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

ICH HARMONISED GUIDELINE

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

Addendum to M7(R2)

Final version

Adopted on 3 April 2023

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of ICH regions.

Addendum to M7(R2)
Document History

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M7(R2) Addendum	Endorsement by the Members of the ICH Assembly under *Step 2* and release for public consultation, following the separation of the M7(R2) Addendum from the M7(R2) draft Guideline.	6 October 2021
M7(R2) Addendum	Adoption by the Regulatory Members of the ICH Assembly under *Step 4*.	3 April 2023

Application of the Principles of the ICH M7 Guideline to Calculation of Compound-Specific Acceptable Intakes

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AI	Acceptable Intakes
ACGIH	American Conference of Governmental Industrial Hygienists
API	Active Pharmaceutical Ingredient
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
DP	Drug Product
EC	European Commission
ECHA	European Chemical Agency
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
GRAS	Generally Recognized As Safe
HSDB	Hazardous Substance Database
IARC	International Agency for Research on Cancer
I.P.	Intraperitoneal
IPCS	International Programme 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; individual tumour 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
OSHA	Occupational Safety and Health Administration
PCE	Polychromatic Erythrocytes
PDE	Permissible Daily Exposure
RfC	Reference Concentration
ROS	Reactive Oxygen Species
S.C.	Subcutaneous
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCE	Sister Chromatid Exchanges
SIDS	Screening Information Dataset
TBA	Tumor Bearing Animal
TD_{50}	Chronic dose-rate in mg/kg body weight/day which would cause tumors in half of the animals at the end of a standard lifespan for the species taking into account the frequency of that tumor type in control animals
TDI	Tolerable Daily Intake
TTC-based	Threshold of Toxicological Concern-based
UDS	Unscheduled DNA Synthesis
UNEP	United Nations Environmental Programme
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

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, AIs or Permissible Daily Exposures (PDEs) have been derived for a set of chemicals that are considered to be mutagens and carcinogens and are common in pharmaceutical manufacturing or are useful to illustrate the principles for deriving compound-specific intakes described in ICH M7[1]. The set of chemicals include compounds in which the primary method used to derive AIs 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}. Some chemicals that are mutagens and carcinogens (classified as Class 1 in ICH M7) may induce tumors through a non-mutagenic mode of action. Therefore, additional compounds are included to highlight alternative principles to deriving compound-specific intakes (i.e. PDE, see below). Other compounds (e.g., aniline) are included even though the available data indicates that they are non-mutagenic; nevertheless, the historical perception has been that they are genotoxic carcinogens.

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)…) 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 included in this Addendum to illustrate assessments of mode of action for some Class 1 chemicals that justify derivation of a PDE calculated using uncertainty factors as described in ICH Q3C(R5) (Ref. 1). These chemicals include hydrogen peroxide, which induces oxidative stress, and aniline which induces tumors secondary to hemosiderosis as a consequence of methemoglobinemia.

It is emphasized that the AI or PDE values presented in this Addendum address carcinogenic risk. Other considerations, such as quality standards, may affect final product specifications. For example, the ICH M7 guidance (Section 7.2.2) notes that when calculating acceptable intakes from compound-specific risk assessments, an upper limit would be 0.5%, or, for example, 500 µg in a drug with a maximum daily dose of 100 mg.

Methods

The general approach used in this addendum for deriving AIs included a literature review, selection of cancer potency estimate [TD_{50}], taken from the CPDB (Carcinogenicity Potency Database (Ref. 2), 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. Based on the description of DNA-reactive mutagens in ICH M7, results from the standard bacterial reverse mutation assay (Ames test) were used as the main criterion for determining that a chemical was mutagenic. Other genotoxicity data, especially in vivo, were considered in assessing a likely mode of action for tumor induction. Any national or international regulatory values for acceptable exposure levels (e.g., US EPA, US FDA, EMA, ECHA, WHO) 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 (Ref. 3). The assumptions for animal body weight, respiratory volume, and water consumption for calculation of doses were adopted from ICH Q3C and ICH Q3D (Ref. 1, 4).

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 additional criteria were applied when studies were of lesser quality. Studies of lesser quality are 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) but there was evidence that higher doses would not have been tolerated, i.e., a Maximum Tolerated Dose (MTD) as defined by the National Toxicology Program (NTP) or ICH S1C(R2) (Ref. 5) was attained. Calculations of potency take intermittent or less-than-lifetime dosing such as that for benzyl chloride 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 lowest TD_{50} value 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 AI for a certain route, but only tumorigenicity is considered in this Addendum similar to M7. 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 that do not induce hemosiderosis. In the case of *p*-chloroaniline, liver tumors, with a higher TD_{50}, were used for the linear extrapolation to calculate the AI because a mutagenic mode of action could not be ruled out for liver tumors. 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, with species difference in metabolism.

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 Programme 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 proposed for mutagenicity *in vitro* and *in vivo* is ethyl methane sulfonate (Ref. 6, 7). A PDE calculation using uncertainty factors, instead of linear extrapolation is appropriate in such cases where a threshold has been established.

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/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(R5) (Ref. 1) and ICH Q3D (Ref. 4).

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 (Ref. 8), WHO IPCS (Ref. 9) and US Environmental Protection Agency (EPA) (Ref. 10) recommend an oral limit of 0.2 mg/kg/day, or 10 mg/day for a 50 kg person.

References

1. International Conference on Harmonisation (2011). Q3C(R5): Impurities: Guideline for Residual Solvents

2. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

3. Carcinogenicity Potency Database (CPDB): [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

4. International Conference on Harmonisation (2014). Q3D: Impurities: Guideline for Elemental Impurities

5. International Conference on Harmonisation (2008). S1C(R2): Dose Selection for Carcinogenicity Studies of Pharmaceuticals

6. 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.

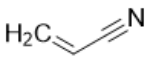
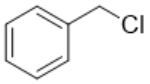
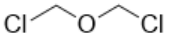
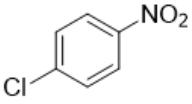
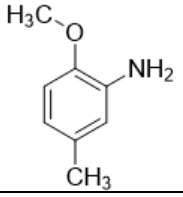
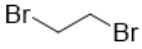
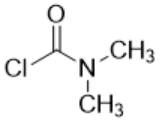
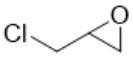
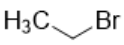
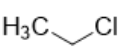
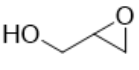
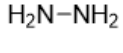
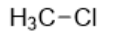
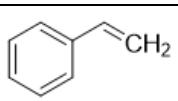
7. Cao X, Mittelstaedt RA, Pearce MG, Allen BC, Soeteman-Hernández LG, Johnson GE, et al. Quantitative dose-response analysis of ethyl methanesulfonate genotoxicity in adult gpt-delta transgenic mice. Environ Mol Mutagen 2014;55:385-99.

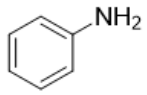
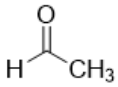
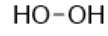
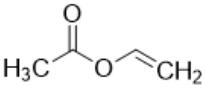
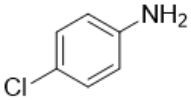
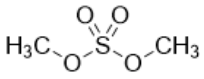
8. Health Canada. 2001 Priority substances list assessment report: Formaldehyde. Ottawa. Ministry of Public Works and Government Services. February. [Online]. Available from: URL: http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/index_e.html

9. World Health Organization (WHO). International Programme on Chemical Safety (IPCS). 2002. Concise International Chemical Assessment Document 40. Formaldehyde. [Online]. Available from: URL: http://www.who.int/ipcs/publications/cicad/en/cicad40.pdf

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

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

Compound	CAS#	Chemical Structure	AI or PDE (µg/day)	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
1,2-Dibromoethane	106-93-4		2	TD_{50} linear extrapolation
Dimethylcarbamyl chloride	79-44-7		0.6 (inhalation)* 5 (all other routes)	TD_{50} linear extrapolation
Epichlorohydrin	106-89-8		3	TD_{50} linear extrapolation
Ethyl bromide	74-96-4		32	TD_{50} linear extrapolation
Ethyl chloride	75-00-3		1,810	TD_{50} linear extrapolation
Glycidol	556-52-5		4	TD_{50} linear extrapolation
Hydrazine	302-01-2		0.2 (inhalation)* 39 (all other routes)	TD_{50} linear extrapolation
Methyl Chloride	74-87-3		1,361	TD_{50} linear extrapolation
Styrene	100-42-5		154	TD_{50} linear extrapolation

Threshold-based PDE				
Aniline Aniline HCl	62-53-3 142-04-1		720	PDE based on threshold mode of action (hemosiderosis)
Endogenous and/or Environmental Exposure				
Acetaldehyde	75-07-0		2,000 (oral)* 185 (all other routes)	Oral PDE is based on average food intake; all other routes based on TD_{50} linear extrapolation from an inhalation study
Formaldehyde	50-00-0		8,000 or 215 ppb, whichever is lower (inhalation)* 10,000 (all other routes)	Inhalation route based on TD_{50} linear extrapolation or local irritation; all other routes based on average food intake
Hydrogen peroxide	7722-84-1		68,000 or 0.5%, whichever is lower	68 mg/day is 1% of estimated endogenous production
Vinyl acetate	108-05-4		2,000 (oral)* 758 (all other routes)	Oral PDE is based on average food intake for acetaldehyde; all other routes based on TD_{50} linear extrapolation from an inhalation study
Other Cases				
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
Dimethyl Sulfate	77-78-1		1.5	Carcinogenicity data available, but inadequate to derive AI. Default to TTC

* Route specific limit

Acetaldehyde (CAS# 75-07-0)

Potential for human exposure

Acetaldehyde is formed endogenously in the human body from the metabolism of ethanol and carbohydrates as well as from bacteria in the alimentary tract. Humans are exposed to acetaldehyde mainly in food, alcoholic beverages, cigarette smoke and to a lesser extent from environmental emissions (Ref. 1, 2). The determination of endogenous acetaldehyde in blood, breath and saliva is challenging as the techniques are prone to artifacts and contaminants (Ref. 3, 4). Nevertheless, a daily endogenous production of 360 mg/day of acetaldehyde was calculated based on a constant endogenous total acetaldehyde concentration in the blood of 2.2 ±1.1 μmol/L (Ref. 3) and acetaldehyde clearance of 0.95 L/min (Ref. 5). Average acetaldehyde consumption of up to 48 mg/day comes from consumption of alcoholic beverages (Ref. 6). Endogenous acetaldehyde concentrations and the associated cancer risk are significantly higher in individuals with an acetaldehyde dehydrogenase-2 (ALDH2) genetic polymorphism (Ref. 7). The exogenous exposure from food (without alcoholic beverages or added acetaldehyde as flavoring agent) was estimated to be around 2 mg/day on average and 8 mg/day at the 95th percentile of the German population (Ref. 8). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) estimated food additive consumption to be 9.7 mg/day in the USA and 11 mg/day in Europe although this estimate is restricted to consumers who eat foods in which acetaldehyde is added as a flavor (Ref. 9). The Japanese Food Safety Committee (FSC) estimated domestic consumption to be 9.6 mg/day in Europe and 19.2 mg/day in the USA (Ref. 10). Acetaldehyde is used in synthesis of pharmaceuticals.

Mutagenicity/genotoxicity

The genotoxicity of acetaldehyde has been previously reviewed by the Chemical Evaluation and Research Institute, Japan (Ref 11) and other authors (Ref. 1, 5, 12-18). Acetaldehyde was negative in Ames mutation assays, but induced increases in mutations at the hypoxanthine-guanine-phosphoribosyl transferase (*HPRT*) *locus* in mammalian cells, which included point mutations as demonstrated by sequencing (Ref. 13). DNA- and DNA-protein adducts were observed in cultured cells treated with acetaldehyde (Ref. 14, 15), and DNA adducts were measured in urine of healthy volunteers and in blood cells from persons who abuse alcohol (Ref. 5). Acetaldehyde is primarily an inducer of larger scale chromosomal effects. It induces chromosomal aberrations and micronuclei *in vitro* and was positive in the mouse lymphoma L5178Y *TK*+/- assay (Ref 13). Acetaldehyde induced increases in micronuclei in the bone marrow of rats and mice (Ref 17).

Carcinogenicity

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

In humans, acetaldehyde is the primary metabolite of alcohol and both high and low alcohol consumption has been correlated with an increased relative risk for certain human cancers (e.g. oral cavity, pharynx cancer and breast cancer) (Ref. 19, 20). The relative risk was increased in smokers with a high alcohol consumption and a possible contribution of acetaldehyde derived from cigarette smoke (Ref. 19). Also, geographical regions with consumption of alcoholic beverages containing high acetaldehyde concentrations showed a tendency for higher incidence of squamous-cell cancer and cancer of the esophagus (Ref. 21). Furthermore, available epidemiological data indicate that there is an increased risk for development of alcohol-related cancers for those individuals who are deficient in detoxifying acetaldehyde to acetate by ALDH.

Especially the genetic variant ALDH2*1/*2 is strongly associated with alcohol-related cancers in not only heavy drinkers but those with moderate levels of alcohol consumption (Ref. 1, 7, 19). Meta analyses and large cohort studies report conflicting conclusions about whether there are increased risks of head, neck and mammary tumors associated with moderate alcohol consumption in the U.S. populations where ALDH deficiency is relatively infrequent. The literature on the elevated risk of head and neck cancers associated with acetaldehyde exposure in heavy drinkers, smokers, and in moderate drinkers with ALDH deficiency does not include discussion of whether those exposures are also associated with histopathological changes consistent with irritation or tissue proliferation (Ref. 22).

In rodents, only inhalation carcinogenicity studies are available in the Carcinogenic Potency Database (CPDB) (Ref. 23). The most robust study was conducted with Wistar rats (Ref. 24) with whole-body inhalation exposure to 0, 750, 1500 or 3000/1000 ppm (reduced after 11 months due to toxicity), 6 h/day at 5 days/week for up to 28 months. The corresponding doses in the CPDB were 0, 70.8, 142 and 147 mg/kg for male rats and 0, 101, 202 and 209 mg/kg for female rats. In the high-dose group, 50% of the male and 42% of the female animals had died by week 67 and no high-dose animals were alive by week 102. An increased incidence of tumors at the site of contact, i.e. nasal squamous cell carcinomas, was observed in males (1/49, 1/52, 10/53 and 15/49 in control, low, mid and high dose groups) and females (0/50, 0/48, 5/53 and 17/53, respectively) at the end of the study. There were also increases in nasal adenocarcinomas at all doses, the incidences were 0/49, 16/52, 31/53 and 21/49 in males and 0/50, 6/48, 26/53 and 21/53 in females, respectively. Based on these data, the TD_{50} value in the CPDB was estimated to be 185 mg/kg for nasal adenocarcinoma in male rats in the most sensitive sex and tissue.

An oral carcinogenicity study (Ref. 25) was conducted in Sprague Dawley rats with acetaldehyde administration in drinking water. In this study, 50 rats per group were given 0, 50, 250, 500, 1500 and 2500 mg/L acetaldehyde in drinking water for 104 weeks and the experiment was terminated when the last animal died at 161 weeks of age. The concentrations correspond to 0, 5, 25, 49, 147 and 246 mg/kg/day for male rats and 0, 5, 27, 53, 155 and 260 mg/kg/day for female rats, respectively. Incidences of adenocarcinomas, lymphomas and leukemias, mammary tumors, and cranial osteosarcomas, were described by the investigators as significantly greater in at least one group of exposed rats, relative to control. There was no increase in malignant tumors at the site of contact organs, i.e. the oral cavity and gastrointestinal tract, or in the liver. This study suggests that acetaldehyde may be carcinogenic after intake via drinking water. However, there was no clear dose-response relationship and therefore, many evaluators found that no clear conclusion can be drawn from this study (Ref. 5, 12, 19). In another evaluation of the same data, two different dose-response models were used to estimate cancer potency and the authors concluded that their quantitative risk assessment indicates the need to lower acetaldehyde exposure in the general population but also acknowledged that naturally occurring acetaldehyde cannot be reduced (Ref. 21). In this model, the carcinogenic potency was calculated for all tumor bearing animals because the authors found that there was insufficient statistical power to generate a model for any specific cancer site. A TD_{50} related to oral administration of acetaldehyde was not calculated.

Acetaldehyde – Details of carcinogenicity studies

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

Studies listed are in Cancer Potency Database (CPDB) (Ref. 24)
NC = not calculated;
[a] TD_{50} taken from the CPDB, carcinogenicity study selected for AI derivation
[b] The TD50 was not calculated due to lack of dose-response and sufficient statistical power; the study is not presented in the CPDB

Mode of action for carcinogenicity

Acetaldehyde is a strong electrophile and is capable of reacting with strong nucleophiles, for example DNA bases or amino acid residues on proteins. Although not mutagenic in the standard bacterial reversion assay, evidence for DNA-reactivity and mutagenicity was shown for acetaldehyde by the presence of DNA and DNA-protein adducts *in vitro* and *in vivo*, as well as by the positive result in the *in vitro HPRT* mutagenicity assay in mammalian cells. Despite its reactive nature, there is evidence for a non-linear dose response associated with the genotoxicity and carcinogenicity of acetaldehyde (Ref. 14). The dose-response of acetaldehyde-induced adducts at concentrations between 1 and 1000 µM has been measured in a cell culture system allowing the discrimination between endogenous and exogenous adducts induced by added acetaldehyde. These concentrations are comparable to salivary acetaldehyde concentrations measured before and after consumption of beverages containing alcohol with or without acetaldehyde (Ref. 27, 28). The exogenous adducts only exceeded the endogenous background level of adducts above a critical concentration.

ALDH, which efficiently detoxifies acetaldehyde, is responsible for the non-linear dose response relationship. ALDH enzymes are expressed in the mitochondria and cytosol of most tissues (e.g., liver, gastrointestinal tract, kidneys, nasal epithelium/olfactory epithelium, lung) and they metabolize acetaldehyde to form acetate and one proton (Ref. 29). The release of protons can reduce cellular pH and thus cause non-specific cytotoxicity with subsequent proliferative effects. The importance of detoxification was shown in ALDH deficient animal models. For example,

acetaldehyde induced chromosome damage and mutation is observed in mice deficient in ALDH2 activity following inhalation and oral (gavage) exposure, but not in ALDH2-proficient mice (Ref. 30). Similarly, more acetaldehyde derived DNA adducts were seen in alcoholics with a deficient aldehyde dehydrogenase genotype (allelic variant type ALDH2*1/2*2 with about 10% residual ALDH activity) compared to those with efficient genotype ALDH2*1/2*1 (Ref. 31) and moderate drinkers with the genotype are at increased risk of head and neck cancers (Ref. 19).

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

Regulatory and/or published limits

Acetaldehyde is listed in the US FDA's 'generally recognized as safe' (GRAS) list for flavoring substances and adjuvants – 21 CFR 182.60 (Ref 32). The Japanese FSC has no safety concerns when acetaldehyde is used as a flavoring agent as it is completely metabolized into non-reactive acetic acid and finally CO_2, and thus, its level as a flavoring agent is presumed not to exceed the physiological range (Ref. 10). The JECFA evaluation has concluded that there are no safety concerns at current levels of intake when used as a flavoring agent (Ref. 9).

The Committee on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants (Ref. 33) recommended a Continuous Exposure Guidance Level (CEGL) of 2 ppm corresponding to 3.6 mg/m^3. This represents an exposure of 3.6 mg/m^3 x 28.8 m^3 (24 hours in a day – ICH Q3C assumption) = 104 mg/day.

The US Environmental Protection Agency (EPA) did not consider a threshold for acetaldehyde carcinogenicity and has calculated that a concentration of 5 μg/m^3 acetaldehyde represents a 10^{-5} excess lifetime cancer risk based on the rat inhalation carcinogenicity study and application of linear extrapolation (Ref. 34). For a 24 h exposure, this represents 5 μg/m^3 x 28.8 m^3 = 144 μg/day. EPA did not consider the risk via the oral route.

Permissible Daily Exposure (PDE) for oral exposure

Rationale for selection of study for PDE calculation

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

PDE (oral) = 2 mg/day

Acceptable intake (AI) for all other routes

Rationale for selection of study for AI calculation

The inhalation study in rats by Woutersen et al. (Ref. 24) was used to derive the AI for all other routes. This study comprises group sizes of 55/sex/dose and animals were treated for life time

(i.e., 28 months). According to ICH M7 recommendations for selecting the most relevant study for deriving an AI, this is considered the most appropriate and robust study available for acetaldehyde. The inhalation carcinogenicity data and mechanistic study data suggest acetaldehyde cancer risk to be associated with cytotoxicity at the site of contact as nasal tumors were only found at doses also associated with cytotoxicity and severe irritation causing regenerative proliferation a promotion of growth of mutated cells.

Calculation of AI

Lifetime AI = TD_{50}/50000 x 50 kg

Lifetime AI =185 mg/kg/day/50000 x 50 kg

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

References

1. IARC. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Acetaldehyde. 1999;71:319–35.

2. O'Brien PJ, Siraki AG, et al. Aldehyde sources, metabolism, molecular toxicity mechanisms, and possible effects on human health. Crit Rev Toxicol 2005;35:609-62.

3. Fukunaga T, Sillanaukee P, Eriksson CJP. Problems involved in the determination of endogenous acetaldehyde in human blood. Alcohol and Alcoholism 1993;28:535-541.

4. Jones AW. Measuring and reporting the concentration of acetaldehyde in human breath. Alcohol and Alcoholism 1995;30:271-285.

5. Maximum Workplace Concentration Commission (MAK). The MAK Collection for Occupational Health and Safety. Acetaldehyde. 2013;1–58.

6. Lachenmeier DW, Gill JS, Chick J, Rehm J. The total margin of exposure of ethanol and acetaldehyde for heavy drinkers consuming cider or vodka. Food Chem Toxicol 2015;83:210–214.
7. Väkeväinen S, Tillonen J, Agarwal DP, Srivastava N, Salaspuro M. High salivary acetaldehyde after a moderate dose of alcohol in ALDH2-deficient subjects: strong evidence for the local carcinogenic action of acetaldehyde. Alcohol Clin Exp Res 2000;24:873-7.
8. Uebelacker M, Lachenmeier DW. Quantitative determination of acetaldehyde in foods using automated digestion with simulated gastric fluid followed by headspace gas chromatography. J Autom Methods Manag Chem 2011;2011:907317.

9. Joint FAO/WHO Expert Committee on Food Additives (JECFA). Evaluation of certain food additives and contaminants. Forty-Ninth Report of the Joint FAO/WHO Expert Committee on Food Additives. 1999; WHO Technical Report Series 884:30p.

10. Food Safety Commission (FSC). Evaluation of Food Additives: Acetaldehyde, Tokyo, Japan. 2005.

11. Chemicals Evaluation and Research Institute Japan (CERI). Hazard Assessment Report Acetaldehyde, Japan. 2007.

12. European Commission (EC). Scientific Committee on Consumer Safety: SCCS/1468/12: Opinion On: Acetaldehyde. 2012; SCCS/1468/12.

13. Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP). Opinion of the Scientific Committee on Cosmetic Products and non-food products for consumers concerning acetaldehyde. 2004; SCCNFP/0821/04.

14. Albertini RJ. Vinyl acetate monomer (VAM) genotoxicity profile: Relevance for carcinogenicity. Critical Reviews in Toxicology 2013;43:671-706.

15. Grafström RC, Dypbukt JM, Sundqvist K, Atzori L, Nielsen I, Curren RD, Harris CC. Pathobiological effects of acetaldehyde in cultured human epithelial cells and fibroblasts. Carcinogenesis 1994;15:985–990.

16. Moeller BC, Recio L, Green A, Sun W, Wright FA, Bodnar WM, Swenberg JA. Biomarkers of Exposure and Effect in Human Lymphoblastoid TK6 Cells Following [$^{13}C_2$]-Acetaldehyde Exposure. Toxicological Sciences 2013;133:1-12.

17. Morita T, Asano N, Awogi T, Sasaki YF, Sato S-I, Shimada H, Sutou S, Suzuki T, Wakata A, Sofuni T, Hayashi M. Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS MMS. Mutation Research 1997;389:3–122

18. Speit G, Froehler-Keller M, Schuetz P, Neuss S. Low sensitivity of the comet assay to detect acetaldehyde-induced genotoxicity. Mutation Research 2008;657:93–97.

19. IARC. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Personal habits and indoor combustions. 2012; Volume 100 E.

20. Shield KD, Soerjomataram I, Rehm J. Alcohol Use and Breast Cancer: A Critical Review. Alcohol Clin Exp Res 2016;40:1166-81.

21. Lachenmeier DW, Kanteres F, Rehm J. Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism. Addiction 2009;104:533-50.

22. Bagnardi V, Rota M, Botteri E, Tramacere I, Islami F, Fedirko V, Scotti L, Jenab M, Turati F, Pasquali E, Pelucchi C, Galeone C, Bellocco R, Negri E, Corrao G, Boffetta P, La Vecchia C. Alcohol consumption and site-specific cancer risk: a comprehensive dose-response meta-analysis. Br J Cancer 2015;112:580-93.

23. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

24. Woutersen RA, Appelman LM, Van Garderen-Hoetmer a and Feron VJ. Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. Toxicology 1986;41:213-231.

25. Soffritti M, Belpoggi F, Lambertini L, Lauriola M, Padovani M, Maltoni C. Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. Ann N Y Acad Sci 2002;982:87–105.

26. Feron VJ, Kruysse A, Woutersen RA. Respiratory tract tumours in hamsters exposed to acetaldehyde vapour alone or simultaneously to benzo(a)pyrene or diethylnitrosamine. European Journal of Cancer Clinical Oncology 1982;1:13-31.

27. Homann N, Jousimies-Somer H, Jokelainen K, Heine R, Salaspuro M. High acetaldehyde levels in saliva after ethanol consumption: methodological aspects and pathogenetic implications. Carcinogenesis 1997;18:1739-43.

28. Salaspuro V, Hietala J, Kaihovaara P, Pihlajarinne L, Marvola M, Salaspuro M. Removal of acetaldehyde from saliva by a slow-release buccal tablet of L-cysteine. Int J Cancer 2002;97:361-4.

29. Sladek NE. Human aldehyde dehydrogenases: Potential pathological, pharmacological and toxicological impact. Journal of Biochemical Molecular Toxicology 2003;17:8-23.

30. Kunugita N, Isse T, Oyama T, Kitagawa K, Ogawa M, Yamaguchi T, Kinaga T and Kawamoto T. Increased frequencies of micronucleated reticulocytes and T-cell receptor mutation in ALDH2 knockout mice exposed to acetaldehyde. Journal of Toxicological Science 2008;33:31-6.

31. Matsuda T, Yabushita H, Kanaly RA, Shibutani S, Yokoyama A. Increased DNA damage in ALDH2-deficient alcoholics. Chemical Research in Toxicology 2006;19:1374-1378.

32. United States Food and Drug Administration (US FDA) Title 21- Subchapter B - Food for human consumption Part 182 -Substances generally recognized as safe. 21CFR182.60]

33. Committee on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants (CECEGL). Emergency and continuous exposure guidance levels for selected submarine contaminants, Volume 3, National Academies Press, Washington. 2009.

34. United States Environmental Protection Agency (USEPA). Integrated Risk Information System (IRIS). Acetaldehyde (CASRN 75-07-0) Carcinogenicity Assessment. 1988; Available from: URL: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0290_summary.pdf

Acrylonitrile (CAS# 107-13-1)

Potential for human exposure

No data are available for exposure of the general population.

Mutagenicity/Genotoxicity

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

The World Health Organization (WHO) Concise International Chemical Assessment Document (CICAD, Ref. 1), provided a thorough risk assessment of acrylonitrile. In this publication, oxidative metabolism was indicated as 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 (Ref. 1) 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.

In vivo genotoxicity studies are negative or inconclusive, and reports of DNA binding are consistently positive in liver but give conflicting results in brain.

Carcinogenicity

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

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 (Ref. 3) and the results from three additional oral studies are summarized in Ref. 1. Of these seven studies only one is negative but this study tested only a single dose administered for short duration (Ref. 4).

The NCI/NTP (National Cancer Institute) study in the CPDB of acrylonitrile in mice (Ref. 5) was selected for derivation of the oral AI, based on robust study design and the most conservative TD_{50} value. In this 2 year-study, 3 doses of acrylonitrile were administered by oral gavage to male and female mice. There were statistically significant increases in tumors of the Harderian gland and forestomach.

In the 1980 study of Quast *et al* (Ref. 6), cited in the CPDB as a report from Dow Chemical, it appears that the most sensitive TD_{50} is for astrocytomas in female rats (5.31 mg/kg/day). However, this same study was later described in detail (Ref. 7) and the calculated doses in that published report are higher than those listed in the CPDB. Quast (Ref. 7) 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. (The TD_{50}'s calculated from the dose estimates by Quast (Ref. 7) for forestomach tumors are also higher than those in the CPDB based on the same study, as shown in the Table below). Central Nervous System (CNS), tumors are described (Ref. 7), but the most sensitive TD_{50} was for stomach tumors, as shown in the Table below.

Studies considered less robust included three rat drinking water studies. The largest study (Ref. 8) 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 by WHO (Ref. 1) and by US EPA (Ref. 9) 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. Two studies (Ref. 10, 11) each had only two dose levels and individual tumor types are not reported (Ref. 1), although tumors of stomach, Zymbal gland and brain were observed.

Acrylonitrile has also been studied by the inhalation route. Fifty rats per sex per dose were exposed for 2 years to acrylonitrile, and brain tumors were observed (Ref. 12). This study however, tested only 2 dose levels. 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/type/sex**	**TD_{50} (mg/kg/d)**
Ref. 5[a]	50 B6C3F1 Mice (F)	2 years Gavage	50	**3:** 1.79; 7.14; 14.3 mg/kg/d	Forestomach	6.77[c]
	50 B6C3F1 Mice (M)	2 years Gavage	50	**3:** 1.79; 7.14; 14.3 mg/kg/d	Forestomach	5.92[c]
Ref. 6	~50 SD Spartan rats (F)	2 years Drinking water	~80	**3:** 2.00; 5.69; 15.4 mg/kg/d	Astrocytoma	5.31[d] (20.8)
	~50 SD Spartan rats (M)	2 years Drinking water	~80	**3:** 1.75; 4.98; 14.9 mg/kg/d	Stomach, non-glandular	6.36[d] (9.0)
Ref 7 (report of Ref. 6)	~50 female SD Spartan rats	2 years Drinking water	~80	**3:** 4.4; 10.8; 25 mg/kg/d	Stomach, non-glandular	19.4
	~50 SD male Spartan rats	2 years Drinking water	~80	**3:** 3.4; 8.5; 21.3 mg/kg/d	Stomach, non-glandular	9.0
Ref. 8[e]	100 male rats	~2 years Drinking water	~200	**5:** 0.1-8.4 mg/kg/d	Brain astrocytoma	(22.9)[c]

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/d)
	100 female rats	~2 years Drinking water	~200	**5:** 0.1-10.9 mg/kg/d	Brain astrocytoma	(23.5)[c]
Ref. 11[e]	100/sex Rats	19-22 mo Drinking water	~98	**2:** ~0.09; 7.98 mg/kg/d	Stomach, Zymbal's gland, brain, spinal cord	NC
Ref. 10[e]	50/sex Rats	18 mo Drinking water	No	**2:** 14; 70 mg/kg/d	Brain, Zymbal's gland, forestomach	NC[b]
Ref. 13	20 male CD rats	2 years Drinking water	No	**3:** 1; 5; 25 mg/kg/d	Zymbal's gland	30.1
Ref. 4	40/sex SD rats	1 year 3d/wk Gavage	75/sex	**1:** 1.07 mg/kg/d	Neg in both sexes	NA
Ref. 12	100/sex SD Spartan rat	2 years 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
Ref. 4	30/sex SD rats	1 year 5d/wk 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
Ref. 4	54 female SD rats	2 years 5d/wk Inhalation	60	**1:** 11.1 mg/kg/d	Brain glioma	(132)[f]

Studies listed are in CPDB (Ref. 3) unless otherwise noted.
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.
[a]Carcinogenicity study selected for AI calculation; in CPDB.
[b]NC= Not calculated as individual tumor type incidences not provided in WHO (Ref. 1).
[c]TD_{50} calculated based on astrocytoma incidence implied as most significant site by WHO (Ref. 1). Serial sampling reduced number of animals exposed for 2 years, so tumor incidences may be underestimates.
[d]Taken from the CPDB. Note that based on the dose calculations by the author (Ref. 7) the TD_{50} for astrocytomas and stomach tumors in Spartan rats (20.8 and 9.0) are higher than those in the CPDB.
NA= Not applicable.
[e] Not in CPDB. Summarized in Refs. 1 and 9.
[f] 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 (Ref. 1). CNS tumors were seen in multiple carcinogenicity

studies in rats, in addition to forestomach tumors; forestomach tumors were also the most sensitive tumor type in mice.

Forestomach tumors are associated with local irritation and inflammation, and Quast (Ref. 7) 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 at low concentrations that are non-irritating (Ref. 14). 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 and tongue. Forestomach tumors were seen after administration of acrylonitrile to rats in drinking water, and to mice by gavage. The AI for acrylonitrile was derived based on mouse forestomach tumors.

Regulatory and/or published limits

The US EPA (Ref. 9) 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 drinking water limit 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 routes of administration, and acrylonitrile is rapidly absorbed *via* all routes of exposure and distributed throughout examined tissues (Ref. 1), so that a specific inhalation AI was not considered necessary. All of the carcinogenicity studies that were used by the US EPA (Ref. 9) 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. The NCI/NTP study (Ref. 5) was selected to calculate the AI based on the TD_{50} derived from administering acrylonitrile by oral gavage to male and female mice since 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 (Ref. 9) for drinking water.

Calculation of AI

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

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

Lifetime AI = 5.9 µg/day (6 µg/day)

References

1. World Health Organization (WHO). Concise International Chemical Assessment Document (CICAD) 39. Acrylonitrile. [Online]. Geneva. 2002; Available from: URL: http://www.inchem.org/documents/cicads/cicads/cicad39.htm

2. International Agency for Research on Cancer (IARC). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. International Agency for Research on Cancer, World Health Organization, Lyon. Acrylonitrile 1999; Vol. 71, 43.

3. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

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

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

6. Quast JF, Wade CE, Humiston CG, Carreon RM, Hermann EA, Park CN et al, Editors. A Two-Year Toxicity and Oncogenicity Study with Acrylonitrile Incorporated in the Drinking Water of Rats, Final Report. Dow Chemical USA, Midland, MI; 1980.

7. Quast, JF Two-year toxicity and oncogenicity study with acrylonitrile incorporated in the drinking water of rats. Toxicol Lett 2002;132:153-96.

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

9. US EPA. Acrylonitrile (CAS# 107-13-1). Integrated Risk Information System (IRIS) [Online].1987. Available from: URL: https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=206

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

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

12. Quast JF, Schuetz DJ, Balmer MF, Gushow TS, Park CN, McKenna MJ, editors. A Two-Year Toxicity and Oncogenicity Study with Acrylonitrile Following Inhalation Exposure of Rats, Final Report. Dow Chemical USA, Midland, MI; 1980.

13. Gallagher GT, Maull EA, Kovacs K, Szab S. Neoplasms in rats ingesting acrylonitrile for two years. J Am Col Toxicol 1988;7:603-15.

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

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*. Aniline is included in this Addendum because of the historical perception that aniline is a genotoxic carcinogen, since some *in vitro* and *in vivo* genotoxicity tests are positive.

Aniline is not mutagenic in the 5 standard strains of *Salmonella* or in *E.coli* WP2 *uvrA*, with or without S9 (Ref. 1, 2, 3, 4, 5, 6, 7, 8).

Aniline was positive in the mouse lymphoma L5178Y cell *tk* assay with and without S9 at quite high concentrations, such as 0.5 to 21 mM (Ref. 9, 10, 11).

Chromosomal aberration tests gave mixed results, with some negative reports and some positive results in hamster cell lines at very high, cytotoxic concentrations, e.g., about 5 to 30 mM, with or without S9 metabolic activation (Ref. 1, 12, 13, 14, 15).

In vivo, chromosomal aberrations were not increased in the bone marrow of male CBA mice after two daily intraperitoneal (i.p.) doses of 380 mg/kg (Ref. 16), but a small increase in chromosomal aberrations 18 h after an oral dose of 500 mg/kg to male PVR rats was reported (Ref. 17).

Most studies of micronucleus induction are positive in bone marrow after oral or i.p. treatment of mice (Ref. 18, 19, 20, 21) or rats (Ref. 17, 22), 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 (Ref. 23).

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 i.p. dose of 61 to 420 mg/kg aniline (Ref. 24, 25). DNA strand breaks were not detected in the mouse bone marrow by the alkaline elution assay in this study.

Carcinogenicity

Aniline is classified by IARC as Group 3, not classifiable as to its carcinogenicity in humans (Ref. 4).

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, Ref. 26) 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 (Ref. 27) 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 (Ref. 27), no statistically significant increase in any type of tumor was observed at very high doses.

Aniline itself did not induce tumors in rats when tested in a less robust study design (Ref. 28).

Aniline and Aniline HCl – Details of carcinogenicity studies

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/d)
Ref. 26[a] 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 sarcoma (high dose). NOEL at low dose	Not reported
Ref. 27[b] Aniline HCl	50/sex/group, F344 rats	103 weeks (107-110 wk study) Diet	50	**2:** 3000 and 6000 ppm in diet (F: 144;268 M: 115;229 mg/kg/d)	Spleen hemangio-sarcoma/Male	160 (Male)
Ref. 27[b] Aniline HCl	50/sex/group B6C3F1 mice	103 weeks (107-110 wk study) Diet	50	**2:** 6000 and 12000 ppm in diet (F: 741;1500 M: 693;1390 mg/kg/d)	Negative	NA
Ref. 28[b] Aniline	10-18/group, male Wistar rats	80 weeks Diet	Yes	**3:** 0.03, 0.06 and 0.12% in diet (15;30;60 mg/kg/d)	Negative	NA

[a]Carcinogenicity study selected for PDE calculation. Not in CPDB.
[b]Taken from CPDB (Ref. 29). The TD_{50} values represent the TD_{50} from the most sensitive tumor site.
NA = Not applicable

Mode of action for carcinogenicity

In animal studies, aniline caused methemoglobinemia and hemolysis at high doses, the latter of which could indirectly lead to increases in micronuclei by inducing erythropoiesis (Ref. 19, 30, 31). Micronuclei are induced in both rats and 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 (Ref. 32). 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 (Ref. 33). 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 (Ref. 32, 34). The lack of tumorigenicity in mice may be due to less severe toxicity observed in spleen compared to that in rats (Ref. 17, 35).

In support of this toxicity-driven mode of action for carcinogenicity, the dose response for aniline-induced tumorigenicity in rats is non-linear (Ref. 36). When considering the NCI and CIIT studies which both used the same rat strain, no tumors 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 (Ref. 37) support the conclusion that a threshold exists for aniline carcinogenicity (Ref. 36). The weight of evidence supports the conclusion that these tumors do not result from a primary mutagenic mode of action (Ref. 38).

Regulatory and/or published limits

The US EPA (Ref. 39) outlines a quantitative cancer risk assessment for aniline based on the CIIT study (Ref. 26). 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 (Ref. 39). 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 tumors observed in rats.

Permissible daily exposure (PDE)

It is considered inappropriate to base an AI for aniline on linear extrapolation for spleen tumors observed in rats, since these have a non-linear dose response, aniline is not mutagenic, and genotoxicity is not central to the mode of action of aniline-induced carcinogenicity. The PDE is derived using the process defined in ICH Q3C (Ref. 40).

Rationale for selection of study for PDE calculation

Data from the CIIT 2-year rat carcinogenicity study (Ref. 26) have been used. 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 Effect Level for tumors (NOEL).

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 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 NOEL)

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

Lifetime PDE = 720 µg/day

References

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

2. IARC. Some aromatic amines, anthraquinones and nitroso compounds, and inorganic fluorides used in drinking water and dental preparations. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. International Agency for Research on Cancer, World Health Organization, Lyon. 1982; 27:39.

3. IARC. Genetic and related effects: An update of selected IARC Monographs from volumes 1 to 42. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. International Agency for Research on Cancer, World Health Organization, Lyon.1987. Addendum 6: 68.

4. IARC. Overall evaluation of carcinogenicity: An update of IARC monographs volumes 1 to 42. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. International Agency for Research on Cancer, World Health Organization, Lyon. 1987. Addendum 7: pp 99 and 362.

5. Jackson MA, Stack HF, Waters MD. The genetic toxicology of putative nongenotoxic carcinogens. Mutat Res 1993;296:241-77.

6. Brams A, Buchet JP, Crutzen-Fayt MC, De Meester C, Lauwerys R, Leonard A. A Comparative Study, With 40 Chemicals, of The Efficiency of the Salmonella Assay and the SOS Chromotest (Kit Procedure). Toxicol Lett 1987;38:123-33.

7. Rashid KA, Arjmand M, Sandermann H, Mumma RO. Mutagenicity of chloroaniline / lignin metabolites in the Salmonella/microsome assay. J Environ Sci Health 1987;Part B B22(6):721-9.

8. Gentile JM, Gentile GJ and Plewa M. Mutagenicity of selected aniline derivatives to Salmonella following plant activation and mammalian hepatic activation. Mutat Res 1987;188:185-96.

9. Wangenheim J, Bolcsfoldi G. Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds; Mutagenesis 1988;3(3):193-205.

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

11. McGregor DB, Brown AG, Howgate S, Mcbride D, Riach C, Caspary WJ. Responses of the L5178y mouse lymphoma cell forward mutation assay. V: 27 Coded Chemicals. Environ Mol Mutagen 1991;17:196-219.

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

13. Ishidate M, Jr, Odashima S. Chromosome tests with 134 compounds on Chinese hamster cells *in vitro* – A screening for chemical carcinogens. Mutat Res 1977;48:337-54.

14. Ishidate M Jr. The data book of chromosomal aberration tests *in vitro* on 587 chemical substances using Chinese hamster fibroblast cell line (CHL cells). Tokyo . The Realize Inc. 1983;p26.

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

16. Jones E, Fox V. Lack of clastogenicity activity of aniline in the mouse bone marrow. Mutagenesis 2003;18:283-6.

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

18. Westmoreland C, Gatehouse DG. Effects of aniline hydrochloride in the mouse bone marrow micronucleus test after oral administration. Carcinogenesis 1991;12:1057-9.

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

20. Sicardi SM, Martiarena JL, Iglesian MT. Mutagenic and analgesic activities of aniline derivatives. J Pharm Sci 1991;80:761-4.

21. Ress NB, Witt KL, Xu J, Haseman JK, Bucher JR. Micronucleus induction in mice exposed to diazoaminobenzene or its metabolites, benzene and aniline: implications for diazoaminobenzene carcinogenicity. Mutat Res 2002;521:201-8.

22. George E, Andrews M, and Westmoreland C. Effects of azobenzene and aniline in the rodent bone marrow micronucleus test. Carcinogenesis 1990;11:1551-5.

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

24. Parodi S, Pala M, Russo P, Zunino A, Balbi C, Albini A, et al. DNA damage in liver, kidney, bone marrow, and spleen of rats and mice treated with commercial and purified aniline as determined by alkaline elution assay and sister chromatid exchange induction. Cancer Res 1982;42:2277-83.

25. Parodi S, Zunino A, Ottaggio L, De Ferrari M, Santi L. Lack of correlation between the capability of inducing sister chromatid exchanges *in vivo* and carcinogenic potency for 16 aromatic amines and azo derivatives. Mutat Res 1983;108:225-38.

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

27. NCI (National Cancer Institute) National Toxicology Program. Technical report on the bio-assay for Aniline hydrochloride for possible carcinogenicity. (CAS No., 142-04-1). NCI-CG-TR-130. 1978. Available from: URL: https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr130.pdf

28. Hagiwara A, Arai M, Hirose M, Nakanowatari J-I, Tsuda H and Ito N. Chronic effects of norharman in rats treated with aniline. Toxicol Lett 1980;6:71-5.

29. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

30. Steinheider G, Neth R, Marguardt H. Evaluation of nongenotoxic and genotoxic factors modulating the frequency of micronucleated erythrocytes in the peripheral blood of mice. Cell Biol Toxicol 1985;1:197-211.

31. Tweats D, Blakey D, Heflich RH, Jacobs A, Jacobsen SD, Nohmi TT, et al. Report of the IWGT working group on strategies and interpretation of regulatory *in vivo* tests. I. Increases in micronucleated bone marrow cells in rodents that do not indicate genotoxic hazards. Mutat Res 2007;627:78-91.

32. Khan MF, Wu X, Boor PJ, Ansari GAS. Oxidative modification of lipids and proteins in aniline induced splenic toxicity. Toxicol Sci 1999;48:134-40.

33. Khan MF, Wu X, Wang JL. Upregulation of transforming growth factor-beta 1 in the spleen of aniline-induced rats. Toxicol Appl Pharmacol 2003;187:22-8.

34. Weinberger MA, Albert RH, Montgomery SB. Splenotoxicity associated with splenic sarcomas in rats fed high doses of D & C Red No. 9 or aniline hydrochloride. J Natl Cancer Inst 1985; 5:681-7.

35. Smith RP, Alkaitis AA, Shafer PR. Chemically induced methemoglobinemias in the mouse. Biochem. Pharmacol 1967;16:317-28.

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

37. Robertson O, Cox MG, Bus JS. Response of the erythrocyte and spleen to aniline insult in Fischer 344 rats. Toxicologist 1983;3:128.

38. Bomhard EM, Herbold BA. Genotoxic activities of aniline and its metabolites and their relationship to the carcinogenicity of aniline in the spleen of rats. Crit Rev Toxicol 2005;35:783-835.

39. US Environmental Protection Agency. Aniline (CAS No 62-53-3). Integrated Risk Information System (IRIS). [Online]. 1988. Available from: URL: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0350_summary.pdf

40. International Conference on Harmonisation (2011). Q3C(R5): Impurities: Guideline for Residual Solvents.

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 (Ref. 1). Some of the key conclusions are summarized here.

Benzyl chloride is mutagenic in:
Microbial reverse mutation assay (Ames) in *Salmonella typhimurium* strain TA100. Results of the standard assay are inconsistent across and within laboratories, but clear increases are obtained when testing in the gaseous phase (Ref. 2);
Chinese hamster cells (Ref. 1).

Benzyl chloride did not induce micronuclei *in vivo* in mouse bone marrow following oral, intraperitoneal or subcutaneous administration, but did form DNA adducts in mice after i.v. administration (Ref. 1).

Carcinogenicity

Benzyl chloride is classified as Group 2A, probably carcinogenic to humans (Ref. 3).

Benzyl chloride was administered in corn oil by gavage 3 times/week for 104 weeks to F-344 rats and B6C3F1 mice (Ref. 4). 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 for thyroid C-cell adenoma/carcinoma in the female high-dose group (27% versus 8% for control). A discussion of whether these thyroid tumors were treatment-related is included below. Several toxicity studies were conducted but C-cell hyperplasia was noted only in this lifetime study and only in female rats.

In mice (Ref. 4), 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 dose (54% 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).

Additional studies to assess carcinogenic potential were conducted but were not considered of adequate study design for use in calculating an AI. In one of three topical studies (Ref. 5) skin carcinomas were increased, although not statistically significantly (15% versus 0% in benzene controls). Initiation-promotion studies to determine the potential of benzyl chloride to initiate

skin cancer, using croton oil and the phorbol ester TPA (12-O-tetradecanoyl- phorbol-13-acetate) as promoters (Ref. 6, 7, 8) were of limited duration and the published reports were presented as preliminary findings, but no final results have been located in the literature. Injection site sarcomas were seen after subcutaneous administration (Ref. 9).

Benzyl chloride – Details of carcinogenicity studies

Study	**Animals/dose group**	**Duration/ Exposure**	**Controls**	**Doses**	**Most sensitive tumor site/type/sex or tumor observations**	**TD_{50} (mg/kg/d)**
Ref. 4[a]	52/sex/group F344 rat	2 year 3 times/wk Gavage	52	**2:** 15 and 30 mg/kg (6 and 12 mg/kg/d)	Thyroid C-cell neoplasm/ Female	40.6
Ref. 4	52/sex/group B6C3F1 mouse	2 year 3 times/wk Gavage	52	**2:** 50 and 100 mg/kg (21 and 42 mg/kg/d)	Forestomach papilloma, carcinoma/ Male	49.6
Ref. 5	11/group female ICR mouse	9.8 mo 3 times/wk for 4 wks, 2 times/wk Dermal	Yes (benzene treated)	**1:** 10 µL	No skin tumors	NC [b]
Ref. 5	20/group female ICR mouse	50 weeks 2 times/wk Dermal	20 (benzene treated)	**1:** 2.3 µL	Skin squamous cell carcinoma	NC [b]
Ref. 6	20/group male ICI Swiss albino mouse	>7 mo 2 times/wk Dermal, in toluene	20	**1:** 100 µg/mouse	No skin tumors	NC [b]
Ref. 9	14 (40 mg/kg), and 8 (80 mg/kg) BD rat	51 weeks 1 time/wk Subcutaneous	Yes	**2:** 40 and 80 mg/kg/wk	Injection site sarcoma	NC [b]
Ref. 7	40/sex/group Theiler's Original mouse	10 mo 1 dose (in toluene); wait 1 wk Promoter (croton oil)	40	**1:** 1 mg/ mouse	No skin tumors	NC [b]

Study	Animals/dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex or tumor observations	TD_{50} (mg/kg/d)
		2 times/wk				
Ref. 8	Sencar mice	6 mo dose; Promoter (TPA) 2 times/wk	Yes	3: 10; 100 and 1000 µg/ mouse	20% skin tumors [5% in TPA controls] (DMBA controls had skin tumors by 11 weeks)	NC [b]

Studies listed are in CPDB (Ref. 10) unless otherwise noted.
[a]Carcinogenicity study selected for AI calculation.
[b]NC= Not calculated; small group size, limited duration. Not included in CPDB as route with greater likelihood of systemic exposure is considered more relevant.

Mode of action for carcinogenicity

The tumor types with the lowest calculated TD_{50} (highest potency) in the CPDB (Ref. 10) 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* (Ref. 11) proposed 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 (Ref. 12). Groups of 10 Fischer 344 rats of both sexes died within 2-3 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 (Ref. 4). 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* (Ref. 4) 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 (Ref. 12) 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 (Ref. 13, 14), males and females show comparable levels of C-cell adenomas plus carcinomas 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 (Ref. 15) 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 2 μg/L or 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 (Ref. 4) 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 (Ref. 4), 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)

References

1. IARC. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. International Agency for Research on Cancer, World Health Organization, Lyon. [Online] 1972-PRESENT. (Multivolume work). 1999. Available from: URL: http://monographs.iarc.fr/ENG/Monographs/vol71/mono71-19.pdf

2. Fall M, Haddouk H, Morin JP, Forster R. Mutagenicity of benzyl chloride in the *Salmonella*/microsome mutagenesis assay depends on exposure conditions. Mutat Res 2007;633:13-20.

3. IARC. An update of selected IARC Monographs from volumes 1 to 42. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. International Agency for Research on Cancer, World Health Organization, Lyon.1987. Suppl. 7: 126–7; 148–9.

4. Lijinsky W. Chronic Bioassay of Benzyl Chloride in F344 Rats and (C57BL/6J X BALB/c) F1 Mice. J Natl Cancer Inst 1986;76:1231-6.

5. Fukuda K, Matsushita H, Sakabe H, Takemoto K. Carcinogenicity of benzyl chloride, benzal chloride, benzotrichloride and benzoyl chloride in mice by skin application. Gann 1981;72(5):655-64.

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

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

8. Coombs MM. The UKEMS Genotoxicity Trial: A summary of the assays for skin tumour induction in mice, the subcutaneous implant test and the sebaceous gland suppression test. Mutat Res 1982;100:407-9.

9. Druckrey H, Kruse H, Preussmann R, Ivankovic S, Landschuetz C. Cancerogenic alkylating substances. III. Alkyl-halogenides, - sulfates, - sulfonates and strained heterocyclic compounds. 1970;74(3):241-73.

10. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

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

12. OECD Chemicals Screening Information Dataset (SIDS) for high volume chemicals benzyl chloride report published by the United Nations Environmental Programme (UNEP). [Online]. Available from: URL:http://www.chem.unep.ch/irptc/sids/OECDSIDS/100447.pdf

13. Haseman JK, Huff J, Boorman GA. Use of historical control data in carcinogenicity studies in rodents., Toxicol Pathol 1984;12:126-35.

14. Haseman JK, Hailey JR, Morris RW. Spontaneous neoplasm incidence in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: A National Toxicology Program update, Toxicol Pathol 1998;26:428-41.

15. US Environmental Protection Agency. Benzyl chloride (CAS 100-44-7). Integrated Risk Information System (IRIS). [Online] 1989. Available from: URL: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0393_summary.pdf

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

Potential for human exposure

Industrial use, mainly via inhalation with minimal environmental exposure as result of rapid degradation in the environment, which is supported by the reported absence of BCME in ambient air or water (Ref. 1).

Mutagenicity/genotoxicity

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

BCME is mutagenic in:
Microbial reverse mutation assay (Ames), *Salmonella typhimurium* (Ref. 2).

In vivo, BCME did not cause chromosomal aberrations in bone-marrow cells of rats exposed by inhalation for six months (Ref. 3). A slight increase in the incidence of chromosomal aberrations was observed in peripheral lymphocytes of workers exposed to BCME (Ref. 4).

Carcinogenicity

BCME is classified by US EPA as a Group A, known human carcinogen (Ref. 5), and by IARC as a Group 1 compound, carcinogenic to humans (Ref. 6).

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* (Ref. 3) 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 h/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, tumors of the olfactory epithelium, 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.

In an inhalation study, male Sprague-Dawley rats were exposed to BCME at a single dose level of 0.1 ppm (100 ppb) 6 h/day, 5 days/week for 10, 20, 40, 60, 80, or 100 days, then observed for the remainder of their lifetimes (Ref. 7). 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 (Ref. 8) and skin tumors in mice, (Ref. 9); it also induces lung adenomas in newborn mice following sub-cutaneous application (Ref. 10).

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

Study	Animals/dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/d)
Ref. 3[a]	~104/group Rat, male Sprague-Dawley.	28 weeks 6 h/d, 5 d/wk Inhalation	104	**3:** 1; 10; 100 ppb (53;528; 7780 ng/ kg/d)	Nasal passage - esthesioneuro-epitheliomas	0.00357
Ref. 3	138-144/ group Mouse, male ICR/Ha.	25 weeks 6 h/d, 5 d/wk Inhalation	157	**3:** 1; 10; 100 ppb (0.295; 2.95;33.6 ng/kg/d)	Lung adenomas	No significant increases
Ref. 7	30-50 treated for different durations with same concentration, male Sprague Dawley rats.	6h/d, 5d/wk, for 10, 20, 40, 60, 80, and 100 exposures. Inhalation	240	**1:** 0.1 ppm	Lung and nasal cancer	NC[b]
Ref. 7	100/group male Golden Syrian Hamsters.	Lifetime 6h/d, 5d/wk, Inhalation	NA	**1:** 1 ppm	One undifferentiated in the lung	NC[b]
Ref. 9	50/group female ICR/Ha Swiss mice.	424-456 days, once weekly Intra-peritoneal	50	**1:** 0.114 mg/kg/d	Sarcoma (at the injection site)	0.182

Studies listed are in CPDB (Ref. 11) unless otherwise noted.
[a]Carcinogenicity study selected for AI calculation
[b]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

BCME is a mutagenic carcinogen, and the acceptable intake is calculated by linear extrapolation from the TD50.

Regulatory and/or published limits

The US EPA (Ref. 5), 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* (Ref. 7). The inhaled (and oral) dose associated with a 1 in 100,000 lifetime cancer risk is 3.2 ng/day (1.6 x 10^{-8} mg/m^3 for inhalation, 1.6 x 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, so that 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) in male rats in the inhalation carcinogenicity study (Ref. 3), with a TD_{50} of 3.57µg/kg/day. The AI derived by linear extrapolation from that $TD_{50,}$ ~4ng/day, is essentially the same as the 3.2 ng/day recommendation of the US EPA. The study (Ref. 3) had a reliable design 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 (Ref. 10) 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 kg

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

References

1. NIH ROC. National Institutes of Health. Report on Carcinogens, Twelfth Edition [Online]. 2011. Available from: URL: http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/bis(chloromethyl)ether.pdf

2. Nelson N. The chloroethers - occupational carcinogens: A summary of laboratory and epidemiology studies. Ann. NY Acad Sci 1976;271:81-90.

3. Leong BKJ, Kociba RI, Jersey GC. A lifetime study of rats and mice exposed to vapors of bis(chloromethy1) ether. Toxicol Appl Pharmacol 1981;58:269-81.

4. IARC. Bis(chloromethyl)ether and chloromethyl methyl ether (technical-grade). Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans.

International Agency for Research on Cancer, World Health Organization. Lyon. 1987;Addendum 7: 131-3.

5. US Environmental Protection Agency. Bis(chloromethyl)ether (CAS# 542-88-1). Integrated Risk Information System (IRIS). [Online]. 1999. Available from: URL: http://www.epa.gov/iris/subst/0375.htm

6. IARC. Chemicals, industrial processes and industries associated with cancer in humans. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. International Agency for Research on Cancer, World Health Organization, Lyon. 1982;Volumes 1 to 29, Addendum 4.

7. Kuschner M, Laskin S, Drew RT, Cappiello V, Nelson N. Inhalation carcinogenicity of alpha halo ethers. III. Lifetime and limited period inhalation studies with bis(chloromethyl)ether at 0.1 ppm. Arch Environ Health 1975;30:73-7.

8. Van Duuren BL, Sivak A, Goldschmidt BM, Katz C, Melchionne S. Carcinogenicity of halo-ethers. J Nat Cancer Inst 1969;43:481-6.

9. Van Duuren BL, Goldschmidt BM, Seidman I. Carcinogenic activity of di- and trifunctional α-chloro ethers and of 1,4-dichlorobutene-2 in ICR/HA swiss mice. Cancer Res 1975;35:2553-7.

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

11. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

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

Potential for human exposure

Industrial exposure is primarily derived from the dye, textile, rubber and other industries (Ref. 1). If released into the environment, it is inherently biodegradable in water under aerobic conditions (Ref. 2).

Mutagenicity/Genotoxicity

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

A detailed review of genotoxicity testing in a range of systems is provided by WHO (Ref. 3) with references, so only key conclusions are summarized here.

p-Chloroaniline is mutagenic in:
Microbial reverse mutation assay (Ames); 2 to 3-fold increase in revertants was seen in some laboratories but not in others.
Positive results reported in the mouse lymphoma L5178Y cell *tk* assay (Ref. 3) are small increases, associated with substantial cytotoxicity, and do not meet the current criteria for a positive assay using the "global evaluation factor" (Ref. 4).

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 by IARC as Group 2B, possibly carcinogenic to humans with adequate evidence of carcinogenicity in animals and inadequate evidence in humans (Ref. 5).

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

The NTP (Ref. 6) 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 (Ref. 6, 7). 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 in 0 mg/kg/day; 4/49 in 2.1 mg/kg/day; l/50 in 7.1 mg/kg/day; 10/50 in 21.4 mg/kg/day). The incidences of hepatocellular adenomas or carcinomas (combined) were increased in dosed male mice; of these, the numbers of hepatocellular carcinomas were (3/50 in 0 mg/kg/day; 7/49 in 2.1 mg/kg/day; 11/50 in 7.1 mg/kg/day; 17/50 in 21.4 mg/kg/day). The female mouse

study was negative. The final conclusion of NTP (Ref. 6) 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 (Ref. 8). 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 (Ref. 3). 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/type/sex**	**TD_{50} (mg/kg/d)**
Ref. 6[a] *p*-chloroaniline HCl	50/group male B6C3F1 mice	103 weeks 5 times/ wk Gavage	50	**3:** 3; 10; 30 mg/kg (2.1; 7.1; 21.4 mg/kg/d)	Hepatocellular adenomas or carcinomas	33.8
Ref. 6 *p*-chloroaniline HCl	50/group female B6C3F1 mice	103 weeks 5 times/ wk Gavage	50	**3:** 3; 10; 30 mg/kg (2.1; 7.1; 21.4 mg/kg/d)	Negative	NA
Ref. 6 *p*-chloroaniline HCl	50/group male Fischer 344 rat	103 weeks 5 times/ wk Gavage	50	**3:** 2; 6;18 mg/kg (1.4; 4.2; 12.6 mg/kg/d)	Spleen fibrosarcoma, haemangiosarcoma, osteosarcoma	7.62
Ref. 6 *p*-chloroaniline HCl	50/group female Fischer 344 rat	103 weeks 5 times/ wk Gavage	50	**3:** 2; 6; 18 mg/kg (1.4; 4.2; 12.6 mg/kg/d)	No significant increases; equivocal	NA
Ref. 8	50/group male Fischer 344 rat	78 weeks (study duration: 102 wk) Diet	20	**2:** 250; 500 ppm (7.7; 15.2 mg/kg/d)	Mesenchymal tumors (fibroma, fibrosarcoma, haemangiosarcoma, osteosarcoma, sarcoma not otherwise specified)	72

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/d)
					of the spleen or splenic capsule	
Ref. 8	50/group female Fischer 344 rat	78 weeks (study duration: 102 wk) Diet	20	**2:** 250; 500 ppm (9.6, 19 mg/kg/d)	Negative	NA
Ref. 8	50/group male B6C3F1 mice	78 weeks (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 tumors	Not significant (CPDB)
Ref. 8	50/group female B6C3F1 mice	78 weeks (study duration: 102 wk) Diet	20	**2:** 2500; 5000 ppm (278, 558 mg/kg/d)	Haemangiosarcomas (liver and spleen). Increased incidence of combined vascular tumors	1480

Studies listed are in CPDB (Ref. 9)
[a]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 (Ref. 6, 8). These effects occur secondary to excessive compound-induced hemolysis and are consistent with a regenerative anemia (Ref. 3). The evidence supports an indirect mechanism for tumorigenesis, secondary to methemoglobinemia, splenic fibrosis and hyperplasia (Ref. 10), and not tumor induction related to a direct interaction of *p*-chloroaniline or its metabolites with DNA. Similarly, the reported induction of micronuclei *in vivo* is likely to be secondary to regenerative anemia/altered erythropoeisis, as with aniline (Ref. 11,12).

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, spleen tumors were not used to calculate the acceptable intake. Based on non-neoplastic (hematotoxic) effects, WHO (Ref. 3) recommends a level of 2 µg/kg/day, i.e., 100 µg/day for a 50 kg human.

Although the *in vitro* mutagenicity data for *p*-chloroaniline indicate small increases in mutations that are not reproducible across laboratories, a mutagenic component to a mode of action for liver tumors cannot be ruled out.

Regulatory and/or published limits

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

Acceptable intake (AI)

Because a mutagenic component to the mode of action for male mouse liver tumors cannot be ruled out, the AI was derived by linear extrapolation from the TD_{50} of 33.8 mg/kg/day for combined numbers of adenomas and carcinomas.

Calculation of AI

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

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

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

Lifetime AI = 34 µg/day

References

1. Beard RR, Noe JT. Aromatic nitro and amino compounds, Clayton GD, Clayton FE, editors. Patty's Industrial Hygiene and Toxicology. New York. John Wiley 1981; 2A:2413–89.

2. BUA. *p*-Chloroaniline. Beratergremium für Umweltrelevante Altstoffe (BUA) der Gesellschaft Deutscher Chemiker. Weinheim, VCH, 1995;171. (BUA Report 153).

3. World Health Organization (WHO). International Programme on Chemical Safety (IPCS). 2003. Concise International Chemical Assessment Document 48. 4-chloroaniline. [Online]. Available from: URL: http://www.inchem.org/documents/cicads/cicads/cicad48.htm

4. Moore, MM, Honma, M, Clements J, Bolcsfoldi G, Burlinson B, Cifone M, et al.Mouse Lymphoma Thymidine Kinase GeneMutation Assay: Follow-upMeeting of the International Workshop on Genotoxicity Testing_Aberdeen, Scotland, 2003_Assay Acceptance Criteria, Positive Controls, and Data Evaluation. Environ Mol Mutagen 2006;47:1-5.

5. IARC. Para-chloroaniline. In: Occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. International Agency for Research on Cancer, World Health Organization, Lyon. 1993;57: 305-21.

6. NTP. Technical report on the toxicology and carcinogenesis studies of para-chloroaniline hydrochloride (CAS No. 20265-96-7) in F344/N rats and B6C3F1 mice (gavage studies). National Toxicology Program, Research Triangle Park, NC. 1989. NTP TR 351.

7. Goodman DG, Ward JM, Reichardt WD. Splenic fibrosis and sarcomas in F344 rats fed diets containing aniline hydrochloride, *p*-Chloroaniline, azobenzene, o-toluidine hydrochloride, 4,4'-sulfonyldianiline, or D & C Red No. 9. J Natl Cancer Inst 1984;3:265-73.

8. NCI. Bioassay of *p*-Chloroaniline for possible carcinogenicity, CAS No. 106-47-8. US National Cancer Institute, Bethesda, MD. 1979;NCI-CG-TR-189.

9. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

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

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

12. Tweats D, Blakey D, Heflich RH, Jasobs A, Jacobsen SD, Nohmi TT, et al. Report of the IWGT working group on strategies and interpretation of regulatory *in vivo* tests. I. Increases in micronucleated bone marrow cells in rodents that do not indicate genotoxic hazards. Mutat Res 2007;627:78-91.

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

Chloro-4-nitrobenzene is mutagenic and genotoxic *in vitro* and *in vivo*.
Chloro-4-nitrobenzene was mutagenic in:
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* WP2*uvrA* (Ref. 1, 2, 3, 4). It was also weakly positive without metabolic activation in TA1535 in 2 of 4 studies (Ref. 4).

In vivo, DNA strand breaks were induced in the liver, kidney, and brain of male Swiss mice when chloro-4-nitrobenzene was administered intraperitoneally (Ref. 5, 6).

Carcinogenicity

1-Chloro-4-nitrobenzene is classified by IARC as a Group 2 carcinogen, not classifiable as to its carcinogenicity in humans (Ref. 7) and US EPA considers it to be a Group B2 carcinogen or probable human carcinogen (Ref. 8).

Animal carcinogenicity studies have been conducted with 1-chloro-4-nitrobenzene by administration in the feed to rats and mice (Ref. 9, 10) or by gavage in male rats (Ref. 12).

In a 2-year diet study (Ref. 9), 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 another diet study (Ref. 10), 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 diet 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).

The same laboratory (Ref. 10) also investigated the carcinogenic potential of 1-chloro-4-nitrobenzene in male and female CD-1 mice, given in the diet 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 an oral study (Ref. 11), 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/type/sex**	**TD_{50} (mg/kg/d)**
Ref. 9[ac]	50/group male F344 rats (SPF)	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 female F344 rats (SPF)	2 years (Diet)	50	**3:** 40; 200; 1000 ppm. (1.9; 9.8;53.8 mg/kg/d)	Pheochromo-cytoma/Female 53.8 mg/kg/d	116.9[b]
	50/group male Crj:BDF1 (SPF)	2 years (Diet)	50	**3:** 125;500; 2000 ppm. (15.3; 60.1;240 .1 mg/kg/d)	NA	
	50/group female Crj:BDF1 (SPF)	2 years (Diet)	50	**3:** 125;500; 2000 ppm. (17.6; 72.6; 275.2 mg/kg/d)	Hepatic hemangiosarcomas 275.2 mg/kg/d	1919.9
Ref. 10	14-15/ group male CD-1	18 mo Diet; sacrificed	16	**2:** Average 17 and	NA	Negative[d]

	rats	6 mo after last dose		33 mg/kg; (see text) (22.6 and 45.2 mg/kg/d)		
	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/ hemangio-sarcomas)/Male	430[d]
Ref. 11[c]	60/sex/ group Sprague Dawley rat	24 mo 5 d/ wk, Gavage	Yes	**3:** 0.1; 0.7; 5 mg/kg/d	NA	Negative

Studies listed are in CPDB (Ref. 12) unless otherwise noted..
[a]Carcinogenicity study selected for AI/PDE calculation.
[b]TD50 calculated based on carcinogenicity data (see Note 1)
[c]Not in CPDB.
[d] Histopathology limited to 11-12 tissues.
NA = Not applicable

Mode of action for carcinogenicity

1-Chloro-4-nitrobenzene is significantly metabolized by reduction to 4-chloroaniline (*p*-chloroaniline) in rats (Ref. 13), rabbits (Ref. 14) and humans (Ref. 15). *p*-Chloroaniline has been shown to produce hemangiosarcomas and spleen tumors in rats and mice, similar to 1-chloro-4-nitrobenzene (Ref. 16). Like aniline, an indirect mechanism for vascular tumorigenesis in liver and spleen was indicated, secondary to oxidative erythrocyte injury and splenic fibrosis and hyperplasia, both for 4-chloroaniline (Ref. 16) and 1-chloro-4-nitrobenzene (Ref. 17). 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 oral gavage study (Ref. 11), carried out at lower doses than the diet studies (Ref. 9, 10), methemoglobinemia and hemosiderin were seen but there was no increase in tumors.

The tumor type with the lowest TD_{50} was adrenal medullary pheochromocytomas in female rats (Ref. 9). 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 (Ref. 18). It has been proposed that these tumors 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 phosphorylation (Ref. 18) or perhaps hypoxia.

Overall, there is substantial evidence for a non-mutagenic mode of action as follows:
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 negative results of the lower-dose study (Ref. 11).

However, 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, and the pattern of mutagenicity is different from its metabolite *p*-chloroaniline, which was not consistently detected as mutagenic across laboratories, and was reproducibly mutagenic only in *Salmonella* TA98 with rat liver S9 (Ref. 19) indicating differences in mutagenic metabolites or mechanism. *In vivo* genotoxicity data are lacking to help assess potential for a mutagenic mode of action.

Since 1-chloro-4-nitrobenzene is mutagenic, and a mutagenic mode of action cannot be ruled out, an AI calculation was performed.

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

The most sensitive TD_{50} is for adrenal medullary pheochromocytomas in female rats (Ref. 9).

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

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

Lifetime AI = 117 µg/day

References

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

2. Japan Chemical Industry Ecology-Toxicology & information Center (JETOC). Japan: Mutagenicity test data of existing chemical substances based on the toxicity investigation system of the Industrial Safety and Health law. 2005 Addendum 3.

3. Kawai A, Goto S, Matsumoto Y, Matsushita H. Mutagenicity of aliphatic and aromatic nitro compounds. Sangyoigaku 1987; 29: 34-55.

4. NTP. Technical Report on Toxicity Studies on 2-Chloronitrobenzene and 4-Chloronitrobenzene (CAS Nos. 88-73-3 and 100-00-5) Administered by Inhalation to F344/N Rats and B6C4F1 Mice. National Toxicology Program, Research Triangle Park, NC. 1993;NTP TR 33.

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

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

7. IARC. Printing processes and printing inks, carbon black and some nitro compounds. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. World Health Organization, Lyon. 1996.Vol. 65.

8. US Environmental Protection Agency (USEPA). Health Effects Assessment Summary Tables. Office of Solid Waste and Emergency Response, US Environmental Protection Agency, Washington DC. 1995; No. PB95-921199.

9. Matsumoto M., Aiso S, Senoh H, Yamazaki K, Arito H, Nagano K, et al. Carcinogenicity and chronic toxicity of *para*-chloronitrobenzene in rats and mice by two-year feeding. J. Environ Pathol Toxicol Oncol 2006;25:571-84.

10. Weisburger EK, Russfield AB, Homburger F, Weisburger JH, Boger E, Van Dongen, et al. Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J Environ Pathol Toxicol 1978;2:325-56.

11. Schroeder RE, Daly JW. A chronic oral gavage study in rats with p-nitrochlorobenzene. Biodynamics Inc. 1984. Project No. 80-2487. NTIS/OTS 0536382.

12. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

13. Yoshida T, Andoh K, Tabuchi T. Identification of urinary metabolites in rats treated with *p*-chloronitrobenzene. Arch Toxicol 1991;65: 52-8.

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

15. Yoshida T, Tabuchi T, Andoh K. Pharmacokinetic study of p-chloronitrobenzene in humans suffering from acute poisoning. Drug Metab Dispos 1993;21:1142-6.

16. IARC. Occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. International Agency for Research on Cancer, World Health Organization, Lyon. 1993;57.

17. Travlos GS, Mahler J, Ragan HA, Chou BJ, Bucher JR. Thirteen-week inhalation toxicity of 2- and 4-chloronitrobenzene in F344/N rats and B6C3F1 mice. Fundam Appl Toxicol 1996;30:75-92.

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

19. WHO. CICAD 48: Concise International Chemical Assessment Document 48 *p*-Chloroaniline. Geneva. [Online]. 2003; Available from: URL: http://www.inchem.org/documents/cicads/cicads/cicad48.htm

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 (Ref. 1, 2, 3).
Big Blue transgenic mouse model with the lamda cII gene; *p*-cresidine was administered a diet of 0.25 and 0.5%, comparable to the doses in the carcinogenicity study, for 180 days (Ref. 4).

In vivo, *p*-cresidine did not induce micronuclei in bone marrow of mice (Ref. 5. 6, 7), or in p53 heterozygous or nullizygous mice (Ref. 8). Increases in micronuclei in another study in p53 heterozygous mice may be secondary to methemobolinemia and regenerative anemia as with aniline and related compounds (Ref. 9).

DNA strand breaks were not observed using the alkaline elution method in several tissues including bladder (Ref. 6; 7) but 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 (Ref. 10).

Carcinogenicity

p-Cresidine is classified by IARC as a Group 2B carcinogen, or possibly carcinogenic in humans (Ref. 11).

There is only one set of carcinogenicity studies in the standard rodent model. In NTP studies (Ref. 5) *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 (Ref. 12), were 198 and 396 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 (Ref. 13). 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 was given in feed.

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

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/ sex	TD_{50} (mg/kg/d)
Ref. 5[a]	50/sex/ group B6C3F1 mice	2 year Feed	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
Ref. 5	50/sex/ group Fisher 344 rats	2 year Feed	50	0.5 and 1% M: 198;396. F: 245;491 mg/kg/d	Urinary bladder /Male	88.4

[a]Carcinogenicity study selected for AI calculation.
Studies listed are in CPDB (Ref. 12).

Mode of action for carcinogenicity

p-cresidine is a mutagenic carcinogen, and the acceptable intake is calculated by linear extrapolation from the TD_{50}.

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 NCI/NTP (Ref. 5). 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 NCI/NTP 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

References

1. Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. Environ Mol Mutagen 1988;11 Suppl 12:1-158.

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

3. Japan Chemical Industry Ecology-Toxicology and Information Center (JETOC); Mutagenicity test data of existing chemical substances based on the toxicity investigation of the Industrial Safety and Health law; 1997; Suppl.

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

5. NCI. Technical report on the Bioassay of *p*-cresidine for possible carcinogenicity. National Toxicology Program, Research Triangle Park, NC. 1979; TR 142.

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

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

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

9. Stoll RE, Blanchard KT, Stoltz JH, Majeski JB, Furst S, Lilly PD et al. Phenolphthalein and nisacodyl: Assessment of genotoxic and carcinogenic responses in heterozygous p53 $^{(+/-)}$ mice and Syrian Hamster Embryo (SHE) assay. Toxicol Sci 2006;90:440-50.

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

11. IARC. para-Cresidine. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. World Health Organization, Lyon. 1982;27:92. reviewed in Suppl 7 1987.

12. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

13. Storer RD, French JE, Haseman J, Hajian G, LeGran EK, Long GD, et al. $p53^{+/-}$ hemizygous knockout mouse: Overview of available data. Toxicologic Pathol 2001; 29 Suppl:30-50.

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

Potential for human exposure

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

Mutagenicity/genotoxicity

1,2-Dibromoethane is mutagenic/genotoxic *in vitro* and *in vivo*. 1,2-Dibromoethane was mutagenic in the Ames mutation assay both in the presence and absence of added metabolic activation (Ref. 3-7). 1,2-Dibromoethane was positive in the mouse lymphoma assay, with and without metabolic activation (Ref. 8). It caused a dose-dependent increase in DNA repair in both spermatocytes and hepatocytes *in vitro* (Ref. 9) and induced mutations in Chinese hamster ovary (CHO) cells (Ref. 10). 1,2-Dibromoethane increased the frequencies of chromosome aberrations in a dose-dependent manner in CHO cells (Ref. 11). *In vivo* in the comet assay in rats, positive results were obtained in liver and glandular stomach following treatment with 1,2-dibromoethane at 100 mg/kg. 1,2-Dibromoethane was negative in the bone marrow erythrocyte micronucleus test in rats when tested up to 100 mg/kg (Ref. 12).

Carcinogenicity

1,2-Dibromoethane is classified by IARC as probably carcinogenic to humans (Group 2A) (Ref. 13). Inhalation and oral carcinogenicity studies are cited in CPDB (Ref. 14). 1,2-Dibromoethane was carcinogenic following both routes of administration in male and female rats and mice (Ref. 16-21). The most sensitive tumor sites were forestomach following oral administration (gavage or drinking water) and nasal cavity following inhalation. Other tumor sites include blood vessels, lung, liver and mammary glands. There was more than one positive experiment in both species.

1,2-Dibromoethane – Details of carcinogenicity studies

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses[a]	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/day)[b]
Ref. 16	30/sex/ group B6C3F1 mice	M: 65 weeks F: 73 weeks, drinking water	50	**1:** 4 mmol/L M: 116 F: 103 mg/kg/day	Forestomach/ Squamous carcinoma/Male	11.8
Ref. 17	50/sex/ group B6C3F1 mice	78 weeks, drinking water	100	**1:** M: 46.7 F: 48 mg/kg/day	Forestomach/ Squamous carcinoma/Male	9.44
Ref. 18	50/sex/ group B6C3F1 mice	53 weeks, gavage	20	**2:** M: 30, 53 F: 26, 52 mg/kg/day	Forestomach/ Squamous cell carcinoma/ Male	2.38

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses[a]	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/day)[b]
Ref. 18	50/sex/ group Osborne-Mendel rats	M: 40 weeks F: 50 weeks, gavage	20	**2:** M: 27.4, 29.2 F: 26.7, 28.1 mg/kg/day	Stomach/ Squamous cell carcinoma/ Female	1.26
Ref. 19	50/sex/ group B6C3F1 mice	M: 78 weeks, F: 96 weeks, inhalation	50	**2:** M: 19.9, 79.5 F: 23.9, 95.6 mg/kg/day	Lung/ Multiple tumor types/ Male	18.2
Ref. 19[c]	50/sex/ group F344 rats	M: 95 weeks F: 97 weeks, inhalation	50	**2:** M: 4.0, 15.9 F: 5.71, 22.8 mg/kg/day	Nasal cavity/ Carcinomas and adenocarcinoma / Female	2.33
Ref. 20	48/sex/ group Sprague-Dawley rats	78 weeks, inhalation	48	**1:** M: 9.39 F: 13.4 mg/kg/day	Nasal cavity/Multiple tumor types/Male	1.19
Ref. 21[d]	50/sex/ group B6C3F1 mice	103 weeks (10 ppm) / 90 weeks (40 ppm), inhalation	50	**2:** M and F: 3.55, 14.18 mg/kg/day	Nasal cavity/Multiple tumor types/Female	NC

NC = Not calculated

[a] mg/kg/day values stated in CPDB (Ref. 14)

[b] Individual TD_{50} values are the CPDB TD_{50} values as reported in the Lhasa carcinogenicity database (Ref. 15). TD_{50} values represent the TD_{50} from the most sensitive tumor site.

[c] Carcinogenicity study selected for AI derivation

[d] This study was conducted specifically to evaluate the types of tumors formed in the nasal cavity of mice following inhalation exposure. No other tissues were evaluated for carcinogenicity.

The most robust carcinogenicity study is the inhalation study conducted by the NTP (Ref. 19) in F344 rats. This study (duration 95 weeks in males and 97 weeks in females) included two test article treatment groups with adequate dose spacing (M: 4.0, 15.9 mg/kg/day, F: 5.71, 22.8 mg/kg/day with 50 rats/sex/group) and a control group (50/sex). The TD_{50} from the most sensitive sex and site is 2.33 mg/kg/day. Another study with inhalation exposure conducted in Sprague Dawley rats (Ref. 20) resulted in a lower TD_{50}, however the study comprised only one dose group, only 78 weeks exposure duration, and 48 animals/dose; therefore, this study was considered inferior to the NTP study with respect to estimating the TD_{50}.

The study in B6C3F1 mice with 1,2-dibromoethane administered by gavage for 53 weeks (Ref. 18) is the most robust study using the oral route of exposure. This study employed two test article dose groups (50/sex/group) in addition to a control group (20/sex). The TD_{50} from the most sensitive sex and site is 2.38 mg/kg/day. Another oral study was conducted in Osborne-Mendel rats included two dose groups, however due to insufficient dose spacing (Ref. 18) and less than

one year exposure, the study is considered less useful as it limits characterization of the dose-response relationship and estimation of the TD_{50} (Ref. 18).

Mode of action for carcinogenicity

1,2-Dibromoethane is a mutagenic carcinogen, which is expected to be mutagenic based on an alkylating mechanism of action. Therefore, the acceptable intake (AI) can be calculated by linear extrapolation from the TD_{50}. The tumor types with the lowest calculated TD_{50} (highest potency) for 1,2-dibromoethane following oral exposure are forestomach tumors in mice and rats (Ref. 18). Following inhalation exposure, the lowest calculated TD_{50} values are associated with the lung and nasal cavity for mice and rats, respectively. High concentrations of orally dosed non-mutagenic chemicals have been shown to cause inflammation and irritation after contact with the forestomach leading to hyperplasia and ultimately tumors. Substances that are dosed 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. Hence, such tumor induction is considered not relevant to humans at non-irritating doses (Ref. 22, 23). The same inflammatory and hyperplastic effects are also seen with mutagenic chemicals. However, in the case of 1,2-dibromoethane, which is a directly DNA-reactive alkylating agent and a reported multi-site, multi-species carcinogen, it is difficult to discriminate between the contribution to the mode of action of these non-mutagenic, high-dose effects compared with direct mutation induction.

Regulatory and/or published limits

No regulatory limits have been published.

Acceptable intake (AI)

Rationale for selection of study for AI calculation

1,2-Dibromoethane is a mutagenic carcinogen via the inhalation and oral routes of exposure. 1,2-Dibromoethane is considered to be a carcinogen in both mice and rats. The available toxicological data indicate that absorption of inhaled 1,2-dibromoethane occurs in several animal species. In rats, oral absorption is nearly complete within 30 minutes (Ref. 1). Therefore, it can be reasonably assumed that complete systemic exposure to 1,2-dibromoethane occurs following oral and inhalation exposure. This assumption is also supported by the observation of distal tumors in animals exposed to 1,2-dibromoethane by both routes of exposure. TD_{50} values tend to be similar across species and routes of administration.

Taking into consideration the carcinogenicity data generated by NTP in both mice and rats, the TD_{50} for the most sensitive sex/site from the most appropriate study is 2.33 mg/kg/day. This is the TD_{50} value derived from F344 female rats based on the incidence of nasal cavity tumors.

Given that the TD_{50} values recommended for the derivation of an inhalation AI and an oral AI are very similar (2.33 and 2.38 mg/kg/day, respectively), a single AI for both routes of administration is calculated below using a TD_{50} value of 2.3 mg/kg/day.

Calculation of AI

Lifetime AI = TD_{50}/50000 x 50 kg

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

Lifetime AI = 2 µg/day

References

1. ATSDR. U.S. Department of Human Health and Services, Agency for Toxic Substances and Disease Registry, Toxicological Profile for 1,2-Dibromoethane, September 2018.

2. EU Council Directive 87/ 1 81 /EEC, amending the Annex to Directive 79/117/EEC prohibiting the placing on market and use of plant protection products containing certain active substances. Official Journal of the European Communities 1987; L 71/33.

3. Moriya M, Ohta T, Watanabe K, Miyazawa T, Kato K, Shirasu, Y. Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat Res 1983;116:185-216.

4. McCann J, Choi E, Yamasaki E, Ames B. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Natl Acad Sci USA 1975;72:5135-5139.

5. Rannug U, Sundvall A and Ramel C. The mutagenic effect of 1,2-dichloroethane on *Salmonella typhimurium* 1. Activation through conjugation with glutathione in vitro. Chem Biol Interact 1978;20:1-16.

6. Strobel K, Grummt T. Aliphatic and Aromatic Halocarbons as Potential Mutagens in Drinking Water. iii. Halogenated Ethanes and Ethenes. Toxicol Environ Chem 1987;15:101-128.

7. Dunkel VC, Zeiger E, Brusick D, Mccoy E, McGregor D, Mortelmans K, Rosenkranz HS, Simmon, VF. Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and Escherichia coli. Environ Mutagen 1985;7(Suppl. 5):1-248.

8. Clive D, McCuen R, Spector JFS, Piper C, Mavournin KH. Specific gene mutations in L5178Y cells in culture: A Report of The U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 1983;115:225-251.

9. Smith-Oliver T, Working PK, White RD, Butterworth BE. Induction of DNA repair in rat spermatocytes and hepatocytes by 1,2-dibromoethane: the role of glutathione conjugation. Carcinogenesis 1986;7:467-472.

10. Tan EL, Hsie AW. Mutagenicity and cytotoxicity of haloethanes as studied in the CHO/HGPRT system. Mutat Res 1981;90:183-191.

11. Asita A. A comparative study of the clastogenic activity of ethylating agents. Mutagenesis 1989;4:432-436.

12. Takasawa H, Takashima R, Narumia K, Kawasako K, Hattoria A, Kawabata M, Shuichi Hamada S. Results of the International Validation of the *in vivo* rodent alkaline comet assay for the detection of genotoxic carcinogens: Individual data for 1,2-dibromoethane, p-anisidine, and o-anthranilic acid in the 2nd step of the 4th phase Validation Study under the JaCVAM initiative. Mutat Res 2015;786–788:144–150.

13. IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, World Health Organization (WHO). Re-Evaluation of some organic chemicals, hydrazine and hydrogen peroxide. 1999;71:641.

14. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL:https://files.toxplanet.com/cpdb/index.html

15. Lhasa Carcinogenicity Database. [Online]. Available from: URL: https://carcdb.lhasalimited.org/carcdb-frontend/

16. van Duuren BL, Melchionne S, Kline SA, Seidman I. Carcinogenicity bioassays of bromoacetaldehyde and bromoethanol- Potential metabolites of dibromoethane. Teratogenesis, Carcinogenesis and Mutagenesis 1985;5:393-403.

17. van Duuren BL, Melchionne S, Seidman I, Pereira MA. Chronic bioassays of chlorinated humic acids in B6C3F1 mice. Environmental Health Perspectives 1986;69:109-117.

18. National Toxicology Program. Bioassay of 1,2-dibromoethane for possible carcinogenicity (CAS No. 106-93-4). National Toxicology Program Technical Report 1978: TR-86.

19. National Toxicology Program. Carcinogenesis bioassay of 1,2-dibromoethane (CAS No. 106-93-4) in F344 rats and B6C3F1 mice (inhalation study). National Toxicology Program Technical Report 1982: TR-210.

20. Wong LCK, Winston JM, Hong CB, Plotnick H. Carcinogenicity and toxicity of 1,2-dibromoethane in the rat. Toxicology and Applied Pharmacology 1982;63:155-165.

21. Stinson SF, Reznik G, Ward JM. Characteristics of proliferative lesions in the nasal cavities of mice following chronic inhalation of 1,2-dibromoethane. Cancer Letters 1981;12:121-129.

22. Proctor DM, Suh M, Chappell G, Borghoff SJ, Thompson CM, Wiench K, Finch L, Ellis-Hutchings R. An Adverse Outcome Pathway (AOP) for forestomach tumors induced by non-genotoxic initiating events. Regul Toxicol Pharmacol 2018;96:30-40.

23. Proctor DM, Gatto NM, Hong SJ, Allamneni KP. Mode-of-action framework for evaluating the relevance of rodent forestomach tumors in cancer risk assessment. Toxicol Sci 2007;98:313-326.

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 with and without metabolic activation (Ref. 1, 2);

In vivo, positive results were seen in the micronucleus assay (Ref. 3).

Carcinogenicity

DMCC is classified by IARC as a Group 2A compound, or probably carcinogenic to humans (Ref. 4).

No deaths from cancer were reported in a small study of workers exposed for periods ranging from 6 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 (Ref. 5). 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% (Ref. 5).

DMCC was tested for carcinogenic activity in female ICR/Ha Swiss mice by skin application, subcutaneous injection and i.p. injection (Ref. 6; 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 i.p. 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 i.p. 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	Tumor observations	TD_{50} (mg/kg/d)
Ref. 6[a]	30 female ICR/Ha Swiss mice	64 weeks Once/wk Intra-peritoneal	30	**1:** 1 mg 5.71 mg/kg/d	Injection site : malignant tumors/Female	4.59 [e]
Ref. 5[b]	99 male Syrian golden hamsters	Lifetime 6 h/d, 5 d/wk Inhalation	50 sham treated 200 untreated	**1:** 1 ppm 0.553 mg/kg/d	Squamous cell carcinoma of nasal cavity	0.625
Ref. 6	50 female ICR/Ha Swiss mice	70 weeks 3 times/wk Skin	50	**1:** 2 mg	Skin: Papillomas and carcinomas/ Female	NA[c]
Ref. 6	50 female ICR/Ha Swiss mice	61 weeks Once/wk Subcutaneous	50	**1:** 5 mg	Injection site: Fibrosarcomas; Squamous cell carcinomas/ Female	NA[c]
Ref. 7	Male Sprague-Dawley rats	6 weeks 6 h/d, 5 d/wk Inhalation; examined at end of life	Yes	**1:** 1 ppm	Nasal tumors/Male	NA[f]
Ref. 8	30-50 female ICR/Ha Swiss mice	18-22 mo 3 times/wk Skin	Yes	**2:** 2 and 4.3 mg	Skin. Mainly skin squamous carcinoma/Female	NA[c]
Ref. 8	Female ICR/Ha Swiss mice	18-22 mo Once/wk Subcutaneous	Yes	**1:** 4.3 mg	Site of administration. Mainly sarcoma. Hemangioma, squamous carcinoma and papilloma also seen/Female	NA[d]
Ref. 8	Female ICR/Ha Swiss mice	12 mo Once/wk Subcutaneous; examined at end of life	Yes	**2:** 0.43 and 4.3 mg		NA[d]

Studies listed are in CPDB (Ref. 9) unless otherwise noted.
[a]Carcinogenicity study selected for non-inhalation AI.
[b]Carcinogenicity study selected for inhalation AI.
NA= Not applicable

[c]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.
[d]Subcutaneous and skin painting studies are not included in CPDB as route with greater likelihood of whole body exposure is considered more valuable.
[e]Histopathology only on tissues that appeared abnormal at autopsy.
[f]Examined only for nasal cancer. Does not meet criteria for inclusion in CPDB of exposure for at least one fourth of the standard lifetime.

Regulatory and/or published limits

No regulatory limits have been published.

Acceptable intake (AI)

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 AI 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* (Ref. 6), with administration by i.p. injection, was used. The TD_{50} was 4.59 mg/kg/day based on mixed tumor incidences (CPDB).

The lifetime AI is calculated as follows:

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

The inhalation AI is calculated as follows:

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 inhalation AI = 0.6 µg/day

References

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

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

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

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

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

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

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

8. Van Duuren BL, Melchionne S, Seidman I. Carcinogenicity of acylating agents: chronic bioassays in mice and Structure-Activity Relationships (SARC). J Am Col Toxicol 1987;6:479-487.

9. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

Dimethyl Sulfate (CAS# 77-78-1)

Potential for human exposure

Dimethyl sulfate (DMS) is found in ambient air with mean concentration of 7.4 µg per cubic meter or 1.4 ppb based on 1983 data compiled from a single site by the US EPA (Ref. 1).

Mutagenicity/genotoxicity

DMS is mutagenic/genotoxic *in vitro* and *in vivo* (Ref. 2).

DMS is mutagenic in:
The microbial reverse mutation assay (Ames), *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with and without activation (Ref. 3).

In vivo, DMS forms alkylated DNA bases and is consistently positive in genotoxicity assays (Ref. 4). Elevated levels of chromosomal aberrations have been observed in circulating lymphocytes of workers exposed to DMS (Ref. 4).

Carcinogenicity

DMS is classified by IARC as a Group 2A carcinogen, probably carcinogenic to humans (Ref. 4).

No epidemiological studies were available for DMS although a small number of cases of human exposure and bronchial carcinoma have been reported. DMS is carcinogenic in animals by chronic and subchronic inhalation, and single and multiple subcutaneous injections; however, DMS has not been tested by the oral route of exposure. DMS is carcinogenic in rats, mice, and hamsters (Ref. 4). 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 US EPA, Ref. 5).

DMS- Details of carcinogenicity studies

Study	Animals	Duration/ Exposure	Controls	Doses	Tumor observations	TD_{50} (mg/kg/d)
Ref. 6	Golden hamsters, Wistar rats, and NMRI mice male and female (number not clearly specified)	15 mo 6 h/d, 2 d/wk followed by 15 mo observation period Inhalation	Yes	**2:** 0.5; 2.0 ppm	Tumors in lungs, thorax and nasal passages at both doses	NA[a]
Ref. 7	20-27 BD rats Sex not specified	130 days 1 h/d, 5 d/wk followed by 643 day observation period Inhalation	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[b]
Ref. 8	8-17 BD Rats Sex not specified	394 days The duration of the study was not reported but mean tumor induction time was 500 days Subcutaneous	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[c]
Ref. 7	15 BD Rats Sex not specified	Up to 740 day evaluation Following single injection Subcutaneous	No	**1:** 50 mg/kg	Local sarcomas of connective tissue in 7/15 rats; multiple metastases to the lungs in three cases	NA[c]
Ref. 7	12 BD rats Sex not specified	800 days Once/wk Intravenous	No	**2:** 2; 4 mg/kg	No tumors reported	NA[c]

Study	Animals	Duration/ Exposure	Controls	Doses	Tumor observations	TD_{50} (mg/kg/d)
Ref. 7	8 BD rats (pregnant females)	1 year offspring observation following single dose, gestation day 15 Intravenous	No	**1:** 20 mg/kg	4/59 offspring had malignant tumors of the nervous system while 2/59 had malignant hepatic tumors.	NA[d]
Ref. 9	90 female CBAX57 Bl/6 mice	Duration not reported 4 h/d, 5 d/wk Inhalation	Not indicated	**3:** 0.4; 1; 20 mg/m^3	Increase in lung adenomas at high dose	NA[e]
Ref. 10	20 ICR/Ha Swiss mice[g]	475 days 3 times/wk Dermal	Not indicated	**h** 0.1 mg	No findings	NA[f]

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

Mode of action for carcinogenicity

Dimethyl Sulfate is a mutagenic carcinogen, and the acceptable intake is calculated by linear extrapolation from the TD50.

Regulatory and/or published limits

The European Union (EU) Institute for Health and Consumer Protection (ECHA, Ref.11) developed a carcinogenicity slope curve based on the inhalation carcinogenicity data for DMS. ECHA calculated a T_{25} (dose that resulted in a 25% increase in tumors) using the rat inhalation study (Ref. 7). 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 mortality, no control animals, only 2 dose groups and minimal pathological evaluations; therefore, the study was 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 (TTC) lifetime level of 1.5 µg/day.

Lifetime AI = 1.5 µg/day

References

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

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

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

4. IARC. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. International Agency for Research on Cancer, World Health Organization, Lyon. 1999;71:575

5. US Environmental Protection Agency. Dimethyl sulfate (CASRN 77-78-1). Integrated Risk Information System (IRIS). [Online]. 1988. Available from: URL: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0365_summary.pdf#nameddest=woe

6. Schlogel FA, Bannasch P. Carcinogenicity and Chronic Toxicity of Inhaled Dimethyl Sulfate. (In German) (Inaugural Dissertation) Julius-Maximilians University, Würzburg 1972. (data in Ref. 11).

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

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

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

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

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

Epichlorohydrin (CAS# 106-89-8)

Potential for human exposure

Epichlorohydrin is used in the synthesis of active pharmaceutical ingredients.

Mutagenicity/genotoxicity

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

Carcinogenicity

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

In an oral study, Wester et al. (Ref. 5) treated rats by oral gavage with epichlorohydrin, 5 times per week for lifetime at 2 and 10 mg/kg; when converted to an average daily dose for 7 days per week, the doses shown in the CPDB (Ref. 6) are 1.43 and 7.14 mg/kg/day, respectively. In the surviving rats at the end of the study, squamous cell carcinomas were found in the forestomach of all 24 females and 35 of 43 males at the high dose, and in 2 of 27 females and 6 of 43 males at the low dose. The tumors were considered low grade and there was no evidence of metastasis; no increase in tumors was found at other sites. At both dose levels, there were proliferative changes in the forestomach mucosa with ulceration and necrosis observed in some cases at the high dose. A TD_{50} of 2.55 mg/kg/day is reported in the CPDB. The findings are consistent with the squamous cell carcinomas seen in the forestomach of male Wistar rats treated with epichlorohydrin in drinking water for up to 81 weeks (Ref. 7). The Konishi et al. study is not included in the CPDB and not considered in this monograph because of technical deficiencies, and poor condition of the animals.

In an inhalation study, Laskin et al. (Ref. 8) treated male Sprague Dawley rats with epichlorohydrin by inhalation, 6 hours/day, 5 days/week, either for a short-term regimen (30 exposures at 100 ppm) with lifetime observation (140 rats per group), or throughout lifetime at lower doses, 10 and 30 ppm (100 rats per group). After the shorter-term and high dose exposure, squamous cell carcinomas of the nasal cavity in 15/140 rats and respiratory tract papillomas in 3/140 rats were observed and were associated with severe inflammation in the nasal turbinates, the larynx, and the trachea. After lifetime exposure, tumors were seen in 2/100 animals exposed to a dose of 30 ppm and only in the nasal cavity (1 nasal carcinoma and 1 nasal papilloma). Despite the low tumor incidence, a TD_{50} of 421 mg/kg/day is reported in the CPDB.

In a subcutaneous study, Van Duuren et al. (Ref. 9) found sarcomas at the injection site after subcutaneous injection of epichlorohydrin in mice, but no increase in tumor incidence after dermal application, and weekly i.p. injections for over 64 weeks

Epichlorohydrin – Details of carcinogenicity studies

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/day)
Ref. 5[a]	50/sex Wistar rat	104 weeks, Gavage	50	**2:** 1.43, 7.14 mg/kg/day	Forestomach /Squamous cell carcinomas / Female	2.55[b,c]
Ref. 7	18/ group Male Wistar rat	81 weeks, Drinking water	18	**3:** 375, 750, 1500 ppm. 375, 750, 1500 mg/kg/day	Forestomach / Squamous cell carcinomas	NC[d]
Ref. 8	140 Male Sprague Dawley rat	30 days, Inhalation	140	**1:** 100 ppm. 10.2 mg/kg/day	Nasal / Squamous cell Carcinomas / Male	NC[e]
Ref. 8	100 Male Sprague Dawley rat	136 weeks, Inhalation	150	**2:** 10, 30 ppm. 0.729, 2.88 mg/kg/day	Nasal / Squamous cell carcinoma / Male	421[b]
Ref. 9	50 Female ICR/Ha Swiss mouse	61 weeks, SC	150	**1:** 1 mg/once a week	Injection site sarcomas	NC[f]
Ref. 9	50 Female ICR/Ha Swiss mouse	70 weeks, Skin	150	**1:** 2 mg/3 times/week	No skin papillomas or carcinomas	NC[f]
Ref. 9	50 Female ICR/Ha Swiss mouse	64 weeks, i.p.	30	**1:** 5.71 mg/kg/day	No tumors (including no injection site sarcomas)	NC[g]

NC – Not Calculated, s.c. – Subcutaneous, i.p. - Intraperitoneal

[a] Carcinogenicity study selected for AI calculation

[b] The TD_{50} values are taken from CPDB (Ref. 6)

[c] The TD_{50} value represents the TD_{50} from the most sensitive tumor site

[d] Not calculated due to short term exposure

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

[f] Not calculated: Although TD_{50} is listed in CPDB, there was no increase in tumors

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

Mode of action for carcinogenicity

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

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

Regulatory and/or published limits

The World Health Organization (Ref. 12) published a provisional total daily intake of 0.14 µg/kg/day or 8.4 µg/day (for a 60 kg adult), based on the assumption of a non-linear dose-response for this site-of-contact carcinogen. The US EPA used linear extrapolation to derive a drinking water level (1 in 10^5 risk of excess cancer) of 30 µg/L or about 60 µg/day (Ref. 13), using data from Konishi et al. (Ref. 7). US EPA also calculated an inhalation concentration of 8 µg/m^3 for a 1 in 10^5 excess cancer risk, or 230 µg/day, using ICH Q3C assumptions for human daily breathing volume (Ref. 13).

FDA/CFSAN calculated the Unit Cancer Risk of 2.7 x 10^{-3} $(mg/kg/day)^{-1}$ using the data in Konishi et al. cited in the table above (Ref. 14). A food additive contaminant migrating into human food at an exposure of over 0.37 µg/kg or 22 µg/day would result in an estimated cancer risk over 1:1,000,000.

Acceptable intake (AI)

Rationale for selection of study for AI calculation

The oral gavage study of Wester et al. (Ref. 5) is the most robust study for calculation of the AI and the most sensitive species and tissue is rat forestomach in the gavage carcinogenicity study. The study included an appropriate dose range for measuring tumor incidence, demonstrated a clear dose-response, and provided sufficient data for the calculation of a compound specific AI.

Calculation of AI

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

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

Lifetime AI = 3 µg/day

References

1. IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 1987;71:603-628. Available from: URL: https://publications.iarc.fr/89

2. Srám RJ, Landa L, et al. Effects of occupational exposure to epichlorohydrin on the frequency

of chromosome aberrations in peripheral lymphocytes. Mutat Res 1983;122:59-64.

3. Giri AK. Genetic toxicology of epichlorohydrin: A review. Mutat Res 1977;386:25-38.

4. Canter DA, Zeiger E, et al. Comparative mutagenicity of aliphatic epoxides in *Salmonella*. Mutat Res 1986;172:105-38.

5. Wester PW, van der Heijden CA, et al. Carcinogenicity study with epichlorohydrin (CEP) by gavage in rats. Toxicology 1985;36:325-39.

6. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

7. Konishi Y, Kawabata A, et al. Forestomach tumors induced by orally administered epichlorohydrin in male Wistar rats. Gann 1980;71:922-3.

8. Laskin S, Sellakumar AR, et al. Inhalation carcinogenicity study of epichlorohydrin in noninbred Sprague-Dawley rats. J Natl Cancer Inst 1980;65:751-7.

9. Van Duuren BL, Goldschmidt BM, et al. Carcinogenic activity of alkylating agents. J Natl Cancer Inst 1974;53:695-700.

10. Stoner GD, Conran PB, Greisiger EA, Stober J, Morgan M, Pereira MA. Comparison of two routes of chemical administration on the lung adenoma response in strain A/J mice. Toxicol Appl Pharmacol 1986;82:19-31.

11. Daniel FB, Robinson M, et al. Toxicity studies of epichlorohydrin in Sprague-Dawley rats. Drug Chem Toxicol 1996;19:41–58.

12. World Health Organization (WHO). Epichlorohydrin in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. 2004; WHO/SDE/WSH/03.04/94.

13. United States Environmental Protection Agency (USEPA). Integrated Risk Information System (IRIS). Epichlorohydrin (CASRN 106-89-8) Cancer Assessment.1988; Available from: URL: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=50

14. United States Food and Drug Administration (USFDA). Indirect food additives: Paper and paperboard components. Available from URL: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-176

Ethyl bromide (CAS# 74-96-4)

Potential for human exposure

Ethyl bromide (bromoethane) is a colorless volatile and flammable liquid. It is an alkylating agent used primarily as a reagent in synthesis of pharmaceuticals. Its close analog, ethyl chloride, which also has a monograph in ICH M7, is a mutagenic carcinogen.

Mutagenicity/genotoxicity

Ethyl bromide was mutagenic in the Ames test Ames in *Salmonella typhimurium* TA98, TA100, TA104 with metabolic activation and mutagenic in TA97 with and without metabolic activation using the plate incorporation method (Ref.1). As ethyl bromide is a volatile and hydrophobic compound, it was also tested in a modified Ames mutation assay as vapor in a closed desiccator. In this system, ethyl bromide was mutagenic in TA100 and TA1535, but not in TA98, with and without metabolic activation (Ref. 2, 3, 4). Other Ames mutation assays with rat and hamster S9 using the preincubation method showed negative results, most likely due to the volatile nature of ethyl bromide (Ref. 4, 5, 6).

In cultured CHO cells, ethyl bromide induced sister chromatid exchanges (SCEs) but not chromosomal aberrations in both the presence and absence of exogenous metabolic activation (Ref. 7).

Carcinogenicity

The IARC determined that ethyl bromide is not classifiable as to its carcinogenicity to humans (Ref. 8). There are no epidemiological data relevant to carcinogenicity and limited evidence in experimental animals for the carcinogenicity of ethyl bromide.

In animals, evidence of carcinogenicity was identified from a 2-year bioassay from the National Toxicology Program (NTP) that evaluated ethyl bromide by inhalation administration in rats and mice. A variety of effects (dependent on species and sex) were seen with exposures of 100, 200, or 400 ppm 6 hours/day, 5 days/week (Ref. 9).

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

Ethyl Bromide – Details of carcinogenicity studies

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses [a]	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/day)[b]
Ref. 9	50/sex/ group B6C3F1 mice	105 weeks, Inhalation	50	**3:** M: 115, 229, 458 F: 137, 275, 550 mg/kg/day	Uterus / Female	535
Ref. 9	50/sex/ group F344/N Rats	106 weeks, Inhalation	50	**3:** M: 22.9, 45.8, 91.7 F: 32.7, 65.5, 131 mg/kg/day	Adrenal / Male	149 [c]
Ref. 9	50/sex/ group F344/N Rats	106 weeks, Inhalation	50	**3:** M: 22.9, 45.8, 91.7 F: 32.7, 65.5, 131 mg/kg/day	Liver	670

[a] mg/kg/day values stated in CPDB (Ref. 10) and calculated by method used to standardize average daily dose levels from variety of routes of administration, dosing schedules, species, strains and sexes; values stated in CPDB accounted for exposure duration of 24 h per day for 7 days per week. (Dose rate = (administered dose × intake/day × number of doses/week) / (animal weight × 7 days/week))
[b] TD_{50} calculated in CPDB
[c] Carcinogenicity study selected for AI calculation

Mode of action for carcinogenicity

Ethyl bromide is an alkylating agent. It is a mutagenic carcinogen, and the AI is calculated by linear extrapolation from the TD_{50}.

Regulatory and/or published limits

For ethyl bromide, the ACGIH threshold limit value-time-weighted average (TLV-TWA) for ethyl bromide is 5 ppm (22 mg/m^3), while OSHA and NIOSH indicate the TWA as 200 ppm (890 mg/m^3) (Ref. 11). The ACGIH estimates this value with a notation for skin absorption, but OSHA and NIOSH estimates are based on inhalation studies.

Acceptable intake (AI)

Rationale for selection of study for AI calculation

Ethyl bromide is a mutagenic carcinogen via the inhalation route of exposure. Although no information on the inhaled bioavailability of ethyl bromide was found, organic solvents have high inhalation bioavailability values (Ref. 12) and systemic exposure via inhalation route has been demonstrated in multiple studies by clinical observations (Ref. 13). Neoplastic lesions were observed in multiple organs where systemic exposure is indicated in mice and rats in addition to the site-of-contact tissues (e.g., lung). Therefore, it is reasonable to apply the AI derived from inhalation studies for other routes of administration.

Considering all the available data from the inhalation studies in rats and mice, the most sensitive tumor endpoint was the combined pheochromocytoma and malignant pheochromocytomas of the adrenal gland in male F344/N rats, The TD_{50} calculated by CPDB for this endpoint was based on tumor incidences which were not statistically significant by trend test . However, the tumor incidence of each dose was statistically different from the tumor incidence of the control . Therefore, the effect is considered relevant. The calculated TD_{50} values for each dose are 32.2 mg/kg/day for low dose, 115 mg/kg/day for mid dose, 162 mg/kg/day for high dose (Note 2). The lowest TD_{50} value of 32.2 mg/kg/day is used as it is considered to conservatively yield the most sensitive potency estimate for calculating the AI.

Calculation of AI

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

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

Lifetime AI = 32 µg/day

References

1. Strubel K, Grummt T. Aliphatic and aromatic halocarbons as potential mutagens in drinking water. III. Halogenated ethanes and ethenes. Toxicology and Environmental Chemistry 1987;15:101–128.

2. Barber ED, Donish, WH, Mueller KR, A procedure for the quantitative measurement of the mutagenicity of volatile liquids in the Ames Salmonella/microsome assay, Mutation Research, 1981; 90:31-48

3. Simmon VF. Applications of the Salmonella/Microsome Assay. In: Stich HF, San RHC (eds) Short-Term Tests for Chemical Carcinogens. Topics in Environmental Physiology and Medicine, 1981;120-126

4. Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. Salmonella Mutagenicity tests:V. Results from the Testing of 311 Chemicals, Environ Molec Mutagen 1992;19:Suppl 21:2-141

5. Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E. Salmonella Mutagenicity Test Results for 250 Chemicals. Environ Mut 1983; Suppl 1:3-142

6. WHO, Bromoethane, Concise International Chemical Assessment Document 42, 2002

7. Loveday KS, Lugo MH, Resnick MA, Anderson BE, Zeiger E. Chromosome aberration and sister chromatid exchange tests in vitro in Chinese hamster ovary cells in vitro: II. Results with 20 chemicals. Environ. Molec Mutagen 1989; 13: 60-94

8. NTP. Toxicology and Carcinogenesis Studies of Bromoethane (EthylBromide) (CAS NO. 74-96-4) in F344/N Rats and B6C3F Mice (Inhalation Studies). National Toxicology Program, US Dept Health and Human Services. 1989; NTP-TR 363.

9. IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 1999;71:1305-1307.

10. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL:

https://files.toxplanet.com/cpdb/index.html

11. NIOSH. The National Institute for Occupational Safety and Health (NIOSH) Publications & Products. Available from: URL: https://www.cdc.gov/niosh/idlh/74964.html

12. Fiserova-Bergerova, V. Toxicokinetics of organic solvents. Scand J Work Environ Health 1985;11:suppl 1, 7-21.

13. Sayers RR, Yant WP, Thomas BGH, Berger LB. Physiological response attending exposure to vapors of methyl bromide, methyl chloride, ethyl bromide and ethyl chloride. Public Health Bull 1929;185:1-5

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

Potential for human exposure

Low levels (parts-per-trillion) from contaminated ambient air and drinking water. Dermal contact as a topical anesthetic.

Mutagenicity/genotoxicity

Ethyl chloride is mutagenic and genotoxic *in vitro* but not *in vivo*. IARC (Ref. 1) 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 (Ref. 2, 3, 4);
CHO cell *hprt* assay with and without metabolic activation.

In vivo ethyl chloride was negative in a mouse bone marrow micronucleus test after inhalation at approximately 25,000 ppm for 3 days, and in an Unscheduled DNA Synthesis (UDS) assay in female mouse liver (Ref. 5).

Carcinogenicity

Ethyl chloride was designated by IARC as Class 3, or not classifiable as to its carcinogenicity (Ref. 1).

Only one carcinogenicity study was found for ethyl chloride, NTP studies (Ref. 6) in rats and mice of both sexes *via* inhalation for 6 h/day, 5 days/week for 100 weeks. The single exposure concentration (15,000 ppm) tested 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 assessed by US EPA (Ref. 7), 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)
Ref. 6, 7[a]	50/sex/ group B6C3F1 mice	100 weeks 6 h/d, 5 d/wk Inhalation	50	**1:** M: 10.4 F: 12.4 g/kg/d	Uterus/Female	1810

Ref. 6, 7	50/sex/ group Fischer 344 rats	100 weeks 6 h/d, 5 d/wk Inhalation	50	**1:** M: 2.01 F: 2.88 g/kg/d	Negative	NA

[a]Carcinogenicity study selected for AI calculation. Studies listed are in CPDB (Ref. 8).
NA = Not applicable

Mode of action of carcinogenicity

Holder (Ref. 7) 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 lead to adrenal stimulation. It was proposed that 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 (Ref. 9).

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 affected compared with 0/49 controls) suggest a strong carcinogenic response. The observation is supported by the fact that the same type of tumors (mouse uterine tumors) was seen with a comparator molecule ethyl bromide, in a more robust carcinogenicity study with 3 doses and a control (Ref. 10).

Ethyl chloride is considered to be a mutagenic carcinogen. Based on the NTP inhalation study the most sensitive species/site is female mouse uterus. Since the number of tumors is high, it is possible to calculate a TD_{50} even though only one dose was tested. The authors of the CPDB (Ref. 8) converted 0 and 15,000 ppm to doses of 0 and 12.4 g/kg and calculated a TD_{50} of 1810 mg/kg/day for mouse uterine tumors.

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

References

1. IARC. Chloroethane. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. International Agency for Research on Cancer, World Health Organization, Lyon. 1999;71:1345.

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

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

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

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

6. NCI/NTP Technical Report on the toxicology and carcinogenesis studies of chloroethane. National Toxicology Program, Research Triangle Park, NC. NTP TR 346 1989. [Online]. 1989; Available from: URL: https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr346.pdf

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

8. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

9. US Environmental Protection Agency. Ethyl Chloride (CAS# 75-00-3). Integrated Risk Information System (IRIS)... [Online] 1991. Available from: URL: https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=523

10. NTP. Technical Report on the toxicology and carcinogenesis studies of Ethyl Bromide. National Toxicology Program, Research Triangle Park, NC. NTP TR 363. [Online]. 1989; Available from: URL: http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr363.pdf

Formaldehyde (CAS# 50-00-0)

Potential for human exposure

Formaldehyde exposure occurs in air, water, and food, and it is a common endogenous component of biological materials and is a naturally occurring component of many foods such as meat, dairy products, fruit and vegetables. Levels of daily exposure to formaldehyde via the dietary route have been estimated in the range of 1.5-119 mg/day (Ref. 1,2). Formaldehyde is also a product of normal human metabolism and is essential for the biosynthesis of certain amino acids. The human body produces and uses 53-92 g (878-1210 mg/kg b.w./day for a 60-70 kg person) of formaldehyde per day, which is rapidly metabolized and cleared from blood plasma (Ref. 2). Formaldehyde is used in the synthesis and formulation of pharmaceuticals. In some cases, formaldehyde can function as the active ingredient in a drug (Ref. 3). Formaldehyde is also found as a component of some consumer products and can be produced during cooking or smoking (Ref. 1).

Mutagenicity/genotoxicity

Formaldehyde is a mutagenic compound (Ref. 4,5). Formaldehyde induced mutations in the Ames test with and without S9 activation. It induced deletions, point mutations, insertions, and cell transformations in mammalian cells (Ref. 4-5). Formaldehyde is also clastogenic causing chromosomal aberrations, micronuclei, and sister chromatid exchanges in rodent and human primary cell lines. *In vivo* studies have also detected genotoxic effects primarily at the site of contact (Ref. 4).

Carcinogenicity

IARC considers formaldehyde to be a Group 1 carcinogen, that is carcinogenic in humans based on cancer of the nasopharynx and leukemia (Ref. 6). The European Committee for Risk Assessment has assigned formaldehyde to Category 1B (may cause cancer) based on sufficient evidence from animal studies, but only limited evidence of carcinogenicity in humans (Ref. 7). There are several oral and inhalation animal studies conducted with formaldehyde (summarized in table below). The carcinogenicity of formaldehyde is specific to inhalation, whereas the weight of evidence indicates formaldehyde is not carcinogenic via the oral route (Ref. 6, 8-10). Formaldehyde was negative in oral carcinogenicity studies in rodents. In carcinogenicity studies conducted by the inhalation route, tumors in the nasal cavity have been observed in rodents.

The nasal tumors observed following inhalation of formaldehyde have been attributed to continuous cycles of tissue degeneration and regeneration (cytolethality/regenerative cellular proliferation; CRCP) rather than to a direct genotoxic effect (Ref. 11). Formation of DNA-protein crosslinks (DPX) is probably involved in the cytolethality. Predicted additional cancer risks for an 80-year continuous environmental exposure to formaldehyde was modeled, with the risk predictions obtained from what Conolly et al. (Ref. 11) expected to be significant overestimates of real-world exposures to formaldehyde.

In agreement with IARC (Ref. 6) the US EPA (Ref. 12) and NTP 15th Report on Carcinogens conclude that nasopharyngeal cancer and myeloid leukemia (ML) in humans can be attributed to formaldehyde exposure (Ref. 13). The conclusion that formaldehyde causes cancer has been peer reviewed by the National Academy of Science (Ref. 14). The reviews acknowledged that hazard identification for formaldehyde was not straightforward, especially with respect to possible leukemogenicity, in part due to its endogenous production and high reactivity. The most useful studies on the risk of formaldehyde causing ML are the large cohort studies of chemical workers

and embalmers (Ref. 15, 16), which conclude that there is a causal association between formaldehyde exposure and mortality from ML (Ref. 15, 16). In contrast the European Committee for Risk Assessment concluded that formaldehyde is not a human systemic carcinogen (Ref. 7). Albertini and Kaden (Ref. 17) concluded that overall, the available literature on genetic changes following formaldehyde exposure did not provide convincing evidence that exogenous exposure, and specifically exposure by inhalation, induces mutations as a direct DNA-reactive effect at sites distant from the portal-of-entry tissue. This would include proposed mode of actions that involve a stem cell effect at the port of entry with circulation back to the bone marrow. Mutations in the bone marrow or in any other tissues beyond the point of contact have not been observed.

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

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

Formaldehyde – Details of carcinogenicity studies

Study	**Animals/ dose group**	**Duration/ Exposure**	**Controls**	**Doses**	**Most sensitive tumor site/type/sex**	**TD_{50} (mg/kg/day)**
Ref. 23	42-60/ group C3H Mouse	35- or 64-weeks, Inhalation	59	**3:** 50, 100, 200 mg/m^3	No tumors	NC
Ref. 24	40-54/sex / group B6C3F1 Mouse	2 years, Inhalation	50-62	**3:** 2, 5.6, 14.3 ppm M: 0.644, 1.93, 4.83	Nasal turbinates/ Squamous cell carcinoma/ Male	43.9[b]

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/day)
				F: 0.686, 2.06, 5.15 mg/kg/day[a]		
Ref. 24	73-80/sex/ group F344 Rat	2 years, Inhalation	79	**3:** 2, 5.6, 14.3 ppm M: 0.129, 0.386, 0.965 F: 0.184, 0.552, 1.38 mg/kg/day[a]	Nasal turbinates/ Squamous cell carcinoma/ Male	0.798[b]
Ref. 25	100/ group Male Sprague Dawley Rat	Lifetime, Inhalation	99	**1:** 14.8 ppm 0.952 mg/kg/day[a]	Nasal mucosa / Squamous cell carcinoma/ Male	1.82[b]
Ref. 26	45/group Male Wistar Rat	4, 8 or 13 weeks, Inhalation	134	**2:** 10, 20 ppm	Nasal cavity / Male	NC[c]
Ref. 27	30/group (Undamaged) Male Wistar Rat	3- or 28-months, Inhalation	30	**3:** 0.1, 1.0; 10 ppm	No tumors for undamaged animals[d]	NC
Ref. 28	15-16/ group Female Sprague Dawley Rat	24 months, Inhalation	16	**1:** 12.4 ppm	Nasal cavity/ One squamous cell carcinoma	NC
Ref. 29	47-97/ group Male F344 Rat	24 months, Inhalation	46	**5:** 0.7, 2, 6, 10, 15 ppm 0.045, 0.129, 0.386, 0.643, 0.965 mg/kg/day[a]	Nasal cavity /Squamous cell carcinoma/ Male	0.48[b]
Ref. 30	20-22/ group Male F344 Rat	28 months, Inhalation	22	**3:** 0.3, 2, 15 ppm 0.0193, 0.129, 0.965 mg/kg/day[a]	Nasal cavity /Mixed tumor type/ Male	0.98[b]

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/day)
Ref. 31	88/ group Male Syrian Golden Hamster	Lifetime, Inhalation	132	**1:** 10 ppm	No tumors	NC
Ref. 32	70/sex/ group Wistar Rat	2 years, Drinking water	70	**3:** M: 1.2, 15, 82 F: 1.8, 21, 109 mg/kg/day	No tumors	NC
Ref. 33	50/sex/ group Sprague Dawley Rat	Lifetime, Drinking water	50	**7:** 10, 50, 100, 500, 1000, 1500, 2500 ppm M: 0.359, 1.79, 3.59, 17.9, 35.9, 53.8 F: 0.410, 2.05, 4.10, 20.5, 41.0, 61.5 mg/kg/day[a]	Lymphoblastic leukemia-lymphosarcoma / Male[e]	424[b]
Ref. 34	20/sex/ group Wistar Rat	24 months, Drinking water	20	**3:** 10, 50, 300 mg/kg/day	No tumors	NC

NC – Not Calculated

[a] mg/kg/day doses are taken from CPDB

[b] TD_{50} taken from the CPDB (Ref. 35)

[c] Not calculated given the limited duration of dosing

[d] After 28 months of exposure animals damaged by electrocoagulation experienced an increase in nasal cavity tumors

[e] There were concerns about study design (pooling of lymphomas and leukemias diagnosed, lack of reporting of non-neoplastic lesions and historical control data, discrepancies of data between this study and Sofritti (Ref. 36) [second report of this study], and lack of statistical analysis) (Ref. 4, 6, 10).

Mode of action for carcinogenicity

Formaldehyde was only clearly carcinogenic in studies conducted by the inhalation route in rodents. Tumors in the nasal cavity have been observed and are considered a site of contact effect in rodents. The nasal tumors observed following inhalation of formaldehyde were attributed to continuous cycles of tissue degeneration and regeneration (cytolethality/regenerative cellular proliferation) rather than to a direct genotoxic effect (Ref. 11). Formation of DPX is probably involved in the cytolethality of formaldehyde but not considered as the driving mechanism to carcinogenicity. In recent reviews of the mode of action of formaldehyde and relevance of rat nasal tumors to humans, the role of cytotoxicity and regenerative cell proliferation was reaffirmed.

The reviews indicate that although DPX are a good biomarker of exposure, they may not meaningfully contribute to cancer via genotoxic effects except at concentrations that result in tissues levels well above endogenous levels (Ref. 37, 38).

Regulatory and/or published limits

For oral exposure to the general population, the ATSDR, Health Canada, International Programme on Chemical Safety (IPCS), and US EPA limit for formaldehyde is 0.2 mg/kg/day or 10 mg/day for a 50 kg person, which is based on a non-cancer endpoint (reduced weight gain and histological changes to the gastrointestinal tract and kidney) (Ref. 10, 12, 39, 40). No oral carcinogenicity risk estimates exist for formaldehyde, since carcinogenicity is specific to the inhalation route of exposure.

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

For inhalation exposure to the general population, the US EPA, IPCS, and Health Canada have developed inhalation cancer risk values (Ref. 12, 10, 40). The US EPA limit is based on a linear cancer model, and Health Canada/IPCS developed nonlinear and linear cancer models. Using the linear method from all three agencies, a daily inhaled dose of 16-32 µg/day would result in a 1 in 10^5 excess risk of cancer. However, more recent scientific analysis supports the use of the Health Canada/IPCS nonlinear model, which incorporates mechanistic data (Ref. 11, 41-43). Conolly et al. (Ref. 11, 41) developed a nonlinear / linear mechanistic-based model using empirical rodent and human data for the two modes of action with formaldehyde tumorigenicity: CRCP and DPX.

Acceptable intake (AI) for inhalation exposure

Rationale for selection of study for AI calculation

The AI for inhalation is based on the carcinogenicity model developed by Conolly et al. (Ref. 11). Figure 1 represents the dose-response hockey stick-shaped model developed by Conolly et al., for a mixed population of smokers and non-smokers. The rat dose-response for CRCP/DPX was used by Connolly for the human model in absence of an alternative model. Since the exposure related tumor risk predicted by clonal growth models was extremely sensitive to cell kinetics, Conolly decided to evaluate human cancer risk associated with formaldehyde exposure using both the raw J-shaped dose-response and a hockey stick–shaped transformation of the rat data. This model incorporates the non-linear-based mechanism at the high dose region (CRCP) and the linear mechanism at the low dose region (DPX). As noted above, the translation of DPX into mutations and an assumed linear dose-response emerging from such mutations is not established experimentally. Moreover, experimental results suggest that DPX are not leading to mutations in a linear fashion. Thus, the linear dose-response model at low doses reflects a conservative and practical approach and is not dictated by experimental data.

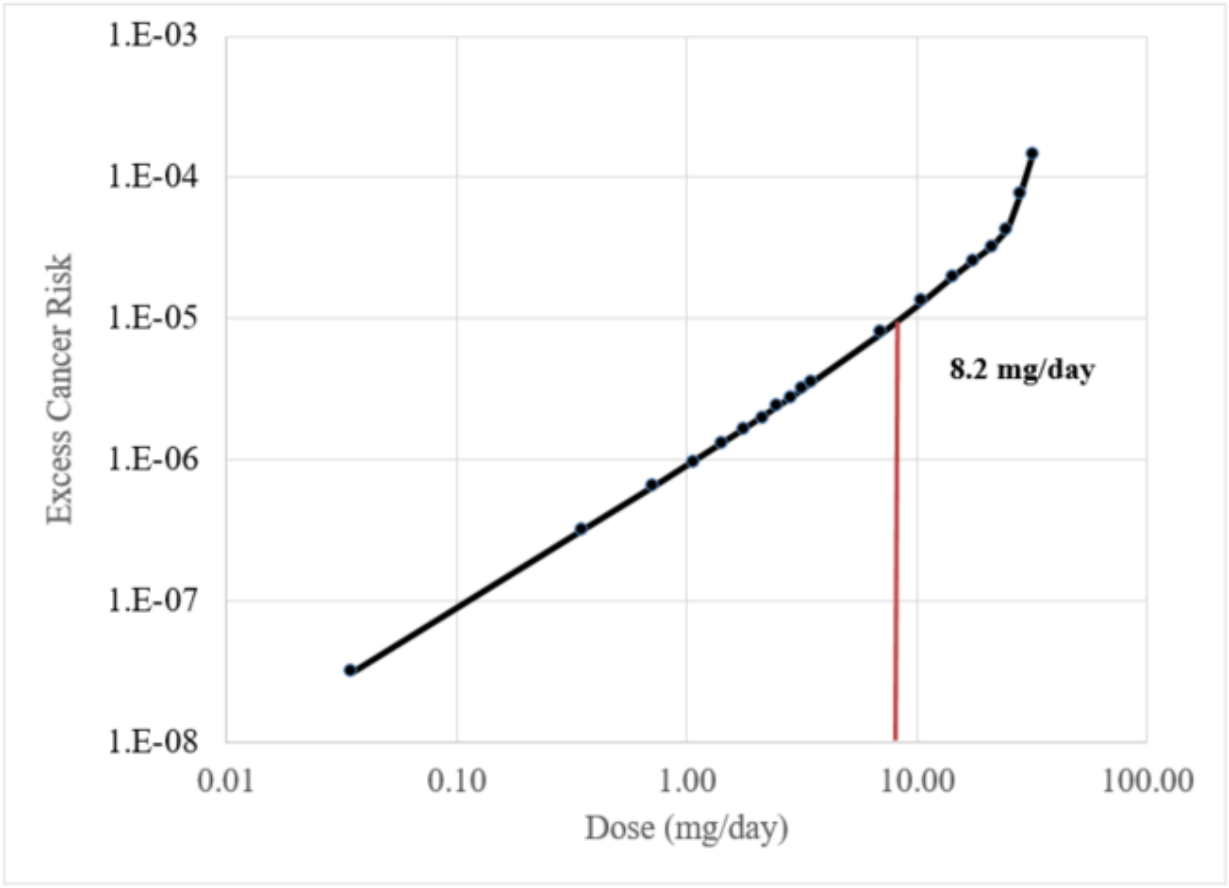


Figure 1. Dose-response model hockey stick-shaped model from (Ref. 11) representing mixed smokers and non-smokers. The dose (mg/day) was based on converting air concentration (ppm) to daily dose using ICH Q3C assumptions for human breathing volume (28,800 L/day).

Calculation of inhalation AI

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

Risk (y) = 1.62E-06x(dose) – 3.27E-06
0.00001 = 1.62E-06x – 3.27E-06
x = (0.00001 + 3.27E-06) / 1.62E-06
Dose (x) = 8.2 mg/day

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

Rationale for the concentration limit

Formaldehyde is considered a mutagenic carcinogen by the inhalation route of exposure. The acceptable intake of 8 mg/day represents an upper limit over a 24 hour time period which is

considered acceptable as it limits excess cancer risk to 1 in 100,000. As described in the introduction section of Appendix 3 of this guideline, "other considerations" may affect final product specifications. Formaldehyde is known to cause local irritation and sensitization effects. Therefore, WHO recommends a limit of 81.4 ppb in air as a 30 minute average (Ref. 44) and Health Canada recommends a short-term exposure limit of 100 ppb as a 1 hour average (Ref. 45). These recommended values provide at least a 10-fold margin of exposure to the lowest level at which symptoms have been observed. To protect patients from the potential for local irritation and sensitization effects of formaldehyde by the inhalation route of exposure, a concentration-based limit of 215 ppb is recommended. [8 mg/day over 24 hours of exposure is equal to a concentration limit of 215 ppb]. That is:

(0.008 g/day / 28.8 m^3/day) * 1 / 1293 g/m^3) = 215 ppb

- Human breathing volume/day = 28.8 m^3 /day
- Air mass/m^3 at standard conditions = 1293 g

The limit of 215 ppb could either be interpreted as the concentration of formaldehyde in air (which is the basis of the exposure limit), or the concentration of formaldehyde relative to drug substance. See Note 3 for examples to clarify how the 215 ppb limit in air relates to a limit in API or DP.

Permissible Daily Exposure (PDE) for all other routes

See Section 4 of the Introduction to this Addendum that addresses formaldehyde exposure from the environment.

PDE (all other routes) = 10 mg/day

References

1. Organization for Economic Co-operation and Development (OECD). SIDS Initial Assessment Report. Formaldehyde. 2002.

2. EFSA 2014. Scientific Report. Endogenous formaldehyde turnover in humans compared with exogenous contribution from food sources. EFSA Journal 2014;12(2):3550. Available online, https://www.efsa.europa.eu/de/efsajournal/pub/3550

3. Bingham E, Cohrssen B, Powell CH. Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001) p. 5:981.

4. IARC (2006). Formaldehyde, 2-butoxyethanol and 1-tertbutoxypropan-2-ol. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 88:1–478. PMID:17366697.

5. Ma TH, Harris MM. Review of the genotoxicity of formaldehyde. Mutat Res 1988;196:37-59.

6. IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Chemical agents and related occupations. Volume 100 F. 2012.

7. Committee for Risk Assessment. RAC Opinion proposing harmonised classification and labelling at EU level of Formaldehyde. https://echa.europa.eu/documents/10162/b8dfa022-9544-72e8-dcaa-7491dff3c0

8. World Health Organization (WHO). Background document for development of WHO guidelines for drinking-water quality. 2005; WHO/SDE/WSH/05.08/48.

9. Scheuplein, RJ. Formaldehyde: The Food and Drug Administration's perspective. Chapter 16. in: Turoski, V (Ed.), Formaldehyde: Analytical Chemistry and Toxicology. American Chemical Society, Washington DC. 1985;210:237-45.

10. International Programme on Chemical Safety (IPCS) Concise International Chemical Assessment Document 40. Formaldehyde. 2002.

11. Conolly RB, Kimbell JS, et al. Human respiratory tract cancer risks of inhaled formaldehyde: dose-response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. Toxicol Sci 2004;82:279-96.

12. United States Environmental Protection Agency (USEPA). Integrated Risk Information System (IRIS). Formaldehyde (CASRN 50-00-0) 1990.

13. National Toxicology Program. 2021. Formaldehyde CAS No. 50-00-0. Report on Carcinogens, Fifteenth Edition.; Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service.

14. National Research Council. 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Washington, DC: The National Academies Press, ISBN 978-0-309-21193-2.

15. Hauptmann M, Lubin JH, Stewart PA, Hayes RB, Blair A. Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries. J Natl Cancer Inst 2003;95:1615–1623.

16. Hauptmann M, Stewart PA, Lubin JH, Freeman LEB, Hornung RW, Herrick RF, Hoover RN, Fraumeni JF, Blair A, Hayes, RB. Mortality from lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde. J Natl Cancer Inst 2009;101:1696–1708.

17. Albertini RJ, Kaden DA. Do chromosome changes in blood cells implicate formaldehyde as a leukemogen? Crit Rev Toxicol 2017;47:145-184.

18. Morgan DL, Dixon D, King DH, Travlos GS, Herbert RA, French JE, Tokar EJ, Waalkes MP, Jokinen MP. NTP Research Report on Absence of Formaldehyde-Induced Neoplasia in Trp53 Haploinsufficient Mice Exposed by Inhalation: Research Report 3. Durham (NC): National Toxicology Program; 2017. PMID: 30016014

19. Lu K, Collins LB, Ru H, Bermudez E, Swenberg JA. Distribution of DNA adducts caused by inhaled formaldehyde is consistent with induction of nasal carcinoma but not leukemia. Toxicol Sci 2010;116:441-451.

20. Edrissi B, Taghizadeh K, Moeller BC, Kracko D, Doyle-Eisele M, Swenberg JA, Dedon PC. Dosimetry of N^6-formyllysine adducts following [$^{13}C^2H_2$]-formaldehyde exposures in rats.

Chem Res Toxicol 2013;26:1421-1423.

21. Yu R, Lai Y, Hartwell HJ, Moeller BC, Doyle-Eisele M, Kracko D, Bodnar WM, Starr TB, Swenberg JA. Formation, accumulation, and hydrolysis of endogenous and exogenous formaldehyde-induced DNA damage. Toxicol Sci 2015;146:170-182.

22. Moeller BC, Lu K, Doyle-Eisele M, McDonald J, Gigliotti A, Swenberg JA. Determination of N2-hydroxymethyl-dG adducts in the nasal epithelium and bone marrow of non-human primates following $^{13}CD_2$-formaldehyde inhalation exposure. Chem Res Toxicol 2011;24:162-164.

23. Horton AW, Tye R, Stemmer KL. Experimental carcinogenesis of the lung. Inhalation of gaseous formaldehyde or an aerosol of coal tar by C3H mice. J Natl Cancer Inst 1963;30:31-43.

24. Kerns WD, Pavkov KL, Donofrio DJ, Gralla EJ, Swenberg JA. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Res 1983;43:4382-4392.

25. Sellakumar AR, Snyder CA, Solomon JJ, Albert RE. Carcinogenicity of formaldehyde and hydrogen chloride in rats. Toxicol Appl Pharmacol 1985;81:401-406.

26. Feron VJ, Bruyntjes JP, Woutersen RA, Immel HR, Appelman LM. Nasal tumours in rats after short-term exposure to a cytotoxic concentration of formaldehyde. Cancer Lett 1988;39:101-11.

27. Woutersen RA, van Garderen-Hoetmer A, Bruijntjes JP, Zwart A, Feron V J. Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. J Appl Toxicol 1989;9:39-46.

28. Holmström M, Wilhelmsson B, Hellquist H. Histological changes in the nasal mucosa in rats after long-term exposure to formaldehyde and wood dust. Acta Oto-Laryngologica 1989;108:274-283.

29. Monticello TM, Swenberg JA, Gross EA; Leininger JR, Kimbell JS, Seilkop S, Starr TB, Gibson JE, Morgan KT. Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. Cancer Res 1996;56:1012-1022.

30. Kamata E, Nakadate M, Uchida O, Ogawa Y, Suzuki S, Kaneko T, Saito M, Kurokawa Y. Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats. J Toxicol Sci 1997;22:239-254.

31. Dalbey WE. Formaldehyde and tumors in hamster respiratory tract. Toxicology 1982;24:9-14.

32. Til HP, Woutersen R, Feron VJ, Hollanders VHM, Falke HE. Two-year drinking-water study of formaldehyde in rats. Fd Chem Toxic 1989;27:77-87.

33. Soffritti M, Maltoni C, Maffei F, Biagi R. Formaldehyde: an experimental multipotential carcinogen. Toxicol Ind Health 1989;5:699-730.

34. Tobe M, Naito K, Kurokawa Y. Chronic toxicity study on formaldehyde administered orally

to rats. Toxicology 1989;56:79-86.

35. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

36. Soffritti M, Belpoggi F, et al. Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. Ann N Y Acad Sci 2002;982:87–105.

37. Thompson CM, Gentry R, Fitch S, Lu K, Clewell HJ. An updated mode of action and human relevance framework evaluation for Formaldehyde-Related nasal tumors, Critical Reviews in Toxicology 2020;50:919-952.

38. SCOEL/REC/125 Formaldehyde Recommendation from the Scientific Committee on Occupational Exposure Limits, 2016. https://op.europa.eu/en/publication-detail/-/publication/7a7ae0c9-c03d-11e6-a6db-01aa75ed71a1/language-en

39. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Formaldehyde. U.S. Department of Health and Human Services, Public Health Service. 1999; Available from: URL: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=220&tid=39

40. Health Canada. Priority substances list assessment report: Formaldehyde. Ottawa. Ministry of Public Works and Government Services. 2001; Available from: URL: http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/index_e.html

41. Conolly RB, Kimbell JS, Janszen D, Schlosser, PM, Kalisak D, Preston J, Miller FJ. Biologically motivated computational modeling of formaldehyde carcinogenicity in the F344 rat. Toxicol Sci 2003;75:432-447.

42. Starr TB, Swenberg JA. A novel bottom-up approach to bounding low-dose human cancer risks from chemical exposures. Regul Toxicol Pharmacol 2013;65:311-315.

43. Swenberg JA, Moeller BC, Lu K, Rager JE, Fry RC, Starr TB. Formaldehyde carcinogenicity research: 30 years and counting for mode of action, epidemiology, and cancer risk assessment. Toxicol Pathol 2013;41:181-189.

44. World Health Organization (WHO). WHO Guidelines for Indoor Air Quality: Selected Pollutants. 3 Formaldehyde. 2010. Available from: URL: https://www.euro.who.int/__data/assets/pdf_file/0009/128169/e94535.pdf

45. Formaldehyde in your home. Available from: URL: https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/air-quality/formaldehyde-indoor-air-environment-workplace-health.html.

Glycidol (CAS# 556-52-5)

Potential for human exposure

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. Potential daily glycidol exposure in food has been estimated at 20-80 µg/day (Ref. 1).

Mutagenicity/genotoxicity

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

IARC (Ref. 2) and CCRIS (Ref. 3) 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 WP2*uvrA*/pKM101 in a preincubation assay with and without rat liver S9.

In vivo, glycidol was positive in a mouse micronucleus assay by oral gavage in male and female P16Ink4a/p19Arf haploinsufficient mice.

Carcinogenicity

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

In NTP studies (Ref. 4, 5), 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 (mammary gland tumors in females), and mice (Harderian gland). 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 group 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) (Ref. 6). 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 (Ref. 5) 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 (Ref. 7).

Glycidol – Details of carcinogenicity studies

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/sex	TD_{50} (mg/kg/d)
Ref. 5[a]	50/sex/ group F344/N rats	2 years 5 days/wk Oral gavage	50	**2:** 26.5; 53.8 mg/kg/d	Mammary gland/Female	4.15
Ref. 5	50/sex/ group B6C3F1 mice	2 years 5 days/wk Oral gavage	50	**2:** 17.7; 35.4 mg/kg/d	Harderian gland /Female	32.9
Ref. 8	12-20/ sex/group Syrian Golden Hamsters	60 weeks Twice/wk Gavage	Yes	**1:** M: 15.8 F: 17.9 mg/kg/d	Spleen/Female	56.1[c]
Ref. 9 ([b]Cited in Ref. 2)	20 ICR/Ha Swiss mice	520 days 3 times/wk Skin Painting	Yes	**1:** 5%	No Tumors	NA[c]

Studies listed are in CPDB (Ref. 10) unless otherwise noted.
[a]Carcinogenicity study selected for AI calculation.
[b]Not in CPDB.
NA= Not applicable.
[c]Not a standard carcinogenicity design. Only one dose, intermittent dosing, and small sample size (Ref.7).

Mode of action of carcinogenicity

Glycidol is a mutagenic carcinogen, and the acceptable intake is calculated by linear extrapolation from the TD_{50}.

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 (Ref. 5). 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

References

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

2. IARC. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Geneva: International Agency for Research on Cancer, World Health Organization. [Online]. 1972-PRESENT. (Multivolume work). 2000;77:469; Available from: URL: http://monographs.iarc.fr/index.php.

3. CCRIS. Chemical Carcinogenesis Research Information System. National Library of Medicine. [Online]. 2013. Available from: URL: https://www.ncbi.nlm.nih.gov/pcsubstance?term=%22Chemical%20Carcinogenesis%20Research%20Information%20System%20(CCRIS)%22%5BSourceName%5D%20AND%20hasnohold%5Bfilt%5D and search on CAS number.

4. Irwin RD, Eustis SL, Stefanski S, Haseman JK. Carcinogenicity of Glycidol in F344 rats and $B6C3F_1$ mice. J Appl Toxicol 1996;16 (3):201-9.

5. NTP. Technical report on the toxicology and carcinogenesis studies of glycidol (CAS No. 556-52-5) in F344/N Rats and B6C3F1 Mice (Gavage Studies). National Toxicology Program, Research Triangle Park, NC. 1990. NTP TR 374.

6. NTP. Toxicology and Carcinogenesis Studies of Glycidol (CAS No. 556-52-5) in genetically modified haploinsufficient p16 (Ink4a)/p19 (Arf) mice (gavage study). Natl Toxicol Program Genet Modif Model Rep 2007;13:1-81.

7. California Environmental Protection Agency (CalEPA). No Significant Risk Level (NSRL) for the Proposition 65 carcinogen Glycidol. [Online]. 2010. Available from: URL: https://oehha.ca.gov/media/downloads/proposition-65/chemicals/glycidolnsrl073010.pdf

8. Lijinsky W, Kovatch RM. A study of the carcinogenicity of glycidol in Syrian hamsters. Toxicol Ind Health 1992;8(5):267-71.

9. Van Duuren BL, Langseth L, Goldschmidt BM, Orris L. Carcinogenicity of epoxides, lactones, and peroxy compounds. VI. Structure and carcinogenic activity. J Natl Cancer Inst 1967;39:1217–28.

10. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

Hydrazine (CAS# 302-01-2)

Potential for human exposure

Hydrazine is used in the synthesis of pharmaceuticals, pesticides and plastic foams (Ref. 1). Hydrazine sulphate has been used in the treatment of tuberculosis, sickle cell anemia and other chronic illnesses (Ref. 2). There is limited information on the natural occurrence of hydrazine and derivatives (Ref. 3). Humans may be exposed to hydrazine from environmental contamination of water, air and soil (Ref. 1); however, the main source of human exposure is in the workplace (Ref. 4). Small amounts of hydrazine have also been reported in tobacco products and cigarette smoke (Ref. 1, 5).

Mutagenicity/genotoxicity

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

IARC (Ref. 6) 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.

In vivo, (Ref. 6) hydrazine induced micronuclei but not chromosome aberrations in mouse bone marrow. DNA adducts have been reported in several tissues *in vivo*.

Carcinogenicity

Hydrazine is classified by IARC as Group 2B, or possibly carcinogenic to humans (Ref. 6) and by US EPA as Group B2 or a probable human carcinogen (Ref. 7).

There are seven hydrazine carcinogenicity studies cited in the CPDB (Ref. 8): Three inhalation studies that included 1-year dosing duration, three studies in drinking water and one by oral gavage. 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 studies based on group size and dose levels were published in Refs. 9 and 10. The most robust inhalation study with the lowest TD_{50} is in Ref. 11. The most sensitive tumor 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 (Ref. 8) are not shown here as they included <50 animals per group (and a single dose level in one case), and the calculated TD_{50} values were higher (less potent) than those for the drinking water study of hydrazine (Ref. 9). Given the similarity between the outcomes from the two robust drinking water studies (Ref. 9, 10), the more recent study with the higher tested doses (Ref. 10) was selected for the non-inhalation AI calculation for hydrazine.

Hydrazine – Details of carcinogenicity studies

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/d)
Ref. 9	50/sex/ group Wistar rats	Lifetime Drinking water	50	**3:** M: 0.1; 1.5, 2.5. F: 0.11, 0.57, 2.86 mg/kg/d	Liver/Female	41.6
Ref. 11[a]	100/sex/ group F344 rats	1 year with 18 mo observation Inhalation	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
Ref. 12	50/sex/ group Bor:NMRI, SPF-bred NMRI mice	2 year Drinking water	50	**3:** M: 0.33, 1.67, 8.33. F: 0.4, 2.0, 10.0 mg/kg/d	Negative	NA, negative study
Ref. 11	200 male Golden Syrian hamsters	1 year with 12 mo observation Inhalation	Yes	**3:** 0.02, 0.08, 0.41 mg/kg/d	Nasal adenomatous polyps/Male	4.16
Ref. 11	400 female C57BL/6 Mice	1 year with 15 mo observation Inhalation	Yes	**1:** 0.18 mg/kg/d	Negative	NA
Ref. 13	50/sex/ group Swiss mice	Lifetime Drinking water	Not concurrent	**1:** ~1.7-2 mg/kg/d	Lung/Male	2.20[c]
Ref. 14	25 female Swiss mice	40 weeks 5d/wk Gavage	85 Untreated	**1:** ~5 mg/kg/d	Lung/Female	5.67[d]
Ref. 10[be]	50/sex/ F344/DuCrj rats	Lifetime Drinking water	Yes	**3:** M: 0.97, 1.84, 3.86 F:1.28, 2.50, 5.35 mg/kg/d	Liver/Female	38.7
Ref. 10[e]	50/sex Crj:BDF1 mice	Lifetime Drinking water		**3:** M: 1.44, 2.65, 4.93 F: 3.54,	Liver/Female	52.4

				6.80, 11.45 mg/kg/d		

Studies listed are in CPDB (Ref. 8).
[a]Carcinogenicity study selected for inhalation AI calculation.
[b]Carcinogenicity study selected for non-inhalation TD_{50} (see Note 4) and AI calculations.
NA= Not applicable.
[c] Excluded by US EPA (Ref. 7); no concurrent controls. Liver negative.
[d] Animal survival affected. Liver negative.
[e]Not in CPDB

Mode of action of carcinogenicity

Not defined. DNA adducts have been detected *in vivo*, (Ref. 15, 16, 17, 18, 19, 20) 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 US EPA (Ref. 7) has published an oral slope factor of 3.0 per mg/kg/day and a drinking water unit risk of 8.5×10^{-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 (Ref. 21) where hydrazine sulfate was administered to mice for 25 weeks followed by observation throughout their lifetime (Ref. 7). Additional studies were identified that were published after the oral slope factor was calculated (Ref. 9, 10, 17, 22). These studies could potentially produce a change in the oral slope factor but it has not yet been re-evaluated by US EPA.

The US EPA (Ref. 7) 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 where hydrazine was administered 6 hours/day, 5 days/week for 1 year followed by an 18-month observation period (cited in Ref. 7). Only the US 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* (Ref. 11).

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 4 mouse studies and 2 rat studies. The most sensitive effect in the oral studies was based on hepatocellular adenomas and carcinomas of the liver in female rats (Ref. 10).

All of the inhalation carcinogenicity studies that were used by the US 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 by MacEwen *et al* used by US EPA (Ref. 7) was proprietary but is likely the same one described in Vernot *et al* (Ref. 11). 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 US 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 (Ref. 11). 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, after being adjusted, as standard practice, to account for 1 vs 2 years of exposure.

Calculation of AI

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

Lifetime AI = 38.7 (mg/kg/day)/50,000 x 50 kg

Lifetime AI = 39 µg/day

Calculation of inhalation AI

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

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

Lifetime inhalation AI = 0.2 µg/day

References

1. Choudary G, Hansen H. Human health perspective on environmental exposure to hydrazines: A review. Chemosphere 1998;37:801-43.

2. Von Burg R, Stout T. Hydrazine. J Appl Toxicol 1991;11:447–50.

3. Toth B. A review of the natural occurrence, synthetic production and use of carcinogenic hydrazines and related chemicals. *In vivo*. 2000;14(2):299-319.

4. Hazardous Substance Database (HSDB): Hydrazine (302-01-2); [Online]. 2005 June 24 [cited 2013 February 27]; Available from: URL: https://pubchem.ncbi.nlm.nih.gov/compound/9321

5. Liu YY, Schmeltz I, Hoffman D. Chemical studies on tobacco smoke. Quantitative analysis of hydrazine in tobacco and cigarette smoke. Anal Chem 1974;46: 885–9.

6. IARC. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Geneva. International Agency for Research on Cancer, World Health Organization, [Online] 1972-PRESENT. (Multivolume work). 1999; Available from: URL: http://monographs.iarc.fr/index.php p. V71 1006.

7. US Environmental Protection Agency. Hydrazine/Hydrazine sulfate (302-01-2). Integrated Risk Information System (IRIS). [Online]. 1991. Available from: URL: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0352_summary.pdf

8. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

9. Steinhoff D, Mohr U. The question of carcinogenic effects of hydrazine. Exp Pathol 1988;33:133-40.

10. Matsumoto M, Kano H, Suzuki M, Katagiri T, Umeda Y, Fukushima S. Carcinogenicity and chronic toxicity of hydrazine monohydrate in rats and mice by two-year drinking water treatment. Regul Toxicol Pharmacol 2016;76:63-73.

11. Vernot EH, MacEwen JD, Bruner RH, Haun CC, Kinkead ER, Prentice DE, et al. Long-term inhalation toxicity of hydrazine. Fundam Appl Toxicol 1985;5:l050-64.

12. Steinhoff D, Mohr U, Schmidt WM. On the question of the carcinogenic action of hydrazine - evaluation on the basis of new experimental results. Exp Pathol 1990;39:1-9.

13. Toth B. Hydrazine, methylhydrazine and methylhydrazine sulfate carcinogenesis in Swiss mice. Failure of ammonium hydroxide to interfere in the development of tumors. Int J Cancer 1972;9:109-18.

14. Roe FJC, Grant GA, Millican DM. Carcinogenicity of hydrazine and 1,1-dimethylhydrazine for mouse lung. Nature 1967;16:375-6.

15. Becker RA, Barrows LR, Shank RC. Methylation of liver DNA guanine in hydrazine hepatotoxicity: dose-response and kinetic characteristics of 7-methylguanine and O^6-methylguanine formation and persistence in rats. Carcinogenesis 1981;2:1181-8.

16. Bosan WS, Shank RC. Methylation of liver DNA guanine in hamsters given hydrazine. Toxicol Appl Pharmacol 1983;70:324-34.

17. Bosan WS, Shank RC, MacEwen JD, Gaworski CL, Newberne PM. .Methylation of DNA guanine during the course of induction of liver cancer in hamsters by hydrazine or dimethylnitrosamine. Carcinogenesis 1987;8:439-44.

18. Saffhill R, Fida S, Bromley M, O'Connor PJ. Promutagenic alkyl lesions are induced in the tissue DNA of animals treated with isoniazid. Human Toxicol 1988;7:311-7.

19. Leakakos T, Shank RC. Hydrazine genotoxicity in the neonatal rat. Toxicol Appl Pharmacol 1994;126:295-300.

20. Mathison B, Murphy SE, Shank RC. Hydralazine and other hydrazine derivatives and the formation of DNA adducts. Toxicol Appl Pharmacol 1994;127:91-8.

21. Biancifiori, C. Hepatomas in CBA/Cb/Se mice and liver lesions in golden hamsters induced by hydrazine sulfate. J Natl Cancer Inst 1970;44:943.

22. FitzGerald BE, Shank RC. Methylation status of DNA cytosine during the course of induction of liver cancer in hamsters by hydrazine sulphate. Carcinogenesis 1996;17:2703-9.

Hydrogen Peroxide (CAS# 7722-84-1)

Potential for human exposure

Hydrogen peroxide can be present in green tea and instant coffee, in fresh fruits and vegetables and naturally produced in the body (Ref. 1). It is estimated up to 6.8 g is produced endogenously per day (Ref. 2). Other common sources of exposure are from disinfectants, some topical cream acne products, and oral care products which can contain up to 4% hydrogen peroxide (Ref. 2).

Mutagenicity/genotoxicity

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

IARC (Ref. 3) and European Commission Joint Research Centre (Ref. 4) 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;
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 by IARC as Group 3, not classifiable as to its carcinogenicity to humans (Ref. 3).

There is only one carcinogenicity report (Ref. 5) cited in the CPDB (Ref. 6), in which mice were treated with hydrogen peroxide in drinking water at 0.1 or 0.4% for approximately 2 years. The study included two treatment groups and about 50 animals per dose group. Statistically significant increases in tumors of the duodenum ($p<0.005$) were observed in both dose groups in the mouse carcinogenicity study (Ref. 5) although only the duodenal tumors at the high dose in females are noted as significant in the CPDB (Ref. 6). Thus, 0.1% hydrogen peroxide administered in drinking water was defined as the Lowest Observed Adverse Effect Level (LOAEL), equivalent to an average daily dose-rate per kg body weight per day of 167 mg/kg/day.

Studies of 6-month duration or longer are summarised in the following table (adapted from Ref. 2); they are limited in the numbers of animals and used a single dose level. Most studies did not meet the criteria for inclusion with a TD_{50} calculation in the CPDB. DeSesso *et al* (Ref. 2) noted that, out of 14 carcinogenicity studies (2 subcutaneous studies in mice, 2 dermal studies in mice, 6 drinking water studies [2 in rats and 4 in mice], 1 oral intubation study in hamsters, and 3 buccal pouch studies), only 3 mouse drinking water studies (Ref. 5, 8, 9) demonstrated increases in tumors (of the proximal duodenum) with hydrogen peroxide. These mouse studies were thoroughly evaluated by the Cancer Assessment Committee (CAC) of the US FDA (Ref. 10). The conclusion was that the studies did not provide sufficient evidence that hydrogen peroxide is a carcinogen (Ref. 10).

In Europe, the Scientific Committee on Consumer Products reviewed the available data for

hydrogen peroxide and concluded that hydrogen peroxide did not meet the definition of a mutagen (Ref.11) They also stated that the weak potential for local carcinogenic effects has an unclear mode of action, but a genotoxic mechanism could not be excluded (Ref. 11). In contrast, DeSesso *et al* (Ref. 2) suggested that dilute hydrogen peroxide would decompose before reaching the target site (duodenum) and that the hyperplastic lesions seen 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. The lack of a direct effect is supported by the lack of tumors in tissues directly exposed *via* drinking water (mouth, oesophagus and stomach), and the fact that in studies up to 6 months in the hamster (Ref. 14), in which hydrogen peroxide was administered by gastric intubation (water intake was not affected), the stomach and duodenal epithelia appeared normal; this was the basis for the US FDA conclusion above (Ref. 10).

Hydrogen Peroxide – Details of oral carcinogenicity studies

Study	**Animals/ dose group**	**Duration/ Exposure**	**Controls**	**Doses**	**Notes**
Ref. 5[a]	48-51/sex/ group C57BL/6J mice	100 weeks Drinking water	Yes	**2:** 0.1; 0.4% M: 167; 667 F: 200; 800 mg/kg/d	TD_{50} 7.54 g/kg/d for female duodenal carcinoma
Ref. 7	29 mice C57BL/6J total male & female (additional groups sampled at intervals from 7 to 630 days of treatment; or 10 – 30 days after cessation of treatment at 140 days)	700 days Drinking water	No	**1:** 0.4%	No tumors reported. Time-dependent induction of erosions and nodules in stomach and nodules and plaques in duodenum. After a recovery period following 140 days of H_2O_2 treatment, by 10 to 30 days without treatment there were fewer mice with lesions.
Ref. 8	18 C3H/HeN mice total male & female	6 mo Drinking water	No	**1:** 0.4%	2 mice with duodenal tumors (11.1%)
Ref. 8	22 B6C3F1 mice total male & female	6 mo Drinking water	No	**1:** 0.4%	7 mice with duodenal tumors (31.8%)
Ref. 8	21 C57BL/6N [¢] mice total male &	7 mo Drinking water	No	**1:** 0.4%	21 mice with duodenal tumors (100%)

	female				
Ref. 8	24 C3HCb/s ¢ mice total male & female	6 mo Drinking water	No	0.4% only	22 mice with duodenal tumors (91.7%)
Ref. 9	21 female C3H/HeN mice	6 mo Drinking water	11	**1:** 0.4%	2 mice with duodenal tumors (9.5%). None in controls
Ref. 9	22 female B6C3F1 Mice	6 mo Drinking water	12	**1:** 0.4%	7 mice with duodenal tumors (31.8%) None in controls
Ref. 9	24 female C3HCb/s ¢ mice	6 mo Drinking water	28	**1:** 0.4%	22 mice with duodenal tumors (91.7%). None in controls
Ref. 12	3 male rats	21 weeks Drinking water	3	**1:** 1.5%	No tumorigenic effect observed
Ref. 13	Male and female rats (50/sex/group)	2 years Drinking water	Yes	**2:** 0.3% 0.6%	No tumorigenic effect observed
Ref. 14	Hamsters, sex not reported (20/group)	15 weeks and 6 mo Oral gavage (5 d/wk)	Yes	**1:** 70 mg/kg/d	No tumorigenic effect observed

*[a] their studies are not in the CPDB but are summarized in Ref. 2

Catalase deficient

Mode of action for carcinogenicity

Hydrogen peroxide is one of the reactive oxygen species (ROS) that is formed as part of normal cellular metabolism (Ref. 4). The toxicity of hydrogen peroxide is attributed to the production of ROS and subsequent oxidative damage resulting in cytotoxicity, DNA strand breaks and genotoxicity (Ref. 15). 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 (Ref. 16). 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 (Ref. 17). 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 (Ref. 16). 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 (Ref. 11). 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, Ref. 4) 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 (Ref. 18) provided acceptable levels of hydrogen peroxide in oral hygiene and tooth whitening products. For oral products sold over the counter, including mouth rinse, toothpaste 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. The EC SCCP (Ref. 11) estimated that 3 g of mouthwash or 0.48 g of toothpaste could be ingested per day. With 0.1% hydrogen peroxide in the product, the amount of hydrogen peroxide potentially ingested would be 3 mg from mouthwash or 0.48 mg from toothpaste. These values may overestimate ingestion as it is likely that most of the hydrogen peroxide is decomposed during use of oral care products and is not ingested (Ref. 4).

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 (Ref. 19).

Permissible daily exposure (PDE)

Hydrogen peroxide is genotoxic *via* a mode of action with a threshold (i.e., oxidative stress) and is endogenously produced in the body at high levels that exceed the levels encountered in oral care and other personal care products. Therefore, it was not considered appropriate to derive a PDE based on carcinogenicity data. Even an intake 1% of the estimated endogenous production of 6.8 g/day, that is, 68 mg/day (or 68,000 µg/day) would not significantly add to background exposure, but would usually exceed limits based on quality, in a pharmaceutical. The ICH M7 guideline notes that when calculating acceptable intakes from compound-specific risk assessments, an upper limit would be determined by a quality limit of 0.5%, or, for example, 500 µg in a drug with a maximum daily dose of 100 mg.

References

1. Halliwell B, Clement MV, Long LH. Hydrogen peroxide in the human body. FEBS Lett 2000;486:10-13.

2. DeSesso JM, Lavin AL, Hsia SM, Mavis RD. Assessment of the carcinogenicity associated with oral exposures to hydrogen peroxide. Food and Chem Toxicol 2000;38:1021-41.

3. IARC. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. International Agency for Research on Cancer, World Health Organization, Lyon. 1999 Vol. 71.

4. European Commission Joint Research Center. EU Risk Assessment report. Hydrogen Peroxide. CASRN 7722-84-1). 38. [Online]2003. Available from: URL: https://echa.europa.eu/documents/10162/a6f76a0e-fe32-4121-9d9d-b06d9d5f6852

5. Ito A, Watanabe H, Naito M, Naito Y. Induction of duodenal tumors in mice by oral administration of hydrogen peroxide. Gann the Japanese Journal of Cancer Research 1981;72: 174-5.

6. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

7. Ito A, Naito M, Naito Y, Watanabe H. Induction and characterization of gastro-duodenal lesions in mice given continuous oral administration of hydrogen peroxide. Gann the Japanese Journal of Cancer Research 1982;73: 315-322.

8. Ito A, Watanabe H, Naito M, Naito Y, Kawashima K. Correlation between induction of duodenal tumor by hydrogen peroxide and catalase activity in mice. Gann the Japanese Journal of Cancer Research 1984;75: 17-21.

9. Ito A, Watanabe H, Aoyama H, Nakagawa Y, Mori M. Effect of 1,2-dimethylhydrazine and hydrogen peroxide for the duodenal tumorigenesis in relation to blood catalase activity in mice. Hiroshima Journal of Medical Science 1986;35:197-200.

10. US FDA. Irradiation in the production, processing, and handling of food. Food and Drug Administration. Federal Register 1988; Vol. 53, No. 251:53198-9.

11. SCCP. European Commission. Scientific Committee on Consumer Products. Opinion on Hydrogen peroxide, in its free form or when released, in oral hygiene products and tooth whitening products. SCCP/1129/07 [Online] 2007. Avalable from: URL: https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_122.pdf

12. Hiroto N. and Yokoyama T. Enhancing effect of hydrogen peroxide upon duodenal and upper jejunal carcinogenesis in rats. Gann 1981; 72: 811-812. Cited in Ref. 2.

13. Ishikawa T. and Takayama S. (1984) Hydrogen peroxide. In Information Bulletin on the Survey of Chemicals being Tested for Carcinogenicity. International Agency for Research on Cancer, Lyon. 1984; 11:86. (Cited in Ref. 2).Li Y, Noblitt T, Zhang A, Origel A, Kafrawy A, Stookey G. Effect of long-term exposure to a tooth whitener [Abstract]. Journal of Dental Research 1993;72:1162. (Cited in Ref. 2).

14. Tredwin CJ, Naik S, Lewis NJ, Scully C. Hydrogen peroxide tooth-whitening (bleaching) products: Review of adverse effects and safety issues. British Dental Journal 2006;200:371-6.

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

16. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000;408:239-47.

17. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products.

18. US FDA. Oral health care drug products for over-the-counter human use; antigingivitis/antiplaque drug products; establishment of a monograph. Federal Register 2003; 68:32232-86.

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

Potential for human exposure

Low levels of methyl chloride occur in the environment, since thousands of tons of methyl chloride are produced naturally every day, e.g., by marine phytoplankton, by microbial fermentation, and from biomass fires (burning in grasslands and forest fires) and volcanoes, greatly exceeding release from human activities.

WHO (Ref. 1) reports that the methyl chloride concentration in the air in rural sites is in general below 2.1 µg/m^3 (1.0 ppb) while in urban cities it is equal to 0.27 to 35 µg/m^3 (0.13-17 ppb), corresponding to approximately 20-700 µg daily intake (human respiratory volume of 20 m^3 per day). A wide range of concentrations is reported in rivers, ocean water, ground water and drinking water, with the maximum drinking water level reported at 44 µg/L in a well sample (Ref. 1).

Mutagenicity/Genotoxicity

Methyl chloride is mutagenic and genotoxic *in vitro* but equivocal *in vivo*. WHO (Ref. 1) and US EPA (Ref. 2) 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 (Ref. 1) 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".

Carcinogenicity

Methyl chloride is classified by IARC as Group 3: "Inadequate evidence for the carcinogenicity to humans" (Ref. 3), and by US EPA as a Category D compound not classifiable as to human carcinogenicity (Ref. 2).

In animals, the only evidence of carcinogenicity comes from a single 2-year bioassay that used the inhalation route of administration in rats and mice (Ref. 4). 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 (Ref. 4). 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. Methyl chloride is metabolized by glutathione conjugation and to a lesser extent by p450 oxidation (Ref. 1, 2). Renal tumors in 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 (Ref. 2), 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 US EPA (Ref. 2) and WHO (Ref. 1), 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; Ref. 5). In addition, based on the limitations of human relevance, US 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)
Ref. 4 (summarized in Ref. 1 and Ref. 2)[a]	120/sex/ group B6C3F1 mice	24 mo 6h/d, 5d/wk Inhalation	Yes	**3:** 103; 464; 2064 mg/m^3 (50; 225; 1000 ppm)	Kidney tumors in males only. No finding in females.	1,360.7[b]
Ref. 4 (summarized in Ref. 1 and Ref. 2)	120/sex/ group Fisher 344 rats	24 mo 6h/d, 5d/wk Inhalation	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.
[a]Carcinogenicity study selected for AI calculation.
[b]TD_{50} calculated based on carcinogenicity data (see Note 5).
NA = Not applicable

Regulatory and/or published Limits

WHO (Ref. 1) developed a guideline value for the general population of 0.018 mg/m^3 and US EPA (Ref. 2) developed a reference concentration of 0.09 mg/m^3. Both were based on the potential for adverse 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 = TD_{50}/50,000 x 50 kg

Lifetime AI = 1,360.7 mg/kg/day /50,000 x 50 kg

Lifetime AI = 1,361 µg/day

References

1. World Health Organization (WHO). Concise International Chemical Assessment Document (CICAD) 28. Methyl chloride. [Online]. 2000; Available from: URL: http://www.inchem.org/documents/cicads/cicads/cicad28.htm

2. US EPA. Methyl chloride. (CAS No. 74-87-3). Integrated Risk Information System (IRIS). [Online]. 2001; Available from: URL: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/1003tr.pdf

3. IARC. Methyl Chloride. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. International Agency for Research on Cancer, World Health Organization, Lyon. 1999 Vol. 71.

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

5. EFSA. European Food Safety Authority. Endogenous formaldehyde turnover in humans compared with exogenous contribution from food sources. EFSA Journal 2014; 12 Suppl 2:3550.

Styrene (CAS# 100-42-5)

Potential for human exposure

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

Mutagenicity/genotoxicity

Styrene has produced contradictory findings in the *in vitro* Ames test and it is predominantly inactive in the *in vivo* chromosome aberration, micronucleus and UDS assays when conducted according to OECD guidelines. Inconsistent results in the Ames test were attributed to styrene volatility, poor solubility, and different metabolic systems (Ref. 5). Styrene was positive for mutagenicity in the Ames test only with metabolic activation (Ref. 5), where it is converted to electrophilic intermediates (e.g., styrene-7,8-oxide) to enable formation of covalent adducts with DNA. Most of the genetic damage associated with styrene exposure is thought to be due to styrene-7,8-oxide, the main metabolite of styrene, which is further detoxified to styrene glycol. Styrene exposure elevated DNA adducts (N^7-guanine, O^6-guanine, and N^1-adenine) and sister chromatid exchanges (SCEs) in both animal models and in humans, and DNA strand breaks in humans (Ref. 5, 6). In a critical review of styrene genotoxicity based on the requirements outlined in the current OECD guidelines, Moore et al. (Ref. 7) concluded that it is unclear whether unmetabolized styrene is mutagenic in the Ames test, while the styrene-7,8-oxide metabolite is clearly mutagenic. The authors also noted that most styrene-7,8-oxide Ames positive data was collected without using exogenous metabolic activation, meaning that styrene-7,8-oxide was not further metabolized to styrene glycol.

Styrene was mutagenic in glycophorin A variant frequencies in erythrocytes from 28 workers exposed via inhalation to ≥ 85 mg/m^3 styrene (Ref. 8). Lymphocytes from styrene-exposed workers had increased mutation frequencies (MFs) at the hypoxanthine-guanine phosphoribosyl transferase *HPRT* locus (Ref. 9).

Two *in vitro* mammalian gene mutation studies were identified. In the *HPRT* assay, styrene induced only small increases in *HPRT* MFs in V79 cells (Ref. 10). Similarly, in V79 cells, styrene induced increases in mutations at the *HPRT* locus with exogenous metabolic activation system (Ref. 11). No rodent *in vivo* mutation studies evaluating styrene or styrene-7,8-oxide were identified.

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

Carcinogenicity

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

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

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

IARC evaluated nine studies each (with various routes of application) in mice and rats for styrene and three each in mice and rats for styrene-7,8-oxide. For styrene, one study with transplacental exposure followed by gavage using O20 mice demonstrated an increase of lung carcinoma and adenoma in pups whereas a second study in C57BL mice was negative (Ref. 18). Two out of five studies with inhalation in CD-1 mice reported an increase in lung bronchoalveolar tumors (Ref. 16, 19) whereas the other three (in C57BL/6 mice) were negative (Ref. 19). One study in mice with oral application found increased lung tumors and a positive trend for hepatocellular carcinoma (Ref. 16). Another study with i.p. application gave negative results (Ref. 20). In two studies in CD (SD derived) or SD rats with whole body inhalation exposure, styrene produced negative results in one (Ref. 21) but increased mammary gland tumors in the other (Ref. 22), whereas four oral studies, three with gavage (Ref. 17, 22) and one via drinking water (Ref. 23), were negative. The observed increase in mammary gland tumors was not dose-dependent and was not considered reliable evidence of carcinogenicity by NIH ROC (Ref. 1); IARC (Ref. 5) also noted short treatment duration and incomplete reporting of the study. Other studies in rats with transplacental exposure followed by gavage (Ref. 17), i.p. application, or s.c. application (Ref. 22) were also negative.
Styrene-7,8-oxide was tested in three studies in mice, one by gavage (Ref. 24) and two by skin application (Ref. 25, 26). In the oral gavage study styrene-7,8-oxide increased squamous cell tumors in forestomach in males and females and hepatocellular tumors in males. The studies by skin application were inadequate for evaluation due to the limited reporting of study details and the lack of controls. In rats, styrene-7,8-oxide was tested in two studies with oral exposure by gavage (Ref. 22, 24) and one by transplacental exposure followed by gavage (Ref. 27). In both studies by gavage, squamous cell tumors of the forestomach were increased and in one of the studies

mammary gland tumors where also increased in males. In the study by transplacental exposure followed by gavage, forestomach tumors were increased. IARC concluded that there is sufficient evidence for carcinogenicity of styrene and styrene-7,8-oxide in experimental animals (Ref. 5).

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

Styrene – Details of the most relevant carcinogenicity studies

Study	**Animals/ dose group**	**Duration/ Exposure**	**Controls**	**Doses**	**Most sensitive tumor site/type/sex**	**TD_{50} (mg/kg/day)[a]**
Ref. 16	50/sex/ group M&F B6C3F1 mouse	78 weeks, Oral Gavage	20	**2:** 150, 300 mg/kg/day	Lung/ Male [b]	360
Ref. 17	70/sex/ group CD1 mouse	98-104 weeks, Inhalation	70	**4:** 20, 40, 80, 160 ppm, 22.3, 44.6, 89.3, 179 mg/kg/day	Lung/ Male	154[c]
Ref. 16	70/sex/ group Fischer 344 rats	78 -107 weeks, Oral Gavage	40	**3:** 500, 1000, 2000 mg/kg/day	No Tumors	NC
Ref. 21	70/sex/ group CD rats	104 weeks, Inhalation	70	**4:** 50, 200, 500, 1000 ppm	No Tumors	NC
Ref. 22	30/sex/ group SD rats	52 weeks, Inhalation	60	**5:** 25, 50, 100, 200, 300 ppm	Mammary tissue/ Female [d]	23.3
Ref. 22	40/sex/ group SD Rats	52 weeks, Gavage	40	**2:** 50, 250 mg/kg/day	No Tumors	NC
Ref. 22	40/sex/ group SD Rats	SC once, then i.p. 4 times at 2-month intervals	40	**1:** 50 mg (s.c.), 50 mg (i.p.)	No Tumors[e]	NC

NC – Not Calculated, s.c. – Subcutaneous Injection, i.p. – Intraperitoneal Injection, SD – Sprague Dawley

[a] The TD_{50} values are taken from CPDB (Ref. 15)
[b] Despite having a statistically significant dose-trend per CPDB, the author concluded that there was no convincing evidence of carcinogenicity in mice
[c] Carcinogenicity study selected for the AI calculation, mg/kg/day dose conversion taken from CPDB
[d] Author opinion: Styrene, was found to cause an increase in total (benign & malignant) and malignant mammary tumors. Cruzan et al., (Ref. 21) noted no obvious dose-response in the data. Furthermore, the study findings were not considered reliable evidence of carcinogenicity by NIH ROC (Ref. 1) and IARC (Ref. 5) noted short treatment duration and incomplete reporting of the study.
[e] Study limited to acute exposures and a non-standard study design

Mode of action for carcinogenicity

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

The genotoxic potential and human relevance of the observed mouse tumors has been questioned (Ref. 28, 29, 30, 31). Other possible mechanisms contributing to the carcinogenic activity of styrene include oxidative stress, immunosuppression and chronic inflammation. The mechanism suggested by Cruzan et al. (Ref. 28) as the main cause of mice lung tumors includes styrene metabolites inducing gene expression for metabolism of lipid, lipoprotein, cell cycle and mitotic M-M/G1 phases, mild cytotoxicity and strong mitogenicity in mice lung cells, leading to excessive cell proliferation and hyperplasia. On the other hand, the authors assume that it would not be relevant in humans due to limited lung metabolism (by CYP2F2). IARC concluded that the evidence for these mechanisms of action is moderate to weak. The various perspectives were evaluated in determining the overall conclusions regarding the genotoxic potential and human relevance of tumors associated with styrene administration. Ultimately, the IARC conclusions were used in supporting the derivation of the AI for styrene.

Regulatory and/or published limits

The WHO defined a Tolerable Daily Intake (TDI) for styrene via the oral route of 7.7 µg/kg/day (i.e., 0.385 mg per day based on 50 kg body weight) from which a drinking water guideline value of 20 µg/L has been defined (i.e., 40 µg per day based on consumption of 2 L water per day) (Ref. 32). This WHO limit was based on reduced body weight gain in a two-year rat drinking water study. The US EPA oral reference dose (RfD) (Ref. 33) for styrene is 200 µg/kg/day (i.e., 10 mg/day based on 50 kg body weight), based on non-cancer endpoints. The associated US EPA drinking water limit is 100 µg/L (i.e., 200 µg per day based on consumption of 2 L water per day). The JECFA maximum TDI for styrene (Ref. 34) from migration from food packaging is 0.04 mg/kg/day (i.e., a maximum of 2 mg per day based on 50 kg body weight). A Specific Migration Limit of 60 ppm styrene into foods in polystyrene packaging (i.e., 60 mg per day assuming the consumption of 1 kg food/day for adult humans) is considered permissible in the European Union (Ref. 4).

Acceptable intake (AI)

Rationale for selection of study for AI calculation

Since styrene is not considered to be a rat carcinogen, mouse lung tumor data were used to derive the AI. The most sensitive TD_{50} was in the inhalation study of Cruzan et al. (Ref. 17). The AI derived from this inhalation study was considered suitable for all routes of administration as an increase in lung tumors were also seen in the carcinogenicity study in mice with gavage treatment (Ref. 16). The AI is expected to be a conservative limit as the mouse is known to have higher levels of CYP2F enzymes in comparison to humans, which is key to tumor formation (Ref. 28).

Calculation of AI

Lifetime AI = TD_{50}/50000 x 50 kg

Lifetime AI =154 mg/kg/day/50000 x 50 kg

Lifetime AI = 154 µg/day

References

1. National Institutes of Health. Report on Carcinogens (NIH ROC). 14th edition. 2016; Available from: URL: https://ntp.niehs.nih.gov/ntp/roc/content/profiles/styrene.pdf

2. Capella KM, Roland K, et al. Ethylbenzene and styrene exposure in the United States based on urinary mandelic acid and phenylglyoxylic acid: NHANES 2005–2006 and 2011–2012. Environ Res 2019;171:101-110.

3. World Health Organization (WHO). Chapter 5.12 Styrene. Air Quality Guidelines – 2nd Edition. 2000; Available from: URL: http://www.euro.who.int/__data/assets/pdf_file/0018/123066/AQG2ndEd_5_12Styrene.pdf

4. Gelbke HP, Banton M, et al. Derivation of safe health-based exposure limits for potential consumer exposure to styrene migrating into food from food containers. Food Chem Toxicol 2014;64:258–69.

5. IARC (International Agency on Research of Cancer). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Styrene, stryrene-4,8-oxide and quinoline. Volume 121. Lyon, 2019.

6. NRC. Review of the Styrene Assessment in the National Toxicology Program 12th Report on Carcinogens. Washington, DC: The National Academies Press. 2014; Available from: URL: https://doi.org/10.17226/18725

7. Moore MM, Pottenger LH, House-Knight T. Critical review of styrene genotoxicity focused on the mutagenicity/clastogenicity literature and using current organization of economic cooperation and development guidance. Environ Mol Mutagen 2019;60:624-663.

8. Compton-Quintana PJ, Jensen RH, Bigbee WL, Grant SG, Langlois RG, Smith MT, Rappaport SM. Use of the glycophorin A human mutation assay to study workers exposed to styrene. Environ Health Perspect 1993;99:297-301.

9. Vodicka P, Soucek P, Tates AD, Dusinska M, Sarmanova J, Zamecnikova M, Vodickova L, Koskinen M, de Zwart FA, Natarajan AT, Hemminki K. Association between genetic polymorphisms and biomarkers in styrene-exposed workers. Mutat Res 2001;482:89-103.

10. Loprieno N, Abbondandolo A, Barale R, Baroncelli S, Bonatti S, Bronzetti G, Cammellini A, Corsi C, Corti G, Frezza D, Leporini C, Mazzaccaro A, Nieri R, Rosellini D, Rossi AM. Mutagenicity of industrial compounds: styrene and its possible metabolite styrene oxide. Mutat Res 1976;40:317-24.

11. Beije B, Jenssen D. Investigation of styrene in the liver perfusion/cell culture system. No indication of styrene-7,8-oxide as the principal mutagenic metabolite produced by the intact rat liver. Chem Biol Interact 1982;39:57-76.

12. NTP (National Toxicology Program). 12th Report on Carcinogens. Rep Carcinog 2011; 12: iii-499.

13. NTP (National Toxicology Program). Report on Carcinogens, Fourteenth Edition.; Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service. 2016; Available from: URL: https://ntp.niehs.nih.gov/go/roc14

14. Collins JJ, Delzell E. A systematic review of epidemiologic studies of styrene and cancer. Critical Reviews in Toxicology 2018;48:443-470.

15. Carcinogenicity Potency Database (CPDB). National Institutes of Health. Carcinogenic Potency Database. Available from: URL: https://files.toxplanet.com/cpdb/index.html

16. National Cancer Institute (NCI). Technical Report Series No. 185. Bioassay of styrene for possible carcinogenicity. NCI-CG-TR-185. 1979; Available from: URL: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr185.pdf

17. Cruzan G, Cushman JR, et al. Chronic Toxicity/Oncogenicity Study of Styrene in CD-1 Mice by Inhalation Exposure for 104 Weeks. J Appl Toxicol 2001;21:185–98.

18. Ponomarkov V, Tomatis L. Effects of long-term oral administration of styrene to mice and rats. Scand J Work Environ Health 1978;4:127–35.

19. Cruzan G, Bus JS, Banton MI, Sarang SS, Waites R, Layko DB, et al. Complete attenuation of mouse lung cell proliferation and tumorigenicity in CYP2F2 knockout and CYP2F1 humanized mice exposed to inhaled styrene for up to 2 years supports a lack of human relevance. Toxicol Sci 2017;159:413–21.

20. Brunnemann KD, Rivenson A, Cheng SC, Saa V, Hoffmann D. A study of tobacco carcinogenesis. XLVII. Bioassays of vinylpyridines for genotoxicity and for tumorigenicity in A/J mice. Cancer Lett 1992;65:107–13.

21. Cruzan G, Cushman JR, et al. Chronic Toxicity/Oncogenicity Study of Styrene in CD

rats by Inhalation Exposure for 104 Weeks. Toxicol Sci 1998;46:266–81.

22. Conti B, Maltoni C, et al. Long-term carcinogenicity bioassays on styrene administered by inhalation, ingestion and injection and styrene oxide administered by ingestion in Sprague-Dawley rats, and para-methylstyrene administered by ingestion in Sprague-Dawley rats and Swiss mice. Ann N Y Acad Sci 1988;534:203-34.

23. Beliles RP, Butala JH, Stack CR, Makris S. Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats. Fundam Appl Toxicol 1985;5:855–68.

24. Lijinsky W. Rat and mouse forestomach tumors induced by chronic oral administration of styrene oxide. J Natl Cancer Inst 1986;77:471–6.

25. Weil CS, Condra N, Haun C, Striegel JA. Experimental carcinogenicity and acute toxicity of representative epoxides. Am Ind Hyg Assoc J 1963;24:305–25.

26. Van Duuren BL, Nelson N, Orris L, Palmes ED, Schmitt FL. Carcinogenicity of epoxides, lactones, and peroxy compounds. J Natl Cancer Inst 1963;31:41–55.

27. Ponomarkov V, Cabral JRP, Wahrendorf J, Galendo D. A carcinogenicity study of styrene-7,8-oxide in rats. Cancer Lett 1984;24:95–101.

28. Cruzan G, Bus JS, et al. Based on an analysis of mode of action, styrene-induced mouse lung tumors are not a human cancer concern. Reg Tox Pharm 2018;95:17-28.

29. European Food Safety Authority (EFSA). EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) Assessment of the Impact of the IARC Monograph Vol. 121 on the Safety of the Substance Styrene (FCM No 193) for its Use in Plastic Food Contact Materials. EFSA Journal 2020;18(10):6247.

30. European Chemicals Authority (ECHA). CLH report for styrene, Proposal for Harmonised Classification and Labelling, Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2, Substance Name: Styrene, September 2011. Available from: URL: https://echa.europa.eu/documents/10162/fd574447-2888-e637-ae30-77df1b043a2f

31. European Chemicals Authority (ECHA). Registry of CLH intentions until outcome, Styrene. 2021. Available from: URL: https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e180a0fa4f

32. World Health Organization (WHO). Styrene in Drinking Water; Background Document for Development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WHS/03.04/27. 2003; Available from: URL: http://www.who.int/water_sanitation_health/dwq/chemicals/styrene.pdf

33. United States Environmental Protection Agency (USEPA). Integrated Risk Information System (IRIS). Styrene (CASRN 100-42-5). 1990; Available from: URL: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0104_summary.pdf

34. Joint Expert Committee on Food Additives (JECFA). Styrene. WHO Food Additives Series 19. 1984; Available from: URL: http://www.inchem.org/documents/jecfa/jecmono/v19je15.htm

Vinyl Acetate (CAS# 108-05-4)

Potential for human exposure

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

Mutagenicity/genotoxicity

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

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

Carcinogenicity

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

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

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

There are two inhalation carcinogenicity reports cited in the CPDB (Ref. 5). Vinyl acetate was not carcinogenic to CD-1 mice but induced nasal tumors in Sprague-Dawley rats (Ref. 13). All but one of the 11 nasal tumors in rats (benign endo and exophytic papillomas and squamous-cell carcinomas) were observed at the terminal sacrifice at the high dose of 600 ppm, indicating a late life dependency of tumor formation. One benign tumor, of questionable relationship to exposure, was observed at the 200 ppm concentration (Ref. 13). In both species and both sexes, vinyl acetate induced morphological non-neoplastic lesions in the nasal cavity of the 200 and 600 ppm groups and in the trachea (mice only) and in the lungs of the 600 ppm groups.

Vinyl Acetate – Details of carcinogenicity studies

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

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/day)
				mg/kg/day F and 0.062 mg/kg/day M), 2500 mg/L (0.04 mg/kg/day F and 0.025 mg/kg/day M)		
Ref. 9	50/sex/ group Crj:BDF_1 Mice	2 years, drinking water	50	**3:** 400 ppm (63 mg/kg F and 42 mg/kg/day M), 2000 ppm (301 mg/kg/day F and 202 mg/kg/day M), 10000 ppm (1418 mg/kg/day F and 989 mg/kg/day M)	Oral cavity, Male	1854[c]
Ref. 9	50/sex/ group F344/Du Crj Rats	2 years, drinking water	50	**3:** 400 ppm (31 mg/kg/day F and 21 mg/kg/day M), 2000 ppm (146 mg/kg/day F and 98 mg/kg/day M), 10000 ppm (575 mg/kg/day F and 442 mg/kg/day M)	Oral cavity, Male	3057[c]
Ref. 11	37F and 14M/ group, Breeders	2 years, drinking water	Breeders 14M and 37F; Offspring	**2:** 1000 ppm (70.6 mg/kg/day), 5000 ppm	Oral cavity and lips, Male	983[c]

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/day)
	(17 wk old); 53 or 83M and 57 or 87F Sprague-Dawley Rat Offspring (12 day old)		107M and 99F	(353 mg/kg/day)[a]		
Ref. 12	60/sex/ group Crl:CD(SD)BR Rats	2 years, drinking water	60	**3:** 200 ppm (16 mg/kg/day F and 10 mg/kg/day M), 1000 ppm (76 mg/kg/day F and 47 mg/kg/day M), 5000 ppm (302 mg/kg/day F and 202 mg/kg/day M)	No tumors	NC
Ref. 13	60/sex/ group Charles River CD1 Mice	2 years, inhalation	60	**3:** 50 ppm (55.3 mg/kg/day F and 46.1 mg/kg/day M), 200 ppm (221 mg/kg/day F and 184 mg/kg/day M), 600 ppm (664 mg/kg/day F and 554 mg/kg/day M)	No tumors	NC

Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most sensitive tumor site/type/sex	TD_{50} (mg/kg/day)
Ref. 13	60/sex/ group Charles River CD (Sprague-Dawley) Rats	2 years, inhalation	20	**3:** 50 ppm (13.3 mg/kg/day F and 9.32 mg/kg/day M), 200 ppm (52.7 mg/kg/day F and 36.9 mg/kg/day M), 600 ppm (158 mg/kg/day F and 111 mg/kg/day M)	Nasal, Male	758[b]

NC – Not Calculated

[a] Calculated based on ICH Q3C assumptions

[b] Taken from the CPDB (Ref. 14). Carcinogenicity study selected for the AI calculation

[c] Study not reported in CPDB, therefore TD_{50} value calculated based on carcinogenicity data

Mode of action for carcinogenicity

Vinyl acetate has been reviewed by the European Commission's Scientific Committee on Health and Environmental Risks (SCHER), who published a Risk Assessment Report in 2008 (Ref. 1). Overall, SCHER supports the conclusion that the carcinogenic potential of vinyl acetate is expressed only when tissue exposure to acetaldehyde is high and when cellular proliferation is simultaneously elevated. This mode of action suggests that exposure levels, which do not increase intracellular concentrations of acetaldehyde will not produce adverse cellular responses. As long as the physiological buffering systems are operative, no local carcinogenic effect by vinyl acetate should be expected at the NOAEL for histological changes in respiratory rodent tissues. However, the SCHER indicated that local levels at or below the NOAEL are not free of carcinogenic risk, although the risk may be negligibly low. Hengstler et al. (Ref. 8) presented the case for vinyl acetate as a DNA-reactive carcinogen with a threshold dose-response, which has also been described by Albertini (Ref. 2). Like acetaldehyde, vinyl acetate is not mutagenic in the standard bacterial reversion assay; evidence for DNA-reactivity and site of contact carcinogenicity of vinyl acetate is that it occurs because of metabolic conversion to acetaldehyde.

The genotoxicity profiles for acetaldehyde and vinyl acetate are almost identical and vinyl acetate is not active as a clastogen without the addition of carboxylesterase (Ref. 8). Therefore, the clastogenic activity of vinyl acetate is attributed to metabolic formation of acetaldehyde. At high concentrations, enzyme activities are not able to oxidize all the generated acetaldehyde, and a low pH microenvironment is the result (Ref. 12). From consistent endogenous acetic acid exposure, tissues may sustain a reduction of 0.15 units in pH following vinyl acetate treatment without adverse effects (i.e., cytotoxicity and genotoxicity) (Ref. 15). However, when this practical threshold is exceeded, DNA damage, cytotoxicity, and regenerative cellular proliferation occur, resulting in tumor formation at the site of contact.

There is clear evidence for the carcinogenicity of vinyl acetate in two animal species, in both sexes and for both inhalation and oral administration. Following both oral and inhalation administration, vinyl acetate is rapidly hydrolyzed at the site of contact by carboxylesterases, to acetic acid and acetaldehyde (Ref. 3, 16). Vinyl acetate exposure produces tumors at the site of first contact along the exposure routes. The dose-response is thought to be non-linear, with the observed tumor responses reflecting the target tissue-specific enzyme activities for activation and detoxification (Ref. 2). However, as noted in the acetaldehyde monograph, there are no published measurements which would allow discrimination between the irritating effect and the potential mutagenic effect ion cancer development.

Regulatory and/or published limits

For vinyl acetate, the US EPA IRIS database calculated an inhalation Reference Concentration (RfC) for non-carcinogenic effects of 0.2 mg/m^3, or 5.8 mg/day assuming a respiratory volume of 28.8 m^3. The RfC was based on a human equivalent concentration of 5 mg/m^3 derived from Owen et al. 1988 which identified both a NOAEL and a LOAEL for histopathological effects of the nasal olfactory epithelia in rats and mice in a chronic 2-year study. A total adjustment factor of 30 was applied (Ref. 17). The US EPA report did not include a carcinogenicity assessment for lifetime exposure to vinyl acetate. It is stated that RfCs can be derived for the noncarcinogenic health effects of substances that are carcinogens and to refer to other sources of information concerning the carcinogenic potential.

Permissible Daily Exposure (PDE) for oral exposure

Rationale for selection of study for PDE calculation

Following oral administration, vinyl acetate is rapidly hydrolyzed at the site of contact by carboxylesterases, to acetic acid and acetaldehyde. Given the weight of evidence for a non-linear dose-response for the carcinogenicity of both vinyl acetate and acetaldehyde following oral administration and considering high background exposure to acetaldehyde from a wide variety of foods, the oral PDE recommended is based on that derived for acetaldehyde of 2 mg/day.

PDE (oral) = 2 mg/day

Acceptable intake (AI) for all other routes

Rationale for selection of study for AI calculation

For routes of administration other than the oral route, the inhalation carcinogenicity study in rats (Ref. 13) was used for derivation of an AI. In this study, there were 3 treatment groups with 60 animals per sex per treatment group. Animals were exposed 6 hours per day, 5 days per week for 2 years to vinyl acetate. Carcinogenicity was observed in the nasal cavity of rats, with male being the more sensitive sex. The TD_{50} for the nasal cavity in male rats is 758 mg/kg/day, as reported in CPDB. The only other carcinogenicity study that is available with vinyl acetate administered via the inhalation route in mice is negative (Ref. 13). Therefore, the rat inhalation study was selected for derivation of an AI.

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

Calculation of AI

Lifetime AI = TD_{50}/50000 x 50 kg

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

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

References

1. Scientific Committee on Health and Environmental Risks (SCHER). Risk Assessment Report on Vinyl acetate. 2008. Available from: URL: https://ec.europa.eu/health/archive/ph_risk/committees/04_scher/docs/scher_o_108.pdf

2. Albertini RJ. Vinyl Acetate Monomer (VAM) Genotoxicity Profile: Relevance for Carcinogenicity. Crit Rev Toxicol 2013;43:671-706.

3. European Chemicals Agency (ECHA). Summary Risk Assessment Report. Vinyl Acetate CASRN 108-05-4. 2008. Available from: URL: https://echa.europa.eu/documents/10162/a3c24f78-4c8d-44e9-a424-24ac30c9c8aa

4. International Agency for Research on Cancer (IARC). IARC monographs on the evaluation 403 of carcinogenic risks to humans. Vinyl Acetate. 1995;63:443. Available from: URL: http://www.inchem.org/documents/iarc/vol63/vinyl-acetate.html

5. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

6. Maltoni C, Ciliberti A, Lefemine G, Soffritti M. Results of a long-term experimental study on the carcinogenicity of vinyl acetate monomer in mice. Ann N Y Acad Sci 1997;837:209-38.

7. Lijinsky W, Reuber MD. Chronic toxicity studies of vinyl acetate in Fischer rats. Toxicol Appl Pharmacol 1983;68:43-53.

8. Hengstler JG, Bogdanffy MS, et al. Challenging Dogma: Thresholds for genotoxic carcinogens? The case of vinyl acetate. Annu Rev Pharmacol Toxicol 2003;43:485–520.

9. Umeda Y, Matsumoto M, et al. Carcinogenicity and chronic toxicity in mice and rats administered vinyl acetate monomer in drinking water. J Occup Health 2004;46:87-99.

10. Organization for Economic Co-operation and Development (OECD). OECD 453 Guideline for the Testing of Chemicals: Combined Chronic Toxicity\Carcinogenicity Studies. 2009.

11. Minardi F, Belpoggi B, et al. Results of long-term carcinogenicity bioassay on vinyl acetate monomer in Sprague- Dawley rats. Ann N Y Acad Sci 2002:982;106-22.

12. Bogdanffy MS, Tyler TR, et al. Chronic toxicity and oncogenicity study with vinyl acetate in the rat: in utero exposure in drinking water. Fundam Appl Toxicol 1994;23:206–14.

13. Bogdanffy MS, Dreef-van der Meulen HC, Beems RB, Feron VJ, Cascieri TC, Tyler TR, Vinegar MB, Rickard RW. Chronic toxicity and oncogenicity inhalation study with vinyl acetate in the rat and mouse. Fundam Appl Toxicol. 1994;23:215-29.

14. Carcinogenicity Potency Database (CPDB). [Online]. Available from: URL: https://files.toxplanet.com/cpdb/index.html

15. Nedergaard M, Goldman SA, et al. Acid-induced death in neurons and glia. J Neurosci 1991;11:2489-97.

16. Robinson DA, Bogdanffy MS, et al. Histochemical localisation of carboxylesterase activity in rat and mouse oral cavity mucosa. Toxicology 2002;180:209-20.

17. United States Environmental Protection Agency (USEPA). Integrated Risk Information System (IRIS). Vinyl acetate (CASRN 108-05-4). 1990. Available from: URL: http://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0512_summary.pdf

Note 1

The calculated TD_{50} for 1-chloro-4-nitrobenzene is illustrated below since it was not listed in the CPDB. 1-Chloro-4-nitrobenzene calculations were based on the most sensitive tumor type: female rat pheochromocytoma (Ref. 1). The doses and incidences are listed below.

ppm	Dose (mg/kg/day)	Number of Positive Animals	Total Number of Animals
0	0	3	50
50	1.9	6	50
225	9.8	4	50
1000	53.8	16	50

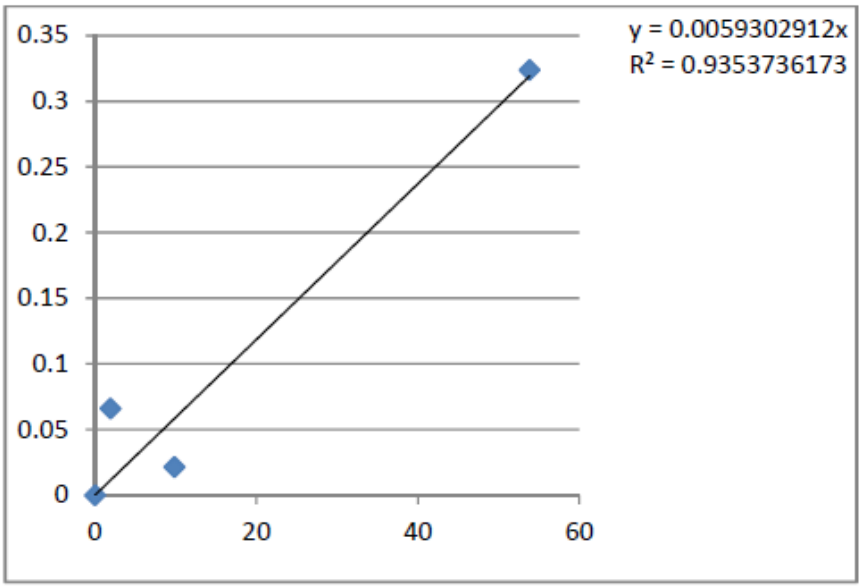
The TD_{50} is calculated from crude summary data of tumor incidence over background with the following equation (Ref. 2, 3):

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

Where P is the proportion of animals with the specified tumor type observed at a certain dose (D in the equation) and P_0 is the proportion of animals with the specified tumor type for the control. Converting β and D into a simple linear equation results in the following:

$$-\ln\left(\frac{p - p_0}{1 - p_0} - 1\right) = \beta \cdot D$$

Plotting the results and using the slope to represent β results in the following graph for the dose-response and β = 0.0059302912.



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

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

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

$$TD_{50} = \frac{0.693}{\beta}$$

Therefore, the TD_{50} = 0.693 / 0.0059302912 or 116.9 mg/kg/day.

References

1. Matsumoto M, Aiso S, Senoh H, Yamazaki K, Arito H, Nagano K, et al. Carcinogenicity and chronic toxicity of *para*-chloronitrobenzene in rats and mice by two-year feeding. J. Environ Pathol Toxicol Oncol 2006;25:571-84.

2. Gaylor DW, Gold LS. Quick estimate of the regulatory virtually safe dose based on the maximum tolerated dose for rodent bioassays. Regul Toxicol Pharmacol 1995;22:57-63.

3. Sawyer C, Peto R, Bernstein L, Pike MC. Calculation of carcinogenic potency from long-term animal carcinogenesis experiments. Biometrics 1984;40:27-40.

Note 2

The calculated TD_{50} for ethyl bromide is illustrated below since it was decided to use the same study data but not the TD_{50} calculated by CPDB as the positive dose response was not statistically significant (see monograph for ethyl bromide).

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

A TD_{50} is calculated for each dose separately with the following equation (Ref. 1, 2):

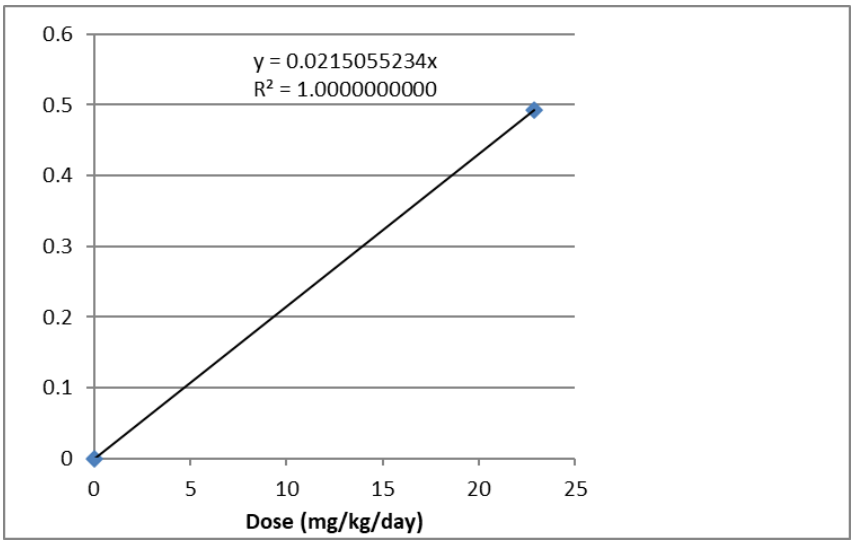
$$\frac{P - P_0}{1 - P_0} = 1 - \exp(-\beta \cdot D)$$

Where P is the proportion of animals with the specified tumor type observed at a certain dose (D in the equation) and P_0 is the proportion of animals with the specified tumor type for the control. Converting β and D into a simple linear equation results in the following:

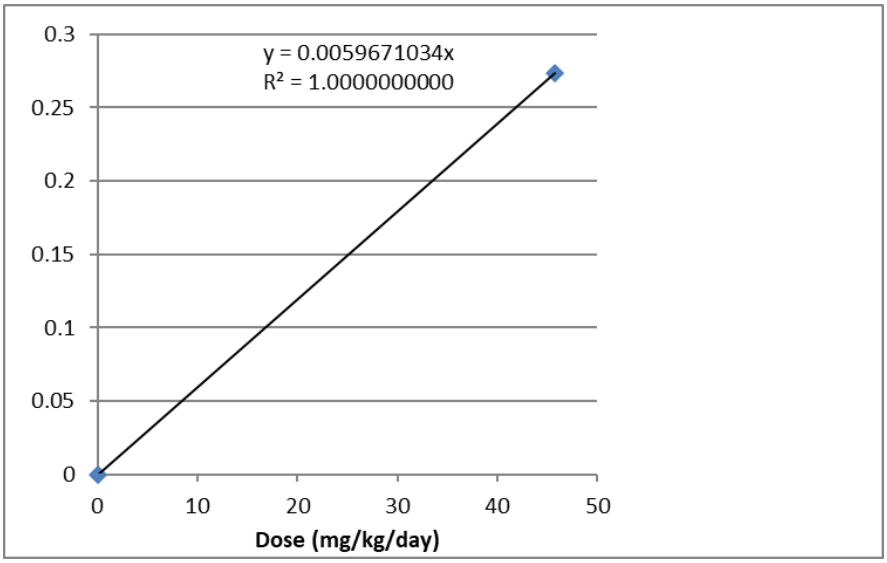
$$-\ln\left(\frac{p - p_0}{1 - p_0} - 1\right) = \beta \cdot \mathrm{D}$$

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

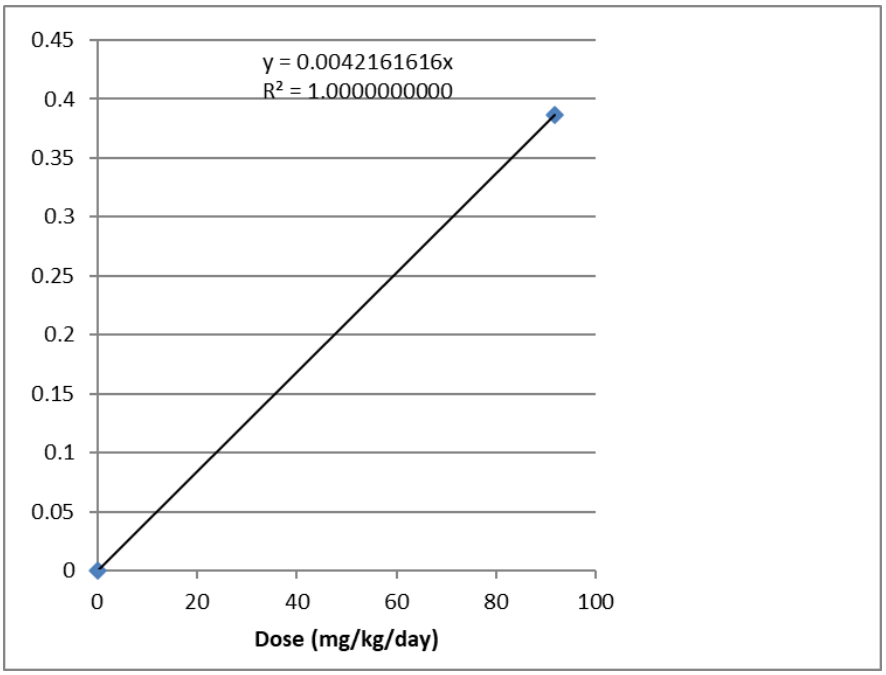
Low Dose



Mid Dose



High Dose



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

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

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

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

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

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

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

References

1. Gaylor DW, Gold LS. Quick estimate of the regulatory virtually safe dose based on the maximum tolerated dose for rodent bioassays. Regul Toxicol Pharmacol 1995;22:57-63.

2. Sawyer C, Peto R, Bernstein L, Pike MC. Calculation of carcinogenic potency from long-term animal carcinogenesis experiments. Biometrics 1984;40:27-40.

Note 3

For formaldehyde the limit is 215 ppb or 8 mg/day whichever is lower. As written, this may be misunderstood. That is, the limit of 215 ppb could either be interpreted as the concentration of formaldehyde in air (which is the basis of the exposure limit), or the concentration of formaldehyde relative to drug substance. The following example could clarify how the limit in API or DP would be derived:

Example: Albuterol sulfate actuator with formaldehyde as an impurity in the API

- Each actuator of albuterol delivers 90 µg of API.

- The API and any impurities will be diluted into air which is inhaled with each actuator. The tidal volume of air is 500 mL for adult males and 400 mL for adult females Physiology, Tidal Volume - StatPearls - NCBI Bookshelf (nih.gov). The more conservative dilution of formaldehyde into air would be for adult females with a lower tidal volume.

- Convert the concentration limit of formaldehyde in air (215 ppb) to an absolute amount of formaldehyde based on the female tidal volume: 215 ppb formaldehyde = 0.215 x 30 g/mol (MW of formaldehyde)/24.45 = 0.263 mg/m^3. 0.263 mg/m^3 x 1 m^3/1000 L x 0.4 L (tidal volume women) = 0.105 µg formaldehyde.

- Calculate the corresponding API limit: 0.105 µg formaldehyde / 90 µg API = 0.12%

Example: Albuterol sulfate actuator with formaldehyde as a drug product impurity

- Each actuator of albuterol delivers 35 mg of drug product.
- The absolute amount of formaldehyde associated with the 215 ppb in air limit will be the same, that is, 0.105 µg formaldehyde
- Calculate the corresponding drug product limit: 0.105 µg formaldehyde/35 mg drug product = 3 ppm.

Note 4

The calculated TD_{50} for hydrazine is illustrated below since it was not listed in the CPDB. Hydrazine calculations were based on the most sensitive tumor type: female rats, hepatocellular adenoma and/or carcinoma (Ref. 1). The doses and incidences are listed below

ppm	Dose (mg/kg/day)	Number of Positive Animals	Total Number of Animals
0	0	1	50
20	1.28	0	50
40	2.50	3	50
80	5.35	6	50

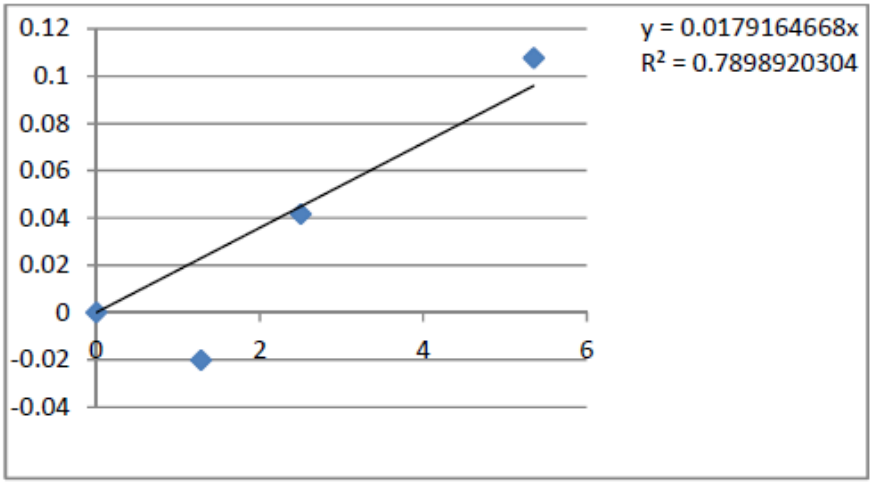
The TD_{50} is calculated from crude summary data of tumor incidence over background with the following equation (Ref. 2, 3):

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

Where P is the proportion of animals with the specified tumor type observed at a certain dose (D in the equation) and P_0 is the proportion of animals with the specified tumor type for the control. Converting β and D into a simple linear equation results in the following:

$$-\ln\left(\frac{p - p_0}{1 - p_0} - 1\right) = \beta \cdot \mathrm{D}$$

Plotting the results and using the slope to represent β results in the following graph for the dose-response and β = 0.0179164668.



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

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

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

$$TD_{50} = \frac{0.693}{\beta}$$

Therefore, the TD_{50} = 0.693 / 0.0179164668 or 38.7 mg/kg/day.

References

1. Matsumoto M, Kano H, Suzuki M, Katagiri T, Umeda Y, Fukushima S. Carcinogenicity and chronic toxicity of hydrazine monohydrate in rats and mice by two-year drinking water treatment. Regul Toxicol Pharmacol 2016;76:63-73.

2. Gaylor DW, Gold LS. Quick estimate of the regulatory virtually safe dose based on the maximum tolerated dose for rodent bioassays. Regul Toxicol Pharmacol 1995;22:57-63.

3. Sawyer C, Peto R, Bernstein L, Pike MC. Calculation of carcinogenic potency from long-term animal carcinogenesis experiments. Biometrics 1984;40:27-40.

Note 5

The calculated TD_{50} for methyl chloride is illustrated below since it was not listed in the CPDB. Since the methyl chloride study (Ref. 1, 2) is based on inhalation, the inhaled ppm concentrations need to be converted to dose.

ppm	Dose (mg/kg/day)[1]	Number of Positive Animals	Total Number of Animals
0	0	0	67
50	28	0	61
225	127	2	57
1000	566	22	86

[1] ppm to mg/kg/day conversion – X ppm x 50.5 g/mol (mol weight)/24.45 x 0.043 (breathing volume) x 6/24 hours x 5/7 days / 0.028 kg (mouse weight) = dose mg/kg/day

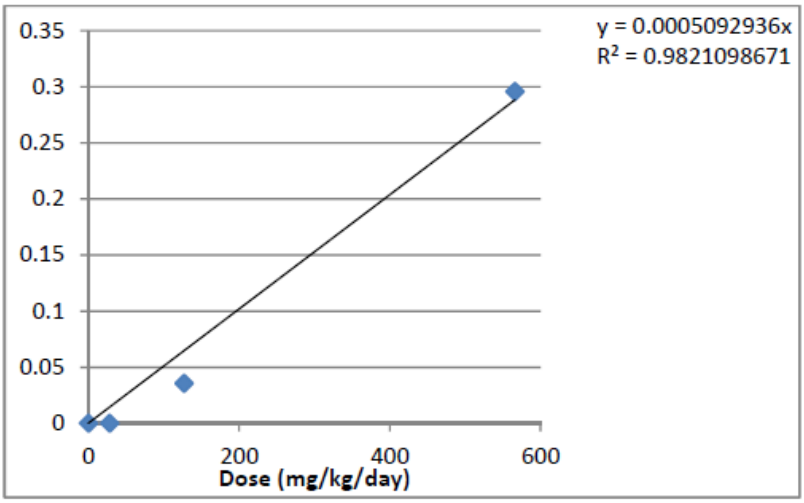
The TD_{50} is calculated from crude summary data of tumor incidence over background with the following equation (Ref. 3, 4):

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

Where P is the proportion of animals with the specified tumor type observed at a certain dose (D in the equation) and P_0 is the proportion of animals with the specified tumor type for the control. Converting β and D into a simple linear equation results in the following:

$$-\ln\left(\frac{p - p_0}{1 - p_0} - 1\right) = \beta \cdot \mathrm{D}$$

Plotting the results and using the slope to represent β results in the following graph for the dose-response and β = 0.0005092936.



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

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

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

$$TD_{50} = \frac{0.693}{\beta}$$

Therefore, the TD_{50} = 0.693 / 0.0005092936 or 1360.7 mg/kg/day.

References

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

2. US EPA. Toxicological review of methyl chloride. (CAS No. 74-87-3). In Support of Summary Information on the IRIS. EPA/635/R01/003. 2001.

3. Gaylor DW, Gold LS. Quick estimate of the regulatory virtually safe dose based on the maximum tolerated dose for rodent bioassays. Regul Toxicol Pharmacol 1995;22:57-63.

4. Sawyer C, Peto R, Bernstein L, Pike MC. Calculation of carcinogenic potency from long- term animal carcinogenesis experiments. Biometrics 1984;40:27-40.