Additional Comments

The REACH registration dossier states that this study is not compliant with GLP and does not follow a guideline. They also comment that the data given in the study report are basic, but they nevertheless consider the study to be reliable with restrictions and it is the main study supporting the carcinogenicity assessment in the dossier. Due to the limited information reported, the reasons for the different doses and dosing schedules are not clear, and there is only limited reporting of the results.

Klimisch Rating

3 (unreliable)

Reference 1.18

Reference
Holland, J.M., Smith, L.H., Frome, E. et al. (1978) Test of Carcinogenicity in Mouse Skin: Methylenedianiline, gamma Glycidyloxytrimethyloxysilane, gamma Aminopropyltriethoxysilane and a mixture of M-phenylenediamine, methylenedianiline, and diglycidylether of Bisphenol-A. Govt Reports Announcements & Index (GRA&I), Issue 23. Cited in: ATSDR (1998). Toxicological Profile for Methylenedianiline. Agency for Toxic Substances and Disease Registry, Public Health Service, US Department of Health and Human Services.
Study Design/Details
4,4'-MDA (in ethanol) was applied to the clipped skin of male and female C3Hf/Bd mice three times per week for 24 months, at estimated doses of 0, 5.3, 10.7 or 21.3 mg/kg bw/day. Benzo[a]pyrene was used as a positive control.
Study Findings
Tumours were not observed at the site of application in MDA treated mice. However, a dose-dependent increase in incidence of hepatic tumours was observed in females (11%, 22%, 25% and 85% in the negative control and three MDA dose groups, respectively).
Study Deficiencies, Questions and Additional Comments
No statistical analysis of the results was provided and the study authors report that this strain of mice is unusually susceptible to liver tumours, and therefore the significance of the results needs further study. There was also no information available on the number of animals used. ATSDR used the lowest dose of 5.3 mg/kg bw/day as their dermal cancer effect level (CEL) in the absence of any other data.
Klimisch Rating
3 (unreliable)

Appendix B MOCA

B1 Epidemiology Studies

Reference 2.1

Reference
Hogan, T.J. (1993). Case study “carcinogens”: the MBOCA TLV example. *Am. Ind. Hyg. Assoc.*, 54, 458–460. Ward, E., Halperin, W., Thun, M., Grossman, H.B., Fink, B., Koss, L., Osorio, A.M. and Schulte, P. (1988). Bladder tumors in two young males occupationally exposed to MBOCA. *Am. J. Ind. Med.*, 14, 267–272. Ward, E., Halperin, W., Thun, M., Grossman, H.B., Fink, B., Koss, L., Osorio, A.M. and Schulte, P. (1990). Screening workers exposed to 4,4′-methylenebis(2-chloroaniline) for bladder cancer by cystoscopy. *J. Occup. Med.*, 32, 865–868.
Study Design/Details
A retrospective bladder cancer incidence study was conducted on 540 occupational workers exposed to MOCA during its manufacture in Michigan, USA from 1968 to 1979 (median duration of exposure to MOCA was 3.2 months). The authors reported a latency period of 11.5 years. 89.5% of the exposed individuals were males, 91.4% were white and 82.7% were under the age of 30 at the time of initial employment. Of the 540 workers 385 of the exposed workers participated in a urine screening study.
Study Findings
The percentage of urine samples with <25 µg MOCA/l increased from 77% to 86% from 1985 to 1990, while the percentage of urine samples with >5 µg MOCA/l decreased from 12% to 8% in the same time frame. Cytology examinations of 200 urine samples identified three men with bladder tumours. It was anticipated that workers were heavily exposed to MOCA as the highest MOCA urine concentration detected was 50 mg/l even after several months of MOCA production was arrested. One man was identified with a papillary tumour, while the other two men were reported to have low-grade papillary transitional cell tumours. Two of the men were less than 30 years old. The time between initial exposure to MOCA and diagnosis was 11.5 years. The study concluded that bladder carcinomas in young men was uncommon and therefore the results suggest a possible association of exposure to MOCA increasing the risk of bladder tumours in humans.

Study Deficiencies, Questions and Additional Comments
There were several limitations to the study including no available controls, to be able compare the bladder tumour incidences in an unexposed population. In addition no statistical evidence was provided to relate exposure of MOCA to increased risk of bladder tumours, consequently the statistical significance of three reported bladder tumours could not be evaluated. The duration and daily concentrations of MOCA exposure in workers was not reported. Additionally, the route of exposure was not identified, although it is anticipated that inhalation and dermal exposure are the most likely routes. The authors reported that the population was not “ideal”, due to a high turnover in the plant (median duration of exposure to MOCA was only 3.2 months). By the end of the study, 80% of the occupational workers were no longer working at the plant and 40% of the study population were no longer living in the local area. It was also reported that the latency period for bladder cancers is 20 years and consequently the period of the study (11.5 years) was too short. There is also the possibility that the workers were exposed to other chemicals such as 4,4’-methylenedianiline, 4-chloro-*ortho*-toluidine, aniline and *ortho*-toluidine, as well as MOCA. This generates uncertainty as to the carcinogenicity of MOCA because of these additional confounding factors.

Reference 2.2

Reference
Chen, H.I., Liou, S.H., Loh, C.H., Uang, S.N., Yu, Y.C. and Shih, T.S. (2005). Bladder cancer screening and monitoring of 4,4’-methylenebis(2-chloroaniline) exposure among workers in Taiwan. *Urology*, 66, 305–310.
Study Design/Details
In Taiwan, China, a cohort study of male and female workers at four MOCA manufacturing factories were assessed for bladder cancer. 76 workers from the four plants, which were either directly involved in the reaction, neutralization, washing, purification, packing and laboratory research and development of MOCA were included in the study. 70 of the 76 workers were assessed in parallel with 92 workers who were not directly involved with the production of MOCA. The manufacture of MOCA was a closed system, however the authors reported leakage of products from pipes and tanks. Questionnaires were provided to the exposed and control group about occupational history and habits, such as smoking.

Study Findings
The air concentration of MOCA was measured in various different sections of the plants. The greatest air concentration of MOCA was detected in the purification area; the concentration detected ranged between 0.23 to 0.41 mg/m^3. The MOCA air concentrations were also reported in the reaction, neutralisation, washing and packing sections, these concentrations ranged from <0.018-0.025, <0.05-0.06, <0.02-0.08 and <0.026-0.03 mg/m^3, respectively. The exposure duration of the majority of MOCA-exposed workers was less than 10 years. There was no significant difference in the prevalence of abnormal urinary cells between the exposed workers and the control workers, however there was a significant difference in the incidence of positive urine occult blood in male exposed workers (17%) compared to male control workers (7%). From the 70 workers one individual was reported with suspected malignant cells from the urine sample, and another individual with atypical cytology and haematuria. One other worker was diagnosed with bladder cancer. The bladder cancer was identified as a grade 3/3 invasive transitional cell carcinoma. The exposed worker with bladder cancer was identified as working in the MOCA purification process for 14 years (1987–2001). Concentrations of 0.23–0.41 mg/m^3 were reported in the air of the purification area. No personal protective equipment was worn by the individual. The authors reported the worker with the bladder cancer was a non-smoker and the mostly likely routes of MOCA exposure were dermal and inhalation.
Study Deficiencies, Questions and Additional Comments
Any potential confounding factors such as exposure to other chemicals were considered by the authors. Before the worker was involved in the manufacture of MOCA, there was the potential of exposure to pesticides while doing agricultural work.

Reference 2.3

Reference
Mason, T.J. and Vogler, W.J. (1990). Bladder cancer screening at the Dupont Chambers Works: a new initiative. *J. Occup. Med.*, 32, 874–877. Mason, T.J., Walsh, W.P., Lee, K. and Vogler, W. (1992). New opportunities for screening and early detection of bladder cancer. *J. Cell. Biochem. Supp.*, 16I (S16I), 13–22.
Study Design/Details
1723 employees of a manufacturer of MOCA, in New Jersey, USA were assessed in a 3-year case-control screening study between 1954 and 1982. Quarterly microscopic examinations of urine were conducted to detect red blood cells. The urine was tested in a home self-test for microhaematuria using the Ames HemastixR, for 14 consecutive days every other quarter. On the alternating quarters, cytology assessments (using the Papanicolaou test) were performed. Individuals who tested positive (haematuria and/or abnormal cytology) had a

complete urologic assessment. Additionally, age-matched controls were recruited from the local community.
Study Findings
From the first 7 screening assessments, two new cases and one recurrence of transitional cell carcinomas of the bladder were reported. No additional information on the analysis of the study was located and therefore a statistical evaluation was not conducted.
Study Deficiencies, Questions and Additional Comments
In addition to the production of MOCA, the manufacturer was also a major producer of two known human bladder carcinogens, beta-naphthylamine and benzidine. Consequently, there is the potential for workers to be exposed to a mixture of carcinogenic chemicals, which is therefore a confounding factor.

Reference 2.4

Reference
Dost, A., Straughan, J.K. and Sorahan, T. (2009). Cancer incidence and exposure to 4,4′-methylene-bis-*ortho*-chloroaniline (MbOCA). *Occup. Med. (Lond.)*, 59, 402–405.
Study Design/Details
A cohort study was conducted reviewing seven polymer-manufacturing companies, in the UK, which uses MOCA in the manufacturing process. The workers were mainly involved in the production of polyurethane elastomers polymers. Work histories, urine samples and smoking statuses for 308 male maintenance workers were reported during 1973–2000. Office workers were excluded from the study. The major route of exposure to MOCA was reported to be dermal skin absorption, although exposure via oral and inhalation were possible. All individuals involved in the study were employed for a minimum of 12 months and routine urine cytology tests were carried out on individuals in five of the seven polymer manufacturers. The observed and expected numbers of deaths and cancer registrations were reported in the study.
Study Findings
The authors calculated standardised mortality ratios (SMRs) and standardised registration ratios (SRRs) for cancer morbidities. There were two cases of non-significant excess of a malignant bladder cancer, one death (Obs 1, SMR 560; 14-3122 95% CI) and two cancer registrations (Obs 2, SRR 328; 40-1184 95% CI). However the two reported bladder cancer registrations were employed at manufacturers that were not participating in routine urine cytology. One of the reported bladder cancer registrations was employed for 10 years and the cancer was diagnosed 23 years after first employment; however, no information was available on smoking status.

The second reported bladder cancer registration was employed for 6 years and the cancer was diagnosed 12 years after first employment. The individual was a former smoker, which is a potential confounding factor. Overall, the cancer incidence and mortality was below average. There was a total of four mortalities with cancer, these included one incidence of lung and bladder cancer and two incidences of other non-reported neoplasms. No cancer registrations were reported for stomach and kidney cancer.
Study Deficiencies, Questions and Additional Comments
The study was well conducted but is in the early stages of follow-up. No further details of further follow-up data were reported.

B2 Experimental Animal Studies

B2.1 Initiation-promotion Studies

Reference 2.5

Reference
Nesnow, S., Triplett, L.L. and Slaga, T.J. (1985). Studies on the tumor initiating, tumor promoting, and tumor co-initiating properties of respiratory carcinogens. *Carcinog*. *Compr*. *Surv*., 8, 257–277.
Study Design/Details
SENCAR mice (80/sex/dose), 7–9 weeks old, were administered a single dermal application of 0, 0.1, 1, 10, 100 or 200 mg MOCA (purity not specified) to the dorsal area of the body. MOCA was evaluated as a tumour initiator. Following a one week interval, 2 µg of the promoter 12-*O*-tetradecanobylphorbol-13-acetate (TPA) was applied twice a week for 26 weeks.
Study Findings
There was no significant incidence of mice skin papillomas.
Study Deficiencies, Questions and Additional Comments
The study was well conducted with large numbers of mice and doses used to assess MOCA as a tumour initiator. However, the only information available is from a secondary source.
Klimisch Rating
4 (not assignable)

Reference 2.6

Reference
Rozinova, E., Khalil, M. and Bonin, A.M. (1998). MOCA and some proposed substitutes (Cyanacure, Conacure, Polacure 740M and Ethacure 300) as two-stage skin carcinogens in HRA/Skh hairless mice. *Mutat*. *Res*., 398, 111–121.
Study Design/Details
Female hairless albino HRA/Skh mice (20 mice), approximately six weeks old, were administered a single dermal application of 0, 12.5, 25, 50 or 100 mg MOCA (purity ≥ 90–100%). MOCA was assessed as a tumour initiator. Following a one week interval, 5 µg of 12-*O*-tetradecanonylphorbol-13-acetate (TPA) was applied twice a week for 21 weeks as a promoter and the mice were observed for one year.
Study Findings
The minimum diameter of reported papillomas were approximately 1 mm, however no statistically significant difference of tumour incidence was observed between control and treated mice.
Study Deficiencies, Questions and Additional Comments
In the study, only one gender of mice was used to assess MOCA as a tumour initiator, rather than evaluating both male and female mice. However, no significant differences in tumour incidences were reported between control and treated females.
Klimisch Rating
2 (reliable with restrictions)

Reference 2.7

Reference
Rozinova, E., Khalil, M. and Bonin, A.M. (1998). MOCA and some proposed substitutes (Cyanacure, Conacure, Polacure 740M and Ethacure 300) as two-stage skin carcinogens in HRA/Skh hairless mice. *Mutat*. *Res*., 398, 111–121.
Study Design/Details
Female hairless albino HRA/Skh mice (20 mice), approximately six weeks old, were administered a single dermal application of 2.56 µg 7,12-dimethylbenz[*a*]anthracene (DMBA). A negative control group were administered a single application of DMBA. Following a one week interval, dermal applications of 2.5 or 5 mg of MOCA (purity ≥ 90–100%) were

applied twice a week for twenty weeks and were observed for one year. The study was to assess MOCA as a tumour promoter.

Study Findings

The minimum diameter of reported papillomas were approximately 1 mm.

In the low dose MOCA-promoted group, the first tumour occurrence was observed at week 9 and the maximum tumour yield was 0.65 ± 0.17 tumours/mouse.

In the top dose MOCA-promoted group, the first tumour occurrence was observed at week 11 and the maximum tumour yields was 0.30 ± 0.14 tumours/mouse.

The first tumour occurrence in the control group was reported at week 19 and the maximum tumour yield was 0.55 ± 0.21 tumours/mouse.

Study Deficiencies, Questions and Additional Comments

In the study, only one gender of mice was used to assess MOCA as a tumour promotor rather than assessing both genders. No significant differences between tumour incidences; however, promotional activity was observed in the low dose group.

Klimisch Rating

2 (reliable with restrictions)

B2.2 Chronic Studies

Reference 2.8

Reference

Russfield, A.B., Homburger, F., Boger, E., Van Dongen, C.G., Weisburger, E.K. and Weisburger, J.K. (1975). The carcinogenic effect of 4,4-methylene-bis-(2-chloroaniline) in mice and rats. *Toxicol. Appl. Pharmacol.*, 31, 47–54.

Study Design/Details

Groups of HaM/ICR mice 6–8 weeks of age (25/sex/dose) were administered diets containing 0, 130 or 260 mg/kg bw/day MOCA hydrochloride salt (purity 97%) for 18 months.

The doses were chosen based on preliminary tests and the highest dose was the maximum tolerated dose in the mice.

Following 18 months of treatment the mice were observed for a further 6 months on a control diet.

Study Findings
At the end of the study the remaining mice for evaluation were 18, 13 and 20 male mice, and 20, 21 and 14 female mice in the control, low- and high-dose groups, respectively. Combined haemangiomas or haemangiosarcomas (mainly subcutaneous) occurred in 0/18, 3/13 (23%) and 8/20 (40%) of male mice, and 1/20 (5%), 0/21 and 6/14 (43%) of female mice in the control, low- and high-dose groups, respectively. A significant increase in the incidence of hepatomas was observed in both dose groups of treated females. No significant increase in hepatomas in male mice was reported. Hepatomas occurred in 3/18 (17%), 3/13 (23%), 4/20 (20%) of male mice and 0/20, 9/21 (43%) (P < 0.01, Fisher exact test), 7/14 (50%) (P < 0.01, Fisher exact test) of female mice in the control, low-dose, and high-dose groups, respectively. The authors reported MOCA having a potential gender effect, causing a significant increase in hepatomas in female mice.
Study Deficiencies, Questions and Additional Comments
The study was fairly well conducted; however, there are limitations. Only 25 mice/dose/sex were used in the study but to have a thorough statistical analysis and to decrease the uncertainty of the results, at least 50 mice/dose/sex and at least three dose groups and a concurrent control group are required.
Klimisch Rating
3 (not reliable)

Reference 2.9

Reference
Grundmann, E. and Steinhoff, D. (1970). Liver and lung tumors following 3,3′-dichloro-4,4′-diaminodiphenylmethane in rats. *Z. Krebsforsch*, 74, 28–39.
Study Design/Details
Wistar rats (25/sex/dose), 100 days of age, were administered 0 or 54 mg/kg bw/day MOCA (purity unspecified) in a protein-deficient diet for 500 days. Following treatment, rats were fed protein-deficient diet for an observation period. A group of 50 control rats were used in the study.
Study Findings
There was a decrease in survival rate of treated rats. Mean survival of treated males and females were 565 and 535 days, respectively, while mean survival of male and female controls on the protein-deficient diet was 730 days. 23 of the 25 treated males died with tumours. Hepatomas were identified in 22/25 (88%) (P < 0.001, Fisher exact test), and lung tumours (primarily carcinomas) in 8/25 (32%) (P = 0.002, Fisher exact test). 20 of the 25 treated females died with tumours. Hepatomas were reported in 18/25 (72%) (P < 0.001

Fisher exact test), and lung tumours occurred in 5/25 (20%) (P = 0.025, Fisher exact test). No hepatomas or lung tumours were reported in the controls.
Study Deficiencies, Questions and Additional Comments
Only 25 rats/dose/sex were used in the study but to have a thorough statistical evaluation and to decrease the uncertainty of the results, at least 50 rats/dose/sex, at least three dose groups and a concurrent control group are required to determine a potential dose-related response following exposure to MOCA. Additionally, very few rats survived the study, which suggests that 54 mg/kg bw/day was too high a dose.
Klimisch Rating
3 (not reliable)

Reference 2.10

Reference
Russfield, A.B., Homburger, F., Boger, E., Van Dongen, C.G., Weisburger, E.K. and Weisburger, J.K. (1975). The carcinogenic effect of 4,4-methylene-bis-(2-chloroaniline) in mice and rats. *Toxicol*. *Appl*. *Pharmacol*., **31** (1), p47–54.
Study Design/Details
Male Charles River CD-1 rats (25/dose), 6–8 weeks of age, were administered standard protein diets containing 0, 25 or 50 mg/kg bw/day MOCA hydrochloride salt (purity 97%) for 18 months. Preliminary tests were carried out to determine the doses of the study; the highest dose was the maximum tolerated dose. Surviving rats were sacrificed 6 months following 18 months of MOCA treatment.
Study Findings
Approximately 55% of the control and treated animals had survived at 20–22 months. The survival numbers were 22, 22 and 19 in the control, low- and high-dose group, respectively. Hepatomas were reported in 0/22, 1/22 (5%) and 4/19 (21%) rats in the control, low- and high-dose group, respectively (P < 0.05, Cochran-Armitage trend test). The results from the treated rats were not statistically significant from the control group. 3/22 and 4/19 rats in the low- and high-dose treated groups, respectively, developed adenomatosis (pre-neoplastic lesion).

Study Deficiencies, Questions and Additional Comments
50/sex/dose and at least three doses are required to be able to report reliable statistical evaluations and associations between exposure to MOCA and cancer. In this study there are insufficient number of doses and rats per dose.
Klimisch Rating
3 (not reliable)

Reference 2.11

Reference
Stula, E.F., Sherman, H., Zapp, J.A. and Clayton, J.W. (1975). Experimental neoplasia in rats from oral administration of 3,3′-dichlorobenzidine, 4,4′-methylene-bis-bis(2-chloroaniline), and 4,4′-methylene-bis(2-methylaniline). *Toxicol. Appl. Pharmacol.*, 31, 159–176.
Study Design/Details
In a 2-year study, Charles River CD rats (50/sex/dose), 38 days of age, were administered 0 or 50 mg/kg bw/day MOCA (purity approximately 95%) in a standard-protein diet (23% protein). Six rats from each dose group were sacrificed after one year for an interim evaluation.
Study Findings
21/44 (48%) ($P < 0.05$, $\chi 2$-test) and 27/44 (61%) ($P < 0.05$, $\chi 2$-test) lung adenocarcinomas were reported in treated males and females, respectively. Squamous-cell carcinoma of the lung was reported in one treated male and female. No lung tumour was observed among control animals. Lung adenomatosis (pre-neoplastic lesion) was observed in 1/44 (2%) and 14/44 (32%) ($P < 0.05$, $\chi 2$-test) control and treated males, respectively. Lung adenomatosis was identified in 1/44 (2%) and 11/44 (25%) ($P < 0.05$, $\chi 2$-test) control and treated females ($P < 0.05$, $\chi 2$-test). Pleural mesotheliomas were identified in 4/44 (9%) and 2/44 (5%) treated males and females. Hepatocellular adenomas and carcinomas occurred in 3/44 (7%) and 3/44 (7%) treated males, respectively and 2/44 (5%) and 3/44 (7%) treated females, respectively. Pleural mesotheliomas and hepatocellular adenomas and carcinomas tumours were not observed in the control group. A lower incidence of pituitary tumours was identified in MOCA-treated females (1/44 (2%) compared to controls (12/44 (27%)).
Study Deficiencies, Questions and Additional Comments
Only one dose was used in the study and therefore a dose-response relationship cannot be determined.

Klimisch Rating
3 (not reliable)

Reference 2.12

Reference
Stula, E.F., Sherman, H., Zapp, J.A. and Clayton, J.W. (1975). Experimental neoplasia in rats from oral administration of 3,3′-dichlorobenzidine, 4,4′-methylene-bis-bis(2-chloroaniline), and 4,4′-methylene-bis(2-methylaniline). *Toxicol. Appl. Pharmacol.*, 31, 159–176.
Study Design/Details
Charles River CD rats (25/sex/dose), 36 days of age, were administered 0 or 50 mg/kg bw/day MOCA (purity approximately 95%) in a low-protein diet (7%) for 16 months. Six rats from each dose group were sacrificed after one year of treatment for an interim evaluation.
Study Findings
Lung adenocarcinomas were observed in 5/21 (24%) ($P < 0.05$, $\chi 2$-test) and 6/21 (29%) ($P < 0.05$, $\chi 2$-test) in treated males and females, respectively ($P < 0.05$, $\chi 2$-test). No lung adenocarcinomas were identified in the 21 control rats. Lung adenomatosis (pre-neoplastic lesion) were observed in 8/21 (38%) ($P < 0.05$, $\chi 2$-test) and 14/21 (67%) ($P < 0.05$, $\chi 2$-test) in treated male and female rats, respectively. One male and one female control rat were identified ($P < 0.05$, $\chi 2$-test). Hepatocellular adenomas and carcinomas were reported in 5/21 (24%) ($P < 0.05$, $\chi 2$-test) and 11/21 (52%) ($P < 0.05$, $\chi 2$-test) in exposed males and 2/21 (10%) and 1/21 (5%) exposed female rats, respectively. Hepatocellular tumours were not reported in the control group. Mammary gland adenocarcinomas were observed in 6/21 (29%) ($P < 0.05$, $\chi 2$-test) treated and in 0/21 control females. The results of Stula *et al*. 1975 low-protein diet study identified a reduction in lung tumours in exposed animals compared with the standard-protein diet study. Lung adenocarcinomas were observed in 24% and 48% treated male rats in the low- and standard-protein diet, respectively. Lung adenocarcinomas were observed in 29% and 61% treated female rats in the low- and standard-protein diet, respectively.
Study Deficiencies, Questions and Additional Comments
There are insufficient number of animals/dose and number of doses in the study to reliably evaluate the statistical significance of MOCA exposure causing cancer and its potential dose-response.
Klimisch Rating
3 (not reliable)

Reference 2.13

Reference
Kommineni, C., Groth, D.H., Frockt, I.J., Voelker, RW. and Stanovick, R.P. (1979). Determination of the tumorigenic potential of methylene-bis-*ortho*-chloroaniline. *J*. *Environ*. *Pathol*. *Toxico*., 2, 149–171.
Study Design/Details
In an 18-month study, male Charles River CD rats, 35 days of age, were administered either a protein-adequate (27%) diet (Group A) containing 0, 25, 50 or 100 mg/kg bw/day MOCA (industrial grade) or a protein-deficient (8%) diet (Group B) containing 0, 12.5, 25 and 50 mg/kg bw/day MOCA in groups of 100, 100, 75 and 50, respectively. Following MOCA exposure, the treated rats in both groups were maintained on their diets without MOCA for 6 month and an additional 32-week observation period.
Study Findings
Group A The number of male rats surviving after 104 weeks were 20/100, 14/100, 10/75 and 0/50 in the control, low-, mid- and high-dose groups, respectively. At 84 weeks 6/50 rats were alive in the high-dose group. Lung adenocarcinomas reported in male rats were 0/100, 14/100 (P < 0.001, two-tailed test), 20/75 (P < 0.001, two-tailed test) and 31/50 (P < 0.001, two-tailed test) in the control, low-, mid- and high-dose groups, respectively. All types of lung tumours in the rats were reported as 1/100, 23/100 (P < 0.001, two-tailed test), 28/75 (P < 0.001, two-tailed test) and 35/50 (P < 0.001, two-tailed test) in the control, low-, mid- and high-dose groups, respectively. The number of mammary gland adenocarcinomas were 1/100, 5/100, 8/75 (P < 0.01, two-tailed test), 14/50 (P < 0.001, two-tailed test) in the control, low-, mid- and high-dose groups, respectively. Zymbal gland carcinomas were identified as 1/100, 8/100 (P < 0.05, two-tailed test), 5/75 and 11/50 (P < 0.01, two-tailed test) in the control, low-, mid- and high-dose groups, respectively. The number of hepatocellular carcinomas reported in the control, low-, mid- and high-dose groups were 0/100, 3/100, 3/75 and 18/50 (P < 0.001, two-tailed test) No significant increases of haemangiosarcomas were reported in treated rats. *Group B* The number of male rats surviving after 104 weeks were 34/100, 22/100, 14/75 and 5/50 in the control, low-, mid- and high-dose groups, respectively. The number of all types of lung tumours were reported as 0/100, 6/100 (P < 0.01, two-tailed test), 11/75 (P < 0.001, two-tailed test) and 13/50 (P < 0.001, two-tailed test) in the control, low-, mid- and high-dose groups, respectively. Of these lung tumours 0/100, 3/100, 7/75 and 8/50 were reported as lung adenocarcinomas in the control, low-, mid- and high-dose groups, respectively. The number of identified mammary gland adenocarcinomas were 0/100, 1/100, 3/75, 3/50 (P < 0.05,

two-tailed test) in the control, low-, mid- and high-dose groups, respectively.

Zymbal gland carcinomas reported in the control, low-, mid- and high-dose groups were identified as 0/100, 0/100, 4/75 ($P < 0.05$, two-tailed test) and 6/50 ($P < 0.001$, two-tailed test), respectively.

Hepatocellular carcinomas observed in the rats were reported as 0/100, 0/100, 0/75 and 9/50 ($P < 0.001$, two-tailed test), in the control, low-, mid- and high-dose groups, respectively.

A significant increase in haemangiosarcomas were reported in the high dose group. 4/50 rats ($P < 0.005$, two-tailed test) were identified in the highest dose with haemangiosarcomas.

A dose-related increase in the incidences of lung tumours, mammary adenocarcinomas, Zymbal gland carcinomas and hepatocellular carcinomas were reported in both the protein-adequate diet (Group A) and the protein-deficient diet (Group B). The highest tumour incidence was identified in the lung of treated animals.

In the low-dose group, tumour incidence was decreased in rats fed the protein-deficient diet (Group B), compared with protein-adequate diet (Group A). The only exception is the incidences of haemangiosarcomas were the same in both Group A and B.

In the mid-dose group, the incidences of lung tumours and mammary gland adenocarcinomas were lower in the protein-deficient diet group (Group B), compared with the protein-adequate diet group (Group A), suggesting the diet affects the tumour incidence. However, the incidences of Zymbal gland carcinomas, hepatocellular carcinomas and haemangiosarcomas were greater in Group B compared with Group A.

Study Deficiencies, Questions and Additional Comments

The study identifies that a very large number of the treated rats did not survive until the end of the study, suggesting that the doses were too high. There was also a high number of controls that did not survive for 104 weeks.

Klimisch Rating

2 (reliable with restrictions)

Reference 2.14

Reference

Stula, E.F., Barnes, J.R., Sherman, H., Reinhardt, C.F. and Zapp, J.A. Jr. (1978). Urinary bladder tumors in dogs from 4,4'-methylene-bis (2-chloroaniline) (MOCA). *J. Environ. Pathol. Toxico.*, 1, 31–50.

Study Design/Details

Female Beagle dogs (6/dose) (approximately one-year-old), were administered a daily oral dose of 100 mg MOCA (approximately 90% purity) in a gelatine capsule for 3 days/week for 6 weeks, then 5 days/week for 9 years. On average a dose of 10 mg/kg bw/day was administered to the dogs. An untreated control group of 6 female dogs was also used.

Study Findings
One of the treated dogs died at 3.4 years of age due to pyelonephritis infection (unrelated to MOCA exposure), while the other five dogs were sacrificed between 8.3 and nine years. Urinary bladder transitional cell carcinomas were reported in 4/5 (80%) of the treated female dogs. A transitional cell adenocarcinoma tumour of the urethra also developed in one of the treated dogs. No tumours were reported in the untreated control dogs ($P < 0.025$, Fisher exact test).
Study Deficiencies, Questions and Additional Comments
The study, which covers a 9 year exposure period suggests a potential association with MOCA exposure and urinary carcinomas, however, there are limited number of dogs used in only one dose group.
Klimisch Rating
3 (not reliable)

Reference 2.15

Reference
Steinhoff, D. and Grundmann, E. (1969). Carcinogenic effect of 3,3′-dichloro-4,4′-diaminodiphenylmethane in rats. *Naturwissenschaften*, 56, 215-216.
Study Design/Details
Wistar rats (17/sex/dose) were subcutaneously injected with 500 or 1000 mg/kg bw MOCA (94% purity) as a suspension in saline solution either once a week or at longer time intervals for 88 weeks. There was an additional observation period for 23 weeks. The rats were fed a normal laboratory protein content diet and an untreated control group of 25/sex were also used and observed for 148 weeks.
Study Findings
9/34 (26%) ($P < 0.0042$, Fisher exact test) hepatocellular carcinomas and 7/34 (20%) ($P < 0.016$, Fisher exact test) malignant lung tumours (six adenocarcinomas and one carcinoma) were observed in the experimental animals of the top dose group. A malignant lung tumour was found in one rat in the low dose group. 13/50 control animals developed malignant tumours, including one lung tumour. No hepatocellular carcinomas were observed, however no additional information on the types of tumours were reported. No NOAEL was reported from the study.
Study Deficiencies, Questions and Additional Comments

The study has a low number of test doses and experimental animals/dose, therefore increasing the uncertainty of the statistical analysis of the results. There is also very limited data on the carcinogenic effects observed in each sex and the rats were exposed to MOCA by subcutaneous injection, which is not a relevant exposure route for humans.
Klimisch Rating
3 (not reliable)

Appendix C 1,2-Dichloroethane

C1 Epidemiology studies

Reference 3.1

Reference
Benson, L.O. and Teta, M.J. (1993) Mortality due to pancreatic lymphopoietic cancers in chlorohydrin production workers. British Journal of Industrial Medicine, 50, 710-716
Study Design/Details
278 Men assigned to the chlorohydrin unit of Union Carbide's South Charleston plant in West Virginia were followed up for mortality between 1940 and 1988. The chlorohydrin unit primarily produced ethylene chlorohydrin between 1925 and 1967 (unit was shut down in December 1967) and produced ethylene dichloride and bis-chloroethyl ether as by-products. The 278 males all worked on the chlorohydrin unit between 1st January 1940 and 31st December 1967 for varying lengths of time. Vital status (1979-1988) was obtained from company records and the US National Death Index. Work history data were not updated beyond 1978. Standard Mortality Ratio (SMR) analyses were conducted with general United States population mortality through 1988 to calculate expected deaths. Person-years were accrued from the first date of assignment or 1st January 1940 if assignment was earlier than 1940. Person-years were accumulated into 5 year intervals beginning at age 15 and 5 year calendar intervals beginning in 1940. Internal comparison groups were also conducted for comparison: 29 965 workers who never worked in the chlorohydrin unit or any other unit producing ethylene oxide before 1978; and 1986 workers who worked with ethylene oxide but never worked in the chlorohydrin unit. Linear trends in relative risk (RR) for total malignancies, pancreatic cancer, all lymphatic and haematopoietic cancers, and leukaemia were evaluated over levels of duration of assignment to the chlorohydrin unit, stratified by age, calendar year, and interval since first assignment to the chlorohydrin unit.
Study Findings
Of the 278 workers in the cohort, 53% were deceased at the close of the study. Mean duration in the chlorohydrin unit was 5.9 years and the mean duration of follow-up was 36.5 years. A statistically significant excess of deaths due to pancreatic cancer was observed, as well as a statistically significant excess of deaths due to lymphatic or haematopoietic tissue cancers. When the cohort was limited to workers who had worked in the chlorohydrin unit for more than 2 years an excess of total malignant neoplasms was observed as well as an almost eightfold excess of pancreatic cancer. Based on comparison with the internal groups pronounced increases in total cancer, pancreatic cancer, all lymphatic and haematopoietic cancers and leukaemia with increasing durations of assignment to the chlorohydrin unit. Those who died of pancreatic cancer were all hired before 1947 with six of the eight cases hired in the 1920's and 1930's. These cases had a mean latency period of 36.8 years. The four who died from leukaemia had a shorter mean latency of 31.8 years.

Study Deficiencies, Questions and Additional Comments
The study cohort was small and the effects rare but follow-up period was long almost 50 years. The carcinogens present in the chlorohydrin unit have yet to be identified. Ethylene oxide can be excluded as the carcinogen as internal comparisons revealed no correlation between cancers and exposure to ethylene oxide. The findings are best interpreted in the context of the chemicals involved, the production of ethylene chlorohydrin, ethylene dichloromethane and bis-chloroethyl ether. There are numerous suggestions that ethylene dichloride may be significant in the occurrence of pancreatic cancer. The study concluded that the study was insufficient to confirm which exposure or combination of exposures caused the cancer excesses. The weight of evidence based on probable exposure levels, known toxicity and data from animal experiments suggests that ethylene dichloride, perhaps in combination with other chlorinated hydrocarbons, is the most likely explanation.

Reference 3.2

Reference
Isacson, P., Bean, J.A., Splinter, R., Olson, D.B. and Kohler, J. (1985) Drinking water and cancer incidence in Iowa: III. Association of cancer with indices of contamination. *American journal of epidemiology*, 121, 856–869.
Study Design/Details
An ecological study looking at the potential associations between drinking water from groundwater contaminated with particular substances (including 10 volatile organics and 43 in organic substances) and cancer. The study aimed to compare the cancer rates in small Iowa towns with the presence of contaminants in the water supply. A number of towns in Iowa were selected that had populations of between 1000 and 10 000 in 1970 and had a drinking water supply originating from one source prior to 1965. Associations between cancer incidence rates and drinking water treatment variables in towns where the sole drinking water source comes from groundwater. This is because surface and ground water supplies have very different treatment process, levels of chlorination and types and quantity of contaminants especially as surface water has a higher potential for more contamination by potential carcinogens. Towns with populations above 10 000 were not included in order to decrease possible confounding effects of occupation and other variables associated with urban density. Cancer incidence was derived from the Iowa Health Registry. Yearly population projections by sex and five-year age group were made, allowing for calculation of direct age-adjusted, sex and site specific cancer incidence rates grouped by variables of interest. Water quality was determined using finished drinking water samples collected in 1979 as part of a study into bladder cancer and artificial sweetener. Finished drinking water samples from each town in Iowa with a population >1000 were taken and analysed for Volatile Organic Compounds (VOCs), trace elements and metals, and specific VOCs such as trihalomethanes (chloroform, bromodichloromethane,

bromoform, and dibromochloromethane), trichloroethylene, tetrachloroethylene, 1,2-dichloroethane, 1,1,1,trichloroethane, carbon tetrachloride, and cis- and trans-1,2dichloroethylene. Other pertinent data such as occupation and other socio demographic parameters were obtained from the Iowa portion of the United State Census.

Study towns were grouped according to the variables of interest and direct age-adjusted sex specific incidence rates for major cancer sites were calculated. Each town or grouping was considered to be a single-population.

The municipalities were grouped by presence or absence of individual volatile organic compounds in their finished ground water supplies and the cancer incidence rates for major sites were calculated.

Study Findings

The average annual age-adjusted incidence (1969-1981) incidence of colon and rectal cancer was statistically greater (p = 0.009 and p = 0.02, respectively) in men >55 years of age whose drinking water contained >0.1 µg/l 1,2-dichloroethane compared to those with <0.1 µg/l. Rectal cancer in males was associated with chlorination of drinking water. No Significant differences between groups of towns with respect to eight socioeconomic factors, except that of the percentage change in population between 1970 and 1980 was significantly less in towns with >0.1 µg/l 1,2-dichloroethane. The authors did not suggest a causal link between rectal cancer and 1,2-dichloroethane but that incidence may be elevated in populations consuming water from wells with anthropogenic contamination.

Table 1. Average annual age adjusted cancer incidence per 100,000 in towns by presence or absence of detectable VOCs compounds in finished ground water drinking supply, Iowa, 1969-1981.

Average annual age adjusted cancer incidences (number of cases)	**1,2-dichloroethane drinking water concentration**	
Site and sex	<0.10 µg/l	≥0.10 µg l
Bladder – male	139.7 (515)	137.3 (122)
Bladder – female	32.5 (181)	27.1 (34)
Breast – female	300.2 (1,534)	273.4 (332)
Colon – male	170.3 (633)	222.8 (193)
Colon – female	181.5 (987)	171.8 (219)
Lung – males	343.1 (1,193)	346.7 (287)
Lung – females	57.9 (288)	50.4 (60)
Prostate – male	378.1 (1,463)	388.8 (360)
Rectum – male	92.9 (337)	126.5 (106)
Rectum – female	56.1 (301)	63.0 (80)

Note: Only ages 55 and over were used and the year 1972 was excluded.

Study Deficiencies, Questions and Additional Comments

Sampling and testing of finished water was over a very short period of time (April to May 1979). Samples tested may not have been representative of exposure over a complete lifetime.

The “ecologic fallacy” of the assumption that all residents in each municipality drink from the local water supply was addressed by a survey of residents. However the details of this survey were not detailed.

The number of potential cancer sites and water quality variables is vast and this was addressed by

analysing chemicals that are potential carcinogens and selecting based on evidence of human contamination. A large number of potential carcinogens were omitted from the study as they were not examined during the sampling period.

Even though mobility in Iowa is believed to be low it is thought that up to a third of the study population changed water supplies in a fifteen year period prior to clinical detection of the cancers.

Levels of 1,2-dichloroethane were very low and well below any no adverse effects levels in animals experimentation or human occupational exposures. It is thought that the data are more indicative of effects correlated to groundwater supplies contaminated by anthropogenic activities rather than effects correlated to the compounds themselves and considers them as indicator compounds for human contamination.

The role of chlorination in these water supplies has not been assessed. It is though that as they are groundwater sources there may be less chlorination treatment. There was some correlation with chlorinated supplies but chlorination is not used as a sole treatment and this varies between sites and situations. It is thought that non-treated supplies may have a wider range of potential carcinogens present. The dataset was not sufficient to draw conclusions of the effect of treatment processes and chlorination on cancer incidence.

Reference 3.3

Reference
Austin, S.G. and Schnatter, A.R.J. (1983) A case-control study of chemical exposures and brain tumors in petrochemical workers. Occup Med., 25, 313-20.
Study Design/Details
Case control study looking at exposure of 21 males at a petrochemical plant in Texas, USA whose deaths were attributed to brain cancer were compared to two control groups of 80 workers from the same plant. Group 1 consisted of males who had died of non-neoplastic causes and Group 2 consisted of males who died of all other causes. Employees were classified as having been exposed to 1,2-dichloroethane if they had ever worked in a department that uses the compound, or unexposed workers who had never worked in those departments, and the exposure of others were considered unknown.
Study Findings
When the workers with unknown exposures were excluded from the analysis there was no significant difference between the exposed and non-exposed groups for occurrence of brain cancer. When a 15-year latency period was considered there was no significant difference between the reported cases and controls.
Study Deficiencies, Questions and Additional Comments

Reference 3.4

Reference
Austin, S.G. and Schnatter, A.R.J. (1983) A cohort mortality study of petrochemical workers. J Occup Med. 25, 304-312.
Study Design/Details
A cohort study of 6588 workers at a petrochemical plant in Texas, USA looked at mortality rates compared with national rates.
Study Findings
There were no significant increases in brain tumours in the general population of the plant but there was a borderline increase in hourly employees with >6 months employment.
Study Deficiencies, Questions and Additional Comments
1,2-Dichloroethane was not specifically considered in this study and there were a number of confounding substances such as benzene, diethyl sulphate, ethylene oxide and vinyl chloride.

Reference 3.5

Reference
Deschamps, M. and Band, P. (1993) Study of a cluster of Childhood Leukaemia. Health Rep., 5, 81-85
Study Design/Details
Study designed to investigate the possible association between a spill of 1,2-dichloroethane in 1982 into a river supplying drinking water to parts of Vancouver, Canada and a cluster of 15 child leukaemia cases.
Study Findings
It was determined that none of the 15 cases diagnosed between 1975 and 1988 had lived in areas of the city serviced by the contaminated supply.
Study Deficiencies, Questions and Additional Comments

Reference 3.6

Reference
Goldberg, M.S., Al-Homsi, N. and Goulet, L. *et al.* (1995) Incidence of cancer among persons living near a municipal solid waste landfill in Montreal, Quebec. Arch Environmental Health, 50, 416-424.
Study Design/Details
Study looking at incidences of cancer in a population residing near a municipal solid waste site in Montreal, Quebec, Canada, emitting airborne 1,2-dichloroethane and other volatile substances.
Study Findings
Males residing near the site showed a significant increase in stomach cancers, liver and bile duct cancers, trachea and bronchus, and lung cancer. Females showed increased risk of stomach cancer and cervix uteri cancers.
Study Deficiencies, Questions and Additional Comments

C2 Experimental Animal Studies

C2.1 Oral studies

Reference 3.7

Reference
Klaunig, J.E., Ruch, R.J. and Periera, M.A. (1986) Carcinogenicity of chlorinated methane and ethane compounds in drinking water in mice. *Environ. Health Perspect.,* 69, 89-95
Study Design/Details
Groups of 25 $B6C3F_1$ mice from 30 days of age, were administered *N*-nitrosadiethylamine (NDEA) for four weeks. Animals were then given drinking water containing 1,2-dichloroethane at concentrations of 0, 835 or 2500 mg/l for 52 weeks. The highest concentration was selected as it was the highest concentration that failed to cause mortality in 8 week old $B6C3F_1$ mice after 4 weeks of administration. A complete autopsy was conducted and histological examination was carried out on the liver, kidney and lung.

Study Findings

There were no significant differences in either tumour incidence or number of tumours per mouse in any organ between the controls and the treated groups.

	Control, 0 mg/l	835 mg/l	2500 mg/l
Incidences of liver tumours	25/25	25/25	23/25
Incidences of liver tumours per mouse	29.30 ± 15.40	34.50 ± 17.40	25.20 ± 16.70
Incidences of lung tumours	18/25	12/25	23/25
Incidences of lung tumours per mouse	1.40 ± 1.40	1.00 ± 1.10	2.60 ± 2.00

Study Deficiencies, Questions and Additional Comments

IARC Working Group noted that the incidences of tumours were too high for evaluation of a promoting effect of 1,2-dichloroethane.

Klimisch Rating

2 (reliable with restrictions)

Reference 3.8

Reference

Milman, H.A., Story, D.L., Riccio, E.S., Sivak, A., Tu, A.S., Williams, G.M., Tong, C. and Tyson, C.A. (1988) Rat liver Foci and *in vitro* assays to detect initiating and promoting effects of chlorinated ethanes and ethylene. *Ann. N.Y. Acad. Sci.,* 534, 521-530.

Study Design/Details

In an initiation study one group of 10 male Osbourne-Mendel rats, weighing 180-230g, were given a two-thirds partial hepatectomy and 24 hours later, a single dose of 100 mg/kg bw of 1,2-dichloroethane in corn oil by gavage. Similar groups were given corn oil alone or 30 mg/kg bw *N*-nitrosodiethylamine (NDEA) as positive controls, followed by a two-thirds partial hepatectomy. Starting 6 days after the partial hepatectomy the rats received a control 500 mg/kg of diet phenobarbital for seven weeks, then a control diet of seven days before they were killed and their livers examined for histologically for γ-glutamyltranspeptidase (γ-GT) positive foci.

Study Findings
There was no significant increase in the number of total γ-GT-positive foci (1.01 ± 0.55 and 0.27 ± 0.19/cm^2 in the 1,2-dichloroethane or vehicle only control groups. NDEA increased the number of γ-GT-positive foci (4.04 ± 1.47/ cm^2).
Study Deficiencies, Questions and Additional Comments
The IARC working group noted the small number of animals.
Klimisch Rating
2 (reliable with restrictions)

Reference 3.9

Reference
Milman, H.A., Story, D.L., Riccio, E.S., Sivak, A., Tu, A.S., Williams, G.M., Tong, C. and Tyson, C.A. (1988) Rat liver Foci and *in vitro* assays to detect initiating and promoting effects of chlorinated ethanes and ethylene. *Ann. N.Y. Acad. Sci.,* 534, 521-530.
Study Design/Details
In a promotion study one group of 10 male Osbourne-Mendel rats, weighing 180-230 g were given a two-thirds partial hepatectomy and 24 hours after that they were given an interperitoneal injection of 30 mg/kg *N*-nitrosodiethylamine (NDEA). Starting 6 days later the rats were given a 100 mg/kg bw 1,2-dichloroethane via gavage in corn oil for five days per week for seven weeks. Control groups received corn oil alone. After the promotion phase they were killed and their livers were examined histologically for γ-glutamyltranspeptidase (γ-GT)-positive foci.
Study Findings
There was no significant difference in the number of γ-GT-positive foci between the 1,2-dichloroethane group and the controls (1.54 ± 0.54 and 1.62 ± 0.33/cm^3, respectively).
Study Deficiencies, Questions and Additional Comments
The IARC working group noted the small number of animals.
Klimisch Rating
2 (reliable with restrictions)

Reference 3.10

Reference
NCI (1978) National Cancer Institute (1978). Bioassay of 1,2-dichloroethane for possible carcinogenicity. NCI Tech. Rep. 55. NTIS/PB 285-968.

Study Design/Details
In a 78 week study, groups of Osbourne-Mendel rats (50/sex/dose), 9 weeks old at start of study) were administered 1,2-dichloroethane dissolved in corn oil via gavage for 5 days per week at doses of 47 or 95 mg/kg bw/day (time weighted doses). Further groups of rats (20/sex/dose), administered corn oil alone or were untreated, were considered controls. Clinical signs such as body weight, food consumption and observations on body condition, behaviour and signs of toxic effects were monitored at weekly intervals for the first 10 weeks and monthly thereafter. A necropsy was performed on all animals regardless of whether they died, were killed because they were moribund or sacrificed at the end of the experiment. Histopathological examination consisted of gross and microscopic examination of major tissues, organs, or gross lesions taken from sacrificed animals, and whenever possible from animals found dead. The number of animals for which particular organs, tissues or lesions were examined microscopically varies and does not necessarily represent the number of animals placed in each group.

Study Findings
There was a statistically significant association between increased dosage and elevated mortality. Squamous-cell carcinomas of the forestomach were observed in 3/50 males in the low dose group, 9/50 males in the high dose group and 1/49 females in the low dose group but none in the high dose females or any of the control animals. A significant dose-positive trend was identified for squamous-cell carcinomas of the forestomach in males but not females. Haemangiosarcomas occurred at a variety of body sites in all treated groups of both sexes. A significant dose-positive trend was identified for hemangiosarcomas in male rats but not females. Adenocarcinomas of the mammary gland were observed in 0/20 vehicle control, 1/50 in the low dose groups and 18/50 in the high dose group.

Sex	Effect	Exposure concentration (mg/kg bw/day)		
		0 (vehicle)	47	95
Male	Subcutaneous fibroma	0/20	5/50	6/50
	Tunica vaginalis mesothiolioma	0/20	3/50	0/50
	Haemangiosarcoma	0/20	9/50	7/50
	Stomach, squamous-cell carcinoma	0/20	3/50	9/50
	Pituitary chromophobe adenoma	2/20	1/50	4/49
Female	Pituitary chromophobe adenoma	7/20	7/50	5/49
	Thyroid follicular-cell adenoma	0/20	3/50	0/50
	Mammary gland adenocarcinoma	0/20	1/50	18/50

	Mammary gland fibroadenoma	0/20	14/50	8/50
	Mammary gland adenocarcinoma or fibroadenoma	0/20	15/50	24/50
	Haemangiosarcoma	0/20	4/50	4/50

Under the conditions of this study 1,2-dichloroethane is carcinogenic to Osbourne-Mendel rats, causing squamous-cell carcinomas of the forestomach, haemangiosarcomas and subcutaneous fibromas in male rats and causing mammary adenocarcinomas in female rats.

Study Deficiencies, Questions and Additional Comments

The data on tumour incidence do not take into account the increased early mortality in the high dose groups. The incidence of tumours may have been higher if the animals survived for a longer period of time.

Klimisch Rating

2 (reliable with restrictions)

Reference 3.11

Reference

NCI (1978) National Cancer Institute (1978). Bioassay of 1,2-dichloroethane for possible carcinogenicity. NCI Tech. Rep. 55. NTIS/PB 285-968.

Study Design/Details

In a 78 week study, groups of B6C3F1 mice (50/sex/dose, 9 weeks old at start of study) were administered 1,2-dichloroethane dissolved in corn oil via gavage for 5 days per week at doses of 97 or 195 mg/kg bw/day for males, and 149 or 299 mg/kg bw/day for females (time weighted doses). Further groups of mice (20/sex/dose) were administered corn oil alone or were untreated, were considered controls.

Clinical signs such as body weight, food consumption and observations on body condition, behaviour and signs of toxic effects were monitored at weekly intervals for the first 10 weeks and monthly thereafter. A necropsy was performed on all animals regardless of whether they died, were killed because they were moribund or sacrificed at the end of the experiment. Histopathological examination consisted of gross and microscopic examination of major tissues, organs, or gross lesions taken from sacrificed animals, and whenever possible from animals found dead.

The number of animals for which particular organs, tissues or lesions were examined microscopically varies and does not necessarily represent the number of animals placed in each group.

Study Findings

A significant increase in mortality was seen in the top dose females (low dose females had similar mortality rates to control groups). During the second year of the study mortality rates for males were higher in the low dose and untreated control compared to the high dose group. Despite this

accelerated mortality positive associations were established between 1,2-dichloroethane administration and the incidences of several neoplasms.

Adenocarcinomas of the mammary gland in female mice were observed (9/50 in low dose females, 7/48 in the high dose females and none in the controls.

Hepatocellular carcinomas were observed in 2/17 untreated males, 1/19 vehicle control males, 6/47 low dose males, 12/48 high dose males, 1/20 vehicle control females, 1/47 high dose females).This indicated a significant positive dose-response association males but not females.

The increased incidence of endometrial stromal polyps and sarcomas was not statistically significant but a significant positive association between administration of 1,2-dichloroethane and tumour incidence was demonstrated.

Alveolar/bronchiolar adenomas in mice were observed in 1/47 untreated males, 15/48 low dose males, 15/38 high dose males, 1/19 untreated females, 1/20 vehicle control females, 15/48 high dose females.

The statistical association between 1,2-dichloroethane and alveolar/bronchiolar adenomas in mice was significantly positive for both sexes.

Sex	Effect	Exposure concentration (mg/kg bw/day)		
		0 (vehicle)	47	95
Male	Subcutaneous fibroma	0/19	0/47	4/48
	Lung, bronchiolar/alveolar adenoma	0/19	1/47	15/48
	Malignant lymphoma	2/19	8/47	5/48
	Live, hepatocellular carcinoma	1/19	6/47	12/48
	Stomach squamous-cell carcinoma	1/19	1/46	2/46
Female	Lung, bronchiolar/alveolar adenoma	1/20	7/50	15/48
	Malignant lymphoma	4/20	10/50	2/48
	Stomach squamous-cell carcinoma	1/20	2/50	5/48
	Mammary gland adenocarcinoma	0/20	9/50	7/48
	Uterus, adenocarcinoma	0/20	3/49	4/47
	Endometrial stromal polyp	0/20	3/49	2/47
	Endometrial stromal sarcoma	0/20	2/49	3/47
	Endometrial stromal polyp or sarcoma	0/20	5/49	5/47

Under the conditions of this study 1,2-dichloroethane is carcinogenic to B6C3F1 rats, causing mammary adenocarcinomas and endometrial tumours in female mice and alveolar/bronchiolar adenomas in mice of both sexes.

Study Deficiencies, Questions and Additional Comments

The data on tumour incidence does not take into account the increased early mortality in the high dose groups. The incidence of tumours may have been higher if the animals survived for a longer period of time.

Klimisch Rating
2 (reliable with restrictions)

C2.2 Dermal studies

Reference 3.12

Reference
Van Duuren, B.L., Goldsmidt, B.M., Loewengart, G., Smith, A.C., Melchionne, S., Seidman, I. and Roth, D. (1979) Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. *J. Natl Cancer Inst.,* 63, 1443-1439.
Study Design/Details
A group of 30 female Ha:ICR swiss mice, six to eight weeks old received skin applications of 1,2-dichloroethane at doses of 0 (untreated), 0 (vehicle), 42 or 126 mg/animal with 0.2 ml acetone, three times a week for life (440-594 days). 100 animals were also used as a naïve control. The control animals received applications of 0.2 ml acetone. At death, a complete autopsy was carried out and histological examinations were performed on the skin, liver, stomach, kidney and all abnormal–appearing tissues and organs.
Study Findings
Increased incidences of lung tumours (benign papillomas) were observed in the highest dose animals compared to controls (26/30 126 mg/animal, 11/30 vehicle control, and 30/100 untreated controls). No skin tumours were observed.
Study Deficiencies, Questions and Additional Comments
Study was poorly reported.
Klimisch Rating
3 (not reliable)

Reference 3.13

Reference
Van Duuren, B.L., Goldsmidt, B.M., Loewengart, G., Smith, A.C., Melchionne, S., Seidman, I. and Roth, D. (1979) Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. *J. Natl Cancer Inst.* 63, 1443-1439.
Study Design/Details
In a two-stage mouse study a group of 30 female Ha:ICR Swiss mice received a single dose of 126 mg 1,2-dichloroethane/animal in 0.2 ml of acetone by direct skin application followed 14 days later by 5 µg phorbol myristyl acetate/animal in 0.2ml of acetone three times a week for life. Animals dosed with phorbol myristyl acetate alone served as controls.
Study Findings
Survival was described as 'excellent', and the median survival for the various groups in the study (which included some groups exposed to chemicals other than 1,2-dichloroethane) ranged from 429-576 days. There were no significant differences in the occurrence of skin tumours between controls and treated groups. (7 papillomas in 6/90 mice for controls and 3 papillomas in 3/30 for treated rats).
Study Deficiencies, Questions and Additional Comments
Study was poorly reported.
Klimisch Rating
3 (not reliable)

C2.3 Inhalation studies

Reference 3.14

Reference
Maltoni, C, Valgimigli, L, Scarnato, C. (1980) Long-term carcinogenic bioassays on ethylene dichloride administered by inhalation to rats and mice. In: Ames BN, Infante P, Reitz R, eds. Ethylene dichloride: a potential health risk? Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 3–33 (Banbury Report No. 5).
Study Design/Details
Swiss mice (90/sex/dose) were exposed to 1,2,dichloroethane in air at concentrations of 0, 5, 10, 50 or 250 ppm (reported to be 0, 20, 40, 200 or 1000 mg/m^3) for 7 hours a day 5 times a week for 78 weeks. A group of 115 males and 134 females were kept in an adjacent room as controls. At the end of the

treatment period animals were kept until spontaneous death. The experiment lasted 119 weeks and a complete autopsy was carried out on all animals and histological examination was carried out on almost all organs.

Study Findings

After several days of exposure to 250 ppm (reported to be 1000 mg/m^3) the concentration was reduced to 150 ppm because of severe toxic effects. Table 1 presents the survival rates of each group at 78 weeks.

Table 1. Survival rates at 78 weeks

Concentration	Male survival at 78 weeks	Female survival at 78 weeks
Control group	42/115	76/134
5 ppm	26/90	68/90
10 ppm	34/90	50/90
50 ppm	30/90	49/90
150-250 ppm	26/90	44/90

No specific types of tumour or changes in the incidence of tumours normally occurring in this strain of mice were observed in the treated animals.

Study Deficiencies, Questions and Additional Comments

Mortality was high in this study and incidence rates were not adjusted for differential mortality among groups. This is also pre-GLP.

Klimisch Rating

2 (reliable with restrictions)

Reference 3.15

Reference

Maltoni, C., Valgimigli, L., Scarnato, C. (1980) Long-term carcinogenic bioassays on ethylene dichloride administered by inhalation to rats and mice. In: Ames BN, Infante P, Reitz R, eds. Ethylene dichloride: a potential health risk? Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 3–33 (Banbury Report No. 5).

Study Design/Details

Sprague Dawley Rats (90/sex/dose) were exposed to 1,2,dichloroethane in air at concentrations of 0, 5, 10, 50 or 250 ppm (reported to be 0, 20, 40, 200 or 1000 mg/m^3) for 7 hours a day 5 times a week for 78 weeks. A group of 90 males and 90 females were kept in exposure chambers under the same condition for the same period of time and served as chamber controls, and another group of 90 males and 90 females were kept in a separate room served as untreated controls. At the end of the treatment period

animals were kept until spontaneous death. The experiment lasted 148 weeks and a complete autopsy was carried out on all animals and histological examination was carried out on almost all organs.

Study Findings

After several days of exposure to 250 ppm (reported to be 1000 mg/m^3) the concentration was reduced to 150 ppm because of severe toxic effects. Table 1 presents the survival rates of each group at 104 weeks.

Table 1. Survival rates at 104 weeks

Concentration	Male survival at 104 weeks	Female survival at 104 weeks
Control group	16/90	36/90
Chamber control	12/90	22/90
5 ppm	45/90	48/90
10 ppm	13/90	26/90
50 ppm	17/90	29/90
150-250 ppm	10/90	21/90

Table 2. presents the incidences of females with mammary fibromas and fibroadenomas. The incidence of fibromas and fibroadenomas was significant in the 5 ppm, 50 ppm and 150-250 ppm groups. The differences between the two control groups were also significant.

Table 2. incidences of fibromas and fibroadenomas in females

Concentration	Incidence
Control group	47/90
Chamber control	27/90
5 ppm	56/90
10 ppm	33/90
50 ppm	49/90
150-250 ppm	47/90

Study Deficiencies, Questions and Additional Comments

Mortality was high and variable in this study. Mortality was not related to concentration. The incidence rates were not adjusted for differential mortality between groups.

Also the differences between the two control groups were significant. This is also pre-GLP.

Klimisch Rating

2 (reliable with restrictions)

Reference 3.16

Reference
Cheever, K.L., Cholakis, J.M., El-Hawari, A.M., Kovatch, R.M., Weisburger, E.K. (1990) Ethylene dichloride: the influence of disulfiram or ethanol on oncogenicity, metabolism and DNA covalent binding in rats. Fundamental and applied toxicology, 14:243–261.
Study Design/Details
In a 2-year study, equivalent/similar to OECD Guideline 452, six groups of Sprague-Dawley Rats (50/group/sex/dose, 5.6-6 weeks old at start of study) were exposed to 1,2-dichloroethane via whole body inhalation at concentrations of 0, 50 ppm for 7 hours a day 5 days a week (except holidays). The groups were housed separately in stainless steel exposure chambers and fed on a powdered diet and distilled water or were either given either disulfiram in their diet or ethanol in their water (5% ethanol) at levels previously shown to influence the carcinogenicity of halogenated compounds. The powdered diet was removed during the exposure period and food and water consumption was monitored during the study. At the end of the 24-month period groups of five males and five females were used for the determination of either 1,2-dichloroethane elimination from the blood, metabolism and DNA covalent bonding. Dosing solutions of radiolabelled 1,2-dichloroethane were administered by gavage using corn oil as a vehicle at concentration of 75 mg/ml for the metabolism and DNA covalent binding studies. All groups were examined twice daily for signs of toxicity. The rats were examined for palpable masses prior to the initial exposure and at weekly intervals after 4 months. The rats were also weighted weekly for the first 8 weeks then monthly after that. All rats were necropsied at 24 months and subjected to complete gross examination and histopathological examination was undertaken on most tissues. Examination of blood, metabolism, urinary metabolites and DNA covalent binding. Absolute and relative organ weights, body weights, food and water consumption, control and test values for 1,2-dichloroethane blood levels, metabolism, and DNA covalent binding were evaluated by analysis of variance and Dunnett's test. Incidences of mortality and histopathological observations were evaluated by Fisher's Exact Probability Test. The level of significance chosen was $p < 0.05$.
Study Findings
NOAEL = 206 mg/m^3. The mortality rates of the various groups of male and female rats showed no significant difference compared to the controls, there was no sex related difference between groups either. Survival to 24 months was relatively high (58% and 60% in the control and treated males and 54% and 64% for the control and treated females). Exposure to disulfiram or ethanol showed no discernable effect on the appearance of the animals. Increased incidences of certain tissue lesions and masses were noted at necropsy in groups of both sexes exposed to 1,2-dichloroethane than controls. Male rats in certain groups were found to have increase liver masses mostly related to bile duct cysts (32% in the 1,2-dichloroethane groups v 8% in controls), kidney lesions including chronic nephropathy, calculi of the renal pelvis (30% in the 1,2-dichloroethane groups v 8% for controls), or testicular lesions (24% in the 1,2-dichloroethane groups v

10% for controls). Females rats showed increased incidence of liver masses mainly bile duct cysts (46% in the 1,2-dichloroethane groups v 6% for controls).

It was concluded that there was no significant increase in incidences of tumours between the control and treated groups. There were increased incidences of intrahepatic bile duct cholangiomas and intrahepatic bile duct cysts in the treated male and female groups compared to the untreated controls. There were also increased incidences of neoplastic nodules in the liver and interstitial cell tumours in the testis of the treated male group and increased incidence of mammary gland adenocarcinomas in the treat female group, when compared to controls (see Table 1).

Table 1. Results of histological examination of untreated and treated groups of rats.

	Untreated control males	Treated males	Untreated control females	Treated Females
Liver – intraheptatic bile duct cholangiomas	0/50	9/49	0/50	17/50
Liver – intrahepatic bile duct cysts	1/50	12/49	1/50	24/50
Liver – neoplastic nodules	0/50	6/49	-	-
Mammary gland – adenocarcinomas	-	-	4/50	12/48
Testis – interstitial-cell tumours	2/50	11/50	-	-

Study Deficiencies, Questions and Additional Comments

IARC working group noted a low exposure level. There is only 1 dosed group at a low exposure level. This is also pre-GLP.

Klimisch Rating

2 (reliable with restrictions)

Reference 3.17

Reference

Nagano, K., Nishizawa, T., Yamamoto, S. and Matsushima, T. (1998) Inhalation carcinogenesis studies of six halogenated hydrocarbons in rats and mice. In: Chiyotani K., Hosoda Y. and Aizawa Y., eds, *Advances in the Prevention of Occupational Respiratory Diseases*, Amsterdam, Elvesier, pp. 741-746.

Study Design/Details

BDF_1 mice (50/sex/dose), from six weeks of age were exposed by whole body inhalation to 1,2-dichloroethane at concentrations of 0, 10, 30, and 90 ppm (reported to be 0, 40, 120 and 360 mg/m^3) for six hours per day, five days a week for 104 weeks. The top dose was selected on the basis of a 13-week range finding study.

Study Findings

In males, significant increased incidence of liver haemangiosarcomas was observed the mid and high doses. In females, increased incidence of bronchiolar-alveolar adenomas and carcinomas, hepatocellular adenomas, adenocarcinomas of the mammary gland and endometrial stromal polyps, with a significantly positive trend (See Table 1.)

Table 1. Tumour incidence in mice administered 1,2-dichloroethane by inhalation exposure

Mice	Effect	Exposure concentration (ppm)			
		0	10	30	90
Males	Liver haemangiosarcoma	0-50	4/49	6/50	5/50
Females	Hepatocellular adenoma	1/49	1/50	1/50	6/50
	Bronchiolar-alveolar adenoma carcinoma	5/49	1/50	4/50	11/50
	Mammary gland adenocarcinoma	1/49	2/50	1/50	6/50
	Endometrial stromal polyp	2/49	0/50	1/50	6/50

Study Deficiencies, Questions and Additional Comments

Klimisch Rating

2 (reliable with restriction)

Reference 3.18

Reference

Nagano, K., Nishizawa, T., Yamamoto, S. and Matsushima, T. (1998) Inhalation carcinogenesis studies of six halogenated hydrocarbons in rats and mice. In: Chiyotani K., Hosoda Y. and Aizawa Y., eds, *Advances in the Prevention of Occupational Respiratory Diseases*, Amsterdam, Elvesier, pp. 741-746.

Study Design/Details

Sprague-Dawley rats (50/sex/dose), from six weeks of age were exposed by whole body inhalation to 1,2-dichloroethane at concentrations of 0, 10, 40, or 160 ppm (reported to be 0, 40, 160 and 640 mg/m^3) for six hours per day, five days a week for 104 weeks. The top dose was selected on the basis of a 13-week range finding study.

Study Findings

In males, significant increased incidence of liver haemangiosarcomas was observed the mid and high doses. In males, increased incidence of fibromas of the subcutis, fibroadenomas of the mammary gland and mesotheliomas of the peritoneum occurred with a significant positive trend. In females, increased incidences of fibromas of the subcutis and fibroadenomas, adenomas and adenocarcinomas of the

mammary gland occurred (See Table 1.)

Table 1. Tumour incidence in rats administered 1,2-dichloroethane by inhalation exposure

Mice	Effect	Exposure concentration (ppm)			
		0	10	40	160
Males	Mammary fibroadenoma	0/50	0/50	1/50	5/50
	Subcutaneous fibroma	6/50	9/50	12/50	15/50
	Peritoneal mesothelioma	1/50	1/50	1/50	5/50
Females	Mammary adenoma	3/50	5/50	5/50	11/50
	Mammary fibroadenoma	4/50	1/50	6/50	13/50
	Mammary adenocarcinoma	1/50	2/50	0/50	5/50
	Subcutaneous fibroma	0/50	0/50	1/50	5/50

Study Deficiencies, Questions and Additional Comments

Klimisch Rating

2 (reliable with restriction)

Reference 3.19

Reference

Nagano, K., Umeda, Y., Senoh, H., Gotoh, K., Arito, H., Yamamoto, S. and Matsuhima, T. (2006) Carcinogenicity and Chronic Toxicity in Rats and Mice exposed by Inhalation to 1,2-Dichloroethane for Two Years. *Journal of Occupational Health*., 48, 242-436.

Study Design/Details

In a 2-year study, F344/DuCrj (SPF) rats (50/sex/dose) were exposed to 1,2-dichloroethane for 6 hours a day 5 days a week by whole body inhalation at atmospheric concentrations of 0, 10, 40 or 160 ppm. The maximum concentrations were selected in reference to a 13-week inhalation study in which these maximum concentrations did not cause mortality or overt signs of toxicity. The lowest dose was selected based on the Occupational Exposure Limit for 1,2-dichlorethane in Japan.

Animals were examined daily for clinical signs and mortality. Body weights and food consumption were measured once a week for the first 14 weeks and every 4 weeks after that. All of the rats that died within the 2-year period or were killed in a moribund state received a complete necropsy. At necropsy blood and organs were removed for examination.

The incidence of neoplastic lesions were analysed for a dose response relationship indicated by a positive trend by Peto's test and for significant difference from the concurrent controls by Fisher's exact test. Incidences of non-neoplastic lesions and urinary parameters were analysed by Chi-square test. The data was also compared to historical control data compiled from a similar inhalation studies run by

the same laboratory.

This study was conducted according to OECD Guidelines 453 and GLP.

Study Findings

There was no significant difference in survival in any of the dose groups when compared to controls. Survival rates were: 74, 70, 64 and 74% for males, and 70, 82, 74 and 76% for females at concentrations of 0, 10, 40 or 160 ppm respectively. Incidences of subcutaneous masses were found in the breast, back and abdominal and perigential areas tended to increase in groups exposed to 1,2-dichloroethane. No significant differences in haematological, blood biochemical or urinary parameters were detected in any of the 1,2-dichlorethane dosed groups.

Increased incidences of subcutaneous masses in a dose-related manner were observed upon macroscopic examination at necropsy (See table 1). In male rats incidences of fibromas in the subcutis, fibroadenomas in the mammary gland and mesotheliomas in the peritoneum showed a significant positive trend. The incidences of subcutaneous fibromas in the 40 and 160 ppm dose groups exceeded the maximum tumour incidence in the historical controls but was not statistically different from the concurrent controls. The incidence of mammary gland fibroadenomas was significantly increased when compared to the concurrent control and exceeded the historical control ranges. Mammary fibroadenomas and mammary gland adenomas were benign tumours but incidences of these combined exhibited a significant positive trend. The combined mammary fibroadenomas and mammary gland adenomas incidence and the incidence of peritoneal mesotheliomas in the 160 ppm dose group were significantly increased above historical controls but not concurrent controls. The mesotheliomas were malignant tumours involving the surface of the peritoneal cavity.

In female rats, incidences of fibromas in the subcutis and adenomas, fibroadenomas and adenocarcinomas in the mammary gland showed a significant positive trend. The incidence of subcutaneous fibromas in the 160 ppm dose group was statistically increased and exceeded the historical control data. The incidence of mammary gland adenomas and fibroadenomas in the 160 ppm female groups were significantly increased and the combined incidences of the benign mammary tumours were statistically increased and exceed the historical controls. Mammary adenocarcinomas exceeded the historical controls but were not significantly increased from the concurrent controls. The combined incidence of the mammary gland fibromas, adenomas and adenocarcinomas showed a significant positive trend with dose, and the 160ppm female group showed significant increases in incidence when compared with concurrent controls.

No incidences of non-neoplastic lesions were observed in and rat dosed with 1,2-dichloroethane.

Table 1. Tumour incidence in rats administered 1,2-dichloroethane by inhalation exposure

	Effect	**Exposure concentration (ppm)**				**Historical Control Data**	
		0	**10**	**40**	**160**	**Incidence**	**Min-Max**
		Number of animals (%)					
Males	Subcutaneous masses	8/50	13/50	20/50	18/50		
	Subcutis fibroma	6/50 (12.0)	9/50 (18.0)	12/50 (24.0)	15/50 (30.0)	55/749 (7.3)	1/50-10/50 (2.0-20.0)
	Mammary gland adenoma	1/50 (2.0)	2/50 (4.0)	0/50 (0.0)	5/50 (2.0)	7/749 (0.9)	0/50-2/50 (0.0-4.0)
	Mammary gland	0/50	0/50	1/50	5/50*	13/749	0/50-3/50

	fibroadenoma	(0.0)	(0.0)	(2.0)	(10.0)	(1.7)	(0.0-6.0)
	Mammary combined fibroadenoma and adenoma	1/50 (2.0)	2/50 (4.0)	1/50 (2.0)	7/50* (14.0)	19/749 (2.5)	0/50-4/50 (0.0-8.0)
	Peritoneum mesothelioma	1/50 (2.0)	1/50 (2.0)	1/50 (2.0)	5/50 (10.0)	16/749 (2.1)	0/50-4/50 (0.0-8.0)
Females	Subcutaneous masses	12/50	12/50	14/50	30/50		
	Subcutis fibroma	0/50 (0.0)	0/50 (0.0)	1/50 (2.0)	5/50* (10.0)	8-747 (1.1)	0/50-4/50 (0.0-8.0)
	Mammary gland adenoma	3/50 (6.0)	5/50 (10.0)	5/50 (10.0)	11/50* (22.0)	28/747 (3.7)	0/50-9/50 (0.0-18.0)
	Mammary gland fibroadenoma	4/50 (8.0)	1/50 (2.0)	6/50 (12.0)	13/50* (26.0)	76/747 (10.2)	0/50-8/50 (0.0-16.0)
	Mammary gland combined fibroadenoma and adenoma	7/50 (14.0)	6/50 (12.0)	11/50 (22.0)	22/50* (44.0)	103/747 (13.8)	2/50-9/50 (4.0-18.0)
	Mammary gland adenocarcinoma	1/50 (2.0)	2/50 (4.0)	0/50 (0.0)	5/50 (10.0)	5/747 (0.7)	0/50-2/50 (0.0-4.0)
	Mammary gland combined fibroadenoma, adenoma and adenocarcinomas	8/50 (16.0)	8/50 (16.0)	11/50 (22.0)	25/50* (50.0)	104/747 (13.9)	2/50-10/50 (4.0-10.0)

* Statistically different from control group.

The study demonstrated that 2-year inhalation exposure to 1,2-dichloroethane produced dose-dependent increases in the incidences of benign and malignant tumours in various organs of rats. In addition the concentrations of inhalation exposure to 1,2-dichloroethane that induce carcinogenic responses were found to be only slightly higher than the levels to which humans are expected to be exposed to occupationally.

Study Deficiencies, Questions and Additional Comments

Klimisch Rating

1 (reliable without restrictions)

Reference 3.20

Reference
Nagano, K., Umeda, Y., Senoh, H., Gotoh, K., Arito, H., Yamamoto, S. and Matsuhima, T. (2006) Carcinogenicity and Chronic Toxicity in Rats and Mice exposed by Inhalation to 1,2-Dichloroethane for Two Years. *Journal of Occupational Health*., 48, 242-436.
Study Design/Details
In a 2-year study, Crj:BDF1 (SPF) mice (50/sex/dose) were exposed to 1,2-dichloroethane for 6 hours a day, 5 days a week by whole body inhalation at atmospheric concentrations of 0, 10, 30 or 90 ppm. The maximum concentrations were selected in reference to a 13-week inhalation study in which these maximum concentrations did not cause mortality or overt signs of toxicity. The lowest dose was selected based on the Occupational Exposure Limit for 1,2-dichlorethane in Japan. Animals were examined daily for clinical signs and mortality. Body weights and food consumption were measured once a week for the first 14 weeks and every 4 weeks after that. All of the rats that died within the 2-year period or were killed in a moribund state received a complete necropsy. At necropsy blood and organs were removed for examination. The incidence of neoplastic lesions were analysed for a dose response relationship indicated by a positive trend by Peto's test and for significant difference from the concurrent controls by Fisher's exact test. Incidences of non-neoplastic lesions and urinary parameters were analysed by Chi-square test. The data was also compared to historical control data compiled from a similar inhalation studies run by the same laboratory.
Study Findings
There was no significant difference between survival in the male dose groups when compared to controls. Survival rates were: 78, 65, 70 and 74% for males at concentrations of 0, 10, 40 or 160 ppm respectively. Survival rates for females were slightly decreased and 69, 56, 38 and 52% for females at concentrations of 0, 10, 40 or 160 ppm respectively and the 30 ppm dose group showed a significant decrease in survival when compared to controls. In the female group dosed with 30 ppm of 1,2-dichloroethane 19 animals died (38%) of malignant lymphoma before the end of the 2-year period this was increased compared to deaths from malignant lymphoma in controls and other female dose groups with 6/50, 12/50 and 9/50 for 0, 10 and 90 ppm, respectively. The lower survival rates and increased incidences of malignant lymphoma were not treatment related. Incidences of subcutaneous masses were found in the breast, back and abdominal area by clinical examination and tended to increase in females dosed with 90 ppm. groups exposed to 1,2-dichloroethane. No significant difference in haematological, blood biochemical or urinary parameters was detected in any of the 1,2-dichlorethane dosed groups. Macroscopic examination at necropsy observe increases in the incidence of tumour associated nodules in the lung, uterus and subcutis in the 90 ppm female dose group. In male mice, incidences of hemangiosarcomas in the liver of the 30 and 90 ppm groups were significantly increased when compared with concurrent controls and tumour incidences in males at the 30 ppm male groups exceeded the historical controls. These incidences did not show a significant positive trend.

In female mice a significant positive trend was observed for incidences of bronchioalveolar adenomas and carcinomas in the lung, endometrial stromal polyps in the uterus adenocarcinomas in the mammary gland and hepatocellular adenomas as well as combined incidences of bronchiolo-alveolar adenomas and carcinomas and of hepatocellular adenomas and carcinomas. The incidences of bronchiolo-alveolar adenomas and carcinomas, endometrial stromal polyps in the uterus, mammary gland adenocarcinomas and hepatocellular adenomas in the 90 ppm female dose group exceeded the historical controls but were not statistically significant compared to concurrent controls. Malignant lymphoma incidence was significantly increased but were within the range for the historical controls.

No incidences of non-neoplastic lesions were observed in any rat dosed with 1,2-dichloroethane.

Table 1. Tumour incidence in rats administered 1,2-dichloroethane by inhalation exposure

	Effect	**Exposure concentration (ppm)**				**Historical Control Data**	
		0	**10**	**30**	**90**	**Incidence**	**Min-Max**
		Number of animals (%)					
Males	Liver haemangiosarcoma	0/50 (0.0)	4/49 (8.2)	6/50* (12.0)	5/50* (10.0)	27/748 (3.6)	0/50-5/50 (0.0-10.0)
Females	Lung bronchiolo-alveolar adenoma	4/49 (8.2)	1/50 (2.0)	3/50 (6.0)	8/50 (16.0)	29/749 (3.9)	0/50-5/50 (0.0-10.0)
	Lung bronchiolo-alveolar carcinoma	1/49 (10.2)	0/50 (0.0)	1/50 (2.0)	3/50 (6.0)	49/749 (3.9)	0/50-6/50 (0.0-0)
	Lung combined bronchiolo-alveolar adenoma and carcinoma	5/49 (10.2)	1/50 (2.0)	4/50 (4.0)	11/50 (22.0)	49/749 (3.9)	0/50-6/50 (0.0-12.0)
	Uterus endometrial stromal polyp	2/49 (4.1)	0/50 (0.0)	1/50 (2.0)	6/50 (12.0)	26/749 (3.5)	0/50-4-50 (0.0-8.0)
	Mammary gland adenocarcinoma	1/49 (2.0)	2/50 (4.0)	1/50 (2.0)	6/50 (12.0)	20/749 (2.7)	0/50-4/50 (0.0-8.0)
	Liver hepatocelluar adenoma	1/49 (2.0)	1/50 (2.0)	1/50 (2.0)	6/50 (12.0)	33/749 (4.4)	1/50-4-50 (2.0-8.0)
	Liver hepatocelluar carcinoma	1/49 (2.0)	0/50 (0.0)	1/50 (2.0)	0/50 (0.0)	23/731 (3.1)	0/50-4/50 (0.0-8.0)
	Liver hepatocelluar adenoma and carcinoma	2/49 (4.1)	1/50 (2.0)	2/50 (4.0)	6/50 (12.0)	54/749 (7.2)	1/50-6/50 (2.0-12.0)
	Lymph node malignant lymphoma	6/49 (12.2)	17/50* (34.0)	22/50* (44.0)	12/50 (24.0)	214/749 (28.6)	7/50-23/50 (14.0-46.0)

* Statistically different from control group.

The study demonstrated that 2-year inhalation exposure to 1,2-dichloroethane produced dose-dependent increases in the incidences of benign and malignant tumours in various organs of mice. In addition, the concentrations of inhalation exposure to 1,2-dichloroethane that induce carcinogenic responses were found to be only slightly higher than the levels to which humans are expected to be exposed to occupationally.

Study Deficiencies, Questions and Additional Comments
Klimisch Rating
1 (reliable without restrictions)

Appendix D Diglyme

D1 Reproductive toxicity

D1.1 Epidemiology Studies

Reference 4.1

Reference
Gray, R.H. *et al*. (1996) Ethylene glycol ethers and reproductive health in semiconductor workers. *Occupational hygiene*, 2, 331-338,
Study Design/Details
Retrospective studies of workers in semiconductor factories were completed in eastern USA. These studies examined the reproductive health of male and female employees, and the wives of male employees. Ethylene glycol ethers (EGEs) were used in semiconductor fabrication, mainly as components of photoresist mixtures. Workers were potentially exposed to EGEs during chemical mixing and photo/apply processes in semiconductor clean rooms. Potential exposure to EGEs was defined as high or medium required use, and no required use and determined by questionnaire administered to the employees in addition to company records. Prospective studies were also completed in female employees. Early-morning urine samples were assayed for hCG and ovarian steroid hormones to detect early pregnancy loss.
Study Findings
For 581 pregnancies to female employees, the rates of spontaneous abortion were 33.3%, 18.9% and 14.8 for pregnancies exposed to high, intermediate or no EGE exposure, respectively. This trend was statistically significant. The relative risks of abortion were 2.8 (95% CI 1.4-5.6) for the high exposure, and 1.4 (95% CI 0.8-2.6) for the intermediate exposure groups. Paternal exposure did not affect miscarriage rates in 589 pregnancies. The odds ratio for delayed conception greater than or equal to one year in female workers with high potential EGE exposure was 3.9 (95% CI 1.4-11.4). In the prospective study, foetal loss among 148 women was 2.5 (95% CI 0.8-8.5) (not significant) for women working with EGEs, compared with non clean room employees. No evidence of a decrease in conception rate was found.
Study Deficiencies, Questions and Additional Comments
Exposure concentrations, times and exposure to diglyme itself were not defined. Location of study and the control groups were not identified.

Rating
These data are not considered suitable for quantitative human risk assessment due to inadequate categorisation of exposure.

Reference 4.2

Reference
Hammond, S.K. *et al*. (1995) Tired exposure-assessment strategy in the semiconductor health study. American Journal of Industrial Medicine, 28, 661-680. Swan, S.H. and Forest, W. (1996) Reproductive risks of glycol ethers and other agents used in semiconductor manufacturing. Occupational hygiene, 2, 373-385. Eskenazi, B. *et al*. (1995) Prospective monitoring of early fetal loss and clinical spontaneous abortion among female semiconductor workers. American journal of industrial medicine, 28, 833-846.
Study Design/Details
A cohort of semiconductor workers from 14 different companies was examined in both retrospective and prospective studies. Exposure to ethylene glycol ethers (EGEs) was determined using questionnaires looking at the work performed and an assessment of the work environment by industrial hygienists. No measurements of personal or area exposures were made. For the retrospective study, information on pregnancy outcomes and potential confounders (age, smoking, ethnicity, education, income, year of pregnancy, and stress) was obtained through a comprehensive interviewer administered interview of female employees. The prospective study of early foetal loss and fecundity was conducted in a subset of female employees from five plants. Daily diaries and measurements of daily urinary human chorionic gonadotrophin levels for 6 months were collected in addition to the comprehensive interview.
Study Findings
891 medically verified pregnancies were identified for the study, of which 774 were live births, 113 were spontaneous abortions and 4 were stillbirths. The overall unadjusted relative risk for spontaneous abortions was 1.45 (95% CI 1.02-2.05), adjusted for confounders it was 1.43, 95% CI 0.95-2.09). The risk of spontaneous abortion was statistically significantly increased in female workers in the photolithography group (1.67, 95% CI 1.04-2.55) and in the etching group (2.08, 95% CI 1.27-3.19). For women working with higher levels of EGE only in masking, the risk of spontaneous abortion was increased 3 fold (3.38, 95% CI 1.61-5.73). In the prospective study, no statistically significant differences were detected in the overall rate of spontaneous abortions between fabrication and non-fabrication workers or when pregnancy outcomes were examined by work group. However, the ability to conceive was lower among female workers exposed to EGEs (fertility rate 0.37, 95% CI 0.11-1.19).
Study Deficiencies, Questions and Additional Comments

Exposure concentrations, times and exposure to diglyme itself were not defined. Location of study and the control groups were not identified.
Rating
These data are not considered suitable for quantitative human risk assessment due to inadequate categorisation of exposure.

Reference 4.3

Reference
Pastides, H. *et al*. (1988) Spontaneous abortion and general illness symptoms among semiconductor manufacturers. Journal of occupational medicine, 30, 543-551.
Study Design/Details
Pregnancies of women employed in the diffusion area of a semiconductor manufacturing plant were studied. No measurement of workplace exposure was conducted. Exposures included several glycol ethers and other chemicals, such as arsine, phosphine, diobrane, xylene, toluene and hexamethyldisilane.
Study Findings
An increased risk of spontaneous abortion occurred in females from 18 pregnancies in the diffusion area (2.2, 95% CI 1.1-3.6), and 16 pregnancies in the photolithographic area (1.8, 95% CI 0.8-3.3), compared with controls (398 pregnancies).
Study Deficiencies, Questions and Additional Comments
Exposure concentrations, times and exposure to diglyme itself were not defined. Location of study and the control groups were not identified.
Rating
These data are not considered suitable for quantitative human risk assessment due to inadequate categorisation of exposure.

Reference 4.4

Reference
Welch, *et al*. (1988) Effects of exposure to ethylene glycol ethers on shipyard painters: II. Male

reproduction. American journal of industrial medicine, 14, 509-526.

Study Design/Details

Semen samples from 73 painters and 40 controls from a shipyard were analysed. The painters were exposed to 0-17.7 mg /m³ 2-methoxy ethanol and to 0-80.5 mg/m³ 2-ethoxyethanol. Skin contact with these substances was also possible. Exposure to other substances (including organic solvents and metals) was also possible.

Study Findings

No effects were observed in hormone levels or in sperm viability, motility and morphology. The proportion of men with a sperm density < 100 million/cm³ was higher in the exposed group than the unexposed group. The proportion of those with oligospermia who did not smoke compared with controls was 36% versus 16%. The proportion of those with oligospermia was similar between painters and controls who smoked (30% versus 38%). The proportion of painters with azoospermia was 5% compared with 0% in controls.

Study Deficiencies, Questions and Additional Comments

Exposure concentrations, times and exposure to diglyme itself were not defined.

Location of study and the control groups were not identified.

Rating

These data are not considered suitable for quantitative human risk assessment due to inadequate categorisation of exposure.

D1.2 Experimental animal studies

Reference 4.5

Reference
Cheever, K.L. *et al*. (1988) Testicular effects of bis(2-methoxyethyl)ether in the adult male rat: equimolar dose comparison with 2-methoxyethanol and 2-ethoxyethanol. Toxicologist 5, 140. Cheever, K.L. et al. (1989) Testicular effects of bis(2-methoxyethyl)ether in the adult male rat. Toxicology and industrial health. 5, 1099-1109.
Study Design/Details
Groups of five Sprague-Dawley rats were administered up to 20 doses of 0 or 684 mg/kg bw diglyme via the oral route. A recover period of 8 weeks was incorporated into the study, and testicular changes were analysed.
Study Findings
Primary and secondary spermatocyte degeneration and spermatidic giant cells were observed after 6-8 treatments. From treatment day 12 until 8 weeks after cessation of treatment, the testes to bodyweight ration was significantly reduced. A pachytene spermatocyte marker enzyme was significantly decreased in animals by day 18 of treatment.
Study Deficiencies, Questions and Additional Comments
Only one dose and not performed to guidelines.
Klimisch Rating
3 (Unreliable)

Reference 4.6

Reference
Driscoll *et al*. (1998) Developmental toxicity of diglyme by inhalation in the rat. Drug chem toxicol. 21, 119-36. DuPont (1988a) Teratogenicity study of diglyme in the rat. Newark, NJ, EI Du Pont de Nemours Co., 289.
Study Design/Details
Pregnant female CD rats (25 - 26/group) were administered 0, 25, 100 or 400 ppm diglyme (reported to be 0, 140, 558 and 2232 mg/m^3) via inhalation (nose-only) for 6 hours/day during days 7-16 of gestation.

Controls were exposed to air only.
Study Findings
100% resorptions occurred at the top dose. Mean foetal weights were significantly decreased at the mid dose. Malformations occurred at all doses and included; abnormally formed tails, distended lateral ventricles of the brain, axial skeletal malformations, and appendicular malformations. Delayed ossification also occurred. A slightly increased incidence of variations was noted at the lowest dose. These were not statistically different from control values; however, the pattern, type and incidence of variations was similar to those seen at the mid dose (which were statistically significantly different from controls). The authors concluded that a NOAEL could not be clearly identified for the foetus. Increased relative liver weights were found in the dams at the mid dose, therefore the maternal NOAEL was identified as 25 ppm (140 mg/m³).
Study Deficiencies, Questions and Additional Comments
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Klimisch Rating
1 (reliable)

Reference 4.7

Reference
DuPont (1988b) Subchronic inhalation toxicity study with diglyme. Newark, NJ, EI Du Pont de Nemours Co., 445. Valentine, R. *et al*. (1999) subchronic inhalation toxicity of diglyme. Food and chemical toxicology, 37, 75-86.
Study Design/Details
CD rats (20 male and 10 female/dose) were exposed to 0, 110, 370 or 1100 ppm diglyme (reported to be 0, 614, 2065 and 6138 mg/m³, respectively) via nose only inhalation for 6 hours/day, 5 days/week for two weeks. Exposed rats were killed at either 10 days after exposure, 14, 42 or 84 days post-exposure. A control group was exposed to air, and a positive control group was exposed to 2-methylether at 300 ppm in air.
Study Findings
At the top dose, 7 males had coloured ocular discharge and 17 males and 1 female had diarrhoea during exposure. Diarrhoea was also observed in 8 males at the mid dose. Bodyweight gain was decreased in a dose-dependent manner in males. Reversible anaemia occurred in both sexes at the top dose. Mean leucocyte counts were reduced in male rats in all test groups in a dose-dependent manner

and females at the top dose. Serum enzymes were significantly reduced at the dose for both sexes. At the mid and top dose, absolute weights of the testes, epidiymides, seminal vesicles and prostate were statistically reduced. Relative weights of the testes were also reduced at the top dose. Stage-specific germ cell damage was dose and time dependent. Increased mean absolute and relative liver weights were observed in females at the top dose. At the lowest dose, spermatocytes in pachytene and meiotic division at spermatogenic stages XII-XIV were mainly affected. At the mid dose, affected germ cells were similar to those at the low dose, but round spermatids and spermatogenic stages I-VIII were also affected. At the top dose, marked testicular atrophy was found, affecting all spermatogenic stages. The effects were reversible within 84 days at the low and mid dose. A LOAEC of 110 ppm (reported to be 614 mg/m³) was identified from this study.
Study Deficiencies, Questions and Additional Comments
The study has a short duration (2 weeks).
Klimisch Rating
1 (Reliable)

Reference 4.8

Reference
DuPont. Subchronic inhalation toxicity study with diglyme. Newark, NJ, EI Du Pont de Nemours Co., 187, 1989
Study Design/Details
Male CD rats (10/dose) were exposed to 0, 3, 10, 30 or 100 ppm diglyme (reported to be 0, 16.7, 55.8, 167 and 558 mg/m³, respectively) via inhalation for 6 hours/day, 5 days/week for two weeks. Exposed rats were killed 14 days post-exposure.
Study Findings
Mean bodyweights of the animals at the top dose were significantly lower than controls. The weights of testes, seminal vesicles, prostate and epidiymides were similar to those of controls. Minimal/mild atrophy of the testicles occurred at the top dose. Degenerative germ cells in epididymal tubules, spermatic granuloma in the epididymis and prostatitis occurred at lower concentrations (10 ppm and higher) at the end of exposure and after 14 days. Most lesions were minimal to mild and occurred in 1/10 animals. Sufficient data is not available in the CICAD to determine if these are substance related effects. A NOAEL of 30 ppm (167 mg/m³) was identified by the authors. Data only available from a CICAD – further details from the original study are not available as it is

proprietary data.
Study Deficiencies, Questions and Additional Comments
No information is presented on historical controls, although this was used to determine the NOAEL. It is unknown if the lesions occurred in the same or different animals. A NOAEL of 30 ppm was set by the authors, and while the indication of minimal testicular and epidiymal atrophy is present in the results at doses lower than the NOAEL, sufficient data are not currently available to challenge the NOAEL.
Klimisch Rating
2 (Reliable with restrictions)

Reference 4.9

Reference
Hardin, B.D. *et al*. (1986) Relative potency of four ethylene glycol ethers for induction of paw malformations in the mouse. Teratology 33, 85c.
Study Design/Details
CD mice (group size not provided) were administered 0 or 537 mg diglyme/kg bw/day via oral gavage as a single application on day 11. Animals were examined for gross external malformations and foetal bodyweight.
Study Findings
Malformations in the paws and digits of pups occurred at the top dose.
Study Deficiencies, Questions and Additional Comments
Only one dose on one day. Limited study details and examinations.
Klimisch Rating
3 (Unreliable)

Reference 4.10

Reference
McGregor, D.B. *et al*. (1981) Bis-2-methoxyethyl ether and 2-methoxy ethanol results from multiple assays for genotoxic potential. Environmental mutagenesis, 3, 381.
Study Design/Details
Male B6C3F1 mice (10/dose) were exposed to 0, 250 or 1000 ppm diglyme (reported to be 0, 1395 and 5580 mg/m³) for 7 hours/day for 4 days. Sperm was isolated 35 days post exposure.
Study Findings
Four mice at the top dose died on exposure day 4. Bodyweight gain was reduced in both exposure groups. A significant increase in morphologically altered sperm occurred at the top dose (32%, compared with 5% in the controls). A LOAEC of 250 ppm (1395 mg/m³) was identified.
Study Deficiencies, Questions and Additional Comments
Non guideline study. Short duration.
Klimisch Rating
3 (Unreliable)

Reference 4.11

Reference
McGregor, D.B. *et al*. (1983) Genetic effects of methoxy ethanol and bis(2-methoxyethyl)ether. Toxicology and applied pharmacology, 70, 303-316.
Study Design/Details
Sprague-Dawley CD adult male rats (10/group) were exposed via inhalation to 0, 250 or 1000 ppm digylme (reported to be 0, 1395 and 5580 mg/m³) for 7 hours/day for 5 days/week. Following exposure, the males were mated with untreated females. Females were sacrificed 17 days later and examined for evidence of pregnancy. The number of corpora lutea per pregnancy, the live implants and the number of implantation sites were assessed using the Freeman-Tukey Poisson and binomial transformation for evidence of early deaths indicating dominant lethal effects.

Study Findings
A significant reduction in pregnancy frequency (measured as females with implantations) was observed at the top dose. 10% of the mated females had implantations when sacrificed on day 17. No changes in pregnancy frequency were observed at the low dose. Preimplantation losses, manifested as reductions in corpora lutea, were statistically significantly reduced at the top dose. Significant increases in early deaths per pregnancy also occurred at the top dose, and were significantly reduced at the low dose. A NOAEC of 250 ppm (1395 mg/m³) can be identified from this study.
Study Deficiencies, Questions and Additional Comments
Very low numbers of implantations. Animals not exposed throughout gestation. Non guideline study. Short duration.
Klimisch Rating
3 (Unreliable)

Reference 4.12

Reference
NTP (1985) Teratologic evaluation of diethylene glycol dimethyl ether (CAS No 111-96-6) administered to CD-1 mice on gestation day 6 through 15. Research Triangle Park, NC, National Institute of Environmental Health Sciences, National Toxicology Program (NTP-85-5-255, PB86-135233). Price *et al* (1987). The developmental toxicity of diethylene glycol dimethyl ether in mice. Fundamental and applied toxicology, 8, 115-126
Study Design/Details
Pregnant female CD-1 mice (20-24/group) were administered diglyme via gavage at doses of 0, 62.5, 125, 250 or 500 mg/kg bw/day on gestation days 6-15. Food and water were available *ad libitum*. Mice were monitored daily for clinical signs of toxicity. The study was conducted to GLP.
Study Findings
Maternal clinical signs of toxicity, mortality and bodyweight gain did not occur in treated dams. Absolute bodyweight was reduced in dams in the two highest dose groups. A significant dose-related increase in the percentage of non-live implants per litter occurred at the top two doses (4.88%, 8.41%, 7.05%, 12.02% and 50.41% in the control, 62.5, 125, 250 and 500 mg/kg bw/day groups, respectively). The percentage of adversely affected implants per litter was also significantly increased in a dose-dependent manner (5.25%, 8.41%, 9.35%, 32.29% and 96.93% in the control 62.5, 125, 250 or 500 mg/kg bw/day groups, respectively). The mean live litter size was significantly less than controls at the top dose, and was marginally reduced at all lower doses. The mean foetal bodyweight per litter was reduced at and above 125 mg/kg bw/day. Anatomical differences were present in all doses, and were statistically significant in the top two doses.

The mean percentage of malformed foetuses was 0.37%, 0.00%, 2.47%, 23.86% and 95.82% for the control, 62.5, 125, 250 and 500 mg/kg bw/day groups, respectively). Major malformations affected development of the neural tube, limbs and digits, craniofacial structures, abdominal wall, cardiovascular system, urogenital organs, and both the axial and appendicular system. The two most frequently observed malformations were fused ribs in 74% of foetuses at the high dose and exencephaly in 54% foetuses at the high dose. Diglyme is considered a specific developmental toxin in the mouse, producing adverse effects on the conceptus at doses not associated with maternal toxicity. A maternal NOAEL of 500 mg/kg bw/day was identified from this study. A foetal NOAEL of 62.5 mg/kg bw/day was identified from this study.
Study Deficiencies, Questions and Additional Comments
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Klimisch Rating
1 (Reliable)

Reference 4.13

Reference
NTP (1987) Teratologic evaluation of diethylene glycol dimethyl ether (CAS No 111-96-6) administered to New Zealand White rabbits on gestation day 6 through 19. Research Triangle Park, NC, National Institute of Environmental Health Sciences, National Toxicology Program (NTP-87-108, PB87-209532).
Study Design/Details
Pregnant New Zealand white rabbits (15-25/dose) were administered diglyme via gavage at doses of 0, 25, 50, 100 or 175 mg/kg bw/day on gestation days 6 to 19. The study was performed to GLP.
Study Findings
Mortality of the dams occurred at the top dose (15% at 175 mg/kg bw/day, compared with 15% in controls), and also in the second highest dose (mainly from resorptions). Reduced weight gain (attributed to decreased uterine weight) was observed at 50 mg/kg bw/day, and decreased uterine weight was also observed at 100 mg/kg bw/day. The number of implants per litter was significantly adversely affected at 50 mg/kg bw/day (21.4% compared with 7.9% in controls). Abnormal development of the kidneys and axial skeleton and clubbing of the limbs occurred at 100 mg/kg bw/day. The number of resorptions was also increased at this dose. Maternal and foetal NOAELs of 100 and 25 mg/kg bw/day, respectively, have been identified. However, maternal and foetal NOAELs of 25 and 50 mg/kg bw/day, respectively, have also been proposed by Schwertz *et al*.

Study Deficiencies, Questions and Additional Comments
There are differing interpretations of the NOAEL. The Schwertz paper identifies 50 mg/kg bw/day as the foetal NOAEL; however, a significant decrease in the number of implants per litter occurred at this dose, corresponding to the NOAEL identified by the NTP of 25 mg/kg bw/day.
Klimisch Rating
2 (Reliable with restrictions)

Reference 4.14

Reference
Schuler, *et al*. (1984) Results of testing fifteen glycol ethers in a short-term *in vivo* reproduction assay. Environmental Health Perspectives, 57, 141-146.
Study Design/Details
Pregnant female CD-1 mice (50/dose) were administered 0 or 3000 mg/kg bw/day diglyme via gavage on days 7-14 of gestation. Maternal bodyweight was measured on day 7 of gestation immediately prior to dosing, on day 18 of gestation and on day 3 postpartum. Animals were housed individually throughout the reproductive study. Food and water were available *ad libitum*.
Study Findings
Maternal mortality occurred in the treatment group in 20/49 animals (41%) and no viable litters were produced, compared with controls, where no deaths occurred, and 42/43 viable litters were produced (98%).
Study Deficiencies, Questions and Additional Comments
This is a screening study and therefore the data are not sufficient for hazard determination. Only one dose was used.
Klimisch Rating
3 (Unreliable)