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APPLICATION FOR AUTHORISATION: ESTABLISHING A REFERENCE DOSE RESPONSE RELATIONSHIP FOR CARCINOGENICITY OF 1,2-DICHLOROETHANE

Background

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

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

Reference values in the form of DNEL's for threshold substances and/or dose response relationships for non-threshold substances (mainly carcinogens) are published in advance of applications, for authorisation, so providing greater consistency and better use of the legally defined periods of opinion-development in the Committee for Risk Assessment (RAC).

Annex 1: Reference dose response relationship for carcinogenicity of 1,2-dichloroethane

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

Annex 1 Reference dose-response relationship for carcinogenicity of 1,2-dichloroethane

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

Relevance of endpoints

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

For applicants aiming at authorisation based on Article 60(4) (socio-economic analysis route), i.e. where there is no adequate control, Article 62(4)(d) applies and is also focussed on the risks related to the intrinsic properties specified in Annex XIV. The SEA should in turn consider the impacts related to such risks. In practice the applicant is expected to provide this information in their (Chemical Safety Report) CSR. However, for an authorisation to be granted, the applicant should also demonstrate that there are no suitable alternatives. In this latter analysis other endpoints than those for which the substance was listed in Annex XIV may become relevant in order to demonstrate that no suitable alternative is available.

1,2-dichloroethane was included on Annex XIV due to its carcinogenic properties. The reference dose-response relationships proposed in the present document are only developed for carcinogenicity arising from 1,2-dichloroethane exposure[3].

Carcinogenicity

Table 1 below provides an overview of expert assessments on the carcinogenic mode of action, the assumed carcinogenic mechanism and the low-dose extrapolation approaches that were used:

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

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

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

Expert evaluation	Primary mechanistic concern	Threshold / Non-threshold approach	Studies / effects of most concern for Point Of Departure	Unit risk/ Slope factor / Threshold dose
CSR A **[These are CSRs submitted under the REACH process by registrants]**	Genotoxicity	Non 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) However, registrants considered 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 mg/m^3) for a number of reasons including comparison with some occupational exposure limits, practical measures being taken to minimise exposure, and that a more stringent DMEL would not be technically feasible at the current time. Dermal DMEL (lifetime cancer risk 10^{-5}) = 0.156 mg/kg bw/day The registrants proposed 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) The registrants use 2.9 µg/m^3 in risk assessment

Expert evaluation	Primary mechanistic concern	Threshold / Non-threshold approach	Studies / effects of most concern for Point Of Departure	Unit risk/ Slope factor / Threshold dose
CSR B **[This is a CSR submitted under the REACH process by a registrant]**	Carcinogenicity	Non-threshold	Nagano *et al*., 2006	**Workers** Inhalation DMEL (2014) = 6.6 mg/m^3 Dermal DMEL = 62.4 mg/kg bw/day **General population** Inhalation DMEL = 2.9 µg/m^3
US EPA (2014, last revision 1993)	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 (potency) factor = 9 x 10^{-2} per mg/kg/day (based on tumour findings in rats in the NCI study). This oral slope factor corresponded to the following values: Drinking water unit risk =2.6 x 10^{-6} µg/l Inhalation unit risk = 2.6 x 10^{-5} µg/m^3
EC Regulation No. 1272/2008 on classification labelling and packaging of substances and mixtures	Not applicable	Not applicable	Classification according to part 3 of Annex VI, Table 3.1 (list of harmonised classification and labelling of hazardous substances) = Carc. 1B. Described as being presumed to have carcinogenic potential for humans, classification largely based on animal evidence.	Not applicable

Expert evaluation	Primary mechanistic concern	Threshold / Non-threshold approach	Studies / effects of most concern for Point Of Departure	Unit risk/ Slope factor / Threshold dose
WHO (2003)	Not specified	Non-threshold	Group 2B possible carcinogen to humans. NCI (1978) Male mice (gavage, 78 weeks) • haemangiosarcomas	Applying the linearised multistage model, concentrations in drinking water of 300, 30 and 3 µg/l, corresponded to upper-bound excess cancer risks of 10^{-4}, 10^{-5}, 10^{-6}, respectively. Guideline for Drinking Water Quality = 30 µg/l.
OECD (2002)	Genotoxicity	Non-threshold	Classified as a suspected human carcinogen	Not specified
US Agency for Toxic Substances and Disease Registry (US ATSDR, 2001)	Genotoxic metabolites DNA adducts Mutagenicity	Threshold (possible that detoxification pathways are saturated at high doses)	Described as a probable human carcinogen. Cheever *et al*., 1990 Rats (inhalation) • liver histopathology NTP (1991) Rats and mice (drinking water or gavage) • kidney effects	No cancer unit risk values provided. Minimal Risk Levels (MRL) MRL for chronic duration inhalation exposure = 0.6 ppm (based on liver histopathology in rats after 2 years exposure) MRL for intermediate oral exposure periods (15-365 days) = 0.2 mg/kg bw/day (based on kidney damage in rats in a 13-week drinking water study)
IARC (1999)	Mutagenicity DNA damage	Not specified	*Inadequate evidence* in humans for carcinogenicity *Sufficient evidence* in experimental animals for carcinogenciity	Group 2B possible carcinogen to humans
WHO (1998)	Genotoxic metabolites	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.	Doses associated with a 5% increase in tumour incidence $TD_{0.05}$ = 6.2–34 mg/kg bw Guidance values: Air = 3.6-20 mg/m^3 or

Expert evaluation	Primary mechanistic concern	Threshold / Non-threshold approach	Studies / effects of most concern for Point Of Departure	Unit risk/ Slope factor / Threshold dose
			Mice (gavage) • alveolar/bronchiolar adenomas, hepatocellular carcinomas, mammary gland adenocarcinomas and endometrial stromal polyps	0.36-2 mg/m^3 Ingestion = 1.2-6.8 mg/kg bw or 0.12-0.68 mg/kg bw.
WHO (1995) (IPCS, 1995)	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. Induction of common and rare tumours, production of intermediate that alkylates DNA and positive results of genotoxicity in vitro and in vivo.	Not specified
Health Canada (1994) (ECHC, 1994)	Genotoxic metabolites	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	Doses associated with a 5% increase in tumour incidence $TD_{0.05}$s = 6.2–34 mg/kg bw

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.

The evidence suggests that a genotoxic non-threshold mechanism of action of 1,2-dichloroethane is appropriate.

Genotoxicity
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 ASTDR, 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.

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.

Animal 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 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 1) 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 2). 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 rather on genotoxic potential and the best dose-response rather than its relevance to a specific human cancer.

Table 2 Total mammary tumour incidence in 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

Bioavailability

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).

Inhalation

It has been reported that 1,2-dichloroethane is rapidly absorbed through the lungs of humans and experimental animals upon inhalation exposure (ATSDR, 2001). In old 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 the breast milk (Urusova, 1953; US EPA, 1980). 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 1-3 hours of continuous inhalation exposure. 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.

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 peak blood levels in Osborne-Mendel rats occurred within 10-15 minutes of administration (Reitz *et al*., 1982; Spreafico *et al*., 1980). 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 molecule 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 only be considered if there was convincing justification that absorption was different in the application being reviewed.

Summary of Bioavailability

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.

Carcinogenicity risk assessment

The review of the carcinogenicity and genotoxicity 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 definitive proof of carcinogenicity in humans is lacking. Review of the available 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 estimations are based on the most relevant, robust study in experimental animals.

The Point of Departure (PoD) selected for risk assessment is the T25 value 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.

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 2: 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

$$\mathbf{T25_{(inhalation,\ rat)} = 160 \times (0.25)/(0.50 - 0.16) \times (1\text{-}0.16)/1}$$

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

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 3.

Table 3 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/m^3)	**6.0×10^{-7} per µg/m^3**
	General population	$T25_{(inhalation, human)}$ 17.6 ppm (72.5 mg/m^3)	**3.45×10^{-6} per µg/m^3**
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/m^3	6.0×10^{-7}
2 µg/m^3	1.2×10^{-6}
10 µg/m^3	6.0×10^{-6}
100 µg/m^3	6.0×10^{-5}
1000 µg/m^3	6.0×10^{-4}

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