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Provisional Peer Reviewed Toxicity Values for
Benzoic Acid
(CASRN 65-85-0)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
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U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level

MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

**PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR
BENZOIC ACID (CASRN 65-85-0)**

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).
2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.
3. Other (peer-reviewed) toxicity values, including:
 - ▶ Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
 - ▶ California Environmental Protection Agency (CalEPA) values, and
 - ▶ EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions (or the EPA HQ Superfund Program) sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

The HEAST (U.S. EPA, 1997) lists a subchronic oral RfD value of 4E+0 mg/kg-day for benzoic acid, with a notation that the chronic oral RfD was adopted for this reference value. The HEAST references IRIS (U.S. EPA, 2003) for the chronic oral RfD. Benzoic acid and its sodium salt are used as food preservatives. The chronic oral RfD is based on the estimated human daily per capita intakes of benzoic acid (0.9 - 34 mg/day) and sodium benzoate (34-328 mg/day) derived from estimates of their production for use in food (U.S. FDA, 1973). The upper range of each estimate was considered a NOAEL for the compound, based on the absence of reported adverse effects at these levels and because these compounds are Generally Recognized as Safe (GRAS) by U.S. FDA (Informatics, Inc., 1972). U.S. EPA (2003) converted the upper range of sodium benzoate intake to a benzoic acid equivalent intake of 278 mg/day. The combined daily intake (34 mg + 278 mg) as benzoic acid was 312 mg/kg. The RfD of 4E+0 was calculated using

an uncertainty factor (UF) and modifying factor (MF) of one each and a default adult body weight of 70 kg.

The Health and Environmental Effects Document (HEED) (U.S. EPA, 1987a) assigned benzoic acid to carcinogenicity group D (not classifiable as to human carcinogenic potential), and no carcinogenicity classification or quantitative assessment of cancer risk is reported in the HEAST. IRIS (U.S. EPA, 2003) does not list an RfC or quantitative cancer assessment for benzoic acid, but includes a weight of evidence characterization of D (not classifiable as to human carcinogenicity). Benzoic acid is not included in the Drinking Water Standards and Health Advisories List (U.S. EPA, 2002). The CARA list (U.S. EPA, 1991, 1994a) reports the HEED (U.S. EPA, 1987a) and a Reportable Quantities Document (U.S. EPA, 1987b). ATSDR (2002) has not published a Toxicological Profile for benzoic acid. WHO (2000) has published a Concise International Chemical Assessment Document (CICAD) on Benzoic Acid and Sodium Benzoate and has recently conducted an evaluation of benzoate intake assessments prepared by nine countries, including the United States (WHO, 1999). The Joint FAO/WHO Expert Committee on Food Additives (WHO, 1996) lists a Group Acceptable Daily Intake (ADI) of 0-5 mg/kg-bw for benzoic acid and its salts, including benzyl alcohol and related benzyl derivatives used as flavorings. The European Commission Scientific Committee on Food (ECSCF, 2002) recently re-evaluated the safety of benzoic acid as a food additive and also adopted a Group ADI of 0 - 5 mg/kg bw - day for benzoic acid and its salts. The Cosmetic Ingredient Review (CIR) Expert Panel conducted a safety assessment on the use of benzoic acid and sodium benzoate in cosmetics and concluded that these compounds could be safely used at concentrations up to 5% in products intended for adults (CIR, 2001). Neither ACGIH (2001), NIOSH (2002), nor OSHA (2002) have adopted occupational exposure limits for benzoic acid. Neither NTP (2002) nor IARC (2002) have evaluated the carcinogenicity of benzoic acid.

Literature searches were conducted from 1988 through March, 2002 for studies relevant to the derivation of provisional toxicity values for benzoic acid. Databases searched included: TOXLINE, MEDLINE, TSCATS, RTECS, CCRIS, DART, EMIC/EMICBACK, HSDB, GENETOX and CANCERLIT. Additional literature searches from April 2002 through September 2004 were conducted by NCEA-Cincinnati using MEDLINE, TOXLINE, Chemical and Biological Abstracts databases.

REVIEW OF PERTINENT DATA

Human and animal toxicity data on benzoic acid and sodium benzoate were reviewed for potential use in derivation of provisional reference values and quantitative cancer assessment. Consideration of data on sodium benzoate is appropriate for oral exposure because the acid

environment of the stomach ($\text{pH} = 2$) favors conversion of ionized benzoate to undissociated benzoic acid ($\text{pK}_a = 4.2$) (WHO, 2000).

Human Studies

No subchronic or chronic data on human health effects resulting from inhalation exposure to benzoic acid were located in the literature examined.

Human data on benzoic acid toxicity are available from early oral exposure studies conducted in human volunteers. Chittenden et al. (1909) observed no apparent effects in six adult male human volunteers given 300 to 400 mg/day via the diet for up to 62 days. Assuming an average body weight of 70 kg, the average daily doses of benzoic acid were approximately 4 to 6 mg/kg-day. Gerlach (1909) reported no apparent effects in human volunteers (number not specified) ingesting daily doses of benzoic acid of 500 or 1000 mg/day for 44 consecutive days; 82 doses of 1000 mg/day over 86 days; or 88 doses of 1000 mg/day over 92 days. These dosing schedules resulted in average daily doses of up to 14 mg/kg-day, as reported in U.S. EPA (1987a). Wiley and Bigelow (1908) observed irritation, discomfort, weakness, and malaise in human volunteers given oral bolus doses of benzoic acid over a 20 day period. Only 3 of 12 volunteers completed the scheduled dose regimen as a result of the observed effects. The total dose to individuals over the course of the experiment ranged from 13.5 to 35 grams. U.S. EPA (1987a) estimated an average daily dose of 25 mg/kg-day in this study.

Sodium benzoate is used in the treatment of urea cycle enzymopathies to facilitate alternative pathways of nitrogen excretion (WHO, 2000). Therapeutic doses are reported to be in the range of 250 to 500 mg/kg-day and are given over several years. Clinical signs of toxicity are reported to be rare at this dose level and in most cases limited to anorexia and vomiting, particularly after bolus intravenous doses.

Some individuals may show greater sensitivity to benzoic acid or sodium benzoate exposure. Cases of urticaria, asthma, rhinitis or anaphylactic shock have been reported after oral, dermal, or inhalation exposure to these compounds (WHO, 2000). These symptoms are reported to occur shortly after exposure and disappear within a few hours (WHO, 2000).

No human data on the carcinogenicity of benzoic acid or sodium benzoate were identified in the literature examined.

Animal Studies

A single inhalation study is available for benzoic acid. IRDC (1981) reported a study in which young Sprague-Dawley rats (10/sex/dose) were exposed to benzoic acid dust by whole body inhalation at target concentrations of 0, 0.02, 0.2, or 2.00 mg/L, 6 hours/day, 5 days/week

for four weeks. The test material was generated as a dust aerosol with an equivalent aerodynamic diameter (EAD) of 4.7 μm when averaged across the three exposure groups. The individual EAD values for the low-, mid, and high-exposure groups were 4.6, 4.4, and 5.2 μm , respectively, with corresponding geometric standard deviations (σ_g) of 3.1, 2.1, and 2.1. Animal care and environmental conditions were maintained according to standard guidelines. Information on animal bedding (a potential confounding factor in evaluation of fibrosis) was not provided in the methods section. The parameters evaluated included clinical signs, morbidity, mortality, body weight gain, hematology, serum biochemistry (focused on indicators of liver function and damage), gross pathology, organ weights, extensive histopathology of control and high-dose rats, and histopathological examination of the lungs of mid- and low-dose animals. The lungs were removed while in an inflated state. Post sacrifice lungs were deflated and re-inflated, with buffered 10% neutral formalin. Lung histopathology was evaluated using hematoxylin and eosin stained paraffin sections taken from each of the five lobes of the lung. The methods section of the report did not indicate use of a collagen-specific stain for assessment of fibrosis.

Measured concentrations of benzoic acid in the test chambers were 0.025, 0.25, and 1.2 mg/L (0, 25, 250, and 1200 mg/m³). A reddish discharge was observed around the nares of exposed animals. The occurrence of this discharge was dose-related. All animals in the mid- and high-dose groups exhibited this sign on study day 4, with the discharge more pronounced in the high-dose group. The discharge appeared in the mid- and high-dose groups throughout the study with varying intensity. The discharge was noted in low-dose animals only on study day 13 and was not observed in control animals. Two compound-related deaths (one male and one female) occurred in the high-dose groups. High-dose rats of both sexes showed significantly decreased body weights throughout the study (Table 1). Platelet counts were significantly reduced in high-dose males and females. No compound-related effects were noted for clinical chemistry parameters. Decreased absolute organ weights and organ-to-brain weight ratios were observed for the liver in high-dose males and the kidney and trachea/lungs of high-dose females. Reduced absolute kidney weight was observed in mid-dose females. No compound-related gross lesions were observed in any of the test animals. Compound-related microscopic lesions were confined to the lung and consisted of 1) an increase in the extent and intensity of interstitial inflammatory cell infiltrate (males and females) and 2) an increase in the incidence (males) and intensity (males and females) of interstitial cell. Incidence data for pulmonary lesions are summarized in Table 2. Interstitial inflammatory cell infiltrate was observed in all exposed animals, all female control animals, and 9/10 male control animals. The study authors did not comment on the high incidence of this lesion in the control groups. Review of the individual animal data by Syracuse Research Corporation (SRC) indicates that slides from four of the nine males included in the control incidence showed only subpleural inflammatory cell infiltrate (i.e., the pathologist did not specifically record interstitial cell involvement for these animals). Compound-related changes in the extent of interstitial inflammatory cell infiltrate were assessed by recording the progression from focal to multifocal and generalized lesions (Table 3). The

Table 1. Group Mean Body Weight (grams) in the 4-Week Inhalation Study Conducted by IRDC (1981)

Measured Concentration mg/L (mg/m ³)	Week of Exposure				
	0 (Pre-exposure)	1	2	3	4
Males					
0 (0)	199±11.6 ^a	262±15.5	310±23.7	339±31.0	369±37.3
0.025 (25)	199±17.3	261±10.8	311±14.6	349±19.3	381±20.3
0.25 (250)	194±11.0	251±10.6	301±13.5	335±14.9	364±20.6
1.2 (1200)	201±13.8	242±9.4**	273±14.9**	294±19.2**	315±29.5**
Females					
0 (0)	163±7.3	194±10.4	218±14.6	234±16.3	249±19.6
0.025 (25)	156±8.1	185±11.7	206±15.8	222±17.0	235±19.6
0.25 (250)	148±8.4**	184±8.7	205±10.9	218±10.2*	234±15.7
1.2 (1200)	157±10.9	176±8.5**	200±12.9*	206±13.8**	219±14.1**

Source: Table 6 in IRDC (1981).

^a Mean and standard deviation

* Statistically significant from control group mean, p<0.05

** Statistically significant from control group mean, p<0.001

Table 2. Terminal Incidence of Compound-Related Pulmonary Lesions in the 4-Week Inhalation Study Reported by IRDC (1981)

Measured Concentration mg/L (mg/m ³)	Lesion	
	Interstitial Inflammatory Cell Infiltrate	Interstitial Fibrosis
Males		
0 (0)	9/10	1/10
0.025 (25)	10/10	5/10
0.25 (250)	10/10	5/10 ^b
1.2 (1200) ^a	9/9	5/9*
Females		
0 (0)	10/10	3/10
0.025 (25)	10/10	5/10 ^b
0.25 (250)	10/10	6/10
1.2 (1200) ^a	9/9	3/9

Source: Table 20 (Individual Animal Data) in IRDC (1981).

^a One male and one female died on study and were not included in this evaluation.

^b Incidences determined from individual animal data differed from summary data reported in Table 21 of the study report.

* Statistically significant from control group (p=0.046) when analyzed by SRC using Fisher's Exact Test.

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Table 3. Incidence of Compound-Related Pulmonary Lesions Categorized By Extent and Severity (IRDC, 1981)

Measured Concentration mg/L (mg/m ³)	n	Interstitial Inflammatory Cell Infiltrate						Interstitial Fibrosis					
		Focal		Multifocal		Generalized		Focal		Multifocal		Generalized	
		Very Slight	Slight	Very Slight	Slight	Very Slight	Slight	Very slight	Slight	Very Slight	Slight	Very Slight	Slight
Males													
0 (0)	10	4	3	1	1			1					
0.025 (25)	10			1	6	1	2			1	4		
0.25 (250)	10				6		4		1		2		2
1.2 (1200) ^a	9				2		7		1		1		3
Females													
0 (0)	10	8		2				2		1			
0.025 (25)	10	1	1	1	7						5		
0.25 (250)	10				5	1	4		2		3		1
1.2 (1200) ^a	9				1	2	6		2				1

Source: Data are from an unnumbered table on page 18 of the study report; some incidences have been corrected based on review of individual animal data presented in Table 20 of the study report.

^a One male and one female in the high dose group died on study and were not included in this evaluation.

number of animals with lesions classified as multifocal or generalized increased with dose in both sexes. The intensity of the lesion increased with dose as judged by the greater number of animals with slight versus very slight severity. The incidence of interstitial fibrosis was increased in exposed males when compared to the control group (Table 2). This response reached statistical significance in high dose males only ($p=0.046$ as determined by SRC using Fisher's Exact Test). The intensity of this lesion appeared to increase with dose in males and females (Table 3). The alterations in both types of pulmonary lesions were attributed to a persistent irritating effect of benzoic acid on the lung. The weight of evidence suggests a LOAEL of 0.025 mg/L (25 mg/m^3) based on the occurrence of clinical signs of respiratory irritation and changes in the extent and/or incidence of histopathological changes in the lungs of exposed animals. A NOAEL could not be determined.

Subchronic data are available from oral exposure studies of benzoic acid and sodium benzoate. Shtenberg and Ignat'ev (1970) administered daily 80 mg/kg gavage doses of benzoic acid (vehicle not reported) to male and female white mice (50/sex) for three months. A control group was included in the experiment, but details on treatment and number of animals maintained were not provided by the study authors. Clinical signs, survival, food and water intake and body weight gain were monitored during the study. Responses of the test animals to stressors including starvation and carbon tetrachloride poisoning were assessed at the end of the exposure period. Body weight gain was significantly reduced by 63% in dosed males and 71% in dosed females relative to the controls, although food intake by control and dosed animals was similar. Dosed mice showed greater mortality when stressed by poisoning with carbon tetrachloride.

Kreis et al. (1967) fed diets containing 1.1 % benzoic acid to Royal Wistar rats for 35 days and observed significantly decreased weight gain. No effects were evident on behavior, gross pathology, or histopathology of the heart, liver, kidneys or brain.

U.S. EPA (1987a) summarized several subchronic dietary studies that examined the effect of sodium benzoate on growth. Griffith (1929) fed diets containing sodium benzoate at concentrations of 0, 1.5, 2.0, 2.5, or 3 % to young male white rats for 40 days. Exposure to sodium benzoate had no effect on food consumption at any dietary level. Growth was reduced at the high dose of 3% and one third of the rats in that exposure group died. The incorporation of glycine or gelatin in the 3% diet resulted in a normal growth rate (sodium benzoate is detoxified by glycine conjugation). White (1941) observed marked stunting in rats fed 5% sodium benzoate in the diet after three to six weeks of treatment. Gross signs of toxicity were observed in a few rats that did not tolerate the sodium benzoate. Harshbarger (1942) pair-fed diets containing 3% sodium benzoate to young male white rats for four to five weeks and observed marked reduction in growth rate and food conversion efficiency in the treated groups. Two of eight animals on the 3% diet died. Addition of 1% sodium benzoate to a basal diet had no effect on growth or survival. In a range-finding study, Smyth and Carpenter (1948) fed Sherman rats (5/sex/dose)

diets containing doses of sodium benzoate ranging from 16 to 1009 mg/kg-day for 30 days. No adverse effects were reported on survival, appetite, or growth rate or on histopathology of a limited number of tissues.

Deuel et al. (1954) fed male and female Sherman rats (5/sex/dose) diets containing 0, 1, 2, 4, or 8% sodium benzoate for 90 days. The tested dietary levels corresponded to doses of approximately 0, 640, 1320, 2620, and 6290 mg/kg-day, respectively, as calculated by the investigators. Experimental results for both sexes were combined. Four study animals died from infections. In addition, four treatment-related deaths occurred at the 8% level. A decreased rate of body weight gain and increased relative liver and kidney weights occurred in rats on the 8% diet. The investigators reported "frequent pathological lesions" in the 8% group but did not report incidences or provide descriptions of the lesions.

Fanelli and Halliday (1963) fed diets containing 0, 2, or 5% sodium benzoate to young Sherman rats (6/sex/dose) for 28 days. The estimated equivalent doses of sodium benzoate at the 2% level were 2002 and 2171 mg/kg-day for males and females, respectively. All rats on the 5% diet died between the first and second weeks of the study after exhibiting severe signs of central nervous system toxicity. The rate of body weight gain was slightly, but significantly ($p < 0.05$), depressed in males fed the 2% diet.

Limited data for chronic oral exposure are available from studies of benzoic acid in rats and mice. No chronic oral exposure data were located for sodium benzoate. Marquardt (1960) exposed Wistar rats (20 females and 30 males) to a diet containing 1.5% benzoic acid (approximately 750 mg/kg-day). The control group included 12 females and 13 males. At 18 months, animals fed benzoic acid showed increased mortality, decreased food intake, and suppressed growth. In a second experiment, Marquardt (1960) fed male Wistar and male Osborne-Mendel rats (20/strain) a diet containing 1.5% benzoic acid. The control group contained 10 male rats of each strain. Exposed animals gained less weight and had reduced feed intake, as observed in the previous experiment.

Ignat'ev (1965) conducted an 18 month oral exposure study of benzoic acid in male and female mice. As reported by Informatics, Inc. (1972), the mice (25/sex/dose) were fed 0, 40, or 80 mg/kg-day for 3, 8, or 18 months. The parameters evaluated included general appearance, viability (survival), reproduction, food and water consumption, weight gain, blood and urine parameters, histopathology, carcinogenicity, and response to various stressors. Numerical data were presented for body weight gain, food and water consumption, and viability in the 80 mg/kg-day group. Statistical analysis of these data was not reported. Relative body weight gain was reduced in male and female mice treated with 80 mg/kg-day relative to the controls. There were possible compound-related decreases in food and water consumption and viability in 80 mg/kg-day females relative to the control. Dosed mice were reported (without supporting data) to have increased liver weights; enlarged spleens, ovaries, and lungs; and decreased detoxifying capacity

for carbon tetrachloride. In assessing the quality of this study, Informatics, Inc. (1972) noted that “This study was reported in several other papers, none of which provided data sufficient to justify the conclusions reached.” In a parallel study, Ignat’ev (1965) fed male and female rats 0, 40, or 80 mg/kg-day for 3, 8, or 18 months. There were no apparent effects on body weight gain, viability, or gross appearance or histopathology of “parenchymatous” organs after treatment with 80 mg/kg-day for 18 months.

Shtenberg and Ignat’ev (1970) gave 40 mg/kg-day of benzoic acid to male and female white mice (25/sex) for 17 months. The benzoic acid was administered daily as a paste before the main feeding. A control group of unspecified size was included in the study. Although data for body weight, food consumption, clinical signs, survival, organ weight, and response to stressors were collected, only the results for stress response at 17 months were reported. Animals treated with benzoic acid showed increased mortality (50.0% vs. 12.5%) and weight loss (26.0% vs. 17.8%) relative to control mice when subjected to a 5-day fast period during which benzoic acid treatment was continued by gavage. Mice treated with benzoic acid required 2.7 days to regain lost weight compared to 1.6 days for control mice when feeding was resumed.

Ohno et al. (1978) fed Sprague-Dawley rats (20/sex/dose) a diet containing 0.5% or 2% benzoic acid for one year. No effect of treatment was observed at 0.5%. A slight reduction of growth was noted at the 2% level.

Limited information is available on the carcinogenicity of benzoic acid or sodium benzoate in animals. Toth (1984) exposed albino Swiss mice (50/sex/dose, 99/sex/control) to 0 or 2% sodium benzoate in drinking water from 39 days of age throughout their lifetime. The estimated daily dose was approximately 5920 to 6200 mg/kg-day. No effects were observed on survival or tumor incidence. Sodemoto and Enomoto (1980) fed sodium benzoate to male and female F344 rats (50 male and 52 female rats/sex/dose, 25 male and 43 female rats/sex/control) at levels of 0, 1, or 2% in the diet for 18-24 months. The estimated doses of sodium benzoate were 0, 700, or 1400 mg/kg-day in males and 0, 290, or 580 mg/kg-day in females. Survival was poor in all dose groups as a result of sialodacryoadenitis and mycoplasma infections. No treatment-related adverse clinical signs were observed and only negligible differences in mortality and body weight occurred in exposed and control animals. No compound-related changes in tumor incidence were identified. Shtenberg and Ignat’ev (1970) reported an increased incidence of malignant tumors in mice administered 40 mg/kg-day, but the results of the study were inadequately documented.

Few data are available on the reproductive toxicity of benzoic acid or sodium benzoate. No adverse effects on fertility or lactation were observed in Bayer-Elberfeld rats in a four-generation study where benzoic acid was administered in the diet at concentrations up to 1% (Kieckebusch and Lang, 1960). No adverse effects on the testes were observed in albino rats treated with benzoic acid for 4 weeks at doses up to 647 mg/kg-day via the diet (Bio-Fax, 1973)

or in albino Swiss mice after lifetime exposure to 6200 mg/kg-day doses of sodium benzoate via the drinking water (Toth, 1984).

The developmental toxicity of benzoic acid or sodium benzoate has been evaluated in several studies. Pregnant rats given a single 510 mg/kg gavage dose of benzoic acid on gestation day 9 showed no indication of an increase in resorption rates or malformations (Kimmel et al., 1971). In a series of tests conducted by U.S. FDA, no effects were observed in dams or offspring of rats, mice, rabbits, or hamsters given gavage doses of up to 175-300 mg/kg-day (high dose differed by species) during gestation (FDRL, 1972). CIR (2001) cited data from developmental studies conducted in golden hamsters and Wistar rats by the Polish Academy of Sciences (1977). Survival of pregnant golden hamsters was reported to be unaffected by gavage doses of benzoic acid of up to 600 mg/kg-day given on gestation days 6 to 10. The incidence of resorptions in this study was significantly ($p < 0.05$) increased at doses of 30 mg/kg-day and above and the number of malformations was significantly increased at 600 mg/kg-day. In a study of pregnant Wistar rats conducted by the same group, no effect on survival was seen at benzoic acid doses of up to 500 mg/kg-day when given by gavage on gestation days 6 to 15 (Polish Academy of Sciences, 1977). The incidence of resorptions was significantly ($p < 0.05$) increased at doses of 25 mg/kg-day and above. In a dietary study, severe maternal toxicity (including loss of body weight, failure to gain weight, and increased mortality) was observed in rats provided with feed containing 4% or 8% sodium benzoate (Onodera et al., 1978). The estimated doses at these concentrations were 1875 and 965 mg/kg-day, respectively. Embryo- and fetotoxic effects and malformations observed in the offspring may have been secondary to maternal malnutrition resulting from decreased food intake. WHO (2000) identified a NOAEL of approximately 1310 mg/kg-day for this study.

Other Data

Benzoic acid has tested negative for reverse mutation in *Salmonella typhimurium* strains TA 92, TA94, TA 97, TA98, TA 100, TA1535, TA1537, and TA1538 (Litton Bionetics, 1974; McCann et al., 1975; Ishidate et al., 1984; and Zeiger et al., 1988). Negative results were obtained for a DNA damage test in *S. typhimurium* strain TA1535/pSK 1002 (Nakamura et al., 1987). Positive results have been reported for a recombination assay in *Bacillus subtilis*, but no information on the test conditions was provided (Nonaka, 1989). Benzoic acid was negative for sister chromatid exchange (SCE) in transformed human lymphoblastoid cells (Tohda et al., 1980), human lymphocytes (Jansson et al., 1988), and Chinese hamster ovary (CHO) cells (Oikawa et al., 1980). Equivocal results were obtained for chromosome aberration in Chinese hamster lung (CHL) cells (Ishidate et al., 1984). No *in vivo* studies of benzoic acid genotoxicity were identified in the literature examined.

Sodium benzoate has tested negative for reverse mutation in *S. typhimurium* strains TA 92, TA94, TA98, TA 100, TA1535, TA1537, and TA1538 (Ishidate et al., 1984; Prival et al.,

1991) and *Escherichia coli* WP2 (Prival et al., 1991). Positive (Nonaka, 1989) or weakly positive (Ishizaki and Ueno, 1989) results have been reported for a recombination assay in *Bacillus subtilis*. Negative results were obtained in a cytogenetic assay performed in WI-38 cells (Litton Bionetics, 1974). Positive results were obtained for chromosome aberrations in CHL (Ishidate et al., 1984, 1988; Ishidate and Odashima, 1977) and Chinese hamster DON cells (Abe and Sasaki, 1977). A weakly positive response without dosage effect was obtained for SCE in Chinese hamster DON cells (Abe and Sasaki, 1977). Sodium benzoate gave a positive response for SCE in human lymphocytes (Xing and Zhang, 1990). Negative results were obtained in an *in vivo* bone marrow cytogenetic assay and a host-mediated assay in mice (Litton Bionetics, 1974). Positive results were obtained in a dominant lethal assay conducted in rats (Litton Bionetics, 1974).

Information on the absorption, distribution, metabolism, and excretion of benzoic acid following oral exposure have been reviewed by WHO (2000). No toxicokinetic data were located for the inhalation route. Rapid absorption occurs after oral ingestion; excretion data suggest that 100% absorption can be assumed for humans. After uptake, benzoic acid is metabolized in the liver by conjugation with glycine, resulting in the formation of hippuric acid. The rate of biotransformation to hippuric acid in humans is high and is independent of dose for oral doses of 40 mg/kg or higher. The hippuric acid formed by biotransformation of benzoic acid is rapidly excreted in the urine. In humans, 75-100% of applied oral doses of up to 160 mg/kg were excreted as hippuric acid within six hours of administration. The remainder of the dose was excreted within 2-3 days. Experiments conducted in rats using ¹⁴C-labeled benzoate have shown no evidence for accumulation in the body (WHO, 2000).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR BENZOIC ACID

A **chronic p-RfD of 4E+0 mg/kg-day** is listed for benzoic acid on IRIS (U.S. EPA, 2003) based on human daily per capita intakes and human health experience, which precludes derivation of a provisional chronic RfD for this chemical. The available animal data on the oral toxicity of benzoic acid and sodium benzoate were evaluated with regard to derivation of a provisional subchronic RfD, but were found to be of limited use for assessment of subchronic health effects. The majority of existing studies were not conducted using current methodologies, assessed a limited number of endpoints, and were poorly documented. The predominate health effect identified in these studies was reduction of body weight gain. Target organ effects were not clearly identified in any of the reviewed toxicity studies. Developmental effects were observed in adequately documented studies only at high doses and in association with severe maternal toxicity. None of these data were considered adequate for derivation of a provisional

subchronic RfD value. A provisional **subchronic RfD of 4E+0 mg/kg-day** is derived by adopting the chronic p-RfD of 4E+0 mg/kg-day as a health protective estimate of the subchronic RfD.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR BENZOIC ACID

No human inhalation data on benzoic acid or sodium benzoate were available for derivation of a subchronic inhalation p-RfC. The database for inhalation toxicity consists of a single four week exposure study of benzoic acid conducted in rats (IRDC, 1981). To calculate the provisional subchronic RfC, the LOAEL of 25 mg/m³ in rats identified in this study is first adjusted for intermittent exposure as follows (U.S. EPA, 1994b):

$$\begin{aligned} \text{LOAEL}_{\text{ADJ}} &= (\text{LOAEL}_{\text{RAT}}) (\text{hours}/24 \text{ hours}) (\text{days}/7 \text{ days}) \\ &= (25 \text{ mg}/\text{m}^3) (6 \text{ hours}/24 \text{ hours}) (5 \text{ days}/7 \text{ days}) \\ &= 4.46 \text{ mg}/\text{m}^3 \end{aligned}$$

For pulmonary effects caused by benzoic acid dust aerosol, the LOAEL_{HEC} (human equivalent concentration) is calculated as follows (U.S. EPA, 1994b):

$$\text{LOAEL}_{\text{HEC}} = (\text{LOAEL}_{\text{ADJ}}) (\text{RDDR}_p)$$

where:

$$\begin{aligned} \text{LOAEL}_{\text{ADJ}} &= \text{LOAEL adjusted for intermittent exposure} \\ \text{RDDR}_p &= \text{Regional Deposited Ratio, rat/human for pulmonary effects} \end{aligned}$$

In the IRDC (1981) study, the mean EAD at 25 mg/m³ was 4.7 μm with a geometric standard deviation (σ_g) of 3.1. Using version 2.3 of the RDDR program, an RDDR_p of 0.419 for pulmonary effects was calculated from the EAD and the σ_g reported by IRDC (1981), the average body weight estimated from reported data (300 g), and the default values for the other rat and human parameters. The human equivalent LOAEL is calculated as follows:

$$\text{LOAEL}_{\text{HEC}} = (4.46 \text{ mg}/\text{m}^3) (0.419) = 1.9 \text{ mg}/\text{m}^3$$

The following uncertainty factors were applied to the LOAEL_{HEC}: 3 for extrapolation to humans using dosimetric adjustments, 3 for use of a subacute study, 3 for use of a conservative LOAEL (minimal compound-related effects observed at LOAEL concentration), 10 to protect sensitive individuals (reactions to benzoate and structurally-related compound have been documented), and 3 for lack of developmental and reproductive data by the inhalation route (no

studies are available). The total uncertainty factor was 1000. A provisional **subchronic RfC of 2E-3 mg/m³** for benzoic acid was derived as follows:

$$\begin{aligned} \text{subchronic p-RfC} &= \text{LOAEL}_{\text{HEC}} \div (\text{UF}) \\ &= 1.9 \text{ mg/m}^3 \div (1000) \\ &= 0.002 \text{ or } 2\text{E-3 mg/m}^3 \end{aligned}$$

Confidence in the principal study is low. The study was GLP-compliant and appropriate endpoints were evaluated in an adequate number of animals, but the duration of exposure was short (28 days). Perfusion of the lungs and use of a collagen-specific stain to facilitate evaluation of pulmonary fibrosis were not reported. High control incidences for interstitial inflammatory cell infiltrate (9/10, males; 10/10 females) were observed, but possible causes were not addressed. Comparison of data reported for individual animals with lesion incidences reported in the summary tables of the study report revealed several discrepancies, suggesting possible quality assurance issues in spite of GLP-compliance. Confidence in the database is low because there are no subchronic, chronic, developmental, or reproductive toxicity studies by the inhalation route of exposure. Low confidence in the subchronic p-RfC follows.

No human inhalation data or longer-term animal inhalation data on benzoic acid or sodium benzoate were available for derivation of a provisional chronic inhalation RfC. The four-week inhalation exposure study of benzoic acid conducted in rats was considered inadequate for derivation of a chronic p-RfC for benzoic acid because of the short (less than subchronic) duration of exposure. In the absence of suitable inhalation data, a provisional **chronic RfC cannot be derived**.

DERIVATION OF A PROVISIONAL CARCINOGENICITY ASSESSMENT FOR BENZOIC ACID

Human data on the carcinogenic effects of benzoic acid or sodium benzoate are not available. The carcinogenicity of sodium benzoate has been evaluated in three oral exposure studies conducted in rats or mice (Shtenburg and Ignat'ev, 1970; Sodemoto and Enomoto, 1980; Toth, 1984). These studies are insufficient for quantification of carcinogenic effects because they either used too few doses, were poorly documented and were compromised by low survival of the test animals, or did not observe a compound-related tumor response. Therefore, quantitative cancer assessments cannot be performed for the oral or inhalation exposure routes.

The available *in vitro* assays indicate low genotoxic potential for benzoic acid. No *in vivo* genotoxicity data for benzoic acid were identified. Genotoxicity assays of sodium benzoate have yielded mixed results. Negative results were obtained for mutagenicity in bacteria, a host-mediated assay in mice, and an *in vivo* bone marrow cytogenetic assay in mice, while positive or

weakly positive results were obtained for chromosome aberrations and SCE in cultured mammalian cells and for a dominant lethal assay in rats. Under the proposed guidelines (U.S. EPA, 1999), the data for carcinogenicity of benzoic acid *are inadequate for an assessment of human carcinogenic potential*.

Derivation of quantitative estimates of cancer risk for benzoic acid is precluded by the absence of reliable carcinogenicity data for this chemical.

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