
**M7(R2) Addendum:
Application of the Principles
of the ICH M7 Guidance to
Calculation of Compound-
Specific Acceptable Intakes
Guidance for Industry**

**U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)**

**July 2023
ICH-Multidisciplinary**

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FOREWORD

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has the mission of achieving greater regulatory harmonization worldwide to ensure that safe, effective, and high-quality medicines are developed, registered, and maintained in the most resource-efficient manner. By harmonizing the regulatory expectations in regions around the world, ICH guidelines have substantially reduced duplicative clinical studies, prevented unnecessary animal studies, standardized safety reporting and marketing application submissions, and contributed to many other improvements in the quality of global drug development and manufacturing and the products available to patients.

ICH is a consensus-driven process that involves technical experts from regulatory authorities and industry parties in detailed technical and science-based harmonization work that results in the development of ICH guidelines. The commitment to consistent adoption of these consensus-based guidelines by regulators around the globe is critical to realizing the benefits of safe, effective, and high-quality medicines for patients as well as for industry. As a Founding Regulatory Member of ICH, the Food and Drug Administration (FDA) plays a major role in the development of each of the ICH guidelines, which FDA then adopts and issues as guidance to industry.

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M7(R2) Addendum: Application of the Principles of the ICH M7 Guidance to Calculation of Compound-Specific Acceptable Intakes Guidance for Industry¹

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA office responsible for this guidance as listed on the title page.

INTRODUCTION

The International Council for Harmonisation (ICH) guidance for industry *M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* (ICH M7(R2)) (July 2023) discusses the derivation of acceptable intakes (AIs) for mutagenic impurities with positive carcinogenicity data, (section VII.B.1 (7.2.1)) and states:²

Compound-specific risk assessments to derive acceptable intakes should be applied instead of the [threshold of toxicological concern-based] TTC-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(R2), 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(R2).³ 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(R2) of linear extrapolation

¹ This guidance was developed within the Expert Working Group (*Multidisciplinary*) of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document has been endorsed by the ICH Assembly at *Step 4* of the ICH process, April 2023. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the ICH regions.

² We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents>.

³ 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 AI will not typically be needed.

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from the calculated cancer potency estimate, the TD₅₀.⁴ Some chemicals that are mutagens and carcinogens (classified as Class 1 in ICH M7(R2)) may induce tumors through a nonmutagenic 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 nonmutagenic; nevertheless, the historical perception has been that they are genotoxic carcinogens.

ICH M7(R2) states in section VII.B.2 (7.2.2):

The existence of mechanisms leading to a dose response that is nonlinear 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 (ICH guidance for industry *Q3C(R8) Impurities: Guidance for Residual Solvents* [ICH Q3C(R8)] (December 2021)) to calculate a permissible daily exposure (PDE) when data are available.

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(R8) (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(R2) guidance (section VII.B.2 (7.2.2)) notes that when calculating AIs from compound-specific risk assessments, an upper limit would be 0.5 percent, or, for example, 500 micrograms in a drug with a maximum daily dose of 100 milligrams (mg).

METHODS

The general approach used in this addendum for deriving AIs included a literature review, selection of cancer potency estimate (TD₅₀), taken from the CPDB (Carcinogenic 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 III (3) of this addendum). The literature review focused on data relating to exposure of the general population (i.e., food, water, air), mutagenicity/genotoxicity, and carcinogenicity. Based on the description of DNA-reactive mutagens in ICH M7(R2), 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., U.S. Environmental Protection Agency (EPA), U.S. Food and Drug Administration, European Medicines Agency, European Chemicals Agency, World Health

⁴ TD₅₀ is defined as the chronic dose-rate in milligrams per kilograms of body weight per day, which would cause tumors in half of the animals at the end of a standard life span for the species taking into account the frequency of that tumor type in control animals.

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Organization (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, methemoglobinemia).

I. STANDARD METHOD (1)⁵

A. Linear Mode of Action and Calculation of AI (1.1)

Note 4 of ICH M7(R2) states:

It is possible to calculate a compound-specific acceptable intake based on rodent carcinogenicity potency data such as TD₅₀ values (doses giving a 50 percent 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 TD₅₀ by 50,000. This procedure is similar to that employed for derivation of the TTC.

Thus, linear extrapolation from a TD₅₀ 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 nonlinear 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 precalculated TD₅₀ 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 TD₅₀ value was not recalculated.

If robust data were available in the literature but not in the CPDB, then a TD₅₀ 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(R8) and the ICH guidance for industry *Q3D(R2) Elemental Impurities* (ICH Q3D(R2)) (September 2022) (Ref. 1, 4).

B. Selection of Studies (1.2)

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:

- Less than 50 animals per dose per sex
- Less than three dose levels
- Lack of concurrent controls
- Intermittent dosing (less than 5 days per week)
- Dosing for less than lifetime

⁵ The numbers in parentheses reflect the organizational breakdown of the document endorsed by the ICH Assembly at Step 4 of the ICH process, April 2023.

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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 as defined by the National Toxicology Program or the ICH guidance for industry *S1C(R2) Dose Selection for Carcinogenicity Studies of Pharmaceuticals* (September 2008) (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 three 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 TD₅₀ 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.

C. Selection of Tumor and Site (1.3)

The lowest TD₅₀ 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 TD₅₀, but in this addendum the lowest TD₅₀ was considered a more conservative estimate. Data compiled as *all tumor-bearing animals* were not considered in selecting an appropriate TD₅₀ from the CPDB; mixed tumor types (e.g., adenomas and carcinomas) in one tissue (e.g., liver) were used where appropriate because this often gives a more sensitive potency estimate.

D. Route of Administration (1.4)

Section VII.E (7.5) of ICH M7(R2) states:

The above risk approaches described in section VII (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 TD₅₀ from the route with the lowest TD₅₀ 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 ICH M7(R2). Here, if tumors were considered site specific (e.g., inhalation exposure resulting in respiratory tract tumors with no tumors at distal sites) and the TD₅₀ was lower than for other routes, then a separate AI was developed for that route (e.g., dimethyl carbamoyl chloride, hydrazine).

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E. Calculation of AI From the TD₅₀ (1.5)

Calculating the AI from the TD₅₀ is as follows (see Note 4 of ICH M7(R2) for example):

$$\text{AI} = \text{TD}_{50}/50,000 \times 50 \text{ kilograms (kg)}$$

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.

II. CONSIDERATION OF ALTERNATIVE METHODS FOR CALCULATION OF AI (2)

A. Human Relevance of Tumors (2.1)

Note 4 of ICH M7(R2) states:

As an alternative of using the most conservative TD₅₀ 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 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 nonlinear dose response are not relevant to humans at the low, nontoxic 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 nonlinear 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 TD₅₀, 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.

B. Published Regulatory Limits (2.2)

Note 4 of ICH M7(R2) also states:

Compound-specific acceptable intakes can also be derived from published recommended values from internationally recognized bodies such as the 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.

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In this addendum, available regulatory limits are described (omitting occupational health limits because they are typically regional and may use different risk levels). However, the conservative linear extrapolation from the TD₅₀ was generally used as the primary method to derive the AI, as the default approach of ICH M7(R2), and for consistency across compounds. It is recognized that minor differences in methodology for cancer risk assessment can result in different recommended limits (e.g., adjusting for body surface area in calculations), but the differences are generally quite small when linear extrapolation is the basis of the calculation.

III. NONLINEAR (THRESHOLD) MODE OF ACTION AND CALCULATION OF PDE (3)

ICH M7(R2) states in section VII.B.2 (7.2.2):

The existence of mechanisms leading to a dose response that is nonlinear 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 (ICH guidance for industry *Q3C(R8) Impurities: Guidance for Residual Solvents* (December 2021)) 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 II.A (2.1) of this addendum) that lack human relevance at low doses, based upon their association with a nonlinear dose response for tumor induction:

- Chemicals that induce methemoglobinemia, hemosiderin deposits in tissues such as spleen, and subsequent inflammation and tumors (e.g., aniline, 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, nonirritating 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 because abundant endogenous protective mechanisms exist (e.g., hydrogen peroxide)

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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(R8) (Ref. 1) and ICH Q3D(R2) (Ref. 4).

IV. ACCEPTABLE LIMIT BASED ON EXPOSURE IN THE ENVIRONMENT (E.G., IN THE DIET) (4)

As noted in ICH M7(R2) section VII.E (7.5), “Higher acceptable intakes may be justified when human exposure to the impurity will be much greater from other sources e.g., food, endogenous metabolism (e.g., formaldehyde).”

For example, formaldehyde is not a carcinogen orally, so regulatory limits have been based on noncancer endpoints. Health Canada (Ref. 8), WHO IPCS (Ref. 9), and the U.S. EPA (Ref. 10) recommend an oral limit of 0.2 mg/kg/day, or 10 mg/day for a 50 kg person.

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LIST OF ABBREVIATIONS

AI	acceptable intake
ACGIH	American Conference of Governmental Industrial Hygienists
API	active pharmaceutical ingredient
ATSDR	Agency for Toxic Substances and 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	Carcinogenic Potency Database
CYP	cytochrome P450
DMCC	Dimethylcarbamyl chloride
DMS	dimethyl sulfate
DNA	deoxyribose nucleic acid
DP	drug product
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EPA	Environmental Protection Agency

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EU	European Union
FDA	Food and Drug Administration
GRAS	generally recognized as safe
HSDB	Hazardous Substance Data Bank
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 tumor type incidences not provided in the World Health Organization, 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	Organization for Economic Co-operation and Development
OSHA	Occupational Safety and Health Administration
PCE	polychromatic erythrocytes
PDE	permissible daily exposure
RfC	reference concentration
ROS	reactive oxygen species

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s.c.	subcutaneous
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCE	sister chromatid exchange
SIDS	Screening Information Dataset
TBA	tumor-bearing animal
TD ₅₀	Chronic dose-rate in milligrams per kilograms of body weight per day, which would cause tumors in half of the animals at the end of a standard life span 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 Environment Programme
U.S. EPA	United States Environmental Protection Agency
WHO	World Health Organization

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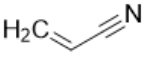
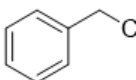
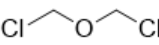
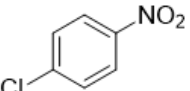
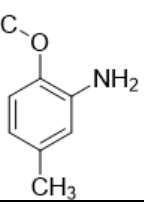
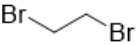
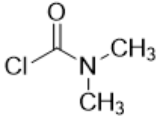
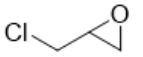


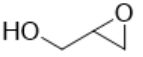
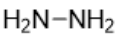
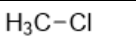
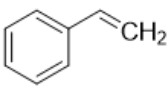
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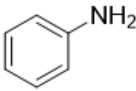
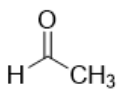

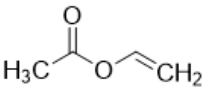
¹ We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents>.

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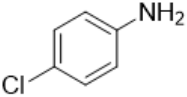
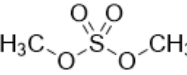
ACCEPTABLE INTAKES OR PERMISSIBLE DAILY EXPOSURES

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

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Compound	CAS#	Chemical Structure	AI or PDE (µg/day)	Comment
Threshold-Based PDE				
Aniline Aniline hydrochloride	62-53-3 142-04-1		720	PDE based on threshold mode of action (hemosiderosis)
Endogenous and/or Environmental Exposure				
Acetaldehyde	75-07-0		2,000 (oral)* 185 (all other routes)	Oral PDE is based on average food intake; all other routes based on TD ₅₀ linear extrapolation from an inhalation study
Formaldehyde	50-00-0		8,000 or 215 ppb, whichever is lower (inhalation)* 10,000 (all other routes)	Inhalation route based on TD ₅₀ linear extrapolation or local irritation; all other routes based on average food intake
Hydrogen peroxide	7722-84-1	HO-OH	68,000 or 0.5%, whichever is lower	68 mg/day is 1% of estimated endogenous production
Vinyl acetate	108-05-4		2,000 (oral)* 758 (all other routes)	Oral PDE is based on average food intake for acetaldehyde; all other routes based on TD ₅₀ linear extrapolation from an inhalation study

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Compound	CAS#	Chemical Structure	AI or PDE (µg/day)	Comment
Other Cases				
<i>p</i> -Chloroaniline <i>p</i> -Chloroaniline hydrochloride	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

CAS = Chemical Abstracts Service; AI = acceptable intake; PDE = permissible daily exposure; µg = microgram; TD₅₀ = chronic dose-rate in milligrams per kilogram of body weight per day, which would cause tumors in half of the animals at the end of a standard life span for the species; ppb = parts per billion; mg = milligrams; TTC = threshold of toxicological concern.

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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, and 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 milligrams (mg)/day of acetaldehyde was calculated based on a constant endogenous total acetaldehyde concentration in the blood of 2.2 ± 1.1 micromole/liter (L) (Ref. 3) and acetaldehyde clearance of 0.95 L/minute (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 Food and Agriculture of the United Nations and the World Health Organization Expert Committee on Food Additives (JECFA) estimated food additive consumption to be 9.7 mg/day in the United States 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 United States (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 to 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 \pm assay (Ref 13). Acetaldehyde induced increases in micronuclei in the bone marrow of rats and mice (Ref 17).

Carcinogenicity

Acetaldehyde is an International Agency for Research on Cancer (IARC) Group 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, breast cancer) (Ref. 19, 20). The relative risk was increased

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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 and 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 parts per million (ppm) (reduced after 11 months due to toxicity), 6 hours/day at 5 days/week for up to 28 months. The corresponding doses in the CPDB were 0, 70.8, 142, and 147 mg/kilogram (kg) for male rats and 0, 101, 202, and 209 mg/kg for female rats. In the high-dose group, 50 percent of the male and 42 percent 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 and 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 TD₅₀ 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

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model for any specific cancer site. A TD₅₀ 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	TD₅₀ (mg/kg/ day)
Ref. 24	55/sex/ group Wistar rat	28 months, inhalation	55	3: M: 70.8; 142; 147 mg/kg/day 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 mg/kg/day 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 mg/kg/day F: 391 mg/kg/day	Larynx / Mixed tumor type / Male	461

Studies listed are in the Carcinogenic Potency Database (CPDB) (Ref. 24).

mg = milligram; kg = kilogram; NC = not calculated.

^a TD₅₀ taken from the CPDB, carcinogenicity study selected for acceptable intake derivation.

^b The TD₅₀ was not calculated because of 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 nonlinear dose response associated with the genotoxicity and carcinogenicity of acetaldehyde (Ref. 14). The dose-response of acetaldehyde-induced adducts at concentrations between 1 and 1,000 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.

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ALDH, which efficiently detoxifies acetaldehyde, is responsible for the nonlinear 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 nonspecific 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 percent 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, nonirritating doses. However, there are no published measurements that 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 U.S. Food and Drug Administration's (FDA's) *generally recognized as safe* 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 because it is completely metabolized into nonreactive acetic acid and finally carbon dioxide, 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 of 2 ppm corresponding to 3.6 mg/cubic meter (m³). This represents an exposure of 3.6 mg/m³ times 28.8 m³ (24 hours in a day — the International Council for Harmonisation (ICH) guidance for industry *Q3C(R8) Impurities: Guidance for Residual Solvents* (December 2021) assumption)¹ equals 104 mg/day.

The U.S. Environmental Protection Agency (EPA) did not consider a threshold for acetaldehyde carcinogenicity and has calculated that a concentration of 5 micrograms (µg)/m³ acetaldehyde represents a 10⁻⁵ (1 in 100,000) excess lifetime cancer risk based on the rat inhalation carcinogenicity study and application of linear extrapolation (Ref. 34). For a 24-

¹ We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents>.

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hour exposure, this represents $5 \mu\text{g}/\text{m}^3$ times 28.8 m^3 equals $144 \mu\text{g}/\text{day}$. 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 nonlinear dose response for the carcinogenicity of acetaldehyde following oral administration and high background exposure from a wide variety of foods, a PDE of $2 \text{ mg}/\text{day}$ is identified for oral limit based on the estimated average intake of acetaldehyde from food of around $2 \text{ mg}/\text{day}$ (Ref. 8).

PDE (oral) = $2 \text{ mg}/\text{day}$

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 lifetime (i.e., 28 months). According to the ICH guidance for industry *M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* 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 = $\text{TD}_{50}/50,000 \times 50 \text{ kg}$

Lifetime AI = $185 \text{ mg}/\text{kg}/\text{day}/50,000 \times 50 \text{ kg}$

Lifetime AI (all other routes) = $185 \mu\text{g}/\text{day}$

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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 (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 the International Agency for Research on Cancer as a Group 2B carcinogen, possibly carcinogenic to humans (Ref. 2).

Acrylonitrile is a multiorgan carcinogen in mice and rats, with the brain being the primary target organ in rat. There are four oral carcinogenicity studies cited in the Carcinogenic Potency Database (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/National Toxicology Program) study in the CPDB of acrylonitrile in mice (Ref. 5) was selected for derivation of the oral acceptable intake (AI), based on robust study design and the most conservative TD₅₀ value. In this 2-year study, three 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 TD₅₀ is for astrocytomas in female rats (5.31 milligrams (mg)/kilogram (kg)/day). However, this same study was later described in detail (Ref. 7), and

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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 parts per million, adjusting for body weight and the decreased water consumption in the study. The TD₅₀ 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 TD₅₀'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 TD₅₀ 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 the U.S. Environmental Protection Agency (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 two 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/S ex	TD₅₀ (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)

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Study	Animals/ dose group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD₅₀ (mg/kg/d)
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
	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 months, 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 months, 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 the Carcinogenic Potency Database (CPDB) (Ref. 3) unless otherwise noted.

The TD₅₀ values represent the TD₅₀ from the most sensitive tumor site.

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

mg = milligram; kg = kilogram; d = day; wk = week; F = female; M = Male; NA = not applicable.

^a Carcinogenicity study selected for acceptable intake (AI) calculation; in CPDB.

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^b NC = Not calculated as individual tumor type incidences not provided in the World Health Organization (WHO) (Ref. 1).

^c TD₅₀ 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 TD₅₀ for astrocytomas and stomach tumors in Spartan rats (20.8 and 9.0) are higher than those in the CPDB.

^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 nonirritating (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 U.S. EPA (Ref. 9) calculated an oral slope factor of 0.54/mg/kg/day and a drinking water limit of 0.6 micrograms (µg)/liter at the 1 in 100,000 risk level, based on the occurrence of multiorgan tumors in a drinking water study in rats. This drinking water limit equates to a daily dose of about 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 U.S. 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 TD₅₀ derived from administering acrylonitrile by oral gavage to male and female mice because the tumor type with the lowest TD₅₀ was forestomach tumors in male mice, with a TD₅₀ value of 5.92 mg/kg/day. As discussed in the Methods section II.B (2.2), linear extrapolation from the TD₅₀ 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 U.S. EPA (Ref. 9) for drinking water.

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Calculation of AI

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

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

$$\text{Lifetime AI} = 5.9 \text{ }\mu\text{g/day (6 }\mu\text{g/day)}$$

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ANILINE (CAS# 62-53-3) AND ANILINE HYDROCHLORIDE (CAS# 142-04-1)

Potential for Human Exposure

Aniline occurs naturally in some foods (i.e., corn, grains, beans, 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 because some in vitro and in vivo genotoxicity tests are positive.

Aniline is not mutagenic in the five standard strains of *Salmonella* or in *Escherichia coli* WP2uvrA, 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 millimolar (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 milligrams (mg)/kilogram (kg) (Ref. 16), but a small increase in chromosomal aberrations 18 hours 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, 1,000, and 2,000 parts per million (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, reaching a maximum of twofold increase over the background, was observed in the bone marrow of male Swiss mice 24 hours 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 the International Agency for Research on Cancer (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 α -naphthylamine, benzidine, and other amines.

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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 2,000 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 permissible daily exposure (PDE) for aniline based on the robust study design with three 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 U.S. National Cancer Institute (NCI) (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 Hydrochloride (HCl) – Details of Carcinogenicity Studies

Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD₅₀ (mg/kg/d)
Ref. 26 ^a Aniline HCl	130/sex/ group, CD-F rats	2 years, diet	130	3: 200, 600, and 2,000 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 week study), diet	50	2: 3,000 and 6,000 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 week study), Diet	50	2: 6,000 and 12,000 ppm in diet (F: 741, 1,500 M: 693, 1,390 Mg/kg/d)	Negative	NA

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Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD ₅₀ (mg/kg/d)
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 permissible daily exposure calculation. Not in the Carcinogenic Potency Database (CPDB).

^b Taken from CPDB (Ref. 29). The TD₅₀ values represent the TD₅₀ from the most sensitive tumor site. HCl = hydrochloride; mg = milligram; kg = kilogram; d = day; ppm = parts per million; NOEL = no-observed effect level; M = male; F = female; 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, and 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 (greater than 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 nonlinear (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 percent (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).

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Regulatory and/or Published Limits

The U.S. Environmental Protection Agency (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 micrograms (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

It is considered inappropriate to base an acceptable intake for aniline on linear extrapolation for spleen tumors observed in rats; because these have a nonlinear 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 the ICH guidance for industry *Q3C(R8) Impurities: Guidance for Residual Solvents* (ICH Q3C(R8)) (December 2021) (Ref. 40).¹

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 2,000 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 (NOEL) for tumors.

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

The following safety factors as outlined in ICH Q3C(R8) have been applied to determine the PDE for aniline:

F1 = 5 (rat to human)

F2 = 10 (interindividual variability)

F3 = 1 (study duration at least half lifetime)

F4 = 10 (severe toxicity — nongenotoxic 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

¹ We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents>.

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¹ We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents>.

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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 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 intravenous 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 three times a week for 104 weeks to F-344 rats and B6C3F1 mice (Ref. 4). Rats received doses of 0, 15, or 30 milligrams (mg)/kilogram (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 percent versus 8 percent 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 percent and 37 percent, respectively, compared with 0 percent 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 percent versus 0 percent in controls) at the high dose and in carcinoma or adenoma in the liver but only at the low dose (54 percent versus 33 percent in controls). In female, but not male, mice there were significant increases in the incidence of alveolar-

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bronchiolar adenoma or carcinoma at the high dose (12 percent versus 1.9 percent in controls).

Additional studies to assess carcinogenic potential were conducted but were not considered of adequate study design for use in calculating an acceptable intake (AI). In one of three topical studies (Ref. 5) skin carcinomas were increased, although not statistically significantly (15 percent versus 0 percent 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	TD₅₀ (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 months 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

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Study	Animals/Dose Group	Duration/Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex or Tumor Observations	TD ₅₀ (mg/kg/d)
Ref. 6	20/group male ICI Swiss albino mouse	>7 months 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 months 1 dose (in toluene); wait 1 wk Promoter (croton oil) 2 times/wk	40	1: 1 mg/mouse	No skin tumors	NC ^b
Ref. 8	Sencar mice	6 months 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 Carcinogenic Potency Database (CPDB) (Ref. 10) unless otherwise noted.

^a Carcinogenicity study selected for acceptable intake 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.

mg = milligram; kg = kilogram; d = day; wk = week; µL = microliter; µg = microgram; DMBA = dimethylbenzanthracene.

Mode of Action for Carcinogenicity

The tumor types with the lowest calculated TD₅₀ (highest potency) in the Carcinogenic Potency Database (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, nonirritating 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 nonmutagenic 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

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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 nonirritating 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 nonmutagenic, 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 nonlinear 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 maximum tolerated dose (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 Organization for Economic Co-operation and Development (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 to 3 weeks from severe acute and chronic gastritis of the forestomach, often with ulcers, following oral administration three times a week of doses greater than 250 mg/kg for males and greater than 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 (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 (TD₅₀ 454 mg/kg/day), and in thyroid C-cell adenomas or carcinomas in the female rats (TD₅₀ 40.6 mg/kg/day). The levels of thyroid C-cell tumors in

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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 National Toxicology Program (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 percent).

Regulatory and/or Published Limits

The U.S. Environmental Protection Agency (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 micrograms (μg)/liter or approximately 4 μg a day using U.S. EPA assumptions.

Acceptable Intake

Rationale for selection of study for AI calculation

The most robust evaluation of the carcinogenic potential of benzyl chloride was the Lijinsky 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 National Cancer Institute /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 10 rats of each sex given 125 or 250 mg/kg (3 days per week) died within 2 to 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 (three times per week). At this dose, there was no difference from controls in survival in the 2-year carcinogenicity study, but three 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 II.B (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 because 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 because they were associated with the lowest TD_{50} : 40.6 mg/kg/day.

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Calculation of AI

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 40.6 \text{ (mg/kg/day)}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 40.6 \text{ }\mu\text{g/day (41 }\mu\text{g/day)}$$

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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 bis(chloromethyl)ether (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 6 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 the U.S. Environmental Protection Agency (EPA) as a Group A, known human carcinogen (Ref. 5), and by the International Agency for Research on Cancer 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 acceptable intake (AI) based on the most robust study design and the lowest TD₅₀ value. Groups of male Sprague-Dawley rats and Ha/ICR mice were exposed by inhalation to 1, 10, and 100 parts per billion (ppb) of BCME 6 hours a day, 5 days a week for 6 months and subsequently observed for the duration of their natural life span (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 percent of the surviving rats, which had been exposed to 100 ppb (7780 nanograms (ng)/kilogram (kg)/day, or about 8 micrograms (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 percent 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 parts per million (100 ppb) 6 hours a day, 5 days a week for 10, 20, 40, 60, 80, or

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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 subcutaneous application (Ref. 10).

Bis(chloromethyl)ether – Details of Carcinogenicity Studies

Study	Animals/Dose Group	Duration/Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD₅₀ (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 Carcinogenic Potency Database (CPDB) (Ref. 11) unless otherwise noted.

^a Carcinogenicity study selected for acceptable intake calculation.

^b NC= Not calculated due to nonstandard carcinogenicity design. Not in CPDB. NA= Not available since controls were not reported in the study.

mg = milligram; kg = kilogram; d = day; h = hour; wk = week; ppb = parts per billion; ng = nanogram; ppm = parts per million; NA = not applicable.

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Mode of Action for Carcinogenicity

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

Regulatory and/or Published Limits

The U.S. EPA (Ref. 5) calculated an oral cancer slope factor of 220 per milligram (mg)/kg/day based on linearized 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 times 10⁻⁸ mg/cubic meter for inhalation, 1.6 times 10⁻⁶ mg/liter for oral exposure).

Acceptable Intake

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 TD₅₀ of 3.57 μg/kg/day. The AI derived by linear extrapolation from that TD₅₀, about 4 ng/day, is essentially the same as the 3.2 ng/day recommendation of the U.S. EPA. The study (Ref. 3) had a reliable design with multiple dose levels and greater than 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 threshold of toxicological concern of 1.5 μg/day). The AI is also similar to the limit derived by the U.S. EPA (based on inhalation data) that is recommended both for inhalation and ingestion (drinking water) of BCME (4 ng/day versus 3.2 ng/day).

Calculation of AI

Lifetime AI = TD₅₀/50,000 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

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***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 the World Health Organization (WHO) (Ref. 3) with references, so only key conclusions are summarized here.

p-Chloroaniline is mutagenic in:

- Microbial reverse mutation assay (Ames); two- to three-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 milligrams (mg)/kilogram (kg), but a significant increase was reported at 300 mg/kg/day after three daily doses in mice.

Carcinogenicity

p-Chloroaniline is classified by the International Agency for Research on Cancer 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 National Toxicology Program (NTP) (Ref. 6) oral gavage study was used to calculate the acceptable intake (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

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increased. In male mice, the incidence of hemangiosarcomas of the liver or spleen in high-dose groups was greater than that in the vehicle controls (4/50 in 0 mg/kg/day; 4/49 in 2.1 mg/kg/day; 1/50 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; and 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 hemangiomas in mice. Although the incidences of these tumors are strongly suggestive of carcinogenicity, the National Cancer Institute concluded that sufficient evidence was not found to establish the carcinogenicity of *p*-chloroaniline in rats or mice under the conditions of these studies. Because *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 Hydrochloride – Details of Carcinogenicity Studies**

Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD₅₀ (mg/kg/d)
Ref. 6 ^a <i>p</i> -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 <i>p</i> -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 <i>p</i> -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, hemangiosarcoma, osteosarcoma.	7.62
Ref. 6 <i>p</i> -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

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Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD ₅₀ (mg/kg/d)
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, hemangiosarcoma, osteosarcoma, sarcoma not otherwise specified) of the spleen or splenic capsule.	72
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)	Hemangiosarcoma's (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)	Hemangiosarcoma's (liver and spleen). Increased incidence of combined vascular tumors.	1480

Studies listed are in the Carcinogenic Potency Database (Ref. 9).

^a Carcinogenicity study selected for acceptable intake calculation.

mg = milligram; kg = kilogram; d = day; HCl = hydrochloride; wk = week; NA = not applicable; ppm = parts per million; CPDB = Carcinogenic Potency Database.

Mode of Action for Carcinogenicity

p-Chloroaniline-induced tumors in male rats, such as spleen fibrosarcomas and osteosarcomas, typical for aniline 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 does not support tumor induction related to a direct interaction of *p*-chloroaniline or its metabolites with DNA.

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Similarly, the reported induction of micronuclei in vivo is likely to be secondary to regenerative anemia/altered erythropoiesis, as with aniline (Ref. 11,12).

The tumor type with the lowest TD₅₀ was spleen tumors in male rats. However, since this tumor type is associated with a nonlinear dose relation, spleen tumors were not used to calculate the AI. Based on nonneoplastic (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

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 TD₅₀ 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 = TD₅₀/50,000 x 50 kg

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

Lifetime AI = 34 µg/day

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1-CHLORO-4-NITROBENZENE (PARA-CHLORONITROBENZENE, CAS# 100-00-5)

Potential for Human Exposure

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

Mutagenicity/Genotoxicity

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 *Escherichia coli* WP2uvrA (Ref. 1, 2, 3, 4). It was also weakly positive without metabolic activation in TA1535 in two of four 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 the International Agency for Research on Cancer as a Group 2 carcinogen, not classifiable as to its carcinogenicity in humans (Ref. 7), and the U.S. Environmental Protection Agency (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, which were statistically significant in males at the mid and high doses (7.7 and 41.2 milligrams (mg)/kilogram (kg)/day). Nonneoplastic 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 hematocrit, 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 2,000 parts per million (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 4,000 ppm for the first 3 months, 500 ppm for next 2 months, and 1,000 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

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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 a 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	TD₅₀ (mg/kg/d)
Ref. 9 ^{ac}	50/group male F344 rats (SPF)	2 years, diet	50	3: 40; 200; 1,000 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; 1,000 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; 2,000 ppm. (15.3; 60.1; 240 .1 mg/kg/d)	NA	

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Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD ₅₀ (mg/kg/d)
Ref. 9 ^{ac}	50/group female Crj:BDF1 (SPF)	2 years, diet	50	3: 125;500; 2,000 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 rats	18 months, diet; sacrificed 6 months after last dose	16	2: Average 17 and 33 mg/kg; (see text) (22.6 and 45.2 mg/kg/d)	NA	Negative ^d
	14-20/sex group CD-1 mice	18 months, diet; sacrificed 3 months 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 months 5 d/ wk, gavage	Yes	3: 0.1; 0.7; 5 mg/kg/d	NA	Negative

Studies listed are in the Carcinogenic Potency Database (CPDB) (Ref. 12) unless otherwise noted.

^a Carcinogenicity study selected for acceptable intake/permissible daily exposure calculation.

^b TD₅₀ calculated based on carcinogenicity data (see Note 1).

^c Not in CPDB.

^d Histopathology limited to 11 to 12 tissues.

mg = milligram; kg = kilogram; d = day; wk = week; ppm = parts per million; NA = not applicable; M = male; F = female.

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

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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 nonlinear 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 TD₅₀ 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 nonmutagenic (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 nonmutagenic 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 nonlinear 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.

Because 1-chloro-4-nitrobenzene is mutagenic, and a mutagenic mode of action cannot be ruled out, an acceptable intake (AI) calculation was performed.

Regulatory and/or Published Limits

No regulatory limits have been published, for example by the U.S. EPA, the World Health Organization, or the Agency for Toxic Substances and Disease Registry.

Calculation of AI

The most sensitive TD₅₀ is for adrenal medullary pheochromocytomas in female rats (Ref. 9).

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 117 \text{ mg/kg/day} / 50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 117 \text{ } \mu\text{g/day}$$

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P-CRESIDINE (2-METHOXY-5-METHYL ANILINE, CAS# 120-71-8)

Potential for Human Exposure

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

Mutagenicity/Genotoxicity

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

p-Cresidine is mutagenic in:

- Several *Salmonella* strains in the presence of metabolic activation (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 percent, 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 the International Agency for Research on Cancer 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 the National Toxicology Program (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 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 milligram (mg) per kilogram (kg) per day in the Carcinogenic Potency Database 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

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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 increased instances 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 interlaboratory 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	TD₅₀ (mg/kg/d)
Ref. 5 ^a	50/sex/ group B6C3F1 mice	2 year, feed	50	2: 0.5 and 1% reduced after 21 weeks 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

Contains Nonbinding Recommendations

^a Carcinogenicity study selected for acceptable intake calculation. Studies listed are in the Carcinogenic Potency Database (Ref. 12). mg = milligram; kg = kilogram; M = male; F = female.

Mode of Action for Carcinogenicity

p-Cresidine is a mutagenic carcinogen, and the acceptable intake (AI) is calculated by linear extrapolation from the TD₅₀.

Regulatory and/or Published Limits

No regulatory limits have been published

Acceptable Intake

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 the National Cancer Institute (NCI)/NTP (Ref. 5). The study in mice was selected for derivation of the AI because the most sensitive TD₅₀ was based on urinary bladder tumors in male mice.

Calculation of AI

The most sensitive TD₅₀ values from the NCI/NTP studies are for the urinary bladder in both sexes of rats and mice; in rats the TD₅₀ was 110 mg/kg/day for females and 88.4 mg/kg/day for males; in mice the TD₅₀ 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:

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 44.7 \text{ mg/kg/day} / 50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 45 \text{ } \mu\text{g/day}$$

Contains Nonbinding Recommendations

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Contains Nonbinding Recommendations

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Contains Nonbinding Recommendations

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. Environmental Protection Agency and the European Commission 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 (milligrams) (mg)/kilogram (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 the International Agency for Research on Cancer as probably carcinogenic to humans (Group 2A) (Ref. 13). Inhalation and oral carcinogenicity studies are cited in the Carcinogenic Potency Database (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/d) ^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/Ma le	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/Ma le	9.44

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Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses^a	Most Sensitive Tumor Site/Type/Sex	TD₅₀ (mg/kg/d)^b
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
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 adenocarcino ma/ 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/Multipl e 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/Multipl e tumor types/Female	NC

mg = milligram; kg = kilogram; d = day; M = male; F = female; mmol = millimole; L = liter; ppm = parts per million; NC = not calculated.

^a mg/kg/day values stated in the Carcinogenic Potency Database (CPDB) (Ref. 14).

^b Individual TD₅₀ values are the CPDB TD₅₀ values as reported in the Lhasa carcinogenicity database (Ref. 15). TD₅₀ values represent the TD₅₀ from the most sensitive tumor site.

^c Carcinogenicity study selected for acceptable intake 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 National Toxicology Program (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 (male: 4.0, 15.9 mg/kg/day, female: 5.71, 22.8 mg/kg/day with 50 rats/sex/group) and a control group (50/sex). The TD₅₀ from the most sensitive sex and site is 2.33 mg/kg/day. Another study with inhalation exposure conducted in Sprague Dawley rats (Ref.

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20) resulted in a lower TD₅₀; 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 TD₅₀.

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 TD₅₀ 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 1 year exposure, the study is considered less useful as it limits characterization of the dose-response relationship and estimation of the TD₅₀ (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 TD₅₀. The tumor types with the lowest calculated TD₅₀ (highest potency) for 1,2-dibromoethane following oral exposure are forestomach tumors in mice and rats (Ref. 18). Following inhalation exposure, the lowest calculated TD₅₀ values are associated with the lung and nasal cavity for mice and rats, respectively. High concentrations of orally dosed nonmutagenic 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 nonirritating 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 multisite, multispecies carcinogen, it is difficult to discriminate between the contribution to the mode of action of these nonmutagenic, high-dose effects compared with direct mutation induction.

Regulatory and/or Published Limits

No regulatory limits have been published.

Acceptable Intake

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. TD₅₀ values tend to be similar across species and routes of administration.

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

Given that the TD₅₀ 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 TD₅₀ value of 2.3 mg/kg/day.

Calculation of AI

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 2.3 \text{ mg/kg/day}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 2 \text{ micrograms } (\mu\text{g})/\text{day}$$

Contains Nonbinding Recommendations

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Contains Nonbinding Recommendations

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 the International Agency for Research on Cancer 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 acceptable intake (AI) derivation used inhalation and intraperitoneal administration.

Syrian golden hamsters were exposed to 1 parts per million 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 percent 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 percent (Ref. 5).

DMCC was tested for carcinogenic activity in female ICR/Ha Swiss mice by skin application, subcutaneous injection and intraperitoneal (i.p.) injection (Ref. 6; this study was selected to calculate the AI). In the skin application, 2 milligrams (mg) of DMCC was applied three 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.

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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/week, 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/week, 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/week, Skin	50	1: 2 mg	Skin: Papillomas and carcinomas/ Female	NA ^c
Ref. 6	50 female ICR/Ha Swiss mice	61 weeks once/week, 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/week, 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 months 3 times /week, 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 months once/week, 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 months once/week, subcutaneous; examined at end of life	Yes	2: 0.43 and 4.3 mg		NA ^d

Studies listed are in the Carcinogenic Potency Database (CPDB) (Ref. 9) unless otherwise noted.

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^a Carcinogenicity study selected for noninhalation acceptable intake (AI).

^b Carcinogenicity study selected for inhalation AI.

^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.

mg = milligram; kg = kilogram; d = day; h = hour; ppm = parts per million; NA = not applicable.

Regulatory and/or Published Limits

No regulatory limits have been published.

Acceptable Intake

Based on the above data, DMCC is considered to be a mutagenic carcinogen. As a result, linear extrapolation from the most sensitive TD₅₀ 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 TD₅₀ was 4.59 mg/kg/day based on mixed tumor incidences (Carcinogenic Potency Database).

The lifetime AI is calculated as follows:

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 4.59 \text{ mg/kg/day} / 50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 5 \text{ micrograms } (\mu\text{g})/\text{day}$$

Inhalation AI

The inhalation AI is calculated as follows:

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

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 0.625 \text{ mg/kg/day} / 50,000 \times 50 \text{ kg}$$

$$\text{Lifetime inhalation AI} = 0.6 \mu\text{g/day}$$

Contains Nonbinding Recommendations

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Contains Nonbinding Recommendations

DIMETHYL SULFATE (CAS# 77-78-1)

Potential for Human Exposure

Dimethyl sulfate (DMS) is found in ambient air with mean concentration of 7.4 (micrograms) µg per cubic meter or 1.4 parts per billion based on 1983 data compiled from a single site by the U.S. Environmental Protection Agency (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 the International Agency for Research on Cancer 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 Carcinogenic Potency Database. The studies evaluating carcinogenicity of DMS are described below (excerpted from U.S. EPA, Ref. 5).

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Dimethyl Sulfate (DMS) - Details of Carcinogenicity Studies

Study	Animals	Duration/ Exposure	Controls	Doses	Tumor Observation	TD₅₀ (mg/kg/d)
Ref. 6	Golden hamsters, Wistar rats, and NMRI mice male and female (number not clearly specified)	15 months 6 h/d, 2 d/wk followed by 15 months 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

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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 the Carcinogenic Potency Database.

^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.

mg = milligram; kg = kilogram; d = day; h = hour; wk = week; ppm = parts per million; NA = not applicable; m = meter.

Mode of Action for Carcinogenicity

DMS is a mutagenic carcinogen, and the acceptable intake (AI) 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 T₂₅ (dose that resulted in a 25 percent 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 two dose groups, and minimal pathological evaluations; therefore, the study was not suitable for linear extrapolation.

Acceptable Intake

Although DMS is considered to be a likely oral carcinogen and probable human carcinogen, there are no oral carcinogenicity studies from which to derive a TD₅₀ value. Moreover, the

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inhalation studies that are available are limited for a variety of reasons and are not suitable for TD₅₀ extrapolation. Given this, it is reasonable to limit DMS to the threshold of toxicological concern lifetime level of 1.5 µg/day.

Lifetime AI = 1.5 µg/day

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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. Although genotoxicity in vitro is seen both with and without liver S9 metabolic activation, activity tends to be suppressed by S9 (Ref. 1, 2, 3). Epichlorohydrin is mutagenic in the Ames test in several strains of *Salmonella typhimurium* and in *Escherichia coli* WP2uvrA 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 the International Agency for Research on Cancer 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, five times per week for lifetime at 2 and 10 milligrams (mg)/kilogram (kg); when converted to an average daily dose for seven days per week, the doses shown in the Carcinogen Potency Database (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 TD₅₀ 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 parts per million (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 (one nasal carcinoma and one nasal papilloma). Despite the low tumor incidence, a TD₅₀ of 421 mg/kg/day is reported in the CPDB.

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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 intraperitoneal injections for over 64 weeks.

Epichlorohydrin – Details of Carcinogenicity Studies

Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD₅₀ (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, s.c.	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

mg = milligram; kg = kilogram; d = day; ppm = parts per million; NC = not calculated; i.p. = intraperitoneal; s.c. = subcutaneous.

^a Carcinogenicity study selected for acceptable intake calculation.

^b The TD₅₀ values are taken from the Carcinogenic Potency Database (CPDB) (Ref. 6).

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

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^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 TD₅₀ 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 micrograms (µg)/kg/day or 8.4 µg/day (for a 60 kg adult), based on the assumption of a nonlinear dose-response for this site-of-contact carcinogen. The U.S. Environmental Protection Agency (EPA) used linear extrapolation to derive a drinking water level (1 in 10⁵ risk of excess cancer) of 30 µg/liter or about 60 µg/day (Ref. 13), using data from Konishi et al. (Ref. 7). The U.S. EPA also calculated an inhalation concentration of 8 µg/cubic meter for a 1 in 10⁵ excess cancer risk, or 230 µg/day, using the International Council for Harmonisation guidance for industry *Q3C(R8) Impurities: Guidance for Residual Solvents* (December 2021)¹ assumptions for human daily breathing volume (Ref. 13).

The Food and Drug Administration/Center for Food Safety and Applied Nutrition calculated the unit cancer risk of 2.7×10^{-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.

¹ We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents>.

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Calculation of AI

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 2.55 \text{ mg/kg/day}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = \mathbf{3 \text{ micrograms } (\mu\text{g})/\text{day}}$$

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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 the International Council for Harmonisation *M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*,¹ is a mutagenic carcinogen.

Mutagenicity/Genotoxicity

Ethyl bromide was mutagenic in the Ames test 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 Chinese hamster ovary cells, ethyl bromide induced sister chromatid exchanges but not chromosomal aberrations in both the presence and absence of exogenous metabolic activation (Ref. 7).

Carcinogenicity

The International Agency for Research on Cancer 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 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 parts per million (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

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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	TD₅₀ (mg/kg/d)^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 The milligrams per kilogram per day values stated in the Carcinogenic Potency Database (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 hours per day for 7 days per week. (Dose rate = (administered dose × intake/day × number of doses/week) / (animal weight × 7 days/week))

^b TD₅₀ calculated in CPDB.

^c Carcinogenicity study selected for acceptable intake calculation.
mg = milligram; kg = kilogram; d = day; M = male; F = female.

Mode of Action for Carcinogenicity

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

Regulatory and/or Published Limits

For ethyl bromide, the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value-time-weighted average (TLV-TWA) for ethyl bromide is 5 ppm (22 milligram (mg)/cubic meter (m³)), while Occupational Safety and Health Administration (OSHA) and National Institute for Occupational Safety and Health (NIOSH) indicate the TWA as 200 ppm (890 mg/m³) (Ref. 11). The ACGIH estimates this value with a notation for skin absorption, but OSHA and NIOSH estimates are based on inhalation studies.

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Acceptable Intake

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 TD₅₀ calculated by the Carcinogenic Potency Database 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 TD₅₀ values for each dose are 32.2 mg/kilogram (kg)/day for low dose, 115 mg/kg/day for mid dose, 162 mg/kg/day for high dose (Note 2). The lowest TD₅₀ value of 32.2 mg/kg/day is used because it is considered to conservatively yield the most sensitive potency estimate for calculating the AI.

Calculation of AI

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 32.2 \text{ mg/kg/day}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 32 \text{ micrograms } (\mu\text{g})/\text{day}$$

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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. The International Agency for Research on Cancer (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* WP2uvrA with and without metabolic activation when tested in conditions that enable exposure to gas (Ref. 2, 3, 4);
- Chinese hamster ovary 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 parts per million (ppm) for 3 days, and in an unscheduled DNA synthesis 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, National Toxicology Program (NTP) studies (Ref. 6) in rats and mice of both sexes via inhalation for 6 hours/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 the U.S. Environmental Protection Agency (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.

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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).

Mg = milligram; kg = kilogram; d = day; h = hour; wk = week; M = male; F = female; g = gram; 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 U.S. EPA established an inhalation reference concentration for noncarcinogenic effects of 10 milligrams (mg)/cubic meter, or 288 mg/day assuming a respiratory volume of 28,800 liters /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 origin 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 three 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. Because the number of tumors is high, it is possible to calculate a TD₅₀ even though only one dose was tested. The authors of the Carcinogenic Potency Database (Ref. 8) converted 0 and 15,000 ppm to doses of 0 and 12.4 grams /kilogram (kg) and calculated a TD₅₀ of 1810 mg/kg/day for mouse uterine tumors.

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

Lifetime AI = $1,810 \text{ mg/kg/day} / 50,000 \times 50 \text{ kg}$

Lifetime AI = 1,810 micrograms (μg)/day

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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 to 119 milligrams (mg) per 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 to 92 grams (g) (878 to 1210 mg/kilogram (kg) body weight/day for a 60 to 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

The International Agency for Research on Cancer (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, 9, 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 U.S. Environmental Protection Agency (EPA) (Ref. 12) and the National Toxicology Program (NTP) 15th Report on Carcinogens conclude that nasopharyngeal cancer and myeloid leukemia (ML) in humans can be attributed to

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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 parts per million (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.

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Formaldehyde – Details of Carcinogenicity Studies

Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD₅₀ (mg/kg/d)
Ref. 23	42-60/ group C3H Mouse	35- or 64- weeks, inhalation	59	3: 50, 100, 200 mg/m ³	No tumors	NC
Ref. 24	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 F: 0.686, 2.06, 5.15 mg/kg/d ^a	Nasal turbinates/ Squamous cell carcinoma/ Male	43.9 ^b
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/d ^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/d ^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 (Undama ged) Male Wistar Rat	3 or 28months, 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

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Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD₅₀ (mg/kg/d)
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/d ^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/d ^a	Nasal cavity /Mixed tumor type/ Male	0.98 ^b
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- lymphosarcom a / Male ^c	424 ^b
Ref. 34	20/sex/ group Wistar Rat	24 months, drinking water	20	3: 10, 50, 300 mg/kg/d	No tumors	NC

^a The milligrams per kilogram per day doses are taken from the Carcinogenic Potency Database (CPDB).

^b TD₅₀ 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.

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^c There were concerns about study design (pooling of lymphomas and leukemias diagnosed, lack of reporting of nonneoplastic 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).

mg = milligram; kg = kilogram; d = day; m = meter; NC = not calculated; M = male; F = female; ppm = parts per million.

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 (CRCP) rather than to a direct genotoxic effect (Ref. 11). Formation of DPX is probably involved in the cytotoxicity 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 tissue levels well above endogenous levels (Ref. 37, 38).

Regulatory and/or Published Limits

For oral exposure to the general population, the Agency for Toxic Substances & Disease Registry, Health Canada, International Programme on Chemical Safety (IPCS), and U.S. EPA limit for formaldehyde is 0.2 mg/kg/day or 10 mg/day for a 50 kg person, which is based on a noncancer 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 because carcinogenicity is specific to the inhalation route of exposure.

Occupational limits have been set for air at workplaces by the National Institute for Occupational Safety and Health (recommended exposure limit time-weighted average (TWA) 0.016 ppm), American Conference of Governmental Industrial Hygienists (TWA 0.1 ppm), DFG MAKs (TWA 0.3 ppm), European Union (binding occupational exposure limit 0.3 ppm) and Occupational Safety and Health Administration (permissible exposure limit TWA 0.75 ppm).

For inhalation exposure to the general population, the U.S. EPA, IPCS, and Health Canada have developed inhalation cancer risk values (Ref. 12, 10, 40). The U.S. 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 to 32 micrograms (μg)/day would result in a 1 in 105 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 nonsmokers. The rat dose response for CRCP/DPX was used by Conolly 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 nonlinear-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.

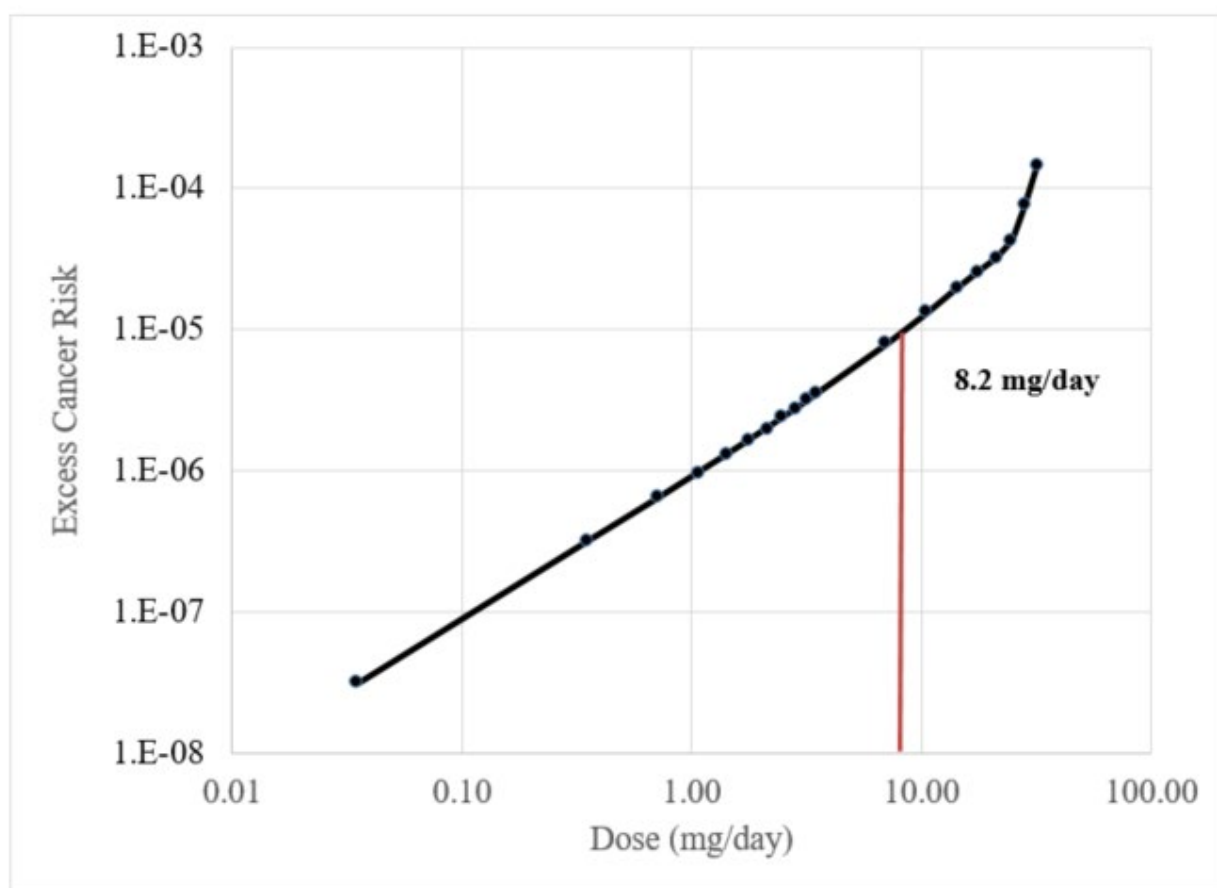


Figure 1. Dose-response model hockey stick-shaped model from (Ref. 11) representing mixed smokers and nonsmokers. The dose (milligrams per day) was based on converting air concentration (parts per million) to daily dose using the International Council for Harmonisation guidance for industry *Q3C(R8) Impurities: Guidance for Residual Solvents* (December 2021) assumptions for human breathing volume (28,800 liters/day).¹

¹ We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents>.

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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 less than or equal to 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 AI of 8.2 mg/day (see Figure 1 dose equivalent to the 1:100,000 risk).

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

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

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

$$\text{Dose (x)} = 8.2 \text{ mg/day}$$

Lifetime AI (inhalation) = 8 mg/day or 215 parts per billion (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 the International Council for Harmonisation guidance for industry *M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*, “other considerations” may affect final product specifications. Formaldehyde is known to cause local irritation and sensitization effects. Therefore, the World Health Organization 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:

$$(0.008 \text{ g/day}/28.8 \text{ cubic meters (m}^3\text{)/day}) * 1 / 1293 \text{ g/m}^3 = 215 \text{ ppb}$$

- Human breathing volume/day = 28.8 m³ /day
- Air mass/m³ at standard conditions = 1,293 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 active pharmaceutical ingredient (API) or drug product.

Permissible Daily Exposure (PDE) for All Other Routes

See section IV (4) of the introduction to this addendum that addresses formaldehyde exposure from the environment.

PDE (all other routes) = 10 mg/day

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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 to 80 micrograms (μg)/day (Ref. 1).

Mutagenicity/Genotoxicity

Glycidol is mutagenic/genotoxic in vitro and in vivo.

The International Agency for Research on Cancer (IARC) (Ref. 2) and Chemical Carcinogenesis Research Information System (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 WP2uvrA/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 National Toxicology Program (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 milligrams (mg)/kilogram (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

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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 2-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	TD₅₀ (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 the Carcinogenic Potency Database (CPDB) (Ref. 10) unless otherwise noted.

^a Carcinogenicity study selected for acceptable intake calculation.

^b Not in CPDB.

^c Not a standard carcinogenicity design. Only one dose, intermittent dosing, and small sample size (Ref.7).

mg = milligram; kg = kilogram; d = day; wk = week; M = male; F = female; NA = not applicable.

Mode of Action of Carcinogenicity

Glycidol is a mutagenic carcinogen, and the acceptable intake (AI) is calculated by linear extrapolation from the TD₅₀.

Regulatory and/or Published Limits

No regulatory limits have been published, for example by the U.S. Environmental Protection Agency, the World Health Organization, or the Agency for Toxic Substances and Disease Registry.

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Acceptable Intake

Rationale for selection of study for AI calculation

The most suitable carcinogenicity data for human cancer potency assessment come from the 2-year oral studies conducted in F344/N rats and B6C3F1 mice by the NTP (Ref. 5). The most sensitive organ site was female mammary glands with a TD₅₀ of 4.15 mg/kg/day.

Calculation of AI

Lifetime AI = TD₅₀/50,000 x 50 kg

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

Lifetime AI = 4 µg/day

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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.

The International Agency for Research on Cancer (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 the U.S. Environmental Protection Agency (EPA) as Group B2 or a probable human carcinogen (Ref. 7).

There are seven hydrazine carcinogenicity studies cited in the Carcinogenic Potency Database (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 TD₅₀ 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 less than 50 animals per group (and a single dose level in one case), and the calculated TD₅₀ values were higher (less potent) than those for the drinking water study of

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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 noninhalation acceptable intake (AI) calculation for hydrazine.

Hydrazine – Details of Carcinogenicity Studies

Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/S ex	TD₅₀ (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-month observation, inhalation	150	4: M:1.37, 6.87, 27.5, 137 F: 1.96, 9.81, 39.3, 196 µg/kg/d	Nasal adenomatous polyps/Male	0.194
Ref. 12	50/sex/ group Bor:NMRI, SPF-bred NMRI mice	2 years, 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-month 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-month observation, inhalation	Yes	1: 0.18 mg/kg/d	Negative	NA
Ref. 13	50/sex/ group Swiss mice	Lifetime, drinking water	Not concurr ent	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

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Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/S ex	TD₅₀ (mg/kg/d)
Ref. 10 ^{bc}	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, 6.80, 11.45 mg/kg/d	Liver/Female	52.4

Studies listed are in the Carcinogenic Potency Database (CPDB) (Ref. 8).

^a Carcinogenicity study selected for inhalation acceptable intake (AI) calculation.

^b Carcinogenicity study selected for noninhalation TD₅₀ (see Note 4) and AI calculations.

^c Excluded by the U.S. Environmental Protection Agency (Ref. 7); no concurrent controls. Liver negative.

^d Animal survival affected. Liver negative.

^e Not in CPDB

mg = milligram; kg = kilogram; d = day; wk = week; M = male; F = female; NA = not applicable.

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 U.S. 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 times 10⁻⁵ per microgram (µg)/liter (L). At the 1 in 100,000 risk level, this equates to a concentration of 0.1 µg of hydrazine/L of water or about 0.2 µg/day for a 50 kg human. This limit is a linearized multistage extrapolation based on the observation of hepatomas in a multidose 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 reevaluated by the U.S. EPA.

The U.S. EPA (Ref. 7) has also published an inhalation slope factor of 17 per milligrams (mg)/kilogram (kg)/day and an inhalation unit risk of 4.9x10⁻³ per µg/cubic meter (m³). At the 1 in 100,000 risk level, this equates to an air concentration of 2 x 10⁻³ µg/m³ of hydrazine or 0.04 µg/day assuming a person breathes 20 m³/day. This limit is a linearized multistage extrapolation based on the observation of nasal cavity adenoma or adenocarcinoma in male rats in a multidose 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

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U.S. EPA review of this data was accessible; however, the results appear to be very similar to, if not the same as, those of Vernot et al (Ref. 11).

Acceptable Intake

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 four mouse studies and two 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 U.S. EPA in the derivation of the inhalation carcinogenicity limit for hydrazine were taken into consideration when selecting the most robust carcinogenicity study for the derivation of an AI for inhaled pharmaceuticals. The critical study by MacEwen et al. used by U.S. EPA (Ref. 7) was proprietary but is likely the same one described in Vernot et al. (Ref. 11). Given that the threshold of toxicological concern was derived via linear extrapolation from TD₅₀ values for hundreds of carcinogens, that same approach was used in the derivation of a compound-specific AI for hydrazine. The methodology used by the U.S. EPA and the method used here are both highly conservative in nature. However, given that the methodologies do differ, it is reasonable to expect some slight differences. The AI was calculated based on the TD₅₀ derived from a study in which male and female rats were administered hydrazine via inhalation for 1 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 TD₅₀ value of 0.194 mg/kg/day, after being adjusted, as standard practice, to account for 1 versus 2 years of exposure.

Calculation of AI

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 38.7 \text{ (mg/kg/day)}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 39 \text{ }\mu\text{g/day}$$

Calculation of Inhalation AI

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 0.194 \text{ (mg/kg/day)}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime inhalation AI} = 0.2 \text{ }\mu\text{g/day}$$

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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 grams (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 percent hydrogen peroxide (Ref. 2).

Mutagenicity/Genotoxicity

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

The International Agency for Research on Cancer (IARC) (Ref. 3) and European Commission's 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 milligrams (mg)/kilogram (kg), or to catalase-deficient C57BL/6NCr1BR mice in drinking water at 200; 1,000; 3,000; and 6,000 parts per million for 2 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 Carcinogenic Potency Database (CPDB) (Ref. 6), in which mice were treated with hydrogen peroxide in drinking water at 0.1 or 0.4 percent 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 less than 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 percent hydrogen peroxide administered in drinking water was defined as the lowest observed adverse effect level, equivalent to an average daily dose-rate per kilogram body weight per day of 167 mg/kg/day.

Studies of 6-month duration or longer are summarized in the following table (adapted from Ref. 2); they are limited in the numbers of animals and used a single dose level. Most studies

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did not meet the criteria for inclusion with a TD₅₀ calculation in the CPDB. DeSesso et al. (Ref. 2) noted that, out of 14 carcinogenicity studies (two subcutaneous studies in mice, two dermal studies in mice, six drinking water studies (two in rats and four in mice), one oral intubation study in hamsters, and three buccal pouch studies), only three 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 of the U.S. Food and Drug Administration (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 (SCCP) 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, esophagus, 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 U.S. 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	TD ₅₀ 7.54 g/kg/d for female duodenal carcinoma.
Ref. 7	29 mice C57BL/6J total male and female (additional groups sampled at intervals from 7 to 630 days of treatment; or 10 to 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 ₂ O ₂ treatment, by 10 to 30 days without treatment there were fewer mice with lesions.

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Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Notes
Ref. 8	18 C3H/HeN mice total male and female	6 months, Drinking water	No	1: 0.4%	2 mice with duodenal tumors (11.1%).
Ref. 8	22 B6C3F1 mice total male and female	6 months, drinking water	No	1: 0.4%	7 mice with duodenal tumors (31.8%).
Ref. 8	21 C57BL/6N ^ε mice total male and female	7 months, drinking water	No	1: 0.4%	21 mice with duodenal tumors (100%).
Ref. 8	24 C3H/Cb/s ^ε mice total male and female	6 months, drinking water	No	0.4% only	22 mice with duodenal tumors (91.7%).
Ref. 9	21 female C3H/HeN mice	6 months, drinking water	11	1: 0.4%	2 mice with duodenal tumors (9.5%). None in controls.
Ref. 9	22 female B6C3F1 Mice	6 months, drinking water	12	1: 0.4%	7 mice with duodenal tumors (31.8%). None in controls.
Ref. 9	24 female C3H/Cb/s ^ε mice	6 months, 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 months, oral gavage (5 d/wk)	Yes	1: 70 mg/kg/d	No tumorigenic effect observed.

^a Their studies are not in the Carcinogenic Potency Database but are summarized in Ref. 2
Catalase deficient.

M = male; F = female; g = grams; kg = kilogram; d = day; wk = week; H₂O₂ = hydrogen peroxide;

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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 nonlinear, threshold effect.

Regulatory and/or Published Limits

Annex III of the European Union 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 percent. Higher levels up to 6 percent are also permitted providing products are prescribed by dental practitioners to persons older than 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 percent 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).

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U.S. FDA — hydrogen peroxide is generally recognized as safe up to 3 percent for long-term over-the-counter-use as an antigingivitis/antiplaque 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 percent of the estimated endogenous production of 6.8 g/day, that is, 68 mg/day (or 68,000 micrograms (µg)/day) would not significantly add to background exposure, but would usually exceed limits based on quality, in a pharmaceutical. The International Council for Harmonisation guidance for industry *M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* notes that when calculating acceptable intakes from compound-specific risk assessments, an upper limit would be determined by a quality limit of 0.5 percent, or, for example, 500 µg in a drug with a maximum daily dose of 100 mg.¹

¹ We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents>.

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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.

The World Health Organization (WHO) (Ref. 1) reports that the methyl chloride concentration in the air in rural sites is in general below 2.1 micrograms (μg)/cubic meter (m^3) (1.0 parts per billion), and in urban cities it is equal to 0.27 to 35 $\mu\text{g}/\text{m}^3$ (0.13 to 17 ppb), corresponding to approximately 20 to 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 $\mu\text{g}/\text{liter}$ in a well sample (Ref. 1).

Mutagenicity/Genotoxicity

Methyl chloride is mutagenic and genotoxic in vitro but equivocal in vivo. WHO (Ref. 1) and U.S. Environmental Protection Agency (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* WP2uvrA 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 the International Agency for Research on Cancer (IARC) as Group 3, “inadequate evidence for the carcinogenicity to humans” (Ref. 3), and by the U.S. 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 parts per million (ppm)). Although not of statistical significance, cortical adenoma was also seen at 464 milligrams (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.

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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 P450 (CYP) isozyme believed to be responsible, CYP2E1, is present in male mouse kidney and is androgen-dependent; female mice had CYP2E1 levels only 20 to 25 percent 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 P450 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), and it was not 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 P450 enzymes found at significant levels in human renal microsomes are CYP4F2 and CYP3A. Moreover no commonly known environmental chemicals appear to be metabolized by the CYP4A family. The lack of detectable CYP2E1 protein in human kidney (in contrast to mice, which have high levels) suggests that the metabolism of methyl chloride by P450 (presumably leading to elevated formaldehyde concentrations) that is likely responsible for the induction of male mouse kidney tumors are not likely relevant to humans.

However, as highlighted by the U.S. EPA (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 to 1310 mg/kilogram (kg)/day; Ref. 5). In addition, based on the limitations of human relevance, the U.S. EPA classified methyl chloride as a group D compound, that is Not Classifiable as to Human Carcinogenicity.

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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 months 6 h/d, 5 d/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 months 6 h/d, 5 d/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 the Carcinogenic Potency Database.

^a Carcinogenicity study selected for acceptable intake calculation.

^b TD₅₀ calculated based on carcinogenicity data (see Note 5).

mg = milligram; kg = kilogram; d = day; h = hour; wk = week; m³ = cubic meter; ppm = parts per million; NA = not applicable.

Regulatory and/or Published Limits

The WHO (Ref. 1) developed a guideline value for the general population of 0.018 mg/m³, and the U.S. EPA (Ref. 2) developed a reference concentration of 0.09 mg/m³. Both were based on the potential for adverse central nervous system 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.

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 1,360.7 \text{ mg/kg/day} / 50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 1,361 \text{ } \mu\text{g/day}$$

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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 to 4 micrograms (μg) in the United Kingdom, 2 to 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 unscheduled DNA synthesis assays when conducted according to Organization for Economic Co-operation and Development (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 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 greater than or equal to 85 milligrams (mg)/cubic meters 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-

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oxide) has been proposed as a possible mode of action for styrene-induced carcinogenicity in experimental animals and humans (Ref. 1).

Carcinogenicity

The International Agency for Research on Cancer (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 National Institutes of Health (NIH) (Ref. 1). Possible modes of action for styrene-induced carcinogenicity involve genotoxic and cytotoxic effects together with immunosuppression (Ref. 1). The U.S. National Toxicology Program (NTP) listed styrene as “reasonably anticipated to be a human carcinogen” in its 12th and 14th Reports on Carcinogens (Ref. 12, 13). The National Research Council 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 Carcinogenic Potency Database, styrene is reported to be carcinogenic in mice via the oral and inhalation routes and rats via the inhalation route (Ref. 15). The NIH Report on Carcinogens (Ref. 1) considered the most robust studies to be the 2-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).

The 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 intraperitoneal (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 Report on Carcinogens (Ref. 1); the 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 subcutaneous application (Ref. 22) were also negative.

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

The 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/Se x	TD₅₀ (mg/kg/d)^a
Ref. 16	50/sex/ group M&F B6C3F1 mouse	78 weeks, oral gavage	20	2: 150, 300 mg/kg/d	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/d	Lung/ Male	154 ^c
Ref. 16	70/sex/ group Fischer 344 rats	78 -107 weeks, oral gavage	40	3: 500, 1,000, 2,000 mg/kg/d	No Tumors	NC
Ref. 21	70/sex/ group CD rats	104 weeks, inhalation	70	4: 50, 200, 500, 1,000 ppm	No Tumors	NC

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Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Se x	TD ₅₀ (mg/kg/d) ^a
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	s.c. once, then i.p. 4 times at 2-month intervals	40	1: 50 mg (s.c.), 50 mg (i.p.)	No Tumors ^e	NC

^a The TD₅₀ values are taken from the Carcinogenic Potency Database (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 acceptable intake calculation, milligrams per kilogram per day dose conversion taken from CPDB

^d Author opinion: Styrene, was found to cause an increase in total (benign and 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 the National Institutes of Health Report on Carcinogens (Ref. 1) and the International Agency for Research on Cancer (Ref. 5) noted short treatment duration and incomplete reporting of the study.

^e Study limited to acute exposures and a nonstandard study design

mg = milligram; kg = kilogram; d = day; ppm = parts per million; SD = Sprague-Dawley; NC = not calculated; s.c. = subcutaneous injection; i.p. = intraperitoneal injection.

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, the 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 N⁷-guanine. Styrene-7,8-oxide DNA adducts have been observed in vitro, in rodents and in humans exposed to styrene. The 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, and mild cytotoxicity and strong mitogenicity in mice

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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). The 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 acceptable intake (AI) for styrene.

Regulatory and/or Published Limits

The World Health Organization (WHO) defined a tolerable daily intake (TDI) for styrene via the oral route of 7.7 µg/kilogram (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 liters (L) of water per day) (Ref. 32). This WHO limit was based on reduced body weight gain in a 2-year rat drinking water study. The U.S. Environmental Protection Agency (EPA) oral reference dose (Ref. 33) for styrene is 200 µg/kg/day (i.e., 10 mg/day based on 50 kg body weight), based on noncancer endpoints. The associated U.S. EPA drinking water limit is 100 µg/L (i.e., 200 µg per day based on consumption of 2 L water per day). The Joint FAO/WHO Expert Committee on Food Additives 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 parts per million 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

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 TD₅₀ 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

$$\text{Lifetime AI} = \text{TD}_{50}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 154 \text{ mg/kg/day}/50,000 \times 50 \text{ kg}$$

$$\text{Lifetime AI} = 154 \text{ µg/day}$$

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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 intraperitoneal (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 intracellular homeostasis (Ref. 2). Given its response in mammalian cells, and rapid conversion to acetaldehyde, vinyl acetate is considered mutagenic. See Mode of Action for Carcinogenicity 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 Carcinogenic Potency Database (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 were observed in Fischer 344 rats. A number of nonsite-of-contact tumors (e.g., Zymbal gland, lung, liver, uterine, 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 parts per million (ppm). There were no tumors among mice administered 1,000 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 percent per day. Therefore, by the end of the week the animals in the 2,500 ppm group, for example, were exposed to approximately 1,300 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

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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 the Organization for Economic Cooperation and Development guideline 453, including three 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 treatment 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.

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Vinyl Acetate – Details of Carcinogenicity Studies

Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD₅₀ (mg/kg/d)
Ref. 6	37 F and 13 M/ group Swiss mice	2 years, drinking water	37 F, 14 M	2: 1,000 ppm (103 mg/kg/d F and 96.3 mg/kg/d M), 5,000 ppm (578 mg/kg/d F and 546 mg/kg/d M)	Uterine, Female	3920 ^b
Ref. 7	20/sex/ group F344 rat	2 years, drinking water	20	2: 1,000 mg/L (0.1 mg/kg/d F and 0.062 mg/kg/d M), 2,500 mg/L (0.04 mg/kg/d F and 0.025 mg/kg/d M)	Liver, Male	132 ^b
Ref. 9	50/sex/ group Crj:BDF ₁ mice	2 years, drinking water	50	3: 400 ppm (63 mg/kg F and 42 mg/kg/d M), 2,000 ppm (301 mg/kg/d F and 202 mg/kg/d M), 10,000 ppm (1,418 mg/kg/d F and 989 mg/kg/d M)	Oral cavity, Male	1854 ^c

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Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD₅₀ (mg/kg/d)
Ref. 9	50/sex/ group F344/Du Crj rats	2 years, drinking water	50	3: 400 ppm (31 mg/kg/d F and 21 mg/kg/d M), 2,000 ppm (146 mg/kg/d F and 98 mg/kg/d M), 10,000 ppm (575 mg/kg/d F and 442 mg/kg/d M)	Oral cavity, Male	3057 ^c
Ref. 11	37F and 14M/ group, Breeders (17 wk old); 53 or 83M and 57 or 87F Sprague- Dawley rat offspring (12 day old)	2 years, drinking water	Breeders 14M and 37F; Offspring 107M and 99F	2: 1,000 ppm (70.6 mg/kg/d), 5,000 ppm (353 mg/kg/d) ^a	Oral cavity and lips, Male	983 ^c
Ref. 12	60/sex/ group CrI:CD(S D)BR rats	2 years, drinking water	60	3: 200 ppm (16 mg/kg/d F and 10 mg/kg/d M), 1,000 ppm (76 mg/kg/d F and 47 mg/kg/d M), 5,000 ppm (302 mg/kg/d F and 202 mg/kg/d M)	No tumors	NC

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Study	Animals/ Dose Group	Duration/ Exposure	Controls	Doses	Most Sensitive Tumor Site/Type/Sex	TD₅₀ (mg/kg/d)
Ref. 13	60/sex/ group Charles River CD1 mice	2 years, inhalation	60	3: 50 ppm (55.3 mg/kg/d F and 46.1 mg/kg/d M), 200 ppm (221 mg/kg/d F and 184 mg/kg/d M), 600 ppm (664 mg/kg/d F and 554 mg/kg/d M)	No tumors	NC
Ref. 13	60/sex/ group Charles River CD (Sprague- Dawley) Rats	2 years, inhalation	20	3: 50 ppm (13.3 mg/kg/d F and 9.32 mg/kg/d M), 200 ppm (52.7 mg/kg/d F and 36.9 mg/kg/d M), 600 ppm (158 mg/kg/d F and 111 mg/kg/d M)	Nasal, Male	758 ^b

^a Calculated based on the International Council for Harmonisation guidance for industry *Q3C(R8) Impurities: Guidance for Residual Solvents* (December 2021) assumptions.¹

^b Taken from the Carcinogenic Potency Database (CPDB) (Ref. 14). Carcinogenicity study selected for the acceptable intake calculation

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

mg = milligram; kg = kilogram; d = day; M = male; F = female; ppm = parts per million; L = liter; NC = not calculated.

Mode of action for carcinogenicity

Vinyl acetate has been reviewed by the European Commission’s Scientific Committee on Health and Environmental Risks (SCHER), which 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 no-observed adverse

¹ We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents>.

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effect level (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 nonlinear, 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 on cancer development.

Regulatory and/or Published Limits

For vinyl acetate, the U.S. Environmental Protection Agency (EPA) integrated risk information system database calculated an inhalation reference concentration (RfC) for noncarcinogenic effects of 0.2 milligrams (mg)/cubic meter (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 lowest-observed adverse effect level 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 U.S. 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

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nonlinear 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 three 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 TD₅₀ for the nasal cavity in male rats is 758 mg/kilogram (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 nonlinear, as stated above, there are no published measurements which allow discrimination between a true threshold versus a nonlinear inflection point. Therefore, the AI was calculated using linear extrapolation.

Calculation of AI

Lifetime AI = TD₅₀/50,000 x 50 kg

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

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

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NOTE 1

The calculated TD₅₀ for 1-chloro-4-nitrobenzene is illustrated below because it was not listed in the Carcinogenic Potency Database. 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
1,000	53.8	16	50

ppm = parts per million; mg = milligram; kg = kilogram.

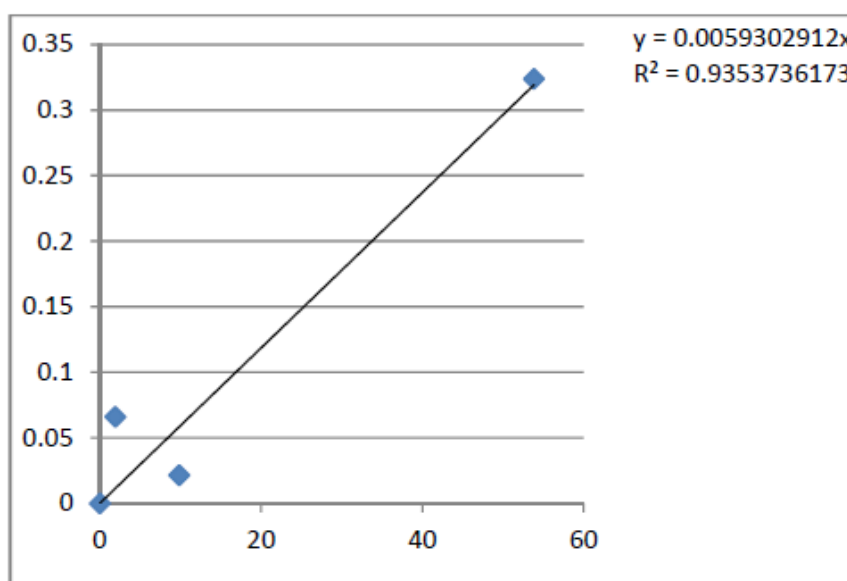
The TD₅₀ is calculated from crude summary data of tumor incidence over background with the following equation (Ref. 2, 3):

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

Where P is the proportion of animals with the specified tumor type observed at a certain dose (D in the equation) and P₀ 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 $\beta = 0.0059302912$.



Contains Nonbinding Recommendations

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 milligrams/kilogram/day.

Contains Nonbinding Recommendations

REFERENCES (FOR NOTE 1)

1. Matsumoto M, Aiso S, Senoh H, Yamazaki K, Arito H, Nagano K, et al., 2006, Carcinogenicity and Chronic Toxicity of *para*-Chloronitrobenzene in Rats and Mice by Two-Year Feeding, *J. Environ Pathol Toxicol Oncol*, 25:571–584.
2. Gaylor DW and Gold LS, 1995, Quick Estimate of the Regulatory Virtually Safe Dose Based on the Maximum Tolerated Dose for Rodent Bioassays, *Regul Toxicol Pharmacol*, 22:57–63.
3. Sawyer C, Peto R, Bernstein L, and Pike MC, 1984, Calculation of Carcinogenic Potency From Long-Term Animal Carcinogenesis Experiments, *Biometrics*, 40:27–40.

Contains Nonbinding Recommendations

NOTE 2

The calculated TD₅₀ for ethyl bromide is illustrated below since it was decided to use the same study data but not the TD₅₀ calculated by the Carcinogenic Potency Database because 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

ppm = parts per million; mg = milligram; kg = kilogram.

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

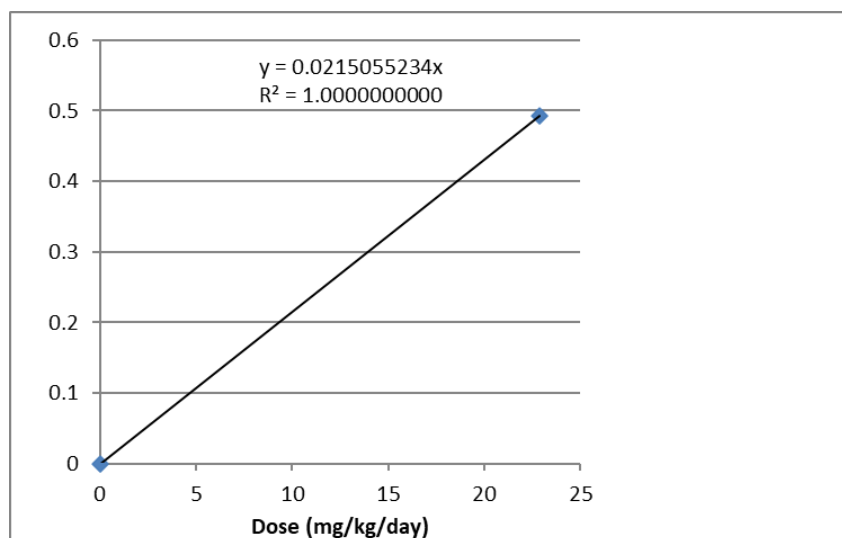
$$\frac{P - P_0}{1 - P_0} = 1 - \exp(-\beta \cdot D)$$

Where P is the proportion of animals with the specified tumor type observed at a certain dose (D in the equation) and P₀ 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:

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

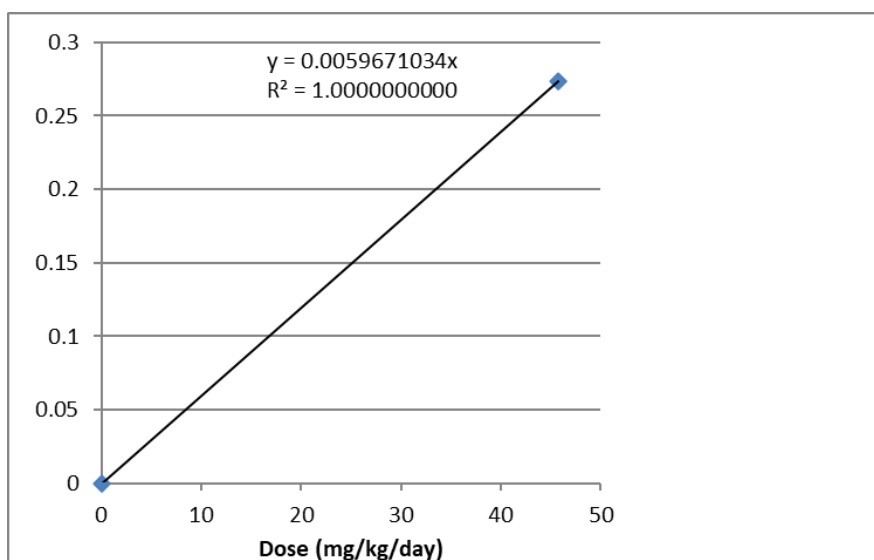
Plotting the results and using the slope to represent β results in the following graphs for the dose response and $\beta = 0.0215055234$ for low dose, 0.0059671034 for mid dose, and 0.0042161616 for the high dose.

Low Dose

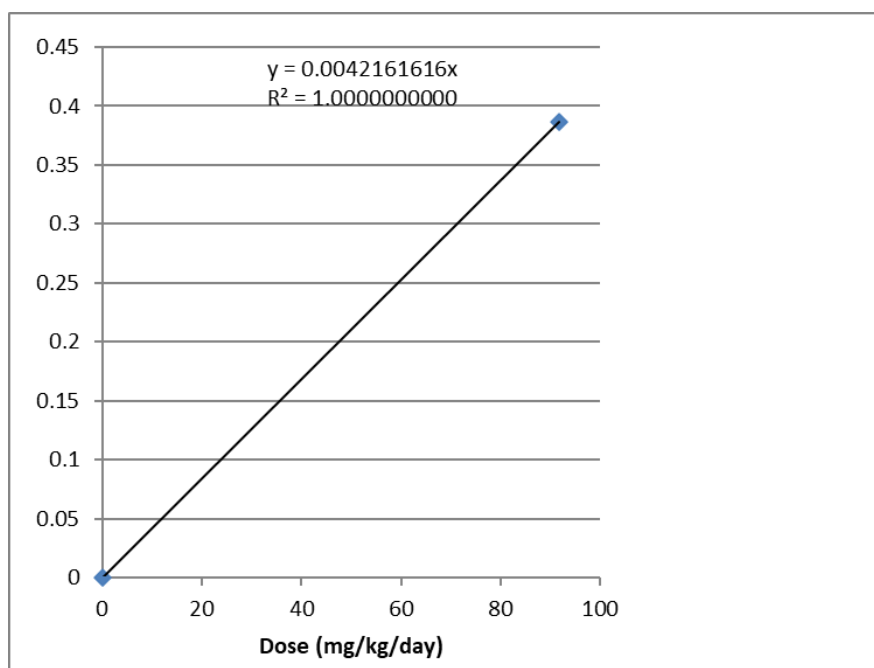


Contains Nonbinding Recommendations

Mid Dose



High Dose



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.

Contains Nonbinding Recommendations

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

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

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

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

Contains Nonbinding Recommendations

REFERENCES (FOR NOTE 2)

1. Gaylor DW and Gold LS, 1995, Quick Estimate of the Regulatory Virtually Safe Dose Based on the Maximum Tolerated Dose for Rodent Bioassays, *Regul Toxicol Pharmacol*, 22:57–63.
2. Sawyer C, Peto R, Bernstein L, and Pike MC, Calculation of Carcinogenic Potency From Long-Term Animal Carcinogenesis Experiments, *Biometrics*, 40:27–40.

Contains Nonbinding Recommendations

NOTE 3

For formaldehyde the limit is 215 parts per billion (ppb) or 8 milligrams (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 active pharmaceutical ingredient (API) or drug product would be derived:

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

- Each actuator of albuterol delivers 90 micrograms (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 milliliters (mL) for adult males and 400 mL for adult females (see *Physiology, Tidal Volume*, available at StatPearls - NCBI Bookshelf (NIH.gov), <https://www.ncbi.nlm.nih.gov/books/NBK482502/>). 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: $215 \text{ ppb formaldehyde} = 0.215 \times 30 \text{ grams (g)/mole (molecular weight of formaldehyde)/24.45} = 0.263 \text{ mg/cubic meter (m}^3\text{)}$. $0.263 \text{ mg/m}^3 \times 1 \text{ m}^3/1,000 \text{ liter (L)} \times 0.4 \text{ L (tidal volume women)} = 0.105 \text{ }\mu\text{g formaldehyde}$.
- Calculate the corresponding API limit: $0.105 \text{ }\mu\text{g formaldehyde}/90 \text{ }\mu\text{g API} = 0.12\%$.

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: $0.105 \text{ }\mu\text{g formaldehyde}/35 \text{ mg drug product} = 3 \text{ parts per million}$.

Contains Nonbinding Recommendations

NOTE 4

The calculated TD₅₀ for hydrazine is illustrated below since it was not listed in the Carcinogenic Potency Database. 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

ppm = parts per million; mg = milligram; kg = kilogram.

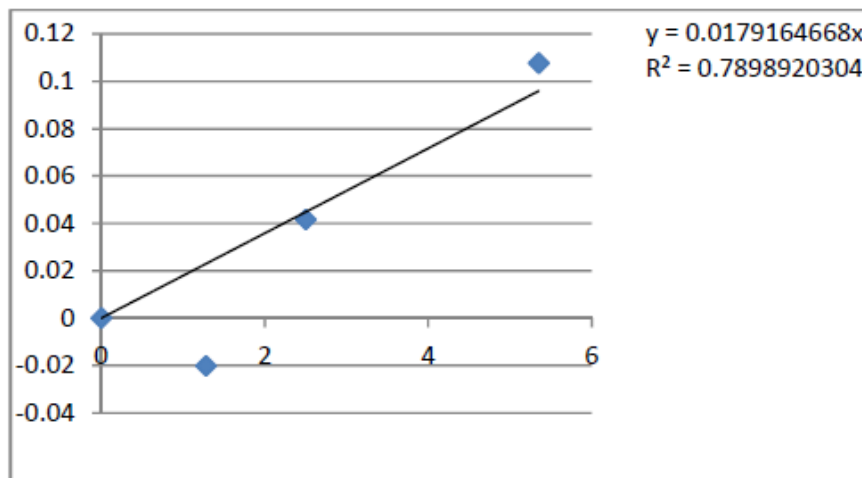
The TD₅₀ is calculated from crude summary data of tumor incidence over background with the following equation (Ref. 2, 3):

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

Where P is the proportion of animals with the specified tumor type observed at a certain dose (D in the equation) and P₀ 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 $\beta = 0.0179164668$.



Contains Nonbinding Recommendations

The TD₅₀ can then be calculated as follows.

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

Solving for TD₅₀ results in the following equation.

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

Therefore, the TD₅₀ = 0.693 / 0.0179164668 or 38.7 milligram/kilogram/day.

Contains Nonbinding Recommendations

REFERENCES (FOR NOTE 4)

1. Matsumoto M, Kano H, Suzuki M, Katagiri T, Umeda Y, and Fukushima S, 2016, Carcinogenicity and Chronic Toxicity of Hydrazine Monohydrate in Rats and Mice by Two-Year Drinking Water Treatment, *Regul Toxicol Pharmacol*, 76:63–73.
2. Gaylor DW and Gold LS, 1995, Quick Estimate of the Regulatory Virtually Safe Dose Based on the Maximum Tolerated Dose for Rodent Bioassays, *Regul Toxicol Pharmacol*, 22:57–63.
3. Sawyer C, Peto R, Bernstein L, and Pike MC, 1984, Calculation of Carcinogenic Potency From Long-Term Animal Carcinogenesis Experiments, *Biometrics*, 40:27–40.

Contains Nonbinding Recommendations

NOTE 5

The calculated TD₅₀ for methyl chloride is illustrated below because it was not listed in the Carcinogenic Potency Database. Because the methyl chloride study (Ref. 1, 2) is based on inhalation, the inhaled parts per million 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

ppm = parts per million; mg = milligram; kg = kilogram; mol = mole.

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

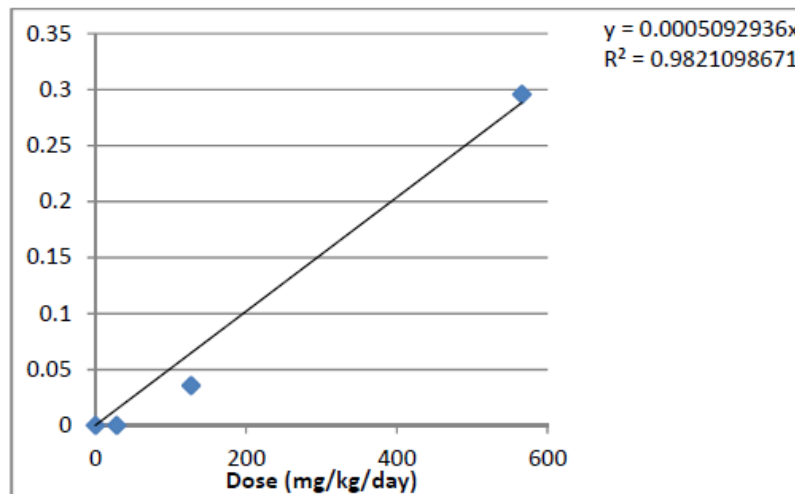
The TD₅₀ is calculated from crude summary data of tumor incidence over background with the following equation (Ref. 3, 4):

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

Where P is the proportion of animals with the specified tumor type observed at a certain dose (D in the equation) and P₀ 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 = 0.0005092936.



Contains Nonbinding Recommendations

The TD₅₀ can then be calculated as follows.

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

Solving for TD₅₀ results in in the following equation.

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

Therefore, the TD₅₀ = 0.693 / 0.0005092936 or 1360.7 milligram/kilogram/day.

Contains Nonbinding Recommendations

REFERENCES (FOR NOTE 5)

1. Chemical Industry Institute of Toxicology, 1981, 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. EPA/OTS Doc #878212061, NTIS/OTS0205952.
2. U.S. Environmental Protection Agency, 2001, Toxicological Review of Methyl Chloride (CAS No. 74-87-3): In Support of Summary Information on the Integrated Risk Information System (IRIS), available at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/1003tr.pdf.
3. Gaylor DW and Gold LS, 1995, Quick Estimate of the Regulatory Virtually Safe Dose Based on the Maximum Tolerated Dose for Rodent Bioassays, Regul Toxicol Pharmacol, 22:57–63.
4. Sawyer C, Peto R, Bernstein L, and Pike MC, 1984, Calculation of Carcinogenic Potency From Long-Term Animal Carcinogenesis Experiments, Biometrics, 40:27–40.