

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL
REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

**ADDENDUM TO ICH M7: ASSESSMENT AND CONTROL OF DNA
REACTIVE (MUTAGENIC) IMPURITIES IN PHARMACEUTICALS TO
LIMIT POTENTIAL CARCINOGENIC RISK**

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

M7(R1)

Current *Step 2* version

dated 9 June 2015

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 Steering Committee to the regulatory authorities of the ICH regions (European Union, Japan, USA, Canada and Switzerland) for internal and external consultation, according to national or regional procedures.

M7
Document History

Code	History	Date
M7	Approval by the Steering Committee under <i>Step 2</i> and release for public consultation.	6 February 2013
M7	Approval by the Steering Committee under <i>Step 4</i> and recommendation for adoption to the three ICH regulatory bodies.	5 June 2014

M7 Step 4 version

M7	Corrigendum to fix typographical errors and replace word “degradants” with “degradation products” throughout the document.	23 June 2014
----	--	--------------

Current M7(R1) Addendum Step 2 version

M7(R1)	Approval by the Steering Committee under <i>Step 2</i> and release for public consultation.	9 June 2015
--------	---	-------------

Legal notice: *This document is protected by copyright and may be used, reproduced, incorporated into other works, adapted, modified, translated or distributed under a public license provided that ICH's copyright in the document is acknowledged at all times. In case of any adaption, modification or translation of the document, reasonable steps must be taken to clearly label, demarcate or otherwise identify that changes were made to or based on the original document. Any impression that the adaption, modification or translation of the original document is endorsed or sponsored by the ICH must be avoided.*

The document is provided "as is" without warranty of any kind. In no event shall the ICH or the authors of the original document be liable for any claim, damages or other liability arising from the use of the document.

The above-mentioned permissions do not apply to content supplied by third parties. Therefore, for documents where the copyright vests in a third party, permission for reproduction must be obtained from this copyright holder.

ADDENDUM TO ICH M7: ASSESSMENT AND CONTROL OF DNA REACTIVE (MUTAGENIC) IMPURITIES IN PHARMACEUTICALS TO LIMIT POTENTIAL CARCINOGENIC RISK

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

M7(R1)

Draft ICH Consensus Guideline

Released for Consultation on 9 June 2015, at *Step 2* of the ICH Process

TABLE OF CONTENTS

Application Of The Principles Of The ICH M7 Guideline To Calculation Of Compound-Specific Acceptable Intakes	1
Acceptable Intakes (AIs) or Permissible Daily Exposures (PDEs)	6
** for future compounds	7
Acrylonitrile (CAS# 107-13-1).....	8
Aniline (CAS# 62-53-3) and Aniline Hydrochloride (CAS# 142-04-1).....	14
Aniline and Aniline HCl – Details of carcinogenicity studies.....	16
Benzyl Chloride (α -Chlorotoluene, CAS# 100-44-7)	22
Bis(chloromethyl)ether (BCME, CAS# 542-88-1)	28
<i>p</i> -Chloroaniline (CAS# 106-47-8) and <i>p</i> -Chloroaniline HCl (CAS# 20265-96-7)	32
1-Chloro-4-nitrobenzene (para-Chloronitrobenzene, CAS# 100-00-5).....	37
<i>p</i> -Cresidine (2-Methoxy-5-methyl aniline, CAS# 120-71-8)	43
Dimethylcarbonyl chloride (CAS# 79-44-7).....	48
Dimethyl Sulfate (CAS# 77-78-1).....	53
Ethyl chloride (Chloroethane, CAS# 75-00-3)	57
Glycidol (CAS# 556-52-5)	60
Hydrazine (CAS# 302-01-2).....	64
Hydrogen peroxide (CAS# 7722-84-1).....	70
Hydroxylamine (CAS# 7803-49-8)	75
Methyl chloride (Chloromethane, CAS# 74-87-3).....	80

LIST OF ABBREVIATIONS

AI	Acceptable Intakes
ATSDR	Agency for Toxic Substances & Disease Registry
BC	Benzyl Chloride
BCME	Bis(chloromethyl)ether
BUA	Biodegradable in water Under Aerobic conditions
CAC	Cancer Assessment Committee
CCRIS	Chemical Carcinogenesis Research Information System
CHL	Chinese Hamster Lung fibroblast cell line
CICAD	Concise International Chemical Assessment Document
CIIT	Chemical Industry Institute of Toxicology
CNS	Central Nervous System
CPDB	Carcinogenicity Potency Database
CYP	Cytochrome P-450
DMCC	Dimethylcarbanyl Chloride
DMS	Dimethyl Sulfate
DNA	Deoxyribose Nucleic Acid
EC	European Commission
ECHA	European Chemical Agency
EFSA	European Food Safety Autortiy
EMA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration
GRAS	Generally Recognised As Safe
HSDB	Hazardous Substance Database
IARC	International Agency for Research on Cancer
IPCS	International Program on Chemical Safety
IRIS	Integrated Risk Information System
JETOC	Japan Chemical Industry Ecology-Toxicology & information Center
JRC	Joint Research Centre
LOAEL	Lowest Observed Adverse Effect Level
MTD	Maximum Tolerated Dose
NA	Not applicable
NC	Not calculated as individual tumor type incidences not provided in WHO, 2002
NCI	National Cancer Institute
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NSRL	No Significant Risk Level
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
PCE	Polychromatic Erythrocytes
PDE	Permissible Daily Exposure
RfC	Reference Concentration
ROS	Reactive Oxygen Species
SARC	Structure-Activity Relationships
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCE	Sister Chromatid Exchanges
SIDS	Screening Information Dataset

TBA	Tumor Bearing Animal
TTC-based	Threshold of Toxicological Concern-based
UDS	Unscheduled DNA Synthesis
UNEP	United Nations Environmental Programm
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

1 **ADDENDUM TO ICH M7: ASSESSMENT AND CONTROL OF DNA**
2 **REACTIVE (MUTAGENIC) IMPURITIES IN PHARMACEUTICALS TO**
3 **LIMIT POTENTIAL CARCINOGENIC RISK**

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

6 **M7(R1)**

7
8 **Application Of The Principles Of The ICH M7 Guideline To Calculation Of**
9 **Compound-Specific Acceptable Intakes**

10 **Introduction**

11 The ICH M7 Guideline discusses the derivation of Acceptable Intakes (AIs) for mutagenic
12 impurities with positive carcinogenicity data, (Section 7.2.1) and states: *“Compound-specific*
13 *risk assessments to derive acceptable intakes should be applied instead of the TTC-based*
14 *(Threshold of Toxicological Concern-based) acceptable intakes where sufficient*
15 *carcinogenicity data exist. For a known mutagenic carcinogen, a compound-specific*
16 *acceptable intake can be calculated based on carcinogenic potency and linear extrapolation*
17 *as a default approach. Alternatively, other established risk assessment practices such as*
18 *those used by international regulatory bodies may be applied either to calculate acceptable*
19 *intakes or to use already existing values published by regulatory authorities.”*
20

21 In this Addendum to ICH M7, acceptable intakes have been derived for a set of chemicals that
22 are considered to be mutagens and carcinogens and were selected because they are common in
23 pharmaceutical manufacturing, or are useful to illustrate the principles for deriving
24 compound-specific intakes described in ICH M7¹. Compounds are included in which the
25 primary method used to derive acceptable intakes for carcinogens with a likely mutagenic
26 mode of action is the “default approach” from ICH M7 of linear extrapolation from the
27 calculated cancer potency estimate, the TD₅₀. Compounds are also included which highlight
28 alternative principles to deriving compound-specific intakes (see below).
29

30 Chemicals that are mutagens and carcinogens (Classified as Class 1 in ICH M7) include
31 chemicals that induce tumors through a non-mutagenic mode of action.

32 ICH M7 states in Section 7.2.2: *“The existence of mechanisms leading to a dose response that*
33 *is non-linear or has a practical threshold is increasingly recognized, not only for compounds*
34 *that interact with non-DNA (Deoxyribose Nucleic Acid) targets but also for DNA-reactive*
35 *compounds, whose effects may be modulated by, for example, rapid detoxification before*
36 *coming into contact with DNA, or by effective repair of induced damage. The regulatory*
37 *approach to such compounds can be based on the identification of a No-Observed Effect*
38 *Level (NOEL) and use of uncertainty factors (see ICH Q3C(R5), Ref. 7) to calculate a*
39 *Permissible Daily Exposure (PDE) when data are available.”*

¹ Some chemicals are included whose properties (including chemical reactivity, solubility, volatility, ionizability) allow efficient removal during the steps of most synthetic pathways, so that a specification based on an acceptable intake will not typically be needed.

40 Examples are provided in this Addendum to illustrate assessments of mode of action that
41 justify exclusion of some Class 1 chemicals from the linear extrapolation approach, and
42 derivation instead of a PDE calculated using uncertainty factors as described in ICH Q3C(R5).
43 These include hydrogen peroxide, which induces oxidative stress, and compounds that induce
44 tumors secondary to hemosiderosis as a consequence of methemoglobinemia, such as aniline
45 and hydroxylamine.

46 It is emphasized that the AI or PDE values presented here address carcinogenic risk. Other
47 toxicological considerations, along with quality standards, may affect final product
48 specifications.

49

50 **Methods**

51 The general process for deriving acceptable intakes included a literature review, selection of
52 cancer potency estimate [TD₅₀, taken from the Carcinogenicity Potency Database (CPDB -
53 <http://toxnet.nlm.nih.gov/cpdb/>), or calculated from published studies using the same method
54 as in the CPDB] and ultimately calculation of an appropriate AI or PDE in cases with
55 sufficient evidence for a threshold mode of action (see Section 3). The literature review
56 focused on data relating to exposure of the general population (i.e., food, water, and air),
57 mutagenicity/genotoxicity, and carcinogenicity. Any national or international regulatory
58 values (e.g., US EPA, US FDA, EMA, ECHA, WHO, etc.) are described in the compound-
59 specific assessments. Toxicity information from acute, repeat-dose, reproductive,
60 neurological, and developmental studies was not reviewed in depth except to evaluate
61 observed changes that act as a carcinogenic precursor event (e.g., irritation/inflammation, or
62 methemoglobinemia).

63

64 **1. Standard Method**

65 ***1.1 Linear Mode of Action and Calculation of AI***

66 Note 4 of ICH M7 states: *“It is possible to calculate a compound-specific acceptable intake*
67 *based on rodent carcinogenicity potency data such as TD₅₀ values (doses giving a 50% tumor*
68 *incidence equivalent to a cancer risk probability level of 1:2). Linear extrapolation to a*
69 *probability of 1 in 100,000 (i.e., the accepted lifetime risk level used) is achieved by simply*
70 *dividing the TD₅₀ by 50,000. This procedure is similar to that employed for derivation of the*
71 *TTC.”*

72

73 Thus, linear extrapolation from a TD₅₀ value was considered appropriate to derive an AI for
74 those Class 1 impurities (known mutagenic carcinogens) with no established “threshold
75 mechanism”, that is, understanding of a mode of action that results in a non-linear dose-
76 response curve. In many cases, the carcinogenicity data were available from the CPDB; the
77 conclusions were based either on the opinion of the original authors of the report on the
78 carcinogenicity study (“author opinion” in CPDB) or on the conclusions of statistical analyses
79 provided in the CPDB. When a pre-calculated TD₅₀ value was identified in the CPDB for a
80 selected chemical, this value was used to calculate the AI; the relevant carcinogenicity data
81 were not reanalyzed and the TD₅₀ value was not recalculated.

82

83 If robust data were available in the literature but not in the CPDB, then a TD₅₀ was calculated
84 based on methods described in the CPDB (<http://toxnet.nlm.nih.gov/cpdb/td50.html>). The
85 assumptions for animal body weight, respiratory volume, and water consumption for
86 calculation of doses were adopted from ICH Q3C and ICH Q3D.

87 **1.2 Selection of Studies**

88 The quality of studies in the CPDB is variable, although the CPDB does impose criteria for
89 inclusion such as the proportion of the lifetime during which test animals were exposed. For
90 the purposes of this Addendum further criteria were applied. Studies of lesser quality were
91 defined here as those where one or more of the following scenarios were encountered:

- 92 • < 50 animals per dose per sex;
- 93 • < 3 dose levels;
- 94 • Lack of concurrent controls;
- 95 • Intermittent dosing (< 5 days per week);
- 96 • Dosing for less than lifetime.

97
98 The more robust studies were generally used to derive limits. However studies that did not
99 fulfill all of the above criteria were in some cases considered adequate for derivation of an AI
100 when other aspects of the study were robust, for example when treatment was for 3 days per
101 week (e.g., Benzyl Chloride [BC]) but there was evidence that higher doses would not have
102 been tolerated, i.e., a Maximum Tolerated Dose (MTD) as defined by NTP or ICH S1C was
103 attained. Calculations of potency take intermittent or less-than-lifetime dosing into account;
104 for example, in the CPDB the dose levels shown have been adjusted to reflect the estimated
105 daily dose levels, such that the daily dose given 3 times per week is multiplied by 3/7 to give
106 an average daily dose; a comparable adjustment is made if animals are treated for less than 24
107 months. Use of less robust data can sometimes be considered acceptable when no more
108 complete data exist, given the highly conservative nature of the risk assessment in which TD₅₀
109 was linearly extrapolated to a 1 in 100,000 excess cancer risk. In these cases, the rationale
110 supporting the basis for the recommended approach is provided in the compound-specific
111 assessments.

112

113 **1.3 Selection of Tumor and Site**

114 The lowest TD₅₀ of a particular organ site for an animal species and sex was selected from the
115 most robust studies. When more than one study exists, the CPDB provides a calculated
116 harmonic mean TD₅₀, but in this Addendum the lowest TD₅₀ was considered a more
117 conservative estimate. Data compiled as “all Tumor Bearing Animals” (TBA) were not
118 considered in selecting an appropriate TD₅₀ from the CPDB; mixed tumor types (e.g.,
119 adenomas and carcinomas) in one tissue (e.g., liver) were used where appropriate as this often
120 gives a more sensitive potency estimate.

121

122 **1.4 Route of Administration**

123 Section 7.5 of ICH M7 states: “The above risk approaches described in Section 7 are
124 applicable to all routes of administration and no corrections to acceptable intakes are
125 generally warranted. Exceptions to consider may include situations where data justify route-
126 specific concerns that should be evaluated case-by-case.”

127

128 In this Addendum, when robust data were available from carcinogenicity studies for more
129 than one route, and the tumor sites did not appear to be route- specific, the TD₅₀ from the
130 route with the lower TD₅₀ was selected for the AI calculation and is thus usually considered
131 suitable for all routes. Exceptions may be necessary case by case; for example, in the case of
132 a potent site-of-contact carcinogen a route-specific AI or PDE might be necessary. Other
133 toxicities such as irritation might also limit the acceptable intake for a certain route, but only

134 tumorigenicity is considered in this Addendum. Here, if tumors were considered site-specific
135 (e.g., inhalation exposure resulting in respiratory tract tumors with no tumors at distal sites)
136 and the TD₅₀ was lower than for other routes, then a separate AI was developed for that route
137 (e.g., dimethyl carbamoyl chloride, hydrazine).
138

139 **1.5 Calculation of AI from the TD₅₀**

140 Calculating the AI from the TD₅₀ is as follows (see Note 4 of ICH M7 for example):

141
142
$$AI = TD_{50} / 50,000 \times 50 \text{ kg}$$

143
144 The weight adjustment assumes an arbitrary adult human body weight for either sex of 50 kg.
145 This relatively low weight provides an additional safety factor against the standard weights of
146 60 kg or 70 kg that are often used in this type of calculation. It is recognized that some adult
147 patients weigh less than 50 kg; these patients are considered to be accommodated by the
148 inherent conservatism (i.e., linear extrapolation of the most sensitive organ site) used to
149 determine an AI.
150

151 **2. Consideration of Alternative Methods for Calculation of AI**

152 **2.1 Human relevance of tumors**

153 Note 4 of ICH M7 states: *“As an alternative of using the most conservative TD₅₀ value from*
154 *rodent carcinogenicity studies irrespective of its relevance to humans, an in-depth*
155 *toxicological expert assessment of the available carcinogenicity data can be done in order to*
156 *initially identify the findings (species, organ, etc.) with highest relevance to human risk*
157 *assessment as a basis for deriving a reference point for linear extrapolation.”*

158
159 Human relevance of the available carcinogenicity data was considered for deriving AIs.
160 Effects in rodents associated with toxicities that occur with a non-linear dose response are not
161 relevant to humans at the low, non-toxic concentrations associated with a pharmaceutical
162 impurity. For example, in the case of *p*-Chloroaniline, the most sensitive site for tumor
163 induction was the spleen, but these tumors were associated with hemosiderosis, considered to
164 be a mode of action with a non-linear dose response, and thus not relevant to humans at low
165 doses. In the case of *p*-Chloroaniline, liver tumors, with a higher TD₅₀, were used for the
166 linear extrapolation to calculate the AI.

167 A second category of tumors considered not to be relevant to humans is tumors associated
168 with a rodent-specific mode of action e.g., methyl chloride.
169

170 **2.2 Published regulatory limits**

171 Note 4 of ICH M7 also states: *“Compound-specific acceptable intakes can also be derived*
172 *from published recommended values from internationally recognized bodies such as World*
173 *Health Organization (WHO, International Program on Chemical Safety (IPCS) Cancer Risk*
174 *Assessment Programme) and others using the appropriate 10⁻⁵ lifetime risk level. In general,*
175 *a regulatory limit that is applied should be based on the most current and scientifically*
176 *supported data and/or methodology.”*

177
178 In this Addendum, available regulatory limits are described (omitting occupational health
179 limits as they are typically regional and may use different risk levels). However the
180 conservative linear extrapolation from the TD₅₀ was generally used as the primary method to

181 derive the AI, as the default approach of ICH M7, and for consistency across compounds. It
182 is recognized that minor differences in methodology for cancer risk assessment can result in
183 different recommended limits (for example adjusting for body surface area in calculations),
184 but the differences are generally quite small when linear extrapolation is the basis of the
185 calculation.
186

187 3. Non-linear (Threshold) Mode of Action and Calculation of PDE

188 ICH M7 states in Section 7.2.2: “The existence of mechanisms leading to a dose response
189 that is non-linear or has a practical threshold is increasingly recognized, not only for
190 compounds that interact with non-DNA targets but also for DNA-reactive compounds, whose
191 effects may be modulated by, for example, rapid detoxification before coming into contact
192 with DNA, or by effective repair of induced damage. The regulatory approach to such
193 compounds can be based on the identification of a No-Observed Effect Level (NOEL) and use
194 of uncertainty factors (see ICH Q3C(R5)) to calculate a Permissible Daily Exposure (PDE)
195 when data are available.”
196

197 An example of a DNA-reactive chemical for which a threshold has been established for
198 mutagenicity *in vitro* and *in vivo* is ethyl methane sulfonate (Müller *et al.* 2009; Cao *et al.*
199 2014). A PDE calculation using uncertainty factors, instead of linear extrapolation is
200 appropriate in such cases.
201

202 This threshold approach was considered appropriate in the compound-specific assessments for
203 carcinogens with modes of action (Section 2.1) that lack human relevance at low doses, based
204 upon their association with a non-linear dose response for tumor induction:

- 205 • Chemicals that induce methemoglobinemia, hemosiderin deposits in tissues such as
206 spleen, and subsequent inflammation and tumors (e.g., aniline and related compounds);
 - 207 ○ Supporting information includes evidence that mutagenicity was not central to the
208 mode of action, such as weak evidence for mutagenicity e.g., aniline and
209 hydroxylamine; and/or lack of correlation between sites or species in which *in vivo*
210 genotoxicity (such as DNA adducts) and tumor induction were seen.
- 211 • Chemicals that induce tumors associated with local irritation/inflammation (such as
212 rodent forestomach tumors) and are site-of-contact carcinogens may be considered not
213 relevant to human exposure at low, non-irritating concentrations as potential impurities in
214 pharmaceuticals (e.g., benzyl chloride);
- 215 • Chemicals that act through oxidative damage, so that deleterious effects do not occur at
216 lower doses since abundant endogenous protective mechanisms exist, (e.g., hydrogen
217 peroxide).
218

219 Acceptable exposure levels for carcinogens with a threshold mode of action were established
220 by calculation of PDEs. The PDE methodology is further explained in ICH Q3C and ICH
221 Q3D.
222

223 4. Acceptable Limit Based on Exposure in the Environment, e.g., in the Diet

224 As noted in ICH M7 Section 7.5, “Higher acceptable intakes may be justified when human
225 exposure to the impurity will be much greater from other sources e.g., food, or endogenous
226 metabolism (e.g., formaldehyde).” For example, formaldehyde is not a carcinogen orally, so
227 that regulatory limits have been based on non-cancer endpoints. Health Canada, IPCS and US

228 EPA (Integrated Risk Information System [IRIS]) recommend an oral limit of 0.2 mg/kg/day,
229 or 10 mg/day for a 50 kg person.
230

231 **References**

232 Cao X, Mittelstaedt RA, Pearce MG, Allen BC, Soeteman-Hernández LG, Johnson GE, et al.
233 Quantitative dose-response analysis of ethyl methanesulfonate genotoxicity in adult gpt-delta
234 transgenic mice. *Environ Mol Mutagen* 2014; 55:385-99.
235

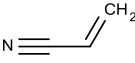
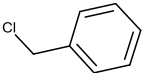
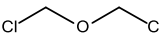
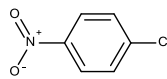
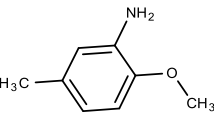
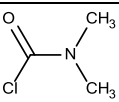
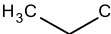
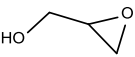

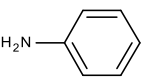
236 Health Canada. 2001 Priority substances list assessment report: Formaldehyde. Ottawa.
237 Ministry of Public Works and Government Services. February. [Online]. Searched October 10,
238 2013; Available from: URL: [http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-](http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/index_e.html)
239 [lsp2/index_e.html](http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/index_e.html)
240

241 World Health Organization (WHO). International Programme on Chemical Safety (IPCS).
242 2002. Concise International Chemical Assessment Document 40. Formaldehyde.
243

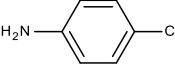
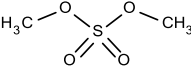
244 IRIS (Integrated Risk Information System). U.S. Environmental Protection Agency. [Online].
245 1990; Available from: URL: <http://www.epa.gov/iris/>
246

247 Müller L, Gocke E, Lave T, Pfister T. Ethyl methanesulfonate toxicity in Viracept-A
248 comprehensive human risk assessment based on threshold data for genotoxicity. *Toxicol. Lett*
249 2009; 190:317-29.
250

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

Compound	CAS#	Chemical Structure	AI or PDE (µg/d)	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
p-Cresidine	120-71-8		45	TD ₅₀ linear extrapolation
Dimethylcarbamoyl chloride	79-44-7		5 0.6 (inhalation) *	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		42 Inhalation: 0.2*	TD ₅₀ linear extrapolation
Methyl Chloride	74-87-3	Cl-CH ₃	1,360	Defaulted to TD ₅₀ linear extrapolation even though tumors were likely
Threshold-based PDE				
Aniline Aniline HCl	62-53-3 142-04-1		720	PDE based on threshold mode of action (hem siderosis)
Hydrogen peroxide	7722-84-1	HO-OH	6,960	PDE based on threshold (oxidant stress where protective antioxidant

Addendum to ICH M7: Application Of The Principles Of The ICH M7 Guideline To Calculation Of Compound-Specific Acceptable Intakes

Compound	CAS#	Chemical Structure	AI or PDE (µg/d)	Comment
				mechanisms overwhelmed)
Hydroxylamine	7803-49-8	HO—NH ₂	2	PDE based on threshold mode of action (hemosiderosis)
Endogenous and food exposure**				
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 (not most sensitive site, which was spleen tumors associated with hemosiderosis)
Dimethyl Sulfate	77-78-1		1.5	Carcinogenicity data available, but inadequate to derive AI. Default to TTC.

252 *Route specific limit

253 ** for future compounds

254

Acrylonitrile (CAS# 107-13-1)

255 **Potential for human exposure**

256 Industrial use. No data are available for exposure of the general population.

257

258 **Mutagenicity/Genotoxicity**

259 Acrylonitrile is mutagenic and genotoxic *in vitro* and *in vivo*.

260

261 The World Health Organization (WHO) published Concise International Chemical
262 Assessment Document (CICAD) 39 in 2002, providing a thorough risk assessment of
263 acrylonitrile. In this publication, the reviewers indicated that oxidative metabolism is a
264 critical step for acrylonitrile to exert genotoxic effects, implicating cyanoethylene oxide as a
265 DNA-reactive metabolite. A detailed review of genotoxicity testing in a range of systems is
266 provided in CICAD 39 (WHO, 2002) with references, so only a few key conclusions are
267 summarized here.

268 Acrylonitrile is mutagenic in:

- 269 • Microbial reverse mutation assay (Ames) in *Salmonella typhimurium* TA 1535 and TA
270 100 only in the presence of rat or hamster S9 and in several *Escherichia coli* strains in
271 the absence of metabolic activation;
- 272 • Human lymphoblasts and mouse lymphoma cells, reproducibly with S9, in some cases
273 without S9;
- 274 • Splenic T cells of rats exposed *via* drinking water.

275

276 Studies of structural chromosome aberrations and micronuclei in rodent bone marrow and
277 blood are negative or inconclusive. There are consistent reports of DNA binding in the liver
278 following acrylonitrile administration, but reports are conflicting for the brain, which is the
279 primary target of carcinogenesis.

280

281 **Carcinogenicity**

282 Acrylonitrile is classified as a Group 2B carcinogen, possibly carcinogenic to humans (IARC,
283 1999).

284

285 Acrylonitrile is a multi-organ carcinogen in mice and rats, with the brain being the primary
286 target organ in rat. There are four oral carcinogenicity studies cited in the CPDB (Gold and
287 Zeiger, 1997) and the results from three additional oral studies are summarized in CICAD 39
288 (WHO, 2002). Of these seven studies only one is negative but this study tested only a single
289 dose administered for short duration (Maltoni *et al.* 1988).

290 The NCI/NTP (National Cancer Institute) study in the CPDB of acrylonitrile in mice was
291 selected for derivation of the oral and inhalation AI, based on robust study design and the
292 most conservative TD₅₀ value. In this 2 year-study, 3 doses of acrylonitrile were administered
293 byoral gavage to male and female mice. There were statistically significant increases in
294 tumors of the Harderian gland and forestomach.

295

296 In the CPDB, it appears that the most sensitive TD₅₀, slightly lower than that for forestomach
297 tumors in mice, is for astrocytomas in female rats (5.31 mg/kg/day) in the study of Quast *et al.*
298 1980a, cited in the CPDB as a report from Dow Chemical. There were 46-48 animals per
299 treatment group and 80 animals in controls. This study was later described in detail in a

300 publication by Quast (2002) and the calculated doses in that published report are higher than
 301 those in the CPDB. Quast (2002) describes the derivation of doses in mg/kg/day from the
 302 drinking water concentrations of 35, 100 and 300 ppm, adjusting for body weight and the
 303 decreased water consumption in the study. The TD₅₀ for astrocytomas derived from these
 304 numbers is 20.2 mg/kg/day for males and 20.8 for females, in contrast to the calculated values
 305 in the CPDB of 6.36 and 5.31 mg/kg/day.

306 Studies considered less robust included three rat drinking water studies. The largest
 307 (Bio/Dynamics, 1980b), included five acrylonitrile treated groups with 100 animals per dose
 308 and 200 control animals, but serial sacrifices of 20 animals per treatment group occurred at 6,
 309 12, 18 and 24 months. Data summaries presented in CICAD 39 (WHO, 2002) and IRIS
 310 present tumor incidence based on data from all time points combined. Therefore, the
 311 incidence of tumors reported may be an underestimate of the total tumors that would be
 312 observed if all animals were kept on study for 2 years. Studies by Bigner *et al.* (1986) and
 313 BioDynamics (1980a), had only two dose levels and individual tumor types are not reported
 314 (WHO, 2002), although tumors of stomach, Zymbal gland and brain were observed.
 315

316 Acrylonitrile has also been studied by the inhalation route. The study by Quast *et al.* 1980b
 317 exposed 50 rats per sex per dose for 2 years to acrylonitrile, and observed brain tumors. This
 318 study however, tested only 2 doses. The other inhalation studies were deficient in number of
 319 animals per group, duration of exposure, or administration of a single dose, although brain
 320 tumors were observed.

321 Acrylonitrile – Details of carcinogenicity studies

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
NCI/NTP*	50 B6C3F1 Mice (F)	2 year/ Gavage	50	3: 1.79;7.14;1 4.3 mg/kg/d	Fore- Stomach	6.77 ⁺⁺
	50 B6C3F1 Mice (M)	2 year/ Gavage	50	3: 1.79;7.14;1 4.3 mg/kg/d	Fore- Stomach	5.92 ⁺⁺
Quast, <i>et al.</i> 1980a In CPDB	~50 SD Spartan rats (F)	2 year/ Water	~80	3: 2.00;5.69;1 5.4 mg/kg/d	CNS	5.31 ⁺⁺
	~50 SD Spartan rats (M)	2 year/ Water	~80	3: 1.75;4.98;1 4.9 mg/kg/d	Stomach, non- glandular	6.36 ⁺⁺
Quast, 2002 Report of Quast 1980a	~50 SD Spartan rats (F)	2 year/ Water	~80	3: 4.4;10.8; 25 mg/kg/d	Stomach, non- glandular	19.4
	~50 SD Spartan rats	2 year/ Water	~80	3: 3.4;8.5;21.	Stomach, non-	9.0

Addendum to ICH M7: Application Of The Principles Of The ICH M7 Guideline To Calculation Of Compound-Specific Acceptable Intakes

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
	(M)			3 mg/kg/d	glandular	
Bio/Dynamics 1980b [¥]	100 male rats	~2 year/ Water	~200	5: 0.1-8.4 mg/kg/d	Brain astrocytoma	(22.9) ⁺
	100 female rats	~2 year/ Water	~200	5: 0.1-10.9 mg/kg/d	Brain astrocytoma	(23.5) ⁺
Bio/Dynamics 1980a [¥]	100/sex rats	19-22 months/ Water	~98	2: ~0.09; 7.98 mg/kg/d	Stomach, Zymbal's gland, brain, spinal cord	NC
Bigner, <i>et al.</i> 1986 [¥]	50/sex rats	18 months/ Water	No	2: 14;70 mg/kg/d	Brain, Zymbal's gland, forestomach	NC [^]
Gallagher, <i>et al.</i> 1988	20 CD rats (M)	2 year/ Water	No	3: 1; 5; 25 mg/kg/d	Zymbal's gland	30.1
Maltoni <i>et al.</i> , 1988	40/sex SD rats	1 year/ 3d/week Gavage	75/sex	1: 1.07 mg/kg/d	Neg in both sexes	NA
Quast, <i>et al.</i> 1980b	100/sex SD Spartan rat	2 year 6 h/d; 5d/wk Inhalation	~100	2: M: 2.27; 9.1 F: 3.24; 13.0 mg/kg/d	Brain astrocytoma Male	32.4
Maltoni <i>et al.</i> 1988	30/sex SD rats	1 yr 5d/wk; 1 year observation Inhalation	30	4: M: 0.19; 0.38; 0.76; 1.52 F: 0.27;0.54;1 .0; 2.17 mg/kg/d	Brain glioma Male	19.1
Maltoni <i>et al.</i> 1988	54 female SD rats	2 yr 5d/wk inhalation	60	1: 11.1 mg/kg/d	Brain glioma	(132) ^W

322 Studies listed are in CPDB unless otherwise noted [Cancer Potency Database
323 <http://toxnet.nlm.nih.gov/cpdb/>].

324 *Carcinogenicity study selected for AI calculation; in CPDB

325 ^NC= Not calculated as individual tumor type incidences not provided in WHO, 2002.

326 ⁺TD₅₀ calculated based on astrocytoma incidence implied as most significant site in WHO,
327 2002. Serial sampling reduced number of animals exposed for 2 years, so tumor incidences
328 may be underestimates.

329 ⁺⁺Taken from the CPDB. The TD₅₀ values represent the TD₅₀ from the most sensitive tumor
330 site.

331 TD₅₀ values in parentheses are considered less reliable as explained in footnotes.

332 NA= Not applicable.

333 [¥]Not in CPDB. Summarized by WHO, 2002 and National Library of Medicine IRIS database.

334 ^Ψ Single dose-level study.

335

336 **Mode of action for carcinogenicity**

337 Although the mechanism of carcinogenesis remains inconclusive, a contribution of DNA
338 interaction cannot be ruled out (WHO, 2002). Carcinogenicity Studies (CNS) tumors were
339 seen in multiple studies in rats, and forestomach tumors were also prominent; this was the
340 most sensitive tumor type in mice.

341 Forestomach tumors are associated with local irritation and inflammation, and Quast (2002)
342 notes the typical association between these tumors in rats and hyperplasia and/or dyskeratosis,
343 with other inflammatory and degenerative changes. Forestomach tumors in rodents
344 administered high concentrations orally, a type of site-of-contact effect, may not be relevant to
345 human exposure to low concentrations that are non-irritating (for discussion see, for example,
346 Proctor *et al.* 2007). However, acrylonitrile is not only a site-of contact carcinogen. Tumors
347 were seen in the CNS, in addition to tissues likely to be exposed directly (such as the
348 gastrointestinal tract, tongue and Zymbal gland). Forestomach tumors were seen after
349 administration of acrylonitrile to rats in drinking water, and by gavage. Thus, the AI was
350 derived here based on mouse forestomach tumors.

351

352 **Regulatory and/or Published Limits**

353 The US EPA (01/01/1991) calculated an oral slope factor of 0.54 /mg/kg/day and a drinking
354 water limit of 0.6 µg/L at the 1/100,000 risk level, based on the occurrence of multi-organ
355 tumors in a drinking water study in rats. This equates to a daily dose of ~1 µg/day for a 50 kg
356 human.

357

358 **Acceptable Intake (AI)**

359 Rationale for selection of study for AI calculation

360

361 Both inhalation and oral studies (gavage and drinking water) are available. Tumors of the
362 CNS were seen by both route of administration, and acrylonitrile is rapidly absorbed *via* all
363 routes of exposure and distributed throughout examined tissues (WHO, 2002), so that a
364 specific inhalation AI was not considered necessary. All of the carcinogenicity studies that
365 were used by the US EPA in the derivation of the drinking water limit for acrylonitrile were
366 reviewed when selecting the most robust carcinogenicity study for the derivation of an AI.
367 Here, the NCI/NTP study was selected to calculate the AI based on the TD₅₀ derived from
368 administering acrylonitrile by oral gavage to male and female mice. The tumor type with the
369 lowest TD₅₀ was forestomach tumors in male mice, with a TD₅₀ value of 5.92 mg/kg/day. As
370 discussed in the Methods Section 2.2, linear extrapolation from the TD₅₀ was used here to
371 derive the AI, and it is expected that minor differences in methodology can result in different

372 calculated limits; thus the AI calculated below for potential pharmaceutical impurities is
373 slightly higher than that derived by US EPA for drinking water.

374

375 Calculation of AI:

376

377 Lifetime AI = $TD_{50}/50,000 \times 50\text{kg}$

378

379 Lifetime AI = $5.92 \text{ (mg/kg/day)}/50,000 \times 50 \text{ kg}$

380

381 **Lifetime AI = 5.9 µg/day (6 µg/day)**

382

383 **References**

384 Bigner DD, Bigner SH, Burger PC, Shelburne JD, Friedman HS. Primary brain tumors in
385 Fischer 344 rats chronically exposed to acrylonitrile in their drinking water. Food and
386 Chemical Toxicology 1986; 24:129–37.

387

388 Bio/Dynamics Inc. Monsanto Company, Division of Biology and Safety evaluation. 1980a. A
389 twenty-four month oral toxicity/carcinogenicity study of acrylonitrile administered to Spartan
390 rats in the drinking water. Final report. Two volumes. St. Louis, MO. Project No. 77-1745;
391 BDN-77-28.

392 Bio/Dynamics Inc. Monsanto Company. 1980b. A twenty-four month oral
393 toxicity/carcinogenicity study of acrylonitrile administered in the drinking water to Fischer
394 344 rats. Final report. Four volumes. St. Louis, MO. Project No. 77-1744; BDN-77-27.

395 Gallagher GT, Maull EA, Kovacs K, Szab S. Neoplasms in rats ingesting acrylonitrile for two
396 years. J Am Coll Toxicol 1988 7(5):603-15.

397

398 Gold S, Zeiger E, editors. Handbook of Carcinogenic Potency and Genotoxicity Databases,
399 Boca Raton, FL: CRC Press. [Online]. 1997; Available from: URL:
400 <http://toxnet.nlm.nih.gov/cpdb/td50.html>.

401

402 International Agency for Research on Cancer (IARC). IARC monographs on the evaluation
403 of carcinogenic risks to humans. Acrylonitrile 1999; Vol. 71, 43.

404

405 Maltoni C, Ciliberti A, Cotti G, Perino G. Long-term carcinogenicity bioassays on
406 acrylonitrile administered by inhalation and by ingestion to Sprague-Dawley rats. Annals of
407 the New York Academy of Sciences 1988; 534:179–202.

408

409 National Toxicology Program (NTP) Toxicology and Carcinogenesis Studies of Acrylonitrile
410 (CAS No. 107-13-1) In B6C3F1 Mice (Gavage Studies). NTP TR 506 NIH Publication No.
411 02-4440. 2001; 198.

412

413 Proctor DM, Gatto NM, Hong SJ, Allamneni KP. Mode-of-action framework for evaluation
414 of the relevance of rodent forestomach tumors in cancer risk assessment. Toxicol. Sci 2007;
415 98:313-26.

416

- 417 Quast JF, Wade CE, Humiston CG, Carreon RM, Hermann EA, Park CN *et al.* editors. A
418 Two-Year Toxicity and Oncogenicity Study with Acrylonitrile Incorporated in the Drinking
419 Water of Rats, Final Report. Dow Chemical USA, Midland, MI; 1980a.
- 420 Quast JF, Schuetz DJ, Balmer MF, Gushow TS, Park CN, McKenna MJ, editors. A Two-Year
421 Toxicity and Oncogenicity Study with Acrylonitrile Following Inhalation Exposure of Rats,
422 Final Report. Dow Chemical USA, Midland, MI; 1980b.
- 423 Quast, JF Two-year toxicity and oncogenicity study with acrylonitrile incorporated in the
424 drinking water of rats. *Toxicol. Lett.* 2002; 132:153-96.
- 425 U.S. EPA, Integrated Risk Information System (IRIS) Carcinogenicity Assessment
426 Acrylonitrile. Accessed *via* National Library of Medicine IRIS Database [Online]. Revised
427 01/01/1991b; Available from: URL: <http://toxnet.nlm.nih.gov/>
428
- 429 World Health Organization (WHO). CICADS 39: Concise International Chemical
430 Assessment Document 39 Acrylonitrile. [Online]. Geneva. 2002; Available from: URL:
431 <http://www.inchem.org/documents/cicads/cicads/cicad39.htm>
432
433

434 **Aniline (CAS# 62-53-3) and Aniline Hydrochloride (CAS# 142-04-1)**

435 **Potential for human exposure**

436 Aniline occurs naturally in some foods (i.e., corn, grains, beans, and tea), but the larger source
437 of exposure is in industrial settings.
438

439 **Mutagenicity/genotoxicity.**

440 Aniline is not mutagenic in the microbial reverse mutation assay (Ames) in *Salmonella* and is
441 considered weakly mutagenic and genotoxic. A discussion is included here because of the
442 historical perception that aniline is a genotoxic carcinogen.
443

444 Aniline is not mutagenic in *Salmonella* with or without S9 or in *E.Coli* WP2 uvrA with S9 up
445 to 3000 µg/plate (Chung *et al.* 1996; IARC Monographs, 1982, 1987a & b; Jackson *et al.*
446 1993). Further Ames study data are described in both the Chemical Carcinogenesis Research
447 Information System (CCRIS) and IRIS databases (Brams *et al.* 1987; Rashid *et al.*, 1987;
448 Gentile *et al.* 1987) and show aniline to be negative in all 5 standard strains.
449

450 Aniline was mutagenic in the mouse lymphoma L5178Y cell *tk* assay with and without S9 at
451 quite high concentrations (Wangenheim and Bolcsfoldi, 1988; Amacher *et al.* 1980;
452 McGregor *et al.* 1991).
453

454 Chromosomal aberration tests gave mixed results; both negative and some weakly positive
455 results are reported in hamster cell lines at very high, cytotoxic concentrations, e.g., about 5 to
456 30 mM, with or without S9 metabolic activation (Abe and Sasaki, 1977; Ishidate and
457 Odashima, 1977; Galloway *et al.* 1987; Ishidate, 1983; Chung *et al.* 1996).
458

459 *In vivo*, chromosomal aberrations were not increased in the bone marrow of male CBA mice
460 after two daily i.p. doses of 380 mg/kg (Jones and Fox, 2003), but a small increase in
461 chromosomal aberrations 18 h after an oral dose of 500 mg/kg to male PVR rats was reported
462 by Bomhard (2003).
463

464 Most studies of micronucleus induction are weakly positive in bone marrow after oral or
465 i.p.treatment of mice (Westmoreland and Gatehouse, 1991; Ashby *et al.* 1991; Sicardi *et al.*
466 1991; Ress *et al.* 2002) or rats (George *et al.* 1990; Bomhard 2003), and most commonly at
467 high doses, above 300 mg/kg. Dietary exposure to 500, 1000 and 2000 ppm for 90 days was
468 associated with increases in micronuclei in peripheral blood of male and female B6C3F1 mice
469 (Witt *et al.* 2000).
470

471 *In vivo*, a weak increase in Sister Chromatid Exchanges (SCE), reaching a maximum of 2-fold
472 increase over the background, was observed in the bone marrow of male Swiss mice 24 h after
473 a single intraperitoneal dose of 61 to 420 mg/kg aniline (Parodi *et al.* 1982; 1983). DNA
474 strand breaks were not detected in the mouse bone marrow by the alkaline elution assay in this
475 study.
476
477

478 **Carcinogenicity**

479 Aniline is classified as Group 3, not classifiable as to its carcinogenicity in humans (IARC,
480 1987b).

481
482 Bladder cancers in humans working in the dye industry were initially thought to be related to
483 aniline exposure but were later attributed to exposures to intermediates in the production of
484 aniline dyes, such as β -naphthylamine, benzidine, and other amines.

485
486 The Chemical Industry Institute of Toxicology (CIIT, 1982) performed a study in which
487 aniline hydrochloride was administered in the diet for 2 years to CD-F rats (130
488 rats/sex/group) at levels of 0, 200, 600, and 2000 ppm. An increased incidence of primary
489 splenic sarcomas was observed in male rats in the high dose group only. This study was
490 selected for derivation of the PDE for aniline based on the robust study design with 3 dose
491 groups and a large group size (130/sex/group).

492
493 The results of the CIIT study are consistent with those of the dietary study by the US National
494 Cancer Institute (NCI, 1978) of aniline hydrochloride in which male rats had increases in
495 hemangiosarcomas in multiple organs including spleen, and a significant dose-related trend in
496 incidence of malignant pheochromocytoma. In mice (NCI 1978), no statistically significant
497 increase in any type of tumor was observed at very high doses.

498
499 With aniline itself, no tumors were seen in male rats, with a less robust study design
500 (Hagiwara *et al.* 1980).

501
502

503

Aniline and Aniline HCl – Details of carcinogenicity studies

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
CIIT, 1982* Aniline HCl	130/sex/ group, CD-F rats	2 years (diet)	130	3: 200, 600 and 2000 ppm in diet (M;7.2;22;7 2 mg/kg/d)	Spleen (high dose) NOEL at low dose	Not reported
NCI 1978** Aniline HCl	50/sex/group, F344 rats	103 wk treatment (diet), 107-110 wk study	50	2: 3000 and 6000 ppm in diet (F: 144;268 M: 115;229 mg/kg/d)	Hemangiosarco ma in multiple organs including spleen/ Male	146 (Male)
NCI, 1978** Aniline HCl	50/sex/group B6C3F1 mice	103 wk treatment (diet), 107-110 wk study	50	2: 6000 and 12000 ppm in diet (F: 741;1500 M: 693;1390 mg/kg/d)	Negative	Not applicable
Hagiwar a <i>et al.</i> 1980 ⁺⁺ Aniline	10-18/group, Wistar rats (M)	80 wk Treatment (diet)	Yes	2: 0.03, 0.06 and 0.12% in diet (15;30;60 mg/kg/d)	Negative	Not applicable

504 * Carcinogenicity study selected for PDE calculation. Not in CPDB.

505 ++ Taken from CPDB. The TD₅₀ values represent the TD₅₀ from the most sensitive tumor site.

506

507 **Mode of action for carcinogenicity**

508 In animal studies, aniline induces methemoglobinemia and hemolysis at high doses, the latter
509 of which could indirectly lead to increases in micronuclei by inducing erythropoiesis
510 (Steinheider *et al.* 1985; Ashby *et al.* 1991; Tweats *et al.* 2007). Micronuclei are induced in
511 mice, while aniline induced tumors are seen in rats but not mice, adding to the evidence that
512 genotoxicity is not key to the mode of action for aniline-induced tumors.

513

514 Aniline-induced toxicity in the spleen appears to be a contributory factor for its
515 carcinogenicity *via* free radical formation and tissue injury (Khan *et al.* 1999). High doses
516 (>10 mg/kg) of aniline lead to iron accumulation in the spleen resulting from the preferential
517 binding of aniline to red blood cells and damaged cells accumulating in the spleen. Iron-
518 mediated oxidative stress in the spleen appears to induce lipid peroxidation, malondialdehyde-

519 protein adducts, protein oxidation, and up-regulation of Transforming Growth Factor- β 1, all
520 of which have been detected in the rat spleen following aniline exposure (Khan *et al.* 2003).
521 Increased oxidative stress may be a continual event during chronic exposure to aniline and
522 could contribute to the observed cellular hyperplasia, fibrosis, and tumorigenesis in rats
523 (Weinberger *et al.* 1985; Khan *et al.* 1999). The lack of tumorigenicity in mice may be due to
524 reduced toxicity observed in spleen compared to that in the rats (Smith *et al.* 1967; Bomhard,
525 2003).

526
527 In support of this toxicity-driven mode of action for carcinogenicity, the dose response for
528 aniline-induced tumorigenicity in rats is non-linear (Bus and Popp, 1987). When considering
529 the NCI and CIIT studies which both used the same rat strain, no tumours were observed
530 when aniline hydrochloride was administered in the diet at a concentration of 0.02% (equal to
531 approximately 7.2 mg/kg/day aniline in males). This, together with studies evaluating the
532 pattern of accumulation of bound radiolabel derived from aniline in the spleen (Roberston *et*
533 *al.* 1983) support the conclusion that a threshold exists for aniline carcinogenicity (Bus and
534 Popp, 1987). The weight of evidence supports the conclusion that these tumours do not result
535 from a primary mutagenic mode of action (Bomhard and Herbold 2005).

536

537 **Regulatory and/or Published Limits**

538 The US EPA IRIS database outlines a quantitative cancer risk assessment for aniline based on
539 the CIIT study and use of a linearised multistage procedure (IRIS, 2008). The resulting
540 cancer potency slope curve was 0.0057/mg/kg/day and the dose associated with a 1 in 100,000
541 lifetime cancer risk is calculated to be 120 μ g/day. However, the assessment states that this
542 procedure may not be the most appropriate method for the derivation of the slope factor as
543 aniline accumulation in the spleen is nonlinear (IRIS, 2008). Minimal accumulation of aniline
544 and no hemosiderosis is observed at doses below 10 mg/kg and as already described,
545 hemosiderosis may be important in the induction of the splenic tumours observed in rats.

546

547 **Permissible Daily Exposure (PDE)**

548 It is considered inappropriate to base an AI for aniline on linear extrapolation for spleen
549 tumours observed in rats, since these have a non-linear dose response, and
550 mutagenicity/genotoxicity is not central to the mode of action of aniline-induced
551 carcinogenicity. The PDE is derived using the process defined in ICH Q3C.

552

553 Rationale for selection of study for PDE calculations.

554

555 Data from the CIIT 2-year rat carcinogenicity study have been used to derive risk-based dose
556 levels. Dose levels of 200, 600 and 2000 ppm for aniline hydrochloride in the diet were
557 equivalent to dose levels of aniline of 7.2, 22 and 72 mg/kg/day. Tumors were observed in
558 high dose males and one stromal sarcoma of the spleen was identified at 22 mg/kg/day. Based
559 on these data the lowest dose of 7.2 mg/kg/day was used to define the No Observed Adverse
560 Effect Level (NOAEL).

561

562 The PDE calculation is: (NOAEL x body weight adjustment (kg)) / F1 x F2 x F3 x F4 x F5

563

564 The following safety factors as outlined in ICH Q3C have been applied to determine the PDE
565 for aniline:

566

567 F1 = 5 (rat to human)
568 F2 = 10 (inter- individual variability)
569 F3 = 1 (study duration at least half lifetime)
570 F4 = 10 (severe toxicity – non-genotoxic carcinogenicity)
571 F5 = 1 (using a NOAEL)

572
573 Lifetime PDE = $7.2 \times 50 \text{ kg} / (5 \times 10 \times 1 \times 10 \times 1)$

574
575 **Lifetime PDE = 720 µg/day**
576

577 **References**

578 Abe S, Sasaki M. Chromosome aberrations and sister chromatic exchanges in Chinese
579 hamster cells exposed to various chemicals. J. Natl: Cancer Inst. 1977; 58: 1635-41.

580
581 Amacher DE, Paillet SC, Turner GN, Ray VA, Salsburg DS. Point mutations at the thymidine
582 kinase locus in L5178Y mouse lymphoma cells. Mutat. Res. 1980 72: 447-74.

583
584 Ashby J, Vlachos DA, Tinwell H. Activity of aniline in the mouse bone marrow micronucleus
585 assay. Mutat. Res. 1991; 263: 115-7.

586
587 Bomhard EM. High-dose clastogenic activity of aniline in the rat bone marrow and its
588 relationship to the carcinogenicity in the spleen of rats. Arch. Toxicol. 2003 77: 291-7.

589
590 Bomhard EM, Herbold BA. Genotoxic activities of aniline and its metabolites and their
591 relationship to the carcinogenicity of aniline in the spleen of rats. Crit. Rev. Toxicol. 2005;
592 35(10):783-835.

593
594 Brams A, Buchet JP, Crutzen-Fayt MC, De Meester C, Lauwerys R, Leonard A. A
595 Comparative Study, With 40 Chemicals, Of The Efficiency Of The Salmonella Assay And
596 The SOS Chromotest (Kit Procedure). Toxicol. Lett. 1987; 38(1-2):123-33.

597
598 Bus JS, Popp JA. Perspectives on the mechanism of action of the splenic toxicity of aniline
599 and structurally-related compounds. Food. Chem. Toxicol. 1987; 25(8): 619-26.

600
601 Chung KT, Murdock CA, Zhou Y, Stevens SE, Li YS, Wei CI, et al. Effects of the nitro-
602 group on the mutagenicity and toxicity of some benzamines. Environ. Molecul. Mutagen.
603 1996; 27:67-74.

604
605 CIIT. 1982. 104-week chronic toxicity study in rats with aniline hydrochloride. Final report.
606 Report prepared for CIIT by Hazleton Laboratories America, Inc. CIIT Docket No. 11642.
607 CIIT, Research Triangle Park, NC.

608
609 Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, et al. Chromosome
610 aberrations and sister chromatid exchanges in Chinese Hamster Ovary cells: Evaluations Of
611 108 Chemicals; Environ. Mol. Mutagen. 1987; 10 Suppl 10:1-175.

612
613 Gentile JM, Gentile GJ and Plewa M. Mutagenicity of selected aniline derivatives to
614 Salmonella following plant activation and mammalian hepatic activation; Mutat. Res. 1987;
615 188(3):185-96.

- 616
617 George E, Andrews M, and Westmoreland C. Effects of azobenzene and aniline in the rodent
618 bone marrow micronucleus test. *Carcinogenesis* 1990; 11: 1551-5.
619
- 620 Hagiwara A, Arai M, Hirose M, Nakanowatari J-I, Tsuda H and Ito N. Chronic effects of
621 norharman in rats treated with aniline. *Toxicol Lett.* 1980; 6:71-5.
622
- 623 IARC. Monographs on the evaluation of carcinogenic risks to humans: Genetic and related
624 effects – an updating of selected IARC Monographs from volumes 1 to 42. IARC
625 Publications, 1987a. Addendum 6, pp. 68, Lyon, France.
626
- 627 IARC. Monographs on the evaluation of carcinogenic risks to humans: Overall evaluation of
628 carcinogenicity – an updating of IARC Monographs volumes 1 to 42. IARC Publications,
629 1987b. Addendum 7, pp. 99 and 362, Lyon, France.
630
- 631 IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Some
632 aromatic amines, anthraquinones and nitroso compounds, and inorganic fluorides used in
633 drinking water and dental preparations. IARC Publications, 1982. Vol. 27, pp. 39, Lyon,
634 France.
635
- 636 IRIS. United States Environmental Protection Agency (U.S. EPA) Integrated Risk
637 Information System monograph on aniline (CAS No 62-53-3). Version last updated January
638 10th 2008; Available from: URL: <http://www.epa.gov/ncea/iris/subst/0350.htm>
639
- 640 Ishidate M Jr. The data book of chromosomal aberration tests *in vitro* on 587 chemical
641 substances using Chinese hamster fibroblast cell line (CHL cells). The Realize Inc., Tokyo,
642 1983; 607 pp.
643
- 644 Ishidate M, Jr, Odashima S. Chromosome tests with 134 compounds on Chinese hamster cells
645 *in vitro* – A screening for chemical carcinogens. *Mutat.*
646 *Res.* 1977; 48: 337-54.
647
- 648 Jackson MA, Stack HF, Waters MD. The genetic toxicology of putative nongenotoxic
649 carcinogens. *Mutat Res* 1993; 296:241-77.
650
- 651 Jones E, Fox V. Lack of clastogenicity activity of aniline in the mouse bone marrow.
652 *Mutagenesis* 2003; 18:283-6.
653
- 654 Khan MF, Wu X, Wang JL. Upregulation of transforming growth factor-beta 1 in the spleen
655 of aniline-induced rats. *Toxicol Appl Pharmacol* 2003; 187:22-8.
656
- 657 Khan MF, Wu X, Boor PJ, Ansari GAS. Oxidative modification of lipids and proteins in
658 aniline induced splenic toxicity. *Toxicol Scis* 1999; 48:134-40.
659
- 660 McGregor DB, Brown AG, Howgate S, McBride D, Riach C, Caspary WJ. Responses of the
661 L5178y mouse lymphoma cell forward mutation assay. V: 27 Coded Chemicals; *Environ Mol*
662 *Mutagen* 1991; 17(3):196-219.
663
- 664 NCI (National Cancer Institute) National Toxicology Program. 1978. Bio-assay for Aniline
665 hydrochloride for possible carcinogenicity. CAS No., 142-04-1, NCI-CG-TR-130. US

- 666 Department of Health, Education, and Welfare, National Institutes of Health.ITS.
667 Carcinogenesis Technical Report Ser No. 130. US DHEW, PHS, NIH, Bethesda, MD. DHEW
668 Publ. No (NIH) 78-1385.
669
- 670 Parodi S, Pala M, Russo P, Zunino A, Balbi C, Albini A, et al. DNA damage in liver, kidney,
671 bone marrow, and spleen of rats and mice treated with commercial and purified aniline as
672 determined by alkaline elution assay and sister chromatid exchange induction. *Cancer Res.*
673 1982; 42:2277-83.
674
- 675 Parodi S, Zunino A, Ottaggio L, De Ferrari M, Santi L. Lack of correlation between the
676 capability of inducing sister chromatid exchanges *in vivo* and carcinogenic potency for 16
677 aromatic amines and azo derivatives. *Mutat Res* 1983; 108:225-38.
678
- 679 Rashid KA, Arjmand M, Sandermann H, Mumma RO. Mutagenicity of chloroaniline / lignin
680 metabolites in the Salmonella/microsome assay; *J Environ Sci Health* 1987; Part B
681 B22(6):721-9.
682
- 683 Ress NB, Witt KL, Xu J, Haseman JK, Bucher JR. Micronucleus induction in mice exposed
684 to diazoaminobenzene or its metabolites, benzene and aniline: implications for
685 diazoaminobenzene carcinogenicity. *Mutat Res* 2002; 521:201-8.
686
- 687 Robertson O, Cox MG, Bus JS. Response of of the erythrocyte and spleen to aniline insult in
688 Fischer 344 rats. *Toxicologist* 1983; 3:128.
689
- 690 Sicardi SM, Martiarena JL, Iglesias MT. Mutagenic and analgesic activities of aniline
691 derivatives. *J Pharm Sci* 1991; 80:761-4.
692
- 693 Smith RP, Alkaitis AA, Shafer PR. Chemically induced methemoglobinemias in the mouse.
694 *Biochem. Pharmacol* 1967; 16:317-28.
695
- 696 Steinheider G, Neth R, Marguardt H. Evaluation of nongenotoxic and genotoxic factors
697 modulating the frequency of micronucleated erythrocytes in the peripheral blood of mice.
698 *Cell Biol. Toxicol* 1985; 1:197-211.
699
- 700 Tweats D, Blakey D, Heflich RH, Jacobs A, Jacobsen SD, Nohmi TT, et al. Report of the
701 IWGT working group on strategies and interpretation of regulatory *in vivo* tests. I. Increases
702 in micronucleated bone marrow cells in rodents that do not indicate genotoxic hazards.
703 *Mutat Res* 2007; 627:78-91
704
- 705 Wangenheim J, Bolcsfoldi G. Mouse lymphoma L5178Y thymidine kinase locus assay of 50
706 compounds; *Mutagenesis* 1988; 3(3):193-205.
707
- 708 Weinberger MA, Albert RH, Montgomery SB. Splenotoxicity associated with splenic
709 sarcomas in rats fed high doses of D & C Red No. 9 or aniline hydrochloride. *J Natl Cancer*
710 *Inst* 1985; 75:681-7.
711
- 712 Westmoreland C, Gatehouse DG. Effects of aniline hydrochloride in the mouse bone marrow
713 micronucleus test after oral administration. *Carcinogenesis* 1991; 12:1057-9.
714

715 Witt KL, Knapton A, Wehr CM, Hook GJ, Mirsalis J, Shelby MD et al. Micronucleated
716 erythrocyte frequency in peripheral blood of B6C3F1 mice from short-term, prechronic and
717 chronic studies of the NTP carcinogenesis bioassay program. *Environ Mol Mutagen* 2000; 36,
718 163–94.
719

720 **Benzyl Chloride (α -Chlorotoluene, CAS# 100-44-7)**

721 **Potential for human exposure**

722 Human exposure is mainly occupational *via* inhalation while less frequent is exposure from
723 ingesting contaminated ground water.
724

725 **Mutagenicity/Genotoxicity**

726 Benzyl chloride is mutagenic and genotoxic *in vitro* but not in mammalian systems *in vivo*.
727

728 The International Agency for Research on Cancer (IARC) published a monograph performing
729 a thorough review of the mutagenicity/genotoxicity data for benzyl chloride (IARC, 1999). A
730 few key conclusions are summarized here.
731

- 732 • Mutagenic in the microbial reverse mutation assay (Ames) in *Salmonella typhimurim*
733 strain TA100 with and without metabolic activation produced weak and inconsistent
734 increase in mutation frequency. The results are more convincing when testing in the
735 gaseous phase (Fall *et al.* 2007).
- 736 • Benzyl chloride induced sister chromatid exchanges, chromosomal aberrations, mutations,
737 and DNA strand breaks in cultured rodent cells and induced DNA strand breaks, but not
738 chromosomal aberrations in cultured human cells. Benzyl chloride did not induce
739 micronuclei *in vivo* in bone marrow of mice (IARC, 1999).
740

741 **Carcinogenicity**

742 Benzyl chloride is classified as Group 2A, probably carcinogenic to humans (IARC, 1982,
743 1987).
744

745 Lijinsky (1986) administered benzyl chloride in corn oil by gavage 3 times/week for 104
746 weeks to F-344 rats and B6C3F1 mice. Rats received doses of 0, 15, or 30 mg/kg (estimated
747 daily dose: 0, 6.4, 12.85 mg/kg); mice received doses of 0, 50, or 100 mg/kg (estimated daily
748 dose: 0, 21.4, 42.85 mg/kg). In rats, the only statistically significant increase in the tumor
749 incidence was thyroid C-cell adenoma/carcinoma in the female high-dose group (27% versus
750 8% for control). Control incidence for this tumor type in males was 23% and there was no
751 difference in C-cell hyperplasia with treatment between treated rats and controls of either sex.
752 Several toxicity studies were conducted but C-cell hyperplasia was noted only in this lifetime
753 study and only in female rats.
754

755 In mice, there were statistically significant increases in the incidence of forestomach
756 papillomas and carcinomas (largely papillomas) at the high dose in both males and females
757 (62% and 37%, respectively, compared with 0% in controls). Epithelial hyperplasia was
758 observed in the stomachs of animals without tumors. There were also statistically significant
759 increases in male but not female mice in hemangioma or hemangiosarcoma (10% versus 0%
760 in controls) at the high dose and in carcinoma or adenoma in the liver but only at the low, not
761 the high, dose (54% and 39%, respectively, versus 33% in controls). In female, but not male,
762 mice there were significant increases in the incidence of alveolar-bronchiolar adenoma or
763 carcinoma at the high dose (12% versus 1.9% in controls).
764

765 Additional studies to assess carcinogenic potential were conducted but were not considered to
 766 be adequate in terms of study design for use in calculating an AI. In one of three topical
 767 studies (Fukuda *et al.* 1981) skin carcinomas were increased, although not statistically
 768 significantly (15% versus 0% in benzene controls). Initiation-promotion studies to determine
 769 the potential of benzyl chloride to initiate skin cancer, using croton oil and the phorbol ester
 770 TPA (12-O-tetradecanoyl-phorbol-13-acetate) as promoters (Ashby, 1982; Coombs, 1982a
 771 and b) were of limited duration and the published reports were presented as preliminary
 772 findings, but no final results have been located in the literature. Injection site sarcomas were
 773 seen after subcutaneous administration (Druckrey *et al.* 1970).
 774

775 **Benzyl chloride – Details of carcinogenicity studies**

Study	Animals/dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
Lijinsky <i>et al.</i> 1986*	52/sex/group F344 rat	3 times/wk, 2 year. Gavage	52	2: 15 and 30 mg/kg (6 and 12 mg/kg/d)	Thyroid C-cell neoplasm Female	40.6 ⁺⁺
Lijinsky <i>et al.</i> 1986	52/sex/group B6C3F1 mouse	3 times/wk, 2 year. Gavage	52	2: 50 and 100 mg/kg (21 and 42 mg/kg/d)	Forestomach papilloma, carcinoma Male	49.6 ⁺⁺
Fukuda <i>et al.</i> 1981	11/ group ICR mouse female	3 times/wk for 4 wks, 2 times/wk 9.8 months Dermal	Yes (benzene treated)	1: 10 µL	No skin tumors	NC [^]
Fukuda <i>et al.</i> 1981	20/ group ICR mouse (F)	2 times/wk for 50 wks, Dermal	20 (benzene treated)	1: 2.3 µL	Skin squamous cell carcinoma	NC [^]
Ashby 1982	20 / group ICI Swiss albino mouse (M)	2 times/wk for >7 months Dermal, in toluene	20	1: 100 µg/mouse	No skin tumors	NC [^]
Druckrey <i>et al.</i> 1970	14 (40 mg/kg), and 8 (80 mg/kg) BD rat	1/wk for 51 wks subcutaneous	Yes	2: 40 and 80 mg/kg/wk	Injection site scarcoma	NC [^]
Coombs 1982a	40/sex/ group Theiler's Original mouse	1 dose (in tolene); wait 1 wk Promoter	40	1: 1 mg/mouse	No skin tumors	NC [^]

Study	Animals/dose group	Duration/Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
		(croton oil) 2 times/wk for 10 months				
Coombs 1982b	Sencar mice	1 dose; Promoter (TPA) 2 times/wk for 6 months	Yes	3: 10; 100 and 1000 µg/mouse	20% skin tumors [5% in TPA controls] (DMBA controls had skin tumors by 11 weeks)	NC [^]

776 Studies listed are in CPDB [Cancer Potency Database <http://toxnet.nlm.nih.gov/cpdb/>].

777 * Carcinogenicity study selected for AI calculation.

778 ^NC= Not calculated; small group size, limited duration. Not included in CPDB as route with
779 greater likelihood of systemic exposure is considered more relevant.

780 ++ Taken from CPDB. The TD₅₀ values represent the TD₅₀ from the most sensitive tumor site.

781

782 Mode of action for carcinogenicity

783 The tumor types with the lowest calculated TD₅₀ (highest potency) in the CPDB for benzyl
784 chloride are forestomach tumors in mice and thyroid C-cell tumors in female rats. The
785 relevance of the forestomach tumors to human risk assessment for low, non-irritating doses
786 such as those associated with a potential impurity is highly questionable.

787

788 Forestomach tumors in rodents have been the subject of much discussion in assessment of risk
789 to humans. With non-mutagenic chemicals, it is recognized that after oral gavage
790 administration, inflammation and irritation related to high concentrations of test materials in
791 contact with the forestomach can lead to hyperplasia and ultimately tumors. (Material
792 introduced by gavage can remain for some time in the rodent forestomach before discharge to
793 the glandular stomach, in contrast to the rapid passage through the human esophagus). Such
794 tumor induction is not relevant to humans at non-irritating doses. The same inflammatory and
795 hyperplastic effects are also seen with mutagenic chemicals, where it is more complex to
796 determine relative contribution to mode of action of these non-mutagenic, high- dose effects
797 compared with direct mutation induction. However, often a strong case can be made for site-
798 of contact tumorigenesis that is only relevant at concentrations that cause
799 irritation/inflammation, potentially with secondary mechanisms of damage. Cell proliferation
800 is expected to play an important role in tumor development such that there is a non-linear dose
801 response and the forestomach (or other site-of-contact) tumors are not relevant to low-dose
802 human exposure.

803

804 Proctor *et al.* (2007) propose a systematic approach to evaluating relevance of forestomach
805 tumors in cancer risk assessment, taking into account whether any known genotoxicity is
806 potentially relevant to human tissues (this would include whether a compound is genotoxic *in*

807 *vivo*), whether tumors after oral administration of any type are specific to forestomach, and
808 whether tumors are observed only at doses that irritate the forestomach or exceed the MTD.
809 As described above and in the table, benzyl chloride predominantly induces tumors at the site
810 of contact in rats and mice following exposure to high doses by gavage (forestomach tumors),
811 by injection (injection site sarcoma) and by topical application in a skin tumor initiation-
812 promotion model in sensitive Sencar mice. An OECD report in the Screening Information
813 Dataset (SIDS) for high volume chemicals describes benzyl chloride as intensely irritating to
814 skin, eyes, and mucous membranes in acute and repeat dose studies. Groups of 10 Fischer
815 344 rats of both sexes died within 2 weeks from severe acute and chronic gastritis of the
816 forestomach, often with ulcers, following oral administration 3 times/week of doses ≥ 250
817 mg/kg for males and ≥ 125 mg/kg for females (Lijinsky *et al.* 1986). Proliferative changes
818 observed in female rats at lower doses included hyperplasia of the forestomach (62 mg/kg),
819 and hyperkeratosis of the forestomach (30 mg/kg). The incidence of forestomach tumors was
820 high in mice in the carcinogenicity study, and Lijinsky *et al.* (1986) also observed non-
821 neoplastic lesions in the forestomach of the rat in the subchronic range-finding study, but few
822 forestomach neoplasms developed in the rat carcinogenicity assay. Due to the steepness of
823 the dose-response curve and the difficulty establishing the MTD for rats, the author speculates
824 that it was possible that the dose used in the rat study was marginally too low to induce a
825 significant carcinogenic effect in rats.

826
827 In the case of benzyl chloride, other tumor types were discussed as possibly treatment-related
828 besides those at the site of contact. In the mouse oral bioassay, Lijinsky characterized the
829 carcinogenic effects other than forestomach tumors as “marginal”, comprising an increase of
830 endothelial neoplasms in males, alveolar-bronchiolar neoplasms of the lungs only in female
831 mice (neither of these is statistically significant) and hepatocellular neoplasms only in low
832 dose male mice (this tumor type was discounted as not dose related). It is of note that OECD
833 SIDS reports observations of severe to moderate dose-related liver hyperplasia in a 26-week
834 oral toxicity study in mice.

835
836 Statistically significant increases were reported in hemangiomas/hemangiosarcomas of the
837 circulatory system in the male mice (TD₅₀ 454 mg/kg/day), and in thyroid C-cell adenomas or
838 carcinomas in the female rats (TD₅₀ 40.6 mg/kg/day). The levels of thyroid C-cell tumors in
839 female rats in the high dose group, while higher than female concurrent controls, (14/52
840 versus 4/52 in controls) were similar to the levels in the male concurrent controls (12/52). In
841 males, thyroid C- cell tumor levels were lower in treated than in control rats. In a compilation
842 of historical control data from Fisher 344 rats in the NTP studies, Haseman *et al.* (1984;
843 1998) show comparable levels of C-cell adenomas plus carcinomas in males and females in
844 this rat strain, although the range is wider in males. Thus it is likely justifiable to compare the
845 thyroid tumor levels in female rats treated with benzyl chloride with the concurrent controls of
846 both sexes, and question whether the female thyroid tumors are treatment-related, although
847 they were higher than the historical control range cited at the time (10%).

848

849 **Regulatory and/or Published Limits**

850 The US EPA derived an Oral Slope Factor of 1.7×10^{-1} per (mg/kg)/day, which corresponds to
851 a 1 in 100,000 risk level of approximately 4 $\mu\text{g}/\text{day}$ using US-EPA assumptions.

852 **Acceptable Intake (AI)**

853 Rationale for selection of study for AI calculation

854
855 The most robust evaluation of the carcinogenic potential of benzyl chloride was the Lijinsky
856 *et al.* study (1986) that utilized oral (gavage) administration. In this study, the animals were
857 treated 3 days a week rather than 5 days a week as in a typical NCI/NTP study. Overall,
858 however, the rat study is considered adequate for calculation of an AI because there was
859 evidence that the top dose was near the maximum tolerated dose. In a 26-week range finding
860 study described in the same report (Lijinsky *et al.* 1986), all ten rats of each sex given 125 or
861 250 mg/kg (3 days per week) died within 2-3 weeks. The cause of death was severe gastritis
862 and ulcers in the forestomach; in many cases there was also myocardial necrosis. At 62
863 mg/kg, only 4 of 26 females survived to 26 weeks, and myocardial necrosis and forestomach
864 hyperplasia were seen; hyperkeratosis of the forestomach was seen in some females at 30
865 mg/kg. At 62 mg/kg benzyl chloride, there was a decrease in body weight gain in both sexes,
866 which was statistically significant in males. Thus, the high dose chosen for the
867 carcinogenicity study was 30 mg/kg (3 times per week). At this dose, there was no difference
868 from controls in survival in the 2-year carcinogenicity study, but 3 male rats had squamous
869 cell carcinomas and papillomas of the forestomach, so it is unlikely that a lifetime study could
870 have been conducted at a higher dose.

871
872 As described in the Methods Section 2.2., linear extrapolation from the TD₅₀ was used to
873 derive the AI. As described above, it is highly unlikely that benzyl chloride poses a risk of
874 site-of-contact tumors in humans exposed to low concentrations as impurities in
875 pharmaceuticals, well below concentrations that could cause irritation/inflammation.
876 Therefore, the observed forestomach tumors in male mice are not considered relevant for the
877 AI calculation. The significance of the thyroid C-cell tumors in female rats is also
878 questionable since these tumors occur commonly in control rats. However, given the
879 uncertain origin of these tumors, the thyroid C-cell tumors were used to derive the AI since
880 they were associated with the lowest TD₅₀; 40.6 mg/kg/day.

881
882 Calculation of AI

883
884 Lifetime AI = TD₅₀/50,000 x 50 kg

885
886 Lifetime AI = 40.6 (mg/kg/day)/50,000 x 50 kg

887
888 **Lifetime AI = 40.6 µg/day (41 µg/day)**

889
890 **References**

891 Ashby J, Gaunt C, Robinson M. Carcinogenicity bioassay of 4-chloromethylbiphenyl (4CMB),
892 4-hydroxymethylbiphenyl (4HMB) and benzyl chloride (BC) on mouse skin: Interim (7
893 month) report. *Mutat Res* 1982; 100:399-401.

894
895 Coombs MM. Attempts to initiate skin tumours in mice in the 2-stage system using 4-
896 chloromethylbiphenyl (4CMB), -hydroxymethylbiphenyl (4HMB) and benzyl chloride (BC),
897 Report of the experiment at 10 months, *Mutat Res* 1982a; 100:403-5.

- 898 Coombs MM. The UKEMS Genotoxicity Trial: A summary of the assays for skin tumour
899 induction in mice, the subcutaneous implant test and the sebaceous gland suppression test,
900 *Mutat Res* 1982b; 100:407-9.
901
- 902 Druckrey H, Kruse H, Preussmann R, Ivankovic S, Landschuetz C. Cancerogenic alkylating
903 substances. III. Alkyl-halogenides, - sulfates, - sulfonates and strained heterocyclic
904 compounds. 1970; 74(3):241-73
905
- 906 Fall M, Haddouk H, Morin JP, Forster R. Mutagenicity of benzyl chloride in the
907 *Salmonella*/microsome mutagenesis assay depends on exposure conditions, *Mutat Res* 2007;
908 633:13-20.
909
- 910 Fukuda K, Matsushita H, Sakabe H, Takemoto K. Carcinogenicity of benzyl chloride, benzal
911 chloride, benzotrichloride and benzoyl chloride in mice by skin application, *Gan* 1981; 72(5):
912 655-64.
913
- 914 Haseman JK, Huff J, Boorman GA. Use of historical control data in carcinogenicity studies in
915 rodents, *Toxicol Pathol* 1984; 12:126-35.
916
- 917 Haseman JK, Hailey JR, Morris RW. Spontaneous neoplasm incidence in Fischer 344 rats and
918 B6C3F1 mice in two-year carcinogenicity studies: A National Toxicology Program update,
919 *Toxicol Pathol* 1998; 26:428-41.
920
- 921 IARC. Monographs on the evaluation of the carcinogenic risk of chemicals to man. Geneva:
922 WHO, International Agency for Research on Cancer, [Online] 1972-PRESENT.
923 (Multivolume work). 1999. Accessed on April 25, 2014. Available from: URL:
924 [http://monographs.iarc.fr/ENG/Monographs/vol71/mono71-](http://monographs.iarc.fr/ENG/Monographs/vol71/mono71-19.pdf)
925 [19.pdf](http://monographs.iarc.fr/ENG/Monographs/vol71/volume71.pdf)<http://monographs.iarc.fr/ENG/Monographs/vol71/volume71.pdf>
926
- 927 Lijinsky W. Chronic Bioassay of Benzyl Chloride in F344 Rats and (C57BL/6J X BALB/c)
928 F1 Mice. *J Natl Cancer Inst* 1986; 76(6):1231-6.
929
- 930 OECD Chemicals Screening Information Dataset (SIDS) for high volume chemicals benzyl
931 chloride report published by the United Nations Environmental Programm (UNEP) [Online].
932 Accessed on April 25, 2014; Available from: URL:
933 <http://www.chem.unep.ch/irptc/sids/OECDSEIDS/100447.pdf>
934
- 935 Proctor DM, Gatto NM, Hong SJ, Allamneni KP. Mode-of-action framework for evaluation
936 of the relevance of rodent forestomach tumors in cancer risk assessment. *Toxicol Sci* 2007;
937 98:313-26.

938

Bis(chloromethyl)ether (BCME, CAS# 542-88-1)

939 Potential for human exposure

940 Potential for exposure is in industrial use, mainly *via* inhalation. Environmental exposure is
941 predicted to be minimal, as result of its low industrial usage and rapid degradation in the
942 environment, which is supported by the reported absence of BCME in ambient air or water
943 (NIH ROC, 2011).
944

945 Mutagenicity/genotoxicity

946 BCME is mutagenic and genotoxic *in vitro* and *in vivo*.

947

- 948 • BCME is mutagenic in the microbial reverse mutation assay (Ames), *Salmonella*
949 *typhimurium* (Nelson, 1976).
- 950 • *In vivo*, BCME did not cause chromosomal aberrations in bone-marrow cells of rats
951 exposed to BCME vapors for six months (Leong *et al.* 1981). A slight increase in the
952 incidence of chromosomal aberrations was observed in peripheral lymphocytes of
953 workers exposed to BCME in the preparation of ion-exchange resins (IARC, 1987).
954

955 Carcinogenicity

956 BCME is classified as Group A, known human carcinogen (USEPA, 1999), and a Group 1
957 compound, carcinogenic to humans (IARC, 1982).
958

959 As described in the above reviews, numerous epidemiological studies have demonstrated that
960 workers exposed to BCME (*via* inhalation) have an increased risk for lung cancer. Following
961 exposure by inhalation, BCME is carcinogenic to the respiratory tract of rats and mice as
962 described in the following studies:
963

964 The study of Leong *et al.* (1981) was selected for derivation of the AI based on the most
965 robust study design and the lowest TD₅₀ value. Groups of male Sprague-Dawley rats and
966 Ha/ICR mice were exposed by inhalation to 1, 10 and 100 ppb of BCME 6 hr/day, 5
967 days/week for 6 months and subsequently observed for the duration of their natural lifespan
968 (about 2 years). Evaluation of groups of rats sacrificed at the end of the 6-month exposure
969 period revealed no abnormalities in hematology, exfoliative cytology of lung washes, or
970 cytogenetic parameters of bone marrow cells. However, 86.5% of the surviving rats which
971 had been exposed to 100 ppb (7780 ng/kg/day, or 8 µg/kg/day) of BCME subsequently
972 developed nasal tumors (esthesioneuroepitheliomas, which are similar to the rare human
973 neuroblastoma) and approximately 4% of the rats developed pulmonary adenomas. Tumors
974 were not observed in rats exposed to 10 or 1 ppb of BCME. Mice exposed to 100 ppb of
975 BCME did not develop nasal tumors, but showed a significant increase in incidence of
976 pulmonary adenomas over the control mice. Mice exposed to 10 or 1 ppb of BCME did not
977 show a significant increase in incidence of pulmonary adenomas.
978

979 Kuschner *et al.* (1975) conducted an inhalation study of male Sprague-Dawley rats exposed to
980 BCME at a single dose level of 0.1 ppm (100 ppb) 6 hours/day, 5 days/week for 10, 20, 40,
981 60, 80, or 100 days, then observed the animals for the remainder of their lifetimes. There was
982 a marked increase in the incidence of several types of respiratory tract tumors in the treated
983 animals compared with the controls.

984
 985 BCME is a site of contact carcinogen, producing injection site sarcomas (Van Duuren *et al.*
 986 1969) and skin tumors in mice, (Van Duuren *et al.* 1975); it also induces lung adenomas in
 987 newborn mice following skin application (Gargus *et al.* 1969).
 988

989 **Bis(chloromethyl)ether (BCME) – Details of carcinogenicity studies**

Study	Animals/dose group	Duration/Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
Leong <i>et al.</i> 1981*	~104/group Rat, Sprague-Dawley, (M).	6 h/d, 5 d/wk 28 wk. Inhalation	104	3: 1; 10; 100 ppb (53;528; 7780 ng/kg/d)	Nasal passage - esthesioneuroepitheliomas	0.00357
Leong <i>et al.</i> 1981	138-144/group Mouse, ICR/Ha, (M).	6 h/d, 5 d/wk 25 wk. Inhalation	157	3: 1; 10; 100 ppb (0.295; 2.95;33.6 ng/kg/d)	Lung adenomas	No significant increases
Kuschner <i>et al.</i> 1975	30 – 50 treated for different durations with same concentration, Sprague Dawley rats, (M).	6h/d, 5d/wk, for 10, 20, 40, 60, 80, and 100 exposures. Inhalation	240	1: 0.1 ppm	Lung and nasal cancer	NC [^]
Kuschner <i>et al.</i> 1975	100/group Golden Syrian Hamsters, (M),	6h/d, 5d/wk, for a lifetime. Inhalation	NA	1: 1 ppm	One undifferentiated in the lung	NC [^]
Van Duuren <i>et al.</i> 1975	50/group ICR/Ha Swiss mice (F).	424-456 d Intra-peritoneal injection, once weekly.	50	1: 0.114 mg/kg/d	Sarcoma (at the injection site)	0.182

990 Studies listed are in CPDB unless otherwise noted [Cancer Potency Database

991 <http://toxnet.nlm.nih.gov/cpdb/>].

992 *Carcinogenicity study selected for AI calculation

993 [^]NC= Not calculated due to non-standard carcinogenicity design. Not in CPDB.

994 NA= Not available since controls were not reported in the study

995

996 **Mode of action for carcinogenicity**

997 Not defined.
998

999 **Regulatory and/or Published Limits**

1000 The US EPA IRIS database (EPA 1988), calculated an oral cancer slope factor of 220 per
1001 mg/kg/day based on linearised multistage modelling of the inhalation study data by Kuschner
1002 *et al.* 1975. The inhaled (and oral) dose associated with a 1 in 100,000 lifetime cancer risk is
1003 3.2 ng/day (1.6×10^{-8} mg/m³ for inhalation, 1.6×10^{-6} mg/L for oral exposure).
1004

1005 **Acceptable Intake (AI)**

1006 Rationale for selection of study for AI calculation

1007
1008 BCME is an *in vitro* mutagen, causes cancer in animals and humans and is classified as a
1009 known human carcinogen. Oral carcinogenicity studies were not conducted, therefore,
1010 intraperitoneal injection and inhalation studies are considered as a basis for setting an AI. The
1011 most sensitive endpoint was an increase in nasal tumors (esthesioneuroepitheliomas, tumors
1012 of the olfactory epithelium) in male rats in the inhalation carcinogenicity study of Leong *et al*
1013 (1981), with a TD₅₀ of 3.57 µg/kg/day. The AI derived by linear extrapolation from the TD₅₀
1014 from Leong *et al.* 4 ng/day, is essentially the same as the 3.2 ng/day recommendation of the
1015 USEPA. The Leong *et al.* (1981) study is a reliable study with multiple dose levels and >50
1016 animals per dose group.
1017

1018 Evidence for tumors at other sites than those exposed by inhalation is lacking; the study cited
1019 above (Gargus *et al.* 1969) that describes lung tumors in newborn mice following skin
1020 application may not be definitive if inhalation may have occurred as a result of skin
1021 application. However, the AI derived here from inhalation data is considered applicable to
1022 other routes, because it is highly conservative (orders of magnitude below the default TTC of
1023 1.5 µg/day). The AI is also similar to the limit derived by US EPA (based on inhalation data)
1024 that is recommended both for inhalation and ingestion (drinking water) of BCME (4 ng /day
1025 vs 3.2 ng/day).
1026

1027 Calculation of AI

1028
1029 Lifetime AI = TD₅₀/50,000 x 50 kg
1030

1031 Lifetime AI = 3.57 µg/kg/day/50,000 x 50
1032

1033 **Lifetime AI = 0.004 µg/day or 4 ng/day**
1034

1035 **References**

1036 EPA. Integrated risk information system (IRIS). Printout for bis(chloromethyl) ether.
1037 Washington, DC: U.S. Environmental Protection Agency 1988 November.
1038

1039 Gargus JL, Reese WH, Rutter HA. Induction of lung adenomas in newborn mice by
1040 bis(chloromethyl) ether. Toxicol Appl Pharmacol 1969; 15:92-96.
1041

- 1042 IARC. Monographs on the evaluation of the carcinogenic risk of chemicals to humans.
1043 Chemicals, industrial processes and industries associated with cancer in humans. IARC
1044 Monographs, 1982; Volumes 1 to 29, Addendum 4.
1045
- 1046 IARC. Bis(chloromethyl)ether and chloromethyl methyl ether (technical-grade). In *Overall*
1047 *Evaluations of Carcinogenicity*. IARC Monographs on the Evaluation of Carcinogenic Risk of
1048 Chemicals to Humans. International Agency for Research on Cancer 1987; Addendum 7, pp.
1049 131-133, Lyon, France.
1050
- 1051 Kuschner M, Laskin S, Drew RT, Cappiello V, Nelson N. Inhalation carcinogenicity of alpha
1052 halo ethers. III. Lifetime and limited period inhalation studies with bis(chloromethyl)ether at
1053 0.1 ppm. Arch Environ Health 1975; 30:73-7.
1054
- 1055 Leong BKJ, Kociba RI, Jersey GC. A lifetime study of rats and mice exposed to vapors of
1056 bis(chloromethyl) ether. Toxicol Appl Pharmacol 1981; 58:269-81.
1057
- 1058 Nelson N. The chloroethers - occupational carcinogens: A summary of laboratory and
1059 epidemiology studies. Ann. NY Acad Sci 1976; 271:81-90.
1060
- 1061 NIH ROC. National Institutes of Health. Report on Carcinogens, Twelfth Edition [Online].
1062 2011 [Cited 2014 May 1]; Available from: URL:
1063 [http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/bis\(chloromethyl\)ether.pdf](http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/bis(chloromethyl)ether.pdf)
1064
- 1065 U.S. Environmental Protection Agency. National Center for Environmental Assessment,
1066 Office of Research and Development, Washington, DC. Integrated Risk Information System
1067 (IRIS) on Bis(chloromethyl)ether. [Online]. 1999; Available from: URL:
1068 <http://www.epa.gov/iris/subst/0375.htm>
1069
- 1070 Van Duuren BL, Goldschmidt BM, Seidman I. Carcinogenic activity of di- and trifunctional
1071 α -chloro ethers and of 1,4-dichlorobutene-2 in ICR/HA swiss mice. Cancer Res 1975;
1072 35:2553-7.
1073
- 1074 Van Duuren BL, Sivak A, Goldschmidt BM, Katz C, Melchionne S. Carcinogenicity of halo-
1075 ethers. J Nat Cancer Inst 1969; 43: 481-6.

1076
1077

***p*-Chloroaniline (CAS# 106-47-8) and
p-Chloroaniline HCl (CAS# 20265-96-7)**

1078 **Potential for human exposure**

1079 Industrial exposure to *p*-Chloroaniline is primarily derived from the dye, textile, rubber and
1080 other industries (Beard and Noe, 1981). If released into the environment, it is inherently
1081 Biodegradable in water Under Aerobic conditions (BUA, 1995).
1082

1083 **Mutagenicity/Genotoxicity**

1084 *p*-Chloroaniline is weakly mutagenic *in vitro*, with limited evidence for genotoxicity *in vivo*.

1085
1086 A detailed review of genotoxicity testing in a range of systems is provided in CICAD 48
1087 (WHO, 2003) with references, so only key conclusions are summarized here.
1088

- 1089 • *p*-Chloroaniline was reproducibly mutagenic in the microbial reverse mutation assay
1090 (Ames), *Salmonella typhimurium* only in strain TA98 with S9 metabolic activation,
1091 although there are conflicting data in multiple studies.
- 1092 • Weak mutagenicity has been reported in several mouse lymphoma (L6178Y) cell *tk*
1093 mutation assays in the presence of metabolic activation (WHO 2003); however the
1094 increases were very small, associated with substantial cytotoxicity, and do not meet the
1095 up-to-date criteria for a positive assay using the “global evaluation factor” (Moore *et al.*
1096 2006).
- 1097 • Small increases in chromosomal aberrations in Chinese hamster ovary cells were not
1098 consistent between two laboratories.
- 1099 • *In vivo*, a single oral treatment did not induce micronuclei in mice at 180 mg/kg, but a
1100 significant increase was reported at 300 mg/kg/day after 3 daily doses in mice.
1101

1102 **Carcinogenicity**

1103 *p*-Chloroaniline is classified as Group 2B, possibly carcinogenic to humans with adequate
1104 evidence of carcinogenicity in animals and inadequate evidence in humans (IARC, 1993).

1105
1106 Carcinogenicity studies in animals have been conducted for *p*-Chloroaniline or its
1107 hydrochloride salt, *p*-Chloroaniline HCl.
1108

1109 The NTP (1989) oral gavage study was used to calculate the AI, where *p*-Chloroaniline HCl
1110 was carcinogenic in male rats, based on the increased incidence of spleen tumors: (Combined
1111 incidence of sarcomas: vehicle control, 0/49; low dose, 1/50; mid dose, 3/50; high dose,
1112 38/50). Fibrosis of the spleen, a preneoplastic lesion that may progress to sarcomas, was seen
1113 in both sexes (Goodman *et al.* 1984; NTP, 1989). In female rats, splenic neoplasms were seen
1114 only in one mid-dose rat and one high-dose rat. Increased incidences of pheochromocytoma
1115 of the adrenal gland in male and female rats may have been related to *p*-Chloroaniline
1116 administration; malignant pheochromocytomas were not increased. In male mice, the
1117 incidence of hemangiosarcomas of the liver or spleen in high dose group was greater than that
1118 in the vehicle controls (4/50; 4/49; 1/50; 10/50). The incidences of hepatocellular adenomas or
1119 carcinomas (combined) were increased in dosed male mice; of these, the numbers of
1120 hepatocellular carcinomas were (3/50; 7/49; 11/50; 17/50). The female mouse study was
1121 negative. The final conclusion of NTP (1989) was that there was clear evidence of

1122 carcinogenicity in male rats, equivocal evidence of carcinogenicity in female rats, some
 1123 evidence of carcinogenicity in male mice, and no evidence of carcinogenicity in female mice.

1124
 1125 An earlier study used *p*-Chloroaniline administered in feed to rats and mice (NCI, 1979).
 1126 Splenic neoplasms were found in dosed male rats and hemangiomas in mice.
 1127 While the incidences of these tumors are strongly suggestive of carcinogenicity, NCI
 1128 concluded that sufficient evidence was not found to establish the carcinogenicity of *p*-
 1129 Chloroaniline in rats or mice under the conditions of these studies. Since *p*-Chloroaniline is
 1130 unstable in feed, the animals may have received the chemical at less than the targeted
 1131 concentration (WHO, 2003). Therefore, this study is deemed inadequate.
 1132

1133 ***p*-Chloroaniline and *p*-Chloroaniline HCl – Details of carcinogenicity studies**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
NTP, 1989* <i>p</i> -chloroaniline HCl	50/group B6C3F1 mice (M)	Gavage 5X/wk, 103 wk	50	3: 3; 10; 30 mg/kg (2.1; 7; 21.1 mg/kg/d)	Hepatocellular adenomas or carcinomas	33.8
NTP, 1989 <i>p</i> -chloroaniline HCl	50/group B6C3F1 mice (F)	Gavage 5X/wk, 103 wk	50	3: 3; 10; 30 mg/kg (2.1; 7; 21.1 mg/kg/d)	Negative	NA
NTP, 1989 <i>p</i> -chloroaniline HCl	50/group Fischer 344 rat (M)	Gavage 5X/wk, 103 wk	50	3: 2; 6; 18 mg/kg (1.4; 4.2; 12.6 mg/kg/d)	Spleen fibrosarcoma, haemangiosarcoma, osteosarcoma	7.62
NTP, 1989 <i>p</i> -chloroaniline HCl	50/group Fischer 344 rat (F)	Gavage 5X/wk, 103 wk	50	3: 2; 6; 18 mg/kg (1.4; 1.2; 12.6 mg/kg/d)	No significant increases; equivocal	NA
NCI, 1979	50/group Fischer 344 rat (M)	78 wk (study duration: 102 wk) Diet	20	2: 250; 500 ppm (7.7; 15.2 mg/kg/d)	Mesenchymal tumours (fibroma, fibrosarcoma, haemangiosarcoma, osteosarcoma, sarcoma not otherwise specified) of the spleen or	72

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
					splenic capsule	
NCI, 1979	50/group Fischer 344 rat (F)	78 wk (study duration: 102 wk) Diet	20	2: 250; 500 ppm (9.6, 19 mg/kg/d)	Negative	NA
NCI, 1979	50/group B6C3F1 mice (M)	78 wk (study duration: 91 wk) Diet	20	2: 2500; 5000 ppm (257;275 mg/kg/d)	Haemangiosarcomas (subcutaneous tissue, spleen, liver, kidney) Increased incidence of all vascular tumours	Not significant (CPDB)
NCI, 1979	50/group B6C3F1 mice (F)	78 wk (study duration: 102 wk) Diet	20	2: 2500; 5000 ppm (278, 558 mg/kg/d)	Haemangiosarcomas (liver and spleen) Increased incidence of combined vascular tumours	1480

1134 Studies listed are in CPDB [Cancer Potency Database <http://toxnet.nlm.nih.gov/cpdb/>].

1135 *Carcinogenicity study selected for AI calculation.

1136 NA = Not applicable

1137

1138 Mode of action for carcinogenicity

1139 *p*-Chloroaniline induced tumors in male rats, such as spleen fibrosarcomas and osteosarcomas,
1140 typical for aniline and related chemicals. Repeated exposure to *p*-Chloroaniline leads to
1141 cyanosis and methemoglobinemia, followed by effects in blood, liver, spleen, and kidneys,
1142 manifested as changes in hematological parameters, splenomegaly, and moderate to severe
1143 hemosiderosis in spleen, liver, and kidney, partially accompanied by extramedullary
1144 hematopoiesis (NCI, 1979; NTP, 1989). These effects occur secondary to excessive
1145 compound-induced hemolysis and are consistent with a regenerative anemia (WHO, 2003).
1146 The evidence supports an indirect mechanism for tumorigenesis, secondary to
1147 methemoglobinemia, splenic fibrosis and hyperplasia (e.g., Bus and Popp, 1987), and not
1148 tumor induction related to a direct interaction of *p*-Chloroaniline or its metabolites with DNA.

1149

1150 The tumor type with the lowest TD₅₀ was spleen tumors in male rats. However, since this
1151 tumor type is associated with a non-linear dose relation, a PDE calculation was done (see
1152 below). The result (143 µg/day) is comparable to the recommendation for a level of 0.2
1153 µg/kg/day, based on non-neoplastic (hematotoxic) effects (WHO 2003), i.e., 100 µg/day for a
1154 50 kg human.

1155

1156 For male mouse liver tumors, the TD₅₀ based on the combined numbers of adenomas and
1157 carcinomas was 33.8 mg/kg/day. *p*-Chloroaniline is not reproducibly mutagenic. There is
1158 one positive study *in vivo* (micronucleus test), but this was positive only at a dose level in the
1159 range of the LD₅₀ and given the known methemoglobinemia, this might be secondary to
1160 regenerative anemia/altered erythropoiesis, as with aniline (Ashby *et al.* 1991; Tweats *et al.*
1161 2007).

1162
1163 A Permissible Daily Exposure (PDE) for *p*-Chloroaniline was calculated as follows:
1164 (NOEL x body weight adjustment (kg) / F1 x F2 x F3 x F4 x F5
1165
1166 The following safety factors as outlined in ICH Q3C have been applied:
1167
1168 F1 = 5 (rat to human)
1169 F2 = 10 (inter- individual variability)
1170 F3 = 1 (study duration at least half lifetime)
1171 F4 = 10 (severe toxicity – non-genotoxic carcinogenicity)
1172 F5 = 1 (using a NOEL)
1173
1174 In the rat study of *p*-Chloroaniline HCl (NTP, 1989) the lowest dose was clearly a No
1175 Observed Effect Level (NOEL): (2 mg/kg 5 days per week, or 1.43 mg/kg/day).
1176
1177 On this basis the PDE is calculated as follows:
1178 Lifetime PDE = 1.43 x 50 kg / (5 x 10 x 1 x 10 x 1)
1179 **Lifetime PDE = 143 µg/day**
1180
1181 Conclusion
1182 Overall, there is very limited evidence for a mutagenic mode of action, but *in vivo* information
1183 is lacking. Thus, a mutagenic mode of action cannot be entirely ruled out and calculation of
1184 an AI was considered appropriate. Other single-ring aromatic amines have been associated
1185 with tumors in liver, urinary bladder and kidney (CPDB). Because a mutagenic component to
1186 the mode of action for liver tumors cannot be ruled out, the linear extrapolation AI is
1187 recommended.
1188
1189 **Regulatory and/or Published Limits**
1190 No regulatory limits have been published for *p*-Chloroaniline or the hydrochloride salt.
1191
1192 **Calculation of AI**
1193 Calculation of AI
1194
1195 Based on male mouse liver tumors for *p*-Chloroaniline HCl
1196
1197 Lifetime AI = TD₅₀/50,000 x 50kg
1198
1199 Lifetime AI = 33.8mg/kg/day /50,000 x 50 kg
1200
1201 **Lifetime AI = 34 µg/day**
1202
1203

1204 **References**

- 1205 Ashby J, Vlachos DA, Tinwell H. Activity of aniline in the mouse bone marrow micronucleus
1206 assay.- *Mutat Res* 1991 263:115-7.
1207
- 1208 Beard RR, Noe JT. Aromatic nitro and amino compounds, Clayton GD, Clayton FE, editors.
1209 Patty's Industrial Hygiene and Toxicology John Wiley, New York, 1981; 2A:2413-89.
1210
- 1211 BUA. *p*-Chloroaniline. Beratergremium für Umweltrelevante Altstoffe (BUA) der
1212 Gesellschaft Deutscher Chemiker. Weinheim, VCH, 1995; 171. (BUA Report 153).
1213
- 1214 Bus JS, Popp JA. Perspectives on the mechanism of action of the splenic toxicity of aniline
1215 and structurally-related compounds, *Food Chem Toxicol* 1987; 25:619-26.
1216
- 1217 Goodman DG, Ward JM, Reichardt WD. Splenic fibrosis and sarcomas in F344 rats fed diets
1218 containing aniline hydrochloride, *p*-Chloroaniline, azobenzene, o-toluidine hydrochloride, 4,4'-
1219 sulfonyldianiline, or D & C Red No. 9; *J Natl Cancer Inst* 1984; 73(1):265-73.
1220
- 1221 IARC. Monographs on the evaluation of carcinogenic risks to humans: Para-chloroaniline. In:
1222 Occupational exposures of hairdressers and barbers and personal use of hair colourants; some
1223 hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. International Agency
1224 for Research on Cancer, 1993; Vol. 57, pp. 305-321, Lyon, France.
1225
- 1226 NCI. Bioassay of *p*-Chloroaniline for possible carcinogenicity, CAS No. 106-47-8. Bethesda,
1227 MD, US National Cancer Institute, 1979; 88 pp. (NCI-CG-TR-189).
1228
- 1229 NTP. Toxicology and carcinogenesis studies of para-chloroaniline hydrochloride (CAS No.
1230 20265-96-7) in F344/N rats and B6C3F1 mice (gavage studies). Research Triangle Park, NC,
1231 National Toxicology Program (NTP TR 351; NIH Publication No. 89-2806). 1989.
1232
- 1233 Tweats D, Blakey D, Heflich RH, Jasobs A, Jacobsen SD, Nohmi TT, et al. Report of the
1234 IWGT working group on strategies and interpretation of regulatory *in vivo* tests. I. Increases
1235 in micronucleated bone marrow cells in rodents that do not indicate genotoxic hazards.
1236 *Mutat Res* 2007; 627:78-91
1237
- 1238 WHO. CICADS 48: Concise International Chemical Assessment Document 48
1239 *p*-chloroaniline. Geneva. [Online]. 2003; Available from: URL:
1240 <http://www.inchem.org/documents/cicads/cicads/cicad48.htm>
1241

1242 **1-Chloro-4-nitrobenzene (para-Chloronitrobenzene, CAS# 100-00-5)**

1243 **Potential for human exposure**

1244 Potential for exposure is in industrial use. No data are available for exposure of the general
1245 population.
1246

1247 **Mutagenicity/genotoxicity**

1248 1-Chloro-4-nitrobenzene is mutagenic and genotoxic *in vitro* and *in vivo*.

- 1249 • 1-Chloro-4-nitrobenzene was mutagenic in the microbial reverse mutation assay (Ames)
1250 *Salmonella typhimurium* strains TA100 and TA1535 in the presence of S9 metabolic
1251 activation, and was negative in TA1537, TA1538, TA98, and *E.coli* WP2uvrA (Haworth
1252 *et al.* 1983; Japan, 2005; Kawai *et al.* 1987; NTP, 1993). It was also weakly positive
1253 without metabolic activation in TA1535 in 2 of 4 studies (NTP, 1993).
- 1254 • Positive results have been reported for induction of structural chromosome aberrations
1255 and sister chromatid exchanges in Chinese hamster ovary (CHO) cells; the increase was
1256 weaker without than with S9 (Galloway *et al.* 1987; NTP 1993). Structural chromosome
1257 aberrations were also reported in CHL cells with and without S9 (Japan, 1996).
- 1258 • It induced single-strand DNA breaks, measured by the alkaline elution technique, in rat
1259 hepatocytes *in vitro*, and in the liver, kidney, and brain of male Swiss mice when
1260 administered intraperitoneally (Cesarone *et al.* 1983; 1984).
1261

1262 **Carcinogenicity**

1263 1-Chloro-4-nitrobenzene is classified as a Group 2 carcinogen, not classifiable as to its
1264 carcinogenicity in humans (IARC, 1996) and US EPA considers it to be a Group B2
1265 carcinogen or probable human carcinogen (US EPA, 1995).
1266

1267 Animal carcinogenicity studies have been conducted with 1-chloro-4-nitrobenzene by
1268 administration in the feed in rats and mice (Matsumoto *et al.* 2006; Weisburger *et al.* 1978;
1269 CPDB) or by gavage in male rats (Schroeder and Daly, 1984).
1270

1271 In the study of Matsumoto *et al.* (2006), there were significant increases in spleen tumors
1272 (fibroma, fibrosarcoma, osteosarcoma and sarcoma) in rats of both sexes, and there were
1273 increases in spleen hemangiosarcomas in both sexes, that were statistically significant in
1274 males at the mid and high doses (7.7 and 41.2 mg/kg/day). Non-neoplastic changes of the
1275 spleen such as fibrosis, and capsule hyperplasia were seen. An increase in adrenal medullary
1276 pheochromocytomas was seen at the high dose that was statistically significant in females
1277 (53.8 mg/kg/day). In mice, the only significant increase in tumors was in liver
1278 hemangiosarcomas at the high dose in females (275.2 mg/kg/day). Hematologic disturbances
1279 such as decreases in red blood cell numbers and haematocrit, and extramedullary
1280 hematopoiesis, were seen both in rats and in mice.
1281

1282 In the study of Weisburger *et al.* (1978), 1-chloro-4-nitrobenzene did not induce tumors in
1283 male CD-1 rats when fed in the diet for 18 months. The concentration in the feed was
1284 adjusted during the 18-month period due to toxicity as follows: The low dose group received
1285 2000 ppm for the first 3 months, 250 ppm for next 2 months, and 500 ppm from 6 to 18
1286 months; the high dose group received 4000 ppm for the first 3 months, 500 ppm for next 2
1287 months, and 1000 ppm from 6 to 18 months. The average daily exposure was approximately

1288 17 and 33 mg/kg for the low and high dose groups, respectively. Rats were sacrificed 6
 1289 months after the last dose and examined for tumors. No treatment-related increases in tumors
 1290 were observed in the 11 tissues examined (lung, liver, spleen, kidney, adrenal, heart, bladder,
 1291 stomach, intestines, testes and pituitary).
 1292

1293 Weisburger *et al.* (1978) also investigated the carcinogenic potential of 1-chloro-4-
 1294 nitrobenzene in male and female CD-1 mice, given in the feed for 18 months. Mice were
 1295 sacrificed 3 months after the last exposure and 12 tissues (lung, liver, spleen, kidney, adrenal,
 1296 heart, bladder, stomach, intestines, and reproductive organs) were examined for tumors. A
 1297 dose-dependent increase in vascular tumors (hemangiomas or hemangiosarcomas) of liver,
 1298 lung, and spleen was observed in both male and female mice.
 1299

1300 In another study (Schroeder and Daly, 1984), male and female Sprague-Dawley rats (n = 60)
 1301 were given 1-chloro-4-nitrobenzene by gavage 5 days/week for 24 months. In both sexes,
 1302 toxicity was observed: methemoglobinemia in mid- and high-dose groups, and hemosiderin
 1303 and anemia in the high-dose group.
 1304

1305 **1-Chloro-4-nitrobenzene – Details of carcinogenicity studies**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
Matsumoto <i>et al.</i> 2006 ^{*,+}	50/ group F344 rats (SPF) (M)	2 years (diet)	50	3: 40; 200; 1000 ppm. (1.5; 7.7; 41.2 mg/kg/d)	Spleen hemangiosarcomas 7.7 mg/kg/d	173.5
	50/ group F344 rats (SPF) (F)	2 years (diet)	50	3: 40; 200; 1000 ppm. (1.9; 9.8;53.8 mg/kg/d)	Female pheochromocytom a 53.8 mg/kg/d	116.9
	50/ group Crj:BDF 1 (SPF) (M)	2 years (diet)	50	3: 125;500; 2000 ppm. (15.3; 60.1;240 .1 mg/kg/d)	Not applicable	
	50/ group Crj:BDF 1 (SPF) (F)	2 years (diet)	50	3: 125;500; 2000 ppm.	Hepatic hemangiosarcomas 275.2 mg/kg/d	1919.9

				(17.6; 72.6; 275.2 mg/kg/d)		
Weisberger <i>et al.</i> 1978	14-15/group CD-1 rats (M)	18 mo diet; sacrificed 6 mo after last dose	16	2: Average 17 and 33 mg/kg; (see text) (22.6 and 45.2 mg/kg/d)	Not applicable	Negative [^]
	14-20/sex group CD-1 mice	18 mo diet; sacrificed 3 mo after last dose	15/sex	2: M: 341; 720. F: 351; 780 mg/kg/d	Vascular (hemangiomas/Hemangiosarcomas)/Male	430 [^]
Schroeder and Daly, 1984 ⁺	60/sex/group Sprague Dawley rat	Gavage, 5 d/wk: 24 mo	Yes	3: 0.1; 0.7; 5 mg/kg/d	Not applicable	Negative

1306 Studies listed are in CPDB unless otherwise noted. [Cancer Potency Database
1307 <http://toxnet.nlm.nih.gov/cpdb/>.
1308

1309 *Carcinogenicity study selected for AI/PDE calculation.

1310 ⁺Not in CPDB.

1311 [^] Histopathology limited to 11-12 tissues.

1312

1313 Mode of action for carcinogenicity

1314 1-Chloro-4-nitrobenzene is significantly metabolized by reduction to 4-chloroaniline (*p*-
1315 Chloroaniline) in rats (Yoshida *et al.* 1991), rabbits (Bray *et al.* 1956) and humans (Yoshida *et al.*
1316 1993). *p*-Chloroaniline has been shown to produce hemangiosarcomas and spleen tumors
1317 in in rats and mice, similar to 1-chloro-4-nitrobenzene (IARC, 1993). Like aniline, an indirect
1318 mechanism for vascular tumorigenesis in liver and spleen is indicated, secondary to oxidative
1319 erythrocyte injury and splenic fibrosis and hyperplasia, both for 4-chloroaniline (IARC, 1993)
1320 and 1-chloro-4-nitrobenzene (Travlos *et al.* 1996). Methemoglobinemia and associated
1321 toxicity is a notable effect of 1-chloro-4-nitrobenzene. A non-linear mechanism for tumor
1322 induction is supported by the fact that in the study of Schroeder and Daly (1984), carried out
1323 at lower doses than the studies of Matsumoto *et al.* (2006) and Weisberger *et al.* (1978),
1324 methemoglobinemia and hemosiderin were seen but there was no increase in tumors.
1325

1326 The tumor type with the lowest TD₅₀ was adrenal mudullary pheochromocytomas in female
1327 rats (Matsumoto *et al.* 2006). This tumor type is common as a background tumor in F344 rats,
1328 especially males, and is seen after treatment with a number of chemicals, many of them non-
1329 mutagenic (Greim *et al.* 2009). It has been proposed that they are associated with various

1330 biochemical disturbances, and the mode of action for induction of pheochromocytomas by
1331 chemicals such as aniline and *p*-Chloroaniline that are toxic to red blood cells may be
1332 secondary to uncoupling of oxidative phosphorylation (Greim *et al.* 2009) or perhaps hypoxia.
1333

1334 Two models were considered for deriving an acceptable intake for 1-chloro-4-nitrobenzene.
1335 First is the linear extrapolation model. It was noted that in mutagenicity studies in *Salmonella*,
1336 1-chloro-4-nitrobenzene was mutagenic in *Salmonella* TA100 and TA1535 (but not TA98 and
1337 other strains). This may indicate a mutagenic component to the mode of action for tumor
1338 induction by 1-chloro-4-nitrobenzene, but the pattern of mutagenicity is different from its
1339 metabolite *p*-Chloroaniline, which was reproducibly mutagenic only in *Salmonella* TA98
1340 with rat liver S9 (WHO, 2003) indicating differences in mutagenic metabolites or mechanism.
1341 *In vivo* genotoxicity data are lacking to help assess potential for a mutagenic mode of action.
1342

1343 Second, a non linear model was considered based on the following:

- 1344 • The most notable types of tumors induced were those associated with
1345 methemoglobinemia, (spleen and vascular tumors);
- 1346 • Adrenal medullary pheochromocytomas may be associated with the same perturbations;
- 1347 • There is clearly a non-linear dose relation (based on no-effect doses and on the the
1348 negative results of the lower-dose study of Schroeder and Daly (1984)).
1349

1350 Thus a PDE calculation was performed.

1351

1352 Calculation of Permissible Daily Exposure (PDE)

1353

1354 The PDE calculation is: (NOEL x body weight adjustment (kg)) / F1 x F2 x F3 x F4 x F5

1355

1356 The following safety factors as outlined in ICH Q3C have been applied to determine the PDE:

1357

1358 F1 = 5 (rat to human)

1359 F2 = 10 (inter- individual variability)

1360 F3 = 1 (study duration at least half lifetime)

1361 F4 = 10 (severe toxicity – non-genotoxic carcinogenicity)

1362 F5 = 1 (using a NOEL)

1363

1364 The NOAEL for changes in red blood cell parameters and for male rat spleen
1365 hemangiosarcomas in the study of Matsumoto *et al.* (2006) was 1.5 mg/kg/day. This is also
1366 below the no-effect dose for female rat pheochromocytomas.
1367

1368 Lifetime PDE = 1.5 x 50 kg / (5 x 10 x 1 x 10 x 1)

1369

1370 **Lifetime PDE = 150 µg/day**

1371

1372 Conclusion

1373 The linear and non-linear models in this case result in similar values, 117 and 150 µg/day,
1374 although the safety factor used for non-genotoxic carcinogenicity (F4 = 10) may be higher
1375 than necessary, and the PDE correspondingly lower. Because we cannot rule out a mutagenic
1376 component to the mode of action for pheochromocytomas, the linear extrapolation AI is
1377 recommended.
1378

1379 **Regulatory and/or Published Limits**

1380 No regulatory limits have been published, for example by US EPA, WHO, or Agency for
1381 Toxic Substances & Disease Registry (ATSDR).

1382

1383 **Calculation of AI**

1384 Calculation of AI

1385

1386 The most sensitive TD₅₀ is that for adrenal medullary pheochromocytomas in female rats
1387 (Matsumoto *et al.* 2006).

1388

1389 Lifetime AI = TD₅₀/50,000 x 50kg

1390

1391 Lifetime AI = 117 mg/kg/day /50,000 x 50 kg

1392

1393 **Lifetime AI = 117 µg/day**

1394

1395 **References**

1396 Bray HG, James SP, Thorpe WV. The metabolism of the monochloronitrobenzenes in the
1397 rabbit. *Biochem J* 1956; 64:38-44.

1398

1399 Cesarone CF, Bolognesi C, Santi L. DNA damage induced *in vivo* in various tissues by
1400 nitrobenzene derivatives. *Mutat Res* 1983; 116:239-46.

1401

1402 Cesarone CF, Fugassa E, Gallo G, Voci A, Orunesu M. Influence of the culture time on DNA
1403 damage and repair in isolated rat hepatocytes exposed to nitrochlorobenzene derivatives.
1404 *Mutat Res* 1984; 131:215-22.

1405

1406 CPDB (Carcinogenic Potency Database) data on 1-chloro-4-nitrobenzene. [Online]. Available
1407 from: URL: [http://toxnet.nlm.nih.gov/cpdb/chempages/1-CHLORO-4-](http://toxnet.nlm.nih.gov/cpdb/chempages/1-CHLORO-4-NITROBENZENE.html)
1408 [NITROBENZENE.html](http://toxnet.nlm.nih.gov/cpdb/chempages/1-CHLORO-4-NITROBENZENE.html)

1409

1410 Galloway S, Armstrong M, Reuben C, Colman S, Brown B, Cannon C, et al. Chromosome
1411 aberration and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108
1412 chemicals. *Environ Mol Mutagen* 1987; 10 Suppl 10:1-175.

1413

1414 Greim H, Hartwig A, Reuter U, Richter-Reichel HB, Thielman HW. Chemically induced
1415 pheochromocytomas in rats: mechanisms and relevance for human risk assessment. *Crit Rev*
1416 *Toxicol* 2009; 39:695-718.

1417

1418 Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E. Salmonella mutagenicity test
1419 results for 250 chemicals. *Environ Mutagen* 1983; 5 Suppl 1:1-142

1420

1421 IARC. Monographs on the evaluation of carcinogenic risks to humans. WHO. Occupational
1422 exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes,
1423 cosmetic colourants, industrial dyestuffs and aromatic amines. 1993; Vol. 57, Lyon, France.

1424

- 1425 IARC. Monographs on the evaluation of carcinogenic risks to humans. WHO. Printing
1426 processes and printing inks, carbon black and some nitro compounds. 1996 Vol. 65, Lyon,
1427 France.
- 1428
- 1429 Japan Chemical Industry Ecology-Toxicology & information Center (JETOC). Japan:
1430 Mutagenicity test data of existing chemical substances based on the toxicity investigation
1431 system of the Industrial Safety and Health law. 2005 Addendum 3.
- 1432
- 1433 JETOC. Japan: Mutagenicity test data of existing chemical substances based on the toxicity
1434 investigation system of the industrial safety and health law. 1996.
- 1435
- 1436 Kawai A, Goto S, Matsumoto Y, Matsushita H. Mutagenicity of aliphatic and aromatic nitro
1437 compounds. Sangyoigaku 1987; 29: 34-55.
- 1438
- 1439 Matsumoto M., Aiso S, Senoh H, Yamazaki K, Arito H, Nagano K, et al. Carcinogenicity and
1440 chronic toxicity of *para*-chloronitrobenzene in rats and mice by two-year feeding. J. Environ
1441 Pathol Toxicol Oncol 2006; 25:571-84.
- 1442
- 1443 NTP. Technical Report on Toxicity Studies on 2-Chloronitrobenzene and 4-
1444 Chloronitrobenzene (CAS Nos. 88-73-3 and 100-00-5) Administered by Inhalation to F344/N
1445 Rats and B6C4F1 Mice. Toxic Rep Ser 1993; 33: NIH Publication 93-3382. Research
1446 Triangle Park, NC.
- 1447
- 1448 Schroeder RE, Daly JW. A chronic oral gavage study in rats with p-nitrochlorobenzene.
1449 Biodynamics Inc. 1984. Project No. 80-2487. NTIS/OTS 0536382.
- 1450
- 1451 Travlos GS, Mahler J, Ragan HA, Chou BJ, Bucher JR. Thirteen-week inhalation toxicity of
1452 2- and 4-chloronitrobenzene in F344/N rats and B6C3F1 mice. Fundam Appl Toxicol 1996;
1453 30:75-92.
- 1454
- 1455 U.S. Environmental Protection Agency (USEPA). Health Effects Assessment Summary
1456 Tables. Office of Solid Waste and Emergency Response, US Environmental Protection
1457 Agency, Washington DC. Pub. 1995; No. PB95-921199.
- 1458
- 1459 Weisburger EK, Russfield AB, Homburger F, Weisburger JH, Boger E, Van Dongen, et al.
1460 Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or
1461 carcinogenicity. J Environ Pathol Toxicol 1978; 2:325-56.
- 1462
- 1463 WHO. CICAD 48: Concise International Chemical Assessment Document
1464 48 *p*-Chloroaniline. Geneva. [Online]. 2003; Available from: URL:
1465 <http://www.inchem.org/documents/cicads/cicads/cicad48.htm>
- 1466
- 1467 Yoshida T, Andoh K, Tabuchi T. Identification of urinary metabolites in rats treated with
1468 *p*-chloronitrobenzene. Arch Toxicol 1991; 65: 52-8.
- 1469
- 1470 Yoshida T, Tabuchi T, Andoh K. Pharmacokinetic study of *p*-chloronitrobenzene in humans
1471 suffering from acute poisoning. Drug Metab Dispos 1993; 21: 1142-6.
- 1472

1473

p-Cresidine (2-Methoxy-5-methyl aniline, CAS# 120-71-8)

1474 Potential for human exposure

1475 Potential for exposure is in industrial use. No data are available for exposure of the general
1476 population.
1477

1478 Mutagenicity/Genotoxicity

1479 p-Cresidine is mutagenic/genotoxic *in vitro* with equivocal evidence for genotoxicity *in vivo*.
1480

1481 p-Cresidine is mutagenic in:

- 1482 • Several *Salmonella* strains in the presence of metabolic activation (Zeiger *et al.* 1988;
1483 Dunkel *et al.* 1985; Japan 1997).
- 1484 • Big Blue transgenic mouse model with the lamda cII gene; p-cresidine administered a
1485 diet of 0.25 and 0.5%, comparable to the doses in the carcinogenicity study, for 180 days
1486 (Jakubczak *et al.* 1996).
1487

1488 Weakly positive results were reported for induction of structural chromosome aberrations and
1489 sister chromatid exchanges in CHO cells with rat liver S9 U.S. National Toxicology Program
1490 (NTP) and structural chromosome aberrations in CHL cells (Japan 2005).
1491

1492 *In vivo*, p-cresidine did not induce micronuclei in bone marrow of male B6C3F1 mice given 3
1493 daily intraperitoneal injections in two separate studies up to 300 mg/kg/day (NCI), or in p53
1494 heterozygous or nullizygous mice after oral gavage treatment for 7 weeks (Delker *et al.* 2000).
1495 Increases in micronuclei were seen in blood Polychromatic Erythrocytes (PCE) after dosing
1496 with p-cresidine by oral gavage to p53+/- mice for 39 to 183 days (Stoll *et al.* 2006). Since
1497 there were indications of the well characterized methemobolinemia and regenerative anemia
1498 associated with aniline and related compounds, (decreased hematocrit, dark urine, increased
1499 percentage of circulating PCEs) the authors noted it is not possible to determine whether the
1500 increase in micronuclei reflects hematological disturbance rather than genotoxicity (Stoll *et al.*
1501 2006).
1502

1503 Extensive experiments in multiple strains of rodents by oral and intraperitoneal routes after 1
1504 to 6 administrations failed to demonstrate *in vivo* genotoxicity in several tissues including
1505 bladder, by induction of DNA single-strand breaks measured by the alkaline elution assay, or
1506 of micronuclei (Ashby *et al.* 1991; Morita *et al.* 1997). Concomitant methemoglobinemia
1507 demonstrated that the p-cresidine was absorbed and oxidized in these negative studies.
1508 However, DNA strand breaks assessed by the Comet assay were reported in bladder mucosa,
1509 but not other tissues, after oral treatment of mice with p-cresidine (Sasaki *et al.* 1998).
1510

1511 Carcinogenicity

1512 p-Cresidine is classified as a Group 2B carcinogen, or possibly carcinogenic in humans
1513 (IARC 1982; 1987).
1514

1515 There is only one set of carcinogenicity studies in the standard rodent model. In NTP studies
1516 (NCI technical report 142) p-cresidine induced tumors in lifetime studies in Fischer 344 rats
1517 and B6C3F1 mice, with p-cresidine administered in the feed. No carcinogenicity data are
1518 available for other routes of exposure.

1519 p-Cresidine was administered in the feed, to groups of 50 male and 50 female animals of each
1520 species. There were also 50 control animals of each sex. The concentrations of p-cresidine
1521 were 0.5 or 1.0 percent in the diet, but in mice the concentrations administered were reduced
1522 after 21 weeks to 0.15 and 0.3 percent. The dose levels, converted to mg/kg/day in the CPDB,
1523 were 198 and 368 mg/kg/day for male rats; 245 and 491 mg/kg/day for female rats; 260 and
1524 552 mg/kg/day for male mice and 281 and 563 mg/kg/day for female mice.

1525
1526 All dosed animals, except for high dose male mice, were administered p-cresidine in the diet
1527 for 104 weeks and observed for an additional period of up to 2 weeks. All high dose male
1528 mice were dead by the end of week 92. Mortality rates were dose-related for both sexes of
1529 both species. That incidences of certain tumors were higher in low dose than in high dose
1530 groups was probably due to accelerated mortality in the high dose groups.

1531
1532 In dosed rats of both sexes, statistically significant incidences of bladder carcinomas
1533 (combined incidences of papillary carcinomas, squamous-cell carcinomas, transitional-cell
1534 papillomas, transitional-cell carcinomas, and undifferentiated carcinomas) and olfactory
1535 neuroblastomas were observed. The combined incidence of neoplastic nodules of the liver,
1536 hepatocellular carcinomas, or mixed hepato/cholangio carcinomas was also significant in low
1537 dose male rats. In both male and female dosed mice, the incidence of bladder carcinomas
1538 (combined incidence of carcinomas, squamous-cell carcinomas, and transitional-cell
1539 carcinomas) was significant. The incidence of hepatocellular carcinomas was significant in
1540 dosed female mice.

1541
1542 In summary, p-cresidine was carcinogenic to Fischer 344 rats, causing increased incidences of
1543 carcinomas and of papillomas of the urinary bladder in both sexes, increased incidences of
1544 olfactory neuroblastomas in both sexes, and of liver tumors in males. p-Cresidine was also
1545 carcinogenic in B6C3F1 mice, causing carcinomas of the urinary bladders in both sexes and
1546 hepatocellular carcinomas in females.

1547
1548 Induction of bladder tumors was also seen in a short-term carcinogenicity model in p53+/-
1549 hemizygous mice. p-Cresidine was used as a positive control in a large inter-laboratory
1550 assessment of the mouse model (Storer *et al.* 2001). Increases in bladder tumors were seen in
1551 18 of 19 studies in which p-cresidine was administered by gavage at 400 mg/kg/day for 26
1552 weeks, and in the single study where compound as given in feed.

1553 **p-Cresidine – Details of carcinogenicity studies**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
NCI*	50/sex/ group B6C3F1 mice	Feed 2 year	50	2: 0.5 and 1% Reduced after 21 wk to 0.15 and 0.3%. M: 260;552. F: 281; 563 mg/kg/d	Urinary Bladder /Male	44.7
NCI/NTP	50/sex/ Group Fisher 344 rats	Feed 2 year	50	0.5 and 1% M: 198;396. F: 245;491 mg/kg/d	Urinary Bladder /Male	88.4

1554 *Carcinogenicity study selected for AI calculation.

1555 Studies listed are in CPDB [Cancer Potency Database <http://toxnet.nlm.nih.gov/cpdb/>].

1556 **Mode of action for carcinogenicity:**

1557 Not defined.

1558

1559 **Regulatory and/or Published Limits**

1560 No regulatory limits have been published

1561

1562 **Acceptable intake (AI)**

1563 Rationale for selection of study for AI calculation:

1564

1565 The only adequate carcinogenicity studies of p-cresidine were those reported in the CPDB and
1566 conducted by NTP/NCI. The study in mice was selected for derivation of the AI since the
1567 most sensitive TD₅₀ was based on urinary bladder tumors in male mice.

1568

1569 **Calculation of AI:**

1570

1571 The most sensitive TD₅₀ values from the NTP/NCI studies are for the urinary bladder in both
1572 sexes of rats and mice; in rats the TD₅₀ was 110 mg/kg/day for females and 88.4 mg/kg/day
1573 for males; in mice the TD₅₀ was 69 mg/kg/day for females and 44.7 mg/kg/day for males. The
1574 most conservative value is that identified for male mice.

1575

1576 The lifetime AI is calculated as follows:

1577

1578 Lifetime AI = TD₅₀/50,000 x 50 kg

1579

1580 Lifetime AI = 44.7 mg/kg/day /50,000 x 50 kg

1581
1582 **Lifetime AI = 45 µg/day**
1583

1584 **References**

1585 Ashby J, Lefevre PA, Tinwell H, Brunborg G, Schmezer P, Pool-Zobel B, et al. The non-
1586 genotoxicity to rodents of the potent rodent bladder carcinogens *o*-anisidine and *p*-cresidine.
1587 *Mutat Res* 1991; 250:115-133.

1588
1589 CPDB data on *p*-cresidine. [Online]; Available from: URL:
1590 <http://toxnet.nlm.nih.gov/cpdb/chempages/p-CRESIDINE.html>
1591

1592 Delker DA, Yano BL, Gollapudi BB. Evaluation of cytotoxicity, cell proliferation, and
1593 genotoxicity induced by *p*-cresidine in hetero- and nullizygous transgenic *p53* mice.
1594 *Toxicolog Sci* 2000; 55:361-9.

1595
1596 Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, et al.
1597 Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and
1598 noncarcinogens in *Salmonella typhimurium* and *Escherichia coli*. *Environ Mutagen* 1985; 7
1599 Suppl 5:1-248.

1600
1601 IARC. *para*-Cresidine 27, 92, 1982; reviewed in Suppl 7 1987.
1602

1603 Jakubczak JL, Merlino G, French JE, Muller WJ, Paul B, Adhya S et al. Analysis of genetic
1604 instability during mammary tumor progression using a novel selection-based assay for *in vivo*
1605 mutations in a bacteriophage λ transgene target. *Proc Natl Acad Sci (USA)* 1996;
1606 93(17):9073-8.

1607
1608 Japan Chemical Industry Ecology-Toxicology and Information Center Japan; Mutagenicity
1609 test data of existing chemical substances based on the toxicity investigation of the Industrial
1610 Safety and Health law; 1997; Suppl.

1611
1612 JETOC. Japan; Mutagenicity test data of existing chemical substances based on the toxicity
1613 investigation of the Industrial Safety and Health law: 2005; Suppl 3.
1614

1615 Morita T, Norihide A, Awogi T, Sasaki Yu F, Sato-S-I, Shimada H, et al. Evaluation of the
1616 rodent micronucleus assay in the screening of IARC carcinogens (Groups 1, 2A and 2B). The
1617 summary report of the 6th collaborative study by CSGMT/JEMS.MMS. *Mutat Res* 1997;
1618 389:3-122.

1619
1620 NCI. Carcinogenesis Technical Report Series No. 142 Bioassay of *p*-cresidine for possible
1621 carcinogenicity. 1979.

1622
1623 NTP. [Online]; Available from: URL: [http://ntp.niehs.nih.gov/testing/status/agents/ts-10556-](http://ntp.niehs.nih.gov/testing/status/agents/ts-10556-y.html)
1624 [y.html](http://ntp.niehs.nih.gov/testing/status/agents/ts-10556-y.html)
1625

1626 Sasaki YF, Nishidate E, Su YQ, Matsusaka N, Tsuda S, Susa N, et al. Organ-specific
1627 genotoxicity of the potent rodent bladder carcinogens *o*-anisidine and *p*-cresidine. *Mutat Res*
1628 1998; 412:155-60.

- 1629 Storer RD, French JE, Haseman J, Hajian G, LeGran EK, Long GD, et al. p53^{+/-} hemizygous
1630 knockout mouse: Overview of available data. Toxicologic Pathol. 2001; 29 Suppl:30-50.
1631
1632 Stoll RE, Blanchard KT, Stoltz JH, Majeski JB, Furst S, Lilly PD et al. Phenolphthalein and
1633 nisacodyl: Assessment of genotoxic and carcinogenic responses in heterozygous p53^(+/-) mice
1634 and Syrian Hamster Embryo (SHE) assay. Toxicol Sci 2006; 90(2):440-50.
1635
1636 Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. *Salmonella* mutagenicity tests:
1637 IV. Results from the testing of 300 chemicals. Environ Mol Mutagen 1988; 11 Suppl 12:1-158.
1638

1639 **Dimethylcarbamyl chloride (CAS# 79-44-7)**

1640 **Potential for human exposure**

1641 Potential for exposure is in industrial use. No data are available for exposure of the general
1642 population.
1643

1644 **Mutagenicity/Genotoxicity**

1645 Dimethylcarbamyl Chloride (DMCC) is considered mutagenic and genotoxic *in vitro* and *in*
1646 *vivo*.

1647

1648 DMCC was mutagenic in:

1649 • *Salmonella typhimurium* TA100, TA1535, TA1537, TA98 and TA1538 Ames positive
1650 with and without metabolic activation (Dunkel *et al.* 1984, Kier *et al.* 1986);
1651 • Mouse lymphoma L5178Y cell *tk* mutation assay (Myhr *et al.* 1988).
1652

1653 DMCC was positive in a chromosomal aberration test with CHO cells (Galloway *et al.* 1985)
1654 and the micronucleus assay *in vivo* (Heddle *et al.* 1983).
1655

1656 **Carcinogenicity**

1657 DMCC is classified as a Group 2A compound, or probably carcinogenic to humans (IARC,
1658 1999).
1659

1660 No deaths from cancer were reported in a small study of workers exposed for periods ranging
1661 from six months to 12 years, and there is inadequate evidence in humans for the
1662 carcinogenicity of DMCC. There is evidence that DMCC induced tumors in rodents.
1663

1664 Since oral studies are lacking, the studies considered for AI derivation used inhalation and
1665 intraperitoneal administration.
1666

1667 Syrian golden hamsters were exposed to 1 ppm DMCC by inhalation for 6 hours/day,
1668 5 days/week until the end of their lives or sacrifice due to moribundity (Sellakumar *et al.*
1669 1980). Squamous cell carcinoma of the nasal cavity was seen in 55% of the animals whereas
1670 no spontaneous nasal tumors were seen in the controls, or historical controls. When early
1671 mortality was taken into consideration, the percentage of tumor bearing animals was
1672 calculated to be 75% (Sellakumar *et al.* 1980).
1673

1674 DMCC was tested for carcinogenic activity in female ICR/Ha Swiss mice by skin application,
1675 subcutaneous injection and intraperitoneal injection (Van Duuren *et al.* 1974; this study was
1676 selected to calculate the AI). In the skin application, 2 mg of DMCC was applied 3 times a
1677 week for 492 days; this was seen to induce papillomas in 40/50 mice and carcinomas in 30/50
1678 mice. Subcutaneous injection once weekly was continued for 427 days at a dose of 5
1679 mg/week. Sarcomas and squamous cell carcinomas were seen in 36/50 and 3/50 mice,
1680 respectively, after the subcutaneous injection. In the intraperitoneal experiment, the mice
1681 were injected weekly with 1 mg DMCC for a total duration of 450 days. The treatment
1682 induced papillary tumors of the lung in 14/30 animals and local malignant tumors in 9/30
1683 animals (8/30 were sarcomas). In the control groups, no tumors were seen by skin application,
1684 1/50 sarcoma by subcutaneous injection, and 1/30 sarcoma and 10/30 papillary tumors of lung

1685 by intraperitoneal injection. Overall, only the local (injection site) tumors were significantly
 1686 increased; tumors at distant sites were not statistically significantly increased compared with
 1687 controls.
 1688

1689 **Dimethylcarbamyl chloride – Details of carcinogenicity studies**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
Van Duuren <i>et al.</i> 1974*	30 ICR/Ha Swiss mice (F)	Intra- peritoneal 64 wk once/wk	30	1: 1 mg 5.71 mg/kg/d	Injection site: malignant tumors/Female	4.59 ^{^^^}
Sellakumar <i>et al.</i> 1980**	99 Syrian golden hamsters (M)	Inhalation Lifetime 6 h/d, 5 d/wk	50 sham treated 200 untreated	1: 1 ppm 0.553 mg/kg/d	Squamous cell carcinoma of nasal cavity	0.625
Van Duuren <i>et al.</i> 1974	50 ICR/Ha Swiss mice (F)	Skin. 70 wk 3 times/wk	50	1: 2 mg,	Skin: Papillomas and carcinomas /Female	NA [^]
Van Duuren <i>et al.</i> 1974	50 ICR/Ha Swiss mice (F)	Subcutaneous 61 wk once/wk	50	1: 5 mg	Injection site: Fibrosarcomas; Squamous cell carcinomas/Femal e	NA [^]
Snyder <i>et al.</i> 1986	Sprague- Dawley rats (M)	Inhalation 6 wk. 6 h/d, 5 d/wk Examined at end of life	Yes	1: 1 ppm	Nasal tumors/Male	NA ^{^^^^}
Van Duuren <i>et al.</i> 1987	30 - 50 ICR/Ha Swiss mice (F)	Skin 18 – 22 mo 3 times/wk	Yes	2: 2 and 4.3 mg	Skin. Mainly skin squamous carcinoma/Female	NA [^]
Van Duuren <i>et al.</i> 1987	ICR/Ha Swiss mice (F)	Subcutaneous once/wk 18 – 22 mo	Yes	1: 4.3 mg	Site of administration. Mainly sarcoma. Hemangioma, squamous carcinoma and papilloma also seen/Female	NA ^{^^}

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
Van Duuren <i>et al.</i> 1987	ICR/Ha Swiss mice (F)	Subcutaneous 12 mo; once/wk examined at end of life	Yes	2: 0.43 and 4.3 mg		NA ^{^^}

1690 Studies listed are in CPDB unless otherwise noted. [Cancer Potency Database
1691 <http://toxnet.nlm.nih.gov/cpdb/>].

1692 *Carcinogenicity study selected for non-inhalation AI. In CPDB.

1693 **Carcinogenicity study selected for inhalation AI. In CPDB.

1694 NA= Not applicable

1695 ^Did not examine all tissues histologically. Subcutaneous and skin painting studies are not
1696 included in CPDB as route with greater likelihood of whole body exposure is considered more
1697 valuable.

1698 ^^Subcutaneous and skin painting studies are not included in CPDB as route with greater
1699 likelihood of whole body exposure is considered more valuable.

1700 ^^^Histopathology only on tissues that appeared abnormal at autopsy.

1701 ^^^^Examined only for nasal cancer. Does not meet criteria for inclusion in CPDB of
1702 exposure for at least one fourth of the standard lifetime

1703

1704 **Mode of Action of Carcinogenicity**

1705 Not defined.

1706

1707 **Regulatory and/or Published Limits**

1708 No regulatory limits have been published

1709

1710 **Acceptable Intake**

1711 Based on the above data, DMCC is considered to be a mutagenic carcinogen. As a result,
1712 linear extrapolation from the most sensitive TD₅₀ in carcinogenicity studies is an appropriate
1713 method with which to derive an acceptable risk dose. Since DMCC appears to be a site-of-
1714 contact carcinogen, it was appropriate to derive a separate acceptable intake for inhalation
1715 exposure compared with other routes of exposure.

1716

1717 No information from oral administration is available, so that for routes of exposure other than
1718 inhalation, the study by Van Duuren *et al.* (1974), with administration by intraperitoneal
1719 injection, was used. The TD₅₀ was 4.59 mg/kg/day based on mixed tumor incidences (CPDB).

1720

1721 Lifetime AI = TD₅₀/50,000 x 50 kg

1722

1723 Lifetime AI = 4.59 mg/kg/day /50,000 x 50 kg

1724

1725 **Lifetime AI = 5 µg/day**

1726 **Inhalation AI**

1727 After inhalation of DMCC, nasal cancer in hamsters is the most sensitive endpoint and the
1728 TD₅₀ was 0.625 mg/kg/day.

1729
1730 Lifetime AI = TD₅₀/50,000 x 50 kg

1731
1732 Lifetime AI = 0.625 mg/kg/day /50,000 x 50 kg

1733
1734 **Lifetime AI = 0.6 µg/day**

1735

1736 **References**

1737 Dunkel V, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, et al. Reproducibility
1738 of microbial mutagenicity assays. I. Tests with *Salmonella* typhimurium and *Escherichia coli*
1739 using a standardized protocol. Environ Mutagen 1984; 6 Suppl 2: 1-251.

1740 Heddle JA, Hite M, Kirkhart B, Mavournin K, MacGregor JT, Newell GW, et al. The
1741 induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental
1742 Protection Agency Gene-Tox Program. Mutat Res 1983; 123:61-118.

1743 IARC. Monographs on the evaluation of the Carcinogenic Risk of Chemicals to Man. Geneva:
1744 WHO, International Agency for Research on Cancer, [Online] 1972-PRESENT.
1745 (Multivolume work). 1999; 71:539; Available from: URL:
1746 <http://monographs.iarc.fr/index.php>

1747 Kier LD, Brusick DJ, Auletta AE, Von Halle ES, Brown MM, Simmon VF, et al. The
1748 *Salmonella* typhimurium/mammalian microsomal assay. A report of the U.S. Environmental
1749 Protection Agency Gene-Tox Program. Mutat Res 1986; 168: 69-240.

1750 Myhr BC, Caspary WJ. Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay:
1751 Intralaboratory results for sixty-three coded chemicals tested at Litton Bionetics, Inc. Environ
1752 Mol Mutagen 1988; 12 Suppl 13:103-94.

1753 NTP. DHHS/National Toxicology Program; Report on Carcinogens, twelfth edition:
1754 Dimethylcarbamoyl Chloride (79-44-7). [Online]. 2012 [as of September 30, 2013]; Available
1755 from: URL: <http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/DimethylcarbamoylChloride.pdf>

1756
1757 Galloway S, Bloom A, Resnick M, Margolin B, Nakamura F, Archer P, et al. Development of
1758 a standard protocol for *in vitro* cytogenetic testing with CHO cells: Comparison of results for
1759 22 compounds in two laboratories. Environ Mutagen 1985; 7 1-52.

1760
1761 Sellakumar AR, Laskin S, Kushner M, Rush G, Katz GV, Snyder CA, et al. Inhalation
1762 carcinogenesis by dimethylcarbamoyl chloride in Syrian golden hamsters, J Environ Pathol
1763 Toxicol 1980; 4:107-15.

1764
1765 Snyder CA, Garte SJ, Sellakumar AR, Albert RE. Relationships between the levels of binding
1766 to DNA and the carcinogenic potencies in rat nasal mucosa for three alkylating agents, Cancer
1767 Lett 1986 33, 175-81.

1768

- 1769 Van Duuren BL, Goldschmidt BM, Katz C, Seidman I, Paul JS. Carcinogenic activity of
1770 alkylating agents, J Natl Cancer Inst 1974; 53:695-700.
1771
1772 Van Duuren BL, Melchionne S, Seidman I. Carcinogenicity of acylating agents: chronic
1773 bioassays in mice and Structure-Activity Relationships (SARC), J Am Col Toxicol 1987; 6:
1774 479-487.

1775

Dimethyl Sulfate (CAS# 77-78-1)

1776 Potential for human exposure

1777 In 1983, the U.S. EPA compiled ambient air data from one United States urban location and
1778 the mean ambient air concentration for Dimethyl Sulfate (DMS) was measured at 7.4 µg per
1779 cubic meter or 1.4 ppb (U.S. EPA, 1985).

1780

1781 Mutagenicity/Genotoxicity

1782 DMS is mutagenic/genotoxic *in vitro* and *in vivo*.

1783

1784 Results have been extensively reviewed by Hoffmann (1980). DMS is mutagenic in:

1785

1786 • The microbial reverse mutation assay (Ames), *Salmonella typhimurium* strains TA98,
1787 TA100, TA1535, TA1537 and TA1538 with and without activation (Skopek *et al.* 1978).

1788 • DMS is a potent alkylating agent for cellular macromolecules and forms a variety of
1789 alkylated bases with DNA *in vitro* and the same alkylated bases are formed *in vivo*
1790 (IARC, 1999).

1791

1792 DMS has also consistently produced positive responses in the small number of *in vivo* tests to
1793 which it has been subjected. Workers exposed to DMS have developed chromosomal
1794 aberrations are reported to be increased in their circulating lymphocytes of workers exposed to
1795 DMS (IARC, 1999).

1796

1797 Carcinogenicity

1798 DMS is classified as a Group 2A carcinogen, probably carcinogenic to humans (IARC, 1999).

1799

1800 No epidemiological studies were available for DMS although a small number of cases of
1801 human exposure and bronchial carcinoma have been reported. DMS has tested positive for
1802 carcinogenicity in animals by chronic and subchronic inhalation, and single and multiple
1803 subcutaneous injection. DMS is carcinogenic in rats, mice, and hamsters (IARC, 1999).
1804 DMS has not been tested by oral exposure. The carcinogenicity studies for DMS were limited
1805 for a variety of reasons and this is likely why DMS is not listed on the Carcinogenicity
1806 Potency Database (CPDB). The studies evaluating carcinogenicity of DMS are described
1807 below (excerpted from IRIS):

1808

1809 DMS- Details of carcinogenicity studies

Study	Animals	Duration/ Exposure	Controls	Doses	Most sensitive site/sex	TD ₅₀ (mg/kg/d)
Schlogel and Bannasch, 1972 (in ECHA 2002)	Golden hamsters, Wistar rats, and NMRI mice male and female (number not clearly specified)	Inhalation, 6 h/d, 2 d/wk for 15 mo 15-mo observation period.	Yes	2: 0.5; 2.0 ppm	Tumors in lungs, thorax and nasal passages.	NA [^]
Druckrey <i>et al.</i> (1970)	20 – 27 BD rats Sex not specified	Inhalation 1 h/d, 5 d/wk, and 130 d; followed for 643 d	No	2: 3; 10 ppm	Squamous cell carcinoma in nasal epithelium at 3 ppm. Squamous cell carcinomas in nasal epithelium and lympho-sarcoma in the thorax with metastases to the lung at 10 ppm.	NA ^{^^}
Druckrey <i>et al.</i> (1966)	8 – 17 BD Rats Sex not specified	Subcutaneously for up to 394 d. The duration of the study was not reported but mean tumor induction time was 500 d.	No	2: 8; 16 mg/kg/wk	Injection-site sarcomas in 7/11 at low dose and 4/6 at high dose; occasional metastases to the lung. One hepatic carcinoma.	NA ^{^^^}
Druckrey <i>et al.</i> (1970)	15 BD Rats Sex not specified	Single Subcutaneous injection up to 740 d evaluation	No	1: 50 mg/kg	Local sarcomas of connective tissue in 7/15 rats; multiple metastases to the lungs in three cases	NA ^{^^^}
Druckrey <i>et al.</i> (1970)	12 BD rats	Intravenous, for 800 d once/wk	No	2: 2; 4 mg/kg	No tumors reported	NA ^{^^^}

Study	Animals	Duration/ Exposure	Controls	Doses	Most sensitive site/sex	TD ₅₀ (mg/kg/d)
	Sex not specified					
Druckrey <i>et al.</i> (1970)	8 BD rats (pregnant females,)	Single intravenous dose, gestation day 15, offspring observed for 1 yr	No	1: 20 mg/kg	4/59 offspring had malignant tumors of the nervous system while 2/59 had malignant hepatic tumors.	NA ^{^^^^}
Fomenko <i>et al.</i> (1983)	90 CBAX57 BI/6 mice (F)	Inhalation, duration not reported. 4 h/d, 5 d/wk	Not indicated	3: 0.4; 1; 20 mg/m ³	increase in lung adenomas at high dose	NA*
Van Duuren (1974)	20 ICR/Ha Swiss mice [¥]	Dermal, 3 times/wk for up to 475 d	Not indicated	1: 0.1 mg	No findings	NA**

1810 Studies listed are in not in CPDB.

1811 NA = Not applicable

1812 [^] Control data not reported. Tumor incidences not tabulated by species or dose

1813 ^{^^} Small group size. No concurrent control group. One rat at high dose had a cerebellar tumor and two at low dose had nervous system tumors which are very rare and distant from exposure.

1815 ^{^^^} Small group size, no concurrent control group.

1816 ^{^^^^} No concurrent control group.

1817 * Duration not reported

1818 ** Limited number of animals. Only one dose tested. Even when DMS was combined with tumor promoters no tumors were noted.

1820 [¥] Sex not specified

1821

1822 Mode of Action of Carcinogenicity:

1823 Not defined.

1824

1825 Regulatory and/or Published Limits

1826 The European Union Institute for Health and Consumer Protection developed a
1827 carcinogenicity slope curve based on the inhalation carcinogenicity data for DMS (ECHA
1828 2002). Using the Druckrey inhalation study to assess a more systemic exposure by the EU
1829 calculated estimated a T₂₅ (dose that resulted in a 25% increase in tumors). Systemic effects
1830 (nervous system) and local nasal tumors were observed in this limited carcinogenicity study.
1831 However, as with other studies listed, this study was severely limited with high death level, no
1832 control animals, few dose groups and minimal pathological evaluations, and therefore, not
1833 suitable for linear extrapolation.

1834

1835 **Acceptable Intake (AI)**

1836 While DMS is considered to be a likely oral carcinogen and probable human carcinogen, there
1837 are no oral carcinogenicity studies from which to derive a TD₅₀ value. Moreover, the
1838 inhalation studies that are available are limited for a variety of reasons and are not suitable for
1839 TD₅₀ extrapolation. Given this, it is reasonable to limit DMS to the threshold of toxicological
1840 concern level (TTC) of 1.5 µg/day.

1841

1842 **Lifetime AI = 1.5 µg/day**

1843 **References**

1844 Druckrey H. Carcinogenic alkylating compounds: I. Dimethyl sulfate, carcinogenic effect in
1845 rats and probable cause of occupational cancer. (Article in German) Z. Krebsforsch 1966;
1846 68:103–111.

1847

1848 Druckrey H. Carcinogenic alkylating compounds: III. Alkyl halogenids, sulfates, sulfonates,
1849 and heterocyclics. (Article in German) Z. Krebsforsch 1970; 74:241–273.

1850

1851 ECHA (European Chemical Agency). European Union Risk Assessment Report: Institute for
1852 Health and Consumer Protection. Dimethyl Sulphate. [Online]. 2002 Vol. 12. Available from:
1853 URL: <http://echa.europa.eu/documents/10162/3d2e4243-8264-4d09-a4ab-92dde5abfadd>

1854

1855 Fomenko VN, Katasova LD, Domshlak MG (1983); USSR Minist Health All-Union Sci Soc
1856 Med Genet 1:348-49 as cited in WHO; Environ Health Criteria 1985; Dimethyl Sulfate p.36

1857

1858 Hoffmann GR. Genetic effects of dimethyl sulfate, diethyl sulfate, and related compounds.
1859 Mutat Res 1980; 75:63-129.

1860

1861 IARC. 1999; Vol 71, p. 575.

1862

1863 Schlogel FA, Bannasch P. Carcinogenicity and Chronic Toxicity of Inhaled Dimethyl Sulfate.
1864 (In German) (Inaugural Dissertation) Julius-Maximilians University, Würzburg (data shown
1865 in ECHA 2002). 1972.

1866

1867 Skopek TR, Liber HL, Kaden DA, Thilly WG. Relative sensitivities of forward and reverse
1868 mutation assays in Salmonella typhimurium. Proc Natl Acad Sci USA 1978; 75:4465-9.

1869

1870 US EPA. Health and Environmental effects profile for dimethyl sulfate. Prepared by the
1871 Office of Health and Environmental Assessment, Environmental Criteria and Assessment
1872 Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington,
1873 DC. 1985.

1874

1875 Van Duuren BL, Goldschmidt BM, Katz C, Seidman I, Paul JS. Carcinogenic activity of
1876 alkylating agents. J Natl Cancer Inst 1974; 53:695-700.

1877

Ethyl chloride (Chloroethane, CAS# 75-00-3)

1878 Potential for human exposure

1879 The general population may be exposed to low levels (parts-per-trillion, ppt) of ethyl chloride
1880 through inhalation of contaminated ambient air and consumption of contaminated drinking
1881 water. Dermal contact can occur as a result of the intentional use of ethyl chloride as a topical
1882 anesthetic. It is possible that ethyl chloride forms in some waste-water streams as a result of
1883 disinfection by chlorination. Because of its volatility, the majority of ethyl chloride released
1884 to surface water is expected to enter the atmosphere. This compound can leach into
1885 groundwater from waste disposal sites, and it may form in groundwater as an anaerobic
1886 biodegradation product of chlorinated solvents (e.g., 1, 1, 1-trichloroethane and cis-1, 1-
1887 dichloroethylene). No data were located that indicate that ethyl chloride is found in food.
1888

1889 Mutagenicity/Genotoxicity

1890 Ethyl chloride is mutagenic and genotoxic *in vitro* but not *in vivo*. IARC (1999) has reviewed
1891 the mutagenicity data for ethyl chloride; key points are summarized here.
1892

1893 Ethyl chloride was mutagenic in:

- 1894 • Microbial reverse mutation assay (Ames), *Salmonella typhimurium* strains TA100 and
1895 TA1535 and in *Escherichia coli* WP2 uvrA with and without metabolic activation when
1896 tested in conditions that enable exposure to gas (Goto *et al.* 1995; Zeiger *et al.* 1992;
1897 Araki *et al.* 1994).
- 1898 • CHO cell *hprt* assay with and without metabolic activation.
1899

1900 Ethyl chloride was not genotoxic in B6C3F1 mice following 6 hour exposures for 3
1901 consecutive days *via* nose-only inhalation at approximately 25,000 ppm in a male and female
1902 bone marrow micronucleus test and in a Unscheduled DNA Synthesis (UDS) female mouse
1903 liver test (2-4 h and 12-14 h time points) (Ebert *et al.* 1994).
1904

1905 Carcinogenicity

1906 IARC considers ethyl chloride to be an IARC Class 3 compound, or not classifiable as to its
1907 carcinogenicity (IARC, 1999).
1908

1909 Only one carcinogenicity study was found for ethyl chloride, NTP studies in rats and mice of
1910 both sexes *via* inhalation for 6 hr/day, 5 days/week for 100 weeks. The exposure
1911 concentration (15,000 ppm) was limited by safety concern (explosion risk) and on the lack of
1912 obvious effect in a 3 month range-finding study up to 19,000 ppm. These data were later
1913 published by Holder (2008) comparing ethyl chloride with ethyl bromide. Ethyl chloride was
1914 notable because, along with structurally similar ethyl bromide, it induced very high numbers
1915 of uncommon uterine tumors (endometrial carcinomas) in mice, but not rats. Ethyl chloride
1916 produced clear evidence of carcinogenicity in female mice (uterus) and equivocal evidence of
1917 carcinogenicity in male and female rats. Due to poor survival, the male mouse study was
1918 considered inadequate although there was an increased incidence of lung tumors.
1919

1920 **Ethyl Chloride – Details of carcinogenicity studies**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
NCI/NTP TR-346; Holder, 2008*	50/sex/ group B6C3F1 Mice	Inhalation 6 h/d, 5 d/wk for 100 wk	50	1: M: 10.4 F: 12.4 g/kg/d	Uterus/Female	1810
NCI/NTP TR-346; Holder, 2008	50/sex/ group Fischer 344 Rats	Inhalation 6 h/d, 5 d/wk for 100 wk	50	1: M: 2.01 F: 2.88 g/kg/d	Negative	Not Applicable

1921 *Carcinogenicity study selected for AI calculation. Studies listed are in CPDB [Cancer
1922 Potency Database <http://toxnet.nlm.nih.gov/cpdb/>].

1923

1924 **Mode of Action of Carcinogenicity**

1925 Holder (2008) proposes reactive metabolites may contribute to carcinogenicity, but notes
1926 female mice have a marked stress response to ethyl chloride exposure at the high
1927 concentrations used in the carcinogenicity study; such stress has been shown to stimulate
1928 adrenal stimulation. He proposes high corticosteroid production could promote development
1929 of endometrial cancers in mice.

1930

1931 **Regulatory and/or Published Limits**

1932 The US EPA established an inhalation Reference Concentration (RfC) for non-carcinogenic
1933 effects of 10 mg/m³, or 288 mg/day assuming a respiratory volume of 28,800 L/day (USEPA,
1934 1991).

1935

1936 **Acceptable Intake (AI)**

1937 Rationale for selection of study for AI calculation

1938

1939 Although the studies are not robust in design, having a single dose group, the high level of a
1940 specific rare type of uterine carcinoma of endometrial origin in mice (43/50 compared with
1941 0/49 controls), suggest a strong carcinogenic response. A comparator molecule, ethyl
1942 bromide, was tested in a more robust carcinogenicity study (3 doses and a control) and had a
1943 similar response in female mouse uterine tumors (NTP, 1989). The lowest TD₅₀ for ethyl
1944 bromide uterine tumors was 535 mg/kg.

1945

1946 Ethyl chloride was considered to be a mutagenic carcinogen. Based on the NTP inhalation
1947 study the most sensitive species/site is female mouse uterus. The CPDB converted 0 and
1948 15,000 ppm to doses of 0 and 12.4 g/kg and calculated a TD₅₀ = 1810 mg/kg/day for mouse
1949 uterus.

1950

1951 Lifetime AI = TD₅₀/50,000 x 50 kg

1952

1953 Lifetime AI = 1810 mg/kg/day /50,000 x 50 kg

1954

1955 **Lifetime AI = 1,810 µg/day**

1956

1957 **References**

1958 Araki A, Noguchi T, Kato F, Matsushima T. Improved method for mutagenicity testing of
1959 gaseous compounds by using a gas sampling bag. *Mutat Res* 1994; 307(1):335-44.

1960

1961 Ebert R, Fedtke N, Certa H, Wiegand HJ, Regnier JF, Marshall R, et al. SW. Genotoxicity
1962 Studies With Chloroethane. *Mutat Res* 1994; 322(1):33-43.

1963

1964 Goto S, Shiraishi F, Tanabe K, Endo O, Machii K, Tezuka Y, et al. Mutagenicity Detection
1965 Method for Vinyl Chloride and Vinylidene Chloride Gases. *Kankyo Kagaku* 1995; 5(2):235-
1966 40.

1967

1968 Holder JW. Analysis of Chloroethane Toxicity and Carcinogenicity Including a Comparison
1969 With Bromoethane. *Toxicology and Industrial Health* 2008; 24(10):655-675.

1970

1971 IARC. Chloroethane. 1999; Vol. 71. p.1345

1972

1973 NTP. Toxicology and Carcinogenesis Studies of Ethyl Bromide. Technical Report Series No.
1974 363. [Online]. 1989; Available from: URL:

1975 http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr363.pdf

1976

1977 NCI/NTP Carcinogenesis Technical Report Series; National Cancer Institute/National
1978 Toxicology Program; U.S. Department Of Health And Human Services, Tr-346 Y89.

1979

1980 USEPA. Integrated Risk Information System. Ethyl Chloride. 1991.

1981

1982 Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. *Salmonella* mutagenicity tests. V.
1983 Results from the testing of 311 chemicals. *Environ Mol Mutagen* 1992; 19 Suppl 21:2-141.

1984

1985

Glycidol (CAS# 556-52-5)

1986 **Potential for human exposure**

1987 The primary routes of potential human exposure to glycidol are inhalation, eye and dermal
1988 contact, and ingestion (NTP Report on Carcinogens, 12th Edition, 2011). Heating of glycerol
1989 and sugars causes the formation of glycidol. Glycidol is a metabolite of
1990 3-monochloropropane-1, 2-diol, a chloropropanol found in many foods and food ingredients,
1991 including soy sauce and hydrolyzed vegetable protein. Toxicological assessments for glycidol
1992 in food have calculated a potential daily glycidol exposure to be 20-80 µg/day (Bakhiya *et al.*
1993 2011). Glycidol has been detected in the urine of rats exposed to 1-bromopropane by
1994 inhalation (Ishidao *et al.* 2002).
1995

1996 **Mutagenicity/Genotoxicity**

1997 Glycidol is mutagenic/genotoxic *in vitro* and *in vivo*.

1998

1999 IARC (2000) and CCRIS (2013) contain reviews of the mutagenicity/genotoxicity data for
2000 glycidol; key conclusions are summarized here.

2001

2002 Glycidol is mutagenic in:

- 2003 • Microbial reverse mutation assay (Ames), *Salmonella* strains TA100, TA1535, TA98,
2004 TA97 and TA1537 both with and without rat liver S9 activation and in standard plate and
2005 preincubation assays.
- 2006 • *Escherichia coli* strain WP2uvrA/pKM101 in a preincubation assay with and without rat
2007 liver S9.
- 2008 • Mouse lymphoma I5178Y cell *tk* assay without metabolic activation.

2009

2010 Glycidol was positive in an *in vitro* chromosome aberration assay in CHL cells with and
2011 without rat liver S9, and *in vivo* in a mouse micronucleus assay by oral gavage in male and
2012 female P16Ink4a/p19Arf haploinsufficient mice.
2013

2014 **Carcinogenicity**

2015 Glycidol is classified as Group 2A, or probably carcinogenic in humans (IARC, 2000).

2016

2017 In NTP studies (also published by Irwin *et al.* 1996), glycidol was administered by gavage in
2018 water to male and female F344/N rats and B6C3F1 mice. Rats received 0, 37.5 or 75 mg/kg
2019 and mice received 0, 25 or 50 mg/kg daily, 5 days per week for 2 years. The average daily
2020 doses were calculated by multiplying the administered dose by 5/7 to account for the 5 days
2021 per week dosing schedule and 103/104 to account for the less-than-lifetime duration of dosing.
2022 The resulting average daily doses were 0, 26.5, and 53.1 mg/kg/day in male and female rats,
2023 and 0, 17.7, and 35.4 mg/kg/day in male and female mice.

2024

2025 Exposure to glycidol was associated with dose-related increases in the incidences of
2026 neoplasms in various tissues in both rats and mice. Survival of treated rats and mice was
2027 markedly reduced compared to controls because of the early induction of neoplastic disease.

2028

2029 The oral gavage study in hamsters was less robust due to small group size, single dose levels
2030 and shorter duration. Further oral gavage chronic studies with glycidol were conducted by the

2031 NTP in genetically modified mice lacking two tumor suppressor genes (*i.e.*, haploinsufficient
 2032 p16Ink4a/p19Arf mice) (NTP, 2007). Although there was clear evidence of carcinogenic
 2033 activity in males (based on the occurrence of histiocytic sarcomas and alveolar/bronchiolar
 2034 adenomas) and some evidence of carcinogenic activity in female mice (based on the
 2035 occurrence of alveolar/bronchiolar adenomas), these studies are considered less suitable for
 2036 dose-response assessment than the two-year bioassays (NTP, 1990) for reasons including the
 2037 short duration, the small number of animals used per treatment group, and limited
 2038 understanding of how dose-response relationships observed in genetically modified animals
 2039 correspond with those observed in standard long-term carcinogenicity bioassays (CalEPA,
 2040 2010).

2041

2042 **Glycidol – Details of carcinogenicity studies**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
NTP 1990*	50/sex/ group F344/N rats	Oral gavage, 5 d/wk for 2 yr	50	2: 26.5; 53.8 mg/kg/d	Mammary gland /Female	4.15
NTP 1990	50/sex/ group B6C3F1 mice	Oral gavage, 5 d/wk for 2 yr	50	2: 17.7; 35.4 mg/kg/d	Harderian gland /Female	32.9
Lijinsky and Kovatch, 1992	12 – 20/sex/ group Syri an Golden Hamsters	Gavage Twice/wk for 60 wk	Yes	1: M: 15.8 F: 17.9 mg/kg/d	Spleen / Female	56.1 [^]
Van Duuren <i>et</i> <i>al.</i> 1967 (*Cited in IARC, 2000)	20 ICR/Ha Swiss mice	Skin Painting 3 times/wk for 520 d	Yes	1: 5%	No Tumors	NA [^]

2043 Studies listed are in CPDB unless otherwise noted. [Cancer Potency Database

2044 <http://toxnet.nlm.nih.gov/cpdb/>].

2045 *Carcinogenicity study selected for AI calculation.

2046 **Not in CPDB.

2047 NA= Not applicable.

2048 ^Not a standard carcinogenicity design. Only one dose, intermittent dosing, and small sample
2049 size (CalEPA, 2010).
2050

2051 **Mode of Action**

2052 Not defined.
2053

2054 **Regulatory and/or Published Limits**

2055 No regulatory limits have been published, for example by US EPA, WHO, or ATSDR.
2056

2057 **Acceptable Intake (AI)**

2058 Rationale for selection of study for AI calculation:
2059

2060 The most suitable carcinogenicity data for human cancer potency assessment come from the
2061 two-year oral studies conducted in F344/N rats and B6C3F1 mice by NTP (1990). The most
2062 sensitive organ site was female mammary glands with a TD₅₀ of 4.15 mg/kg/day.
2063

2064 Calculation of AI:
2065

2066 Lifetime AI = TD₅₀/50,000 x 50 kg
2067

2068 Lifetime AI = 4.15 (mg/kg/day)/50,000 x 50 kg
2069

2070 **Lifetime AI = 4 µg/day**
2071

2072 Note that this is lower than the estimated daily glycidol exposure from food of 20-80 µg/day
2073 (Bakhiya *et al.* 2011).
2074

2075 **References**

2076 Bakhiya N, Abraham K, Gürtler R, Appel KE, Lampen A. Toxicological assessment of 3-
2077 chloropropane-1,2-diol and glycidol fatty acid esters in food. Mol Nutr Food Res 2011;
2078 55:509-21.
2079

2080 CalEPA. No Significant Risk Level (NSRL) for the Proposition 65 carcinogen Glycidol.
2081 [Online]. 2010; Available from: URL:

2082 http://www.oehha.ca.gov/prop65/CRNR_notices/pdf_zip/GlycidolNSRL073010.pdf
2083

2084 CCRIS. Chemical Carcinogenesis Research Information System. National Library of
2085 Medicine. [Online]. 2013 [last accessed: 2013 October 10]; Available from: URL:

2086 <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS> and search on CAS number.
2087

2088 IARC. Monographs on the evaluation of the carcinogenic risk of chemicals to Man. Geneva:
2089 WHO, International Agency for Research on Cancer, [Online]. 1972-PRESENT.
2090 (Multivolume work). 2000; 77:469; Available from: URL:
2091 <http://monographs.iarc.fr/index.php>.

- 2092 Irwin RD, Eustis SL, Stefanski S, Haseman JK. Carcinogenicity of Glycidol in F344 rats and
2093 B6C3F₁ mice. *J Appl Toxicol* 1996; 16 (3):201-9.
2094
- 2095 Ishidao T, Kunugita N, Fueta Y, Arashidani K, Hori H. Effects of inhaled 1-bromopropane
2096 vapor on rat metabolism. *Toxicol Lett* 2002; 134(1-3):237-43.
2097
- 2098 Lijinsky W, Kovatch RM. A study of the carcinogenicity of glycidol in Syrian hamsters.
2099 *Toxicol Ind Health* 1992; 8(5):267-71.
2100
- 2101 NTP. Toxicology and Carcinogenesis Studies of Glycidol (CAS No. 556-52-5) In F344/N
2102 Rats and B6C3F₁ Mice (Gavage Studies). Research Triangle Park, NC: Natl Toxicol Program
2103 Tech Rep Ser. 1990; 374-229.
2104
- 2105 NTP. Toxicology and Carcinogenesis Studies of Glycidol (CAS No. 556-52-5) in genetically
2106 modified haploinsufficient p16 (Ink4a)/p19 (Arf) mice (gavage study). *Natl Toxicol Program*
2107 *Genet Modif Model Rep* 2007; 13:1-81.
2108
- 2109 NTP. DHHS/National Toxicology Program; Twelfth Report on Carcinogens: Glycidol (556-
2110 52-5). [Online]. 2011 [cited 2013 October]; Available from: URL:
2111 <http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/Glycidol.pdf>.
2112
- 2113 Van Duuren BL, Langseth L, Gldschmidt BM, Orris L. Carcinogenicity of epoxides, lactones,
2114 and peroxy compounds. VI. Structure and carcinogenic activity. *J Natl Cancer Inst* 1967;
2115 39:1217–28.

2116

Hydrazine (CAS# 302-01-2)

2117 Potential for human exposure

2118 Hydrazine has been used as fuel for rockets and spacecraft, to treat boiler water to reduce
2119 corrosion, as a reducing agent, and to speed up chemical reactions (Choudary and Hansen,
2120 1998). It is also used in the synthesis of pharmaceuticals, pesticides and plastic foams
2121 (Choudary and Hansen, 1998). Hydrazine sulphate has been used in the treatment of
2122 tuberculosis, sickle cell anemia and other chronic illnesses (von Burg and Stout, 1991). There
2123 is limited information on the natural occurrence of hydrazine and derivatives (Toth, 2000).
2124 Humans may be exposed to hydrazine from environmental contamination of water, air and
2125 soil (Choudary and Hansen, 1998); however, the main source of human exposure is in the
2126 workplace (HSDB, 2005). Small amounts of hydrazine have also been reported in tobacco
2127 products and cigarette smoke (Choudary and Hansen, 1998; Lui *et al.* 1974).
2128

2129 Mutagenicity/Genotoxicity

2130 Hydrazine is mutagenic/genotoxic *in vitro* and *in vivo*.

2131
2132 IARC (1999) has reviewed the mutagenicity of hydrazine. Key observations are summarized
2133 here.

2134
2135 Hydrazine was mutagenic in:

- 2136 • Microbial reverse mutation assay (Ames), *Salmonella typhimurium* strains TA 1535, TA
2137 102, TA 98 and TA 100, and in *Escherichia coli* strain WP2 uvrA, with and without
2138 activation.
- 2139 • *In vitro* mouse lymphoma L5178Y cells, in *tk* and *hprt* genes.

2140
2141 Hydrazine induced sister chromatid exchanges and chromosomal aberrations in Chinese
2142 Hamster cells and *in vivo*, induced micronuclei but not chromosome aberrations, in mouse
2143 bone marrow (IARC, 1999). DNA adducts have been reported in several tissues *in vivo*.
2144

2145 Carcinogenicity

2146 Hydrazine is classified as Group 2B, or possibly carcinogenic to humans (IARC, 1999).
2147 Group B2 or a probable human carcinogen (U.S. EPA, 1991).

2148 There are seven hydrazine carcinogenicity studies cited in the Carcinogenic Potency Database
2149 (CPDB); three inhalation studies that included 1-year dosing duration, three studies in
2150 drinking water and one by oral gavage (Gold and Zeiger, 1997). Five of the seven hydrazine
2151 carcinogenicity studies were deemed positive by the authors of the original reports.

2152 The main target organs for oral carcinogenicity of hydrazine in rodents are the liver and lungs.
2153 The most robust oral study based on group size and dose levels was that of Stienhoff and
2154 Mohr (1988). The most robust inhalation study with the lowest TD₅₀ was that of Vernot *et al.*
2155 (1985). The most sensitive targets for inhalation carcinogenicity of hydrazine in rodents are
2156 sites of initial contact such as the nasal cavity and lungs.

2157 The studies done on hydrazine sulphate in the CPDB are not shown here as they included <50
2158 animals per group (and a single dose level in one case), and the calculated TD₅₀'s were higher

2159 (less potent) than those for the drinking water study of hydrazine (Steinhoff and Mohr, 1988)
 2160 that was selected as the most robust for AI calculation.

2161 **Hydrazine – Details of carcinogenicity studies**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
Steinhoff & Mohr, 1988*	50/sex/ group Wistar rats	Lifetime, water	50	3: M: 0.1; 1.5, 2.5. F: 0.11, 0.57, 2.86 mg/kg/d	Liver/Female	41.6
Vernot <i>et al.</i> 1985**	100/sex/ group F344 rats	1 yr inhalation with 18 mo observation	150	4: M:1.37, 6.87, 27.5, 137 F: 1.96, 9.81, 39.3, 196 µg/ /kg/d	Nasal adenomatous polyps/Male	0.194
Steinhoff <i>et al.</i> 1990	50/sex/ group Bor:NMR I, SPF- bred NMRI mice	2 yr, water	50	3: M: 0.33, 1.67, 8.33. F: 0.4, 2.0, 10.0 mg/kg/d	Negative	NA, negative study
Vernot <i>et al.</i> 1985	200 Golden Syrian hamsters (M)	1 yr inhalation with 12 mo observation	Yes	3: 0.02, 0.08, 0.41 mg/kg/d	Nasal adenomatous polyps/Male	4.16
Vernot <i>et al.</i> 1985	400 C57BL/6 Mice (F)	1 yr inhalation with 15 mo observation	Yes	1: 0.18 mg/kg/d	Negative	NA
Toth, 1972	50/sex/ group Swiss mice	Lifetime, water	Not concurr ent	1: ~1.7-2 mg/kg/d	Lung/Male	2.20 [¥]
Roe <i>et al.</i> 1967	25 Swiss mice (F)	Gavage 5X/wk, 40 wk	85 Untreated	1: ~5 mg/kg/d	Lung/Female	5.67 ^{¥¥}

2162 Studies listed are in CPDB [Cancer Potency Database <http://toxnet.nlm.nih.gov/cpdb/>].

2163 *Carcinogenicity study selected for non-inhalation AI calculation.

2164 **Carcinogenicity study selected for inhalation AI calculation.

2165 NA= Not applicable.

2166 ‡ Excluded by U.S. EPA (no concurrent controls). Liver negative.

2167 ‡‡ Animal survival affected; Liver negative.

2168 Vernot *et al.* 1985 = MacEwen *et al.* 1981 & summarized in U.S. EPA IRIS database, last
2169 revision 04/01/1991.

2170 Used by U.S. EPA (1986) for derivation of inhalation unit risk.

2171

2172 **Mode of Action of Carcinogenicity**

2173 Not defined. DNA adducts have been detected *in vivo*, (Becker, *et al.* 1981; Bosan and Shank,
2174 1983; Bosan *et al.* 1987; Saffhill *et al.* 1988; Leakakos and Shank, 1994; Mathison *et al.*
2175 1994) although they are reported in tissues that do not develop tumors, so their contribution to
2176 tumorigenicity is not known.

2177

2178 **Regulatory and/or Published Limits**

2179 The U.S. EPA (1991) has published an oral slope factor of 3.0 per mg/kg/day and a drinking
2180 water unit risk of 8.5E-5 per µg/L. At the 1 in 100,000 risk level, this equates to a
2181 concentration of 0.1 µg of hydrazine/L of water or ~0.2 µg/day for a 50 kg/human. This limit
2182 is a linearized multistage extrapolation based on the observation of hepatomas in a multi-dose
2183 gavage study (Biancifiori, 1970) where hydrazine sulfate was administered to mice for 25
2184 weeks and observed throughout their lifetime (U.S. EPA, 1991). In a U.S. EPA (2002)
2185 literature review for hydrazine and hydrazine sulphate, three additional studies were identified
2186 that were published after the oral slope factor was calculated (Steinhoff and Mohr, 1988;
2187 FitzGerald and Shank, 1996; Bosan *et al.* 1987). It was noted that these studies could
2188 potentially produce a change in the oral slope factor but it has not been re-evaluated.

2189

2190 The U.S. EPA (1986) has also published an inhalation slope factor of 17 per mg/kg/day and
2191 an inhalation unit risk of 4.9×10^{-3} per µg/m³. At the 1 in 100,000 risk level, this equates to an
2192 air concentration of 2×10^{-3} µg/m³ of hydrazine or 0.04 µg/day assuming a person breathes 20
2193 m³/day. This limit is a linearized multistage extrapolation based on the observation of nasal
2194 cavity adenoma or adenocarcinoma in male rats in a multi-dose inhalation study (MacEwen *et*
2195 *al.*, 1986) where hydrazine was administered 6 hours/day, 5 days/week for 1 year followed by
2196 an 18-month observation period (U.S. EPA, 1986). Only the U.S. EPA review of this data
2197 was accessible; however, the results appear to be very similar to, if not the same as, those of
2198 Vernot *et al.* (1985).

2199

2200 **Acceptable Intake (AI)**

2201 Rationale for selection of study for AI calculation

2202

2203 Both oral and inhalation carcinogenicity studies for hydrazine were reviewed to determine if a
2204 separate limit is required specific for inhalation carcinogenicity. Given the more potent
2205 carcinogenicity specific to the first site of contact observed in inhalation studies, it was
2206 determined that a separate AI for inhalation exposure was appropriate.

2207

2208 For oral hydrazine, carcinogenicity has been reported in 3 mouse studies and one rat study.

2209 Only one mouse study (Steinhoff *et al.* 1990) and the rat study (Steinhoff and Mohr, 1988)

2210 meet currently acceptable study design criteria (50 animals per sex/group, minimum of 3
2211 treatment groups, both sexes included, and concurrent controls). The mouse study by
2212 Steinhoff and Mohr (1988) was negative with a high dose of 10 mg/kg/day. The rat study
2213 included doses of up to 3 mg/kg/day and was positive for hepatocellular neoplasms in both
2214 sexes at a similar dose level. The rat study (Steinhoff and Mohr, 1988) is deemed the most
2215 sensitive robust study available, with a TD₅₀ of 41.6 mg/kg/day. Both of these studies were
2216 conducted after the U.S. EPA oral slope factor and drinking water limit was derived.

2217
2218 All of the inhalation carcinogenicity studies that were used by the U.S.EPA in the derivation
2219 of the inhalation carcinogenicity limit for hydrazine were taken into consideration when
2220 selecting the most robust carcinogenicity study for the derivation of an AI for inhaled
2221 pharmaceuticals. The critical study used by U.S. EPA was proprietary (i.e., MacEwen *et al.*
2222 1981), but is likely the same data as in Vernot *et al.* 1985. Given that the TTC was derived
2223 *via* linear extrapolation from TD₅₀ values for hundreds of carcinogens, that same approach
2224 was used in the derivation of a compound specific AI for hydrazine. The methodology used
2225 by the U.S. EPA and the method used here are both highly conservative in nature. However,
2226 given that the methodologies do differ, it is reasonable to expect some slight differences. The
2227 AI was calculated based on the TD₅₀ derived from a study in which male and female rats were
2228 administered hydrazine *via* inhalation for one year with an 18-month observation period
2229 (Vernot *et al.* 1985). While a 1-year study is not a standard design for carcinogenicity, a
2230 positive response was observed demonstrating that the window for carcinogenicity was not
2231 missed. The most sensitive target tissue was the male nasal region, with a TD₅₀ value of
2232 0.194 mg/kg/day, which was lowered as standard practice to account for 2-year lifetime
2233 exposure.

2234 2235 Calculation of AI

2236 2237 ➤ AI

2238
2239 Lifetime AI = TD₅₀/50,000 x 50 kg

2240
2241 Lifetime AI = 41.6 (mg/kg/day)/50,000 x 50 kg

2242
2243 **Lifetime AI = 42 µg/day**

2244 2245 ➤ Inhalation AI

2246
2247 Lifetime AI = TD₅₀/50,000 x 50 kg

2248
2249 Lifetime AI = 0.194 (mg/kg/day)/50,000 x 50 kg

2250
2251 **Lifetime AI = 0.2 µg/day**

2252 2253 **References**

2254 Becker RA, Barrows LR, Shank RC. Methylation of liver DNA guanine in hydrazine
2255 hepatotoxicity: dose-response and kinetic characteristics of 7-methylguanine and O⁶-
2256 methylguanine formation and persistence in rats. *Carcinogenesis* 1981; 2(11):1181-8.

2257

- 2258 Bosan WS, Shank RC. Methylation of liver DNA guanine in hamsters given hydrazine.
2259 Toxicol Appl Pharmacol 1983; 70:324-34.
2260
- 2261 Bosan WS, Shank RC, MacEwen JD, Gaworski CL, Newberne PM. Methylation of DNA
2262 guanine during the course of induction of liver cancer in hamsters by hydrazine or
2263 dimethylnitrosamine. Carcinogenesis 1987; 8(3):439-44.
2264
- 2265 Choudary G, Hansen H. Human health perspective on environmental exposure to hydrazines:
2266 A review. Chemosphere 1998; 37(5):801-43.
2267
- 2268 FitzGerald BE, Shank RC. Methylation status of DNA cytosine during the course of induction
2269 of liver cancer in hamsters by hydrazine sulphate. Carcinogenesis 1996; 17(12):2703-9.
2270
- 2271 Gold S, Zeiger E, editors. Handbook of Carcinogenic Potency and Genotoxicity Databases.
2272 CRC Press, Boca Raton, FL; 1997.
2273
- 2274 Hazardous Substance Database (HSDB): Hydrazine (302-01-2); [Online]. 2005 June 24 [cited
2275 2013 February 27]; Available from: URL: <http://toxnet.nlm.nih.gov/>
2276
- 2277 IARC. Monographs on the evaluation of the carcinogenic risk of chemicals to man. Geneva:
2278 WHO, International Agency for Research on Cancer, [Online] 1972-PRESENT.
2279 (Multivolume work). 1999; Available from: URL: <http://monographs.iarc.fr/index.php> p. V71
2280 1006.
2281
- 2282 Leakakos T, Shank RC. Hydrazine genotoxicity in the neonatal rat. Toxicol Appl Pharmacol
2283 | 1994; 126 (2):-295-300.
2284
- 2285 Lui YY, Schmeltz I, Hoffman D. Chemical studies on tobacco smoke. Quantitative analysis of
2286 hydrazine in tobacco and cigarette smoke. Anal Chem 1974; 46: 885-9.
2287
- 2288 Mathison B, Murphy SE, Shank RC. Hydralazine and other hydrazine derivatives and the
2289 | formation of DNA adducts. Toxicol Appl Pharmacol 1994; 127 (1):-91-8.
2290
- 2291 Roe FJC, Grant GA, Millican DM. Carcinogenicity of hydrazine and 1,1-dimethylhydrazine
2292 for mouse lung. Nature 1967; 216:375-376.
2293
- 2294 Saffhill R, Fida S, Bromley M, O'Connor PJ. Promutagenic alkyl lesions are induced in the
2295 tissue DNA of animals treated with isonizid. Human Toxicol 1988; 7:311-7.
2296
- 2297 Steinhoff D, Mohr U. The question of carcinogenic effects of hydrazine. Exp Pathol 1988; 33:
2298 133-40.
2299
- 2300 Steinhoff D, Mohr U, Schmidt WM. On the question of the carcinogenic action of hydrazine -
2301 evaluation on the basis of new experimental results. Exp Pathol 1990; 39: 1-9.
2302
- 2303 Toth B. Hydrazine, methylhydrazine and methylhydrazine sulfate carcinogenesis in Swiss
2304 mice. Failure of ammonium hydroxide to interfere in the development of tumors. Int J Cancer
2305 | 1972; 9:-109-18.
2306

- 2307 Toth B. A review of the natural occurrence, synthetic production and use of carcinogenic
2308 hydrazines and related chemicals. *In vivo*. 2000; 14(2):299-319.
2309
- 2310 U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS).
2311 Summary on Hydrazine/Hydrazine sulfate (302-01-2). [Online]. 1991 January 4 [cited 2013
2312 February 27]; Available from: URL: <http://www.epa.gov/iris/>.
2313
- 2314 U.S. Environmental Protection Agency's IRIS. Screening-Level Literature Review.
2315 Hydrazine/Hydrazine sulfate, CASRN: 302-01-2., [Online]. 2002 January 1 [cited 2012
2316 August 30]; Available from: URL: hotline.iris@epa.gov.
2317
- 2318 Vernot EH, MacEwen JD, Bruner RH, Haun CC, Kinkead ER, Prentice DE, et al. Long-term
2319 inhalation toxicity of hydrazine. *Fundam Appl Toxicol* 1985; 5:1050-64.
2320
- 2321 Von Burg R, Stout T. Hydrazine. *J Appl Toxicol* 1991 ; 11:447–450.

2322

Hydrogen peroxide (CAS# 7722-84-1)

2323

Potential for Human Exposure

2324

Hydrogen peroxide (HSDB, 2005) can be present in green tea and instant coffee, in fresh fruits and vegetables and naturally produced in the body (Halliwell *et al.* 2000). It is estimated up to 6.8 g is produced endogenously per day (Desesso *et al.* 2000). Other common sources of exposure are from disinfectants, some topical cream acne products, and oral care products up to which can contain up to 4% hydrogen peroxide (Desesso *et al.* 2000).

2329

2330

Mutagenicity/Genotoxicity

2331

Hydrogen peroxide is mutagenic/genotoxic *in vitro* but not *in vivo*.

2332

2333

IARC (1999) and Joint Research Centre (JRC) (2003) reviewed the mutagenicity data for hydrogen peroxide, and key observations are summarized here.

2334

2335

2336

Hydrogen peroxide is mutagenic in:

2337

- *Salmonella typhimurium* strains TA96, TA97, SB1106p, SB1106, and SB1111 and *Escherichia coli* WP2 in the absence of exogenous metabolic activation;

2338

2339

- L5178Y mouse lymphoma cell sublines at the *hprt* locus (weak increase);

2340

- Chinese hamster V79 cells at the *hprt* locus, in only one of six studies.

2341

2342

In vivo, micronuclei were not induced after administration of hydrogen peroxide to mice intraperitoneally at up to 1,000 mg/kg, or to catalase-deficient C57BL/6NCr1BR mice in drinking water at 200, 1,000, 3,000, and 6,000 ppm for two weeks.

2343

2344

2345

2346

Carcinogenicity

2347

Hydrogen peroxide is classified as Group 3 (not classifiable as to its carcinogenicity to humans) (IARC, 1999).

2348

2349

2350

There is only one carcinogenicity report cited in the CPDB (Ito *et al.* 1981), in which mice were treated with hydrogen peroxide in drinking water for approximately 2 years. The study included two treatment groups and about 50 animals per dose group. Hydrogen peroxide induced small intestinal tumours in C57BL female mice (Ito *et al.* 1981). Statistically significant increases in tumours ($p < 0.005$) were observed in both dose groups in the mouse carcinogenicity study (Ito *et al.* 1981) although only the duodenal tumors at the high dose in females are noted as significant in the CPDB. Thus, 0.1% hydrogen peroxide administered in drinking water was defined as the (Lowest Observed Adverse Effect Level) LOAEL, equivalent to an average daily dose-rate per kg body weight per day of 200 mg/kg/day (CPDB).

2351

2352

2353

2354

2355

2356

2357

2358

2359

2360

2361

Several carcinogenicity studies are not reported in the CPDB. Studies of 6-month duration or longer are summarised in the following table (adapted from Desesso *et al.* 2000); they are limited in the numbers of animals and used a single dose level.

2362

2363

2364

2365

The results of the Ito mouse carcinogenicity studies, conducted in 1981, 1982, 1984, 1986, were thoroughly evaluated by the Cancer Assessment Committee (CAC) of the US Food and

2366

2367 Drug Administration (FDA) and published in the Federal Register. The conclusion was that
 2368 the studies did not provide evidence that hydrogen peroxide is a carcinogen (FDA, 1988).
 2369

2370 In Europe the Scientific Committee on Consumer Products (SCCP), now the Scientific
 2371 Committee on Consumer Safety (SCCS), reviewed the available carcinogenicity data for
 2372 hydrogen peroxide and concluded the carcinogenic mechanism of action is unknown and
 2373 believe that a genotoxic mechanism cannot be excluded (SCCP, 2005). In contrast, Desesso
 2374 *et al.* (2000) suggested that dilute hydrogen peroxide would not reach the target site and that
 2375 the hyperplastic lesions seen at the LOAEL dosage were due to irritation from food pellets
 2376 accompanying a decrease in water consumption which is often noted with exposure to
 2377 hydrogen peroxide in drinking water. This is supported by life time studies in the hamster in
 2378 which hydrogen peroxide was administered by gastric intubation (water uptake was not
 2379 affected) in which the duodenal epithelia appeared normal; this was the basis for the CAC
 2380 conclusion above (FDA, 1988).
 2381

2382 **Hydrogen Peroxide – Details of carcinogenicity studies**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Notes
Ito <i>et al.</i> 1981*	48- 51/sex/group C57BL/6J mice	100 wk Drinking water	Yes	2: 0.1; 0.4% M: 200; 800 F: 167; 667 mg/kg/d	CPDB study with TD ₅₀ of 7.54 g/kg/d for female duodenal carcinoma
Ito <i>et al.</i> 1982**	29 mice (No. of M and F not reported)	700 d Drinking water	No	1: 0.4%	Cessation of H ₂ O ₂ treatment decreased percent of mice with stomach erosions and percent of mice with duodenal lesions (plaques and nodules)
Ito <i>et al.</i> 1984**	18 mice (No. of M and F not reported)	6 mo Drinking water	No	1: 0.4%	2 duodenal tumours (11.1%)
Ito <i>et al.</i> 1984**	22 mice (No. of M and F not reported)	6 mo Drinking water	No	1: 0.4%	7 duodenal tumours (31.8%)
Ito <i>et al.</i> 1984**	21 mice (No. of M and F not reported)	7 mo Drinking water	No	1: 0.4%	21 duodenal tumours (100%)
Ito <i>et al.</i> 1984**	24 mice (No. of M and F not reported)	6 mo Drinking water	No	0.4% only	22 duodenal tumours (91.7%)
Ito <i>et al.</i> 1986**	Female mice (11 control, 21 treatment)	6 mo Drinking water	Yes	1: 0.4%	No duodenal tumours in control mice, 2 (9.5%) in treatment group

Ito <i>et al.</i> 1986 ^{**}	Female mice (12 control, 22 treatment)	6 mo Drinking water	Yes	1: 0.4%	No duodenal tumours in control mice, 7 (31.8%) in treatment group
Ito <i>et al.</i> 1986 ^{**}	Female mice (28 control, 24 treatment)	6 mo Drinking water	Yes	1: 0.4%	No duodenal tumours in control mice, 22 (91.7%) in treatment group

2383 *Carcinogenicity study selected for PDE calculation

2384 ** All other studies are not in the CPDB but are discussed in the reference FDA, 1988 and not
2385 cited separately.

2386

2387 **Mode of action for carcinogenicity**

2388 Hydrogen peroxide is a Reactive Oxygen Species (ROS) that is formed as part of normal
2389 cellular metabolism (JRC, 2003). The toxicity of hydrogen peroxide is attributed to the
2390 production of ROS and subsequent oxidative damage resulting in cytotoxicity, DNA strand
2391 breaks and genotoxicity (Tredwin *et al.* 2006). Due to the inevitable endogenous production
2392 of ROS, the body has evolved defense mechanisms to limit their levels, involving catalase,
2393 superoxide dismutases and glutathione peroxidase.

2394
2395 Oxidative stress occurs when the body's natural antioxidant defense mechanisms are exceeded,
2396 causing damage to macromolecules such as DNA, proteins and lipids. ROS also inactivate
2397 antioxidant enzymes, further enhancing their damaging effects (De Bont and Larebeke, 2004).
2398 During mitochondrial respiration, oxygen undergoes single electron transfer, generating the
2399 superoxide anion radical. This molecule shows limited reactivity but is converted to hydrogen
2400 peroxide by the enzyme superoxide dismutase. Hydrogen peroxide is then reduced to water
2401 and oxygen by catalase and glutathione peroxidase (Finkel and Holbrook, 2000). However, in
2402 the presence of transition metals, such as iron and copper, hydrogen peroxide is reduced
2403 further to extremely reactive hydroxyl radicals. They are so reactive they do not diffuse more
2404 than one or two molecular diameters before reacting with a cellular component (De Bont and
2405 Larebeke, 2004). Therefore, they must be generated immediately adjacent to DNA to oxidize
2406 it. Antioxidants provide a source of electrons that reduce hydroxyl radicals back to water,
2407 thereby quenching their reactivity. Clearly, antioxidants and other cellular defenses that
2408 protect against oxidative damage are limited within an *in vitro* test system. Consequently,
2409 following treatment with hydrogen peroxide these protective mechanisms are readily
2410 overwhelmed inducing cytotoxicity and genotoxicity in bacterial and mammalian cell lines.
2411 Diminution of the *in vitro* response has been demonstrated by introducing elements of the
2412 protective mechanisms operating in the body; for example, introducing hydrogen peroxide
2413 degrading enzymes, such as catalase or adjusting the level of transition metals (SCCP, 2005).
2414 Unsurprisingly *in vivo*, where the cellular defense mechanisms are intact, hydrogen peroxide
2415 is not genotoxic following short-term exposure. This suggests that a threshold exists below
2416 which the cellular defense mechanisms can regulate ROS maintaining homeostasis.

2417
2418 Based on the comprehensive European Commission (EC) risk assessment, the weight of
2419 evidence suggests hydrogen peroxide is mutagenic *in vitro* when protective mechanisms are
2420 overwhelmed. However, it is not genotoxic in standard assays *in vivo*. Its mode of action has
2421 a non-linear, threshold effect.

2422

2423 **Regulatory and/or Published Limits**

2424 Annex III of the European Cosmetic Regulation ([EC] No 1223/2009) was updated to include
2425 acceptable levels of hydrogen peroxide with regard to tooth whitening products. For oral
2426 products sold over the counter, including mouth rinse, tooth paste and tooth whitening or
2427 bleaching products, the maximum concentrations of hydrogen peroxide allowed (present or
2428 released) is 0.1%. Higher levels up to 6% are also permitted providing products are
2429 prescribed by dental practitioners to persons over 18 years old. Cosmetics Europe estimated
2430 that 1 g of mouthwash is ingested per application, and that frequency of application is 5 per
2431 day. Therefore, assuming mouthwash products contain 0.1% hydrogen peroxide, the daily
2432 exposure is 5 mg/day, or 0.1 mg/kg of body weight per day for a 50 kg adult. According to
2433 the Scientific Committee on Consumer Safety (SCCS) Notes for Guidance on the Safety
2434 Evaluation of Cosmetic Products ([EC] No 1223/2009), a typical amount of toothpaste per
2435 application is 2.75g. The Joint Research Centre published Risk Assessment Report considers
2436 17% a reasonable value for accidental ingestion. This is equivalent to 9.35 mg/day, assuming
2437 a frequency of application of twice per day or 0.19 mg/kg/day for a 50 kg adult. These
2438 estimated ingestion values are considered conservative as it is likely that most of the hydrogen
2439 peroxide is decomposed after using oral care products and is not ingested (JRC, 2003).

2440
2441 US FDA - hydrogen peroxide is Generally Recognized As Safe (GRAS) up to 3% for long-
2442 term over the counter use as an anti-gingivitis/anti-plaque agent (FDA 2003).

2443

2444 **Permissible Daily Exposure (PDE)**

2445 It is considered that hydrogen peroxide acts *via* a mode of action with a threshold (i.e.,
2446 oxidative stress). An increase in tumors was observed in female mice at ≥ 167 mg/kg/day
2447 (0.1% dose group). Thus, the Lowest Observed Adverse Effect Level (LOAEL) in the 2 year
2448 rat studies was 0.2 mg/kg/day.

2449

2450 The PDE calculation is: (NOEL x body weight adjustment (kg)) / F1 x F2 x F3 x F4 x F5

2451

2452 The following safety factors as outlined in ICH Q3C have been applied to determine the AI
2453 for hydrogen peroxide, these are:

2454

2455 F1 = 12 (mouse to man)

2456 F2 = 10 (inter-individual variability)

2457 F3 = 1 (study duration at least half lifetime)

2458 F4 = 1 (endogenous product, so severe toxicity not expected at low doses)

2459 F5 = 10 (using a LOAEL)

2460

2461 On this basis the PDE is calculated as follows:

2462

2463 Lifetime PDE = 167 mg/kg/day x 50 kg / (12 x 10 x 1 x 1 x 10)

2464

2465 **Lifetime PDE = 6,960 μ g/day**

2466

2467 **References**

2468 De Bont R, Larebeke N. Endogenous DNA damage in humans: a review of quantitative data.
2469 Mutagenesis 2004; 19(3):169-85.

- 2470 Desesso JM, Lavin AL, Hsia SM, Mavis RD. Assessment of the carcinogenicity associated
2471 with oral exposures to hydrogen peroxide. *Food and Chem Toxicol* 2000; 38:1021-41.
2472
- 2473 FDA. Irradiation in the production, processing, and handling of food. Food and Drug
2474 Administration. *Federal Register* 1988; Vol. 53, 251:53198-9.
2475
- 2476 FDA. Oral health care drug products for over-the-counter human use; antigingivitis/antiplaque
2477 drug products; establishment of a monograph. *Federal Register* 2003; 68:32232-32286.
2478
- 2479 Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature* 2000;
2480 408:239-47.
2481
- 2482 CPDB, Gold S, Zeiger E, editors. *Handbook of Carcinogenic Potency and Genotoxicity*
2483 *Databases*, Boca Raton, FL: CRC Press. 1997.
2484
- 2485 Halliwell B, Clement MV, Long LH. Hydrogen peroxide in the human body. *FEBS Letters*
2486 2000; 486:10-13.
2487
- 2488 HSDB, Hazardous Substance Data Bank. Hydrogen Peroxide. [Online]. 2005 June 24 [cited
2489 2013 October 4]; Available from: URL:
2490 <http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~6kxo2y:1>.
2491
- 2492 IARC. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. 1999 Vol.
2493 71.
2494
- 2495 Ito A, Watanabe H, Naito M, Naito Y. Induction of duodenal tumours in mice by oral
2496 administration of hydrogen peroxide. *Gann the Japanese journal of cancer research* 1981; 72:
2497 174-5.
2498
- 2499 JRC. Hydrogen Peroxide. Summary Risk Assessment Report, Special Publication 2003;
2500 I.03.148.
2501
- 2502 Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30
2503 November 2009 on cosmetic products.
2504
- 2505 SCCP: European Commission Health & Consumer Directorate General Scientific Committee
2506 on consumer products opinion on hydrogen peroxide in tooth whitening products. 2005
2507 SCCP/0844/04.
2508
- 2509 Tredwin CJ, Naik S, Lewis NJ, Scully C. Hydrogen peroxide tooth-whitening (bleaching)
2510 products: Review of adverse effects and safety issues. *British Dental Journal* 2006; 200:371-6.
2511

2512 **Hydroxylamine (CAS# 7803-49-8)**

2513 **Potential for human exposure**

2514 The most common source of exposure is in industrial settings, and there are no data available
2515 for exposure to the general population. Hydroxylamine is reported to be a product of normal
2516 cellular metabolism (Gross, 1985).

2518 **Mutagenicity/Genotoxicity**

2519 Based on weight of evidence from genotoxicity assays generally used in standard test batteries,
2520 hydroxylamine is not mutagenic in the *in vitro* bacterial reverse mutation test, has weak or no
2521 genotoxic activity *in vitro* in mammalian cells, it is not genotoxic in bone marrow when given
2522 orally to rodents.

2523
2524 Hydroxylamine has little or no mutagenic activity in the *Salmonella* and *Escherichia coli*
2525 reverse mutation assay (Ames), and has not been shown to be genotoxic *in vivo*. However,
2526 hydroxylamine is often described as a mutagen because at high molar concentrations it has
2527 been used as a diagnostic mutagen (Freese *et al.* 1961) and the compound has been reported to
2528 be positive in diverse genotoxicity assays (Marfey and Robinson, 1981) that are not in the
2529 standard set of assays used for regulatory purposes (e.g., those described in OECD guidelines).

2530
2531 In contrast, hydroxylamine was reported to be negative in the majority of “standard”
2532 genotoxicity assays (namely the bacterial reverse mutation assay (Ames), and the *in vivo*
2533 rodent bone marrow micronucleus test). Hydroxylamine sulphate (CAS No: 10039-54-0) was
2534 not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 and TA102
2535 with and without metabolic activation at test concentrations limited by toxicity to ≤ 1000
2536 $\mu\text{g}/\text{plate}$ (NTP, 1991). Hydroxylamine hydrochloride (CAS No: 5470-11-1) was reported to
2537 be weakly mutagenic (dose related increases < 2 fold) in the presence, but not absence, of
2538 metabolic activation in TA100 at concentrations of > 100 and < 330 $\mu\text{g}/\text{plate}$ (NTP, 1988).
2539 Hydroxylamine hydrochloride was not mutagenic in TA98, TA100, TA1535, TA1537,
2540 TA1538 and *Escherichia coli* WP2 *uvrA* in the presence and absence of metabolic activation
2541 ≤ 333 $\mu\text{g}/\text{plate}$ – the highest dose tested in the assay (Dunkel *et al.* 1984).

2542
2543 Hydroxylamine hydrochloride was reported to be mutagenic in the mouse lymphoma *tk*
2544 mutation assay, with and without metabolic activation (NTP, 1988), but the data do not
2545 convincingly meet the up-to-date criteria for positive results in this assay (Moore *et al.* 2006).
2546 Hydroxylamine hydrochloride was not genotoxic in an oral bone-marrow micronucleus assay
2547 when tested in male and female rats at doses ≤ 125 $\text{mg}/\text{kg}/\text{day}$, where the maximum dose was
2548 limited by adverse clinical signs (Getman, 2014). Hydroxylamine sulfate was not genotoxic
2549 in an oral bone-marrow micronucleus assay when tested in male and female mice at doses \leq
2550 1200 $\text{mg}/\text{kg}/\text{day}$ where the maximum dose was limited by adverse clinical signs (ECHA, no
2551 date).

2553 **Carcinogenicity**

2554 No studies were identified in the CPDB. The details of a 2-year drinking water study are
2555 described in a European Union Risk Assessment Report (ECHA, 2008). Hydroxylamine
2556 sulphate (bis [hydroxylammonium] sulphate; CAS 10039-54-0) was carcinogenic in male and
2557 female rats *via* the oral route (hydroxylamine was administered by giving bis

2558 (hydroxylammonium) sulphate, which dissociates in water to a hydroxyl-ammonium ion
 2559 which converts to the reactive free hydroxylamine base). The administration of
 2560 hydroxylamine sulphate in the drinking water for 2 years to rats was associated with an
 2561 increased incidence of hemangiosarcomas in males and hemangioma development in females,
 2562 both in the spleen. In groups of 50 rats, the incidence of hemgiosarcomas in males was 4 in
 2563 controls, and 7, 9 and 8 in the 0.2, 1.0 and 3.7 mg/kg/day treated groups. Although the
 2564 increase in number of tumours in the spleen of male and female rats was low, not dose-related
 2565 and the difference did not attain statistical significance, the levels were above those in the
 2566 concurrent control groups and above the ranges of historical control background data (ECHA,
 2567 2008).
 2568

2569 **Mode of action for carcinogenicity**

2570 A critical review of the data concluded that the mechanism of carcinogenicity had a threshold
 2571 and that there was no indication that these tumors were related to a primary genotoxic
 2572 mechanism (ECHA, 2008). The tumor induction is not related to initial mutagenicity, but
 2573 secondary to methemoglobinemia and accumulation of hemosiderin in the spleen. This can
 2574 lead to iron overload of the spleen resulting in iron-catalyzed free radical reactions, damage,
 2575 and corresponding hyperplasia (Bus and Popp, 1987). Evidence for this also comes from
 2576 short-term and long-term studies demonstrating that hydroxylamine induces hemolytic anemia
 2577 and hemosiderosis that results in precursor damage to the spleen. In subacute and 90-day rat
 2578 studies, exposure to hydroxylamine induced hemolytic anemia, and splenomegaly with
 2579 changes to red blood parameters (enhanced levels of methemoglobin, Heinz bodies and a shift
 2580 in blood cell pattern, e.g., increase in reticulocytes and leukocytes). Increased decomposition
 2581 of erythrocytes was seen as hemosiderin deposits and iron pigment deposition in the spleen.
 2582 Damage to the spleen was observed by sinus dilation together with congestion, splenomegaly,
 2583 and increased organ weight (ECHA, 2008). Administration over 1-2 years in rats also
 2584 resulted in hemosiderin storage in the spleen, and signs of hemolysis. No hematotoxic effects or
 2585 other systemic effects were detected at a dose of 0.2 mg/kg/day in male rats or 0.4 mg/kg/day
 2586 in female rats. An increased incidence of a precursor lesion (i.e., angiomatous hyperplasia)
 2587 was observed in low and high male dose groups and the high female dose group (ECHA,
 2588 2008).
 2589

2590 In addition, hydroxylamine is the reactive moiety for the hemosiderosis-induced spleen
 2591 tumors observed with aniline and its analogues. These effects occur mainly in male rats, and
 2592 exhibit a non-linear response. Aniline and related structures form phenylhydroxylamine
 2593 which is taken up by erythrocytes resulting in hemosiderosis and ultimately spleen tumors
 2594 (Bus and Popp, 1987).
 2595

2596 **Hydroxylamine – Details of carcinogenicity studies**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
ECHA, 2008 ^{*^} Bis (hydrox ylammo	50/sex/ group Wistar rat	Drinking water 104 wk	Yes	3: 5; 20; 80 ppm M: 0.2; 1; 3.7 mg/kg/d	Spleen Hemangiosarcomas/ Male	22 ^{**¥}

niium)sulphate, CAS [10039-54-0]				F: 0.4, 1.6, 6.2 mg/kg/d		
Yamamoto <i>et al.</i> 1967	Mice: Swiss Webster (5 M) and C3H/HeN (10 F)	Drinking Water 52 wk	Yes	2: 100; 200 mg/kg/d	No Tumors Found	NA ^{^^}
Stenk <i>et al.</i> 1987	40 C3H/HeN (F)	Drinking Water 105 wk	Yes	1: 246 mg/kg/d	Hemangioma (Spleen)	524 ^{^^^}
	50/sex C3H/HeJ(+)	Drinking Water 105 wk	Yes	1: 246 mg/kg/d	Hemangioma (Lymph Node)	540 ^{^^^}

2597 Note: Studies in the table are not in the CPDB.
 2598 *Carcinogenicity study selected for AI calculation.
 2599 **TD₅₀ calculated based on carcinogenicity data.
 2600 †Small increase in number of tumours, not dose-related & not statistically significant.
 2601 However, levels above control groups and historical control background data.
 2602 ^ Study details given in ECHA 2008.
 2603 NA= Not applicable.
 2604 ^^ Limited number of animals and duration.
 2605 ^^^ Limited number of doses, mice carry germinal provirus (MMTV; mouse mammary tumor virus) and develop a moderately high incidence of mammary tumors late in life.
 2606
 2607

2608 **Regulatory and/or Published Limits**

2609 No regulatory limits have been published, for example by U.S. EPA, WHO.
 2610

2611 **Permissible Daily Exposure (PDE)**

2612 Rationale for selection of study for PDE calculation:
 2613

2614 It is considered that hydroxylamine induces tumors *via* a mode of action with a threshold (i.e.,
 2615 hemosiderosis of the spleen). An increase in tumors was observed in male rats at ≥ 5 ppm or
 2616 0.2 mg/kg/day for hemangiosarcomas and females at the high dose of 80 ppm or 6.2
 2617 mg/kg/day (hemangiosarcomas and hemangiomas). Thus, the lowest observed adverse effect
 2618 level (LOAEL) in the 2-year rat study was 0.2 mg/kg/day in males.
 2619

2620 **Calculation of PDE:**

2621
 2622 The PDE calculation is: (NOEL x body weight adjustment (kg)) / F1 x F2 x F3 x F4 x F5
 2623

2624 The following safety factors as outlined in ICH Q3C Guideline Appendix 3 have been applied
 2625 to determine the PDE for hydroxylamine, these are:

2626 F1 = 5 (rat to man)

2627 F2 = 10 (inter-individual variability)

2628 F3 = 1 (study duration at least half lifetime)
2629 F4 = 10 (severe toxicity – non-genotoxic carcinogenicity)
2630 F5 = 10 (using a LOAEL, but percent response close to threshold 4% versus 7%)

2631
2632 On this basis the PDE is calculated as follows:

2633
2634 Lifetime PDE = 0.2 mg/kg/day x 50 kg / (5 x 10 x 1 x 10 x 10)

2635
2636 **Lifetime PDE = 2 µg/day**

2637

2638 **References**

2639 Bus JS, Popp JA. Perspectives on the mechanism of action of the splenic toxicity of aniline
2640 and structurally-related compounds. Food Chem Toxicol 1987; 25:619–26.

2641
2642 Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, et al.
2643 Reproducibility of Microbial Mutagenicity Assays: I. Tests With Salmonella typhimurium and
2644 Escherichia coli using a Standardized Protocol. Environ Mutagen 1984; 6 Suppl 2:1-254.

2645
2646 Freese E, Bautz- Freese E, Bautz E. Hydroxylamine as a mutagenic and inactivating agent. J
2647 Mol Biol 1961; 3:133-43.

2648
2649 ECHA. European Union Risk Assessment Report - [bis(hydroxylammonium)sulphate] CAS
2650 [10039-54-0] [Online]. 2008; Available from: URL:
2651 <http://echa.europa.eu/documents/10162/a94c5d98-4ecb-459d-9dab-594fd516e30a>

2652
2653 ECHA. Registered Substances. Accessed [bis(hydroxylammonium)sulphate] CAS [10039-
2654 54-0] [Online]. 2015 March 19; Available from: URL: <http://echa.europa.eu/information-on-chemicals/registered-substances>.

2655
2656
2657 Getman SM. Hydroxylamine: Oral micronucleus study in rats (Study No. DS03096). Bristol-
2658 Myers Squibb Company. 2014.

2659
2660 Gross P. Biologic activity of hydroxylamine: a review. Crit Rev in Toxicol 1985; 14:87-99.

2661
2662 Marfey P, Robinson E. The genetic toxicology of hydroxylamines. Muta Res 1981; 86:155-91.

2663
2664 Moore MM, Honma M, Clements J, Bolcsfoldi G, Burlinson B, Cifone M, et al. Mouse
2665 lymphoma thymidine kinase mutation assay: Follow-up meeting of the International
2666 Workshop on Genotoxicity Testing- Aberdeen Scotland, 2003- Assay Acceptance Criteria,
2667 Positive controls and Data Evaluation. Environ Mol Mutagen 2006; 47:105.

2668
2669 NTP study 689679. [Online]. 1991 [cited 2014 May 1]; Available from: URL:
2670 http://tools.niehs.nih.gov/ntp_tox/index.cfm

2671
2672 NTP. [Online]. 1988; Available from: URL: [http://ntp.niehs.nih.gov/testing/status/agents/ts-
2673 m20240.html](http://ntp.niehs.nih.gov/testing/status/agents/ts-m20240.html)

2674
2675 Stenbäck F, Weisburger JH, Williams GM. Hydroxylamine effects on cryptogenic neoplasm
2676 development in C3H mice. Cancer Lett 1987; 38:73-85.

2677 Yamamoto RS, Weisburger EK, Korzis J. Chronic administration of Hydroxylamine and
2678 derivatives in mice. Proceedings of the Society for Experimental Biology and Medicine 1967;
2679 124:1217-20.

2680

Methyl chloride (Chloromethane, CAS# 74-87-3)

2681 Potential for human exposure

2682 Methyl chloride is found ubiquitously in nature. Low levels of methyl chloride occur
2683 naturally in the environment (thousands of tons of methyl chloride are produced naturally
2684 every day). The vast majority comes from natural sources. Methyl chloride is formed in the
2685 oceans by natural processes (e.g., marine phytoplankton), by microbial fermentation and from
2686 biomass fires (burning in grasslands and forest fires) and volcanoes.

2687
2688 Methyl chloride has been detected at low levels all over the world in air, in groundwater,
2689 surface water, streams, lakes, seawater, effluents, and sediments. It has also been detected at
2690 low levels in drinking water, in fish samples and in human milk. Methyl chloride is present in
2691 the troposphere at a concentration of approximately $1.2 \mu\text{g}/\text{m}^3$ (0.6 ppb). The methyl chloride
2692 concentration in the air in rural sites is in general below $2.1 \mu\text{g}/\text{m}^3$ (1.0 ppb) while in urban
2693 cities it is equal to $1.0\text{-}35 \mu\text{g}/\text{m}^3$ (0.5-17 ppb), corresponding to approximately 20 - 700 μg
2694 daily intake (human respiratory volume of 20 m^3 per day). The maximum concentration
2695 found in drinking water is $44 \mu\text{g}/\text{litre}$ which is an exposure of $88 \mu\text{g}/\text{day}$ assuming a person
2696 drinks 2 L of water a day.
2697

2698 Mutagenicity/Genotoxicity

2699 Methyl chloride is mutagenic and genotoxic *in vitro* but equivocal *in vivo*. WHO (2000) and
2700 U.S. EPA (2001) reviewed the mutagenicity data for methyl chloride; key observations are
2701 summarized here.

2702
2703 Methyl chloride is mutagenic in:

- 2704 • Microbial reverse mutation assay (Ames), *Salmonella typhimurium* TA100, TA1535 and
2705 in *Escherichia coli* WP2 uvrA both in the presence and absence of metabolic activation;
- 2706 • TK6 human lymphoblasts.

2707
2708 *In vivo*, WHO 2000 concluded that “though data from standard *in vivo* genotoxicity studies
2709 are not available, methyl chloride might be considered a very weak mutagen *in vivo* based on
2710 some evidence of DNA–protein crosslinking at higher doses”. For other genotoxicity
2711 endpoints, induction of SCE by methyl chloride has been observed in human lymphoblasts
2712 (U.S. EPA, 2001).
2713

2714 Carcinogenicity

2715 Methyl chloride is classified as Group 3 “inadequate evidence for the carcinogenicity of
2716 methyl chloride to humans” (IARC, 1999). Category D compound not classifiable as to
2717 human carcinogenicity (U.S. EPA 2001).

2718
2719 In animals, the only evidence of carcinogenicity comes from a single 2-year bioassay that
2720 used the inhalation route of administration. A statistically significant increased incidence of
2721 renal benign and malignant tumors was observed only in male B6C3F1 mice at the high
2722 concentration ($1,000 \text{ ppm}$). Although not of statistical significance, cortical adenoma was
2723 also seen at $464 \text{ mg}/\text{m}^3$ (225 ppm), and development of renal cortical microcysts in mice was
2724 seen in the $103 \text{ mg}/\text{m}^3$ (50 ppm) dose group and to some extent in the $464 \text{ mg}/\text{m}^3$ (225 ppm)
2725 group (CIIT, 1981). However, no concentration–response relationship could be established.

2726 Renal cortical tubuloepithelial hyperplasia and karyomegaly were also confined to the 1,000-
2727 ppm group of male mice. Neoplasias were not found at lower concentrations or at any other
2728 site in the male mouse, or at any site or concentration in female mice or F-344 rats of either
2729 sex. Renal adenocarcinomas have been shown to occur only in male mice at a level of
2730 exposure unlikely to be encountered by people.

2731
2732 These renal tumors of the male mouse are not likely to be relevant to humans. Renal tumors
2733 in the male mouse are thought to be related to the production of formaldehyde during methyl
2734 chloride metabolism. The cytochrome P-450 (CYP) isozyme believed to be responsible,
2735 CYP2E1, is present in male mouse kidney and is androgen-dependent; female mice had
2736 CYP2E1 levels only 20%-25% of those in males. Generation of formaldehyde has been
2737 demonstrated in renal microsomes of male CD-1 mice that exceed that of naive (androgen-
2738 untreated) female mice, whereas kidney microsomes from the rat did not generate
2739 formaldehyde. Additionally, species-specific metabolic differences in how the kidney
2740 processes methyl chloride strongly suggest that renal mouse neoplasms *via* P-450 oxidation
2741 are not biologically relevant to humans given that human kidney lacks the key enzyme
2742 (CYP2E1) known to convert methyl chloride to toxic intermediates having carcinogenic
2743 potential. In the rat, renal activity of CYP2E1 was very low. No CYP2E1 activity was
2744 detected in human kidney microsomal samples, nor was it detected in freshly isolated
2745 proximal tubular cells from human kidney. CYP4A11 was detected in human kidney, but its
2746 ability to metabolize methyl chloride is unknown. In addition to CYP4A11, the only other P-
2747 450 enzymes found at significant levels in human renal microsomes are CYP4F2 and CYP3A.
2748 Moreover no commonly known environmental chemicals appear to be metabolized by the
2749 CYP4A family. The lack of detectable CYP2E1 protein in human kidney (in contrast to mice,
2750 which have high levels) suggests that the metabolism of methyl chloride by P450 (presumably
2751 leading to elevated formaldehyde concentrations) that is likely responsible for the induction of
2752 male mouse kidney tumors are not likely relevant to humans.

2753
2754 However, as highlighted by the U.S. EPA and WHO, the role of hepatic (and/or kidney)
2755 metabolism (leading to potential genotoxic metabolites) *via* the predominant glutathione
2756 (GSH)-dependent pathway (metabolism of methyl chloride to formate in liver is GSH-
2757 dependent, *via* the GSH-requiring formaldehyde dehydrogenase that oxidizes formaldehyde to
2758 formate) or even by P450 isozymes other than CYP2E1 in this regard cannot be discounted.
2759 Nonetheless, production of formaldehyde *via* low doses of methyl chloride would be
2760 negligible compared with the basal formation of formaldehyde in the body (i.e., 878 – 1310
2761 mg/kg/day; EFSA [European Food Safety Authority], 2014). In addition, based on the
2762 limitations of human relevance, U.S. EPA classified methyl chloride as a group D compound,
2763 that is, “Not Classifiable as to Human Carcinogenicity”.

2764

2765 **Methyl Chloride – Details of carcinogenicity studies (only inhalation studies available)**

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD ₅₀ (mg/kg/d)
CIIT 1981 (summarized by WHO 2000 and EPA 2001)*	120/sex/ group B6C3F1 mice	Inhalation for 6h/d, 5d/wk 24 mo	Yes	3: 103; 464; 2064 mg/m ³ (50; 225; 1000 ppm)	Kidney tumors in males only. No finding in females.	1,360**^
CIIT 1981 (summarized by WHO 2000 and EPA 2001)	120/sex/ group Fisher 344 rats	Inhalation for 6h/d, 5d/wk 24 mo	Yes	3: 103; 464; 2064 mg/m ³ (50; 225; 1000 ppm)	No findings in males and females	NA

2766 Note: Studies not listed in CPDB.

2767 *Carcinogenicity study selected for AI calculation.

2768 **TD₅₀ calculated based on carcinogenicity data.

2769 ^ Not statistically significant at 225 ppm but considered induced by methyl chloride because
2770 similar to those seen at 1000 ppm where a clear significant increase was noted.

2771 NA = Not applicable

2772

2773 **Regulatory and/or published Limits**

2774 WHO developed a guideline value for the general population of 0.018 mg/m³ and U.S. EPA
2775 developed a reference concentration of 0.09 mg/m³. Both were based on the potential for
2776 adverse Central Nervous System (CNS) effects following inhaled methyl chloride.

2777

2778 **Acceptable Intake (AI)**

2779 While the data indicate the tumors observed in male mice are likely not relevant to humans, an
2780 AI was developed because of the uncertainties in data.

2781

2782 Lifetime AI = TD₅₀/50,000 x 50 kg

2783

2784 Lifetime AI = 1,360 mg/kg/day /50,000 x 50 kg

2785

2786 **Lifetime AI = 1,360 µg/day**

2787

2788 **References**

2789 CIIT. Final report on a chronic inhalation toxicology study in rats and mice exposed to methyl
2790 chloride. Report prepared by Battelle Columbus Laboratories for the CIIT. 1981 EPA/OTS
2791 Doc #878212061, NTIS/OTS0205952.

- 2792 EFSA. Endogenous formaldehyde turnover in humans compared with exogenous contribution
2793 from food sources. EFSA Journal 2014; 12 Suppl 2:3550.
2794
2795 IARC. Methyl Chloride. 1999; Vol. 71.
2796
2797 US EPA. Toxicological review of methyl chloride. (CAS No. 74-87-3). In Support of
2798 Summary Information on the IRIS. EPA/635/R01/003. 2001.
2799
2800 WHO. Concise International Chemical Assessment Documents (CICADS) n 28, Methyl
2801 chloride. [Online]. 2000; Available from: URL:
2802 <http://www.inchem.org/documents/cicads/cicads/cicad28.htm>