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Committee for Medicinal Products for Human Use

ICH guideline M7 on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk - addendum

Step 2b

Transmission to CHMP	16 September 2021
Adoption by CHMP	16 September 2021
Release for public consultation	08 October 2021
Deadline for comments	08 December 2021

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INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL
REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE (ICH)

ICH HARMONISED GUIDELINE

**APPLICATION OF THE PRINCIPLES OF THE ICH M7 GUIDELINE TO
CALCULATION OF COMPOUND-SPECIFIC ACCEPTABLE INTAKES**

Addendum to M7(R2)

Draft version

Endorsed on 6 October 2021

Currently under public consultation

Note: This document contains only the list of the revisions to the M7(R1) Guideline as well as the new monographs for the 7 new compounds Acetaldehyde, Dibromoethane, Epichlorohydrin, Ethyl Bromide, Formaldehyde, Styrene, and Vinyl Acetate, which are submitted for public consultation. Further to reaching *Step 4*, these revisions would be integrated into a complete M7(R2) Guideline and Addendum documents.

At Step 2 of the ICH Process, a consensus draft text or guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Assembly to the regulatory authorities of the ICH regions for internal and external consultation, according to national or regional procedures.

Addendum to M7(R2)
Document History

Current *Step 2* version

M7(R2) Addendum	Endorsement by the Members of the ICH Assembly under <i>Step 2</i> and release for public consultation	6 October 2021
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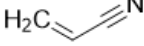
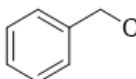
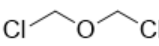
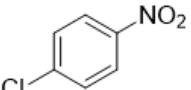
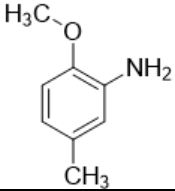
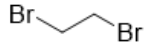
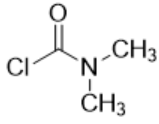
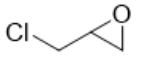
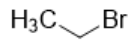
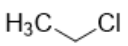
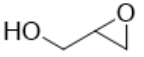
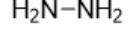
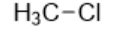
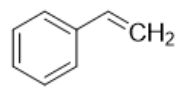
1 **List of changes to the M7 Guideline and Addendum in line with the ICH process for the**
2 **maintenance of the M7 Guideline:**

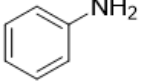
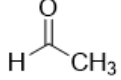

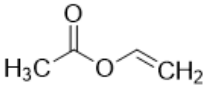
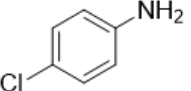
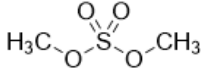
- 3 1. The M7 document was physically separated into a main Guideline and a separate Addendum
4 including the monographs;
- 5 2. In the main M7 Guideline, the HIV duration was changed from “>1-10 years to >10 years” to
6 “lifetime”;
- 7 3. In the main M7 Guideline, the monograph table was edited to include the 7 new monographs
8 and 1 note;
- 9 4. In the Addendum, 7 new monographs and 1 note were added (see pages 4-51 of this
10 document):
- 11 a. Acetaldehyde, Dibromoethane, Epichlorohydrin, Ethyl Bromide, Formaldehyde,
12 Styrene, Vinyl Acetate;
- 13 b. Note 2;
- 14 5. In the main M7 Guideline and Addendum, standard grammatical and formatting edits were
15 made;
- 16 6. In the main M7 Guideline and Addendum, additional corrections in content were made that
17 were determined to be minor by the M7(R2) Maintenance Expert Working Group.

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Acceptable Intakes (AIs) or Permissible Daily Exposures (PDEs)

Compound	CAS#	Chemical Structure	AI or PDE (µg/day)	Comment
Linear extrapolation from TD₅₀				
Acrylonitrile	107-13-1		6	TD ₅₀ linear extrapolation
Benzyl chloride	100-44-7		41	TD ₅₀ linear extrapolation
Bis(chloromethyl)ether	542-88-1		0.004	TD ₅₀ linear extrapolation
1-Chloro-4-nitrobenzene	100-00-5		117	TD ₅₀ linear extrapolation
<i>p</i> -Cresidine	120-71-8		45	TD ₅₀ linear extrapolation
1,2-Dibromoethane	106-93-4		2	TD ₅₀ linear extrapolation
Dimethylcarbamyl Chloride	79-44-7		0.6 (inhalation)* 5 (all other routes)	TD ₅₀ linear extrapolation
Epichlorohydrin	106-89-8		3	TD ₅₀ linear extrapolation
Ethyl bromide	74-96-4		32	TD ₅₀ linear extrapolation
Ethyl chloride	75-00-3		1,810	TD ₅₀ linear extrapolation
Glycidol	556-52-5		4	TD ₅₀ linear extrapolation
Hydrazine	302-01-2		0.2 (inhalation)* 39 (all other routes)	TD ₅₀ linear extrapolation
Methyl Chloride	74-87-3		1,361	TD ₅₀ linear extrapolation
Styrene	100-42-5		154	TD ₅₀ linear extrapolation
Threshold-based PDE				

Aniline Aniline HCl	62-53-3 142-04-1		720	PDE based on threshold mode of action (hemosiderosis)
Endogenous and/or Environmental Exposure				
Acetaldehyde	75-07-0		2,000 (oral)* 185 (all other routes)	Oral PDE is based on average food intake; all other routes based on TD ₅₀ linear extrapolation from an inhalation study
Formaldehyde	50-00-0		8,000 or 215 ppb, whichever is lower (inhalation)* 10,000 (all other routes)	Inhalation route based on TD ₅₀ linear extrapolation or local irritation; all other routes based on average food intake
Hydrogen peroxide	7722-84-1	HO-OH	68,000 or 0.5%, whichever is lower	68 mg/day is 1% of estimated endogenous production
Vinyl acetate	108-05-4		2,000 (oral)* 758 (all other routes)	Oral PDE is based on average food intake for acetaldehyde; all other routes based on TD ₅₀ linear extrapolation from an inhalation study
Other Cases				
<i>p</i> -Chloroaniline <i>p</i> -Chloroaniline HCl	106-47-8 20265-96-7		34	AI based on liver tumors for which mutagenic mode of action cannot be ruled out
Dimethyl Sulfate	77-78-1		1.5	Carcinogenicity data available, but inadequate to derive AI. Default to TTC

* route specific limit

Acetaldehyde (CAS# 75-07-0)

Potential for human exposure

Acetaldehyde is formed endogenously in the human body from the metabolism of ethanol and carbohydrates as well as from bacteria in the alimentary tract. Humans are exposed to acetaldehyde mainly in food, alcoholic beverages, cigarette smoke and to a lesser extent from environmental emissions (Ref. 1, 2). The determination of endogenous acetaldehyde in blood, breath and saliva is challenging as the techniques are prone to artifacts and contaminants (Ref. 3, 4). Nevertheless, a daily endogenous production of 360 mg/day of acetaldehyde was calculated based on a constant endogenous total acetaldehyde concentration in the blood of $2.2 \pm 1.1 \mu\text{mol/L}$ (Ref. 3) and acetaldehyde clearance of 0.95 L/min (Ref. 5). Average acetaldehyde consumption of up to 48 mg/day comes from consumption of alcoholic beverages (Ref. 6). Endogenous acetaldehyde concentrations and the associated cancer risk are significantly higher in individuals with an ALDH II genetic polymorphism (Ref. 7). The exogenous exposure from food (without alcoholic beverages or added acetaldehyde as flavoring agent) was estimated to be around 2 mg/day on average and 8 mg/day for the upper 95% of the German population (Ref. 8), JECFA estimated food additive consumption to be 9.7 mg/day in the USA and 11 mg/day in Europe although this estimate is restricted to consumers who eat foods in which acetaldehyde is added as a flavor (Ref. 9) and the Japanese Food Safety Committee estimated domestic consumption between 9.6 – 19.2 mg/day (Ref. 10). Acetaldehyde is used in synthesis of pharmaceuticals.

Mutagenicity/genotoxicity

The genotoxicity of acetaldehyde has been previously reviewed by the Chemical Evaluation and Research Institute and others (Ref. 1, 5, 11-18). Acetaldehyde was negative in comprehensive bacterial Ames reverse mutation assays, but induced increases in mutations at the hypoxanthine-guanine-phosphoribosyl transferase (*hprt*) locus in mammalian cells, which included point mutations demonstrated by sequencing (Ref. 13). DNA- and DNA-protein adducts were observed in cultured cells treated with acetaldehyde and DNA adducts were measured in urine of healthy volunteers and in blood cells from persons who abuse alcohol. Acetaldehyde is primarily an inducer of larger scale chromosomal effects. It induces chromosomal aberrations and micronuclei *in vitro* and was positive in the mouse lymphoma L5178Y *tk*^{+/-} assay. Acetaldehyde induced increases in micronuclei in the bone marrow of rats and mice.

Carcinogenicity

Acetaldehyde is an IARC 2B carcinogen and “acetaldehyde associated with the consumption of alcoholic beverages” is an IARC 1 carcinogen, i.e. “carcinogenic to humans.” Acetaldehyde was carcinogenic in rats and hamsters after inhalation exposure (Ref. 1).

In humans, acetaldehyde is the primary metabolite of alcohol and high as well as low alcohol consumption has been correlated with an increased relative risk for certain human cancers (e.g. oral cavity, pharynx cancer and breast cancer) (Ref. 19, 20). The relative risk was increased in smokers showing a tobacco-alcohol synergism and a possible contribution of acetaldehyde derived from cigarette smoke (Ref. 19). Also, geographical regions with consumption of alcoholic beverages containing high acetaldehyde concentrations showed a tendency for higher incidence of squamous-cell cancer and cancer of the esophagus (Ref. 21). Furthermore, available epidemiological data indicate that there is an increased risk for development of alcohol-related cancers for those individuals who are deficient in detoxifying acetaldehyde to acetate by ALDH. Especially the genetic variant ALDH2*1/*2 is strongly associated with alcohol-related cancers in not only heavy drinkers but those with moderate levels of alcohol consumption (Ref. 1, 5, 19).

72 Meta analyses and large cohort studies report conflicting conclusions about whether there are
73 increased risks of head, neck and mammary tumors associated with moderate alcohol consumption
74 in the U.S. populations where ALDH deficiency is relatively infrequent (Ref. 22, 23). The
75 literature on the elevated risk of head and neck cancers associated with acetaldehyde exposure in
76 heavy drinkers, smokers, and in moderate drinkers with ALDH deficiency does not include
77 discussion of whether those exposures are also associated with histopathological changes
78 consistent with irritation or tissue proliferation.

79
80 In rodents, only inhalation carcinogenicity studies are available in the Carcinogenic Potency
81 Database (CPDB) (Ref. 24). The most robust study was conducted with Wistar rats (Ref. 25) with
82 whole-body inhalation exposure to 0, 750, 1500 or 3000/1000 ppm (reduced after 11 months due
83 to toxicity), 6 h/day at 5 days/week for up to 28 months. The doses shown in the CPDB were 0,
84 70.8, 142 and 147 mg/kg for male rats and 0, 101, 202 and 209 mg/kg for female rats. In the high-
85 dose group, 50% of the male and 42% of the female animals had died by week 67 and no high-
86 dose animals were alive by week 102. An increased incidence of tumors at the site of contact, i.e.
87 nasal squamous cell carcinomas, was observed in males (1/49, 1/52, 10/53 and 15/49
88 corresponding to control, low, mid and high dose groups) and females (0/50, 0/48, 5/53 and 17/53,
89 respectively) at the end of the study. There were also increases in nasal adenocarcinomas at all
90 doses, the incidences were 0/49, 16/52, 31/53 and 21/49 in males and 0/50, 6/48, 26/53 and 21/53
91 in females, respectively. Based on these data, the TD₅₀ value shown in the CPDB was estimated
92 to be 185 mg/kg for nasal adenocarcinoma in male rats in the most sensitive sex and tissue.

93
94 An oral carcinogenicity study (Ref. 26) was conducted in Sprague Dawley rats with acetaldehyde
95 administration in drinking water. In this study, 50 rats per group were given 0, 50, 250, 500, 1500
96 and 2500 mg/L acetaldehyde in drinking water for 104 weeks and the experiment was terminated
97 when the last animal died at 161 weeks of age. The concentrations correspond to 0, 5, 25, 49, 147
98 and 246 mg/kg/day for male rats and 0, 5, 27, 53, 155 and 260 mg/kg/day for female rats,
99 respectively. Incidences of adenocarcinomas, lymphomas and leukemias, mammary tumors, and
100 cranial osteosarcomas, were described by the investigators as significantly greater in at least one
101 group of exposed rats, relative to control. There was no increase in malignant tumors at the site of
102 contact organs, i.e. the oral cavity and gastrointestinal tract, or in the liver.

103
104 This study suggests that acetaldehyde may be carcinogenic after intake via drinking water.
105 However, there was no clear dose-response relationship and therefore, many evaluators found that
106 no clear conclusion can be drawn from this study (Ref. 5, 12, 19). In another evaluation of the
107 same data, two different dose-response models were used to estimate cancer potency and the
108 authors concluded that their quantitative risk assessment indicates the need to lower acetaldehyde
109 exposure in the general population but also acknowledged that naturally occurring acetaldehyde
110 cannot be reduced (Ref. 21). In this model, the carcinogenic potency was calculated for all tumor
111 bearing animals because the authors found that there was insufficient statistical power to generate
112 a model for any specific cancer site. A TD₅₀ related to oral administration of acetaldehyde was
113 not calculated.

114

115

116 **Acetaldehyde – Details of carcinogenicity studies**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
Ref. 26	50/sex/ group Sprague Dawley rat	24 months, drinking water	50	5: M: 5, 25, 49, 147 and 246 mg/kg/d F: 5, 27, 53, 155 and 260 mg/kg/d	Not identifiable	NC ^a
Ref. 25	55/sex/ group Wistar rat	28 months, Inhalation	55	3: M: 70.8, 142, 147 mg/kg/d F: 101, 202, 209 mg/kg/d	Male Nasal adenocarcinoma	185 ^b
Ref. 27	30/sex/ group Syrian golden hamster	52 weeks, Inhalation	30	1: M: 344 mg/kg/d, F: 391 mg/kg/d	Male Larynx	461 ^c

117 Studies listed are in Cancer Potency Database (CPDB) (Ref. 24)

118 NC = not calculated;

119 ^a Not in CPDB and given the lack of dose-response and insufficient statistical power no TD₅₀ was
120 calculated.

121 ^b TD₅₀ taken from the CPDB

122 ^c In CPDB but not used in evaluation because of small group size and single treatment group.

123

124 **Mode of action for carcinogenicity**

125 Acetaldehyde is a strong electrophile and is capable of reacting with strong nucleophiles, for
126 example DNA bases or amino acid residues on proteins. Although not mutagenic in the standard
127 bacterial reversion assay, evidence for DNA-reactivity and mutagenicity was shown for
128 acetaldehyde by the presence of DNA and DNA-protein adducts *in vitro* and *in vivo*, as well as
129 the positive result in the *in vitro* *hprt* mutagenicity assay in mammalian cells. Despite its reactive
130 nature, there is evidence for a non-linear dose response associated with the genotoxicity and
131 carcinogenicity of acetaldehyde (Ref. 14). The dose-response of acetaldehyde-induced adducts at
132 concentrations between 1 and 1000 uM has been measured in a cell culture system allowing the
133 discrimination between endogenous and exogenous adducts induced by added acetaldehyde.
134 These concentrations are comparable to salivary acetaldehyde concentrations measured before
135 and after consumption of beverages containing alcohol with or without acetaldehyde (Ref. 28, 29).
136 The exogenous adducts only exceeded the endogenous background level of adducts above a
137 critical concentration.

138 Aldehyde hydrogenase (ALDH), which efficiently detoxifies acetaldehyde, is responsible for the
139 non-linear dose response relationship. ALDH enzymes are expressed in the mitochondria and
140 cytosol of most tissues (e.g., liver, gastrointestinal tract, kidneys, nasal epithelium/olfactory
141 epithelium, lung) and they metabolize acetaldehyde to form acetate and one proton (Ref. 30). The

142 release of protons can reduce cellular pH and thus cause non-specific cytotoxicity with subsequent
143 proliferative effects. The importance of detoxification is shown in ALDH deficient animal models.
144 For example, acetaldehyde induced chromosome damage and mutation is observed in mice
145 deficient in ALDH2 activity following inhalation and oral (gavage) exposure, but not in ALDH2-
146 proficient mice (Ref. 31). Similarly, more acetaldehyde derived DNA adducts were seen in
147 alcoholics with a deficient aldehyde dehydrogenase genotype (allelic variant type ALDH2*1/2*2
148 with about 10% residual ALDH activity) compared to those with efficient genotype
149 ALDH2*1/2*1 (Ref. 32) and moderate drinkers with the genotype are at increased risk of head
150 and neck cancers (IARC).

151 The inhalation carcinogenicity data and mechanistic study data suggest that acetaldehyde cancer
152 risk is highest at and possibly limited to the site-of-contact. The nasal tumors in inhalation
153 carcinogenicity studies were only found at inhalation doses also associated with cytotoxicity and
154 severe irritation causing regenerative proliferation consistent with the hypothesis that there could
155 be promotion of growth of mutated cells (Ref. 5, 14). Detoxification of acetaldehyde by ALDH,
156 in airway cells may make tumor induction less likely at lower, non-irritating doses. However,
157 there are no published measurements which would allow discrimination between the irritating
158 effect and the potential mutagenic effect in cancer development.

159

160 **Regulatory and/or published limits**

161 Acetaldehyde is listed in the US Food and Drug Administration's (FDA's) 'generally recognized
162 as safe' (GRAS) list for flavoring substances and adjuvants – 21 CFR 182.60. The Japanese FSC
163 confirmed the absence of safety concerns when used as a flavoring agent as it is completely
164 metabolized into non-reactive acetic acid and finally CO₂, and thus, its level as a flavoring agent
165 is presumed not to exceed the physiological range (Ref. 10). The Joint FAO/WHO Expert
166 Committee on Food Additives (JECFA) evaluation has concluded that there are no safety concerns
167 at current levels of intake when used as a flavoring agent, which was 11 mg/day in Europe and
168 9.7 mg/day in the United States (Ref. 9).

169 The Committee on Emergency and Continuous Exposure Guidance Levels for Selected
170 Submarine Contaminants (Ref. 33) recommended a Continuous Exposure Guidance Level
171 (CEGL) of 2 ppm corresponding to 3.6 mg/m³. This represents an exposure of 3.6 mg/m³ x 28.8
172 m³ (24 hours in a day – ICH Q3C assumption) = 104 mg/day.

173 The US EPA did not consider a threshold for acetaldehyde carcinogenicity and has calculated that
174 a concentration of 5 µg/m³ acetaldehyde represents a 10⁻⁵ excess lifetime cancer risk based on the
175 rat inhalation carcinogenicity study and application of linear extrapolation (Ref. 34). For a 24 h
176 exposure, this represents 5 µg/m³ x 28.8 m³ = 144 µg/day. EPA did not consider the risk via the
177 oral route.

178

179 **Permissible Daily Exposure (PDE) for oral exposure**

180 Rationale for selection of study for PDE calculation

181 Given the weight of evidence for a non-linear dose-response for the carcinogenicity of
182 acetaldehyde following oral administration and high background exposure from a wide variety of
183 foods, a permissible daily exposure (PDE) of 2 mg/day is identified for oral limit based on the
184 estimated average intake of acetaldehyde from food around 2 mg/day (Ref. 8).

185

186 **PDE (oral) = 2 mg/day**

187

188

189 **Acceptable intake (AI) for all other routes**

190 Rationale for selection of study for AI calculation

191 The inhalation study in rats by Woutersen et al. (Ref. 25) was used to derive the AI for all other
192 routes. This study comprises group sizes of 50/sex/dose and animals were treated for life time (i.e.,
193 24 months). According to M7's recommendations for selecting the most relevant study for
194 deriving an AI, this is considered the most appropriate and robust study available for acetaldehyde.
195 The inhalation carcinogenicity data and mechanistic study data suggest acetaldehyde cancer risk
196 to be associated with cytotoxicity at the site of contact as nasal tumors were only found at doses
197 also associated with cytotoxicity and severe irritation causing regenerative proliferation a
198 promotion of growth of mutated cells.

199

200 **Calculation of AI**

201 Lifetime AI = $TD_{50}/50000 \times 50 \text{ kg}$

202

203 Lifetime AI = $185 \text{ mg/kg/day}/50000 \times 50 \text{ kg}$

204

205 **Lifetime AI (all other routes) = 185 µg/day**

206

207

208 **References**

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1,2-Dibromoethane (CAS# 106-93-4)

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Potential for human exposure

1,2-Dibromoethane was previously used as an insect fumigant and soil nematocide but was banned by the U.S. EPA and the EC due to toxicity concerns (Ref. 1, 2). 1,2-Dibromoethane is used in the synthesis of active pharmaceutical ingredients.

Mutagenicity/genotoxicity

1,2-Dibromoethane is mutagenic/genotoxic *in vitro* and *in vivo*. The mutagenicity of 1,2-dibromoethane was evaluated in *Salmonella* tester strains TA 1535, TA 1537, TA 98, TA 100, TA 1538 and in *E. coli* WP2, both in the presence and absence of added metabolic activation by Aroclor-induced rat liver S9 fraction (Ref. 3-7). 1,2-Dibromoethane was mutagenic in *Salmonella typhimurium* strains TA 100, TA 1535, TA 98 and *E. coli* WP2, with and without metabolic activation. 1,2-Dibromoethane was positive in the mouse lymphoma assay, with and without metabolic activation (Ref. 8). It caused a dose-dependent increase in DNA repair in both spermatocytes and hepatocytes *in vitro* (Ref. 9) and induced mutations in Chinese hamster ovary (CHO) cells (Ref. 10). 1,2-Dibromoethane increased the frequencies of chromosome aberrations in a dose-dependent manner in CHO cells (Ref. 11). *In vivo* in the Comet assay in rats, positive results were obtained in liver and glandular stomach following treatment with 1,2-dibromoethane at 100 mg/kg. 1,2-Dibromoethane was negative in the bone marrow and erythrocyte micronucleus test in rats when tested up to 100 mg/kg (Ref. 12). At this dose, a 7% body weight reduction and 25 % reduction in immature erythrocytes was observed indicating slight to moderate toxicity.

Carcinogenicity

1,2-Dibromoethane is classified by IARC as probably carcinogenic to humans (Group 2A) (Ref. 13). Inhalation and oral carcinogenicity studies are cited in CPDB (Ref. 14). 1,2-Dibromoethane was carcinogenic following both routes of administration in male and female rats and mice (Table 1). The most sensitive tumor sites were forestomach following oral administration (gavage or drinking water) and nasal cavity following inhalation. Other tumor sites include, blood vessels, lung, liver and mammary glands. There was more than one positive experiment in both species.

1,2-Dibromoethane – Details of carcinogenicity studies

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses*	Most sensitive tumor site/type/sex	TD ₅₀ (mg/kg/d) *
Ref. 16	30/sex/ group B6C3F1 mice	M: 65 weeks F: 73 weeks, drinking water	50	1: 4 mmol M: 116 mg/kg/d F: 103 mg/kg/d	Squamous carcinoma of forestomach	11.8
Ref. 17	50/sex/ group B6C3F1 mice	78 weeks, drinking water	100	1: M: 1.4 mg F: 1.2 mg	Forestomach papilloma	9.44

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses*	Most sensitive tumor site/type/sex	TD ₅₀ (mg/kg/d) *
Ref. 18	50/sex/ group B6C3F1 mice	53 weeks, gavage	20	2: M: 26, 52 mg/kg/d F: 30, 53 mg/kg/d	Squamous-cell carcinomas of forestomach	2.36
Ref. 18	50/sex/ group Osborne- Mendel rats	M: 40 weeks F: 50 weeks, gavage	20	2: M: 27.4, 29.2 mg/kg/d F: 26.7, 28.1 mg/kg/d	Squamous-cell carcinomas of forestomach	1.26
Ref. 19	50/sex/ group B6C3F1 mice	M: 78 weeks, F: 96 weeks, inhalation	50	2: M: 19.9, 79.5 mg/kg/d F: 23.9, 95.6 mg/kg/d	Alveolar/bronch iolar carcinomas and adenomas	18.2
Ref. 19	50/sex/ group F344 rats	M: 95 weeks F: 97 weeks, inhalation	50	2: M: 4, 15.9 mg/kg/d F: 5.71, 22.8 mg/kg/d	Carcinomas, adenocarcinoma s, adenomas of nasal cavity	2.33
Ref. 20	48/sex/ group Sprague- Dawley rats	78 weeks, inhalation	48	1: M: 9.39 mg/kg/d F: 13.4 mg/kg/d	Nasal cavity	1.19
Ref. 21	50/sex/ group B6C3F1 mice	103 weeks (10 ppm) / 90 weeks (40 ppm), inhalation	50	2: 10, 40 ppm for 6 h/d, 5 d/wk	Focal epithelial hyperplasia	Not available

338 * mg/kg/d values stated in CPDB (Ref. 14) and calculated by method used to standardize average daily
339 dose levels from variety of routes of administration, dosing schedules, species, strains and sexes; values
340 stated in CPDB accounted for exposure duration of 24 h per day for 7 days per week. (Dose rate =
341 (administered dose × intake/day × number of doses/week) / (animal weight × 7 days/week))

342 * Individual TD₅₀ values are the CPDB TD₅₀ values as reported in the Lhasa carcinogenicity database
343 (Ref. 15). TD₅₀ values represent the TD₅₀ from the most sensitive tumor site.
344

345 Mode of action for carcinogenicity

346 1,2-Dibromoethane is a mutagenic carcinogen, which is expected to be mutagenic based on an
347 alkylating mechanism of action. Therefore, the acceptable intake can be calculated by linear
348 extrapolation from the TD₅₀. The tumor types with the lowest calculated TD₅₀ (highest potency)
349 for 1,2-dibromoethane following oral exposure are forestomach tumors in mice and rats (Ref 18).
350 Following inhalation exposure, the lowest calculated TD₅₀ values are associated with the lung and

351 nasal cavity for mice and rats, respectively. High concentrations of orally dosed non-mutagenic
352 chemicals have been shown to cause inflammation and irritation after contact with the
353 forestomach leading to hyperplasia and ultimately tumors. Substances that are dosed by gavage
354 can remain for some time in the rodent forestomach before discharge to the glandular stomach, in
355 contrast to the rapid passage through the human esophagus. Hence, such tumor induction is
356 considered not relevant to humans at non-irritating doses (Ref. 22, 23). The same inflammatory
357 and hyperplastic effects are also seen with mutagenic chemicals. However, in the case of 1,2-
358 dibromoethane, which is a directly DNA reactive alkylating agent and reported multi-site, multi-
359 species carcinogen, it is difficult to discriminate between the contribution to mode of action of
360 these non-mutagenic, high-dose effects compared with direct mutation induction.

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362 **Regulatory and/or published limits**

363 No regulatory limits have been published.

364

365 **Acceptable intake (AI)**

366 Rationale for selection of study for AI calculation

367 1,2-Dibromoethane is a mutagenic carcinogen via the inhalation and oral routes of exposure. 1,2-
368 Dibromoethane is considered to be a carcinogen in both mice and rats. The available toxicological
369 data indicate that absorption of inhaled 1,2-dibromoethane occurs in several animal species. In
370 rats, oral absorption has been shown to be nearly complete within 30 minutes (Ref. 1). Therefore,
371 it can be reasonably assumed that complete systemic exposure to 1,2-dibromoethane occurs
372 following oral and inhalation exposure. This is also supported by the observation of distal tumors
373 in animals exposed to 1,2-dibromoethane by both routes of exposure. TD₅₀ values tend to be
374 similar across species and route of administration.

375

376 The most appropriate and robust carcinogenicity data for derivation of an AI is the inhalation
377 study conducted by the NTP (Ref. 19) in F344 rats. This study (duration 95 weeks in males and
378 97 weeks in females) included two test article treatment groups with adequate dose spacing (M:
379 4, 15.9 mg/kg/d, F: 5.71, 22.8 mg/kg/d with 50 rats/sex/group) and a control group (50/sex).
380 Another study with inhalation exposure conducted in Sprague Dawley rats (Ref. 20) resulted in a
381 lower TD₅₀, however the study comprised only one dose group and only 78 weeks duration and
382 48 animals/dose and therefore was considered inferior to the NTP study with respect to AI
383 calculation. Therefore, the TD₅₀ value for the most sensitive species/sex/site of the most
384 appropriate study is 2.33 mg/kg/d.

385

386 For the oral route of exposure the study in B6C3F1 mice with 1,2-dibromoethane administered by
387 gavage for 53 weeks (Ref. 18) is the most extensive study. This study employed two test article
388 dose groups (50 sex/group) in addition to a control group (20 sex). The TD₅₀ from the most
389 sensitive sex and site is 2.36 mg/kg/day. Another oral study was conducted in Osborne-Mendel
390 rats including two dose groups, however due to insufficient dose spacing (Ref. 18) and less than
391 one year exposure, the study is considered inferior as it limits characterization of the dose-
392 response relationship and estimation of the TD₅₀ (Ref. 18).

393

394 Taking into consideration the carcinogenicity data generated by NTP in both mice and rats, the
395 TD₅₀ for the most sensitive sex/site from the most appropriate study is 2.33 mg/kg/day. This is the
396 TD₅₀ value derived from F344 female rats based on the incidence of nasal cavity tumors (Table
397 1).

398

399 Given that the TD₅₀ values recommended for the derivation of an inhalation AI and an oral AI are
400 very similar (2.33 and 2.36 mg/kg/day, respectively), a single AI for both routes of administration
401 is calculated below using a TD₅₀ value of 2.3 mg/kg/day.

402

403 **Calculation of AI**

404 Lifetime AI = TD₅₀/50000 x 50 kg

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406 Lifetime AI = 2.3 mg/kg/day/50000 x 50 kg

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408 **Lifetime AI = 2 µg/day**

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Epichlorohydrin (CAS# 106-89-8)

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Potential for human exposure

Epichlorohydrin is used in the synthesis of active pharmaceutical ingredients.

Mutagenicity/genotoxicity

The genotoxicity of epichlorohydrin has been reviewed (Ref. 1-3). Epichlorohydrin is mutagenic and genotoxic *in vitro*, with mixed results of genotoxicity tests *in vivo*. While genotoxicity *in vitro* is seen both with and without liver S9 metabolic activation, activity tends to be suppressed by S9 (Ref. 3). Epichlorohydrin is mutagenic in several strains of *Salmonella typhimurium* and in *Escherichia coli* WP2 *uvrA* with and without metabolic activation using both plate incorporation and preincubation protocols (Ref. 4). *In vitro*, epichlorohydrin is positive in mammalian cells for mutation, and for chromosome and DNA damage.

Carcinogenicity

Epichlorohydrin is classified as a Group 2A carcinogen, probably carcinogenic to humans (Ref. 1). Epichlorohydrin is a site-of contact carcinogen, by oral, subcutaneous and inhalation routes.

In an oral study, Wester et al. (Ref. 5) treated rats by oral gavage with epichlorohydrin, 5 times per week for lifetime at 2 and 10 mg/kg, when converted to an average daily dose for 7 days per week, the doses shown in the CPDB (Ref. 6) are 1.43 and 7.14 mg/kg/d, respectively. In the surviving rats at the end of the study, squamous cell carcinomas were found in the forestomachs of all 24 females and 35 of 43 males at the high dose, and in 2 of 27 females and 6 of 43 males at the low dose. The tumors were considered low grade and there was no evidence of metastasis; no increase in tumors was found at other sites. At both dose levels, there were proliferative changes in the forestomach mucosa, in some cases with ulceration and necrosis at the high dose. A TD₅₀ of 2.55 mg/kg/day is reported in the CPDB. The findings are consistent with the squamous cell carcinomas seen in forestomachs of male Wistar rats treated with epichlorohydrin in drinking water for up to 81 weeks (Ref. 7). The Konishi et al. study is not included in the CPDB and not considered in this monograph because of technical deficiencies, and poor condition of the animals.

In an inhalation study, Laskin et al. (Ref. 8) treated male Sprague Dawley rats with epichlorohydrin by inhalation, 6 hours/day, 5 days/week, either for a short-term regimen (30 exposures at 100 ppm) with lifetime observation (140 rats per group), or throughout lifetime at lower doses, 10 and 30 ppm (100 rats per group). After the shorter-term and high dose exposure, squamous cell carcinomas of the nasal cavity in 15/140 rats and respiratory tract papillomas in 3/140 rats were observed associated with severe inflammation in the nasal turbinates, the larynx and the trachea. After lifetime exposure, tumors were seen in 2/100 animals exposed to a dose of 30 ppm and only in the nasal cavity (1 nasal carcinoma and 1 nasal papilloma). Despite the low tumor incidence, a TD₅₀ of 421 mg/kg/day is reported in the CPDB.

In a subcutaneous study, Van Duuren et al. (Ref. 9) found sarcomas at the injection site after subcutaneous injection of epichlorohydrin in mice, but no increase in tumor incidence after dermal application, and weekly i.p. injections for over 64 weeks. Storrer et al. (Ref. 10) injected mice (AJ strain), with total doses of 20, 50 or 100 mg/kg epichlorohydrin given three times per week for eight weeks. There was a significant increase in the number of lung tumors in males treated with the highest dose (0.80 ± 0.68 , compared with 0.47 ± 0.63 in controls; $p < 0.01$), but not in other groups.

Epichlorohydrin – Details of carcinogenicity studies

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD ₅₀ (mg/kg/d)
Ref. 5 ^a	50/sex Wistar rat	104 weeks, Gavage	50	1: 2 and 10 mg/kg	Forestomach; squamous cell carcinomas female	2.55 ^{b,c}
Ref. 8	140 Male Sprague Dawley rat	30 exposures, Inhalation	140	1: 100 ppm	Nasal squamous cell carcinomas	NC ^d
	100 Male Sprague Dawley rat	Lifetime, Inhalation	150	2: 10 and 30 ppm	Nasal squamous cell carcinoma	421 ^b
Ref. 9	50 Female ICR/Ha Swiss mice	61 weeks, SC	150	1: 1 mg/once a week	Injection site sarcomas	NC ^e
Ref. 9	50 Female ICR/Ha Swiss mice	70 weeks, Skin	150	1: 2 mg/ 3 times/ week	No skin papillomas or carcinomas	NC ^e
Ref. 9	50 Female ICR/Ha Swiss mice	64 weeks, IP	130	1: 5.71 mg/ once a week	No tumors (including no injection site sarcomas)	NC ^f
Ref. 7	18/ group Male Wistar rats	81 weeks, Drinking water	yes	3: 375, 750 and 1500 ppm	Forestomach Squamous cell carcinomas	NC ^g

549 NC – Not Calculated, SC – Subcutaneous, IP - Intraperitoneal

550 ^a Carcinogenicity study selected for AI calculation

551 ^b The TD₅₀ values are taken from CPDB (Ref. 6)

552 ^c The TD₅₀ value represents the TD₅₀ from the most sensitive tumor site

553 ^d Not calculated due to short term exposure

554 ^e Not calculated due to limitations of the study design (injection, single dose level, and did not examine all tissues histologically). The skin painting studies showed no increase in skin papillomas or carcinomas.

556 ^f Not calculated: Although TD₅₀ is listed in CPDB, there was no increase in tumors

557 ^g Not calculated because the group size was small, the rats were in poor condition, dosing had to be stopped intermittently, and there was body weight loss in all dose groups

559

560 **Mode of action for carcinogenicity**

561 Epichlorohydrin caused tumors only at the site of contact; forestomach and oral cavity tumors
562 after oral exposure, nasal tumors after inhalation and injection site sarcomas after subcutaneous
563 injection.

564
565 Epichlorohydrin is mutagenic in vitro in bacteria and mammalian cells (Ref. 4). It is highly
566 irritating to the exposed tissues. For example, dose-related lesions of the forestomach were
567 observed in rats given epichlorohydrin by gavage at 3, 7, 19 and 46 mg/kg/day for 10 days, or 1,
568 5 and 25 mg/kg/day for 90 days (Ref. 11). There were a range of inflammatory and epithelial
569 alterations; most pronounced were dose-related increase in mucosal hyperplasia and
570 hyperkeratosis. Data indicate that epichlorohydrin is absorbed, and its metabolites enter systemic
571 circulation; however, tumors are seen only at sites of direct contact. For more details on relevance
572 of forestomach tumors see acrylonitrile and benzyl chloride monographs in the ICH M7
573 Addendum (ICH M7 (R1), 2018).

574

575 **Regulatory and/or published limits**

576 The World Health Organization (Ref. 12) has published a provisional total daily intake of 0.14
577 µg/kg/day or 8.4 µg/day (for a 60 kg adult), based on the assumption of a non-linear dose response
578 for this site-of-contact carcinogen. The US EPA used linear extrapolation to derive a drinking
579 water level (1 in 10⁵ risk of excess cancer) of 30 µg/L or about 60 µg/day (Ref. 13), using data
580 from Konishi et al. (Ref. 7). US EPA also calculated an inhalation concentration of 8 µg/m³ for a
581 1 in 10⁵ excess cancer risk, or 230 µg/day, using ICH Q3C assumptions for human daily breathing
582 volume (Ref. 13).

583

584 FDA/CFSAN calculated the Unit Cancer Risk of $2.7 \times 10^{-3} \text{ (mg/kg/day)}^{-1}$ using the data in Konishi
585 et al. cited in the table above (Ref. 14). A food additive contaminant migrating into human food
586 at an exposure of over 0.37 µg/kg or 22 µg/day would result in an estimated cancer risk over 1 in
587 10⁶.

588

589 **Acceptable intake (AI)**

590 Rationale for selection of study for AI calculation

591 The oral gavage study of Wester et al. (Ref. 5) is the most robust study for calculation of the AI
592 and the most sensitive species and tissue is rat forestomach in the gavage carcinogenicity study.
593 The study included appropriate dose range for measuring tumor incidence demonstrating a clear
594 dose response and provides sufficient data for the calculation of a compound specific AI.

595

596 **Calculation of AI**

597 Lifetime AI = TD₅₀/50,000 x 50 kg

598

599 Lifetime AI = 2.55 mg/kg/day/50,000 x 50 kg

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601 **Lifetime AI = 3 µg/day**

602

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Ethyl bromide (CAS# 74-96-4)

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Potential for human exposure

Ethyl bromide (bromoethane) is a colorless volatile and flammable liquid. It is an alkylating agent used primarily as a reagent in synthesis of pharmaceuticals. Its close analog, chloroethane, listed in M7, is a mutagenic carcinogen.

Mutagenicity/genotoxicity

Ethyl bromide is mutagenic per the principles of ICH M7 and genotoxic *in vitro*. The mutagenicity of ethyl bromide was evaluated in Salmonella tester strains TA 97, TA 98, TA 100 and TA 104, both in the presence and absence of added metabolic activation by Aroclor-induced rat liver S9 fraction (Ref. 1). Ethyl bromide is a volatile and hydrophobic compound, it was tested in both the standard Salmonella assay and in the same assay modified by incubation in a desiccator. In the standard assay, at concentrations of 5, 10, 50, 100, 500, and 1000 µg/plate ethyl bromide was not mutagenic. However, ethyl bromide was mutagenic in bacterial reverse mutation assays in *Salmonella typhimurium* TA98, TA100, TA104 with metabolic activation and mutagenic in TA 97 with and without metabolic activation. TA100, TA1535 and *Escherichia coli* WP2 with and without metabolic activation when tested as a gas in sealed desiccators (Ref. 2, 3).

In cultured CHO cells, ethyl bromide induced sister chromatid exchanges (SCEs) but not chromosomal aberrations in both the presence and absence of exogenous metabolic activation (Ref. 4).

Carcinogenicity

The IARC has determined that ethyl bromide is not classifiable as to its carcinogenicity to humans (Ref. 5). There is no epidemiological data relevant to carcinogenicity and limited evidence in experimental animals for the carcinogenicity of ethyl bromide.

In animals, evidence of carcinogenicity was identified from a 2-year bioassay from the National Toxicology Program (NTP) that evaluated the inhalation route of ethyl bromide administration in rats and mice. A variety of effects (dependent on species and sex) were seen with exposures of 100, 200, or 400 ppm 6 hours/day, 5 days/week (Ref. 3).

There was some evidence of carcinogenic activity of ethyl bromide for male F344/N rats, as indicated by increased incidences of pheochromocytomas and malignant pheochromocytomas, combined, of the adrenal medulla (control, 8/40; 100 ppm, 23/45; 200 ppm, 18/46; 400 ppm, 21/46). In female rats, the incidences of gliomas in the brain and adenomas in the lung were increased. However, the incidence of the former was within historical control and the latter the incidence was not statistically significant by trend test or pairwise comparisons. For male B6C3F1 mice, there was equivocal but statistically significant increase in incidences of neoplasms of the lung (alveolar/bronchiolar adenomas or carcinomas). There was clear evidence of carcinogenic activity for female B6C3F1 mice, as indicated by neoplasms of the uterus (adenomas or adenocarcinomas).

684 Ethyl Bromide – Details of carcinogenicity studies

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses*	Most sensitive tumor site/type/sex	TD ₅₀ (mg/kg/d)
Ref. 3	50/sex/ group B6C3F1 mice	105 weeks, Inhalation	50	3: 100, 200, 400 ppm M: 115, 229, 458 F: 137, 275, 550 mg/kg/d	Uterus / Female	535 [^]
Ref. 3	50/sex/ group F344/N Rats	106 weeks, Inhalation	50	3: 100, 200, 400 ppm M: 22.9, 45.8, 91.7 F: 32.7, 65.5, 131 mg/kg/d	Adrenal / Male	149 [^]
Ref. 3	50/sex/ group F344/N Rats	106 weeks, Inhalation	50	3: 100, 200, 400 ppm M: 22.9, 45.8, 91.7 F: 32.7, 65.5, 131 mg/kg/d	Liver	670 [^]

685 * mg/kg/d values stated in CPDB (Ref. 6) and calculated by method used to standardize average daily dose
686 levels from variety of routes of administration, dosing schedules, species, strains and sexes; values stated
687 in CPDB accounted for exposure duration of 24 h per day for 7 days per week. (Dose rate = (administered
688 dose × intake/day × number of doses/week) / (animal weight × 7 days/week))

689 [^] TD₅₀ calculated in CPDB

690

691 **Mode of action for carcinogenicity**

692 Ethyl bromide is an alkylating agent. It is a mutagenic carcinogen, and the acceptable intake is
693 calculated by linear extrapolation from the TD₅₀.

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695 **Regulatory and/or published limits**

696 For ethyl bromide, the ACGIH threshold limit value-time-weighted average (TLV-TWA) for
697 ethyl bromide is 5 ppm (22 mg/m³), while OSHA and NIOSH indicate the TWA as 200 ppm (890
698 mg/m³) (Ref. 7). The ACGIH estimates this value with a notation for skin absorption, but OSHA
699 and NIOSH estimates are based on inhalation studies.

700

701 **Acceptable intake (AI)**702 Rationale for selection of study for AI calculation

703 Ethyl bromide is a mutagenic carcinogen via the inhalation route of exposure. Although no
704 information on the inhaled bioavailability of ethyl bromide was found, organic solvents have high
705 inhalation bioavailability values (Ref. 8) and systemic exposure via inhalation route has been
706 demonstrated in multiple studies by clinical observations (Ref. 9). Neoplastic lesions were
707 observed in multiple organs where systemic exposure is indicated in mice and rats in addition to

708 the site-of-contact tissues (e.g., lung). Therefore, it is reasonable to apply the AI derived from
709 inhalation studies for other routes of administration.

710
711 Considering all the available data from the inhalation studies in rats and mice, the most sensitive
712 tumor endpoint was the combined pheochromocytoma and malignant pheochromocytomas of the
713 adrenal gland in male F344/N rats. The TD₅₀ calculated by CPDB for this endpoint was, however,
714 not statistically significant. This is due to the lack of a significant dose response trend test for the
715 endpoint. However, calculating a TD₅₀ for each dose separately results in statistically significant
716 TD₅₀ values for each dose (TD₅₀ = 32.2 mg/kg/d for low dose, 115 mg/kg/d for mid dose, 162
717 mg/kg/d for high dose – Note 2). Therefore, the effect is considered relevant and the lowest TD₅₀
718 value of 32.2 mg/kg/d is used as it was considered to conservatively yield the most sensitive
719 potency estimate for calculating the AI.

720

721 **Calculation of AI**

722 Lifetime AI = TD₅₀/50,000 x 50 kg

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724 Lifetime AI = 32.2 mg/kg/day/50,000 x 50 kg

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726 **Lifetime AI = 32 µg/day**

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Formaldehyde (CAS# 50-00-0)

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Potential for human exposure

Formaldehyde exposure occurs in air, water, and food, and is a common endogenous component of biological materials and is a naturally occurring component of many foods such as meat, dairy products, fruit and vegetables. Levels of daily exposure to formaldehyde via the dietary route have been estimated in the range of 1.5-14 mg/day (Ref. 1-3). Formaldehyde is also a product of normal human metabolism and is essential for the biosynthesis of certain amino acids. The human body produces and uses approximately 50 g of formaldehyde per day, which is rapidly metabolized and cleared from blood plasma (Ref. 3-5). Formaldehyde is used in the synthesis and formulation of pharmaceuticals. In some cases, formaldehyde can function as the active ingredient in a drug. Formaldehyde is also found as a component of some consumer products and can be produced during cooking or smoking.

Mutagenicity/genotoxicity

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Formaldehyde is a mutagenic compound (Ref. 6). Formaldehyde induced mutations in the bacterial reverse mutation assay with and without S9 activation. It induced deletions, point mutations, insertions, and cell transformations in mammalian cells (Ref. 7). Formaldehyde is also clastogenic causing chromosomal aberrations, micronuclei, and sister chromatid exchanges in rodent and human primary cell lines. *In vivo* studies have also detected genotoxic effects primarily at the site of contact (Ref. 8).

Carcinogenicity

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IARC considers formaldehyde to be a Group 1 carcinogen, or carcinogenic in humans based on cancer of the nasopharynx and leukemia (Ref. 6). There are several oral and inhalation animal studies (summarized in Table 1) conducted with formaldehyde. The carcinogenicity of formaldehyde is specific to inhalation and formaldehyde is not carcinogenic via the oral route (Ref. 6, 9-11).

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Formaldehyde was negative in oral carcinogenicity studies in rodents. In carcinogenicity studies conducted by the inhalation route, tumors in the nasal cavity have been observed in rodents.

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The nasal tumors observed following inhalation of formaldehyde were attributed to continuous cycles of tissue degeneration and regeneration (cytolethality/regenerative cellular proliferation; CRCP) rather than to a direct genotoxic effect (Ref. 12). Formation of DNA-protein crosslinks is probably involved in the cytolethality. Predicted additional cancer risks for an 80-year continuous environmental exposure to formaldehyde was modeled using CRCP. The risk predictions were obtained from what Conolly et al. (Ref. 12) expected to be significant overestimates of real-world exposures to formaldehyde.

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Both IARC and US EPA concluded formaldehyde causes leukemia, in agreement with the conclusion of the NTP 14th Report on carcinogens that formaldehyde causes nasopharyngeal cancer and myeloid leukemia (ML), (Ref. 13). The conclusion that formaldehyde causes cancer was peer reviewed by the National Academy of Science (Ref. 14). The reviews acknowledged that hazard identification for formaldehyde was not straightforward, especially with respect to possible leukemogenicity, in part due to its endogenous production and high reactivity. The most useful studies on the risk of formaldehyde causing ML are the large cohort studies of chemical workers and embalmers (Ref. 15). The conclusion was that there is a causal association between

799 formaldehyde exposure and mortality from ML (Ref. 16). Albertini and Kaden (Ref. 17)
 800 concluded that overall, the available literature on genetic changes following formaldehyde
 801 exposure did not provide convincing evidence that exogenous exposure, and specifically exposure
 802 by inhalation, induces mutations as a direct DNA-reactive effect at sites distant from the portal-of-
 803 entry tissue. This would include proposed mode of actions that involve a stem cell effect at the
 804 port of entry with circulation back to the bone marrow. Such exposures have not been shown to
 805 induce mutations in the bone marrow or in any other tissues beyond the point of contact.

806
 807 Since 2010, two short-term carcinogenicity studies have been conducted and published by the NTP
 808 in strains of genetically predisposed mice (male C3B6·129F1-Trp53tm1Brdp53 haplo-insufficient
 809 mice and male B6.129- Trp53tm1Brd) (Ref. 18). These short-term carcinogenicity studies were
 810 conducted to test the hypothesis that formaldehyde inhalation would result in an increased
 811 incidence and/or shortened latency to nasal and lymphohematopoietic tumors and to investigate
 812 hypotheses that formaldehyde may induce leukemia by a mechanism not involving DNA adduct
 813 formation. This proposed mechanism assumes that inhaled formaldehyde could cause significant
 814 genetic damage to stem cells in the nasal epithelium or circulating in local blood vessels. These
 815 damaged stem cells could reach the general circulation, undergo lodgment and become leukemic
 816 stem cells. The animals were exposed to 7.5 or 15 ppm formaldehyde 6 hours/day, 5 days/week,
 817 for 8 weeks and mice were monitored for approximately 32 weeks. At the highest concentrations,
 818 significant cell proliferation and squamous metaplasia of the nasal epithelium were observed;
 819 however, no nasal tumors were observed. No cases of leukemia were seen in either strain and a
 820 low incidence of lymphoma in exposed mice was not considered related to exposure. In addition,
 821 no significant changes in hematological parameters were noted. Under the conditions of these
 822 studies, the authors concluded that formaldehyde inhalation did not cause leukemia in these strains
 823 of genetically predisposed mice (Ref. 18).

824
 825 Moreover, multiple studies in rats (Ref. 19-21) monkeys (Ref. 21, 22) conducted with sensitive
 826 analytical methods that can measure endogenous versus exogenous formaldehyde DNA or protein
 827 adducts have demonstrated that inhaled exogenous formaldehyde is not systemically absorbed or
 828 reaches sites distant from the point of initial contact. In addition to these studies, the available data
 829 on the toxicokinetics of formaldehyde suggest that no significant amount of “free” formaldehyde
 830 would be transported beyond the portal of entry.

831
 832 **Formaldehyde – Details of carcinogenicity studies**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD ₅₀ (mg/kg/d)
Ref. 23	42-60/ group C3H Mouse	35- or 64- weeks, Inhalation	59	3: 50, 100, 200 mg/m ³	No tumors	NC
Ref. 24	120/sex / group B6C3F1 Mouse	2 years, Inhalation	120	3: 2, 5.6, 14.3 ppm	Nasal Turbinates/ Male	43.9 ^a
Ref. 24	120/sex/ group F344 Rat	2 years, Inhalation	120	3: 2, 5.6, 14.3 ppm	Nasal Turbinates/ Male	0.798 ^a
Ref. 25	100/ group	Lifetime, Inhalation	99	1: 14.8 ppm	Nasal Mucosa / Male	1.82 ^a

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD ₅₀ (mg/kg/d)
	Male Sprague Dawley Rat					
Ref. 26	45/group Male Wistar Rat	4, 8 or 13 weeks, Inhalation	134	2: 10, 20 ppm	Nasal Cavity / Male	NC ^b
Ref. 27	30/group (Undama ged) Male Wistar Rat	3- or 28- months, Inhalation	30	4: 0, 0.1, 1.0, 10 ppm	No Tumors for Undamaged animals ^c	NC
Ref. 28	15-16/ group Female Sprague Dawley Rat	24 months, Inhalation	16	1: 12.4 ppm	No Tumors	NC
Ref. 29	90 or 147/ group Male F344 Rat	24 months, Inhalation	90	5: 0.7, 2, 6, 10, 15 ppm	Nasal Cavity / Male	0.48 ^a
Ref. 30	32/ group Male F344 Rat	28 months, Inhalation	32	3: 0.3, 2, 15 ppm	Nasal Cavity / Male	0.98 ^a
Ref. 31	88/ group Male Syrian Golden Hamster	Lifetime, Inhalation	132	1: 10 ppm	No Tumors	NC
Ref. 32	70/sex/ group Wistar Rat	2 years, Drinking water	70	3: 1.2, 15, 82 mg/kg/d (M), 1.8, 21, 109 mg/kg/d (F)	No Tumors	NC
Ref. 33	50/sex/ group Sprague Dawley Rat	Lifetime, Drinking water	50	7: 10, 50, 100, 500, 1000, 1500, 2500 ppm (0.7, 3.5, 7, 35, 71, 106 176 mg/kg/d ^d)	Lymphoblastic leukemia- lymphosarcoma / Male ^e	424 ^a

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD ₅₀ (mg/kg/d)
Ref. 34	20/sex/ group Wistar Rat	24 months, Drinking water	20	3: 10, 50, 300 mg/kg/d	No Tumors	NC

833 NC – Not Calculated

834 ^a TD₅₀ taken from the CPDB (Ref. 35)

835 ^b Not calculated given the limited duration of dosing

836 ^c After 28 months of exposure animals damaged by electrocoagulation experienced an increase in nasal
837 cavity tumors

838 ^d Calculated based on ICH Q3C assumptions for respiratory parameters

839 ^e There were concerns about study design (pooling of lymphomas and leukemias diagnosed, lack of
840 reporting of non-neoplastic lesions and historical control data, discrepancies of data between this study
841 and Sofritti (Ref. 36) [second report of this study], and lack of statistical analysis) (Ref. 6, 11, 37).

842

843 **Mode of action for carcinogenicity**

844 Formaldehyde was carcinogenic only in studies conducted by the inhalation route in rodents.
845 Tumors in the nasal cavity have been observed and are considered a site of contact effect in
846 rodents. The nasal tumors observed following inhalation of formaldehyde were attributed to
847 continuous cycles of tissue degeneration and regeneration (cytotoxicity/regenerative cellular
848 proliferation; CRCP) rather than to a direct genotoxic effect. Formation of DNA-protein
849 crosslinks (DPX) is probably involved in the cytotoxicity of formaldehyde but not considered as
850 the driving mechanism to carcinogenicity. In a recent review of the mode of action of
851 formaldehyde and relevance of rat nasal tumors to humans, the role of cytotoxicity and
852 regenerative cell proliferation was reaffirmed. The authors also indicate that although DNA-
853 protein crosslinks are a good biomarker of exposure, they may not meaningfully contribute to
854 cancer via genotoxic effects except at concentrations that result in tissues levels well above
855 endogenous levels (Ref. 38).

856

857 **Regulatory and/or published limits**

858 For oral exposure to the general population, the ATSDR, Health Canada, International Programme
859 on Chemical Safety (IPCS), and US EPA limit for formaldehyde is 0.2 mg/kg/day or 10 mg/day
860 for a 50 kg person, which is based on a non-cancer endpoint (reduced weight gain and histological
861 changes to the gastrointestinal tract and kidney) (Ref. 39-41). No oral carcinogenicity risk
862 estimates have been performed with formaldehyde, since carcinogenicity is specific to the
863 inhalation route of exposure.

864

865 Occupational limits have been set for air at work places by NIOSH (REL TWA 0.016 ppm),
866 ACGIH (TWA 0.1 ppm), DFG MAKs (TWA 0.3 ppm), and OSHA (PEL TWA 0.75 ppm).

867

868 For inhalation exposure to the general population, the US EPA, IPCS, and Health Canada have
869 developed inhalation cancer risk values (Ref. 11, 40, 41). The US EPA limit is based on a linear
870 cancer model, and Health Canada/IPCS developed nonlinear and linear cancer models. Using the
871 linear method from all three agencies, a daily inhaled dose of 16-32 µg/day would result in a 1 in
872 10⁵ excess risk of cancer. However, more recent scientific analysis supports the use of the Health
873 Canada/IPCS nonlinear model, which incorporates mechanistic data (Ref. 42-44). Conolly et al.
874 (Ref. 12) developed a nonlinear / linear mechanistic-based model using empirical rodent and
875 human data for the two modes of action with formaldehyde tumorigenicity: CRCP and DNA-

876 protein cross-links (DPX).

877

878

879 **Acceptable intake (AI) for inhalation exposure**

880 Rationale for selection of study for AI calculation

881

882 The AI for inhalation is based on the carcinogenicity model developed by Conolly et al. (Ref. 12).

883 Figure 1 represents the dose-response hockey stick-shaped model developed by Conolly et al.,

884 (Ref. 12) for a mixed population of smokers and non-smokers. The rat dose response for

885 CRCP/DPX was used by Connolly for the human model in absence of an alternative model. Since

886 the exposure related tumor risk predicted by clonal growth models was extremely sensitive to cell

887 kinetics, Conolly et al. decided to evaluate human cancer risk associated with formaldehyde

888 exposure using both the raw J-shaped dose-response and a hockey stick-shaped transformation of

889 the rat data. This model incorporates the non-linear-based mechanism at the high dose region

890 (CRCP) and the linear mechanism at the low dose region (DPX). As noted above, the translation

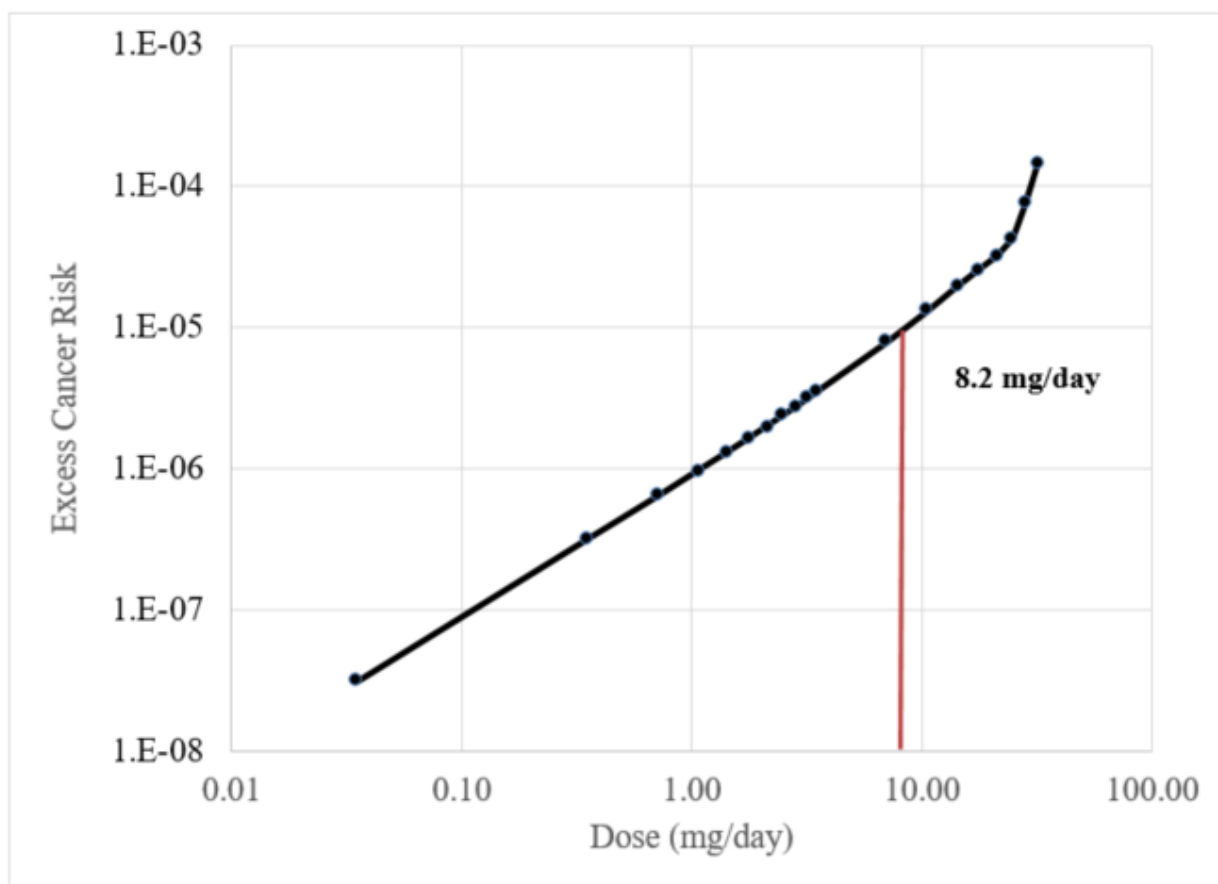
891 of DPX into mutations and an assumed linear-dose response emerging from such mutations is not

892 established experimentally. Moreover, experimental results suggest that DPX are not leading to

893 mutations in a linear fashion. Thus, the linear dose-response model at low doses reflect a

894 conservative and practical approach and is not dictated by experimental data.

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897

898 Figure 1. Dose-response model hockey stick-shaped model from (Ref. 12) representing mixed smokers and non-

899 smokers. The dose (mg/day) was based on converting air concentration (ppm) to daily dose using ICH Q3C

900 assumptions for human breathing volume (28,800 L/day).

901

902

903 **Calculation of inhalation AI**

904

905 The linear low dose region of Figure 1 was used to determine the dose at a 1 in 100,000 excess
906 cancer risk. Linear regression at the low dose region, which is ≤ 24.74 mg/day (converted from
907 0.7 ppm) results in an equation of $y = 1.62E-06x - 3.27E-06$. The dose of 24.74 mg/day was the
908 point at which there is a predicted upward inflection of additional risk. Solving for a 1 in 100,000
909 excess cancer risk in the regression line (y) results in an acceptable intake of 8.2 mg/day (see
910 Figure 1 dose equivalent to the 1:100,000 risk).

911

912 Risk (y) = $1.62E-06x(\text{dose}) - 3.27E-06$

913 $0.00001 = 1.62E-06x - 3.27E-06$

914 $x = (0.00001 + 3.27E-06) / 1.62E-06$

915 Dose (x) = 8.2 mg/day

916

917 **Lifetime AI (inhalation) = 8 mg/day or 215 ppb, whichever is lower**

918

919 *Formaldehyde is considered a mutagenic carcinogen by the inhalation route of exposure. The
920 acceptable intake of 8 mg/day represents an upper limit over a 24 hour time period. As described
921 in the introduction section of Appendix 3 of this guideline, "other considerations" may affect
922 final product specifications. WHO recommends a limit of 77 ppb in air as a 30 min average and
923 Health Canada recommends a short-term exposure limit of 100 ppb based as a 1 hour average.
924 These recommended values provide at least a 10-fold margin of exposure to the lowest level at
925 which symptoms were observed. To protect patients from the local irritation and sensitization
926 effects of formaldehyde by the inhalation route of exposure, a lower concentration-based limit of
927 215 ppb is recommended [8 mg/day over 24 hours of exposure is equal to a concentration limit
928 of 215 ppb ($0.008 \text{ g/day} / 28.8 \text{ m}^3/\text{day} * 1 / 1293 \text{ g/m}^3$)].

929

930 human breathing volume/d - 28.8 m^3

931 air mass/m³ at standard conditions - 1293 g

932

933 **Permissible Daily Exposure (PDE) for all other routes**

934 See section 4 of the introduction to this Addendum that addresses formaldehyde exposure from the
935 environment.

936

937 **PDE (all other routes) = 10 mg/day**

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Styrene (CAS# 100-42-5)

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Potential for human exposure

Styrene exposure to the general population occurs via environmental contamination and dietary exposure (Ref. 1). In the general population, indoor and outdoor air account for the largest exposures. However, smoking one pack of cigarettes would likely lead to the inhalation of milligram quantities of styrene (Ref. 2). Styrene has been detected as a natural constituent of a variety of foods and beverages, the highest levels occurring in cinnamon. Polystyrene and its copolymers are widely used as food-packaging materials and monomers such as styrene can migrate to food at low levels. The daily intake of styrene from dietary sources has been estimated to be 1-4 µg in the United Kingdom, 2-12 µg in Germany and 9 µg in the United States (Ref. 3, 4). Styrene is used in the synthesis of active pharmaceutical ingredients.

Mutagenicity/genotoxicity

Styrene has produced contradictory findings in the *in vitro* bacterial reverse mutation assay and it is predominantly inactive in the *in vivo* chromosome aberration, micronucleus and UDS assays when conducted according to OECD guidelines. Inconsistent results in the bacterial reverse mutation (Ames) test were attributed to styrene volatility, poor solubility, and different metabolic systems (Ref. 5). Styrene was positive for mutagenicity in the Ames test only with metabolic activation (Ref. 5), where it is converted to electrophilic intermediates (e.g., styrene 7,8-oxide) to enable formation of covalent adducts with DNA. The main metabolite of styrene is styrene 7, 8-oxide. Most of the genetic damage associated with styrene exposure is thought to be due to styrene 7, 8-oxide, which is further detoxified to styrene glycol. Styrene exposure elevated DNA adducts (N⁷-guanine, O⁶-guanine, and N¹-adenine) and SCEs in both animal models and in humans, and DNA strand breaks in humans (Ref. 5, 6). In a critical review of styrene genotoxicity based on the requirements outlined in the current OECD guidelines, Moore et al. (Ref. 7) concluded that it is unclear whether unmetabolized styrene is mutagenic in the Ames test, while the styrene 7, 8-oxide metabolite is clearly mutagenic. The authors also noted that most styrene 7, 8-oxide Ames positive data was collected without using exogenous metabolic activation, meaning that styrene 7, 8-oxide was not further metabolized to styrene glycol.

Styrene was mutagenic in glycophorin A (GPA) variant frequencies in erythrocytes from 28 workers inhalation-exposed to ≥ 85 mg/m³ styrene (Ref. 8). Lymphocytes from styrene exposed workers had increased mutation frequencies (MFs) at the *HPRT* locus (Ref. 9).

Two *in vitro* mammalian gene mutation studies were identified. In the hypoxanthine-guanine phosphoribosyl transferase (*Hprt*) assay, styrene induced only small increases in *HPRT* MFs in V79 cells (Ref. 10). Similarly, in V79 cells, styrene induced small increases in *Hprt* MFs with large variability observed in a liver perfusion system and little to no increases with or without S9 (Ref. 11). No rodent *in vivo* mutation studies evaluating styrene or styrene 7, 8-oxide were identified.

Based on standard regulatory tests, there is no convincing evidence that styrene possesses significant genotoxic potential *in vivo* from the available data in experimental animals. However, genotoxicity associated with styrene exposure (related to formation of styrene-7, 8-oxide) has been proposed as a possible mode of action for styrene induced carcinogenicity in experimental animals and humans (Ref. 1).

1100 **Carcinogenicity**

1101 The IARC has classified styrene and the metabolite styrene 7,8-oxide in Group 2A, “probably
1102 carcinogenic to humans based on limited evidence in humans and sufficient evidence in
1103 experimental animals” (Ref. 5). Styrene is also reasonably anticipated to be a human carcinogen
1104 by the NIH (Ref. 1). Possible modes of action for styrene-induced carcinogenicity involve
1105 genotoxic and cytotoxic effects together with immunosuppression (Ref. 1). NTP listed styrene
1106 as “reasonably anticipated to be a human carcinogen” in its 12th and 14th Reports on Carcinogens
1107 (Ref. 12, 13). The NRC concluded “reasonably anticipated to be a human carcinogen” was an
1108 appropriate carcinogenicity classification for styrene, due to limited carcinogenicity evidence in
1109 humans, sufficient evidence in animal studies, and other mechanistic data supporting
1110 carcinogenicity (Ref. 6).

1111
1112 A recent systematic review of epidemiologic studies of exposure to styrene concluded that
1113 besides some limitations of available research as lack of quantitative estimates of styrene, the
1114 risk of specific cancers found no strong and consistent evidence of a causal association between
1115 styrene and Non-Hodgkin lymphoma and its subtypes, all leukemia, subtypes of leukemia or
1116 cancers of the esophagus, pancreas, lung, kidney or other sites (Ref. 14).

1117
1118 In the CPDB, styrene is reported to be carcinogenic in mice via the oral and inhalation routes and
1119 rats via the inhalation route (Ref. 15). The National Institutes of Health Report on Carcinogens
1120 (Ref. 1) considered the most robust studies to be the two-year studies via (1) oral exposure in
1121 B6C3F1 mice and (2) inhalation exposure in CD-1 mice. In male B6C3F1 mice, oral exposure
1122 to styrene increased the combined incidence of alveolar and bronchiolar adenomas and
1123 carcinomas (Ref. 16). In the inhalation study, in male and female CD-1 mice, there was an
1124 increase in the incidence of pulmonary adenomas and also an increase in pulmonary carcinomas
1125 in females in the high-dose group (Ref. 17).

1126
1127 IARC evaluated nine studies each (with various routes of application) in mice and rats for styrene
1128 and three each in mice and rats for styrene-7,8-oxide. For styrene in mice in one study with
1129 transplacental exposure followed by gavage using O20 mice, an increase of lung carcinoma and
1130 adenoma was observed in pups whereas a second study in C57BL mice was negative (Ref. 18).
1131 Two out of five studies with inhalation in mice reported an increase in lung bronchoalveolar
1132 tumors in CD-1 mice (Ref. 16, 19) whereas the other three (in C57BL/6 mice) were negative
1133 (Ref. 19). One study with oral application found increased lung tumors and a positive trend for
1134 hepatocellular carcinoma (Ref. 16). One study with i.p. application gave negative results (Ref.
1135 20). In two studies in SD-rats with inhalation exposure, styrene increased mammary gland tumors
1136 (Ref. 21, 22), whereas four oral studies, three with gavage (Ref. 17, 22) and one via drinking
1137 water (Ref. 23), were negative as well as one study with transplacental exposure followed by
1138 gavage (Ref. 17), one study with i.p. application and one with s.c. application (Ref. 22). Styrene-
1139 7-8-oxide was tested in three studies in mice, one by gavage (Ref. 24) and two by skin application
1140 (Ref. 25, 26). In the oral study by gavage styrene-7-8-oxide increased squamous cell tumors in
1141 forestomach in males and females and hepatocellular tumors in males. The studies by skin
1142 application were inadequate for evaluation. In rats, styrene-7-8-oxide was tested in two studies
1143 with oral exposure by gavage (Ref. 22, 24) and one by transplacental exposure followed by
1144 gavage (Ref. 27). In both studies by gavage, squamous cell tumors of the forestomach were
1145 increased and in one of the studies mammary gland tumors were also increased in males. In the
1146 study by transplacental exposure followed by gavage, forestomach tumors were increased.
1147 IARC concluded that there is sufficient evidence for carcinogenicity of styrene and styrene-7,8-
1148 oxide in experimental animals (Ref. 5).

1149

1150 US NTP concluded that the evidence from studies in rats was insufficient for reaching a
 1151 conclusion concerning the carcinogenicity of styrene (Ref. 1). An evaluation of the available data
 1152 from eight oncogenicity studies by Cruzan et al., (Ref. 21) concluded that there was clear
 1153 evidence that styrene did not induce cancer in rats. It has been proposed that the reason for lung
 1154 tumor induction in mice but not rats may involve differential metabolism of styrene in the two
 1155 species (Ref. 1).

1156 **Styrene – Details of carcinogenicity studies**
 1157

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD ₅₀ (mg/kg/d) *
Ref. 16	50/sex/ group M&F B6C3F1 mouse	78 weeks, Oral Gavage	20	2: 150, 300 mg/kg/d	Lung/ Male ^	360
Ref. 17	70/sex/ group CD1 mouse	98-104 weeks, Inhalation	70	4: 20, 40, 80, 160 ppm	Lung/ Male	154 ⁺
Ref. 16	70/sex/ group Fischer 344 rats	78 -107 weeks, Oral Gavage	40	3: 500, 1000, 2000 mg/kg/d	No Tumors	NC
Ref. 21	70/sex/ group CD rats	104 weeks, Inhalation	70	4: 50, 200, 500, 1000 ppm	No Tumors	NC
Ref. 22	30/sex/ group SD rats	52 weeks, Inhalation	60	5: 25, 50, 100, 200, 300 ppm	Mammary tissue/ Female ⁺⁺	23.3
Ref. 22	40/sex/ group SD Rats	52 weeks, Gavage	40	2: 50, 250 mg/kg/d	No Tumors	NC
Ref. 22	40/sex/ group SD Rats	SC once, then IP 4 times at 2-month intervals	40	1: 50 mg (SC), 50 mg (IP)	No Tumors [¥]	NC

1158 NC – Not Calculated, SC – Subcutaneous Injection, IP – Intraperitoneal Injection, SD – Sprague
 1159 Dawley

1160 * The TD₅₀ values are taken from CPDB (Ref. 15)

1161 ^ Despite having a statistically significant dose-trend per CPDB, the author concluded that there was no
 1162 convincing evidence of carcinogenicity in mice

1163 + Carcinogenicity study selected for the AI calculation

1164 ++ Author opinion: Styrene, was found to cause an increase in total (benign & malignant) and malignant
1165 mammary tumors. Cruzan et al., (Ref. 21) noted no obvious dose-response in the data. Furthermore, the
1166 study findings were not considered reliable evidence of carcinogenicity by NIH ROC (Ref. 1) and
1167 IARC (Ref. 5) noted short treatment duration and incomplete reporting of the study.

1168 † Study limited to acute exposures and a non-standard study design
1169

1170 **Mode of action for carcinogenicity**

1171 A comprehensive review of the mechanisms that contribute to the carcinogenicity of styrene is
1172 presented in the IARC Monograph (Ref. 5). Taking into consideration the available in vitro and
1173 in vivo genotoxicity data, IARC concludes that there is strong evidence that styrene is genotoxic,
1174 and that the mechanism is relevant to humans. Styrene is metabolically activated in animals and
1175 in humans to an electrophile, styrene-7,8-oxide which interacts with nucleophilic
1176 macromolecules, such as proteins and DNA. DNA adducts-are formed primarily by alkylation of
1177 N⁷-guanine. Styrene-7,8-oxide DNA adducts have been observed in vitro, in rodents and in
1178 humans exposed to styrene. IARC also indicates that there is strong evidence that both styrene
1179 and styrene-7,8-oxide alter cell proliferation and that styrene modulates receptor-mediated
1180 effects based on increased serum prolactin in humans exposed occupationally.

1181 Other possible mechanisms contributing to the carcinogenic activity of styrene include oxidative
1182 stress, immunosuppression and chronic inflammation. The mechanism suggested by Cruzan et
1183 al. (Ref. 28) as main cause of mice lung tumor includes styrene metabolites inducing gene
1184 expression for metabolism of lipid, lipoprotein, cell cycle and mitotic M-M/G1 phases, mild
1185 cytotoxicity and strong mitogenicity in mice lung cells, leading to excessive cell proliferation
1186 and hyperplasia. On the other hand, authors assume that it would not be relevant in humans due
1187 to limited lung metabolism (by CYP2F2). IARC concludes that the evidence for these
1188 mechanisms of action are moderate to weak.
1189

1190 **Regulatory and/or published limits**

1191 The WHO defined a Tolerable Daily Intake (TDI) for styrene via the oral route of 7.7 µg/kg/day
1192 (i.e., 0.385 mg per day based on 50 kg body weight) from which a drinking water guideline value
1193 of 20 µg/L has been defined (i.e., 40 µg per day based on consumption of 2 L water per day)
1194 (Ref. 29). This WHO limit was based on reduced body weight gain in a two-year rat drinking
1195 water study. The US EPA oral reference dose (RfD) (Ref. 30) for styrene is 200 µg/kg/day (i.e.,
1196 10 mg/day based on 50 kg body weight), based on non-cancer endpoints. The associated US
1197 EPA drinking water limit is 100 µg/L (i.e., 200 µg per day based on consumption of 2 L water
1198 per day). The JECFA maximum TDI for styrene (Ref. 31) from migration from food packaging
1199 is 0.04 mg/kg/day (i.e., a maximum of 2 mg per day based on 50 kg body weight). A Specific
1200 Migration Limit of 60 ppm styrene into foods in polystyrene packaging (i.e., 60 mg per day
1201 assuming the consumption of 1 kg food/day for adult humans) is considered permissible in the
1202 European Union (Ref. 4).
1203

1204 **Acceptable intake (AI)**

1205 Rationale for selection of study for AI calculation 1206

1207 Since styrene is considered not to be a rat carcinogen, mouse lung tumors were used to derive
1208 the AI. The more sensitive TD₅₀ was in the inhalation study of Cruzan et al. (Ref. 17). The AI
1209 derived from this inhalation study was considered suitable for all routes of administration as an
1210 increase in lung tumors were also seen in the carcinogenicity study in mice with gavage treatment.
1211 The AI is expected to be a conservative limit as the mouse is known to have higher levels of
1212 CYP2F enzymes in comparison to human which is key to tumor formation (Ref. 28).

1213

1214 **Calculation of AI**

1215 Lifetime AI = $TD_{50}/50000 \times 50 \text{ kg}$

1216

1217 Lifetime AI = $154 \text{ mg/kg/day}/50000 \times 50 \text{ kg}$

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1219 **Lifetime AI = 154 $\mu\text{g/day}$**

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Vinyl Acetate (CAS# 108-05-4)

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1316 **Potential for human exposure**

1317 Human exposure occurs primarily in the occupational setting with very little exposure to vinyl
1318 acetate in the general population (Ref. 1). Vinyl acetate is used in the synthesis of
1319 pharmaceuticals.

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1321 **Mutagenicity/genotoxicity**

1322 The mutagenicity and genotoxicity of vinyl acetate has been reviewed by Albertini (Ref. 2).
1323 Vinyl acetate is not mutagenic in the microbial reversion assay (i.e., Ames tests) in multiple
1324 strains of *Salmonella* or in *Escherichia coli* and vinyl acetate mutagenicity in mammalian cells
1325 (at the *tk* locus human TK6 cells) appears to reflect mainly chromosome level or large mutational
1326 events, but “normal growth” mutants thought to reflect smaller, gene mutations were also
1327 reported. Vinyl acetate also induced micronuclei and chromosome aberrations *in vitro* and
1328 chromosome aberrations *in vivo* and was positive in one out of five *in vivo* micronucleus studies.
1329 Small increases of micronuclei in mouse bone marrow were induced following i.p. administration,
1330 but the genotoxicity was associated with elevated toxicity and mortality (Ref. 3).

1331

1332 There is extensive evidence that vinyl acetate genotoxicity is mediated by its metabolite
1333 acetaldehyde. Acetaldehyde is produced endogenously and detoxification by aldehyde
1334 dehydrogenase is required to maintain intra-cellular homeostasis (Ref. 2). Given its response in
1335 mammalian cells, and rapid conversion to acetaldehyde, vinyl acetate is considered mutagenic.
1336 See Mode of Action information below for further details.

1337

1338 **Carcinogenicity**

1339 Vinyl acetate is classified as Group 2B, possibly carcinogenic to humans (Ref. 4). There are two
1340 oral carcinogenicity reports cited in the CPDB (Ref. 5). One mouse and one rat study, in which
1341 vinyl acetate was administered in drinking water, are limited as there are only two treatment
1342 groups and less than 50 animals per group. Uterine, esophageal and forestomach tumors were
1343 observed in Swiss mice; and liver, thyroid and uterine tumors in Fisher 344 rats. A number of
1344 non-site of contact tumors (e.g., Zymbal gland, lung, liver, uterine, and mammary gland) were
1345 observed in the oral carcinogenicity studies conducted by Maltoni et al. (Ref. 6) and Lijinsky et
1346 al. (Ref. 7). These tumors in Maltoni et al. (Ref. 6) all occurred with high background incidence.
1347 Therefore, without adjusting for age, these tumor data cannot be evaluated with certainty.
1348 Squamous cell carcinoma of the oral cavity, tongue, esophagus, and forestomach were all
1349 treatment related at 5000 ppm. There were no tumors among mice administered 1000 ppm (Ref.
1350 8). In the oldest published oral carcinogenicity study, Lijinsky et al. (Ref. 7) there are a number
1351 of deficiencies in the study design, most notably that the drinking water solutions were prepared
1352 only once per week. The authors recognized a decomposition rate of approximately 8.5% per
1353 day. Therefore, by the end of the week the animals in the 2500 ppm group, for example, were
1354 exposed to approximately 1300 ppm vinyl acetate and significant quantities of breakdown
1355 products, including acetaldehyde and acetic acid. The authors also did not purify the vinyl acetate
1356 prior to preparation of the drinking water solutions. Thus, the rats were also exposed to
1357 unspecified impurities. In addition, only 20 rats were in each group, so the statistical power for
1358 detecting true positive responses and for discriminating against false positive and false negative
1359 outcomes is compromised (Ref. 8).

1360

1361 In addition to the CPDB, other carcinogenicity studies are available in the literature. An oral
 1362 drinking water study was conducted by the Japan Bioassay Research Centre in accordance with
 1363 OECD guideline 453, including 3 treatment groups and 50 animals per group (Ref. 9, 10).
 1364 Increases in tumors of the oral cavity, esophagus and forestomach in Crj:BDF1 mice and
 1365 statistically significant increases of tumors in the oral cavity of female F344:DuCrj rats at all
 1366 doses are reported following drinking water administration of vinyl acetate. In another lifetime
 1367 study, Minardi et al. (Ref. 11) report increases in tumors in oral cavity and lips in 17-week old
 1368 and 12-day old Sprague-Dawley rats also administered vinyl acetate in the drinking water. Two
 1369 treatment groups are included with more than 50 animals per group for the 12-day old rats
 1370 (offspring) but less than 50 per group for the 17-week old animals (breeders). The 12-day old
 1371 rats are more sensitive with tumors in the oral cavity and lips, whereas an increase tumor response
 1372 is not evident in the 17-week old animals.

1373
 1374 Finally, Bogdanffy et al. (Ref. 12) administered vinyl acetate in drinking water for 10 weeks to
 1375 male and female rats that were subsequently mated. The offspring were then culled into two
 1376 groups of 60 for the main study and 30 for satellite groups and exposure in the drinking water
 1377 continued to 104 weeks. The authors concluded that in the offspring there were no non-neoplastic
 1378 or neoplastic lesions observed that were compound related. Two squamous carcinomas were
 1379 observed in the oral cavity of treated males, but the incidence of these tumors was within
 1380 historical control ranges. Therefore, they were not considered related to vinyl acetate treatment.

1381
 1382 There are two inhalation carcinogenicity reports cited in the CPDB (Ref. 5). Vinyl acetate is not
 1383 carcinogenic to CD-1 mice but induces nasal tumors in Sprague-Dawley rats (Ref. 12). All but
 1384 one of the 11 nasal tumors in rats (benign endo and exophytic papillomas and squamous-cell
 1385 carcinomas) were observed at the terminal sacrifice at the high dose of 600 ppm, indicating a late
 1386 life dependency of tumor formation. One benign tumor, of questionable relationship to exposure,
 1387 was observed at the 200 ppm concentration (Ref. 12). In both species and both sexes, vinyl
 1388 acetate induced morphological non-neoplastic lesions in the nasal cavity of the 200 and 600 ppm
 1389 groups and in the trachea (mice only) and in the lungs of the 600 ppm groups.

1390

1391 **Vinyl Acetate – Details of carcinogenicity studies**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD ₅₀ (mg/kg/d)
Ref. 6	37 F and 13 M/ group Swiss Mice	2 years in drinking water	37 F, 14 M	2: 1000 ppm (103 mg/kg/d F and 96.3 mg/kg/d M), 5000 ppm (578 mg/kg/d F and 546 mg/kg/d M)	Uterine, Female	3920 ^b
Ref. 7	20/sex/ group F344 Rat	2 years, drinking water	20	2: 1000 mg/L (0.1 mg/kg/d F and 0.062 mg/kg/d M), 2500 mg/L (0.04	Liver, Male	132 ^b

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD ₅₀ (mg/kg/d)
				mg/kg/d F and 0.025 mg/kg/d M)		
Ref. 9	50/sex/ group Crj:BDF ₁ Mice	2 years, drinking water	50	3: 400 ppm (63 mg/kg F and 42 mg/kg/d M), 2000 ppm (301 mg/kg/d F and 202 mg/kg/d M), 10000 ppm (1418 mg/kg/d F and 989 mg/kg/d M)	Oral cavity, Male	1854 ^c
Ref. 9	50/sex/ group F344/Du Crj Rats	2 years, drinking water	50	3: 400 ppm (31 mg/kg/d F and 21 mg/kg/d M), 2000 ppm (146 mg/kg/d F and 98 mg/kg/d M), 10000 ppm (575 mg/kg/d F and 442 mg/kg/d M)	Oral cavity, Male	3057 ^c
Ref. 11	37F and 14M/ group, Breeders (17 wk old); 53 or 83M and 57 or 87F Sprague- Dawley Rat Offspring (12 day old)	2 years, drinking water	Breeders 14M and 37F; Offspring 107M and 99F	2: 1000 ppm (70.6 mg/kg/d), 5000 ppm (353 mg/kg/d) ^a	Oral cavity and lips, Male	983 ^c
Ref. 12	60/sex/ group Crl:CD(S	2 years, drinking water	60	3: 200 ppm (16 mg/kg/d F	No tumors	NC

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD ₅₀ (mg/kg/d)
	D)BR Rats			and 10 mg/kg/d M), 1000 ppm (76 mg/kg/d F and 47 mg/kg/d M), 5000 ppm (302 mg/kg/d F and 202 mg/kg/d M)		
Ref. 12	60/sex/ group Charles River CD1 Mice	2 years, inhalation	60	3: 50 ppm (55.3 mg/kg/d F and 46.1 mg/kg/d M), 200 ppm (221 mg/kg/d F and 184 mg/kg/d M), 600 ppm (664 mg/kg/d F and 554 mg/kg/d M)	No tumors	NC
Ref. 12	60/sex/ group Charles River CD (Sprague- Dawley) Rats	2 years, inhalation	20	3: 50 ppm (13.3 mg/kg/d F and 9.32 mg/kg/d M), 200 ppm (52.7 mg/kg/d F and 36.9 mg/kg/d M), 600 ppm (158 mg/kg/d F and 111 mg/kg/d M)	Nasal, Male	758 ^b

1392 NC – Not Calculated

1393 ^a Calculated based on ICH Q3C assumptions

1394 ^b Taken from the CPDB (Ref. 13)

1395 ^c Study not reported in CPDB, therefore TD₅₀ value calculated based on carcinogenicity data

1396

1397 **Mode of action for carcinogenicity**

1398 Vinyl acetate has been reviewed by the European Commission's Scientific Committee on Health
1399 and Environmental Risks (SCHER), who published a Risk Assessment Report in 2008 (Ref. 1).

1400 Overall, SCHER supports the conclusion that the carcinogenic potential of vinyl acetate is
1401 expressed only when tissue exposure to acetaldehyde is high and when cellular proliferation is
1402 simultaneously elevated. This mode of action suggests that exposure levels, which do not

1403 increase intracellular concentrations of acetaldehyde will not produce adverse cellular responses.
1404 As long as the physiological buffering systems are operative, no local carcinogenic effect by
1405 vinyl acetate should be expected at the NOAEL for histological changes in respiratory rodent
1406 tissues. However, the SCHER indicated that local levels at or below the NOAEL are not free of
1407 carcinogenic risk, although the risk may be negligibly low. Hengstler et al. (Ref. 8) presented the
1408 case for vinyl acetate as a DNA-reactive carcinogen with a threshold dose-response, which has
1409 also been described by Albertini (Ref. 2). Like acetaldehyde, vinyl acetate is not-mutagenic in
1410 the standard bacterial reversion assay; evidence for DNA-reactivity and site of contact
1411 carcinogenicity of vinyl acetate is that it occurs because of metabolic conversion to acetaldehyde.
1412

1413 The genotoxicity profiles for acetaldehyde and vinyl acetate are almost identical and vinyl acetate
1414 is not active as a clastogen without the addition of carboxylesterase (Ref. 8). Therefore, the
1415 clastogenic activity of vinyl acetate is attributed to metabolic formation of acetaldehyde. At high
1416 concentrations, enzyme activities are not able to oxidize all the generated acetaldehyde, and a
1417 low pH microenvironment is the result (Ref. 12). From consistent endogenous acetic acid
1418 exposure, tissues may sustain a reduction of 0.15 units in pH following vinyl acetate treatment
1419 without adverse effects (i.e. cytotoxicity and genotoxicity) (Ref. 14). However, when this
1420 practical threshold is exceeded, DNA damage, cytotoxicity, and regenerative cellular
1421 proliferation occur, resulting in tumor formation at the site of contact.
1422

1423 There is clear evidence for the carcinogenicity of vinyl acetate in two animal species, in both
1424 sexes and for both inhalation and oral administration. Following both oral and inhalation
1425 administration, vinyl acetate is rapidly hydrolyzed at the site of contact by carboxylesterases, to
1426 acetic acid and acetaldehyde (Ref. 3, 15). Vinyl acetate exposure produces tumors at the site of
1427 first contact along the exposure routes. The dose-response is thought to be non-linear, with the
1428 observed tumor responses reflecting the target tissue-specific enzyme activities for activation and
1429 detoxification (Ref. 2). However, as noted in the acetaldehyde monograph, there are no published
1430 measurements which would allow discrimination between the irritating effect and the potential
1431 mutagenic effect on cancer development.
1432

1433 **Regulatory and/or published limits**

1434 For vinyl acetate, the US EPA IRIS database calculated an inhalation Reference Concentration
1435 (RfC) for non-carcinogenic effects of 0.2 mg/m³, or 5.8 mg/day assuming a respiratory volume
1436 of 28.8 m³. The RfC was based on a human equivalent concentration of 5 mg/m³ derived from
1437 Owen et al. 1988 which identified both a NOAEL and a LOAEL for histopathological effects of
1438 the nasal olfactory epithelia in rats and mice in a chronic 2-year study. A total adjustment factor
1439 of 30 was applied (Ref. 16). The US EPA report did not include a carcinogenicity assessment for
1440 lifetime exposure to vinyl acetate. It is stated that RfCs can be derived for the noncarcinogenic
1441 health effects of substances that are carcinogens and to refer to other sources of information
1442 concerning the carcinogenic potential.
1443

1444

1445 **Permissible Daily Exposure (PDE) for oral exposure**

1446 Rationale for selection of study for PDE calculation

1447

1448 Following oral administration, vinyl acetate is rapidly hydrolyzed at the site of contact by
1449 carboxylesterases, to acetic acid and acetaldehyde. Given the weight of evidence for a non-linear
1450 dose response for the carcinogenicity of both vinyl acetate and acetaldehyde following oral

1451 administration and considering high background exposure to acetaldehyde from a wide variety
1452 of foods, the oral PDE recommended is based on that derived for acetaldehyde of 2 mg/day.

1453

1454 **PDE (oral) = 2 mg/day**

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1457 **Acceptable intake (AI) for all other routes**

1458 Rationale for selection of study for AI calculation

1459 For routes of administration other than the oral route, the inhalation carcinogenicity study in rats
1460 (Ref. 12) was used for derivation of an AI. In this study, there were 3 treatment groups with 60
1461 animals per sex per treatment group. Animals were exposed 6 hours per day, 5 days per week
1462 for 2 years to vinyl acetate. Carcinogenicity was observed in the nasal cavity of rats, with male
1463 being the more sensitive sex. The TD₅₀ for the nasal cavity in male rats is 758 mg/kg/day, as
1464 reported in CPDB. The only other carcinogenicity study that is available with vinyl acetate
1465 administered via the inhalation route in mice is negative (Ref. 12). Therefore, the rat inhalation
1466 study was selected for derivation of an AI.

1467

1468 Although the dose-response relationship for carcinogenicity is thought to be non-linear, as stated
1469 above, there are no published measurements which allow discrimination between a true threshold
1470 versus a non-linear inflection point. Therefore, the AI was calculated using linear extrapolation.

1471

1472 **Calculation of AI**

1473 Lifetime AI = TD₅₀/50000 x 50 kg

1474

1475 Lifetime AI = 758 mg/kg/day x 50 kg

1476

1477 **Lifetime AI (all other routes) = 758 µg/day**

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1479

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- 1518
- 1519

1521

Note 2

1522

1523 The calculated TD₅₀ for ethyl bromide is illustrated below since it was decided to use the same
1524 study data but not the TD₅₀ calculated by CPDB as the positive dose response was not statistically
1525 significant (see monograph for ethyl bromide).

1526

ppm	Dose (mg/kg/day) ¹	Number of Positive Animals	Total Number of Animals
0	0	8	40
100	22.9	23	45
200	45.8	18	46
400	91.7	21	46

1527

1528

1529 A TD₅₀ is calculated for each dose separately with the following equation (Ref. 1, 2):

$$\frac{P - P_0}{1 - P_0} = 1 - \exp(-\beta \cdot D)$$

1530

1531 Where P is the proportion of animals with the specified tumor type observed at a certain dose (D
1532 in the equation) and P₀ is the proportion of animals with the specified tumor type for the control.
1533 Converting β and D into a simple linear equation results in the following:

$$\ln\left(-\left[\frac{P - P_0}{1 - P_0} - 1\right]\right) = \beta \cdot D$$

1534

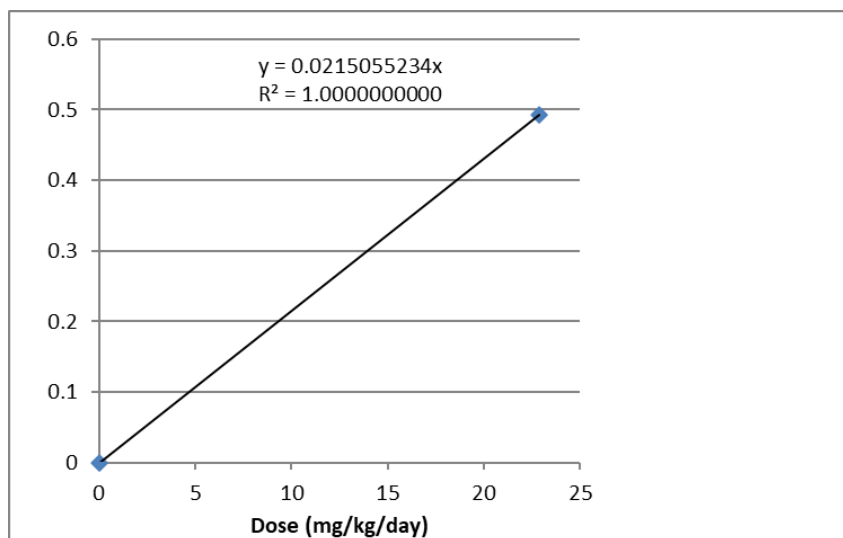
1535 Plotting the results and using the slope to represent β results in the following graphs for the dose-
1536 response and β = 0.0215055234 for low dose, 0.0059671034 for mid-dose and 0.0042161616 for
1537 the high dose.

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Low Dose



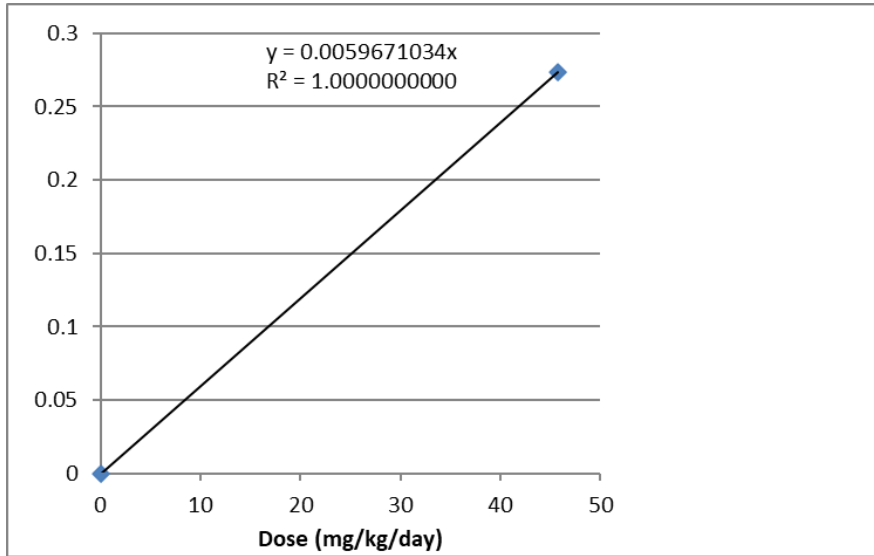
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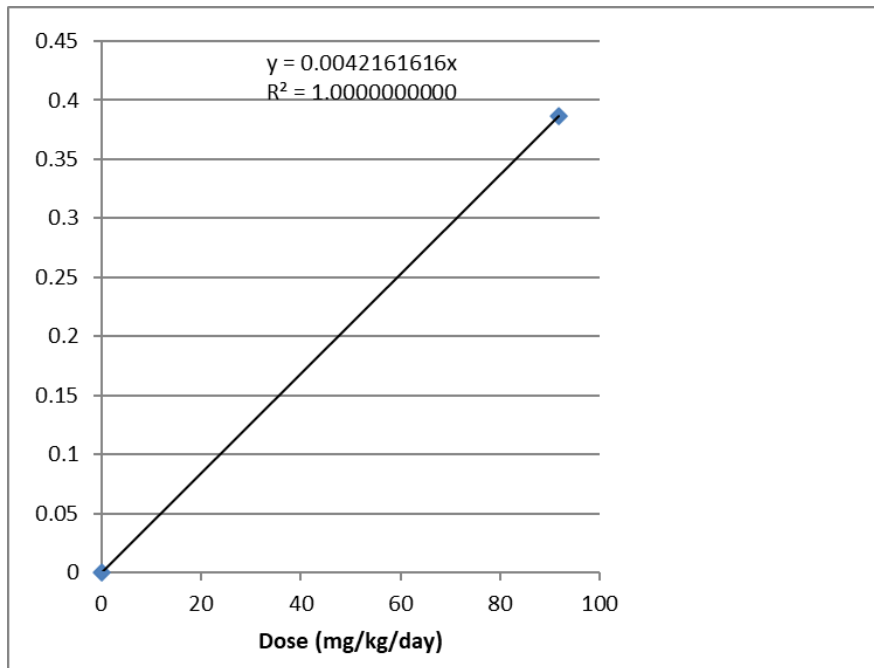
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Mid Dose



1545
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High Dose



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The TD_{50} can then be calculated as follows.

$$0.5 = 1 - \exp(-\beta \cdot TD_{50})$$

1553
1554
1555

Solving for TD_{50} results in in the following equation.

$$TD_{50 \text{ low dose}} = \frac{0.693}{0.0215055234}$$

$$TD_{50 \text{ mid dose}} = \frac{0.693}{0.0059671034}$$

$$TD_{50 \text{ high dose}} = \frac{0.693}{0.0042161616}$$

1556
1557
1558

Therefore, the lowest $TD_{50} = 0.693 / 0.0215055234$ or 32.2 mg/kg/day.

1559
1560

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