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2-chloroethanol

Re-assessment of its mutagenic potential and derivation of provisional acute and chronic oral reference values (ARfD, ADI)

2-Chloroethanol (2-CE) is a degradation product of ethylene oxide, a fumigant that has been banned in food production in the EU for several decades due to its mutagenic (genotoxic) and carcinogenic effects. However, ethylene oxide continues to be used in some countries outside the EU and can therefore occasionally enter the European market. Since 2020, excessive levels of the active substance have repeatedly been detected in treated foods (e.g., sesame seeds or spices).

Due to inconsistent data on the potential genotoxicity of 2-CE, the degradation product has so far been treated in the same way as ethylene oxide. Through advances in analytical technology and new data on mutagenic effects, the German Federal Institute for Risk Assessment (BfR) has now been able to assess the potential health risks of 2-CE itself. The BfR concludes that a mutagenic effect of 2-CE is unlikely with relevant exposure via food.

In addition, the BfR proposes a provisional acute reference dose (ARfD) of 0.13 mg/kg body weight and a provisional acceptable daily intake (ADI) of 0.02 mg/kg bw/day for 2-CE. The ARfD indicates the estimated maximum amount of a substance that can be ingested with food in one meal or several meals over the course of a day without any discernible health risk. The ADI indicates the amount of a substance that can be ingested orally on a daily basis over a lifetime without any discernible health risk.

A harmonised assessment of human toxicology of 2-CE at EU level, based on the new information, is pending. The BfR recommends further analyses and comprehensive systematic literature searches to substantiate the results obtained and to confirm the proposed reference values.

The BfR also proposes initiating a procedure within the EU to create a separate residue definition for ethylene oxide and 2-CE as well as harmonized MRLs for the European market.

1 Subject of the assessment

From September 2020 onwards, the maximum residue level for the active substance ethylene oxide in sesame seeds and other foodstuff was repeatedly exceeded in the EU. Although ethylene oxide has been banned for use in the EU since 1991, it is still used as a fumigant in non-European countries. Goods treated with ethylene oxide and entering the EU market may contain residues of the substance. Ethylene oxide forms the degradation product 2-chloroethanol (2-CE) when it reacts with natural chlorides after the fumigation of food. Hence, the residue definition for ethylene oxide therefore includes 2-CE.

While ethylene oxide is regarded as known genotoxic carcinogen for which a safe threshold has not been established, the database for 2-CE was inconsistent, meaning it was not possible to draw a conclusion about its genotoxic potential or to set health-based guidance values with sufficient confidence (BfR, 2021[1]). Accordingly, in a worst-case assumption, 2-CE has previously been treated as ethylene oxide and a joint residue definition of ethylene oxide plus 2-CE was established. Recent advances in analytical technology do now allow for separate quantification of ethylene oxide and 2-CE on a routine basis.

The German Federal Institute for Risk Assessment (BfR) has now re-evaluated the mutagenic potential of 2-CE on the basis of new mutagenicity data supported by physiologically based kinetic (PBK) modelling submitted by the Food Federation Germany and evaluated the possibility of deriving health-based guidance values (HBGV) supporting the setting of a refined residue definition.

2 Results

Based on the new information provided, a biologically significant mutagenic activity of 2 CE at relevant dietary exposures is considered unlikely. Accordingly, the general toxicity database was reviewed with regard to their suitability for deriving acute and chronic dietary reference values. Taking into account the limited quality and availability of original information, a provisiona9l

- Acute Reference Dose (ARfD) of 0.13 mg/kg bw and
- Acute Daily Intake (ADI) of 0.02 mg/kg bw/d are proposed.

Further mechanistic analyses are required to substantiate the non-relevance of the weak in vitro mutagenicity findings. A full systematic literature review should be performed and original study reports for unpublished repeated-dose toxicity and multi-generation studies should be made available to allow for independent review and confirmation and refinement

[1] Health risk assessment of ethylene oxide residues in sesame seeds. Updated BfR Opinion No 024/2021 issued 01 September, 2021. DOI 10.17590/20210428-072544

of the proposed reference values. In the meantime, residue data could be collected and analytical methods validated to derive maximum residue levels (MRL). Finally, a peer-review process could be initiated within the EU, with the aim of creating a separate residue definition for ethylene oxide and 2-CE, alongside harmonised MRLs for the European market.

3 Rationale

3.1 Summary

According to the BfR opinion published 2021[2], "no reliable regulatory conclusion can be drawn on the carcinogenic properties of 2-CE. While there are numerous indications for genotoxic activity, clarification of the in vivo relevance or the existence of a potential threshold value are pending" (Updated BfR Opinion No. 024/2021).

Since then, a set of new in vitro genotoxic tests has become available. These tests were performed under GLP and according to current OECD test guidelines using optimised condition for metabolic activation to adequately reflect oxidation of 2-CE into 2-chloroacetaldehyde by aldehyde dehydrogenases, which was supported by comparisons of physiologically based kinetic (PBK) modelling to in vivo data.

While the in vitro mammalian cell gene mutations test (OECD TG 476) and the in vitro mammalian cell micronucleus assay (OECD TG 487) were clearly negative, dose-dependent increases in revertant counts were observed in the Ames test with metabolic activation in strains TA1535, TA100 and E.coli WP2uvrA. Although the max. increase over control at the top dose of 5000 µg/plate was 2.7-, 1.5- and 1.9-fold, respectively and did thus not meet the criteria frequently applied for a positive call (3-fold or greater response for TA1535 and 2-fold or greater response with the TA100 and WP2uvrA), benchmark dose (BMD) modelling (Bayesian Benchmark Dose Modelling, EFSA WebTool version 0.1.17) confirmed the effect, resulting in acceptable BMDL50 values of 524 to 3812 µg/plate, i.e. below the recommended top dose for all three tester strains detecting base pair substitutions.

Overall, 2-CE is considered to be a weak in vitro mutagen in bacteria. As the activity was limited to experimental conditions including metabolic activation, it is concluded that the mutagenicity of 2-CE involves prior activation to the corresponding aldehyde. No such activity was seen in the mammalian cell gene mutation assays and observations were strictly limited to very high doses overlapping with the recommended limit dose. In vivo relevance of such findings is considered as unlikely, in particular with regard to the low dietary exposure via residues. Therefore, health-based guidance values (HBGV) can be derived. Nevertheless, these should be considered provisional until the working hypothesis has been substantiated by further mechanistic and/or statistical evidence.

For the derivation of acute and chronic dietary reference values, key studies were selected from the available dataset, taking into account quality of the reporting, methodological reliability and potential species differences. Provisional reference values were derived from

[2] https://www.bfr.bund.de/cm/349/health-risk-assessment-of-ethylene-oxide-residues-in-sesame-seeds.pdf

No Observed Adverse Effect Levels (NOAELs) using adequate uncertainty and extrapolation factors (Table 1).

Table 1 Summary of the derivation of the provisional Acceptable Daily Intake (ADI) and Acute Reference Value (ARfD) with the corresponding No Observed Adverse Effect Level (NOAEL) and the necessary Uncertainty / Extrapolation factor (UF).

Reference value	Point of departure (PoD)	Uncertainty / Extrapolation factor (UF)	Reference value (numerical)
ARfD	90-day dog study, dietary NOAEL: 13.3 mg/kg bw/d LOAEL: 18.4 mg/kg bw/d based on vomiting supported by human case reports	Intra- and Interspecies: UF = 100	0.13 mg/kg bw
ADI	90-day dog study, dietary NOAEL: 13.3 mg/kg bw/d LOAEL: 18.4 mg/kg bw/d based on reduced body weight	Intra- and Interspecies: UF = 100 Extrapolation sub- chronic to chronic: UF = 2 quality of dataset: UF = 3	0.02 mg/kg bw/d

3.2 Regulatory background

2-CE (CAS 107-07-3) has a harmonised EU classification as Acute Tox 2* (H300, fatal if swallowed; H330, fatal if inhaled) and as Acute Tox 1 (H310, fatal in contact with skin).

So far 2-CE has not been assessed in the context of either the EU biocidal products regulation (EU) No 528/2012 or the EU plant protection products regulation (EC) No 1107/2009.

Due to its industrial use as intermediate and processing aid, 2-CE was registered under REACH regulation (EC) No 1907/2006 by different registrants (Reg. ID 01-2119514297-39-0002, 01 2119907149-37-0000, 01-2119907149-37-0001, 01-2119907149-37-0002) with the last update of the publicly available summary dossier in 2019.

In the US, the substance has been registered under a number of different regulations, including the Superfund Amendments and Reauthorization Act (SARA) establishing the Extremely Hazardous Substance (EHS) List and the Toxic Substances Control Act (TSCA). In 2020, US EPA evaluated 2-CE in the context of its assessment for ethylene oxide.

3.3 Data sources

The following sources of information were considered for this assessment:

- REACH dossier
- study data as reported by US EPA (2012, 2020) and corresponding EPA reports
- the outcome of a targeted literature search
- a toxicological profile submitted by Food Federation Germany
- new study reports of Ames, HPRT and MN assays submitted by Food Federation Germany, supported by documentation for IVIVE.

To date, the information contained in the REACH dossier has not yet undergone an independent, comprehensive analysis by an EU Member State authority or ECHA. It primarily consists of summaries that cannot be independently verified without access to the original data. Neither Derived No Effect Levels (DNEL) nor any other reference values were derived in the registration dossier. Furthermore, information on genotoxicity was partly conflicting and could not been independently reviewed due to a lack of access to the original study reports.

In 2012, US EPA published Provisional Peer-Reviewed Toxicity Values for 2-CE (EPA/690/R 12/007F) and provided a short 3-page evaluation of 2-CE as degradation product of ethylene oxide in support of the respective registration review (US EPA, 2020). However, the short study summaries do not allow for independent review.

Therefore, a targeted literature search was performed in PubMed using the following search string: ((repeat*) OR (long term) OR (dev*) OR (repro*) AND ((mouse) OR (mice) OR (rat) OR (dog) OR (rabbit) AND ("2-Chloroethanol"[Title/Abstract] OR "chloroethanol"[Title/Abstract] OR "ethylene chlorohydrin"[Title/Abstract] OR "glycol chlorohydrin"[Title/Abstract]). Seven of 75 references were considered in detail (see References).

3.4 Toxicology

3.4.1 Toxicokinetics and Metabolism

Two main pathways can contribute to the potential mutagenicity of 2-CE:

1. Direct DNA alkylation: Alkylhalogenides like 2-CE act as electrophiles capable of directly alkylating DNA. This is often responsible for the mutagenic effect in bacterial Ames tests with and without metabolic activation. 2-CE is reported to form adducts with glutathione in vivo, which is typical for electrophilic alkylating agents.
2. As a primary alcohol, 2-CE can be oxidised to carboxylic acid via protein- and DNA-reactive aldehyde intermediates. Notably, biotransformation of 2-CE to 2-chloro acetaldehyde has been demonstrated (Grunow and Altmann, 1982).

In the context of these pathways, a feasibility study was conducted with the aim of optimising the co-factors of the liver enzyme mixture S9 mix in the in vitro studies and modelling was performed to address in vivo relevance of the resulting test conditions.

Four documents were examined:

D1: Procter & Gamble (2023) 2-Chloroethanol In Vitro Metabolism and In-Vitro-to-In-Vivo Extrapolation, dated 27.10.2023.

D2: Procter & Gamble, (2023b) 2-Chloroacetic Acid Formation: Review of In Vitro Data and use of In-Vitro-to-In Vivo Extrapolation, dated 27.10.2023.

D3: Lebensmittelverband (2023) 2-Chloroethanol Project on Genotoxicity Tests Overview. Lebensmittelverband Deutschland, dated 12.10.2023.

D4: ICCR Roßdorf (2023): Report 2-Chloroethanol: Feasibility Study aiming to demonstrate the transformation of 2-chloroethanol to 2-chloroacetic acid, dated 18.7.2023.

Document D1 was used to prove that the metabolism of 2-CE is extensive enough under the conditions intended to be used in the in vitro genotoxicity assay. This was done by

incubating the test item (2-CE) with rat liver S9 and regenerating co-factors (NAD/NADP) for 4 hours (240 mins). The depletion of the test item (parent compound, 2-CE) and the formation of the metabolite 2-chloracetic acid (2-CAA) were measured at timepoint 0 and after the end of the incubation (240 mins). The results of this assay were used to compute the in vitro hepatic clearance (CLhep) and extrapolate this value to a whole-body half-life for rat and compare it to in vivo rat toxicokinetic (TK) data (Grunow and Altmann, 1982). In the absence of a designated guidance document on the performance of in vitro intrinsic clearance experiments with rat S9, we used the OECD TG 319B on the "Determination of in vitro intrinsic clearance using rainbow trout liver S9 subcellular fraction (RT S9)" as a main read across reference. We consider the following elements of the experiments and analysis presented in document D1 (in combination with the evidence provided also in documents D2 and D3) well justified:

- S9 protein concentration: 0.374 mg/mL is within the suggested range (OECD TG 319B, section 25: 0.25-2 mg/mL).
- incubation temperature: 37°C, in line with experimental procedures elsewhere
- use of co-factors: NAD/NADP are necessary for the oxidation of the alcohol (2-chlorethanol, 2-CE) to acetaldehyde (chloroacetaldehyde) and the oxidation of acetaldehyde to carboxyl (chloroacetic acid, 2-CAA). The combination of co-factors and their concentration used in the assay presented in document D1 (2 millimolar (mM) NADP & 2mM NAD) are well justified also by the data presented in document D3, which show that addition of NAD to NADP boosters the reaction.
- use of negative controls (2-CE + buffer to demonstrate stability, 2-CE + prequenched rat liver (i.e. de-activated S9) to examine non-specific binding of 2-CE)
- IVIVE Equations 1 to 4 are well established and acceptable, while Grunow & Altmann 1982 in vivo rat data could have been used to derive total Clearance and Volume of distribution (e.g. via non-compartmental and/or one/more compartment model analysis) and in equation 4 the use of the in vivo hepatic clearance CLhep as a surrogate for total clearance bears the assumption that none of the parent compound is cleared unchanged renally although Grunow & Altmann 1982 states that approx. 80 % are excreted as metabolites leaving 20 % unaccounted.
- Table 1 values for in vitro-in vivo extrapolation (IVIVE) are well acceptable, although some literature reported higher values of 209 mg S9 per g liver than the value of 165 mg/g used.

Some aspects of the IVIVE are considered critical:

1. The IVIVE performed in document D1 is based on a kinetic study that examines the parent compound depletion at only one time point. A kinetic profile cannot be constructed based on only one measurement. OECD TG 319B recommends at least 6 time points.
2. Multiple testing concentrations at similar testing conditions are highly recommended in order to characterise the saturation of the system via Michaelis Menten kinetic modelling. While some preliminary analysis was provided, the reported rate values relate to different experimental conditions (incubation time, co-factor composition).
3. Reporting limitations making it difficult to characterise uncertainties associated with the estimation of the depletion kinetic rate constant(s) kelm and the maximum rate

Vmax (e.g. incubations performed in duplicates but data reported as single values; estimated values lacking their estimated errors; plots without confidence ranges).

4. the use of positive controls
5. reporting of the LOQ for 2-CE (only LOQ for 2-CAA reported as 0.21 µM)
6. characterisation of the rat S9 mix

Nevertheless, the primary aim of the studies was to investigate whether the S9 system is able to metabolise 2-CE via 2-chloroacetaldehyde to its acid, optimise the conditions with this respect and evaluate whether resulting metabolic activation may be of a magnitude that is relevant for the in vivo situation. Based on the information obtained, the protocol for metabolic activation in a set of OECD TG compliant in vitro tests was optimised.

3.4.2 New *in vitro* genotoxicity data

This includes the following studies performed using optimised conditions for metabolic activation as described above:

- bacterial reverse mutation test (OECD TG 471)
- in vitro mammalian cell gene mutation test (OECD TG 476)
- in vitro mammalian cell micronucleus test (OECD TG 487)

The bacterial reverse mutation test (ASB2024-10963) included the plate incorporation and preincubation method with five tester strains (TA98, TA100, TA1535, TA1537, WP2 uvrA) in the presence and absence of S9-mix. The test substance 2-CE in an appropriate non-cytotoxic dose range (3-5000 µg) was used together with the appropriate positive- and vehicle controls. All positive controls as well as vehicle control values in this test was within acceptable limits. No biologically relevant increases in the revertant colony counts were observed in the absence of metabolic activation. The same applies to tester strains TA98 and TA1537. For TA1535, TA100 and E.coli WP2 uvrA, dose-dependent increases in revertant counts were reported as shown below.

Table 2 Mutagenic Response of 2-CE in a bacterial reverse mutation test of Salmonella typhimurium strains (TA1535, TA100) and an Escherichia coli strain.

TA1535	microgram per plate	mean revertants	SD	N	Exp. no.
1	0	13.7	4.5	3	1
2	3	13.3	3.5	3	1
3	10	12.7	1.2	3	1
4	33	12.3	3.1	3	1
5	100	11.3	4.9	3	1
6	333	15.0	2.6	3	1
7	1000	13.7	1.5	3	1
8	2500	24.3	2.5	3	1
9	5000	31.7	4.0	3	1
28	0	13.3	4.2	3	2
29	33	13.7	3.5	3	2
30	100	16.0	5.2	3	2
31	333	14.7	6.5	3	2
32	1000	15.0	2.6	3	2
33	2500	27.7	4.0	3	2
34	5000	34.0	8.9	3	2

TA100	microgram per plate	mean revertants	SD	N	Exp. no.
10	0	141.7	17.9	3	1
11	3	131.7	16.4	3	1
12	10	126.7	13.1	3	1
13	33	133.0	9.5	3	1
14	100	136.7	16.0	3	1
15	333	135.3	15.5	3	1
16	1000	128.0	8.7	3	1
17	2500	165.3	1.5	3	1
18	5000	216.7	2.1	3	1
35	0	143.3	4.0	3	2
36	33	159.0	6.6	3	2
37	100	143.7	19.5	3	2
38	333	152.7	4.7	3	2
39	1000	188.3	15.9	3	2
40	2500	189.3	16.6	3	2
41	5000	213.3	10.2	3	2

E.coli WP2uvrA	microgram per plate	mean revertants	SD	N	Exp. no.
19	0	69.0	10.5	3	1
20	3	66.0	15.6	3	1
21	10	73.7	6.1	3	1
22	33	70.7	7.2	3	1
23	100	73.3	5.5	3	1
24	333	68.7	3.1	3	1
25	1000	76.0	3.6	3	1
26	2500	86.7	3.1	3	1
27	5000	101.3	7.1	3	1
42	0	64.0	15.1	3	2
43	33	62.3	5.0	3	2
44	100	69.7	4.7	3	2
45	333	78.3	6.0	3	2
46	1000	77.7	7.6	3	2
47	2500	89.3	8.0	3	2
48	5000	122.3	17.8	3	2

Although the increase in revertant counts over controls did not reach the 2- or 3-fold trigger applied by most laboratories, the internal historical control range was exceeded (according to the full report) at least for TA 1535 and there was a clear dose-dependency allowing for benchmark dose calculations using the Bayesian EFSA Webtool (version 0.1.17):

Table 3 Benchmark dose modelling (BMD) calculations with BMDL-benchmark dose lower limit and BMDU-benchmark dose upper limit

BMR=50 %	BMDL (µg/plate)	BMD	BMDU (µg/plate)	Ratio BMDU/BMDL
TA1535 Exp.1	542	1953	7306	13,5
TA1535 Exp.2	980	1593	2555	2,6
TA100 Exp.1	3812	4395	4939	1,3
TA100 Exp.2	2316	3509	5513	2,4
E.coli Exp.1	3769	5088	6769	1,8
E.coli Exp.2	2806	3528	4398	1,6

This weak positive result is in line with historic reports for Ames tests using 2-CE as discussed in previous BfR opinions on the subject matter.

The *in vitro* mammalian cell gene mutation test (ASB2024-2781) was performed in V79 cells in the presence and absence of S9-mix. The test substance 2-CE was used in an appropriate non-cytotoxic dose range (50.4-806 µg/mL) together with the corresponding positive- and vehicle controls. No biologically relevant increases in mutant frequency were observed, neither with nor without metabolic activation. The positive controls showed distinct increases, confirming the performance of the test system. The study is considered negative-acceptable.

The i*n vitro* mammalian cell micronucleus test (ASB2024-5121) was performed in primary human lymphocytes in the presence and absence of S9-mix. The test substance 2-CE was used in an appropriate non-cytotoxic dose range (477-806 µg/mL) together with the corresponding positive- and vehicle controls. No biologically relevant increases in micronuclei numbers were observed, neither with nor without metabolic activation. The positive controls showed distinct increases, confirming the performance of the test system. The test is considered negative-acceptable.

3.4.3 General Toxicity

In the context of dietary risk assessment, only studies with oral exposure to 2-CE were considered. Overall, five toxicity studies were regarded as relevant for the derivation of health-based guidance values. None of the studies was, based on the limited information available, performed under Good Laboratory Practice (GLP) and/or following OECD test guidelines. Of these, one represented a meta-analysis of human case reports (Deng *et al.*, 2001), while the other four were repeated dose or 2-generation *in vivo* animal studies.

Human data

In Deng *et al*. (2001), 17 cases (between 2-70 years old) were analysed that were exposed to 2-CE either suicidal (5 cases), accidental (9 cases) or occupationally (3 cases). These patients were orally exposed to a single dose in the range between 83 and 9900 mg/kg bw. Following exposure, the majority of patients (16 out of 17) developed the first symptoms within 2 h. In the further course, the symptoms were categorised in:

- fatal/severe effects: metabolic acidosis, respiratory failure, shock, and/or coma, death
- mild/moderate effects: gastrointestinal-, cardiovascular-, respiratory-, or neurologic-effects, and sore throat/oral discomfort, dizziness, chest tightness, transient hypertension, chilliness, hypokalemia, impaired renal function

Mild-to-moderate effects were reported from the dose of 83 mg/kg bw, while severe effects were noted at and above 412 mg/kg bw, indicating a steep dose-response relationship. For the respective effects categories, the authors calculated mean doses of 2427 mg/kg for fatal/severe effects and 91 mg/kg for mild/moderate effects.

Table 4 Summary of the chronic oral toxicity studies including the species, exposure duration, rout of application, dose, effects and the corresponding No Observed Adverse Effect Level (NOAEL) and Lowest Observed Adverse Effect Level (LOAEL)

Species, Duration, Route of application	Dose [mg/kg bw/d]	Effects	NOAEL/ LOAEL [mg/kg bw/d]	Reference
Beagle Dog (4/sex/dose), 15 weeks, Diet	0, 13.3, 18.4 and 18.3 in males and 0, 16.9, 20.3 and 19.3 in females	Mid and high dose group: Severe vomiting (within hours after ingestion) without histopathological correlate in the stomach; Body weight ↓; Transient haemoglobin haematocrit↓ Doses freshly prepared.	Male: 13.3/18.3 Female: 16.9/20.3	Oser et al. 1975 Key study (published)
Rat (FDRL, 25/sex/dose), 12 weeks, diet and gavage	0, 30, 45 and 67.5	Changed dosing regimen to gavage from week 6 due to instability of the test substance in the diet 67.5 mg/kg bw: Mortality of 17/25 (male) and 19/25 (female) (within 3 weeks); Body weight gain ↓ All decedents: Relative organ weight↑ (all organs, no details given), Dark liver, red GI tissues and lungs	45/67.5 (both sexes)	
Monkey (2/sex/dose), 12 weeks, Bolus	0, 30, 45 and 62.5	Body weight ↓ (Dose not given)	Not established	
Rat (strain/number unspecified), 2-generation, Diet reliability not assignable	Males: 0, 27.3, 82.4 and 160.6 Females: 0, 31.3, 95.8, 209.6	Parental effects (male): Body weight↓; Liver weight↑; Spleen, kidney, and adrenal gland weight↓ Parental (female): Body weight↓; Ovary, uterus/cervix/oviduct, adrenal gland, pituitary, spleen and kidney weight ↓	Parental (male): 82.4/160.6 Parental (female): 95.8/209.6 Offspring: 82.4/160.6 Reproductive: 95.8/209.6	US-EPA 2020 (study summary only)

Species, Duration, Route of application	Dose [mg/kg bw/d]	Effects	NOAEL/ LOAEL [mg/kg bw/d]	Reference
		Offspring (male/female): Bodyweight↓; Spleen and thymus weight ↑; Incidence of runts↑ Reproductive: total number of follicular counts ↓; ovary/uterus/cervix/oviduct weights ↓; delayed sexual maturation Test item stability in diet not reported.		
Mouse (CD-1, 10-16 /dose), GD6-GD16 (prenatal), Gavage	50, 100, 150	High dose: 75 % mortality (after 2-4 treatments), surviving 25 % not-pregnant Mid dose: maternal weight gain ↓ fetal findings: fetal body weight gain ↓; fetal liver weight gain ↓; bilateral 14th rib	50/100 (both maternal and developmental)	Courtney et al. 1982
Mouse (CD-1, 10-16 /dose), GD6-GD16 (prenatal), Drinking water	16, 43, 77, 227	No mortality No dose-related effects neither on dams nor on foetuses reported. Test item stability in water not reported.	227/Not established	
Rat (male, strain not specified, 5/dose), 220 days, Diet	0, 9, 18, 36, 72, 108, 144, 216	>108 mg/kg: bodyweight↓ >144 mg/kg: Food consumption↓ Test item stability in diet not reported.	72/108	Ambrose, 1950

In vivo animal data

Four reports describe *in vivo* animal studies in rats, mice, dogs and monkeys. Individual animal data was not available for any of these. One report represents secondary literature, i.e. a summary in US EPA, 2020 and does not contain sufficient study detail.

The only study that has documented verification of the dose in the diet is the rat study by Oser *et al.* (1975). Notably, it was found that 2-CE is not stable in the diet and a gavage administration was required. From this point of view, diet studies that did not document any dose-related effects were not considered as sufficiently reliable. This includes the mouse drinking water studies by Courtney *et al.* (1982) and the monkey study of Oser *et al.* (1975). Dietary studies without dose verification but showing a treatment related effect may still be suitable for hazard identification, but are severely compromised with regard to the quantitative assessment (Ambrose, 1950).

Also, studies in which the Lowest Observed Adverse Effect Level (LOAEL) is based on lethality are not considered suitable for derivation of a reference value due to the severe nature of the effect.

This selection process leaves the dog study by Oser *et al.* (1975) for reference value derivation, while the rat study by Ambrose (1950) is severely compromised due to lack of dose verification. Also, based on the reported NOAELs/LOAELs, the male dog seems to be the most sensitive species with a NOAEL of 13.3 mg/kg. While it may be questioned whether the vomiting in dogs reported by Oser *et al.* (1975) represents a relevant systemic effect, it is noted that Martis *et al.* (1982) did describe emesis also after intravenous application of 46 mg/kg bw to dogs.

In principle, the database could be enriched by grouping and read-across of information from structurally related substances such as propylene chlorohydrin. However, based on the information currently available, this read-across hypothesis would not be supported. For example, the target substance 2-CE showed lethal effects in various repeated dose studies while such was not reported for the potential source substance propylene chlorohydrin even at higher doses.

3.5 Derivation of the acute and chronic oral reference values

Acute Reference Dose (ARfD)

With regard to the acute nature of some of the effects reported, the derivation of an acute reference value is warranted. In the retrospective case report study by Deng *et al.* (2001), first symptoms appeared within two hours for the majority of poisoning cases. Acute effects were also reported by Oser *et al.* (1975) in dogs.

The rationale for using the NOAEL of 13.3 mg/kg from the dog study by Oser *et al.* (1975) to derive a provisional ARfD is based on:

- dose verification
- dog being the most sensitive species is in the current dataset
- severe vomiting as acute effects occurring confirmed in an in vivo dog study
- original report (publication) available for review

Using a default uncertainty factor (UF = 100) for intra- and interspecies, the provisional ARfD is set at 0.13 mg/kg.

The provisional ARfD of 0.13 mg/kg bw provides a margin of 500 over the lowest lethal dose in animals and approx. 600 over the lowest reported dose with mild effects in humans. Mortality was reported in Courtney *et al*. (1982) for mice at 150 mg/kg bw and Oser *et al.* (1975) for rat from 67.5 mg/kg bw. While in Courtney *et al.* (1982), mortality occurred from day 2-4 of treatment, death was reported in Oser *et al.* (1975) at unspecified time points during a period of 3 weeks.

Note: US-EPA (2012) in their provisional assessment recommend a similar acute reference value of 0.1 mg/kg bw based on lack of mortality at 100 mg/kg bw in the study of Courtney *et al. (1982)* and an UF of 1000. In the re-registration assessment for ethylene oxide (US EPA, 2020), the reliability of Oser *et al.* (1975) was questioned and a higher value was proposed.

Acceptable Daily Intake (ADI)

Based on the rationale described above, the NOAEL of 13.3 mg/kg for the male dogs in Oser *et al.* (1975) is also chosen as the most appropriate starting point for derivation of the ADI. In analogy to the COM guidance document SANCO/221/2000 rev. 1, compensation for differences in exposure duration and the limited database can be achieved by application of an UF of 1000, resulting in an ADI of 0.013 mg/kg. However, in view of the additional data available for 2-chloroethanol, the use of specific factors for extrapolation from sub-chronic to chronic exposure and the limitations in the quality of the available data of the documents D2 and D3 (see page 5), respectively, was considered more appropriate. Thus, an ADI of 0.02 mg/kg bw/d is proposed.

Note: In 2012, the US-EPA derived an identical provisional chronic dietary reference value (cRfD) of 0.02 mg/kg/day based on the rat data from the publication of Oser *et al.* (1975) using an UF of 3000. In their re-registration assessment for ethylene oxide, the same study was regarded as unacceptable due to a number of deficiencies, such as lack of information on test item and individual animal data (US-EPA, 2020). The chronic reference value was then set to 0.824 mg/kg bw/d on the basis of the parental and offspring effects in the 2-generation study from 0.02 mg/kg/day using a Point of departure (PoD) of 82.4 mg/kg bw/d and a default uncertainty factor of 100, not accounting for duration of exposure or quality of the database. This latter value is not supported, in particular based on lack of dose verification, choice of the uncertainty factor and lack of access to study data.

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