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

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

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

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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	Dimethylcarbamyl 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 Environemental Protection Agency
WHO	World Health Organization

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

M7(R1)

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

Introduction

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

In this Addendum to ICH M7, acceptable intakes have been derived for a set of chemicals that are considered to be mutagens and carcinogens and were selected because they are common in pharmaceutical manufacturing, or are useful to illustrate the principles for deriving compound-specific intakes described in ICH M7[1]. Compounds are included in which the primary method used to derive acceptable intakes for carcinogens with a likely mutagenic mode of action is the "default approach" from ICH M7 of linear extrapolation from the calculated cancer potency estimate, the TD_{50}. Compounds are also included which highlight alternative principles to deriving compound-specific intakes (see below).

Chemicals that are mutagens and carcinogens (Classified as Class 1 in ICH M7) include chemicals that induce tumors through a non-mutagenic mode of action.
ICH M7 states in Section 7.2.2: *"The existence of mechanisms leading to a dose response that is non-linear or has a practical threshold is increasingly recognized, not only for compounds that interact with non-DNA (Deoxyribose Nucleic Acid) targets but also for DNA-reactive compounds, whose effects may be modulated by, for example, rapid detoxification before coming into contact with DNA, or by effective repair of induced damage. The regulatory approach to such compounds can be based on the identification of a No-Observed Effect Level (NOEL) and use of uncertainty factors (see ICH Q3C(R5), Ref. 7) to calculate a 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.

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

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

Methods

The general process for deriving acceptable intakes included a literature review, selection of cancer potency estimate [TD_{50}, taken from the Carcinogenicity Potency Database (CPDB - http://toxnet.nlm.nih.gov/cpdb/), or calculated from published studies using the same method as in the CPDB] and ultimately calculation of an appropriate AI or PDE in cases with sufficient evidence for a threshold mode of action (see Section 3). The literature review focused on data relating to exposure of the general population (i.e., food, water, and air), mutagenicity/genotoxicity, and carcinogenicity. Any national or international regulatory values (e.g., US EPA, US FDA, EMA, ECHA, WHO, etc.) are described in the compound-specific assessments. Toxicity information from acute, repeat-dose, reproductive, neurological, and developmental studies was not reviewed in depth except to evaluate observed changes that act as a carcinogenic precursor event (e.g., irritation/inflammation, or methemoglobinemia).

1. Standard Method

1.1 Linear Mode of Action and Calculation of AI

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

Thus, linear extrapolation from a TD_{50} value was considered appropriate to derive an AI for those Class 1 impurities (known mutagenic carcinogens) with no established "threshold mechanism", that is, understanding of a mode of action that results in a non-linear dose-response curve. In many cases, the carcinogenicity data were available from the CPDB; the conclusions were based either on the opinion of the original authors of the report on the carcinogenicity study ("author opinion" in CPDB) or on the conclusions of statistical analyses provided in the CPDB. When a pre-calculated TD_{50} value was identified in the CPDB for a selected chemical, this value was used to calculate the AI; the relevant carcinogenicity data were not reanalyzed and the TD_{50} value was not recalculated.

If robust data were available in the literature but not in the CPDB, then a TD_{50} was calculated based on methods described in the CPDB (http://toxnet.nlm.nih.gov/cpdb/td50.html). The assumptions for animal body weight, respiratory volume, and water consumption for calculation of doses were adopted from ICH Q3C and ICH Q3D.

1.2 Selection of Studies

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

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

The more robust studies were generally used to derive limits. However studies that did not fulfill all of the above criteria were in some cases considered adequate for derivation of an AI when other aspects of the study were robust, for example when treatment was for 3 days per week (e.g., Benzyl Chloride [BC]) but there was evidence that higher doses would not have been tolerated, i.e., a Maximum Tolerated Dose (MTD) as defined by NTP or ICH S1C was attained. Calculations of potency take intermittent or less-than-lifetime dosing into account; for example, in the CPDB the dose levels shown have been adjusted to reflect the estimated daily dose levels, such that the daily dose given 3 times per week is multiplied by 3/7 to give an average daily dose; a comparable adjustment is made if animals are treated for less than 24 months. Use of less robust data can sometimes be considered acceptable when no more complete data exist, given the highly conservative nature of the risk assessment in which TD_{50} was linearly extrapolated to a 1 in 100,000 excess cancer risk. In these cases, the rationale supporting the basis for the recommended approach is provided in the compound-specific assessments.

1.3 Selection of Tumor and Site

The lowest TD_{50} of a particular organ site for an animal species and sex was selected from the most robust studies. When more than one study exists, the CPDB provides a calculated harmonic mean TD_{50}, but in this Addendum the lowest TD_{50} was considered a more conservative estimate. Data compiled as "all Tumor Bearing Animals" (TBA) were not considered in selecting an appropriate TD_{50} from the CPDB; mixed tumor types (e.g., adenomas and carcinomas) in one tissue (e.g., liver) were used where appropriate as this often gives a more sensitive potency estimate.

1.4 Route of Administration

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

In this Addendum, when robust data were available from carcinogenicity studies for more than one route, and the tumor sites did not appear to be route- specific, the TD_{50} from the route with the lower TD_{50} was selected for the AI calculation and is thus usually considered suitable for all routes. Exceptions may be necessary case by case; for example, in the case of a potent site-of-contact carcinogen a route-specific AI or PDE might be necessary. Other toxicities such as irritation might also limit the acceptable intake for a certain route, but only

tumorigenicity is considered in this Addendum. Here, if tumors were considered site-specific (e.g., inhalation exposure resulting in respiratory tract tumors with no tumors at distal sites) and the TD_{50} was lower than for other routes, then a separate AI was developed for that route (e.g., dimethyl carbamoyl chloride, hydrazine).

1.5 Calculation of AI from the TD_{50}

Calculating the AI from the TD_{50} is as follows (see Note 4 of ICH M7 for example):

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

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

2. Consideration of Alternative Methods for Calculation of AI

2.1 Human relevance of tumors

Note 4 of ICH M7 states: *"As an alternative of using the most conservative TD_{50} value from rodent carcinogenicity studies irrespective of its relevance to humans, an in-depth toxicological expert assessment of the available carcinogenicity data can be done in order to initially identify the findings (species, organ, etc.) with highest relevance to human risk assessment as a basis for deriving a reference point for linear extrapolation."*

Human relevance of the available carcinogenicity data was considered for deriving AIs. Effects in rodents associated with toxicities that occur with a non-linear dose response are not relevant to humans at the low, non-toxic concentrations associated with a pharmaceutical impurity. For example, in the case of *p*-Chloroaniline, the most sensitive site for tumor induction was the spleen, but these tumors were associated with hemosiderosis, considered to be a mode of action with a non-linear dose response, and thus not relevant to humans at low doses. In the case of *p*-Chloroaniline, liver tumors, with a higher TD_{50}, were used for the linear extrapolation to calculate the AI.
A second category of tumors considered not to be relevant to humans is tumors associated with a rodent-specific mode of action e.g., methyl chloride.

2.2 Published regulatory limits

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

In this Addendum, available regulatory limits are described (omitting occupational health limits as they are typically regional and may use different risk levels). However the conservative linear extrapolation from the TD_{50} was generally used as the primary method to

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

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

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

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

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

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

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

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

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

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

References

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

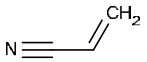
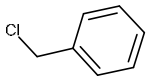
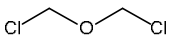
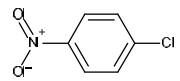
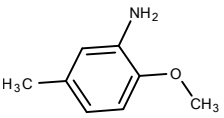
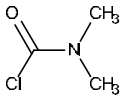
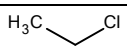
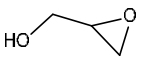
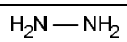
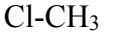
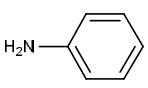
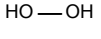
Health Canada. 2001 Priority substances list assessment report: Formaldehyde. Ottawa. Ministry of Public Works and Government Services. February. [Online]. Searched October 10, 2013; Available from: URL: http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/index_e.html

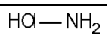
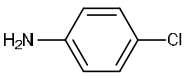
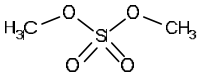
World Health Organization (WHO). International Programme on Chemical Safety (IPCS). 2002. Concise International Chemical Assessment Document 40. Formaldehyde.

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

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

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

Compound	CAS#	Chemical Structure	AI or PDE (µg/d)	Comment
Linear extrapolation from TD_{50}				
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		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 (hemosiderosis)
Hydrogen peroxide	7722-84-1		6,960	PDE based on threshold (oxidant stress where protective antioxidant

Compound	CAS#	Chemical Structure	AI or PDE (µg/d)	Comment
				mechanisms overwhelmed)
Hydroxylamine	7803-49-8		2	PDE based on threshold mode of action (hemosiderosis)
Endogenous and food exposure**				
Other Cases				
p-Chloroaniline *p*-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.

[*]Route specific limit

[**] for future compounds

Acrylonitrile (CAS# 107-13-1)

Potential for human exposure

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

Mutagenicity/Genotoxicity

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

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

Acrylonitrile is mutagenic in:

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

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

Carcinogenicity

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

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

The NCI/NTP (National Cancer Institute) study in the CPDB of acrylonitrile in mice was selected for derivation of the oral and inhalation AI, based on robust study design and the most conservative TD_{50} value. In this 2 year-study, 3 doses of acrylonitrile were administered byoral gavage to male and female mice. There were statistically significant increases in tumors of the Harderian gland and forestomach.

In the CPDB, it appears that the most sensitive TD_{50}, slightly lower than that for forestomach tumors in mice, is for astrocytomas in female rats (5.31 mg/kg/day) in the study of Quast *et al.* 1980a, cited in the CPDB as a report from Dow Chemical. There were 46-48 animals per treatment group and 80 animals in controls. This study was later described in detail in a

publication by Quast (2002) and the calculated doses in that published report are higher than those in the CPDB. Quast (2002) describes the derivation of doses in mg/kg/day from the drinking water concentrations of 35, 100 and 300 ppm, adjusting for body weight and the decreased water consumption in the study. The TD_{50} for astrocytomas derived from these numbers is 20.2 mg/kg/day for males and 20.8 for females, in contrast to the calculated values in the CPDB of 6.36 and 5.31 mg/kg/day.

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

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

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;14.3 mg/kg/d	Fore-Stomach	6.77^{++}
	50 B6C3F1 Mice (M)	2 year/ Gavage	50	**3:** 1.79;7.14;14.3 mg/kg/d	Fore-Stomach	5.92^{++}
Quast, *et al*. 1980a In CPDB	~50 SD Spartan rats (F)	2 year/ Water	~80	**3:** 2.00;5.69;15.4 mg/kg/d	CNS	5.31^{++}
	~50 SD Spartan rats (M)	2 year/ Water	~80	**3:** 1.75;4.98;14.9 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

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, *et al*. 1986[¥]	50/sex rats	18 months/ Water	No	**2:** 14;70 mg/kg/d	Brain, Zymbal's gland, forestomach	NC[^]
Gallagher, *et al*. 1988	20 CD rats (M)	2 year/ Water	No	**3:** 1; 5; 25 mg/kg/d	Zymbal's gland	30.1
Maltoni *et al*., 1988	40/sex SD rats	1 year/ 3d/week Gavage	75/sex	**1:** 1.07 mg/kg/d	Neg in both sexes	NA
Quast, *et al*. 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 *et al*. 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 *et al*. 1988	54 female SD rats	2 yr 5d/wk inhalation	60	**1:** 11.1 mg/kg/d	Brain glioma	$(132)^{\Psi}$

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

[*]Carcinogenicity study selected for AI calculation; in CPDB

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

[+]TD_{50} calculated based on astrocytoma incidence implied as most significant site in WHO, 2002. Serial sampling reduced number of animals exposed for 2 years, so tumor incidences may be underestimates.
[++]Taken from the CPDB. The TD_{50} values represent the TD_{50} from the most sensitive tumor site.
TD_{50} values in parentheses are considered less reliable as explained in footnotes.
NA= Not applicable.
[¥] Not in CPDB. Summarized by WHO, 2002 and National Library of Medicine IRIS database.
[ψ] Single dose-level study.

Mode of action for carcinogenicity

Although the mechanism of carcinogenesis remains inconclusive, a contribution of DNA interaction cannot be ruled out (WHO, 2002). Carcinogenicity Studies (CNS) tumors were seen in multiple studies in rats, and forestomach tumors were also prominent; this was the most sensitive tumor type in mice.
Forestomach tumors are associated with local irritation and inflammation, and Quast (2002) notes the typical association between these tumors in rats and hyperplasia and/or dyskeratosis, with other inflammatory and degenerative changes. Forestomach tumors in rodents administered high concentrations orally, a type of site-of-contact effect, may not be relevant to human exposure to low concentrations that are non-irritating (for discussion see, for example, Proctor *et al*. 2007). However, acrylonitrile is not only a site-of contact carcinogen. Tumors were seen in the CNS, in addition to tissues likely to be exposed directly (such as the gastrointestinal tract, tongue and Zymbal gland). Forestomach tumors were seen after administration of acrylonitrile to rats in drinking water, and by gavage. Thus, the AI was derived here based on mouse forestomach tumors.

Regulatory and/or Published Limits

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

Acceptable Intake (AI)

Rationale for selection of study for AI calculation

Both inhalation and oral studies (gavage and drinking water) are available. Tumors of the CNS were seen by both route of administration, and acrylonitrile is rapidly absorbed *via* all routes of exposure and distributed throughout examined tissues (WHO, 2002), so that a specific inhalation AI was not considered necessary. All of the carcinogenicity studies that were used by the US EPA in the derivation of the drinking water limit for acrylonitrile were reviewed when selecting the most robust carcinogenicity study for the derivation of an AI. Here, the NCI/NTP study was selected to calculate the AI based on the TD_{50} derived from administering acrylonitrile by oral gavage to male and female mice. The tumor type with the lowest TD_{50} was forestomach tumors in male mice, with a TD_{50} value of 5.92 mg/kg/day. As discussed in the Methods Section 2.2, linear extrapolation from the TD_{50} was used here to derive the AI, and it is expected that minor differences in methodology can result in different

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

Calculation of AI:

Lifetime AI = TD_{50}/50,000 x 50kg

Lifetime AI = 5.92 (mg/kg/day)/50,000 x 50 kg

Lifetime AI = 5.9 µg/day (6 µg/day)

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

Potential for human exposure

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

Mutagenicity/genotoxicity.

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

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

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

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

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

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

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

Carcinogenicity

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

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

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

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

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

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;72 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)	Hemangiosarcoma 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
Hagiwara *et al.* 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

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

++ Taken from CPDB. The TD_{50} values represent the TD_{50} from the most sensitive tumor site.

Mode of action for carcinogenicity

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

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

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

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

Regulatory and/or Published Limits

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

Permissible Daily Exposure (PDE)

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

<u>Rationale for selection of study for PDE calculations.</u>

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

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

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

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

Lifetime PDE = 7.2 x 50 kg / (5 x 10 x 1 x 10 x 1)

Lifetime PDE = 720 µg/day

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719

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

Potential for human exposure

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

Mutagenicity/Genotoxicity

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

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

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

Carcinogenicity

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

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

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

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 *et al.* 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 *et al*. 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 *et al.* 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 *et al*. 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 *et al*. 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 ^

Studies listed are in CPDB [Cancer Potency Database http://toxnet.nlm.nih.gov/cpdb/].
* Carcinogenicity study selected for AI calculation.
^NC= Not calculated; small group size, limited duration. Not included in CPDB as route with greater likelihood of systemic exposure is considered more relevant.
++ Taken from CPDB. The TD_{50} values represent the TD_{50} from the most sensitive tumor site.

Mode of action for carcinogenicity

The tumor types with the lowest calculated TD_{50} (highest potency) in the CPDB for benzyl chloride are forestomach tumors in mice and thyroid C-cell tumors in female rats. The relevance of the forestomach tumors to human risk assessment for low, non-irritating doses such as those associated with a potential impurity is highly questionable.

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

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

vivo), whether tumors after oral administration of any type are specific to forestomach, and whether tumors are observed only at doses that irritate the forestomach or exceed the MTD.

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

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

Statistically significant increases were reported in hemangiomas/hemangiosarcomas of the circulatory system in the male mice (TD_{50} 454 mg/kg/day), and in thyroid C-cell adenomas or carcinomas in the female rats (TD_{50} 40.6 mg/kg/day). The levels of thyroid C-cell tumors in female rats in the high dose group, while higher than female concurrent controls, (14/52 versus 4/52 in controls) were similar to the levels in the male concurrent controls (12/52). In males, thyroid C- cell tumor levels were lower in treated than in control rats. In a compilation of historical control data from Fisher 344 rats in the NTP studies, Haseman *et al.* (1984; 1998) show comparable levels of C-cell adenomas plus carcinomas in males and females in this rat strain, although the range is wider in males. Thus it is likely justifiable to compare the thyroid tumor levels in female rats treated with benzyl chloride with the concurrent controls of both sexes, and question whether the female thyroid tumors are treatment-related, although they were higher than the historical control range cited at the time (10%).

Regulatory and/or Published Limits

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

Acceptable Intake (AI)

Rationale for selection of study for AI calculation

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

As described in the Methods Section 2.2., linear extrapolation from the TD_{50} was used to derive the AI. As described above, it is highly unlikely that benzyl chloride poses a risk of site-of-contact tumors in humans exposed to low concentrations as impurities in pharmaceuticals, well below concentrations that could cause irritation/inflammation. Therefore, the observed forestomach tumors in male mice are not considered relevant for the AI calculation. The significance of the thyroid C-cell tumors in female rats is also questionable since these tumors occur commonly in control rats. However, given the uncertain origin of these tumors, the thyroid C-cell tumors were used to derive the AI since they were associated with the lowest TD_{50}; 40.6 mg/kg/day.

Calculation of AI

Lifetime AI = TD_{50}/50,000 x 50 kg

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

Lifetime AI = 40.6 µg/day (41 µg/day)

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

Potential for human exposure

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

Mutagenicity/genotoxicity

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

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

Carcinogenicity

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

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

The study of Leong *et al*. (1981) was selected for derivation of the AI based on the most robust study design and the lowest TD_{50} value. Groups of male Sprague-Dawley rats and Ha/ICR mice were exposed by inhalation to 1, 10 and 100 ppb of BCME 6 hr/day, 5 days/week for 6 months and subsequently observed for the duration of their natural lifespan (about 2 years). Evaluation of groups of rats sacrificed at the end of the 6-month exposure period revealed no abnormalities in hematology, exfoliative cytology of lung washes, or cytogenetic parameters of bone marrow cells. However, 86.5% of the surviving rats which had been exposed to 100 ppb (7780 ng/kg/day, or 8 µg/kg/day) of BCME subsequently developed nasal tumors (esthesioneuroepitheliomas, which are similar to the rare human neuroblastoma) and approximately 4% of the rats developed pulmonary adenomas. Tumors were not observed in rats exposed to 10 or 1 ppb of BCME. Mice exposed to 100 ppb of BCME did not develop nasal tumors, but showed a significant increase in incidence of pulmonary adenomas over the control mice. Mice exposed to 10 or 1 ppb of BCME did not show a significant increase in incidence of pulmonary adenomas.

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

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

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 *et al*. 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 *et al*. 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 *et al.* 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 *et al*. 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 *et al*. 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

Studies listed are in CPDB unless otherwise noted [Cancer Potency Database http://toxnet.nlm.nih.gov/cpdb/].
*Carcinogenicity study selected for AI calculation
^NC= Not calculated due to non-standard carcinogenicity design. Not in CPDB.
NA= Not available since controls were not reported in the study

Mode of action for carcinogenicity

Not defined.

Regulatory and/or Published Limits

The US EPA IRIS database (EPA 1988), calculated an oral cancer slope factor of 220 per mg/kg/day based on linearised multistage modelling of the inhalation study data by Kuschner *et al*. 1975. The inhaled (and oral) dose associated with a 1 in 100,000 lifetime cancer risk is 3.2 ng/day (1.6×10^{-8} mg/m^3 for inhalation, 1.6×10^{-6} mg/L for oral exposure).

Acceptable Intake (AI)

Rationale for selection of study for AI calculation

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

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

Calculation of AI

Lifetime AI = TD_{50}/50,000 x 50 kg

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

Lifetime AI = 0.004 µg/day or 4 ng/day

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

Potential for human exposure

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

Mutagenicity/Genotoxicity

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

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

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

Carcinogenicity

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

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

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

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

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

***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* *p*-chloraniline 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 *p*-chloraniline 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 *p*-chloraniline 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 *p*-chloraniline 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

Studies listed are in CPDB [Cancer Potency Database http://toxnet.nlm.nih.gov/cpdb/].
*Carcinogenicity study selected for AI calculation.
NA = Not applicable

Mode of action for carcinogenicity

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

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

For male mouse liver tumors, the TD_{50} based on the combined numbers of adenomas and carcinomas was 33.8 mg/kg/day. *p*-Chloroaniline is is not reproducibly mutagenic. There is one positive study *in vivo* (micronucleus test), but this was positive only at a dose level in the range of the LD_{50} and given the known methemoglobinema, this might be secondary to regenerative anemia/altered erythropoeisis, as with aniline (Ashby *et al.* 1991; Tweats *et al.* 2007).

A Permissible Daily Exposure (PDE) for *p*-Chloroaniline was calculated as follows:
(NOEL x body weight adjustment (kg) / F1 x F2 x F3 x F4 x F5

The following safety factors as outlined in ICH Q3C have been applied:

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

In the rat study of *p*-Chloroaniline HCl (NTP, 1989) the lowest dose was clearly a No Observed Effect Level (NOEL): (2 mg/kg 5 days per week, or 1.43 mg/kg/day).

On this basis the PDE is calculated as follows:
Lifetime PDE = 1.43 x 50 kg / (5 x 10 x 1 x 10 x 1)
Lifetime PDE = 143 µg/day

Conclusion
Overall, there is very limited evidence for a mutagenic mode of action, but *in vivo* information is lacking. Thus, a mutagenic mode of action cannot be entirely ruled out and calculation of an AI was considered appropriate. Other single-ring aromatic amines have been associated with tumors in liver, urinary bladder and kidney (CPDB). Because a mutagenic component to the mode of action for liver tumors cannot be ruled out, the linear extrapolation AI is recommended.

Regulatory and/or Published Limits

No regulatory limits have been published for *p*-Chloroaniline or the hydrochloride salt.

Calculation of AI

Calculation of AI

Based on male mouse liver tumors for *p*-Chloroaniline HCl

Lifetime AI = TD_{50}/50,000 x 50kg

Lifetime AI = 33.8mg/kg/day /50,000 x 50 kg

Lifetime AI = 34 µg/day

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

Potential for human exposure

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

Mutagenicity/genotoxicity

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

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

Carcinogenicity

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

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

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

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

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

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

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

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 *et al.* 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 pheochromocytoma 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 *et al.* 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

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

*Carcinogenicity study selected for AI/PDE calculation.
+Not in CPDB.
^ Histopathology limited to 11-12 tissues.

Mode of action for carcinogenicity

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

The tumor type with the lowest TD_{50} was adrenal mudullary pheochromocytomas in female rats (Matsumoto *et al*. 2006). This tumor type is common as a background tumor in F344 rats, especially males, and is seen after treatment with a number of chemicals, many of them non-mutagenic (Greim *et al*. 2009). It has been proposed that they are associated with various

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

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

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

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

Thus a PDE calculation was performed.

Calculation of Permissible Daily Exposure (PDE)

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

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

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

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

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

Lifetime PDE = 150 µg/day

Conclusion

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

Regulatory and/or Published Limits

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

Calculation of AI

Calculation of AI

The most sensitive TD_{50} is that for adrenal medullary pheochromocytomas in female rats (Matsumoto *et al*. 2006).

Lifetime AI = TD_{50}/50,000 x 50kg

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

Lifetime AI = 117 µg/day

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

Potential for human exposure

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

Mutagenicity/Genotoxicity

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

p-Cresidine is mutagenic in:

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

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

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

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

Carcinogenicity

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

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

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

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

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

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

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

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

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

Mode of action for carcinogenicity:

Not defined.

Regulatory and/or Published Limits

No regulatory limits have been published

Acceptable intake (AI)

Rationale for selection of study for AI calculation**:**

The only adequate carcinogenicity studies of p-cresidine were those reported in the CPDB and conducted by NTP/NCI. The study in mice was selected for derivation of the AI since the most sensitive TD_{50} was based on urinary bladder tumors in male mice.

Calculation of AI:

The most sensitive TD_{50} values from the NTP/NCI studies are for the urinary bladder in both sexes of rats and mice; in rats the TD_{50} was 110 mg/kg/day for females and 88.4 mg/kg/day for males; in mice the TD_{50} was 69 mg/kg/day for females and 44.7 mg/kg/day for males. The most conservative value is that identified for male mice.

The lifetime AI is calculated as follows:

Lifetime AI = TD_{50}/50,000 x 50 kg

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

Lifetime AI = 45 µg/day

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Dimethylcarbamyl chloride (CAS# 79-44-7)

Potential for human exposure

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

Mutagenicity/Genotoxicity

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

DMCC was mutagenic in:

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

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

Carcinogenicity

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

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

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

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

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

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

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 *et al*. 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 *et al*. 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 *et al*. 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 *et al*. 1974	50 ICR/Ha Swiss mice (F)	Subcutaneous 61 wk once/wk	50	**1:** 5 mg	Injection site: Fibrosarcomas; Squamous cell carcinomas/Female	NA^
Snyder *et al*. 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 *et al*. 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 *et al*. 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 *et al*. 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^^

Studies listed are in CPDB unless otherwise noted. [Cancer Potency Database http://toxnet.nlm.nih.gov/cpdb/].
*Carcinogenicity study selected for non-inhalation AI. In CPDB.
**Carcinogenicity study selected for inhalation AI. In CPDB.
NA= Not applicable
^Did not examine all tissues histologically. Subcutaneous and skin painting studies are not included in CPDB as route with greater likelihood of whole body exposure is considered more valuable.
^^Subcutaneous and skin painting studies are not included in CPDB as route with greater likelihood of whole body exposure is considered more valuable.
^^^Histopathology only on tissues that appeared abnormal at autopsy.
^^^^Examined only for nasal cancer. Does not meet criteria for inclusion in CPDB of exposure for at least one fourth of the standard lifetime

Mode of Action of Carcinogenicity

Not defined.

Regulatory and/or Published Limits

No regulatory limits have been published

Acceptable Intake

Based on the above data, DMCC is considered to be a mutagenic carcinogen. As a result, linear extrapolation from the most sensitive TD_{50} in carcinogenicity studies is an appropriate method with which to derive an acceptable risk dose. Since DMCC appears to be a site-of-contact carcinogen, it was appropriate to derive a separate acceptable intake for inhalation exposure compared with other routes of exposure.

No information from oral administration is available, so that for routes of exposure other than inhalation, the study by Van Duuren *et al.* (1974), with administration by intraperitoneal injection, was used. The TD_{50} was 4.59 mg/kg/day based on mixed tumor incidences (CPDB).

Lifetime AI = TD_{50}/50,000 x 50 kg

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

Lifetime AI = 5 µg/day

Inhalation AI

After inhalation of DMCC, nasal cancer in hamsters is the most sensitive endpoint and the TD_{50} was 0.625 mg/kg/day.

Lifetime AI = TD_{50}/50,000 x 50 kg

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

Lifetime AI = 0.6 µg/day

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Dimethyl Sulfate (CAS# 77-78-1)

Potential for human exposure

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

Mutagenicity/Genotoxicity

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

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

- The microbial reverse mutation assay (Ames), *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with and without activation (Skopek *et al*. 1978).
- DMS is a potent alkylating agent for cellular macromolecules and forms a variety of alkylated bases with DNA *in vitro* and the same alkylated bases are formed *in vivo* (IARC, 1999).

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

Carcinogenicity

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

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

1810 **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 *et al*. (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 *et al*. (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 *et al*. (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 *et al*. (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 *et al*. (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 *et al*. (1983)	90 CBAX57 Bl/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**

Studies listed are in not in CPDB.
NA = Not applicable
^ Control data not reported. Tumor incidences not tabulated by species or dose
^^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.
^^^ Small group size, no concurrent control group.
^^^^ No concurrent control group.
* Duration not reported
** Limited number of animals. Only one dose tested. Even when DMS was combined with tumor promoters no tumors were noted.
¥ Sex not specified

Mode of Action of Carcinogenicity:

Not defined.

Regulatory and/or Published Limits

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

Acceptable Intake (AI)

While DMS is considered to be a likely oral carcinogen and probable human carcinogen, there are no oral carcinogenicity studies from which to derive a TD_{50} value. Moreover, the inhalation studies that are available are limited for a variety of reasons and are not suitable for TD_{50} extrapolation. Given this, it is reasonable to limit DMS to the threshold of toxicological concern level (TTC) of 1.5 µg/day.

Lifetime AI = 1.5 µg/day

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

Potential for human exposure

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

Mutagenicity/Genotoxicity

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

Ethyl chloride was mutagenic in:

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

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

Carcinogenicity

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

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

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

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

Mode of Action of Carcinogenicity

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

Regulatory and/or Published Limits

The US EPA established an inhalation Reference Concentration (RfC) for non-carcinogenic effects of 10 mg/m^3, or 288 mg/day assuming a respiratory volume of 28,800 L/day (USEPA, 1991).

Acceptable Intake (AI)

Rationale for selection of study for AI calculation

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

Ethyl chloride was considered to be a mutagenic carcinogen. Based on the NTP inhalation study the most sensitive species/site is female mouse uterus. The CPDB converted 0 and 15,000 ppm to doses of 0 and 12.4 g/kg and calculated a TD_{50} = 1810 mg/kg/day for mouse uterus.

Lifetime AI = TD_{50}/50,000 x 50 kg

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

Lifetime AI = 1,810 µg/day

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Glycidol (CAS# 556-52-5)

Potential for human exposure

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

Mutagenicity/Genotoxicity

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

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

Glycidol is mutagenic in:

- Microbial reverse mutation assay (Ames), *Salmonella* strains TA100, TA1535, TA98, TA97 and TA1537 both with and without rat liver S9 activation and in standard plate and preincubation assays.
- *Escherichia coli* strain WP2uvrA/pKM101 in a preincubation assay with and without rat liver S9.
- Mouse lymphoma l5178Y cell *tk* assay without metabolic activation.

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

Carcinogenicity

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

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

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

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

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

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/ groupSyrian 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 *et al*. 1967 (**Cited in IARC, 2000)	20 ICR/Ha Swiss mice	Skin Painting 3 times/wk for 520 d	Yes	**1:** 5%	No Tumors	NA^

Studies listed are in CPDB unless otherwise noted. [Cancer Potency Database http://toxnet.nlm.nih.gov/cpdb/].
*Carcinogenicity study selected for AI calculation.
**Not in CPDB.
NA= Not applicable.

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

Mode of Action

Not defined.

Regulatory and/or Published Limits

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

Acceptable Intake (AI)

Rationale for selection of study for AI calculation**:**

The most suitable carcinogenicity data for human cancer potency assessment come from the two-year oral studies conducted in F344/N rats and B6C3F1 mice by NTP (1990). The most sensitive organ site was female mammary glands with a TD_{50} of 4.15 mg/kg/day.

Calculation of AI:

Lifetime AI = TD_{50}/50,000 x 50 kg

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

Lifetime AI = 4 µg/day

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

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Hydrazine (CAS# 302-01-2)

Potential for human exposure

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

Mutagenicity/Genotoxicity

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

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

Hydrazine was mutagenic in:

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

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

Carcinogenicity

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

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

The main target organs for oral carcinogenicity of hydrazine in rodents are the liver and lungs. The most robust oral study based on group size and dose levels was that of Stienhoff and Mohr (1988). The most robust inhalation study with the lowest TD_{50} was that of Vernot *et al*. (1985). The most sensitive targets for inhalation carcinogenicity of hydrazine in rodents are sites of initial contact such as the nasal cavity and lungs.

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

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

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 *et al*. 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 adenamatous polyps/Male	0.194
Steinhoff *et al*. 1990	50/sex/ group Bor:NMRI, 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 *et al*. 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 *et al*. 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 concurrent	**1:** ~1.7-2 mg/kg/d	Lung/Male	2.20¥
Roe *et al.* 1967	25 Swiss mice (F)	Gavage 5X/wk, 40 wk	85 Untreated	**1:** ~5 mg/kg/d	Lung/Female	5.67¥¥

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

[*]Carcinogenicity study selected for non-inhalation AI calculation.
[**]Carcinogenicity study selected for inhalation AI calculation.
NA= Not applicable.
[¥] Excluded by U.S. EPA (no concurrent controls). Liver negative.
[¥¥] Animal survival affected; Liver negative.
Vernot *et al.* 1985 = MacEwen *et al*. 1981 & summarized in U.S. EPA IRIS database, last revision 04/01/1991.
Used by U.S. EPA (1986) for derivation of inhalation unit risk.

Mode of Action of Carcinogenicity

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

Regulatory and/or Published Limits

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

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

Acceptable Intake (AI)

Rationale for selection of study for AI calculation

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

For oral hydrazine, carcinogenicity has been reported in 3 mouse studies and one rat study. Only one mouse study (Steinhoff *et al*. 1990) and the rat study (Steinhoff and Mohr, 1988)

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

All of the inhalation carcinogenicity studies that were used by the U.S.EPA in the derivation of the inhalation carcinogenicity limit for hydrazine were taken into consideration when selecting the most robust carcinogenicity study for the derivation of an AI for inhaled pharmaceuticals. The critical study used by U.S. EPA was proprietary (i.e., MacEwen *et al*. 1981), but is likely the same data as in Vernot *et al*. 1985. Given that the TTC was derived *via* linear extrapolation from TD_{50} values for hundreds of carcinogens, that same approach was used in the derivation of a compound specific AI for hydrazine. The methodology used by the U.S. EPA and the method used here are both highly conservative in nature. However, given that the methodologies do differ, it is reasonable to expect some slight differences. The AI was calculated based on the TD_{50} derived from a study in which male and female rats were administered hydrazine *via* inhalation for one year with an 18-month observation period (Vernot *et al*. 1985). While a 1-year study is not a standard design for carcinogenicity, a positive response was observed demonstrating that the window for carcinogenicity was not missed. The most sensitive target tissue was the male nasal region, with a TD_{50} value of 0.194 mg/kg/day, which was lowered as standard practice to account for 2-year lifetime exposure.

Calculation of AI

- AI

Lifetime AI = TD_{50}/50,000 x 50 kg

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

Lifetime AI = 42 µg/day

- Inhalation AI

Lifetime AI = TD_{50}/50,000 x 50 kg

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

Lifetime AI = 0.2 µg/day

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Hydrogen peroxide (CAS# 7722-84-1)

Potential for Human Exposure

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).

Mutagenicity/Genotoxicity

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

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

Hydrogen peroxide is mutagenic in:

- *Salmonella typhimurium* strains TA96, TA97, SB1106p, SB1106, and SB1111 and *Escherichia coli* WP2 in the absence of exogenous metabolic activation;
- L5178Y mouse lymphoma cell sublines at the *hprt* locus (weak increase);
- Chinese hamster V79 cells at the *hprt* locus, in only one of six studies.

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.

Carcinogenicity

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

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 Aadverse Effect Level) LOAEL, equivalent to an average daily dose-rate per kg body weight per day of 200 mg/kg/day (CPDB).

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.

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

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

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

Hydrogen Peroxide – Details of carcinogenicity studies

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Notes
Ito *et al*. 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 *et al*. 1982**	29 mice (No. of M and F not reported)	700 d Drinking water	No	**1:** 0.4%	Cessation of H_2O_2 treatment decreased percent of mice with stomach erosions and percent of mice with duodenal lesions (plaques and nodules)
Ito *et al*. 1984**	18 mice (No. of M and F not reported)	6 mo Drinking water	No	**1:** 0.4%	2 duodenal tumours (11.1%)
Ito *et al*. 1984**	22 mice (No. of M and F not reported)	6 mo Drinking water	No	**1:** 0.4%	7 duodenal tumours (31.8%)
Ito *et al*. 1984**	21 mice (No. of M and F not reported)	7 mo Drinking water	No	**1:** 0.4%	21 duodenal tumours (100%)
Ito *et al.* 1984**	24 mice (No. of M and F not reported)	6 mo Drinking water	No	0.4% only	22 duodenal tumours (91.7%)
Ito *et al*. 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 *et al*. 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 *et al*. 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

*Carcinogenicity study selected for PDE calculation

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

Mode of action for carcinogenicity

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

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

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

Regulatory and/or Published Limits

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

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

Permissible Daily Exposure (PDE)

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

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

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

F1 = 12 (mouse to man)
F2 = 10 (inter-individual variability)
F3 = 1 (study duration at least half lifetime)
F4 = 1 (endogenous product, so severe toxicity not expected at low doses)
F5 = 10 (using a LOAEL)

On this basis the PDE is calculated as follows:

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

Lifetime PDE = 6,960 µg/day

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Hydroxylamine (CAS# 7803-49-8)

Potential for human exposure

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

Mutagenicity/Genotoxicity

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

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

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

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

Carcinogenicity

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

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

Mode of action for carcinogenicity

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

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

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 (hydroxylammo	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**¥

nium)sulphate, CAS [10039-54-0]				F: 0.4, 1.6, 6.2 mg/kg/d		
Yamamoto *et al*. 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^^
Stenbäck *et al*. 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 ^^^

Note: Studies in the table are not in the CPDB.
*Carcinogenicity study selected for AI calculation.
**TD_{50} calculated based on carcinogenicity data.
¥Small increase in number of tumours, not dose-related & not statistically significant. However, levels above control groups and historical control background data.
^ Study details given in ECHA 2008.
NA= Not applicable.
^^Limited number of animals and duration.
^^^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.

Regulatory and/or Published Limits

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

Permissible Daily Exposure (PDE)

Rationale for selection of study for PDE calculation:

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

Calculation of PDE:

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

The following safety factors as outlined in ICH Q3C Guideline Appendix 3 have been applied to determine the PDE for hydroxylamine, these are:
F1 = 5 (rat to man)
F2 = 10 (inter-individual variability)

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

On this basis the PDE is calculated as follows:

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

Lifetime PDE = 2 µg/day

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

Potential for human exposure

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

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

Mutagenicity/Genotoxicity

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

Methyl chloride is mutagenic in:

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

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

Carcinogenicity

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

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

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

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

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

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

Note: Studies not listed in CPDB.
*Carcinogenicity study selected for AI calculation.
**TD_{50} calculated based on carcinogenicity data.
^ Not statistically significant at 225 ppm but considered induced by methyl chloride because similar to those seen at 1000 ppm where a clear significant increase was noted.
NA = Not applicable

Regulatory and/or published Limits

WHO developed a guideline value for the general population of 0.018 mg/m^3 and U.S. EPA developed a reference concentration of 0.09 mg/m^3. Both were based on the potential for adverse Central Nervous System (CNS) effects following inhaled methyl chloride.

Acceptable Intake (AI)

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

Lifetime AI = TD50/50,000 x 50 kg

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

Lifetime AI = 1,360 µg/day

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