



Toxicological profile for Benzoic acid

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Benzoic acid (PubChem)

1.2. Synonyms

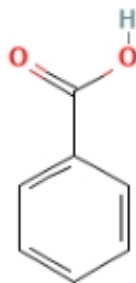
benzoic acid; Dracrylic acid; benzenecarboxylic acid; Carboxybenzene; Benzeneformic acid; phenylformic acid; Benzenemethanoic acid; Phenylcarboxylic acid; benzoate; FEMA No. 2131; Diacrylic acid; Oracrylic acid; Benzoicum acidum; E 210; CHEBI:30746; EINECS 200-618-2; Aromatic carboxylic acid; benzene-2-carboxylic acid; (PubChem); JECFA 850; CoE 21; Flavis 08.021

1.3. Molecular formula

C₇H₆O₂ (PubChem)

1.4. Structural Formula

(ChemIDplus (a))



(PubChem)

1.5. Molecular weight (g/mol)

122.122 (HSDB, 2014)

1.6. CAS registration number

65-85-0

1.7. Properties

1.7.1. Melting point

(°C): 122.4 (Pubchem; ECHA, 2012a; EPISuite, 2017; HSDB, 2014); 121–125 (ChemSpider; Pubchem)

1.7.2. Boiling point

(°C): 249-249.2 (PubChem; ChemSpider; ECHA, 2012a; EPISuite, 2017; HSDB, 2014); 250 (IGS, 2021); 255 (Finnish Environment Institute (a))

1.7.3. Solubility

3.4 g/L at 25°C (PubChem; EPISuite, 2017); 0.29 g/L of benzoic acid in water at 20 deg C (HSDB, 2014); In water, 3.5 g/L at 25 deg C (HSDB, 2014); 2.9, 5 and 15 g/L at pH 2.9, 5 and 9, respectively (at 20°C) (ECHA, 2012a); 0.29 g/L at 20 °C (PubChem); 3 g/L (Finnish Environment Institute (a)); 6.93 g/L at 20°C (PPDB, 2024); 3.4 g/L at 20°C (GESTIS)

1.7.4. pKa

4.2 at 25°C (ECHA, 2012a; HSDB, 2014); 4.19 at 25 °C or 3.98 (PubChem)

1.7.5. Flashpoint

(°C): 250°F (121°C) (closed cup) (ChemSpider; HSDB, 2014); 121-131 (ECHA, 2012a); 140 (closed cup) (GESTIS)

1.7.6. Flammability limits (vol/vol%)

Not flammable (European Commission, 2013a,b); Lower explosion limit 15 vol.% (GESTIS)

1.7.7. (Auto)ignition temperature

(°C): 570 (1058°F) (HSDB, 2014); 573 (ECHA, 2012a); “not expected to autoignite” (PPDB, 2021)

1.7.8. Decomposition temperature

(°C): Decarboxylation above 370 (European Commission, 2013a,b)

1.7.9. Stability

Stable, combustible, incompatible with strong bases, strong oxidizing agents, alkalies (ChemSpider); Undergoes typical reactions of an organic acid, e.g. with alkalis or heavy metals; the powder burns rapidly in oxygen; can react with oxidizing materials; a 0.1% w/v aqueous solution of benzoic acid has been reported to be stable for at least 8 weeks when stored in polyvinyl chloride bottles, at room temperature; stable during transport (HSDB, 2014); Vapor from molten benzoic acid may form explosive mixture with air, concentrated dust may form explosive mixture (PubChem); The substance can react dangerously with fluorine, strong oxidizing agents and oxygen (powder) (GESTIS)

1.7.10. Vapor pressure

7.00E-04 mm Hg at 25°C (PubChem; EPISuite, 2017; HSDB, 2014); 0.11-0.53 Pa at 20°C (ECHA, 2012a); 320 mPa at 20°C (PPDB, 2021); 10 mmHg (ChemSpider); 0.0043 mm Hg at 20°C (Finnish Environment Institute (a)); 1.3 mbar [0.98 mmHg] (IGS, 2021); 1.3 mbar [0.98 mmHg] at 20°C (GSBL, 2021); 0.1 Pa at 25 °C (PubChem)

1.7.11. log Kow

1.87 (PubChem; ChemSpider; ECHA, 2012a; EPISuite, 2017; HSDB, 2014); 0.982 (ChemSpider); 1.9 or 2.03 (Finnish Environment Institute (a)); 1.88 at 20°C (GSBL, 2021)

2. General information

2.1. Exposure

Food Category	Usual (ppm)	Max. (ppm)
Alcoholic beverages	0.00	0.01
Baked goods	0.01	0.01
Chewing gum	0.03	0.04
Condiments, relishes	1.00	1.00
Frozen dairy	0.00	0.01
Gelatins, puddings	0.00	0.01
Imitation dairy	0.10	0.36
Nonalcoholic beverages	0.22	0.25
Soft candy	0.08	0.11
Sugar substitutes	0.75	0.75

Estimated intake from flavouring use: 0.004505 mg/kg bw/day

As taken from Burdock, 2010.

The average daily intake of benzoic acid is 312 mg of which 278 mg is absorbed as sodium benzoate and 34 mg as benzoic acid(1). [(1) USEPA; Health and Environmental Effects Document for Benzoic Acid. p.15 ECAO-CIN-G007 (1987)]

According to the 2006 TSCA Inventory Update Reporting data, the number of persons reasonably likely to be exposed in the industrial manufacturing, processing, and use of benzoic acid is 1000 or greater; the data may be greatly underestimated(1). [(1) US EPA; Inventory Update Reporting (IUR). Non-confidential 2006 IUR Records by Chemical, including Manufacturing, Processing and Use Information. Washington, DC: U.S. Environmental Protection Agency. Available from, as of Aug, 19, 2013: <http://cfpub.epa.gov/iursearch/index.cfm> **PEER REVIEWED**

Occupational exposure to benzoic acid may occur through inhalation and dermal contact with this compound at workplaces where benzoic acid is produced or used. Monitoring data indicate that the general population may be exposed to benzoic acid via inhalation of ambient air, ingestion of food and drinking water, dermal contact with consumer products containing benzoic acid and exposure to certain wood and cooking smoke(SRC).

As taken from HSDB, 2014.

“The aim of this study is to estimate dietary intake of benzoic acid and its salts through food additives in adult population of South East Serbia. Information on dietary intake among 620 adults (aged 18-65) was collected using a food frequency questionnaire, and 748 food samples were analyzed. The mean estimated intake of benzoic acid -0.32 mg/kg of body weight (bw) per day was below acceptable daily intake (ADI). Dietary exposure to benzoic acid (0.36 mg/kg of bw/day; 7.2% ADI) (consumer only), also did not exceed ADI. The main contributors of benzoic acid to dietary intake were non alcoholic beverages (43.1%), ketchup and tomato products (36.1%), and domestic pickled vegetables (19.4%). The results of this study indicate that dietary exposure to benzoic acid and its salts through food preservatives does not represent a public health risk for the adult population of South East Serbia.” As taken from Lazarevic K et al. 2011. Cent. Eur. J. Public Health 19(4), 228-31. PubMed, 2013.

“An exposure assessment was performed to estimate the potential intake of preservatives in the Austrian population. Food consumption data of different population groups, such as preschool

children aged 3-6 years, female and male adults aged 19-65 years were used for calculation. Levels of the preservatives in food were derived from analyses conducted from January 2007 to August 2010. Dietary intakes of the preservatives were estimated and compared to the respective acceptable daily intakes (ADIs). In the average-intake scenario, assuming that consumers randomly consume food products that do or do not contain food additives, estimated dietary intakes of all studied preservatives are well below the ADI for all population groups. Sulphite exposure accounted for 34%, 84% and 89% of the ADI in preschool children, females and males, respectively. The mean estimated daily intake of benzoic acid was 32% (preschool children), 31% (males) and 36% (females) of the ADI. Sorbic acid intakes correspond to 7% of the ADI in preschool children and 6% of the ADI in adults. In the high-intake scenario assuming that consumers always consume food products that contain additives and considering a kind of brand loyalty of consumers, the ADI is exceeded for sulphites among adults (119 and 124%, respectively). Major contributors to the total intake of sulphites were wine and dried fruits for adults. Mean estimated dietary intakes of benzoic acid exceeded the ADI in all population groups, 135% in preschool children, 124% in females and 118% of the ADI in males, respectively. Dietary intakes of sorbic acid are well below the ADI, accounting for a maximum of 30% of the ADI in preschool children. The highest contributors to benzoic and sorbic acid exposure were fish and fish products mainly caused by high consumption data of this large food group, including also mayonnaise-containing fish salads. Other important sources of sorbic acid were bread, buns and toast bread and fruit and vegetable juices." As taken from Mischek D et al. 2012. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 29(3), 371-82. PubMed, 2013.

"As stated in the International Cosmetic Ingredient Dictionary and Handbook, benzoic acid, benzyl alcohol, sodium benzoate, calcium benzoate, magnesium benzoate, potassium benzoate, and benzyl benzoate may function as fragrance ingredients, pesticides, pH adjusters, preservatives, solvents, viscosity decreasing agents, and pH adjusters in cosmetic products ...

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2010, the following ingredients were being used in personal care products: benzoic acid, benzyl alcohol, sodium benzoate, potassium benzoate, and benzyl benzoate. Uses of calcium benzoate and magnesium benzoate were not reported to the VCRP...

Independent of the VCRP data, a survey of ingredient use concentrations was conducted by the Personal Care Products Council in 2010. The results of this survey indicated that the following ingredients were being used in cosmetic products: benzoic acid (0.000002 to 5%), benzyl alcohol (0.000006 to 10%), sodium benzoate (0.000001 to 1%), calcium benzoate (0.002 to 0.004%), potassium benzoate (0.002 to 0.003%), and benzyl benzoate (0.000005 to 4%). No uses of magnesium benzoate were reported in the Council survey.

Personal care products containing the ingredients reported as being used may be applied to the skin, nails, or hair, or, incidentally, may come in contact with the eyes and mucous membranes. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin, nails, or hair for variable periods following application. Daily or occasional use may extend over many years. In the European Union, the maximum authorized concentrations for benzoic acid and sodium benzoate as preservatives in cosmetic products are: rinse-off products, except oral care products (2.5%, as acid), oral care products (1.7%, as acid), and leave-on products (0.5%, as acid). For salts of benzoic acid other than sodium benzoate, the maximum authorized concentration is 0.5% (as acid).

The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP) was asked to review the data submitted to support the safety of benzoic acid and its salts and esters, when used at concentrations other than those laid down in Annex VI to Directive 76/768/EEC as preservatives, for other specific non-preservative purposes apparent from the presentation of the products and to answer the following question: Can benzoic acid and its salts and esters be used safely for non-preservative purposes in cosmetic rinse-off products at a

maximum concentration of 2.5% and in cosmetic oral-care products at a maximum concentration of 1.7%. Data were provided to the SCCNFP in support of the use of benzoic acid as a non-preservative. The SCCNFP did not find the submission appropriate for a safety evaluation of benzoic acid and sodium benzoate for the applied "other uses" in cosmetic products. Subsequently, a second data set was provided, and these data were considered adequate for arriving at a conclusion on the safety of benzoic acid and sodium benzoate, but not the other salts or esters. Based on these data, the Scientific Committee on Consumer Products (SCCP) issued the following opinion: The SCCP is of the opinion that benzoic acid and sodium benzoate are safe for use for preservative and non-preservative purposes in cosmetic rinse-off products at a maximum concentration of 2.5 % and in cosmetic oral-care products at a maximum concentration of 1.7%, and in leave-on products up to 0.5%"

As taken from CIR, 2011.

/HUMAN EXPOSURE STUDIES/ Of 100 patients with asthma undergoing provocation tests with benzoic acid, 47 showed positive reactions. [Reynolds, J.E.F., Prasad, A.B. (eds.) Martindale-The Extra Pharmacopoeia. 28th ed. London: The Pharmaceutical Press, 1982., p. 1283] **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ ... The aim of this study is to determine the incidence of intolerance to sodium benzoate among subjects who experienced repeated episodes of acute urticaria/angio-oedema following the ingestion of a meal or a product containing this substance. This was a retrospective study based on the analysis of data from patients reported to have experienced episodes of urticaria, with or without angio-oedema, after ingesting meals or products containing sodium benzoate. At the first visit to the outpatients clinic, a careful history was taken. Patients were then given the following diagnostic tests: tests for IgE for common inhalant allergens and food allergens, and a double-blind, placebo-controlled challenge with sodium benzoate. A total of 47 subjects were enrolled in the study; five (11%) showed at least one relevant positive reaction to an IgE test for food allergy. Only one subject (2%) had a reaction after the ingestion of 75 mg of sodium benzoate without an adverse reaction to placebo. This study shows that the percentage of repeated episodes of acute urticaria/angio-oedema reactions induced by sodium benzoate is very low (2%). ... /Sodium Benzoate/ [Nettis E et al; Br J Dermatol 151 (4): 898-902 (2004)] **PEER REVIEWED** PubMed Abstract

/HUMAN EXPOSURE STUDIES/ Daily intake of 4 to 6 g does not cause toxic symptoms aside from slight gastric irritation. Larger doses have systemic effects not unlike those of salicylates. [Gilman, A.G., L.S.Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 7th ed. New York: Macmillan Publishing Co., Inc., 1985., p. 961] **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ Chamber-Scarification-Test threshold irritating concentration: 1) normal skin: 30% in ethanol 2) scarified skin: 7.5 % in ethanol: moderate irritations; application of 15% in ethanol leads to marked irritation with erosions. [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ 16 mM benzoic acid (in petrolatum) produced an erythematous reaction in 12 of 13 healthy volunteers on the cheek and in 6 subjects on the forehead, neck and upper back. 8 mM and 4 mM benzoic acid produced only a reaction on cheek. [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ Administration of 0.5 to 1.0 g/day (approximately 14 mg/kg BW) for 44 consecutive days or for 3 months produced no evident signs of toxicity in humans. ... Irritation, discomfort, weakness, and malaise were observed in individuals given oral bolus doses of less than or equal to 1.75 g/day (approximately, 25 mg/kg BW) over a 20 day period. [Sheftel, V.O.;

Indirect Food Additives and Polymers. Migration and Toxicology. Lewis Publishers, Boca Raton, FL. 2000., p. 448] **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ Benzoic acid (5% in petrolatum) did not elicit an allergic reaction when applied to the skin of 10 panelists who, in a previous Kligman-maximization assay, had tested positive for benzoyl peroxide sensitivity. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ /Investigators/ reported results of a cosmetic intolerance assay that patch tested 5202 patients with possible allergic contact dermatitis (537 of the patients had a history of "intolerance," allergy, or irritation to cosmetics). ...A reaction to benzoic acid was noted in 34 (0.7% incidence) /patients/. A reaction was /also/ noted in 1 of the 155 patients with cosmetic allergy. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ Benzoic acid (in 50% aqueous isopropanol) was applied to the medial cheek of adult volunteers; a 2% solution led to wheals (11/11), a 0.04% solution lead to erythema (11/11) and pruritus (4/11). [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ ... Nine patients on penicillin treatment received 1200 mg benzoic acid daily, in eight doses, over five days for eight subjects and for 14 days in one case. No effect was observed; in particular, no significant change was seen in endogenous creatinine clearance, and routine urine analysis showed no abnormality. [WHO; Food Additive Series 37: Toxicological evaluation of ceratin food additives: Benzyl acetate, Benzyl alcohol, Benzaldehyde, and Benzoic acid and its Salts (1996). Available from, as of March 24, 2005: <http://www.inchem.org/documents/jecfa/jecmono/v37je05.htm> **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ Forty-eight female panelists participated in an in-use study that investigated the acnegenic and irritation potential of a liquid/ powder foundation containing 0.2% benzoic acid. ...Approximately half of the test population had "mild to moderate" acne. Panelists were instructed to apply the product to the entire face and neck area at least twice a day for 45 days. Acne lesions and irritation were evaluated by a dermatologist on days 0, 3, 7, 10, 28, and 45. Objective and subjective evaluations of irritation were made by a nurse or technician on days 15, 21, and 35. Panelists also maintained daily response logs. The dermatologist noted "no significant changes the lesion counts of the non acne subjects and the acne subjects had a decrease. ... Panelists' logs recorded occasional instances of dryness, itching, and flakiness. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ An eye shadow plus base containing 0.1% benzoic acid was tested in a 28-day in-use study using 52 women. Half of the panelists were contact lens wearers. Panelists were instructed to use the product at least twice a day. Examinations were made at the beginning and end of the study by an ophthalmologist, weekly by a nurse or technician, and the panelists maintained daily logs. Slight conjunctival hyperemia without chemosis was noted in eight women; the condition was nonpersistent in all cases. Another two women presented with slight hyperemia and were also experiencing allergy symptoms; erythema was noted around the eye of one of these two panelists. One panelist had an incidence of red and mildly swollen caruncles at the fifth observation. Four other panelists reported occasional itching; dryness was reported by another two panelists. One panelist reported redness, puffiness, and irritation of the left upper eyelid that prevented her from wearing her contact lenses for 2 days. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

0.2.1 SUMMARY OF EXPOSURE

0.2.1.1 ACUTE EXPOSURE

A) USES: Benzoates are widely used as preservatives in foods and pharmaceuticals; they are also used as buffering agents. Benzoic acid in combination with salicylic acid has been used as an antifungal agent. Sodium benzoate in combination with sodium phenylacetate is approved as adjunctive therapy for the treatment of acute hyperammonemia in adult and pediatric patients with urea cycle enzyme deficiencies.

B) TOXICOLOGY: Benzoic acid is thought to inhibit oxidative enzymes in the tricarboxylic acid cycle, similar to salicylates, and may result in secondary lactic acid production. Metabolic acidosis may also be explained by a direct action of benzoic acid. Metabolism of benzyl alcohol to benzoic acid is postulated as the mechanism for toxicity in premature infants.

C) EPIDEMIOLOGY: Benzoate exposures are extremely common due to their frequent use as preservatives and buffering agents, however, significant toxicity is rare.

D) WITH POISONING/EXPOSURE

1) MILD TO MODERATE TOXICITY: The primary effect expected from ingestion of moderate amounts of benzoates is gastrointestinal irritation with symptoms including nausea, vomiting, diarrhea, and abdominal pain. Benzoic acid is more irritating than benzoate salts. A non-immunologic contact dermatitis commonly occurs following dermal exposure. Renal tubular dysfunction and elevated free bilirubin levels may occur in a small number of cases.

2) SEVERE TOXICITY: Large ingestions of benzoates may cause metabolic acidosis, elevated serum lactate levels, and associated tachypnea and Kussmaul respirations. Hypokalemia and hypocalcemia may also occur. Administration of benzyl alcohol to neonates has been associated with metabolic acidosis and gasping respirations. Seizures have been reported in experimental animals.

Laboratory:

A) Monitor serum electrolytes, renal function, liver enzymes, blood gases, and lactate levels in patients with evidence of toxicity.

B) Institute cardiac monitoring and obtain an ECG in patients with toxicity.

C) Benzoate concentrations are not widely available or clinically useful for acute management.

Treatment Overview:

0.4.2 ORAL EXPOSURE

A) MANAGEMENT OF MILD TO MODERATE TOXICITY

1) Treatment of mild to moderate toxicity consists of predominantly symptomatic and supportive care.

B) MANAGEMENT OF SEVERE TOXICITY

1) Treatment for severe toxicity consists of predominantly symptomatic and supportive care. Treat severe metabolic acidosis (pH less than 7.1) with sodium bicarbonate 1 to 2 mEq/kg to maintain hemodynamic stability, however the long-term benefit of this treatment has not been shown. Seizures should be treated with benzodiazepines as needed; NOTE: diazepam IV contains 5% sodium benzoate and benzoic acid as buffering agents, therefore, an alternative benzodiazepine such as lorazepam or midazolam may be a better choice.

C) DECONTAMINATION

1) PREHOSPITAL: Gastrointestinal decontamination is not generally recommended as acute toxicity is limited. Remove contaminated clothing and wash exposed skin with soap and water. Irrigate exposed eyes with copious amounts of water.

2) HOSPITAL: Gastrointestinal decontamination is not generally recommended as acute toxicity is limited. Remove contaminated clothing and wash exposed skin with soap and water. Irrigate exposed eyes with copious amounts of 0.9% saline or water.

D) AIRWAY MANAGEMENT

1) Rarely, patients with marked CNS depression or acidosis may need intubation for respiratory support.

E) ANTIDOTE

1) None

F) ENHANCED ELIMINATION

1) Efficacy of enhanced elimination techniques is unknown. Hemodialysis may be useful to treat severe acidosis or in cases that develop renal failure.

G) PATIENT DISPOSITION

1) OBSERVATION CRITERIA: Patients with known large ingestions may be watched clinically for the development of toxicity such as gastrointestinal irritation and acidosis.

2) ADMISSION CRITERIA: Patients who develop gastrointestinal irritation or other signs of benzoate toxicity after a known exposure should be admitted to the hospital. Patients who develop evidence of severe toxicity with acidosis, tachypnea, altered mental status, or seizures should be admitted to an ICU.

3) CONSULT CRITERIA: Contact your local poison center for a toxicology consult for any patient with suspected benzoate toxicity and acidosis, seizures, or other end-organ toxicity.

H) PITFALLS

1) Missing an ingestion of another chemical or other possible etiologies for a patient's symptoms.

I) PHARMACOKINETICS

1) Benzoic acid is rapidly absorbed from the gastrointestinal tract. The volume of distribution is small (estimated 0.14 L/kg in neonates). Benzoic acid is conjugated in the liver with glycine to form hippuric acid, the major metabolite. In healthy adults, up to 97% of the dose of sodium benzoate is excreted as hippuric acid in the urine within 4 hours.

J) TOXICOKINETICS

1) Benzoate exhibits nonlinear kinetics (prolonged elimination half-life) at higher doses.

K) DIFFERENTIAL DIAGNOSIS

1) Includes other agents that may cause metabolic acidosis.

0.4.4 EYE EXPOSURE

A) Irrigate exposed eyes with copious amounts of 0.9% saline or water.

B) Patients with persistent irritation, pain, swelling, lacrimation, or photophobia after irrigation should have a thorough ophthalmologic exam, including slit lamp.

0.4.5 DERMAL EXPOSURE

A) OVERVIEW

1) Remove contaminated clothing and wash exposed skin with soap and water.

2) Dermal exposure commonly causes an acute non-immunologic contact urticaria. Urticaria typically occurs within 30 minutes of exposure, and is transient, lasting only 20 to 40 minutes. Administration of oral indomethacin has reportedly blocked this reaction.

Range of Toxicity:

A) TOXICITY: ADULT: Ingestion of benzoic acid in amounts of 1 to 1.5 g in adults produced gastric pain, nausea, and vomiting; Sodium benzoate ingestions of up to 20 to 60 g daily have been tolerated. PEDIATRIC: 200 to 300 mg/kg/day orally, given to infants, caused lactic acidosis. Inadvertent oral administration of 800 mg/kg of sodium benzoate over 24 hours to a neonate with hyperammonemia resulted in vomiting and irritability. Oral administration of 1000 mg/kg/day to a 5.5-month-old child with nonketotic hyperglycemia resulted in metabolic acidosis, renal tubular dysfunction, hypokalemia, and hypocalcemia. Anion gap metabolic acidosis, agitation, and tachypnea developed in 3 pediatric patients (ages ranging from 2 to 6 years) following inadvertent IV overdoses of sodium benzoate and sodium phenylacetate, ranging from 750 mg/kg infused over 10 hours to 1750 mg/kg infused over 18 hours. Despite aggressive supportive care, 2 of the 3 patients developed cerebral edema and died within hours.

B) THERAPEUTIC DOSE: ADULT: 55 mL/m(2) of Ammonul(R) IV over 90 to 120 minutes (loading dose), followed by the same dose over 24 hours (maintenance dose); provides 5.5 g/m(2) sodium phenylacetate, 5.5 g/m(2) sodium benzoate. CHILD: 0 TO 20 KG: 2.5 mL/kg of Ammonul(R) IV over 90 to 120 minutes (loading dose), followed by the same dose over 24 hours (maintenance dose); provides 250 mg/kg sodium phenylacetate, 250 mg/kg sodium benzoate. GREATER THAN 20 KG: 55 mL/m(2) of Ammonul(R) IV over 90 to 120 minutes (loading dose), followed by the same dose over 24 hours (maintenance dose); provides 5.5 g/m(2) sodium phenylacetate, 5.5 g/m(2) sodium benzoate.

[Rumack BH POISINDEX(R) Information System Micromedex, Inc., Englewood, CO, 2015; CCIS Volume 164, edition expires May, 2015. Hall AH & Rumack BH (Eds): TOMES(R) Information System Micromedex, Inc., Englewood, CO, 2015; CCIS Volume 164, edition expires May, 2015.]

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Non-Human Toxicity Excerpts:

/LABORATORY ANIMALS: Acute Exposure/ In a limit test with rabbits, no mortality or signs of intoxication were seen after dermal application of 10,000 mg/kg bodyweight. The gross autopsy gave no significant findings. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 ****PEER REVIEWED****

/LABORATORY ANIMALS: Acute Exposure/ In rats, exposure by inhalation to 26 mg/cu m over 1 hr caused no mortality, but generalized inactivity and lacrimation were noted. The gross autopsy gave no significant findings. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 ****PEER REVIEWED****

/LABORATORY ANIMALS: Acute Exposure/ In a maximization test, none of 15 guinea-pigs reacted positively after induction and challenge with a 10-20% solution of benzoic acid in water. In addition, the substance also tested negative in a Buehler test with guinea-pigs and in an ear swelling test and local lymph node assay with mice. The concentrations used for induction and challenge were 10-20% in acetone or water. However, a dose-dependent positive result was obtained in an ear swelling test with 5 guinea-pigs (induction with 0.2, 1, 5, or 20% in absolute ethyl alcohol; no challenge) used as a model for detecting agents causing non-immunological contact urticaria in humans. At several other regions (back, abdomen, flank site), a concentration of 20% failed to produce any reactions. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 ****PEER REVIEWED****

/LABORATORY ANIMALS: Acute Exposure/ In four cats given diets containing 0 or 1% benzoic acid (approximately 0 or 450-890 mg/kg bw), aggression, hyperesthesia, and collapse starting 14-16 hr after feed uptake were seen at a dose level equal to 630 mg/kg bw. The duration of the syndrome was about 18-176 hr, and the mortality rate was 50%. The histopathological examination of the two cats that died revealed degenerative changes in liver, kidneys, and lung, but no pathological findings in brain or spinal cord. The authors attributed the higher toxicity of benzoic acid in cats compared with other species to the low capacity of cats for glucuronidation. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ Clinical signs of intoxication (reported for rats only) included diarrhea, muscular weakness, tremors, hypoactivity, and emaciation. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ The effects of benzoic acid and its analogues on insulin and glucagon secretion were investigated in conscious sheep. Intravenous injections of benzoic acid increased plasma insulin and glucagon concentrations in a dose-dependent manner between 39-1250 $\mu\text{mol/kg}$, with ED50s for increasing both hormones of about 625 $\mu\text{mol/kg}$. Various derivatives of benzoic acid (625 $\mu\text{mol/kg}$) were administered and structure-activity relationships were examined. A single carboxylic group was essential for stimulating insulin and glucagon secretion, since both hormone responses were abolished with compounds in which the carboxylic group was replaced by sulfonic or phosphoric groups, or in which another carboxylic element was introduced (phthalic acids). Most of the compounds which introduced other elements (amino and hydroxy groups, and halogens) onto the benzene ring had an altered stimulating activity. Thus the pancreatic endocrine system can recognize the chemical structure of benzoic acid and its derivatives in detail and induce insulin and glucagon secretion in sheep. [Mineo,H et al; Eur J Pharmacol 280 (2): 149-154 (1995)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ A 4% soln ... was injected iv daily /in brown rabbits/. ... Animals were killed with a gas embolus after 12 hr to 3 days. Histologically, exudative detachment of the retinal neuroepithelium from the pigment epithelium was found. The toxic /effects/ appeared ... predominantly on the layer of rods and cones. ... Acid mucopolysaccharides appeared to be increased in the rod and cone layer, but also irregularly in other parts of the retina. [Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 142] **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ Na benzoate, which dissociates intravascular bilirubin-albumin complexes, might thus have the potential for driving bilirubin from the plasma into tissues such as the skin. /The authors/ investigated this hypothesis in the adult Gunn rat. As others, /investigators/ observed an early fall in blood bilirubin concentration after intraperitoneal Na benzoate injection, without any change in skin bilirubin content. Nevertheless, /investigators/ observed a significant decrease (p less than 0.01) in skin bilirubin content at 24 and then 48 hr after Na benzoate injection, at which times blood bilirubin remained at a low level. The phenomenon was reversible since, at 8 days after Na benzoate injection, blood and skin bilirubin contents had returned to control values. Regarding the decrease in skin bilirubin content after Na benzoate injection we described a dose-effect relationship. So, /the authors/ could not confirm /this/ hypothesis. /The authors/ concluded that the phenomenon observed is probably due to a shift in the distribution equilibrium of the pigment between serum, skin and other tissues. /Sodium benzoate/ [Bessard G et al; Biol Neonate 44 (5): 315-20 (1983)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ Royal Wistar rats dosed with 3% for 1, 2, 3, or 5 days (approx.1500 mg/kg/day); basal diet followed for 19 to 30 days. Fourteen of 35 rats dosed for 5

days died; necrosis of parenchymal cells noted in brain in all 5-day treated rats and occasionally in 3-day treated rats. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ In an acute eye irritation/corrosion study with rabbits conducted according to OECD Guideline 405, some eye irritation was reported after application of benzoic acid in the form of a paste. Within 72 hr, the scores for chemosis, reddening of the conjunctivae, iritis, and keratitis always remained at <2. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ From the available studies, it can be assumed that the toxicity of benzoic acid after short-term oral exposure is low. In high-dosed rats given approximately 2250 mg/kg body weight per day via diet over 5 days, excitation, ataxia, convulsions, and histopathological changes in the brain were seen. The mortality was about 50%; in some cases, bleeding into the gut was noted. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005**PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ A dose-dependent positive result was obtained in an ear swelling test with five guinea-pigs (induction with 0.2, 1, 5, or 20% in absolute ethyl alcohol; no challenge) used as a model for detecting agents causing non-immunological contact urticaria in humans. At several other regions (back, abdomen, flank site), a concentration of 20% failed to produce any reactions. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ 0.5% /benzoic acid was given/ in diet (approx. 300-420 mg/kg/day) /to/ cats /for/ 3-4 days continuously in diet. 4 cats were tested; initial body weight: 1.42-2.0 kg. /Effects include/ convulsions, hyperaesthesia, apprehension, swollen hepatocytes with infiltrations of macrophages and fibroblasts, swollen kidney tubules, no pathological findings in brain and spinal cord; mortality: 2/4. [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ In two other studies with rats dosed with approximately 825 mg/kg body weight per day over 7-35 days or with 65-647 mg/kg bodyweight per day over 28 days, no clear treatment-related effects occurred. The reduced weight gain at 2250 and 825 mg/kg body weight per day may be attributed to reduced food intake... . The relevance of the reduced relative kidney weight at 324 mg/kg body weight per day, which was not dose-related and not accompanied by changes in histopathological examinations, is unclear. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ F344 rats received 1.81, 2.09, or 2.4% sodium benzoate, and B6C3F1 /mice/ received 2.08, 2.50, or 3.0% sodium benzoate in their diet for 10 days. In male rats of the 2.4% group, relative liver and kidney weight, serum levels of albumin, total protein, and gamma-glutamyl transpeptidase were significantly increased; enlarged hepatocytes with glassy cytoplasm were seen. In male mice of the 3.0% group, absolute liver weights and serum cholesterol and phospholipid levels were increased, and enlargement, vacuolation and necrosis of hepatocytes were evident. /Sodium benzoate/ [Sheftel, V.O.; Indirect Food Additives and Polymers. Migration and Toxicology. Lewis Publishers, Boca Raton, FL. 2000., p. 448] **PEER REVIEWED**

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Mice /were given/ daily 80 mg/kg/day /benzoic acid/. /Effects include/ reduced weight gain without reduced food intake;

mortality rate at week 10: 32% in males and females. [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Ten CD rats per sex per group were exposed to 0, 25, 250, or 1200 mg benzoic acid dust aerosol/cu m (analytical concentration; mass aerodynamic diameter [MAD]/sigma g (standard deviation): 0, 4.6/3.1, 4.4/2.1, 5.2/2.1; mass median aerodynamic diameter [MMAD]: 4.7 um) for 6 hr/day and 5 days per week over 4 weeks. After this time, various serum biochemical, hematological, organ weight, and histopathological examinations were conducted. At >25 mg/cu m, an increased incidence of interstitial inflammatory cell infiltrate and interstitial fibrosis in the trachea and lungs in treated animals compared with controls was seen. Although the number of these microscopic lesions was higher in treated animals than in controls, there was no clear dose dependency for this effect. A concentration of >250 mg/cu m resulted in upper respiratory tract irritation, as indicated by inflammatory exudate around the nares, and significantly decreased absolute kidney weights in females. In the highest dose group, one rat per sex died, and the body weight gain was significantly decreased in males and females compared with controls. In addition, a significant decrease in platelets (males/females), absolute/relative liver weights (males), and trachea/lung weights (females) was noted. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

/LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ In two studies with rats given 1.5% benzoic acid via diet (approximately 750 mg/kg body weight per day), the animals showed a reduced weight gain with decreased feed intake after dosing over 18 months. In one of these studies, mortality was increased (15/50 rats of both sexes versus 3/25 in controls). [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

/LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ Seventeen dogs given diets containing sodium benzoate or benzoic acid at 1000 mg/kg bw for 250 days showed no effect on growth, appetite, or well-being; but at higher doses ataxia, epileptic convulsions, and death occurred. [WHO; Food Additive Series 37: Toxicological evaluation of ceratin food additives: Benzyl acetate, Benzyl alcohol, Benzaldehyde, and Benzoic acid and its Salts (1996). Available from, as of March 24, 2005: <http://www.inchem.org/documents/jecfa/jecmono/v37je05.htm> **PEER REVIEWED**

/LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ 40 Sprague-Dawley rats (20 each sex) received feed containing either 0.5% or 2% for 1 year. Some other groups also received sorbic acid. No effect noted at 0.5%; slight reduction of growth rate noted at 2%. No additive toxicity noted of benzoic acid plus sorbic acid. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

/LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ Administration of 80 mg /benzoic acid/ (BA)/kg body weight for 18 months caused no adverse effects on body weight gain, survival, or gross microscopic pathology in rats and mice. Mice were fed 40 mg BA/kg body weight for 1.5 years. The treatment caused a decrease in the resistance to stress and reduction in food and water intake and body weight gain. Rats received 1.5% BA in their diet (750 mg/kg body weight). The treatment decreased food intake and body weight gain. [Sheftel, V.O.; Indirect Food Additives and Polymers. Migration and Toxicology. Lewis Publishers, Boca Raton, FL. 2000., p. 448] **PEER REVIEWED**

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ In a study with pregnant rats given only one oral dose of benzoic acid (510 mg/kg body weight on gestation day 9), there was no indication of an increase in resorption rates or malformations. [International Programme on

Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ In a four-generation study with rats, no effects on life span, growth rate, or organ weights were reported after dosing with up to 1% in the diet (approximately 500 mg/kg body weight per day). Only animals of the third generation were autopsied after 16 weeks, but it is not clear if a complete histopathological investigation was performed. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

As taken from HSDB, 2014

The main route of exposure of the general population to benzoic acid or sodium benzoate is likely via foodstuffs that contain the substances naturally or added as antimicrobial agents. There are a few analyses of processed foodstuffs available. They refer to different types of food items (juice, soft drinks, soya sauce varieties) from the Philippines (a total of 44 samples) and from Japan (a total of 31 samples) and to orange drinks sampled in England. The concentrations of sodium benzoate in the Philippine dietary samples ranged from 20 to >2000 mg/litre. The range in the Japanese products was 50-200 mg/litre, thus reflecting the lower maximum level of sodium benzoate allowed to be added to food in Japan as compared with the Philippines (Villanueva et al., 1994). Orange drinks from England contained sodium benzoate at concentrations ranging from 54 to 100 mg/litre (mean 76.7 mg/litre; n = 6) (Freedman, 1977).

Generally, the actual uptake depends on the individual's choice of food to be consumed and the different limit values in different countries. Several intake estimations have been published. Three Japanese studies reported average daily intakes of benzoic acid from processed foodstuffs to be 10.9 mg per person (Toyoda et al., 1983a) and 1.4 mg per person (Toyoda et al., 1983b; Yomota et al., 1988), corresponding to 0.02-0.2 mg/kg body weight (for persons with a body weight of 50-70 kg). Both of the latter studies used the market basket method for intake calculations, whereas the first-mentioned study calculated intakes using the results of a national nutrition survey. The concentrations of benzoic acid in 3319 food samples analysed for this study (Toyoda et al., 1983a) ranged from not detected to 2100 mg/kg food. The maximum was found in salted fish (n = 7; mean 754 mg/kg). Another survey refers to the United Kingdom, where analyses of benzoic acid in foods and drinks in which it is permitted as well as intake estimates have been performed (UK MAFF, 1995). Sixty-five out of 122 samples tested contained detectable benzoic acid. The highest concentrations were found in sauces (mean 388 mg/kg; n = 20; range 71-948 mg/kg), reduced sugar jam (mean 216 mg/kg; n = 4; range <20-333 mg/kg), non-alcoholic drinks (mean 162 mg/kg; n = 20; range 55-251 mg/kg), and semipreserved fish product (653 mg/kg; n = 1). The survey found that the concentrations of benzoic acid detected would lead to a dietary intake below 5 mg/kg body weight per day, even for adults with an above-average consumption.

A frequent contributor to dietary exposure is soft drinks. A rough estimation based on the average daily consumption in Germany of such drinks (372 ml non-alcoholic beverages, excluding bottled water; BAGS, 1995) by 19- to 24-year-old men, assuming the concentration of benzoic acid present corresponds to a maximum allowable level of 150 mg/litre (EC, 1995), would result in a mean daily intake of 55.8 mg benzoic acid per person (or 0.80 mg/kg body weight, assuming a 70-kg body weight). For comparison, a similar calculation with sugar-free marmalade, jam, and similar spreads, which are allowed to contain higher levels of benzoic acid (500 mg/kg; EC, 1995), would result in a possible intake of 4.1 mg per person per day, or 0.06 mg/kg body weight per day (assumes a daily consumption of 8.2 g, according to BAGS, 1995). This was more than a possible intake via fruits containing natural benzoic acid. For example, a daily consumption of 40.4 g of fruits (BAGS, 1995) would lead to a possible intake of 0.57 mg benzoic acid per person per day (or 0.008 mg/kg body weight for a 70-kg person), if the reported maximum of 14 mg benzoic acid/kg (see section 6.1) were present.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) assessed the intake of benzoates from information provided by nine countries (Australia, China, Finland, France, Japan, New Zealand, Spain, United Kingdom, and USA) (WHO, 1999). Because diets differ among countries, the foods that contribute to benzoate intake would be expected to vary. The food category that contributed most to benzoate intake was soft drinks (carbonated, water-based, flavoured drinks) for Australia/New Zealand, France, the United Kingdom, and the USA. In Finland, 40% was in soft drinks. Soya sauce was the main source of benzoate in China and the second most important in Japan. The best estimates of national mean intakes of benzoates by consumers ranged from 0.18 mg/kg body weight per day in Japan to 2.3 mg/kg body weight per day in the USA. These estimates were based on analyses involving either model diets or individual dietary records and maximum limits specified by national governments or the European Union. The estimated intake by high consumers of benzoates, based on food additive levels in national standards, was 7.3 mg/kg body weight per day in the USA and 14 mg/kg body weight per day in China.

Benzoates have been detected in groundwater, but not in drinking-water.

Quantitative information on (oral or dermal/mucosal) exposure via cosmetic, hygienic, or medical products is rare, but the data available indicate a remarkable contribution to exposure. There are reports on leaching of benzoic acid from denture-base acrylic resins. After 10 days of immersion in artificial saliva, concentrations of up to about 3 mg/litre have been observed for benzoic acid, which is formed as a degradation product of the benzoyl peroxide that is added as a polymerization initiator (Koda et al., 1989, 1990). In Japan, commercial toothpastes have been found to contain benzoic acid at concentrations ranging from 800 to 4450 mg/kg (n = 18). Use of the toothpaste with the highest concentration (by 40 20-year-old female students) would result in a calculated daily intake of about 2.23 mg per person. This was about the same amount as their estimated intake from diet (Ishida, 1996). Benzoic acid is also used in dermatology as a fungicidal topical treatment for ringworm (*Tinea* spp.). The emulsifying ointment preparation contains benzoic acid at 6% and is applied twice daily (Goodman et al., 1990; BMA, 1998).

Recent quantitative monitoring data on concentrations of benzoic acid or salts in ambient or indoor air are not available. Considering the few (low) levels of benzoic acid measured in urban air in the past, with a maximum of 0.38 µg/m³ (see section 6.1), inhalation may contribute only marginally to exposure of the general population. Using this maximum, a daily inhalative dose of 8.74 µg per person (or 0.12 µg/kg body weight) is obtained (assuming a daily inhalation volume of 23 m³ for a 70-kg adult male; WHO, 1994).

Few quantitative data on occupational exposure have been identified. Nevertheless, there is a potential for inhalation or dermal contact in the chemical and allied product industries as well as in workplaces where these products are used. Air samples (n = 50) collected in an industrial environment (no further details given) over a year's time showed benzoic acid concentrations ranging from not detected to 1.5 mg/m³ (Halvorson, 1984). On the basis of the latter value, an inhalative dose of 14.4 mg per person per 8-h working time (or 0.2 mg/kg body weight) would result (assuming an inhalation volume of 9.6 m³ for an 8-h exposure with light activity; WHO, 1994). However, because of the lack of information on specific working operations and conditions involved (e.g., duration of exposure, use of protective clothes, etc.), it is impossible to derive a realistic estimate of occupational exposure.

As taken from INCHEM, 2000, CONCISE INTERNATIONAL CHEMICAL ASSESSMENT DOCUMENT NO. 26, BENZOIC ACID AND SODIUM BENZOATE.

TYPES OF HAZARD/ EXPOSURE	ACUTE HAZARDS/ SYMPTOMS	PREVENTION	FIRST AID/ FIRE FIGHTING
FIRE	Combustible.	NO open flames.	Powder, water spray, foam, carbon dioxide.
EXPLOSION	Finely dispersed	Prevent deposition of dust;	In case of fire: keep drums,

	particles form explosive mixtures in air.	closed system, dust explosion-proof electrical equipment and lighting.	etc., cool by spraying with water.
EXPOSURE			
•INHALATION	Cough. Sore throat.	Local exhaust or breathing protection.	Fresh air, rest.
•SKIN	Redness. Burning sensation. Itching.	Protective gloves.	Remove contaminated clothes. Rinse and then wash skin with water and soap.
•EYES	Redness. Pain.	Safety goggles.	First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then take to a doctor.
•INGESTION	Abdominal pain. Nausea. Vomiting.	Do not eat, drink, or smoke during work. Wash hands before eating.	Rinse mouth. Induce vomiting (ONLY IN CONSCIOUS PERSONS!). Refer for medical attention.

As taken from NIOSH, 1999.

Maximised Survey-derived Daily Intakes (MSDI-EU): 34 µg/capita/day

Maximised Survey-derived Daily Intakes (MSDI-USA): 340 µg/capita/day

As taken from EFSA, 2008.

Benzoic acid is listed as a fragrance ingredient by the IFRA and on the US EPA InertFinder Database (2024).

Benzoic acid is listed as an ingredient in inside the home, personal care (at 0.1-1.0% where specified), pet care, and commercial/institutional products by the CPID.

“By setting a default assessment factor of 100, an Acute Acceptable Exposure Level (AEL_{acute}) of 5 mg/kg bw/d, and a Medium-term Acceptable Exposure Level (AEL_{medium-term}) of 5 mg/kg bw/d are proposed for acute and repeated, medium-term exposure to benzoic acid.

....By setting a default assessment factor of 100, a Long-term Acceptable Exposure Level (AEL_{long-term}) of 5 mg/kg bw/d is proposed for chronic exposure towards benzoic acid. AELs for local effects are not required as toxic effects after dermal application are covered by the systemic AELs.

....consumer exposure to benzoic acid residues in food or feed items via biocidal products will be much lower compared to consumer exposure to benzoic acid occurring as natural food ingredient and from use as registered food additive and authorised feeding stuff additive.”

“.....a rough estimate of the inhalation exposure from evaporating surface disinfectants showed that inhalation exposure to benzoic acid vapours is negligible (calculation as proposed by Human Exposure Expert Group (HEEG)). Exposure to benzoic dusts is not expected but any risk would be sufficiently addressed by general exposure limits for dusts, e.g. national OELs such as MAK for fine dust (DFG, 2011). This conclusion was based on the observation that effects after dust inhalation

are most likely due to pneumoconiosis and are not substance-specific. A need is not seen to revise the AEL setting and preference is given to derive AELs on the basis of the oral studies.”

As taken from European Commission, 2013a, b.

“In the current risk assessment, the intake of benzoic acid for two-year-old children and 18-70 year old men and women were calculated. When it comes to benzoic acid, the calculated mean and high intake for adults was below the ADI in all scenarios. The mean intake for 2-year-olds was below ADI in all scenarios, whereas high consumers among the two-year-old children in scenario 3 and 4 exceeded the ADI (not in scenario 1 and 2). The comparison of the current calculated intake of benzoic acid (in scenario 1) with the intake reported by VKM in 2007, showed that for 2-year-olds there was an increase in the intake of benzoic acid. For both men and women the intake of benzoic acid were about equal. An increase in the benzoic acid concentration in some products within the beverage categories contributed to the increased intake for the 2-year-olds (scenarios 3 and 4).

VKM....concludes that the benzoic acid intake in 2-year-old-children (in scenarios 3 and 4) is of concern for high consumers since their intake have increased compared to 2007 and now reach the ADI for high consumers of soft drinks and “saft”, although the ADI is not a threshold for toxicity. For the other age groups, there is no concern related to the intake of benzoic acid from beverages. However, it should be noted that a considerable intake of benzoic acid also is expected from other sources such as food and cosmetics. High consumers of soft drinks, “saft” or flavoured water in all age groups could be at risk for exceeding or approaching ADI if the exposures from foods are taken into account. This is especially of concern for 2-year-old children, since high consumers of soft drinks and “saft” already have reached the ADI.”

As taken from VKM, 2013.

“The purpose was to assess the health risk to general consumers in Taiwan associated with dietary intake of benzoic acid and sorbic acid by conducting a total diet study (TDS). The hazard index (HI) in percent acceptable daily intake (%ADI) of benzoic acid and sorbic acid for eight exposure groups classified by age were calculated. In high-intake consumers, the highest HI of benzoic acid was 54.1%ADI for males aged 1-2 years old at the 95th percentile, whereas for females, the HI was 61.7%ADI for aged over 66 years old. The highest HI of sorbic acid for male and female consumers aged 3-6 years old at the 95th percentile were 14.0%ADI and 12.2%ADI, respectively. These results indicate that the use of benzoic acid and sorbic acid as preservatives at the current level of use in the Taiwanese diet does not constitute a public health and safety concern..” As taken from Ling MP et al. 2015. J. Agric. Food Chem.63(7), 2074-82. PubMed, 2017 available at <http://www.ncbi.nlm.nih.gov/pubmed/25633072>

“Benzoic acid and sorbic acid are widely used for food preservation. These preservatives are generally recognised as safe. The aim of this study was to determine the level of benzoic and sorbic acid in food samples that are usually consumed in Iran. Therefore, 54 samples, including 15 soft drinks, 15 ultra-high-temperature milk, 15 ketchup sauces and 9 bread samples, were analysed by high-performance liquid chromatography with UV detection. Benzoic acid was detected in 50 (92.5%) of the samples ranging from 3.5 to 1520 µg mL(-1), while sorbic acid was detected in 29 (50.3%) samples in a range of 0.8 and 2305 µg mL(-1). Limits of detection and limits of quantification for benzoate were found to be 0.1 and 0.5 µg mL(-1), respectively, and for sorbate 0.08 and 0.3 µg mL(-1), respectively. The results showed that benzoic acid and sorbic acid widely occur in food products in Iran.” As taken from Javanmardi Fet al. 2015.Food Addit. Contam. Part B Surveill.8(1), 32-9. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/25135626>

“....foods of animal origin are likely to provide only a very minor contribution if any to the overall dietary intake of benzoic acid, which is mostly provided by foods of vegetable origin and food additives.”

As taken from EFSA, 2018.

“The chemical is reported to be used in cosmetic/domestic products overseas (up to a maximum concentration of 5 % in cosmetics, (INCI, 2010), although its use in cosmetic/domestic products in Australia is not known. The likely route of exposure to general public is through dermal use of consumer products containing the chemical. However, in such formulations it is expected that the chemical will be used at very low concentrations and will be buffered to the physiological pH that will mitigate the risk, so no likely harm from the use of cosmetics is expected.

The chemical has reported cosmetic use in:

- bulking and masking agents; and
- fragrance ingredients, preservatives and pH adjusters.

The chemical has reported domestic use including in:

- adhesives, binding agents;
- cleaning/washing agents;
- colouring agents;
- corrosion inhibitors;
- fillers;
- flame retardants and extinguishing agents; and
- paints, lacquers and varnishes.

The chemical has reported commercial use including in:

- fixing agents;
- lubricants and additives;
- reprographic agents;
- softeners; and
- process regulators for polymerisation processes in production of resins, rubbers and polymers.

The chemical has reported site-limited use including as:

- intermediates; and
- laboratory chemicals.

The chemical has reported non-industrial use including in:

- non-agricultural pesticides and preservatives; and
- pharmaceuticals, milk, food/foodstuff flavourings, plant extracts and nutrients.

Exposure standards:

An exposure limit (TWA) of 5–10 mg/m³ in different countries such as USA (California, Tennessee), Canada and England.

As taken from AICIS, 2013.

“Food consumption data from national dietary surveys were combined with brand-specific-use levels reported by beverage manufacturers to calculate the exposure to benzoic acid and its salts (INS Nos 210-213) from non-alcoholic beverages in Brazil, Canada, Mexico and the United States. These four jurisdictions were identified as having some of the most prevalent use of benzoates in beverages globally. Use levels were weighted according to the brand's market volume share in the respective countries. Benzoates were reported to be used primarily in 'water-based flavoured drinks' (Codex General Standard for Food Additives (GSFA) category 14.1.4). As such, the assessments focused only on intakes from these beverage types. Two different models were established to determine exposure: probabilistic (representing non-brand loyal consumers) and

distributional (representing brand-loyal consumers). All reported-use levels were incorporated into both models, including those above the Codex interim maximum benzoate use level (250 mg kg⁻¹). The exception to this was in the brand-loyal models for consumers of regular carbonated soft drinks (brand loyal category) which used (1) the interim maximum use level for beverages with a pH ≤ 3.5 and (2) all reported use levels for beverages pH > 3.5 (up to 438 mg kg⁻¹). The estimated exposure levels using both models were significantly lower than the ADI established for benzoates at the mean level of intake (4-40% ADI) and lower than - or at the ADI only for toddlers/children - at the 95th percentile (23-110% ADI). The results rendered in the models do not indicate a safety concern in these jurisdictions, and as such provide support for maintaining the current Codex interim maximum benzoate level of 250 mg kg⁻¹ in water-based beverages.” As taken from Martyn D et al. 2017. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 34(9), 1485-1499. PubMed, 2018.

“BACKGROUND AND OBJECTIVES: The prevalence and potential health effects of common food antimicrobials in processed foods and beverages are relatively unknown in Singapore. The occurrence of chemical antimicrobials in processed foods and beverages and their effects on inflammation and oxidative stress in vitro were examined. METHODS AND STUDY DESIGN: The occurrence of antimicrobials in 1605 processed food and 359 beverage items were examined by surveying the ingredients on the product labels. Human neutrophils were exposed to physiologically relevant concentrations of common antimicrobials. Established markers of inflammation, I Leukotriene B4 and oxidative stress, F2-isoprostanes were measured using stable-isotope dilution gas chromatography-mass spectrometry. RESULTS: Antimicrobials were added to 23.2% of the processed foods and beverages. Sorbic, benzoic, lactic, propionic and acetic acids accounted for 84.8% of the added antimicrobials in the processed foods and beverages. 92.5% of the bread contained propionic acid. Lactic acid was the most common antimicrobial (44.4%) in cheeses. Sorbic acid was added to 63.2% of the margarines selected. Sauces (31.5%), energy drinks (50.0%), soft drinks (70.7%) and fruit cordials (66.6%) contained added benzoic acid. Benzoic and propionic acids at physiologically relevant concentrations augmented leukotriene B4 formation (benzoic acid, EC₅₀ = ~100 µg L⁻¹ and propionic acid, >200 µg L⁻¹). Lactic and sorbic acids dose-dependently inhibited the F2-isoprostanes production (IC₅₀ values ~100 µg L⁻¹) and myeloperoxidase activity (IC₅₀ values ~100 µg L⁻¹). CONCLUSIONS: Our results demonstrate that Singapore consumers are significantly exposed to food antimicrobials, and these molecules, in physiologically relevant concentrations, exert significant and differential effects in vitro.” As taken from Loong C et al. 2018. Asia Pac. J. Clin. Nutr. 27(1), 113-120. PubMed, 2018.

Acceptable operator exposure level: 5 mg/kg/day (PubChem).

ACGIH TLV: Notice of intended change: 0.5 mg/m³ (measured as inhalable fraction and vapour)

TLV basis: Eye, upper and lower respiratory tract irritation; lung damage

DFG MAK: TWA 0.5 mg/m³ (measured as respirable fraction of the aerosol; can also occur as vapor and aerosol)

MAK excursion factors, maximum duration of peaks, maximum number per shift, and minimum interval between peaks:

Category	Excursion factor	Duration	Number per shift	Interval
II: Substances with systemic effects	4	15 min, average value	4	1 hour

As taken from ACGIH, 2021a,b

Benzoic acid (CAS RN 65-85-0) is used as a flavour enhancer, fragrance ingredient and preservative antimicrobial in topical non-medicinal natural health products, at concentrations up to 5%. It has an upper limit toxicity restriction of 5 mg/kg bw/day (Health Canada, 2021).

“Preservatives are essential additives in dishwashing detergents and wet wipes. Ensuring the safe use of preservatives in products is important for public health, as some preservatives are associated with health issues. In this study, the content of 12 preservatives in 105 dishwashing detergents and 105 wet wipes was determined, where these compounds are commonly found, among consumer products. A realistic exposure estimation was considered by using Korean consumer-specific exposure parameters for adults and babies. A probabilistic risk assessment was conducted by applying the Monte Carlo simulation method. Benzoic acid (41%) and cetylpyridinium chloride (30%) were the most commonly used preservatives in dishwashing detergents and wet wipes, respectively, although their content in different products (benzoic acid: 0.28-19.4 mg/g in dishwashing detergents; cetylpyridinium chloride: 0.003-0.64 mg/g in wet wipes) varied widely. The calculated median and upper-limit margin of safety (MOS) values related to systemic health effects and skin sensitization from exposure to preservatives largely exceeded the target MOS, which confirmed the safety of the products. Exposure to preservatives from wet wipes was several times higher in babies than in adults. Sensitivity analysis revealed that the amount of the product used, frequency of use, and weight fraction of the preservative were the major contributors to the exposure to preservatives from dishwashing detergents and wet wipes.” As taken from Pack EC et al. 2021. Sci. Total Environ. 782, 146829. PubMed, 2021.

Overview of estimated dietary exposure of the total population to benzoates (as benzoic acid)a from their use as food additives

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2004) J. Anal. Appl. Pyrolysis, 71: 223-311.

Ingredients CAS Number	Chemical Class	Mol. Wt. (M) bp or mp (°C)	Max cig Appln. Level (ppm)	Purity of sample Pyrolysed (%)	Composition of pyrolysate (Compound %)	Max level in smoke (n++-ig)
Benzoic acid CAS 65-85-0	Aryl acid	M=122 Mp 121-123 Bp 249	140	99.5	Benzoic acid (97.2) Isopropyl benzoate (1.6) Phenylacetic acid (1.1) Phenyl benzoate (0.1)	68 1 0.8 0.007

Results of GC/MS Pyrolysis Analysis of Benzoic acid (Free acid of Sodium Benzoate present in tobacco as a preservative):

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
benzoic acid	3618255736	97.36	3800403002	96.86
Total ion chromatogram	3716367847	100	3923685191	100

In a pyrolysis study, 98.6% of Benzoic acid added to cigarettes was transferred intact to the smoke. (Purkis et al. 2011).

2.3. Ingredient(s) from which it originates

Natural Pollution Sources:

Benzoic acid is widely distributed in nature(1) and occurs in nature in free and combined forms(2). Gum benzoin (Styrax benzoin) may contain as much as 20% free acid; Acroid resin (Xanthorrhoca

haslilis) contains 4.5 to 7%. Smaller amounts of the free acid are found in the scent glands of beavers, cherry tree bark, cranberries, prunes, ripe cloves, and oil of anise seed. Peru and Tolu balsams contain benzyl benzoate. The urine of herbivores contains a small proportion of the glycine derivative of benzoic acid, hippuric acid(1). [(1) Opgrande JL et al; Benzoic acid. Kirk-Othmer Encyclopedia of Chemical Technology. (1999-2013). New York, NY: John Wiley & Sons. Online Posting Date: 16 May 2003 (2) O'Neil MJ, ed; The Merck Index.15th ed, Cambridge, UK: Royal Soc Chem, p. 192 (2013)] **PEER REVIEWED**

Cranberries, prunes, ripe cloves, bark of wild black cherry tree, scent glands of beavers, and oil of anise seeds. [Verschuere, K. Handbook of Environmental Data on Organic Chemicals. Volumes 1-2. 4th ed. John Wiley & Sons. New York, NY. 2001, p. 286] **PEER REVIEWED**

Food Survey Values:

Apple wine and apple essence contain 0.329 and 40 ppm of benzoic acid, respectively(1). Benzoic acid and sodium benzoate are common food additives, being used as food preservatives at a concentration of 0.1% and as antimicrobial agents in food at concentration levels of 0.29-0.00001%(1). Most berries contain about 0.05% of benzoic acid(2). Benzoic acid has been detected but not quantified in pine sprout tea(3). Benzoic acid has been detected in concentrations of 10, 50, 10, and <5 ppb in canned creamed, canned kernel, frozen kernel, and fresh kernel sweet corn, respectively(4). Benzoic acid was detected in fine particle organic matter emitted from Chinese cooking. Four representative restaurants in Guangzhou city, China: Cantonese style (fresh local ingredients, lightly seasoned, stir fry, peanut and blended oil); Hunan style (spicy and sour dishes, steamed, peanut oil); Sichuan style (sharp and spicy, sauteing, blended oil), and Dongbei style (home-cooking, pot roast, simmering, marinating, canola and peanut oil) Average benzoic acid concentrations in fine particulates were 39, 17, 30, and 20 ng/mg of POM (particulate organic matter), respectively(5). [(1) USEPA; Health and Environmental Effects Document for Benzoic Acid. ECAO-CIN-G007 (1987) (2) O'Neil MJ, ed; The Merck Index. 15th ed, Cambridge, UK: Royal Soc Chem, p. 192 (2013) (3) Kim KY; Chung HJ; J Agric Food Chem 48: 1268-72 (2000) (4) Buttery RG et al; J Agric Food Chem 42: 781-5 (1994) (5) Zhao Y et al; Environ Sci Technol 41: 99-105 (2007)] **PEER REVIEWED**

Fish/Seafood Concentrations:

Benzoic acid has been detected but not quantified in mollusks and crustaceans(1). [(1) Livingstone DR; Persistent Pollution of Marine Ecosystems. New York, NY; Pergamon pp. 3-34 (1992)] **PEER REVIEWED**

Other Environmental Concentrations:

Used motor oil contained 45.3 umol/L of benzoic acid, although new motor oil did not contain detectable quantities(1). Benzoic acid was present at concentrations of 370, 200, and 160 ng/mg equivalent organic carbon on respirable airborne particles (PM2.5) collected in Rochester, NY, Quabbin Summit, Massachusetts, and Kenmore Square, Boston, MA, respectively(2). Benzoic acid is released during the burning of firewood. The compound was detected but not quantified in the combustion of red maple, red oak, hemlock and balsam fir; it was found in the combustion of paper birch (0.464 mg/g) and white pine (0.185 mg/g)(3). [(1) Kawamura K et al; Environ Sci Technol 19: 1082-6 (1985) (2) Pedersen DU et al; Environ Sci Technol 39: 9547-9560 (2005) (3) Fine, PM et al; Environ Sci Technol 35: 2665-75 (2001)] **PEER REVIEWED**

As taken from HSDB, 2014.

“Used to make other chemicals, as a food preservative, and for other uses.

Substances capable of inhibiting, retarding or arresting the process of fermentation, acidification or other deterioration of foods.”

As taken from PubChem.

Benzoic acid is produced by many plants as an intermediate in the formation of other compounds (Goodwin, 1976). High concentrations are found in certain berries (see section 6.1). Benzoic acid has also been detected in animals (see section 6.1). Benzoic acid therefore occurs naturally in many foods, including milk products (Sieber et al., 1989, 1990).

Benzoic acid is produced exclusively by the liquid-phase oxidation of toluene (Srour, 1998).

According to Srour (1998), the estimated global production capacity of benzoic acid is 638 000 tonnes per year, although over half of this is converted directly to phenol. The major producers of benzoic acid are the Netherlands (220 000 tonnes per year) and Japan (140 000 tonnes per year), followed by the USA (125 000 tonnes per year). Another reference gives the total European capacity as less than 153 000 tonnes (SRI, 1998).

Benzoic acid is detected in car exhaust gases, presumably as an oxidation product of toluene (Kawamura et al., 1985), and in Japanese cigarettes (12 and 28 µg per cigarette in mainstream and sidestream smoke, respectively; Sakuma et al., 1983). It can also be produced through the photochemical degradation of benzoic acid esters used as fragrance ingredients (Shibamoto & Umamo, 1985; Shibamoto, 1986). Benzoic acid has been detected in wastewater from the wood production industry in Norway and Sweden (Carlberg et al., 1986; Lindström & Österberg, 1986) and in foundry waste leachates (Ham et al., 1989), as well as in extracts of fly ash from municipal incinerators (Tong et al., 1984).

Owing to its occurrence in many organisms, benzoic acid is naturally present in foods (review in Sieber et al., 1989, 1990). Some typical examples specifying reported ranges of means in selected foods have been compiled from Sieber et al. (1989) as follows:

Milk	traces - 6 mg/kg
Yoghurt	12-40 mg/kg
Cheese	traces - 40 mg/kg
Fruits (excluding	traces - 14 mg/kg
Vaccinium species)	
Potatoes, beans, cereals	traces - 0.2 mg/kg
Soya flour, nuts.....	1.2-11 mg/kg

Honeys from different floral sources (n = 7) were found to contain free benzoic acid at concentrations of <10 mg/kg (n = 5) and of <100 mg/kg (n = 2) (Steeg & Montag, 1987).

Because benzoic acid and its compounds are used as food preservatives (see section 4), some processed foods contain artificially elevated concentrations of these substances (see section 6.2).

INCHEM, 2000, CONCISE INTERNATIONAL CHEMICAL ASSESSMENT DOCUMENT NO. 26, BENZOIC ACID AND SODIUM BENZOATE.

Synthesised by oxidation of toluene with nitric acid or sodium bichromate or from benzonitrile.

Reported found in fresh apple, apricot, strawberry, cherry, plum, butter, cooked beef, pork fat, white wine, black and green tea, mushroom, Bourbon vanilla and other natural sources. Reported as being a constituent of various oils, resins and flower absolutes; hyacinth, tuberose, neroli bigarade, Chinese cinnamon, cinnamon leaves, anise, vertiver, ylang-ylang, Tolu balsam and clove; it is contained in fairly sizable amounts in gum benzoin, from which benzoic acid is extracted by sublimation.

As taken from Burdock, 2010.

“Natural sources (water and air): cranberries, prunes, ripe cloves, bark of wild black cherry tree, scent glands of beavers, and oil of anise seeds (Verschueren 1983”

As taken from the Finnish Environment Institute (a).

3. Status in legislation and other official guidance

FIFRA Requirements:

Residues of benzoic acid are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest. Use: preservative for formulations. [40 CFR 180.910 (USEPA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

Residues of benzoic acid are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to animals. Use: preservative for formulations. [40 CFR 180.930 (USEPA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

As the federal pesticide law FIFRA directs, EPA is conducting a comprehensive review of older pesticides to consider their health and environmental effects and make decisions about their continued use. Under this pesticide reregistration program, EPA examines newer health and safety data for pesticide active ingredients initially registered before November 1, 1984, and determines whether the use of the pesticide does not pose unreasonable risk in accordance to newer safety standards, such as those described in the Food Quality Protection Act of 1996. Pesticides for which EPA had not issued Registration Standards prior to the effective date of FIFRA '88 were divided into three lists based upon their potential for human exposure and other factors, with List B containing pesticides of greater concern than those on List C, and with List C containing pesticides of greater concern than those on List D. Benzoic acid is found on List D. Case No: 4013; Pesticide type: Insecticide, fungicide; Case Status: OPP is reviewing data from the pesticide's producers regarding its human health and/or environmental effects, or OPP is determining the pesticide's eligibility for reregistration and developing the Reregistration Eligibility Decision (RED) document.; Active ingredient (AI): Benzoic acid; AI Status: The active ingredient is no longer contained in any registered pesticide products ... "cancelled." [United States Environmental Protection Agency/ Prevention, Pesticides and Toxic Substances; Status of Pesticides in Registration, Reregistration, and Special Review. (1998) EPA 738-R-98-002, p. 297] **PEER REVIEWED**

Acceptable Daily Intakes:

EPA RfD= 4.0 mg/kg [USEPA/OPP; Health Effects Div RfD/ADI Tracking Report p.8 (8/26/91)]
PEER REVIEWED

CERCLA Reportable Quantities:

Persons in charge of vessels or facilities are required to notify the National Response Center (NRC) immediately, when there is a release of this designated hazardous substance, in an amount equal to or greater than its reportable quantity of 5000 lb or 2270 kg. The toll free number of the NRC is (800) 424-8802. The rule for determining when notification is required is stated in 40 CFR 302.4 (section IV. D.3.b). [40 CFR 302.4 (USEPA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

Atmospheric Standards:

This action promulgates standards of performance for equipment leaks of Volatile Organic Compounds (VOC) in the Synthetic Organic Chemical Manufacturing Industry (SOCMI). The intended effect of these standards is to require all newly constructed, modified, and reconstructed SOCMI process units to use the best demonstrated system of continuous emission reduction for

equipment leaks of VOC, considering costs, non air quality health and environmental impact and energy requirements. Benzoic acid is produced, as an intermediate or a final product, by process units covered under this subpart. [40 CFR 60.489 (USEPA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

Clean Water Act Requirements:

Benzoic acid is designated as a hazardous substance under section 311(b)(2)(A) of the Federal Water Pollution Control Act and further regulated by the Clean Water Act Amendments of 1977 and 1978. These regulations apply to discharges of this substance. This designation includes any isomers and hydrates, as well as any solutions and mixtures containing this substance. [40 CFR 116.4 (USEPA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

State Drinking Water Guidelines:

(FL) Florida 28,000 ug/L [USEPA/Office of Water; Federal-State Toxicology and Risk Analysis Committee (FSTRAC). Summary of State and Federal Drinking Water Standards and Guidelines (11/93) To Present] **PEER REVIEWED**

(ME) Maine 28,000 ug/L [USEPA/Office of Water; Federal-State Toxicology and Risk Analysis Committee (FSTRAC). Summary of State and Federal Drinking Water Standards and Guidelines (11/93) To Present] **PEER REVIEWED**

(MN) Minnesota 30000 ug/L [USEPA/Office of Water; Federal-State Toxicology and Risk Analysis Committee (FSTRAC). Summary of State and Federal Drinking Water Standards and Guidelines (11/93) To Present] **PEER REVIEWED**

(NH) New Hampshire 28,000 ug/L [USEPA/Office of Water; Federal-State Toxicology and Risk Analysis Committee (FSTRAC). Summary of State and Federal Drinking Water Standards and Guidelines (11/93) To Present] **PEER REVIEWED**

FDA Requirements:

Substance added directly to human food affirmed as generally recognized as safe (GRAS). Current usage results in a max level of 0.1% in food. [21 CFR 184.1021 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

This substance is generally recognized as safe for use at a level not exceeding 0.1 percent in accordance with good manufacturing or feeding practice. [21 CFR 582.3021 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. A number of active ingredients have been present in OTC drug products for various uses, as described below. However, based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses: Benzoic acid is included in topical acne drug products. [21 CFR 310.545(a)(1) (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. A number of active ingredients have been present in OTC drug products for various uses, as described below. However, based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses: Benzoic acid is included in skin protectant drug products. [21 CFR 310.545(a)(18) (USFDA);

U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. A number of active ingredients have been present in OTC drug products for various uses, as described below. However, based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses: Benzoic acid is included in topical antifungal drug products. [21 CFR 310.545(a)(22) (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr> **PEER REVIEWED**

As taken from HSDB, 2014.

Benzoic acid is listed on the US EPA InertFinder Database (2024) as approved for food, non-food and fragrance use pesticide products.

OCCUPATIONAL EXPOSURE LIMITS:

OEL-RUSSIA: STEL 5 mg/m³, JUN2003

As taken from RTECS, 2019.

JECFA has established a Group ADI of 0-5 mg/kg bw/day (expressed as benzoic acid) that is applicable for benzoic acid and its salts, benzyl acetate, benzyl alcohol and benzaldehyde (JECFA 1996, 2005). The European Commission's Scientific Committee on Food has established the same value (SCF 2002). At its 57th meeting, JECFA concluded that the use of benzoic acid to flavour food was of "no safety concern", based on current intakes from such use, which were estimated to be 39 and 340 ug/person per day in Europe and the USA, respectively (JECFA, 2005).

"Benzoic acid and its salts were evaluated by SCF in 1994 and 2002, and in 2002 SCF established an ADI of 5 mg/kg bw for benzoic acid and its salts, including benzyl alcohol and related benzyl derivatives used as flavourings (SCF, 2002)

As taken from VKM, 2013.

"....consumer exposure to benzoic acid residues in food or feed items via biocidal products will be much lower compared to consumer exposure to benzoic acid occurring as natural food ingredient and from use as registered food additive and authorised feeding stuff additive. Therefore, the derivation of an Acceptable Daily Intake (ADI) is considered not necessary in the context of biocidal use. For the use as a Plant Protection Product an ADI of 5 mg/kg bw was derived by JECFA (1983) and EU (2002, 2005), based on the NOAEL of 500 mg/kg bw/d from subacute/subchronic and teratogenicity studies and an assessment factor of 100.

According to an EU guidance document (Doc. 7199/VI/99; EU, 2001) and a publication, an ARfD is not allocated because of low acute oral toxicity of benzoic acid and the cut off value at 5 mg/kg bw."

"The overall conclusion from the evaluation of benzoic acid for use in Product Type 3 (veterinary hygiene biocidal products), is that it may be possible to issue authorisations of products containing benzoic acid in accordance with the conditions laid down in Article 5(1) b), c) and d) of Dir. 98/8/EC. It is therefore proposed to approve benzoic acid as an active substance for use in product-type 3 (veterinary hygiene), subject to" specific conditions.

"The overall conclusion from the evaluation of benzoic acid for use in Product Type 4 (food and feed area disinfectants), is that it may be possible to issue authorisations of products containing benzoic acid in accordance with the conditions laid down in Article 5(1) b), c) and d) of Dir. 98/8/EC. It is therefore proposed to approve benzoic acid as an active substance for use in product-type 4 (food and feed area), subject to" specific conditions.

“It is considered that the evaluation has shown that sufficient data have been provided to verify the outcome and conclusions, and permit the proposal for the approval of Benzoic acid in accordance with Article 9 of Regulation (EU) No 528/2012.”

As taken from European Commission, 2013a, b.

“Oral RfD Summary:

Critical Effect	Experimental Doses*	UF	MF	RfD
No adverse effects observed Human daily per capita intakes FDA, 1973; Selected Committee on Review of the GRAS List	NOAEL: 34 mg/day benzoic acid and 328 mg/day for sodium benzoate (converted to 312 mg/day benzoic acid) LOAEL: none	1	1	4E+0 mg/kg/day

*Conversion Factors -- $328 \text{ mg/day sodium benzoate} \times [122.12 \text{ (MW benzoic acid)} / 144.11 \text{ (MW sodium benzoate)}] = 278 \text{ mg/day benzoic acid}$. $278 \text{ mg/day benzoic acid from sodium benzoate} + 34 \text{ mg/day benzoic acid} = 312 \text{ mg/day}$; assuming adult human body weight of 70 kg, the exposure dose is 312 divided by 70 = 4.4 mg/kg/day.

Principal and Supporting Studies (Oral RfD):

FDA (Food and Drug Administration). 1973. Evaluation of the Health Aspects of Benzoic Acid and Sodium Benzoate as Food Ingredients. DHEW, Washington, DC. Report No. SCOGS-7. NTIS PB-223837/6.

Early studies (Gerlach, 1909) indicate that laboratory animals are inappropriate models for studying the toxicity of benzoic acid in humans (FDRL, 1972) (see Additional Comments). Based on data regarding the amounts of benzoic acid and sodium benzoate produced as a food preservative, FDA (1973) estimated a daily per capita intake of 0.9-34 mg for benzoic acid and 34-328 mg for sodium benzoate. At these levels, there are no reports of toxic effects in humans. These compounds have Generally Recognized as Safe (GRAS) status by FDA. Therefore, the upper ranges can be considered NOAELs for benzoic acid and sodium benzoate. In the stomach, both benzoic acid and sodium benzoate exist in their ionized form, benzoate, which is absorbed rapidly and completely by the GI tract. Therefore, exposure to sodium benzoate is comparable to exposure to benzoic acid if molecular weight differences are corrected for; here, 328 mg sodium benzoate is equivalent to 278 mg benzoic acid. Adding 278 to the daily intake for benzoic acid of 34 mg yields a total of 312 mg benzoic acid (see Conversion Factors). If no uncertainty factor is used, the RfD is 312 mg/day for a 70 kg human or 4 mg/kg/day.

Uncertainty and Modifying Factors (Oral RfD):

UF — An uncertainty factor of 10 for the protection of sensitive subgroups was considered unnecessary; although reactions to benzoate and structurally related compounds do occur, an uncertainty factor of 10 would be of little value to the sensitive individuals.

MF — None

Additional Studies/Comments (Oral RfD):

Confidence in the Oral RfD:

Study — Medium

Database — Medium

RfD — Medium

Medium confidence is placed in the FDA (1973) estimate of per capita intake. Medium confidence in the database reflects the inappropriateness of using animal data as the basis of the RfD for humans and the lack of reported effects in humans at the estimated intakes. Thus, confidence in the RfD is medium.”

As taken from IRIS (1988)

“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).

A provisional subchronic RfC of $2\text{E-}3 \text{ mg/m}^3$ for benzoic acid was derived as follows:

subchronic p-RfC = $\text{LOAELHEC} \div (\text{UF})$

= $1.9 \text{ mg/m}^3 \div (1000)$

= 0.002 or $2\text{E-}3 \text{ mg/m}^3$

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.”

As taken from US EPA, 2005.

Benzoic acid and sodium benzoate are used as food preservatives and are most suitable for foods, fruit juices, and soft drinks that are naturally in an acidic pH range. Their use as preservatives in food, beverages, toothpastes, mouthwashes, dentifrices, cosmetics, and pharmaceuticals is regulated. The estimated global production capacity for benzoic acid is about 600 000 tonnes per year. Worldwide sodium benzoate production in 1997 can be estimated at about 55 000-60 000 tonnes. Benzoic acid occurs naturally in many plants and in animals. It is therefore a natural constituent of many foods, including milk products. Anthropogenic releases of benzoic acid and sodium benzoate into the environment are primarily emissions into water and soil from their uses as preservatives. Concentrations of naturally occurring benzoic acid in several foods did not exceed average values of 40 mg/kg of food. Maximum concentrations reported for benzoic acid or sodium benzoate added to food for preservation purposes were in the range of 2000 mg/kg of food.

INCHEM, 2000, CONCISE INTERNATIONAL CHEMICAL ASSESSMENT DOCUMENT NO. 26, BENZOIC ACID AND SODIUM BENZOATE

Substance (No.)	Most recent annual volume (kg)	Intake ^a		Annual volume in naturally occurring foods (kg) ^b	Consumption ratio ^c
		µg/day	µg/kg bw per day		
Benzoic acid (850)					
Europe	280	39	0.6	+	
USA	2 600	340	5.7		NA

As taken from JECFA, 2002

There is a REACH dossier on benzoic acid (ECHA).

Benzoic acid (CAS RN65-85-0) is classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023a).

Benzoic acid is listed on the US EPA Toxic Substances Control Act (TSCA) inventory and also on the US EPA 2024 CDR list (Chemical Data Reporting Rule). The Chemical Data Reporting (CDR) Rule requires companies that manufacture (including import) certain chemicals at certain volumes in the U.S. to report to EPA every four years through its CDR.

US EPA Substance Registry Services (SRS)

Currently, the use of a mixture of sorbates (E 200, E 202 and E 203) and benzoates (E 210 to E 213) is authorised in cooked shrimps for preservation. It is appropriate to extend that authorisation to its use in all cooked crustaceans and molluscs.

DIRECTIVE 2006/52/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 5 July 2006.

“Benzoic acid is a naturally occurring organic acid, which is found in many different fruits, often at levels far higher than would be allowed as a food additive! (the Scandinavian cloudberry actually contains 50 times the legal limit!) It is used to prevent the growth of yeasts, moulds and some bacteria in acidic foods such as fruit juices, carbonated drinks and pickles, and is used either as the free acid (E210), or as its sodium (E211), potassium (E212) or calcium (E213) salt. It has been suggested that a benzoate-free diet may help selected patients with persistent asthma, but this approach has not been evaluated in published controlled trials.”

As taken from Food Additives and Ingredients Association.

Restrictions: Maximum authorized concentration 0.5 % (calculated as benzoic acid) in the finished product (VI/1,1).

SCCNFP (2000).

Benzoic acid is included on the FDA's inventory of “Substances Added to Food (formerly EAFUS)” as an antimicrobial agent, flavouring agent or adjuvant and pH control agent, is affirmed as generally recognized as safe under 21 CFR 184.1021 and is also included under 21 CFR sections 166.110 (Margarine), 175.300 (Resinous and polymeric coatings) and 177.1390 (Laminate structures for use at temperatures of 250 deg. F and above) (FDA, 2024a,b).

ADI 0-5 mg/kg bw (1996)

As taken from WHO, 2021.

“According to FDA's Select Committee on Generally Recognized as Safe (GRAS) substances, benzoic acid (21 CFR [Code of Federal Regulations] 184.1021)28 and sodium benzoate (21 CFR

184.1733) are classified as GRAS food substances. Sodium benzoate has also been approved by FDA for use as an antimycotic when migrating from foodpackaging material (21 CFR 181.23). Other CFR citations relating to FDA-approved direct/indirect food additive ingredient uses include: benzyl alcohol (21 CFR 172.515, 175.105, 175.300, and 177.1210); benzoic acid (21 CFR 150.141, 150.161, 166.110, 175.300, and 177.1390)35,36,37,33,38 ; sodium benzoate (21 CFR 150.141, 150.161, and 166.110); calcium benzoate (21 CFR 166.110 and 178.2010); potassium benzoate (21 CFR 166.110 and 177.1210); and benzyl benzoate (21 CFR 172.515 and 175.105). Benzyl alcohol is an FDA-approved diluent in color additive mixtures for external drug use. In 2009, the FDA approved benzyl alcohol lotion, 5%, as a prescription medication for the treatment of head lice (*Pediculosis capitis*) infestation in patients 6 months of age and older. The results of clinical trials supporting benzyl alcohol lotion 5% (Ulesfia™) as a safe and effective topical treatment for head lice are included in the section on Skin Irritation and Sensitization later in the report text. Benzyl benzoate (10%) has been used in the treatment of scabies in developing countries...

Benzoic acid has been classified by FDA regarding its use in the following types of OTC drug products:

- acne (final monograph: Category III)
- antifungal (final monograph: Category III)
- oral health care - (Category IIIE pending)
- skin protectant - (final monograph: Category III)

Sodium benzoate has been classified by FDA regarding its use in menstrual/diuretic OTC drug products (final monograph: Category III), and benzyl benzoate has been approved for use in OTC pediculicides (final monograph: Category II).

Calcium benzoate, magnesium benzoate, and potassium benzoate are inert non-food ingredients that, under the Federal Insecticide, Fungicide, and Rodenticide Act, are permitted by the Environmental Protection Agency (EPA) for use in non-food use pesticide products. Pesticide products containing benzoic acid as the active ingredient have been registered with EPA for use in the extermination of dust mites”

As taken from CIR, 2017.

Benzoic acid is listed as authorised for use as a flavouring substance in all categories of flavoured foods in the EU under Regulation no. 872/2012 (European Commission, food flavourings database).

Benzoic acid is included on the US EPA's list of Safer Chemical Ingredients with functional use: preservatives and antioxidants (US EPA, 2024).

The European Scientific Committee on Consumer Products has concluded that benzoic acid and sodium benzoate are safe for use for preservative and non-preservative purposes in cosmetic rinse-off products at a maximum concentration of 2.5% and in cosmetic oral-care products at a maximum concentration of 1.7%, and in leave-on products up to 0.5% (SCCP, 2005).

Maximum levels of benzoic acid-benzoates (E 210–213) have been defined in Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives, as amended. These levels are defined by the Panel as maximum permitted levels (MPLs) in this document.

Currently, benzoic acid-benzoates (E 210–213) are authorised as food additives in the EU with MPLs ranging from 150 to 6,000 mg/kg in foods. The Panel noted that benzoic acid-benzoates (E 210–213) are authorised in combination with sorbic acid-sorbates (E 200, 202, 203) and p-hydroxybenzoates (PHB; E 214, 215, 218, 219) (Regulation (EC) No 1333/2008).

As taken from EFSA, 2016a.

“Benzyl alcohol, benzoic acid and its salts, and benzyl benzoate function mostly as fragrance ingredients/preservatives in cosmetic products. The Cosmetic Ingredient Review Expert Panel previously established concentration limits for benzyl alcohol, benzoic acid, and sodium benzoate in cosmetics and determined that the available data were insufficient to support the safety of these ingredients during inhalation exposure. After reviewing newly available data, it was concluded that benzyl alcohol, benzoic acid and its salts, and benzyl benzoate are safe in the present practices of use and concentration described in this safety assessment.” As taken from Johnson W et al. 2017. *Int. J. Toxicol.* 36(3_suppl.), 5S-30S. PubMed, 2018.

Benzoic acid is listed by the US EPA Office of Pesticide Programs and was first registered as an “antimicrobial” and “conventional chemical” pesticide on 1 July 1987. It is noted as being under registration review (case #5107) and reregistration under the benzyl benzoate group (case #4013).

“The current acceptable daily intake (ADI) for benzoic acid and its salts as food additives is 0-5 mg/kg body weight. This accounts for a total uncertainty factor (UF) of 100, which includes a default factor of 10 for interspecies differences. Based on pharmacokinetic data in rodents and humans, we derived a chemical-specific adjustment factor (CSAF) of 2 for the pharmacokinetic component of the interspecies UF. Additional analyses indicate that this CSAF is conservative and interspecies differences between rats and humans are likely closer to unity. Human clinical studies indicate that the pharmacokinetics of benzoic acid and its salts are similar in children and adults, and that there is a lack of adverse events in humans at doses comparable to the no observed adverse effect level (NOAEL) in rodents; this suggests that the pharmacokinetic UF for intraspecies variability, as well as the pharmacodynamic components of the UFs, may also be reduced, although we did not calculate to what degree. In conclusion, the total UF can be reduced to 50 (2 for interspecies differences in pharmacokinetics, 2.5 for interspecies differences in pharmacodynamics, and 10 for intraspecies variability), which would increase the ADI to 0-10 mg/kg body weight.” As taken from Zu K et al. 2017. *Regul. Toxicol. Pharmacol.* 89, 83-94. PubMed, 2018.

Benzoic acid is authorised to be used as monomer and additive to manufacture plastics with no particular restriction on its migration.”

As taken from EFSA, 2018.

Benzoic acid (E210) is authorised for use as a food additive in the EU under Regulations 1129/2011, 0570/2012, 2015/0538, 2015/0647, 2018/1497 and 2018/0098 (European Commission, food Additives database).

Benzoic acid (FEMA No. 2131) has been designated as GRAS (generally recognized as safe) for use in food by FEMA (Hall RL and Oser BL, 1965).

“The CIR considers the chemical (benzoic acid and its salts) to be safe for use at a maximum allowed concentrations of 5 % (as acid).

The chemical is controlled in the New Zealand Cosmetic Products Group Standard - Schedule 7 - list of preservatives allowed in cosmetics. It states that benzoic acid and sodium benzoate are safe for use for preservative and non-preservative purposes in cosmetic rinse-off products at a maximum concentration of 2.5 % and in cosmetic oral-care products at a maximum concentration of 1.7 %, and in leave-on products up to 0.5 %. For other salts of benzoic acid other than listed above, maximum authorised concentrations is 0.5 %.

Currently, there are no restrictions on the use of this chemical in Australia.”

Benzoic acid was assigned a Tier II assessment “This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.”

As taken from AICIS.

Benzoic acid is included on the US FDA's list of inactive ingredients for approved drug products. It is permitted for use as an ingredient in various products, at the following maximum potencies per unit dose and maximum daily exposures:

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose	Maximum Daily Exposure (MDE)
BENZOIC ACID	BUCCAL	MOUTHWASH	65850	8SKN0B0MIM	NA	
BENZOIC ACID	INTRAMUSCULAR	INJECTION	65850	8SKN0B0MIM	5 %w/v	
BENZOIC ACID	INTRAMUSCULAR	INJECTION, SOLUTION	65850	8SKN0B0MIM	0.2 %w/v	
BENZOIC ACID	INTRAVENOUS	INJECTION	65850	8SKN0B0MIM	5 %w/v	
BENZOIC ACID	IRRIGATION	SOLUTION	65850	8SKN0B0MIM	0.02 %w/v	
BENZOIC ACID	ORAL	CONCENTRATE	65850	8SKN0B0MIM	12.5 mg/5ml	
BENZOIC ACID	ORAL	ELIXIR	65850	8SKN0B0MIM		90 mg
BENZOIC ACID	ORAL	LIQUID	65850	8SKN0B0MIM	2 mg/1ml	
BENZOIC ACID	ORAL	SOLUTION	65850	8SKN0B0MIM		100 mg
BENZOIC ACID	ORAL	SUSPENSION	65850	8SKN0B0MIM	15 mg	
BENZOIC ACID	ORAL	SYRUP	65850	8SKN0B0MIM		20 mg
BENZOIC ACID	ORAL	TABLET, COATED	65850	8SKN0B0MIM	NA	
BENZOIC ACID	ORAL	TABLET, FILM COATED	65850	8SKN0B0MIM	NA	
BENZOIC ACID	RECTAL	GEL	65850	8SKN0B0MIM		11 mg
BENZOIC ACID	RECTAL	SUSPENSION	65850	8SKN0B0MIM	0.1 %w/w	

BENZOIC ACID	TOPICAL	AEROSOL, FOAM	65850	8SKN0B0MIM		1 mg
BENZOIC ACID	TOPICAL	CREAM	65850	8SKN0B0MIM		2 mg
BENZOIC ACID	TOPICAL	GEL	65850	8SKN0B0MIM	0.1 %w/w	
BENZOIC ACID	TOPICAL	LOTION	65850	8SKN0B0MIM	0.2 %w/w	
BENZOIC ACID	TOPICAL	SOLUTION	65850	8SKN0B0MIM	0.1%w/w	
BENZOIC ACID	TOPICAL	SOLUTION	65850	8SKN0B0MIM	0.1 %w/w	
BENZOIC ACID	VAGINAL	AEROSOL, FOAM	65850	8SKN0B0MIM	0.1 %w/w	
BENZOIC ACID	VAGINAL	CREAM	65850	8SKN0B0MIM	0.25 %w/w	
BENZOIC ACID	VAGINAL	SUPPOSITORY	65850	8SKN0B0MIM	2 mg	

As taken from FDA, 2024c.

“Based on the NOAEC for lung toxicity of 12.6 mg/m³ in a 4-week inhalation study in rats, a MAK value of 0.5 mg/m³ for the strongly irritating benzoic acid has been derived.”

“Benzoic acid and alkali benzoates are assigned to Peak Limitation Category II, because systemic effects are critical, and excursion factors of 2 for alkali benzoates and of 4 for benzoic acid are set.”

“MAK Value: For the strongly irritating benzoic acid, the MAK value is derived on the basis of its local effects. In the 4-week inhalation study with rats (Velsicol Chemical Company 1981), benzoic acid caused local pulmonary toxicity in the form of interstitial inflammation and fibrosis at concentrations of 25 mg/m³ and above. From another 4-week inhalation study with benzoic acid in rats, a NOAEC of 12.6 mg/m³ for local pulmonary toxicity was calculated (The Personal Care Products Council 2010). Assuming an increase in effects over time from the short-term study (1:6), the increased respiratory volume at the workplace (1:2) and the extrapolation of the data from experimental studies with animals to humans (1:2), the MAK value is derived from this NOAEC of 12.6 mg/m³. As the vapour saturation concentration of benzoic acid is about 4.4 mg/m³, at this MAK value the substance can be present in vapour form, and the MAK value is given in ml/m³. For benzoic acid, therefore, a MAK value of 0.1 ml/m³ has been established. This value corresponds to 0.5 mg/m³ for the R (respirable) fraction, as the lungs are the target organ.

Peak limitation: As the interstitial inflammation in the lungs with fibrosis is the critical effect for determining the threshold limit value and this effect does not occur immediately, but requires a longer exposure period before it manifests itself, benzoic acid is classified in Peak Limitation Category II. As in the 4-week inhalation study with rats no irritation occurred at a concentration of 12.6 mg benzoic acid/m³, an excursion factor of 4 can be set.”

As taken from Hartwig A and MAK Commission, 2018

Benzoic acid (CAS RN 65-85-0) is classified as a natural health product (NHP) under Schedule 1, item 2 (an isolate) of the Natural Health Products Regulations (Health Canada, 2021).

Substance	Benzoic acid and its sodium salt
CAS #	65-85-0 / 532-32-1

EC #	200-618-2 / 208-534-8
Name of Common Ingredients Glossary	BENZOIC ACID; SODIUM BENZOATE
INN/ISO/AN	
Regulation	(EC) 2009/1223
Regulated By	2007/17/EC
Other Directives/Regulations	
Annex/Ref #	V/1
Product Type, body parts	a) Rinse-off products, except oral care products b) Oral care products c) Leave-on products
Maximum concentration in ready for use preparation	a) 2.5% (acid) b) 1.7% (acid) c) 0.5% (acid)
Other	
Wording of conditions of use and warnings	
SCCS opinions	0532/01 - Opinion on Benzoic Acid and Sodium Benzoate 0891/05 - Opinion on Benzoic Acid and Sodium Benzoate 0125/99 - Opinion concerning Restrictions on Materials listed in annex VI of Directive 76/768/EEC on Cosmetic Products
Chemical/IUPAC Name	
Identified INGREDIENTS or substances e.g.	BENZOIC ACID SODIUM BENZOATE
Note	
Current Version	v.1

Benzoic acid is used as a pH adjuster, fragrance and preservative ingredient in cosmetics in the EU.

As taken from CosIng (Cosmetic substances and ingredients database).

Benzoic acid (CAS RN 65-85-0) is on the list of Biocidal Active Substances of ECHA approved to be used in products type: PT03 - Veterinary hygiene and PT04 - Food and feed area (Regulation (EU) No 1035/2013) There is an Initial application for approval in progress regarding it's use in products type: PT09 - Fibre, leather, rubber and polymerised materials preservatives. There is a cancelled application for approval in progress regarding it's use in products type: PT07 - Film preservatives.

ECHA (2024b)

Benzoic acid is included on the FDA's inventory of Inactive Ingredient Database for Food Contact Substances.

FDA (2024d)

BENZOIC ACID	
INS:	210
COE No.:	21
FEMA No.:	2131
JECFA No.:	850
Chemical names:	BENZOIC ACID
Synonyms:	BENZENECARBOXYLIC ACID; CARBOXYBENZENE; PHENYL FORMIC ACID; DRACYLIC ACID
Functional class:	ANTIMICROBIAL PRESERVATIVE; FLAVOURING AGENT
Latest evaluation:	2002
ADI:	0-5 mg/kg bw (1996)
Comments:	No safety concern at current levels of intake when used as a flavouring agent (2002)
Report:	TRS 913-JECFA 59/112
Specifications:	COMPENDIUM ADDENDUM 12/FNP 52 Add. 12/67 (METALS LIMITS) (2004)
Tox monograph:	FAS 48-JECFA 57/227 (2001)
Previous status:	2001, TRS 909-JECFA 57/73, COMPENDIUM ADDENDUM 9/FNP 52 Add.9/140, FAS 48-JECFA 57/227. (EVALUATION AS A FLAVOURING AGENT NOT FINALIZED AT THE FIFTY-SEVENTH MEETING (2001); FURTHER INFORMATION REQUIRED TO DETERMINE WHETHER THIS SUBSTANCE IS IN CURRENT USE AS A FLAVOURING AGENT. THE 1996 GROUP ADI OF 0-5 mg/kg bw FOR BENZOIC ACID, THE BENZOATE SALTS (CALCIUM, POTASSIUM AND SODIUM), BENZALDEHYDE, BENZYL ACETATE, BENZYL ALCOHOL AND BENZYL BENZOATE, EXPRESSED AS BENZOIC ACID EQUIVALENTS, WAS MAINTAINED.) 1996, TRS 868-JECFA 46/41, COMPENDIUM ADDENDUM 4/FNP 52 Add.4/19, FAS 37-JECFA 46/29. 0-5 mg/kg bw (GROUP ADI FOR BENZOIC ACID, THE BENZOATE SALTS (CALCIUM, POTASSIUM AND SODIUM), BENZALDEHYDE, BENZYL ACETATE, BENZYL ALCOHOL AND BENZYL BENZOATE, EXPRESSED AS BENZOIC ACID). FU. R 1983, TRS 696-JECFA 27/20, FNP 4-JECFA 17/109 (1973) (COMPENDIUM/183), FAS 18-JECFA 27/29. 0-5 (GROUP ADI FOR BENZOIC ACID AND ITS CALCIUM, POTASSIUM AND SODIUM SALTS, EXPRESSED AS BENZOIC ACID). FU. R(1973) 1973, NMRS 53/TRS 539-JECFA 17/16, FAS 5/NMRS 53A-JECFA 17/34, FNP 4-JECFA 17/109. 0-5 (AS SUM OF BENZOIC ACID AND SODIUM AND POTASSIUM BENZOATE, EXPRESSED AS BENZOIC ACID). FU. R 1970, NMRS 48/TRS 462-JECFA 14/24, FAS 70.41/NMRS 48C-JECFA 14/8 1965, NMRS 40/TRS 339-JECFA 9/19, FAS 67.29/NMRS 40A,B,C-JECFA 9/14, FAS 67.29/NMRS 40A,B,C-JECFA 9/15. 0-5 (CONDITIONAL, 5-10). CO. S 1961, NMRS 31/TRS 228-JECFA 6/27, NMRS 31/TRS 228-JECFA 6/27. 0-5 (CONDITIONAL, 5-10). CO. S 1958, NMRS VOL I-III/15. N

JECFA (2005)

“The Committee evaluated a new extended one-generation reproductive toxicity study on benzoic acid. This study showed no treatment-related adverse effects, indicating a NOAEL of 1000 mg/kg bw per day, the highest dose tested. Applying a chemical specific adjustment factor of 2 for interspecies toxicokinetics variation instead of the default factor of 4.0, the Committee established a group ADI of 0–20 mg/kg bw, which applies to benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate, expressed as benzoic acid equivalents. The Committee withdrew the previous group ADI of 0–5 mg/kg bw. The Committee noted that the high dietary exposure estimate, expressed as benzoic acid, of 7.1 mg/kg bw per day for children aged 3–9 years does not exceed the group ADI of 0–20 mg/kg bw.”

JECFA (2021)

Excipients and information for the package leaflet:

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Benzoic acid (E 210) and benzoates e.g.: Sodium benzoate (E 211) Potassium benzoate (E 212)	09/10/2017	All	Zero	This medicine contains x mg <benzoic acid/benzoate salt> in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.	
Benzoic acid (E 210) and benzoates e.g.: Sodium benzoate (E 211) Potassium benzoate (E 212)	09/10/2017	Oral, parenteral	Zero	<Benzoic acid/Benzoate salt> may increase jaundice (yellowing of the skin and eyes) in newborn babies (up to 4 weeks old).	Increase in bilirubinaemia following its displacement from albumin may increase neonatal jaundice which may develop into kernicterus (non-conjugated bilirubin deposits in the brain tissue).

Benzoic acid (E 210) and benzoates e.g.: Sodium benzoate (E 211) Potassium benzoate (E 212)	09/10/2017	Topical	Zero	<Benzoic acid/Benzoate salt> may cause local irritation.	May cause non-immunologic immediate contact reactions by a possible cholinergic mechanism.
Benzoic acid (E 210) and benzoates e.g.: Sodium benzoate (E 211) Potassium benzoate (E 212)	09/10/2017	Topical	Zero	<Benzoic acid/Benzoate salt> may increase jaundice (yellowing of the skin and eyes) in newborn babies (up to 4 weeks old).	Absorption through the immature skin of neonates is significant.

EMA

GSFA [General Standard for Food Additives] Provisions for benzoates (including benzoic acid):

Number	Food Category	Max Level	Notes
14.2.7	Aromatized alcoholic beverages (e.g. beer, wine and spirituous cooler-type beverages, low alcoholic refreshers)	1,000 mg/kg	As benzoic acid.
07.0	Bakery wares	1,000 mg/kg	As benzoic acid
04.1.2.7	Candied fruit	1,000 mg/kg	As benzoic acid
06.5	Cereal and starch-based desserts (e.g. rice pudding, tapioca pudding)	1,000 mg/kg	As benzoic acid
05.3	Chewing gum	1,500 mg/kg	As benzoic acid
14.2.2	Cider and perry	1,000 mg/kg	As benzoic acid For use in products containing less than 7% ethanol only.
05.1.3	Cocoa-based spreads, including fillings	1,500 mg/kg,	As benzoic acid Excluding products conforming to the Standard for Cocoa Butter (CODEX STAN 86-1981).
14.1.5	Coffee, coffee substitutes, tea, herbal infusions, and other hot cereal and grain beverages, excluding cocoa	1,000 mg/kg	As benzoic acid
14.1.2.3	Concentrates for fruit juice	1,000 mg/kg	As benzoic acid Singly or in combination: Benzoates and sorbates. Subject to national legislation of the importing country. On the served to the consumer basis.

14.1.3.3	Concentrates for fruit nectar	1,000 mg/kg	As benzoic acid Singly or in combination: Benzoates and sorbates. Subject to national legislation of the importing country. On the served to the consumer basi
14.1.3.4	Concentrates for vegetable nectar	600 mg/kg	As benzoic acid
05.2	Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	1,500 mg/kg	As benzoic acid Excluding products conforming to the Codex Regional Standard for Halawa Tehenia (CODEX STAN 309R-211).
04.1.2.12	Cooked fruit	1,000 mg/kg	As benzoic acid
09.2.4.2	Cooked mollusks, crustaceans, and echinoderms	2,000 mg/kg	As benzoic acid Except for use in shrimp (Crangon crangon and Crangon vulgaris) at 6 000 mg/kg.
04.2.2.8	Cooked or fried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds	1,000 mg/kg	As benzoic acid
08.3.1.2	Cured (including salted) and dried non-heat treated processed comminuted meat, poultry, and game products	1,000 mg/kg	As benzoic acid For use in surface treatment only.
08.2.1.2	Cured (including salted) and dried non-heat treated processed meat, poultry, and game products in whole pieces or cuts	1,000 mg/kg	As benzoic acid For use in surface treatment only.
01.7	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	300 mg/kg	As benzoic acid
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	1,500 mg/kg	As benzoic acid
13.5	Dietetic foods (e.g. supplementary foods for dietary use) excluding products of food categories 13.1 - 13.4 and 13.6	2,000 mg/kg	As benzoic acid
13.3	Dietetic foods intended for special medical purposes (excluding products of food category 13.1)	1,500 mg/kg	As benzoic acid Excluding products conforming to the Guidelines for Ready to Use Therapeutic Foods (CXG 95-2022).
13.4	Dietetic formulae for slimming purposes and weight reduction	1,500 mg/kg	As benzoic acid
04.1.2.2	Dried fruit	800 mg/kg	As benzoic acid
04.2.2.2	Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds	1,000 mg/kg	As benzoic acid
10.4	Egg-based desserts (e.g. custard)	1,000 mg/kg	As benzoic acid
02.3	Fat emulsions mainly of type oil-in-water, including mixed and/or	1,000 mg/kg	As benzoic acid

	flavoured products based on fat emulsions		
02.2.2	Fat spreads, dairy fat spreads and blended spreads	1,000 mg/kg	As benzoic acid Excluding products conforming to the Standard for Dairy Fat Spreads (CODEX STAN 253-2006). For use in products conforming to the Standard for Fat Spreads and Blended Spreads (CXS 256-2007); if benzoates and sorbates are used in combination, the combined use shall not exceed 2000 mg/kg of which the benzoic acid portion shall not exceed 1000 mg/kg.
02.4	Fat-based desserts excluding dairy-based dessert products of food category 01.7	1,000 mg/kg	As benzoic acid
04.1.2.10	Fermented fruit products	1,000 mg/kg	As benzoic acid
04.2.2.7	Fermented vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweed products, excluding fermented soybean products of food categories 06.8.6, 06.8.7, 12.9.1, 12.9.2.1 and 12.9.2.3	1,000 mg/kg	As benzoic acid Excluding products conforming to the Standard for Gochujang (CXS 294-2009).
13.6	Food supplements	2,000 mg/kg	As benzoic acid
04.1.2.11	Fruit fillings for pastries	1,000 mg/kg	As benzoic acid
04.1.2.3	Fruit in vinegar, oil, or brine	1,000 mg/kg	As benzoic acid
14.1.2.1	Fruit juice	1,000 mg/kg	As benzoic acid Singly or in combination: Benzoates and sorbates. Subject to national legislation of the importing country.
14.1.3.1	Fruit nectar	1,000 mg/kg	As benzoic acid Singly or in combination: Benzoates and sorbates. Subject to national legislation of the importing country.
04.1.2.8	Fruit preparations, including pulp, purees, fruit toppings and coconut milk	1,000 mg/kg	As benzoic acid
04.1.2.9	Fruit-based desserts, including fruit-flavoured water-based desserts	1,000 mg/kg	As benzoic acid
04.1.2.6	Fruit-based spreads (e.g. chutney) excluding products of food category 04.1.2.5	1,000 mg/kg	As benzoic acid Except for use in products conforming to the Standard for Mango Chutney (CXS 160-1987): Sodium benzoate (INS 211) and Potassium benzoate (INS 212) only at 250 mg/kg, singly or in combination.
05.1.5	Imitation chocolate, chocolate substitute products	1,500 mg/kg	As benzoic acid
04.1.2.5	Jams, jellies, marmelades	1,000 mg/kg	As benzoic acid
10.2.1	Liquid egg products	5,000 mg/kg	As benzoic acid

14.2.5	Mead	1,000 mg/kg	As benzoic acid
12.4	Mustards	1,000 mg/kg	As benzoic acid
11.4	Other sugars and syrups (e.g. xylose, maple syrup, sugar toppings)	1,000 mg/kg	As benzoic acid
06.4.3	Pre-cooked pastas and noodles and like products	1,000 mg/kg	As benzoic acid Excluding products conforming to the Standard for Instant Noodles (CXS 249-2006).
12.7	Salads (e.g. macaroni salad, potato salad) and sandwich spreads excluding cocoa- and nut-based spreads of food categories 04.2.2.5 and 05.1.3	1,500 mg/kg	As benzoic acid
12.6	Sauces and like products	1,000 mg/kg	As benzoic acid
12.2.2	Seasonings and condiments	1,000 mg/kg	As benzoic acid
09.3	Semi-preserved fish and fish products, including mollusks, crustaceans, and echinoderms	2,000 mg/kg	As benzoic acid Except for use in caviar substitutes at 2 500 mg/kg. Excluding products conforming to the Standard for Sturgeon Caviar (CODEX STAN 291-2010).
09.2.5	Smoked, dried, fermented, and/or salted fish and fish products, including mollusks, crustaceans, and echinoderms	200 mg/kg	Except for use in fermented fish products at 1 000 mg/kg. Excluding products conforming to the Standard for Dried Shark Fins (CODEX STAN 189-1993). As benzoic acid Excluding products conforming to the Standard for Salted Fish and Dried Salted Fish of the Gadidae Family of Fishes (CODEX STAN 167-1989). Excluding products conforming to the Standard for Crackers from Marine and Freshwater Fish, Crustaceans and Molluscan Shellfish (CODEX STAN 222-2001). In foods conforming to the Standard for Smoked Fish, Smoke-Flavoured Fish and SmokeDried Fish (CODEX STAN 311-2013), for use in reduced oxygen packaged products in smoked fish and smoke-flavoured fish products only. Excluding products conforming to the Standard for Boiled Dried Salted Anchovies (CODEX STAN 236-2003).
15.1	Snacks - potato, cereal, flour or starch based (from roots and tubers, pulses and legumes)	1,000 mg/kg	As benzoic acid
12.5	Soups and broths	500 mg/kg	As benzoic acid Excluding use for canned bouillons and consommés. For use in products conforming to the Codex Standard for Bouillons and Consommés (CODEX STAN 117-1981) singly or in combination: sorbic acid (INS 200), potassium sorbate (INS 202), calcium sorbate (INS 203), benzoic acid (INS 210), sodium benzoate (INS

			211), potassium benzoate (INS 212), and calcium benzoate (INS 213) at 500 mg/kg as sorbic acid (INS 200-203) or as benzoic acid (INS 210-213).
11.6	Table-top sweeteners, including those containing high-intensity sweeteners	2,000 mg/kg	As benzoic acid
04.2.2.6	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed pulps and preparations (e.g. vegetable desserts and sauces, candied vegetables) other than food category 04.2.2.5	3,000 mg/kg	As benzoic acid
04.2.2.5	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g., peanut butter)	1,000 mg/kg	As benzoic acid
04.2.2.3	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds in vinegar, oil, brine, or soybean sauce	2,000 mg/kg	As benzoic acid
12.3	Vinegars	1,000 mg/kg	As benzoic acid
14.1.4	Water-based flavoured drinks, including "sport," "energy," or "electrolyte" drinks and particulated drinks	250 mg/kg	As benzoic acid
14.2.4	Wines (other than grape)	1,000 mg/kg	As benzoic acid

Note: Unless otherwise specified, food additive provisions apply to the food category indicated (e.g. Dairy), as well as to all subcategories of that category (e.g. Cheese, Ripened Cheese, etc.).

As taken from Codex Alimentarius, 2023.

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

After oral and dermal uptake, benzoate is metabolized in the liver by conjugation with glycine, resulting in the formation of hippuric acid (Feldmann & Maibach, 1970; US FDA, 1972a; WHO, 1996; Feillet & Leonard, 1998). The rate of biotransformation in humans is high: after oral doses of 40, 80 or 160 mg sodium benzoate/kg body weight, the transformation to hippuric acid was independent of the dose -- about 17-29 mg/kg body weight per hour, corresponding to about 500 mg/kg body weight per day (Kubota & Ishizaki, 1991). Other authors obtained higher values of 0.8-2 g/kg body weight per day (US FDA, 1972a, 1973; WHO, 1996). Hippuric acid is rapidly excreted in urine. In humans, after oral doses of up to 160 mg/kg body weight, 75-100% of the applied dose is excreted as hippuric acid within 6 h after administration, and the rest within 2-3 days (Kubota et al., 1988; Fujii et al., 1991; Kubota & Ishizaki, 1991).

The limiting factor in the biosynthesis of hippuric acid is the availability of glycine. The utilization of glycine in the detoxification of benzoate results in a reduction in the glycine level of the body. Therefore, the ingestion of benzoic acid or its salts affects any body function or metabolic process

in which glycine is involved; for example, it leads to a reduction in creatinine, glutamine, urea, and uric acid levels (US FDA, 1972a, 1973; Kubota & Ishizaki, 1991; WHO, 1996).

Another metabolite of benzoate is the benzoyl glucuronide. For example, the dog excretes considerable amounts of this metabolite in the urine (20% after a single dose of 50 mg/kg body weight; Bridges et al., 1970). In other species, this metabolite appears only after higher doses of about 500 mg/kg body weight (see above) of benzoic acid or sodium benzoate, resulting in a depletion of the glycine pool (Bridges et al., 1970; US FDA, 1972a; Kubota et al., 1988). In cats, glucuronidation is generally very low (Williams, 1967).

In some species, including humans, minor amounts of benzoic acid itself are also excreted in the urine (Bridges et al., 1970; Kubota & Ishizaki, 1991).

As taken from INCHEM, 2000

/OTHER TOXICITY INFORMATION/ Large oral doses of benzoic acid used therapeutically to alleviate the effects of inherited urea cycle disorders were studied with respect to their effect on intermediary metabolism. Documented effects on the urea cycle, gluconeogenesis, fatty acid metabolism, carnitine status, and the tricarboxylic acid cycle were attributed to sequestering of coenzyme A by benzoate as a result of glycine depletion required for the formation of hippuric acid from benzoyl coenzyme A. In addition, binding of benzoate to albumin results in toxic reactions due to the displacement of other metabolites from albumin-binding sites; e.g. bilirubin toxicity and appetite suppression result from increased serum tryptophan levels, which increase the turnover of brain serotonin [WHO; Food Additive Series 37: Toxicological evaluation of ceratin food additives: Benzyl acetate, Benzyl alcohol, Benzaldehyde, and Benzoic acid and its Salts (1996). Available from, as of March 24, 2005: <http://www.inchem.org/documents/jecfa/jecmono/v37je05.htm> **PEER REVIEWED**

Benzoic acid ... conjugated with glycine to give hippuric acid in ... many mammals (man, monkeys, pig, rabbit, rodents, cat, dog, ferret & hedgehog). Dog, ferret, & hedgehog also excreted ... benzoyl glucuronide ... but indian fruit bat excreted almost all dose as benzoyl glucuronide. [The Chemical Society. Foreign Compound Metabolism in Mammals. Volume 2: A Review of the Literature Published Between 1970 and 1971. London: The Chemical Society, 1972., p. 407] **PEER REVIEWED**

/Investigators/ demonstrated that biotransformation of benzoic acid to hippuric acid follows saturable or Michaelis-Menten kinetics in humans following ingestion of sodium benzoate. /Sodium Benzoate/ [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

The availability of glycine was the rate-limiting factor in the formation of hippuric acid. When insufficient glycine was available benzoyl glucuronide was formed. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

/Investigators/ reported that both lipoic acid and valproic acid reduced the clearance of benzoic acid in rats that had been "loaded" with glycine. Both acids reduced the availability of hepatic coenzyme A that is needed for the adenosine triphosphate (ATP)-dependent conjugation with glycine. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

Yields benzoyl-beta-d-glucuronic acid in rabbit, hen, dog, rat, monkey, pig, ferret, hedgehog, bat, pigeon; man; guinea pig, cat, rabbit; duck, goose, pigeon, crow, and parrot; yields benzoyl-beta-d-glucose in cockroach; yields benzoylagmatine in scorpion and in peripatus; yields benzoylarginine in scorpion peripatus; boophilus, eperia, teganaria, mitopus, and Phalangium; yields benzaldehyde in Aspergillus neurospora, and polystictus; yields benzoylglutamine in mitopus; yields benzoylhistidine in peripatus. /From table/ [Goodwin, B.L. Handbook of Intermediary Metabolism of Aromatic Compounds. New York: Wiley, 1976., p. B-7] **PEER REVIEWED**

Yields m-hydroxybenzoic acid in rabbit; yields m-hydroxybenzoic acid, p-hydroxybenzoic acid in Aspergillus; yields p-hydroxybenzoic acid in plants; yields p-hydroxybenzoic acid in Polyporus, Trichoderma, and yeast. /Fom table/ [Goodwin, B.L. Handbook of Intermediary Metabolism of Aromatic Compounds. New York: Wiley, 1976., p. B-8] **PEER REVIEWED**

In birds classed as anseriformes (duck, goose) & galliformes (hen, turkey), glycine is replaced by ornithine so that benzoic acid is excreted as dibenzoylornithine (ornithuric acid). ... Ornithine conjugation does not occur in all classes of birds. [LaDu, B.N., H.G. Mandel, and E.L. Way. Fundamentals of Drug Metabolism and Disposition. Baltimore: Williams and Wilkins, 1971., p. 195] **PEER REVIEWED**

In most animals, the conversion of benzoic acid to hippuric acid has been found to occur in the kidney, with conversion possible in the liver when kidney malfunction exists. The monkey metabolized benzoic acid only in the liver. [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

The rate of biotransformation in humans is high: after oral doses of 40, 80 or 160 mg sodium benzoate/kg bodyweight, the transformation to hippuric acid was independent of the dose -- about 17-29 mg/kg body weight per hour, corresponding to about 500 mg/kg body weight per day. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

The limiting factor in the biosynthesis of hippuric acid is the availability of glycine. The utilization of glycine in the detoxification of benzoate results in a reduction in the glycine level of the body. Therefore, the ingestion of benzoic acid or its salts affects any body function or metabolic process in which glycine is involved; for example, it leads to a reduction in creatinine, glutamine, urea, and uric acid levels. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

Another metabolite of benzoate is the benzoyl glucuronide. For example, the dog excretes considerable amounts of this metabolite in the urine (20% after a single dose of 50 mg/kg body weight). In other species, this metabolite appears only after higher doses of about 500 mg/kg body weight of benzoic acid or sodium benzoate, resulting in a depletion of the glycine pool. In cats, glucuronidation is generally very low. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

As taken from HSDB, 2014.

Flavouring agent (No.)	Species	Route	Dose	24-h urine/ faeces (% dose)	Major urinary metabolite	Minor urinary metabolites	Reference
Benzoic acid (850)	Rats	Oral	0.061, 0.61, 6.1, 61, or 305 mg/kg bw	88–89/1–6	Hippuric acid	Benzoic acid, benzoyl glucuronide, 3-hydroxy-3-phenyl propionic acid	Nutley (1990)
	Mice	Intraperitoneal	0.061, 0.61, 6.1, 61,	92–98/1–10	Hippuric acid	Benzoic acid, benzoyl glucuronide, 3-	Nutley (1990)

			or 305 mg/kg bw			hydroxy--phenyl propionic acid	
	Various	Oral	1–400 mg/kg bw	50–100	Hippuric acid	Benzoyl glucuronide, ornithic acid	Bridges et al. (1970)

As taken from JECFA, 2002

“Benzoic acid and its sodium and potassium salts are rapidly absorbed after oral administration.”

As taken from EFSA, 2016a.

Benzoic acid and sodium benzoate rapidly metabolise and excrete via a common pathway within 24 hours.

As taken from SCENIHR, 2016.

4.2. Absorption, distribution and excretion

After oral ingestion of benzoic acid and sodium benzoate, there is a rapid absorption (of undissociated benzoic acid) from the gastrointestinal tract in experimental animals or humans (US FDA, 1972a, 1973). From the figures on excretion given below, 100% absorption can be assumed. In humans, the peak plasma concentration is reached within 1-2 h (Kubota et al., 1988; Kubota & Ishizaki, 1991).

Benzoic acid is not completely absorbed by the dermal route. In a study with six human subjects, Feldmann & Maibach (1970) found an uptake of 36% of the applied dose (14C-labelled benzoic acid dissolved in acetone; 4 µg/cm²; circular area of 13 cm²; ventral surface of the forearm; non-occlusive) within 12 h. The total uptake within 5 days was 43%. In a second study with 6-7 subjects (comparable method; application of 3, 400 or 2000 µg/cm²), the percent absorption decreased from 35% to 14% within 24 h. However, the total uptake per cm² increased from 1 to 288 µg (Wester & Maibach, 1976).

In vivo dermal studies with benzoic acid in experimental animals (e.g., guinea-pigs, mice, rats, pigs, dogs, rhesus monkeys) confirm the results with humans (Hunziker et al., 1978; Andersen et al., 1980; Wester & Noonan, 1980; Bronaugh et al., 1982a; Reifenrath et al., 1984; Carver & Riviere, 1989; Maibach & Wester, 1989; Bucks et al., 1990). Absorption ranged from 25% in pigs (Reifenrath et al., 1984; Carver & Riviere, 1989) to 89% in rhesus monkeys (Wester & Noonan, 1980; Maibach & Wester, 1989; Bucks et al., 1990). Due to the good database on humans and animals in vivo, in vitro studies performed with animal or human skin are not considered further (Franz, 1975; Bronaugh et al., 1982b; Hotchkiss et al., 1992; MacPherson et al., 1996).

Experiments on the distribution and elimination of 14C-benzoate in the rat have shown no accumulation of sodium benzoate or benzoic acid in the body (US FDA, 1972a, 1973).

As taken from INCHEM, 2000.

After oral ingestion of benzoic acid and sodium benzoate, there is a rapid absorption (of undissociated benzoic acid) from the gastrointestinal tract in experimental animals or humans. ... 100% absorption can be assumed. In humans, the peak plasma concentration is reached within 1-2 hr. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

Experiments on the distribution and elimination of (14)C-benzoate in the rat have shown no accumulation of sodium benzoate or benzoic acid in the body. [International Programme on

Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

Benzoic acid and sodium benzoate are rapidly absorbed from the gastrointestinal tract of mammals and conjugated with glycine in the liver. The resulting hippuric acid is excreted in the urine rapidly (75% to 100% of the dose is excreted within 6 hours; the remaining dose is excreted within 2 to 3 days). [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

/Investigators/ reported that 42.6% +/-16.5% of a dermally applied (14)C-Benzoic acid dose (4 ug/sq cm; in acetone) was excreted in the urine within 24 hours. When applied in petrolatum, 60.5% of the dose was absorbed. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

By quantifying 24-hour urine excretion, /investigators/ demonstrated that dermal application of 1000 nmol (14)C-Benzoic acid (1.0×10^{-3} Ci/nmol) produced the following penetration scale: forehead > abdomen > thigh > chest > arm > back. The 4-day penetration through the forehead (27.65 +/- 3.61 nmol/sq cm) was three times greater than absorption through the back (8.55 +/- 1.32 nmol/sq cm). Benzoic Acid had been applied to two sites of each body area; one site was tape stripped to determine the amount of test material in the stratum corneum. The quantified values from the urine were comparable to predicted values estimated from the tape stripping. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

In a study investigating the effects of aging on dermal absorption, /investigators/ applied (14)C-Benzoic acid (in acetone) to the forearm of two groups of panelists, "young" (22 to 40 years) and "old" (>65 years). A 24-hour protective patch was placed on the skin and the site was washed after patch removal. A second protective patch was then applied and remained in place until day 7. Analysis of 7-day urine excretion indicated that 36.2% +/-4.6% of the applied dose was absorbed by the young panelists, whereas 19.5% +/-1.6% was absorbed by the old panelists. The difference was statistically significant ($p < .01$). [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

No statistical difference ($P > 0.05$) was found /for/ the percutaneous absorption of benzoic acid by Asian, Black, and Caucasian panelists. (14)C-Benzoic acid (1 umol/sq cm) was applied to two sites of the upper arm and the sites were washed after 30 minutes of contact. (The two applications occurred on contralateral arms and were made 48 hours apart.) Urine was collected for 24 hours and one site was tape stripped to measure benzoic acid in the stratum corneum. Amounts absorbed were 1.43% +/-0.27% by Asian skin, 1.07% +/-0.17% by Black skin, and 1.2% +/-0.19% by Caucasian skin. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

(14)C was excreted at different rates from various species following oral dose of (14)C-benzoic acid. In 24-hr urine, man excreted 100%, hamster 99%, dog 94%, guinea pigs 79%, ferret 69%, rabbit 60%, mouse 55%, pig 50% & squirrel monkey 48%. Man received one-fiftieth of dose (52 + or - 4 mg/kg) given to other species. [The Chemical Society. Foreign Compound Metabolism in Mammals. Volume 2: A Review of the Literature Published Between 1970 and 1971. London: The Chemical Society, 1972., p. 160] **PEER REVIEWED**

Biliary excretion ... in different species. Percent of dose excreted in 3 hr: rat 1.2, guinea pig 1.7, rabbit 0.7, dog 0.8, cat 1.2, hen 0.5. [LaDu, B.N., H.G. Mandel, and E.L. Way. Fundamentals of Drug Metabolism and Disposition. Baltimore: Williams and Wilkins, 1971., p. 139] **PEER REVIEWED**

The transdermal absorption of benzoic acid was studied in excised human skin and compared to absorption in living man. In equivalent time, the total absorption (% of applied dose) was 42.6% (in vivo) or 44.9 (in vitro). [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0)]

(2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

The percutaneous absorption and the excretion of benzoic acid were tested in female weanling Yorkshire swine (approximately 20 kg) after topical and intravenous administration. After iv injection of 200 ug (10 uCi)/pig 84.5% of (14)C-activity were excreted with urine and 4.6% in feces within 6 days; the radiolabel recovery in carcass was 0.1%. After topical application of the same dose the radiolabel recovery within 6 days (% of applied doses) was in urine 20%, feces 2.9%, carcass 0.8%, border 40.2%, dosed skin 12.2% and adjacent skin 9.1%. [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

The percutaneous absorption of (14)C-labelled benzoic acid was studied in the Mexican hairless dog and compared to human data. Total absorption and maximum absorption rates were greater in humans than in hairless dogs. Surface counting experiments showed that benzoic acid persisted on the dog skin far longer than on human skin (no further information). [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

The percutaneous absorption of increasing topical doses of benzoic acid was determined in the Rhesus monkey and humans (dosage: 4, 40, 2000 ug/sq cm; dose absorbed: monkey 59.2%, 3.6%, 17.4%; human 42.6%, 25.7%, 14.4%). In vivo percutaneous absorption was similar, also the dose-response curve was similar in the two species (no further information). [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

The percutaneous absorption and metabolism of benzoic acid was determined through hairless guinea pig skin in vitro. The absorption within 48 hr was greater through nonviable skin (60.1% of applied dose) than through viable skin (49.5%). 6.9% of absorbed dose (2 ug/sq cm) were conjugated with glycine to form hippuric acid. [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

Damaging the skin (tape stripping, irritation, delipidization) increased absorption of benzoic acid dissolved in acetone (200 ug/mL, 50 uCi; topical application: 4 ug/sq cm) in hairless guinea pigs: 71.1/73.4/94.1% vs 34.2% absorbed in the group with intact skin. [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

Benzoic acid is not completely absorbed by the dermal route. In a study with 6 human subjects, /investigators/ found an uptake of 36% of the applied dose ((14)C-labelled benzoic acid dissolved in acetone; 4 ug/sq cm; circular area of 13 sq cm; ventral surface of the forearm; non-occlusive) within 12 hr. The total uptake within 5 days was 43%. In a second study with 6-7 subjects (comparable method; application of 3, 400 or 2000 ug/sq cm), the percent absorption decreased from 35% to 14% within 24 hr. However, the total uptake per sq cm increased from 1 to 288 ug. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

Hippuric acid is rapidly excreted in urine. In humans, after oral /benzoic acid/ doses of up to 160 mg/kg bw, 75-100% of the applied dose is excreted as hippuric acid within 6 hr after administration, and the rest within 2-3 days. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

As taken from HSDB, 2014.

Dermal penetration: 100% (PPDB, 2024).

"The stratum corneum (SC), a permeable membrane, limits percutaneous penetration. As SC chemical and structural properties responsible for skin barrier function appear depth-related, we conducted an in vitro dermatopharmacokinetic study on intact and adhesive tape-stripped skin samples to clarify whether SC is a homogeneous barrier for chemical transport. SC concentration-thickness profiles were determined for four C-14 labeled model chemicals, panthenol, benzoic acid, paraoxon and butenafine, using the tape-stripping approach. Data analysis with the unsteady-state diffusion equation of Fick's second law permitted a chemical diffusion coefficient in SC. To evaluate the consistency of SC permeability from its surface to lower levels, the skin was tape-stripped five to 10 times to remove the upper cell layers before chemical application, such that diffusion coefficients could be determined from three SC depth levels (0, 5 and 10 tape strips). Results suggested the depth-dependency of SC permeability to panthenol, benzoic acid and butenafine; the diffusion coefficient of panthenol decreased significantly after the first five tape strips and subsequently remained consistent. A progressive increase in diffusion coefficients of benzoic acid and butenafine was observed as tape-stripping levels increased. The removal of superficial SC did not result in a significant difference in the paraoxon diffusion coefficient. For individual chemicals, a variation in the diffusion coefficient from SC surface to deeper layers agreed with the change of the diffusion coefficient over time in intact skin. Characterization of the SC properties contributing to the depth-dependent SC permeability will hopefully provide further insight to skin penetration/decontamination.." As taken from Cadavona JJ et al. 2016. J. Appl. Toxicol. 36(9), 1207-13. PubMed, 2017 available at <http://www.ncbi.nlm.nih.gov/pubmed/26843137>

"The Panel considered that due to its ionisation properties ($pK_a = 4.19$), the non-ionised form of benzoic acid will be absorbed by a diffusion process in the acid environment of the stomach."

"The ex vivo intestinal absorption of benzoic acid was studied in perfused rat small intestine preparations (Cong et al., 2001). A rapid and uneven absorption was observed among the segmental regions, absorption being highest in the jejunum and slightly lower in the ileum. The absorption pattern paralleled the distribution of the monocarboxylic acid transporter (Mct1), suggesting that this transporter may play a role in the absorption of benzoic acid."

"Following oral administration of 305 mg ^{14}C -benzoic acid/kg bw to rats, 91–94% of the radioactivity was recovered in the urine of rats after 72 h, whereas only 1–6% was present in the faeces. In addition to benzoic acid (0.4–12.8%), the following metabolites were identified: hippuric acid (70.2–84.2%), benzoyl glucuronide (0.7–1.8%) and 3-hydroxy-3-phenylpropionic acid (0.1–0.2%) (Nutley, 1990"

"In humans, benzoic acid is rapidly absorbed and its metabolites are completely excreted in the urine (Schachter, 1957; Schanker et al., 1958; Barnes, 1959 as referred to by JECFA, 1996"

"A dose of 1 mg ^{14}C -benzoic acid/kg bw was excreted in the urine (99.4–99.7% of total dose) entirely as hippuric acid in two males (Bridges et al., 1970"

"In order to investigate the types and quantities of beverages that increase urinary hippuric acid excretion, 137 healthy students were recruited and divided into quintiles based on their consumption of non-alcoholic beverages containing benzoic acid. HPLC was used to determine benzoic acid intake from beverages and urinary hippuric acid before, 1.5 and 3 h after consumption of various beverages. The range of benzoic acid in 13 beverages was 0–1.02 mg/mL and the benzoic acid intakes from the beverages for groups 1–5, respectively, were: 0.4 ± 0.5 mg; 23.4 ± 9.8 mg; 55.2 ± 2.3 mg; 76.3 ± 4.0 mg; and 116.5 ± 16.5 mg. Urinary hippuric acid geometric mean concentrations before consuming beverages in the five groups, respectively, were 0.276, 0.270, 0.207, 0.262 and 0.316 g/L; 1.5 hours after beverage consumption, they were 0.210, 0.603, 1.026, 1.066 and 1.688 g/L, and significantly increased ($P < 0.001$) after adjustment for urinary hippuric acid before ingestion. Three hours after beverage consumption, urinary hippuric acid geometric mean concentrations in the five groups, respectively, were 0.160, 0.232, 0.306, 0.287 and 0.337 g/L ($P < 0.001$). The authors concluded that intakes of more than 100 mg benzoic acid from

consumption of beverages containing the preservative at a single occasion may increase urinary hippuric acid significantly (Chang et al., 2000 as referred to by EFSA CEF Panel, 2012”

“Overall, the Panel considered that the available ADME data in animals and humans indicate that benzoic acid and its sodium and potassium salts are rapidly absorbed, primarily in the proximal part of the gastrointestinal tract, after oral administration. Benzoate is conjugated to glycine to form hippuric acid which is excreted in the urine.”

As taken from EFSA, 2016a.

“Benzoic acid and sodium benzoate rapidly metabolise and excrete via a common pathway within 24 hours.”

As taken from SCENIHR, 2016.

“For an adult, after intravenous administration of a dose of 15 mg/kg, the elimination half-life from plasma is 16 minutes for benzoic acid and 31 minutes for hippuric acid. Eighty per cent of the dose is eliminated in the urine in the form of hippuric acid. Clearance is approximately 600 ml/min. The benzoic acid detoxification process is immature in those pre-term neonates and conjugation of benzoic acid is saturated more quickly. This reaction is furthermore limited by the glycine available. This results in an accumulation of benzoic acid.”

As taken from EMA, 2017.

“INTRODUCTION: Chemical necrectomy of deep burns using 40% benzoic acid has been used extensively by the Department of Burns and Reconstructive Surgery at the University Hospital since its establishment in 1982. In spite of definite advantages for the patient and medical staff, hard data concerning benzoic acid absorption through skin necrosis and patient safety was missing. MATERIAL AND METHODS: We examined 22 burn patients in collaboration with the University Hospital Brno, Department of Clinical Biochemistry. The plasmatic levels of benzoic acid, hippuric acids and glycine, which is consumed during the metabolism of benzoic acid, were measured. Urine samples were collected to determine the total amount of hippuric acid that is excreted. We were able to determine the total amount of absorbed and excreted benzoic acid from these values. RESULTS: We consistently found that there was a rapid and short-term increase of plasmatic levels of benzoic acid (maximum 1.3 mmol/l). This value is about 5 times lower than the minimum toxic level of this acid (6.5 mmol/l). The same course has been observed in hippuric acid. The level of glycine dropped slightly, but was still within the normal range. DISCUSSION: Typical and atypical courses of the levels of both acids were discussed as well as the correlation of the dynamics of elimination with the extent of benzoic acid application in relationship with the clinical status of the patient. The effectiveness and safety of this method was evaluated. CONCLUSION: After summarizing the observations, it was demonstrated that chemical necrectomy using 40% benzoic acid is a selective method comparable with other types of sharp necrectomy. Chemical necrectomy is inexpensive, easy to perform and also reduces blood loss. Toxicity of absorbed benzoic acid is clinically negligible. Furthermore, benzoic acids antimycotic and antibacterial properties prevent the development of wound infection.” As taken from Jelínková Z et al. 2017. Acta Chir. Plast. 59(1), 5-10. PubMed, 2018

Benzyl alcohol is metabolized to benzoic acid. Both benzoic acid and sodium benzoate are rapidly absorbed from the gastrointestinal tract of mammals and conjugated with glycine in the liver; the resulting hippuric acid is rapidly excreted in the urine.² Benzyl alcohol and benzoic acid and its sodium and potassium salt can be considered as a single category regarding human health because they are all rapidly metabolized and excreted via a common pathway within 24 hours (Johnson W et al., 2017).

The penetration of benzoic acid through excised dorsal skin (full thickness and with stratum corneum removed) from guinea pigs was examined. Skin preparations were mounted in a 2-chamber diffusion cell. A suspension of an excess amount of benzoic acid in saline was added to

donor compartments. In full-thickness skin, permeation proceeded with a short lag time. Solubility (Cd, unit $\frac{1}{4}$ mM) and permeability (Kp, unit= 10^{-2} cm/h) coefficients for benzoic acid were 32.7 ± 1.6 and 9.01 ± 1.51 , respectively. Removal of the stratum corneum by tape stripping and its delipidation using an organic solvent mixture enhanced the skin penetration of benzoic acid (Johnson et al., 2017).

"The data presented in this article are related to the research article entitled "Design of in vitro skin permeation studies according to the EMA Guideline on quality of transdermal patches" (<https://doi.org/10.1016/j.ejps.2018.09.014>) (Cilurzo et al., 2018) [1]. In vitro permeation studies are generally carried out by Franz's diffusion cell method using human epidermis as a membrane (Franz, 1975) [2]. The evaluation of membrane integrity is mandatory to assure the quality of the experiments. However, the methods used for this determination are different and the results are strictly dependent on the operative conditions. The article reports the electrical resistance values of human epidermis samples and in vitro skin permeability data of caffeine and benzoic acid. The data are used to establish a cut-off suitable for checking the skin integrity. This information may be useful to enable critical or extended analyses in order to contribute to the development of a compendial method." As taken from Musazzi UM et al. 2018. Data Brief 21, 1258-1262. PubMed, 2019

"Studies of absorption through the skin are available only for benzoic acid. As benzoic acid dissolved in aqueous media is present in equilibrium with its dissociated form (benzoate), the two compounds cannot be differentiated with regard to absorption through the skin; dermal absorption takes place via the undissociated form. Assuming a maximum absorption rate of $166 \mu\text{g}/\text{cm}^2$ and hour (Nielsen and Sørensen 2012), about 332 mg benzoic acid would be absorbed through the skin under standard conditions (2000 cm^2 , 1-hour exposure). The NOAEC for systemic effects after short-term inhalation exposure in rats is $250 \text{ mg}/\text{m}^3$, so that after extrapolation to long-term exposure (1:6), extrapolation to humans ($250 \text{ mg}/\text{m}^3 \times 10 \text{ m}^3$ respiratory volume per 8-hour exposure) and after halving the dose because the results were obtained on the basis of animal experiments, (see List of MAK and BAT Values, Section I) and correction for the increased respiratory volume at the workplace (1:2), a systemically tolerable amount of 104 mg is obtained. The possible contribution of absorption through the skin to systemic toxicity is therefore not negligible, so that both benzoic acid and alkali benzoates are designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts"

As taken from Hartwig A and MAK Commission, 2018

"Introduction: Skin care products are often utilized in conjunction with topical treatment for skin disease. However, there appears insufficient body of experimental data to advise the health care worker or patient. Examine penetration effect (hence potential for altering efficacy and toxicity) - dosing with one topical after another and how quickly such a potential phenomenon might occur. Methods: A marketed moisturizer applied on human skin with an in vitro diffusion system. At 1, 15, and 30 min post application, [^{14}C]-benzoic acid dosed the same skin site for 24 h. Amounts of chemical retained in skin and permeation flux rates were measured to determine penetration effect of the prior moisturizer application. Results: Exposure of a water-enriched moisturizer before a hydrophilic chemical immediate application favors the chemical penetration, especial in 1 and 15 min moisturizer exposure groups. The enhancement effect was expressed as an earlier lag time and a rapid absorption peak when compared to related non-moisturizer control. Conclusion: This experiment opens up a large door: what would be the result with many complex topical products and different tracers was used here, of varying hydrophilicity and lipophilicity. We do not wish to overgeneralize until such studies are confirmed in vivo." As taken from Hui X and Maibach H. 2020. J. Dermatolog. Treat. Epub ahead of print. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/32633593/>

"Danger of cutaneous absorption" (ACGIH, 2021a).

4.3. Interactions

The metabolism of the benzoates depletes glycine concentrations and can therefore alter the glycine-dependent metabolism of other compounds. /Investigators/ demonstrated that benzoic acid or sodium benzoate successfully competed with aspirin for glycine, resulting in increased concentration and persistence of salicylic acid in the body. Almost total inhibition of salicyluric acid formation in humans was achieved using either 2.7 g benzoic acid or 3.2 g sodium benzoate. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

In rat liver microsomes deferoxamine was a potent inhibitor of the oxidation of the scavenging agent, benzoate. Nearly complete inhibition was observed at 33-100 uM. [Cederbaum AI, Dicker E; Oxy Radicals Their Scavenger Syst, Proc Int Conf Superoxide Dismutase 1: 116-21 (1983)] **PEER REVIEWED**

Groups of 25 male and 25 female mice were given benzoic acid at a dose of 40 mg/kg bw/day, sodium bisulfite at 80 mg/kg bw/day, or a mixture of the two for 17 months. Mortality was greater in the groups receiving the mixture (62%) than in those receiving the individual substances (32%) at eight months. [WHO; Food Additive Series 37: Toxicological evaluation of ceratin food additives: Benzyl acetate, Benzyl alcohol, Benzaldehyde, and Benzoic acid and its Salts (1996). Available from, as of March 24, 2005: <http://www.inchem.org/documents/jecfa/jecmono/v37je05.htm> **PEER REVIEWED**

Wistar rats /were administered/ 40 mg benzoic acid/kg/day and 80 mg sodium bisulphite/kg/day once daily /for/ 72 weeks. 50 rats /with/ initial body weight /of/ 100-120g /experienced/ reduced weight gain, kidney function and reaction on stress factors were altered (no further information), /and/ the erythrocyte sedimentation rate was increased. [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

As taken from HSDB, 2014.

“Embryotoxicity and teratogenicity of benzoic acid when administered at a single dose of 510 mg/kg bw/d on day 9 of gestation to 7 pregnant rats were evaluated. Under these experimental conditions, there were no indications for embryotoxic or teratogenic activity of benzoic acid. Interestingly, it was noted that high doses of benzoic acid may increase the teratogenicity of other substances, presumably via toxicokinetic interactions by delaying the elimination of these. Nevertheless, this study can not be regarded suitable as the basis for assessment of developmental toxicity (single dose on day 9 only, low no. of animals)”

As taken from European Commission, 2013a, b.

“Antimicrobial activity of the simultaneous UV-A light and benzoic acid (BA) treatment against stationary phase Escherichia coli O157:H7 was investigated. While 15 mM BA or UV-A light exposure for 30 min alone caused < 1 logarithmic reduction in the bacterial population, > 5 logarithmic reductions were induced by the simultaneous application of UV-A and 15 mM BA in 30 min, demonstrating a synergistic antimicrobial effect. Due to its ability to increase cell membrane permeability, addition of EDTA (1 mM) was able to decrease the required concentration of BA in the simultaneous treatment from 15 to 8 mM. Microbial inactivation was a result of simultaneous membrane damage, intracellular acidification, and intracellular oxidative stress. The simultaneous treatment was effective in the presence of organic load of up to 500 mg/L of chemical oxygen demand (COD) and was able to lower cross-contamination risk during simulated washing of spinach (Spinacia oleracea) without adversely affecting its color.” As taken from Ding Q et al. 2018. Food Microbiol. 72, 39-54. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29407404>

“An antimicrobial effect of benzoic acid (BA) and ethylenediaminetetraacetic acid (EDTA) was evaluated as a potential antimicrobial treatment against *Escherichia coli* O157:H7 and *Listeria innocua*. A 30 min exposure to the combination of 15 mM BA and 1 mM EDTA at 22 °C resulted in approximately 3 logarithmic reductions in stationary phase *E. coli* O157:H7. Logarithmic phase *E. coli* O157:H7 was more sensitive ($P < 0.05$) to the treatment and 1 mM EDTA alone caused more than 5 logarithmic reductions. *L. innocua* was also sensitive to a treatment with 15 mM BA alone, which induced 5 logarithmic reductions. By increasing the temperature of the solution containing 15 mM BA and 1 mM EDTA to 40 °C, more than 5 logarithmic reductions in stationary phase *E. coli* O157:H7 was observed after 5 min of treatment. However, the antimicrobial effect was attenuated (reaching less than 1 logarithmic reductions) at 4 °C. In addition, the combined BA and EDTA treatment retained its antimicrobial effect against *E. coli* O157:H7 for at least 6 cycles of treatment over 6 days at room temperature (22 °C). In a simulated spinach washing study, 15 mM BA and 1 mM EDTA together were able to prevent cross-contamination of *E. coli* O157:H7. The results highlight the potential use of combination of BA (15 mM) and EDTA (1 mM) to address microbial risk from *E. coli* O157:H7 and *L. innocua* in fresh produce industry. PRACTICAL APPLICATION: This study demonstrates the effectiveness of benzoic acid (BA) and EDTA mixture in inactivating bacteria in the water used for produce washing and reducing the incidence of cross-contamination during washing of fresh produce. Use of BA + EDTA mixture has significant benefits such as: (a) ability to be reused, (b) effectiveness in the presence of organic matter, and (c) reduced need of monitoring wash water conditions such as pH, concentration and organic matter.” As taken from Alborzi S et al. 2018. *J. Food Sci.* 83(4), 1032-1040. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29488632>

“Seven selected microbial metabolites of proanthocyanidins (MMP), 3-phenylpropionic, 4-hydroxyphenyl acetic, 3-(4-hydroxyphenyl) propionic, p-coumaric, benzoic acid, pyrogallol (PG), and pyrocatechol (PC) were evaluated for their ability to reduce chemical carcinogen-induced toxicity in human lung epithelial cells (BEAS-2B) and human fetal hepatic cells (WRL-68). Cells pre-treated with MMP were exposed to a known chemical carcinogen, 4-[(acetoxymethyl) nitrosamino]-1-(3-pyridyl)-1-butanone (NNKOA) to assess MMP-mediated cytoprotection and reduction of DNA damage. PG in BEAS-2B and PC in WRL-68 cells mitigated the NNKOA-induced cytotoxicity. Pre-incubation of PG depicted significant protection against NNKOA-induced DNA damage in BEAS-2B cells. PC in WRL-68 cells showed similar activity. To understand the mechanisms of PG- and PC-mediated DNA damage reduction, the effect on DNA damage response (DDR) proteins, cellular reactive oxygen species (ROS), total antioxidant capacity (TAC), glutathione peroxidase (GPx), and caspase activity were studied. PG and PC alter the DDR and may promote ATR-Chk1 and ATM-Chk2 pathways, respectively. Cellular oxidative stress induced by NNKOA was mitigated by PG and PC through enhanced GPx expression and TAC. PG and PC suppressed the activation of the extrinsic apoptotic pathway (caspase 3 and 8) provoked by NNKOA. MMP are beneficial in chemoprevention by reducing cellular DNA damage.” As taken from Thilakarathna WPDW and Rupasinghe HPV. 2019. *Food Chem. Toxicol.* 125, 479-493. PubMed, 2019.

5. Toxicity

5.1. Single dose toxicity

“Benzoic acid shows very low acute toxicity (for all administration routes (oral, inhalation, dermal, intravenous, intraperitoneal) in essentially all species tested (Rat, mouse, rabbit, dog), except for cats (LOEC = 630 mg/kg bw). Cat sensitivity is thought to be related to species-specific deficiencies in benzoate metabolism (no glucuronidation pathway), and is considered not relevant to humans. Therefore, no acute toxicity classification and labelling is proposed for benzoic acid”

As taken from ECHA, 2012a.

Type	of	Route	of	Species/Test	Dose	Toxic Effects	Reference

Test	Exposure or Administration	System	Data		
TDLo - Lowest published toxic dose	Administration onto the skin	Human	6 mg/kg	Lungs, Thorax, or Respiration - dyspnea Skin and Appendages - dermatitis, allergic (after systemic exposure)	JOALAS Journal of Allergy. (St. Louis, MO) V.1-47, 1929-71. For publisher information, see JACIBY. Volume(issue)/page/year: 16,195,1945
LDLo - Lowest published lethal dose	Oral	Human - man	500 mg/kg	Details of toxic effects not reported other than lethal dose value	FCTXAV Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. Volume(issue)/page/year: 17,715,1979
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - rat	1700 mg/kg	Details of toxic effects not reported other than lethal dose value	IPSTB3 International Polymer Science and Technology. (Rapra Technology Ltd., Shawbury, Shrewsbury, Shropshire SY4 4NR, UK) Volume(issue)/page/year: 3,93,1976
LD50 - Lethal dose, 50 percent kill	Intraperitoneal	Rodent - rat	1600 mg/kg	Details of toxic effects not reported other than lethal dose value	BCFAAI Bollettino Chimico Farmaceutico. (Societa Editoriale Farmaceutica, Via Ausonio 12, 20123 Milan, Italy) V.33- 1894- Volume(issue)/page/year: 112,53,1973
LD50 - Lethal dose, 50 percent kill	Intravenous	Rodent - rat	1700 mg/kg	Details of toxic effects not reported other than lethal dose value	APYPAY Acta Physiologica Polonica. (Karger-Libri, POB CH-4009, Warszawa, Switzerland) V.1-41, 1950-90. Volume(issue)/page/year: 12,173,1961
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - mouse	1940 mg/kg	Behavioral - somnolence (general depressed activity) Lungs, Thorax, or Respiration - respiratory depression Gastrointestinal - other changes	IYKEDH Iyaku hin Kenkyu. Study of Medical Supplies. (Nippon Koteisho Kyokai, 12-15, 2-chome, Shibuya, Shibuya-ku, Tokyo 150, Japan) V.1- 1970- Volume(issue)/page/year: 15,359,1984
LD50 - Lethal dose, 50 percent kill	Intraperitoneal	Rodent - mouse	1460 mg/kg	Details of toxic effects not reported other than lethal dose value	CRSBAW Comptes Rendus des Seances de la Societe de Biologie et de Ses Filiales. (SPPIF, B.P.22, F-41353 Vineuil, France) V.1- 1849- Volume(issue)/page/year:

					160,1097,1966
LDLo - Lowest published lethal dose	Oral	Rodent rabbit -	2 gm/kg	Details of toxic effects not reported other than lethal dose value	HBTXAC "Handbook of Toxicology," 4 vols., Philadelphia, W.B. Saunders Co., 1956-59 Volume(issue)/page/year: 5,23,1959
LDLo - Lowest published lethal dose	Subcutaneous	Rodent rabbit -	2 gm/kg	Details of toxic effects not reported other than lethal dose value	HBTXAC "Handbook of Toxicology," 4 vols., Philadelphia, W.B. Saunders Co., 1956-59 Volume(issue)/page/year: 5,23,1959
LDLo - Lowest published lethal dose	Oral	Rodent guinea pig -	2 gm/kg	Details of toxic effects not reported other than lethal dose value	MMWOAU Muenchener Medicinische Wochenschrift. (Munich, Fed. Rep. Ger.) V.33-115, 1886-1973. Volume(issue)/page/year: 77,13,1930
LDLo - Lowest published lethal dose	Intraperitoneal	Rodent guinea pig -	1400 mg/kg	Details of toxic effects not reported other than lethal dose value	HBTXAC "Handbook of Toxicology," 4 vols., Philadelphia, W.B. Saunders Co., 1956-59 Volume(issue)/page/year: 5,23,1959

As taken from RTECS, 2019.

The minimum lethal human oral dose of benzoic acid is 500 mg/kg body-weight. [Rowe, R.C., Sheskey, P.J., Quinn, M.E.; (Eds.), Handbook of Pharmaceutical Excipients 6th edition Pharmaceutical Press, London, England 2009, p. 62] **PEER REVIEWED**

LD50 Cat oral 2000 mg/kg [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 379] **PEER REVIEWED**

LD50 Dog oral 2000 mg/kg [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 379] **PEER REVIEWED**

LD50 Mouse intraperitoneal 1460 mg/kg [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 379] **PEER REVIEWED**

LD50 Mouse oral 1940 mg/kg [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 379] **PEER REVIEWED**

LD50 Rat oral 1700 mg/kg [Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 379] **PEER REVIEWED**

LC50 Rat inhalation >0.026 mg/L/1 hr [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

LD50 Rabbit dermal > 5000 mg/kg bw [European Chemicals Bureau; IUCLID Dataset, Benzoic acid (65-85-0) (2000 CD-ROM edition). Available from, as of March 22, 2005: <http://esis.jrc.ec.europa.eu/> **PEER REVIEWED**

As taken from HSDB, 2014.

Flavouring agent (No.)	Species	Sex	Route	LD50 (mg/kg bw)	Reference
Benzoic acid (850)	Mouse	NR	Oral	1200	Schafer & Bowles (1985)
Benzoic acid (850)	Mouse	NR	Oral	2000	Sado (1973)
Benzoic acid (850)	Mouse	NR	Intragastric	2000	Shell BV (1982)

As taken from JECFA, 2002,.

Record for benzoic acid:

Chemical Purity	Species Scientific Name	Species Common Name	Organism Age	Organism Age Units	Exposure Type	Media Type	Test Location	Conc 1 (Author)	Conc 1 Units (Author)	Effect	Effect Measurement	Endpoint	Exposure Duration Mean (Days)	Response Site	Observed Duration (Days)
NR	Ovis aries	Domestic Sheep	NR	Year (s)	Intravenous	No substrate	Lab	625	uM/kg	Biochemistry	Glucose	LOEL	0.0035	Blood	0.0035
NR	Ovis aries	Domestic Sheep	NR	Year (s)	Intravenous	No substrate	Lab	625	uM/kg	Hormone(s)	Insulin	LOEL	0.0035	Blood	0.0035

As taken from the US EPA ECOTOX database, 2021.

“Direct observations in healthy individuals (oral exposure) - Several studies from the 19th and the beginning of the 20th century regarding the oral exposure of humans to benzoic acid or sodium benzoate are described. However, owing to the low number of individuals exposed, the validity of these studies is limited. No adverse effects were reported after a single oral dose of 10.000 mg benzoic acid....”

“Inhalation toxicity of benzoic acid was evaluated in one rat study using fine benzoic acid dust with a mean aerodynamic particle diameter of 4.7 µm. Evaluation of survival, body weight and organ weight demonstrated significant systemic toxicity including 2/20 mortalities at the top dose of 1.2 mg/L. A reddish discharge around the nares was observed at doses ≥ 0.25 mg/L. In addition to systemic toxicity, compound-related microscopic lesions consisting of multifocal to generalised inflammatory cell infiltrates and interstitial fibrosis of the lung were observed. This was reported for all groups of benzoic acid treated animals with a concentration-dependent increase in intensity and incidence. It is widely accepted, that particles of low-solubility materials retained in the lower lung are cleared by phagocytic cells, mostly alveolar macrophages. Excessive activation of these cells is

linked to the release of ROS (reactive oxygen species) as well as inflammatory and fibrogenic mediators initiating lung remodelling well described for silica pneumoconiosis. The systemic effects observed at higher concentrations differed from those observed after oral exposure at higher concentrations (organ weight decrease, platelet decrease) and are thought to be secondary to local lung toxicity. Because the toxic effects observed in this study are attributed to the physico-chemical properties of these fine low-solubility particles, the study was not considered relevant for the evaluation of human health effects after repeated exposure to fluid benzoic acid formulations used in biocidal applications. In addition, a rough estimate of the inhalation exposure from evaporating surface disinfectants showed that inhalation exposure to benzoic acid vapours is negligible (calculation as proposed by Human Exposure Expert Group (HEEG)). Exposure to benzoic dusts is not expected but any risk would be sufficiently addressed by general exposure limits for dusts, e.g. national OELs such as MAK for fine dust (DFG, 2011). This conclusion was based on the observation that effects after dust inhalation are most likely due to pneumoconiosis and are not substance-specific. A need is not seen to revise the AEL setting and preference is given to derive AELs on the basis of the oral studies.”

As taken from European Commission, 2013a, b.

Rat acute oral LD50 >2100 mg/kg

Rabbit acute dermal LD50 >2000 mg/kg bw

As taken from PPDB, 2024.

An acute study with benzoic acid by gavage in male and female mice was available to the Panel. This unpublished study (Bio-Research Laboratories, 1979 [Doc. provided to EFSA n. 1]) showed a LD50 of 2,250 (1,875–2,700) mg benzoic acid/kg bw.

An acute study by gavage with benzoic acid in albino rats was available to the Panel. This unpublished study (International Research and Development Corporation, 1974 [Doc. provided to EFSA n. 10]) showed a LD50 for males of 2,742 mg benzoic acid/kg bw and for females of 2,565 mg benzoic acid/kg bw.

In conclusion, the acute oral toxicity of benzoic acid is low with LD50 values in the range of 1,200–2,742 mg/kg bw.

As taken from EFSA, 2016a.

“In the acute toxicity studies, benzoic acid is of low toxicity in rat, mouse, rabbit and dog, and of moderate toxicity in cat.”

“Benzoic acid - Low acute inhalation toxicity to rat LC50 inhalation (> 1.2 mg/L air per 6 h (whole body))”.

As taken from EFSA, 2016b.

“Benzoic acid and sodium benzoate have low acute oral and dermal toxicity with LD50 values >2000 mg/kg bw. The 4 hour-inhalation exposure of benzoic acid at 0.026mg/l/h also shows low acute inhalation toxicity.”

As taken from SCENIHR, 2016.

“Oral: LD50 in rats ranged from 1700-3040 mg/kg bw/d and in mouse ranged from 1940-2370 mg/kg bw/d. However, the studies that reported the lower LD50s were all pre-guideline studies and no further information was available to critically assess the data. LD50s reported in two reliable studies were 2250 mg/kg bw/d (mice) and 2565 mg/kg bw/d (rats) (OECD, 2004).

Inhalation: The chemical exhibits low acute toxicity in animal tests following inhalation exposure. No mortalities or toxic effects were observed in rats and mice with the reported median lethal concentration (LC50) > 12.2 mg/L/4-h (ECHA, 2011; OECD, 2004”

As taken from AICIS, 2013.

Rat oral LD50: 2530 mg/kg (IGS, 2021).

	PACs based on AEGLs, ERPGs, or TEELs			
CASRN	PAC-1	PAC-2	PAC-3	Units
65-85-0	13	140	830	mg/m3

As taken from US DoE, 2024.

5.2. Repeated dose toxicity

Type of Test	Route of Exposure or Administration	Species/ Test System	Dose Data	Toxic Effects	Reference
TCLo - Lowest published toxic concentration	Inhalation	Rodent - rat	1200 mg/m3/4W (intermittent)	Lungs, Thorax, or Respiration - fibrosis (interstitial) Related to Chronic Data - death	NTIS** National Technical Information Service. (Springfield, VA 22161) Formerly U.S. Clearinghouse for Scientific & Technical Information. Volume(issue)/page/year: OTS0539172

As taken from RTECS, 2019.

“A 4-week inhalation toxicity study of aerosolized benzyl alcohol and benzoic acid was performed using groups of Crl:CD(SD) rats. Four groups of rats (10/sex/group) were exposed (nose-only) to aerosolized benzyl alcohol 5 days per week (6 h/day) for 4 weeks. Each animal received a minimum of 20 exposures of benzyl alcohol, and target exposure concentrations were 30, 100, 300, and 1,000 mg/m³ for the four groups. Two additional groups of rats were exposed to aerosolized benzoic acid (2.5 and 12.5 mg/m³) according to the same procedure. The group designated for exposure to benzyl alcohol (30 mg/ mg/m³ target) was actually exposed to a mean atmosphere concentration that was 136% of the target concentration. For the remaining groups, all mean atmosphere concentrations were within 96.7% to 107.2% of the respective target concentrations. A concurrent control group was exposed to filtered air only.

There were no test-substance related deaths or effects on any of the following: body weight, food consumption, clinical pathology parameters, and organ weights. Additionally, there were no test substance-related macroscopic or microscopic findings. The (NOEL) and NOAEL were considered to be 1,072 mg/m³ for benzyl alcohol and 12.6 mg/ mg/m³ for benzoic acid.”

As taken from CIR, 2011.

In a 4-generation study 20 rats/sex/group were dosed continuously by diet with 375 or 750 mg/kg/day benzoic acid. In all 4 generations no influence on growth (weight, weight gain and food efficiency (measured by protein efficiency)) and organ weights was found. The animals of the 3rd generation were killed and examined histopathologically after 16 weeks (after lactation of the pups). No histo-pathological findings were found. In the paper, no information is given on the organs investigated; however the robustness of the total study, the reputation of the investigators, as well as the reputation of the Professor who did the histopathologic investigation, a high scientific quality has to be assumed even though the studies were performed many years ago. From other

parameters it can be assumed that as a minimum the brains, heart, liver, kidney, testis and spleen were examined.

Feeding of 375 mg/kg/day led to prolongation of survival compared to controls NOAEL > 750 mg/kg/day

(Kieckebusch & Lang, 1960)

Due to missing hematological and clinical chemistry investigations in all studies only a preliminary NO(A)EL of about 800 mg/kg can be derived for rats which is based on the studies from Kieckebusch & Lang (1960), Kreis et al. (1967) and Bio-Fax (1973)

A 21 day dermal study with male/female New Zealand white rabbits dosed with 100, 500, or 2500 mg/kg bw benzoic acid 5 days/week showed no compound related effects in behavior, body weight organ weights, clinical laboratory tests or survival. Very slight dermal irritation was noted for 1/8 rabbits at the 2500 mg/kg level.

NOAEL = 2500 mg/kg/day (IRDC# 163-675, 1981)

OECD SIDS BENZOATES UNEP PUBLICATIONS 1 FOREWORD INTRODUCTION BENZOATES CAS N°:65-85-0, 532-32-1, 582-25-2, 100-51-6 OECD SIDS BENZOATES UNEP PUBLICATIONS 2 SIDS Initial Assessment Report for 13th SIAM (Bern, 7th - 9th November 2001)

As taken from INCHEM, 2004, Benzoates, SIDS Initial Assessment Report..

“The objective of this study was to compare the effects of sodium-benzoate (NaB) with those of benzoic acid (BAC) on growth performance of piglets as well as nutrient digestibility, nitrogen and mineral balance, urinary pH, and the urinary excretion of BAC and hippuric acid (HAc). The study was conducted with 120 weaning piglets (6.5 kg body weight), divided in four groups (15 replicates of two piglets each), which received (1) a basal diet (Control), or the basal diet supplemented with (2) 4 g NaB per kg (Group 4NaB), (3) 3.5 g BAC per kg (Group 3.5BAC) or (4) 5 g BAC per kg (Group 5BAC). Performance data were monitored over a 42-day period. Urine and faeces were collected from day 28-33 in metabolic cages with five piglets per treatment. Piglets of Groups 3.5BAC and 5BAC had similarly a considerably improved average daily gain and feed intake ($P<0.05$). Performance of Group 4NaB was not significantly different from the other groups. Compared to the Control, the nitrogen retention was only improved in Group 5BAC ($P<0.05$); the other groups showed intermediate values. In the supplemented groups, most of the BAC was excreted as HAc in urine, but only Groups 3.5BAC and 5BAC had reduced urinary pH ($P<0.05$). Daily intake and faecal and urinary excretion of P and Ca were not affected by the treatment. The molar excess of Na in Group 4NaB was reflected by higher renal excretion of Na compared to the other groups ($P<0.05$ ” as taken from Gräber T et al. 2012. Arch. Anim. Nutr. 66(3), 227-36. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22724168>.

Record for benzoic acid:

Chemical Purity	Species Scientific Name	Species Common Name	Organism Age	Organism Age Units	Exposure Type	Media Type	Test Location	Conc 1 (Author)	Conc 1 Units (Author)	Effect	Effect Measurement	Endpoint	Exposure Duration Mean (Days)	Response Site	Observed Duration (Days)
2	Rattus norvegicus	Norway	NR	Not reported	Food	No substrate	Lab	15.2	g/orgr	Growth	Weight	LOEL	224	Not reported	224

	egic us	Rat		rted		te			d					rted	
2	Ratt us norv egic us	Nor way Rat	NR	Not repo rted	Foo d	No sub stra te	Lab	15. 2	g/o rg/ d	Feedi ng beha vior	Food consu mptio n	NO EL	364	Not repo rted	364
2	Ratt us norv egic us	Nor way Rat	NR	Not repo rted	Foo d	No sub stra te	Lab	15. 2	g/o rg/ d	Histol ogy	Hyper plasia	NO EL	364	Not repo rted	364
NR	Sus scro fa	Pig	4	We ek(s)	Foo d	No sub stra te	Lab	1	%	Bioch emist ry	Lactat e	NO EL	12	Inte stin al tract	12

As taken from the US EPA ECOTOX database, 2021.

“A large number of subacute and subchronic oral toxicity studies with benzoic acid or sodium benzoate have been performed in various mammalian species. However, in general these do not meet current standards with regard to study design and reporting. Many of these studies primarily relied on clinical observations.

Typical signs caused by benzoic acid in the rat were similar to those from acute studies and included ataxia, aggressiveness, tremor, convulsions, reduction of food consumption and bw gain, and mortality. With few exceptions, histopathological evaluation was not performed or included only few organs. A 35-d CNS toxicity study reported regional CNS necrosis from daily doses of 2250 mg/kg in the rat. The kidney weight was increased in some studies. Generally, LOAEL values for the rat were between 1200 and 2250 mg/kg bw/d for subacute and subchronic exposure without strict correlation to exposure time. A reliable NOAEL for derivation of threshold limit values may be based on a multigeneration study. In this study, no changes in bw gain (data supplied only for up to 12/8 weeks for M/F) and histopathology of key organs performed after wk 16 were reported at the top dose of 500 mg/kg bw/d benzoic acid. Survival at this dose remained unaffected. NOAELs reported in other studies were between 500 and 1360 mg/kg bw/d. Mortalities and CNS effects observed at doses $\geq$ 1200 mg/kg bw/d are considered to be related to metabolic acidosis after saturation of glycine and glucuronate conjugation pathways. Thus, the threshold dose that leads to metabolic acidosis and its related symptoms is not regarded to deteriorate from subchronic to chronic exposure.

The submitted data (NOAEL/LOAEL = 600/800 mg/kg bw/d, 25-d oral) further supports the conclusion, that dogs are of similar sensitivity to subacute benzoate toxicity as rats and show similar symptoms. In contrast, results by another study suggest that the mouse is less sensitive (NOAEL/LOAEL = 3750/4500 mg/kg bw/d, 10-d oral), but typical adverse effects were similar to that observed in the rat and included tremor/convulsions as well as liver toxicity.

The cat currently represents the most sensitive mammalian species tested. This sensitivity is thought to be related to species-specific deficiencies in benzoate metabolism paired with a cat-typical feeding behaviour. Therefore, the subacute NOAEL/LOAEL derived for cats (200/340 mg/kg bw/d, 15-d feeding) is not considered relevant for human risk assessment.

Four studies that aimed to assess the chronic toxicity of benzoic acid in rat and mice were submitted. Of these, only two rat studies are suitable for risk assessment, although these two studies do neither meet current EU nor OECD standards for chronic toxicity testing. Both did not go

beyond assessment of survival and clinical signs. Gross and histopathology in one study was performed after subchronic exposure and body weight was reported for an initial 12/8 wks (M/F) only. In this study, the top dose of 10,000 ppm, corresponding to approx. 500 mg/kg bw/d, did neither reduce lifetime survival nor produced overt clinical symptoms of benzoic acid poisoning. Feeding of 15,000 ppm (~750 mg/kg bw/d) benzoic acid over 18 months increased mortality and reduced body weight and body weight gain in comparison to control rats. Data obtained at later time points and haematological, biochemical or histopathological reports were not available for this study. In summary, it may be concluded that a dose of 500 mg/kg bw/d, as identified as safe in rodents for subchronic exposure, did neither affect survival nor produced clinical signs of benzoic acid poisoning when exposure time was extended. However, the available chronic toxicity data is not suitable to establish a NOAEL for target organ (liver, kidneys, brain) toxicity.

Mortalities observed at 750 mg/kg bw/d in one study and in subacute/subchronic studies at 1200/1500 mg/kg bw/d are considered to be related to metabolic acidosis after saturation of glycine and glucuronic acid conjugation pathways. This effect was also observed in acute studies and human case reports when high doses of benzoic acid/benzoate were administered repeatedly within 10-24 h. Thus, this effect is not regarded to aggravate from subacute/subchronic to chronic exposure.

Direct observations in healthy individuals (oral exposure) - Several studies from the 19th and the beginning of the 20th century regarding the oral exposure of humans to benzoic acid or sodium benzoate are described. However, owing to the low number of individuals exposed, the validity of these studies is limited. No adverse effects were reported after a single oral dose of 10.000 mg benzoic acid or up to 1000 mg/d over a period of up to 90 days. In another study no abnormalities were found in hematology, urine composition, nitrogen balance, or well-being of six men given 300-400 mg/d in the diet for up to 62 days. In another study with volunteers given 1000 mg/d for 5 days and subsequently increasing the dose in increments of 500 mg/d every 5 days to 2500 mg/d, signs of discomfort (nausea, headache, weakness, burning and irritation of oesophagus) were reported.

In nine patients on penicillin treatment given 12.000 mg benzoic acid divided into eight doses over 5 days in eight subjects and over 14 days in one subject, no adverse effects on blood urea nitrogen or creatinine clearance were reported.

Other authors compiled 1879 27 case reports of patients with acute rheumatic arthritis which were treated with 4 to 25 g/d benzoic acid without adverse effects."

"Adverse toxic effects of benzoic acid were usually observed after repeated bolus doses within a short time. This can be explained by metabolic interference following saturation of the conjugation pathways and/or depletion of glycine/glucuronate. Thus, it appears unlikely that prolongation of exposure from subchronic to chronic aggravates benzoic acid toxicity. In addition, no additional target organs or adverse effects were reported after chronic exposure, neither in animals nor in humans from the use as food additive or therapeutic drug. Therefore, a NOAEL of 500 mg/kg bw/d is considered as the relevant basis for setting a systemic reference dose for long-term exposure to benzoic acid."

As taken from European Commission, 2013a, b.

"Noncancer Oral Synopsis:

IPCS and U.S. EPA have evaluated the noncancer oral data for benzoic acid. EPA derived a reference dose (RfD) of 4 mg/kg-day. The estimated daily per capita intake of benzoic acid is 0.9 to 34 mg; at these levels, there are no reports of toxic effects in humans. Therefore, the upper end of this range, 34 mg, was considered to be the NOAEL for the basis of deriving a RfD. IPCS noted that short-term, subchronic, or chronic oral exposure studies conducted according to current guidelines are not available for benzoic acid or sodium benzoate. However, IPCS did identify a preliminary NOAEL of 500 mg/kg-day (highest dose tested) in a limited four-generation study in rats (Kieckebusch & Lang, 1960). Based on this NOAEL and an uncertainty factor of 100 (10 each for

uncertainty of the database and interspecies variation), IPCS derived a provisional tolerable intake (TI) of 5 mg/kg-day.

Noncancer Inhalation Synopsis:

IPCS has evaluated the noncancer inhalation toxicity data for benzoic acid, but did not derive a tolerable concentration (TC) due to an inadequate database.”

As taken from ITER, 2018.

“The use of benzoic acid in growing-finishing pigs (van der Peet-Schwering et al., 1999) was tested at 0, 10,000 and 20,000 mg/kg complete feed. The experiment was carried out with 60 pigs housed in individual pens, 10 barrows and 10 gilts per each experimental treatment. The study lasted 97 days (from average initial bw of 24.3 kg to an average final bw of 108.9 kg), but was not set up as a standard tolerance study. Data on the occurrence of diseases and/or lesions, therapeutic treatments and culling (date and weight of animal withdrawn and cause) were recorded for each animal. Individual weight and feed intake were recorded periodically, and growth rate and feed to gain ratio were calculated. No adverse effects on pig health or performance were observed in both treatment groups. Pigs supplemented with 10,000 and 20,000 mg benzoic acid/kg complete feed had less diarrhoea problems than control pigs.

In another study, the addition of 10,000 mg benzoic acid/kg feed to low and high protein diets was investigated (Buhler et al., 2006). Treatments were arranged in a 2 levels of protein × 2 rates of benzoic acid (0 vs 10 000 mg/kg feed) factorial design. A total of 24 crossbred barrows (26–106 kg body weight (bw), for 12 weeks) were included in the study, giving six pigs (replicates) per experimental group. The animals were fed restrictively grower and finisher diets, and effects on performance, nitrogen balance and urinary pH were investigated. The addition of benzoic acid did not influence weight gain, feed to gain ratio, nitrogen digestibility or nitrogen balance, reduced the urinary pH and increased the concentration of hippuric acid in urine.

Kluge et al. (2006) investigated the effects of benzoic acid on growth performance, nutrient digestibility, nitrogen balance and gastrointestinal microbiota of piglets. In a performance trial, piglets (28 days of age, 7.5 kg) were fed diets containing 0, 10,000 or 20,000 mg benzoic acid/kg complete feed for 35 days. Each dietary treatment was assigned to nine replicate groups, each consisting of two piglets. Body weight, daily weight gain, feed intake and feed conversion ratio were monitored. Supplementation of the diet with benzoic acid resulted in a dose-dependent increase in feed intake and body weight gain and an improved feed conversion ratio.”

As taken from EFSA, 2016c.

In subacute inhalation toxicity study in rats, the no observed adverse effect concentration (NOAEC) for systemic effects was 0.025 mg/L, whereas pulmonary fibrosis and inflammatory cell infiltrate were observed from 0.025 mg/L.

As taken from EFSA, 2016b

“In a 3-month study, two groups of 50 male and 50 female cross-bred white mice (no available information on strain) were given benzoic acid by oral gavage at a dose of 80 mg/kg bw per day (Shtenberg and Ignat’ev, 1970). Body weight gain was reduced in the mice receiving 80 mg benzoic acid/kg bw per day compared with controls, although food consumption was similar in the two groups. The highest mortality rate was observed in mice given a combination of benzoic acid and sodium bisulphite (30% survival after 2.5 months compared with 60% in controls); however, mortality was slightly lower in animals given benzoic acid compared with controls. A 5-day period of food restriction at 2.5 months induced 85% mortality as compared to a 56% mortality in the control group. The Panel noted that the mortality in the control group of this study is very high; hence, the results of the study are of limited value.”

As taken from EFSA, 2016a

"In subacute and short-term oral toxicity studies with several species including rats, mice, dogs and cats, benzoic acid and its salts were of low toxicity, with no marked organ toxicity. The most sensitive species was the cat, where the relevant no observed adverse effect level (NOAEL) is 200 mg/kg bodyweight (bw) per day (15- and 23-day studies). The cat is, however, considered a non-relevant species for humans. In the rat toxicity studies, the relevant oral NOAEL for benzoic acid is 500 mg/kg bw per day (16-week study), and for benzoate as well, it is 500 mg/kg bw day (5-week study). In subacute inhalation toxicity study in rats, the no observed adverse effect concentration (NOAEC) for systemic effects was 0.025 mg/L, whereas pulmonary fibrosis and inflammatory cell infiltrate were observed from 0.025 mg/L.

As taken from EFSA, 2016b.

"Oral: Based on the weight of evidence the chemical is not considered to cause serious damage to health by repeated oral exposure (no observed adverse effect level (NOAEL) of 825 mg/kg bw/d). Effects observed at > 1000 mg/kg bw/d included increased mortality, reduced weight gain, and liver and kidney effects (OECD, 2004).

The only available rat study for this chemical reported 2/20 mortalities at 1.2 mg/L 6 h/d (5 d/wk over 4 wk). Local reddish discharge around the nostrils and inflammatory cell infiltrates and interstitial fibrosis of the lung secondary to local irritant effects were also observed at 0.25 mg/L. On the basis of systemic effects, the NOAEC is considered to be > 0.25 mg/L 6 h/d (ECHA, 2011"

As taken from AICIS, 2013.

"The present study assessed the long-term daily administration of benzoic acid (BA), potassium sorbate (PS), chlorophyll (CPL), tartrazine (TAZ), and butylated hydroxyanisole (BHA) on hepato-renal changes and DNA damage in rats. Animals were orally administered with the 10 times of the acceptable daily intake (ADI) from each tested substance daily for 60 consecutive days. Blood, liver, and kidney samples were collected to evaluate hematological, biochemical, histopathological, and genotoxic alterations. The extent of liver and kidney damage was evaluated by comet assay and histopathologically. Significant reduction of leukocyte numbers and lymphocytes % in CPL- and TAZ-treated rats. However, significant increases in platelet count in all treated groups after 60 days were detected. The levels of serum transaminases enzymes (ALT, AST), alkaline phosphatase (ALP), and creatinine were significantly increased in all treatments except with BHA group, but no substantial differences were found in urea after 60 days. Aside from BHA, results of DNA damage revealed significant increases in tailed nuclei, tail moment, DNA% in the tail, and tail length in liver and kidney at different degrees. Moreover, the histopathological figures of liver and kidneys affirmed destructive and degenerative changes. The study indicates that most of the tested food additives may provoke genotoxicity and hepato-nephropathy, which could be serious for human health. Therefore, it is necessary to be informed about the hazardous effects of food additives and more attention should be focused towards using natural substitutes." As taken from Abo-El-Sooud K et al. 2018. Environ. Sci. Pollut. Res. Int. 25(26), 26341-26350. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/29981020>

Safety Evaluation

Quantitative Risk Type - RfD

Quantitative Risk Value – 4

Product Use – Not specified

Safety Evaluation Owner – US EPA IRIS

POD Method – NOAEL

POD Value – 4.4

POD Owner – US EPA IRIS

Critical study: RAT (Reproductive/Developmental Toxicity) Oral - dietary exposure for 1 GEN

NOEL/LEL Owner – US EPA IRIS | US FDA CFSAN

Original NOEL – 4.4 mg/kg bw/day

Original LEL – Not established

Critical Sites –

Critical Effects - NO EFFECTS

Safety Evaluation Comments: Human daily per capita intakes | 34 mg/day benzoic acid and 328 mg/day for sodium benzoate (converted to 312 mg/day benzoic acid) | NOAEL=4 mg/kg-bw/day for 70 kg human

Source Document: Regulatory document: #0355 (07/01/1993)

Additional PODs

POD Method	POD Value	POD Owner
NOAEL	Not Calculated	US EPA IRIS

Lowest observed effect:

Owner	Type	Value	Sites	Effects
US EPA IRIS	LOAEL	Not established	• CLINICAL SIGNS	• CLINICAL SIGNS

No-observed effect: US EPA IRIS: NOAEL: 40.0 mg/kg bw/day

Adjustment factors: Critical study: Target Organ Toxicity > Chronic Toxicity (Mouse, Oral - Dietary exposure) for 17 month

POD Method	POD Value	POD Owner
NOAEL	Not Calculated	US EPA IRIS

wner	Type	Value	Sites	Effects
US EPA IRIS	LOAEL	Not established	• FOOD/WATER CONSUMPTION CHANGES	• FOOD/WATER CONSUMPTION CHANGES

Adjustment factors: Critical study: Target Organ Toxicity > Chronic Toxicity (Rat, Oral - Dietary exposure) for 18 month

As taken from the COSMOS database available at <https://ng.cosmosdb.eu>

“Ingredient chemicals like fragrances may cause adverse health effects. Frequent health risk assessments and stringent management of consumer products are of paramount importance to reduce these serious occurrences. In this study, the respiratory and dermal health effects were assessed in relation to air fresheners. Twenty six fragrance ingredients, thirty four biocidal ingredients, and sixteen hazardous ingredients were analyzed and assessed according to their risk to human health on five groups by application type in eighty two air fresheners. For hazard characterization of ingredients, toxicological information on the intrinsic properties of the ingredients was collected, and reference values were determined as chronic NOAEL. Exposure assessment was performed in two steps. The 95th exposure factor values were used to estimate exposure to assume the worst-case scenario and the maximum concentration determined by the product purpose and application type was used type in tiered 1 assessment. The values input into the exposure algorithms were developed via the exposure route. In the tiered 2 assessment, the 75th exposure factor values were used to estimate the assumed reasonable exposure to ingredients. Six ingredients for the inhalation and twelve ingredients for the dermal route were conducted for tiered 2 assessment. This study showed that the assessed ingredients have no health risks at their maximum concentrations in air fresheners. The approach should be used to establish improved guidelines for specific ingredients that may pose inhalation and dermal hazard.” As taken from Kim

JH et al. 2018. Regul. Toxicol. Pharmacol. 98, 31-40. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/29857116>

“There are sufficient repeated dose toxicity data on benzoic acid. In addition to the key study used to determine a conservative NOAEL (below), additional studies on benzoic acid involving other routes of administration and varying lengths are summarized in Table 1 as weight of evidence (WoE). In a GLP-compliant study equivalent or similar to an OECD 412 subacute inhalation toxicity 28-day study, Sprague Dawley CD rats (10 rats/sex/dose) were exposed to benzoic acid (purity not reported) at concentrations of 0, 25, 250, or 1200 mg/m³ (equivalent to 0, 6.48, 64.83, or 311.19 mg/kg/day, respectively) for 6 h/day, 5 days/week, through whole-body exposure over 4 weeks. Parameters that were evaluated included clinical signs (twice daily), body weight (prior to exposure and thereafter weekly), serum biochemistry, hematology, organ weights, and histopathology. At the highest (1200 mg/m³) dose, mortality in 2 rats, decreased body weight, statistically significantly decreased platelets, decreased absolute/relative liver weights, and decreased relative weight of trachea with lungs (females only) were reported. At the highest dose, absolute kidney weight and body weight were reported to be slightly decreased (though not significantly) in females compared to controls. No treatment-related gross lesions were reported in any of the tested doses for the following organs: adrenal, nasal turbinate, brain, pancreas, colon, pituitary, esophagus, prostate/uterus, the eye with the optic nerve, submaxillary salivary gland, testis (both), ovary, jejunum, Harderian glands, spleen, heart, sternum (bone marrow), kidney, stomach, liver, thymus, lungs (5 lobes), thyroid/parathyroid, bronchial lymph node, urinary bladder, and mammary gland. Treatment-related but not dose-dependent microscopic lesions were reported, which included increased inflammatory cell infiltrate and increased incidence, intensity, and extent of interstitial fibrosis in the lungs of animals from the low-, mid-, and high-dose groups. The interstitial fibrosis in the lungs was due to a local corrosive property of benzoic acid through the inhalational route. In both mid- and high-dose groups, reddish discharge around the nares was reported. At the mid (250 mg/m³) dose, upper respiratory tract irritation was observed, which was confirmed by inflammatory exudate around the nares. Based on the presence of systemic effects observed at 1200 (the highest tested dose) and 250 mg/m³, the no observed adverse effect concentration (NOAEC) was considered to be 25 mg/m³, although local effects were observed at low dose predominantly due to the local corrosive property of benzoic acid (ECHA, 2011)”

Api AM et al. (2022). RIFM fragrance ingredient safety assessment, benzoic acid, CAS Registry Number 65-85-0.

“Sodium benzoate is a widely used food and pharmaceutical preservative due to its antibacterial and antifungal activity. In the present study effect of different concentrations of sodium benzoate on hepatic antioxidants, inflammatory cytokines (TNF- α , IFN- γ , IL-1 β and IL-6), biochemical markers and histopathology of liver was evaluated. Twenty five adult rats (aged 1-2 months) with 5 rats per group were randomly distributed into 5 groups. Group 1 rats were used as control and all groups (1-5) were provided with water and fed ad libitum. In addition to usual water and food, rats of group 2, 3, 4 and 5 were treated with 70, 200, 400 and 700 mg/kg b.wt of sodium benzoate once a day via oral gavage for 30 days. Our results showed that activity of glutathione peroxidase (GPx), catalase (CAT), glutathione-s-transferase (GST), glutathione reductase (GR) and superoxide dismutase (SOD) in rats decreased significantly when treated with 200, 400 and 700 mg/kg b.wt of sodium benzoate. Increase in the concentration of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum total protein, albumin, globulin, urea and creatinine was found to be dose dependent. Severe histopathological damage was observed in the hepatic tissue at higher concentrations of sodium benzoate. It was noticed that high concentrations of sodium benzoate (200, 400 and 700 mg/kg b.wt) produce significant increase in inflammatory cytokine markers (TNF- α , IFN- γ , IL-1 β and IL-6) in comparison to control. Sodium benzoate at concentration of 70 mg/kg b.wt did not produce any significant changes in any of the above studied parameters.”

Khan, I. S. et al. (2022). Toxicological impact of sodium benzoate on inflammatory cytokines, oxidative stress and biochemical markers in male Wistar rats.

Male albino Wistar rats (n = 4 per group) were fed increasing amounts of sodium benzoate either alone or in combination with ascorbic acid to study effects on haematological parameters (19). Treatment with sodium benzoate alone consisted of 1.0 or 10 mg/kg bw per day for 21 non-consecutive days (not stated how many days required to achieve 21 treatment days). No statistically significant changes were reported in animals at the lower dose, while the higher dose induced an anaemic condition. The authors also reported high white blood cell counts among animals fed the basal diet alone, suggesting biased statistical significance for this parameter. It was not clear whether the high white blood cell counts indicated an infection in control animals, which would have affected other parameters measured in this study. Short-term studies on sodium benzoate evaluated previously by the Committee showed increased mortality in mice given 3 g/day and in rats at a dose of 5% in the diet (Annex 1, reference 32). Other reported effects in rats were body-weight loss, reduction of phospholipids in the liver and of the potassium concentration in skeletal muscle, loss of coordination, tremor and convulsions. Addition of glycine to the diet reduced the toxic effects. In guinea-pigs, doses of benzoate plus benzoic acid of 150 mg/kg bw (not clear whether this was the only or the highest dose tested) given for 65 days had no adverse effects.

JECFA (2022)

5.3. Reproduction toxicity

“Overall, there is no indication for developmental or reproductive toxicity of benzoic acid in rats below the high dose that revealed severe maternal toxicity (1610 mg/kg bw/d”

As taken from ECHA, 2012a.

In a four-generation study with male and female rats, no adverse effects on fertility or lactation (only investigated parameters) were seen after dosing with benzoic acid at up to 1% in the diet (approximately 500 mg/kg body weight per day) Kieckebusch & Lang, 1960).

In studies with repeated oral application, no effects on the testes were observed in rats after dosing with benzoic acid at up to 647 mg/kg body weight per day in the diet for 4 weeks or in mice after lifelong application of 6200 mg sodium benzoate/kg body weight per day via drinking-water

In a study with pregnant rats given only one oral dose of benzoic acid (510 mg/kg body weight on gestation day 9), there was no indication of an increase in resorption rates or malformations (Kimmel et al., 1971).

Studies performed with eggs of leghorn hens (single injection of 5 mg per egg), chick embryo neural retina cells (lowest-observed-effect concentration [LOEC] of 34.7 mmol/litre), and a chick embryotoxicity screening test (single injection of <0.1 mg per embryo) gave no indication of embryotoxic or teratogenic effects (Verrett et al., 1980; Jelinek et al., 1985; Daston et al., 1995).

As taken from INCHEM, 2000.

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ Benzoic acid at doses of 6, 30, 60, and 600 mg/kg was administered by stomach tube to groups of 21 to 24 pregnant golden hamsters on GDs 6 to 10. Two negative-control groups were maintained; one was treated with water, the other with 0.5% carboxymethyl cellulose. A positive-control group received either thalidomide or aspirin. Dams were killed on day 16. No adverse effect in maternal survival was noted. A significant number of resorptions was noted in hamsters which received /greater than or equal to/ 30 mg/kg. The incidence of fetal malformations reached statistical significance at >600

mg/kg. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ Benzoic acid at doses of 5, 25, 50, and 500 mg/kg was administered by stomach tube to groups of 20 pregnant Wistar rats on GDs 6 to 15. Two negative-control groups were maintained; one was treated with water, the other with 0.5% carboxymethylcellulose (used to keep the benzoic acid in suspension). A positive control group received either thalidomide or aspirin. Dams were killed on day 21. Maternal survival was similar for treated and control groups. A significant number of resorptions /were/ noted in rats which received /greater than or equal to/ 25 mg/kg. The incidence of fetal malformations in /benzoic acid/ treated rats did not reach statistical significance. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ In a four-generation study with male and female rats, no adverse effects on fertility or lactation (only investigated parameters) were seen after dosing with benzoic acid at up to 1% in the diet (approximately 500 mg/kg bw/day). In studies with repeated oral application, no effects on the testes were observed in rats after dosing with benzoic acid at up to 647 mg/kg bw/day in the diet for 4 weeks or in mice after lifelong application of 6200 mg sodium benzoate/kg bw/day via drinking-water. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

As taken from HSDB, 2014.

"Embryotoxicity and teratogenicity of benzoic acid when administered at a single dose of 510 mg/kg bw/d on day 9 of gestation to 7 pregnant rats were evaluated. Under these experimental conditions, there were no indications for embryotoxic or teratogenic activity of benzoic acid. Interestingly, it was noted that high doses of benzoic acid may increase the teratogenicity of other substances, presumably via toxicokinetic interactions by delaying the elimination of these. Nevertheless, this study can not be regarded suitable as the basis for assessment of developmental toxicity (single dose on day 9 only, low no. of animals).

In a developmental neurotoxicity study, sodium benzoate was administered to rats at a similar top dose of 500 mg/kg bw/d sodium benzoate from day 5 of gestation through lactation and after weaning. Mortality and bw of parental animals, mortality of embryos and fetuses as well as survival, activity and bw development of offspring remained unaffected. No teratogenicity was reported, but this endpoint was not addressed specifically.

Another study reported a slight reduction in offspring survival of 5 % and a decrease in lactation rate at the higher dose of 1000 mg/kg bw/d in Wistar rat.

Overall, there is currently no indication for developmental or reproductive toxicity of benzoic acid in rats at or below the NOAEL of 500 mg/kg bw/d for subchronic toxicity."

As taken from European Commission (2013a,b).

"No effects on reproduction were observed in a 5 generation study on crossbred white mice where animals were administered with 40 mg/kg bw/day benzoic acid for 8 months prior to breeding."

As taken from NICNAS, 2015.

Embryo implantation is the crucial step for a successful pregnancy. Diverse factors, including adhesion molecules, growth factors, and cytokines are important for embryo implantation through improving endometrial receptivity. Benzoic acid (BA), a component of various plants, has been shown to have antifungal and antioxidant effects. However, the effect of BA on embryo implantation remains unknown. Here, we showed the contribution of BA for the enhancement of endometrial receptivity through the leukemia inhibitory factor (LIF)-dependent increase of integrin α V, β 3, and

$\beta 5$ expression. Furthermore, in vivo study using a mifepristone-induced implantation failure model showed that BA definitely improves the numbers of implantation embryos. Taken together, we suggest that BA has a novel function for embryo implantation through the up-regulation of LIF-mediated integrins, and may be a candidate for therapeutic medicine to increase the pregnancy rate.” As taken from Choi HJ et al. 2017. J. Microbiol. Biotechnol. 27(4), 668-677. PubMed, 2018

“In a multi-generation reproductive toxicity study on benzoic acid, which also included limited investigations into the chronic toxicity of benzoic acid, three groups of 20 male and 20 female white rats were pair fed for 8 weeks on a standard rat diet, prepared as a feed mix with either 0, 0.5 or 1% benzoic acid (Kieckebusch and Lang, 1960). The authors did not provide information regarding the strain of the rat used, only stating that they were sourced from Bayer, Elberfeld (Germany). The rats were fed ad libitum over four generations resulting in an approximate intake of benzoic acid of 0, 250 or 500 mg/kg bw per day. Two generations were fed for their whole lifespan, the third and fourth generations were autopsied after 16 weeks. No treatment-related effects were observed on growth, fertility, lactation throughout all four generations and lifespan (for the first two generations). On the contrary, ad libitum ingestion of the 0.5% benzoic acid feed mix resulted in a significantly longer lifespan in the first and second generation of the rats, based on statistical analysis of the two generations combined. In the third generation, autopsied at 16 weeks, no treatment-related effects were reported on organ weights and histopathological findings in organs. The assessment of reproductive parameters was considered to be valid by the Panel, and provided a NOAEL, for both the parental animals and the offspring over four generations, of 500 mg benzoic acid/kg bw per day, the highest dose tested.

“Groups of 25 pregnant rats were given benzoic acid (purity 96.7%) by gavage from GD 7–16 dose levels of 0, 30, 160, or 450 mg benzoic acid/kg of body weight in a 0.5% aqueous suspension of methyl cellulose (EPA, 1992 (Doc. provided to EFSA n. 5)). Overt maternal toxicity occurred at 450 mg/kg as indicated by death in four rats, significant depression in body weight gain and feed consumption during the dosing period, an increased incidence of ruffled fur and increased relative liver weight on post-mortem examination. Haemorrhages of the gastric mucosa were observed in three of the four rats that died. A transient but significant decrease in body weight gain also occurred in dams in the 160 mg/kg group during the first 4 days of dosing. The number of pregnant females at caesarean section was 16, 14, 18 and 19 for the control, low-, mid- and high-dose, respectively.

No compound-related effects on reproductive parameters were demonstrated. Fetal weight was significantly decreased in the 160 and 450 mg/kg groups. The Panel noted that the effect on fetal weight in the mid-dose group was related to the higher number of fetuses per litter in this group when compared with the control group; calculated mean litter weight of the mid-dose group was 10% higher than in the control group. Significant increases in malformations, fetal developmental variations and variations due to retarded development were observed in the group administered 450 mg benzoic acid/kg bw per day.

The authors considered 30 mg benzoic acid/kg bw per day as the no observed effect level (NOEL) for the dams based on the effect on body weight gain at 160 mg/kg bw per day. The NOEL for the foetuses was also 30 mg/kg bw per day based on the decreased fetal weight at the mid-dose. The Panel considered the transitory effect on maternal body weight gain in the mid-dose group not relevant for identification of the NOAEL. Owing to a higher number of fetuses in the low-dose group compared to the control group, the decreased mean fetal weight in combination with the increased mean litter weight was not considered to be a developmental toxic effect by the Panel. The only fetal alterations demonstrated to be dose related and significantly increased above the control incidence occurred in the 450 mg/kg group in the presence of overt toxicity and maternal death. Therefore, the Panel considered that 160 mg benzoic acid/kg bw per day was the NOAEL for maternal and developmental toxicity in this study.”

“In another developmental toxicity study in rats with benzoic acid by gavage, a NOAEL of 160 mg benzoic acid/kg bw per day was observed for maternal and developmental toxicity (EPA, 1992 (Doc. provided to EFSA n. 5))”

“The four-generation reproductive toxicity study (Kieckebusch and Lang, 1960) with benzoic acid in the diet in rats was considered by the Panel as the pivotal study, as this study was the longest exposure period as compared to the developmental studies. This study showed no effect on growth, fertility, lactation or survival, and provided a NOAEL, for both the parental animals and the offspring, of 500 mg benzoic acid/kg bw per day, the highest dose tested.”

As taken from EFSA, 2016a

“In reproductive toxicity studies, fertility and overall reproductive performance were not impaired; developmental toxicity studies in rats with sodium benzoate in the diet showed a NOAEL of 500 mg/kg bw per day comparable to the NOAEL of the dietary four-generation reproductive toxicity in rats with benzoic acid”

As taken from EFSA, 2016b

“As summarised by SCCP (2005), various Committees concluded that the data reviewed for compounds in this group were sufficient to demonstrate lack of teratogenic, reproductive or carcinogenic potential.”

As taken from SCENIHR, 2016.

“In rats, no action on the growth, fertility, lactation or survival has been observed during a multigeneration reproductive study (because of the consumption of glycine occurring during elimination of benzoic acid and the possibility that glycine is essential for growth, a detrimental effect on growth could be envisaged”

As taken from EMA, 2017.

“No evidence of reproductive or developmental toxicity was observed for the chemical.”

As taken from AICIS, 2013.

Safety Evaluation

Quantitative Risk Type - MOS

Quantitative Risk Value - 206

Product Use – Not specified

Safety Evaluation Owner – EU SSC

POD Method - NOAEL

POD Value – 500.0

POD Owner - COSMOS TTC (NON-CANCER)

Critical study: RAT (Reproductive/Developmental Toxicity) Oral - Dietary exposure for 1 GEN

NOEL/LEL Owner - SCCP

Original NOEL - 500.0 mg/kg bw/day

Original LEL – Not established

Critical Sites –

Critical Effects – NO EFFECTS

Safety Evaluation Comments: 100% dermal absorption was assumed

Source Document: Regulatory document: SCC Opinion: SCCP/0891/05

Safety Evaluation

Quantitative Risk Type - Not calculated

Quantitative Risk Value - Not calculated

Safety Evaluation Owner - COSMOS TTC (NON-CANCER)

Critical study: RAT (Reproductive/Developmental Toxicity) Oral - Dietary exposure for 1 GEN

Safety Evaluation Comments: Benzoic acid is registered at FDA as 21 CFR 184.1021 (antimicrobial agent). In September 2013, a report "Regulation (EU) n°528/2012 concerning the making available on the market and use of biocidal products" was published. The derivation of an Acceptable Daily Intake (ADI) is considered not necessary in the context of biocidal use. For the use as a Plant Protection Product an ADI of 5 mg/kg bw was derived by JECFA (1983) and EU (2002, 2005), based on the NOAEL of 500 mg/kg bw/d from subacute/subchronic and teratogenicity studies and an assessment factor of 100.

Source Document: no source document available.

Additional PODs:

POD Method	POD Value	POD Owner
NOEL	887.0	MUNRO

Lowest observed effect:

Owner	Type	Value	Sites	Effects
MUNRO	LOEL	Not established	• NO EFFECTS	• NO EFFECTS

No-observed effect: MUNRO: NOEL: 887.0 mg/kg bw/day

Adjustment factors: Critical study: Reproductive/Developmental Toxicity > Multigeneration Reproductive Toxicity (Rat, Oral - Dietary exposure) for 4 GEN

POD Method		POD Value	POD Owner	
NOAEL		Not Calculated	SCF	
Owner	Type	Value	Sites	Effects
SCF	LOAEL	Not established	• NO EFFECTS	• NO EFFECTS

No-observed effect: SCF: NOAEL: 750.0 mg/kg bw/day

POD Method	POD Value		POD Owner	
NOAEL	Not Calculated		US FDA CFSAN	
Owner	Type	Value	Sites	Effects
US FDA CFSAN	LOAEL	Not established	• NO EFFECTS	• NO EFFECTS

No-observed effect: US FDA CFSAN: NOAEL: 175.0 mg/kg bw/day

Adjustment factors: Critical study: Reproductive/Developmental Toxicity > Developmental Toxicity (Rat, Oral - Gavage/Intubation exposure) for 10 GDAs taken from the COSMOS database available at <https://ng.cosmosdb.eu>

The estrogenic activity of benzoic acid was evaluated in the recombinant yeast human estrogen receptor (ERα) assay in vitro and in uterotrophic assays involving immature female Alpk:APfSD rats (21-22 days old) and immature female Alpk:APfCD-1 mice. 70 Immature mouse uterotrophic assays involved 3 daily SC injections of benzoic acid in corn oil (100 and 1,000 mg/kg doses; 5 mL/kg =

dose volume) ending on day 4. The same protocol was used for the rat assays, with the exception that benzoic acid in arachis oil was injected (doses of 10, 100, and 1,000 mg/kg). Benzoic acid (10^{-7} to 10^{-3} M) was negative in the recombinant yeast human ER α assay in vitro. Overall, benzoic acid produced one statistically significant and 3 statistically non-significant increases in average uterine weight (rat assay) and 1 significant and 6 non-significant decreases in average uterine weight (mouse assay). Therefore, in all uterotrophic assays, benzoic acid did not produce any reproducible evidence of estrogenic activity. Results for vehicle controls were negative and the positive control, estradiol, was uterotrophic (Jonhson et al., 2017).

“In a developmental toxicity study with sodium benzoate in rats, foetotoxic effects were observed at 1850 mg/kg body weight and day. The NOAEL was 1340 mg/kg body weight and day. In mice, rabbits and hamsters, no developmental toxicity from sodium benzoate was detected in the foetuses of dams treated with up to 175, 250 or 300 mg/kg body weight and day, respectively. The differences between the NOAEL for rats, mice, rabbits and hamsters scaled to an inhalation concentration at the workplace and the MAK value are considered so large that damage to the embryo or foetus is unlikely when the MAK value is observed. Therefore, benzoic acid and alkali benzoates are classified in Pregnancy Risk Group C.”

“Prenatal toxicity: For benzoic acid, only three studies are available (Kimmel et al. 1971; Nair 2001). These were not carried out according to valid guidelines and have serious shortcomings, such as the use of only one dose and treatment on only one gestation day or the lack of information about early or late resorptions. The systemic effects of benzoic acid, however, are expected to be similar to those of its salts, as these effects are most likely mediated via the benzoate formed endogenously. In addition, the MAK value for benzoic acid of 0.5 mg/m³ is lower than that for the benzoates, so that the difference between this and the NOAEL for the developmental toxicity of the benzoate is greater by a factor of 20. In view of this greater difference, benzoic acid is likewise classified in Pregnancy Risk Group C.”

As taken from Hartwig A and MAK Commission, 2018

“The use of bisphenol A (BPA) is restricted due to its reproductive toxicity and endocrine disrupting (ED) properties. The public concern and regulatory restrictions on BPA stimulated the development of alternative substances to replace BPA. The aim of this study is to review the available data on carcinogenic, mutagenic, reproductive toxicity, and ED properties of BPA alternatives used in consumer products. The focus is on the potential hazard for (young) children and/or pregnant women. An inventory of known potential alternative substances (n = 99) was made, of which 20 were prioritized based on reported use by the general population. For all the selected alternatives, data on ED potential, carcinogenicity and reproductive toxicity was very limited or even absent (i.e. Tefacid Stearic 95, Bisphenol C, AP, and P). For the alternative substances bisphenol S (BPS), bisphenol AF (BPAF), p-tert-butylphenol and to a lesser extent bisphenol F (BPF), fluorine-9-bisphenol (BHPF), bisphenol E, M, and Z (BPE, BPM, BPZ), Irganox 1076, and butylated hydroxytoluene (BHT), the data indicates a reproductive toxicity hazard with a possible ED mode of action. 3,3',5,5'-Tetrabromobisphenol A (TBBPA) tested positive for carcinogenicity. Data gaps are present for most of these substances. In this study, data on reproductive toxicity and/or ED potential were only negative, although not complete, for benzoic acid and Irganox 1010, tetra methyl bis phenol F (TMBPF) and bisphenol-A bis(diphenyl phosphate) (BDP). A full evaluation of all data, including in vitro data, is recommended to guide targeted testing prioritization.” As taken from den Braver-Sewradj SP et al. 2020. Crit. Rev. Toxicol. 50(2), 128-147. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/32031044/>

MAK pregnancy risk group classification: Group C: There is no reason to fear damage to the embryo or foetus when MAK and BAT values are observed (ACGIH, 2021a).

“Benzoic acid (BA) was administered in the diet to male and female Sprague Dawley Crl:CD(SD) rats in an OECD Test Guideline 443 Extended One-Generation Reproductive Toxicity (EOGRT) study to test for effects that may occur as a result of pre- and postnatal exposure. The study

included cohorts of F1 offspring to evaluate potential effects of benzoic acid on reproduction, the developing immune system, and the developing neurological system with the inclusion of learning and memory assessments. Benzoic acid was incorporated in the diet at concentrations of 0, 7,500, 11,500, and 15,000 mg/kg diet (ppm). These concentrations were selected based on the results of preliminary studies, and, based on average food consumption, were intended to supply BA doses of approximately 0, 500, 750, and 1000 mg/kg bw/day. To avoid exceeding these target dose levels, the dietary concentrations were adjusted (based on historical control body weight and food consumption data) to maintain the target mg/kg bw/day dose levels during those life periods when food intake per unit of body weight was increased to support milk production by females (gestation and lactation) and rapid pup growth (post-weaning). In the parental (F0) generation, survival, clinical observations, organ weights, pathology, hematology, serum chemistry, urinalysis, and bile acids were unaffected by BA administration. Reproductive parameters were also unaffected by BA administration. In the F1 generation, survival, growth and developmental landmarks, organ weights, pathology, immunotoxicity assessment, and neurotoxicity and neurobehavioral parameters such as auditory startle response, locomotor activity, learning and memory assessments were unaffected by BA administration, as were clinical pathology (hematology, serum chemistry, urinalysis, bile acids and thyroid hormones) and reproductive performance. Similarly, no adverse effects or systemic toxicity were observed in the F2 generation. Overall, the highest dietary concentration (15,000 ppm), providing a dosage of approximately 1000 mg/kg bw/day, was the NOAEL for benzoic acid in this EOGRT study.” As taken from Turnbull D et al. 2021. Regul. Toxicol. Pharmacol. 122, 104897. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33639256/>

“There are sufficient reproductive toxicity data on benzoic acid. In an OECD 443/GLP Extended One-Generation Reproductive Toxicity (EOGRT) study, conducted in Sprague Dawley rats, groups of 25 rats/sex/dose were administered benzoic acid in the diet at concentrations 0, 7500, 11,500, or 15,000 ppm (Equivalent to 526, 821, and 1069 mg/kg/day for P0 males and females, 499, 747, and 987 mg/kg/day for F1 males and 535, 790, and 1024 mg/kg/day for F1 females). Males were administered benzoic acid in the diet for 2 weeks prior to mating and continuing until euthanasia (10–11 weeks) for 6 weeks (2 weeks prior to mating) and females were treated for 6 weeks (2 weeks before mating, throughout gestation, and for 4 days after parturition). For females, benzoic acid was continuously administered in the diet for 2 weeks prior to mating and continuing throughout mating (2 weeks), gestation (3 weeks), and lactation (3 weeks), until euthanasia (10–11 weeks). In addition, the offspring selected for the F1 and F2 generations were administered benzoic acid in the diet beginning at weaning and continuing until euthanasia. In the parental generation, no treatment-related effects were seen on survival, clinical observations, organ weights, and necropsy/histopathology. Reproductive parameters were also unaffected by benzoic acid administration. In the F1 generation, no treatment-related effects were seen on survival, growth and developmental landmarks, organ weights, or necropsy/histopathology. In addition, clinical pathology (hematology, serum chemistry, urinalysis, bile acids, and thyroid hormones) and reproductive performance were also unaffected. Similarly, no adverse effects were observed in the F2 generation. Therefore, the NOAEL for developmental toxicity and fertility in the current study was 15,000 ppm (equivalent to 1069 mg/kg/day in males and females), the highest dose tested (Turnbull et al., 2021).

There are sufficient reproductive toxicity data on benzoic acid. In a non-GLP, 4-generation oral reproductive toxicity study, groups of 20 rats/sex/dose/generation were fed diets containing benzoic acid at doses of 0%, 0.5%, and 1% (equivalent to 0, 450, and 900 mg/kg/day for males and 0, 600, and 1176 mg/kg/day for females, as per the ECHA Dossier). The first generation was exposed for 8 weeks and then allowed to mate (1:1 for a period of 14 days). Mating was repeated in week 48 to raise a second litter. The first and second generations were treated for a lifetime; the third generation was treated for 16 weeks, and the fourth generation was treated up to breeding. No treatment-related adverse effects on fertility or the development of pups were reported in all 4 generations. The NOAEL for fertility effects and maternal and developmental toxicity was considered to be 1%, the highest dose tested (Kieckebusch and Lang, 1960; also available on

ECHA, 2011). The most conservative NOAEL of 900 mg/kg/day was selected for the reproductive toxicity endpoint for this study.

Benzoic acid did not cause any adverse effects in the fertility or the development of pups in the 4-generation study. In addition, sodium benzoate has also been extensively reviewed by SCCP (2005), OECD (2001), WHO (2005), and EFSA, in which studies on different species have been conducted; see below for a summary. Results from these studies indicate that embryotoxic and fetotoxic effects, as well as malformations, were observed only at doses that induced severe maternal toxicity. In a dietary study in rats, a NOAEL of 1310 mg/kg/day was established for sodium benzoate. Thus, sodium benzoate is unlikely to have adverse developmental effects at dose levels not toxic to the mother.”

Api AM et al. (2022). RIFM fragrance ingredient safety assessment, benzoic acid, CAS Registry Number 65-85-0.

“Although benzaldehyde, an aromatic aldehyde, has been declared safe for uses in food, conflicting reports exist regarding its genotoxic and cytotoxic potentials in organisms. Our present study is the first attempt to evaluate the effects of exposure of benzaldehyde on the entire course of development of a eukaryote model organism, *Drosophila melanogaster*. Total time required for the initial appearance of the third instar larvae, pupae and adults increased dose dependently with the increasing dietary concentration of benzaldehyde. Exposure of flies to each concentration of benzaldehyde caused dose-dependent and significant reductions in the population of pupae and young adults of the fly. Developmental inhibition was associated with dose dependent and significant structural aberrations of larval polytene chromosomes like ectopic pairing, inversion, fusion, etc., and deformities of hemocytes and neuroblasts and death of hemocytes. As much as 34% (SD $\pm$ 1.76)-52% (SD $\pm$ 1.7) and 18% (SD $\pm$ 2.5)-40% (SD $\pm$ 3.38) hemocytes and neuroblasts, respectively, underwent nuclear deformations in response to dietary exposures of flies to BA 100-1000 mg/l. Moreover, 16% (SD $\pm$ 0.52)-31% (SD $\pm$ 1.97) and 19% (SD $\pm$ 0.3)-33% (SD $\pm$ 1.78) hemocyte mortalities in response to BA 100-1000 mg/l were determined by two cell viability assays. Thus our study revealed that benzaldehyde was genotoxic to *Drosophila melanogaster* larvae that might be responsible for larval cell death and their subsequent developmental retardation. As this fly possesses substantial genetic homology with human, possibility of developmental inhibition of the later due to exposure of this chemical during pregnancy may not be ruled out.”

Kumar Ghosh, S. et al. (2022). Benzaldehyde-induced developmental genotoxicity triggers both neural and non-neuronal cells including the cells of immunity in *Drosophila melanogaster*.

Reproduction / development effects: yes, known to cause a problem. PPDB, 2024.

5.4. Mutagenicity

“There is no evidence for mutagenic potential in vivo. Most in vitro tests are also negative and only chromosomal aberration tests in mammalian cell cultures scored repetitively as positive.”

As taken from ECHA, 2012a.

Mutagenicity of Benzoic Acid 65-85-0:	
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA97A
Metabolic Activation:	NONE
Method:	PREINCUBATION
Results:	NEGATIVE

Reference: [FUJITA,H AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97A AND TA102. (I); KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 37:447-452, 1986]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA102

Metabolic Activation: NONE

Method: PREINCUBATION

Results: NEGATIVE

Reference: [FUJITA,H AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97A AND TA102. (I); KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 37:447-452, 1986]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA97A

Metabolic Activation: RAT, LIVER, S-9, AROCLOR 1254

Method: PREINCUBATION

Results: NEGATIVE

Reference: [FUJITA,H AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97A AND TA102. (I); KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 37:447-452, 1986]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA102

Metabolic Activation: RAT, LIVER, S-9, AROCLOR 1254

Method: PREINCUBATION

Results: NEGATIVE

Reference: [FUJITA,H AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97A AND TA102. (I); KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 37:447-452, 1986]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA100

Metabolic Activation: NONE

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS; ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA100

Metabolic Activation: HAMSTER, LIVER, S-9, AROCLOR 1254 (10 OR 30%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;

ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA100

Metabolic Activation: RAT, LIVER, S-9, AROCLOR 1254 (10 OR 30%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA1535

Metabolic Activation: NONE

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA98

Metabolic Activation: NONE

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA1535

Metabolic Activation: HAMSTER, LIVER, S-9, AROCLOR 1254 (10 OR 30%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA1535

Metabolic Activation: RAT, LIVER, S-9, AROCLOR 1254 (30%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA1537

Metabolic Activation: HAMSTER, LIVER, S-9, AROCLOR 1254 (10 OR 30%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA1537

Metabolic Activation: RAT, LIVER, S-9, AROCLOR 1254 (30%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA1535

Metabolic Activation: RAT, LIVER, S-9, AROCLOR 1254 (10%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA1537

Metabolic Activation: RAT, LIVER, S-9, AROCLOR 1254 (10%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA97

Metabolic Activation: NONE

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA97

Metabolic Activation: HAMSTER, LIVER, S-9, AROCLOR 1254 (10 OR 30%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA97

Metabolic Activation: RAT,LIVER, S-9, AROCLOR 1254 (10 OR 30%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA1537

Metabolic Activation: NONE

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA98

Metabolic Activation: HAMSTER, LIVER, S-9, AROCLOR 1254 (10 OR 30%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA98

Metabolic Activation: RAT, LIVER, S-9, AROCLOR 1254 (30%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K;
SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS;
ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA98

Metabolic Activation: RAT, LIVER, S-9, AROCLOR 1254 (10%)

Method: PREINCUBATION

Results: NEGATIVE

Reference: [ZEIGER,E, ANDERSON,B, HAWORTH,S, LAWLOR T AND MORTELMANS,K; SALMONELLA MUTAGENICITY TESTS: IV. RESULTS FROM THE TESTING OF 300 CHEMICALS; ENVIRON. MOL. MUTAGEN. 11(SUPPL.12):1-158, 1988]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA98

Metabolic Activation: NONE

Method: PREINCUBATION

Results: NEGATIVE

Reference: [KUBOYAMA,N AND FUJII,A; MUTAGENICITY OF ANALGESICS, THEIR DERIVATIVES, AND ANTI- INFLAMMATORY DRUGS WITH S-9 MIX OF SEVERAL ANIMAL SPECIES; J. NIHON UNIV. SCH. DENT. 34(3):183-195, 1992]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA98

Metabolic Activation: RAT, LIVER, S-9, PCB

Method: PREINCUBATION

Results: NEGATIVE

Reference: [KUBOYAMA,N AND FUJII,A; MUTAGENICITY OF ANALGESICS, THEIR DERIVATIVES, AND ANTI-INFLAMMATORY DRUGS WITH S-9 MIX OF SEVERAL ANIMAL SPECIES; J. NIHON UNIV. SCH. DENT. 34(3):183-195, 1992]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA98

Metabolic Activation: MOUSE, LIVER, S-9, PCB

Method: PREINCUBATION

Results: NEGATIVE

Reference: [KUBOYAMA,N AND FUJII,A; MUTAGENICITY OF ANALGESICS, THEIR DERIVATIVES, AND ANTI-INFLAMMATORY DRUGS WITH S-9 MIX OF SEVERAL ANIMAL SPECIES; J. NIHON UNIV. SCH. DENT. 34(3):183-195, 1992]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA98

Metabolic Activation: GUINEA PIG, LIVER, S-9, PCB

Method: PREINCUBATION

Results: NEGATIVE

Reference: [KUBOYAMA,N AND FUJII,A; MUTAGENICITY OF ANALGESICS, THEIR DERIVATIVES, AND ANTI-INFLAMMATORY DRUGS WITH S-9 MIX OF SEVERAL ANIMAL SPECIES; J. NIHON UNIV. SCH. DENT. 34(3):183-195, 1992]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA98

Metabolic Activation: HAMSTER, LIVER, S-9, PCB

Method: PREINCUBATION

Results: NEGATIVE

Reference: [KUBOYAMA,N AND FUJII,A; MUTAGENICITY OF ANALGESICS, THEIR DERIVATIVES, AND ANTI-INFLAMMATORY DRUGS WITH S-9 MIX OF SEVERAL ANIMAL SPECIES; J. NIHON UNIV.

SCH. DENT. 34(3):183-195, 1992]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA100

Metabolic Activation: NONE

Method: PREINCUBATION

Results: NEGATIVE

Reference: [KUBOYAMA,N AND FUJII,A; MUTAGENICITY OF ANALGESICS, THEIR DERIVATIVES, AND ANTI INFLAMMATORY DRUGS WITH S-9 MIX OF SEVERAL ANIMAL SPECIES; J. NIHON UNIV. SCH. DENT. 34(3):183-195, 1992]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA100

Metabolic Activation: RAT, LIVER, S-9, PCB

Method: PREINCUBATION

Results: NEGATIVE

Reference: [KUBOYAMA,N AND FUJII,A; MUTAGENICITY OF ANALGESICS, THEIR DERIVATIVES, AND ANTI-INFLAMMATORY DRUGS WITH S-9 MIX OF SEVERAL ANIMAL SPECIES; J. NIHON UNIV. SCH. DENT. 34(3):183-195, 1992]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA100

Metabolic Activation: MOUSE, LIVER, S-9, PCB

Method: PREINCUBATION

Results: NEGATIVE

Reference: [KUBOYAMA,N AND FUJII,A; MUTAGENICITY OF ANALGESICS, THEIR DERIVATIVES, AND ANTI-INFLAMMATORY DRUGS WITH S-9 MIX OF SEVERAL ANIMAL SPECIES; J. NIHON UNIV. SCH. DENT. 34(3):183-195, 1992]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA100

Metabolic Activation: GUINEA PIG, LIVER, S-9, PCB

Method: PREINCUBATION

Results: NEGATIVE

Reference: [KUBOYAMA,N AND FUJII,A; MUTAGENICITY OF ANALGESICS, THEIR DERIVATIVES, AND ANTI-INFLAMMATORY DRUGS WITH S-9 MIX OF SEVERAL ANIMAL SPECIES; J. NIHON UNIV. SCH. DENT. 34(3):183-195, 1992]

Test System: AMES SALMONELLA TYPHIMURIUM

Strain Indicator: TA100

Metabolic Activation: HAMSTER, LIVER, S-9, PCB

Method: PREINCUBATION

Results: NEGATIVE

Reference: [KUBOYAMA,N AND FUJII,A; MUTAGENICITY OF ANALGESICS, THEIR DERIVATIVES, AND ANTI- INFLAMMATORY DRUGS WITH S-9 MIX OF SEVERAL ANIMAL SPECIES; J. NIHON UNIV. SCH. DENT. 34(3):183-195, 1992]

As taken from CCRIS, 2008.

Gene-Tox Evaluation A Benzoic Acid (Pre-1980):

Species/Cell Type: *Saccharomyces cerevisiae*

Assay Type: Mitotic recombination or gene conversion

Assay Code: YEH-

Results: Negative

Panel Report: EMICBACK/52546; MUTAT RES 133:199-244,1984

Species/Cell Type: *Salmonella typhimurium* (one or more of the five standard strains: TA98, TA100, TA1535, TA1537, and TA1538)

Assay Type: Histidine reverse gene mutation, Ames assay

Assay Code: SAL-

Panel Report: EMICBACK/63689; MUTAT RES 168:69-240,1986

Reference: EMICBACK/21338; PROC NATL ACAD SCI USA 72:5135-5139,1975 ;

EMICBACK/31656; REPORT (LSU-5612):14 PP,1978

Gene-Tox Evaluation B (Post-1980)

Species/Cell Type: Human

Assay Type: Sister-chromatid exchange (SCE) in vitro

Assay Code: SC1+

Results: Positive

Panel Report: EMIC/91392; Mutat Res 297:101-180,1993

Reference: EMICBACK/37545; CANCER RES 40:4775-4780,1980

As taken from GENETOX, 1998.

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reference
Mutation in microorganisms		Bacteria - <i>Escherichia coli</i>	10 mmol/L	ZBPIA9 Zentralblatt fuer Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung II. Naturwissenschaftliche: Allgemeine, Landwirtschaftliche und Technische Mikrobiologie. (Jena, Ger. Dem. Rep.) V.107-132, 1952-77. Volume(issue)/page/year: 112,226,1959
DNA inhibition		Human Lymphocyte	5 mmol/L	PNASA6 Proceedings of the National Academy of Sciences of the United States of America. (National Academy of Sciences, Printing & Pub. Office, 2101

				Constitution Ave., Washington, DC 20418) V.1- 1915- Volume(issue)/page/year: 79,1171,1982
Mutation test systems - not otherwise specified	Oral	Insect - Drosophila melanogaster	50 mmol/L	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 46,1034,2008
DNA damage		Human Lymphocyte	5 mmol/L	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 48,1239,2010

As taken from RTECS, 2019.

/GENOTOXICITY/ Benzoic acid tested negative in several Ames tests and in one DNA damage assay with different Salmonella typhimurium strains in the presence or absence of metabolic activation. Only in one recombination assay with Bacillus subtilis H17 and M45 was a positive result obtained. There was no indication of genotoxic activity (chromosome aberrations, sister chromatid exchange) in tests with mammalian cells (Chinese hamster CHL and CHO cells, human lymphoblastoid cells, human lymphocytes) without metabolic activation. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

/ALTERNATIVE and IN VITRO TESTS/ In an in vitro study hippurate and its parent compound sodium benzoate had antitumor effects on cells derived from Skalsky lymphoma, N'emeth-Kellner lymphoma (LYNK), L-asparaginase-sensitive 6C3HED Gardner lymphoma (GS), and LP-2 plasmacytoma. /Sodium Benzoate/ [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

As taken from HSDB, 2014.

No.	Flavouring agent	End-point	Test system	Concentration	Results	Comments	Reference
In vitro							
850	Benzoic acid	Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1538 (plate incorporation)	2500 µg/plate	Negative	Assay performed with and without S9	Anderson & Styles (1978)
		Reverse mutation ^a	S. typhimurium TA98, TA100, TA1535, TA1536	3.6 µg/plate	Negative	Assay performed with and without S9	Cotruvo et al. (1977)
		Reverse mutation	S. typhimurium TA97, TA98, TA100, TA1535, TA1537 (preincubation)	10 mg/plate	Negative	Assay performed with and without S9	Zeiger et al. (1988)

		Reverse mutation ^a	<i>S. typhimurium</i> TA100	1 mg/plate	Negative	Use of S9 not reported	Rapson et al. (1980)
		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 (plate incorporation)	1 mg/plate	Negative	Assay performed with S9	McCann et al. (1975)
		Reverse mutation	<i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, TA1537 (preincubation)	10 mg/plate	Negative	Assay performed with and without S9	Ishidate et al. (1984)
		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538 (plate incorporation)	100 µg/plate	Negative	Assay performed without S9	Milvy & Garro (1976)
		Reverse mutation	<i>S. typhimurium</i> TA1535, TA1537, TA1538 (plate incorporation)	0.5%	Negative	Assay performed with and without S9	Food & Drug Administration (1975)
		Mutation (umu gene expression)	<i>S. typhimurium</i> TA1535/ pSK1002	1.7 mg/ml	Negative	Assay performed with and without S9	Nakamura et al. (1987)
		DNA repair	<i>B. subtilis</i> H17, H45	NR	Positive	Abstract; methods and test concentration(s) not reported	Nonaka (1989)
		Mutation	<i>Saccharomyces cerevisiae</i> D3	0.18%	Negative	Assay performed with and without S9	Cotruvo et al. (1977)
		Mutation	<i>S. cerevisiae</i> D4	0.15%	Negative	Assay performed with and without S9	Food & Drug Administration (1975)
		Indirect DNA repair (induction of beta-galactosidase)	<i>E. coli</i> PQ37	400 µg/ml	Negative		Glosnicka & Dziadziusko (1986)
		Chromosomal aberration	Chinese hamster fibroblasts	1.5 mg/ml	Weakly positive	Total incidence of cells with aberrations, 5–9%; performed	Ishidate et al. (1984)

						without S9	
		Sister chromatid exchange	Human lymphocytes	2.0 mmol/L	Negative	Assay performed without S9	Jansson et al. (1988)

As taken from JECFA, 2002.

This study presents the first application of an in vivo alkaline comet assay using hemocytes in the hemolymph of *Drosophila melanogaster* larvae for the genotoxic evaluation of benzyl derivatives. Haemocytes are playing a function similar to mammalian blood. In this study, different concentrations. (5, 10, 25 and 50 mM) of benzyl alcohol, benzyl acetate, benzaldehyde and benzoic acid used as flavor ingredients were investigated for genotoxicity as in vivo. Haemolymph was extracted from the larvae, and hemocytes were isolated by centrifugation and used in the comet assay. In the comet assay with hemocytes for benzyl derivatives observed significantly and dose-dependently induced DNA damage formation. The highest concentration of each tested benzyl derivative was significantly induced DNA damage. Their genotoxic potencies according to the induced % DNA tail in the highest concentration are as follows: benzyl acetate (35.51 ± 0.31), benzyl alcohol (34.87 ± 2.01), benzaldehyde (33.27 ± 0.95) and benzoic acid (28.21 ± 0.31). These results confirm the benefit of the comet assay with hemocytes as an in vivo model and support the possibility that there is a genotoxic risk associated with benzyl derivatives exposure (Esref Demir & Bülent Kaya, 2013).

“The use of food additives has increased enormously in modern food technology but they have adverse effects in human healthy. The aim of this study was to investigate the DNA damage of some food additives such as citric acid (CA), benzoic acid (BA), brilliant blue (BB) and sunset yellow (SY) which were investigated in human male germ cells using comet assay. The sperm cells were incubated with different concentrations of these food additives (50, 100, 200 and 500 µg/mL) for 1 h at 32 °C. The results showed for CA, BA, BB and SY a dose dependent increase in tail DNA%, tail length and tail moment in human sperm when compared to control group. When control values were compared in the studied parameters in the treatment concentrations, SY was found to exhibit the highest level of DNA damage followed by BB > BA > CA. However, none of the food additives affected the tail DNA%, tail length and tail moment at 50 and 100 µg/mL. At 200 µg/mL of SY, the tail DNA% and tail length of sperm were 95.80 ± 0.28 and 42.56 ± 4.66 , for BB the values were 95.06 ± 2.30 and 39.56 ± 3.78 , whereas for BA the values were 89.05 ± 2.78 and 31.50 ± 0.71 , for CA the values were 88.59 ± 6.45 and 13.59 ± 2.74 , respectively. However, only the highest concentration of the used food additives significantly affected the studied parameters of sperm DNA. The present results indicate that SY and BB are more harmful than BA and CA to human sperm in vitro. As taken from Pandir D. 2016. Cytotechnology 68(4), 725-33. PubMed, 2017

“In vitro genotoxic effects of antioxidant additives, such as citric acid (CA) and phosphoric acid (PA) and their combination, as well as antimicrobial additives, such as benzoic acid (BA) and calcium propionate (CP), on human lymphocytes were determined using alkaline single-cell gel electrophoresis. There was a significant increase in the DNA damage in human lymphocytes after 1 h of in vitro exposure to CA, PA, BA and CP (200, 25-200, 50-500, 50-1000 µg/mL, respectively). The combination of CA and PA significantly increased the mean tail intensity at all the concentrations used (25-200 µg/mL) and significantly increased the mean tail length mainly after higher concentrations (100 and 200 µg/mL). Data in this study showed that the concentrations of food additives used induce DNA damage and PA was the most genotoxic and CA was less genotoxic additives among them.” As taken from Yilmaz Set al. 2014.Toxicol. Ind. Health30(10), 926-37. PubMed, 2015 available at <http://www.ncbi.nlm.nih.gov/pubmed/23188648>

“JECFA at its 46th meeting (JECFA, 1997) evaluated the genotoxicity of benzoic acid in different in vitro and in vivo genotoxicity tests and concluded that benzoic acid was not mutagenic in the Ames test, either with or without metabolic activation but showed weak clastogenic activity in vitro which was not reproduced in vivo.”

“JECFA’s conclusion regarding the clastogenicity of benzoic acid was based on the results from two in vitro chromosome aberration studies in Chinese hamster fibroblasts (Ishidate et al., 1984) and in root tips of *Vicia faba* (Njagi and Gopalan, 1982) which showed weak cytogenetic effects. However, the Panel noted that the assay on root tips of *V. faba* does not belong to the assays recommended for regulatory purposes and that the study by Ishidate et al. (1984) was of limited value because in the evaluation of clastogenic effects only results for total aberrations were reported, which included gaps not normally considered for the evaluation of clastogenicity. In addition, the study was poorly reported. The SCF in its 2002 evaluation (SCF, 2002) of benzoic acid and its salts noted that all the relevant studies in vivo were negative at somatic or germ cell levels and concluded that it is very unlikely that benzoic acid would interact with chromosomes in vivo and recommended The observations of clastogenic activity of benzoic acid in vitro indicate that it should be tested for clastogenic activity in vivo in peripheral lymphocytes or bone marrow in animals and that blood or bone marrow levels respectively of benzoic acid should be measured in such a study.”

“TemaNord (2002) has evaluated the genotoxicity of benzoic acid, sodium benzoate, potassium benzoate and calcium benzoate, and concluded that benzoic acid/sodium benzoate was without mutagenic effect in in vitro mutagenicity tests.”

“In the framework of classification, labelling and packaging of substances and mixtures according to Regulation (EC) No 1272/2008, benzoic acid was evaluated by the German Federal Institute for Occupational Safety and Health (BAuA, 2011). The BAuA considered that while the reverse mutation assays and sister chromatid exchange assays (except one equivocal result) with benzoic acid, sodium benzoate and the metabolite hippuric acid were negative, weak genotoxic effects or equivocal results were observed in most of the chromosome aberration assays in mammalian cell lines with benzoic acid and sodium benzoate. The BAuA further considered that All the in vivo genotoxicity tests were negative at somatic or germ cell level. Accordingly, BAuA concluded that On this basis and the negative results obtained in two carcinogenicity studies in rats and mice for sodium benzoate, notwithstanding some limitations, it is very unlikely that benzoic acid would interfere with chromosomes in vivo. In the absence of a genotoxic potential in in vivo studies and negative results in carcinogenicity studies, no classification and labelling regarding mutagenicity is required.”

“In a study by the EG&G Mason Research Institute (1979 [Doc. provided to EFSA n. 3]) benzoic acid (purity 99.5%) was tested for its mutagenicity in the Ames test with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 using the plate incorporation method both in the absence and presence of rat liver S9 metabolic activation up to 1,000 µg/plate. The highest concentration was based on a toxicity test with strain TA100 in which the background bacterial lawn was slightly reduced at 976.6 µg/plate and markedly reduced at 3,125 and 10,000 µg/plate. No increases in the frequency of revertant colonies were observed with benzoic acid under any experimental condition.”

“Benzoic acid, sodium benzoate and potassium benzoate were investigated for their potential to cause chromosomal aberrations, sister chromatid exchanges (SCEs) and micronuclei formation in cultured human peripheral lymphocytes (Yilmaz et al., 2009; Zengin et al., 2011). The potential of sodium benzoate and potassium benzoate to induce DNA damage was also investigated in the alkaline Comet assay. Test concentrations were 50, 100, 200 and 500 µg/mL for benzoic acid, 6.25, 12.5, 25, 50 and 100 µg/mL for sodium benzoate and 62.5, 125, 250, 500 and 1,000 µg/mL for potassium benzoate. Cells were exposed for 24 or 48 h for the chromosomal aberration and SCE assays, for 48 h in the micronucleus assay and for 1 h for comet assay. The mitotic index was reduced in a dose-related manner in all treatment groups compared to the negative controls, but it was not lower than 50% of control in any of the cultures, even at the highest dose for each benzoate, indicating that cytotoxicity was not a major factor in the induction of chromosome aberrations. None of the substances affected the replication index of the lymphocyte cultures. Chromosome aberrations were significantly increased compared to controls in cultures treated with benzoic acid and its sodium and potassium salts at all test concentrations. The frequencies of

SCEs were similarly increased in a dose-related manner by all three benzoates, benzoic acid showing a slightly weaker response than either sodium or potassium benzoate. Micronuclei formation was significantly increased in a dose-related manner following treatment of the lymphocyte cultures with 200 or 500 µg/mL benzoic acid, or with 25, 50 and 100 µg/mL sodium benzoate, or with 125, 250, 500 and 1,000 µg/mL potassium benzoate. In the comet assay, a positive response was seen in the presence of sodium benzoate but not potassium benzoate. The authors concluded that benzoic acid, sodium benzoate and potassium benzoate are clastogenic, mutagenic and cytotoxic to human lymphocytes in vitro (Yilmaz et al., 2009; Zengin et al., 2011). The Panel noted that treatment and sampling times used in the chromosomal aberration and SCE assays from both studies deviate from relevant OECD TG Guideline 473. Furthermore, the different concentrations used for sodium and potassium benzoate (the concentration of potassium benzoate was 10 times higher than the concentration of sodium benzoate) appear to be inexplicable as the difference between the two compounds is only in the cation which is not expected to elicit any genotoxicity per se and no justification was reported for the application of these different concentrations. Overall, the Panel considered these studies as not adequate for risk assessment.

“Demir et al. (2010) investigated the effects of benzoic acid, benzyl alcohol, benzyl acetate and benzaldehyde in an in vitro comet assay. Lymphocytes from two human volunteers were exposed to concentrations of 0, 0.05, 0.1, 0.3, 1 or 5 mM benzoic acid or 0, 1, 5, 10, 25 or 50 mM benzyl alcohol, benzyl acetate and benzaldehyde. Control cultures were exposed to distilled water alone, while ethylmethanesulphonate (EMS, 5 mM) was used as a positive control. Tail moment and % tail DNA were used as tail parameters in analysing the results of the study. Benzoic acid at a concentration of 5 mM produced a significant increase in both tail moment (4.28 ± 1.11) and % tail DNA (13.08 ± 2.03), with no effect being seen at lower concentrations of benzoic acid compared with control. Positive results were also seen with benzyl alcohol at concentrations of 25 and 50 mM, with benzyl acetate at 50 mM and with benzaldehyde at 10, 25 and 50 mM. EMS induced both tail moment (16.59 ± 2.71) and % tail DNA (28.73 ± 3.87). The authors concluded that these benzyl compounds showed evidence of genotoxicity at high test concentrations in the in vitro comet assay. However, the Panel noted that statistically significant increases observed for benzoic acid were modest and similar to the values observed for the negative controls used for the other substances assessed in this study. Statistical significance was only achieved due to the low value obtained in the concurrent control. The outcome obtained is therefore of no biological relevance.”

“In the study by Yilmaz et al. (2014), benzoic acid was assessed for its potential to induce DNA breakage in the alkaline comet assay in purified human lymphocytes (one male and one female healthy subject). Concentrations used were 50, 100, 200 and 500 µg/mL and cells were treated for 1 h at 37°C. Cell viability was evaluated by the incorporation of trypan blue dye in cells. Two slides were prepared for each concentration of benzoic acid and 200 selected cells (100 cells from each of the two replicate slides) were analysed per treatment using the image analysis system (Comet Assay IV, Perceptive Instruments Ltd., UK). The tail length and tail intensity (%) parameters were used to estimate the extent of DNA breakage. The results obtained indicated moderate, no dose-related increases in both tail length and tail intensity (%) compared to the relevant solvent control culture, which achieved statistical significance at all concentrations employed. However, the Panel noted some limitations in the study, which include the absence of historical negative control values, the lack of dose-response over a 10-fold dose range and only 200 cells analysed per test point compared to 300 normally scored. Furthermore, the Panel noted that in a previous study (Zengin et al., 2011), the same authors reported negative results for potassium benzoate in the comet assay even at higher “concentrations.”

As taken from EFSA, 2016a.

“Based on available genotoxicity studies, the substance is unlikely to be genotoxic.”

As taken from EFSA, 2016b.

"It was shown that benzoic acid significantly increased the chromosomal aberration, sister chromatid exchange and micronucleus frequency (at 200 and 500 µg/mL) without changing the pH of the medium in a dose-dependent manner. This additive also significantly decreased the mitotic index (MI) at the highest concentration for 24 hours and 100, 200 and 500µg/mL for 48 hours (Y--^aImaz et al., 2009). SCE negative in study (Jansson et al., 1988"

As taken from SCENIHR, 2016.

"Overall, the results confirm previous assessments that benzoic acid and its derivatives may induce genotoxic changes in vitro; however, in vivo genotoxicity studies are consistently negative."

As taken from EFSA, 2017.

"Based on the weight of the evidence of the in vitro and in vivo genotoxicity data, the chemical is not considered mutagenic or clastogenic.

In vitro data using the reverse mutation assays with various strains of *Salmonella typhimurium* (with and without metabolic activation) and sister chromatid exchange assays (except one equivocal result) were negative. Weak genotoxic effects or equivocal results were observed in most of the chromosome aberration assays in three mammalian cell lines and two of the recombination assays in *Bacillus subtilis* (no further information available, only summary given) (REACH). No genotoxicity was observed in the in vivo cytogenetic, micronucleus, or other assays at either somatic or germ cell level (OECD, 2004"

As taken from AICIS, 2013.

"The present study assessed the long-term daily administration of benzoic acid (BA), potassium sorbate (PS), chlorophyll (CPL), tartrazine (TAZ), and butylated hydroxyanisole (BHA) on hepato-renal changes and DNA damage in rats. Animals were orally administered with the 10 times of the acceptable daily intake (ADI) from each tested substance daily for 60 consecutive days. Blood, liver, and kidney samples were collected to evaluate hematological, biochemical, histopathological, and genotoxic alterations. The extent of liver and kidney damage was evaluated by comet assay and histopathologically. Significant reduction of leukocyte numbers and lymphocytes % in CPL- and TAZ-treated rats. However, significant increases in platelet count in all treated groups after 60 days were detected. The levels of serum transaminases enzymes (ALT, AST), alkaline phosphatase (ALP), and creatinine were significantly increased in all treatments except with BHA group, but no substantial differences were found in urea after 60 days. Aside from BHA, results of DNA damage revealed significant increases in tailed nuclei, tail moment, DNA% in the tail, and tail length in liver and kidney at different degrees. Moreover, the histopathological figures of liver and kidneys affirmed destructive and degenerative changes. The study indicates that most of the tested food additives may provoke genotoxicity and hepato-nephropathy, which could be serious for human health. Therefore, it is necessary to be informed about the hazardous effects of food additives and more attention should be focused towards using natural substitutes." As taken from Abo-El-Sooud K et al. 2018. Environ. Sci. Pollut. Res. Int. 25(26), 26341-26350. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/29981020>

"Benzoic acid and its alkali salts are not genotoxic.

In the *Salmonella* mutagenicity test, benzoic acid was not mutagenic in vitro. The clastogenic effects found in vitro were not confirmed in tests for chromosomal aberrations in vivo in rats. In the dominant lethal test with rats, benzoic acid was not genotoxic in germ cells. There is thus no evidence of germ cell mutagenicity, so that benzoic acid and its alkali salts are not classified in one of the categories for germ cell mutagens."

As taken from Hartwig A and MAK Commission, 2018.

Record for benzoic acid:

Chemical Purity	Species Scientific Name	Species Common Name	Organism Age	Organism Age Units	Exposure Type	Media Type	Test Location	Conc 1 (Author)	Conc 1 Units (Author)	Effect	Effect Measurement	Endpoint	Exposure Duration Mean (Days)	Response Site	Observed Duration (Days)
99.5	Mus musculus	House Mouse	7	Week(s)	Diet, unspecified	No substrate	Lab	1000/	mg/kg bwt	Genetics	Damage	NOEL	1	Colon	0.125

As taken from the US EPA ECOTOX database, 2021.

“The mutagenic activity of benzoic acid has been evaluated in a bacterial reverse mutation assay conducted in an equivalent manner to OECD TG 471 using the standard plate incorporation method. Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA97 were treated with benzoic acid in dimethyl sulfoxide (DMSO) at concentrations up to 10,000 µg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (Zeiger et al., 1988). Under the conditions of the study, benzoic acid was not mutagenic in the Ames test.

The clastogenicity of benzoic acid was assessed in an in vitro chromosome aberration study. Chinese hamster lung cells were treated with benzoic acid in DMSO at a concentration of 1.5 mg/mL (1500 µg/mL) in the absence of metabolic activation. Ambiguous statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed at this concentration of the test material without S9 metabolic activation (ECHA, 2011). Under the conditions of the study, benzoic acid was considered to be ambiguous in the in vitro chromosome aberration assay.

Due to the results and the limited concentrations tested of the chromosome aberration study, the clastogenic activity of benzoic acid was evaluated in an in vitro micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Mouse lymphoma L5178Y were treated with benzoic acid in DMSO, and micronuclei analysis was conducted at concentrations up to 1000 µg/mL in the presence and absence of metabolic activation. Benzoic acid did not induce binucleated cells with micronuclei when tested in either the presence or absence of an S9 activation system (ECHA, 2011). Under the conditions of the study, benzoic acid was considered to be non-clastogenic in the in vitro micronucleus test.

The results of the in vitro chromosome aberration study for benzoic acid can be considered not biologically relevant due to only being performed with 1 concentration and due to the use of Chinese hamster lung cells. Chinese hamster lung cells are a p53-deficient cell line that has been shown to result in a higher frequency of “misleading” positive results (Fowler et al., 2012). As an additional weight of evidence (WoE), sodium benzoate (CAS # 532-32-1) was found to be negative in an in vivo mammalian bone marrow chromosome aberration test, which followed guidelines in an equivalent manner to OECD 475 (ECHA, 2011).

Based on the data available, benzoic acid does not present a concern for genotoxic potential. (...)

In a GLP and OECD 412-compliant study, 10 Crl:CD(SD) rats/sex/dose were administered benzoic acid via inhalation (nose-only) at doses of 0, 2.5, and 12.5 mg/m³ (equivalent to 0, 0.65, and 3.24 mg/kg/day) for 4 weeks (5 days/week; 6 h/day). Based on no effects seen up to the highest dose, the NOAEL was considered to be 3.24 mg/kg/day (RIFM, 2009; see Table 1).

The NOAEL was determined from the OECD 412 study on Sprague Dawley rats (ECHA, 2011). A default safety factor of 3 was used when deriving a NOAEL from the 28-day repeated dose study (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*. Thus, the derived NOAEL for the repeated dose toxicity data is 6.48/3 or 2.16 mg/kg/day. (...)

*The Expert Panel for Fragrance Safety is composed of scientific and technical experts in their respective fields. This group provides advice and guidance."

Api AM et al. (2022). RIFM fragrance ingredient safety assessment, benzoic acid, CAS Registry Number 65-85-0.

Genotoxic: A3, A = (Chromosomal aberration (EFSA database), 3 = negative. PPDB (2024).

5.5. Cytotoxicity

Type of Test	Route of Exposure or Administration	Species/ Test System	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Human skin -	>2000 umol/L/48H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	TXAPA9 Toxicology and Applied Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1959- Volume(issue)/page/year: 245,281,2010
IC30 - Inhibitor Concentration 30	In vitro	Human lymphocyte -	>200 mg/L/45H	In Vitro Toxicity Studies - cell viability (dye exclusion): trypan blue assay etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,901,2015

As taken from RTECS, 2019.

Toxicity threshold (cell multiplication inhibition test): bacteria (*Pseudomonas putida*): 480 mg/l (Bringmann & Kühn 1980).

As taken from the Finnish Environment Institute (a).

"AIMS: The aim of this study was a challenge testing the effect of lower concentrations of micronized benzoic acid against two strains of *Alicyclobacillus*. METHODS AND RESULTS: The effect of micronized benzoic acid was compared with the usual levels of untreated commercial sodium benzoate and benzoic acid, at the challenge temperature of 45°C. The size of the benzoic acid particles was determined by scanning electron microscopy. The diameter of the micronized particles was around 10 µm with a maximum length of 200 µm, while the untreated preservative structures were irregular with lengths up to 500 µm. A continuous bactericidal effect against two *Alicyclobacillus* strains, throughout the 28-day period, was observed with 50 mg l(-1) of micronized benzoic acid, but when the untreated preservative was used, the same lethal effect was not achieved even after doubling its concentration. CONCLUSIONS: The antimicrobial activity of benzoic acid was improved by micronization. The process proved to be an effective alternative to reduce the benzoic acid concentration necessary to ensure stability of an orange juice matrix.

SIGNIFICANCE AND IMPACT OF THE STUDY: The results proved that the micronization process represents an alternative to reduce the required food preservative concentration; this method increased the stability of the compound, which maintains its bioavailability.” As taken from Kawase KY et al. 2013. J. Appl. Microbiol. 115(2), 466-74. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23594315>

“The product Menno Florades is a soluble concentrate of the active substance benzoic acid.

The biocidal product Menno Florades contains 90g/L benzoic acid and other ingredients.

The product Menno Florades shows a basic bactericidal effectiveness on samples of the target organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) under clean conditions by a 1% solution (corresponds to 0.9 g/L benzoic acid) after a contact time of 60 min.

The product shows a basic fungicidal effectiveness on samples of the target organisms under clean conditions by a 2% solution (contains 1.8 g/L benzoic acid) against *Candida albicans* and by a 4% solution (contains 3.6 g/L benzoic acid) against *Aspergillus brasiliensis* contact time of 60 min.

The product shows a basic virucidal effectiveness on the model organism for the genus picornavirus Bovine enterovirus Type 1 (Enteric Cytopathogenic Bovine Orphan Virus – ECBO) by a 2% solution (corresponds to 1.8 g/L benzoic acid) at conditions without organic soiling after a contact time of 60 min.

Mode of action: In solution, benzoic acid exists in a pH-dependent equilibrium between the undissociated and dissociated form. Only in its undissociated state, the acid is able to pass the cells membrane. At a relatively low pH, the uncharged acid enters the cell. Inside the cell, the benzoic acid dissociates due to the higher pH. The molecules remain inside the cell, because the resulting ions cannot pass the membrane. The pH inside the cell is lowered and metabolic reactions are inhibited. Further effects are also reported: Decrease of the membrane permeability for amino acids, organic acids, phosphates resulting in uncoupling of both substrate transport and oxidative phosphorylation from the electron transport system. Furthermore, an inhibition of the citric acid cycle is observed.”

As taken from European Commission, 2013a, b.

“**CONTEXT:** It is necessary to advance the field of alternative treatments for chronic wounds that are financially accessible to the least economically developed countries. Previously we demonstrated that topical applications of *Lactobacillus plantarum* culture supernatants (LAPS) on human-infected chronic wounds reduce the pathogenic bioburden, the amount of necrotic tissue, and the wound area, as well as promote debridement, granulation tissue, and wound healing. **OBJECTIVE:** To study LAPS chemically and biologically and to find potential molecules responsible for its pro-healing and anti-pathogenic properties in chronic wounds. **MATERIALS AND METHODS:** (1) Chemical analysis: extracts were subjected to a column chromatography and the fractions obtained were studied by GCMS. (2) Quantification: dl-lactic acid (commercial kit), phenolic compounds (Folin-Ciocalteu), H₂O₂ (micro-titration), and cations (flame photometry). (3) Biological analysis: autoinducers type 2 (AI-2) (*Vibrio harveyi* BB170 bioassay), DNAase activity (Agar DNAase), and *Pseudomonas aeruginosa* biofilm inhibition (crystal violet technique). **RESULTS:** According to its biological activity, the most significant molecules found by GCMS were the following: antimicrobials (mevalonolactone, 5-methyl-hydantoin, benzoic acid, etc.); surfactants (di-palmitin, distearin, and 1,5-monolinolein); anesthetics (barbituric acid derivatives), and AI-2 precursors (4,5-dihydroxy-2,3-pentanedione and 2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran). Concentrations measured (µg/mL): DL-lactic acid (11.71 ± 1.53) and H₂O₂ (36 ± 2.0); phenolic compounds (485.2 ± 15.20); sodium (370 ± 17); potassium 920 ± 24; calcium (20 ± 4); and magnesium (15 ± 3). DNAase from LAPS had activity on genomic DNA from PMNs and *P. aeruginosa*. **DISCUSSION AND CONCLUSION:** The molecules and biological activities found in LAPS could explain the observed effects in human chronic wounds.” As taken from Ramos AN et al. 2015. Pharm. Biol. 53(3), 350-8. PubMed, 2016

“Benzoic acid is a bacteriostatic antiseptic that is only active in an acidic environment (pH 2.5 to 4.5”

As taken from EMA, 2017.

“Chlorogenic acid (CGA) decreases colon cancer-cell proliferation but the combined anti-cancer effects of CGA with its major colonic microbial metabolites, caffeic acid (CA), 3-phenylpropionic acid (3-PPA) and benzoic acid (BA), needs elucidation as they occur together in colonic digesta. Caco-2 cancer cells were treated for 24 h with the four compounds individually (50-1000 μ M) and as an equimolar ratio (1:1:1:1; MIX). The effective concentration to decrease cell proliferation by 50% (EC50) was lower for MIX (431 ± 51.84 μ M) and CA (460 ± 21.88) versus CGA (758 ± 19.09 μ M). The EC50 for cytotoxicity measured by lactate dehydrogenase release in MIX (527 ± 75.34 μ M) showed more potency than CA (740 ± 38.68 μ M). Cell proliferation was decreased by 3-PPA and BA at 1000 μ M with no cytotoxicity. Cell-cycle arrest was induced at the S-phase by CA (100 μ M), MIX (100 μ M), CGA (250 μ M) and 3-PPA (500 μ M) with activation of caspase-3 by CGA, CA, MIX (500 and 1000 μ M). Mitochondrial DNA content was reduced by 3-PPA (1000 μ M). The anti-cancer effects occurred at markedly lower concentrations of each compound within MIX than when provided singly, indicating that they function together to enhance anti-colon cancer activities.” As taken from Sadeghi Ekbatan S et al. 2018. Int. J. Mol. Sci. 19(3), E723. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29510500>

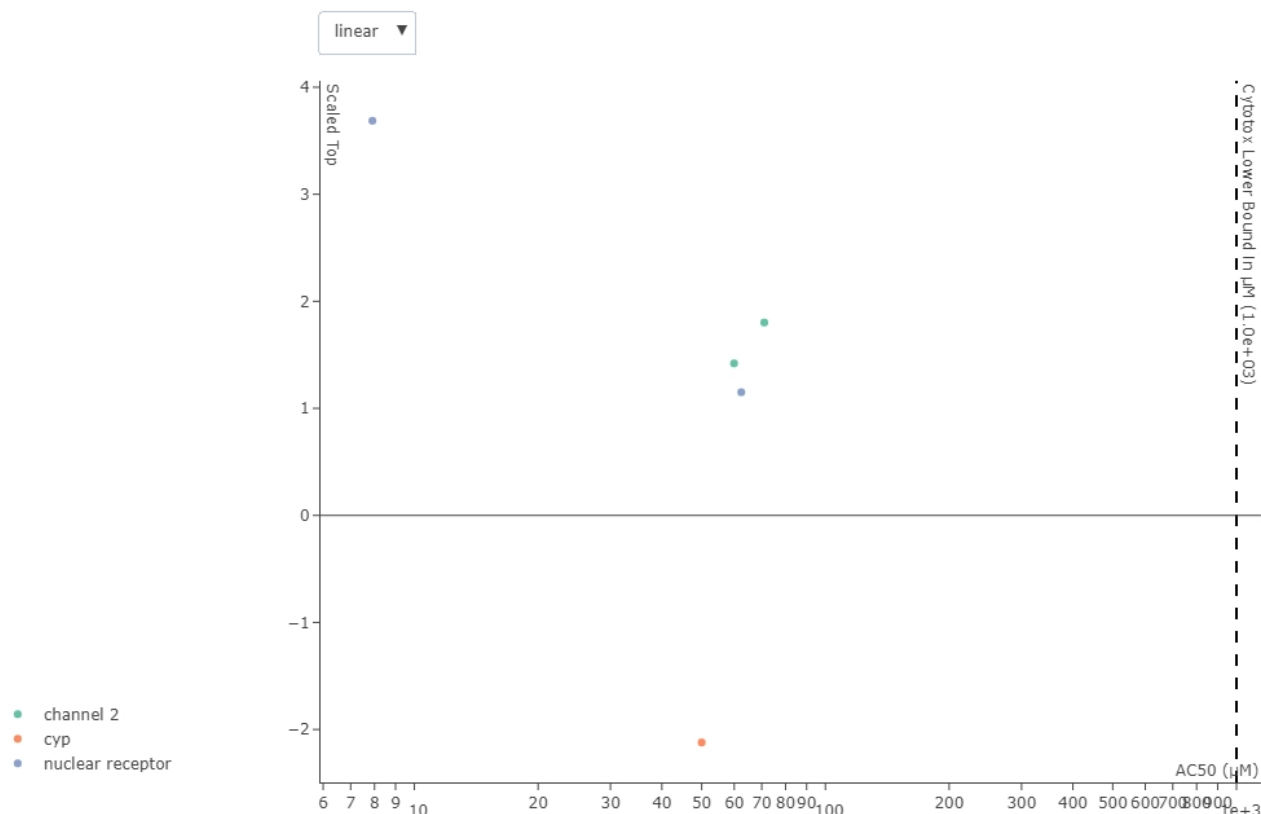
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated benzoic acid in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: <https://comptox.epa.gov/dashboard>

US EPA provides the following data use considerations for ToxCast data: “The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve.”

A summary of the ToxCast assay data on benzoic acid is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 1



“Background: Rapid lifestyle, especially among people living in urban areas, has led to increasing reliance on the processed food market. Unfortunately, harmful effects caused by the excessive use of food additives in such type of industry are often neglected. Objective: This proposal investigates in vitro cytotoxic and apoptotic effects of three food preservatives commonly consumed in daily meals; sodium sulphite, boric acid, and benzoic acid. Methods: The effect of the three preservatives on cell viability was tested on two different cell lines; normal liver cell line THLE2 and human hepatocellular carcinoma cancer cell line HepG2 using MTT assay. Cell cycle arrest was measured using flow cytometry by propidium iodide. Measurement of expression levels of two central genes, p53 and bcl-2 that play key roles in cell cycle and apoptosis was carried out in HepG2 cells using real time-PCR. Results: Although the effect was more significantly realized in the HepG2 cell line, the viability of both cell lines was decreased by all of the three tested compounds. Flow cytometric analysis of HepG2 cells treated with sodium sulphite, boric acid, and benzoic acid has revealed an increase in G2/M phase cell cycle arrest. In Sodium sulphite and boric acid-treated cells, expression levels of p53 were up-regulated, while that of the Bcl2 was significantly down-regulated. On the other hand, Benzoic acid has shown an anti-apoptotic feature based on the increased expression levels of Bcl-2 in treated cells. Conclusion: In conclusion, all of the tested compounds have decreased the cell line viability and induced both cell cycle arrest and apoptotic events indicating their high potential of being cytotoxic and genotoxic materials.” As taken from El-Hefny IM et al. 2020. Recent Pat. Food Nutr. Agric. 11(3), 193-201. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32065108/>

5.6. Carcinogenicity

“Carcinogenicity studies in rat and mice did not provide concern for a potential carcinogenic potential of benzoate when given with the diet or the drinking water at high dosages.”

As taken from ECHA, 2012a.

Cancer Classification: Group D Not Classifiable as to Human Carcinogenicity [USEPA Office of Pesticide Programs, Health Effects Division, Science Information Management Branch: "Chemicals Evaluated for Carcinogenic Potential" (April 2006)] **PEER REVIEWED**

CLASSIFICATION: D; not classifiable as to human carcinogenicity. BASIS FOR CLASSIFICATION: No human data and inadequate data from animal bioassays. HUMAN CARCINOGENICITY DATA: None. ANIMAL CARCINOGENICITY DATA: Inadequate. [U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS). Summary on Benzoic acid (65-85-0). Available from, as of March 15, 2000: <http://www.epa.gov/iris/> **PEER REVIEWED**

As taken from HSDB, 2014.

Rats and Mice: Cancer Test Summary

Rat Target Sites		Mouse Target Sites		TD50 (mg/kg/day)	
Male	Female	Male	Female	Rat	Mouse
no positive	no test	no test	no test	no positive	no test

Positivity: For each chemical with a positive (carcinogenic) experiment in the Carcinogenic Potency Database (CPDB), results are included on carcinogenic potency (TD50) in each species and target sites in males and females. Positivity is determined by an author's opinion in a published paper. If all experimental results in the CPDB are negative in a sex-species group, "no positive" appears. If the CPDB has no experiments in the sex-species group, "no test" appears. The summary presents the strongest evidence of carcinogenicity in each group. If there are both positive and negative experiments in a sex-species, the negative results are ignored in this Summary Table.

Target Site Codes: Target sites are listed if any author of published experimental results concluded that tumors were induced in that organ by the test agent. If there is more than one positive experiment in a sex-species, target sites listed may be from more than one experiment, e.g. if liver and lung are both listed, then liver may have been a target in one experiment and lung in another.

TD50: Our standardized measure of carcinogenic potency, TD50, is the daily dose rate in mg/kg body weight/day to induce tumors in half of test animals that would have remained tumor-free at zero dose. Whenever there is more than one positive experiment in a species, the reported TD50 value is a Harmonic Mean calculated using the TD50 value from the most potent target site in each positive experiment.

The Carcinogenic Potency Database (CPDB) is a unique and widely used international resource of the results of 6540 chronic, long-term animal cancer tests on 1547 chemicals. The CPDB provides easy access to the bioassay literature, with qualitative and quantitative analyses of both positive and negative experiments that have been published over the past 50 years in the general literature through 2001 and by the National Cancer Institute/National Toxicology Program through 2004. The CPDB standardizes the diverse literature of cancer bioassays that vary widely in protocol, histopathological examination and nomenclature, and in the published author's choices of what information to provide in their papers. Results are reported in the CPDB for tests in rats, mice, hamsters, dogs, and nonhuman primates.

For each experiment, information is included on species, strain, and sex of test animal; features of experimental protocol such as route of administration, duration of dosing, dose level(s) in mg/kg body weight/day, and duration of experiment; experimental results are provided on target organ, tumor type, and tumor incidence; carcinogenic potency (TD50) and its statistical significance; shape of the dose-response, author's opinion as to carcinogenicity, and literature citation.

Only tests with dosing for at least $\frac{1}{4}$ the standard lifespan of the species and experiment length at least $\frac{1}{2}$ the lifespan are included in the CPDB. Only routes of administration with whole body exposure are included. Doses are standardized, average dose rates in mg/kg/day. A description of methods used in the CPDB to standardize the diverse literature of animal cancer tests is presented

for: 1) Criteria for inclusion of experiments 2) Standardization of average daily dose levels and 3) TD50 estimation for a standard lifespan. See Methods for other details.

TD50 provides a standardized quantitative measure that can be used for comparisons and analyses of many issues in carcinogenesis. The range of TD50 values across chemicals that are rodent carcinogens is more than 100 million-fold. More than half the chemicals tested are positive in at least one experiment.

A plot of all results on each experiment in the CPDB for this chemical is presented below. These results are the source information for the Cancer Test Summary table above.

Benzoic acid: All Experiments and Citations in CPDB

The definition of each code in the plot below will appear in a pop-up window when the field name in the header line is clicked, e.g., Strain, Site, Path. Each numbered line starts a new experiment and reports protocol information in black. Average daily dose-rates per kg body weight per day are in green. Remaining lines report experimental results in blue.

Abbreviations of fields in header line: # = the line number in the plot of all CPDB chemicals; Xpo = duration of dosing; Xpt = duration of experiment; Site = tissue; Path = tumor type; DR = dose-response; AuOp = author's opinion about carcinogenicity; LoConf, UpConf = confidence limits (99%) on TD50; Inc = tumor incidence for each dose group.

As taken from CPDB, 2007.

“Classification — D; not classifiable as to human carcinogenicity

Basis — No human data and inadequate data from animal bioassays.

Animal Carcinogenicity Data:

Inadequate. In a lifetime study, Toth (1984) administered sodium benzoate (of 99% purity) to 50 male and 50 female 5 week-old albino Swiss mice at a level of 2% in the drinking water. Control groups consisted of 100 mice/sex. The dose level was selected based on results of a subchronic study in which levels of 4 and 8% were considered to be too toxic. The 2% level was equivalent to sodium benzoate doses of 4133 mg/kg/day for males and 3973 mg/kg/day for females. Based on average measured daily water consumptions of 6.2 mL for males and 5.9 mL for females and an assumed average body weight of 0.03 kg. The equivalent benzoic acid doses, adjusted for molecular weight differences between sodium benzoate and benzoic acid, are 3502 mg/kg/day and 3367 mg/kg/day for males and females, respectively. Histopathologic examinations of all mice included 11 organs and all gross lesions. The treatment had no apparent effect on survival or tumor incidence.

As part of a 5-generation reproduction study, Shtenberg and Ignat'ev (1970) administered test compounds in a paste in daily doses of 40 mg/kg benzoic acid combined with 80 mg/kg sodium bisulfite in a paste before feeding an otherwise unspecified basic diet to a group of 50 white cross-bred mice/sex for 17 months. Another group received benzoic acid only; no further details were given. An unspecified number of control animals received only basic diet. Malignant tumors (not otherwise specified) occurred in 8/100 treated mice and 1/8 mice in the third generation of the treated group. Tumor incidences were not reported for untreated mice.

Dinerman and Ignat'ev (1966) reported that a 3-month exposure to 0.2% benzoic acid in the diet increased the susceptibility of mice to the development of carcinomas following intraperitoneal inoculation with Erlich ascites carcinoma cells. Tumors developed in 62/90 (68.8%) of benzoic acid-treated mice and in 16/49 (32.6%) of the control mice.

Benzoic acid and sodium benzoate have been tested for mutagenicity or genotoxicity in prokaryotes (McCann et al., 1975), eukaryotes (Litton Bionetics, Inc., 1974), and several mammalian test systems (Litton Bionetics, Inc., 1974, 1975; Oikawa et al., 1980). No positive results have been reported.

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the cancer assessment for Benzoic acid conducted in August 2003 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or 202-566-1676.”

As taken from IRIS (1988)

“Cancer Oral Synopsis:

IPCS and U.S. EPA have evaluated the oral carcinogenicity data for benzoic acid. EPA classifies this chemical as D - not classifiable as to human carcinogenicity. The cancer weight of evidence classification is based on all routes of exposure. IPCS noted that studies on the precursors of benzoic acid support the notion that benzoic acid is unlikely to be carcinogenic. In addition, two chronic studies of benzoic acid in rats and mice gave no indication of a carcinogenic effect in either species. However, some positive genotoxicity test results suggest that the possible genotoxic activity of sodium benzoate cannot be ruled out.

Cancer Inhalation Synopsis:

IPCS and U.S. EPA have evaluated the inhalation carcinogenicity data for benzoic acid. EPA classifies this chemical as D - not classifiable as to human carcinogenicity. The cancer weight of evidence classification is based on all routes of exposure. IPCS noted that studies on the precursors of benzoic acid support the notion that benzoic acid is unlikely to be carcinogenic. In addition, two chronic studies of benzoic acid in rats and mice gave no indication of a carcinogenic effect in either species. However, some positive genotoxicity test results suggest that the possible genotoxic activity of sodium benzoate cannot be ruled out.”

As taken from ITER, 2018.

“In long-term toxicity and carcinogenicity studies with rats and mice, benzoic acid and its salts were of low toxic ity, with no marked organ toxicity, and showed no carcinogenic potential in both species. The relevant NOAEL is 500 mg/kg bw per day (2-year rat study)”

As taken from EFSA, 2016b

“As summarised by SCCP (2005), various Committees concluded that the data reviewed for compounds in this group were sufficient to demonstrate lack of teratogenic, reproductive or carcinogenic potential.”

As taken from SCENIHR, 2016.

“JECFA (joint FAO/WHO expert committee on food additives) considered that, although long term carcinogenicity and toxicity studies carried out with sodium benzoate in rats and mice were not very good quality, all of the available data, in particular new studies with benzyl acetate, lead to the conclusion that compounds from the benzoic acid family are not carcinogenic.”

As taken from EMA, 2017.

“Based on the available data, the chemical is not considered carcinogenic.

The chemical was not carcinogenic (NOAEL 500 mg/kg bw/d) in a lifetime 3-generation study in rats when given with the diet at doses up to 500 mg/kg bw/d. No increase in the lifetime tumour incidence, clinical abnormalities or histopathological changes were observed (OECD, 2004”

As taken from AICIS, 2013.

“Because of various shortcomings, the available long-term studies with sodium benzoate in rats and mice can only be used for the evaluation to a limited extent. Nevertheless, there is no evidence of carcinogenic effects in the studies. Benzoic acid and its alkali salts are therefore not classified in one of the categories for carcinogens.”

As taken from Hartwig A and MAK Commission, 2018

ACGIH TLV-A5: Not Suspected as a Human Carcinogen — The agent is not suspected to be a human carcinogen on the basis of properly conducted epidemiologic studies in humans. These studies have sufficiently long follow-up, reliable exposure histories, sufficiently high dose, and adequate statistical power to conclude that exposure to the agent does not convey a significant risk of cancer to humans; or, the evidence suggesting a lack of carcinogenicity in experimental animals is supported by mechanistic data demonstrating a lack of the key characteristics of carcinogenicity.

As taken from ACGIH, 2021a

A screening method based on gene expression (CARCINOscreen®) was used to predict hepatic carcinogenicity of sodium benzoate and 2,6-diaminotoluene in a 28-day repeated dose study in which CrI:CD(SD) and CrI:WI(HAN) male rats (n = 3/group) were fed 100 mg/kg bw sodium benzoate per day by gavage in a 28-day repeated dose study in which CrI:CD(SD) and CrI:WI(HAN) male RNA. Predictive scores obtained in Sprague-Dawley and Wistar rats indicated that sodium benzoate is not carcinogenic in either strain. The predictive results were confirmed by negative histopathology in the livers of both strains of rats.

JECFA (2022)

Carcinogen: no, known to not cause a problem. PPDB (2024).

5.7. Irritation/immunotoxicity

“Benzoic acid shows no skin or respiratory sensitization activity.”

As taken from ECHA, 2012a.

Type of Test	Route of Exposure or Administration	Species/ Test System	Dose Data	Reaction Severity	Reference
Standard Draize test	Administration onto the skin	Human	22 mg/3D (intermittent)	Moderate	85DKA8 "Cutaneous Toxicity, Proceedings of the 3rd Conference, 1976," Drill, V.A., and P. Lazar, eds., New York, Academic Press, Inc. 1977 Volume(issue)/page/year: - ,127,1977
Open irritation test	Administration onto the skin	Human	0.25%/1H		ADVEA4 Acta Dermato-Venereologica. (Almqvist & Wiksell, POB 45150, S-10430 Stockholm, Sweden) V.1- 1920- Volume(issue)/page/year: 26,734,1984
Standard Draize test	Administration onto the skin	Human	0.76%/40M	Mild	CODEDG Contact Dermatitis. Environmental and Occupational Dermatitis. (Munksgaard International Pub., c/o Publications Expediting Inc., 200 Meacham Ave., Elmont, NY 11003) V.1- 1975- Volume(issue)/page/year: 33,177,1995

As taken from RTECS, 2019.

/CASE REPORTS/ The case of a trained chemical worker highly sensitive to benzoic acid is described. He suffered from allergic reactions of increasing intensity while being constantly exposed to benzoic acid during work. After oral exposure to benzoic acid he suffered a severe anaphylactic shock and later on showed similar but milder reactions when eating food containing benzoic acid. No allergic reactions reoccurred avoiding every contact with benzoic acid especially observing a strict benzoic acid-free diet. [Pevny I et al; Derm Beruf Umwelt 29 (5): 123-30 (1981)]
PEER REVIEWED PubMed Abstract

/HUMAN EXPOSURE STUDIES/ Forty-eight female panelists participated in an in-use study that investigated the acneogenic and irritation potential of a liquid/ powder foundation containing 0.2% benzoic acid. ...Approximately half of the test population had "mild to moderate" acne. Panelists were instructed to apply the product to the entire face and neck area at least twice a day for 45 days. Acne lesions and irritation were evaluated by a dermatologist on days 0, 3, 7, 10, 28, and 45. Objective and subjective evaluations of irritation were made by a nurse or technician on days 15, 21, and 35. Panelists also maintained daily response logs. The dermatologist noted "no significant changes the lesion counts of the non acne subjects and the acne subjects had a decrease. ... Panelists' logs recorded occasional instances of dryness, itching, and flakiness. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)]
PEER REVIEWED

/HUMAN EXPOSURE STUDIES/ An eye shadow plus base containing 0.1% benzoic acid was tested in a 28-day in-use study using 52 women. Half of the panelists were contact lens wearers. Panelists were instructed to use the product at least twice a day. Examinations were made at the beginning and end of the study by an ophthalmologist, weekly by a nurse or technician, and the panelists maintained daily logs. Slight conjunctival hyperemia without chemosis was noted in eight women; the condition was nonpersistent in all cases. Another two women presented with slight hyperemia and were also experiencing allergy symptoms; erythema was noted around the eye of one of these two panelists. One panelist had an incidence of red and mildly swollen caruncles at the fifth observation. Four other panelists reported occasional itching; dryness was reported by another two panelists. One panelist reported redness, puffiness, and irritation of the left upper eyelid that prevented her from wearing her contact lenses for 2 days. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

Dust irritating to nose and throat if inhaled; solid irritating to skin and eyes. At elevated temp, fumes may cause irritation of eyes, resp system, and skin. [U.S. Coast Guard, Department of Transportation. CHRIS - Hazardous Chemical Data. Volume II. Washington, D.C.: U.S. Government Printing Office, 1984-5.] **PEER REVIEWED**

Benzoic acid ... is a mild irritant to the skin. It is also a mild irritant to the eyes and mucus membranes. [Rowe, R.C., Sheskey, P.J., Quinn, M.E.; (Eds.), Handbook of Pharmaceutical Excipients 6th edition Pharmaceutical Press, London, England 2009, p. 62] **PEER REVIEWED**

SIGNS AND SYMPTOMS/ Of 100 patients with asthma undergoing provocation tests with benzoic acid, 47 showed positive reactions. [Reynolds, J.E.F., Prasad, A.B. (eds.) Martindale-The Extra Pharmacopoeia. 28th ed. London: The Pharmaceutical Press, 1982., p. 1283] **PEER REVIEWED**

As taken from HSDB, 2014.

Although there is a wide range of results from mostly non-standardized tests using various scoring systems, it can be concluded that benzoic acid is slightly irritating to the skin and irritating to the eyes.

In different experiments with rabbits, which have not been performed according to current guidelines, benzoic acid applied as dry powder or in the form of a paste was not irritating to slightly irritating to the skin (score 1.66/8: Bio-Fax, 1973; no score given: Bayer AG, 1978; primary skin irritation index 0.5 [no further information available]: RCC Notox, 1988a).

In an acute eye irritation/corrosion study with rabbits conducted according to OECD Guideline 405, some eye irritation was reported after application of benzoic acid in the form of a paste. Within 72 h, the scores for chemosis, reddening of the conjunctivae, iritis, and keratitis always remained at <2 (Bayer AG, 1986).

In different non-standardized experiments with the solid substance, moderately irritating to severely irritating effects on the eye were noted (score 65/110: Bio-Fax, 1973; no score given: Bayer AG, 1978; score up to 108/110 [eyes rinsed after instillation] or up to 50/100 [eyes not rinsed]: Monsanto Co., 1983; score 35 according to the scheme of Kay & Calandra, 1962: RCC Notox, 1988b).

In a maximization test, none of 15 guinea-pigs reacted positively after induction and challenge with a 10-20% solution of benzoic acid in water (Gad et al., 1986). In addition, the substance also tested negative in a Buehler test with guinea-pigs and in an ear swelling test and local lymph node assay with mice (Gad et al., 1986; Gerberick et al., 1992). The concentrations used for induction and challenge were 10-20% in acetone or water.

However, a dose-dependent positive result was obtained in an ear swelling test with five guinea-pigs (induction with 0.2, 1, 5, or 20% in absolute ethyl alcohol; no challenge) used as a model for detecting agents causing non-immunological contact urticaria in humans. At several other regions (back, abdomen, flank site), a concentration of 20% failed to produce any reactions (Lahti & Maibach, 1984).

As taken from INCHEM, 2000, CONCISE INTERNATIONAL CHEMICAL ASSESSMENT DOCUMENT NO. 26, BENZOIC ACID AND SODIUM BENZOATE.

ECHA's committee for risk assessment (RAC) has concluded that benzoic acid should be subject to harmonised classification and labelling as irritating to the skin, and seriously damaging to the eye.

As taken from ECHA, 2012b.

"Benzoic acid is not irritating to the skin (below classification threshold) in standard animal irritation studies but highly irritating to the eyes. The effects observed on the eyes comprised corneal opacity (severe (1/3 animals) and not reversible (2/3 animals), no reaction to light up to d 21 (1/3 animals), non-reversible chemosis (2/3 animals), iridial injection and conjunctival redness as reported in the SCCP report (SCCP/0891/05-Opinion on Benzoic Acid and Sodium Benzoate, EU, 2005).

Benzoic acid and sodium benzoate are not irritating to the skin (below classification threshold) in standard animal irritation studies (cf. Doc. II table 3-5). However, skin effects have frequently been reported for humans (cf. Doc II table 3-15 for selected publications).

Benzoic acid and its salts are capable of causing non-immune immediate contact reactions (NIICR) and non immunogenic contact urticaria (NICU), also known as pseudoallergy.

In an investigation with unselected volunteers (200), double-blind skin tests were run with benzoic acid. Benzoic acid was applied in chambers onto the skin for 20 min and assessed 30 min after the initiation of the application. In this study, a considerable proportion of volunteers (73.5 %) reacted to benzoic acid at 125 mM (ca. 1.5 % dilution) with erythema, while 12.5 % developed oedema at this test concentration. At 500 mM (ca. 6 % dilution), 78.5 % reacted with erythema and 18 % with oedema. The mean erythema severity scores (applying a grading of 0-8) were 1.81 at 125 mM and 2.12 at 500 mM, respectively, while the oedema scores after 30 min were 0.16 at 125 mM and 0.22 at 500 mM.

1976 a new method was investigated. The chamber-scarification test for skin irritancy, as an alternative for the conventional patch test. Benzoic acid was applied once daily for 3 days in chambers on scarified and unscarified skin on the forearm of 5-10 healthy volunteers and rated 24 h after the last exposure. The irritant threshold concentration of benzoic acid on unscarified skin was 30 %, on scarified skin 7.5 %. Applying a five-point grading system of 0-4, a moderate

response (score between 1.5-2.4) was observed at 7.5 % on scarified skin. A marked response, leading to erosions, was observed at 15 % (score between 2.5-4).

Regarding skin testing of benzoates, closed patch tests in patients with an atopic predisposition, asthma or urticaria have been reported (1955: 4 % positive reactions 1987 (0.7 %) 1993 (0.2 %), among others). The vehicle used for dissolving benzoic acid or sodium benzoate is considered to be of great influence on the outcome of the test. A large amount of positive oral provocation tests with benzoates including skin reactions are also provided in a comprehensive literature (1982 (11 %), 1985 (18 %), 1991 (14 %), among others).

Based on the positive results in human skin irritation studies and the underlying non-immunogenic irritant and inflammatory mechanism which does not involve sensitisation, the data are regarded sufficient to justify labelling benzoic acid with R38 resp. H315 in a weight of evidence approach."

As taken from European Commission, 2013a,b.

"BACKGROUND: Irritant contact dermatitis (ICD) is a common diagnosis in patients with occupational contact dermatitis (OCD). Studies are lacking on the usefulness of material safety data sheets (MSDSs) in making the diagnosis of ICD. OBJECTIVE: To characterize irritant exposures leading to the diagnosis of occupational ICD (OICD), and to evaluate the occurrence of concomitant exposures to contact allergens. METHODS: We included 316 patients with suspected occupational hand dermatitis, referred to the Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark during January 2010-August 2011, in a programme consisting of a clinical examination, exposure assessment, and extensive patch/prick testing. RESULTS: OCD was diagnosed in 228 patients. Of these patients, 118 were diagnosed with OICD. The main irritant exposures identified were wet work (n = 64), gloves (n = 45), mechanical traumas (n = 19), and oils (n = 15). Exposure to specific irritant chemicals was found in 9 patients, and was identified from MSDSs/ingredients labelling in 8 of these patients. Review of MSDSs and ingredients labelling showed that 41 patients were exposed to 41 moderate to potent contact allergens, and 18 patients were exposed to 25 weak workplace contact allergens. CONCLUSION: In the present study, the systematic exposure assessment did not reveal any new irritants. MSDSs have a limited role in the investigation of ICD." As taken from Friis UF et al. 2014. Contact Dermatitis 71(6), 364-70. PubMed, 2015

Benzoic acid was not skin sensitizing in several animal models. For sodium benzoate, no data were identified covering this specific end-point.

As taken from INCHEM, 2000.

Various cosmetic formulations containing up to 0.2% benzoic acid have been tested on groups of volunteers for their ability to induce photosensitization. No reactions were noted during the induction or challenge phases or in response to irradiation for any of the tested formulations (CIR, 2001).

/SIGNS AND SYMPTOMS/ Cases of urticaria, asthma, rhinitis, or anaphylactic shock have been reported following oral, dermal, or inhalation exposure to benzoic acid and sodium benzoate. The symptoms appear shortly after exposure and disappear within a few hours, even at low doses. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

As taken from HSDB, 2014.

"BACKGROUND: Recalcitrant non-actinic cheilitis may indicate contact allergy. OBJECTIVES: This study aimed to determine the prevalence of allergic contact cheilitis (ACC) in patients with non-actinic cheilitis and to identify the most relevant allergens. METHODS: We used an institutional database to identify patients with non-actinic cheilitis who underwent patch testing between January 1, 2001, and August 31, 2011, and conducted a retrospective review of patch test results in

these patients. Additional data were obtained from institutional electronic medical records. RESULTS: Ninety-one patients (70 [77%] female; mean age: 51 years) were included in the study. Almost half (41 [45%]) had a final diagnosis of ACC. Patch testing was performed in line with universally accepted methods, with application on day 1, allergen removal and an initial reading on day 3, and the final reading on day 5. The allergens of most significance were fragrance mix, Myroxylon pereirae resin, dodecyl gallate, octyl gallate, and benzoic acid. Nickel was the most relevant metal allergen. CONCLUSIONS: Contact allergy is an important consideration in recalcitrant cheilitis. Fragrances, antioxidants, and preservatives dominated the list of relevant allergens in our patients. Nickel and gold were among the top 10 allergens. Almost half (45%) of these patients had a final diagnosis of ACC. Patch testing beyond the oral complete series should be undertaken in any investigation of non-actinic cheilitis." As taken from O'Gorman SM and Torgerson RR. 2016. Int. J. Dermatol. 55(7), 386-91. PubMed, 2017 available at <http://www.ncbi.nlm.nih.gov/pubmed/26547761>

"Activity-guided isolation of compounds from the fruits of Amomum xanthioides resulted in the purification of fourteen phenolic compounds, 4-hydroxy-benzaldehyde (1), 3,4-dihydroxybenzaldehyde (2), 3,5-dimethoxy-4-methylbenzaldehyde (3), syringic aldehyde (4), benzoic acid (5), 3,4-dihydroxy benzoic acid (6), vanillic acid (7), 3-hydroxy-2-methoxybenzoic acid (8), o-vanillic acid (9), phenylacetic acid (10), tyrosol (11), pyrocatechol (12), 1,2,4,5-tetramethoxybenzene (13), and 3,3',5,5'-tetramethoxybiphenyl-4,4'-diol (14). To evaluate the anti-allergic inflammatory activities of these compounds, we examined the inhibitory effects of the isolates (1-14) on histamine release and on the expressions of tumor necrosis factor (TNF)- α and interleukin (IL)-6 genes by using human mast cells. Of the tested compounds, 9, 11, and 13 suppressed histamine release from mast cells, and all isolates attenuated the expressions of the pro-inflammatory cytokines, TNF- α and IL-6 genes in human mast cells." As taken from Choi HG et al. 2015. Nat. Prod. Commun. 10(4), 631-2. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25973495>

Skin irritant: yes, known to cause a problem. Skin sensitiser: no, known to not cause a problem. Eye irritant: yes, known to cause a problem. May cause urticaria, asthma, rhinitis and anaphylactic shock (PPDB, 2024).

"Several studies including guinea pig maximisation and Buehler tests (Gad et al. 1986) and a local lymph node assay in mice (Gerberick et al., 1992) did not reveal any sensitising potential for benzoic acid."

As taken from EFSA, 2016a

"[Benzoic acid] is a skin and an eye irritant. Benzoic acid is probably not phototoxic. Benzoic acid is not a skin sensitiser in a local lymph node assay (LLNA). However, benzoic acid and its salts may cause non-immune immediate contact reactions.

As taken from EFSA, 2016b.

"Benzoic acid is a mild skin irritant, but sodium benzoate is not a skin irritant (SCCP, 2005). It is classified as an eye irritant.

Clinical data indicated that these ingredients can produce non-immunologic contact urticaria and non-immunologic immediate contact reactions, characterised by the appearance of wheals, erythema, and pruritus (irritant) (Nair, 2000; SCCP, 2005"

As taken from SCENIHR, 2016.

"The critical health effects associated with the chemical (but not the salts) are skin, eye and respiratory tract irritation.

The chemical was highly irritating in rabbit eyes, causing irreversible corneal opacity and chemosis in 2/3 animals, and increasing conjunctival redness severity with white/grey discoloration after 2-day observation. A Draize score of 35 was given based on the effects (REACH).

Positive skin irritant reactions (erythema and oedema) were frequently reported in a number of human studies. The threshold concentrations are strongly dependent on the vehicles (ethanol or petrolatum), skin conditions (scarified/unscarified, dermatitis or urticaria) and application conditions (closed/open patch tests or chamber-scarification tests for skin irritancy) (SCCP, 2005).

Non-immunological contact urticaria has frequently been reported in humans for the chemical. Contact urticarial reactions to the chemical (1 %) were seen in healthy volunteers (SCCP, 2005). Oedematous reaction to the chemical (0.05 %) were also observed in patients during an occlusion test (OECD, 2004).

The negative results seen for the chemical from several skin sensitisation animal studies including guinea pig maximisation test (GPMT), Buehler test and local lymph node assay (LLNA) support a conclusion that the chemical is not a skin sensitizer (REACH).

The chemical did not induce sensitisation in healthy volunteers although some allergic reactions were noted in 34/537 patients with suspected contact dermatitis (at 2 %) (SCCP, 2005) and 9/121 patients with dermatoses and 10/57 patients with chronic urticaria (at 5 %) (ECHA, 2011)

As taken from AICIS, 2013.

"The American Contact Dermatitis Society Core Allergen Series was introduced in 2012. After 4 years of use, changes in our recommended allergens are necessary. For the updated series, we have reordered the first 4 panels to approximately mirror the current TRUE Test and removed parthenolide, triclosan, glutaraldehyde, and jasmine. Polymyxin B, lavender, sodium benzoate, ethylhexylglycerin, and benzoic acid are new additions to the American Contact Dermatitis Society series." As taken from Schalock PC et al. 2017. *Dermatitis* 28(2), 141-143. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28169850>

"A skin and eye irritant; [ICSC] Causes non-immunological contact urticaria; [Kanerva, p. 218] Benzoic acid alkali salts cause pseudoallergic reactions; [MAK]."

As taken from Haz-Map, 2020.

"Benzoic acid is a skin and eye irritant but is not considered to be a skin sensitizer."

As taken from EFSA, 2019a.

"The critical effects of benzoic acid are the strong irritation of the eyes and the pulmonary toxicity after inhalation exposure.

The results from animal experiments for contact sensitization are all negative, and the immediate reactions to benzoic acid and sodium benzoate found in humans and guinea pigs are to be regarded as non-immunological reactions. Therefore, in view of the findings detailed in this supplement, there is still no necessity for designating the substance with "Sh" (for substances which cause sensitization of the skin). As no findings are available for respiratory sensitization, benzoic acid and alkali benzoates are not designated with "Sa" (for substances which cause sensitization of the airways"

As taken from Hartwig A and MAK Commission, 2018

ACGIH TLV basis: Eye, upper and lower respiratory tract irritation; lung damage (ACGIH, 2021b).

"As the topical use of non-steroidal anti-inflammatory drugs (NSAIDs) has gained popularity recently, adverse reactions related to their application have also become more common. The authors present the case of a 49-year-old man, who used etofenamate gel to treat leg pain. Following sun exposure, haemorrhagic, atypical lesions appeared and after rapid spread of the

symptoms, the patient was hospitalized. In the area of the etofenamate application as well as on both legs, arms, trunk and face, confluent, erythematous sero-papules and macules were found, along with petechiae on the oral mucosa. Splenomegaly and thrombocytopenia accompanied the skin symptoms, which prompted an oncohematological workup, and the patient was diagnosed with hairy cell leukaemia. Epicutaneous testing (ET) was performed and found a positive reaction to etofenamate gel as well wood tar, propylen glycol, fragrance mix I, methylisothiazolinone, benzoic acid and balsam of Peru. The lymphocyte transformation test (LTT) and CD69 expression were negative for etofenamate.” As taken from Fábíán M et al. 2020. *Orv. Hetil.* 161(38), 1646-1651. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32924969/>

“Background: Allergic contact dermatitis (ACD) from topical medication often occurs in occluded areas, for example, with wound treatment, but also in certain body locations, such as the anogenital area. Objectives: To investigate the demographics and specific lesion location of patients with ACD from topical drugs applied onto the (peri)anal/genital area, and to identify the respective causal topical pharmaceutical products and ingredients involved. Methods: From January 2000 to December 10, 2018, 532 patients were tested with the baseline series, sometimes with additional series, and the topical medication used along with the ingredients. The relevant data were extracted from our electronic databases developed in-house. Results: Forty-four patients (9%) out of 473 patients suffering from lesions in the (peri)anal/genital area had positive patch test results to topical drug preparations and/or their ingredients, sometimes in association with cosmetics for intimate hygiene. The most frequent sensitizing active principles were local anaesthetics and corticosteroids, while wool alcohols and to a minor extent benzoic acid were the most frequent culprits among the vehicle components and preservative agents, respectively. Conclusions: The local conditions (eg, occlusion, sweating, moist) in the anogenital area may favour skin sensitization to topical medication used to treat various skin diseases.” As taken from Gilissen L et al. 2021. *Contact Dermatitis* 84(6), 431-438. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33350482/>

“Based on the existing data, benzoic acid is not considered to be a skin sensitizer under the current, declared levels of use. The chemical structure of this material indicates that it would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). Benzoic acid was found to be positive in an in vitro direct peptide reactivity assay (DPRA) (Natsch and Haupt, 2013b) and negative in KeratinoSens and U937-CD86 tests (Natsch et al., 2013a; Natsch and Haupt, 2013b). In a murine local lymph node assay (LLNA), benzoic acid was not found to be sensitizing up to 20% (Gerberick et al., 1992; ECHA, 2011). In a guinea pig maximization test, benzoic acid did not present reactions indicative of sensitization up to 20% (Gad et al., 1986; ECHA, 2011). In guinea pig Freund's Complete Adjuvant Test (FCAT), reactions were reported with benzoic acid at 10% (Hausen et al., 1995; Hausen et al., 1992). However, limited details on the study protocol and the reactions were provided. In a human maximization test, no skin sensitization reactions were observed with benzoic acid at 2% (1380 µg/cm²) and 5% (3450 µg/cm²) (RIFM, 1977; Leyden and Kligman, 1977). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 992 µg/cm² of benzoic acid in 3:1 EtOH:DEP, no reactions indicative of sensitization were observed in any of the 113 volunteers (RIFM, 2020).

Based on WoE from structural analysis and animal and human studies, benzoic acid does not present a concern for skin sensitization under the current, declared levels of use. (...)

UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). In in vitro phototoxicity assays with 3T3 Swiss mouse fibroblasts, there was no evidence of phototoxicity (Duffy et al., 1987; Duffy et al., 1989). Based on the lack of absorbance and in vitro study data, benzoic acid does not present a concern for phototoxicity. Based on the lack of absorbance, benzoic acid does not present a concern for photoallergenicity.”

Api AM et al. (2022). RIFM fragrance ingredient safety assessment, benzoic acid, CAS Registry Number 65-85-0.

In patch-testing of 41 patients with a diagnosis of allergic contact cheilitis, benzoic acid gave 12% positive results and allergic-relevant reactions (36). The aim of the study was to determine the prevalence of allergic contact cheilitis in patients with non-actinic cheilitis and to identify the most relevant allergens. Patients without study was to determine the prevalence of allergic contact cheilitis in patients with patch testing of a series of antigens determined by a dermatologist. Benzoic acid was classified as one of the most relevant preservative-derived allergens in the study. The American Contact Dermatitis Society has added benzoic acid to its core allergen series (37), which gives dermatologists who are using TRUE test standard allergens as their baseline series a logical, graded tool to increase the number of allergens tested. In 2016, EFSA (23) reviewed the available data on allergenicity, hypersensitivity and intolerance for sodium benzoate (Table 5). In its previous evaluations, the Committee noted reports of allergic responses to sodium benzoate. The Committee concluded that the human data available indicate that benzoic acid and sodium benzoate can trigger allergic reactions in some individuals when consumed in foodstuffs.

JECFA (2022)

“Benzoic acid was chosen as a non-sensitizer control.” “The results showed a dose–response effect after exposure to all the substances tested, except for benzoic acid. Indeed, benzoic acid is not a sensitizer and did not activate either of the two BMDC markers as expected.” “Phenol was classified as a weak sensitizer while benzoic acid and BPF were both classified as non-sensitizers.”

Chemical IUPAC name	Abbreviation	CAS number	Concentrations tested (µM)	LLNA class	h-CLAT result	Human class	CLP	Supplier	BPA analogue or alternative
Benzoic acid	Ac BZ	65-85-0	4000-5000-6000-7000-8000	Non-sensitizer	Negative	Non-sensitizer	/	SigmaAldrich	NA

As taken from M Mourot-Bousquenaud et al., 2024 available at <https://pubmed.ncbi.nlm.nih.gov/37927141/>

5.8. All other relevant types of toxicity

/SIGNS AND SYMPTOMS/ Twelve men drank apple juice containing 0.1% sodium benzoate and had the following signs and symptoms: burning taste, fullness in the head, headache, nervousness, nausea, vomiting, itching of the skin, unusual perspiration, constipation, decreased urine flow, increased specific gravity of the urine, and albuminuria. /Sodium Benzoate/ [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

/SIGNS AND SYMPTOMS/ In /a/ study participants initially ingested benzoic acid at 1000 mg/day for 5 days and progressed to 1500 then 2000 and finally 2500 mg/day staying at each protocol for 5 days before increasing the dose. Three of the 12 participants took the entire dose of 35 g in 20 days. This total dose produced marked symptoms of discomfort and malaise, which included nausea, headache, weakness, esophageal burning and irritation, hunger, and indigestion. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

In nine patients on penicillin treatment given 12,000 mg benzoic acid divided into eight doses over 5 days in eight subjects and over 14 days in one subject, no adverse effects on blood urea nitrogen or creatinine clearance were reported. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005 **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ /Investigators/found no abnormalities in blood chemistry, urine composition, nitrogen balance, or well-being in six men given 300-400 mg /benzoic acid/ per day

via diet for up to 62 days. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

Nine patients on penicillin treatment received 1200 mg benzoic acid daily, in eight doses, over five days for eight subjects and for 14 days in one case. No effect was observed; in particular, no significant change was seen in endogenous creatinine clearance, and routine urine analysis showed no abnormality. [WHO; Food Additive Series 37: Toxicological evaluation of ceratin food additives: Benzyl acetate, Benzyl alcohol, Benzaldehyde, and Benzoic acid and its Salts (1996). Available from, as of March 24, 2005: <http://www.inchem.org/documents/jecfa/jecmono/v37je05.htm> **PEER REVIEWED**

/HUMAN EXPOSURE STUDIES/ In a study with volunteers given 1000, 1500, 2000, or 2500 mg/day for 5 days each, marked symptoms, signs of discomfort, and malaise (nausea, headache, weakness, burning and irritation of esophagus) were reported. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

/SIGNS AND SYMPTOMS/ Infants given large doses of sodium benzoate have suffered vomiting. Symptoms of overdosage reported in this group have included vomiting, irritability, and in more severe cases, renal tubular dysfunction., hypokalemia, hypocalcemia, and metabolic acidosis. premature infants have been reported to be at risk of metabolic acidosis and kernicterus. [SWEETMAN, S.C. (ed.) Martindale-The Complete Drug Reference. 36th ed. London: The Pharmaceutical Press, 2009., p. 1630] **PEER REVIEWED**

The effects of subchronic exposure to benzoic acid (BA) were evaluated in male and female Sprague-Dawley rats (10/sex/group) exposed by inhalation to 0, 0.025, 0.25 or 1.2 mg BA/L (generated as a dust aerosol with an equivalent aerodynamic diameter of 4.7 um) for 6 hrs/day, 5 days/week for 4 weeks. All high- and mid-dose animals exhibited upper respiratory tract irritation (red material around the nares). Two animals (1/sex) died in the high-dose group. There were statistically significant differences observed between treated and control animals in the following (high-dose group unless noted otherwise): decreased body weight gain, random differences in hematological data and serum biochemical evaluation (not considered to be exposure related except for a related decrease in the number of platelets), and decreased absolute and relative weights of liver (males), kidney (females, high- and mid-dose levels), and trachea/lung (females). The incidence of slight multifocal and generalized interstitial fibrosis and inflammatory cell infiltrate in treated animals were high compared to controls (not dose-related). No compound-related macroscopic lesions were observed in any of the rats. There were no deaths, no significant effects on body weight gain, and there were no significant effects on hematologic or biochemical parameters in the low- or mid-dose animals relative to the negative controls. [International Research and Development Corp.; Four Week Subacute Inhalation Toxicity Study of Benzoic Acid in Rats. (1981), EPA Document No. FYI-OTS-1281-0147, Fiche No. OTS0000147-0] **UNREVIEWED**

/LABORATORY ANIMALS: Neurotoxicity/ Royal Wistar rats (number not stated) dosed with 1.1% for 7, 14, or 35 days (approx. 550 mg/kg/day). Significantly poor weight gain; no signs of neurotoxicity or pathological changes in the brain /were observed/. [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

/LABORATORY ANIMALS: Neurotoxicity/ Groups of eight Wistar dams received feed containing 0.1%, 0.5%, or 1.0% sodium benzoate beginning on gestation day (GD) 5 and continuing throughout pregnancy and lactation. The control group was untreated. At birth, the number of pups in each litter was equalized to eight. Locomotor activity of the pups was measured on various days. One pup from each litter was killed on days 9, 15, and 21 and the brain removed and examined. On

day 22, pups were weaned onto the same diet as their respective dam. On day 24, one male pup from each litter was caged individually and monitored for spontaneous locomotor activity. Rats were killed on day 45 and brain concentrations of norepinephrine, dopamine, and serotonin were measured. No significant difference was noted in feed intake and body weight gain of dams and pups of the treated groups compared to controls. No consistent differences in motor activity and monoamine concentrations were noted. /Sodium Benzoate/ [Cosmetic Ingredient Review Expert Panel; International Journal of Toxicology 20 (suppl. 3): 23-50 (2001)] **PEER REVIEWED**

/OTHER TOXICITY INFORMATION/ Benzoic acid inhibits pepsin digestion, ...but /it has/ no effect on amylase or lipase. ...Benzoic acid is a specific, powerful inhibitor of D-amino acid oxidase, a 10-4 mmol/L solution resulting in 50% inhibition. Concentrations in the range of 10-3 mmol/L have non-specific inhibitory effects on the metabolism of fatty acids, including acetoacetate formation. [WHO; Food Additive Series 37: Toxicological evaluation of ceratin food additives: Benzyl acetate, Benzyl alcohol, Benzaldehyde, and Benzoic acid and its Salts (1996). Available from, as of March 24, 2005: <http://www.inchem.org/documents/jecfa/jecmono/v37je05.htm> **PEER REVIEWED**

/OTHER TOXICITY INFORMATION/ Benzoic acid (2%) as found in some preserved pet foods was toxic to cats. The /investigators/ suggest that the largest amount that could be fed daily to cats was 0.2 g/kg. [Beford PGC, Clarke ECG; Vet Rec 90: 53-8 (1972)] **PEER REVIEWED** PubMed Abstract

/PLANTS/ Flower-inducing effect of benzoic acid in various strains of *Lemna paucicostata* and *L. minor* was investigated. Benzoic acid is more effective than salicylic acid for all strains of *L. paucicostata*, but the contrary is true for two *L. minor* strains. [Kaihara S et al; Plant Cell Physiol 22 (5): 819-25 (1981)] **PEER REVIEWED**

Therapeutic Uses:

Antifungal Agents; Food Preservatives [National Library of Medicine's Medical Subject Headings online file (MeSH, 1999)] **PEER REVIEWED**

Benzoic acid ... has a long history of use as an antifungal agent in topical therapeutic preparations such as Whitfield's ointment (benzoic acid 6% and salicylic acid 3%). [Rowe, R.C., Sheskey, P.J., Quinn, M.E.; (Eds.), Handbook of Pharmaceutical Excipients 6th edition Pharmaceutical Press, London, England 2009, p. 61] **PEER REVIEWED**

... It is used especially in the treatment of athlete's foot and to lesser extent for management of ringworm. [Osol, A. (ed.). Remington's Pharmaceutical Sciences. 16th ed. Easton, Pennsylvania: Mack Publishing Co., 1980., p. 1172] **PEER REVIEWED**

Medication (vet): has been used with salicylic acid as topical antifungal [O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 187] **PEER REVIEWED**

/Experimental Ther:/ Process for controlling waste nitrogen accumulation diseases in humans by administering at least 1 compound selected from group of benzoic acid, phenylacetic acid and their salts is disclosed. Sodium benzoate, (6.2 g/day) given to female patient with carbophosphate synthetase deficiency Increased total urinary nitrogen excretion 58%. [Brusilow SW et al; US Patent Number 4284647 08/18/81 (Johns Hopkins University)] **PEER REVIEWED**

As taken from HSDB, 2014.

"Sodium benzoate appeared to have no maternal toxicity, fetal toxicity, or teratogenicity in mice, rats, hamsters, or rabbits when given orally (FDRL, 1972). The highest doses tested were 175.0 in mice and rats, 300.0 in hamsters, and 250.0 mg/kg/day in rabbits.

The only chronic oral data available involve administration of benzoic acid to rats and mice (Shtenberg and Ignat'ev, 1970; Ignat'ev, 1965; Marquardt, 1960). A dose of 40 mg/kg/day for 17 months was associated with decreased resistance to stress in mice and possibly with reduced food

and water intake in rats after 18 months (Shtenberg and Ignat'ev, 1970). However, another report from this laboratory (Ignat'ev, 1965) indicated that 80 mg/kg/day in rats for 18 months was not associated with adverse effects on body weight, survival, or gross or microscopic pathology. If 40 mg/kg/day in mice in the study by Shtenberg and Ignat'ev (1970) is considered to be the LOAEL, application of an uncertainty factor of 1000 would result in an RfD of 0.04 mg/kg/day or 2.8 mg/day, which is near the lower end of the range of the estimated daily human exposure to benzoic acid (not including exposure to sodium benzoate). The lower RfD based on animal data is not unexpected, however, since application of uncertainty factors is intentionally conservative in the absence of human data. Since human data are available in this case, it is not appropriate to use the animal data for the RfD.

Other long-term dietary studies (Marquardt, 1960) showed decreased food intake and body weight in rats fed 1.5% benzoic acid (750 mg/kg/day); at a dose of 1.0% in the diet (50 mg/kg/day) there were no signs of toxicity or adverse reproductive effects.

Gerlach (1909) reported no externally visible effects in humans ingesting benzoic acid at 0.5-1.0 g/day for 44 consecutive days or for 82/86 or 88/92 days. Assuming a human body weight of 70 kg, this level corresponds to a dose of 14 mg/kg/day. Wiley and Bigelow (1908), however, observed irritation, discomfort, weakness, and malaise in humans given oral bolus doses of less than or equal to 1.75 g/day over a 20-day period (25 mg/kg/day). The RfD (4 mg/kg/day) is well below these doses."

As taken from IRIS (1988).

"The free radical scavenging activity and reducing power of 16 phenolic compounds including four hydroxycinnamic acid derivatives namely ferulic acid, caffeic acid, sinapic acid and p-coumaric acid, benzoic acid and its derivatives namely protocatechuic acid, gallic acid and vanillic acid, benzene derivatives namely vanillin, vanillyl alcohol, veratryl alcohol, veratraldehyde, pyrogallol, guaiacol and two synthetic antioxidants, butylated hydroxy anisole (BHA) and propyl gallate were evaluated using 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH(•)), 2,2'-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical (ABTS(•+)), Hydroxyl radical ((•)OH) and Superoxide radical (O₂ (•-)) scavenging assays and reduction potential assay. By virtue of their hydrogen donating ability, phenolic compounds with multiple hydroxyl groups such as protocatechuic acid, pyrogallol, caffeic acid, gallic acid and propyl gallate exhibited higher free radical scavenging activity especially against DPPH(•) and O₂ (•-). The hydroxylated cinnamates such as ferulic acid and caffeic acid were in general better scavengers than their benzoic acid counter parts such as vanillic acid and protocatechuic acid. All the phenolic compounds tested exhibited more than 85 % scavenging due to the high reactivity of the hydroxyl radical. Phenolic compounds with multiple hydroxyl groups also exhibited high redox potential. Exploring the radical scavenging and reducing properties of antioxidants especially those which are found naturally in plant sources are of great interest due to their protective roles in biological systems." As taken from Mathew S et al. 2015. J. Food Sci. Technol. 52(9), 5790-8. PubMed, 2016.

"Cranberry consumption has shown prophylactic effects against urinary tract infections (UTI), although the mechanisms involved are not completely understood. In this paper, cranberry phenolic compounds and their potential microbial-derived metabolites (such as simple phenols and benzoic, phenylacetic and phenylpropionic acids) were tested for their capacity to inhibit the adherence of uropathogenic *Escherichia coli* (UPEC) ATCC®53503™ to T24 epithelial bladder cells. Catechol, benzoic acid, vanillic acid, phenylacetic acid and 3,4-dihydroxyphenylacetic acid showed anti-adhesive activity against UPEC in a concentration-dependent manner from 100-500 µM, whereas procyanidin A2, widely reported as an inhibitor of UPEC adherence on uroepithelium, was only statistically significant (P<0.05) at 500 µM (51.3% inhibition). The results proved for the first time the anti-adhesive activity of some cranberry-derived phenolic metabolites against UPEC in vitro, suggesting that their presence in the urine could reduce bacterial colonization and progression of UTI." As taken from de Llano DG et al. 2015. Int. J. Mol. Sci. 16(6), 12119-30. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26023719>

“CONTEXT: It is necessary to advance the field of alternative treatments for chronic wounds that are financially accessible to the least economically developed countries. Previously we demonstrated that topical applications of *Lactobacillus plantarum* culture supernatants (LAPS) on human-infected chronic wounds reduce the pathogenic bioburden, the amount of necrotic tissue, and the wound area, as well as promote debridement, granulation tissue, and wound healing. OBJECTIVE: To study LAPS chemically and biologically and to find potential molecules responsible for its pro-healing and anti-pathogenic properties in chronic wounds. MATERIALS AND METHODS: (1) Chemical analysis: extracts were subjected to a column chromatography and the fractions obtained were studied by GCMS. (2) Quantification: dl-lactic acid (commercial kit), phenolic compounds (Folin-Ciocalteu), H₂O₂ (micro-titration), and cations (flame photometry). (3) Biological analysis: autoinducers type 2 (AI-2) (*Vibrio harveyi* BB170 bioassay), DNAase activity (Agar DNAase), and *Pseudomonas aeruginosa* biofilm inhibition (crystal violet technique). RESULTS: According to its biological activity, the most significant molecules found by GCMS were the following: antimicrobials (mevalonolactone, 5-methyl-hydantoin, benzoic acid, etc.); surfactants (di-palmitin, distearin, and 1,5-monolinolein); anesthetics (barbituric acid derivatives), and AI-2 precursors (4,5-dihydroxy-2,3-pentanedione and 2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran). Concentrations measured (µg/mL): DL-lactic acid (11.71 ± 1.53) and H₂O₂ (36 ± 2.0); phenolic compounds (485.2 ± 15.20); sodium (370 ± 17); potassium 920 ± 24; calcium (20 ± 4); and magnesium (15 ± 3). DNAase from LAPS had activity on genomic DNA from PMNs and *P. aeruginosa*. DISCUSSION AND CONCLUSION: The molecules and biological activities found in LAPS could explain the observed effects in human chronic wounds.” As taken from Ramos AN et al. 2015. Pharm. Biol. 53(3), 350-8. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25347359>

“Many phenolic compounds have been described in the scientific literature as inhibitors of tyrosinase. In this work a test is proposed that allows us to distinguish whether a molecule is an enzyme inhibitor or substrate. The test has several stages. First, the degree of inhibition of the studied molecule is determined on the monophenolase activity (i(M)) and on the diphenolase activity (i(D)). If i(M) = i(D), it is an inhibitor. If i(M) ≠ i(D), the molecule could be substrate or inhibitor. Several additional stages are proposed to solve this ambiguity. The study described herein was carried out using the following molecules: benzoic acid, cinnamic acid, guaiacol, isoeugenol, carvacrol, 4-tert-butylphenol, eugenol, and arbutin.” As taken from Ortiz-Ruiz CV et al. 2015. J. Agric. Food Chem. 63(8), 2162-71. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25665009>

“Benzoic acid may be used for managing hyperammonaemia in urea cycle disorders in the neonates. However, the main problem linked to the use of benzoic acid concerns neonates (pre-term and fullterm) who have immature metabolising enzymes leading to accumulation. The risk associated with the accumulation of benzoic acid is an increase in bilirubinaemia following displacement of bilirubin from albumin and which may cause newborn jaundice to develop into kernicterus (non-conjugated bilirubin deposits in the brain tissue)”

As taken from EMA, 2017.

“Benzoic acid was also tested in the US EPA’s ToxCast project, a large screening programme using a panel of high-throughput cell-free assays for CYP activation/inhibition or binding to membrane receptors (mitochondria, norepinephrine, dopaminergic, aminergic, etc.) as well as nuclear receptors (e.g. oestrogen): benzoic acid was consistently negative in all assays, indicating a low likelihood to exert organ/system-specific toxicity (Sipes et al., 2013)”

As taken from EFSA, 2017.

Type of Test	Route of Exposure or Administration	Species/ Test System	Dose Data	Toxic Effects	Reference

IC50 Inhibitor Concentration 50	-	In vitro	Human - liver tumor	21 mmol/L/24H	In Vitro Toxicity Studies - cell protein synthesis	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 3,189,1989
ICLo Inhibitor Concentration Low	-	In vitro	Guinea pig - leukocyte	0.25 gm/L/30M	In Vitro Toxicity Studies - other assays	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 137,914,2011
IC50 Inhibitor Concentration 50	-	In vitro	Human - lymphocyte	>200 mg/L/45H	In Vitro Toxicity Studies - other assays	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,901,2015

As taken from RTECS, 2019.

"Histone deacetylases (HDACs), which modulate the expression of genes, are potential therapeutic targets in several cancers. Targeted inhibition of HDAC prevents the expression of oncogenes thereby help in the treatment of cancers. Hence, several pharmaceutical companies developed inhibitors of HDAC and tested them in preclinical models and in clinical trials. SAHA (suberanilohydroxamic acid) is one such HDAC inhibitor developed for treating breast and colorectal carcinomas. However, due to poor efficacy in clinical trials the utility of SAHA for treating cancers was discouraged. Similarly another HDAC inhibitor Trichostatin-A (TSA) also showed promising results in clinical trials but exhibited severe adverse effects, which dampened the interest of using this molecule for cancer treatment. Therefore, search for developing a potent HDAC inhibitor with minimal side effects still continues. Hence, in this study we have screened benzoic acid and benzoic acid derivatives with hydroxylic (-OH) groups and methoxy (-OCH₃) groups for their efficacy to bind to the TSA binding site of HDAC using molecular docking studies. Molecules that showed much stronger affinity (than TSA) to HDAC were tested for inhibiting HDAC expressing cultured cancer cells. DHBA but not Dimethoxy Benzoic Acid (DMBA) inhibited HDAC activity, leading to cancer cell growth inhibition through the induction of ROS and cellular apoptosis mediated by Caspase-3. In addition, DHBA arrested cells in G2/M phase of the cell cycle and elevated the levels of sub-G0-G1 cell population. In summary, results of this study report that DHBA could be a strong HDAC inhibitor and inhibit cancer cell growth more effectively." As taken from Anantharaju PG et al. 2017. Cancer Biol. Ther. 18(7), 492-504. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28506198>

Benzoic acid is included in EFSA's "list of active substances considered in view of establishing CAGs [cumulative assessment groups] for effects of pesticides on the thyroid (EFSA, 2019b).

"It has a role as an antimicrobial food preservative, an EC 3.1.1.3 (triacylglycerol lipase) inhibitor, an EC 1.13.11.33 (arachidonate 15-lipoxygenase) inhibitor."

As taken from PubChem

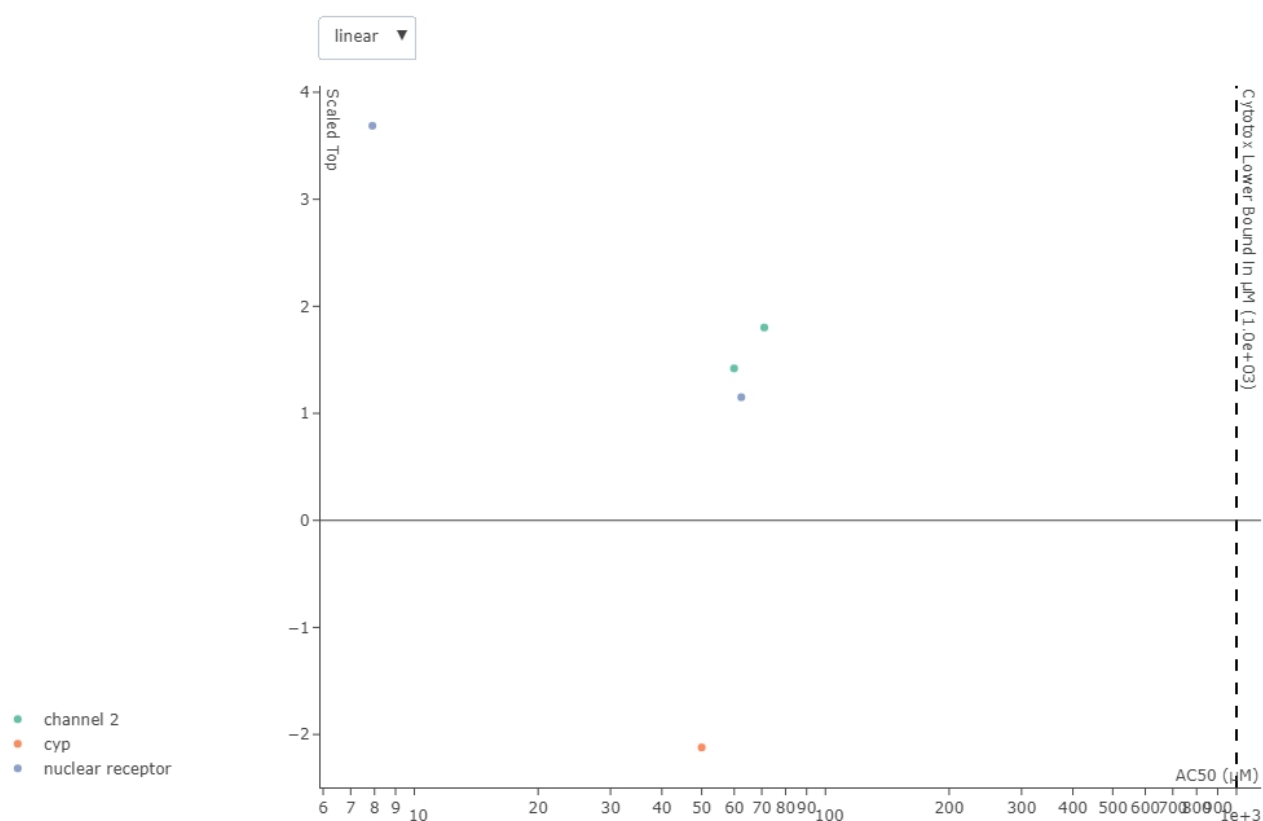
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated benzoic acid in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY).

US EPA provides the following data use considerations for ToxCast data: "The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve."

A summary of the ToxCast assay data on benzoic acid is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 1



6. Functional effects on

6.1. Broncho/pulmonary system

Four groups of 10 CD rats/sex/group were exposed to 0, 25, 250 or 1200 mg benzoic acid dust aerosol/m³ (analytical concentration; MMAD 4.7 μm) for 6 hours/day and 5 days/week over 4 weeks. At ≥ 25 mg/m³ an increased incidence of interstitial cell infiltrate and interstitial fibrosis in the lungs in treated animals compared with controls was seen. However, there was no clear dose dependency. A concentration of ≥ 250 mg/m³ resulted in upper respiratory tract irritation and decreased absolute kidney weights in females. In the highest-dose group one rat/sex died and the body weight gain was decreased in males and females. Other effects included a decrease in

platelets (males/females), absolute/relative liver weights (males) and trachea/lung weights (females).

LOAEC (local effect) = 25 mg/m³ (However no clear dose-response was observed).

NOAEC (systemic) = 25 mg/m³ (IRDC# 163-676, 1981)

OECD SIDS BENZOATES UNEP PUBLICATIONS 1 FOREWORD INTRODUCTION BENZOATES CAS N°:65-85-0, 532-32-1, 582-25-2, 100-51-6 OECD SIDS BENZOATES UNEP PUBLICATIONS 2 SIDS Initial Assessment Report for 13th SIAM (Bern, 7th - 9th November 2001)

As taken from INCHEM, 2004.

Rats survived a 4-hr inhalation exposure to benzoic acid dust at 12.2 mg/l (IRDC, 1974).

Ten rats per sex per group were exposed to an aerosol of benzoic acid dust at 0, 25, 250, or 1200 mg/m³ for 6 hr/day, 5 days/week, for 4 weeks. At 25 mg/m³ and above, there was an increased incidence of interstitial inflammatory cell infiltrate and interstitial fibrosis in the trachea and lungs. Although the number of these microscopic lesions was higher in treated animals, there was no clear relation with exposure concentration. At 250 mg/m³ and above, inflammatory exudates around the nares were indicative of upper respiratory tract irritation, and the females had significantly decreased kidney weights. At 1200 mg/m³, two rats died, and the others showed decreased growth. Additional effects included decreases in platelet numbers (males/females), liver weights (males), and trachea/lung weights (females) (Velsicol Chemical Corp., 1981).

ECHA's committee for risk assessment (RAC) has concluded that benzoic acid should be subject to harmonised classification and labelling as damaging to the lungs after repeated exposure by irritation.

As taken from ECHA, 2012b.

Respiratory tract irritant: yes, known to cause a problem. May cause asthma (PPDB, 2024).

ACGIH TLV basis: Eye, upper and lower respiratory tract irritation; lung damage (ACGIH, 2021b).

"The inhalation exposure estimated for combined exposure was considered along with toxicological data observed in the scientific literature to calculate the MOE from inhalation exposure when used in perfumery. In a 28-day OECD 412, GLP-compliant study, Sprague Dawley CD rats (10/sex/dose) were exposed to benzoic acid at concentrations of 0, 25, 250, or 1200 mg/m³ for 6 h/day, 5 days/week, through whole-body inhalation exposure for 4 weeks (ECHA, 2011). Standard endpoints evaluated included clinical signs, body weight, serum biochemistry, hematology, organ weight, necropsy (heart, kidney, lungs/trachea, brain, liver, and spleen), and histopathological examination. Treatment-related but not dose-dependent microscopic lesions were reported in the lungs of animals from the low-, mid-, and high-dose groups, which included increased inflammatory cell infiltrate and increased incidence, intensity, and extent of interstitial fibrosis. In both mid- and high-dose groups, reddish discharge around the nares was reported. At the 250 mg/m³ dose, upper respiratory tract irritation was observed, which was confirmed by inflammatory exudate around the nares. Additionally, at the 250 mg/m³ dose, decreased relative weight of trachea with lungs (females) were reported. The effects observed in the mid-dose of 250 mg/m³ were confined to local effects observed in the respiratory tract. Based on the observations in the lungs, the local effects LOAEC was identified at 25 mg/m³. Using a safety factor of 10, the estimated NOAEC is 2.5 mg/m³."

Api AM et al. (2022). RIFM fragrance ingredient safety assessment, benzoic acid, CAS Registry Number 65-85-0.

6.2. Cardiovascular system

BIOSIS COPYRIGHT: BIOL ABS. Background: Benzoic acid is one of the most commonly used preservatives in cosmetics, foodstuffs, and drug preparations. Nonetheless, products containing this compound frequently induce cutaneous erythema. Previous studies have suggested that prostaglandins may mediate the cutaneous vasodilation because ingestion of cyclooxygenase inhibitors before the application of benzoic acid markedly diminishes this symptom. However, the prostaglandin responsible has not been conclusively determined. Recently we showed that cutaneous erythema similar to that associated with application of benzoic acid is induced by the topical administration of another preservative, sorbic acid, and is mediated by the increased biosynthesis of prostaglandin (PG)D₂ in the skin. This study was designed to determine whether the cutaneous vasodilation induced by benzoic acid is mediated by this prostaglandin in humans.

DOWNARD CD et al., CLINICAL PHARMACOLOGY & THERAPEUTICS; 57 (4). 1995. 441-445.

6.3. Nervous system

Non-human

Weanling male rats fed 3% benzoic acid in the diet for 3-5 days developed neurological disorders (irritation, ataxia and tonic-clonic convulsions) and histological changes indicating brain damage. No such effects were noted in rats fed 1.1% benzoic acid for 35 days (Kreis et al. 1967).

In short-term studies with rats, disorders of the central nervous system (benzoic acid/sodium benzoate) as well as histopathological changes in the brain (benzoic acid) were seen after feeding high doses (≥ 1800 mg/kg body weight) over 5-10 days.

As taken from INCHEM (2000): CONCISE INTERNATIONAL CHEMICAL ASSESSMENT DOCUMENT NO. 26, BENZOIC ACID AND SODIUM BENZOATE.

“Acute neuroexcitation by benzoic acid was studied mechanistically in rats at a dose of approx. 500 mg/kg bw administered intravenously by infusion over 60 min. At this dose, neither change in the somatosensory evoked potential (SEP) nor SEP oscillations in the frequency band of 30-120 Hz were observed. Other endpoints were not reported.

In another developmental neurotoxicity study the effects of sodium benzoate were evaluated on activity and behavior in neonatal and juvenile rats. Treatment with 0, 0.1, 0.5, and 1.0 % benzoate in the diet commenced on day 5 of gestation of the parental animals and continued through lactation and after weaning. Offspring activity was measured every 3 days preweaning and continuously thereafter. Observations were comparable for all treatment groups. On day 9, 15, and 21, one animal per litter was sacrificed for measurement of brain region weight and serotonin, dopamine and noradrenaline levels. The remaining 5 animals per litter were subjected to the same analysis on day 45. There were no treatment-related changes in weight or neurotransmitter content for any of 5 analysed brain regions. Consequently, the NOAEL for developmental neurotoxicity in rats was considered as 1 % in the diet, corresponding to approx. 500 mg/kg bw/d.

Overall, neurotoxicity observed in benzoic acid intoxications seems to be secondary to metabolic changes (acidosis, acyl-CoA and ATP depletion). In addition, subacute exposure to lethal concentrations was associated with non-reversible histopathologic changes of the brain. However, no evidence for neurofunctional abnormalities or histopathological alterations of the CNS were reported following subacute and subchronic exposure to doses up to approx. 500 mg/kg bw/d.”

As taken from European Commission, 2013a,b.

Possible brain toxicant (PPDB, 2024).

“Neurotoxicity and CNS toxicity were assessed in juvenile rats exposed to the diet for up to 35 days at 0, 1.1, and 3 %, corresponding to approximate doses of 0, 825, and 2250 mg/kg bw/d benzoic acid. At day 4, clinical observation showed signs of neurotoxicity in most animals of the highest dose group including ataxia, tremor, excitation, aggressive behaviour and convulsions. However,

these changes were deemed secondary to metabolic changes (acidosis, acyl-CoA and ATP depletion). Histological evaluation of the brain showed prominent pathological changes in 2/5 animals treated for 3 days, 18/18 animals treated for 5 days and 13/15 animals with 3 weeks of recovery following the 5 days of exposure. These changes included ischaemic necrosis preferentially of ganglial cells in the stratum granulosum of the fascia dentate and the cortex of the lobus piriformis. No histopathology of the brain or clinical abnormality was noted in any of the animals of the low dose group receiving approximately 825 mg/kg bw/d over 7, 14, or 35 days (ECHA, 2011”

As taken from AICIS, 2013.

“D-Amino acid oxidase (DAAO) specifically catalyzes the oxidative deamination of neutral and polar D-amino acids and finally yields byproducts of hydrogen peroxide. Our previous work demonstrated that the spinal astroglial DAAO/hydrogen peroxide (H_2O_2) pathway was involved in the process of pain and morphine antinociceptive tolerance. This study aimed to report mouse strain specificity of DAAO inhibitors on antinociception and explore its possible mechanism. DAAO inhibitors benzoic acid, CBIO, and SUN significantly inhibited formalin-induced tonic pain in Balb/c and Swiss mice, but had no antinociceptive effect in C57 mice. In contrast, morphine and gabapentin inhibited formalin-induced tonic pain by the same degrees among Swiss, Balb/c and C57 mice. Therefore, mouse strain difference in antinociceptive effects was DAAO inhibitors specific. In addition, intrathecal injection of D-serine greatly increased spinal H_2O_2 levels by 80.0% and 56.9% in Swiss and Balb/c mice respectively, but reduced spinal H_2O_2 levels by 29.0% in C57 mice. However, there was no remarkable difference in spinal DAAO activities among Swiss, Balb/c and C57 mice. The spinal expression of glutathione (GSH) and glutathione peroxidase (GPx) activity in C57 mice were significantly higher than Swiss and Balb/c mice. Furthermore, the specific GPx inhibitor D-penicillamine distinctly restored SUN antinociception in C57 mice. Our results reported that DAAO inhibitors produced antinociception in a strain-dependent manner in mice and the strain specificity might be associated with the difference in spinal GSH and GPx activity.” As taken from Liu H et al. 2021. Pharmacol. Res. Perspect. 9(2), e00727. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33710781/>

6.4. Other organ systems, dependent on the properties of the substance

“The liver is the principal source of glutamate in blood plasma. Recently we have discovered that efflux of glutamate from hepatocytes is catalyzed by the transporter OAT2 (human gene symbol SLC22A7). Organic anion transporter 2 (OAT2) is an integral membrane protein of the sinusoidal membrane domain; it is primarily expressed in liver and much less in kidney, both in rats and humans. Many years ago, Häussinger and coworkers have demonstrated in isolated perfused rat liver that benzoic acid or specific 2-oxo acid analogs of amino acids like e.g. 2-oxo-4-methyl-pentanoate ('2-oxo-leucine') strongly stimulate release of glutamate (up to 7-fold); '2-oxo-valine' and the corresponding amino acids were without effect. The molecular mechanism of efflux stimulation has remained unclear. In the present study, OAT2 from human and rat were heterologously expressed in 293 cells. Addition of 1 mmol/l benzoic acid to the external medium increased OAT2-specific efflux of glutamate up to 20-fold; '2-oxo-leucine' was also effective, but not '2-oxo-valine'. Similar effects were seen for efflux of radiolabeled orotic acid. Expression of OAT2 did not increase uptake of benzoic acid; thus, benzoic acid is no substrate, and trans-stimulation can be excluded. Instead, further experiments suggest that increased efflux of glutamate is caused by direct interaction of benzoic acid and specific 2-oxo acids with OAT2. We propose that stimulators bind to a distinct extracellular site and thereby accelerate relocation of the empty substrate binding site to the intracellular face. Increased glutamate efflux at OAT2 could be the main benefit of benzoate treatment in patients with urea cycle defects.” As taken from Pfennig T et al. 2013. Biochim. Biophys. Acta 1828(2), 491-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/22981274>

“...in previous assessments of benzoic acid as feed additive (EFSA 2005, 2007; EFSA FEEDAP Panel, 2012a), it was shown that the ingestion of diets containing benzoic acid at or above 10,000 mg/kg feed could result in the occurrence of ulcers and mucosal lesions in the stomach.”

As taken from EFSA, 2016c.

“Large oral doses may cause abdominal pain, nausea and vomiting.”

“Possible liver, kidney and brain toxicant.”

As taken from PPDB, 2024.

“Systemic toxic effects on liver and kidney were observed.”

As taken from SCENIHR, 2016.

“Objective: Endocrine-disrupting chemicals (EDCs) are viewed as a major potential link between the environment and obesity development. We did a systematic review and meta-analysis to examine the association between exposure to EDCs and obesity. Data sources, design and eligibility criteria: PubMed, Scopus and Web of Science were searched from inception to 6 June 2018 for studies primarily addressing the association between exposure to EDCs after the age of 2 years and anthropometric measures of obesity or body fat. The Newcastle-Ottawa scale was used to assess the risk of bias. Data extraction and synthesis: Two independent reviewers screened and conducted data extraction and synthesis. A third reviewer resolved disagreements. Results: A total of 73 studies investigating bisphenol A (32 286 individuals), organochlorine compounds (34 567 individuals), phthalates (21 401 individuals), polybrominated biphenyls (2937 individuals), polycyclic aromatic hydrocarbons (5174 individuals), parabens (4097 individuals), benzoic acid (3671 individuals) and polyfluoroalkyl substances (349 individuals) met our inclusion criteria. Most had a cross-sectional design and low or medium risk of bias. In qualitative analysis, bisphenol A and phthalates were consistently associated with general and abdominal obesity, in children and adults, and some studies suggested this association was age-dependent and gender-dependent. Meta-analysis indicated a significant association between exposure to bisphenol A and overweight (OR 1.254, 95% CI 1.005 to 1.564), obesity (OR 1.503, 95% CI 1.273 to 1.774) and increased waist circumference (OR 1.503, 95% CI 1.267 to 1.783) in adults, and between exposure to 2,5-dichlorophenol and obesity in children (OR 1.8, 95% CI 1.1018 to 3.184). Conclusion: Most observational studies supported a positive association between obesity and exposure to EDCs. Although causality cannot be determined from these data, they underscore the need to limit human exposure to EDCs in light of the evidence from animal and cell-based studies indicating the effects of these chemicals on adiposity.” As taken from Ribeiro CM et al. 2020. BMJ Open 10(6), e033509. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/32565448/>

“In real life, organisms are exposed to complex mixtures of chemicals at low concentration levels, whereas research on toxicological effects is mostly focused on single compounds at comparably high doses. Mixture effects deviating from the assumption of additivity, especially synergistic effects, are of concern. In an adverse outcome pathway (AOP)-guided manner, we analyzed the accumulation of triglycerides in human HepaRG liver cells by a mixture of eight steatotic chemicals (amiodarone, benzoic acid, cyproconazole, flusilazole, imazalil, prochloraz, propiconazole and tebuconazole), each present below its individual effect concentration at 1-3 µM. Pronounced and significantly enhanced triglyceride accumulation was observed with the mixture, and similar effects were seen at the level of pregnane-X-receptor activation, a molecular initiating event leading to hepatic steatosis. Transcript pattern analysis indicated subtle pro-steatotic changes at low compound concentrations, which did not exert measurable effects on cellular triglycerides. Mathematical modeling of mixture effects indicated potentially more than additive behavior using a model for compounds with similar modes of action. The present data underline the usefulness of AOP-guided in vitro testing for the identification of mixture effects and highlight the need for further research on chemical mixtures and harmonization of data interpretation of mixture effects.” As

taken from Lichtenstein D et al. 2021. Toxicology 459, 152857. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/34273450/>

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to tobacco in the manufacture of cigarettes. An expert panel of toxicologists reviewed the open literature and internal toxicology data of 5 tobacco companies to evaluate a composite list of ingredients used in the manufacture of cigarettes. The conclusion of this report was that these ingredients did not increase the inherent biological activity of tobacco cigarettes, and are considered to be acceptable under conditions of intended use (Doull et al., 1994 & 1998).

Tobacco smoke condensates from cigarettes containing Benzoic acid and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of Benzoic acid. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	164	Baker et al., 2004a
	7,977	Gaworski et al., 2011a & Coggins et al., 2011g
	54	Roemer et al., 2014
In vitro genotoxicity	164	Baker et al., 2004c
	7,977	Gaworski et al., 2011a & Coggins et al., 2011g
	54	Roemer et al., 2014
In vitro cytotoxicity	164	Baker et al., 2004c
	7,977	Gaworski et al., 2011a & Coggins et al., 2011g
	54	Roemer et al., 2014
Inhalation study	164	Baker et al., 2004c
	7,977	Gaworski et al., 2011a & Coggins et al., 2011g
	54	Schramke et al., 2014
In vivo genotoxicity	54	Schramke et al., 2014

In a pyrolysis study, 98.6% of Benzoic acid added to cigarettes was transferred intact to the smoke. (Purkis et al. 2011).

9. Heated/vapor emissions toxicity

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing Benzoic acid was tested in a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic responses upon exposure. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (ppm)	Reference
Aerosol chemistry	7,257	Labstat International Inc. (2021)
In vitro genotoxicity	7,257	Labstat International Inc. (2022)
In vitro cytotoxicity	7,257	Labstat International Inc. (2022)

Aerosol from heated tobacco stick(s) containing benzoic acid was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	2.33	Labstat International Inc. (2020a) Labstat International Inc. (2021a) Labstat International Inc. (2023a) JTI Heated Tobacco Stick Study Report(s)
In vitro genotoxicity	2.33	Labstat International Inc. (2020b) Labstat International Inc. (2021b) Labstat International Inc. (2023b) JTI Heated Tobacco Stick Study Report(s)
In vitro cytotoxicity	2.33	Labstat International Inc. (2020b) Labstat International Inc. (2021b) Labstat International Inc. (2023b) JTI Heated Tobacco Stick Study Report(s)

"BACKGROUND/OBJECTIVE: The heating of the fluids used in electronic cigarettes ("e-cigarettes") used to create "vaping" aerosols is capable of causing a wide range of degradation reaction products. We investigated formation of benzene (an important human carcinogen) from e-cigarette fluids containing propylene glycol (PG), glycerol (GL), benzoic acid, the flavor chemical benzaldehyde, and nicotine. METHODS/MAIN RESULTS: Three e-cigarette devices were used: the JUULTM "pod" system (provides no user accessible settings other than flavor cartridge choice), and two refill tank systems that allowed a range of user accessible power settings. Benzene in the e-cigarette aerosols was determined by gas chromatography/mass spectrometry. Benzene formation was ND (not detected) in the JUUL system. In the two tank systems benzene was found to form from propylene glycol (PG) and glycerol (GL), and from the additives benzoic acid and benzaldehyde, especially at high power settings. With 50:50 PG+GL, for tank device 1 at 6W and 13W, the formed benzene concentrations were 1.9 and 750 µg/m³. For tank device 2, at 6W and 25W, the formed concentrations were ND and 1.8 µg/m³. With benzoic acid and benzaldehyde at ~10 mg/mL, for tank device 1, values at 13W were as high as 5000 µg/m³. For tank device 2 at 25W, all values were ≤~100 µg/m³. These values may be compared with what can be expected in a conventional (tobacco) cigarette, namely 200,000 µg/m³. Thus, the risks from benzene will be lower from e-cigarettes than from conventional cigarettes. However, ambient benzene air concentrations in the U.S. have typically been 1 µg/m³, so that benzene has been named the

largest single known cancer-risk air toxic in the U.S. For non-smokers, chronically repeated exposure to benzene from e-cigarettes at levels such as 100 or higher $\mu\text{g}/\text{m}^3$ will not be of negligible risk.” As taken from Pankow JF et al. 2017. PLoS One 12(3), e0173055. PubMed, 2018 available at

<https://www.ncbi.nlm.nih.gov/pubmed/28273096>

10. Ecotoxicity

10.1. Environmental fate

Benzoic acid's production and use as a plasticizer, chemical intermediate in the production of benzoyl chloride alkyd resins, food preservative, in seasoning tobacco, flavors, perfumes, dentifrices and as a standard in analytical chemistry may result in its release to the environment through various waste streams. Its use as a plant growth regulator will result in its direct release to the environment. Benzoic acid is widely distributed in nature. Gum benzoin (*Styrax benzoin*) may contain as much as 20% free acid. Smaller amounts of the free acid are found in the scent glands of beavers, cherry tree bark, cranberries, prunes, ripe cloves, and oil of anise seed. Peru and Tolu balsams contain benzyl benzoate. The urine of herbivores contains a small proportion of the glycine derivative of benzoic acid, hippuric acid. If released to air, a vapor pressure of 7.0×10^{-4} mm Hg at 25 deg C indicates benzoic acid will exist solely as a vapor in the atmosphere. Vapor-phase benzoic acid will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 9 days. Benzoic acid absorbs light at wavelengths >290 nm and, therefore, may be susceptible to direct photolysis by sunlight. If released to soil, benzoic acid is expected to have very high mobility based upon an estimated Koc of 15. The pKa of benzoic acid is 4.20, indicating that this compound will exist almost entirely in the anion form in the environment and anions generally do not adsorb more strongly to soils containing organic carbon and clay than their neutral counterparts. Volatilization from moist soil is not expected because the compound exists as an anion and anions do not volatilize. Benzoic acid is not expected to volatilize from dry soil surfaces based upon its vapor pressure. Mineralization half-life in Captina silt loam for benzoic acid in solution was 4.5 hr after a 30 minute lag and complete degradation occurred in 1 day using a Niagra silt loam inoculum, suggesting that biodegradation may be an important fate process in soil. If released into water, benzoic acid is not expected to adsorb to suspended solids and sediment based upon the estimated Koc. Biodegradation half-lives of 0.85 and 3.6 days using inoculum from a polluted river and a reservoir, respectively, suggest that biodegradation may be an important fate process in water. The pKa indicates benzoic acid will exist almost entirely in the anion form at pH values of 5 to 9 and, therefore, volatilization from water or moist soil surfaces is not expected to be an important fate process. A BCF range of <10 to 21 suggests bioconcentration in aquatic organisms is low. Hydrolysis is not expected to be an important environmental fate process since this compound lacks functional groups that hydrolyze under environmental conditions (pH 5 to 9). Occupational exposure to benzoic acid may occur through inhalation and dermal contact with this compound at workplaces where benzoic acid is produced or used. Monitoring data indicate that the general population may be exposed to benzoic acid via inhalation of ambient air, ingestion of food and drinking water, dermal contact with consumer products containing benzoic acid as well as exposure to certain wood and cooking smokes. (SRC) **PEER REVIEWED**

ATMOSPHERIC FATE: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere(1), benzoic acid, which has a vapor pressure of 7×10^{-4} mm Hg at 25 deg C(2), is expected to exist solely as a vapor in the ambient atmosphere. Vapor-phase benzoic acid is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals(SRC); the half-life for this reaction in air is estimated to be 9 days(SRC), calculated from its rate constant of 1.2×10^{-12} cu cm/molecule-sec at 25 deg C(SRC) that was derived using a structure estimation method(3). Benzoic acid absorbs light at wavelengths >290 nm(4) and,

therefore, may be expected to be susceptible to direct photolysis by sunlight(SRC). [(1) Bidleman TF; Environ Sci Technol 22: 361-367 (1988) (2) McEachern DM, Sandoval O; J Phys E 6: 155-161 (1973) (3) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993) (4) NIST; NIST Chemistry WebBook. Benzoic acid (65-85-0). NIST Standard Reference Database No. 69, June 2005 Release. Washington, DC: US Sec Commerce. Available from, as of Aug 19, 2013: <http://webbook.nist.gov> **PEER REVIEWED**

As taken from HSDB, 2014.

"In this research, the stability of benzoic acid and three of its derivatives (anthranilic acid, salicylic acid, and syringic acid) under subcritical water conditions was investigated. The stability studies were carried out at temperatures ranging from 50 to 350°C with heating times of 10-630 min. The degradation of the benzoic acid derivatives increased with rising temperature and the acids became less stable with longer heating time. The threebenzoic acid derivatives showed very mild degradation at 150°C. Severe degradation of benzoic acid derivatives was observed at 200°C while their complete degradation occurred at 250°C. However, benzoic acid remained stable at temperatures up to 300°C. The degradation products of benzoic acid and the three derivatives were identified and quantified by HPLC and confirmed by GC/MS. Anthranilic acid, salicylic acid, syringic acid, andbenzoic acid in high-temperature water underwent decarboxylation to form aniline, phenol, syringol, and benzene, respectively." (Lindquist E & Yang Y. 2011. J. Chromatogr. A 1218(15), 2146-52. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/20846661>.

The Ecological Categorization Results from the Canadian Domestic Substances List state that benzoic acid is not persistent in the environment:

Media of concern leading to Categorization	Water
Experimental Biodegradation half-life (days)	Not Available
Experimental Biodegradation result (%)	85
Reference (biodegradation result)	CHEMICALS INSPECTION AND TESTING INSTITUTE.; BIODEGRADATION AND BIOACCUMULATION DATA OF EXISTING CHEMICALS BASED ON THE CSCL JAPAN.; JAPAN CHEMICAL INDUSTRY ECOLOGY - TOXICOLOGY AND INFORMATION CENTER.ISBN 4-89074-101-1.; 1992
Predicted Ultimate degradation half-life (days)	15
MITI probability of biodegradation	0.8855
TOPKAT probability of biodegradation	1
EPI Predicted Ozone reaction half-life (days)	999
EPI Predicted Atmospheric Oxidation half-life (days)	8.612

ROBUST STUDY SUMMARY - Persistence

Item	Yes	No
Reference: Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan, 1992. Chemicals Inspection and Testing Institute (Ed.). Published by: Japan Chemical Industry Ecology-Toxicology & Information Center.		

Test Substance (CAS # and name): The list of the substances is attached (see file on N drive)		
Chemical composition of the substance (including purity, by-products)		X
Method		
References	X	
OECD, EU, national, or other standard method?	X	
Justification of the method/protocol if not a standard method was used	Not applicable	
Test design / conditions		
Study type (photodegradation, hydrolysis, biodegradation, other – specify, do not assess): Biodegradation		
Test type (aerobic or anaerobic - specify, do not assess):		
Test medium (air, water, soil, sediment - specify, do not assess):		
Information on stability of the substance in the media of concern is reported?	X	
Controls (positive or negative) Both	X	
Number of replicates (including controls)		X
Temperature (specify) 25±1 °C	X	
Duration of the experiment (specify) 14 or 28 days	X	
For photodegradation only		
Light source (specify):	Not applicable	
Light spectrum and relative intensity based on sunlight intensity:	Not applicable	
For hydrolysis only		
Measured concentrations reported?	Not applicable	

Basic water properties (pH, hardness, etc.)	Not applicable	
For biodegradation only		
Ready or inherent biodegradation (specify): Both types (see file on N drive)	X	
Innoculum (concentration and source)	X	
Results		
Endpoints: half-life (preferred); degradation, %; etc. (do not assess this item): % biodegradation by BOD or TOC (see file on N drive)		
Information on breakdown products (do not assess this item)		X
Overall score: 8/10 = 80%		
EC Reliability code: 1		
Reliability category (high, satisfactory, low): high		
Comments:		

Data accessed January 2017 on the OECD website

EPISuite provides the following data:

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:

Bond Method :	1.08E-007 atm-m3/mole (1.10E-002 Pa-m3/mole)
Group Method:	4.55E-008 atm-m3/mole (4.62E-003 Pa-m3/mole)
Exper Database:	3.81E-08 atm-m3/mole (3.86E-003 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 1.921E-007 atm-m3/mole (1.946E-002 Pa-m3/mole) VP: 0.00298 mm Hg (source: MPBPVP) WS: 2.49E+003 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:

Log Kow used:	1.87 (exp database)
Log Kaw used:	-5.808 (exp database)
Log Koa (KOAWIN v1.10 estimate):	7.678
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	0.9944
Biowin2 (Non-Linear Model) :	0.9959
Biowin3 (Ultimate Survey Model):	3.0392 (weeks)
Biowin4 (Primary Survey Model) :	3.6842 (days-weeks)
Biowin5 (MITI Linear Model) :	0.7669
Biowin6 (MITI Non-Linear Model):	0.8855
Biowin7 (Anaerobic Linear Model):	0.8427
Ready Biodegradability Prediction:	YES

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:

Vapor pressure (liquid/subcooled):	0.857 Pa (0.00643 mm Hg)
Log Koa (Koawin est):	7.678
Kp (particle/gas partition coef. (m ³ /ug)): Mackay model: Octanol/air (Koa) model:	3.5E-006 1.17E-005

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	0.000126
Mackay model:	0.00028
Octanol/air (Koa) model:	0.000935

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	1.2420 E-12 cm ³ /molecule-sec
Half-Life =	8.612 Days (12-hr day; 1.5E6 OH/cm ³)
Half-Life =	103.345 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 0.000203 (Junge-Pankow, Mackay avg) 0.000935 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	16.55 L/kg (MCI method)

Log Koc:	1.219 (MCI method)
Koc :	15.49 L/kg (Kow method)
Log Koc:	1.190 (Kow method)
Experimental Log Koc:	1.5 (database)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:

Rate constants can NOT be estimated for this structure!

Volatilization from Water:

Henry LC: 3.81E-008 atm-m3/mole (Henry experimental database)

Half-Life from Model River:	1.698E+004 hours (707.6 days)
Half-Life from Model Lake:	1.854E+005 hours (7723 days)

Removal In Wastewater Treatment:

Total removal:	2.14 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	2.05 percent
Total to Air:	0.00 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount	Half-Life	Emissions

	(percent)	(hr)	(kg/hr)
Air	0.694	207	1000
Water	28.6	360	1000
Soil	70.6	720	1000
Sediment	0.0723	3.24e+003	0

Persistence Time: 621 hr

“Biodegradation

Based on a weight of evidence approach, considering a test on ready biodegradability according to OECD 301 C (“MITI-I test”), the averaged results of 128 tests performed according to either OECD 301 B, or OECD 301 D, or OECD 301 E, and the fact that sodium salt of benzoic acid is one of the recommended reference compounds proposed in the OECD 301 test guideline, the a.s. benzoic acid was regarded as “readily biodegradable within the 10-day window”.

Anaerobic biodegradation

An anaerobic biodegradation test was submitted by the applicant as a product type specific (product type 3) data requirement caused by releases of benzoic acid into manure storage facilities. The result shows that more than 75% of theoretical gas production is achieved within 60 days of incubation, which means that complete anaerobic biodegradation, can be assumed for benzoic acid.

Abiotic Degradation

Benzoic acid as an aromatic monocarbon acid possesses no hydrolysable functional groups. For this reason, hydrolysis under environmental conditions is not expected. In real life samples the microbial population will rapidly degrade benzoic acid and compared with biodegradation processes, hydrolysis in surface waters will not be relevant. In conclusion, benzoic acid is stable in pure and sterile water.

Benzoic acid has two absorption bands at wavelength of 228 nm and 274 nm. Thus, a direct photodegradation does not occur for benzoic acid as chemicals with UV/absorption maximum of <290 nm cannot undergo direct photolysis in sunlight. Nevertheless, in studies with added catalysers and high energy input photodegradation of benzoic acid was observed. Photodecomposition of benzoic acid in these systems depends on temperature and pH of the medium. Benzoic acid was shown to be an end-product of photodecomposition of other organic compound, e.g. DDA (2,2-bis-(p-chlorophenyl) acetic acid. In air benzoic acid will be degraded by indirect photodegradation, the half-life was calculated to 12.9 d, which corresponds to a value of 18.6 d for the chemical lifetime in the troposphere.

Distribution and Mobility

Based on log Pow, the log Koc was estimated to 1.4 by application of QSAR models (Sabljic and Güsten in EU TGD on Risk Assessment (2003), Part III, Chapter 4.3, Table 4) and calculations based on the generally accepted model PCKOCWIN v1.66. The value was used for classification of benzoic acid as substance with high mobility in soil and for the environmental exposure assessment.

The Koc value for benzoic acid is dependent on pH variation and solubility. Due to the changes of solubility of chemical substances with variation of pH, several QSAR models allow prediction of Koc values depending on pH. The Koc values for benzoic acid in dependence on environmentally relevant pH-values were estimated applying the model ACD/AdsorptionCoefficient 8.02. The predicted Koc for the benzoic acid decreases slightly with pH-value, i.e. from the non-ionised molecule to the gradually ionised molecule. A calculation of Koc was not possible using the model ACD for the completely ionised benzoate. Nevertheless and in conclusion, benzoic acid is expected to exhibit only a weak adsorption in soils and sediments and is regarded to be a highly mobile substance in soil at environmentally relevant pH values.

The mobility of benzoic acid depends on further soil properties. Lower Fe-contents in several soils may have contributed to weaker bonding and greater mobility of benzoic acid in those soils. Lower Al- and Fe-contents decrease the retention of benzoate anions to positively charged surfaces of Fe- and Al-oxides. Removal of organic matter increased the mobility of benzoic acid in all soils.

In conclusion, benzoic acid mobility was positively correlated with soil pH, and negatively correlated with Al- and Fe-contents.”

As taken from European Commission, 2013a, b.

Identity : benzoic acid

Identifier : Common name

Details on inoculum : - Concentration of sludge:30ppm

Duration : 2

Unit : wk

Initial test substance concentration

Initial concentration : 100

Unit : other:

Unit : ppm

Parameter followed for biodegradation estimation

Details on study design : Standard type

Reference substance

RESULTS AND DISCUSSION

% Degradation of test substance

% degradation : 85

Parameter : other:

Parameter : BOD

% degradation : 98 Parameter : TOC

% degradation : 100

Parameter : UV-VIS

As taken from J-Check, 2017

Total degradation in water :

Biodegradation:

85% by BOD

period: 14d

substance: 100 mg/l

sludge: 30 mg/l

(MITI 1992)

At concentrations of 15-18 mg/l benzoic acid was biodegraded with half-lives of 0.85 and 3.6 days in a polluted river and reservoir, respectively (Banerjee 1984).

At 59 ng/l of benzoic acid, over 98% mineralization had occurred in 7 days in both eutrophic and oligotrophic water (Rubin et al. 1982).

The half-life for mineralization in eutrophic water was 0.22 days over a concentration range of 32 ng/l to 50 000 ng/l. From 63 to 83% of the benzoic acid was lost in 6 hr and >94% in 58hr (Subba-Rao et al. 1982).

Adsorption/desorption: Benzoic acid did not adsorb appreciable to two different sandy soils, a clayey subsoil and montmorillonite (Bailey et al. 1968) (Loekke 1984). Photochemical degradation in air : Benzoic acid adsorbs UV radiation up to approx 310 nm and therefore may photolyze (Sadtler). In a photomineralization test in which the chemical is adsorbed on silica gel and irradiated with light >290 nm, 10.2% mineralization occurred in 17 hr (Freitag 1986). In the vapor phase benzoic acid should react with photochemically produced hydroxyl radicals by aromatic ring addition with an estimated half-life of 2.0 days (GEMS 1987). Photochemical degradation in water: Benzoic acid was stable when exposed to sunlight or a sunlamp for 137 hr in aqueous solution (Ware et al. 1980). When illuminated with a sunlamp for 24 hr in solution containing zinc oxide, 67% degradation occurred in 24 hr. Zinc oxide therefore appears to possess catalytic activity, as does beach sand (Kinney et al. 1969). Hydrolysis in water: The pKa for benzoic acid suggests that it exists almost exclusively in the dissociated form at environmental pHs (Howard 1989). Aerobic degradation in soil: Using a Catina silt loam inoculum, the half-life for mineralization of benzoic acid in solution was 4.5 hr after a 30 min lag (Dao & Lavy 1987). Complete degradation occurred in 1 day with a Niagara silt loam inoculum (Alexander & Lustigman 1966). Aerobic degradation in water: Eleven laboratories testing a respiratory biodegradability test utilizing an unacclimated sludge inoculum found benzoic acid to be readily degradable, obtaining a mean oxygen uptake of 84% of theoretical after 10 days, and no lag period before biodegradation commenced (King & Painter 1983). Some results from degradation of benzoic acid: - 99% COD removal in 5 days with acclimated activated sludge - 67% of theoretical BOD removal in 5 days - 97% degradation in 20

days by activated sludge, where 10% of benzoic acid was replaced every 2 days to acclimate the sludge- 68.2% and 86.9% mineralization in 5 days by acclimated activated sludge in salt solution and simulated industrial effluent, respectively- 65.4% mineralization in 5 days by activated sludge inoculum - 73% of theoretical BOD utilized in 6 days using activated sludge from 3 municipal sewage plants- 74% of theoretical BOD utilized in a 5-day test with a sewage seed- >90% degraded in 2 days using activated sludge.- 84.1 and 74.9% of theoretical BOD in 5 days by the standard and seawater dilution methods, respectively (Howard 1989). Total degradation in soil: When benzoic acid was incubated in an acidic loam soil adjusted to 60% of its water holding capacity, 74 and 81% was mineralized in 1 and 12 weeks. The same experiment using a neutral, sandy loam soil resulted in 55 and 71% mineralization in 1 and 12 weeks. After 12 weeks, 3.0 and 4.4% of the chemical was incorporated into the biomass of the two soils. In an alkaline para-brown soil, 40 and 63% mineralization occurred in 3 days and 10 weeks, respectively (Kassim et al. 1982) (Haider et al. 1974). ¹⁴C-labeled benzoic acid was added to subsurface soil taken from the unsaturated zone beneath the tile of a septic tank and incubated both aerobically and anaerobically. Under aerobic conditions the half-life was 3.9 and 7.3 for carboxyl- and ring-labeled chemical, respectively. The ring-labeled benzoic acid had a mineralization half-life of 18.2 hr when incubated anaerobically (Ward 1985). Benzoic acid is biodegradable under anaerobic conditions, indicated by the fact that >75% of theoretical methane production was obtained when incubated for 8 weeks with 10% sludge from a secondary digester (Shelton & Tiedje 1984). Under anaerobic conditions 91% of benzoic acid was converted to methane and carbon dioxide in 18 days, including an 8-day lag period (Healy & Young 1979). In an experiment 86-93% conversion to methane and carbon dioxide occurred in 14 days with a sewage sludge inoculum (Nottingham 1969). Benzoic acid was completely mineralized in a week when incubated anaerobically with municipal digested sludge or in anoxic sediment from a hypereutrophic lake in Kalamazoo County, MI (Horowitz 1982). Decomposition period by a soil microflora: 1 day (Verschueren 1983).

Lag period for degradation of 16 mg/l by waste water or by soil at pH 7.3 and 30 °C: less than 1 day (Haller 1978).

As taken from the Finnish Environment Institute (b).

Soil degradation (days) (aerobic)

DT50 (typical) 0.5. Interpretation: Non-persistent.

DT50 (lab at 20°C) 0.5 Interpretation; Non-persistent.

DT90 (lab at 20°C) 2.1

EU dossier (RAR 2015) lab studies DT50 range 0.23-0.62 days, Soils=3; General literature give DT50 1-10 days

Bioconcentration factor 3.16 l/kg "low potential"

As taken from PPDB, 2016

10.2. Aquatic toxicity

/AQUATIC SPECIES/ 0.5 mg/L of benzoic acid did not affect growth of blue-green alga, *Anabaena flos-aquae*. [Phipps et al; J Water Pollut Control Fed 56 (6): 725-58 (1984)] **PEER REVIEWED**

/AQUATIC SPECIES/ Lethal to goldfish in 7-96 hr at 200 mg/L; lethal to orange spotted sunfish in 1 hr at 550-570 mg/L. /Conditions of bioassay not specified in source examined/ [Verschueren, K. Handbook of Environmental Data on Organic Chemicals. Volumes 1-2. 4th ed. John Wiley & Sons. New York, NY. 2001, p. 288] **PEER REVIEWED**

/AQUATIC SPECIES/ EC50/LC50 values (24-96 hr) for most of the other aquatic species tested (protozoa, molluscs, crustaceans, fish, amphibians) were in the range of 100-1291 mg/L. As seen with daphnids, the pH value of the test medium influences the toxicity of benzoic acid, which proved to be more toxic at lower pH levels. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

/AQUATIC SPECIES/ Developmental toxicity effects seen in frog (*Xenopus*) embryos were craniofacial defects, especially microcephaly, and abnormal gut coiling. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

/AQUATIC SPECIES/ A recently developed cytotoxicity assay with cultured fathead minnow (*Pimephales promelas*) cells resulted in a PI50 (the concentration required to induce a 50% reduction in total protein content) of 1450 mg/L. [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

AQUATIC FATE: Based on a classification scheme(1), an estimated Koc value of 15(SRC), determined from a structure estimation method(2), indicates that benzoic acid is not expected to adsorb to suspended solids and sediment(SRC). A pKa of 4.20(3) indicates benzoic acid will exist almost entirely in the anion form at pH values of 5 to 9 and, therefore, volatilization from water or moist soil surfaces is not expected to be an important fate process(SRC). According to a classification scheme(4), a BCF range of <10 to 21(5) suggests the potential for bioconcentration in aquatic organisms is low(SRC). Biodegradation half-lives of 0.85 and 3.6 days using inoculum from a polluted river and a reservoir, respectively(6), suggest that biodegradation may be an important fate process in water(SRC). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of Aug 20, 2013: <http://www.epa.gov/oppt/exposure/pubs/episuite.html> (3) Haynes WM, ed; CRC Handbook of Chemistry and Physics. 91st ed. Boca Raton, FL: CRC Press Inc., p 8-47 (2010) (4) Franke C et al; Chemosphere 29: 1501-14 (1994) (5) Freitag D et al; Chemosphere 14: 1589-616 (1985) (6) Rubin HE et al; Appl Environ Microbiol 43: 1133-8 (1982)] **PEER REVIEWED**

Environmental Water Concentrations:

GROUND WATER: Benzoic acid was found in 2 aquifers at the Hoe Creek underground coal gasification site 15 mo after gasification was completed at concentration of 16-860 ppb(1). Concentrations of benzoic acid in the plumes in shallow, sandy aquifers emanating from landfills in Ontario were 17->1000 ppb in one aquifer and not detected to 8.8 ppb in another(2). The concentration in background monitoring wells was at trace levels (<0.1 ppb) in the first aquifer and was not determined in the second(2). Two wells monitoring near-surface groundwater adjacent to an unlined surface impoundment at a wood-preserving facility at Pensacola, FL contained 3.1 and 27.5 ppm of benzoic acid while wells 150 m away contained 0-0.01 ppm of the chemical(3). It is believed that the benzoic acid was rendered from the wood during treatment or was a degradation product of creosote solutes(3). Benzoic acid was found in groundwater in Australia underlying an area where acid wastes from a manufacturing process of a chemical company was stored in unlined ponds(4). Since the chemical was only found in the aquifer down-gradient from the believed source of pollution and not closer to this source, it was either formed by bacterial action or came from another source(4). Benzoic acid was detected in one of three samples taken from superfund sites at a concentration of 2.5 mg/L(5). Benzoic acid was detected not quantified in drinking water from Jefferson Parish, LA in 1994(6). The compound was detected at 0.6 to 6.2 ug/L in contaminated groundwater samples from a former ammunition destruction site, Susten-Steingletscher, Switzerland, where operations ceased in 1997. Benzoic acid is a degradation

product of the explosives disposed of(7). [(1) Stuermer DH et al; Environ Sci Technol 16: 582-7 (1982) (2) Reinhard M et al; Environ Sci Technol 18: 953-61 (1984) (3) Goerlitz DF et al; Environ Sci Tech 19: 955-61 (1985) (4) Stepan S et al; Austral Water Resources Council Conf Ser 1: 415-24 (1981) (5) Betowski LD et al; Environ Sci Technol 30: 3558-64 (1996) (6) Richardson SD et al; Environ Sci Technol 33: 3368-77 (1999) (7) Godejohann M et al; Environ Sci Technol 43: 7056-7061 (2009)] **PEER REVIEWED**

DRINKING WATER: In a five city survey of drinking water, 15 ppm benzoic acid was found in the tap water of Otumwa, IA but not in that of Miami, FL, Seattle, WA, Philadelphia, PA or Cincinnati, OH(1). Another study found the compound in water from the Torresdale water treatment plant in Philadelphia, PA(2). Benzoic acid was detected, but not quantified, in treated drinking water in England whose source was a lowland river containing relatively high levels of wastewater(3). [(1) USEPA; Interim Report To Congress, June, 1975 Washington, DC (1975) (2) Suffet IH et al; Water Res 14: 853-67 (1980) (3) Fielding M et al; Organic Micropollutants In Drinking Water TR-159 Medmenham, England: Water Res Cent (1981)] **PEER REVIEWED**

SURFACE WATER: Benzoic acid was detected, but not quantified, in a Norwegian river downstream from an industrial treatment facility(1). [(1) Schou L et al; Total Environ 20: 277-86 (1981)] **PEER REVIEWED**

RAIN/SNOW: Benzoic acid was found in the particulate fraction of four samples of rain and snow in Norway; no concentrations were indicated(1). Analysis of rain and snow samples collected at the Japanese cities of Nagano (360 m altitude) and Okaya (790 altitude) in the central mountainous region identified benzoic acid as a major organic component. The concentration ranged from <0.02 to 6.0 ug/L and showed some variation in summer and winter(2).

As taken from HSDB, 2014.

From valid test results available on the toxicity of benzoic acid and sodium benzoate to various aquatic organisms, these compounds appear to exhibit low to moderate toxicity in the aquatic compartment. The lowest EC50 value of 9 mg/litre (cell multiplication inhibition) reported in a chronic study was observed in the cyanobacterium *Anabaena inaequalis*. EC50/LC50 values for the other aquatic species tested were in the range of 60-1291 mg/litre. Immobilization of *Daphnia magna* has been demonstrated to be pH dependent, with a lower 24-h EC50 (102 mg/litre) at acidic pH. For the freshwater fish golden ide (*Leuciscus idus*), a 48-h LC50 of 460 mg/litre has been determined. Developmental effects have been found in frog (*Xenopus*) embryos at a concentration of 433 mg/litre (96-h EC50 for malformation). For sodium benzoate, exposure of juvenile stages of aquatic organisms in a multispecies test (including *Daphnia magna*, *Gammarus fasciatus*, *Asellus intermedius*, *Dugesia tigrina*, *Helisoma trivolvis*, and *Lumbriculus variegatus*) resulted in 96-h LC50 values of greater than 100 mg/litre. A 96-h LC50 of 484 mg/litre has been determined in the freshwater fish fathead minnow (*Pimephales promelas*). Owing to the limited available data on exposure levels in water, a quantitative risk characterization with respect to aquatic organisms in surface waters could not be performed. Taking into account the rapid biodegradability, the low to moderate bioaccumulation potential, the low toxicity to most aquatic species, and the rapid metabolism of these substances, benzoic acid and sodium benzoate will -- with the exception of accidental spills -- pose only a minimal risk to aquatic organisms.

The n-octanol/water partition coefficient (log Kow) of 1.9 (see section 2) indicates a low potential for bioaccumulation. Consistently, measured bioconcentration factors (BCFs) found in aquatic biota were low. BCFs of <10 (based on wet weight) have been determined for fish (golden ide, *Leuciscus idus melanotus*) and green algae (*Chlorella fusca*) after 3 and 1 days, respectively (Freitag et al., 1985). A 6-day BCF of 7.6 has been reported for another green alga (*Selenastrum capricornutum*) (Mailhot, 1987), and a 5-day BCF of 1300 (based on dry weight) in activated sludge (Freitag et al., 1985). The following 24-h bioaccumulation factors (focusing on uptake via medium plus feed within food chain members) have been obtained in aquatic model ecosystems operated with 0.01-0.1 mg of radiolabelled benzoic acid per litre: 21 (mosquitofish, *Gambusia affinis*), 102 (green alga,

Oedogonium cardiacum), 138 (mosquito larvae, Culex quinquefasciatus), 1772 (water flea, Daphnia magna), and 2786 (snail, Physa sp.). Except for Daphnia and snail, the values were low (Lu & Metcalf, 1975). However, the very low exposure concentrations could likely have resulted in the calculation of the high BCF values, even with moderate uptake. Moreover, because this was a radiolabel study, it remains unclear if the label was still the parent compound.

As taken from INCHEM, 2000, CONCISE INTERNATIONAL CHEMICAL ASSESSMENT DOCUMENT NO. 26, BENZOIC ACID AND SODIUM BENZOATE.

The Ecological Categorization Results from the Canadian Domestic Substances List state that benzoic acid is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	500
Experimental result iT (mg/l)	500
Exposure duration iT (hours)	24
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	1,199.507
Toxicity to fish (LC50 in mg/l) as predicted by Aster	116.687305
Toxicity to fish (LC50 in mg/l) as predicted by PNN	129.2144
Toxicity to daphnia (EC50 in mg/l) as predicted by Topkat v6.1	57
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	212.83
Chronic toxicity to daphnia or algae (EC50 in mg/l) as predicted by Ecosar v0.99g	61.717
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	1.20E+002

ROBUST STUDY SUMMARY - Inherent Toxicity

Item	Yes	No
Reference: Bringmann G and R. Kühn. 1982. Ergebnisse der Schadwirkung wassergefährdender Stoffe gegen Daphnia magna in einem weiterentwickelten standardisierten Testverfahren. Z. Abwasser Forsch. 15(1982) 1:1-6.		
Test Substance: CAS # and name: CAS # 65-85-0 Name: Benzoic acid		
*Chemical composition of the substance (including purity, by-products)		x
Persistence/stability of test substance in aquatic solution		x

Method		
References (More details on test).	x	
*OECD, EU, national, or other standard method? Standardised Daphnia toxicity test.	x	
Justification of the method/protocol if not a standard method was used	NA	
*GLP (Good Laboratory Practice) Not applicable.	NA	
Test organisms (specify common and Latin names) Daphnia magna – water flea.		
Latin or both Latin and common names reported?	x	
Life cycle age / stage of test organism Not applicable.	NA	
Sex. Not applicable.	NA	
Length and weight of test organisms. Max. 24 hours old. Not applicable.	NA	
Number of test organisms per replicate. Ten test organisms.	x	
Food type / feeding periods (acclimation/during test) .	x	
Test design / conditions		
Test type – acute or chronic (specify, but do not assess this item): acute 24 h EC50		
Experiment type (laboratory or field) specified?	x	
System type (static, semi-static, flow-through)?	x	
Negative or positive controls (specify)?		x
Number of replicates (including controls) and concentrations 3 replicates.	x	
Exposure pathways (food, water, both)		x
Exposure duration	x	

*Measured concentrations reported? Nominal conc.		x
Exposure media conditions (temperature, pH, electrical conductivity, hardness, TOC, DOC, DO, major cations and anions; other)	x	
Was pH within 6-9 range? (do not assess this item)	x	
Was temperature within 5-28 °C range? (do not assess this item)	x	
Photoperiod and light intensity	x	
Stock and test solution preparation Dilution series.	x	
Use of emulgators / solubilizers (especially for poorly soluble / unstable substances) Not used.	NA	
Analytical monitoring intervals		x
Statistical methods used: Regression.	x	
Results		
Toxicity values (LC50, EC50, or IC50 - EC50 = 500mg/L		
Other endpoints reported - BCF/BAF, LOEC/NOEC (specify, do not assess this item): No		
*Was toxicity value below the chemical's water solubility?	x	
Other adverse effects (carcinogenicity, mutagenicity, etc. Do not assess this item) No.		
Score: major items - 15 yes and 6 no 71% .		
EC Reliability code: 2		
Reliability category (high, satisfactory, low): satisfactory		
Comments:		

Data accessed January 2017 on the OECD website:
<https://hvpchemicals.oecd.org/UI/SponsoredChemicals.aspx>

Record for benzoic acid:

Spec. Sci. Name Spec. Name Common Name	Exp. Type Chem. Anal.	Media Type Loc	Resp. Site Obs. Dur. (Days)	Endpoint BCF	Trend Eff%	Effect Effect Meas.	Conc (Std.) Appl. Rate	Stat. Signif. Sig. Level
CAS #/Chemical: 65850 - Benzoic acid								
Algae, Moss, Fungi								
Chlorella fusca var. vacuolata	S	FW	WO	BCF	INC	ACC	A	
Green Algae	U	LAB	1 d	3 L/kg		RSDE	50 ug/L	
Chlorella fusca	AQUA - NR	FW		BCF	INC	ACC	F	
Green Algae	U	LAB	1 d	3 L/kg		RSDE	~ 50 ug/L	
Chlorella fusca	AQUA - NR	FW		BCF	INC	ACC	F	
Green Algae	U	LAB	1 d	10 L/kg		RSDE	~ 50 ug/L	
Oedogonium cardiacum	S	FW		BCF		ACC	A	
Green Algae	M	LAB	2 d	102 L/kg		RSDE	1.78 ug/L	
Chlorococcales	S	FW		EC10		PHY	F	
Green Algae Order			1 d			ASML	117000 ug/L	
Chlorococcales	S	FW		EC50		PHY	F	
Green Algae Order			1 d			ASML	168000 ug/L	

Anabaena variabilis	AQUA - NR	FW		EC50	DEC	POP	A	
Blue-Green Algae	U	LAB	(12 - 14) d			PGRT	> 10000 ug/L	
Scenedesmus quadricauda	S	FW		EC50	DEC	POP	A	
Green Algae	U	LAB	0.125 d			PSYN	75000 ug/L	
Chlorella pyrenoidosa	S	FW		EC50	DEC	POP	A	
Green Algae	U	LAB	0.125 d			PSYN	60000 ug/L	
Anabaena inaequalis	S	FW		EC50	DEC	POP	A	
Blue-Green Algae	U	LAB	0.125 d			PSYN	5000 ug/L	
Anabaena inaequalis	S	FW		EC50	DEC	ENZ	A	
Blue-Green Algae	U	LAB	0.208 d			NITG	90000 ug/L	
Chlorella pyrenoidosa	AQUA - NR	FW		EC50	DEC	POP	A	
Green Algae	U	LAB	(12 - 14) d			PGRT	> 10000 ug/L	
Anabaena variabilis	AQUA - NR	FW		EC50	DEC	POP	A	
Blue-Green Algae	U	LAB	(12 - 14) d			ABND	> 10000 ug/L	
Scenedesmus quadricauda	AQUA - NR	FW		EC50	DEC	POP	A	

Green Algae	U	LAB	(12 - 14) d			ABND	> 10000 ug/L	
Scenedesmus quadricauda	AQUA - NR	FW		EC50	DEC	POP	A	
Green Algae	U	LAB	(12 - 14) d			PGRT	> 10000 ug/L	
Anabaena inaequalis	AQUA - NR	FW		EC50	DEC	POP	A	
Blue-Green Algae	U	LAB	(12 - 14) d			PGRT	> 10000 ug/L	
Anabaena inaequalis	AQUA - NR	FW		EC50	DEC	POP	A	
Blue-Green Algae	U	LAB	(12 - 14) d			ABND	9000 ug/L	
Anabaena variabilis	S	FW		EC50	DEC	POP	A	
Blue-Green Algae	U	LAB	0.125 d			PSYN	55000 ug/L	
Chlorella pyrenoidosa	AQUA - NR	FW		EC50	DEC	POP	A	
Green Algae	U	LAB	(12 - 14) d			ABND	> 10000 ug/L	
Scenedesmus quadricauda	S	FW		LOEC	DEC	POP	F	SIG
Green Algae	U	LAB	NR			PGRT/	1630000 ug/L	
Microcystis aeruginosa	S	FW		LOEC	DEC	POP	F	SIG
Blue-Green Algae	U	LAB	NR			PGRT/	55000 ug/L	

Entosiphon sulcatum	S	FW			DEC	POP	F	
Flagellate Euglenoid	U	LAB	3 d			ABND	218000 ug/L	
Scenedesmus quadricauda	S	FW			DEC	POP	F	
Green Algae	U	LAB	7 d			ABND	1630000 ug/L	
Chilomonas paramecium	AQUA - NR					POP/	F	
Cryptomonad	U	LAB	2 d			GPOP	356000 ug/L	
Chilomonas paramecium	AQUA - NR	FW			CHG	POP	F	
Cryptomonad		LAB	NR			PGRT/	356000 ug/L	
Entosiphon sulcatum	AQUA - NR	FW			CHG	POP	F	
Flagellate Euglenoid		LAB	NR			PGRT/	218000 ug/L	
Entosiphon sulcatum	AQUA - NR	FW			CHG	POP	F	
Flagellate Euglenoid		LAB	NR			PGRT/	218000 ug/L	
Scenedesmus quadricauda	S					POP/	F	
Green Algae	U	LAB	8 d			GPOP	1630000 ug/L	
Anacystis aeruginosa	S					POP/	F	
Blue-Green Algae	U	LAB	8 d			GPOP	55000 ug/L	

Scenedesmus quadricauda	AQUA - NR	FW			CHG	POP	F	
Green Algae		LAB	NR			PGRT/	1630000 ug/L	
Scenedesmus quadricauda	AQUA - NR	FW			DEC	POP	F	
Green Algae	U	LAB	10 d			BMAS/	1630000 ug/L	
Entosiphon sulcatum	S	FW			DEC	POP	F	
Flagellate Euglenoid	U	LAB	3 d			PGRT/	218000 ug/L	
Algae, Moss, Fungi; Standard Test Species								
Pseudokirchneriella subcapitata	S	FW		BCF		ACC/	F	
Green Algae	U	LAB	6 d	7.6 L/kg		RSDE	29.4 Ci/mol	
Pseudokirchneriella subcapitata	AQUA - NR	FW		EC50	DEC	POP	A	
Green Algae	U	LAB	2 d			ABND/	207500 ug/L	
Pseudokirchneriella subcapitata	AQUA - NR	FW		EC50	DEC	POP	A	
Green Algae	U	LAB	2 d			ABND/	342800 ug/L	
Anabaena cylindrica	S	FW		EC50	DEC	ENZ	A	
Blue-Green Algae	U	LAB	0.208 d			NITG	30000 ug/L	
Anabaena cylindrica	S	FW		EC50	DEC	POP	A	

Blue-Green Algae	U	LAB	0.125 d			PSYN	60000 ug/L	
Anabaena cylindrica	AQUA - NR	FW		EC50	DEC	POP	A	
Blue-Green Algae	U	LAB	(12 - 14) d			ABND	> 10000 ug/L	
Anabaena cylindrica	AQUA - NR	FW		EC50	DEC	POP	A	
Blue-Green Algae	U	LAB	(12 - 14) d			PGRT	> 10000 ug/L	
Pseudokirchneriella subcapitata	AQUA - NR	FW		EC50	DEC	POP	A	
Green Algae	U	LAB	2 d			ABND/	36390 ug/L	
Pseudokirchneriella subcapitata	AQUA - NR	FW		EC50	DEC	POP	A	
Green Algae	U	LAB	2 d			PGRT	83290 ug/L	
Pseudokirchneriella subcapitata	AQUA - NR	FW		EC50	DEC	POP	A	
Green Algae	U	LAB	2 d			ABND/	37100 ug/L	
Pseudokirchneriella subcapitata	AQUA - NR	FW		LOEC	DEC	POP	A	SIG
Green Algae	U	LAB	2 d			ABND/	9620 ug/L	0.05
Pseudokirchneriella subcapitata	AQUA - NR	FW		LOEC	DEC	POP	A	SIG
Green Algae	U	LAB	2 d			PGRT	19240 ug/L	0.05

Pseudokirchneriella subcapitata	AQUA - NR	FW		NOEC	DEC	POP	A	NOSIG
Green Algae	U	LAB	2 d			PGRT	9620 ug/L	0.05
Pseudokirchneriella subcapitata	AQUA - NR	FW		NOEC	DEC	POP	A	NOSIG
Green Algae	U	LAB	2 d			ABND/	4810 ug/L	0.05
Chlorella vulgaris	S	FW			DEC	POP	F	
Green Algae	U	LAB	(3.333 - 6.667) d		0-100	GPOP/	(0.00005-0.001) M	
Chlorella vulgaris	S	FW			INC	PHY	F	
Green Algae	U	LAB	0.208 d		May-95	PSYN/	(0.00005-0.001) M	
Amphibians; Standard Test Species								
Xenopus laevis	R	FW	MT/	EC50	INC	DVP	A	
African Clawed Frog	U	LAB	4 d			GDVP/	433000 (404000-464000) ug/L	
Xenopus laevis	R	FW		LC50	INC	MOR	A	
African Clawed Frog	U	LAB	4 d			MORT	1290800 (1243000-1340000) ug/L	
Crustaceans; Standard Test Species								
Daphnia magna	S	FW		BCF		ACC	A	

Water Flea	M	LAB	1 d	1772 L/kg		RSDE	1.78 ug/L	
Daphnia magna	AQUA - NR	FW		EC0		BEH	F	
Water Flea			1 d			EQUL	260000 ug/L	
Daphnia magna	AQUA - NR	FW		EC100		BEH	F	
Water Flea			1 d			EQUL	1000000 ug/L	
Daphnia magna	S	FW		EC50	INC	ITX	A	
Water Flea	U	LAB	2 d			IMBL	860000 ug/L	
Daphnia magna	AQUA - NR	FW		EC50		BEH	F	
Water Flea			1 d			EQUL	500000 ug/L	
Daphnia magna	S	FW		EC50		ITX	A	
Water Flea		LAB	2 d			IMBL	> 100000 ug/L	
Daphnia magna	S	FW		LC0	NEF	ITX	F	
Water Flea	U	LAB	1 d			IMBL	540000 ug/L	
Daphnia magna	S	FW		LC50	INC	ITX	F	
Water Flea	U	LAB	1 d			IMBL	1540000 ug/L	
Daphnia magna	S	FW		NOEC		ITX	F	NOSIG
Water Flea	U	LAB	0.667 d			IMBL/	146000 ug/L	

Daphnia magna	S	FW		NOEL		ITX	A	NOSIG
Water Flea		LAB	2 d			IMBL	100000 ug/L	
Daphnia magna	S	FW			INC	MOR	F	
Water Flea	U	LAB	2 d			MORT	0.01 mmol/L	
Daphnia magna	S	FW	WO		INC	ACC	F	
Water Flea	U	LAB	2 d			GACC/	0.01 mmol/L	
Daphnia magna	S	FW	WO		INC	ACC	A	
Water Flea	U	LAB	1 d			GACC	0.0016 mmol/L	
Daphnia magna	S	FW			INC	ITX	F	
Water Flea	U	LAB	2 d			IMBL	0.01 mmol/L	
Fish								
Leuciscus idus	AQUA - NR	FW		BCF	INC	ACC	F	
Ide, Silver Or Golden Orfe	U	LAB	3 d	5 L/kg		RSDE	~ 50 ug/L	
Leuciscus idus	AQUA - NR	FW		BCF	INC	ACC	F	
Ide, Silver Or Golden Orfe	U	LAB	3 d	5.3 L/kg		RSDE	~ 50 ug/L	
Leuciscus idus ssp. melanotus	AQUA - NR	FW		LC0	INC	MOR	F	

Carp			2 d			MORT	400000 ug/L	
Leuciscus idus ssp. melanotus	AQUA - NR	FW		LC100	INC	MOR	F	
Carp			2 d			MORT	600000 ug/L	
Leuciscus idus ssp. melanotus	AQUA - NR	FW		LC50	INC	MOR	F	
Carp			2 d			MORT	460000 ug/L	
Lepomis humilis	S	FW		NR-LETH		MOR	F	
Orangespotted Sunfish	U	LAB	0.042 d		100	MORT	(550000-570000) ug/L	
Fish; Standard Test Species								
Lepomis macrochirus	S	FW		LC0*		MOR	F	
Bluegill	U	LAB	4 d			MORT	180000 ug/L	
Ictalurus punctatus	IV	FW	MT/		INC	ACC	A	
Channel Catfish	U	LAB	2 d			GACC	10 mg/kg bdwt	
Ictalurus punctatus	D/	FW	MT/		INC	ACC	A	
Channel Catfish	U	LAB	2 d			GACC	10 ppm	
Fish; U.S. Exotic/Nuisance Species								
Gambusia affinis	S	FW		BCF		ACC	A	
Western	M	LAB	1 d	21 L/kg		RSDE	1.78 ug/L	

Mosquitofish								
Gambusia affinis	S	FW		LC50/	INC	MOR	F	
Western Mosquitofish	U	LAB	4 d			MORT	180000 ug/L	
Gambusia affinis	S	FW		LC50/	INC	MOR	F	
Western Mosquitofish	U	LAB	1 d			MORT	240000 ug/L	
Gambusia affinis	S	FW		LC50/	INC	MOR	F	
Western Mosquitofish	U	LAB	4 d			MORT	180000 ug/L	
Carassius auratus	S	FW		LT50		MOR	F	
Goldfish	U	LAB	0.033 d			MORT	167000 ug/L	
Carassius auratus	S	FW		LT50		MOR	F	
Goldfish	U	LAB	0.052 d			MORT	125000 ug/L	
Carassius auratus	S	FW		LT50		MOR	F	
Goldfish	U	LAB	0.165 d			MORT	100000 ug/L	
Carassius auratus	S	FW		LT50		MOR	F	
Goldfish	U	LAB	0.019 d			MORT	250000 ug/L	
Carassius auratus	S	FW		LT50		MOR	F	
Goldfish	U	LAB	0.01 d			MORT	500000 ug/L	

Carassius auratus	S	FW		LT50		MOR	F	
Goldfish	U	LAB	0.012 d			MORT	333000 ug/L	
Carassius auratus	S	FW		LT50		MOR	F	
Goldfish	U	LAB	0.008 d			MORT	750000 ug/L	
Carassius auratus	S	FW		LT50		MOR	F	
Goldfish	U	LAB	0.025 d			MORT	200000 ug/L	
Carassius auratus	S	FW				MOR/	F	
Goldfish	U	LAB	0.302 d			MORT	200000 ug/L	
Carassius auratus	AQUA - NR					ACC/	F	
Goldfish	U	LAB	NR			GACC	400000* ug/L	
Carassius auratus	S	FW				MOR/	F	
Goldfish	U	LAB	0.02 d			MORT	1000000 ug/L	
Flowers, Trees, Shrubs, Ferns								
Oryza sativa	S	FW		LOEL		BCM	F	ASIG
Rice	U	LAB	1.667 d			NCON	100 uM	
Insects/Spiders								
Culex quinquefasciatus	S	FW		BCF		ACC	A	

Southern House Mosquito	M	LAB	1 d	138 L/kg		RSDE	1.78 ug/L	
Invertebrates								
Tetrahymena pyriformis	AQUA - NR	FW		IC50	DEC	POP	A	
Ciliate	U	LAB	2 d			PGRT/	251670 (197270-298890) ug/L	
Uronema parduczi	AQUA - NR	FW			CHG	POP	F	
Ciliate		LAB	NR			PGRT/	31000 ug/L	
Uronema parduczi	AQUA - NR	FW			CHG	POP	F	
Ciliate		LAB	0.833 d			PGRT/	31000 ug/L	
Molluscs								
Physa sp.	S	FW		BCF		ACC	A	
Pouch Snail	M	LAB	2 d	2786 L/kg		RSDE	1.78 ug/L	
Mytilus californianus	S	SW				BEH/	F	
Mussel	U	LAB	NR			FLTR	4070000000 ug/L	
Mytilus californianus	S	SW				BEH/	F	
Mussel	U	LAB	NR			DTCH	4070000000 ug/L	

Molluscs; U.S. Exotic/Nuisance Species								
Dreissena polymorpha	S	FW				BEH	F	
Zebra Mussel	U	LAB	2 d			DTCH/	(0-50000) ug/L	
Worms								
Meloidogyne arenaria	S	FW		LC50	INC	MOR	F	
Peanut Root-Knot Nematode	U	LAB	1 d			MORT/	2.50 mM	
Meloidogyne arenaria	S	FW		LC50	INC	MOR	F	
Peanut Root-Knot Nematode	U	LAB	1 d			MORT/	2.38 mM	

As taken from the EPA ECOTOX database.

“Short- and long-term tests with fish, daphnids and green algae are available for benzoic acid. The acute effect values for the three trophic levels were all above 100 mg/L. The lowest valid effect value of 25 mg/L was obtained in a long-term test with Daphnia magna. Applying an assessment factor of 10 resulted in a PNECwater of 2.5 mg/L.”

Species	Time-scale	Endpoint	Toxicity
Fish			
Oncorhynchus mykiss	96 h	Mortality	LC50 > 120 mg/L (nominal concentration confirmed by analytical monitoring)
Oncorhynchus mykiss	28 d	Mortality, Growth	NOEC = 92.4 mg/L (mean measured concentration)
Invertebrates			

Daphnia magna	48 h	Immobilisation	EC50 > 120 mg/L (nominal concentration confirmed by analytical monitoring)
Daphnia magna	21 d	Reproduction	NOEC = 25 mg/L (nominal concentration confirmed by analytical monitoring)
Algae			
Desmodesmus subspicatus	72 h	Growth inhibition	ErC50 ≥ 1000 mg/L NOErC ≥ 1000 mg/L (nominal concentration confirmed by analytical monitoring)
Microorganisms			
Activated sludge (municipal sewage treatment plant)	3 h (static)	respiration inhibition	EC50 ≥ 1000 mg a.s./L (nominal)

As taken from European Commission, 2013a, b.

ECOSAR version 1.11 provides the following aquatic toxicity data for CAS RN 65-85-0:

Values used to Generate ECOSAR Profile:

Log Kow: 1.874 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 3400 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations:

Not Related to an Existing ECOSAR Class Definition

Estimates provided below use the Neutral Organics QSAR equations which represent baseline toxicity potential (minimum toxicity) assuming a simple non-polar narcosis model. Without empirical data on structurally similar chemicals, it is uncertain if this substance will present significantly higher toxicity above baseline estimates.

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)

--> Acid moiety found: Predicted values multiplied by 10				
Neutral Organics-acid :	Fish	96-hr	LC50	1300.781
Neutral Organics-acid :	Daphnid	48-hr	LC50	730.075
Neutral Organics-acid :	Green Algae	96-hr	EC50	518.374
Neutral Organics-acid :	Fish		ChV	125.419
Neutral Organics-acid :	Daphnid		ChV	68.937
Neutral Organics-acid :	Green Algae		ChV	132.290
Neutral Organics-acid :	Fish (SW)	96-hr	LC50	1636.355
Neutral Organics-acid :	Mysid	96-hr	LC50	1324.125
Neutral Organics-acid :	Fish (SW)		ChV	164.501
Neutral Organics-acid :	Mysid (SW)		ChV	118.794

LOEC values to algae, mg/l:

55 rpd, schr, *Microcystis aeruginosa*, Bringmann & Kuhn 1976

1630 rpd, schr, *Scenedesmus quadricauda*, Bringmann & Kuhn 1980a

LC50 values to fishes, mg/l:

180 srv, act, 96 hr, *Gambusia affinis*, Jones, 1971

Other information of water organisms :

Toxicity threshold (cell multiplication inhibition test):

Algae (*Microcystis aeruginosa*): 55 mg/l (Bringmann & Kühn 1976)

Green algae (*Scenedesmus quadricauda*): 1630 mg/l

Protozoa (*Entosiphon sulcatum*): 218 mg/l

Protozoa (*Uronema parduczi*): 31 mg/l

(Bringmann & Kühn 1980a)

Arthropoda: *Daphnia magna*: immobilization at 146 mg/l; prolonged exposure (McKee & Wolf 1963).

As taken from the Finnish Environment Institute (b).

“Effects of benzoic acid (BA) on physicochemical properties and ecotoxicities of CuO nanoparticles (CuONPs) in model aqueous media were studied. The CuONPs had larger hydrodynamic sizes and higher surface zeta potentials during 96 h of settling in the presence of BA than when the BA were not present. BA interaction with CuONPs is shown to promote dissolved Cu release from CuONPs in a dose-dependent manner. The contribution of free Cu(2+)-ions to growth inhibition toxicity of the CuONP suspensions at a toxicologically relevant concentration for the green alga *Scenedesmus obliquus* was around 22 %, indicating that dissolved fraction was not the major source of toxicity of CuONPs. The toxicity of CuONPs increased as the BA concentration increased. BA significantly altered total antioxidant capacity of CuONPs-exposed algal cells. The mechanism of the BA effect on the CuONPs toxicity may be mainly associated with degree of agglomeration, dissolved Cu, and particle-induced oxidative stress” As taken from Wang Z et al. 2016. Bull. Environ. Contam. Toxicol. 97(2), 159-65. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27098254>

Fish (*Oncorhynchus mykiss*) - Acute 96 hour LC50 >120 mg/l

Fish (*Oncorhynchus mykiss*) - Chronic 21 day NOEC 90.5 mg/l

Aquatic invertebrates (*Daphnia magna*) - Acute 48 hour EC50 >120 mg/l

Aquatic invertebrates (*Daphnia magna*) - Chronic 21 day NOEC 55 mg/l

Algae (*Scenedesmus quadricauda*) - Acute 72 hour EC50, growth >10mg/l

As taken from PPDB, 2016

10.3. Sediment toxicity

“No test with benthic organisms is available. As benzoic acid is not expected to partition to the sediment compartment, the effects assessment for the sediment is covered by the effects assessment for the surface water. Nevertheless, a PNEC_{sediment} is derived from the PNEC_{water} using the equilibrium partitioning method according to the EU TGD (2003). With a *K_{susp}*-water of 1.589 and a *RHO_{susp}* of 1150 kg/m³, a PNEC_{sediment} of 3.4 mg/kg ww is derived.”

As taken from European Commission, 2013a, b.

10.4. Terrestrial toxicity

TERRESTRIAL FATE: Based on a classification scheme(1), an estimated K_{oc} value of 15(SRC), determined from a structure estimation method(2), indicates that benzoic acid is expected to have very high mobility in soil(SRC). The pK_a of benzoic acid is 4.20(3), indicating that this compound will exist almost entirely in the anion form in the environment and anions generally do not adsorb more strongly to soils containing organic carbon and clay than their neutral counterparts(4). Volatilization from moist soil is not expected because the compound exists as an anion and anions do not volatilize. Benzoic acid is not expected to volatilize from dry soil surfaces(SRC) based upon a vapor pressure of 7.0X10⁻⁴ mm Hg at 25 deg C(5). Mineralization half-life in Captina silt loam for benzoic acid in solution was 4.5 hr after a 30 minute lag(6) and complete degradation occurred in 1 day using a Niagra silt loam inoculum(7), suggesting that biodegradation may be an important fate process in soil(SRC). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) US EPA; Estimation

Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of Aug 20, 2013: <http://www.epa.gov/oppt/exposure/pubs/episuite.html> (3) Haynes WM, ed; CRC Handbook of Chemistry and Physics. 91st ed. Boca Raton, FL: CRC Press Inc., p 8-47 (2010) (4) Doucette WJ; pp. 141-188 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000) (5) McEachern DM, Sandoval O; J Phys E 6: 155-161 (1973) (6) Dao TH, Lavy TL; Soil Sci 143: 66-72 (1987) (7) Alexander M, Lustigman BK; J Agric Food Chem 14: 410-3 (1966)] **PEER REVIEWED**

Soil Adsorption/Mobility:

Koc of benzoic acid is estimated as 15(SRC), using a log Kow of 1.87(1) and a regression-derived equation(2). An experimental log Koc of 1.50 (Koc = 31) has been reported, test details not available(3). According to a classification scheme(4), these Koc values suggest that benzoic acid is expected to have very high mobility in soil. The pKa of benzoic acid is 4.20(5), indicating that this compound will exist in anion form in the environment and anions generally do not adsorb more strongly to organic carbon and clay than their neutral counterparts(6). Freundlich adsorption constants of 0.23, 0 and 0 were reported using Ersum sandy till (pH 4.7; 0.25% OC), Tirstrup melt water sand (pH 6.1; 0.09% OC) and Djursland clayey till (pH 7.6; 0.22% OC), respectively, at 6 deg C. Soils were collected in North Sealand and Djursland, Jutland(7). Benzoic acid displayed negligible adsorption when using a montmorillonite (Volclay bentonite, Upton WY) clay(8). [(1) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Prof Ref Book. Heller SR, consult. ed., Washington, DC: Amer Chem Soc p. 29 (1995) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of Aug 19, 2013: <http://www.epa.gov/oppt/exposure/pubs/episuite.html> (3) Schuurmann G et al; Environ Sci Technol 40: 7005-11 (2006) (4) Swann RL et al; Res Rev 85: 17-28 (1983) (5) Haynes WM, ed; CRC Handbook of Chemistry and Physics. 91st ed. Boca Raton, FL: CRC Press Inc., p 8-47 (2010) (6) Doucette WJ; pp. 141-188 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000) (7) Loekke H; Water Air Soil Pollut 22: 373-87 (1984) (8) Bailey GW et al; Soil Sci Soc Amer Proc 32: 222-34 (1968)] **PEER REVIEWED**

Volatilization from Water/Soil:

A pKa of 4.20(1) indicates benzoic acid will exist almost entirely in the ionized form at pH values of 5 to 9 and, therefore, volatilization from water or moist soil surfaces is not expected to be an important fate process. Benzoic acid is not expected to volatilize from dry soil surfaces(SRC) based upon a vapor pressure of 7.0×10^{-4} mm Hg at 25 deg C(2). [(1) Haynes WM, ed; CRC Handbook of Chemistry and Physics. 91st ed. Boca Raton, FL: CRC Press Inc., p 8-47 (2010) (2) McEachern DM, Sandoval O; J Phys E 6: 155-161 (1973)] **PEER REVIEWED**

As taken from HSDB, 2014.

The few available data indicate that benzoic acid and sodium benzoate have only a low toxicity potential in the terrestrial environment. Except for the antimicrobial action of benzoic acid, characterized by minimum microbiocidal concentrations ranging from 20 to 1200 mg/litre, no data on toxic effects of benzoic acid on terrestrial organisms were available. For sodium benzoate, bacterial and fungal growth were inhibited in a pH-dependent manner by concentrations ranging from 100 to 60 000 mg/litre. Owing to the lack of measured exposure levels, a sample risk characterization with respect to terrestrial organisms could not be performed.

Geoaccumulation of benzoic acid has also been found to be low. Depending on soil depth, sorption coefficients (Kd) of 0.62 (18.9 m) to 1.92 (0.4 m) have been measured (Federle, 1988). Mobility determinations of ¹⁴C-labelled benzoic acid in different soils by means of thin-layer chromatography showed benzoic acid to be moderately mobile. Its mobility was positively correlated with soil pH and negatively correlated with aluminium and iron contents and effective anion exchange capacity (Stolpe et al., 1993).

As taken from INCHEM, 2000, CONCISE INTERNATIONAL CHEMICAL ASSESSMENT DOCUMENT NO. 26, BENZOIC ACID AND SODIUM BENZOATE.

Record for benzoic acid:

Spec. Name	Sci.	Resp. Site	Media Type	Exp. Type	Dose#	Endpoint	Effect	Signif.	Dose
Spec. Common Name		Exp. Dur. (Days)	Test Loc.	Chem. Anal.	Res. Sample Unit	BAF/BCF	Effect Meas.	Sig. Level	Dose Stat. Meth.
Colinus virginianus Northern Bobwhite Quail		8	LAB	FD		LC50	MOR MORT		NC > 5620 ppm
Anas platyrhynchos Mallard Duck		8	LAB	FD		LC50	MOR MORT		NC > 5620 ppm
Anas platyrhynchos Mallard Duck		14	LAB	OR		LD50	MOR MORT		NC > 2510 mg/kg org
Sturnus vulgaris European Starling		4	NONE LAB	CH U	4	LOEL	AVO CHEM	SIG	F 113 uM/g
Sturnus vulgaris European Starling		4	NONE LAB	FD U	4	LOEL	AVO CHEM	SIG	F 113 uM/g
Sturnus vulgaris European Starling		4	NONE LAB	CH U	4	NOEL	AVO CHEM	NOSIG	F 56 uM/g

Sturnus vulgaris European Starling	4	NONE LAB	FD U	4	NOEL	AVO CHEM	NOSIG	F 56 uM/g
Brassica napus Rapeseed	5	FLT LAB	SO U	2	LOEL	GRO WGHT	ASIG 0.01	F 1.0 mM
Hordeum vulgare Barley	RO 5	ART LAB	HP U	2	LOEL	GRO LGTH	ASIG 0.05	F 5 mol/m3
Hordeum vulgare Barley	RO 5	ART LAB	HP U	2	LOEL	GRO LGTH	ASIG 0.05	F 5 mol/m3
Brassica napus Rapeseed	5	FLT LAB	SO U	2	NOEL	REP GERM	ANOSIG	F 1.0 mM
Ipomoea nil Whiteedge Morningglory	RO 35	CUL LAB	GM*	VG		GRO SIZE		NC (1-50) uM
Avena sp. Oat	COL 1	CUL LAB	SO	SL		GRO SIZE		NC 1 1e-4 M
Triticum sp. Wheat	MT (2.667 - 2.833)	HYP LAB	HP U	4		GRO LGTH		F (.002-.05) mM
Lupinus albus Egyptian Lupine	CN 1	CUL LAB	SO	SL		INJ DAMG		NC 16 1e-5 M
Lupinus albus Egyptian Lupine	CN 2	CUL LAB	SO	SL		INJ DAMG		NC 8 1e-5 M

Lupinus albus Egyptian Lupine	CN 3	CUL LAB	SO	SL		INJ DAMG		NC 2 1e-5 M
Lupinus albus Egyptian Lupine	ME 1	CUL LAB	SO	SL		PHY WILT		NC 16 1e-5 M
Lupinus albus Egyptian Lupine	ME 2	CUL LAB	SO	SL		PHY WILT		NC 8 1e-5 M
Lupinus albus Egyptian Lupine	ME 3	CUL LAB	SO	SL		PHY WILT		NC 2 1e-5 M
Ipomoea nil Whiteedge Morningglory	FLW 35	CUL LAB	GM*	VG		REP FLOR		NC (1-50) uM
Lactuca sativa Lettuce		AGR			EC50	REP GERM		NC 0.56 mmol
Lactuca sativa Lettuce	3	AGR LAB	DA U		EC50	REP GERM		F 0.51 mM
Cucumis sativus Cucumber		FLT LAB	SO U	2	LOEL	BCM PRCO	ASIG 0.05	F 1 mM
Cucumis sativus Cucumber		FLT LAB	SO U	2	LOEL	ENZ CTLS	ASIG 0.05	F 1 mM
Cucumis sativus Cucumber		FLT LAB	SO U	2	LOEL	GRO BMAS	ASIG 0.05	F 1 mM

Zea mays Corn	1	HYP LAB	DA U	2	LOEL	PHY PSYN	ASIG	F 0.5 uM
Cucumis sativus Cucumber	8	NAT LAB	DA U	4	LOEL	POP BMAS	ASIG 0.05	F 10 mM
Phaseolus vulgaris Bean	8	NAT LAB	DA U	4	LOEL	POP BMAS	ASIG 0.01	F 10 mM
Cucumis sativus Cucumber		FLT LAB	SO U	2	LOEL	REP GERM	ASIG 0.05	F 1 mM
Avena sativa Common Oat	RO 0.83	CUL LAB	SO	SL		GRO SIZE		NC 1 1e-3 M
Phaseolus vulgaris Bean	RO 6.12		SO	SL		POP ABND		NC 1000 ppm

As taken from the EPA ECOTOX database.

LD50 values to birds in oral exposure, mg/kg :

>100, orl-Agelaius phoeniceus

>100, orl-Sturnus vulgaris

Schafer et al. 1983

As taken from the Finnish Environment Institute (b).

“No tests with terrestrial organisms are available for benzoic acid. According to the TNsG on data requirement, tests with terrestrial organisms need to be performed for this product type, as exposure to the terrestrial compartment via manure occurs from the use in stables. The applicant justified the non-submission of terrestrial studies with the low exposure of the terrestrial compartment. As a first step, the PNECsoil was derived from the PNECwater using the equilibrium partitioning method according to the EU TGD on Risk Assessment (2003). With a ksoil-water of 1.026 and a RHOSoil of 1700 kg/m³, a PNECsoil of 1.5 mg/kg ww is derived from the PNECwater.”

As taken from European Commission, 2013a, b.

ECOSAR version 1.11 provides the following terrestrial toxicity data for CAS RN 65-85-0:

Values used to Generate ECOSAR Profile:

Log Kow: 1.874 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 3400 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations:

Not Related to an Existing ECOSAR Class Definition

Estimates provided below use the Neutral Organics QSAR equations which represent baseline toxicity potential (minimum toxicity) assuming a simple non-polar narcosis model. Without empirical data on structurally similar chemicals, it is uncertain if this substance will present significantly higher toxicity above baseline estimates.

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
--> Acid moiety found: Predicted values multiplied by 10				
Neutral Organics-acid :	Earthworm	14-day	LC50	2187.900

Soil absorption and mobility

Koc 250. "Moderately mobile"

Kf 1.66; Kfoc 547; 1/n; 0.9. "slightly mobile".

EU dossier (2015 RAR) scant & incomplete data Kf range 0.23-3.56 mL/g, Kfoc range 18-1550 mL/g

Earthworms (*Ensenia foetida*) - Acute 14 day LC50 384 mg kg⁻¹

As taken from PPDB, 2016

10.5. All other relevant types of ecotoxicity

Benzoic acid was found in one of twelve breath samples taken from people in New Jersey. Benzoic acid was not found in the drinking water or air samples from this study(1). [(1) Wallace LA et al; Environ Res 35: 293-319 (1984)] **PEER REVIEWED** PubMed Abstract

LC50; Species: *Colinus virginianus* (Northern bobwhite quail) diet >5620 ppm for 8 days

[USEPA, Office of Pesticide Programs; Pesticide Ecotoxicity Database (2000) as cited in the ECOTOX database. Available from, as of October 26, 2013:] **PEER REVIEWED**

LC50; Species: *Anas platyrhynchos* (Mallard duck) diet >5620 ppm for 8 days [USEPA, Office of Pesticide Programs; Pesticide Ecotoxicity Database (2000) as cited in the ECOTOX database. Available from, as of October 26, 2013:] **PEER REVIEWED**

LD50; Species: *Anas platyrhynchos* (Mallard duck) oral via capsule >2510 mg/kg [USEPA, Office of Pesticide Programs; Pesticide Ecotoxicity Database (2000) as cited in the ECOTOX database. Available from, as of October 26, 2013:] **PEER REVIEWED**

EC50; Species: *Anabaena inaequalis* (Cyanobacteria); Conditions: static; Concentration: 9 mg/L for 14 days; Effect: cell multiplication inhibition [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

LC50; Species: *Gambusia affinis* (Mosquitofish); Concentration: 240 mg/L for 24 hr; 255 mg/L for 48 hr; 180 mg/L for 96 hr /Conditions of bioassay not specified in source examined/ [Verschuere, K. Handbook of Environmental Data on Organic Chemicals. Volumes 1-2. 4th ed. John Wiley & Sons. New York, NY. 2001, p. 288] **PEER REVIEWED**

EC50; Species: *Chlorella pyrenoides* (Algae); Concentration: 60 mg/L for 3 hr; Effect: photosynthesis reduction /Conditions of bioassay not specified in source examined/ /from table/ [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

EC50; Species: *Tetrahymena pyriformis* (Protozoa); Concentration: 252 mg/L for 2 days; Effect: cell multiplication inhibition /Conditions of bioassay not specified in source examined/ /from table/ [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

LC50; Species: *Teredo digensis* (Shipworm) larvae; Conditions: static; Concentration: 100 mg/L for 72 hr /from table/ [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

EC50; Species: *Daphnia magna* (Waterflea); Concentration: 500 mg/L for 24 hr, neutral pH; 102 mg/L for 24 hr, acid pH; Effect: immobilization /Conditions of bioassay not specified in source examined/ /from table/ [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

LC50; Species: *Leuciscus idus* (Ide); Concentration: 460 mg/L for 48 hr; pH 7-8 /Conditions of bioassay not specified in source examined/ /from table/ [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

LC50; Species: *Xenopus laevis* (Frog), embryos; Concentration: 1291 mg/L for 96 hr; pH 7.2-7.4 /Conditions of bioassay not specified in source examined/ /from table/ [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

EC50; Species: *Xenopus laevis* (Frog) embryos; 433 mg/L for 96 hr; pH 7.2-7.4; Effect: malformation /Conditions of bioassay not specified in source examined/ /from table/ [International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 26: Benzoic acid and Sodium Benzoate (2000). Available from, as of March 24, 2005: <http://www.inchem.org/pages/cicads.html> **PEER REVIEWED**

EC50; Species: *Anabaena cylindrica* (Blue-green algae); Conditions: static; Concentration: >10 mg/L for 12-14 days; Effect: cell multiplication inhibition [Stratton GW, Corke CT; Environ Poll Ser A Ecol Biol 29 (1): 71-80 (1982). ECOTOX database on Benzaldehyde (100-52-7). Available from, as of March 24, 2005: http://cfpub.epa.gov/ecotox/quick_query.htm **PEER REVIEWED**

EC50; Species: *Anabaena variabilis* (Blue-green algae); Conditions: static; Concentration: >10 mg/L for 12-14 days; Effect: cell multiplication inhibition [Stratton GW, Corke CT; Environ Poll Ser A Ecol Biol 29 (1): 71-80 (1982). ECOTOX database on Benzaldehyde (100-52-7). Available from, as of March 24, 2005: http://cfpub.epa.gov/ecotox/quick_query.htm **PEER REVIEWED**

EC50; Species: *Chlorella pyrenoidosa* (Green algae); Conditions: static; Concentration: >10 mg/L for 12-14 days; Effect: cell multiplication inhibition [Stratton GW, Corke CT; Environ Poll Ser A Ecol Biol 29 (1): 71-80 (1982). ECOTOX database on Benzaldehyde (100-52-7). Available from, as of March 24, 2005: http://cfpub.epa.gov/ecotox/quick_query.htm **PEER REVIEWED**

EC50; Species: *Scenedesmus quadricauda* (Green algae); Conditions: static; Concentration: >10 mg/L for 12-14 days; Effect: cell multiplication inhibition [Stratton GW, Corke CT; Environ Poll Ser A Ecol Biol 29 (1): 71-80 (1982). ECOTOX database on Benzaldehyde (100-52-7). Available from, as of March 24, 2005: http://cfpub.epa.gov/ecotox/quick_query.htm **PEER REVIEWED**

Artificial Pollution Sources:

Benzoic acid's production and use as a plasticizer, chemical intermediate in the production of benzoyl chloride alkyd resins, food preservative, in seasoning tobacco, flavors, perfumes, dentifrices and as a standard in analytical chemistry(1) may result in its release to the environment through various waste streams(SRC). Its use as a plant growth regulator(2) will result in its direct release to the environment(SRC). Benzoic acid is a biodegradation break down product of the herbicide Isoxaflutole, used for weed control in corn (*Zea mays*)(3). [(1) Lewis RJ Sr, ed; Hawley's Condensed Chemical Dictionary. 15th ed. New York, NY: John Wiley & Sons, p. 137 (2007) (2) Crop Protection Handbook 2011. Willoughby, OH: Meister Publishing Co., (2011) (3) Meyer MT et al; Environ Sci Technol 41: 6933-9 (2007)] **PEER REVIEWED**

Environmental Biodegradation:

AEROBIC: Benzoic acid is biodegradable under aerobic conditions by bacteria present in crude municipal wastewater at less than or equal to 200 g/cu m. [Jaroszynski T, Gomolka E; Environ Prot Eng 5 (4): 375 (1979)] **PEER REVIEWED**

AEROBIC: BOD after 5 days at 20 deg C: 69-75% BODT; 70% BODT (standard dilution technique, acclimated sewage seed); after 10 days at 20 deg C: 73% BODT (standard dilution technique, normal sewage seed); after 20 days at 20 deg C: 79% BODT. Chemical oxygen demand: 1.88-1.95; theoretical oxygen demand: 1.96; KMnO4 value: 0.032 [Verschueren, K. Handbook of Environmental Data on Organic Chemicals. Volumes 1-2. 4th ed. John Wiley & Sons. New York, NY. 2001, p. 286] **PEER REVIEWED**

AEROBIC: Benzoic acid, present at 100 mg/L, reached 85% of its theoretical BOD in 2 weeks using an activated sludge inoculum at 30 mg/L and the Japanese MITI test(1). Both ¹³C-labeled and unlabeled benzoic acid were reported to reach 60% of their theoretical CO₂ evolution within 5 days using a soil inoculum(2). [(1) Chemicals Inspection and Testing Institute; Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan. Japan Chemical Industry Ecology - Toxicology and Information Center. ISBN 4-89074-101-1 (1992) (2) Shen J, Bartha R; Appl Environ Microb 62: 1428-30 (1996)] **PEER REVIEWED**

AEROBIC: Benzoic acid has been studied extensively and shown to be biodegradable in screening tests. Eleven laboratories testing a respiratory biodegradability test utilizing an unacclimated sludge inoculum found benzoic acid to be readily degradable, obtaining a mean oxygen uptake of 84% of theoretical after 10 days and no lag period before biodegradation commenced(10). Some results from other investigators are: 99% COD removal in 5 days with acclimated activated sludge(1); 67% of theoretical BOD removal in 5 days(2); 97% degradation in 20 days by activated sludge where 10% of the benzoic acid was replaced every 2 days to acclimate activated the sludge(3); 68.2 and 86.9% mineralization in 5 days by acclimated sludge in salt solution and simulated industrial effluent, respectively(4); 65.4% mineralization in 5 days by activated sludge(5); complete disappearance in 1 day using an activated sludge inoculum(6); 73% of theoretical BOD utilized in 6

days using activated sludge from 3 municipal sewage plants(7); 74% of theoretical BOD utilized in a 5 day test with a sewage seed(8); >90% degraded in 2 days using activated sludge(9); 84.1 and 74.9% of theoretical BOD in 5 days by the standard and sea water dilution methods, respectively(11). [(1) Pitter P; Water Res 10: 231-5 (1976) (2) Heukelekian H, Rand MC; J Water Pollut Contr Assoc 29: 1040-53 (1955) (3) Lund FA, Rodriguez DS; J Gen Appl Microbiol 30: 53-61 (1984) (4) Belly RT, Goodhue CT; pp.1103-7 in Proc Int Biodegrad Symposium 3rd (1976) (5) Freitag D et al; Chemosphere 14: 1589-616 (1985) (6) Digeronimo MJ et al; in Microbial Degradation Of Pollutants In Marine Environments pp. 154-66 EPA-600/9-79-012 (1979) (7) Lutin PA et al; Purdue Univ Eng Bull, Ext Series 118: 131-45 (1965) (8) Young RHF et al; J Water Pollut Contr Fed 40: 354-68 (1968) (9) Zahn R, Wellens H; Wasser Abwasser Forsch 13: 1-7 (1980) (10) King EF, Painter HA; Ring-test Program 1981-1982 Assessment Of Biodegradability Of Chemicals In Water By Manometric Respirometry Comm Eur Communities, Eur 8631 (1983) (11) Takemoto S et al; Suishitsu Odaku Kenkyu 4: 80-90 (1981)] **PEER REVIEWED**

AEROBIC: Using a Captina silt loam inoculum, the half-life for mineralization of benzoic acid in solution was 4.5 hr after a 30 min lag(1). Complete degradation occurred in 1 day with a Niagra silt loam inoculum(2). When benzoic acid was incubated in an acidic loam soil adjusted to 60% of its water holding capacity, 74 and 81% was mineralized in 1 and 12 wks(3). The same experiment using a neutral, sandy loam soil resulted in 55 and 71% mineralization in 1 and 12 wks(3). After 12 wks, 3.0 and 4.4% of the chemical was incorporated into the biomass of the two soils(3). In an alkaline para-brown soil, 40 and 63% mineralization occurred in 3 days and 10 wks, respectively(4). An experiment was performed in which C14-labeled benzoic acid was added to subsurface soil taken from the unsaturated zone beneath the tile of a septic tank and incubated both aerobically and anaerobically(5). Under aerobic conditions the half-life was 3.9 and 7.3 hrs for carboxyl and ring-labeled chemical, respectively(5). [(1) Dao TH, Lavy TL; Soil Sci 143: 66-72 (1987) (2) Alexander M, Lustigman BK; J Agric Food Chem 14: 410-3 (1966) (3) Kassim G et al; Soil Sci Soc Am J 46: 305-9 (1982) (4) Haider K et al; Arch Microbiol 96: 183-200 (1974) (5) Ward TE; Environ Toxicol Chem 4: 727-37 (1985)] **PEER REVIEWED**

AEROBIC: At concentrations of 15-18 mg/L, benzoic acid was biodegraded, with reported half-lives of 0.85 and 3.6 days using inoculums from a polluted river and a reservoir, respectively(4). Low concentrations of benzoic acid are rapidly mineralized in both eutrophic and oligotrophic lake water with the rate of disappearance being proportional to its concentration(1,2). At 59 ng/L, over 98% mineralization had occurred in 7 days in both eutrophic and oligotrophic water(1). The half-life for mineralization in eutrophic water was 0.22 days over a concentration range of 32 ng/L to 50 ug/L(2). From 63-83% of the benzoic acid was lost in 6 hr and >94% in 58 hr(2). Mineralization was not usually affected by montmorillonite or kaolinite in the water(3). [(1) Rubin HE et al; Appl Environ Microbiol 43: 1133-8 (1982) (2) Subba-Rao RV et al; Appl Environ Microbiol 43: 1139-50 (1982) (3) Subba-Rao RV, Alexander M; Appl Environ Microbiol 44: 659 (1982) (4) Banerjee S et al; Environ Sci Technol 18: 416-22 (1984)] **PEER REVIEWED**

ANAEROBIC: Following an 8 day acclimation period, microbial mixtures assimilated benzoic acid within 18 days; 91% of the benzoic acid sample was converted into methane gas and CO₂(1). An experiment was performed in which C14-labeled benzoic acid was added to subsurface soil taken from the unsaturated zone beneath the tile of a septic tank and incubated both aerobically and anaerobically(2). The ring-labeled benzoic acid had a mineralization half-life of 18.2 hrs when incubated anaerobically(2). [(1) Healy JB, Young LY; Appl Environ Microbiol 38: 84-89 (1979) (2) Ward TE; Environ Toxicol Chem 4: 727-37 (1985)] **PEER REVIEWED**

Environmental Abiotic Degradation:

The rate constant for the vapor-phase reaction of benzoic acid with photochemically-produced hydroxyl radicals has been estimated as 1.2×10^{-12} cu cm/molecule-sec at 25 deg C(SRC) using a structure estimation method(1). This corresponds to an atmospheric half-life of about 9 days at an atmospheric concentration of 5×10^5 hydroxyl radicals per cu cm(1). Benzoic acid is not expected to undergo hydrolysis in the environment due to the lack of functional groups that hydrolyze under

environmental conditions(2). Benzoic acid absorbs UV radiation up to approximately 310 nm(3,4), and, therefore, may be susceptible to direct photolysis by sunlight. In a photomineralization test in which the chemical is adsorbed on silica gel and irradiated with light >290 nm, 10.2% mineralization occurred in 17 hrs(5). When illuminated with a sunlamp for 24 hrs in solution containing zinc oxide, 67% degradation occurred in 24 hrs(6). However, it was stable when exposed to sunlight or a sunlamp for 137 hrs in aqueous solution(7). Zinc oxide, therefore, appears to possess catalytic activity as does beach sand(6). [(1) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 7-4, 7-5 (1990) (3) Sadtler; Sadtler Standard Spectra UV 252 Philadelphia PA: Sadtler Research Lab (4) NIST; NIST Chemistry WebBook. Benzoic acid (65-85-0). NIST Standard Reference Database No. 69, June 2005 Release. Washington, DC: US Sec Commerce. Available from, as of Aug 19, 2013: <http://webbook.nist.gov> (5) Freitag D et al; Chemosphere 14: 1589-616 (1985) (6) Kinney LC et al; Photolysis Mechanisms For Pollution Abatement TWRC-13 Robert A Taft Water Res Cent Rep (1969) (7) Ware GW et al; Arch Environ Contam Toxicol 9: 135-46 (1980)] **PEER REVIEWED**

Environmental Bioconcentration:

Measured BCF values of <10, 14, and 21 were reported for Golden ide (*Leuciscus idus melanotus*)(1), trout(2), and mosquito fish (*Gambusia affinis*)(3), respectively. According to a classification scheme(4), this BCF range suggests the potential for bioconcentration in aquatic organisms is low(SRC). Bioconcentration factors of <10(1), 100, 138, 1800, 2800(3) and 10(4) have been reported in algae (*Chorella fusca*)(1), algae (*Oedogonium cardiacum*), mosquito larvae (*Culex quinquefasciatus*), daphnia (*Daphnia magna*) and snail (*Physa*), respectively(3). [(1) Freitag D et al; Chemosphere 14: 1589-616 (1985) (2) Branson DR; pp.55-70 in Predicting the Fate of Chemicals in the Aquatic Environment from Laboratory Data. ASTM STP 657. Philadelphia. PA: Amer Soc Test Mat, pp. 55-70 (1978) (3) Lu PY, Metcalf RL; Environ Health Perspect 10: 269-84 (1975) (4) Franke C et al; Chemosphere 29: 1501-14 (1994)] **PEER REVIEWED**

Effluent Concentrations:

Benzoic acid concentration of 0.003 mg/L was found in primary domestic sewage plant effluent. [Verschuere, K. Handbook of Environmental Data on Organic Chemicals. Volumes 1-2. 4th ed. John Wiley & Sons. New York, NY. 2001, p. 288] **PEER REVIEWED**

In a comprehensive survey of wastewater from 4000 industrial and publicly owned treatment works (POTWs) sponsored by the Effluent Guidelines Division of the U.S. EPA, benzoic acid was identified in discharges of the following industrial category (frequency of occurrence; median concentration in ppb): timber products (15; 57.7), leather tanning (7; 89.6), iron and steel mfg (7; 33.4), petroleum refining (1; 503.3), nonferrous metals (19; 62.5), paint and ink (36; 162.1), printing and publishing (18; 228.9), ore mining (13; 32.6), organics and plastics (35; 669.9), inorganic chemicals (9; 56.6), textile mills (12; 46.9), plastics and synthetics (16; 36.2), pulp and paper (49; 133.3), rubber processing (6; 223.3), soaps and detergents (2; 148.3), auto and other laundries (13; 127.8), pesticides manufacture (7; 44.3), photographic industries (2; 69.7), pharmaceuticals (15; 121.6), explosives (4; 20.8), foundries (19; 61.4), porcelain/enameling (4; 176.5), electronics (19; 80.3), electroplating (1; 2.8), oil and gas extraction (24; 23.8), organic chemicals (16; 241.3), mechanical products (34; 104.2), transportation equipment (6; 163.5), synfuels (24; 96.3), publicly owned treatment works (84; 35.9), rum industry (1; 405.3)(1). The highest effluent concentration was 72,124 ppb in the pesticides mfg industry. The paint and ink, and organics and plastics industries also had maximum effluents exceeding 10,000 ppb(1). [(1) Shackelford WM et al; Analyt Chim Acta 146: 15-27 (1983)] **PEER REVIEWED**

Benzoic acid appeared in the process exhaust from a phthalic anhydride manufacturing plant without pollution abatement equipment at concentration ranging from 5-40 ppm (v/v)(1). Benzoic acid has been found in emissions from gasoline powered vehicles at concentrations of 124 ug/km(2). It has been reported in the exhaust gas from diesel powered vehicles(3) and the

concentration in the exhaust from a 1982 Toyota Corolla was 0.164 ppb(4). Extracts of 5 incinerator effluents contained 6-3500 ppm of benzoic acid(5). Benzoic acid was detected, not quantified in landfill leachate samples from Porto Alegre city, Rio Grande do Sul, Brazil; the landfill receives exclusively municipal solid wastes(6). The chemical was found in fly ash from a municipal waste incinerator in Ontario(7). Benzoic acid was found (34.8 ug/L) in landfill residue from Japan(8). Benzoic acid has been found in emissions from primers and finishing paints used to coat steel at concentrations ranging from 2 to 40 mg/cu m(9). [(1) Fawcett RL; J Am Pollut Control Assoc 20: 461-5 (1970) (9) Schauer JJ; Environ Sci Technol 36: 1169-80 (2002) (3) Hampton CV et al; Environ Sci Technol 16: 287-98 (1982) (4) Kawamura K et al; Environ Sci Technol 19: 1082-6 (1985) (5) James RH et al; in J Proc-APCA 77th Annual Meeting pp. 1-25 (1984) (6) Nascimento Filho I et al; Chemosphere 50: 657-663 (2003) (7) Tong HY et al; J Chrom 285: 423-41 (1984) (8) Yasuhara A; Kankyo Kagaku 3: 356-7 (1993) (9) Henrikis-Eckerman MJ et al; Am Ind Hyg Assoc J 51: 241-4 (1990)] **PEER REVIEWED**

Effluent from the Los Angeles County wastewater treatment plant contained 400 parts/billion of benzoic acid(1). Benzoic acid was detected, but not quantified, in the effluent of the publicly owned treatment works in Addison, IL that accepts waste from over 300 manufacturing and industrial firms, but not in 9 other treatment facilities sampled in the state(2). Leachate from a sanitary landfill contained benzoic acid but it was not quantified(3). Benzoic acid occurred at concentration levels of 1-50 parts/million in settling basins and other standing water at the Valley of the Drums waste site in Bullitt County, KY(4). It was a component of spent bleach liquor from a softwood kraft pulp plant(5) and averaged 54 parts/million in effluent from a dimethyl terphthalate plant near Wilmington, NC that was disposed of by deep well injection(6). [(1) Gossett RW et al; Mar Pollut Bull 14: 387-92 (1983) (2) Ellis DD et al; Arch Environ Contam Toxicol 11: 373-82 (1982) (3) Leenheer JA et al; Environ Sci Technol 10: 445-51 (1976) (4) Stonebraker RD, Smith AJ Jr; pp.1-10 in Control Hazard Mater Spills, Proc Natl Conf Nashville, TN (1980) (5) Lindstrom K, Osterberg F; Environ Sci Technol 20: 133-8 (1986) (6) Albaiges J et al; Water Res 20: 1153-9 (1986)] **PEER REVIEWED**

Benzoic acid was detected but not quantified in groundwater underlying a Florida landfill sampled in 1991(1), in one of 3 samples by a Swedish landfill in 1990(2) and in the residue from Japanese municipal landfill waste after distillation(3). Benzoic acid was also determined to leach out of tire plugs when placed in lakes or canals. It has been reported that <1.0 ug/L of benzoic acid was released when 29 tire plugs, each 10.25 cm in diameter, were placed in 16 liters of water from Lake Mead, Nevada(4). [(1) Eckel WP et al; Groundwater 31: 801-804 (1993) (2) Oman C Hynning PA; Environ Poll 80: 265-271 (1993) (3) Yashura A et al; Kenkyo Kagaku 3: 356-7 (1993) (4) Nelson et al; Bull Environ Contam Toxicol 52: 574-81 (1994)] **PEER REVIEWED**

Atmospheric Concentrations:

URBAN/SUBURBAN: Los Angeles 1-26 parts per trillion, 10 parts per trillion mean(1). An unspecified sample of urban air contained benzoic acid in both the gas and aerosol phases(2). It was contained in the aerosol fraction of air obtained in a suburban area of Japan 60 km NE of Tokyo(3). However, it was not detected in the Allegheny Mountain Tunnel (Pennsylvania Turnpike, southwestern PA), a tunnel that received considerable traffic(4). [(1) Kawamura K et al; Environ Sci Technol 19: 1082-6 (1985) (2) Cautreels W, VanCauwenberghe K; Atmos Environ 12: 1133-41 (1978) (3) Yokouchi Y, Ambe Y; Atmos Environ 20: 1727-35 (1986) (4) Hampton CV et al; Environ Sci Technol 16: 287-98 (1982)] **PEER REVIEWED**

Benzoic acid has been detected but not quantified in mollusks and crustaceans(1). [(1) Livingstone DR; Persistent Pollution of Marine Ecosystems. New York, NY; Pergamon pp. 3-34 (1992)] **PEER REVIEWED**

HSDB, 2014

From data provided by the German producers, emissions of benzoic acid from industrial processes were less than 525 kg per year into the atmosphere, less than 3 tonnes per year into the River

Rhine, and 8 tonnes per year into sewage or water purification plants (BUA, 1995). No data were available from other countries.

Other anthropogenic releases of benzoic acid and sodium benzoate into the environment are emissions into water and soil from their uses as preservatives in food, toothpastes, mouthwashes, dentifrices, and cosmetics. There were no data available on the emission of benzoic acid from the disposal of antifreeze mixtures and waterborne cooling systems and other miscellaneous industrial uses.

The amount of benzoic acid emitted to air from car exhaust gases as an oxidation product is not quantifiable from the available data.

From its use pattern (see section 4), it can be expected that benzoic acid is released to surface waters and (from dumping sites) to leaching water (and groundwater). Minor amounts are expected to be emitted to the atmosphere. From its physicochemical properties (vapour pressure, Henry's law constant; see section 2), a significant volatilization of benzoic acid from water or soil is not expected. Owing to its solubility in water (see section 2), wet deposition from air may occur. Experimental data on wet and dry deposition from air are not available.

INCHEM, 2000, CONCISE INTERNATIONAL CHEMICAL ASSESSMENT DOCUMENT NO. 26, BENZOIC ACID AND SODIUM BENZOATE.

"An approximate estimation of the bioconcentration factor BCF_{fish} on basis of log K_{ow} = 1.87 was conducted using the standard equation (74) given in the EU Technical Guidance Document (TGD) on Risk Assessment (2003), Part II, 3.8.3.2:

$$\text{Log BCF}_{\text{fish}} = 0.85 \log K_{\text{ow}} - 0.70$$

$$\text{Log BCF}_{\text{fish}} = 0.85 (1.87) - 0.70$$

$$\text{Log BCF}_{\text{fish}} = 0.8895$$

$$\text{BCF}_{\text{fish}} = 7.754 \text{ (on wet weight basis)}$$

The result of the calculation suggests that benzoic acid would not bioaccumulate in aquatic organisms"

As taken from ECHA, 2012a.

The Ecological Categorization Results from the Canadian Domestic Substances List state that benzoic acid is not bioaccumulative in the environment:

Empirical Log Kow	1.87
Log Kow predicted by KowWin	1.87
Log BAF T2MTL predicted by Gobas	0.749975478194873
Log BCF 5% T2LTL predicted by Gobas	0.674796832998173
Log BCF Max predicted by OASIS	1.62330062416177
Log BCF predicted by BCFWIN	0.5

Data accessed January 2017 on the OECD website:
<https://hpvchemicals.oecd.org/UI/SponsoredChemicals.aspx>

EPISuite provides the following data:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-0.9745 days (HL = 0.106 days)
Log BCF Arnot-Gobas method (upper trophic):	0.814 (BCF = 6.514)
Log BAF Arnot-Gobas method (upper trophic):	0.814 (BAF = 6.514)
log Kow used:	1.87 (expkow database)

“Bioaccumulation and Secondary Poisoning

Based on the physicochemical properties an approximate estimation of the bioconcentration factors (BCFs) can be calculated according to TGD (EC 2003). Applying the experimentally derived log KOW of 1.87, results in a BCFFish of 7.75 L/kg ww and a BCFEarthworm of 1.73 L/kg ww. Hence, the aquatic and terrestrial bioaccumulation potential of benzoic acid can be assumed as low.

In consequence of the log KOW <3 and the low estimated BCF values, experimental studies are not required. Furthermore, no other indicators point to an intrinsic potential for bioconcentration.

With regard to the low estimated BCF values in aquatic and terrestrial indicator species, benzoic acid is not expected to accumulate in the environment. The risk of secondary poisoning is therefore assumed to be negligible via ingestion of contaminated food by birds or mammals.”

As taken from European Commission, 2013a, b.

The BCF of benzoic acid in golden ide and algae (*Chlorella fusca*) was <10 as determined in a 3- and 1-day static test, respectively (Freitag 1985).

The BCF for trout muscle calculated by regression analysis from its octanol/water partition coefficient is 14 (Branson 1978).

The BCF of mosquito fish, algae and mosquito larvae after 1 day in an aquatic ecosystem is relatively low (21, 100 and 138 respectively), the BCF in daphnia and snail is high, 1800 and 2800, respectively. However the investigators measured total radioactive, not benzoic acid, so a metabolite may have been accumulated (Lu & Metcalf 1975).

As taken from the Finnish Environment Institute (b).

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13. Last audited

November 2024

BLUE

Benzyl Alcohol, Benzoic Acid,
and its Salts and Ester

CIR EXPERT PANEL MEETING
SEPTEMBER 26-27, 2011

Cosmetic Ingredient Review

Commitment . . . Credibility
Since 1976



September 1, 2011

Memorandum

To: CIR Expert Panel

From: Wilbur Johnson, Jr.
Manager/Lead Specialist

Subject: Draft Amended Final Report on Benzyl Alcohol,
Benzoic Acid, and its Salts and Benzyl Benzoate

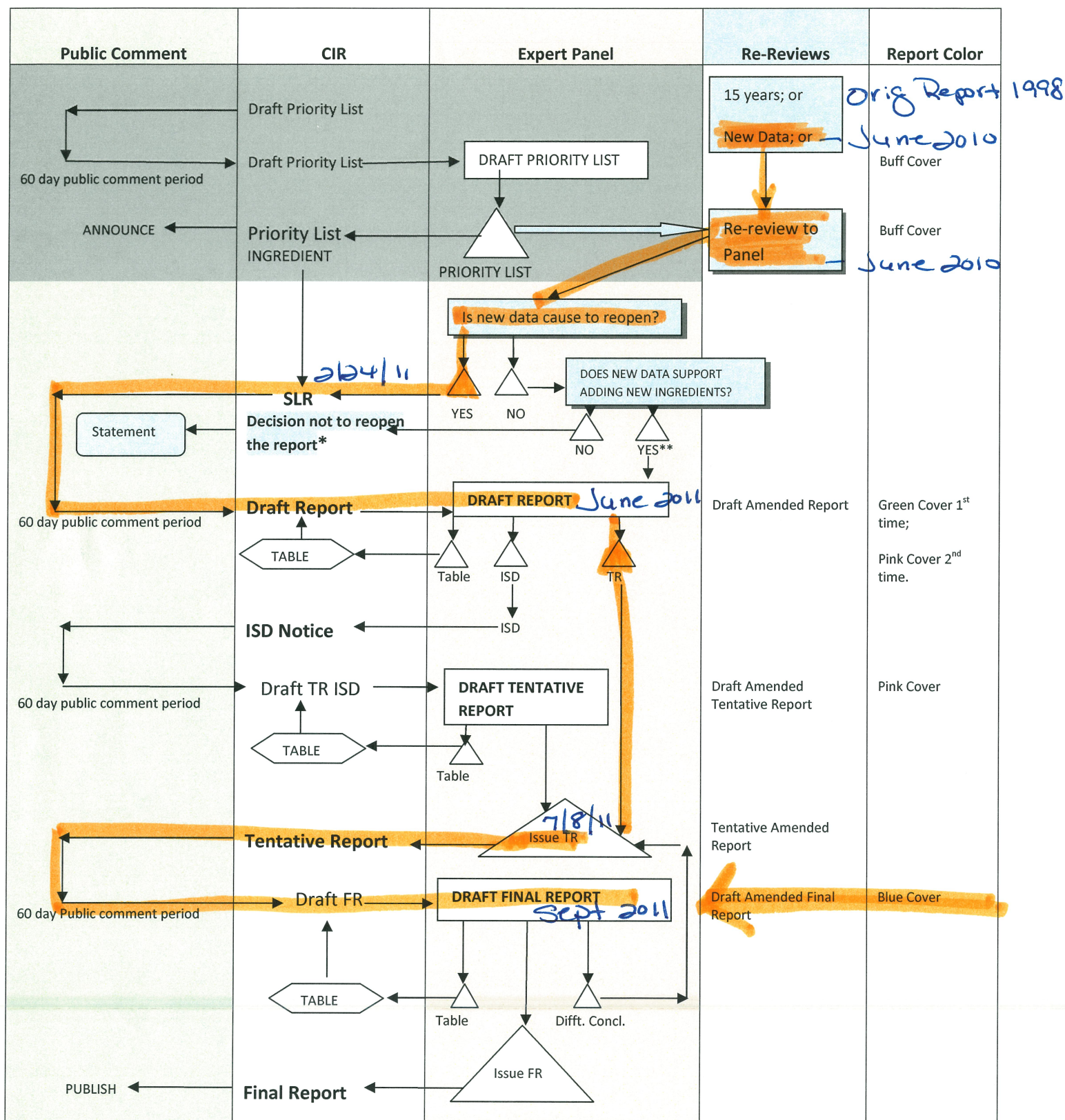
At the June 27-28, 2011 Expert Panel meeting, the Panel issued a tentative amended final report with a conclusion stating that benzyl alcohol, benzoic acid and its salts, and benzyl benzoate are safe in the present practices of use and concentration. Comments were received during the 60-day comment period following announcement of this report, and the report was revised to include pertinent comments.

A copy of the draft amended final report on these ingredients is included along with the following: CIR report history, Minutes from the June 2011 Panel meeting, Literature search strategy, Data profile, and the Council's technical comments on the tentative amended final report (pdf file).

We are seeking Panel input on the suggestion that the abstract should mention dermal irritation and sensitization data, e.g., current use concentrations of these ingredients are below concentrations that may result in dermal irritation or sensitization. We have been keeping abstracts with a safe as used conclusion to a bare minimum, so this would be a departure.

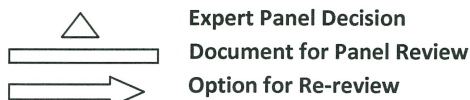
Also, the Council has suggested that the discussion should note that, as stated in the Toxicokinetics section, these ingredients are metabolized and excreted via a common pathway. Therefore, the data on Benzyl Alcohol are directly relevant to Benzoic Acid and its salts. It should also be noted that additional data are summarized in the original report.

If the Panel agrees, these would be editorial changes. After reviewing the draft amended final report to make certain that the discussion reflects the Panel's rationale, the Panel needs to issue an amended final report at this meeting.



*The CIR Staff notifies of the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

****If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.**



CIR History of:

Benzyl Alcohol, Benzoic Acid, and its Salts and Benzyl Benzoate

In 1998, the CIR Expert Panel issued a final safety assessment of Benzyl Alcohol, Benzoic Acid and Sodium Benzoate, concluding that they are safe for use in cosmetics up to 5%, but that the data are insufficient to support the safety of these ingredients in which the primary route of exposure is inhalation. The report was published in 2001.

In June, 2010, the Expert panel reviewed new data provided by the Council on the safety of benzyl alcohol and benzoic acid for inhalation use. The Panel decided to reopen its review of this report to consider the new data.

New Data Review, Belsito and Marks Teams/Panel: June 28-29, 2010

The Council provided data in response to the insufficient data determination on the inhalation use of benzyl alcohol, benzoic acid and sodium benzoate. The Personal Care Products Council provided a 4-week inhalation toxicity study (rats) of aerosolized benzyl alcohol and benzoic acid. After reviewing this study, the Expert Panel determined that the final report on the safety assessment of benzyl alcohol, benzoic acid, and sodium benzoate should be reopened to amend the original conclusion. Furthermore, the Panel determined that data available in the original safety assessment, with the addition of the new inhalation toxicity data, were likely sufficient to support the safety of other simple salts of benzoic acid (calcium, magnesium, and potassium benzoate) and the benzyl benzoate ester. Thus, it was agreed that the safety of these ingredients and those reviewed in the original final report would be reviewed in the SLR that will be developed.

SLR in February, 2011

The availability of a scientific literature review (SLR) on this group of ingredients was announced on February 24, 2011. Technical comments and use concentration data from the Council were received during the 60-day comment period.

Draft Report, Belsito and Marks Teams/Panel: June 27-28, 2011

Technical comments on the SLR and use concentration data, received from the Council during the 60-day comment period have been added to the draft report. The draft report also contains an inhalation toxicity study on benzyl alcohol and benzoic acid and studies from a RIFM synopsis of data on benzyl benzoate that were received in 2010.

The Expert Panel concluded that the newly available inhalation toxicity data and the safety test data already available support the safety of benzyl alcohol, benzoic acid, calcium benzoate, magnesium benzoate, potassium benzoate, sodium benzoate, and benzyl benzoate, and agreed that a tentative amended safety assessment with the conclusion that these 7 ingredients are safe in the present practices of use and concentration should be issued.

The Expert Panel discussed the finding of adenomas of the adrenal cortex of B6C3F1 mice receiving a high dose of benzyl alcohol in an oral carcinogenicity study. Such tumors were considered benign and benzyl alcohol was not considered carcinogenic. In earlier safety assessments, a 5% concentration limit for benzyl alcohol, benzoic acid, and sodium benzoate and a 10% concentration limit for benzyl alcohol in hair dyes were established. Currently available information demonstrates that the present practices of use and concentration of these ingredients are below those levels and that the limits are no longer needed. The Expert Panel did note the presence of benzene as an impurity due to photo-degradation of benzoic acid, but at current use concentrations, the levels of benzene in cosmetic products would be negligible.

Draft Amended Final Report, Belsito and Marks Teams/Panel: September 26-27, 2011

Technical comments on the tentative amended final report, from the Personal Care Products Council, were received during the 60-day comment period. The document was revised to include pertinent comments.

Literature Search on Benzyl Alcohol and Related Ingredients*

Ingre- dients	Toxline &PubMed	ChemIDplus	Multidatabase (See legend*)	DART	Household Products	Beilstein	Registry	Kosmet	Napralert	RTECS	CAplus
BZA	1098 (1997 to 2011)	1	5	44 (1997 to 2011)	1	1	1	5	341	674	1534
BA	1610 (1997 to 2011)	1	6	293 (1997 to 2011)	1	4	1	19	1263	3830	3648
SB	226 1997 to 2011)	1	4	21	1	2	1	0	5	13	643
CB	6	1	0	0	0	1	1	0	0	1	26
MB	0	1	0	0	0	1	1	0	0	1	12
PB	23	1	1	0	0	1	1	0	1	0	58
BB	331	1	1	11 (1997 to 2011)	1	1	1	1	117	31	227

*Data in Table: Total no. publications in search; Multidatabase = HSDB, CCRIS, ITER, IRIS, Gene-Tox, and LacMed;

Searches Performed on 7/9 and 12/2010, and 3/2011 (1 chemical name and 1 CAS No.; Toxline added additional chemical names to search term); Any limitations on years searched indicated. Website list also searched. Searches updated on 8/3/2011 using PubMed and Toxline.

Ingredients

(BZA) Benzyl Alcohol OR 100-51-6

(BA) Benzoic Acid OR 65-85-0

(SB) Sodium Benzoate OR 532-32-1

(CB) Calcium Benzoate OR 2090-05-3

(MB) Magnesium Benzoate OR 553-70-8

(PB) Potassium Benzoate OR 582-25-2

(BB) Benzyl Benzoate OR 120-51-4

Benzyl Alcohol OR 100-51-6 OR Benzoic Acid OR 65-85-0 OR Sodium Benzoate OR 532-32-1 OR Calcium Benzoate OR 2090-05-3 OR Magnesium Benzoate OR 553-70-8 OR Potassium Benzoate OR 582-25-2 OR Benzyl Benzoate OR 120-51-4

Benzyl Alcohol Group Check List for September, 2011. Writer – Wilbur Johnson

			Acute toxicity					Repeated dose toxicity				Irritation			Sensitization					
			ADME	Oral	Parenteral	Dermal	Inhale	Oral	Parenteral	Dermal	Inhale	Ocular Irritation	Dermal Irr. Animal	Dermal Irr. Human	Sensitization Animal	Sensitization Human	Repro/Devel toxicity	Genotoxicity	Carcinogenicity	Phototoxicity
Benzyl alcohol	X		X	X		X	X	X			X	X	X	X	X	X	X	X	X	
Benzoic acid	X		X	X		X	X	X			X	X	X	X	X	X	X	X	X	
Sodium benzoate			X	X		X		X				X	X		X	X	X	X	X	
Calcium benzoate																				
Magnesium benzoate																				
Potassium benzoate			X	X		X		X				X					X	X		
Benzyl benzoate	X		X	X	X	X		X	X	X		X	X	X	X	X	X	X		X

Day of the June 27-28, 2011 CIR Expert Panel Meeting -- Dr. Belsito's Team

Benzyl Alcohol, Benzoic Acid, and its Salts and Ester

Dr. Belsito: Benzoic acid. So we agreed to reopen the report because we had data on inhalation. And then we included in addition to the original three, calcium, benzoic, magnesium, potassium, and sodium benzoate to the list. My comment here was that there seem to be new data or data that I didn't see before about if you package it in such a way that there is UV exposure to the product that then benzene is formulated or is formed in the product itself. And so the question I have is it's okay, but do we need to specify UV packaging for the products or is the concentration of these ingredients and the potential -- therefore potential formation of benzene so low that it really doesn't matter? Regardless of how we approach this I think we need to say something in the discussion about that since it's part of the document.

DR. LIEBLER: I agree. I just don't know -- I don't have a suggestion on how to do that. I guess you put in the discussion?

DR. BERGFELD: That's a good place.

DR. LIEBLER: That's what I would suggest.

DR. BELSITO: Then how do

DR. BERGFELD: There is no discussion here, so you'd have to add that.

DR. LIEBLER: Right.

DR. BELSITO: Right.

DR. LIEBLER: In other words, as the discussion is formulated that can be referred to there. I'm looking for the -- this is under the chemistry section. Yeah, this would be Panel page 8, 9, and 10.

SPEAKER: (inaudible) and benzoic acid.

DR. LIEBLER: Yeah, I mean, the benzene production from benzoic acid in the presence of a hydroxyl radical generating system has been demonstrated. True and unrelated, in my opinion.

I mean, I don't think that's relevant to the circumstances that these compounds would be in. And you're basically adding, you know, a metal catalyzed hydroxyl radical generating system. Of course you can get some detectable benzene out of that, but that's not going to be the circumstance that you'd be dealing with in a product formulation that contains benzoic acid.

DR. BELSITO: So you think that there is no potential for a -- that a cosmetic product would be formulated such that this would be an issue?

DR. LIEBLER: Not based on that reference. Not based on that scenario. The other scenario is the issue of UV stabilized and non-UV stabilized packaging. This is a more realistic scenario, I think, where you do have the ingredient, it may be exposed to light. And then the question is benzene can be detected, does that pose a hazard or a risk? And I would say probably not based on the levels that would be produced; they're very low. That could be -- this study, which is the top of page -- I've got the Panel Book -- 10, top of Panel Book 10 -- top of page 10 in the Panel Book, that could be referenced in the discussion.

DR. SNYDER: Well, under Impurities we did -- it's mentioned that it's always less than one part per billion, so.

DR. LIEBLER: Right.

DR. BELSITO: Okay. So, again, but I think it bears --

DR. LIEBLER: Just mentioning, yeah.

DR. BELSITO: -- mentioning, okay.

DR. LIEBLER: Not really a safety issue.

MR. JOHNSON: Excuse me, Dr. Liebler, specifically what do you want mentioned regarding that first paragraph.

DR. BELSITO: Just that photodegradation of benzoic acid has been reported to result under whatever circumstances in the release of benzene. However, as used in cosmetic products this would be highly unlikely. In addition, the levels of benzoic acid that are being used in cosmetic products are such that the amount of benzene that could potentially be liberated is negligible. Is that pretty much what you're saying?

DR. LIEBLER: Yeah, basically, right.

DR. BELSITO: But just there needs to be something in the discussion to note -- that indicates that we noted this issue of potential photodegradation, we dealt with it, and we felt that it was a non-issue for cosmetics. DR.

LIEBLER: Wilbur, it's the stuff that's referred to under Reference 18. And on the top of page 10 it refers to, you know, UV -- intense UV light increasing benzene levels by as much as 53 percent in model solutions. Now that

sounds big, but actually 53 percent increase in a very small number is still a very small number. So, you know, there's not enough information here to, I think, raise any real concern. And down at the bottom of the paragraph you do say about another survey that the levels were (inaudible) formulated beverage tested were shown to contain less than 1. nanogram per gram. That's very low. If you do cite the Reference 18 where the model system is used and it showed benzene levels going up by 53 percent it'd be good to just mention what the absolute amount was in that study, so that we could see that that -- it was indeed a low number.

DR. BELSITO: Any other issues on the generation of benzene? Does the Cosmetic Ingredient Dictionary really state that these ingredients may function as pesticides in cosmetics?

DR. EISENMANN: Probably (inaudible).

DR. BELSITO: Okay. I mean, I just -- I've never seen that.

DR. EISENMANN: It's an international --

DR. SNYDER: (inaudible) microbial.

DR. BELSITO: What?

DR. EISENMANN: It's an international dictionary, so sometimes things get in from other jurisdictions that seem strange to us.

DR. BELSITO: Okay. And then this is, I guess, a generic question in a sense. The Personal Care Product Council may have provided us with a copy of the inhalation toxicity data, but that was actually performed by RIFM. So in your first -- or third paragraph you say, "In response to the need for inhalation toxicity data the Personal Care Product Council provided a four-week inhalation toxicity study."

DR. EISENMANN: Actually it was joint study from -- we helped pay for it, but RIFM monitored the study and placed it.

DR. BELSITO: Okay, fine.

DR. EISENMANN: But it was a joint study.

DR. BELSITO: Okay. I didn't realize that. And then you go on in that sentence, "and the results of the study were discussed at this panel meeting." I don't think that needs to be part of the report. There's a lot of editorial stuff here that I did, but then in that report there was a large number of references to studies -- patch test studies, provocative use, RIPT studies -- that were referenced by the people who did them. And in your summary, Wilbur, you referenced the report that Carol or PCPC provided you. I don't think that's the appropriate way to reference all of those patch test studies. It's a secondary reference. Quite honestly, I think you need to go back and reference the actual studies that were referenced in that report of which there are a large number. Because it made it very difficult for me when I was reviewing Table 5, I know you like to quote the -- what they said, but if you go to page 25 of the report, CIR Panel Book page 32, just a couple of issues. One, the benzyl benzoate in a maximization test was not a provocative test treatment. That was -- that actually is under the wrong label. That's a maximization test as a predictive test, so it needs to be moved under Predictive Tests. But throughout all the provocative test treatments you keep saying, "sensitization rate of." As a dermatologist who does this what that means to me is that in the course of patch testing these people you sensitized 3 percent of them, and that's not what these studies are reporting. They're reporting positive reactions in 3 percent. So I stopped changing it, but under all of the provocative test treatments instead of using the word "sensitization" it gets boring, but "3 percent of patients tested positive" or "had responses that were determined to be allergic" or however you want to phrase it. But when you use the term "sensitization" it typically means you have induced an allergy that didn't exist before, and that's not the case with a provocative test. Okay. And again, you use a lot of the Reference 18 or whatever that RIFM study was rather than the actual true references, so you're going to have to go in and expand, I think, that reference list.

MR. JOHNSON: Dr. Belsito, I know in some cases the primary references are unpublished data. So in the event that it's not, you know, a typical (inaudible) --

DR. BELSITO: Where it's unpublished, you know, for instance, I mean, RIFM has a lot of HRIPT data that has not been published and it would be appropriate to put it under 58. But when I started checking some of the references actually they were studies that had been done in Japan, they were studies that had been done in -- by Walt Larson and other people that were referenced to the conjoint RIFM-PCPC report when actually they were studies that had been done that were included in that report. I mean, if you go back to the report, which is in the back of a page here someplace you'll see what I'm talking about.

DR. EISENMANN: Dr. Belsito, to clarify,

I mistook the synopsis of data of benzyl benzoate. That is RIFM. The inhalation study was joint.

DR. BELSITO: Okay.

DR. EISENMANN: Okay.

DR. BELSITO: Okay. So then the synopsis on benzyl benzoate, I mean, there's -- I don't know where it is in the data here, I think it was towards the end of the book, but you'll see that there are -- it's Cosmetic Ingredient Review. Okay. Yeah, so under the benzyl benzoate that was from RIFM?

DR. EISENMANN: Yes, it was (inaudible).

DR. BELSITO: Okay. So you'll see that there are a whole slew of references that just seem to -- you just reference the RIFM report 2 rather than referencing the primary authors of 2 those studies.

MR. JOHNSON: That's just for Table 5?

DR. BELSITO: Yeah, Table 5 for the dermal irritation. So basically the RIFM Reference 58, what you need to do, Wilbur, is everything that you referenced as 58, which has got Research Institute for Fragrance Material, you need to go back and actually find what reference they used to quote that rather than the 58.

MR. JOHNSON: Sure, okay.

DR. BELSITO: Paul, you had some comments?

DR. SNYDER: A few editorial ones, but the one was on page 6 under benzyl alcohol and benzyl benzoate, octanol-water partition coefficient.

1 MR. JOHNSON: Which page?

DR. SNYDER: Page 6 of the report, Panel Book page 13, under benzyl alcohol, benzyl benzoate, you've got an octanol-water partition coefficient there of 9,333. The last sentence of 2 that first paragraph, the 7.4 and 9,333.

DR. LIEBLER: Take the log.

DR. BELSITO: And then, Wilbur, on page 1 --

MR. JOHNSON: Panel Book 11.

DR. BELSITO: Panel Book 18, page 11, under the synopsis of irritation and sensitization.

MR. JOHNSON: Yes.

DR. BELSITO: You say fourth line down, "was classified as a non-sensitizer at 5 percent and a weak sensitizer at percent." I think you mean 10 percent.

MR. JOHNSON: Okay.

DR. BELSITO: And then the next header under Benzyl Alcohol Benzoic Acid, the third line towards the end it says, "In the same report benzoic did not induce." Is that benzoic acid or benzoic alcohol?

MR. JOHNSON: Are you in the same paragraph?

DR. BELSITO: Under -- no, no, no, under -- no. Your first paragraph below the italicized Benzyl Alcohol Benzoic Acid, that one.

MR. JOHNSON: Yes, uh-huh.

DR. BELSITO: Okay. Third line.

MR. JOHNSON: Okay.

DR. BELSITO: Go to the end, fourth word in from the end, "benzoic." Acid or alcohol? You see what I'm talking about?

MR. JOHNSON: Okay, yeah, "In the same report benzoic," okay.

DR. BELSITO: Right.

MR. JOHNSON: I'll check that.

DR. BELSITO: Paul, I'm sorry, I cut you off. You had some other comments?

DR. SNYDER: No. He'll pick them up in the report.

DR. LIEBLER: One comment from me, the title. Why not just replace "ester" with "benzyl benzoate" since there's only one ester we're considering?

DR. BRONAUGH: I had a comment, Paul, that octanol-water partition coefficient of 9,000 and something, that's what we calculated. It's not the log. That's the actual octanol-water partition coefficient. The log would be like 10 to the 3 or something like that.

DR. SNYDER: Okay. It just kind of struck me (inaudible).

DR. BRONAUGH: Yeah. We did that a long time ago and we just didn't -- it just seemed like the way to go then, but now we do the logarithm.

DR. SNYDER: Okay. Thanks for clarifying that.

MR. JOHNSON: So I probably should indicate that that isn't the logarithm, but it's the actual --

DR. BRONAUGH: It's the actual partition coefficient.

MR. JOHNSON: Partition coefficient, right.

DR. LIEBLER: Could take the log of it and just report that if people are more familiar with seeing that --

SPEAKER: That's what we typically report.

DR. LIEBLER: -- I would do that.

DR. BRONAUGH: Yeah, that would be good.

DR. KLAASSEN: Bob did that before logs were invented.

(Laughter)

DR. BELSITO: Ouch. Table 5 again, Wilbur, Panel Book 30, page 23 of the report, benzyl benzoate. So the third time you talk about benzyl benzoate going down that column sort of towards the end of the middle, 40 percent? Your conclusion was non-sensitizer at 5 and 10 percent benzyl benzoate, mild sensitizer at 40 percent, 4 of 10 animals. Do you see what I'm talking about?

1 MR. JOHNSON: I'm trying to find it now. You're on page 33?

DR. BELSITO: No, page 23 of the report, page 30 of the Panel Book.

MR. JOHNSON: Okay.

DR. BELSITO: Okay. Scan down the list and when you get to benzyl benzoate it's the third mention of benzyl benzoate.

MR. JOHNSON: Okay.

DR. BELSITO: Okay. And your conclusion there was non-sensitizer at 5 and 10 percent, mild sensitizer at 40 percent. Do you see what I'm talking about?

MR. JOHNSON: Yes, mm-hmm.

DR. BELSITO: Okay. But the induction

was only done at 10 percent, so the sensitization would have been induced at 10 percent, but wasn't elicited unless you put 40 percent on the skin. So I'm not sure that that statement is correct unless the synopsis of what you said is not correct. So if they did induction at 10 and 40 percent, and then they were able to elicit only in the group that was induced at 40 percent, that is what you're saying in your summary. But under your test methods the induction was 10 percent, so it was inducing some -- DR. SNYDER: I think he's referring to a sensitization rate of 40 percent, 4 of 10 animals, afterwards.

DR. BELSITO: No. Mild sensitization at -- see, if you read it, the challenge concentrations were done up to 40 percent. So they sensitized with 10 percent and then they did various concentrations to try and elicit the sensitization. But if they elicited it at 40 percent, then the animals were sensitized. Some animals had low levels of sensitivity at 10 percent, I would think. I don't know. Next time you come around to doing that I'd like to see that study. Again, it's a 58 study and I got tired of trying to go through the RIFM report to figure out whether it was a RIFM study or whether it was published in the literature, but if we could try and get the details of that study. Because I think the next thing that we need to grapple with in this report is, I think, we probably -- I don't know, we haven't said that yet -- but we probably will remove the "insufficient for aerosol use," but we previously had put levels, restricted levels, on these compounds and leave-ons and wash-offs and are those levels still valid. So we gave a 5.2 percent limit for cosmetic formulations for all 2 ingredients and a 10 percent limit for benzyl alcohol in hair dyes. And that was based upon sensitization and irritation data that I think still holds, but.

DR. BERGFELD: I'm confused. So your conclusion is moving it to safe, taking the restricted concentrations off?

DR. BELSITO: No, I don't want to take the restricted concentrations off, but I want to make sure that, you know, we have everything -- you know, there is data that suggests, I think it's in the guinea pig, that 10 percent, at least in some animals, can sensitize and 5 percent was fine. So this is a little misleading in the sense that it would suggest in this study for benzyl benzoate -- I mean, there's only 10 animals -- that 10 percent was fine and 40 percent wasn't. Just trying to get all the facts straight, so.

DR. SNYDER: So essentially 10 percent for that one and 5 percent for all the rest.

DR. BELSITO: Yeah, I've got to go back 2 and look at how it's used. I don't think that -- 2 I mean, if -- we could also go "safe as used," depending upon what the concentrations are. Okay. So benzoic acid we have leave-ons to 1, wash-offs to 5. For benzyl alcohol we have leave-ons to 3, wash-offs to 10. For sodium benzoate it's 1 percent. For calcium benzoate it's negligible. For potassium benzoate -- for benzyl benzoate we have leave-ons to 4; we have wash-offs to 2. And for the others they're either negligible or not reported.

So quite honestly, "as used" is below our prior restrictions, so we could either keep the restrictions or go "safe as used," since none of the reported uses are exceeding the restrictions we had previously set for this group.

DR. LIEBLER: Safe as used.

DR. BELSITO: I mean, that would actually imply a lower restriction than we had given before. Curt? Paul?

DR. BERGFELD: Politically it looks better.

DR. BELSITO: To go safe as used?

DR. SNYDER: I agree.

DR. BELSITO: Okay. So then the document needs to be cleaned up a little bit and we're going to go as "safe as used," get rid of the prior use concentrations, and the discussion obviously probably needs to focus on why we now think the respiratory use is okay based upon the study that was provided to us.

DR. BERGFELD: Anything about the benzoate?

DR. BELSITO: Yes, we've already discussed that.

DR. BERGFELD: Okay.

DR. BELSITO: That needs to go in the discussion, the photodegradation and why we felt that was a non-issue. And maybe, Dan, you could craft some kind of language between now and tomorrow morning?

DR. LIEBLER: Okay.

DR. BELSITO: In terms of not the right circumstances in a cosmetic and even if it were, it'd be negligible.

DR. LIEBLER: Right. I can.

MR. JOHNSON: Dr. Belsito, given the panel's published conclusion with limitations, should there be any explanation as to why those limitations are no longer valid?

DR. BELSITO: Sure. I mean, in a brief sentence the current use is below the level of restriction that we had previously given.

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DR. MARKS: Mm-hmm. Okay. Next is Green Book, benzoic acid, alcohol, salts, and esters. And these are in 88. Let me take a look at the notes in here. So June of last year -- so about a year ago -- the Panel reopened the safety assessment to expand the group. Those are listed, and I'll refer to page 37 with the chemical structures. So it's the alcohol, the acid, the benzoate, sodium benzoate, and then there are four more. We have a new document, and there was concern in the past about inhalation. Has that been resolved at this point? I'll ask you, Ron.

DR. HILL: Yes.

DR. MARKS: And is it okay to expand to those four add-ons? So inhalation okay, Ron?

DR. HILL: It seems to be. The study was done at up to gram per cubic meter inhalation, and that was okay. It's used at 4 percent, and I don't know how to convert that to consumer using any of the ingredients. But it has to be in the close enough area, so I don't think inhalation is an issue. There was one other issue. EPA considered benzyl alcohol as equivocal. Well, they looked at adrenal cortex adenomas. I think this was in mice, and I think those are benign too. But the EPA said this was equivocal evidence of carcinogenic activity, and NTP said no. I would go with the NTP interpretation that these are not in evidence for carcinogenic activity. That should be in the discussion. DR. MARKS: So the discussion will be the inhalation that is okay now.

DR. HILL: Yes.

DR. MARKS: Inhalation is okay. Obviously we need to address that since that was insufficient in a previous conclusion. NTP carcinogenicity addressed --

DR. HILL: Well, there was an NTP study, and the high-dose mice had adenomas of the adrenal cortex, which are benign tumors, are not cancers. EPA used that evidence as equivocal evidence for carcinogenicity and NTP said it is not.

DR. MARKS: Okay. So we addressed that in the discussion.

DR. HILL: Just in the discussion.

DR. MARKS: Adenoma carcinoma. Okay.

DR. SLAGA: No. Not carcinoma, adenoma.

DR. MARKS: No. But the issue of course of carcinogenicity.

DR. SLAGA: No. Okay.

DR. MARKS: So I would say consider safe as used. And then the other then when you look at the original conclusion on these three, lead the alcohol, the acid, and the benzoate, sodium benzoate.

DR. HILL: Mm-hmm.

DR. MARKS: There were limits of concentration, and I wonder if this is similar to what you talked about, Tom, historically for the 5 percent and in the benzyl alcohol and hair dyes up to 10 percent. And when you look at there's this combination of contact urticaria and sensitization. When I look at the present uses of concentration in products, it meets those. It's 5 to 2 percent, 10 percent. So I would be okay with just saying safe as used, and in discussion just refer back to that one.

DR. SLAGA: Right. I agree.

DR. MARKS: Okay. So the third portion of the discussion that should deal with this, the sensitization, contact urticaria, and the 5 and 10 percent limit. Any other things about the discussion?

DR. SHANK: Not for me.

DR. HILL: I have a couple questions for the toxicologist. We don't have any inhalation toxicology on benzyl benzoate, at least per the table. Does that concern anybody? It doesn't really concern me. I just thought I'd raise it

for people to think about. And also, there are some repro toxicity findings for benzyl benzoate, although it was unclear because we -- both places it's written it describes dermal doses up to 1 percent in diet. I'm not sure what to make of that.

DR. MARKS: What page?

DR. HILL: Well, if you look on PanelBook page 20, it appears -- yeah, that's the summary section.

DR. MARKS: I didn't look that closely.

On benzyl benzoate none of those are addressed in the RIFM paper because that's a large document. MR.

JOHNSON: That was a mistake. That should have been daily doses greater than 0.5 grams per kilogram per day.

DR. MARKS: All right.

MR. JOHNSON: Instead of up to 1 percent.

DR. HILL: Okay.

MR. JOHNSON: That's what that --

DR. HILL: Also there are these findings that a whopping huge dose compared to art of use that's -- because of that I wasn't able to make that correlation. Benzyl benzoate up to 4 percent possible dermal contact, according to the use survey.

DR. MARKS: Okay. So I'll move that a tentative amended report be issued with the said ingredients, three of the original and four add-on safe as used.

Okay. Any other?

MR. JOHNSON: Just one question.

DR. MARKS: Sure.

MR. JOHNSON: Per the discussion, Dr. Marks, you mentioned, I guess, addressing sensitization and contact urticaria potential in the 5 and 10 percent limits that were established in the published file report.

DR. MARKS: Yeah.

MR. JOHNSON: Specifically, what do you want to say there?

DR. MARKS: I think what we should do is point out that these are the present use concentrations and products. So we're not contradicting that by saying "safe as used." And I would -- there is obviously a lot of thought and effort reaching when we read the discussion and the data coming up with the 5 percent was, I think, a compromise in terms of what percentage.

MR. JOHNSON: Okay.

DR. MARKS: Yeah. That's all I -- to point out that this previous document did set limits. We're just saying present use of concentration.

MR. JOHNSON: But you're not saying anything specifically about sensitization or contact urticaria?

DR. MARKS: No.

MR. JOHNSON: Okay.

DR. MARKS: No. I don't think so. You would just refer back. You can just mention that that was considered.

MR. JOHNSON: Okay.

DR. MARKS: Okay. Anything else with the benzyl alcohol, acids, salts, and esters? If not, we'll move onto methylene glycol, which is the cosmetic ingredient, and the formaldehyde, which is not.

Day 2 of the June 27-28, 2011 CIR Expert Panel Meeting

Benzyl Alcohol, Benzoic Acid, and its Salts and Ester

DR. MARKS: So, in 1998, the CIR Expert

Panel issued a final safety assessment on the

alcohol, acid, and sodium benzoate with concerns about inhalation and labeled it as an insufficient for safety in inhalation as the primary root of exposure of these three ingredients. In June of last year, this was reopened considering new data about inhalation and then also we considered adding more ingredients. And so for this draft report we have the benzyl alcohol, benzyl acid, sodium benzoate, which were in the 1998 report, plus we have the salts, calcium, magnesium, potassium, benzoate, and also benzyl -- benzoate the ester. And so we felt that review of this now that the concerns about inhalation were met and we felt that we could have these seven ingredients all in one report with a 2 -- a tentative amended report with a conclusion 2 safe as used.

DR. BERGFELD: And that's a motion?

DR. MARKS: Yes.

DR. BERGFELD: Is there a second?

DR. BELSITO: Second.

DR. BERGFELD: Second. Further discussion?

DR. MARKS: Yes, I think in the discussion section we went through several points. One, obviously, is dealing with the inhalation insufficiency had to be addressed in the discussion from the original report. Then, Tom and Ron, was it the NTP that an adenoma was found in some of the experimental animals, but the EPA did not feel that it was carcinogenic? So we can --

1 DR. SLAGA: It's a benign tumor.

DR. MARKS: Yeah, it's a benign tumor and so we can address that in the discussion. And then the other -- when you look at the original report there were limits set, 5 percent and 10 2 percent, and when you look at the concentration of 2 use in products now as we -- safe as used will actually address those concerns from the original report. So, that should be in the discussion.

DR. BERGFELD: Don.

DR. BELSITO: We would agree with that. The other thing we added to the discussion was their mention in the report that photodegradation can result in release of free benzene in these products and we felt that at the current level of use that really would not be of any toxicologic consequence. The other issue that we raised is in the list of provocative studies that were done -- there were two issues on benzyl benzoate, that was provided by the Research Institute for Fragrance Material, and they were all referenced to that article, but that article referenced primary articles regarding that data and they should be referenced rather than as secondary review article. And the second issue that I think we 2 need to be very cognizant of is that in the 2 provocative testing they use words like "three patients sensitized." The implication of that is that three patients were sensitized during the process of patch testing and that's -- to any dermatologist who does that -- and that's not the case. Three patients were positive, so I think that word "sensitized" throughout that provocative table has to be removed and put "positive." Otherwise, you get the implication that at that concentration you're sensitizing individuals during the course of patch testing and that's not the case regardless of how the authors might have phrased it.

DR. BERGFELD: Any other discussion or comment? If not, I'm moving the question then, all those in favor of a safe conclusion?

Final Amended Report

Benzyl Alcohol, Benzoic Acid, and its Salts and Benzyl Benzoate

September 26, 2011

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ABSTRACT: Benzyl alcohol, benzoic acid, and its salts and benzyl benzoate function mostly as fragrance ingredients/preservatives in cosmetic products. The Expert Panel noted that benzoic acid, benzyl alcohol, and benzyl benzoate are dermally absorbed, and it is likely that the same is true for benzoic acid salts. The Panel concluded that negative oral carcinogenicity data on benzyl alcohol and negative inhalation toxicity data on benzoic acid and benzyl alcohol support the safety of all of the ingredients that are being reviewed. Thus, it was concluded that these ingredients are safe in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This report assesses the safety of benzyl alcohol, and benzoic acid and its salts and benzyl ester as used in cosmetics. In cosmetics, these ingredients are reported to function as fragrance ingredients, pesticides, pH adjusters, preservatives, solvents, and/or viscosity decreasing agents in cosmetic products.

In 2001, the Cosmetic Ingredient Review (CIR) published a final report on the safety assessment of benzyl alcohol, benzoic acid, and sodium benzoate with the conclusion that benzyl alcohol, benzoic acid, and sodium benzoate are safe for use in cosmetic formulations at concentrations up to 5%.¹ Benzyl alcohol was safe for use in hair dyes at concentrations up to 10%. The available data were insufficient, however, to support the safety of these ingredients in cosmetic products in which a primary route of exposure is inhalation. The Expert Panel stated that inhalation toxicity data were needed.

The Personal Care Products Council provided a 4-week inhalation toxicity study (rats) of aerosolized benzyl alcohol and benzoic acid. After reviewing this study, the Expert Panel determined that the final report on the safety assessment of benzyl alcohol, benzoic acid, and sodium benzoate should be reopened to amend the original conclusion. Furthermore, the Panel determined that data available in the original safety assessment, with the addition of the new inhalation toxicity data, were sufficient to support the safety of other simple salts of benzoic acid (calcium, magnesium, and potassium benzoate) and the benzyl ester.

Additional new data reviewed included an Organization for Economic Cooperation and Development (OECD) Screening Information Data Sets (SIDS) for high volume chemicals initial assessment report on benzoic acid, sodium benzoate, potassium benzoate, and benzyl alcohol published by the United Nations Environmental Program Chemicals Branch (UNEP chemicals) in 2001,² the same year that the CIR Final Safety Assessment on benzyl alcohol, benzoic acid, and sodium benzoate was published. In addition to the SIDS data summaries included in this CIR safety assessment, the 2001 SIDS report may be consulted for further details. Further, the European Commissions' International Uniform Chemical Information Database (IUCLID) on benzyl alcohol, benzoic acid, sodium benzoate, and benzyl benzoate was created in the year 2000.³ Data from the OECD SIDS assessment and IUCLID data sets are summarized in the Toxicology section. In the process of selecting new data for inclusion in the current report, efforts were made to eliminate redundancy between OECD SIDS, IUCLID, or other data found in the published literature and data included in the original final report.

CHEMISTRY

Definition and Structure

Definitions, other chemical names, and cosmetic ingredient functions for ingredients reviewed in this safety assessment are included in Table 1. Structural formulas are included in Figure 1.

Chemical and Physical Properties

Chemical and physical properties of benzyl alcohol, benzoic acid and its salts, and the benzyl benzoate ester are included in Table 2.

The photodegradation of benzoic acid aqueous solution in the presence of UV light has been reported. Benzoic Acid absorbs UV light below 300 nm, and its concentration in solution was monitored using reverse-phase high-performance liquid chromatography (HPLC). The mercury lamp used emitted polychromatic radiation between 240 and 540 nm. The photon

flow absorbed by the reaction system varied with the concentration of benzoic acid and with the presence of the products of degradation. When the temperature was increased from 25°C to 50°C, photodegradation was increased by 20%. Photodegradation products were not mentioned.⁴

The conversion of benzyl alcohol to benzaldehyde and benzoic acid in aqueous solution has been demonstrated using liquid chromatography/spectrophotometry.⁵ When benzyl benzoate (in ethanol) was exposed to sunlight for 11 days (at 25°C), 35 products were isolated from exposed samples and identified using gas chromatography/mass spectrometry. Benzyl alcohol and benzoic acid were among the products identified.⁶

Method of Manufacture

Benzyl Alcohol

Large scale production of benzyl alcohol is achieved by the action of sodium or potassium carbonate on benzyl chloride.¹

Benzoic Acid

Benzoic acid is produced via the decarboxylation of phthalic anhydride in the presence of catalysts. Another production method involves the chlorination of toluene to yield benzotrichloride, which is hydrolyzed to benzoic acid.⁷

Sodium Benzoate

Sodium benzoate is produced by the neutralization of benzoic acid with sodium bicarbonate, sodium carbonate, or sodium hydroxide.⁸

Potassium Benzoate

Potassium benzoate can be prepared by reacting methyl benzoate with potassium thioacetate.⁹

Benzyl Benzoate

Benzyl benzoate results from the condensation of benzoic acid and benzyl alcohol. It can also be generated from benzaldehyde via the Tishchenko reaction.¹⁰

UV Absorption

The following UV absorption maxima have been reported for salts and an ester of benzoic acid: 228.6 nm (magnesium benzoate), 226.8 nm (potassium benzoate), and 256 nm (benzyl benzoate).^{11,12}

Analytical Methods

Benzoic acid has been analyzed using mass spectrometry,¹³ and potassium benzoate has been analyzed using IR spectroscopy.¹⁴ Benzyl benzoate has been analyzed using IR and NMR spectroscopy.^{15,16}

Impurities

Benzoic acid can react with ascorbic acid in beverages via metal-catalyzed reduction (cans) or on exposure to solar UV (bottles) to form benzene, but the yield of benzene is < 1 ppb. Limits for heavy metal impurities in food-grade sodium benzoate (as Pb, ≤ 2 mg/kg) and pharmaceutical-grade potassium benzoate (heavy metals, ≤ 0.001%) have been established.

Benzoic Acid and Sodium Benzoate

The effects of UVA exposure on benzene formation have been determined. Benzene formation was examined for samples contained in UV stabilized and non-UV stabilized packaging.¹⁷ Some of the samples selected for UVA testing included model solutions prepared with 0.04% benzoate and 0.025% ascorbic acid in unbuffered water. The results of 24-h irradiation studies indicated that, under intense UV light, benzene levels increased by as much as 53% (to 315 ng/g benzene, compared to model solutions exposed to visible light [206 ng/g benzene]) in model solutions stored in non-UV stabilized bottles.

However, the use of UV stabilized polyethylene terephthalate bottles reduced benzene formation by ~ 13%, relative to the non-UV stabilized bottles. Similar trends were observed following irradiation for 7 days. According to the Food and Drug Administration (FDA), the U.S. beverage industry voluntarily reformulated beverages that were found to contain benzene levels at or above the 5 ng/g maximum contaminant level (mcl) for drinking water established by the U.S. Environmental Protection Agency (EPA).¹⁷ An mcl of 0.005 mg/L (5 ppb) is the EPA's official limit for benzene in drinking water.¹⁸ Furthermore, in a 2008 survey conducted by FDA, all of the reformulated beverages tested were shown to contain ≤ 1.1 ng/g benzene. Most of the beverages were reformulated by removing or reducing either benzoate or ascorbic acid, or by adding EDTA.¹⁹

BenzyI Benzoate, Potassium Benzoate, and Sodium Benzoate

According to the *United States Pharmacopoeia*, benzyI benzoate contains less than 99% and not more than 100.5% $C_{14}H_{12}O_2$ and potassium benzoate contains not less than 100.5% and not more than 99% $C_7H_5KO_2$. Limits for water and heavy metals in potassium benzoate are stated as $\leq 1.5\%$ and $\leq 0.001\%$, respectively.²⁰

The Food Chemicals Codex specification for sodium benzoate includes the following limitations: heavy metals, as Pb (≤ 2 mg/kg), alkalinity, as NaOH ($\leq 0.04\%$), and water ($\leq 1.5\%$).²¹

USE

Cosmetic

As stated in the *International Cosmetic Ingredient Dictionary and Handbook*, benzoic acid, benzyI alcohol, sodium benzoate, calcium benzoate, magnesium benzoate, potassium benzoate, and benzyI benzoate may function as fragrance ingredients, pesticides, pH adjusters, preservatives, solvents, viscosity decreasing agents, and pH adjusters in cosmetic products (See Table 1).²²

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2010, the following ingredients were being used in personal care products: benzoic acid, benzyI alcohol, sodium benzoate, potassium benzoate, and benzyI benzoate.²³ Uses of calcium benzoate and magnesium benzoate were not reported to the VCRP. These data are summarized in Table 3.

Independent of the VCRP data, a survey of ingredient use concentrations was conducted by the Personal Care Products Council in 2010. The results of this survey indicated that the following ingredients were being used in cosmetic products: benzoic acid (0.000002 to 5%), benzyI alcohol (0.000006 to 10%), sodium benzoate (0.000001 to 1%), calcium benzoate (0.002 to 0.004%), potassium benzoate (0.002 to 0.003%), and benzyI benzoate (0.000005 to 4%).²⁴ No uses of magnesium benzoate were reported in the Council survey.

Personal care products containing the ingredients reported as being used may be applied to the skin, nails, or hair, or, incidentally, may come in contact with the eyes and mucous membranes. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin, nails, or hair for variable periods following application. Daily or occasional use may extend over many years.

In the European Union, the maximum authorized concentrations for benzoic acid and sodium benzoate as preservatives in cosmetic products are: rinse-off products, except oral care products (2.5%, as acid), oral care products (1.7%, as acid), and leave-on products (0.5%, as acid). For salts of benzoic acid other than sodium benzoate, the maximum authorized concentration is 0.5% (as acid).²⁵

The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP) was asked to review the data submitted to support the safety of benzoic acid and its salts and esters, when used at concentrations other than those laid down in Annex VI to Directive 76/768/EEC as preservatives, for other specific non-preservative purposes apparent from the presentation of the products and to answer the following question: Can benzoic acid and its salts and esters be used safely for non-preservative purposes in cosmetic rinse-off products at a maximum concentration of 2.5% and in cosmetic oral-care products at a maximum concentration of 1.7%. Data were provided to the SCCNFP in support of the use of benzoic

acid as a non-preservative. The SCCNFP did not find the submission appropriate for a safety evaluation of benzoic acid and sodium benzoate for the applied “other uses” in cosmetic products.²⁶ Subsequently, a second data set was provided, and these data were considered adequate for arriving at a conclusion on the safety of benzoic acid and sodium benzoate, but not the other salts or esters. Based on these data, the Scientific Committee on Consumer Products (SCCP) issued the following opinion: The SCCP is of the opinion that benzoic acid and sodium benzoate are safe for use for preservative and non-preservative purposes in cosmetic rinse-off products at a maximum concentration of 2.5 % and in cosmetic oral-care products at a maximum concentration of 1.7%, and in leave-on products up to 0.5%.²⁷

Noncosmetic

According to FDA’s Select Committee on Generally Recognized as Safe (GRAS) substances, benzoic acid (21 CFR [Code of Federal Regulations] 184.1021)²⁸ and sodium benzoate (21 CFR 184.1733)²⁹ are classified as GRAS food substances. Sodium benzoate has also been approved by FDA for use as an antimycotic when migrating from food-packaging material (21 CFR 181.23).³⁰ Other CFR citations relating to FDA-approved direct/indirect food additive ingredient uses include: benzyl alcohol (21 CFR 172.515, 175.105, 175.300, and 177.1210)^{31,32,33,34}; benzoic acid (21 CFR 150.141, 150.161, 166.110, 175.300, and 177.1390)^{35,36,37,33,38}; sodium benzoate (21 CFR 150.141, 150.161, and 166.110)^{35,36,37}; calcium benzoate (21 CFR 166.110 and 178.2010)^{37,39}; potassium benzoate (21 CFR 166.110 and 177.1210)^{37,34}; and benzyl benzoate (21 CFR 172.515 and 175.105).^{31,32}

Benzyl alcohol is an FDA-approved diluent in color additive mixtures for external drug use.⁴⁰ In 2009, the FDA approved benzyl alcohol lotion, 5%, as a prescription medication for the treatment of head lice (*Pediculosis capitis*) infestation in patients 6 months of age and older.⁴¹ The results of clinical trials supporting benzyl alcohol lotion 5% (UlesfiaTM) as a safe and effective topical treatment for head lice are included in the section on Skin Irritation and Sensitization later in the report text.⁴² Benzyl benzoate (10%) has been used in the treatment of scabies in developing countries.⁴³

Benzyl alcohol has been classified by FDA regarding its use in the following types of over-the-counter (OTC) drug products (Category I: generally recognized as safe (S) and effective (E) for the claimed therapeutic indication; Category II: not generally recognized as safe and effective or unacceptable indications; Category III: insufficient data available to permit final classification):⁴⁴

- anorectal (final monograph: 1 to 4% use concentration range established; Category I)
- external analgesic (both Category I and final monograph pending)
- external analgesic (final monograph: Category III)
- oral health care (Category I pending)
- oral discomfort care (Category II pending)
- oral discomfort care (Category IIISE pending)
- pediculicide (final monograph: Category III)
- skin protectant (final monograph: Category III)

Similarly, benzoic acid has been classified by FDA regarding its use in the following types of OTC drug products:⁴⁴

- acne (final monograph: Category III)
- antifungal (final monograph: Category III)
- oral health care - (Category IIIIE pending)
- skin protectant - (final monograph: Category III)

Sodium benzoate has been classified by FDA regarding its use in menstrual/diuretic OTC drug products (final monograph: Category III), and benzyl benzoate has been approved for use in OTC pediculicides (final monograph: Category II).⁴⁴

Calcium benzoate, magnesium benzoate, and potassium benzoate are inert non-food ingredients that, under the Federal Insecticide, Fungicide, and Rodenticide Act, are permitted by the Environmental Protection Agency (EPA) for use in non-food use pesticide products.⁴⁵ Pesticide products containing benzoic acid as the active ingredient have been registered with EPA for use in the extermination of dust mites.⁴⁶

Benzyl alcohol has been listed as a chemical in photographic developing systems,⁴⁷ and, at concentrations of 0.9 to 2%, is commonly used as an antibacterial agent in a variety of pharmaceutical formulations intended for intravenous administration.⁴⁸ Additionally, benzyl alcohol has been used in parenteral medications commonly administered to critically ill neonates.⁴⁹

TOXICOKINETICS

Oral Studies

Benzyl alcohol and benzoic acid and its salts are rapidly metabolized and excreted via a common pathway within 24 h. Benzyl alcohol is metabolized to benzoic acid via simple oxidation. Benzoic acid and sodium benzoate are rapidly absorbed from the gastrointestinal tract of mammals, conjugated with glycine in the liver, and then excreted as hippuric acid. The urinary excretion of benzyl benzoate as glucuronide conjugates and hippuric acid in mammals has also been reported.

Benzyl Alcohol, Benzoic Acid, Sodium Benzoate, and Potassium Benzoate

Benzyl alcohol is metabolized to benzoic acid. Both benzoic acid and sodium benzoate are rapidly absorbed from the gastrointestinal tract of mammals and conjugated with glycine in the liver; the resulting hippuric acid is rapidly excreted in the urine.¹ Benzyl alcohol and benzoic acid and its sodium and potassium salt can be considered as a single category regarding human health, because they are all rapidly metabolized and excreted via a common pathway within 24 h.²

Benzyl Benzoate

Following oral administration (gavage, 0.5, 1, and 2 ml/kg) of benzyl benzoate to fasted female dogs, urine was collected for 5 to 6 days. All 3 doses resulted in creatinuria and urinary excretion of glucuronate conjugates.⁵⁰

Following the administration of unspecified oral doses of benzyl benzoate to cats and dogs (number and breed not stated), small amounts of hippuric acid were detected in the 24 h urine. The metabolism of benzyl benzoate to benzoic acid *in vivo* has also been reported. Additional details for these 2 studies were not provided.³

In a human subject, urinary excretion of hippuric acid was measured after oral dosing (single dose, 2.02 g) with benzyl benzoate. Urine was collected for 6 h. Approximately 90% of the dose administered was excreted as hippuric acid in the urine. After oral dosing with 1 g, approximately 80% of the administered dose was excreted as hippuric acid. In a second subject, 71% of the administered dose was excreted as hippuric acid during the first 6 h and an additional 14% of the dose was excreted as such during the second 6 h after dosing.⁵¹

Dermal Studies

In in vitro studies, the percutaneous absorption of benzoic acid through guinea pig skin and the percutaneous absorption of benzyl alcohol and benzyl benzoate through human skin was reported. The percutaneous absorption and urinary excretion of benzyl alcohol and benzyl benzoate was observed following dermal application to rhesus monkeys.

Benzoic Acid

The penetration of benzoic acid through excised dorsal skin (full-thickness and with stratum corneum removed) from guinea pigs was examined. Skin preparations were mounted in a two-chamber diffusion cell. A suspension of excess amount of benzoic acid in saline was added to donor compartments. In full-thickness skin, permeation proceeded with a short lag time. Solubility (C_d , unit = mM) and permeability (K_p , unit = $\times 10^{-2} \text{ cm} \cdot \text{h}^{-1}$) coefficients for benzoic acid were 32.7 ± 1.6 and 9.01 ± 1.51 , respectively. Removal of the stratum corneum by tape stripping and its delipidation using an organic solvent mixture enhanced the skin penetration of benzoic acid.⁵²

Benzyl Alcohol and Benzyl Benzoate

The percutaneous absorption of [7-¹⁴C]benzyl alcohol and [7-¹⁴C]benzyl benzoate *in vivo* was evaluated using 4 female Rhesus monkeys (10 to 19 years old). Each chemical was applied (4 µg/cm², without or with occlusion [glass chamber - G or plastic wrap - P]) to a clipped 1 cm² area of abdominal skin; the vehicle was acetone (10 to 20 µl/cm²). The animals remained in metabolism chairs/metabolism cages for 5 days. The amount of absorbed compound in the urine was determined by liquid scintillation counting. Mean values (± SEM, 4 animals) for urinary excretion of the administered dose were: 77.4 ± 4.3% (benzyl alcohol) and 65.3 ± 13.4% (benzyl benzoate). Percutaneous absorption values (absorption, as % of applied dose) were as follows: benzyl alcohol (31.6 ± 4.2% [unoccluded]; 56.3 ± 14.5% [P]; 79.9 ± 7.4% [G]) and benzyl benzoate (57.0 ± 10.4% [unoccluded]; 71.2 ± 4.4% [P]; 64.7 ± 10.2% [G]). Over a 5-day period, the urinary excretion of radioactivity was 65.3 ± 13.4% following dermal application of benzyl benzoate and 56.5 ± 7.7% following dermal application of benzyl alcohol. When the acetone vehicle was replaced with a lotion (10 mg/cm²), percutaneous absorption was increased. The increase was significant for benzyl benzoate (P < 0.05), but not benzyl alcohol. There was no apparent correlation between the skin penetration of benzyl alcohol and benzyl benzoate and their octanol-water partition coefficients of 7.4 and 3.97, respectively.⁵³

The percutaneous absorption of benzyl benzoate *in vitro* was evaluated using human epidermis (from abdominal skin) placed in a glass diffusion chamber. Saline (5 ml) was added to the chamber and was in contact with the bottom of the epidermis. Benzyl benzoate (0.2 ml) was applied to the top of the epidermis. Six experiments were performed and the mean value for skin penetration of benzyl benzoate was 0.018 ± 0.002%.⁵⁴

The penetration of benzyl alcohol through split-thickness cadaver skin was evaluated using nonoccluded Franz diffusion cells. Benzyl alcohol (spiked with ¹⁴C radiolabel) in ethanol was applied to the skin at doses ranging from 0.9 to 10,600 µg/cm². After 24 h, the percentage of radioactivity that penetrated the skin increased gradually with dose, ranging from 19.8 ± 2.9% (lowest dose) to 29.2 ± 3.0% (highest dose). Also, less than 4% of the administered radioactivity was retained in the tissues at 24 h, and evaporation accounted for the remaining percentage. Data from this study were also analyzed in relation to a finite dose diffusion/evaporation model. The results of this analysis indicated that the increase in benzyl alcohol absorption with dose was consistent with a 3-fold increase in diffusivity in the stratum corneum, as the concentration of benzyl alcohol increased from tracer levels to saturation.⁵⁵

In Vitro Study

The rate of hydrolysis of benzyl benzoate (in acetonitrile) by 80% human plasma *in vitro* was studied. The t_{1/2} for the *in vitro* hydrolysis of benzyl benzoate to benzoic acid in human plasma was 19 minutes.⁵⁶

TOXICOLOGICAL STUDIES

An Organization for Economic Cooperation and Development (OECD) Screening Information Data Sets (SIDS) for high volume chemicals initial assessment report on benzoic acid, sodium benzoate, potassium benzoate, and benzyl alcohol was published by the United Nations Environmental Program, Chemicals Branch (UNEP chemicals) in 2001, with the following recommendation relating to human safety: "Taking into account the rapid metabolism and excretion, the non-bioaccumulation, the low toxicity after acute and repeated exposures, the non-reproductive toxicity, the non-genotoxicity and the non-carcinogenicity, the low irritating and non- to very low sensitizing properties of these substances, as well as the controlled (industrial settings) and/or regulated (pharma, cosmetics and/or food) uses, these substances will pose a minimal risk to humans (workers and consumers)." Because the database for this safety assessment is similar to that for the 2001 CIR Final Report on Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate, the available data in the OECD SIDS assessment are summarized with minimal details in sections that follow.

Acute and Repeated Dose Inhalation Toxicity

Neither acute nor repeated inhalation exposures to benzyl alcohol or benzoic acid caused death in rats. The results of a repeated dose inhalation toxicity study indicated no test substance-related macroscopic or microscopic findings for either

test substance, and the no-observed-effect-level (NOEL) and no-observed-adverse-effect level (NOAEL) were considered to be 1,072 mg/m³ for benzyl alcohol and 12.6 mg/m³ for benzoic acid.

Benzyl Alcohol and Benzoic Acid

According to the OECD SIDS initial assessment report on Benzoates, 4 h of inhalation exposure to benzyl alcohol or benzoic acid at 4 and 12 mg/L aerosol/dust, respectively, did not cause death in rats. Thus, low acute toxicity was associated with these compounds.²

A 4-week inhalation toxicity study of aerosolized benzyl alcohol and benzoic acid was performed using groups of Crl:CD(SD) rats.⁵⁷ Four groups of rats (10/sex/group) were exposed (nose-only) to aerosolized benzyl alcohol 5 days per week (6 h/day) for 4 weeks. Each animal received a minimum of 20 exposures of benzyl alcohol, and target exposure concentrations were 30, 100, 300, and 1,000 mg/m³ for the four groups. Two additional groups of rats were exposed to aerosolized benzoic acid (2.5 and 12.5 mg/m³) according to the same procedure. The group designated for exposure to benzyl alcohol (30 mg/m³ target) was actually exposed to a mean atmosphere concentration that was 136% of the target concentration. For the remaining groups, all mean atmosphere concentrations were within 96.7% to 107.2% of the respective target concentrations. A concurrent control group was exposed to filtered air only.

There were no test-substance related deaths or effects on any of the following: body weight, food consumption, clinical pathology parameters, and organ weights. Additionally, there were no test substance-related macroscopic or microscopic findings. The (NOEL) and NOAEL were considered to be 1,072 mg/m³ for benzyl alcohol and 12.6 mg/m³ for benzoic acid.⁵⁷

Acute Oral Toxicity

Some of the oral LD50 values that have been reported for various species include > 10,000 mg/kg for potassium benzoate in rats, mice, and guinea pigs, and the following values for benzyl benzoate: > 2,000 mg/kg (rats), 1400 mg/kg (mice), 1,122 mg/kg (guinea pigs), 1,680 mg/kg (rabbits), 2,240 mg/kg (cats), and 2,244 mg/kg (dogs).

Acute oral toxicity data are summarized in Table 4.

Benzyl Alcohol, Benzoic Acid, Sodium Benzoate, and Potassium Benzoate

According to the OECD SIDS initial assessment report on Benzoates, low acute oral toxicity is associated with benzoic acid and its sodium and potassium salts. The LD50 values were > 2000 mg/kg body weight, except for benzyl alcohol (LD50 = 1610 mg/kg [rats]), which needs to be considered harmful via the oral route.²

Acute Intraperitoneal Toxicity

Benzyl Benzoate

Mice were dosed i.p. with benzyl benzoate (test procedure/number of mice not stated), and an LD50 of > 500 mg/kg body weight was reported.³

Acute Dermal Toxicity

An acute dermal LD50 of 2,000 mg/kg for benzyl alcohol in rabbits and acute dermal LD50s of 4,000 mg/kg (rats) and 4,448 mg/kg (rabbits) for benzyl benzoate have been reported. Death of cats has been observed, following single dermal applications of a benzyl benzoate solution.

Benzyl Alcohol, Benzoic Acid, Sodium Benzoate, and Potassium Benzoate

According to the OECD SIDS initial assessment report on Benzoates, low acute dermal toxicity is associated with benzyl alcohol and benzoic acid and its sodium and potassium salts.²

Benzyl Alcohol and Benzyl Benzoate

The acute dermal toxicity of benzyl benzoate was evaluated using rats (number not stated), and an LD50 of 4,000 mg/kg body weight was reported. Study details were not included. In a study involving rabbits, the acute dermal toxicity of undiluted benzyl benzoate was evaluated according to the Draize procedure (no GLP; number of animals not stated), and an LD50 of 4,448 mg/kg body weight was reported.³ In another study involving rabbits, acute dermal LD50s of 2,000 mg/kg and 4,000 mg/kg have been reported for benzyl alcohol and benzyl benzoate, respectively.⁵⁸

Single dermal applications of 25% benzyl benzoate (20 ml in isopropanol/water) to the backs of cats resulted in death. Additional study details were not provided.³

Acute Subcutaneous Toxicity

Benzyl Benzoate

Groups of adult C57BL/6J mice (5 per group) were injected s.c. with 0.1 ml benzyl benzoate (~111 mg) daily for 4 weeks. An untreated group of sham injected animals served as the untreated concurrent control group. The adrenal glands were prepared for light and electron microscopic examination. The animals died within 3 h of initial injection. Additional study details were not provided.³

Guinea pigs (number not stated) received single s.c. injections of benzyl benzoate, and mortality rates at the administered doses were as follows: 0 (0.5 g/kg), 30% (1 g/kg), 80% (5 g/kg), 100% (10 g/kg), and 100% (20 g/kg).⁵⁹

Acute Intramuscular Toxicity

Benzyl Benzoate

Following benzyl benzoate intramuscular injection into cats (number not stated) i.m., doses of 2 and 10 g/kg were described as lethal.⁵⁹

Repeated Dose Toxicity – Oral Studies

Benzoic acid and its salts and benzyl alcohol have caused a low degree of toxicity in repeated dose oral toxicity studies. NOAEL values of 800 mg/kg/day and > 1,000 mg/kg/day have been reported for benzoic acid and its salts, respectively. An NOAEL of > 400 mg/kg/day has been reported for benzyl alcohol. Toxic effects were not associated with repeated oral doses of benzyl benzoate, and an NOEL of 800 mg/kg was reported.

Benzyl Alcohol, Benzoic Acid, Sodium Benzoate, and Potassium Benzoate

According to the OECD SIDS initial assessment report on Benzoates, repeated dose oral toxicity studies yielded an NOAEL of 800 mg/kg/day for benzoic acid and > 1000 mg/kg/day for the salts. At greater doses, increased mortality, reduced weight gain, and liver and kidney effects were observed. Systemic toxic effects of a similar nature (e.g. liver, kidney) were observed after dosing with benzyl alcohol, benzoic acid, sodium benzoate, and potassium benzoate. However, these effects were observed at higher doses of benzoic acid and its salts when compared to dosing with benzyl alcohol. Long-term studies on benzyl alcohol yielded an NOAEL of > 400 mg/kg body weight per day for rats and > 200 mg/kg body weight per day for mice. At higher doses, effects on body weight and lesions in the brain, thymus, skeletal muscle, and kidneys were observed. It was noted that one should take into account that oral administration was by gavage in these studies, whereby saturation of metabolic pathways is likely to occur. It was concluded that benzoic acid and its salts exhibited very low repeated dose toxicity and that benzyl alcohol exhibited low repeated dose toxicity.²

Benzyl Benzoate

No effects were observed in a 7-month toxicity study in which mice (no. and strain not stated) were dosed orally with benzyl benzoate (800 mg/kg).⁶⁰

Repeated Dose Toxicity – Dermal Studies

Systemic/dermal effects induced by benzyl benzoate were observed in rats receiving repeated dermal doses up to 2.0 g/kg and in rabbits receiving 2.2 g/kg doses. Benzyl benzoate was lethal at doses of ~ 2.0 g/kg (rabbits) and higher (cats).

Benzyl Benzoate

The following effects were observed in groups of rats (3 males/3 females/group; strain not stated) that received dermal applications of 0.188, 0.488, 0.781, 1.25, or 2.0 g/kg for 30 days: hyperplasia of squamous epithelium, degeneration of hair follicles and sebaceous glands, subcutaneous fibrosis, and hyperplasia of the thyroid gland. Seven areas of the shaved back were dosed in rotation. Dosing with 2.0 g/kg also caused 4 deaths, decreased white blood cell numbers, and other blood effects. Body weight changes, decreased white blood cell numbers, and other blood effects were also observed following dosing with 1.25 g/kg. Additional study details were not provided.⁶¹

In a study involving rabbits, (non-GLP; number and strain not stated) dermal doses of benzyl benzoate were applied daily for 90 days.³ The range of doses tested was not stated. Deaths occurred at doses of 2.2 g/kg body weight/day and greater. Testicular atrophy was noted at high doses, and, possibly, there was also an increased incidence of focal nephritis and encephalitis. Very mild skin irritation was also observed. Additional details relating to this study were not provided. Benzyl benzoate was also applied topically to rabbits at doses of 0.5 ml/kg/day daily for 90 days. Slight dermatitis and inanition were observed, and, at higher doses, slight to moderate testicular atrophy was apparent. Increased leucocyte counts were reported and results were also suggestive of kidney damage. Benzyl benzoate also caused death, but the number of mortalities and corresponding toxic doses were not reported. Repeated dermal applications of benzyl benzoate (doses not stated) did not cause toxic symptoms in the following species: dogs, horses, cows (young females), sheep, or pigs. Additional study details were not provided.³

In another study, rabbits (no. and strain not stated) received dermal applications of benzyl benzoate at doses of 0.5, 1.0, 2.0, and 4.0 ml/kg daily for 90 days.⁶² Lethality and effects on the following were reported after dosing: blood effects (at 2.0 ml/kg), kidneys, skin (slight dermatitis and other effects), reproduction, leukocyte (increased 2.5 x normal), testicular damage, and kidney damage. Animal deaths were not preceded by the usual pattern of systemic effects prior to death. Additional details were not included.

Five applications of benzyl benzoate (undiluted) were made to the backs of the following domestic animals: 1 sheep (250 ml dose), 1 pig (200 ml), 1 young cow (500 ml), and 1 horse (1000 ml). The animals were observed for 2 weeks. None of the animals died. In another study, 6 applications of undiluted benzyl benzoate were made to the backs (clipped skin, 4 " x 6" area) of 3 dogs as follows: 1 dog (200 ml volume) and 2 dogs (100 ml). The animals were observed for 2 weeks. No test substance-related effects were observed.⁶³

Benzyl benzoate was rubbed onto cutaneous lesions on the shaved upper thighs of cats (no. not stated). Three applications per animal were made. Within 24 to 36 h, the test substance was lethal at doses of 8.7 to 12.8 g/kg.⁵⁹

Repeated Dose Toxicity – Parenteral Studies

Subcutaneous dosing with benzyl benzoate resulted in death of mice (111 mg doses) and rabbits (2.5 ml/kg doses).

Benzyl Benzoate

Rabbits (1 or 2 animals) were injected s.c. once with benzyl benzoate in olive oil (doses of 1 to 2.5 ml/kg) or received 4 daily 0.25 mg/kg s.c. injections. Effects on leukocyte counts were monitored over an 11-day period and the animals were also examined for mortalities and clinical signs. The 2.5 ml/kg dose was lethal and clinical signs were observed. No effects were associated with the 0.25, 1, or 1.5 ml/kg doses.⁶⁴

Ocular Irritation/Toxicity

Benzyl alcohol (4% aqueous), benzoic acid (undiluted), and its sodium salt (concentration not stated) were irritating to the eyes of rabbits, and it was expected that potassium benzoate would be irritating as well. A vehicle containing benzyl alcohol as a preservative for injected triamcinolone acetonide (TA) was toxic when injected intravitreally into the eyes of rabbits. However, it has been noted that retinal toxicity is not observed following injection of 0.1 ml preserved TA in clinical practice. Benzyl benzoate (undiluted) was irritating to the eyes of rabbits and humans.

Benzyl Alcohol

The ocular toxicity of benzyl alcohol was evaluated using 9 New Zealand rabbits. The following test concentrations of benzyl alcohol (in stock solution of carboxymethylcellulose and polysorbate 80) were injected intravitreally, each into 3 eyes (15 eyes total injected): 0.0073%, 0.022%, 0.073%, 0.222%, and 0.733%. Control eyes (2) were injected intravitreally with 0.9% normal saline. Ocular toxicity was not observed at the lowest concentration; transient clinical changes (retinal hemorrhage and whitening), but no remarkable pathological changes, were noted in 1 of 3 eyes tested with 0.022% benzyl alcohol. Ocular toxicity was observed at the remaining concentrations; changes in the outer retina included loss and shortening of outer segments and receptors.⁶⁵

Triamcinolone acetonide (TA) has been increasingly applied intravitreally in therapy for retinal diseases, and the vehicles of TA injections usually contain benzyl alcohol as a preservative. The ocular toxicity of 2 commercial vehicles (A and B) for drugs was evaluated using 3 groups of 12 New Zealand white rabbits (vehicle A, vehicle B, and balanced saline solution [BSS, negative control] groups). The 2 vehicles were similar in that each also contained benzyl alcohol (9.9 mg/ml), sodium carboxymethylcellulose (CBC, 7.5 mg/ml), and polysorbate 80 (0.4 mg/ml). Each group of 12 was divided into 2 groups, each receiving a different intravitreal dose (0.1 or 0.2 ml). Ocular examination (using ophthalmoscope) results were normal for group A and the negative control group; retinal damage was obvious in group B. However, at microscopic examination, retinal toxicity was observed in groups A and B. Vehicle B induced extensive retinal necrosis and atrophy, while vehicle B induced localized retinal changes.⁶⁶

In a published comment on the preceding publication, the association of the benzyl alcohol preservative with retinal toxicity was acknowledged. However, it was noted that retinal toxicity is not observed at concentrations achieved following injection of 0.1 ml preserved TA in clinical practice.⁶⁷

In a similar study, 4 groups of Chinchilla rabbits (pigmented) were injected intravitreally with the following 4 materials, respectively: 0.1 ml BSS, 0.1 ml TA (1.3 mg/ml), 0.1 ml vehicle (0.99% BA and other excipient) alone, and 0.1 ml TA + vehicle. Given the materials tested, it should be noted that commercially prepared TA was described as a suspension containing 40 mg TA plus vehicle containing 0.99% BA, 0.75% excipient sodium carboxymethylcellulose, and 0.04% polysorbate. At microscopic examination, morphologic changes in the ciliary body, lens, and retina were observed only in 2 groups (TA + vehicle and vehicle alone). Thus, it was concluded that benzyl alcohol was toxic to these tissues when injected intravitreally.⁶⁸

Benzoic Acid, Benzyl Alcohol, Sodium Benzoate, and Potassium Benzoate

According to the OECD SIDS initial assessment report on Benzoates, benzyl alcohol (4% aqueous) and benzoic acid (undiluted) were irritating to the eyes of rabbits and sodium benzoate (concentration not stated) was only slightly irritating. Data on potassium benzoate were not available; however, it was expected that this chemical would induce slight ocular irritation.²

Benzyl Benzoate

Undiluted benzyl benzoate was instilled (0.1 ml) into the eyes of 6 rabbits (3 males, 3 females), and reactions were scored up to 72 h post-instillation. Ocular irritation was observed in 3 rabbits (only reactions reported) at 1 h post-instillation.⁶⁹

In an ocular irritation study involving human subjects (test procedure/number of subjects not stated), undiluted benzyl benzoate was classified as an ocular irritant.³

Skin Irritation and Sensitization

Undiluted benzyl alcohol and benzoic acid were slightly irritating and an unspecified concentration of sodium benzoate was non-irritating to the skin of rabbits. In studies involving guinea pigs, benzyl benzoate was non-irritating over the range of test concentrations, 2.5% to 50%. For the lower concentrations evaluated for sensitization in guinea pigs, benzyl benzoate was classified as a non-sensitizer at 5% and a weak sensitizer at 1%. At a concentration of 10%, results were consistent with classifications ranging from non-sensitizer to moderate sensitizer. Benzyl benzoate was classified as a mild sensitizer in guinea pigs when tested at a concentration of . In rabbits, undiluted and 25% benzyl benzoate were classified as non-irritants. Results for healthy human subjects indicate that benzyl benzoate was classified as a non-irritant undiluted and at concentrations down to 2%, except for its classification as a minimal irritant at 50% in a very small percentage of subjects in one of the studies. Benzyl benzoate was classified as non-sensitizer when tested at a concentration of 30% in healthy human subjects. Most of the skin sensitization studies on benzyl benzoate involved groups of patients, and mixed results regarding sensitization potential were reported.

Skin irritation and sensitization data (human and non-human) are included in Table 5.

Benzyl Alcohol, Benzoic Acid, Sodium Benzoate, and Potassium Benzoate

According to the OECD SIDS initial assessment report on Benzoates, benzoic acid and benzyl alcohol were slightly irritating to the skin, whereas, sodium benzoate was non-irritating. Skin irritation data on potassium benzoate were not available; however, it is not expected that this compound would be irritating to the skin.² In the same report, benzoic acid did not induce sensitization in animal studies; however, a low incidence of positive reactions was observed in dermatologic patients patch tested. Similar results were reported for sodium benzoate, and it has been suggested that the positive reactions observed were actually non-immunologic contact urticaria. Both positive and negative results were reported for benzyl alcohol in animal skin sensitization studies. A maximum skin sensitization incidence of 1% was reported for benzyl alcohol in human patch tests. Occupational exposure to benzyl alcohol, benzoic acid, or sodium benzoate has not resulted in skin sensitization over a period of decades.²

Case Reports

Case reports included positive patch test reactions to benzoic acid, negative patch test reactions to benzyl benzoate, and positive or negative patch or intradermal/prick test reactions to benzyl alcohol and sodium benzoate.

Case reports involving patch, intradermal, or prick testing of benzyl alcohol, benzoic acid, sodium benzoate, or benzyl benzoate are included in Table 6.

Cytotoxicity

Sodium Benzoate

The potential for sodium benzoate and other preservatives to induce lipid peroxidation in normal and α -linolenic acid -loaded cultured hepatocytes from male Wistar rats was evaluated. Cell injury was measured by the release of lactic acid dehydrogenase into the culture medium after 10 h of incubation. At concentrations up to 10 mM, sodium benzoate was not cytotoxic to either group of hepatocytes. Also, lipid peroxidation was not detected in either group of hepatocytes incubated with sodium benzoate.⁷⁰

Benzyl Benzoate

In an *in vitro* assay, benzyl benzoate was toxic to ascites sarcoma BP8 cell cultures. Additional details were not provided.³

Phototoxicity

A slightly positive reaction was observed in a phototoxicity study involving hairless mice tested with an unspecified concentration of benzyl benzoate (4, 24 h exposures). Photo-irritation was not observed in guinea pigs tested with benzyl benzoate at concentrations up to 30%.

Benzyl Benzoate

The phototoxicity of benzyl benzoate was evaluated using hairless mice (number and strain not stated). Details relating to the test protocol were not included. Following test substance application, test sites were irradiated for a total of 3 or 4, 24 h exposures. No reactions were observed after 3 exposures; however, a slightly positive reaction was observed after 4 exposures.³

The phototoxicity of benzyl benzoate was evaluated, using 5 female Hartley guinea pigs, at concentrations of 10%, 30%, and 50% in acetone. At 4 h after depilation, the test substance was applied to a circular area (diameter = 1.5 cm) on both sides of the back. Two applications (left and right side) of each concentration were made to the back of each animal. One side of each animal was covered with aluminum foil and the other was irradiated with 5 Toshiba model FL-40 BLB lamps (320 to 400 nm) at a distance of 10 cm for 70 min. Photo-irritation was not observed at any of the 3 test concentrations.⁷¹

Photohemolysis

Neither benzyl alcohol (10^{-3} mol/l or 0.1%) nor sodium benzoate (10^{-3} mol/l) induced significant photohemolysis of human erythrocytes exposed to UVA/UVB light in vitro. However, in another assay, moderate photohemolysis of human erythrocytes was induced by benzyl alcohol (10^{-4} mol/l) only in the presence of UVA light, and benzyl benzoate did not induce photohemolysis in the presence of UVA or UVB light.

Benzyl Alcohol, Sodium Benzoate, and Benzyl Benzoate

The photohemolytic activity of benzyl alcohol and sodium benzoate *in vitro* was evaluated in a test involving human erythrocyte suspensions, incubated with either chemical (10^{-3} mol/l) for 1 h and exposed to UVA or UVB radiation. The UVA rich light source was a UVASUN 5000 lamp (320 to 460 nm; 42 mW/cm²) and the UVB rich light source was a lamp with TL 20 W/12 light bulbs (between 275 and 365 nm; 1 mW/cm² [UVB] and 0.4 mW/cm² [UVA]). Cultures without either chemical served as negative controls. Neither benzyl alcohol nor sodium benzoate induced significant hemolysis.⁷²

A similar *in vitro* photohemolysis test (human erythrocyte suspensions) was used to evaluate the phototoxicity of benzyl alcohol and benzyl benzoate. The radiation sources described in the preceding study were used. Moderate hemolysis (~6%) was induced by benzyl alcohol (at 10^{-4} mol/l) only in the presence of UVA, classifying it as a weakly phototoxic compound. Benzyl benzoate (test concentration not stated) was not phototoxic in the presence of UVA or UVB light.⁷³

The photohemolytic activity of benzyl benzoate (0.1%) *in vitro* was evaluated using human red blood cells. Cultures were exposed to UVA or UVB radiation for up to 3 h and then centrifuged. Release of hemoglobin in the supernatant was determined as cyanomethaemoglobin. No photohemolytic activity was noted for 0.1% benzyl benzoate.⁷⁴

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In an OECD SIDS assessment report, benzoic acid (up to 750 mg/kg/day, maternal LOAEL) was not a reproductive toxicant in rats in a four-generation reproductive toxicity study, and repeated doses of sodium benzoate (0.01g in diet) did not induce reproductive effect in rats. Developmental effects were observed in the presence of marked maternal toxicity in rats fed sodium benzoate in the diet (≥ 2800 mg/kg/day; NOAEL = 1400 mg/kg/day) in another study; maternal toxicity was not observed in hamsters (doses up to 300 mg/kg/day, also the NOEL), rabbits (doses up to 250 mg/kg/day, also the NOEL), or CD-1 mice (doses up to 175 mg/kg/day, also the NOEL). Benzyl alcohol was not a developmental toxicant in CD-1 mice (doses up to 750 mg/kg/day, maternal LOAEL) or in mice of an unspecified strain (doses up to 550 mg/kg/day, also the NOAEL) dosed by gavage. Benzyl benzoate (up to 1% in the diet) was not found to be teratogenic in rats. However,

testicular atrophy was observed in rabbits that received repeated dermal doses of benzyl benzoate (> 0.5 g/kg/day). There was no evidence of adverse effects on pregnancy outcome in a study on the safety of topical application of benzyl benzoate (25%) lotion in pregnant women. Benzoic acid (up to 1,000 µg/kg, s.c. injection) did not induce any reproducible evidence of estrogenic activity in uterotrophic assays involving immature female Alpk:AP₃SD rats and female Alpk:AP₃CD-1 mice. Benzyl benzoate induced weak estrogenic responses in certain tests using cultured human breast cancer cells in vitro, but not in others.

Benzoic Acid, Sodium Benzoate, Potassium Benzoate, and Benzyl Alcohol

According to the OECD SIDS initial assessment report on benzyl alcohol, benzoic acid, and its sodium and potassium salts, benzoic acid did not induce reproductive effects in a four-generation reproductive toxicity study (NOAEL > 750 mg/kg).² Groups of rats (20 rats/sex/group) received benzoic acid doses of 375 or 750 mg/kg/day in the diet continuously. Animals of the third generation were killed after 16 weeks. Also, test substance-related effects on reproductive organs (based on gross and microscopic examination) were not observed in subchronic studies (rats and mice) on benzyl alcohol and sodium benzoate.

In groups of rats fed sodium benzoate (doses up to 5600 mg/kg/day) during each day of gestation, developmental effects were observed only in the presence of marked maternal toxicity (reduced food intake and decreased body weight; NOAEL = 1400 mg/kg/day). All developmental effects were observed at doses ≥ 2800 mg/kg/day. On days 6 through 10, 15, or 18 of gestation, dosing with sodium benzoate by gavage in hamsters (NOEL = 300 mg/kg body weight), rabbits (NOEL = 250 mg/kg), and CD-1 mice (NOEL = 175 mg/kg), did not result in maternal toxicity; doses > NOEL were not tested. In mice of an unspecified strain dosed with benzyl alcohol by gavage, an NOAEL of 550 mg/kg (only dose) body weight for developmental toxicity was reported. An LOAEL of 750 mg/kg/day (only dose) for developmental toxicity was reported for CD-1 mice dosed orally (gavage) with benzyl alcohol. In this study, maternal toxicity (increased mortality, decreased body weight, and clinical toxicity) was observed.²

Sodium Benzoate and Benzyl Benzoate

The effect of sodium benzoate on biochemical aspects of pregnant female albino rats and survival of their offspring was evaluated using groups of 10 Sprague-Dawley rats.⁷⁵ Two groups of weanling female rats were fed (*ad libitum*) sodium benzoate at doses of 0.01 g and 0.0125 g in the diet, respectively, daily for 12 weeks. Standard diet was fed to the control group. After mating, the animals were fed *ad libitum* during pregnancy. When compared to the control group, both doses of sodium benzoate induced a decrease in serum bilirubin and an increase in serum urea.

Both doses of sodium benzoate also induced an increase in serum uric acid ($p < 0.01$ and $p < 0.05$, respectively). Serum alanine aminotransferase (ALT) activity was significantly greater ($p < 0.01$) in the high dose group, but not in the lower dose group. The high dose did not induce a significant increase in serum creatinine. Statistically significant ($p < 0.01$) decreases in food intake, hemoglobin, and hematocrit were also reported. Pregnant rats that received high doses of sodium benzoate sustained a 13.6% decrease (statistically significant, $p < 0.05$) in mean weight of their pups, when compared to mean pup weight in the corresponding control group.⁷⁵

The teratogenicity of benzyl benzoate was evaluated using 21 rats (strains not stated) per dose; doses administered in the diet were defined as 0.04 or 1.0% (≈ 24 or 595 mg/kg body weight/day).³ The test substance was administered daily from day 0 of gestation to day 21 post-parturition. The results of examinations for external, skeletal, or visceral anomalies indicated that benzyl benzoate did not induce harmful effects in fetuses.

In a reproductive and developmental toxicity study, pregnant rats (no. and strain not stated) were fed diets supplemented with 0.04 or 1.0% benzyl benzoate from day 0 of gestation to day 21 post-parturition.⁷⁶

76Another group of rats was fed a control diet. There were no effects on reproductive or developmental toxicity parameters; no external, skeletal, or visceral anomalies were observed in fetuses from either treatment group. Minor variations were observed. A significantly decreased number of fetuses with incomplete sternebrae was noted in the 1% benzyl benzoate treatment group.

Testicular atrophy was observed in 2 repeated dose dermal toxicity studies (90 days) on benzyl benzoate involving rabbits.³ The exact doses at which this finding occurred were not stated. However, testicular atrophy was observed at daily doses > 0.5 g/kg/day. These studies are summarized in the Repeated Dose Toxicity – Dermal Studies section earlier in the report text.

Pregnant NMRI mice (34 animals) were injected s.c. with an unspecified dose of benzyl benzoate in castor oil on days 1 and 11 of gestation.⁷⁷ The animals were killed on gestation day 17. Untreated mice served as controls. Fetal observations relating to the following were made: counts, sex, weight, and malformations. There was no evidence of test substance-related effects on fetuses. Additional details were not included.

A developmental toxicity study was performed using *Drosophila melanogaster*.⁷⁸ One group of males and females was raised on food medium with 4% benzyl benzoate in ethanol and the other group was raised on control feed medium. Virgin Canton-S males and females were collected daily for 4 days and then mated. The percentage of eggs hatched was 6.5 times greater in flies on feed containing benzyl benzoate, compared to those on control feed.

The safety of benzyl benzoate lotion (25% benzyl benzoate) as a topical treatment for scabies during pregnancy was assessed using a population of 444 pregnant women and their matched controls (1,776 pregnant women).⁷⁹ The study population consisted of refugee and migrant women attending antenatal clinics on the Thai-Burmese border between August of 1993 and April of 2006. Most first treatments took place during the second and third trimesters, and the overall median gestation exposure was 24.5 weeks. Treated women (444) received 559 applications of butyl benzoate lotion (79.5%, 15.5%, 4.5%, and 0.15% receiving 1, 2, 3, and 4 treatments, respectively).

Conditional Poisson regression was used to estimate risk ratios for outcomes of pregnancy (proportion of abortions, congenital abnormalities, neonatal deaths, stillbirths, and premature babies), mean birth weight, and estimated median gestational age for scabies and scabies-free women. Regarding pregnancy outcomes, there were no statistically significant differences between women treated with the lotion and their matched controls. Thus, there was no evidence of adverse effects on pregnancy outcome due to topical application of benzyl benzoate (25%) lotion.⁷⁹

Benzoic Acid, Benzyl benzoate - Estrogenic effects

The estrogenic activity of benzoic acid was evaluated in the recombinant yeast human estrogen (ER_α) receptor assay *in vitro* and in uterotrophic assays involving immature female Alpk:AP₁SD rats (21 to 22 days old) and immature female Alpk:AP₁CD-1 mice.⁸⁰ Immature mouse uterotrophic assays involved 3 daily s.c. injections of benzoic acid in corn oil (100 and 1,000 µg/kg doses; 5 ml/kg = dose volume), ending on day 4. The same protocol was used for the rat assays, with the exception that benzoic acid in arachis oil was injected (doses of 10, 100, and 1,000 µg/kg).

Benzoic acid (10⁻⁷ to 10⁻³ M) was negative in the recombinant yeast human estrogen (ER_α) receptor assay *in vitro*. Overall (rat and mouse assays), benzoic acid produced one statistically significant and 3 statistically nonsignificant increases in average uterine weight and one significant and 6 nonsignificant decreases in average uterine weight. Therefore, in all uterotrophic assays, benzoic acid did not produce any reproducible evidence of estrogenic activity. Results for vehicle controls were negative and the positive control, estradiol, was uterotrophic.⁸⁰

The estrogenic activity of benzyl benzoate (concentration range: 10⁻⁹ to 10⁻⁴ M) *in vitro* was evaluated in the E-screen test using MCF-7 breast cancer cells. Untreated cultures served as controls. In this test, MCF-7 cells proliferate in the presence of estrogen. Cell numbers were assessed by measurement of the total protein content, using the sulforhodamine B assay. Compared to control cultures, benzyl benzoate did not increase the proliferation of MCF-7 cells (p > 0.05) over the range of concentrations tested.⁸¹

The estrogenic activity of benzyl benzoate, benzyl salicylate, and butylphenylmethylpropional (Lilial) in the estrogen-responsive MCF7 human breast cancer cell line was evaluated using the following assays: competitive binding assay to estrogen receptor (ER) of MCF7 cytosol, competitive binding assay to recombinant ER_α and ER_β, and the assay of stably transfected ERE-CAT reporter gene in MCF7 cells.⁸² In the latter assay, The ERE-CAT vector consisted of the estrogen response element (ERE) of the vitellogenin A2 gene from -331 to -295 bp cloned into the pBLCAT2 vector upstream of the thymidine kinase (tk) promoter. Cell proliferation experiments were also performed.

The following results indicate that all 3 chemicals produced estrogenic responses in cultured human breast cancer cells *in vitro*.⁸² At 3,000,000-fold molar excess, each chemical was able to partially displace [³H]estradiol from recombinant human estrogen receptors ER α and ER β , and from cytosolic ER of MCF7 cells.

At concentrations in the 5×10^{-5} to 5×10^{-4} M range, benzyl benzoate and the other 2 chemicals were able to increase the expression of a stably integrated estrogen-responsive reporter gene (ERE-CAT) and of the endogenous estrogen-responsive pS2 gene in MCF7 cells; however, these effects were at a lesser extent when compared to 10^{-8} M 17 β -estradiol (5,000 to 50,000 molar excess of benzyl benzoate).

In cell proliferation experiments, each chemical increased the proliferation of estrogen-dependent cells over a 7-day period. Cell proliferation was inhibited by fulvestrant (antiestrogen), suggesting an ER-mediated mechanism. However, over a 35-day period, the extent of proliferation in the presence of 10^{-4} M benzyl benzoate, benzyl salicylate, or butylphenylmethylpropional increased to the same magnitude as that observed in the presence of 10^{-8} M 17 β -estradiol over a 14-day period (10,000 molar excess of benzyl benzoate).⁸²

GENOTOXICITY

According to the OECD SIDS initial assessment report on benzyl alcohol, benzoic acid, and its sodium and potassium salt, the weight of evidence of the in vitro and in vivo genotoxicity data indicates that these chemicals are not mutagenic or clastogenic. Mixed/equivocal results were apparent in in vitro assays, but negative results were reported in in vivo genotoxicity assays. Other test results appear to be consistent with these findings. Benzoic acid was non-genotoxic in the micronucleus test, but results were positive for sodium benzoate and potassium benzoate in the spore rec-assay to assess DNA damaging activity. Also, sodium benzoate caused a significant increase in sister chromatid exchanges when compared to control cultures. Benzyl benzoate was not mutagenic in the Ames test, but results were positive for benzyl alcohol and benzoic acid in the in vitro comet assay for evaluating DNA-damaging potential.

Benzoic Acid, Sodium Benzoate, Potassium Benzoate, and Benzyl Alcohol

According to the OECD SIDS initial assessment report on benzyl alcohol, benzoic acid, and its sodium and potassium salt, each chemical was not mutagenic in *in vitro* Ames tests. Various results (negative and positive [chromosomal/chromatid responses]) for sodium benzoate, potassium benzoate, and benzyl alcohol were obtained in other *in vitro* genotoxicity assays. However, while some mixed and/or equivocal *in vitro* chromosomal/chromatid responses have been observed, sodium benzoate and benzyl alcohol were not genotoxic in the *in vivo* cytogenetic assay, the micronucleus test, or in other *in vivo* assays. The weight of evidence of the *in vitro* and *in vivo* genotoxicity data indicates that these chemicals are not mutagenic or clastogenic.²

In the *in vitro* comet assay, the DNA-damaging potential of benzoic acid and benzyl alcohol was evaluated using human blood cells (lymphocytes).⁸³ This assay is able to detect single and double strand breaks in DNA. Benzoic acid was evaluated at concentrations ranging from 0.05 to 5 mM and, benzyl alcohol, at concentrations ranging from 1 to 50 mM. Benzoic acid was tested at lower concentrations (≤ 5 mM) because this chemical has apoptotic effects at higher concentrations. The tail moment and % tail DNA in chemicals evaluated were compared to results for the solvent control (distilled water). Significantly increased tail moment and % tail DNA were noted only with 5 mM benzoic acid and with 25 and 50 M benzyl alcohol, indicating genotoxicity. A dose response was not observed.

Benzoic acid (in DMSO) was evaluated in the micronucleus test at concentrations ranging from 250 to 1,000 μ g/ml (with and without metabolic activation) using L5178Y TK+/- clone 3.7.2C mouse lymphoma cells. Cultures without benzoic acid served as negative controls and mitomycin C served as the positive control. Benzoic acid was non-genotoxic over the range of concentrations tested, both with and without metabolic activation. The positive control was genotoxic.⁸⁴

The genotoxicity of sodium benzoate was evaluated in the sister chromatid exchanges assay (SCE assay) using human blood lymphocyte cultures incubated for 72 h. Preparations were scored blindly for cells in their first mitosis, second mitosis, and third and subsequent divisions. Forty SCEs second division cells from each culture were scored for SCEs. Compared to

negative control cultures, sodium benzoate (0.02, 0.2, 2, 4, and 8 mM) caused statistically significant delays in cell division ($p < 0.01$), indicative of weak cytostatic activity, but did not induce cytotoxicity. Also, when compared to negative control cultures, 8 mM sodium benzoate induced a statistically significant increase ($p < 0.01$) in SCEs/cell. Sodium benzoate (2 mM) induced a statistically significant decrease ($p < 0.05$) in SCEs/cell when compared to the 8 mM sodium benzoate culture.⁸⁵

The DNA-damaging activity of sodium benzoate and potassium benzoate was evaluated in the spore rec-assay using *Bacillus subtilis* M45 (rec⁻) and H17 (rec⁺) strains with and without metabolic activation. Sodium benzoate (in water) was tested at doses of 16 and 20 mg/disk and potassium benzoate (in mixed solution of water and ethanol [1:1]) was tested at doses of 15 and 20 mg/disk. Results for both chemicals were judged positive in this assay.⁸⁶

Benzyl Benzoate

In the Ames test, benzyl benzoate was not mutagenic to *Salmonella typhimurium* strains TA98 or TA 100 at doses up to 5,000 µg/plate (plate incorporation assay) or 5,000 mg/plate (pre-incubation assay). Benzyl Benzoate also was not mutagenic to the following strains at doses up to 3 µmol/plate (pre-incubation assay) with or without metabolic activation: TA98, TA100, TA1525, and TA 1537. In the recombination assay, benzyl benzoate was not mutagenic to *Bacillus subtilis* strains H17 or M45 at a concentration of 10 mg/disk.³

In another Ames test, the mutagenicity of benzyl benzoate (in ethanol) was evaluated using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537. Benzyl benzoate was tested at a concentration of 3 µmol/plate with and without metabolic activation and was not found to be mutagenic.⁸⁷

Photogenotoxicity

The photogenotoxicity of benzoic acid (0.5%) and sodium benzoate (0.5%) has been demonstrated in vitro using bacterial cell suspensions.

Benzoic Acid and Sodium Benzoate

The photogenotoxicity of benzoic acid (0.5%) and sodium benzoate (0.5%) and other food additives was evaluated using *Escherichia coli* cell suspensions.⁸⁸ Initially, the cytotoxic and genotoxic potential of each in the absence of sunlight (200 to 3,000 nm; mean intensity = $5.8 \times 10^2 \text{ Wm}^{-2}$) was evaluated by allowing the cells to remain in contact with the additive for 80 min in the dark. Neither benzoic acid nor sodium benzoate affected cell viability (i.e., not cytotoxic) or the number of spontaneous mutations in the absence of sunlight. The number of spontaneous mutations was 2.09 mutations per 10^8 cells. Also, these chemicals did not induce mutations in the absence of sunlight.

Plates containing the cellular suspension (5 ml) and a food additive (0.5% concentration) were exposed to sunlight for periods ranging from 0 min to 60 min. Exposure to sunlight resulted in cell death in the presence or absence of benzoic acid or sodium benzoate. When cells were exposed to direct sunlight in the absence of benzoic acid or sodium benzoate, the number of induced mutations increased with duration of exposure (5.1×10^2 mutations per 10^8 cells after 60 min). In the presence of benzoic acid or sodium benzoate, the number of induced mutations increased with the duration of exposure to sunlight. This increase was almost additive over that observed with sunlight exposure alone. After 60 min, there were approximately 1×10^3 mutations per 10^8 cells.⁸⁸

Effect on DNA Synthesis

Sodium benzoate caused a significant increase in the DNA content of protozoan nuclei, suggestive of stimulation of mitosis.

Sodium Benzoate

Aqueous sodium benzoate, at a concentration of 7.20 mg/ml, was added to *Tetrahymena pyriformis* in an experimental series consisting of six 100 ml cultures. Another experimental series, without sodium benzoate, served as the control. The quantitative analysis of DNA content of the protozoan nuclei was performed using an image analysis system. Sodium

benzoate caused a statistically significant increase ($p < 0.01$) in DNA content, suggestive of stimulation of mitosis. The authors speculated that this observation might represent a direct cytotoxic effect due to increased DNA content of nuclei.⁸⁹

CARCINOGENICITY

Benzy alcohol was not carcinogenic when tested dermally on mice at 2.00% in a nonoxidative hair dye. The National Toxicology Program (NTP) considered benzy alcohol negative for carcinogenicity following 2 years of oral dosing in rats (doses up to 400 mg/kg) and mice (doses up to 200 mg/kg), but the EPA considered the 3 of 48 incidence of adrenal cortex adenoma (male mice) to be equivocal evidence of carcinogenic activity rather than negative. Following dermal application to mice, a nonoxidative hair dye containing 2.0% benzy alcohol and 0.016% benzoic acid was negative for carcinogenicity. Sodium benzoate was negative for carcinogenicity when administered orally at up to 2% to rats (in feed for up to 2 years) or mice (in a life-time drinking water study).

The following carcinogenicity study summaries are included in the CIR final report on benzy alcohol, benzoic acid, and sodium benzoate that was published in 2001.¹

Benzy Alcohol and Benzoic Acid

Groups of 100 F344/N rats (50 each sex) were dosed orally with 200 or 400 mg/kg benzy alcohol in corn oil, 5 days per week for 103 weeks. Groups of 100 B6C3F₁ mice were dosed with 100 or 200 mg/kg benzy alcohol following the same schedule. During week 80, mice were mistakenly dosed for four days with 375 (low-dose group) and 750 mg/kg (high-dose group) of α -methylbenzy alcohol. Dose-related negative trends were noted in the incidences of anterior pituitary gland neoplasms in female rats (vehicle control, 29/50; low dose, 17/47; high dose, 9/49) and of Harderian gland adenomas in male mice (8/50; 3/50; 2/50). Epithelial hyperplasia of the nonglandular stomach was noted in 4 of 50 high-dose male rats; it was not found in controls or low-dose male rats. An increased incidence of adenomas of the adrenal cortex noted in high-dose male mice (0/48; 0/44; 3/48) was within historical range and not considered compound-related. The NTP investigators considered the study negative for benzy alcohol-induced carcinogenicity. However, reviewing the study, the EPA considered the 3 of 48 incidence of adrenal cortex adenoma to be “equivocal evidence of carcinogenic activity rather than negative.”

From the Final Report on benzy alcohol, benzoic acid, and sodium benzoate¹

A skin painting study was performed using groups of 120 Eppley Swiss mice (60 per sex). A non-oxidative hair dye containing 2.0% benzy alcohol and 0.016% benzoic acid was painted onto the skin at a dose of 0.05 ml/application, three times weekly for 20 months. Sites were shaved of hair 24 h before each application and a new bottle of dye was used each week. Two groups of control animals were shaved but not treated. Nine months into the study, 10 mice/sex/group were killed. Body weights and survival differed little between treatment and control groups. Varying degrees of chronic dermal inflammation were noted in all groups, including the controls. A significant ($p < .01$) increase in malignant lymphomas was noted in treated females (23/60). However, the researchers noted that one concurrent control group had a very low incidence (7/60 or 12%) of that tumor type. The rate was 22% for the other control group and had averaged 33% for three control groups in previous studies. Thus, the findings were not considered treatment-related. The incidence of pulmonary adenomas and hepatic hemangiomas, which are common in this mouse strain, were similar between treated and control groups. No unusual neoplasms were observed.

From the Final Report on benzy alcohol, benzoic acid, and sodium benzoate¹

Sodium Benzoate

For 18 to 24 months, groups of Fischer 344 rats (50 males and 52 females per group) received feed containing 2% or 1% sodium benzoate. The doses corresponded to the maximum tolerated dose (MTD) and $\frac{1}{2}$ MTD as determined in 6-week toxicity studies. A control group of 25 male and 43 female rats received untreated feed. Average daily sodium benzoate intake was 280 and 202 mg, respectively, for male and female rats of the 2% group, and 141 and 102 mg, respectively, for male and female rats of the 1% group. No clinical signs of toxicity or differences in average body weight or mortality

rates were noted in treated rats when compared with controls. Neoplasms that were present in treated rats were similar in type and number to those in controls. No evidence of sodium benzoate-related carcinogenicity was observed.

From the Final Report on benzyl alcohol, benzoic acid, and sodium benzoate¹

In a life-time drinking water study, 100 Albino Swiss mice (50 of each sex) were supplied with water containing 2% sodium benzoate. A control group of 200 mice was supplied with untreated water. Average daily intake of sodium benzoate was 124.0 and 119.2 mg for males and females, respectively. Sodium benzoate treatment did not affect survival. No carcinogenic effect attributable to treatment was noted at necropsy.

From the Final Report on benzyl alcohol, benzoic acid, and sodium benzoate¹

SUMMARY

The safety of the following ingredients in cosmetics is reviewed in this safety assessment: Benzyl alcohol, benzoic acid, sodium benzoate, calcium benzoate, magnesium benzoate, potassium benzoate, and benzyl benzoate. Most of these ingredients function as fragrance ingredients/preservatives in cosmetic products. Together, data reported to the Food and Drug Administration's Voluntary Cosmetic Registration Program in 2010 and the results of a 2010 Personal Care Products industry survey indicated use of the following ingredients in cosmetics: benzyl alcohol, benzoic acid, sodium benzoate, calcium benzoate, potassium benzoate, and benzyl benzoate. According to this industry survey, ingredient use concentrations have ranged from 0.000001% (sodium benzoate) to 10% (benzyl alcohol).

Among the methods of manufacture identified are the production of benzyl alcohol via the action of sodium or potassium carbonate on benzyl chloride and the production of benzoic acid via the decarboxylation of phthalic anhydride. Hydroxyl radical generated by the metal-catalyzed reduction of O_2 and H_2O_2 by ascorbic acid can attack benzoic acid to produce benzene under conditions that are prevalent in beverages. Additionally, exposure to UV light and elevated temperature over the shelf life of beverages may result in benzene formation in products containing benzoic and ascorbic acids. The U.S. beverage industry voluntarily reformulated beverages that were found to contain benzene levels at or above the maximum contaminant level for drinking water established by the U.S. Environmental Protection Agency. Limits for heavy metal impurities in food-grade sodium benzoate (as Pb, ≤ 2 mg/kg) and pharmaceutical-grade potassium benzoate (heavy metals, $\leq 0.001\%$) have been established.

Benzyl alcohol is metabolized to benzoic acid via simple oxidation. Benzoic acid and sodium benzoate are rapidly absorbed from the gastrointestinal tract of mammals, conjugated with glycine in the liver, and then excreted as hippuric acid. In *in vitro* studies, the percutaneous absorption of benzoic acid through guinea pig skin and the percutaneous absorption of benzyl alcohol and benzyl benzoate through human skin was reported. The percutaneous absorption and urinary excretion of benzyl alcohol and benzyl benzoate was observed following dermal application to rhesus monkeys.

Benzoic acid (up to 1,000 μ g/kg, s.c. injection) did not induce any reproducible evidence of estrogenic activity in uterotrophic assays involving immature female Alpk:AP₁SD rats and female Alpk:AP₁CD-1 mice. However, benzyl benzoate induced estrogenic responses in cultured human breast cancer cells *in vitro*. At 3,000,000-fold molar excess, benzyl benzoate was able to partially displace [³H]estradiol from recombinant human estrogen receptors ER α and ER β , and from cytosolic ER of MCF7 cells. The antiparasitic activity of benzyl benzoate has been demonstrated *in vitro*.

Neither acute (up to 12 mg/L aerosol/dust) nor repeated inhalation (up to 1,000 mg/m³) exposures to benzyl alcohol or benzoic acid caused death in rats. Results of the repeated dose inhalation toxicity study also indicated no test substance-related macroscopic or microscopic findings for either test substance. Overall, the results of acute oral and dermal toxicity studies indicated a low level of toxicity for benzyl alcohol, benzoic acid, and benzyl benzoate. The same was true for repeated dose oral toxicity studies on benzoic acid and its salts and benzyl alcohol. Systemic/dermal effects induced by benzyl benzoate were observed in rats receiving repeated dermal doses up to 2.0 g/kg and in rabbits receiving repeated dermal doses up to 4.0 ml/kg. However, test substance-related toxic signs were not observed in other species that received repeated doses of benzyl benzoate.

Benzyl alcohol (4% aqueous), benzoic acid (undiluted), and its sodium salt (concentration not stated) were irritating to the eyes of rabbits, and it was expected that potassium benzoate would be irritating as well. Benzyl benzoate (undiluted) was irritating to the eyes of rabbits and humans. Undiluted benzyl alcohol and benzoic acid were slightly irritating and benzyl benzoate was non-irritating to the skin at concentrations up to 50% in animal studies. Undiluted benzyl benzoate was a non-irritant in human subjects. In animal studies, benzyl benzoate induced sensitization reactions ranging from none to moderate. Moderate sensitization was observed at a concentration of 10% benzyl benzoate, but not at lower concentrations, and there was one report of mild sensitization at a concentration of 40%. Benzyl benzoate was classified as non-sensitizer when tested at a concentration of 30% in healthy human subjects. Most of the skin sensitization studies on benzyl benzoate involved groups of patients, and mixed results regarding sensitization potential were reported.

A slightly positive reaction was observed in a phototoxicity study involving hairless mice tested with an unspecified concentration of benzyl benzoate (4, 24 h exposures). Photo-irritation was not observed in guinea pigs tested with benzyl benzoate at concentrations up to 30%. Moderate photohemolytic activity in human erythrocytes was observed in the presence of benzyl benzoate, but significant photohemolytic activity was not associated with sodium benzoate or benzyl alcohol in these cells.

Developmental effects only were observed in the presence of marked maternal toxicity in rats fed sodium benzoate in the diet (≥ 2800 mg/kg/day; NOAEL = 1400 mg/kg/day). Testicular atrophy was observed in rabbits that received repeated dermal doses of benzyl benzoate (> 0.5 g/kg/day). However, overall, benzyl alcohol, benzoic acid, and sodium benzoate were not classified as reproductive/developmental toxicants in oral and dermal animal studies. There was no evidence of adverse effects on pregnancy outcome due to topical application of benzyl benzoate (25%) lotion in pregnant women.

Mixed/equivocal results were apparent in *in vitro* assays, but negative results were reported in *in vivo* genotoxicity assays on benzyl alcohol, benzyl benzoate, benzoic acid, and its sodium and potassium salt. The photogenotoxicity of benzoic acid (0.5%) and sodium benzoate (0.5%) has been demonstrated *in vitro* using bacterial cell suspensions. Benzyl alcohol was negative for carcinogenicity when dermally tested on mice at 2.00% in a nonoxidative hair dye. NTP considered it negative for carcinogenicity following 2 years of oral dosing in rats (up to 400 mg/kg) and mice (up to 200 mg/kg), but the EPA considered the 3 of 48 incidence of adrenal cortex adenoma (male mice) to be equivocal evidence of carcinogenic activity rather than negative. Following dermal application to mice, a nonoxidative hair dye containing 2.0% benzyl alcohol and 0.016% benzoic acid was negative for carcinogenicity. Sodium benzoate was negative for carcinogenicity when administered orally at up to 2% to rats (in feed for up to 2 years) or mice (in a life-time drinking water study).

DISCUSSION

The Expert Panel noted gaps in the available safety data for some of the ingredients in this safety assessment. The available data on many of the ingredients are sufficient, however, and similarity between structures and structure activity relationships suggest that the available data can be extrapolated to support the safety of the entire group.

The negative inhalation toxicity data provided satisfied the CIR Expert Panel's request for data and support the safe use of these ingredients in cosmetic products in which inhalation is a primary route of exposure.

The Panel did note the formation of adenomas in the adrenal cortex of B6C3F₁ mice receiving a high dose of benzyl alcohol in an oral carcinogenicity study. These adenomas were considered benign by the NTP, and the Panel concurs. The Panel acknowledged that EPA reviewed the results of this study and concluded that the results suggest equivocal evidence of carcinogenicity, but it was the Panel's view that there was no evidence of carcinomas in the animals.

Data from model studies, pertaining to the beverage industry, reviewed by the Expert Panel raised the possibility that benzene could be formed from benzoic acid and benzoates in cosmetics. The yields of benzene, however, that could be generated by photodegradation are sufficiently low that they do not constitute a relevant hazard.

In its previous safety assessment of benzyl alcohol, benzoic acid, and sodium benzoate, the Panel established a 5% safe concentration limit for benzyl alcohol, benzoic acid, and sodium benzoate and a 10% safe concentration limit for benzyl

alcohol in hair dyes. On review of the current practices of use and concentration, it was clear that industry has adhered to those limits. Accordingly, reference to present practices of use and concentration is sufficient to assure safety.

CONCLUSION

CIR Expert Panel concluded that the following cosmetic ingredients are safe in the present practices of use and concentration described in this safety assessment:

- benzyl alcohol
- benzoic acid
- sodium benzoate
- calcium benzoate
- magnesium benzoate*
- potassium benzoate
- benzyl benzoate

*Were ingredients in this group not in current use to be in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in the group.

Table 1. Benzyl Alcohol and Benzoic Acid and its Salts and Ester²²

Chemical Names/CAS Nos.	Definitions*	Functions in Cosmetics
Benzyl Alcohol Alcohol benzylicus; benzenemethanol; benzylic alcohol; phenylcarbinol; phenylmethanol; phenylmethyl alcohol; and α -toluenol; CAS No. 100-51-6	An aromatic alcohol	External analgesics; fragrance ingredients; oral health care drugs; preservatives; solvents; and viscosity decreasing agents
Benzoic Acid Acidum benzoicum; benzenecarboxylic acid; benzeneformic acid; benzenemethanoic acid; carboxybenzene; dracyclic acid; phenylcarboxylic acid; and phenylformic acid; CAS No. 65-85-0	An aromatic acid	Fragrance ingredients; preservatives; and pH adjusters
Sodium Benzoate Benzoic acid, sodium salt and natrii benzoas (EP); CAS No. 532-32-1	The sodium salt of benzoic acid	Corrosion inhibitors; fragrance ingredients; and preservatives
Calcium Benzoate Benzoic Acid, Calcium Salt and Calcium Dibenzoate; CAS No. 2090-05-3	The calcium salt of benzoic acid	Preservatives
Magnesium Benzoate Benzoic Acid, magnesium salt and magnesium dibenzoate; CAS No. 553-70-8	The magnesium salt of benzoic acid	Preservatives
Potassium Benzoate Benzoic Acid, Potassium Salt; CAS No. 582-25-2	The potassium salt of benzoic acid	Preservatives
Benzyl Benzoate Benylate; benzoic acid, benzyl ester; benzoic acid, phenylmethyl ester; benzyliis benzoas (EP); and phenylmethyl benzoate; CAS No. 120-51-4	The ester of benzyl alcohol and benzoic	Fragrance ingredients; pesticides; and solvents

*See Figure 1 for structures, completing the definition of each chemical.

Table 2. Properties of Benzyl Alcohol and Benzoic Acid and its Salts and Ester⁹⁰

Properties	Benzyl Alcohol	Benzoic Acid	Sodium Benzoate	Calcium Benzoate	Magnesium Benzoate	Potassium Benzoate	Benzyl Benzoate
Form	Liquid	Monoclinic plates	White granules		White powder	White solid ²	Leaflets/oily liquid
Molecular weight	108.14	122.12	144.1	121.12 ⁹¹	266.53	160.21	212.24
Density	1.045	1.321					1.118
Melting point	-15.19°C	122.4°C	330.6°C ²		200 ⁰	330.6°C ²	21°C
Boiling point	204.7°C	249.2°C	464.9°C ²			464.9°C ²	323-324°C
Water Solubility	dissolves (1g) in ~ 25 ml water	2.9 g/l	1 g dissolves in 1.8 ml water		Soluble in 20 parts water	556 g/l ²	Practically insoluble in water
logP	1.1 ²	1.8 ²	-2.269 ²		1.53 ⁹²	-2.269 ²	3.68 ⁹³
UV absorption max.					228.6 nm ⁹¹	226.8 nm ⁹¹	256 nm ⁹¹

Table 3. Current Frequency and Concentration of Use According to Duration and Type of Exposure Provided in 2011^{23,24}

	Benzoic Acid		Benzyl Alcohol		Sodium Benzoate		Calcium Benzoate	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type								
<i>Eye Area</i>	23	0.00005 to 0.2	109	0.000008 to 0.8	58	0.00001 to 0.4	NR	NR
<i>Incidental Ingestion</i>	64	0.003 to 2	23	0.000006 to 0.9	33	0.001 to 0.6	NR	NR
<i>Incidental Inhalation-sprays</i>	16	0.000003 to 0.5	496	0.0002 to 3	43	0.001 to 0.5	NR	0.004
<i>Incidental Inhalation-powders</i>	4	0.000002 to 0.001	28	0.0001 to 0.3	4	0.3	NR	0.002 to 0.004
<i>Dermal Contact</i>	503	0.000002 to 5	1673	0.000008 to 3	1075	0.000001 to 1	NR	
<i>Deodorant (underarm)</i>	1	0.03 to 0.5	38	0.03 to 0.5	2	0.5		
<i>Hair - Non-Coloring</i>	97	0.00002 to 1	361	0.0002 to 3	431	0.000005 to 1	NR	NR
<i>Hair-Coloring</i>	22	0.004 to 0.03	56	0.00007 to 10	18	0.008 to 0.5	NR	NR
<i>Nail</i>	7	0.0005 to 0.2	3	0.00002 to 0.5	7	0.00001 to 0.001	NR	NR
<i>Mucous Membrane</i>	201	0.000003 to 2	269	0.000006 to 1	407	0.0004 to 1	NR	NR
<i>Baby Products</i>	3	0.000002 to 0.08	25	0.004 to 0.9	26	0.3 to 1	NR	NR
Duration of Use								
<i>Leave-On</i>	378	0.000002 to 5	1491	0.000008 to 3	619	0.000001 to 1	NR	0.002 to 0.004
<i>Rinse off</i>	295	0.000003 to 5	651	0.000006 to 10	936	0.000001 to 1	NR	NR
<i>Diluted for (bath) use</i>	23	0.000003 to 0.08	20	0.001 to 0.9	15	0.004 to 1	NR	NR
Totals/Conc. Range	696	0.000002 to 5	2162	0.000006 to 10	1570	0.000001 to 1	NR	0.002 to 0.004
	Potassium Benzoate		Benzyl Benzoate		Magnesium Benzoate			
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)		
Exposure Type								
<i>Eye Area</i>	NR	NR	5	0.0006 to 0.002	NR	NR		
<i>Incidental Ingestion</i>	NR	NR	82	0.000005 to 0.2	NR	NR		
<i>Incidental Inhalation-sprays</i>	NR	NR	583	0.002 to 4	NR	NR		
<i>Incidental Inhalation-powders</i>	NR	NR	30	0.001 to 0.3	NR	NR		
<i>Dermal Contact</i>	NR	0.002	1321	0.0001 to 4	NR	NR		
<i>Deodorant (underarm)</i>	NR	NR	47	NR	NR	NR		
<i>Hair - Non-Coloring</i>	1	NR	259	0.006 to 4	NR	NR		
<i>Hair-Coloring</i>	NR	NR	21	0.07 to 2	NR	NR		
<i>Nail</i>	1	0.0003	26	0.2 to 0.8	NR	NR		
<i>Mucous Membrane</i>	NR	0.0002	227	0.000005 to 2	NR	NR		
<i>Baby products</i>	NR	NR	3	NR				
Duration of Use								
<i>Leave-On</i>	2	0.0003	1396	0.000005 to 4	NR	NR		
<i>Rinse off</i>	NR	0.0002	282	0.0001 to 2	NR	NR		
<i>Diluted for (bath) use</i>		0.0002 to 0.0003	31	0.0005 to 2	NR	NR		
Totals/Conc. Range	2	0.0002 to 0.0003	1709	0.000005 to 4	NR	NR		

NR = Not Reported; Totals = Rinse-off + Leave-on Product Uses.

Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

Table 4. Acute Oral Toxicity Studies on Potassium and Benzyl Benzoate

Material	Animals	Test Procedure	Results
Potassium Benzoate	Rats, mice, and guinea pigs	Not stated	LD50 > 10,000 mg/kg ²
Benzyl Benzoate (undiluted)	10 rats/dose	Oral dosing followed by 6-day observation period; no GLP*	LD50 = 1891 mg/kg ³
Benzyl Benzoate	20 rats	Single dose by gavage; 14-day observation period; no GLP	LD50 = 2800 mg/kg ³
Benzyl Benzoate	Rats (no. not stated)	Not stated	LD50 = 500 mg/kg ³
Benzyl Benzoate (in arachis oil)	Rats (no. not stated)	OECD guideline 401; 100 to 2000 mg/kg oral doses; GLP study	LD50 > 2000 mg/kg ³
Benzyl Benzoate (in arachis oil)	10 Sprague-Dawley albino rats	Single 5.0 ml/kg dose; 14-day observation period	LD50 > 2000 mg/kg ⁹⁴
Benzyl Benzoate	Rats (no. not stated)	Not stated	LD50 = 1700 mg/kg ⁹⁵
Benzyl Benzoate (1% in gum tragacanth suspension)	5 groups of 10 Sprague-Dawley rats	Doses up to 4000 mg/kg total (i.e., four 250 mg/kg doses at 40 ml/kg dose volume/dose); 14-day observation period	LD50 = 1.16 g/kg ⁹⁶
Benzyl Benzoate (in Tween 80 + gum tragacanth vehicle)	6 groups of 10 Sprague-Dawley rats 5 males, 5 females/group)	6 different dose levels (dose volume = 40 ml/kg)	LD50 = 1160 mg/kg ⁹⁷
Benzyl Benzoate (in tween 80 solution + methylcellulose vehicle)	Rats (25 males, 25 females)	Not stated	LD50 = 1550 mg/kg ⁹⁸
Benzyl Benzoate (in tween 80 solution + gum tragacanth vehicle)	Sprague-Dawley rats (5 males, 5 females)	10 mg/kg total dose (i.e., four 2500 mg/kg doses); 14-day observation period	LD50 > 10 g/kg ⁹⁹
Benzyl Benzoate	10 rats/dose	Dosing followed by 6-day observation period	LD50 = 1.7 ml/kg ¹⁰⁰
Benzyl Benzoate	20 rats	Dosing followed by 2-week observation period	LD50 = 2.8 g/kg ⁶³
Benzyl Benzoate (undiluted)	10 mice/dose	Dosing followed by 6-day observation period; no GLP	LD50 = 1557 mg/kg ³
Benzyl Benzoate	10 mice/dose	Dosing followed by 6-day observation period	LD50 = 1.4 ml/kg ¹⁰⁰
Benzyl Benzoate	Mice (no. not stated)	Not stated	LD50 = 1400 mg/kg ³
Benzyl Benzoate (undiluted)	10 rabbits/dose	Dosing followed by 6-day observation period; no GLP	LD50 = 2002 mg/kg ³
Benzyl Benzoate	10 rabbits/dose	Dosing followed by 6-day observation period	LD50 = 1.8 ml/kg ¹⁰⁰
Benzyl Benzoate	9 rabbits	Single dose by gavage; 14-day observation period; no GLP	LD50 = 1680 mg/kg ³
Benzyl Benzoate	12 rabbits	Dosing followed by 2-week observation period	LD50 = 1.68 g/kg ⁶³
Benzyl Benzoate	11 cats	Single dose by gavage; 14-day observation period; no GLP	LD50 = 2240 mg/kg ³
Benzyl Benzoate	4 dogs	Single dose by gavage; 14-day observation period; no GLP	LD50 > 2244 mg/kg ³
Benzyl Benzoate (undiluted)	10 guinea pigs/dose	Oral dosing followed by 6-day observation period ; no GLP	LD50 = 1112 mg/kg ³
Benzyl Benzoate	Guinea pigs (no. not stated)	Not stated	LD50 = 1000 mg/kg ³
Benzyl Benzoate	10 guinea pigs/dose	Dosing followed by 6-day observation period	LD50 = 1 mg/kg ¹⁰⁰

*GLP = good laboratory practice

Table 5. Skin Irritation and Sensitization Studies

Test Substance	Animals/Subjects	Doses/Concentrations Tested	Procedure	Results
Animal Studies				
Benzyl alcohol (purity not stated)	Rabbits (no. not stated)	undiluted benzyl alcohol (10 mg)	dose applied for 24 h	Slightly irritating ²
Benzoic Acid (purity not stated)	3 albino rabbits	undiluted benzoic acid (0.5 g/kg)	dose applied to flank under semi-occlusive dressing for 4 h	Slightly irritating ²
Sodium Benzoate	Rabbits (no. not stated)	undiluted benzyl alcohol (10 mg)	dose applied for 24 h	Slightly irritating ²
Sodium Benzoate	Rabbits (no. not stated)	Concentration not stated	OECD Guide - line 404 protocol	Non-irritating ²
Benzyl Benzoate	Guinea pigs (no. not stated)	undiluted benzyl benzoate (15 g/kg)	Dose applied to intact abdominal skin (duration not stated)	Non-irritating ⁵⁹
Benzyl Benzoate (50%)	Guinea pigs (no. not stated)	up to 50%	Not stated	Non-irritant ⁷¹
Benzyl Benzoate (40%)	10 female Hartley albino guinea pigs	10% induction concentration; challenge concentrations up to 40%	10% w/v in complete adjuvant (intradermal induction); 10% w/v in Freund's complete adjuvant + saline (topical induction); topical challenge concentrations up to 40% benzyl benzoate in propylene glycol + acetone	No reactions at challenge concentrations of 5% and 10% benzyl benzoate; mild reactions at 40% challenge concentration (4 of 10 animals)
Benzyl Benzoate (25% in acetone/PEG vehicle)	4 male guinea pigs	up to 25%	24-h occlusive patch test	Non-irritant ¹⁰¹
Benzyl Benzoate (10%)	Guinea pigs (no. not stated)	10%	Maximization test	Moderate sensitization ¹⁰²
Benzyl Benzoate (10% in ethanol)	4 female guinea pigs	up to 10%	24-h occlusive patch test; up to 10% during induction and challenge with 2.5%	Non-irritating at 5 and 10% concentrations. Barely perceptible erythema (at 24 and 48 h) at 2.5% concentration ¹⁰³
Benzyl Benzoate (10% in acetone)	10 guinea pigs	10%	Modified Freund's complete adjuvant test	Weak sensitization ¹⁰⁴
Benzyl Benzoate (1 and 10%)	Guinea pigs (no. not stated)	1% and 10% (15 or 30 mg)	Modified Freund's complete adjuvant test: Challenge with 1% and 10% benzyl benzoate (in acetone)	Weak sensitization at both concentrations ¹⁰⁵
Benzyl Benzoate	Guinea pigs (no. not stated)	Not stated	Maximization test (no GLP)	Non-sensitizer ³
Benzyl Benzoate	Guinea pigs (6 to 8 per group)	Not stated	Open epicutaneous test (24 h application)	Non-sensitizer ³
Benzyl Benzoate	4 female New Zealand albino rabbits	Undiluted benzyl benzoate (0.5 ml/2.5 cm ²)	4 h semiocclusive patch test	Non-irritant ¹⁰⁶
Benzyl Benzoate	Rabbits (no. not stated)	Not stated	3-week subacute skin toxicity test	Very mild gross skin irritation ¹⁰⁰
Predictive Tests				
Benzyl Alcohol	31 healthy subjects	0.2 ml benzyl alcohol	Hill Top chamber with Webril pad applied to upper arm for up to 4 h	Skin irritation in 1 subject ¹⁰⁷
Benzyl Alcohol lotion 5% (Ulesfia TM)	485 subjects	Lotion tested as supplied	Topical treatment of head lice program	Low frequency of application site irritation (2.3% incidence) ⁴²

Table 5. Skin Irritation and Sensitization Studies

Test Substance	Animals/Subjects	Doses/Concentrations Tested	Procedure	Results
Benzoic Acid	86 healthy subjects (mean age = 37.3 years)	1 M in petrolatum	Finn chambers applied to volar arm for 20 min	Caused contact urticaria ¹⁰⁸
Benzoic Acid	58 healthy subjects (mean age = 39 years)	1 M in petrolatum (50 µl volume)	Finn chambers applied to volar arm for 20 min	Caused contact urticaria ¹⁰⁹
Benzyl Benzoate	18 subjects (18+ years)	5 to 10 drops of undiluted benzyl benzoate	Closed patch containing benzyl benzoate applied to healthy and locally inflamed skin for up to 48 h	No skin irritation ⁵⁹
Benzyl Benzoate	Male and female subjects (no. not stated; 18+ years)	undiluted benzyl benzoate (1cm-diameter area)	24-h closed patch test	No skin irritation ¹¹⁰
Benzyl Benzoate (50% in diethyl phthalate/ethanol)	21 subjects (24 to 70 years old)	50% (0.3 ml)	24-h occlusive patch test	Minimal erythema in 3 subjects; negligible irritation potential ¹¹¹
Benzyl Benzoate (50% in ethanol/diethyl phthalate)	129 subjects (46 males, 83 females)	50% (0.3 ml)	RIPT (24-h applications)	No effects ¹¹²
Benzyl Benzoate (30% in petrolatum)	Patients (no. not stated)	30%	Maximization test	No positive reaction ³
Benzyl Benzoate (30% in petrolatum)	20 healthy males	30%	Occlusive patch test; 5 48 h applications to evaluate sensitization potential	No positive reaction ¹¹³
Benzyl Benzoate (20% in vaselinum alidum or unguentum hydrophilicum vehicle)	34 healthy male and female subjects	20%	48-h closed patch test	No skin irritation ¹¹⁴
Benzyl Benzoate (4% in petrolatum)	25 healthy male and female subjects	4%	48-h closed patch test	No skin irritation ¹¹⁵
Benzyl Benzoate (2% in unguentum simplex or unguentum hydrophilicum vehicle)	30 healthy male and female subjects (18 + years)	2%	24- to 72-h closed patch test	No skin irritation ¹¹⁴
Provocative Tests/Treatment				
Benzyl Alcohol (1% in petrolatum)	11,373 dermatitis patients (males and females)	1%	24 h or 48 h patch test (Finn chambers)	Questionable/irritant reactions (62 patients); sensitization reactions (46 patients) ¹¹⁶
Benzyl Alcohol (5% in petrolatum)	102 patients	5%	24 h patch tests (Finn chambers)	8 subjects with positive reactions ¹¹⁷
Benzyl Alcohol (1%)	2,166 dermatitis patients	1%	24 or 48 h patch tests	0.3% with positive reactions ¹¹⁸
Benzoic Acid (5% in physiological saline)	7 patients (males and females) with birch pollen allergy	5%	45 min epicutaneous application (AL-tests on Scanpor)	Erythema at application sites of all patients (contact urticaria) ¹¹⁹
Benzoic Acid (5% in petrolatum)	102 patients	5%	24 h patch tests (Finn chambers)	20 subjects with positive reactions ¹¹⁷
Benzoic Acid	1,252 patients with oral mucosal disease	Concentration not stated	Contact urticaria test; Delayed hypersensitivity test	366 patients positive (urticaria test); 124 patients positive (hypersensitivity test) ¹²⁰

Table 5. Skin Irritation and Sensitization Studies

Test Substance	Animals/Subjects	Doses/Concentrations Tested	Procedure	Results
Benzoic Acid	417 patients	Concentration not stated	48 h occlusive patch tests (Finn chambers; 10 mm diameter)	5% of patients with questionable/irritant reactions; 4.3% with allergic reactions ¹²¹
Benzoic Acid	465 patients	Concentration not stated	Not stated	Positive reactions (allergenicity) in 2.1% of patients ¹²²
Sodium Benzoate	465 patients	Concentration not stated	Not stated	Positive reactions (allergenicity) in 1.9% of patients ¹²²
Benzyl Benzoate (20% emulsion)	1,000 scabies patients	20%	Treatment for scabies	No dermatitis after treatment ¹²³
Benzyl Benzoate (0.1, 2, and 20% in vaseline ointment or cream base)	175 patients with and without dermatoses	0.1, 2, and 20%	Closed patch test (no GLP) to evaluate sensitization potential	No positive reactions ³
Benzyl Benzoate (10% in water)	73 dermatitis patients	10%	Patch test (no GLP) to evaluate sensitization potential	No positive reactions ³
Benzyl Benzoate (5%)	11 male and female patients (18+ years)	5%	24 h application	Positive reaction in 3 of 8 patients ¹⁰⁴
Benzyl Benzoate (5% in petrolatum)	20 male and female patients (18+ years)	5%	48-h patch test (aluminum-backed strips)	Positive reaction in 1 of 20 patients ¹²⁴
Benzyl Benzoate (5% in petrolatum)	45 dermatitis patients (18+ years)	5%	48-h closed patch test	No reaction ¹²⁵
Benzyl Benzoate (5% in petrolatum)	73 eczematous dermatitis patients	5%	Patch test (no GLP)	1 positive reaction ³
Benzyl Benzoate (5% in petrolatum)	Eczema patients (no. not stated)	5%	Patch test (no GLP)	No positive reactions ³
Benzyl Benzoate (5% in petrolatum)	8 dermatitis patients	5%	Patch test (no GLP)	1 positive reaction ³
Benzyl Benzoate (5% in petrolatum)	19 dermatitis patients	5%	Patch test (no GLP)	No positive reactions ³
Benzyl Benzoate (5% in yellow soft paraffin)	241 patients	5%	Patch test (no GLP)	No positive reactions ³
Benzyl Benzoate (5% in vaseline)	103 patients	5%	Patch test (no GLP)	12 positive reactions ³
Benzyl Benzoate (5% in vaseline)	115 patients	5%	Patch test (no GLP)	14 positive reactions ³
Benzyl Benzoate (5% in vaseline)	465 dermatitis patients	5%	Patch test (no GLP)	7 positive reactions ³
Benzyl Benzoate	443 patients (383 tested and 60 controls)	5%	Patch test (no GLP)	2 positive reactions (1 cosmetic dermatitis patient; 1 non-cosmetic dermatitis patient) ³
Benzyl Benzoate (5%)	271 patients (225 tested and 46 controls)	5%	Patch test (no GLP)	2 positive reactions (1 cosmetic dermatitis patient; 1 non-cosmetic dermatitis patient) ³
Benzyl Benzoate (5% in petrolatum)	73 patients (18+ years)	5%	Not stated	Positive reaction in 1 patient ³

Table 5. Skin Irritation and Sensitization Studies

Test Substance	Animals/Subjects	Doses/Concentrations Tested	Procedure	Results
Benzyl Benzoate (5% in petrolatum)	5 male and female urticaria patients (18+ years)	5%	30 min closed patch test	No reaction ¹²⁶
Benzyl Benzoate (5% in petrolatum)	12 patients (18+ years)	5%	Patch test to evaluate sensitization potential	No positive reactions ¹²⁷
Benzyl Benzoate (5% in petrolatum)	3 female allergic dermatitis patients	5%	Patch tests (Finn chambers) to evaluate sensitization potential	No positive reactions ¹²⁸
Benzyl Benzoate (5%; vehicle not reported)	50 dermatitis patients	5%	Patch tests to evaluate sensitization potential	Positive reaction in 1 patient ¹²³
Benzyl Benzoate (5%)	70 dermatitis patients; 19 eyelid dermatitis patients	5%	48 h patch tests (Finn chambers) to evaluate sensitization potential	2.9% with positive reactions (70 patients); no positive reactions (19 patients) ¹²⁹
Benzyl Benzoate (5% in petrolatum, vaseline, or alcohol)	115 patients with positive reactions to Balsam of Peru	5%	48 h closed patch tests to evaluate sensitization potential	Positive reactions in 14 patients ¹³⁰
Benzyl Benzoate (5%; vehicle not stated)	225 patients (18+ years)	5%	Patch test to evaluate sensitization potential	Positive reactions in 2 patients ¹³¹
Benzyl Benzoate (5%; vehicle not stated)	658 male and female patients	5%	48 h patch tests (Finn chambers) to evaluate sensitization potential	Positive reaction in 1 patient ¹³²
Benzyl Benzoate (5% in white petrolatum)	102 contact dermatitis patients (18+ years)	5%	24 h patch tests (Finn chambers) to evaluate sensitization potential	Positive reactions in 4 patients ¹¹⁷
Benzyl Benzoate (5% in petrolatum)	317 eczema patients (18+ years)	5%	Patch test to evaluate sensitization potential	No positive reactions ¹³³
Benzyl Benzoate (5% in petrolatum)	73 patients with eczematous dermatitis	5%	Patch test to evaluate sensitization potential	1 positive reaction (allergic contact dermatitis) ¹²³
Benzyl Benzoate (5% in petrolatum)	102 patients	5%	24 h patch tests (Finn chambers)	4 subjects with positive reactions ¹¹⁷
Benzyl Benzoate (2 or 5% in vaseline)	144 patients (14 controls included) per test concentration	2 or 5%	Closed patch test (no GLP) to evaluate sensitization potential	1 positive reaction in cosmetic dermatitis patient ³
Benzyl Benzoate (2 or 5% in petrolatum)	64 dermatitis patients (18+ years)	2 or 5%	Closed patch test to evaluate sensitization potential	Positive reaction in 1 patient (5% concentration); no positive reactions (2%) ¹³⁴
Benzyl Benzoate (2%)	Patients (no. not stated)	2%	Patch test (no GLP)	Negative reaction in contact dermatitis patient ³
Benzyl Benzoate (2%)	198 patients	2%	Patch test (no GLP)	No positive reactions ³
Benzyl Benzoate (2% in petrolatum)	335 patients	2%	Patch test (no GLP)	No positive reactions ³

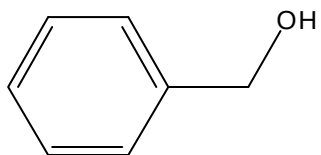
Table 5. Skin Irritation and Sensitization Studies

Test Substance	Animals/Subjects	Doses/Concentrations Tested	Procedure	Results
Benzyl Benzoate (2% in yellow paraffin)	539 patients (18+ years; 50 with photosensitivity dermatitis, 32 with polymorphic light eruption (PLE) and 457 with contact dermatitis)	2%	48 h closed patch test to evaluate sensitization potential	Positive reaction in 1 PLE patient ⁷⁴
Benzyl Benzoate (2% in petrolatum)	284 patients (18+ years)	2%	Patch test to evaluate sensitization potential	1% with positive reactions ¹³⁵
Benzyl Benzoate (2% in petrolatum)	335 patients (18+ years)	2%	Patch test to evaluate sensitization potential	No positive reactions ¹³⁵
Benzyl Benzoate (2% in petrolatum)	198 patients (18+ years)	2%	Patch test to evaluate sensitization potential	No positive reactions ¹³⁶
Benzyl Benzoate (2% in yellow soft paraffin)	241 patients (18+ years)	2%	Finn chamber technique to evaluate sensitization potential	Positive reaction in 1 male patient ¹³⁷
Benzyl Benzoate (2% in white petrolatum)	284 contact dermatitis patients (30 to 67 years)	2%	Patch test to evaluate sensitization potential	No positive reactions ¹³⁸
Benzyl Benzoate (1% in yellow petrolatum)	28 patients allergic to perfumes (18+ years)	1%	Sensitization test (procedure not stated)	No positive reactions ¹³⁹
Benzyl Benzoate (1%)	2,003 dermatitis patients	1%	24 or 48 h patch tests to evaluate sensitization potential	No positive reactions ¹¹⁸
Benzyl Benzoate (0.1% in ethanol or non-irritative cream base)	111 male and female dermatitis patients (18+ years)	0.1%	24-h to 48-h closed patch test to evaluate sensitization potential	No positive reactions ¹¹⁴
Benzyl Benzoate	Cosmetic dermatitis patients (no. not stated)	Concentration not stated	Patch test (no GLP) to evaluate sensitization potential	1 positive reaction ³
Benzyl Benzoate	15 patients	Concentration not stated	Patch test (no GLP)	delayed in reaction in 1 patient sensitized to Peru balsam ³
Benzyl Benzoate	111 patients	Concentration not stated	Patch test (no GLP)	No positive reactions ³
Benzyl Benzoate	142 patients	Concentration not stated	Patch test (no GLP) to evaluate sensitization potential	10 positive reactions in patients sensitive to balsam of Peru ³
Benzyl Benzoate	155 scabies patients	Concentration not stated	Treatment with benzyl benzoate (concentration not stated)	Dermatitis in 17 patients ³
Benzyl Benzoate	460 patients	Concentration not stated	Patch test (no GLP) to evaluate sensitization potential	3 positive reactions in patients sensitive to cosmetics ³
Benzyl Benzoate	25 patients	Concentration not stated	Maximization test (no GLP)	No positive reactions ³
Benzyl Benzoate	465 patients	Concentration not stated	Not stated	Positive reactions (allergenicity) in 1.5% of patients ¹²²

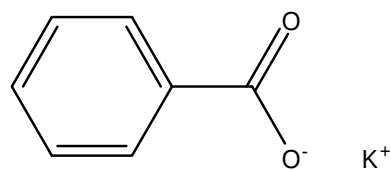
Table 6. Case Reports

Test Substance	Subjects	Procedure/Results
Benzyl Alcohol	30-year-old facial dermatitis patient	Patch testing (no test concentration) produced macular erythema ¹⁴⁰
Benzyl Alcohol	38-year old eczema patient	1% aqueous benzyl alcohol: negative prick test results and positive (++) intradermal injection test results. Negative injection test results in 10 healthy controls ¹⁴¹
Benzyl Alcohol	39-year-old female with pruritic erythema of foot	5% benzyl alcohol in petrolatum: weak (+) reaction in patch test and strong positive reaction in repeated open application test ¹⁴²
Benzyl Alcohol	67-year-old male with leg dermatitis	1% benzyl alcohol in petrolatum: 2+ occlusive patch test reaction. 0.9% benzyl alcohol in saline: negative prick test reaction at 0.5 h reading, but marked induration and proximal spread over arm at days 3 to 8 ¹⁴³
Benzyl Alcohol	53-year-old with stasis dermatitis	1% benzyl alcohol in petrolatum: redness and swelling at 1 h after patch application, wheal 1 day later, and mild urticaria at day 5 ¹⁴⁴
Benzyl Alcohol	16-year-old female with possible anaphylactic reactions after i.m. injection with B12 preparation containing 0.9% benzyl alcohol	Benzyl alcohol preparation: negative in prick tests, but positive in intradermal tests ¹⁴⁵
Benzyl Alcohol	57-year-old female with pruritic dermatitis	Allergic contact dermatitis (+, ++, or +++ reaction) after patch testing with benzyl alcohol ¹⁴⁶
Benzyl Alcohol	40-year-old female with dermatitis	Positive patch test reaction (+++, allergic contact dermatitis) to 9.5% benzyl alcohol in petrolatum ¹⁴⁷
Benzyl Alcohol	65-year-old female with eyelid dermatitis	Macular erythema after patch testing with benzyl alcohol (concentration not stated) ¹⁴⁸
Benzyl Alcohol	30-year-old female with eyelid dermatitis	Positive (1+) patch test reaction to benzyl alcohol (concentration not stated) ¹⁴⁰
Benzoic Acid	30-year-old facial dermatitis patient	Patch testing (no test concentration) produced 1+ reaction ¹⁴⁰
Benzoic Acid	46-year-old female with history of erythema and itching	5% benzoic acid in petrolatum: positive reaction ¹⁴⁹
Sodium Benzoate	64-year-old female with erythema and edema of finger	Positive patch test reaction to 5% sodium benzoate on days 2 and 4; negative patch test results at interdigital area at 2 months ¹⁵⁰
Sodium Benzoate	52-year-old female with hand dermatitis	Negative patch test reaction to sodium benzoate (concentration not stated) ¹⁵¹
Sodium Benzoate	75-year-old female with history of pruritus	Relapse of pruritus after 100 mg oral dose of sodium benzoate ¹⁵²
Benzyl Benzoate	25-year-old male with acute bullous eruption after application of 30% benzyl benzoate preparation	Negative patch test reaction to 1% benzyl benzoate in petrolatum ¹⁵³
Benzyl Benzoate (unspecified concentration or 25% emulsion in aqueous solution of triethanolamine stearate)	4 scabies patients (ages not stated)	Severe skin irritation; pruritic dermatitis within 12 to 20 h after treatment (unspecified concentration or 25% emulsion) ¹⁵⁴
Benzyl Benzoate	Scabies patients (no. not included)	Treatment with benzyl benzoate solution (concentration not stated) did not cause dermatitis ³
Benzyl Benzoate	4 scabies patients	Treatment with benzyl benzoate solution caused dermatitis ³
Benzyl Benzoate (5% in petrolatum)	1 patient (18 + years)	Reaction read at 48 h and 96 h. No sensitization ¹⁵⁵
Benzyl Benzoate 2% in petrolatum)	23-year old male patient	Negative patch test results; no sensitization ¹⁵⁶

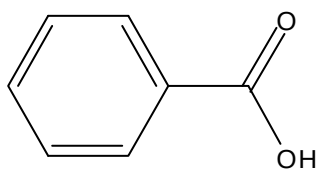
Figure 1. Chemical Structures



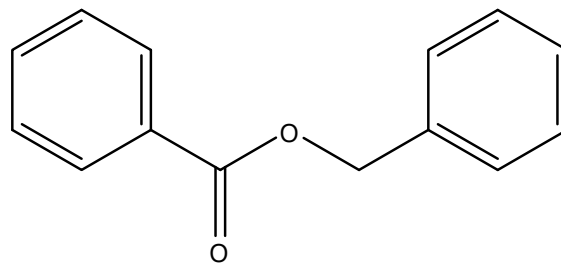
benzyl alcohol



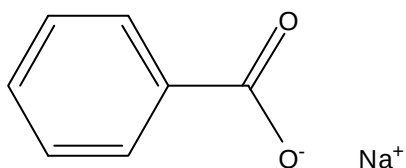
potassium benzoate



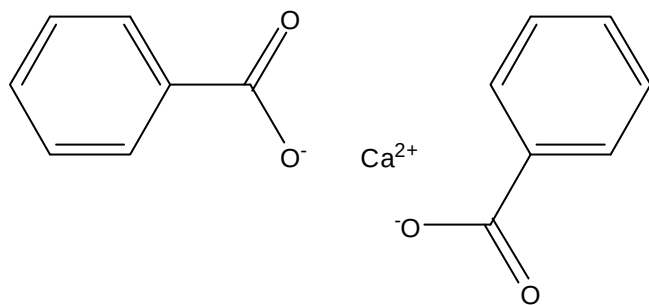
benzoic acid



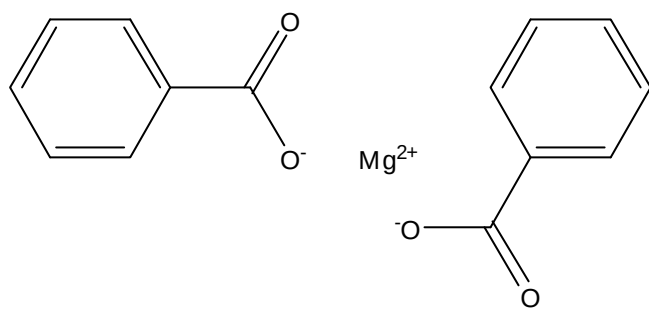
benzyl benzoate



sodium benzoate



calcium benzoate



magnesium benzoate

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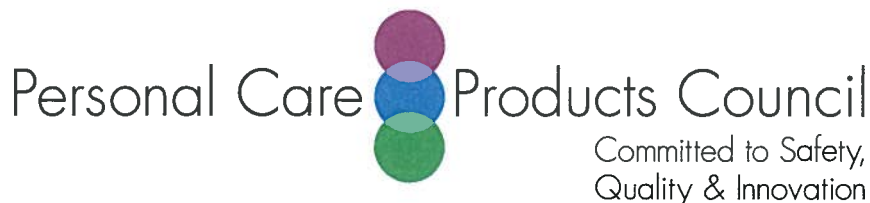
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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D. *JB* 7-14-11
Industry Liaison to the CIR Expert Panel

DATE: July 14, 2011

SUBJECT: Comments on the Tentative Amended Safety Assessment on Benzyl Alcohol, Benzoic Acid and its Salts and Benzyl Ester

Although the disclaimer ("Tentative reports are distributed for comment only -- do not cite") on the CIR website is helpful, it should also be included on every page of a tentative report.

Abstract - It would be helpful to mention dermal irritation and sensitization data in the abstract, e.g., current use concentrations of these ingredients are below concentrations that may result in dermal irritation or sensitization.

p.1 - In the Introduction, in the paragraph concerning the 2001 report, it would be helpful to indicate that all the details of studies in the 2001 report are not included in current report. For a complete review of all the data identified, both reports are needed.

p.1 - It would also be helpful to mention the RIFM synopsis on Benzyl Benzoate in the introduction, as it appears to be the primary source of information for this ingredient.

p.2 - The first sentence under Benzoic Acid and Sodium Benzoate in the Impurities section states: "The effects of UVA exposure on benzene formation were determined in another study." As this is the first study described, it is not clear what is meant by "in another study".

p.3 - The EPA maximum contaminant level (MCL) for drinking water should be cited directly to the EPA (one source <http://water.epa.gov/drink/contaminants/basicinformation/benzene.cfm>). It would also be helpful to explain how MCLs are derived. An MCL is not strictly health based - MCLs "are set as close to the health goals as possible, considering cost, benefits and the ability of public water systems to detect and remove contaminants using suitable treatment technologies."

p.4 - The final monograph for anorectal drugs (<http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DevelopmentResources/Over-the-CounterOTCDrugs/StatusofOTCRulemakings/ucm078704.pdf>) states: "Based on the available evidence, the agency is issuing a final monograph establishing conditions under which OTC anorectal drug products are generally recognized as safe and effective and not misbranded." Benzyl alcohol is included as a local anesthetic. Therefore, "category not stated" needs to be changed to Category I.

p.7 - In the summary of the Acute Oral Exposure section, please add the word "oral" before LD50.

- p.9 - In the third paragraph on this page, “does” should be “doses”
- p.10 - In the third paragraph under Benzyl Alcohol, it should read: “the association of the benzyl alcohol preservative...” (not vehicle as currently stated).
- p.12 - At what concentration was Benzyl Benzoate not phototoxic in the presence of UVA or UVB light?
- p.13 - It is not clear what is meant by “the acceptable dose”.
- p.14, first line - As there is now a Dermal Repeated Dose section, please change: “Parenteral Studies section” to “Dermal Studies section”.
- p.16 - Is the dose “5,000 mg/plate” really correct for the Ames test (pre-incubation assay) of Benzyl Benzoate?
- p.16 - In the last paragraph, units of mg/ml should be called a concentration rather than dose.
- p.17, 19 - In the summary of the Carcinogenicity section and the Summary of the report, it is misleading to present the single study on the non-oxidative hair dye as two separate sentences. It would be more appropriate to state: “A non-oxidative hair dye containing 2% Benzyl Alcohol and 0.016% Benzoic acid tested negative for carcinogenicity following dermal application to mice.”
- p.18 - As no studies on the formation of benzene in foods containing Benzoic Acid are presented earlier in the report, “foods” should be deleted from the second paragraph of the Summary.
- p.19 - The Discussion should note that as stated in the Toxicokinetics section, these ingredients are metabolized and excreted via a common pathway. Therefore, the data on Benzyl Alcohol are directly relevant to Benzoic Acid and its salts. It should also be noted that additional data are summarized in the original report.
- p.19 - The Discussion should note that the studies on benzene formation were in beverages, not cosmetic products.
- p.21, Table 1 - The spelling of Benzyl Alcohol needs to be corrected in the first line of the table.
- p.22, Table 3 - NS can be deleted from the footnotes of this table as all the ingredients were included in a Council concentration of use survey.
- p.24, Table 5 - As the induction concentration was 10%, the results should say that no reactions were observed at a challenge concentrations of 5% and 10%, while mild reactions were observed at challenge concentrations of 40%. The induction concentration(s) used in reference 84 are not clear.
- p.25, Table 5 - It is not clear what is meant by “24-h RIPT” in the Procedure column of reference 92.
- p.32 reference 49, p.33 reference 55 - Reference 49 and reference 55 are the same reference, RIFM’s synopsis of information on Benzyl Bezoate.

SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of benzoic acid (E 210), sodium benzoate (E 211), potassium benzoate (E 212) and calcium benzoate (E 213) as food additives¹

EFSA Panel on Food Additives and Nutrient Sources (ANS)^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating benzoic acid (E 210), sodium benzoate (E 211), potassium benzoate (E 212) and calcium benzoate (E 213) when used as food additives. Benzoic acid and its sodium and potassium salts are rapidly absorbed after oral administration. The Panel considered that the absorption, distribution, metabolism and excretion of calcium benzoate will be similar to sodium or potassium salt and, therefore, read-across between the salts was possible. The results of short-term and subchronic studies on benzoic acid and its salts indicate that their toxicity is low. The Panel considered that the use of benzoic acid and its sodium and potassium salts as food additives does not raise a concern with respect to genotoxicity and, based on read-across, also considered that this conclusion is applicable for calcium benzoate. Moreover, the Panel noted that the available data did not indicate any carcinogenic potential. A four-generation reproductive toxicity study with benzoic acid in the diet in rats was considered by the Panel as the pivotal study and a no observed adverse effect level of 500 mg benzoic acid/kg body weight (bw) per day, the highest dose tested, was identified. From the aforementioned studies, the Panel derived an acceptable daily intake (ADI) of 5 mg/kg bw per day (expressed as benzoic acid) using an uncertainty factor of 100. Taking into account food categories for which direct addition of benzoic acid-benzoates is authorised, the group ADI was exceeded in the *brand-loyal scenario* in particular for toddlers and children consuming on a regular basis flavoured drinks. Considering additional exposure due to carry-over, the intake could be increased by up to two to three fold for all high-level consumers compared to the previous scenario with only direct addition to food. This results in exceedance of the group ADI in toddlers and children for the non-brand-loyal scenario. The main food categories contributing to this exceedance were unprocessed fruits and vegetables and flavoured drinks.

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¹ On request from European Commission, Question Nos EFSA-Q-2011-00001, 2011-00002, 2011-00003 and 2011-00004, adopted on 8 March 2016.

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³ Acknowledgement: The Panel wishes to thank the members of the former Working Group “B” Food Additives and Nutrient Sources (2011–2014) and the members of the Standing Working Group on the re-evaluation of food additives other than gums and colours: Polly Ester Boon, Dimitrios Chrysafidis, Birgit Dusemund, David Gott, Rainer Gürtler, Ursula Gundert-Remy, Claude Lambré, Jean-Charles Leblanc, Daniel Marzin, Peter Moldeus, Pasquale Mosesso, Dominique Parent-Massin, Ivan Stankovic, Paul Tobback, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen and Matthew Wright for the preparatory work on this scientific opinion; and EFSA staff members: Ana Rincon and Alexandra Tard for the support provided to this scientific opinion. The ANS Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific opinion.

Suggested citation: EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2016. Scientific Opinion on the re-evaluation of benzoic acid (E 210), sodium benzoate (E 211), potassium benzoate (E 212) and calcium benzoate (E 213) as food additives. EFSA Journal 2016;14(3):4433, 110 pp. doi:10.2903/j.efsa.2016.4433

Available online: www.efsa.europa.eu/efsajournal

KEY WORDS

food additive, benzoic acid, E 210, CAS No 65-85-0, sodium benzoate, E 211, CAS No 532-31-1, potassium benzoate, E 212, CAS No 582-25-2, calcium benzoate, E 213, CAS No 2090-05-3

SUMMARY

Following a request from the European Commission (EC), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of benzoic acid (E 210), sodium benzoate (E 211), potassium benzoate (E 212) and calcium benzoate (E 213) when used as food additives.

Benzoic acid, sodium benzoate, potassium benzoate and calcium benzoate (E 210, E 211, E 212 and E 213) are authorised food additives in the European Union (EU) and have previously been evaluated by the EU Scientific Committee on Food (SCF) in 1994 and 2002. The SCF established a group acceptable daily intake (ADI) of 0–5 mg/kg body weight (bw) per day for benzoic acid and its salts, also including benzyl alcohol and related benzyl derivatives used as flavourings. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated benzoic acid and its salts as food additives on a number of occasions. In 1974, JECFA established a group ADI of 0–5 mg/kg bw for benzoic acid and its salts, expressed as benzoic acid, which was reconfirmed at its most recent evaluation in 1996. JECFA has additionally reviewed benzoic acid as a flavouring substance.

Specific purity criteria have been defined in Commission Regulation (EU) No 231/2012 for benzoic acid and its salts (E 210–213). The Panel noted that the term ‘benzoic acid’ should be replaced by ‘potassium benzoate’ or ‘calcium benzoate’ in the EU specifications for potassium benzoate (E 212) and calcium benzoate (E 213), respectively. In addition, the Panel considered that if metals are used as catalysts in the manufacturing process of benzoic acid as a food additive, the maximum residual level for each metal should be specified in the EU specifications for benzoic acid (E 210). The Panel also considered that the maximum limits for the impurities of toxic elements (lead, mercury and arsenic) in the EC specification for benzoic acid and its salts (E 210–213) should be revised in order to ensure that benzoic acid and its salts (E 210–213) as food additives will not be a significant source of exposure to those toxic elements in food.

The Panel considered that when benzoic acid or its salts and ascorbic acid are used together, considerations should be given to the storage of the soft drinks and the food contact materials to minimise the formation of benzene in beverages.

The available data on absorption, distribution, metabolism and excretion (ADME) in animals and humans indicate that benzoic acid and its sodium and potassium salts are rapidly absorbed, primarily in the proximal part of the gastrointestinal tract, after oral administration. Benzoate is conjugated to glycine to form hippuric acid which is excreted in the urine. No studies appear to have been conducted on calcium benzoate, but the Panel considered that calcium benzoate also dissociates into its constituents calcium and benzoate ions in the small intestine. Consequently, the ADME of this salt will be similar to that of the sodium or potassium salts, despite differences in water solubility. Therefore, the Panel considered read-across between the salts possible.

The results of the available short-term and subchronic studies on benzoic acid and its salts indicated that their toxicity is low, with no marked target organ toxicity, although high intakes of benzoic acid may lead to disturbances in the acid/base balance, and benzoic acid at high doses may interfere with intermediary metabolism.

The Panel considered that the use of benzoic acid and its sodium and potassium salts as food additives did not raise a concern with respect to genotoxicity. Based on read-across, the Panel considered that this conclusion is also applicable for calcium benzoate.

The Panel noted that the available carcinogenicity studies on benzoic acid and its salts did not indicate any carcinogenic potential, although they were not conducted in accordance with current test guidelines and had deficiencies both in terms of design and reporting. Overall, given supporting

evidence of lack of carcinogenicity of the related substances benzyl alcohol, benzyl acetate and benzaldehyde as described by JECFA, the Panel considered that the data are sufficient to conclude that benzoic acid and its salts did not raise concern with respect to carcinogenicity.

In a developmental toxicity study in rats with benzoic acid by gavage, a no observed adverse effect level (NOAEL) of 160 mg benzoic acid/kg bw per day was observed for maternal and developmental toxicity. The developmental toxicity studies in rats with sodium benzoate in the diet showed a NOAEL of 500 mg/kg bw per day comparable to the NOAEL of the dietary four-generation reproductive toxicity in rats with benzoic acid.

The four-generation reproductive toxicity study with benzoic acid in the diet in rats was considered by the Panel as the pivotal study, as this study was the longest exposure period as compared to the developmental studies. This study showed no effect on growth, fertility, lactation or survival, and provided a NOAEL, for both the parental animals and the offspring, of 500 mg benzoic acid/kg bw per day, the highest dose tested.

The Panel noted that benzoic acid and its salts may enhance hypersensitivity and/or cause skin reactions in sensitive people. Furthermore, anaphylaxis and urticaria have also been observed in sensitive individuals following exposure to benzoic acid and its salts at doses below the ADI. Several studies have shown that subgroups of patients already suffering from atopic dermatitis, pruritus, urticaria or persistent rhinitis may be intolerant even to low doses of benzoate.

The Panel noted the absence of any indication of genotoxicity of benzoic acid and its salts *in vivo*, together with the negative results of limited carcinogenicity studies in rats and mice. The Panel considered that the ADI cannot be derived from a prenatal developmental toxicity study with benzoic acid performed by gavage. The developmental toxicity studies in rats with sodium benzoate in the diet, showed NOAELs of 500 mg/kg bw per day (the highest dose tested) and 1310 mg/kg bw per day, respectively, comparable to or higher than the NOAEL of 500 mg/kg bw per day in the dietary multi-generation reproductive toxicity in rats with benzoic acid. From these reproductive and developmental studies, the Panel derived an ADI of 5 mg/kg bw per day (expressed as benzoic acid) applying an uncertainty factor of 100.

To assess the dietary exposure to benzoic acid-benzoates (E 210–213) from their use as a food additive, the exposure was calculated based on (1) maximum permitted levels (MPLs) set out in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) the reported use levels and analytical data (defined as the *refined exposure assessment scenario*). Dietary exposure through this latter scenario was assessed using two sets of concentration data: reported use levels and analytical data not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised (Annex II to Regulation No 1333/2008); and reported use levels and analytical data not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised and analytical data for food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available.

The exposure estimates in the *regulatory maximum level exposure assessment scenario* exceeded the ADI of 5 mg/kg bw per day for all population groups at the high levels and for toddlers and children also at the mean level.

Considering only food categories for which direct addition of benzoic acid-benzoates to food is authorised, the refined exposure to benzoic acid-benzoates (E 210–213) in toddlers and children, exceeded the ADI of 5 mg/kg bw per day at the high level (95th percentile) in the *refined brand-loyal* exposure estimate, while, for the *non-brand-loyal scenario*, the group ADI was not exceeded in any population group. Considering additional exposure from food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available, in the *refined brand-loyal* exposure scenario, the ADI was exceeded at the high level for all population groups and for infants,

toddlers, children and adolescent at the mean. In the *non-brand-loyal* exposure scenario, the ADI was exceeded only for toddlers and children at the high level; at the mean level, there was no exceedance.

Considering the present toxicological database the Panel identified a NOAEL of 500 mg/kg bw per day and applying an uncertainty factor of 100, the Panel derived a group ADI of 5 mg/kg bw per day, expressed as benzoic acid, for benzoic acid (E 210), sodium benzoate (E 211), potassium benzoate (E 212) and calcium benzoate (E 213).

The Panel concluded that from the use of benzoic acid-benzoates as food additives from direct addition to food, the group ADI was exceeded in the *brand-loyal scenario* for toddlers and children consuming on a regular basis flavoured drinks.

Considering additional exposure from food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available, the Panel concluded that exposure to benzoic acid-benzoates could be increased by up to two to three fold for all high-level consumers compared to the previous scenario for direct addition to food. This results in exceedance of the group ADI in toddlers and children for the *non-brand-loyal scenario*. The main food categories contributing to this exceedance were unprocessed fruits and vegetables and flavoured drinks.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1333/2008⁴ of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under Regulation (EU) No 257/2010⁵. This Regulation also foresees that food additives are re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU⁶ of 2001. The report 'Food additives in Europe 2000',⁷ submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of the adoption of Regulation (EU) 257/2010, the 2003 Terms of Reference are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedure and deadlines that are enshrined in Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

⁴ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

⁵ Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.

⁶ COM(2001) 542 final.

⁷ Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, 560.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of benzoic acid (E 210), sodium benzoate (E 211), potassium benzoate (E 212) and calcium benzoate (E 213) when used as food additives.

Benzoic acid, sodium benzoate, potassium benzoate and calcium benzoate are authorised as food additives in the EU and were previously evaluated by the EU Scientific Committee on food (SCF) in 1994 and 2002 (SCF, 1994, 2002). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated benzoic acid and its salts as food additives on a number of occasions, most recently in 1996 (JECFA, 1996, 1997). JECFA has additionally reviewed benzoic acid as a flavouring substance (JECFA 2001, 2002). Benzoic acid and its salts have also been reviewed by TemaNord (2002).

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following an EFSA public call for scientific data.^{8,9,10} To assist in identifying any emerging issue, EFSA has outsourced a contract to deliver an updated literature review on toxicological end-points, dietary exposure and occurrence levels of benzoic acid-benzoates (E 210–213) which covered the period from January 2013 up to the end of 2014.

2. Technical data

2.1. Identity of the substance

2.1.1. Benzoic acid

Benzoic acid (E 210) is an aromatic carboxylic acid, the chemical name according to IUPAC nomenclature rules being benzoic acid. The Chemical Abstracts Service (CAS) Registry number is 65-85-0 and the European Inventory of Existing Commercial chemical Substances (EINECS) number is 200-618-2. The molecular formula for benzoic acid is $C_7H_6O_2$ and the molecular weight is 122.1 g/mol. Its structural formula is given below (Figure 1):

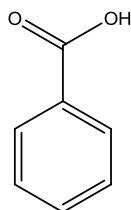


Figure 1: Structural formula of benzoic acid

⁸ Call for scientific data on food additives permitted in the EU and belonging to the functional classes of preservatives and antioxidants. Published: 23 November 2009. Available online: <http://www.efsa.europa.eu/en/dataclosed/call/ans091123a.htm>

⁹ Call for approved food additives (sorbates, benzoates and gallates) occurrence data in food and beverages intended for human consumption. Published: 31 May 2012. Available online: <http://www.efsa.europa.eu/sites/default/files/consultation/120601.pdf>

¹⁰ Call for scientific data on selected food additives permitted in the EU- Extended deadline: 1 September 2014 (batch A), 1 November 2014 (batch B) Available online: <http://www.efsa.europa.eu/en/dataclosed/call/140324.htm>

The most commonly known synonyms are benzenecarboxylic acid, phenylcarboxylic acid, carboxybenzene and dracrylic acid (ChemID Plus, online). Benzoic acid is a white crystalline powder. The melting range is 121.5–123.5°C (Commission Regulation (EU) No 231/2012¹¹). It is slightly soluble in water (2.9 g/l at 20°C) and freely soluble in ethanol (JECFA, 2006). Benzoic acid is a weak acid with a pKa of 4.19 (OECD SIDS, 2001; WHO, 2005) and will be almost completely dissociated at pH 7.

2.1.2. Sodium benzoate

Sodium benzoate (E 211) is the sodium salt of benzoic acid, the chemical name according to IUPAC nomenclature rules being sodium benzoate. The CAS Registry number is 532-32-1 and the EINECS number is 208-534-8. The molecular formula for sodium benzoate is $C_7H_5NaO_2$ and the molecular weight is 144.1 g/mol. Its structural formula is given below (Figure 2):

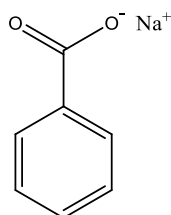


Figure 2: Structural formula of sodium benzoate

The most commonly known synonyms are sodium salt of benzene carboxylic acid, sodium salt of phenyl carboxylic acid and benzoate of soda (ChemID Plus, online). Sodium benzoate is a white, almost odourless, crystalline powder or granule. The compound is freely soluble in water (556 g/l at 20°C) and sparingly soluble in ethanol (OECD SIDS, 2001; Commission Regulation (EU) No 231/2012).

2.1.3. Potassium benzoate

Potassium benzoate (E 212) is the potassium salt of benzoic acid, the chemical name according to IUPAC nomenclature rules being potassium benzoate. The CAS Registry number is 582-25-2 (anhydrous) and the EINECS number is 209-481-3. The molecular formula for anhydrous potassium benzoate is $C_7H_5KO_2$ and the molecular weight is 160.2 g/mol. JECFA specifications refer to both anhydrous and trihydrate forms (JECFA, 2006), while EU specifications refer to the trihydrate only. The structural formula of the anhydrous form is given below (Figure 3):

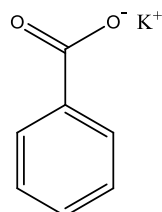


Figure 3: Structural formula of anhydrous potassium benzoate

¹¹ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

The most commonly known synonyms are potassium salt of benzoic acid, potassium salt of benzenecarboxylic acid and potassium salt of phenylcarboxylic acid (ChemID Plus, online). Potassium benzoate is a white crystalline powder (Commission Regulation (EU) No 231/2012). One gram dissolves in 2 mL of water, in 75 mL ethanol and 50 mL alcohol (90 %) (FCC, 2010-2011).

2.1.4. Calcium benzoate

Calcium benzoate (E 213) is the calcium salt of benzoic acid, the chemical name according to IUPAC nomenclature rules being calcium dibenzoate. The CAS Registry number is 2090-05-3 and the EINECS number is 218-235-4. The molecular formula for calcium benzoate (anhydrous) is $C_{14}H_{10}CaO_4$ and the molecular weight is 282.3 g/mol. EC and JECFA specifications refer to the anhydrous, monohydrate and trihydrate forms. The structural formula of the anhydrous form is given below (Figure 4):

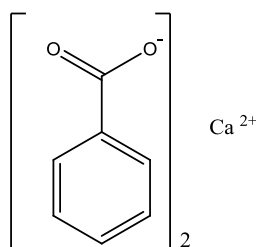


Figure 4: Structural formula of anhydrous calcium benzoate

Synonyms are benzoic acid calcium salt, calcium benzoate and monocalcium benzoate (ChemID Plus, online). Calcium benzoate exists as white or colourless crystals or as a white powder and is sparingly soluble in water (27.2 g/l at 20°C) (Commission Regulation (EU) No 231/2012; JECFA, 2006).

2.2. Specifications

Specifications for benzoic acid and its sodium, potassium and calcium salts have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2006) (Tables 1–4).

Table 1: Specifications for benzoic acid (E 210) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Definition		
Assay	Not less than 99.5% on anhydrous basis	Not less than 99.5% on dry basis
Description	White crystalline powder	White crystalline solid, usually in the form of scales or needles, having not more than a faint characteristic odour
Identification		
Melting range	121.5–123.5 °C	121–123 °C
Sublimation test	Passes test	Place a small amount of the sample in a dry test tube. Wrap the test tube about 4 cm from the bottom with moistened filter paper. Heat the test tube over a low flame. Benzoic acid sublimes and crystals deposit in the colder part of the test tube leaving no residue at the bottom
Test for benzoate	Passes test	Passes test (use 0.1 g of the sample with 0.1 g of calcium carbonate and 5 mL of water)
pH	4 (solution in water)	4 (solution in water)
Solubility	–	Slightly soluble in water, freely soluble in ethanol
Purity		
Loss of drying	Not more than 0.5% (3 h, over sulphuric acid)	Not more than 0.5% (3 h, over sulphuric acid)
Sulphated ash	Not more than 0.05%	Not more than 0.05%
Chlorinated organic compounds	Not more than 0.07% (expressed as chloride corresponding to 0.3% expressed as monochlorobenzoic acid)	Not more than 0.7% (as Cl ₂)
Readily oxidisable substances	Add 1.5 mL of sulphuric acid to 100 mL of water, heat to boiling point and add 0.1 N KMnO ₄ in drops, until the pink colour persists for 30 s. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1 N KMnO ₄ to a pink colour that persists for 15 s. Not more than 0.5 mL should be required	Add 1.5 mL of sulphuric acid to 100 mL of water, heat to boiling point and add 0.1 N KMnO ₄ in drops, until the pink colour persists for 30 s. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1 N KMnO ₄ to a pink colour that persists for 15 s. Not more than 0.5 mL should be required
Readily carbonisable substances	A cold solution of 0.5 g of benzoic acid in 5 mL of 94.5–95.5% sulphuric acid must not show a stronger colouring than that of a reference liquid containing 0.2 mL of cobalt chloride TSC, 0.3 mL of ferric chloride TSC, 0.1 mL of copper sulphate TSC and 4.4 mL of water	Dissolve 0.5 g of the sample, weighed to the nearest mg, in 5 mL of sulphuric acid TS. The colour produced should not be darker than a light pink ('Matching Fluid Q')
Polycyclic acids	On fractional acidification of a neutralised solution of benzoic acid, the first precipitate must not have a different melting point from that of the benzoic acid	
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–

Table 2: Specifications for sodium benzoate (E 211) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Definition		
Assay	Not less than 99% of $C_7H_5O_2Na$, after drying at 105 °C for 4 h	Not less than 99% on dry basis
Description	White, almost odourless, crystalline powder or granules	White, almost odourless, crystalline powder, flakes or granules
Identification		
Solubility	Freely soluble in water, sparingly soluble in ethanol	Freely soluble in water, sparingly soluble in ethanol
Melting range	Melting range of benzoic acid isolated by acidification and not recrystallised (121.5–123.5 °C) after drying in a sulphuric acid desiccator	—
Test for benzoate	Passes test	Passes test
Test for sodium	Passes test	Passes test
Purity		
Loss of drying	Not more than 1.5% (105 °C, 4 h)	Not more than 1.5% (105 °C, 4 h)
Readily oxidisable substances	Add 1.5 mL of sulphuric acid to 100 mL of water, heat to boiling point and add 0.1 N $KMnO_4$ in drops, until the pink colour persists for 30 s. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1 N $KMnO_4$ to a pink colour that persists for 15 s. Not more than 0.5 mL should be required	Add 1.5 mL of sulphuric acid to 100 mL of water, heat to boiling point and add 0.1 N $KMnO_4$ in drops, until the pink colour persists for 30 s. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1 N $KMnO_4$ to a pink colour that persists for 15 s. Not more than 0.5 mL should be required
Readily carbonisable substances	—	Dissolve 0.5 g of the sample, weighed to the nearest mg, in 5 mL of sulphuric acid TS. The colour produced should not be darker than a light pink ('Matching Fluid Q')
Polycyclic acids	On fractional acidification of a (neutralised) solution of sodium benzoate, the first precipitate must not have a different melting range from that of benzoic acid	
Chlorinated organic compounds	Not more than 0.06% (expressed as chloride corresponding to 0.25% expressed as monochlorobenzoic acid)	Not more than 0.7% (as chlorine)
Acidity and alkalinity	Neutralisation of 1 g of sodium benzoate, in the presence of phenolphthalein, must not require more than 0.25 mL of 0.1 N NaOH or 0.1 N HCl	Dissolve 2 g of the sample, weighed to the nearest mg, in 20 mL of freshly boiled water. Not more than 0.5 mL of either 0.1 N NaOH or 0.1 N HCl should be required for neutralisation, using phenolphthalein TS as indicator
Arsenic	Not more than 3 mg/kg	—
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	—

Table 3: Specifications for potassium benzoate (E 212) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Definition		
Assay	Not less than 99% of $C_7H_5KO_2$, after drying at 105 °C to constant weight	Not less than 99% on dry basis
Description	White crystalline powder	White crystalline powder
Identification		
Solubility		Freely soluble in water, soluble in ethanol
Melting range	Melting range of benzoic acid isolated by acidification and not recrystallised (121.5–123.5 °C) after vacuum drying in a sulphuric acid desiccator	-
Test for benzoate	Passes test	Passes test
Test for sodium	Passes test	Passes test
Purity		
Loss of drying	Not more than 26.5% (105 °C, 4 h)	Not more than 26.5% (105 °C, 4 h)
Readily oxidisable substances	Add 1.5 mL of sulphuric acid to 100 mL of water, heat to boiling point and add 0.1 N $KMnO_4$ in drops, until the pink colour persists for 30 s. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1 N $KMnO_4$ to a pink colour that persists for 15 s. Not more than 0.5 mL should be required	Add 1.5 mL of sulphuric acid to 100 mL of water, heat to boiling point and add 0.1 N $KMnO_4$ in drops, until the pink colour persists for 30 s. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1 N $KMnO_4$ to a pink colour that persists for 15 s. Not more than 0.5 mL should be required
Readily carbonisable substances	A cold solution of 0.5 g of benzoic acid in 5 mL of 94.5–95.5% sulphuric acid must not show a stronger colouring than that of a reference liquid containing 0.2 mL of cobalt chloride TSC, 0.3 mL of ferric chloride TSC, 0.1 mL of copper sulphate TSC and 4.4 mL of water	Dissolve 0.5 g of the sample, weighed to the nearest mg, in 5 mL of sulphuric acid TS. The colour produced should not be darker than a light pink ('Matching Fluid Q')
Polycyclic acids	On fractional acidification of a (neutralised) solution of potassium benzoate, the first precipitate must not have a different melting range from that of benzoic acid	
Chlorinated organic compounds	Not more than 0.06% (expressed as chloride corresponding to 0.25% expressed as monochlorobenzoic acid)	Not more than 0.7% (as chlorine)
Acidity and alkalinity	Neutralisation of 1 g of potassium benzoate, in the presence of phenolphthalein, must not require more than 0.25 mL of 0.1 N NaOH or 0.1 N HCl	Dissolve 2 g of the sample, weighed to the nearest mg, in 20 mL of freshly boiled water. Not more than 0.5 mL of either 0.1 N NaOH or 0.1 N HCl should be required for neutralisation, using phenolphthalein TS as indicator
Arsenic	Not more than 3 mg/kg	-
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	-

Table 4: Specifications for calcium benzoate (E 213) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Definition		
Assay	Not less than 99% after drying at 105 °C	Not less than 99% on dry basis
Description	White or colourless crystals, or white powder	White or colourless crystals, or white powder
Identification		
Solubility		Sparingly soluble in water
Melting range	Melting range of benzoic acid isolated by acidification and not recrystallised (121.5–123.5 °C) after vacuum drying in a sulphuric acid desiccator	-
Test for benzoate	Passes test	Passes test
Test for sodium	Passes test	Passes test
Purity		
Loss of drying	Not more than 17.5% (105 °C, to constant weight)	Not more than 17.5% (105 °C, 4 h)
Water-insoluble matter	Not more than 0.3%	Not more than 0.3%
Readily oxidisable substances	Add 1.5 mL of sulphuric acid to 100 mL of water, heat to boiling point and add 0.1 N KMnO ₄ in drops, until the pink colour persists for 30 s. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1 N KMnO ₄ to a pink colour that persists for 15 s. Not more than 0.5 mL should be required	Add 1.5 mL of sulphuric acid to 100 mL of water, heat to boiling point and add 0.1 N KMnO ₄ in drops, until the pink colour persists for 30 s. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1 N KMnO ₄ to a pink colour that persists for 15 s. Not more than 0.5 mL should be required
Readily carbonisable substances	A cold solution of 0.5 g of benzoic acid in 5 mL of 94.5–95.5% sulphuric acid must not show a stronger colouring than that of a reference liquid containing 0.2 mL of cobalt chloride TSC, 0.3 mL of ferric chloride TSC, 0.1 mL of copper sulphate TSC and 4.4 mL of water	Dissolve 0.5 g of the sample, weighed to the nearest mg, in 5 mL of sulphuric acid TS. The colour produced should not be darker than a light pink ('Matching Fluid Q')
Polycyclic acids	On fractional acidification of a (neutralised) solution of calcium benzoate, the first precipitate must not have a different melting range from that of benzoic acid	
Chlorinated organic compounds	Not more than 0.06 % (expressed as chloride corresponding to 0.25 % expressed as monochlorobenzoic acid)	Not more than 0.7% (as Cl ₂)
Acidity and alkalinity	Neutralisation of 1 g of calcium benzoate, in the presence of phenolphthalein, must not require more than 0.25 mL of 0.1 N NaOH or 0.1 N HCl	Dissolve 2 g of the sample, weighed to the nearest mg, in 20 mL of freshly boiled water. Not more than 0.5 mL of either 0.1 N NaOH or 0.1 N HCl should be required for neutralisation, using phenolphthalein TS as indicator
Fluoride	Not more than 10 mg/kg	Not more than 10 mg/kg
Arsenic	Not more than 3 mg/kg	-
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	-

The Panel noted that in the EU specifications for potassium benzoate (E 212) and calcium benzoate (E 213), 'Readily carbonisable substances' are described as 'a solution of 0.5 g of benzoic acid'; however, the term 'benzoic acid' should be replaced by 'potassium benzoate' or 'calcium benzoate', respectively.

From the general information on the manufacturing process (see Section 2.3), several by-products might be formed (benzaldehyde, benzyl alcohol, benzyl benzoate, benzyl formate, benzyl acetate, biphenyl, methyl biphenyls and phthalic acid) next to residual toluene used as raw material. The Panel considered that a maximum limit for toluene and any by-products produced during the manufacturing process of benzoic acid as a food additive should be included in the EU specifications. In addition, the Panel noted that some catalysts (e.g. cobalt naphthenate) can be used in the manufacturing process. Therefore, the Panel also considered that if metals are used as catalysts in the manufacturing process of benzoic acid as a food additive, the maximum residual level for each metal should be specified in the EU specifications.

The Panel noted that according to the EU specifications for benzoic acid and its salts, impurities of the toxic elements lead, mercury and arsenic are accepted up to a concentration of 2, 1 and 3 mg/kg, respectively. Contamination at those levels would have a significant impact on the exposure to these metals, for which the exposure already are close to the health-based guidance values established by EFSA (EFSA CONTAM Panel 2009, 2010, 2012).

2.3. Manufacturing process

According to Maki and Takeda (2000), benzoic acid is produced, worldwide, by liquid-phase oxidation of toluene with molecular oxygen. Originally the oxidation reaction was carried out at 140 °C and ca. 0.2 MPa with a cobalt naphthenate catalyst (0.1%). Other oil-soluble cobalt salts were also used as catalysts. The purity of toluene is critical because sulphur compounds, nitrogen compounds, phenols and olefins inhibit the oxidation reaction. The oxidation reaction is a free-radical chain process. Peroxides are reaction intermediates. In a typical modern process, the oxidation is conducted at 165 °C and 0.9 MPa. The pressure of the liquid discharged from the reactor is reduced to atmospheric, and unreacted toluene is recovered. Benzoic acid is purified by rectification. The bottom residue is extracted to recover the cobalt catalyst. In the oxidation reaction, several by-products are formed: benzaldehyde, benzyl alcohol and benzyl benzoate. Other esters, including benzyl formate and benzyl acetate, are also present. Biphenyl and methyl biphenyls are formed in smaller amounts. Small amounts of phthalic acid can also be present. For food and pharmaceutical uses, benzoic acid is upgraded by further processing. Sublimation, recrystallisation and neutralisation processes have been proposed. To remove phthalic acid, whose presence is not allowed for food uses, treatment with amines and rinsing is required.

According to industry (Doc. provided to EFSA n. 2), 'production of benzoic acid is by liquid-phase oxidation of toluene in the presence of a cobalt catalyst. Air is the source of oxygen in this free radical reaction. Reaction by-products include benzaldehyde, acetic acid, formic acid and benzyl alcohol. Crude benzoic acid is recovered by distillation' and 'for sodium benzoate production sodium hydroxide (caustic soda) is added to benzoic acid'.

Sodium benzoate is produced by the neutralisation of benzoic acid with sodium hydroxide (Maki and Takeda, 2000; Doc. provided to EFSA n. 2). Potassium benzoate is obtained in high yield by reacting an aromatic hydrocarbon solution of benzoic acid, such as that which is obtained from the toluene oxidation process, with potassium hydroxide preferably in concentrated aqueous solution, thereby precipitating solid potassium benzoate (Hills, 1975). No information was available regarding the manufacture of calcium benzoate; the ANS Panel assumed that it is manufactured similarly, by reaction of benzoic acid with calcium hydroxide, or other calcium salt. The Panel noted that if limestone is the source of calcium carbonate which is used in the production of calcium benzoate,

then calcium benzoate could be contaminated with aluminium. In its opinion on the re-evaluation of calcium carbonate (E 170) as a food additive (EFSA ANS Panel, 2011), the ANS Panel noted that limestone may contain aluminium at concentrations up to 190 mg/kg. Therefore, specifications for the maximum level of aluminium in calcium benzoate may be required.

2.4. Methods of analysis in food

The Panel noted that a vast number of methods have been described for the determination of benzoic acid and benzoate salts in foods. From the available literature it is evident that the decision on the appropriate method of analysis that should be used may depend primarily on the matrix in which the benzoic acid and benzoate salts are present, although there are some analytical methods which appear to apply to all matrices. High-performance liquid chromatography (HPLC) (Mandrou et al. 1998; Pylypiw and Grether 2000, Tfouni and Toledo 2002; Techakriengkrai and Surakarnkul 2007; Mota et al. 2003), ion chromatography (Xie et al. 1999) and capillary electrophoresis (CE) (Han et al. 2008; Wei et al. 2011) techniques were frequently applied for the analysis of preservatives including benzoic acid and benzoate salts in foodstuffs. Gas chromatographic (GC) methods have also been used where sample pre-treatment involving liquid-liquid extraction, anion-exchange clean-up and precipitation of proteins was often needed (De Luca et al. 1995; González et al. 1999; Dong and Wang 2006).

Kokya et al. (2012) have developed a dispersive liquid-liquid micro-extraction (DLLME) method based on the dispersion of tiny droplets of the extraction liquid within aqueous solution which is followed by GC analysis.

The Panel decided to refer to these publications for more details on the method of analysis of benzoic acid and its salts in a specific food item.

More specifically, the simultaneous determination of sorbic and benzoic acids in tomato sauce and ketchup using HPLC was performed by Nour et al. (2009).

A validated HPLC method for the measurement of benzoic acid in soft drinks has been described by Ene and Diacu (2009).

A novel reversed-phase HPLC method for simultaneous determination of potassium sorbate and sodium benzoate in soft drinks was described by Can et al. (2011) and in beverage, vinegar and fruit jam by CE after online pre-concentration by dynamic pH junction by Zhang et al. (2011).

The determination of benzoic acid residue from fruit juice by gas chromatography with mass spectrometry detection technique was described by Sen et al. (2011).

The analysis of nine food additives, including benzoic acid, in red wine by ion-suppression reversed-phase HPLC using trifluoroacetic acid and ammonium acetate as ion-suppressors was described by Zhao et al. (2012). The simultaneous determination of 11 preservatives in foods, including benzoic acid in various foodstuffs by using ultrasound-assisted emulsification micro-extraction followed by gas chromatography-mass spectrometry (GC-MS), was described by Yang et al. (2012).

The evaluation of analytical performance parameters and uncertainty budget of the HPLC method with UV diode array detection on a reverse phase column for traceable determination of benzoic acid in soft drinks was described by Jurcovan et al. (2012).

The quantification for benzoic acid in processed foods using quantitative proton nuclear magnetic resonance spectroscopy was described by Ohtsuki et al. (2012).

The determination of benzoic acid in milk by solid-phase extraction and ion chromatography with conductivity detection was described by Wang et al. (2013).

The determination of five organic acid preservatives, including benzoic acid, in fruits and beverages diluted with a phosphate buffer solution followed by liquid chromatography analysis was described by Lin et al. (2013).

HPLC and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) methods were developed and validated for the quantitative analysis of sodium benzoate and potassium sorbate in tomato ketchup and orange juice by Gören et al. (2015) and the outcome of a proficiency testing of a number of accredited laboratories was presented.

2.5. Reaction and fate in food

Benzoic acid and its salts are primarily used as food additives (preservatives) in acidified foodstuffs and beverages such as fruit juice, soft drinks and carbonated drinks. Information on the stability of the salts of the benzoic acid in food is very limited, but the benzoate anion is chemically stable, and degradation in food or reactions with food components are not anticipated, with the exception of the reaction with ascorbic acid under certain conditions described below.

2.5.1. Formation of benzene from benzoic acid in the presence of ascorbic acid

Small amounts of benzene can be formed from benzoic acid in the presence of ascorbic acid and transition metal ions like copper(II) and iron(III) (Chang and Ku 1993; Gardner and Lawrence 1993; McNeal et al., 1993). The chemical reaction is catalysed by metal ions, which are present at low concentrations in drinking water. The studies performed by Chang and Ku (1993), Gardner and Lawrence (1993) and McNeal et al. (1993) were summarised by the German Federal Institute for Risk Assessment (BfR, 2005).

In 2006, the European Commission and Member States had become aware of the potential formation of benzene under certain conditions in soft drinks from the reaction of benzoic acid and other ingredients. The issue was considered in the meetings of the Standing Committee of the Food Chain and Animal Health in July and December 2007 (EC, 2007a, b). ‘The Committee noted that the reformulation work by the industry appeared to be working given the limited number of samples in which levels of benzene were above 10 µg/L. However it was considered that further monitoring would be useful although no further formal action was considered necessary by the Commission at this time’.

The Norwegian Scientific Committee for Food Safety reported results of analysis of benzene content in 16 soft drinks containing both sodium benzoate and ascorbic acid; 13 samples contained non-detectable levels of benzene and three samples contained benzene at levels between 0.7 and 4.7 mg/L (VKM, 2007).

In Germany, the concentrations of benzene measured in non-alcoholic beverages were below the EU drinking water limit of 1 µg/L while higher concentrations were found in previous years (Steinbrenner et al., 2010).

Therefore, the Panel considered that combining ascorbic acid with benzoic acid in soft drinks could lead to the formation of benzene from benzoic acid. The Panel noted that benzene is a genotoxic carcinogen classified by the International Agency for Research on Cancer as *carcinogenic to humans* (Group 1) (IARC, 2012), that the World Health Organization (WHO) has derived a guideline value

for benzene in drinking water of 10 µg/L (WHO, 2011), that according to the Council Directive 98/83/EC¹² the maximum level of benzene in drinking water is 1 µg/L in the European Union and that the German Federal Institute for Risk Assessment considered that the concentration of benzene in beverages should be as low as reasonably achievable (BfR 2013).

2.6. Case of need and proposed uses

Maximum levels of benzoic acid-benzoates (E 210–213) have been defined in Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives, as amended. These levels are defined by the Panel as maximum permitted levels (MPLs) in this document.

Currently, benzoic acid-benzoates (E 210–213) are authorised as food additives in the EU with MPLs ranging from 150 to 6,000 mg/kg in foods. The Panel noted that benzoic acid-benzoates (E 210–213) are authorised in combination with sorbic acid-sorbates (E 200, 202, 203) and *p*-hydroxybenzoates (PHB; E 214, 215, 218, 219) (Regulation (EC) No 1333/2008).

Table 5 summarises foods that are permitted to contain benzoic acid-benzoates (E 210–213) and the corresponding MPLs as defined in Annex II to Regulation (EC) No 1333/2008.

Table 5: MPLs of benzoic acid-benzoates (E 210–213) in foods according to Annex II to Regulation (EC) No 1333/2008

FCS category number	FCS food category	E -number	MPL (mg/L or mg/kg as appropriate)	Restrictions/exception
01.4	Flavoured fermented milk products including heat-treated products	E 200–213	300 ^{(a),(b)}	Only non-heat treated dairy-based desserts
04.2.2	Fruit and vegetables in vinegar, oil or brine	E 200–213	2,000 ^{(a),(b)}	Only vegetables (excluding olives)
04.2.2	Fruit and vegetables in vinegar, oil or brine	E 210–213	500 ^{(a),(b)}	Only olives and olive-based preparations
04.2.4.1	Fruit and vegetable preparations excluding compote	E 210–213	500 ^{(a),(b)}	Only seaweed preparations, olives and olive-based preparations
04.2.4.1	Fruit and vegetable preparations excluding compote	E 210–213	2,000 ^{(a),(b)}	Only cooked red beet
04.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EEC	E 210–213	500 ^{(a),(b)}	Only low-sugar and similar low-calorie or sugar-free products, <i>mermeladas</i>
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC	E 210–213	500 ^{(a),(b)}	Only low-sugar and similar low-calorie or sugar-free products; <i>mermeladas</i>
04.2.5.3	Other similar fruit or vegetable spreads	E 200–213	1,500 ^{(a),(b)}	Only <i>marmelada</i>
04.2.5.3	Other similar fruit or vegetable spreads	E 210–213	500 ^{(a),(b)}	Other fruit-based spreads, <i>mermeladas</i>
04.2.5.3	Other similar fruit or vegetable spreads	E 210–213	1,000 ^{(a),(b)}	Only <i>dulce de membrillo</i>

FCS category number	FCS food category	E -number	MPL (mg/L or mg/kg as appropriate)	Restrictions/exception
05.2	Other confectionery including breath refreshing microsweets	E 200–219	1,500 ^{(a),(b),(c)}	Except candied, crystallised or glacé fruit and vegetables
05.2	Other confectionery including breath refreshing microsweets	E 200–213	1,000 ^{(a),(b)}	Only candied, crystallised or glacé fruit and vegetables
05.3	Chewing gum	E 200–213	1,500 ^{(a),(b)}	
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	E 200–219	1,500 ^{(a),(b),(c)}	
08.3.1	Non-heat-treated processed meat	E 200–219	<i>Quantum satis</i> ^{(a),(b)}	Only surface treatment of dried meat products
08.3.2	Heat-treated processed meat	E 210–213	500 ^{(a),(b)}	Only aspic
09.2	Processed fish and fishery products including molluscs and crustaceans	E 200–213	200 ^{(a),(b)}	Only salted, dried fish
09.2	Processed fish and fishery products including molluscs and crustaceans	E 200–213	2,000 ^{(a),(b)}	Only semi-preserved fish and fisheries products including crustaceans, molluscs, surimi and fish/crustacean paste; cooked crustaceans and molluscs
09.2	Processed fish and fishery products including molluscs and crustaceans	E 200–213	6000	Only cooked <i>Crangon crangon</i> and <i>Crangon vulgaris</i>
09.2	Processed fish and fishery products including molluscs and crustaceans	E 210–213	1,000 ^{(a),(b)}	Only cooked Crustaceans and molluscs
09.2	Processed fish and fishery products including molluscs and crustaceans	E 210–213	1,500 ^{(a),(b)}	Only cooked shrimps in brine
09.3	Fish roe	E 200–213	2,000 ^{(a),(b)}	Only semi-preserved fish products including fish roe products
10.2	Processed eggs and egg products	E 200–213	5,000 ^{(a),(b)}	Only liquid egg (white, yolk or whole egg)
11.4.1	Table-top sweeteners in liquid form	E 200–219	500 ^{(a),(b)}	Only if the water content higher than 75 %
12.2.2	Seasonings and condiments	E 200–213	1,000 ^{(a),(b)}	
12.4	Mustard	E 200–213	1,000 ^{(a),(b)}	
12.5	Soups and broths	E 200–213	500 ^{(a),(b)}	Only liquid soups and broths (excluding canned)
12.6	Sauces	E 210–213	1,000 ^{(a),(b)}	Only emulsified sauces with a fat content of less than 60%
12.6	Sauces	E 200–213	1,000 ^{(a),(b)}	Only emulsified sauces with a fat content of 60% or more; non-emulsified sauces
12.6	Sauces	E 210–213	500 ^{(a),(b)}	Only emulsified sauces with a fat content of 60% or more
12.7	Salads and savoury-based sandwich spreads	E 200–213	1,500 ^{(a),(b)}	

FCS category number	FCS food category	E -number	MPL (mg/L or mg/kg as appropriate)	Restrictions/exception
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	E 200–213	1,500 ^{(a),(b)}	
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	E 200–213	1,500 ^{(a),(b)}	
14.1.2	Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices	E 200–213	2,000 ^{(a),(b)}	Only grape juice, unfermented, for sacramental use
14.1.2	Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices	E 210–213	200 ^{(a),(b)}	Only <i>Sød.....saft</i> and <i>sødet.....saft</i>
14.1.3	Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and similar products	E 210–213	150 ^{(a),(b)}	Only traditional Swedish and Finnish fruit syrups
14.1.4	Flavoured drinks	E 210–213	150 ^{(a),(b)}	Excluding dairy-based drinks
14.1.5.2	Other	E 200–213	600 ^{(a),(b)}	Only liquid tea concentrates and liquid fruit and herbal infusion concentrates
14.2.1	Beer and malt beverages	E 210–213	200 ^{(a),(b)}	Only alcohol-free beer; beer in kegs containing more than 0.5% added fermentable sugar and/or fruit juices or concentrates
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts	E 210–213	200 ^{(a),(b)}	Only alcohol-free
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	E 210–213	200 ^{(a),(b)}	Only alcoholic drinks with less than 15% of alcohol
16	Desserts excluding products covered in category 1, 3 and 4	E 200–213	300 ^{(a),(b)}	Only non-heat-treated dairy-based desserts
16	Desserts excluding products covered in category 1, 3 and 4	E 210–213	500 ^{(a),(b)}	Only <i>frugtgrød</i> and <i>Rote Grütze</i>
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms	E 200–213	1,000 ^{(a),(b)}	Only when supplied in dried form and containing preparations of vitamin A and of combinations of vitamins A and D
17.2	Food supplements supplied in a liquid form	E 200–213	2,000 ^{(a),(b)}	

FCS, Food Categorisation System (food nomenclature) presented in Annex II to Regulation (EC) No 1333/2008; E 210–213: benzoic acid-benzoates; E 200–213: sorbic acid-sorbates; benzoic acid-benzoates; E 200–219: sorbic acid-sorbates; benzoic acid-benzoates; PHB.

(a): The additives may be added individually or in combination.

(b): The maximum level is applicable to the sum and the levels are expressed as the free acid.

(c): E 214–219: PHB, maximum 300 mg/kg.

According to Annex II, Part B, benzoic acid may be present in certain fermented products resulting from the fermentation process following good manufacturing practice.

In addition, benzoic acid, sodium benzoate and potassium benzoate (E 210–211–212) are permitted to be used as food additives other than carriers in food additives according to Annex III to Regulation (EC) No 1333/2008 (Part 2). Thus, these three food additives may be used at concentrations up to 1,500 mg/kg in food additives (colour preparations) individually or in combination (with sorbic acid-sorbates) in the preparation resulting in a maximum of 15 mg/kg in the final product expressed as the free acid. Added to that, benzoic acid (E 210) and sodium benzoate (E 211) are also permitted to be used as food additives including carriers in food enzymes according to Annex III to Regulation (EC) No 1333/2008 (Part 3). They may be used in enzyme preparations up to 5,000 mg/kg individually or in combination (expressed as the free acid) resulting in a maximum of 1.7 mg/kg in the final food, except for beverages, where a maximum level of 0.85 mg/L is authorised. The maximum level in enzyme preparation is 12,000 mg/kg in rennet, and thus maximum levels are 5 mg/kg in cheese where rennet has been used and 2.5 mg/L in whey-based beverages where rennet has been used. Finally, according to Annex III, Part 4, to Regulation (EC) No 1333/2008, benzoic acid-benzoates (E 210–213) are authorised as food additives including carriers in food flavourings in all food flavourings up to 1,500 mg/kg (singly or in combination, expressed as free acid).

The Panel is aware that additional usages from the existing authorisation of benzoic acid-benzoates (E 210–213) in applications for colour preparations, enzyme preparations and flavourings may add substantially to the overall exposure to benzoic acid-benzoates. The Panel noted that, from a methodological point of view, it was not feasible to differentiate between all contributions (i.e. uses as food additives and in enzyme/colour preparations and flavouring) in the overall exposure to benzoic acid-benzoates. Therefore, the Panel considered that the use of analytical data in the refined exposure assessment would be the most appropriate approach to capture all uses and to address the overall safety assessment of benzoic acid-benzoates (E 210–213).

2.7. Reported use levels or data on analytical levels of benzoic acid-benzoates (E 210–213) in food

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels might be required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to *quantum satis* (QS).

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010¹³ regarding the re-evaluation of approved food additives, EFSA issued a public call^{14,15} for occurrence data on benzoic acid-benzoates (E 210–213). In response to this public call, Member States submitted analytical data on benzoic acid to EFSA. In addition, information on the use levels of benzoic acid-benzoates (E 210–213) in foods was made available to EFSA by industry.

¹³ Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19.

¹⁴ Call for scientific data on food additives permitted in the EU and belonging to the functional classes of preservatives and antioxidants. Published: 23 November 2009. Available online: <http://www.efsa.europa.eu/en/dataclosed/call/ans091123a.htm>

¹⁵ Call for approved food additives (sorbates, benzoates and gallates) occurrence data in food and beverages intended for human consumption. Published: 31 May 2012. Available online: <http://www.efsa.europa.eu/sites/default/files/consultation/120601.pdf>

2.7.1. Summarised data on reported use levels in foods provided by industry

Information on the actual use levels of benzoic acid-benzoates (E 210–213) in foods was made available to EFSA by FoodDrinkEurope (FDE), the Union of European Soft Drinks Associations (UNESDA) and Mars Chocolate UK. EFSA was provided with data on use levels for 9 out of the 45 food categories in which benzoic acid-benzoates (E 210–213) are authorised.

Appendix A provides data on the use levels in foods as reported by industry.

2.7.1.1. Summarised data extracted from the Mintel GNPD

Mintel's Global New Products Database (GNPD) is an online database which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over 2 million food and beverage products with more than 800,000 of those in the European food market. Mintel started covering European Union's food markets in 1996, having 20 out of its 28 member countries presented in the GNPD.¹⁶

The Panel noted that, according to the Mintel GNPD database,¹⁷ more than 10,000 products labelled to contain benzoic acid-benzoates (E 210–213) were put on the European market between 1996 and 2015. The usage of benzoic acid-benzoates highly increased in this time period, especially from 2013. The main food categories to which benzoic acid-benzoates are added are carbonated soft drinks, table sauces and fish products.

2.7.2. Summarised data on concentration levels in food submitted by Member States

In total, 39,303 analytical results were reported to EFSA by 12 countries: Slovakia (n = 15,899), Germany (n = 12,722), Denmark (n = 3,209), Austria (n = 2,644), Hungary (n = 1,472), Cyprus (n = 1,679), the Czech Republic (n = 614), Ireland (n = 637), Spain (n = 327), Portugal (n = 49), Bulgaria (n = 41) and Luxembourg (n = 10). All analytical results referred to benzoic acid. Foods were sampled between 2000 and 2015 and the majority of them (97%) were analysed in the year of collection. Analytical results of benzoic acid were not quantified (less than the limit of quantification (LOQ)) in 16,943 samples and not detected (less than the limit of detection (LOD)) in 8,398 samples. In total, 13,956 samples were numerical values (quantified). Six results were qualitative (binary results) and gave only indication of presence or absence of benzoic acid. These six samples were therefore removed from the final database. Only 86 of all these samples came from a non-accredited laboratory. The Panel noted that complete information on the methods of analysis (e.g. validation) was not made available to EFSA.

The data were mainly on flavoured drinks (Food Categorisation System (FCS) 14.1.4), fine bakery wares (FCS 07.2) and salads and savoury-based sandwich spreads (FCS 12.7).

In order to include only recent data from the 10-year period, analytical results sampled before 2004 (n = 1603) were excluded from further analyses.

In total, 16,382 samples were classified either at upper level 1 of the FCS (therefore, it was not possible to classify them according to the FCS food categories properly) or in a subgroup other than those authorised and were, therefore, also excluded. This related to the following food categories: dairy products and analogues (FCS 01); fats and oils, and fat emulsions (FCS 02); edible ices (FCS 03); fruit and vegetables (FCS 04); cereals and cereal products (FCS 06); bakery wares (FCS 07);

¹⁶ Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.

¹⁷ Mintel Global New Products Database (<http://www.mintel.com/global-new-products-database>) [Accessed: 18 December 2015].

meat (FCS 08); fish and fishery products (FCS 09); sugars, syrups, honey and table-top sweeteners (FCS 11); salts, spices, soups, sauces, salads and protein products (FCS 12); foods intended for particular nutritional uses (FCS 13); non-alcoholic beverages (FCS 14.1); and alcoholic beverages (FCS 14.2).

Data (n = 328) above the MPL set for authorised uses of benzoic acid-benzoates (E 210–213) as food additives were reported mainly in the following food categories: flavoured drinks (FCS 14.1.4) (n = 232) and sauces (FCS 12.6) (n = 35). The Panel considered exposure resulting only from authorised uses with occurrence levels not exceeding the MPLs because results over MPL are part of risk management measures, e.g. non-compliance purpose. For this reason, such analytical results over the MPLs were not considered in the exposure assessment.

Overall, 20,984 out of the 39,303 total analytical results reported for benzoic acid in foods were considered by the Panel for the exposure estimates after discarding the following: the data sampled before 2004 (n = 1,603), the qualitative results (n = 6), the provided analytical results on foods in which the direct addition of benzoic acid-benzoates (E 210–213) is not authorised according to Annex II to Regulation (EC) No 1333/2008 (n = 16,382) and the samples exceeding the MPL (n = 328).

Appendix B shows the analytical results of benzoic acid in foods as reported by Member States (whole set of analytical data reported (Appendix B1) and dataset excluding results above the MPLs (Appendix B2)).

2.8. Information on existing authorisations and evaluations

Benzoic acid (E 210), sodium benzoate (E 211), potassium benzoate (E 212) and calcium benzoate (E 213) are authorised as food additives in the EU in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives. Specific purity criteria have been defined in Commission Regulation (EU) No 231/2012.

Benzoic acid, sodium benzoate, potassium benzoate and calcium benzoate (E 210, E 211, E 212 and E 213) have been previously evaluated by the SCF in 1994 and 2002 (SCF, 1994, 2002). JECFA evaluated benzoic acid and its salts as food additives at its sixth, ninth, seventeenth, twenty-seventh and forty-sixth meetings (JECFA, 1962, 1966, 1974a, 1974b, 1983, 1996, 1997). JECFA have additionally reviewed benzoic acid as a flavouring substance (JECFA, 2001, 2002). Benzoic acid and its salts have also been reviewed by TemaNord (2002).

In its 1994 opinion on benzoic acid and its salts, the SCF (1994) set a temporary acceptable daily intake (ADI) of 5 mg/kg body weight (bw) based on an overall no observed adverse effect level (NOAEL) of 500 mg/kg bw per day from long-term and multi-generation studies. Further studies on developmental toxicity and genotoxicity were requested. In 2002, the SCF evaluated additional *in vivo* genotoxicity, reproductive and developmental toxicity studies with benzoic acid, benzyl alcohol, benzyl acetate or sodium benzoate (SCF, 2002). A NOAEL of 500 mg benzyl acetate/kg bw per day was identified by the SCF in a developmental toxicity study in rats, based on effects on fetal weight and this NOAEL was considered by the SCF to be consistent with the NOAELs seen in the reproduction and developmental toxicity studies with the other benzyl derivatives. The SCF (2002) established a group ADI of 5 mg/kg bw per day expressed as benzoic acid for benzoic acid and its salts including benzyl alcohol and related benzyl derivatives used as flavourings.

In 1974, JECFA established a group ADI of 0–5 mg/kg bw for benzoic acid and its salts, expressed as benzoic acid (JECFA, 1974a, b). This ADI was based on the results of a multi-generation study in rats in which a dose level of 1% in the diet, equivalent to 500 mg/kg bw per day, had no effect on growth, fertility, lactation or lifespan (JECFA, 1974b). In 1993, at its 41st meeting, as part of its evaluation of

benzyl acetate as a flavouring agent, JECFA noted the absence of reproduction/teratology studies for the substances in the general group of benzyl derivatives and recommended that benzoic acid, the benzoates, benzyl alcohol, benzaldehyde and benzyl acetate should be re-evaluated to determine whether these or other studies were required (JECFA, 1993). After receipt of new information, a comprehensive review was carried out at its 46th meeting (JECFA, 1996, 1997). JECFA concluded that the data reviewed on the compounds in this group were *sufficient to demonstrate lack of carcinogenic, developmental and reproductive potential* and the group ADI of 0–5 mg/kg bw was maintained (JECFA, 1997), based on the same multi-generation study in rats used in 1974, supported by the results of other reproductive and developmental toxicity studies.

JECFA has evaluated the dietary exposure to benzoates and concluded that *the 95th percentile exposures for the consumers-only group exceeded the upper bound of the ADI in some cases: up to 10.9 mg/kg bw per day for toddlers and young children and up to 7.0 mg/kg bw per day for other children, including adolescents. Additionally, the Committee noted that in some countries, the overall dietary exposure to benzoates for toddlers, young children and adolescents also exceeds the upper bound of the ADI at the high percentiles. Reduction of those exposures exceeding the upper bound of the ADI would require consideration of dietary patterns for both beverage and non-beverage foods containing benzoates and typical/allowed benzoate use levels in those countries* (JECFA, 2015).

In 2008, the EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC) assessed the results of McCann et al. (2007) study that has concluded that exposure to two mixtures of four synthetic colours plus sodium benzoate, as a preservative, in the diet resulted in increased hyperactivity in 3-year-old and 8- to 9-year-old children in the general population. The AFC Panel concluded that the study *provides limited evidence that the two different mixtures of synthetic colours and sodium benzoate tested had a small and statistically significant effect on activity and attention in some children selected from the general population, although the effects were not observed for all children in all age groups and were not consistent for the two mixtures. The findings may thus be relevant for specific individuals within the population, showing sensitivity to food additives in general or to food colours in particular* (EFSA, 2008).

Benzoic acid (FL-No. 08.021) is included in the Union list of flavourings (Commission Implementing Regulation (EU) No 872/2012¹⁸). JECFA reviewed benzoic acid as a flavouring substance at its 59th meeting, as one of a group of benzyl derivatives, at which the group ADI of 0–5 mg/kg bw for benzoic acid and related compounds was reconfirmed (JECFA 2001, 2002). EFSA has evaluated benzoic acid as a supporting substance in its opinion on benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters from chemical group 23 and 30 (EFSA CEF Panel, 2011, 2012), in which it was concluded on the basis of the default maximised survey-derived daily intake (MSDI) approach that these substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

Benzoic acid as a biocide was evaluated by Germany (BAuA, 2011; Assessment report, 2013a, 2013b) and is permitted for this use according to Commission Implementing Regulation (EU) 1035/2013.¹⁹

Benzoic acid and sodium benzoate have also been evaluated by the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP, 2002) and the Scientific

¹⁸ Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting a list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1–161

¹⁹ Commission implementing Regulation (EC) No 1035/2013 of 24 October 2013 approving benzoic acid as an existing active substance for use in biocidal products for products – types 2 and 4. OJ L 283, 25.10.2013, p. 31–34.

Committee on Consumer Products (SCCP, 2005). Additionally, benzoic acid has been reviewed as a pesticide-active substance (EC, 2003).

Benzoic acid and sodium benzoate are included in the European Union Register²⁰ of feed additives (Regulation (EC) No 1831/2003²¹). Benzoic acid is authorised as technological feed additive (acidity regulator) for pigs for fattening (10,000 mg/kg), as zootechnical additive for weaned piglets (5,000 mg/kg) and pigs for fattening (10,000 mg/kg) and as a chemically defined flavouring (125 mg/kg) (EFSA FEEDAP Panel, 2016).

2.9. Exposure assessment

2.9.1. Food consumption data used for exposure assessment

2.9.1.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a)). New consumption surveys recently²² added in the Comprehensive database were also taken into account in this assessment.²³

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 6).

²⁰ Available online: http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm_register_feed_additives_1831-03.pdf

²¹ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29–43.

²² Available online: <http://www.efsa.europa.eu/en/press/news/150428.htm>

²³ Available online: <http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm>

Table 6: Population groups considered for the exposure estimates of benzoic acid-benzoates (E 210–213)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 4 up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, the Netherlands, Spain, UK
Children ^(a)	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly ^(a)	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a): The terms ‘children’ and ‘the elderly’ correspond, respectively, to ‘other children’ and the merge of ‘elderly’ and ‘very elderly’ in the Guidance of EFSA on the ‘Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment’ (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the FCS as presented in Annex II to Regulation (EC) No 1333/2008, part D, to perform exposure calculations.

2.9.1.2. Food categories considered for the exposure assessment of benzoic acid-benzoates (E 210–213)

The food categories in which the use of benzoic acid-benzoates (E 210–213) are authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system food codes) at the most detailed level possible (up to FoodEx level 4) (EFSA, 2011b).

Some food categories are not referenced in the EFSA Comprehensive Database and could therefore not be taken into account in the present exposure assessment. This may have resulted in an underestimation of the exposure. The food categories which were not taken into account are described below (in ascending order of the FCS codes):

- 04.2.5.3. Other similar fruit or vegetable spreads, only *dulce de membrillo*, only *marmeladas*
- 05.4. Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4
- 08.3.1. Non-heat-treated processed meat, only surface treatment of dried meat products
- 14.1.2. Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices, only grape juice, unfermented, for sacramental use
- 14.1.2. Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices, only *Sød.....saft* and *sødet.....saft*
- 14.1.3. Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and similar products, only traditional Swedish and Finnish fruit syrups
- 14.1.5.2. Other, only liquid tea concentrates and liquid fruit and herbal infusion concentrates

- 14.2.2. Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts, only alcohol-free
- 16. Desserts excluding products covered in category 1, 3 and 4, only *frugtgrød* and *Rote Grütze*

For the following food categories, the restrictions which apply to the use of benzoic acid-benzoates (E 210–213) could not be taken into account. Therefore, the whole food category was considered for the present exposure estimates. This may have resulted in an overestimation of the exposure:

- 01.4. Flavoured fermented milk products including heat-treated products, only non-heat-treated dairy-based desserts
- 04.2.5.1. Extra jam and extra jelly as defined by Directive 2001/113/EC,²⁴ only low-sugar and similar low calorie or sugar-free products, *mermeladas*
- 04.2.5.2. Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC, only low-sugar and similar low-calorie or sugar-free products, *mermeladas*
- 09.3. Fish roe, only semi-preserved fish products including fish roe products
- 11.4.1. Table-top sweeteners in liquid form, only if the water content higher than 75%
- 12.6. Sauces, only emulsified sauces with a fat content of less than 60%, or only emulsified sauces with a fat content of 60% or more
- 17.1. Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms, only when supplied in dried form and containing preparations of vitamin A and of combinations of vitamins A and D and 17.2. Food supplements supplied in a liquid form

For the FCS 09.2. ‘Processed fish and fishery products including molluscs and crustaceans’, only salted, dried fish, the fish which are usually salted or dried were taken into account (herring, sprat, anchovy, salmon and trout, cod and whiting).

For the FCS 09.2. ‘Processed fish and fishery products including molluscs and crustaceans’, two restrictions relate to shrimps: ‘only cooked *Crangon crangon* and *Crangon vulgaris*’ and ‘only cooked shrimps in brine’. As shrimps cannot be differentiated in FoodEx, all shrimps were applied the same concentration level.

Overall, 10 food categories were not taken into account in the exposure assessment because they are not referenced in the EFSA Comprehensive Database. Eight food categories were included in the exposure assessment without considering the restrictions/exceptions as set out in Annex II to Regulation No 1333/2008. For the remaining food categories (n = 25), the refinements considering the restrictions/exceptions as set out in Annex II to Regulation No 1333/2008 were applied. Finally, 35 food categories were included in the present assessment of exposure to benzoic acid-benzoates (E 210–213) (Appendix C).

2.9.2. Exposure to benzoic acid-benzoates (E 210–213) from their use as food additives

The Panel estimated chronic exposure to benzoic acid-benzoates (E 210–213) for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. Dietary exposure to benzoic acid-benzoates (E 210–213) was calculated by multiplying benzoic acid-benzoates (E 210–

²⁴ Council Directive 2001/113/EC of 20 December 2001 relating to fruit jams, jellies and marmalades and sweetened chestnut purée intended for human consumption. OJ L 10, 12.1.2002, p. 67–72.

213) concentrations for each food category (Appendix C and H) with their respective consumption amount per kilogram of body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only 1 day per subject were excluded as they are considered as not adequate to assess repeated exposure.

The dietary exposure was assessed for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 6). Based on these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. High percentile exposure was only calculated for those population groups where the sample size was sufficiently large (> 60 subjects) to allow calculation of the 95th percentile of exposure (EFSA, 2011a). Therefore, in the present assessment, high levels of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

Exposure assessment of food additives under re-evaluation was carried out by the ANS Panel based on (1) MPLs as set down in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*); and (2) the reported use levels and analytical data (defined as the *refined exposure assessment scenario*). For benzoic acid-benzoates (E 210–213), considering the important number of data received, dietary exposure through this latter scenario was assessed using two sets of concentration data:

1. reported use levels and analytical data considering levels not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised (Annex II to Regulation No 1333/2008) (**dataset 1**)
2. reported use levels and analytical data considering levels not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised and analytical data for food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available (**dataset 2**).

2.9.2.1. Regulatory maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set out in Annex II to Regulation No 1333/2008 and listed in Table 5.

The exposure estimates derived following this scenario should be considered as the most conservative considering only the use of benzoic acid-benzoates (E 210–213) in food categories listed in Annex II to Regulation No 1333/2008, as this scenario assumes that the consumer will be continuously (over a lifetime) exposed to benzoic acid-benzoates (E 210–213) present in the food at the MPLs.

2.9.2.2. Refined exposure assessment scenario

The refined exposure assessment scenario is based on information on reported use levels by industry and analytical results submitted to EFSA by Member States. This exposure scenario can consider only food categories for which these data were available to the Panel.

Appendices C and H summarise the concentration levels of benzoic acid-benzoates (E 210–213) used in the refined exposure assessment scenario. For the two datasets, the Panel calculated two exposure estimates based on different model populations.

- (1) The brand-loyal consumer scenario: It was assumed that a consumer is exposed over a long period of time to the food additive present at the maximum reported use/analytical level, for one food category. This exposure estimate is calculated as follows:
 - By combining food consumption with the maximum reported use level or the maximum of the analytical results, whichever is highest, for the main contributing food category at the individual level
 - By using the mean of the typical reported use levels or the mean of analytical results, whichever is highest, for the remaining food categories
- (2) The non-brand-loyal consumer scenario: It was assumed that the population is exposed over a long period of time to the food additive present at the mean reported use/analytical levels in food. This exposure estimate is calculated using the mean of the typical reported use levels or the mean of analytical results, whichever is highest, for all food categories.

Considering the high heterogeneity of the foods within food category 18 ‘Processed foods not covered by categories 1–17, excluding foods for infants and young children’, the brand-loyal scenario is considered not applicable to this food category; therefore, the mean level was used instead of the maximum level also in the brand-loyal scenario.

In the brand-loyal consumer scenario including food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available (**dataset 2**), the 95th percentile level of a food category was used instead of the maximum value in order to minimise the impact of possible outliers. However, for food categories listed in Annex II to Regulation (EC) No 1333/2008, in case the 95th percentile level was below the MPL, the maximum value below the MPL as reported in dataset 1 was used also in dataset 2.

In the refined exposure assessment scenario, the concentration levels considered by the Panel were extracted from the whole dataset received (i.e. reported use levels and analytical results). To consider left-censored analytical data (i.e. analytical results < LOD or < LOQ), the substitution method as recommended in the ‘Principles and Methods for the Risk Assessment of Chemicals in Food’ (WHO, 2009) and the EFSA scientific report ‘Management of left-censored data in dietary exposure assessment of chemical substances’ (EFSA, 2010) was used. In the present opinion, analytical data below the LOD or LOQ were assigned half of the LOD or LOQ, respectively (middle-bound). Subsequently, per food category, the mean or median, as appropriate, middle-bound concentration was calculated.

For the reported use levels, the mean typical reported use level for each food category was calculated. If the typical use level was reported as a range, then a normal distribution within the food category was assumed and the mean based on the lower and the upper value of the range was calculated.

2.9.2.3. Exposure to benzoic acid-benzoates (E 210–213)

Table 7 summarises the exposure to benzoic acid-benzoates (E 210–213).

Table 7: Summary of exposure to benzoic acid-benzoates (E 210–213) in six population groups from their use as food additives using the regulatory maximum level exposure assessment scenario and refined exposure assessment scenarios (minimum to maximum across the dietary surveys in mg/kg bw per day)

	Infants (4–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level exposure assessment scenario						
Mean	0.2–1.5	0.8–8.4	0.8–6.5	0.5–3.6	0.6–3.4	0.4–2.8
High level	0.8–5.5	3.3–13.8	3.6–14.7	1.8–8.7	1.7–7.8	1.2–7.0
Refined estimated exposure scenario considering concentration levels not exceeding the MPLs for food categories listed under Annex II to Regulation No 1333/2008 (dataset 1)						
Brand-loyal scenario						
Mean	0.07–0.8	0.6–3.2	0.7–2.6	0.4–1.9	0.4–2.0	0.2–1.6
High level	0.4–3.2	1.9–7.0	2.7–7.1	1.5–5.3	1.2–5.1	0.6–4.3
Non-brand-loyal scenario						
Mean	0.02–0.3	0.1–1.6	0.3–1.5	0.2–1.1	0.1–0.6	0.04–0.4
High level	0.05–1.7	0.2–4.7	1.1–3.8	0.7–2.6	0.5–1.9	0.2–1.0
Refined estimated exposure scenario considering in addition to dataset 1, analytical data for food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available (dataset 2)						
Brand-loyal scenario						
Mean	5.4–7.2	7.3–10.6	4.6–9.8	2.5–5.2	2.9–4.6	3.0–4.7
High level	10.3–15.9	12.3–19.6	8.0–17.6	4.9–11.0	5.3–8.5	5.4–7.8
Non-brand-loyal scenario						
Mean	1.3–2.1	2.2–3.5	1.5–3.1	0.8–1.9	1.0–1.6	0.8–1.5
High level	2.3–4.1	3.4–6.9	3.1–5.6	1.7–3.7	1.7–3.0	1.3–2.6

From the *regulatory maximum level exposure assessment scenario*, mean exposure to benzoic acid-benzoates (E 210–213) from their use as food additives ranged from 0.2 mg/kg bw per day in infants to 8.4 mg/kg bw per day in toddlers. The high level (95th percentile) of exposure to benzoic acid-benzoates (E 210–213) ranged from 0.8 mg/kg bw per day in infants to 14.7 mg/kg bw per day in children.

From the *refined estimated exposure scenario* considering concentration levels not exceeding the MPLs for food categories listed under Annex II to Regulation No 1333/2008, in the *brand-loyal scenario*, mean exposure to benzoic acid-benzoates (E 210–213) from their use as food additives ranged from 0.07 mg/kg bw per day in infants to 3.2 mg/kg bw per day in toddlers. The high exposure to benzoic acid-benzoates (E 210–213) ranged from 0.4 mg/kg bw per day in infants to 7.1 mg/kg bw per day in children. In the *non-brand-loyal scenario*, mean exposure to benzoic acid-benzoates (E 210–213) from their use as food additives ranged from 0.02 mg/kg bw per day in infants to 1.6 mg/kg bw per day in toddlers. The high exposure to benzoic acid-benzoates (E 210–213) ranged from 0.05 mg/kg bw per day in infants to 4.7 mg/kg bw per day in toddlers.

From the *refined estimated exposure scenario* considering concentration levels not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised and analytical data for food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available, in the *brand-loyal scenario*, mean exposure to benzoic acid-benzoates (E 210–213) ranged from 2.5 mg/kg bw per day in adolescents to 10.6 mg/kg bw per day in toddlers. The high exposure to benzoic acid-benzoates (E 210–213) ranged from 4.9 mg/kg bw per day for adolescents to 19.6 mg/kg bw per day in toddlers. In the *non-brand-loyal scenario*, mean exposure to

benzoic acid-benzoates (E 210–213) ranged from 0.8 mg/kg bw per day for adolescents and the elderly to 3.5 mg/kg bw per day in toddlers. The high exposure to benzoic acid-benzoates (E 210–213) ranged from 1.3 mg/kg bw per day for the elderly to 6.9 mg/kg bw per day in toddlers.

The inclusion of analytical results above the MPLs for the food categories listed under Annex II to Regulation No 1333/2008 did not change the results of the scenario using dataset 2. Appendix L shows the anticipated exposure to benzoic acid-benzoates using this last scenario.

2.9.3. Main food categories contributing to exposure to benzoic acid-benzoates (E 210–213)

From the *regulatory maximum level exposure assessment scenario*, the main contributing food categories to the total mean exposure estimates for infants and toddlers were flavoured fermented milk products. For children and adolescents, the main contributing food categories were confectionary, flavoured drinks and processed fish and fishery products; while, for adults and the elderly, the main contributing food categories were processed fruit and vegetables and sauces.

The main contributing food categories from the *refined estimated exposure scenario* considering concentration levels not exceeding the MPLs for food categories listed under Annex II to Regulation No 1333/2008, in the *brand-loyal scenario* were flavoured fermented milk products and sauces for infants, flavoured fermented milk products, processed fish and fishery products and flavoured drinks; for children, adolescents, adults and the elderly, they were flavoured drinks, sauces and processed fish and fishery products. In the *non-brand-loyal scenario*, the main contributing food categories were flavoured drinks and sauces for all population groups.

The main contributing food categories from the *refined estimated exposure scenario* considering concentration levels not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is allowed and analytical data for food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available, the *brand-loyal scenario*, were unprocessed fruits and vegetables for all population groups. When considering the *non-brand-loyal scenario*, the main contributing food categories were also processed fruits and vegetables and processed foods for infants; for toddlers, they were unprocessed fruits and vegetables, flavoured drinks and processed foods; for children and adolescents, the main contributing food categories were flavoured drinks and unprocessed fruits and vegetables and for adults and the elderly, the main contributing food categories were unprocessed fruits and vegetables and coffee, tea, herbal and fruit infusions, chicory.

The main food categories contributing to the combined exposure to benzoic acid-benzoates (E 210–213) are presented in Appendices E, F, G, J and K.

2.9.4. Uncertainty analysis

Uncertainties in the exposure assessment of benzoic acid-benzoates (E 210–213) have been discussed above. According to the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the sources of uncertainty summarised in Table 8 have been considered.

Table 8: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainty	Direction ^(a)
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/-
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels and analytical data to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties as to which types of food the levels refer to	+/-
Food categories selected for the exposure assessment: exclusion of food categories owing to missing FoodEx linkage (n = 10/45 food categories)	-
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception (n = 8/45 food categories)	+
Concentration data: levels considered applicable for all items within the entire food category, exposure calculations based on the maximum or mean levels (reported use from industry or analytical data from Member States)	+/-
Regulatory maximum level exposure assessment scenario:	
- food categories which may contain benzoic acid-benzoates due to carry-over not considered	-
- food categories authorised at MPL according to Annex II to Regulation (EC) No 1333/2008	+
Refined exposure scenarios dataset 1:	
- food categories which may contain benzoic acid-benzoates due to carry-over not considered	-
- concentration data: levels considered applicable for all items within the entire food category, exposure calculations based on the maximum reported use levels/p95 of analytical data or mean levels	+/-
- analytical levels above the MPLs were excluded	-
Refined exposure scenarios dataset 2:	
- food categories which may contain benzoic acid-benzoates due to carry-over considered	+/-
- concentration data: levels considered applicable for all items within the entire food category, exposure calculations based on the maximum reported use levels/p95 of analytical data or mean levels	+/-
- analytical levels above the MPLs were excluded	-
Uncertainty in possible national differences in use levels of food categories, concentration data not fully representative of foods on the EU market	+/-

MPL: maximum permitted level.

(a): + = uncertainty with the potential to cause overestimation of exposure; - = uncertainty with the potential to cause underestimation of exposure.

Overall, the Panel considered that the uncertainties identified would generally lead to an overestimate of the real exposure to benzoic acid-benzoates as food additives in European countries for the *regulatory maximum level exposure scenario* as it is considered that benzoates are used in food categories listed in annex II to Regulation No°1333/2008 at the MPL.

For the refined estimated exposure scenario considering concentration levels not exceeding the MPLs for food categories listed under Annex II to Regulation No 1333/2008 (dataset 1), uncertainties would lead to an underestimation of exposure to benzoic acid-benzoates if it is considered the food additive may be present in foods according to Annex III to Regulation No°1333/2008.

3. Biological and toxicological data

3.1. Absorption, distribution, metabolism and excretion (ADME)

The Panel considered that due to its ionisation properties ($pK_a = 4.19$), the non-ionised form of benzoic acid will be absorbed by a diffusion process in the acid environment of the stomach. The Panel also considered that sodium benzoate, potassium benzoate and calcium benzoate will dissociate into their constituent sodium, potassium or calcium and benzoate ions in the small intestine. In the case of calcium benzoate, while this salt is of lower solubility than sodium or potassium benzoate, the Panel considered that its solubility would be sufficient to allow dissolution and dissociation to benzoate and calcium ions in the gastrointestinal tract.

3.1.1. *In vitro* studies

The *ex vivo* intestinal absorption of benzoic acid was studied in perfused rat small intestine preparations (Cong et al., 2001). A rapid and uneven absorption was observed among the segmental regions, absorption being highest in the jejunum and slightly lower in the ileum. The absorption pattern paralleled the distribution of the monocarboxylic acid transporter (Mct1), suggesting that this transporter may play a role in the absorption of benzoic acid.

3.1.2. *In vivo* studies

Animal data

As summarised by JECFA (1997, 2001, 2002), SCF (1994), SCCP (2005) and EFSA CEF Panel (2011, 2012), after oral intake, benzoates are rapidly and fully absorbed from the gastrointestinal tract, metabolised primarily in the liver, and excreted in the urine as glycine conjugates of benzoic acid derivatives, mainly as hippuric acid (*N*-benzoyl glycine) but also potentially as benzoic acid, benzoyl glucuronide, 3-hydroxy-3-phenylpropionic acid and ornithic acid. After administration of high doses (> 1000 mg/kg bw), formation of the glycine conjugate is limited by the availability of glycine in the species under study. When glycine is depleted, free benzoic acid may sequester acetyl coenzyme A or be excreted unchanged or as the glucuronic acid conjugate. The Panel noted that there is an inter-species difference in relation to the ability to metabolise benzoic acid in that cats have a limited capacity for glucuronidation, resulting in a greater susceptibility of this species to benzoate toxicity compared to other species (WHO, 2005).

When rats were fed 1.5 % sodium benzoate in the diet, they excreted between 94% and 95% as hippuric acid in the urine. As the benzoate in the diet was increased to 3.75%, between 72% and 73% was excreted as hippuric acid in the urine. Addition of glycine (as a percentage of the diet, between 0.39 and 1.56) to a diet containing 3% sodium benzoate increased the percentage excreted as hippuric acid from between 71% and 73% to 86% and 99%. The only other derivative found in significant amounts in the urine was presumed by the authors to be benzoyl glucuronide (Griffith, 1929).

JECFA 1997 stated that *benzoic acid and hippuric acid are generated as a result of phenylalanine and tyrosine metabolism*, likely based on data reported by Bernhard et al (1955). They reported that when guinea pigs, rabbits and humans were given oral doses of deuterio-DL-phenylalanine (guinea pigs 300–520 mg/kg bw per day, rabbits 50–400 mg/kg bw per day, humans 14–28 mg/kg bw per day) for 6–12 days, 0.03–0.15%, 0.2–2.82% and 0.04–0.14%, respectively, of the label were found in the urine as benzoate. From the results the authors concluded that phenylalanine is a source of benzoate formation. However, the authors also discussed the possibility that intestinal bacteria may have formed benzoate. Current textbook knowledge does not indicate that DL-phenylalanine is metabolised to benzoate. Given that experimental analytical details are poorly reported, the Panel considered the

results of Bernhard et al. (1955) not reliable for this risk assessment and considered that there is no evidence that benzoic acid is generated endogenously from phenylalanine or tyrosine.

In studies on the distribution and elimination of $1\text{-}^{14}\text{C}$ -labelled sodium benzoate administered intraperitoneally to rats (50 mg/animal), there was no evidence of bioaccumulation of benzoate (Lang and Lang, 1956). Almost complete excretion occurred in the urine within 1–2 days; less than 1% of radiolabel appeared in the faeces. The authors discussed the possibility that in this study the total detectable radioactivity in the organs was present in the form of the unchanged benzoic acid. The Panel considered that it was more likely that the tissues contain benzoic acid conjugates than the acid itself.

Ring-labelled ^{14}C -benzoic acid was given orally at doses in the range of 1–400 mg/kg bw to various species including primates, pigs, rabbits, rodents, cats, dogs, hedgehogs, bats, birds and reptiles. Hippuric acid was the primary urinary metabolite in most species with 95–100% of ^{14}C -benzoic acid excreted as hippuric acid within 24 h by rodents, rabbits and the capuchin monkey. In rhesus monkey, ^{14}C -benzoic acid at a dose of 20 mg/kg bw was excreted entirely as hippuric acid (Bridges et al., 1970).

Following oral administration of 305 mg ^{14}C -benzoic acid/kg bw to rats, 91–94% of the radioactivity was recovered in the urine of rats after 72 h, whereas only 1–6% was present in the faeces. In addition to benzoic acid (0.4–12.8%), the following metabolites were identified: hippuric acid (70.2–84.2%), benzoyl glucuronide (0.7–1.8%) and 3-hydroxy-3-phenylpropionic acid (0.1–0.2%) (Nutley, 1990).

Human data

In humans, benzoic acid is rapidly absorbed and its metabolites are completely excreted in the urine (Schachter, 1957; Schanker et al., 1958; Barnes, 1959 as referred to by JECFA, 1996). A single healthy male volunteer given 6, 9, 13.9, 34.7, and 69.3 mmol sodium benzoate (864, 1296, 2002, 4996 mg benzoate/day) showed complete elimination in the urine as hippurate and benzoyl glucuronide within 10–14 h (Schachter, 1957).

A dose of 1 mg ^{14}C -benzoic acid/kg bw was excreted in the urine (99.4–99.7% of total dose) entirely as hippuric acid in two males (Bridges et al., 1970).

As reported by EFSA CEF Panel (2012), male volunteers were given oral doses of 2,000 or 5,000 mg sodium benzoate with or without sequential administration of glycine (Amsel and Levy, 1969). Benzoate was excreted in the urine mainly as hippuric acid and no free benzoic acid was detected in the urine for at least 8 h following the administration of sodium benzoate. Minor amounts of benzoyl glucuronide were detected in the urine at both doses. Sequential administration of glycine with benzoate increased the rate of hippuric acid excretion, indicating that at high dose levels, conjugation with glycine is the rate-limiting step in the formation of hippuric acid.

In order to investigate the types and quantities of beverages that increase urinary hippuric acid excretion, 137 healthy students were recruited and divided into quintiles based on their consumption of non-alcoholic beverages containing benzoic acid. HPLC was used to determine benzoic acid intake from beverages and urinary hippuric acid before, 1.5 and 3 h after consumption of various beverages. The range of benzoic acid in 13 beverages was 0–1.02 mg/mL and the benzoic acid intakes from the beverages for groups 1–5, respectively, were: 0.4 ± 0.5 mg; 23.4 ± 9.8 mg; 55.2 ± 2.3 mg; 76.3 ± 4.0 mg; and 116.5 ± 16.5 mg. Urinary hippuric acid geometric mean concentrations before consuming beverages in the five groups, respectively, were 0.276, 0.270, 0.207, 0.262 and 0.316 g/L; 1.5 hours after beverage consumption, they were 0.210, 0.603, 1.026, 1.066 and 1.688 g/L, and significantly increased ($p < 0.001$) after adjustment for urinary hippuric acid before ingestion. Three hours after beverage consumption, urinary hippuric acid geometric mean concentrations in the five groups, respectively, were 0.160, 0.232, 0.306, 0.287 and 0.337 g/L ($p < 0.001$). The authors concluded that

intakes of more than 100 mg benzoic acid from consumption of beverages containing the preservative at a single occasion may increase urinary hippuric acid significantly (Chang et al., 2000 as referred to by EFSA CEF Panel, 2012).

After administration of oral doses of 40, 80 and 160 mg/kg bw of sodium benzoate to humans, the mean plasma area under the curve (AUC) of benzoic acid increased disproportionately to the dose, 3.7 and 12.0 times greater respectively, for the higher dosages than for the lowest dose indicating saturation of the metabolic pathway, while the mean AUC for hippuric acid was proportional to dose (Kubota et al., 1988; Kubota and Ishizaki, 1991, as reported by EFSA CEF Panel, 2012). Peak plasma concentrations of benzoic acid increased with increasing dose, while peak hippuric acid concentrations did not change. According to the authors, the data suggest that the conjugation with glycine to form hippuric acid is a saturable process in humans.

Overall, the Panel considered that the available ADME data in animals and humans indicate that benzoic acid and its sodium and potassium salts are rapidly absorbed, primarily in the proximal part of the gastrointestinal tract, after oral administration. Benzoate is conjugated to glycine to form hippuric acid which is excreted in the urine. No studies have been conducted on calcium benzoate, but the Panel considered that calcium benzoate will dissociate into its constituents calcium and benzoate ions in the small intestine and that the ADME of this salt will be similar to that of the sodium or potassium benzoate. Therefore, the Panel considered read-across between the salts possible.

3.2. Toxicological data

The Panel noted the conclusions of the SCF (2002) and JECFA (1997), that there is evidence of a common and rapid route of metabolism of the salts of benzoic acid and the three benzyl derivatives benzyl alcohol, benzyl acetate and benzaldehyde to benzoic acid and subsequently to hippuric acid. It is therefore reasonable to assume that studies on any of these substances provide valid information for the assessment of the toxicity of benzoic acid and its salts (JECFA, 1997; SCF, 2002). The Panel agreed with this conclusion.

3.2.1. Acute oral toxicity

In its 1996 evaluation, JECFA (1997) presented the results of acute oral toxicity studies in rats, rabbits and dogs, showing LD₅₀ values for benzoic acid or sodium benzoate ranging from 2,000 to 2,700 mg/kg bw. At its 57th meeting JECFA reviewed two further acute oral toxicity studies on benzoic acid in mice, one providing a LD₅₀ value of 1,200 mg/kg bw, and the other a value of 2,000 mg/kg bw (JECFA, 2001).

An acute study with benzoic acid by gavage in male and female mice was available to the Panel. This unpublished study (Bio-Research Laboratories, 1979 [Doc. provided to EFSA n. 1]) showed a LD₅₀ of 2,250 (1,875–2,700) mg benzoic acid/kg bw.

An acute study by gavage with benzoic acid in albino rats was available to the Panel. This unpublished study (International Research and Development Corporation, 1974 [Doc. provided to EFSA n. 10]) showed a LD₅₀ for males of 2,742 mg benzoic acid/kg bw and for females of 2,565 mg benzoic acid/kg bw.

In conclusion, the acute oral toxicity of benzoic acid is low with LD₅₀ values in the range of 1,200–2,742 mg/kg bw.

3.2.2. Short-term and subchronic toxicity

3.2.2.1. Mice

In a 3-month study, two groups of 50 male and 50 female cross-bred white mice (no available information on strain) were given benzoic acid by oral gavage at a dose of 80 mg/kg bw per day (Shtenberg and Ignat'ev, 1970). Body weight gain was reduced in the mice receiving 80 mg benzoic acid/kg bw per day compared with controls, although food consumption was similar in the two groups. The highest mortality rate was observed in mice given a combination of benzoic acid and sodium bisulphite (30% survival after 2.5 months compared with 60% in controls); however, mortality was slightly lower in animals given benzoic acid compared with controls. A 5-day period of food restriction at 2.5 months induced 85% mortality as compared to a 56% mortality in the control group. The Panel noted that the mortality in the control group of this study is very high; hence, the results of the study are of limited value.

Sodium benzoate was administered in the diet to four groups of five male and four or five female B6C3F1 (Crj.B6C3F1) mice over a 10-day period in a study primarily designed to investigate the effect of benzoate anion on the liver (Fujitani, 1993). The animals were 5 weeks old at the start of the study. Mice were fed 0, 2.08, 2.50 or 3.0% (equivalent²⁵ to 0, 3,120, 3,750 and 4,500 mg sodium benzoate/kg bw per day). Investigations included clinical signs of toxicity, body weights, organ weights (selected organs only), blood chemistry and histopathological examination of liver and kidney. One out of five males and two out of five females receiving 4,500 mg/kg bw per day sodium benzoate developed convulsions and the two female mice died on days 5 or 9 respectively of the study. No effect was seen on body weight of mice, but absolute and relative liver weights of male and female mice receiving 4,500 mg/kg bw per day were significantly higher than those of the control group, while relative kidney weight of female mice receiving 4,500 mg/kg bw per day was also increased. Serum cholesterol and phospholipid levels were increased in male mice receiving 4,500 mg/kg bw per day, while serum cholinesterase was increased in male mice receiving 3,750 or 4,500 mg/kg bw per day. Hypertrophy of hepatocytes accompanied by eosinophilic cytoplasm and occasionally single cell necrosis and vacuolation of hepatocytes were seen in liver from all male mice receiving 4,500 mg/kg. A similar effect in females was not mentioned. No treatment-related histopathological effects were apparent in the kidney. The authors concluded that the results observed in male mice suggested a hepatotoxic effect of sodium benzoate and stated that it might be possible that long-term exposure may induce severe damage in the liver. The Panel considered that the results found may also point to an increase in e.g. peroxisome proliferation, which is generally not considered as an adverse effect relevant for humans.

3.2.2.2. Rats

Sodium benzoate was administered in the diet to four groups of six male and six female Fisher (F344/Ducrj) rats over a 10-day period in a study primarily designed to investigate the effect of benzoate anion on the liver (Fujitani, 1993). The animals were 5 weeks old at the start of the study. Rats were fed levels of 0, 1.81, 2.09 or 2.40% sodium benzoate in the diet, equivalent²⁵ to 1,810, 2,090 and 2,400 mg/kg bw per day, respectively. Investigations included clinical signs of toxicity, body weights, organ weights (selected organs only), blood chemistry and histopathological examination of liver and kidney. One male receiving 2,400 mg/kg bw per day sodium benzoate died on day 8 of the study; no other deaths or clinical signs were observed. Mean body weight of both male and female rats receiving 2,400 mg/kg bw per day only was significantly decreased ($p < 0.05$) compared to controls. Relative liver weights of male rats receiving 2,090 or 2,400 mg/kg bw per day and of female rats receiving 2,400 mg/kg bw per day were significantly higher than controls, and

²⁵ Calculated by the Panel according to EFSA Scientific Committee (2012).

relative kidney weight of male and female rats receiving 2,400 mg/kg bw per day was also significantly higher than controls. No treatment-related histopathological effects were apparent in the kidney. Serum albumin, total protein and albumin:globulin ratios were significantly increased compared with controls in male rats receiving 2,090 or 2,400 mg/kg bw per day and serum albumin was increased in female rats receiving 2,400 mg/kg bw per day. Serum cholesterol was decreased in male rats receiving 2,400 mg/kg bw per day and in all treated females. Gamma-glutamyl transpeptidase was increased in males receiving 2,400 mg/kg bw per day. There were no other dose-related effects on blood chemistry in treated animals. Livers from male rats receiving 2,400 mg/kg bw per day showed periportal eosinophilic foci accompanied by enlargement of hepatocytes with glassy cytoplasm. The authors concluded that the results observed in male rats suggested a hepatotoxic effect of sodium benzoate and stated that it might be possible that long-term exposure may induce severe damage in the liver.

In a short-term study focusing on haematological parameters, sodium benzoate was administered orally every 48 h for 14 days to four groups of nine male Wistar albino rats at dose levels of 0, 30, 60 and 120 mg/kg bw (assumed by the Panel to be by gavage but not specifically stated) (Ibekwe et al., 2007). The authors reported a significant ($p < 0.05$) and dose-dependent decrease in haemoglobin at all levels of sodium benzoate (30, 60 and 120 mg/kg bw) administration; however, the data to support this were not provided due to an error in the publication resulting in an omission of the table containing haematological results. The authors reported that white blood cell count was also decreased in animals receiving 60 and 120 mg/kg bw (data not provided), although no significant effect was observed for the lowest dose level of 30 mg/kg bw. Plasma levels of sodium, potassium, chloride, bicarbonate ions and total protein were also measured. No treatment-related effects on plasma protein concentration were noted, but plasma levels of sodium and potassium were reported to increase over the period of the study in animals receiving 60 and 120 mg/kg bw/every 2 days. No changes were seen in levels of plasma chloride or bicarbonate ions.

Twenty-eight young rats (62–70 g at start of study, sex and strain not specified) were given a diet containing 5% sodium benzoate (equivalent²⁵ to 6,000 mg/kg bw per day) for 3 weeks (Kieckebusch and Lang, 1960). Nineteen animals died within 2 weeks and the remainder died in the third week. Food consumption was significantly reduced, and most animals developed severe diarrhoea. The changes seen at autopsy were haemorrhage in the gut and nasal blood crust. Five adult rats on a similar diet died within 5 weeks with severe weight loss.

Three groups of adult male Sprague-Dawley rats were given sodium benzoate at a dietary level of 0 (10 animals) or 5% sodium benzoate (equivalent²⁵ to 6,000 mg/kg bw per day) (15 animals) or 5% sodium benzoate plus 1% glycine (15 animals) for 3 weeks (Kowalewski, 1960). Supplementary glycine corrected the potassium and phospholipid deficiencies. The body weights of animals fed sodium benzoate were reduced, but less so when 1% glycine was added. The total cholesterol content of the liver was unaffected by treatment with benzoate, but the level of liver phospholipids was significantly reduced. The potassium concentration of skeletal muscle was also low in rats on the 5% sodium benzoate diet.

Groups of five male and five female Sherman rats were fed sodium benzoate for 30 days at levels of 16–1,090 mg/kg bw per day (Smyth and Carpenter, 1948). There were no effects on body weight, appetite, or mortality and no histological changes in organs.

Groups of three male and three female Sherman rats were fed diets containing 0, 2 or 5% sodium benzoate (equivalent²⁵ to 2,000 and 6,000 mg/kg bw per day in rats weighing 40–50 g at the start of the study) for 28 days (Fanelli and Halliday, 1963). All animals at the 5,000 mg/kg bw per day group died during the first 2 weeks after showing hyperexcitability, urinary incontinence and convulsions. Male rats at the 2,000 mg/kg bw per day group showed a significant decrease in body weight, and the food intake of male and female animals at this dose was decreased in comparison with controls.

Groups of 4–19 male rats (strain not specified), 4–5 weeks old and weighing 45–60 g, were fed diets containing sodium benzoate at a level of 0, 1.5, 2.0, 2.5, 3, 3.25 or 3.75% (equivalent²⁵ to 0, 1,800, 2,400, 3,000, 3,600, 3,900 and 4,500 mg/kg bw per day) for 40 days (Griffith, 1929). Sodium benzoate in the diet at levels greater than 3,600 mg/kg bw per day resulted in a less than 50% survival rate and surviving animals failed to grow normally. Addition of glycine reduced the toxic effects. Animals died after showing incoordination, tremor or convulsions and severe eye inflammation. Groups of 10–15 young male rats were fed restricted diets containing 0, 1.5, 2.0, 2.5 or 3% sodium benzoate (equivalent²⁵ to 0, 1,800, 2,400, 3,000 and 3,600 mg/kg bw per day). Animals in the 3,600 mg/kg bw per day group showed a reduction in body weight gain compared to the control, and the proportion of deaths was greater than in the non-restricted diet group fed the same concentration of sodium benzoate. Supplementary glycine addition again alleviated the reduction in body weight gain.

Groups of 10 male and 10 female Fischer 344 rats, 4–5 weeks old and weighing 110–150 g, were fed diets containing sodium benzoate at levels of 0, 0.5, 1, 2, 4 or 8% (equivalent²⁵ to 600, 1,200, 2,400, 4,800 and 9,600 mg/kg bw per day) for 6 weeks (Sodemoto and Enomoto, 1980). All rats in the 9,600 mg/kg bw per day group and 19 rats in the 4,800 mg/kg bw per day group died within 4 weeks; one male in the 2,400 mg/kg bw per day group, two males in the 1,200 mg/kg bw per day group and three males in the 600 mg/kg bw per day group also died, but all females at these dose levels survived for 6 weeks. Significant reductions in body weight gain were seen only in animals in the 4,800 and 9,600 mg/kg bw per day groups. No morphological change was seen at necropsy, except for atrophy of the spleen and lymph nodes in rats in the 4,800 and 9,600 mg/kg bw per day groups.

Groups of four to five male and four to five female young Sherman rats were fed diets containing 1, 2, 4 or 8% sodium benzoate (equivalent²⁵ to 900, 1,800, 3,600 and 7,200 mg/kg bw per day) for 90 days (Deuel et al., 1954). Four rats in the 7,200 mg/kg bw per day group died within an average of 13 days. The body weight gain of the four survivors was two-thirds that of controls with an identical food intake. Kidney and liver weights were significantly higher than those of the control group in the 7,200 mg/kg bw per day group.

Conclusion on short-term and subchronic toxicity of benzoic acid and its salts

The results of the available short-term and subchronic studies on benzoic acid and sodium benzoate indicated that the toxicity of benzoic acid and its salts is low with no marked target organ toxicity. The Panel noted however that the majority of available studies were conducted many years ago, and were not in accordance with current test guidelines.

3.2.3. Genotoxicity

3.2.3.1. Previous evaluations

JECFA at its 46th meeting (JECFA, 1997) evaluated the genotoxicity of benzoic acid in different *in vitro* and *in vivo* genotoxicity tests and concluded that benzoic acid was not mutagenic in the Ames test, either with or without metabolic activation but showed weak clastogenic activity *in vitro* which was not reproduced *in vivo*.

JECFA's conclusion regarding the clastogenicity of benzoic acid was based on the results from two *in vitro* chromosome aberration studies in Chinese hamster fibroblasts (Ishidate et al., 1984) and in root tips of *Vicia faba* (Njagi and Gopalan, 1982) which showed weak cytogenetic effects. However, the Panel noted that the assay on root tips of *V. faba* does not belong to the assays recommended for regulatory purposes and that the study by Ishidate et al. (1984) was of limited value because in the evaluation of clastogenic effects only results for total aberrations were reported, which included gaps not normally considered for the evaluation of clastogenicity. In addition, the study was poorly reported. The SCF in its 2002 evaluation (SCF, 2002) of benzoic acid and its salts noted that all the

relevant studies *in vivo* were negative at somatic or germ cell levels and concluded that *it is very unlikely that benzoic acid would interact with chromosomes in vivo* and recommended *The observations of clastogenic activity of benzoic acid in vitro indicate that it should be tested for clastogenic activity in vivo in peripheral lymphocytes or bone marrow in animals and that blood or bone marrow levels respectively of benzoic acid should be measured in such a study.*

TemaNord (2002) has evaluated the genotoxicity of benzoic acid, sodium benzoate, potassium benzoate and calcium benzoate, and concluded that benzoic acid/sodium benzoate was without mutagenic effect in *in vitro* mutagenicity tests. However, *with respect to sodium benzoate – but not benzoic acid – positive or ambiguous results were obtained in some of the in vitro chromosomal aberration tests and tests for effects on DNA. Negative results were seen in two in vivo chromosomal aberration tests with sodium benzoate.*

SCCP (2005), in reviewing the mutagenicity and genotoxicity of benzoic acid and sodium benzoate, evaluated the same studies considered by JECFA and SCF and concluded that *On the basis of provided data, the SCCP is of the opinion that benzoic acid and sodium benzoate are safe for use for preservative and non-preservative purposes in cosmetic rinse-off products.*

In the framework of classification, labelling and packaging of substances and mixtures according to Regulation (EC) No 1272/2008, benzoic acid was evaluated by the German Federal Institute for Occupational Safety and Health (BAuA, 2011). The BAuA considered that *while the reverse mutation assays and sister chromatid exchange assays (except one equivocal result) with benzoic acid, sodium benzoate and the metabolite hippuric acid were negative, weak genotoxic effects or equivocal results were observed in most of the chromosome aberration assays in mammalian cell lines with benzoic acid and sodium benzoate.* The BAuA further considered that *All the in vivo genotoxicity tests were negative at somatic or germ cell level.* Accordingly, BAuA concluded that *On this basis and the negative results obtained in two carcinogenicity studies in rats and mice for sodium benzoate, notwithstanding some limitations, it is very unlikely that benzoic acid would interfere with chromosomes in vivo. In the absence of a genotoxic potential in in vivo studies and negative results in carcinogenicity studies, no classification and labelling regarding mutagenicity is required.*

The EFSA CEF Panel has re-evaluated a number of benzyl derivatives in Flavouring Group Evaluation 20 (FGE.20Rev4) (EFSA CEF Panel, 2012), with a particular focus on genotoxicity. Benzoic acid was a supporting substance in this evaluation. The CEF Panel concluded that *While some of the in vitro studies indicated equivocal weak positive or positive results, considering the weight of evidence from candidate and supporting substances and the in vivo studies, the Panel concluded there was no safety concern with respect to genotoxicity of the substances in the present flavouring group.*

3.2.3.2. Genotoxicity studies not reported in previous evaluations

***In vitro* genotoxicity studies**

In a study by the EG&G Mason Research Institute (1979 [Doc. provided to EFSA n. 3]) benzoic acid (purity 99.5%) was tested for its mutagenicity in the Ames test with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 using the plate incorporation method both in the absence and presence of rat liver S9 metabolic activation up to 1,000 µg/plate. The highest concentration was based on a toxicity test with strain TA100 in which the background bacterial lawn was slightly reduced at 976.6 µg/plate and markedly reduced at 3,125 and 10,000 µg/plate. No increases in the frequency of revertant colonies were observed with benzoic acid under any experimental condition.

Benzoic acid, sodium benzoate and potassium benzoate were investigated for their potential to cause chromosomal aberrations, sister chromatid exchanges (SCEs) and micronuclei formation in cultured human peripheral lymphocytes (Yilmaz et al., 2009; Zengin et al., 2011). The potential of sodium benzoate and potassium benzoate to induce DNA damage was also investigated in the alkaline Comet assay. Test concentrations were 50, 100, 200 and 500 µg/mL for benzoic acid, 6.25, 12.5, 25, 50 and 100 µg/mL for sodium benzoate and 62.5, 125, 250, 500 and 1,000 µg/mL for potassium benzoate. Cells were exposed for 24 or 48 h for the chromosomal aberration and SCE assays, for 48 h in the micronucleus assay and for 1 h for comet assay. The mitotic index was reduced in a dose-related manner in all treatment groups compared to the negative controls, but it was not lower than 50% of control in any of the cultures, even at the highest dose for each benzoate, indicating that cytotoxicity was not a major factor in the induction of chromosome aberrations. None of the substances affected the replication index of the lymphocyte cultures. Chromosome aberrations were significantly increased compared to controls in cultures treated with benzoic acid and its sodium and potassium salts at all test concentrations. The frequencies of SCEs were similarly increased in a dose-related manner by all three benzoates, benzoic acid showing a slightly weaker response than either sodium or potassium benzoate. Micronuclei formation was significantly increased in a dose-related manner following treatment of the lymphocyte cultures with 200 or 500 µg/mL benzoic acid, or with 25, 50 and 100 µg/mL sodium benzoate, or with 125, 250, 500 and 1,000 µg/mL potassium benzoate. In the comet assay, a positive response was seen in the presence of sodium benzoate but not potassium benzoate. The authors concluded that benzoic acid, sodium benzoate and potassium benzoate are clastogenic, mutagenic and cytotoxic to human lymphocytes *in vitro* (Yilmaz et al., 2009; Zengin et al., 2011). The Panel noted that treatment and sampling times used in the chromosomal aberration and SCE assays from both studies deviate from relevant OECD TG Guideline 473. Furthermore, the different concentrations used for sodium and potassium benzoate (the concentration of potassium benzoate was 10 times higher than the concentration of sodium benzoate) appear to be inexplicable as the difference between the two compounds is only in the cation which is not expected to elicit any genotoxicity *per se* and no justification was reported for the application of these different concentrations. Overall, the Panel considered these studies as not adequate for risk assessment.

Demir et al. (2010) investigated the effects of benzoic acid, benzyl alcohol, benzyl acetate and benzaldehyde in an *in vitro* comet assay. Lymphocytes from two human volunteers were exposed to concentrations of 0, 0.05, 0.1, 0.3, 1 or 5 mM benzoic acid or 0, 1, 5, 10, 25 or 50 mM benzyl alcohol, benzyl acetate and benzaldehyde. Control cultures were exposed to distilled water alone, while ethylmethanesulphonate (EMS, 5 mM) was used as a positive control. Tail moment and % tail DNA were used as tail parameters in analysing the results of the study. Benzoic acid at a concentration of 5 mM produced a significant increase in both tail moment (4.28 ± 1.11) and % tail DNA (13.08 ± 2.03), with no effect being seen at lower concentrations of benzoic acid compared with control. Positive results were also seen with benzyl alcohol at concentrations of 25 and 50 mM, with benzyl acetate at 50 mM and with benzaldehyde at 10, 25 and 50 mM. EMS induced both tail moment (16.59 ± 2.71) and % tail DNA (28.73 ± 3.87). The authors concluded that these benzyl compounds showed evidence of genotoxicity at high test concentrations in the *in vitro* comet assay. However, the Panel noted that statistically significant increases observed for benzoic acid were modest and similar to the values observed for the negative controls used for the other substances assessed in this study. Statistical significance was only achieved due to the low value obtained in the concurrent control. The outcome obtained is therefore of no biological relevance. The Panel further noted major shortcomings which include the following:

- i) The use of distilled water as negative solvent control is not recommended due to significant modification of the osmolality of culture medium;
- ii) Cells employed in the study appear to be human lymphocytes, only mentioned in the abstract. No indication whether whole blood cells or purified lymphocytes and relevant procedures followed was reported;

- iii) No indication about treatment and sampling times of the cells, two key points in this study, was provided;
- iv) Cytotoxicity was not assessed;
- v) Concentrations used, in most cases, exceeded the limit dose of 10 mM to be used in *in vitro* studies.

Overall the study is considered not adequate for risk assessment.

In the study by Yilmaz et al. (2014), benzoic acid was assessed for its potential to induce DNA breakage in the alkaline comet assay in purified human lymphocytes (one male and one female healthy subject). Concentrations used were 50, 100, 200 and 500 µg/mL and cells were treated for 1 h at 37°C. Cell viability was evaluated by the incorporation of trypan blue dye in cells. Two slides were prepared for each concentration of benzoic acid and 200 selected cells (100 cells from each of the two replicate slides) were analysed per treatment using the image analysis system (Comet Assay IV, Perceptive Instruments Ltd., UK). The tail length and tail intensity (%) parameters were used to estimate the extent of DNA breakage. The results obtained indicated moderate, no dose-related increases in both tail length and tail intensity (%) compared to the relevant solvent control culture, which achieved statistical significance at all concentrations employed. However, the Panel noted some limitations in the study, which include the absence of historical negative control values, the lack of dose-response over a 10-fold dose range and only 200 cells analysed per test point compared to 300 normally scored. Furthermore, the Panel noted that in a previous study (Zengin et al., 2011), the same authors reported negative results for potassium benzoate in the comet assay even at higher concentrations.

The Panel noted that the genotoxicity of benzoic acid and its salts was investigated *in vitro* in some further studies: in a mutation assay in superoxide dismutase-deficient *S. cerevisiae* (Piper, 1999); in a mutation assay with *Escherichia coli* cells (with no further details given) (Salih, 2006); in an SCE assay in human lymphocytes (Mpountoukas et al. 2008). However, these studies do not belong to the assays recommended for regulatory purposes.

In vivo genotoxicity studies

SCCNFP (2002) and BAuA (2011) evaluated the tests performed by Litton Bionetics (1974 [Doc. provided to EFSA n. 11]) in which sodium benzoate was assessed for its genotoxicity in the host-mediated assay in mice, in the rat bone marrow chromosomal aberration assay and in the dominant lethal assay in rats. In all assays performed, the treatment regime used consisted of three dose levels of 50, 500 and 5,000 mg sodium benzoate/kg bw, administered acutely by oral gavage, as single dose or subacute using the same dosages as those in the acute study, each day for five consecutive days, 24 h apart.

1. In the host-mediated assay, 10 ICR random-bred male mice/group were dosed as indicated above and the indicator organisms used were *S. typhimurium* TA1530 and G46 for mutagenicity and *Saccharomyces cerevisiae* strain D3 for mitotic recombination. The results obtained indicated that, except for a marked increase in mutation frequencies observed in *S. typhimurium* TA1530 in the acute study at the intermediate dose level of 500 mg/kg bw, which was not reproduced at the higher dose level in the absence of any toxicity, no increases in mutant and recombinant frequencies in both *S. typhimurium* TA1530 and G46 and *S. cerevisiae* strain D3 respectively, at both dosing regimens were observed. The authors considered that overall the results were negative. The Panel agreed with this conclusion and noted that the host-mediated assay has not received further validation and it is presently considered to be obsolete.
2. In the rat bone marrow cell chromosomal aberration assay, five male Sprague-Dawley rats for each treatment group were sacrificed at 6, 24 and 48 h from the administration of test compound in the acute treatment, while in the subacute treatment (for 5 days), sampling was

only performed at 6 h from the last administration. Bone marrow cells were used to prepare cytogenetic slides following administration of colcemid at 4 mg/kg by intraperitoneal injection to accumulate cells in metaphase before sacrifice of animals. Fifty metaphase spreads per animal were scored for chromosomal aberration analyses. The mitotic indices were also evaluated by scoring a total of 1,000 cells (mitotic and interphase cells) per animal to assess cytotoxicity. The result obtained showed no relevant increases in chromosomal aberration at any dose level and sampling time employed. No reduction of mitotic indices in the test substance treatment groups was observed indicating no signs of bone marrow toxicity despite the very high dose levels employed. The Panel noted that according to the current version of the OECD guideline TG475 (OECD, 2014), at least 200 metaphases should be analysed for each animal for structural chromosomal aberrations. However, scoring of 50 metaphases per animals was consistent with the recommendations at the time when the study was performed. The Panel further noted that there was no evidence that bone marrow cells were exposed to test substance as no mitotic index reduction was observed at any test point. However, the Panel also considered that the highest dose level applied (5,000 mg/kg bw) significantly exceeded the maximum dose level (2,000 mg/kg bw) recommended by the relevant OECD Guideline TG475.

In the dominant lethal assay, 10 male Sprague-Dawley rats per group following treatments as indicated above, were sequentially mated with two females per week for seven or eight consecutive weeks in the subacute or acute treatment, respectively. Fertility index, total implants (live fetuses plus early and late fetal deaths), total dead (early and late fetal deaths), dead implants per total implants and preimplantation loss (calculated as the difference between the total corpora lutea and total implant counts) were evaluated and the results obtained were considered by the authors to be of no concern with respect to genotoxicity. The Panel agreed with this conclusion.

In the study by Kawachi et al. (1980), a negative result in terms of induction of chromosomal aberrations was observed in the rat bone marrow chromosomal aberration assay. However, the reliability of the study could not be evaluated as details on methods and results were not reported.

In the study by Sasaki et al. (2002), groups of male CD-1 mice were administered once by gavage at a single dose of 1,000 mg/kg bw with benzoic acid or sodium benzoate. Isolated nuclei from glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow were analysed 3 and 24 h after treatment. No DNA migration was observed in nuclei from any of the tissues analysed. Necropsy and histopathological examination revealed no treatment-related effect in any tissue/organ analysed.

In the study by Demir et al. (2008), the genotoxicity of benzoic acid, benzyl alcohol, benzyl acetate and benzaldehyde was investigated in the wing somatic mutation and recombination test in *Drosophila melanogaster* and the authors concluded that benzoic acid, benzyl alcohol, benzyl acetate and benzaldehyde are genotoxic. The Panel noted that the test systems employed have not received any validation and are presently considered to be obsolete and that they do not belong to the assays recommended for regulatory purposes. Therefore, the results of these studies were not further considered in the assessment.

Overall conclusion on genotoxicity of benzoic acid and its salts

Overall, the Panel considered that positive or equivocal results reported for clastogenicity *in vitro* of benzoic acid and its salts sodium and potassium benzoates (Ishidate et al., 1984; Yilmaz et al. 2009; Zengin et al. 2011) have not been reproduced in well performed and relevant *in vivo* studies using the alkaline comet assay (Sasaki et al., 2002), the rodent bone marrow chromosome aberration assay and the dominant lethal assay in rats (Litton Bionetics, 1974). Therefore, the Panel considered that the use of benzoic acid and its sodium and potassium salts as food additives does not raise a concern with respect to genotoxicity. Based on read-across, the Panel considered that this conclusion is also applicable for calcium benzoate.

3.2.4. Chronic toxicity and carcinogenicity

The results of long-term studies of toxicity and carcinogenicity with benzoic acid and sodium benzoate and related substances were reviewed by JECFA at its 46th meeting (JECFA, 1996, 1997). The Panel noted that no haematological and clinical chemistry parameters were determined in these studies.

3.2.4.1 Mice

Sodium benzoate (purity, 99%) was administered as a 2% solution in drinking water to 50 male and 50 female 5-week-old Swiss mice for their lifetime (Toth, 1984). The daily intakes of sodium benzoate were estimated to be 6,200 mg/kg bw per day for males and 5,960 mg/kg bw per day for females, on the basis of daily water consumption of 6.2 and 5.9 mL, respectively. Groups of 100 males and 100 females were used as untreated controls. Body weights were measured weekly, and gross pathological changes were recorded. The animals were either allowed to die or were sacrificed when moribund. Complete necropsies were performed on all animals, and the liver, spleen, kidneys, bladder, thyroid, heart, pancreas, testes, ovaries, brain, nasal turbinates, at least four lobes of the lungs and organs with gross pathological changes were examined histologically. Treatment had no effect on survival or the incidence of tumours in the limited numbers of tissues investigated, and the NOAEL for this study was therefore 5,960 mg sodium benzoate/kg bw per day. The Panel noted the limited number of investigations carried out in this non-guideline study and also the deficiencies in the reporting of the results, including uncertainty about whether histopathological end-points other than neoplasms were investigated. Therefore, the Panel considered that this study was not adequate for the risk assessment.

3.2.4.2 Rats

Groups of 50 male and 52 female Fischer 344 rats, 4–5 weeks old, received diets containing 0%, 1% or 2% sodium benzoate (equivalent²⁶ to 0, 500 and 1,000 mg/kg bw per day) for up to 24 months (Sodemoto and Enomoto, 1980). Controls, consisting of 25 male and 43 female rats, received basal diet. Survival was very poor in all groups, due to intercurrent sialodacryoadenitis and mycoplasma infections. All surviving animals were sacrificed between 18 and 25 months and various tissues were examined histopathologically. No adverse clinical signs directly attributable to treatment were observed, and only negligible differences in average body weight and mortality rate were seen between the treated and control groups. Although a variety of tumours occurred among treated and control rats of each sex, they were of similar type and incidence. The Panel noted that this study had a number of limitations, including poor survival due to the disease status of the animals.

No further data on the carcinogenicity of benzoic acid or its salts were found.

The Panel noted that the available carcinogenicity studies on benzoic acid and its salts did not indicate any carcinogenic potential, although they were not conducted in accordance with current test guidelines and had deficiencies both in terms of design and reporting.

3.2.5. Reproductive and developmental toxicity

3.2.5.1 Reproductive toxicity

In a multi-generation reproductive toxicity study on benzoic acid, which also included limited investigations into the chronic toxicity of benzoic acid, three groups of 20 male and 20 female white

²⁶ Calculated by the Panel according to EFSA Scientific Committee (2012).

rats were pair fed for 8 weeks on a standard rat diet, prepared as a feed mix with either 0, 0.5 or 1% benzoic acid (Kieckebusch and Lang, 1960). The authors did not provide information regarding the strain of the rat used, only stating that they were sourced from Bayer, Elberfeld (Germany). The rats were fed *ad libitum* over four generations resulting in an approximate intake of benzoic acid of 0, 250 or 500 mg/kg bw per day. Two generations were fed for their whole lifespan, the third and fourth generations were autopsied after 16 weeks. No treatment-related effects were observed on growth, fertility, lactation throughout all four generations and lifespan (for the first two generations). On the contrary, *ad libitum* ingestion of the 0.5% benzoic acid feed mix resulted in a significantly longer lifespan in the first and second generation of the rats, based on statistical analysis of the two generations combined. In the third generation, autopsied at 16 weeks, no treatment-related effects were reported on organ weights and histopathological findings in organs. The assessment of reproductive parameters was considered to be valid by the Panel, and provided a NOAEL, for both the parental animals and the offspring over four generations, of 500 mg benzoic acid/kg bw per day, the highest dose tested.

3.2.5.2. Developmental toxicity

Mice

Pregnant CD-1 mice (20–12 per group) were treated by gavage once daily from gestation day (GD) 6 to 15 with doses of 0, 1.75, 8, 38 or 175 mg sodium benzoate/kg bw per day in water (FDRL, 1972 (Doc. provided to EFSA n. 9)). Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. Two females of the control group died before GD 17. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weights of live pups were recorded. All fetuses were examined grossly for external abnormalities (one-third detailed visceral examination and two-third stained and examined for skeletal defects). At necropsy on GD 17 doses up to 175 mg sodium benzoate/kg bw per day, no dose-related effects were observed on maternal toxicity. There was no evidence of developmental toxicity in animals receiving sodium benzoate compared to controls neither on implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions, average implant sites and fetal weights did not differ among the groups. The sex ratio of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the sodium benzoate-treated groups did not differ from the number occurring in vehicle-treated dams of the control group.

Rats

Pregnant Wistar rats (23–24 per group) were treated by gavage once daily from GD 6 to 15 with doses of 0, 1.75, 8, 38 or 175 mg sodium benzoate/kg bw per day in water (FDRL, 1972 (Doc. provided to EFSA n. 9)). Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weights of live pups were recorded. All fetuses were examined grossly for external abnormalities (one-third detailed visceral examination and two-third stained and examined for skeletal defects). At necropsy on GD 20, no dose-related effects were observed on maternal toxicity. There was no evidence of developmental toxicity in animals receiving sodium benzoate compared to controls neither on implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions, average implant sites and fetal weights did not differ among the groups. The sex ratio of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the sodium benzoate-treated groups did not differ from the number occurring in vehicle-treated dams of the control group.

A developmental toxicity study in which groups of 27–30 pregnant Wistar rats were fed diets containing 0, 1, 2, 4, or 8% sodium benzoate on days 1–20 of gestation, equal to 0, 700, 1,310, 1,875 or 965 mg/kg bw per day based on total food intake over the period (Onodera et al., 1978). All but five animals in each group were sacrificed on day 20 of gestation, and the numbers of viable fetuses, dead fetuses, early and late resorptions, and fetal, placental and ovarian weights were measured; abnormalities of maternal organs and fetal appearance were also recorded. About 75% of the fetuses from treated animals were stained with alizarin red S for skeletal examination, and the remainder were fixed with Bouin's solution and examined for visceral anomalies by Wilson's method. The remaining five dams in each group were allowed to deliver naturally, and the number of offspring, survival, body weight, and abnormalities were recorded. Three weeks after birth, all surviving pups were weaned and examined for gross abnormalities, and one-half of the pups and all of the dams were necropsied. The remaining pups were necropsied at 8 weeks of age, body weight and food intake being measured weekly until necropsy.

Maternal body weight and body weight gain were comparable in the controls and in animals receiving 1 and 2% sodium benzoate in the diet; animals at the 4% level did not gain weight and those at 8% lost weight (statistical comparisons not presented). Feed intake was also comparable in the controls and animals at 1% and 2% dietary levels but was markedly reduced in those at 4% and 8%. Two dams at 4% and three at 8% died after convulsions and depressed motor activity. Animals receiving 1% or 2% sodium benzoate showed no differences compared with controls in the average number of implants per female, the numbers of dead, resorbed or viable fetuses, or the average weight of viable fetuses on day 20 of gestation; in the groups at 4% and 8%, the number of dead or resorbed fetuses was significantly increased, and the average body weight of viable fetuses was significantly lower than that of controls. Significant abnormalities and pathological findings were seen only in the fetuses at 4% and 8%; these included mild systemic oedema, anophthalmia, microphthalmia, hydrocephalus, pyelectasis, hydroplasia and cerebral hypoplasia. Delayed ossification, lumbar or cervical ribs, and varied sternabrae were reported in animals in both the control and treated groups. The percentage of animals with these findings was comparable to that in controls among animals at 1% and 2% (each about 37%) but was increased in the groups at 4% (96.5%) and 8% (100%). Additional anomalies seen in the treated groups included a higher incidence of wavy ribs and abnormal vertebrae in the rats at 4%, but not at 8%.

Among pups that were delivered naturally, no differences in the delivery rate, number of perinatal deaths, lactation rate or survival up to week 8 were reported at the 1% and 2% dietary levels, but the groups at 4% and 8% were reported to have delivery rates reduced by 50% and 8.2%, respectively, with complete loss of litters after parturition. The surviving pups in the control group and at 1% and 2% showed no significant differences in birth weight, weight at week 3 or week 8, incidence of abnormalities at week 3 or 8, or organ weights at week 8. The authors suggested that the effects on the dams and fetuses at the 4% and 8% dietary levels were due to reduced maternal feed intake in these groups, leading to malnutrition, as the actual compound intake of the animals at 8% was lower than that of the group at 2%, in which no adverse effects were seen. The authors concluded that the NOAEL was 1,310 mg sodium benzoate/kg bw per day (Onodera et al., 1978). The Panel agreed with the authors.

Groups of 10 pregnant female Wistar rats were administered sodium benzoate at levels of 0%, 0.1%, 0.5% or 1% in the diet (equivalent²⁷ to 0, 50, 250 or 500 mg/kg bw per day) over the whole gestation period, throughout lactation and to pups after weaning up to day 45 of age (Crane and Lachance, 1985). Parameters monitored included body weight change and food consumption; spontaneous locomotor activity (at day 6, 9, 12, 15, 18, 21 of age and thereafter continuously up to termination); brain levels of serotonin, dopamine and norepinephrine (at day 9, 15, 21 of age and at termination), and brain weight. No adverse effect was reported on any of these parameters and the authors

²⁷ Calculated by the Panel according to EFSA Scientific Committee (2012).

concluded that the NOAEL was 500 mg sodium benzoate/kg bw per day, the highest dose tested. The Panel agreed with the authors; however, the Panel noted that this study did not comply with the OECD Guideline 414 (OECD, 2001).

Groups of 25 pregnant rats were given benzoic acid (purity 96.7%) by gavage from GD 7–16 dose levels of 0, 30, 160, or 450 mg benzoic acid/kg of body weight in a 0.5% aqueous suspension of methyl cellulose (EPA, 1992 (Doc. provided to EFSA n. 5)). Overt maternal toxicity occurred at 450 mg/kg as indicated by death in four rats, significant depression in body weight gain and feed consumption during the dosing period, an increased incidence of ruffled fur and increased relative liver weight on post-mortem examination. Haemorrhages of the gastric mucosa were observed in three of the four rats that died. A transient but significant decrease in body weight gain also occurred in dams in the 160 mg/kg group during the first 4 days of dosing. The number of pregnant females at caesarean section was 16, 14, 18 and 19 for the control, low-, mid- and high-dose, respectively.

No compound-related effects on reproductive parameters were demonstrated. Fetal weight was significantly decreased in the 160 and 450 mg/kg groups. The Panel noted that the effect on fetal weight in the mid-dose group was related to the higher number of fetuses per litter in this group when compared with the control group; calculated mean litter weight of the mid-dose group was 10% higher than in the control group. Significant increases in malformations, fetal developmental variations and variations due to retarded development were observed in the group administered 450 mg benzoic acid/kg bw per day.

The authors considered 30 mg benzoic acid/kg bw per day as the no observed effect level (NOEL) for the dams based on the effect on body weight gain at 160 mg/kg bw per day. The NOEL for the fetuses was also 30 mg/kg bw per day based on the decreased fetal weight at the mid-dose. The Panel considered the transitory effect on maternal body weight gain in the mid-dose group not relevant for identification of the NOAEL. Owing to a higher number of fetuses in the low-dose group compared to the control group, the decreased mean fetal weight in combination with the increased mean litter weight was not considered to be a developmental toxic effect by the Panel. The only fetal alterations demonstrated to be dose related and significantly increased above the control incidence occurred in the 450 mg/kg group in the presence of overt toxicity and maternal death. Therefore, the Panel considered that 160 mg benzoic acid/kg bw per day was the NOAEL for maternal and developmental toxicity in this study.

Hamsters

Pregnant Golden hamsters (21–22 animals per group) were treated by gavage once daily from GD 6 to 10 with doses of 0, 3, 14, 65 or 300 mg sodium benzoate/kg bw per day in water (FDRL, 1972 (Doc. provided to EFSA n. 9)). Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weights of live pups were recorded. All fetuses were examined grossly for external abnormalities (one-third detailed visceral examination and two-third stained and examined for skeletal defects). One female of the low-dose group died or aborted before GD 14. At necropsy on GD 14, doses up to 300 mg sodium benzoate/kg bw per day induced no maternal toxicity. There was no evidence of developmental toxicity in animals receiving sodium benzoate compared to controls neither on implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions, average implant sites and fetal weights did not differ among the groups. The sex ratio of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the sodium benzoate treated groups did not differ from the number occurring in vehicle-treated dams of the control group.

Rabbits

Artificially inseminated Dutch-belted rabbits (10–12 animals per group) were treated by gavage once daily from GD 6 to 18 with doses of 0, 2.5, 12, 54 or 250 mg sodium benzoate/kg bw per day in water (FDRL, 1972 (Doc. provided to EFSA n. 9)). Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weights of live pups were recorded. All fetuses were examined grossly for external abnormalities and visceral and skeletal abnormalities. One, 4, 0, 2 and 2 does of the 0, 2.5, 12, 54 and 250 mg sodium benzoate/kg bw per day groups died or aborted before GD 29. Therefore, there were only 9, 10, 8, 5, or 8 litters at necropsy on GD 29. At necropsy on GD 29 doses up to 250 mg sodium benzoate/kg bw per day, no dose-related effects were observed on maternal toxicity. There was no evidence of developmental toxicity in animals receiving sodium benzoate compared to controls neither on implantation nor on maternal and fetal survival. The numbers of live or dead fetuses, resorptions, average implant sites and fetal weights did not differ among the groups. The sex ratio of fetuses was not affected by the treatment. The number of abnormalities seen in either soft tissues or skeletons at fetal pathological examination of the sodium benzoate-treated groups did not differ from the number occurring in vehicle-treated dams of the control group. The Panel noted that the number of pregnant does at caesarean section was very low in all groups, especially in the low-dose group.

Overall, in two developmental studies in rats with sodium benzoate in the diet the NOAEL was 500 mg sodium benzoate/kg bw per day (the highest dose tested) (Crane and Lachance, 1985) and 1,310 mg sodium benzoate/kg bw per day (Onodera et al., 1978). Onodera et al. (1978) also studied the effects of higher concentrations in the diet – doses equal to 1,875 mg sodium benzoate/kg bw per day induced severe maternal toxicity and were associated with embryotoxic and fetotoxic effects and fetal malformations.

In studies in mice, rats, hamsters and rabbits with sodium benzoate administered by gavage (FDRL, 1972 (Doc. provided to EFSA n. 9)), no developmental effects were observed up to doses of 175, 174, 300 and 250 mg sodium benzoate/kg bw per day, the highest doses tested respectively in each species.

In another developmental toxicity study in rats with benzoic acid by gavage, a NOAEL of 160 mg benzoic acid/kg bw per day was observed for maternal and developmental toxicity (EPA, 1992 (Doc. provided to EFSA n. 5)).

The four-generation reproductive toxicity study (Kieckebusch and Lang, 1960) with benzoic acid in the diet in rats was considered by the Panel as the pivotal study, as this study was the longest exposure period as compared to the developmental studies. This study showed no effect on growth, fertility, lactation or survival, and provided a NOAEL, for both the parental animals and the offspring, of 500 mg benzoic acid/kg bw per day, the highest dose tested.

3.2.6. Allergenicity, hypersensitivity and intolerance

3.2.6.1. Animal studies

Several studies including guinea pig maximisation and Buehler tests (Gad et al. 1986) and a local lymph node assay in mice (Gerberick et al., 1992) did not reveal any sensitising potential for benzoic acid.

3.2.6.2. Human data

An anaphylactic reaction was described in a 19-year-old woman in response to ingestion of sodium benzoate in foodstuffs. Adherence to a benzoate-free diet prevented recurrence of the symptoms. An

oral challenge with 20 mg sodium benzoate induced localised itching on the arms and generalised itching (Michils et al., 1991).

In nine patients with atopic dermatitis and positive reactions after oral provocation tests, increased leukotriene production was observed in four of nine patients administered benzoate (Worm et al., 2001). Furthermore, in the presence of benzoate, the mean value of leukotriene production in basophils from the same patients was approximately 10 times higher than in basophils from patients having negative reactions after an oral provocation test.

In a double-blind placebo-controlled study (Pacor et al., 2004), 226 patients (76 males and 150 females) aged 12–60 years (mean age 40.2 ± 16.3 years) were maintained for 1 month on an additive-free diet regimen, followed by an open challenge (food additive-rich diet for 2 weeks). Twenty of the 226 subjects (8.8%) challenged with sodium benzoate induced objective (sneezing and rhinorrhoea) and subjective symptoms (nasal blockage and nasal itching) of rhinitis. The authors concluded that the observation that non-atopic persistent rhinitis may be caused by the repeated ingestion of small doses of a non-tolerated substance suggests that some patients with 'chronic vasomotor rhinitis' may be intolerant to a particular food additive. According to the authors, sodium benzoate can be considered a trigger or an aggravating factor, rather than an aetiological factor.

The incidence of oral intolerance to sodium benzoate was studied among subjects who experienced repeated episodes of acute urticaria/angio-oedema after ingestion of a meal or a product containing sodium benzoate (Nettis et al. 2004). Of the 47 subjects enrolled in the study, five showed at least one positive reaction to an immunoglobulin E (IgE) test for food allergens. Only one subject had a reaction after the ingestion of 75 mg of sodium benzoate without an adverse reaction to placebo. The authors concluded that this study showed that the percentage of episodes of acute urticaria/angio-oedema reactions induced by sodium benzoate is very low.

A 75-year-old woman with a 6-year history of diffuse, severe pruritus, started an elimination diet (free of food additives) and after 1 week, pruritus had totally disappeared (Asero, 2006). Reintroduction of oral sodium benzoate (100 mg) resulted in relapse of diffuse pruritus within 24 h after intake. This finding was confirmed by a second series of two double-blind, placebo-controlled challenges including sodium benzoate and placebo.

3.2.6.3. *In vitro* immunotoxicity

The effect of sodium benzoate on the production of various cytokines by human peripheral blood mononuclear cells was investigated (Maier et al., 2010). Sodium benzoate was able to suppress the Th1 response in a dose-dependent manner, thus suggesting an anti-inflammatory effect. The Panel noted that this apparent beneficial effect might be counterbalanced by an associated possible diminished immune response to pathogens and tumours due to the decreased Th1 type response. In addition, the resulting shift towards Th2 immunity may favour the development of allergic diseases. The Panel also noted that the concentrations used were in the millimolar range and therefore, their relevance to the *in vivo* situation is questionable. These data, although supporting a mechanistic hypothesis for a potential immunomodulatory capacity of sodium benzoate, were considered by the Panel to be of limited relevance for its safety assessment.

Overall, the Panel noted that several studies reported that subgroups of patients already suffering from atopic dermatitis, pruritus, urticaria or persistent rhinitis can react to low doses of benzoate, even below the ADI. The Panel noted that these data, although available for a limited population of individuals following oral exposure, indicated that benzoic acid and its salts used as food additives could trigger the symptoms of asthma and eczema in people who already have these conditions.

3.2.7. Other studies

3.2.7.1. Animals

Noorafshan et al. (2014) dosed Sprague-Dawley rats (10 per group; body weight 180–230 g) by gavage with 0 or 200 mg sodium benzoate /kg bw per day for 4 weeks. At the end of the fourth week, anxiety and motor function were tested in an elevated plus maze and a rotarod test. The sodium benzoate-treated animals spent less time in the open arms and had fewer entrances to the open arms in comparison with the control group. In addition, the performance of the treated animals in the rotarod was impaired. In this studies no other parameters such as clinical signs, body weight, food intake or necropsy data were described.

3.2.7.2. Humans

Lok et al. (2013) tested the effect of 45 mg/person per day of sodium benzoate on the behaviour of one hundred and thirty 8- to 9-year-old schoolchildren and found no significant associations between sodium benzoate in Chinese children's behaviour. The study was a randomised, double-blind, placebo control study in Hong Kong.

Lennerz et al. (2015) studied the acute effects of sodium benzoate on glucose homeostasis and metabolic profiles in humans in a randomised, controlled, cross-over study in 14 overweight subjects. They did not find a statistically significant effect following an oral glucose challenge (75 g glucose solution), in the presence or absence of sodium benzoate (1% benzoate). Glucose, insulin, glucagon as well as temporal mass spectrometry-based metabolic profiles were measured. The Panel noted that this oral glucose tolerance test was performed in overweight subjects (body mass index (BMI) 25–30 kg/m²) who have a high prevalence of pathological response in this test (Shalitin et al., 2005). Therefore, as the study was done in this sensitive subpopulation, the results demonstrate the absence of an endocrine effect at a benzoate dose of 5.65 mg/kg (4.7–7.5 mg/kg) on the glucose homeostasis and metabolic profile in humans.

4. Discussion

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following an EFSA public call for scientific data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Benzoic acid, sodium benzoate, potassium benzoate and calcium benzoate (E 210, E 211, E 212 and E 213) are authorised food additives in the EU and have previously been evaluated by the SCF in 1994 and 2002. The SCF established a group ADI of 0–5 mg/kg bw for benzoic acid and its salts including benzyl alcohol and related benzyl derivatives used as flavourings (SCF, 2002). JECFA has evaluated benzoic acid and its salts as food additives on a number of occasions, most recently in 1996 (JECFA, 1996, 1997). JECFA has additionally reviewed benzoic acid as a flavouring substance (JECFA, 2001, 2002). In 1974, JECFA established a group ADI of 0–5 mg/kg bw for benzoic acid and its salts, expressed as benzoic acid, which was reconfirmed at its most recent evaluation in 1996. In both cases, the ADI was derived from a NOAEL of 500 mg/kg bw per day identified from a multi-generation study in rats (Kieckebusch and Lang, 1960), in which a dose level of 1% in the diet, equivalent to 500 mg/kg bw per day, had no effect on growth, fertility, lactation or lifespan (JECFA, 1974b).

Specific purity criteria have been defined in Commission Regulation (EU) No 231/2012 for benzoic acid and its salts (E 210–213). The Panel noted that the term 'benzoic acid' should be replaced by 'potassium benzoate' or 'calcium benzoate' in the EU specifications for potassium benzoate (E 212) and calcium benzoate (E 213), respectively. In addition, the Panel considered that if metals are used

as catalysts in the manufacturing process of benzoic acid as a food additive, the maximum residual level for each metal should be specified in the EU specifications for benzoic acid (E 210). The Panel also considered that the maximum limits for the impurities of toxic elements (lead, mercury and arsenic) in the EC specification for benzoic acid and its salts (E 210–213) should be revised in order to ensure that benzoic acid and its salts (E 210–213) as food additives will not be a significant source of exposure to those toxic elements in food.

The Panel noted that the formation of benzene in beverages containing benzoic acid or its salts as preservatives is linked to certain formulations, such as ascorbic acid in the presence of metallic ions and also to conditions of storage. Therefore, the Panel considered that when benzoic acid or its salts and ascorbic acid are used together, consideration should be given to the storage of soft drinks and food contact materials to minimise the formation of benzene in beverages.

Benzoic acid is absorbed by a diffusion process in the gastrointestinal tract. Sodium benzoate and potassium benzoate dissociate into their constituent sodium, potassium and benzoate ions in the small intestine. The available ADME data in animals and humans indicate that benzoic acid and its sodium and potassium salts are rapidly absorbed, primarily in the proximal part of the GI, after oral administration. Benzoate is conjugated to glycine to form hippuric acid, which is excreted in the urine. No studies appear to have been conducted on calcium benzoate, but the Panel considered that calcium benzoate also dissociates into its constituents calcium and benzoate ions in the small intestine. Consequently, the ADME of this salt will be similar to that of the sodium or potassium salts, despite differences in water solubility. Therefore, the Panel considered possible read-across between the salts.

The results of the available short-term and subchronic studies on benzoic acid and sodium benzoate indicated that their toxicity is low with no marked target organ toxicity, although high intakes of benzoic acid may lead to disturbances in the acid–base balance, and benzoic acid at high doses may interfere with intermediary metabolism. The Panel noted that most of the available studies were not conducted in accordance with the current test guidelines. The Panel considered that none of these studies were appropriate for identification of a NOAEL for short-term and subchronic toxicity of benzoic acid and its salts.

The Panel considered that positive or equivocal results reported for clastogenicity *in vitro* of benzoic acid and its salts sodium, potassium and calcium benzoates (Ishidate et al., 1984; Yilmaz et al. 2009; Zengin et al. 2011) have not been reproduced in well performed and relevant *in vivo* studies using the alkaline comet assay (Sasaki et al., 2002), the rodent bone marrow chromosome aberration assay and the dominant lethal assay in rats (Litton Bionetics 1974 (Doc. provided to EFSA n. 11)). Therefore, the Panel considered that the use of benzoic acid and its sodium and potassium salts as food additives did not raise a concern with respect to genotoxicity. Based on read-across, the Panel considered that this conclusion is also applicable for calcium benzoate.

The Panel noted that the available carcinogenicity studies on benzoic acid and its salts did not indicate any carcinogenic potential, although they were not conducted in accordance with current test guidelines and had deficiencies both in terms of design and reporting. Overall, given supporting evidence of lack of carcinogenicity of the related substances benzyl alcohol, benzyl acetate and benzaldehyde as described by JECFA (1996, 2001, 2002), the Panel considered that the data are sufficient to conclude that benzoic acid and its salts did not raise concern with respect to carcinogenicity.

In two developmental toxicity studies in rats with sodium benzoate in the diet, the NOAEL was 500 mg sodium benzoate/kg bw per day (the highest dose tested) (Crane and Lachance, 1985) and 1,310 mg sodium benzoate/kg bw per day (Onodera et al., 1978). Onodera et al (1978) studied also the effects of higher concentrations in the diet. Doses equal to 1,875 mg sodium benzoate/kg bw per day induced severe maternal toxicity and were associated with embryotoxic and fetotoxic effects and fetal

malformations. In studies in mice, rats, hamsters and rabbits with sodium benzoate administered by gavage (FDRL, 1972 (Doc. provided to EFSA n. 9)), no developmental effects were observed up to doses of 175, 174, 300 and 250 mg sodium benzoate/kg bw per day, the highest doses tested respectively in each species. In another developmental toxicity study in rats with benzoic acid by gavage, a NOAEL of 160 mg benzoic acid/kg bw per day was observed for maternal and developmental toxicity (EPA, 1992 (Doc. provided to EFSA n. 5)). In relation to developmental toxicity, the SCF noted that hippuric acid, the glycine conjugate of benzoic acid, was the main urinary metabolite and there was evidence in both humans and rodents that large, bolus doses can cause glycine depletion (SCF, 1994). The SCF concluded that: *The data available give adequate reassurance that the use of benzoic acid and its salts as food preservatives is temporarily acceptable. However, the role of glycine in the rate limiting step for hippuric acid formation from benzoic acid suggests that there may be a narrow margin between the metabolic demand for glycine and the rate at which glycine is formed or made available in the body. Glycine is not generally regarded as an essential amino acid but it has been suggested that in rapidly growing organisms glycine may be a conditionally essential amino acid and that this fine balance might be disturbed by benzoic acid. An adequate teratogenicity study using a dietary route of administration is therefore desirable.* The Panel agreed with this conclusion of the SCF.

The four-generation reproductive toxicity study (Kieckebusch and Lang, 1960) with benzoic acid in the diet in rats was considered by the Panel as the pivotal study, as this study was the longest exposure period as compared to the developmental studies. This study showed no effect on growth, fertility, lactation or survival, and provided a NOAEL, for both the parental animals and the offspring, of 500 mg benzoic acid/kg bw per day, the highest dose tested.

The Panel noted that benzoic acid and its salts may enhance hypersensitivity and/or cause skin reactions. Furthermore, anaphylaxis and urticaria have also been observed in sensitive individuals following exposure to benzoic acid and its salts at doses below the ADI. Several studies have shown that subgroups of patients already suffering from atopic dermatitis, pruritus, urticaria or persistent rhinitis may be intolerant even to low doses of benzoate.

The Panel noted that intolerance reactions to benzoic acid and its salts have been reported in the literature, manifesting as gastrointestinal disturbances, skin reactions (urticaria, pruritus, etc.), bronchial hyper reactivity or asthmatic attacks, effects on the central nervous system or even anaphylaxis. The Panel also noted that benzoate-sensitive individuals may show reactions below the ADI.

The Panel noted the absence of any indication of genotoxicity of benzoic acid and its salts *in vivo*, together with the negative results of limited carcinogenicity studies in rats and mice. The Panel considered that the ADI cannot be derived from a prenatal developmental toxicity study with benzoic acid performed by gavage (EPA, 1992 (Doc. provided to EFSA n. 5)). The developmental toxicity studies in rats (Crane and Lachance, 1985 and Onedera et al., 1978) with sodium benzoate in the diet showed NOAELs of 500 (the highest dose tested) and 1,310 mg/kg bw per day, respectively, comparable to or higher than the NOAEL of 500 mg/kg bw per day in the dietary multi-generation reproductive toxicity in rats with benzoic acid (Kieckebusch and Lang, 1960). From these reproductive and developmental studies, the Panel derived an ADI of 5 mg/kg bw per day (expressed as benzoic acid) using an uncertainty factor of 100.

To assess the dietary exposure to benzoic acid-benzoates (E 210–213) from their use as a food additive, the exposure was calculated based on (1) MPLs set out in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) the reported use levels and analytical data (defined as the *refined exposure assessment scenario*). Dietary exposure through this latter scenario was assessed using two sets of concentration data: reported use levels and analytical data considering levels not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised (Annex II to Regulation No 1333/2008) (dataset 1); in addition

to dataset 1, analytical data for food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available (dataset 2).

Based on the available datasets, the Panel calculated two refined exposure estimates based on different assumptions: a *brand-loyal consumer scenario*, where it is assumed that the population is exposed over a long period of time to the food additive present at the maximum reported use/analytical levels for one food category and to a mean reported use/analytical level for the remaining food categories; and a *non-brand-loyal scenario*, where it is assumed that the population is exposed over a long period of time to the food additive present at the mean reported use/analytical levels in all relevant food categories.

The exposure estimates in the *regulatory maximum level exposure assessment scenario* exceeded the ADI of 5 mg/kg bw per day for all population groups at the high level (95th percentile) and for toddlers and children also at the mean level (Table 7). The main contributing food categories to the total mean exposure estimates for infants and toddlers in this scenario were flavoured fermented milk products. For children and adolescents, the main contributing food categories were confectionary and flavoured drinks; while, for adults and the elderly, the main contributing food categories were processed fruit and vegetables and sauces. The Panel noted that the estimated long-term exposures based on this scenario are very likely conservative, as this scenario assumes that all foods and beverages listed under Annex II to Regulation No 1333/2008 contain benzoic acid-benzoates (E 210–213) as food additives at the MPL.

From the *refined estimated exposure scenario* considering only food categories for which direct addition of benzoic acid-benzoates to food is authorised, in the *brand-loyal scenario*, mean exposure to benzoic acid-benzoates (E 210–213) ranged from 0.07 mg/kg bw per day in infants to 3.2 mg/kg bw per day in toddlers. The high exposure to benzoic acid-benzoates (E 210–213) ranged from 0.4 mg/kg bw per day in infants to 7.1 mg/kg bw per day in children. In the *non-brand-loyal scenario*, mean exposure to benzoic acid-benzoates (E 210–213) ranged from 0.02 mg/kg bw per day in infants to 1.6 mg/kg bw per day in toddlers. The high exposure to benzoic acid-benzoates (E 210–213) ranged from 0.05 mg/kg bw per day in infants to 4.7 mg/kg bw per day in toddlers. The main contributing food categories for all population groups were flavoured drinks and sauces in both scenarios.

From the *refined estimated exposure scenario* considering additional exposure from food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available, in the *brand-loyal scenario*, mean exposure to benzoic acid-benzoates (E 210–213) ranged from 2.5 mg/kg bw per day for adolescents to 10.6 mg/kg bw per day for toddlers. The high exposure to benzoic acid-benzoates (E 210–213) ranged from 4.9 mg/kg bw per day for adolescents to 19.6 mg/kg bw per day in toddlers. The main contributing food categories were unprocessed fruits and vegetables for all population groups. In the *non-brand-loyal scenario*, mean exposure to benzoic acid-benzoates (E 210–213) ranged from 0.8 mg/kg bw per day for adolescents to 3.5 mg/kg bw per day for toddlers. The high exposure to benzoic acid-benzoates (E 210–213) ranged from 1.3 mg/kg bw per day in the elderly to 6.9 mg/kg bw per day in toddlers. The main contributing food categories were processed fruits and vegetables and processed foods for infants; for toddlers, they were unprocessed fruits and vegetables, flavoured drinks and processed foods; for children and adolescents, the main contributing food categories were flavoured drinks and unprocessed fruits and vegetables and for adults and the elderly, the main contributing food categories were unprocessed fruits and vegetables and coffee, tea, herbal and fruit infusions, chicory.

Therefore, considering only food categories for which direct addition of benzoic acid-benzoates to food is authorised, the exposure to benzoic acid-benzoates (E 210–213), in toddlers, children, adolescents and adults exceeded the ADI of 5 mg/kg bw per day at the high level (95th percentile) in the *refined brand-loyal* exposure estimate, while, for the *non-brand-loyal scenario*, the group ADI was not exceeded in any population groups (Table 7). Considering additional exposure from food categories which may contain benzoic acid-benzoates due to carry-over and for which data were

available, in the *refined brand-loyal* exposure scenario, the ADI was exceeded at the high level for all population groups and for infants, toddlers, children and adolescent at the mean. In the *non-brand-loyal* exposure scenario, the ADI was exceeded only for toddlers and children at the high level; at the mean level there was no exceedance.

The Panel considered that the refined exposure assessment approach resulted in more realistic long-term exposure estimates compared to the *regulatory maximum level exposure assessment scenario*. This approach is based on the extensive range of analytical data available and assumes that people, in the long term, are exposed to foods and beverages that contain the food additive at a mean concentration level for all products (*non-brand-loyal scenario*) or that one product contains the food additive at the maximum concentration level (*brand-loyal scenario*) and the remaining products contain the additive at a mean concentration level. For benzoic acid-benzoates (E 210–213), reported use/analytical levels were available. However, not all available data could be included in the assessment owing to specific restrictions/exceptions regarding products not referenced in the FoodEx classification. This may have resulted in an underestimation of exposure to benzoic acid and benzoates. On the other hand, several food categories for which usage/analytical data were available were included without considering specific restrictions/exceptions, which may have overestimated the exposure to benzoic acid and benzoates.

The Panel noted that from all contributing sources, exposure from non-alcoholic beverages provided a realistic situation of possible exceedance of the ADI in those population groups due to the fact of the existence of consumer being brand-loyal to non-alcoholic beverage and of the consistency mean value observed from the large number of data that have been submitted (around 7000) from industries or from Member States with a mean of 100 mg/L from reported typical use level vs a mean of 80 mg/L from analytical mean measurement.

The Panel also noted that the exposure assessment considering *brand-loyal* exposure using dataset 1 was concordant with the recent JECFA re-evaluation on the dietary exposure to benzoates where exceedance of the ADI of 5 mg/kg bw per day was only observed at the 95th percentile exposures for the consumers-only group of non-alcoholic beverages for toddlers, children and adolescents (JECFA, 2015).

CONCLUSIONS

Considering the present database, the Panel identified a NOAEL of 500 mg/kg bw per day and applying an uncertainty factor of 100, the Panel derived a group ADI of 5 mg/kg bw per day, expressed as benzoic acid, for benzoic acid (E 210), sodium benzoate (E 211), potassium benzoate (E 212) and calcium benzoate (E 213).

The Panel concluded that from the use of benzoic acid-benzoates as food additives from direct addition to food, the group ADI was exceeded in the *brand-loyal scenario* for toddlers and children consuming on a regular basis flavoured drinks.

Considering additional exposure from food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available, the Panel concluded that exposure to benzoic acid-benzoates could be increased by up to two to three fold for all high-level consumers compared to the previous scenario for direct addition to food. This is resulting in exceedance of the group ADI in toddlers and children for the *non-brand-loyal* scenario. The main food categories contributing to this exceedance were unprocessed fruits and vegetables and flavoured drinks.

RECOMMENDATIONS

The Panel recommended that:

- the maximum limits for the impurities of toxic elements (lead, mercury and arsenic) in the EC specification for benzoic acid and its salts (E 210–213) should be revised in order to ensure that benzoic acid and its salts (E 210–213) as food additives will not be a significant source of exposure to those toxic elements in food.
- When benzoic acid or its salts and ascorbic acid are used together, consideration should be given to the storage of soft drinks and food contact materials to minimise the formation of benzene in beverages as low as reasonably achievable.

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APPENDICES

A. Summary of reported use levels (mg/kg) of benzoic acid-benzoates (E 210–213) provided by industry

FCS Category N°	FCS Food Category	MPL	Restrictions / exceptions	Typical mean (range)	Max	Information provided by	Comments
05.2	Other confectionery including breath refreshing microsweets	1500 / 1000	except candied, crystallised or glacé fruit and vegetables / only candied, crystallised or glacé fruit and vegetables	60 (0-120)	120	FDE (2013)	confectionary (excluding chocolate)
				0	0	FDE (2013)	pralines (reported as not used)
				0	0	FDE (2013)	Bars (reported as not used)
				0	0	FDE (2013)	Dragées (reported as not used)
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	1500		0	0	FDE (2013)	fillings for chocolates (reported as not used)
				0.002	0.002	Mars (2010)	chocolates
12.6	Sauces	1000	non emulsified sauces	132 (0-263)	850*	FDE (2013)	non-emulsified sauces *850 mg/kg in 1 products from few EU countries, not used in the exposure assessment
				0	0	FDE (2013)	ketchup (reported as not used)
		500	only emulsified sauces with a fat content of 60 % or more	0	0	FDE (2013)	sauses for barbecue (reported as not used)
				0	0	FDE (2013)	sauses for pasta (reported as not used)
12.7	Salads and savoury based sandwich spreads	1500		1000	1500	FDE (2013)	prepared salads (reported as 65% of products do not contain these FA)
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	1500		125 (100-150)	150	FDE (2013)	dietetic foods intended for special medical purposes
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	1500		125 (100-150)	150	FDE (2013)	dietetic formulae for weight control: in the product in powder as sold, needs to be diluted

FCS Category N°	FCS Food Category	MPL	Restrictions / exceptions	Typical mean (range)	Max	Information provided by	Comments
14.1.4	Flavoured drinks	150	excluding dairy-based drinks	98 (50-145)	150	FDE (2013)	non-alcoholic flavoured drinks
				98 (50-145)	150	FDE (2013)	carbonates
				100 (50-150)	150	UNESDA (2010)	carbonates
				98 (50-145)	145	FDE (2013)	lemon flavoured carbonates (for non-lemon flavoured: reported as very low use)
				100 (60-140)	150	UNESDA (2010)	still drinks
				125 (110-140)	150	FDE (2013)	still drinks; level reported as representative which does not imply that all or the majority of the products use this food additive
				0	0	UNESDA (2010)	iced coffee (reported as not used)
				100 (60-140)	150	UNESDA (2010)	iced tea
				113 (85-140)	150	FDE (2013)	iced tea
				103 (60-145)	150	UNESDA (2010)	squashes/syrups (concentrates)
				0	0	UNESDA (2010)	fruit powder (reported as not used)
				130 (120-140)	150	FDE (2013)	energy drinks
				113 (85-140)	150	UNESDA (2010)	energy drinks
				70 (0-140)	150	FDE (2013)	sports drinks; reported as occasional use in sports drinks
				138 (130-145)	150	UNESDA (2010)	sports drinks

FCS Category N°	FCS Food Category	MPL	Restrictions / exceptions	Typical mean (range)	Max	Information provided by	Comments
14.2.1	Beer and malt beverages	200	only alcohol free beer; beer in kegs containing more than 0.5 % added fermentable sugar and/or fruit juices or concentrates	125 (100-150)	200	FDE (2013)	alcohol-free beer in keg
16	Desserts excluding products covered in category 1, 3 and 4	300	only non-heat-treated dairy-based desserts	50	50	FDE (2013)	non-heat treated dairy base desserts (limited representation for the Swedish market)

FCS, Food Categorisation System; FDE, FoodDrinkEurope; MPL, maximum permitted level; *QS*, *quantum satis*; UNESDA, Union of European Soft Drinks Associations.

B. Summary of analytical results (mg/kg) of benzoic acid provided by Member States

B1. All data received

FCS Category number	Food category	MPL	restrictions	N	No LC	% LC	No > MPL	% > MPL	LOD		LOQ		Middle-bound				
									Min	Max	Min	Max	Min	Median	Mean	P95	Max
01.1	Unflavoured pasteurised and sterilised (including UHT) milk			16	9	56.3			0.2	3.8	0.5	12.5	0.5	2.2	11.3	52.0	52.0
01.2	Unflavoured fermented milk products, including natural unflavoured buttermilk (excluding sterilised buttermilk) non-heat-treated after fermentation			88	76	86.4			0.0	4.0	0.0	12.5	0.3	1.9	3.2	14.0	20.5
01.3	Unflavoured fermented milk products, heat-treated after fermentation			484	115	23.8			0.0	3.0	0.0	50.0	0.0	9.8	9.9	21.1	73.0
01.4	Flavoured fermented milk products including heat treated products	300	only non-heat treated dairy based desserts	1118	539	48.2	0	0.0	0.0	20.0	0.0	50.0	0.0	2.4	5.2	16.0	116.7
01.5	Dehydrated milk as defined by Directive 2001/114/EC			2	2	100.0			0.3	0.4	1.0	1.0	0.5	0.5	0.5	0.5	0.5
01.6	Cream and cream powder			12	5	41.7			0.0	3.4	0.0	10.1	0.3	5.8	26.9	253.0	253.0
01.6.3	Cream and cream powder			253	184	72.7			0.0	11.3	0.0	33.9	0.0	0.3	2.7	13.5	38.2
01.7	Cheese and cheese products			157	132	84.1			1.0	6.7	5.0	20.0	0.6	1.9	13.6	22.0	1224.4
01.7.1	Unripened cheese excluding products falling in category 16			131	24	18.3			0.0	37.8	0.0	113.3	0.3	8.0	74.1	532.1	1029.0
01.7.2	Ripened cheese			239	107	44.8			0.0	10.0	0.0	50.0	0.0	5.0	12.5	25.0	982.0
01.7.3	Edible cheese rind			13	5	38.5			0.2	0.4	0.5	1.0	0.3	5.0	4.4	9.2	9.2
01.7.4	Whey cheese			168	89	53.0			0.0	37.8	0.0	113.3	0.0	0.5	11.4	35.7	369.3
01.7.5	Processed cheese			858	368	42.9			0.0	20.0	0.0	50.0	0.0	4.8	9.1	23.5	786.6

FCS Category number	Food category	MPL	restrictions	N	No LC	% LC	No > MPL	% > MPL	LOD		LOQ		Middle-bound				
									Min	Max	Min	Max	Min	Median	Mean	P95	Max
01.7.6	Cheese products (excluding products falling in category 16)			8	1	12.5			0.2	0.3	0.6	1.0	0.3	7.3	8.9	22.6	22.6
01.8	Dairy analogues, including beverage whiteners			10	7	70.0			0.0	14.0	0.0	45.0	0.0	4.6	4.1	9.1	9.1
02.1	Fats and oils essentially free from water (excluding anhydrous milk fat)			381	380	99.7			0.0	125.0	0.0	125.0	0.0	0.3	1.5	0.5	66.5
02.2	Fat and oil emulsions mainly of type water-in-oil			24	22	91.7			0.2	11.3	0.5	54.0	0.3	1.9	58.2	317.0	1016.0
02.2.1	Butter and concentrated butter and butter oil and anhydrous milk fat			26	20	76.9			0.0	0.4	0.0	1.0	0.3	0.3	3.1	13.6	16.9
02.2.2	Other fat and oil emulsions including spreads as defined by Regulation (EC) No 1234/2007 and liquid emulsions			189	189	100.0			0.0	20.0	0.0	50.0	0.0	1.9	2.7	10.0	10.0
03	Edible ices			314	244	77.7			0.0	7.6	0.0	75.0	0.0	2.5	12.8	26.0	800.0
04.1	Unprocessed fruit and vegetables			11	9	81.8			1.0	3.0	5.0	50.0	0.5	2.5	12.1	50.0	50.0
04.1.1	Entire fresh fruit and vegetables			92	81	88.0			0.0	10.0	0.0	20.0	0.3	2.5	70.0	766.0	1010.0
04.1.2	Peeled, cut and shredded fruit and vegetables			24	18	75.0			0.0	11.3	0.0	33.9	0.0	0.3	83.7	394.5	548.0
04.1.3	Frozen fruit and vegetables			106	96	90.6			0.0	37.8	0.0	113.3	0.0	0.3	41.7	344.3	872.0
04.2	Processed fruit and vegetables			105	74	70.5			0.3	20.0	0.6	50.0	0.2	7.5	192.5	1271.5	1802.5
04.2.1	Dried fruit and vegetables			1086	982	90.4			0.0	20.0	0.0	60.0	0.0	0.5	9.7	50.0	1425.0
04.2.2	Fruit and vegetables in vinegar, oil, or brine	2000	only vegetables (excluding olives)	1003	624	62.2	3	0.3	0.0	37.8	0.0	113.5	0.0	7.5	267.4	1174.0	2270.0
04.2.2	Fruit and vegetables in vinegar, oil, or brine			27	20	74.1			0.3	56.5	1.0	169.5	0.5	5.0	260.0	1450.0	1640.0

FCS Category number	Food category	MPL	restrictions	N	No LC	% LC	No > MPL	% > MPL	LOD		LOQ		Middle-bound				
									Min	Max	Min	Max	Min	Median	Mean	P95	Max
04.2.2	Fruit and vegetables in vinegar, oil, or brine	500	only olives and olive-based preparations	114	88	77.2	7	6.1	1.0	20.0	3.0	50.0	0.5	7.5	97.4	544.0	1339.0
04.2.3	Canned or bottled fruit and vegetables			1283	893	69.6			0.0	37.8	0.0	115.0	0.0	0.9	241.8	1298.9	3652.4
04.2.4	Fruit and vegetable preparations, excluding products covered by 5.4			87	85	97.7			2.0	20.0	2.0	50.0	1.0	10.0	17.9	10.0	818.0
04.2.4.1	Fruit and vegetable preparations excluding compote			122	111	91.0			0.3	20.0	0.6	50.0	0.2	2.5	21.0	10.0	817.0
04.2.4.1	Fruit and vegetable preparations excluding compote	2000	only cooked red beet	4	3	75.0	0	0.0	0.3	3.3	0.6	15.0	0.3	6.3	193.6	761.7	761.7
04.2.4.1	Fruit and vegetable preparations excluding compote	500	only seaweed preparations, olives and olive-based preparations	13	0	0.0	8	61.5					300.0	540.0	513.8	690.0	690.0
04.2.4.2	Compote, excluding products covered by category 16			402	381	94.8			0.0	20.0	0.0	50.0	0.0	0.3	8.6	10.0	943.0
04.2.5	Jam, jellies and marmalades and similar products			769	598	77.8			0.0	20.0	0.0	75.0	0.0	5.0	50.4	349.0	1139.0
04.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EEC	500	only low-sugar and similar low calorie or sugar-free products , <i>mermeladas</i>	262	257	98.1	0	0.0	0.2	20.0	0.5	50.0	0.3	10.0	7.9	10.0	83.3
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC	500	only low-sugar and similar low calorie or sugar-free products; <i>mermeladas</i>	593	510	86.0	12	2.0	0.3	50.0	1.0	60.0	0.3	2.5	38.7	250.0	1260.0

FCS Category number	Food category	MPL	restrictions	N	No LC	% LC	No > MPL	% > MPL	LOD		LOQ		Middle-bound				
									Min	Max	Min	Max	Min	Median	Mean	P95	Max
04.2.5.3	Other similar fruit or vegetable spreads	500	other fruit-based spreads, <i>mermeladas</i>	468	423	90.4	1	0.2	0.2	50.0	0.5	75.0	0.3	10.0	23.1	92.0	649.0
04.2.5.3	Other similar fruit or vegetable spreads	1000	only <i>dulce de membrillo</i>	0			0										
04.2.5.3	Other similar fruit or vegetable spreads	1500	only <i>marmelada</i>	6	0	0.0	0	0.0	6.7	6.7	20.0	20.0	338.3	589.4	554.0	824.0	824.0
04.2.5.4	Nut butters and nut spreads			7	7	100.0			1.7	1.7	5.0	5.0	2.5	2.5	2.5	2.5	2.5
04.2.6	Processed potato products			93	92	98.9			0.2	20.0	0.5	50.0	0.3	7.5	7.1	10.0	18.5
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC			54	46	85.2			0.0	37.8	0.0	113.5	0.0	0.5	3.3	18.9	22.9
05.2	Other confectionery including breath refreshing microsweets	1500	except candied, crystallised or glacé fruit and vegetables	340	301	88.5	0	0.0	0.0	20.0	0.0	50.0	0.0	2.5	46.0	346.8	1027.0
05.2	Other confectionery including breath refreshing microsweets	1000	only candied, crystallised or glacé fruit and vegetables	164	151	92.1	0	0.0	0.0	10.0	0.0	30.0	0.0	1.9	18.5	110.0	418.0
05.3	Chewing gum	1500		5	4	80.0	0	0.0	0.2	20.0	0.5	50.0	0.5	10.0	89.9	419.0	419.0
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	1500		73	67	91.8	0	0.0	1.7	3.8	5.0	15.0	1.9	1.9	47.0	411.0	780.2
06	Cereals and cereal products			3	3	100.0			3.8	3.8	12.5	12.5	1.9	1.9	1.9	1.9	1.9
06.1	Whole, broken, or flaked grain			6	6	100.0			0.2	10.0	0.6	20.0	0.3	5.0	4.2	7.5	7.5
06.2	Flours and other milled products and starches			2	1	50.0			1.7	1.7	5.0	5.0	2.5	6.8	6.8	11.1	11.1
06.2.1	Flours			18	17	94.4			0.0	6.0	0.0	18.0	0.0	1.8	2.2	9.0	9.0
06.2.2	Starches			1	1	100.0			0.2	0.2	0.6	0.6	0.3	0.3	0.3	0.3	0.3
06.3	Breakfast cereals			67	57	85.1			0.0	22.6	0.0	67.8	0.0	2.5	3.5	15.8	33.9
06.4	Pasta			34	33	97.1			0.0	20.0	0.0	20.0	0.0	7.5	10.8	10.0	180.0

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									Min	Max	Min	Max	Min	Median	Mean	P95	Max
06.4.1	Fresh pasta			3	3	100.0			0.5	5.0	1.5	15.0	0.3	2.5	1.8	2.5	2.5
06.4.2	Dry pasta			3	3	100.0			0.2	0.3	0.5	1.0	0.3	0.3	0.3	0.5	0.5
06.4.4	Potato gnocchi			9	9	100.0			0.0	20.0	0.0	20.0	0.0	5.0	6.1	10.0	10.0
06.6	Batters			3	3	100.0			1.0	10.0	3.0	30.0	0.5	0.5	2.0	5.0	5.0
06.7	Pre-cooked or processed cereals			1	1	100.0					15.0	15.0	7.5	7.5	7.5	7.5	7.5
07.1	Bread and rolls			1501	1475	98.3			0.2	20.0	0.5	30.0	0.3	5.0	5.4	10.0	265.9
07.1.1	Bread prepared solely with the following ingredients: wheat flour, water, yeast or leaven, salt			299	298	99.7			0.0	3.8	0.0	12.5	0.0	0.3	0.5	1.9	17.0
07.1.2	Pain courant français; Friss búzakenyér, fehér és félbarna kenyerek			1	1	100.0			3.3	3.3	10.0	10.0	5.0	5.0	5.0	5.0	5.0
07.2	Fine bakery wares			2937	2526	86.0			0.0	37.8	0.0	113.5	0.0	2.5	18.3	48.0	1900.0
08.1	Unprocessed meat			11	10	90.9			0.2	1.7	0.5	5.0	0.3	2.5	9.8	92.0	92.0
08.2	Meat preparations			6	4	66.7			0.2	1.7	0.5	5.0	0.3	2.5	138.8	423.0	423.0
08.3	Meat products			163	141	86.5			0.0	37.8	0.0	113.3	0.0	0.3	465.4	478.8	68772.6 ^(a)
08.3.1	Non heat treated processed meat		only surface treatment of dried meat products	114	110	96.5			1.3	3.3	1.3	20.0	0.6	6.3	7.9	10.0	147.5
08.3.2	Heat treated processed meat	500	only aspic	85	64	75.3	3	3.5	0.0	37.8	0.0	113.3	0.0	2.5	47.5	198.7	842.1
08.3.3	Casing and Coatings and decorations for meat			1	1	100.0			1.7	1.7	5.0	5.0	2.5	2.5	2.5	2.5	2.5
08.3.4	Traditionally cured meat products with specific provisions concerning nitrites and nitrates			3	3	100.0			1.7	1.7	5.0	5.0	2.5	2.5	2.5	2.5	2.5

FCS Category number	Food category	MPL	restrictions	N	No LC	% LC	No > MPL	% > MPL	LOD		LOQ		Middle-bound				
									Min	Max	Min	Max	Min	Median	Mean	P95	Max
08.3.4.1	Traditional immersion cured products (meat products cured by immersion in a curing solution containing nitrites and/or nitrates, salt and other components)			1	0	0.0							181.0	181.0	181.0	181.0	181.0
08.3.4.2	Traditional dry-cured products (dry curing process involves dry application of curing mixture containing nitrites and/or nitrates, salt and other components to the surface of the meat followed by a period of stabilisation/maturation).			3	3	100.0			0.5	1.0	1.5	3.0	0.3	0.3	0.3	0.5	0.5
08.3.4.3	Other traditionally cured products (immersion and dry cured processes used in combination or where nitrite and/or nitrate is included in a compound product or where the curing solution is injected into the product prior to cooking)			3	3	100.0			0.3	0.3	1.0	1.0	0.5	0.5	0.5	0.5	0.5
09.1.1	Unprocessed fish			42	26	61.9			0.2	10.0	0.5	75.0	0.3	2.2	289.5	1600.0	2029.8
09.1.2	Unprocessed molluscs and crustaceans			47	45	95.7			1.7	3.8	5.0	12.5	1.9	2.5	5.7	5.0	101.1
09.2	Processed fish and fishery products including molluscs and crustaceans			611	322	52.7			0.0	10.0	0.0	30.0	0.0	10.0	353.6	1592.0	2427.0
09.2	Processed fish and fishery products including molluscs and crustaceans	200	only salted, dried fish	39	39	100.0	0	0.0			20.0	20.0	10.0	10.0	10.0	10.0	10.0

FCS Category number	Food category	MPL	restrictions	N	No LC	% LC	No > MPL	% > MPL	LOD		LOQ		Middle-bound				
									Min	Max	Min	Max	Min	Median	Mean	P95	Max
09.2	Processed fish and fishery products including molluscs and crustaceans	2000	only semi-preserved fish and fisheries products including crustaceans, molluscs, surimi and fish / crustacean paste; cooked crustaceans and molluscs	326	135	41.4	2	0.6	1.3	3.3	1.3	15.0	0.6	195.0	410.9	1400.0	3200.0
09.2	Processed fish and fishery products including molluscs and crustaceans	6000	only cooked <i>Crangon crangon</i> and <i>Crangon vulgaris</i>	92	40	43.5	0	0.0	0.2	1.7	0.5	20.0	2.5	337.2	454.6	1200.0	3800.0
09.2	Processed fish and fishery products including molluscs and crustaceans	1000	only cooked crustaceans and molluscs	41	27	65.9	0	0.0	0.2	50.0	0.5	150.0	0.5	10.0	155.6	834.0	881.4
09.2	Processed fish and fishery products including molluscs and crustaceans	1500	only cooked shrimps in brine	0			0										
09.3	Fish roe	2000	only semi-preserved fish products including fish roe products	68	22	32.4	0	0.0	0.0	1.7	0.0	5.0	0.3	663.3	644.2	1686.0	1994.6
09.3	Fish roe			16	9	56.3			1.0	6.7	1.0	20.0	0.5	10.0	463.4	1529.7	1529.7
10.1	Unprocessed eggs			5	3	60.0			1.7	1.7	5.0	5.0	2.5	2.5	295.7	986.0	986.0
10.2	Processed eggs and egg products			16	6	37.5			0.5	37.8	1.5	113.3	0.3	263.0	441.9	4000.0	4000.0
10.2	Processed eggs and egg products	5000	only liquid egg (white, yolk or whole egg)	47	41	87.2	0	0.0	6.7	6.7	20.0	20.0	10.0	10.0	461.6	3855.0	3890.0

FCS Category number	Food category	MPL	restrictions	N	No LC	% LC	No > MPL	% > MPL	LOD		LOQ		Middle-bound				
									Min	Max	Min	Max	Min	Median	Mean	P95	Max
11.1	Sugars and syrups as defined by Directive 2001/111/EC			6	6	100.0			0.3	37.8	1.0	113.4	0.5	7.5	8.2	18.9	18.9
11.2	Other sugars and syrups			219	69	31.5			0.0	37.8	0.0	113.4	0.0	59.0	101.4	675.6	1664.4
11.3	Honey as defined in Directive 2001/110/EC			2	0	0.0			0.2	0.2	0.5	0.5	63.0	85.0	85.0	107.0	107.0
11.4	Table-top sweeteners			2	2	100.0			0.3	0.9	1.0	1.0	0.4	0.5	0.5	0.5	0.5
11.4.1	Table-top sweeteners in liquid form			2	2	100.0			1.0	1.0	3.0	20.0	0.5	5.3	5.3	10.0	10.0
11.4.1	Table-top sweeteners in liquid form	500	only if the water content higher than 75 %	0			0										
12.1.1	Salt			1	1	100.0			0.2	0.2	0.5	0.5	0.3	0.3	0.3	0.3	0.3
12.1.2	Salt substitutes			36	32	88.9			0.0	0.4	0.0	1.0	0.0	0.3	78.5	776.9	988.9
12.2	Herbs, spices, seasonings			30	23	76.7			1.7	2.0	5.0	75.0	2.5	2.5	119.3	850.0	850.0
12.2.1	Herbs and spices			38	34	89.5			0.0	14.0	0.0	45.0	0.1	7.5	78.7	761.8	1156.0
12.2.2	Seasonings and condiments	1000		282	228	80.9	1	0.4	0.0	36.0	0.0	50.0	0.3	7.5	94.1	556.9	3227.0
12.3	Vinegars			67	62	92.5			0.0	10.0	0.0	50.0	0.0	0.3	2.0	7.5	51.2
12.4	Mustard	1000		500	380	76.0	2	0.4	0.0	36.0	0.0	50.0	0.0	1.5	106.6	797.0	1034.0
12.5	Soups and broths			321	307	95.6			0.0	10.0	0.0	30.0	0.0	0.3	5.2	5.0	704.3
12.5	Soups and broths	500	only liquid soups and broths (excluding canned)	47	46	97.9	0	0.0	0.0	20.0	0.2	50.0	0.1	7.5	7.8	10.0	58.0
12.5	Soups and broths			44	43	97.7			0.0	2.2	0.0	6.7	0.0	0.4	0.4	0.5	3.4
12.6	Sauces			839	554	66.0			0.0	36.0	0.0	75.0	0.0	7.5	109.7	670.0	1513.5
12.6	Sauces	1000	only emulsified sauces with a fat content of less than 60 %	887	448	50.5	18	2.0	0.0	37.8	0.0	113.5	0.0	10.0	287.2	896.0	1535.5
12.6	Sauces	500	only emulsified sauces with a fat content of 60 % or more	178	141	79.2	17	9.6	0.5	36.0	1.5	50.0	0.3	2.5	108.8	820.0	990.0

FCS Category number	Food category	MPL	restrictions	N	No LC	% LC	No > MPL	% > MPL	LOD		LOQ		Middle-bound				
									Min	Max	Min	Max	Min	Median	Mean	P95	Max
12.6	Sauces	1000	non emulsified sauces	0			0										
12.7	Salads and savoury based sandwich spreads	1500		3314	1128	34.0	17	0.5	0.0	1345.0	0.0	1345.0	0.0	131.5	255.1	839.7	3675.9
12.9	Protein products, excluding products covered in category 1.8			6	4	66.7			0.2	6.7	0.5	20.0	0.3	4.0	817.6	4800.0	4800.0
13	Foods intended for particular nutritional uses as defined by Directive 2009/39/EC			10	9	90.0			2.0	14.0	2.0	45.0	1.0	5.0	13.6	99.1	99.1
13.1.1	Infant formulae as defined by Commission Directive 2006/141/EC			3	2	66.7			1.5	3.4	4.5	10.1	1.7	2.3	3.5	6.7	6.7
13.1.2	Follow-on formulae as defined by Directive 2006/141/EC			51	47	92.2			0.2	23.9	0.5	71.7	0.3	3.0	5.3	35.9	35.9
13.1.3	Processed cereal-based foods and baby foods for infants and young children as defined by Commission Directive 2006/125/EC			7	7	100.0			0.5	10.0	1.5	30.0	0.3	0.3	1.5	5.0	5.0
13.1.4	Other foods for young children			2	2	100.0			0.5	1.0	1.5	3.0	0.3	0.4	0.4	0.5	0.5
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	1500		216	139	64.4	0	0.0	0.2	100.0	0.4	100.0	0.1	5.0	23.0	112.0	502.0
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	1500		6	6	100.0	0	0.0	0.6	7.5	3.6	22.4	0.3	1.3	3.7	11.2	11.2

FCS Category number	Food category	MPL	restrictions	N	No LC	% LC	No > MPL	% > MPL	LOD		LOQ		Middle-bound				
									Min	Max	Min	Max	Min	Median	Mean	P95	Max
13.4	Foods suitable for people intolerant to gluten as defined by Commission Regulation (EC) No 41/2009			3	3	100.0			5.0	5.0	15.0	15.0	2.5	7.5	5.8	7.5	7.5
14.1	Non-alcoholic beverages			25	12	48.0			1.7	11.3	5.0	33.9	2.5	35.9	48.4	132.0	192.8
14.1.1	Water, including natural mineral water as defined in Directive 2009/54/EC and spring water and all other bottled or packed waters			138	9	6.5			0.0	1.7	0.0	10.0	0.0	117.5	105.8	153.3	250.0
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices			1479	968	65.4			0.0	166.7	0.0	500.0	0.0	2.5	22.9	113.3	1909.2
14.1.2	Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices	2000	only grape juice, unfermented, for sacramental use	0			0										
14.1.2	Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices	200	only <i>Sød.....saft</i> and <i>sødet.....saft</i>	0			0										
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products			308	182	59.1			0.0	10.0	0.0	24.0	0.0	2.0	29.8	119.8	905.0
14.1.3	Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and similar products	150	only traditional Swedish and Finnish fruit syrups	0			0										
14.1.4	Flavoured drinks			10	7	70.0			1.7	11.3	5.0	33.9	2.5	5.0	23.0	92.8	92.8
14.1.4	Flavoured drinks	150	excluding dairy-based drinks	6835	2941	43.0	232	3.4	0.0	37.8	0.0	165.0	0.0	7.5	57.1	143.2	1290.0

FCS Category number	Food category	MPL	restrictions	N	No LC	% LC	No > MPL	% > MPL	LOD		LOQ		Middle-bound				
									Min	Max	Min	Max	Min	Median	Mean	P95	Max
14.1.5	Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products			21	14	66.7			0.0	10.0	0.0	30.0	0.0	3.6	39.6	148.0	208.0
14.1.5.1	Coffee, coffee extracts			8	8	100.0			0.0	3.8	0.0	12.5	0.0	0.5	0.8	1.9	1.9
14.1.5.2	Other	600	only liquid tea concentrates and liquid fruit and herbal infusion concentrates	26	20	76.9	1	3.8	0.2	6.7	0.6	20.0	0.1	10.0	70.8	257.0	845.0
14.2	Alcoholic beverages, including alcohol-free and low-alcohol counterparts			9	3	33.3			3.8	3.8	12.5	12.5	1.9	143.0	109.7	198.0	198.0
14.2.1	Beer and malt beverages			226	158	69.9			0.0	5.0	0.0	15.0	0.0	0.3	2.4	7.5	76.0
14.2.1	Beer and malt beverages	200	only alcohol free beer; beer in kegs containing more than 0.5 % added fermentable sugar and/or fruit juices or concentrates	65	54	83.1	0	0.0	0.2	1.7	1.0	10.0	0.1	2.5	4.9	22.0	60.0
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol free counterparts			679	674	99.3			0.0	10.0	0.0	20.0	0.0	5.0	5.2	5.0	881.6
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts	200	only alcohol-free	0			0										
14.2.3	Cider and perry			1092	1038	95.1			0.0	10.0	0.0	30.0	0.0	0.3	2.1	7.5	121.0

FCS Category number	Food category	MPL	restrictions	N	No LC	% LC	No > MPL	% > MPL	LOD		LOQ		Middle-bound				
									Min	Max	Min	Max	Min	Median	Mean	P95	Max
14.2.4	Fruit wine and made wine			12	11	91.7			0.0	6.7	0.0	20.0	0.3	10.0	16.9	117.1	117.1
14.2.4	Fruit wine and made wine			1	1	100.0			3.3	3.3	10.0	10.0	5.0	5.0	5.0	5.0	5.0
14.2.5	Mead			4	4	100.0			6.7	11.3	5.0	20.0	2.5	6.3	6.3	10.0	10.0
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008 - except: spirit drinks as defined in article 5(1) and sales denominations listed in Annex II, paragraphs 1-14 of Regulation 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, London Gin, Sambuca, Maraschino and Mistrà			62	28	45.2			0.0	0.3	0.0	15.0	0.0	101.3	67.5	157.0	192.2
14.2.7	Aromatised wine-based products as defined by Regulation (EEC) No 1601/91			22	5	22.7			1.7	1.7	5.0	5.0	2.5	160.0	123.3	200.0	200.0
14.2.7.1	Aromatised wines			16	16	100.0			0.0	0.3	0.0	1.0	0.0	0.3	0.3	0.5	0.5
14.2.7.2	Aromatised wine-based drinks except bitter soda, sangria, claria, zurra			4	4	100.0			0.0	10.0	0.0	15.0	0.0	2.6	2.6	5.0	5.0
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	200	only alcoholic drinks with less than 15 % of alcohol	139	63	45.3	1	0.7	0.0	16.7	0.0	50.0	0.0	104.0	76.9	188.0	401.7
15.1	Potato-, cereal-, flour- or starch-based snacks			166	165	99.4			0.0	20.0	0.0	50.0	0.0	2.5	5.7	10.0	207.8
15.2	Processed nuts			4	4	100.0			0.3	1.7	1.0	20.0	0.5	1.5	3.4	10.0	10.0

FCS Category number	Food category	MPL	restrictions	N	No LC	% LC	No > MPL	% > MPL	LOD		LOQ		Middle-bound				
									Min	Max	Min	Max	Min	Median	Mean	P95	Max
16	Desserts excluding products covered in category 1, 3 and 4	300	only non-heat-treated dairy-based desserts	366	330	90.2	2	0.5	1.7	20.0	2.0	50.0	0.8	7.5	12.4	15.0	580.0
16	Desserts excluding products covered in category 1, 3 and 4	500	only <i>frugtgrød</i> and <i>Rote Grütze</i>	0			0										
17	Food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children			236	178	75.4			0.1	100.0	0.3	113.5	0.1	6.2	88.0	678.6	1197.8
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms	1000	only when supplied in dried form and containing preparations of vitamin A and of combinations of vitamin A and D	3	3	100.0	0	0.0	3.3	3.4	9.9	10.1	5.0	5.1	5.0	5.1	5.1
17.2	Food supplements supplied in a liquid form	2000		20	6	30.0	1	5.0	0.5	10.0	1.5	30.0	0.3	716.4	794.2	2040.5	2126.0
18	Processed foods not covered by categories 1 to 17, excluding foods for infants and young children			167	148	88.6			0.0	10.0	0.0	30.0	0.0	2.5	12.4	21.4	723.2

LC, left-censored data; % LC, percentage of left-censored data; FCS, Food Categorisation System; LOD, limit of detection; LOQ, limit of quantification; Max, maximum; MB, middle bound; Min, minimum; MPL, maximum permitted level; N, number of analytical results; P95, 95th percentile.

(a) This level of almost 70 mg/kg meat was considered as an outlier and therefore not use in the exposure assessment

B2. Analytical data on food categories listed under Annex II to Regulation No 1333/2008 excluding data above MPLs

FCS category number	Food category	MPL	restrictions	Data < MPL										
				N	No LC	LOD		LOQ		Middle-bound				
						Min	Max	Min	Max	Min	Median	Mean	p95	Max
01.4	Flavoured fermented milk products including heat treated products	300	only non-heat treated dairy based desserts	1118	539	0.0	20.0	0.0	50.0	0.0	2.4	5.2	16.0	116.7
04.2.2	Fruit and vegetables in vinegar, oil, or brine	2000	only vegetables (excluding olives)	1000	624	0.0	37.8	0.0	113.5	0.0	7.5	261.9	1167.5	1973.0
04.2.2	Fruit and vegetables in vinegar, oil, or brine	500	only olives and olive-based preparations	107	88	1.0	20.0	3.0	50.0	0.5	7.5	44.8	277.0	431.0
04.2.4.1	Fruit and vegetable preparations excluding compote	2000	only cooked red beet	4	3	0.3	3.3	0.6	15.0	0.3	6.3	193.6	761.7	761.7
04.2.4.1	Fruit and vegetable preparations excluding compote	500	only seaweed preparations, olives and olive-based preparations	5	0					300.0	360.0	388.0	490.0	490.0
04.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EEC	500	only low-sugar and similar low calorie or sugar-free products, <i>mermeladas</i>	262	257	0.2	20.0	0.5	50.0	0.3	10.0	7.9	10.0	83.3
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC	500	only low-sugar and similar low calorie or sugar-free products; <i>mermeladas</i>	581	510	0.3	50.0	1.0	60.0	0.3	2.5	25.7	203.0	470.0
04.2.5.3	Other similar fruit or vegetable spreads	500	other fruit-based spreads, <i>mermeladas</i>	467	423	0.2	50.0	0.5	75.0	0.3	10.0	21.8	79.1	490.0
04.2.5.3	Other similar fruit or vegetable spreads	1000	only <i>dulce de membrillo</i>	0										
04.2.5.3	Other similar fruit or vegetable spreads	1500	only <i>marmelada</i>	6	0	6.7	6.7	20.0	20.0	338.3	589.4	554.0	824.0	824.0
05.2	Other confectionery including breath refreshing microsweets	1500	except candied, crystallised or glacé fruit and vegetables	340	301	0.0	20.0	0.0	50.0	0.0	2.5	46.0	346.8	1027.0
05.2	Other confectionery including breath refreshing microsweets	1000	only candied, crystallised or glacé fruit and vegetables	164	151	0.0	10.0	0.0	30.0	0.0	1.9	18.5	110.0	418.0
05.3	Chewing gum	1500		5	4	0.2	20.0	0.5	50.0	0.5	10.0	89.9	419.0	419.0

FCS category number	Food category	MPL	restrictions	Data < MPL										
				N	No LC	LOD		LOQ		Middle-bound				
						Min	Max	Min	Max	Min	Median	Mean	p95	Max
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	1500		73	67	1.7	3.8	5.0	15.0	1.9	1.9	47.0	411.0	780.2
08.3.1	Non heat treated processed meat		only surface treatment of dried meat products											
08.3.2	Heat treated processed meat	500	only aspic	82	64	0.0	37.8	0.0	113.3	0.0	2.5	25.0	198.7	430.0
09.2	Processed fish and fishery products including molluscs and crustaceans	200	only salted, dried fish	39	39			20.0	20.0	10.0	10.0	10.0	10.0	10.0
09.2	Processed fish and fishery products including molluscs and crustaceans	2000	only semi-preserved fish and fisheries products including crustaceans, molluscs, surimi and fish / crustacean paste; cooked crustaceans and molluscs	324	135	1.3	3.3	1.3	15.0	0.6	180.0	395.9	1341.4	1900.0
09.2	Processed fish and fishery products including molluscs and crustaceans	6000	only cooked <i>Crangon crangon</i> and <i>Crangon vulgaris</i>	92	40	0.2	1.7	0.5	20.0	2.5	337.2	454.6	1200.0	3800.0
09.2	Processed fish and fishery products including molluscs and crustaceans	1000	only cooked crustaceans and molluscs	41	27	0.2	50.0	0.5	150.0	0.5	10.0	155.6	834.0	881.4
09.2	Processed fish and fishery products including molluscs and crustaceans	1500	only cooked shrimps in brine	0										
09.3	Fish roe	2000	only semi-preserved fish products including fish roe products	68	22	0.0	1.7	0.0	5.0	0.3	663.3	644.2	1686.0	1994.6
10.2	Processed eggs and egg products	5000	only liquid egg (white, yolk or whole egg)	47	41	6.7	6.7	20.0	20.0	10.0	10.0	461.6	3855.0	3890.0
11.4.1	Table-top sweeteners in liquid form	500	only if the water content higher than 75 %	0										
12.2.2	Seasonings and condiments	1000		281	228	0.0	36.0	0.0	50.0	0.3	7.5	83.0	548.1	865.6
12.4	Mustard	1000		498	380	0.0	36.0	0.0	50.0	0.0	1.5	102.9	790.5	996.9

FCS category number	Food category	MPL	restrictions	Data < MPL										
				N	No LC	LOD		LOQ		Middle-bound				
						Min	Max	Min	Max	Min	Median	Mean	p95	Max
12.5	Soups and broths	500	only liquid soups and broths (excluding canned)	47	46	0.0	20.0	0.2	50.0	0.1	7.5	7.8	10.0	58.0
12.6	Sauces	1000	only emulsified sauces with a fat content of less than 60 %	869	448	0.0	36.0	0.0	101.0	0.0	10.0	266.3	869.4	1000.0
12.6	Sauces	500	only emulsified sauces with a fat content of 60 % or more	161	141	0.5	36.0	1.5	50.0	0.3	2.5	40.5	307.8	500.0
12.6	Sauces	1000	non emulsified sauces	0										
12.7	Salads and savoury based sandwich spreads	1500		3297	1128	0.0	1345.0	0.0	1345.0	0.0	129.1	246.4	822.5	1486.0
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	1500		216	139	0.2	100.0	0.4	100.0	0.1	5.0	23.0	112.0	502.0
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	1500		6	6	0.6	7.5	3.6	22.4	0.3	1.3	3.7	11.2	11.2
14.1.2	Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices	2000	only grape juice, unfermented, for sacramental use	0										
14.1.2	Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices	200	only <i>Sød.....saft</i> and <i>sødet.....saft</i>	0										
14.1.3	Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and similar products	150	only traditional Swedish and Finnish fruit syrups	0										

				Data < MPL										
FCS category number	Food category	MPL	restrictions	N	No LC	LOD		LOQ		Middle-bound				
						Min	Max	Min	Max	Min	Median	Mean	p95	Max
14.1.4	Flavoured drinks	150	excluding dairy-based drinks	6603	2941	0.0	37.8	0.0	165.0	0.0	7.5	46.2	135.1	150.0
14.1.5.2	Other	600	only liquid tea concentrates and liquid fruit and herbal infusion concentrates	25	20	0.2	6.7	0.6	20.0	0.1	10.0	39.9	201.0	257.0
14.2.1	Beer and malt beverages	200	only alcohol free beer; beer in kegs containing more than 0.5 % added fermentable sugar and/or fruit juices or concentrates	65	54	0.2	1.7	1.0	10.0	0.1	2.5	4.9	22.0	60.0
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts	200	only alcohol-free	0										
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	200	only alcoholic drinks with less than 15 % of alcohol	138	63	0.0	16.7	0.0	50.0	0.0	103.5	74.5	182.9	196.0
16	Desserts excluding products covered in category 1, 3 and 4	300	only non-heat-treated dairy-based desserts	364	330	1.7	20.0	2.0	50.0	0.8	7.5	9.5	13.0	199.0
16	Desserts excluding products covered in category 1, 3 and 4	500	only <i>frugtgrød</i> and <i>Rote Grütze</i>	0										
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms	1000	only when supplied in dried form and containing preparations of vitamin A and of combinations of vitamin A and D	3	3	3.3	3.4	9.9	10.1	5.0	5.1	5.0	5.1	5.1
17.2	Food supplements supplied in a liquid form	2000		19	6	0.5	10.0	1.5	30.0	0.3	705.0	724.1	1955.0	1955.0

LC, left-censored data; % LC, percentage of left-censored data; FCS, Food Categorisation System; LOD, limit of detection; LOQ, limit of quantification; Max, maximum; MB, middle bound; Min, minimum; MPL, maximum permitted level; N, number of analytical results; P95, 95th percentile.

C. Concentration levels of benzoic acid-benzoates (E 210–213) used in the MPL scenario and refined exposure scenario using dataset 1^(a) (mg/kg)

FCS Category number	FCS Food category	restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
01.4	Flavoured fermented milk products including heat treated products	only non-heat treated dairy based desserts	300	5	117	Analytical data
04.2.2	Fruit and vegetables in vinegar, oil, or brine	only vegetables (excluding olives)	2000	262	1973	Analytical data
04.2.2	Fruit and vegetables in vinegar, oil, or brine	only olives and olive-based preparations	500	45	431	Analytical data
04.2.4.1	Fruit and vegetable preparations excluding compote	only seaweed preparations, olives and olive-based preparations	500	388	490	Analytical data
04.2.4.1	Fruit and vegetable preparations excluding compote	only cooked red beet	2000	194	762	Analytical data
04.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EEC	only low-sugar and similar low calorie or sugar-free products , <i>mermeladas</i>	500	20	470	Analytical data
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC	only low-sugar and similar low calorie or sugar-free products; <i>mermeladas</i>	500			
04.2.5.3	Other similar fruit or vegetable spreads	other fruit-based spreads, <i>mermeladas</i>	500			
04.2.5.3	Other similar fruit or vegetable spreads	only <i>marmelada</i>	1500	-	-	Not taken into account (no FoodEx code)
04.2.5.3	Other similar fruit or vegetable spreads	only <i>dulce de membrillo</i>	1000	-	-	Not taken into account (no FoodEx code)
05.2	Other confectionery including breath refreshing microsweets	except candied, crystallised or glacé fruit and vegetables	1500	46	1027	Analytical data
05.2	Other confectionery including breath refreshing microsweets	only candied, crystallised or glacé fruit and vegetables	1000	19	418	Analytical data
05.3	Chewing gum		1500	90	419	Analytical data
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4		1500	-	-	Not taken into account (no FoodEx code)

FCS Category number	FCS Food category	restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
08.3.1	Non heat treated processed meat	only surface treatment of dried meat products	QS	-	-	Not taken into account (no FoodEx code)
08.3.2	Heat treated processed meat	only aspic	500	25	430	Analytical data
09.2	Processed fish and fishery products including molluscs and crustaceans	only salted, dried fish	200	10	10	Analytical data
09.2	Processed fish and fishery products including molluscs and crustaceans	only semi-preserved fish and fisheries products including crustaceans, molluscs, surimi and fish / crustacean paste; cooked crustaceans and molluscs	2000	396	1900	Analytical data
09.2	Processed fish and fishery products including molluscs and crustaceans	only cooked <i>Crangon crangon</i> and <i>Crangon vulgaris</i>	6000	455	3800	Analytical data
09.2	Processed fish and fishery products including molluscs and crustaceans	only cooked Crustaceans and molluscs	1000	156	881	Analytical data
09.2	Processed fish and fishery products including molluscs and crustaceans	only cooked shrimps in brine	1500	-	-	Analytical data
09.3	Fish roe	only semi-preserved fish products including fish roe products	2000	663	1995	Analytical data
10.2	Processed eggs and egg products	only liquid egg (white, yolk or whole egg)	5000	462	3855	Analytical data
11.4.1	Table-top sweeteners in liquid form	only if the water content higher than 75 %	500	3	10	Analytical data
12.2.2	Seasonings and condiments		1000	83	866	Analytical data
12.4	Mustard		1000	103	997	Analytical data
12.5	Soups and broths	only liquid soups and broths (excluding canned)	500	8	58	Analytical data
12.6	Sauces	only emulsified sauces with a fat content of less than 60 %	1000	266	1000	Analytical data
12.6	Sauces	only emulsified sauces with a fat content of 60 % or more; non-emulsified sauces	1000			

FCS Category number	FCS Food category	restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
12.6	Sauces	only emulsified sauces with a fat content of 60 % or more	500	41	500	Analytical data
12.7	Salads and savoury based sandwich spreads		1500	246	1486	Analytical data
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		1500	23	502	Analytical data
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		1500	4	11	Analytical data
14.1.2	Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices	only grape juice, unfermented, for sacramental use	2000	-	-	Not taken into account (no FoodEx code)
14.1.2	Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices	only <i>Sød.....saft</i> and <i>sødet.....saft</i>	200	-	-	Not taken into account (no FoodEx code)
14.1.3	Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and similar products	only traditional Swedish and Finnish fruit syrups	150	-	-	Not taken into account (no FoodEx code)
14.1.4	Flavoured drinks	excluding dairy-based drinks	150	100	150	Reported use levels
14.1.5.2	Other	only liquid tea concentrates and liquid fruit and herbal infusion concentrates	600	-	-	Not taken into account (no reported level/no FoodEx code)
14.2.1	Beer and malt beverages	only alcohol free beer; beer in kegs containing more than 0.5 % added fermentable sugar and/or fruit juices or concentrates	200	125	200	Reported use levels
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts	only alcohol-free	200	-	-	Not taken into account (no FoodEx code)
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and	only alcoholic drinks with less than 15 % of alcohol	200	75	196	Analytical data

FCS Category number	FCS Food category	restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
	spirits with less than 15 % of alcohol					
16	Desserts excluding products covered in category 1, 3 and 4	only non-heat-treated dairy-based desserts	300	10	199	Analytical data
16	Desserts excluding products covered in category 1, 3 and 4	only <i>frugtgrød</i> and <i>Rote Grütze</i>	500	-	-	Not taken into account (no FoodEx code)
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms	only when supplied in dried form and containing preparations of vitamin A and of combinations of vitamin A and D	1000	626	1955	Analytical data
17.2	Food supplements supplied in a liquid form		2000			

FCS, Food Categorisation System; MPL, maximum permitted level; *QS*, *quantum satis*.

(a) Considering reported use levels and analytical data not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised (Annex II to Regulation No 1333/2008).

D. Summary of total estimated exposure to benzoic acid-benzoates (E 210–213) from their use as food additives per population group and survey for the MPL scenario and refined exposure scenario using dataset 1^(a): mean and high level (mg/kg bw per day)

	Number of subjects	MPL scenario		Brand-Loyal scenario		Non-Brand-Loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Infants							
Bulgaria (NUTRICHILD)	659	0.153	0.833	0.074	0.359	0.021	0.054
Germany (VELS)	159	0.853	3.661	0.571	2.659	0.303	1.683
Denmark (IAT 2006_07)	826	1.148	4.920	0.531	2.121	0.127	0.583
Finland (DIPP_2001_2009)	500	0.278	1.370	0.241	1.203	0.027	0.078
United Kingdom (DNSIYC_2011)	1366	1.495	5.523	0.783	3.164	0.202	0.997
Italy (INRAN_SCAI_2005_06)	12	1.192		0.237		0.042	
Toddlers							
Belgium (Regional_Flanders)	36	8.389		3.180		1.555	
Bulgaria (NUTRICHILD)	428	0.819	3.333	0.617	1.945	0.162	0.909
Germany (VELS)	348	4.420	9.859	2.509	6.985	1.422	4.699
Denmark (IAT 2006_07)	917	2.731	6.590	1.104	2.779	0.396	1.157
Spain (enKid)	17	2.259		0.824		0.097	
Finland (DIPP_2001_2009)	500	1.630	5.673	0.663	2.234	0.075	0.180
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	4.134	8.724	2.154	5.529	0.874	2.155
United Kingdom (DNSIYC_2011)	1314	3.318	9.276	1.795	5.381	0.623	2.002
Italy (INRAN_SCAI_2005_06)	36	1.744		0.851		0.147	
Netherlands (VCP_kids)	322	6.096	13.809	2.419	6.690	0.728	2.520
Children							
Austria (ASNS_Children)	128	2.567	7.739	1.462	5.224	0.555	1.735
Belgium (Regional_Flanders)	625	6.512	14.682	2.448	5.891	1.085	3.221
Bulgaria (NUTRICHILD)	433	0.829	3.611	0.670	2.729	0.271	1.555
Czech Republic (SISP04)	389	3.099	8.678	1.707	5.279	0.672	2.401
Germany (EsKiMo)	835	2.273	6.135	1.288	3.738	0.594	2.071
Germany (VELS)	293	4.425	9.779	2.386	5.710	1.366	3.780
Denmark (DANSDA 2005-08)	298	2.671	5.351	1.260	2.871	0.733	1.803
Spain (enKid)	156	2.629	6.941	1.312	3.865	0.389	1.649
Spain (NUT_INK05)	399	2.580	6.892	1.192	3.419	0.390	1.252
Finland (DIPP_2001_2009)	750	4.067	11.960	1.970	6.954	0.351	1.230
France (INCA2)	482	2.944	6.477	1.557	3.912	0.646	1.723
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	3.615	7.868	2.032	4.861	0.960	2.164
Greece (Regional_Crete)	838	2.173	7.407	1.278	5.625	0.261	1.113
Italy (INRAN_SCAI_2005_06)	193	1.751	9.460	1.254	7.077	0.301	1.427
Latvia (EFSA_TEST)	187	5.444	11.979	2.609	7.092	0.813	2.163
Netherlands (VCP_kids)	957	5.457	12.860	2.096	5.152	0.703	2.102

	Number of subjects	MPL scenario		Brand-Loyal scenario		Non-Brand-Loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Netherlands (VCPBasis AVL2007_2010)	447	5.508	11.256	2.554	5.298	1.510	3.392
Sweden (NFA)	1473	5.980	11.804	2.649	5.512	1.381	2.947
Adolescents							
Austria (ASNS_Children)	237	1.694	5.503	1.108	3.833	0.446	1.345
Belgium (Diet_National_2004)	576	2.737	6.485	1.632	4.159	0.925	2.392
Cyprus (Childhealth)	303	0.503	1.784	0.424	1.471	0.270	0.954
Czech Republic (SISP04)	298	2.424	6.935	1.502	4.707	0.671	1.948
Germany (National_Nutrition_Survey_II)	1011	1.993	5.688	1.176	3.547	0.542	1.789
Germany (EsKiMo)	393	1.821	5.060	1.143	3.643	0.587	2.103
Denmark (DANSDA 2005-08)	377	1.519	3.301	0.846	2.090	0.514	1.406
Spain (AESAN_FIAB)	86	0.994	3.153	0.621	2.152	0.179	0.740
Spain (enKid)	209	1.450	3.776	0.866	2.562	0.335	1.166
Spain (NUT_INK05)	651	1.489	3.635	0.828	2.302	0.353	1.128
Finland (NWSSP07_08)	306	2.677	8.604	1.476	5.164	0.318	0.954
France (INCA2)	973	1.457	3.500	0.803	2.083	0.361	1.005
United Kingdom (NDNS-RollingProgrammeYears1-3)	666	2.179	4.367	1.318	2.877	0.746	1.675
Italy (INRAN_SCAI_2005_06)	247	1.163	4.995	0.815	3.200	0.237	0.810
Latvia (EFSA_TEST)	453	3.618	8.715	1.914	5.331	0.584	1.627
Netherlands (VCPBasis AVL2007_2010)	1142	3.603	7.667	1.839	4.036	1.127	2.614
Sweden (NFA)	1018	3.631	7.839	1.802	3.986	0.891	2.065
Adults							
Austria (ASNS_Adults)	308	2.114	5.402	1.279	3.190	0.355	1.001
Belgium (Diet_National_2004)	1292	2.361	6.252	1.227	3.721	0.598	1.943
Czech Republic (SISP04)	1666	1.116	3.520	0.789	2.647	0.261	0.987
Germany (National_Nutrition_Survey_II)	10419	1.599	4.486	0.864	2.697	0.329	1.237
Denmark (DANSDA 2005-08)	1739	0.934	2.230	0.497	1.472	0.280	0.947
Spain (AESAN)	410	0.746	2.675	0.498	1.836	0.266	1.083
Spain (AESAN_FIAB)	981	0.644	1.843	0.438	1.333	0.156	0.659
Finland (FINDIET2012)	1295	1.594	5.309	0.843	3.048	0.239	0.864
France (INCA2)	2276	1.070	2.603	0.551	1.371	0.224	0.709
United Kingdom (NDNS-RollingProgrammeYears1-3)	1266	1.408	3.391	0.820	2.080	0.394	1.127
Hungary (National_Repr_Surv)	1074	1.113	3.299	0.958	2.980	0.243	0.764
Ireland (NANS_2012)	1274	0.994	2.582	0.666	1.851	0.308	0.950
Italy (INRAN_SCAI_2005_06)	2313	0.695	3.608	0.452	2.433	0.100	0.471
Latvia (EFSA_TEST)	1271	2.779	6.618	1.598	4.980	0.376	1.060

	Number of subjects	MPL scenario		Brand-Loyal scenario		Non-Brand-Loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Netherlands (VCPBasis AVL2007_2010)	2057	2.198	5.120	1.073	2.919	0.576	1.718
Romania (Dieta_Pilot_Adults)	1254	0.647	1.750	0.360	1.156	0.161	0.515
Sweden (Riksmaten 2010)	1430	3.374	7.847	2.048	5.148	0.618	1.426
The elderly							
Austria (ASNS_Adults)	92	2.123	4.923	1.231	3.274	0.295	0.834
Belgium (Diet_National_2004)	1215	1.955	4.984	0.630	2.080	0.215	0.747
Germany (National_Nutrition_Survey_II)	2496	1.152	3.221	0.502	1.718	0.146	0.554
Denmark (DANSDA 2005-08)	286	0.579	1.471	0.264	0.640	0.107	0.314
Finland (FINDIET2012)	413	0.852	2.959	0.466	1.642	0.130	0.586
France (INCA2)	348	0.883	2.049	0.433	1.027	0.122	0.320
United Kingdom (NDNS-RollingProgrammeYears1-3)	305	1.265	3.301	0.605	1.605	0.219	0.634
Hungary (National_Repr_Surv)	286	0.870	2.578	0.786	2.476	0.143	0.444
Ireland (NANS_2012)	226	0.653	1.983	0.368	1.323	0.127	0.472
Italy (INRAN_SCAI_2005_06)	518	0.378	1.855	0.237	1.295	0.044	0.241
Netherlands (VCPBasis AVL2007_2010)	173	1.783	4.370	0.625	1.636	0.245	0.752
Netherlands (VCP-Elderly)	739	1.764	4.638	0.672	1.978	0.228	0.683
Romania (Dieta_Pilot_Adults)	128	0.498	1.191	0.257	0.701	0.109	0.301
Sweden (Riksmaten 2010)	367	2.782	7.019	1.627	4.290	0.427	1.004

(a) Considering reported use levels and analytical data not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised (Annex II to Regulation No 1333/2008).

E. Main food categories contributing to exposure to benzoic acid-benzoates (E 210–213) using MPLs (> 5 % of the total mean exposure) and the number of surveys in which each food category is contributing

FCS Category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (Number of surveys)(a)					
01.4	Flavoured fermented milk products including heat-treated products	8.7-49.4 (5)	11.6-68.4 (10)	6.2-36.7 (16)	5.6-18.5 (11)	5.4-15.6 (11)	6.8-18.8 (9)
04.1	Unprocessed fruit and vegetables	30.6 (1)				5.4-7.1 (3)	5.0-13 (8)
04.2	Processed fruit and vegetables	73.2 (1)	13.4-31.7 (2)	5.4-19 (4)	5.5-18.5 (4)	5.3-71.5 (8)	5.7-82.1 (12)
05.2	Other confectionery including breath refreshing microsweeteners		7.5-27.1 (5)	5.8-52.1 (16)	6.6-60.0 (13)	5.2-33.8 (5)	6.5-17.0 (3)
09.2	Processed fish and fishery products including molluscs and crustaceans	15.5-15.7 (2)	5.5-30.4 (6)	6.3-72.7 (11)	5.6-68.6 (10)	5.4-67.4 (7)	5.6-61.5 (8)
09.3	Fish roe	15.6 (1)	7.6 (1)				
10.2	Processed eggs and egg products					10.7 (1)	15.2 (1)
12.5	Soups and broths	6.4-83.9 (3)	8.2-30.0 (6)	5.6-38.3 (10)	5.2-35.8 (10)	6.6-34.4 (9)	5.8-52.0 (9)
12.6	Sauces	5.4-20.6 (4)	8.7-18.8 (7)	5.4-26.1 (15)	5.2-30.9 (15)	7.3-44.6 (14)	9.8-33.6 (11)
12.7	Salads and savoury based sandwich spreads			6.8-45.4 (4)	8.7-37.2 (3)	6.5-46.1 (5)	5.9-40.4 (3)
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)				5.9 (1)		
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)						6.0 (1)
14.1.4	Flavoured drinks	14.4-45.1 (2)	9.4-38.7 (7)	5.2-44.2 (18)	9.5-78.6 (17)	6.8-40.4 (16)	5.2-22.6 (9)
16	Desserts excluding products covered in categories 1, 3 and 4	6.6-14.7 (3)	7.5-19.2 (5)	5.1-13.2 (7)	5.6-10.2 (2)	5.3-7 (2)	5.5-8.4 (5)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	13.1 (1)					6.2-11.0 (2)

FCS, Food Categorisation System.

(a): The total number of surveys may be greater than the total number of countries as listed in Table 5, as some countries submitted more than one survey for a specific population.

F. Main food categories contributing to the exposure to benzoic acid-benzoates (E 210–213) using dataset 1^(a) for the “brand-loyal refined exposure scenario” (> 5 % of the total mean exposure) and the number of surveys in which each food category is contributing

FCS Category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (Number of surveys) ^(b)					
01.4	Flavoured fermented milk products including heat-treated products	5.8-35.5 (5)	5.2-65.1 (8)	6.1-26.2 (7)		5.6 (1)	5.4-7.4 (4)
04.1	Unprocessed fruit and vegetables	24.1 (1)					5.1-7.6 (4)
04.2	Processed fruit and vegetables	78.7 (1)	24.9-40.7 (2)	6.9-20.7 (3)	5.1-22.5 (4)	5.9-79.3 (5)	6.3-88.1 (12)
05.2	Other confectionery including breath refreshing microsweeteners	5.9 (1)	9.4-21.9 (3)	5.3-70.3 (12)	8.0-72.7 (8)	13.9-39.2 (4)	5.1-19.1 (5)
09.2	Processed fish and fishery products including molluscs and crustaceans	24.6-26.0 (2)	6.3-53.1 (7)	10.8-82.0 (11)	5.9-69.0 (11)	5.2-70.9 (9)	6.0-67.9 (10)
09.3	Fish roe	29.7 (1)	13.7 (1)				
10.2	Processed eggs and egg products			6.3 (1)		11.3 (1)	16.7 (1)
12.5	Soups and broths	49.0 (1)					7.9 (1)
12.6	Sauces	8.7-34.5 (4)	5.8-26.0 (7)	6.4-49.3 (12)	5.4-46.3 (10)	5.2-54.8 (13)	9.3-51.0 (11)
12.7	Salads and savoury based sandwich spreads			7.4-76.2 (4)	9.6-68.4 (3)	6.7-77.7 (5)	10.3-66.9 (3)
14.1.4	Flavoured drinks	7.9-65.8 (3)	18.5-65.8 (7)	7.4-69.5 (18)	14.8-92.5 (17)	5.1-65.5 (17)	6.9-40.3 (11)
16	Desserts excluding products covered in categories 1, 3 and 4	12.7 (1)	5.3-24.4 (3)	6.1-7.8 (2)			5.0-6.3 (2)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	7-11.6 (2)				5.2 (1)	7.6-15.1 (2)

FCS, Food Categorisation System.

(a) Considering reported use levels and analytical data not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised (Annex II to Regulation No 1333/2008).

(b) The total number of surveys may be greater than the total number of countries as listed in Table 5, as some countries submitted more than one survey for a specific population.

G. Main food categories contributing to the exposure to benzoic acid-benzoates (E 210–213) using dataset 1^(a) for the “non-brand-loyal refined exposure scenario” (> 5 % of the total mean exposure) and the number of surveys in which each food category is contributing

FCS Category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (Number of surveys) ^(b)					
01.4	Flavoured fermented milk products including heat-treated products	5.5-7.8 (2)	5.4-27.6 (4)				
04.1	Unprocessed fruit and vegetables	21.7 (1)					5.4-7.4 (5)
04.2	Processed fruit and vegetables	30.4 (1)	11.6-19.4 (2)	6.0-6.1 (2)	8.1 (1)	11.6-41.5 (3)	6.5-62.6 (5)
05.2	Other confectionery including breath refreshing microsweets			18.5 (1)	7.4-15.5 (2)	6.8 (1)	
09.2	Processed fish and fishery products including molluscs and crustaceans	22.7-27.5 (2)	7.8-57.7 (5)	5.6-60.5 (11)	5.9-36.1 (7)	5.7-44.3 (6)	6.8-51.2 (8)
09.3	Fish roe	46.7 (1)	17.3 (1)				5.6 (1)
10.2	Processed eggs and egg products					5.4 (1)	9.1 (1)
12.5	Soups and broths	37.1 (1)					7.4 (1)
12.6	Sauces	5.1-40.8 (5)	8.2-55.9 (9)	7.6-44.5 (12)	6.9-33.9 (11)	5.3-36.5 (13)	5.8-45.4 (13)
12.7	Salads and savoury based sandwich spreads			11.3-61.8 (3)	5.8-37.8 (3)	10.4-56.0 (4)	5.6-47.7 (3)
14.1.4	Flavoured drinks	7.5-84.4 (4)	14.8-80.3 (9)	28.6-90.1 (18)	39.3-97.6 (17)	18.6-80.1 (17)	13.6-69.2 (14)
14.2	Alcoholic beverages, including alcohol-free and low-alcohol counterparts					9.3 (1)	6.5-10.3 (3)
16	Desserts excluding products covered in categories 1, 3 and 4		14.2 (1)				
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	17.8-42.4 (2)	6.1-19.1 (2)	6.8 (1)		6.7 (1)	6.6-17.7 (3)

FCS, Food Categorisation System.

(a) Considering reported use levels and analytical data not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised (Annex II to Regulation No 1333/2008).

(b): The total number of surveys may be greater than the total number of countries as listed in Table 5, as some countries submitted more than one survey for a specific population.

H. Concentration levels of benzoic acid-benzoates (E 210–213) used in the refined exposure scenario with dataset 2^(a) (mg/kg)

FCS Category number	FCS Food category	restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum / p95 ^(b)	
01.1	Unflavoured pasteurised and sterilised (including UHT) milk ^(c)		-	11	52	Analytical data
01.2	Unflavoured fermented milk products, including natural unflavoured buttermilk (excluding sterilised buttermilk) non-heat-treated after fermentation ^(c)		-	3	14	Analytical data
01.3	Unflavoured fermented milk products, heat-treated after fermentation ^(c)		-	10	21	Analytical data
01.4	Flavoured fermented milk products including heat treated products	only non-heat treated dairy based desserts	300	5	117	Analytical data
01.5	Dehydrated milk as defined by Directive 2001/114/EC ^(c)		-	0.5	0.5	Analytical data
01.6	Cream and cream powder ^(c)		-	4	15	Analytical data
1.7.1	Unripened cheese excluding products falling in category 16 ^(c)		-	74	532	Analytical data
1.7.2	Ripened cheese ^(c)		-	12.5	25	Analytical data
1.7.4	Whey cheese ^(c)		-	11	36	Analytical data
1.7.5	Processed cheese ^(c)		-	9	23.5	Analytical data
1.8	Dairy analogues, including beverage whiteners ^(c)		-	4	9	Analytical data
2.1	Fats and oils essentially free from water (excluding anhydrous milk fat) ^(c)		-	1.5	1.5	Analytical data
2.2	Fat and oil emulsions mainly of type water-in-oil ^(c)		-	8.3	15	Analytical data
3	Edible ices ^(c)		-	13	26	Analytical data
4.1	Unprocessed fruit and vegetables ^(c)		-	56	470	Analytical data
4.2	Processed fruit and vegetables ^(c)		-	110	839	Analytical data

FCS Category number	FCS Food category	restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum / p95 ^(b)	
04.2.1	Dried fruits and vegetables ^(c)			10	50	Analytical data
04.2.2	Fruit and vegetables in vinegar, oil, or brine ^(c)			260	1450	Analytical data
04.2.2	Fruit and vegetables in vinegar, oil, or brine	only vegetables (excluding olives)	2000	262	1973	Analytical data
04.2.2	Fruit and vegetables in vinegar, oil, or brine	only olives and olive-based preparations	500	45	431	Analytical data
04.2.3	Canned or bottled fruits and vegetables ^(c)			242	1299	Analytical data
04.2.4.1	Fruit and vegetable preparations excluding compote	only seaweed preparations, olives and olive-based preparations	500	388	490	Analytical data
04.2.4.1	Fruit and vegetable preparations excluding compote	only cooked red beet	2000	194	762	Analytical data
04.2.4.1	Fruit and vegetable preparations excluding compote ^(c)			10	21	Analytical data
04.2.4.2	Compote ^(c)			9	10	Analytical data
04.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EEC	only low-sugar and similar low calorie or sugar-free products , <i>mermeladas</i>	500	20	470	Analytical data
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC	only low-sugar and similar low calorie or sugar-free products; <i>mermeladas</i>	500			Analytical data
04.2.5.3	Other similar fruit or vegetable spreads	only <i>marmelada</i>	1500			Analytical data
04.2.5.3	Other similar fruit or vegetable spreads	other fruit-based spreads, <i>mermeladas</i>	500			Analytical data
04.2.5.3	Other similar fruit or vegetable spreads	only <i>dulce de membrillo</i>	1000	-	-	Not taken into account (no FoodEx code)
04.2.5.4	Nut butter and nut spreads ^(c)			2.5	2.5	Analytical data

FCS Category number	FCS Food category	restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum / p95 ^(b)	
04.2.6	Processed potato products ^(c)			7	10	Analytical data
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC ^(c)		-	3	19	Analytical data
05.2	Other confectionery including breath refreshing microsweets	except candied, crystallised or glacé fruit and vegetables	1500	46	1027	Analytical data
05.2	Other confectionery including breath refreshing microsweets	only candied, crystallised or glacé fruit and vegetables	1000	19	418	Analytical data
05.3	Chewing gum		1500	90	419	Analytical data
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4		1500	-	-	Not taken into account (no FoodEx code)
06.1	Whole, broken, or flaked grain ^(c)		-	4.2	7.5	Analytical data
6.2	Flours and other milled products and starches ^(c)		-	7	11	Analytical data
6.3	Breakfast cereals ^(c)		-	3	16	Analytical data
6.4	Pasta ^(c)		-	10	11	Analytical data
7.1	Bread and rolls ^(c)		-	5	10	Analytical data
7.2	Fine bakery wares ^(c)		-	18	48	Analytical data
8.1	Unprocessed meat ^(c)		-	10	92	Analytical data
8.2	Meat preparations ^(c)		-	35	204	Analytical data
8.3	Meat products ^(c)		-			Analytical data
08.3.1	Non heat treated processed meat	only surface treatment of dried meat products	QS	-	-	Not taken into account (no FoodEx code)
08.3.2	Heat treated processed meat	only aspic	500	25	430	Analytical data
09.1	Unprocessed fish and fisheries products ^(c)	Unprocessed fish	-	289.5	1600	Analytical data
09.1	Unprocessed fish and fisheries products ^(c)	Unprocessed molluscs and crustaceans	-	5	6	Analytical data

FCS Category number	FCS Food category	restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum / p95 ^(b)	
09.2	Processed fish and fishery products including molluscs and crustaceans	only salted, dried fish	200	10	10	Analytical data
09.2	Processed fish and fishery products including molluscs and crustaceans	only semi-preserved fish and fisheries products including crustaceans, molluscs, surimi and fish / crustacean paste; cooked crustaceans and molluscs	2000	396	1900	Analytical data
09.2	Processed fish and fishery products including molluscs and crustaceans	only cooked <i>Crangon crangon</i> and <i>Crangon vulgaris</i>	6000	455	3800	Analytical data
09.2	Processed fish and fishery products including molluscs and crustaceans	only cooked Crustaceans and molluscs	1000	156	881	Analytical data
09.2	Processed fish and fishery products including molluscs and crustaceans	only cooked shrimps in brine	1500	-	-	Analytical data
09.2	Processed fish and fishery products including molluscs and crustaceans ^(c)		-	354	1592	Analytical data
09.3	Fish roe	only semi-preserved fish products including fish roe products	2000	663	1995	Analytical data
10.1	Unprocessed eggs ^(c)		-	296	986	Analytical data
10.2	Processed eggs and egg products	only liquid egg (white, yolk or whole egg)	5000	462	3855	Analytical data
11.1	Sugars and syrups as defined by Directive 2001/111/EC ^(c)		-	8	19	Analytical data
11.2	Other sugars and syrups ^(c)		-	101	676	Analytical data
11.3	Honey as defined in Directive 2001/110/EC ^(c)		-	85	107	Analytical data
11.4	Table-top sweeteners ^(c)		-	3	10	Analytical data
11.4.1	Table-top sweeteners in liquid form	only if the water content higher than 75 %	500	3	10	Analytical data
12.1	Salt and salt substitutes ^(c)		-	0.3	0.3	Analytical data

FCS Category number	FCS Food category	restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum / p95 ^(b)	
12.2.2	Seasonings and condiments		1000	83	866	Analytical data
12.3	Vinegars ^(c)		-	2	7.5	Analytical data
12.4	Mustard		1000	103	997	Analytical data
12.5	Soups and broths	only liquid soups and broths (excluding canned)	500	8	58	Analytical data
12.6	Sauces	only emulsified sauces with a fat content of less than 60 %	1000	266	1000	Analytical data
12.6	Sauces	only emulsified sauces with a fat content of 60 % or more; non-emulsified sauces	1000			
12.6	Sauces	only emulsified sauces with a fat content of 60 % or more	500	41	500	Analytical data
12.7	Salads and savoury based sandwich spreads		1500	246	1486	Analytical data
12.9	Protein products, excluding products covered in category 1.8 ^(c)		-	818	4800	Analytical data
13.1	Foods for infants and young children ^(c)		-	6	36	Analytical data
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		1500	23	502	Analytical data
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		1500	4	11	Analytical data
14.1.2	Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices ^(c)		-	23	113	Analytical data

FCS Category number	FCS Food category	restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum / p95 ^(b)	
14.1.2	Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices	only grape juice, unfermented, for sacramental use	2000	-	-	Not taken into account (no FoodEx code)
14.1.2	Fruit juices as defined by Council Directive 2001/112/EC and vegetable juices	only Sød....saft and sødet....saft	200	-	-	Not taken into account (no FoodEx code)
14.1.3	Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and similar products		-	30	120	Analytical data
14.1.3	Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and similar products	only traditional Swedish and Finnish fruit syrups	150	-	-	Not taken into account (no FoodEx code)
14.1.4	Flavoured drinks	excluding dairy-based drinks	150	100	150	Reported use levels
14.1.5.2	Other ^(c)		-	48.7	200	Analytical data
14.1.5.2	Other	only liquid tea concentrates and liquid fruit and herbal infusion concentrates	600	-	-	Not taken into account (no reported level/no FoodEx code)
14.2.1	Beer and malt beverages	only alcohol free beer; beer in kegs containing more than 0.5 % added fermentable sugar and/or fruit juices or concentrates	200	125	200	Reported use levels
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts	only alcohol-free	200	-	-	Not taken into account (no FoodEx code)
14.2.2	Wine and other products defined by Regulation (EC) No 1234/2007, and alcohol-free counterparts ^(c)		-	5	5	Analytical data
14.2.3	Cider and perry ^(c)		-	2	7.5	Analytical data
14.2.6	Spirits drinks ^(c)		-	66.5	157	Analytical data

FCS Category number	FCS Food category	restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum / p95 ^(b)	
14.2.7	Aromatised wine-based products ^(c)		-			Analytical data
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol	only alcoholic drinks with less than 15 % of alcohol	200	75	196	Analytical data
15.1	Potato-, cereal-, flour- or starch-based snacks ^(c)		-	6	10	Analytical data
15.2	Processed nuts ^(c)		-	3	10	Analytical data
16	Desserts excluding products covered in category 1, 3 and 4	only non-heat-treated dairy-based desserts	300	10	199	Analytical data
16	Desserts excluding products covered in category 1, 3 and 4	only <i>frugtgrød</i> and <i>Rote Grütze</i>	500	-	-	Not taken into account (no FoodEx code)
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms	only when supplied in dried form and containing preparations of vitamin A and of combinations of vitamin A and D	1000	626	1955	Analytical data
17.2	Food supplements supplied in a liquid form		2000			
17.3	Food supplements supplied in a syrup-type or chewable form ^(c)		-			
18	Processed foods not covered by categories 1 to 17, excluding foods for infants and young children ^(c)		-	12.4	12.4	Analytical data

FCS, Food Categorisation System; MPL, maximum permitted level; *QS*, *quantum satis*.

- (a) Considering reported use levels and analytical data not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised and analytical data for food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available.
- (b) the maximum levels were used for the food categories listed under Annex II to Regulation No 1333/2008, after discarding data above the MPL; for the food categories not listed under Annex II to Regulation No 1333/2008, the p95 was used instead of the mean in order to minimise the impact of possible outliers (as explained in section 2.9.2.2 Refined exposure assessment).
- (c) Food category for which direct addition of benzoic acid - benzoates is not authorised according to Annex II to Regulation (EC) No 1333/2008; however, the use of benzoic acid - benzoates may result in its presence in these food categories because of carry-over.

I. Summary of total estimated exposure to benzoic acid-benzoates (E 210–213) from their use as food additives per population group and survey for the refined exposure scenario using dataset 2^(a): mean and high level (mg/kg bw per day)

	No of subjects	Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level
Infants					
Bulgaria (NUTRICHILD)	659	6.586	14.690	1.903	3.521
Germany (VELS)	159	6.681	14.172	2.106	4.136
Denmark (IAT 2006_07)	826	7.098	14.064	2.051	3.516
Finland (DIPP_2001_2009)	500	5.421	10.308	1.338	2.318
United Kingdom (DNSIYC_2011)	1366	7.241	15.906	2.000	3.854
Italy (INRAN_SCAI_2005_06)	12	6.868		1.676	
Toddlers					
Belgium (Regional_Flanders)	36	8.063		3.341	
Bulgaria (NUTRICHILD)	428	10.627	19.619	2.637	4.467
Germany (VELS)	348	8.409	15.218	3.521	6.881
Denmark (IAT 2006_07)	917	7.593	13.060	2.367	3.676
Spain (enKid)	17	7.803		2.518	
Finland (DIPP_2001_2009)	500	7.328	12.262	2.202	3.411
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	7.930	14.695	2.930	4.723
United Kingdom (DNSIYC_2011)	1314	8.566	16.545	2.795	5.039
Italy (INRAN_SCAI_2005_06)	36	10.046		2.678	
Netherlands (VCP_kids)	322	7.390	12.488	2.647	5.055
Children					
Austria (ASNS_Children)	128	4.608	7.970	1.916	3.248
Belgium (Regional_Flanders)	625	6.714	12.385	2.608	5.116
Bulgaria (NUTRICHILD)	433	9.755	17.580	2.473	4.472
Czech Republic (SISP04)	389	7.684	14.195	2.552	4.562
Germany (EsKiMo)	835	5.762	10.110	2.118	3.728
Germany (VELS)	293	6.988	11.601	3.077	5.595
Denmark (DANSDA 2005-08)	298	6.901	14.030	2.335	4.094
Spain (enKid)	156	5.407	12.256	1.970	4.130
Spain (NUT_INK05)	399	5.206	9.759	1.998	3.463
Finland (DIPP_2001_2009)	750	6.779	12.284	2.224	3.526
France (INCA2)	482	5.307	10.426	2.079	3.758
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	5.942	11.110	2.366	4.143
Greece (Regional_Crete)	838	4.585	9.216	1.514	3.063
Italy (INRAN_SCAI_2005_06)	193	7.134	12.901	2.037	4.123
Latvia (EFSA_TEST)	187	4.912	9.686	1.901	3.926
Netherlands (VCP_kids)	957	5.790	10.846	2.192	3.900
Netherlands (VCPBasis_AVL2007_2010)	447	5.256	9.527	2.613	4.651

	No of subjects	Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level
Sweden (NFA)	1473	5.566	10.758	2.572	4.607
Adolescents					
Austria (ASNS_Children)	237	2.858	5.944	1.212	2.255
Belgium (Diet_National_2004)	576	3.131	6.095	1.536	3.108
Cyprus (Childhealth)	303	2.508	4.909	0.846	1.721
Czech Republic (SISP04)	298	5.200	10.972	1.874	3.664
Germany (National_Nutrition_Survey_II)	1011	2.863	6.168	1.215	2.722
Germany (EsKiMo)	393	4.422	8.860	1.737	3.274
Denmark (DANSDA 2005-08)	377	3.677	7.219	1.392	2.479
Spain (AESAN_FIAB)	86	3.740	7.003	1.092	1.793
Spain (enKid)	209	3.523	6.523	1.326	2.419
Spain (NUT_INK05)	651	3.244	6.337	1.340	2.271
Finland (NWSSP07_08)	306	3.475	7.103	1.223	2.165
France (INCA2)	973	2.704	5.372	1.096	2.021
United Kingdom (NDNS-RollingProgrammeYears1-3)	666	2.989	5.953	1.393	2.511
Italy (INRAN_SCAI_2005_06)	247	4.273	8.466	1.244	2.140
Latvia (EFSA_TEST)	453	3.538	7.278	1.384	2.624
Netherlands (VCPBasis_AVL2007_2010)	1142	3.754	7.022	1.937	3.549
Sweden (NFA)	1018	3.082	6.052	1.533	2.962
Adults					
Austria (ASNS_Adults)	308	3.463	7.167	1.314	2.557
Belgium (Diet_National_2004)	1292	3.290	6.341	1.405	2.766
Czech Republic (SISP04)	1666	2.924	5.322	1.099	2.048
Germany (National_Nutrition_Survey_II)	10419	3.557	6.925	1.352	2.550
Denmark (DANSDA 2005-08)	1739	3.926	6.966	1.500	2.453
Spain (AESAN)	410	3.847	6.907	1.212	2.397
Spain (AESAN_FIAB)	981	4.360	7.594	1.237	2.123
Finland (FINDIET2012)	1295	3.892	7.723	1.436	2.580
France (INCA2)	2276	3.112	6.212	1.174	2.179
United Kingdom (NDNS-RollingProgrammeYears1-3)	1266	3.107	5.938	1.302	2.375
Hungary (National_Repr_Surv)	1074	3.409	6.065	0.991	1.822
Ireland (NANS_2012)	1274	2.875	5.523	1.189	2.211
Italy (INRAN_SCAI_2005_06)	2313	3.706	6.598	0.962	1.706
Latvia (EFSA_TEST)	1271	3.040	6.272	1.106	2.087
Netherlands (VCPBasis_AVL2007_2010)	2057	3.707	6.914	1.640	2.960
Romania (Dieta_Pilot_Adults)	1254	4.551	8.518	1.121	1.996

	No of subjects	Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level
Sweden (Riksmaten 2010)	1430	3.970	7.843	1.593	2.840
The elderly					
Austria (ASNS_Adults)	92	3.019	5.945	1.132	2.124
Belgium (Diet_National_2004)	1215	2.974	5.485	1.068	1.949
Germany (National_Nutrition_Survey_II)	2496	3.507	6.427	1.231	2.179
Denmark (DANSDA 2005-08)	286	3.692	6.938	1.368	2.387
Finland (FINDIET2012)	413	3.342	6.114	1.189	2.144
France (INCA2)	348	3.543	7.012	1.136	2.057
United Kingdom (NDNS-RollingProgrammeYears1-3)	305	3.277	5.385	1.277	2.005
Hungary (National_Repr_Surv)	286	3.350	5.644	0.845	1.341
Ireland (NANS_2012)	226	3.191	5.851	1.177	2.120
Italy (INRAN_SCAI_2005_06)	518	3.908	6.902	0.918	1.540
Netherlands (VCPBasis_AVL2007_2010)	173	3.679	6.259	1.409	2.212
Netherlands (VCP-Elderly)	739	3.707	5.830	1.421	2.266
Romania (Dieta_Pilot_Adults)	128	4.652	7.822	1.139	1.843
Sweden (Riksmaten 2010)	367	3.773	6.859	1.535	2.552

- (a) Considering reported use levels and analytical data not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised and analytical data for food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available.

J. Main food categories contributing to the exposure to benzoic acid-benzoates (E 210–213) using dataset 2^(a) for the “brand-loyal refined exposure scenario” (> 5 % of the total mean exposure) and the number of surveys in which each food category is contributing

FCS Category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (Number of surveys) ^(b)					
01.4	Unflavoured pasteurised and sterilised (including UHT) milk	35.9 (1)	5.1-6.0 (3)	5.0 (1)			
01.7.1	Unripened cheese excluding products falling in category 16	7.2 (1)	5.0 (1)				
04.1	Unprocessed fruit and vegetables	43.3-79.2 (6)	43.4-80.8 (10)	27-81.5 (18)	19.5-68.5 (17)	11.8-82.9 (17)	17-83.2 (14)
04.2	Processed fruit and vegetables			13.0-21.2 (2)	12.1 (1)	6.0-9.1 (2)	5.9-6.4 (2)
05.2	Other confectionery including breath refreshing microsweets			12.3 (1)	23.8 (1)	5.7 (1)	
09.1	Unprocessed fish		21.7-25.9 (2)	7.6-26.7 (5)	5.1-25.7 (9)	5.3-13.3 (10)	5.4-13 (7)
09.2	Processed fish and fishery products including molluscs and crustaceans		5.9 (1)	5.6-9.5 (5)	5.3-10.7 (3)	6.1 (1)	
10.1	Unprocessed eggs		11.2 (1)	6.9-11.3 (3)	8.6-10.5 (2)		
10.2	Processed eggs and egg products	11.1 (1)	14.2-20.4 (2)	13.1-15.9 (2)	13.3-20 (2)	6.2-19.5 (6)	5.1-19.9 (7)
11.2	Other sugars and syrups			5.3 (1)	5-5 (1)		
12.6	Sauces			5.1 (1)	5.2-9.3 (5)	5.2-6.1 (2)	
12.7	Salads and savoury based sandwich spreads			5.4-23.1 (3)	13.4-30.3 (2)	13.5-33.6 (3)	13.0-18.8 (2)
12.9	Protein products, excluding products covered in category 1.8			6.1 (1)	5.4 (1)	5.3 (1)	
13.1	Foods for infants and young children	6.7-18.9 (6)					
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices		5.3-8.4 (2)	5.9-8.4 (2)	10.2 (1)		
14.1.4	Flavoured drinks		6.3-17.1 (4)	7.3-29.5 (12)	6.4-30.8 (15)	5.6-15.8 (8)	
14.1.5	Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	5.0-8.2 (2)	8.3 (1)	5.1-13.3 (5)	5.8-19.4 (6)	6.7-43.4 (13)	12.1-51.2 (11)
18	Processed foods not covered by categories 1 to 17, excluding foods for infants and young children	6.5-8.6 (4)	5-7.6 (2)		5.1 (1)	5.0 (1)	

FCS, Food Categorisation System.

- (a) Considering reported use levels and analytical data not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised and analytical data for food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available.
- (b): The total number of surveys may be greater than the total number of countries as listed in Table 5, as some countries submitted more than one survey for a specific population.

K. Main food categories contributing to the exposure to benzoic acid-benzoates (E 210–213) using dataset 2^(a) for the “non-brand-loyal refined exposure scenario” (> 5 % of the total mean exposure) and the number of surveys in which each food category is contributing

FCS Category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (Number of surveys) ^(b)					
01.1	Unflavoured pasteurised and sterilised (including UHT) milk	7.6-34.9 (2)	6.2-12.8 (7)	5.6-12.6 (5)	5.1-8.4 (3)		
01.7.1	Unripened cheese excluding products falling in category 16	9.0 (1)	6.6 (1)				
04.1	Unprocessed fruit and vegetables	24.5-39.9 (6)	17.6-39.4 (10)	10.2-39.2 (18)	8.3-31.1 (17)	9.0-41 (17)	12.0-43.2 (14)
04.2	Processed fruit and vegetables	7.0 (1)	5.2 (1)	8.6-13.5 (2)	5.6-7.1 (2)	11.0 (1)	5.7-11.7 (2)
09.1	Unprocessed fish		6.5-24.3 (3)	5.1-17.2 (8)	7.0-15.0 (7)	5.3-20.2 (11)	5.7-13.2 (11)
09.2	Processed fish and fishery products including molluscs and crustaceans		6.0-6.9 (2)	5.4-8.9 (4)	5.8-6.9 (3)		
10.1	Unprocessed eggs	8.0 (1)	6.6-19.8 (4)	5.6-17 (13)	5.4-15.6 (10)	5.6-11.1 (8)	6.0-10.7 (5)
10.2	Processed eggs and egg products	6.2 (1)	6.7-9.8 (2)	6.8 (1)	6.2 (1)	5.7-8.0 (4)	5.4-9.1 (6)
12.6	Sauces		5.0-7.1 (4)	5.1-8.7 (9)	5.5-11 (9)	5.6-9.5 (8)	5.3-6.1 (5)
12.7	Salads and savoury-based sandwich spreads			10.7-14.4 (2)	6.2-16 (2)	8.8-19 (3)	10.8-12.4 (2)
13.1	Foods for infants and young children	10.0-18.6 (6)					
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices	5.1 (1)	6.1-11.4 (3)	5.0-11.4 (8)	5.3-9.6 (5)	5.1 (1)	
14.1.4	Flavoured drinks	12.2 (1)	7.2-34 (6)	5.2-47.7 (17)	11.5-47.8 (17)	6.3-30.4 (16)	5.2-8.7 (5)
14.1.5	Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	9.8-12.1 (2)	5.6-8.5 (3)	6.0-17.1 (7)	5.1-19.9 (8)	8.4-36.8 (15)	10.4-43.4 (14)
18	Processed foods not covered by categories 1 to 17, excluding foods for infants and young children	16.0-29.8 (5)	6-25.3 (8)	5.7-12.4 (11)	5.1-12.7 (10)	5.2-13.4 (11)	5.1-10.5 (8)

FCS, Food Categorisation System.

(a) Considering reported use levels and analytical data not exceeding the MPLs for food categories for which direct addition of benzoic acid-benzoates is authorised and analytical data for food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available.

(b): The total number of surveys may be greater than the total number of countries as listed in Table 5, as some countries submitted more than one survey for a specific population.

L. Summary of refined estimated exposure to benzoic acid-benzoates (E 210–213) from their use as food additives considering concentration levels above the MPLs for food categories listed in Annex II to Regulation (EC) No 1333/2008 and analytical data for food categories which may contain benzoic acid-benzoates due to carry-over and for which data were available (min-max across the dietary surveys in mg/kg bw per day).

	Infants (4–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Brand-loyal scenario						
Mean	5.4–7.3	7.3–10.7	4.6–9.8	2.5–5.2	2.9–4.6	3.0–4.7
High level	10.3–15.9	12.3–19.6	8.0–17.6	4.9–11.0	5.4–8.5	5.4–7.8
Non-brand-loyal scenario						
Mean	1.3–2.1	2.2–3.5	1.5–3.1	0.8–2.0	1.0–1.7	0.8–1.6
High level	2.3–4.1	3.4–6.9	3.1–5.6	1.7–3.7	1.7–3.0	1.3–2.6

ABBREVIATIONS

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AFC Panel	EFSA Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
AUC	Area under the curve
BAuA	Federal Institute for Occupational Safety and Health
BfR	Bundesinstitut für Risikobewertung [Federal Institute for Risk Assessment]
BMI	body mass index
bw	body weight
CAS	Chemical Abstracts Service
CE	capillary electrophoresis
CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
DLLME	dispersive liquid–liquid micro-extraction
DNA	deoxyribonucleic acid
EC	European Commission
EINECS	European Inventory of Existing Commercial chemical Substances
EMS	ethylmethanesulphonate
EPA	Environmental Protection Agency
FAO/WHO	Food and Agriculture Organization/World Health Organization
FCC	Food Chemical Codex
FCS	Food Categorisation System
FDA	Food and Drug Administration
FDE	FoodDrinkEurope
FDRL	Food and Drug Research Laboratories
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
GC	gas chromatography
GD	gestation day
GC-MS	gas chromatography-mass spectrometry
GNPD	Mintel's Global New Products Database
HPLC	high-performance liquid chromatography
IARC	International Agency for Research on Cancer
IgE	immunoglobulin E
JECFA	Joint FAO/WHO Expert Committee on Food Additives

LC-MS/MS	liquid chromatography-mass spectrometry/mass spectrometry
LD ₅₀	lethal dose, 50 %, i.e. dose that causes death among 50 % of treated animals
LOD	limit of detection
LOQ	limit of quantification
MPL	maximum permitted level
MS	mass spectrometry
MSDI	maximised survey-derived daily intake
NOAEL	no observed adverse effect level
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
PHB	<i>p</i> -hydroxybenzoates
<i>QS</i>	<i>quantum satis</i>
SCE	sister chromatid exchange
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers
SCCP	Scientific Committee on Consumer Products
SCF	EU Scientific Committee on Food
TemaNord	Nordic Working Group on Food Toxicology and Risk Assessment
UHT	ultra-high temperature processing
UNESDA	Union of European Soft Drinks Associations
VKM	Norwegian Scientific Committee for Food Safety
WHO	World Health Organization

Review

The FEMA GRAS assessment of benzyl derivatives
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Received 8 September 2004; accepted 26 November 2004

Abstract

This publication is the eighth in a series of safety evaluations performed by the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA). In 1993, the panel initiated a comprehensive program to re-evaluate the safety of more than 1700 GRAS flavoring substances under conditions of intended use. Elements that are fundamental to the safety evaluation of flavor ingredients include exposure, structural analogy, metabolism, pharmacokinetics and toxicology. Flavor ingredients are evaluated individually and in the context of the available scientific information on the group of structurally related substances. Scientific data relevant to the safety evaluation of the use of benzyl derivatives as flavoring ingredients is evaluated. The group of benzyl derivatives was reaffirmed as GRAS (GRASr) based, in part, on their self-limiting properties as flavoring substances in food; their rapid absorption, metabolic detoxication, and excretion in humans and other animals, their low level of flavor use, the wide margins of safety between the conservative estimates of intake and the no-observed-adverse effect levels determined from subchronic and chronic studies and the lack of significant genotoxic and mutagenic potential. This evidence of safety is supported by the fact that the intake of benzyl derivatives as natural components of traditional foods is greater than their intake as intentionally added flavoring substances.

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Abbreviations: ABS, chromosomal aberration; AMS, Ames assay; *B. subtilis*, *Bacillus subtilis*; bw, body weight; CHO, Chinese hamster ovary; CoA, coenzyme A; DNA, deoxyribonucleic acid; *E. coli*, *Escherichia coli*; F, Female; FDA, United States Food and Drug Administration; FEMA, The Flavor and Extract Manufacturers Association; GRAS, generally recognized as safe; GRASa, GRAS affirmed; GRASr, GRAS reaffirmed; im, intramuscular; ip, intraperitoneal; iv, intravenous; LD₅₀, median lethal dose; M, Male; MLA, mouse lymphoma cell assay; NAS, National Academy of Science; NCI, National Cancer Institute; NOAEL, No-observed-adverse effect level; NR, not reported; NTP = National Toxicology Program; ppm = parts per million; SMVCE, sperm morphology and vaginal cytology examinations; *S. typhimurium*, *Salmonella typhimurium*; SCE, sister chromatid exchanges; SLR, scientific literature review; UDS, unscheduled DNA synthesis.

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Keywords: Benzyl derivatives; Flavoring ingredients; FEMA GRAS

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1. Chemical identity

This summary presents the key data relevant to the safety evaluation of benzyl alcohol, benzaldehyde, or benzoic acid and 34 structurally related substances for their intended use as flavoring ingredients (see Table 1). All members of this group are aromatic primary alcohols, aldehydes, carboxylic acids, or their corresponding esters or acetals. The structural features common to this group of substances include an oxygenated functional group bonded directly to a benzene ring that may be ring-substituted with alkyl substituents. The group contains the parent saturated alcohol, benzyl alcohol (No. 1), and related benzyl esters (No. 2–12); the corresponding aldehyde, benzaldehyde (No. 13), and related acetals (No. 14–17); the corresponding parent carboxylic acid, benzoic acid (No. 18), and related benzoate esters (No. 19–30). This group also includes 7 additional benzyl derivatives (No. 31–37) containing ring alkyl substituents.

The substances in this group were selected based on the criteria that: (1) all members contain a benzene ring

substituted with a reactive primary oxygenated functional group or can be hydrolyzed to such a functional group, (2) the major pathway of metabolic detoxication involves hydrolysis and oxidation to yield the corresponding benzoic acid derivative which is excreted either as the free acid or the glycine conjugate, (3) they show a consistent pattern of toxicity in both short- and long-term studies, and (4) they exhibit no evidence of genotoxicity in standardized batteries of in vitro and in vivo assays. Metabolism studies in humans and animals and four 2-year bioassays performed on parent substances in this group provide the basis for a comprehensive metabolic and toxicological assessment of the group.

2. Exposure

2.1. Flavor use and natural occurrence

The total annual volume of the 37 benzyl derivatives is approximately 464,110 kg in USA (NAS, 1970, 1982,

Table 1
Identity and exposure data for benzyl derivatives used as flavor ingredients

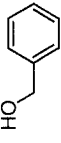
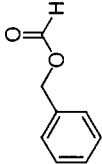
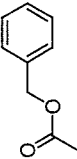
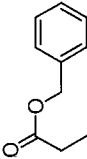
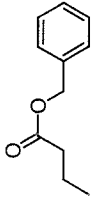
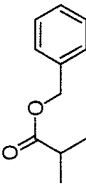
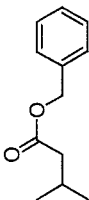
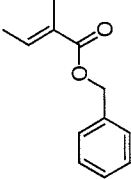
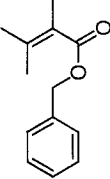
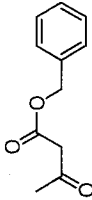
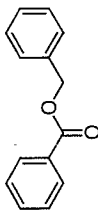
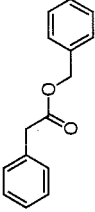
Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume (kg) ^a	Daily per capita intake ("eaters only") ^b		Annual volume in naturally occurring foods (kg) ^c	Consumption ratio ^d
				µg/d	µg/kgbw/d		
1. Benzyl alcohol	2137	100-51-6 	130,635	17,207	287	7099	0.05
2. Benzyl formate	2145	104-57-4 	386	51	1	12	0.03
3. Benzyl acetate	2135	140-11-4 	6486	854	14	3453	0.5
4. Benzyl propionate	2150	122-63-4 	748	99	2	+	NA
5. Benzyl butyrate	2140	103-37-7 	2209	291	5	+	NA
6. Benzyl isobutyrate	2141	103-28-6 	163	21	0.4	+	NA

Table 1 (continued)

Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume (kg) ^a	Daily per capita intake (“eaters only”) ^b		Annual volume in naturally occurring foods (kg) ^c	Consumption ratio ^d
				μg/d	μg/kg bw/d		
7. Benzyl isovalerate	2152	103-38-8 	95	14	0.2	+	NA
8. Benzyl <i>trans</i> -2-methyl-2-butenate	3330	37526-88-8 	0.2	0.03	0.0004	+	NA
9. Benzyl 2,3-dimethyl crotonate	2143	7492-69-5 	9 ^e	2	0.03	–	NA
10. Benzyl acetoacetate	2136	5396-89-4 	0.5 ^f	0.08	0.001	+	NA
11. Benzyl benzoate	2138	120-51-4 	31,797	4188	70	108	0.003
12. Benzyl phenylacetate	2149	102-16-9 	435	57	1	+	NA

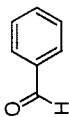
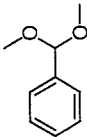
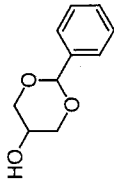
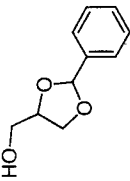
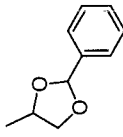
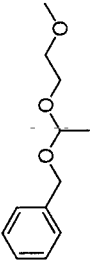
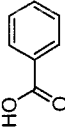
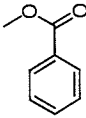
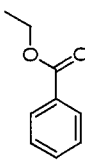
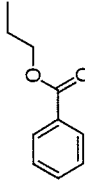
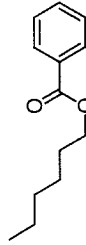
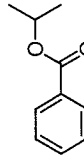
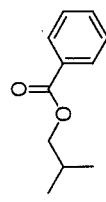
13. Benzaldehyde	2127	100-52-7		273,516	36,027	600	57,956	0.2
14. Benzaldehyde dimethyl acetal	2128	1125-88-8		2 ^g	0.3	0.005	+	NA
15. Benzaldehyde glyceryl acetal	2129	1319-88-6	 or 	2254	297	5	—	NA
16. Benzaldehyde propylene glycol acetal	2130	2568-25-4		821	108	2	+	NA
17. Benzyl methoxyethyl acetal	2148	7492-39-9		10 ^b	2	0.03	—	NA
18. Benzoic acid	2131	65-85-0		2604	343	6	+	NA

Table 1 (continued)

Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume (kg) ^a	Daily per capita intake ("eaters only") ^b		Annual volume in naturally occurring foods (kg) ^c	Consumption ratio ^d
				µg/d	µg/kg bw/d		
19. Methyl benzoate	2683	93-58-3 	1719	226	4	26	0.02
20. Ethyl benzoate	2422	93-89-0 	794	105	2	1070	1
21. Propyl benzoate	2931	2315-68-6 	1 ^e	0.2	0.004	+	NA
22. Hexyl benzoate	3691	6789-88-4 	1	0.2	0.004	1711	1711
23. Isopropyl benzoate	2932	939-48-0 	1	0.2	0.004	+	NA
24. Isobutyl benzoate	2185	120-50-3 	6	1	0.01	+	NA

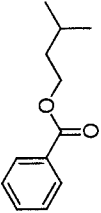
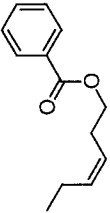
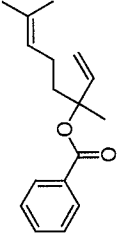
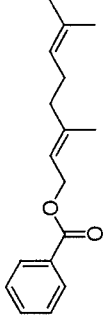
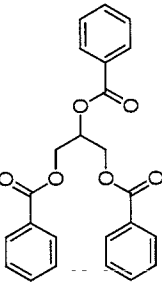
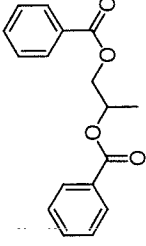
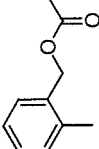
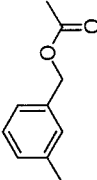
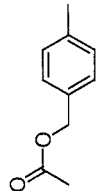
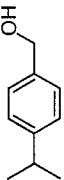
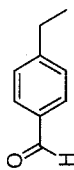
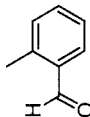
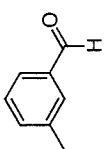
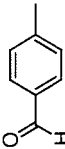
25. Isoamyl benzoate	2058	94-46-2		250	33	1	216	1
26. <i>cis</i> -3-hexenyl benzoate	3688	25152-85-6		1 ^e	0.1	0.002	138	138
27. Linalyl benzoate	2638	126-64-7		14 ^e	2	0.03	+	NA
28. Geranyl benzoate	2511	94-48-4		0.2	0.03	0.0004	—	NA
29. Glycerol tribenzoate	3398	614-33-5		370 ^f	65	1	—	NA
30. Propylene glycol dibenzoate	3419	19224-26-1		104 ^f	18	0.3	—	NA

Table 1 (continued)

Flavoring ingredient	FEMA no.	CAS no. and structure	Most recent annual volume (kg) ^a	Daily per capita intake ("eaters only") ^b		Annual volume in naturally occurring foods (kg) ^c	Consumption ratio ^d
				μg/d	μg/kg bw/d		
31. Methylbenzyl acetate (mixed <i>o</i> , <i>m</i> , <i>p</i>)	3702	17373-93-2 	0.5 ^g	0.08	0.001	+	NA
		17369-57-2 					
		2216-45-7 					
32. <i>p</i> -Isopropylbenzyl alcohol	2933	536-60-7 	2	0.3	0.004	+	NA
33. 4-Ethylbenzaldehyde	3756	4748-78-1 	45 ^h	8	0.1	+	NA
34. Toluinaldehydes (mixed <i>o</i> , <i>m</i> , <i>p</i>)	3068	1334-78-7 (mixture) 529-20-4 	8618	1135	19	+	NA
		620-23-5 					
		104-87-0 					

35. Toluolaldehyde glyceryl acetal (mixed <i>o</i> , <i>m</i> , <i>p</i>)	3067	1333-09-1		5	1	0.01	–	NA
		1333-11-5						
		4757-23-7						
36. Cuminaldehide	2341	122-03-2		7	1	0.02	+	NA
37. 2,4-Dimethylbenzaldehyde	3427	15764-16-6		1	0.1	0.002	+	NA

^a From Lucas et al. (1999) or NAS (1970, 1982, 1987).

^b Intake ($\mu\text{g}/\text{person}/\text{day}$) calculated as follows: $[(\text{annual volume (kg)}) \times (1 \times 10^9 \mu\text{g}/\text{kg})] / [\text{population} \times \text{survey correction factor} \times 365 \text{ days}]$, where population (10%, "eaters only") = 26×10^6 for USA; where correction factor = 0.6 for NAS surveys and 0.8 for the Lucas et al. USA survey representing the assumption that only 60% and 80% of the annual flavor volume, respectively, was reported in the poundage surveys (Lucas et al., 1999; NAS, 1970, 1982, 1987). Intake ($\mu\text{g}/\text{kg bw}/\text{d}$) calculated as follows: $[(\mu\text{g}/\text{person per day})/\text{body weight}]$, where body weight = 60 kg. Slight variations may occur from rounding.

^c Quantitative data for United States reported by Stofberg and Grundschober (1987).

^d The consumption ratio is calculated as follows: (annual consumption via food, kg)/(most recent reported volume as a flavoring substance, kg); NA = data not available.

^e NAS (1970).

^f NAS (1982).

^g NAS (1987).

^h The volume cited is the anticipated annual volume, which was the maximum amount of flavor ingredient estimated to be used annually by the manufacturer at the time the material was proposed for flavor use.

1987; Lucas et al., 1999) (see Table 1). Approximately 94% of the total annual volume in USA is accounted for by three substances in the group [benzyl alcohol (No. 1), benzaldehyde (No. 13), and benzyl benzoate (No. 11)]. Approximately 59% of the total annual volume in USA is accounted for by benzaldehyde. The daily *per capita* intake¹ of each agent is reported in Table 1.

In addition to exposure through food and flavor sources, benzoic acid has been found endogenously in the human body. Endogenous benzoic acid is formed through the phenylalanine–tyrosine pathway (JECFA, 1980, 1997).

Thirty (30) of the 37 substances have been reported to occur naturally in foods. They have been detected in a wide variety of fruits (apple, avocado, blackberry, blueberry, cherry, cranberry, melon, papaya, plum, raspberry, strawberry), vegetables (artichokes, asparagus, beans, cabbage, corn, leek, mushroom, potato, tomato), meats (cooked beef, cooked and roasted chicken, cured pork, shell fish), cheeses (gruyere, cheddar, mozzarella, parmesan), teas, and wines (Maarse et al., 1999).

Quantitative natural occurrence data is reported for 10 substances in the group. Five of these substances show that human oral exposure occurs predominantly in food (i.e., consumption ratio greater than or equal to 1), while the other five substances have consumption ratio less than 1 (See Table 1) (Stofberg and Kirschman, 1985; Stofberg and Grundschober, 1987).

3. Hydrolysis, absorption, distribution, excretion and metabolism

3.1. Hydrolysis

In general, aromatic esters are hydrolyzed *in vivo* through the catalytic activity of carboxylesterases or esterases (Heymann, 1980), the most important of which are the A-esterases. These enzymes are found throughout mammalian tissues (Anders, 1989; Heymann, 1980), but predominate in hepatocytes (Heymann, 1980).

Hydrolysis of benzyl and benzoate esters to yield the corresponding alcohols and carboxylic acids and hydro-

lysis of acetals to yield benzaldehyde and simple aliphatic alcohols have been reported in several *in vitro* experiments (see Fig. 1).

Benzyl acetate (No. 3) when incubated with rat plasma *in vitro* was rapidly hydrolyzed to benzyl alcohol (No. 1) with peak alcohol concentration after 4 minutes. No plasma benzyl alcohol was observed *in vivo* following gavage or feed administration of benzyl acetate to rats; however high plasma levels of hippuric acid and benzoic acid were detected. The absence of benzyl acetate in plasma is evidence that benzyl acetate is rapidly hydrolyzed to benzyl alcohol, which is then rapidly oxidized to benzoic acid *in vivo* (Yuan et al., 1995).

Benzyl acetate was readily hydrolyzed in pig liver homogenate (Heymann, 1980). The plasma half-lives ($t_{1/2}$) for the *in vitro* hydrolysis of a series of four alkyl benzoates [including methyl benzoate (No. 19), ethyl benzoate (No. 20) and propyl benzoate (No. 21)] and two aryl benzoates in 80% human blood plasma decreased from 210 min for ethyl benzoate to 24 min for butyl benzoate and 19 and 15 min for phenyl benzoate and benzyl benzoate, respectively (Nielson and Bundgaard, 1987).

An *in vitro* hydrolysis study found that benzyl phenylacetate (No. 12) was 90% hydrolyzed within 1 h and completely hydrolyzed within 2 h of incubation with a 2% pancreatin solution (Leegwater and van Straten, 1974).

Benzaldehyde related acetals readily hydrolyze to their component alcohols and benzaldehyde under acidic conditions. Benzaldehyde propylene glycol acetal (No. 16) was 97% hydrolyzed after incubation for 5 h with simulated gastric juice and intestinal fluid *in vitro* compared to blank incubation (Morgareidge, 1962).

3.2. Absorption, distribution and excretion

The benzyl derivatives are rapidly absorbed through the gut, metabolized primarily in the liver, and excreted in the urine as glycine conjugates of benzoic acid derivatives (Davison, 1971; Abdo et al., 1985; Temellini, 1993). Recent studies on the effect of dose, species, sex and mode of administration on the absorption, distribution and excretion of these substances are described in detail below.

Groups of 50 male Fischer 344 rats and 50 male B6C3F₁ mice were administered ¹⁴C-benzyl acetate (No. 3) orally at levels of 5–500 mg/kg bw for rats and 10–1000 mg/kg bw for mice for five days per week for a period of two weeks. Urine and feces were collected separately over a period of 24 h following dosing. Carbon dioxide (CO₂) and other volatile substances were collected at 2, 4, 5, and 24 h following each dose. At termination, blood, liver, muscle, adipose, skin, lung, kidney, and stomach were collected and analyzed for

¹ Intake ($\mu\text{g/kg}$) calculated as follows: $[(\text{annual volume, kg}) \times (1 \times 10^9 \mu\text{g/kg})] / [\text{population} \times \text{correction factor} \times 365 \text{ days}]$, where population (10%, “eaters only”) = 26×10^6 for USA; where correction factor = 0.8 for the Lucas et al. and 0.6 for NAS USA surveys, and represents the assumption that only 80% and 60%, respectively, of the flavor volume was reported in the surveys (Lucas et al., 1999; NAS, 1970, 1982, 1987). For agents that were not surveyed the anticipated volume was used with a correction factor of 0.6. Intake ($\mu\text{g/kg bw per day}$) calculated as follows: $[(\mu\text{g per day}) / \text{body weight}]$, where body weight = 60 kg. Slight variations may occur from rounding off.

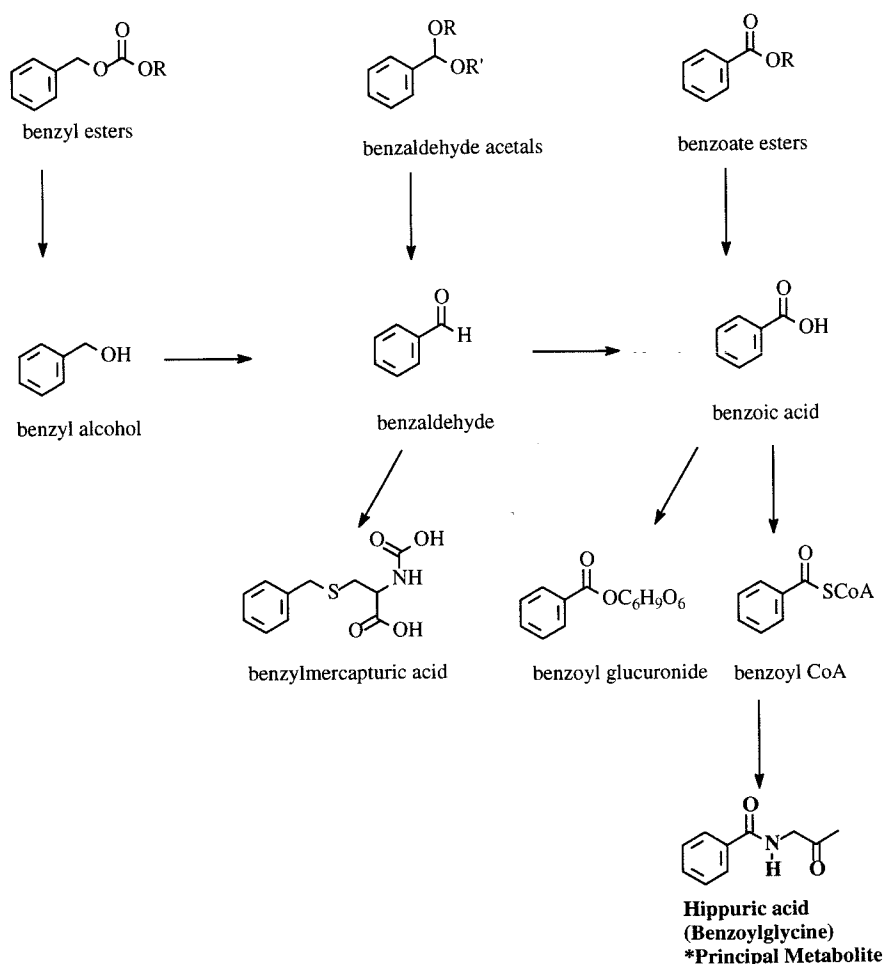


Fig. 1. Metabolism of benzyl derivatives.

radioactivity. The compound was readily absorbed from the gastrointestinal tract of both species, and approximately 90% and 0.3–1.3% of the total dose was recovered as hippuric acid in the urine and feces, respectively, within 24 h. No benzyl acetate-derived radioactivity was detected in any tissue analyzed at 24 h. The clearance pattern was not affected by repeated dosing 5 days/week for 2 weeks at 500 mg/kg bw in rats or 1000 mg/kg bw in mice. Such complete clearance indicates that benzyl acetate is readily absorbed and excreted (Abdo et al., 1985).

Following gavage administration of ^{14}C -benzyl acetate to groups of three or more male Fischer 344 rats at doses of 5, 250 or 500 mg/kg bw as the substance alone, in corn oil, or propylene glycol, 70–89% of the dose was excreted in the urine within 24 h. Approximately 4% radioactivity was detected in the feces after 72 h. The elimination of benzyl acetate and metabolites, regardless of vehicle, was largely complete after 3 days. Only negligible residues were found in tissues. Although no benzyl acetate was detected in the plasma or urine, small amounts of benzyl alcohol were detected in the

plasma. At 500 mg/kg bw, unconjugated benzoic acid was the major plasma metabolite while at 5 mg/kg, hippuric acid was the major urinary metabolite. The proportion of the glucuronic acid conjugate of benzoic acid increased with increasing dose, while low levels (1.0–3.6%) of benzoic acid and benzylmercapturic acid were not affected by dose or vehicle (Chidgey and Caldwell, 1986).

Fischer 344 rats and C57Bl/6N mice were given a single oral dose of ^{14}C benzyl acetate at doses of 5 or 500 mg/kg bw (rats) or 10 mg/kg bw (mice) to determine the effects of age on disposition of benzyl acetate (McMahon et al., 1989). Age groups studied were 3–4, 9, and 25-month-old rats and 2, 13, and 25-month-old mice. In rats, approximately 80% of radioactivity was recovered in the urine in the first 24 h for all age groups. The major urinary metabolite was hippuric acid and a minor urinary metabolite was benzylmercapturic acid in rats. There was no age difference in the percentage of ^{14}C benzyl acetate excreted as hippuric acid, but the amount of excreted benzylmercapturic acid increased slightly in the 25-month-old rats. The percentage of

radioactivity excreted in the feces was slightly decreased in the 25-month-old group.

In mice, hippuric acid was the major urinary metabolite, constituting 93–96% of the total dose. Less radioactivity was excreted in the urine of 25-month-old mice than in the younger groups. Fecal excretion was a minor route and the amount was similar for all age groups. The authors concluded that formation of hippuric acid is not affected by age, but aging does affect the minor routes of metabolism and excretion of benzyl acetate in rats and mice (McMahon et al., 1989).

In a study of the influence of gavage and dietary administration on the toxicokinetics of benzyl acetate, plasma levels of benzyl alcohol, benzoic acid and hippuric acid were measured at 24 h intervals after groups of 6 F344 rats were given 500 mg/kg bw of benzyl acetate by gavage in corn oil, and groups of 12 B6C3F₁ mice were given 1,000 mg/kg bw of benzyl acetate by the same route. Groups of 10 rats and 10 mice were fed diets containing 2700 ppm and 10,800 ppm, respectively, of benzyl acetate for 7 days. The calculated daily dose levels were reported to be approximately 648 mg/kg bw in rats and 900 mg/kg bw in mice. Benzyl acetate was undetectable in the plasma after gavage or dietary administration. After gavage administration, benzyl alcohol could not be detected in the plasma after 5 min in mice and 10 min in rats. Peak plasma levels of benzoic acid and hippuric acid were reached within 3 h of gavage administration. Compared to the gavage mode of administration, peak plasma concentrations of benzoic acid were 40-fold less in rats and 300-fold less in mice after dietary administration. Plasma concentrations of hippuric acid were similar regardless of the mode of administration (Yuan et al., 1995).

Following administration of 375 mg ¹⁴C benzoic acid (No. 18)/kg bw to rats (oral) and mice (ip), 88–89% of the radioactivity was recovered in the urine within 24 h and 91–94% after 72 h, and only 1–6% was present in the feces (Nutley, 1990).

In a study on the effects of benzyl alcohol in neonates, 4 full-term and 9 pre-term infants received intravenous (iv) or intramuscular (im) doses of 0.007–0.222 μmol benzyl alcohol (No. 1)/kg bw in medication. Maximum serum concentration levels of benzoic acid in pre-term infants were approximately 10 times those in full-term infants. Larger percentages of benzyl alcohol doses were found as benzoic acid {75.2% (iv) and 83.8% (im) vs. 38.1% (iv) and 57.7% (im)} and less as hippuric acid {64.2% (iv) and 26.3% (im) vs. 82.3% (iv) and 85.2% (im)} in pre-term infants compared to term infants. The study indicates that glycine conjugation is deficient in pre-term compared to full-term infants (LeBel et al., 1988).

After administration of oral doses of 40, 80, and 160 mg/kg bw of sodium benzoate to humans, the clearance of benzoic acid increased disproportionately to the

dose while the clearance for hippuric acid was proportional to dose. Peak plasma concentrations of benzoic acid increased with increasing dose, while peak hippuric acid concentrations did not change. The data suggest that the conjugation with glycine to form hippuric acid is a saturable process in humans (Kubota et al., 1988; Kubota and Ishizaki, 1991).

Based on these studies, benzyl derivatives are expected to be rapidly absorbed, and rapidly metabolized to benzoic acid. The acid is then conjugated with glycine and excreted mainly in the urine. At high dose levels, the glycine conjugation pathway may be saturated; in which case, free benzoic acid is excreted unchanged.

3.3. Metabolism

3.3.1. Metabolism of benzyl alcohol, benzaldehyde, benzoic acid and related esters

Benzyl esters and acetals are hydrolyzed to benzyl alcohol and benzaldehyde, respectively (see Fig. 1). The alcohol and aldehyde are rapidly oxidized to benzoic acid while benzoate esters are hydrolyzed to benzoic acid. The benzoic acid derivatives are excreted primarily as the glycine conjugate. Benzoic acid is readily conjugated with glycine in liver (321 nmol/min/g) and kidney homogenate (254 nmol/min/g) (Temellini, 1993). At high dose levels formation of the glycine conjugate is glycine limited. When glycine is depleted, free benzoic acid may be excreted unchanged or as the glucuronic acid conjugate (Bray et al., 1951; Diack and Lewis, 1928; Williams, 1959).

Following oral, subcutaneous, or ip administration of ¹⁴C benzyl acetate to mice (10–1000 mg/kg bw) and rats (5–500 mg/kg bw), hippuric acid was the major urinary metabolite. Minor amounts of benzyl alcohol, benzoic acid and benzylmercapturic acid were also detected in the urine (Abdo et al., 1985; Chidgey and Caldwell, 1986; Yuan et al., 1995; Clapp and Young, 1970; McMahon et al., 1989).

Using specific enzyme inhibitors, a pathway was proposed for the metabolic conversion of benzyl acetate to benzylmercapturic acid in male Fischer rats. Five hundred (500) mg/kg bw of ¹⁴C benzyl acetate was administered to groups of 3–5 male Fischer rats by gavage, alone or in conjunction with ip injections of 200 mg/kg bw of pyrazole (an alcohol dehydrogenase inhibitor), 10 mg/kg bw of pentachlorophenol (a sulfotransferase inhibitor) or both. Within 24 h of administration of benzyl acetate alone, 92% of the dose was recovered in the urine, 3% in the feces and <1% in the carcasses. The combination treatment of benzyl acetate and inhibitor delayed the excretion of ¹⁴C in urine compared to treatment with benzyl acetate alone. The pyrazole only group had an 11-fold increase in the amount of benzylmercapturic acid excreted in the first 24 h with a halving of the percentage of benzoyl glucuronide eliminated in the

urine. The elimination of hippuric acid was unaffected in the pyrazole treated group as compared to controls. The amount of benzylmercapturic acid was significantly increased in the combination group, but not to the same levels as the pyrazole only group. Benzyl sulphate has been shown to be converted to benzylmercapturic acid in rats (Clapp and Young, 1970) and S-benzylglutathione is formed in the presence of rat liver cytosol (Gillam, 1971). Benzyl alcohol is converted to benzylmercapturic acid in rats involving an obligatory benzylsulphate intermediate (Van Doorn et al., 1981). In the case of benzyl acetate, these results suggest that benzylmercapturic acid forms via the sulfate ester of benzyl alcohol (Chidgey et al., 1986).

Less than 5 min after single intraperitoneal doses of 770–1100 mg/kg bw of benzyl alcohol given to CD1 mice, benzyl alcohol and benzaldehyde were detected in the plasma. Animals pretreated with an alcohol dehydrogenase inhibitor (pyrazole) resulted in a 200% increase in plasma benzyl alcohol levels, while pretreatment with an aldehyde inhibitor (disulfiram) resulted in a 368% increase in plasma benzaldehyde levels. These data suggest that benzaldehyde and benzyl alcohol are interconvertible in the liver (McCloskey et al., 1986).

The principal pathway of metabolism of benzaldehyde (No. 13) includes oxidation to yield benzoic acid, which is subsequently conjugated with glycine and excreted as hippuric acid (Bray et al., 1951; NTP, 1990a,b). In the rabbit, approximately 83% of single doses of 350 or 750 mg/kg bw of benzaldehyde is excreted in the urine of both dose groups. The aldehyde is oxidized mainly to benzoic acid and excreted predominantly as hippuric acid (~68%). Other urinary metabolites detected are benzoylglucuronic acid (10%), benzoyl glucuronide (3%), free benzoic acid (1.5%), and trace amounts of benzylmercapturic acid (Laham et al., 1988).

To a minor extent, benzaldehyde is reduced to benzyl alcohol, which as the sulfate conjugate may react with glutathione to form benzylmercapturic acid (Laham and Potvin, 1987). Groups of Sprague–Dawley rats (5/group/sex) were given single oral doses of 400, 750, or 1000 mg/kg bw of pure benzaldehyde by gavage daily for 13 consecutive days. Twenty-four (24) h urinary excretion of benzylmercapturic acid was measured after the 2nd, 8th, and 13th dose. Although females in the mid- and high-dose groups exhibited a slight decrease in excretion of benzylmercapturic acid after the 8th dose, all groups showed increased urinary levels of the conjugated acid after 13 doses. An increase in dose from 400 to 1000 mg/kg bw per day resulted in a 7–8-fold increase in excreted benzylmercapturic acid.

Administration of 375 mg ¹⁴C- benzoic acid/kg bw to mice (ip) and rats (oral) resulted in excretion of hippuric acid (70.2–84.2%), benzoyl glucuronide (0.7–1.8%), ben-

zoic acid (0.4–12.8%), and 3-hydroxy-3-phenyl propionic acid (0.1–0.2%) (Nutley, 1990).

¹⁴C-Benzoic acid was administered orally at doses in the range from 1 to 400 mg/kg bw to various species including primates, pigs, rabbits, rodents, cats, dogs, hedgehogs, bats, birds, and reptiles. Hippuric acid was the primary urinary metabolite in most species. The ornithine conjugate of benzoic acid, ornithic acid, was the major urinary metabolite excreted within 24 h in chickens and reptiles. Benzoyl glucuronide was predominant in the fruit bat. In humans, >99% ¹⁴C was excreted as hippuric acid within 24 h (Bridges et al., 1970).

Male volunteers were given oral doses of 2000–5000 mg sodium benzoate. The 5000 mg dose group was given a 5000 mg dose of glycine 1 h later and 2000 mg doses every 2 h thereafter. Benzoate was excreted mainly as hippuric acid. No free benzoic acid was detected. Minor amounts of benzoylglucuronide were detected, with more formed at the 5000 mg dose than at the 2000 mg dose. Co-administration of glycine with benzoate increased the rate of hippuric acid excretion, indicating that at high dose levels, glycine is rate limiting for formation of hippuric acid (Amsel and Levy, 1969).

3.3.2. Metabolism of substituted benzyl alcohols, benzaldehydes and benzoic acid derivatives

Aromatic ring substitution has little or no influence on the principal pathway of metabolism. Oxidation of the alcohol or aldehyde to the benzoic acid derivative may be accompanied by minor amounts of side-chain oxidation or *O*-dealkylation of a ring alkoxy group.

Large doses (2 g) of cuminaldehyde (*p*-isopropylbenzaldehyde, No. 37) were administered orally to male rabbits. Urine was collected for 3 days post-treatment. Cuminaldehyde undergoes a combination of oxidation of the aldehyde function and the oxidation of the alkyl-side chain to yield 9-hydroxycuminic acid, 8-hydroxycuminic acid. Cumyl alcohol and 2-carboxyphenylpropionic acid were minor urinary metabolites (Ishida et al., 1989).

3.3.3. Summary of metabolic data

In summary, benzyl and benzoate esters and benzaldehyde acetals are readily hydrolyzed to the corresponding parent alcohol, aldehyde, or acid. Following hydrolysis, benzyl alcohol is sequentially oxidized to benzaldehyde and then benzoic acid, followed by excretion as hippuric acid. To a minor extent, benzyl alcohol may conjugate with glutathione, benzaldehyde may be reduced to benzyl alcohol, and benzoic acid may conjugate with glucuronic acid. The latter conjugation pathway may become more important as high levels of benzoic acid saturate the glycine (hippurate) conjugation pathway. At very high levels of exposure, free

benzoic acid may sequester significant quantities of acetyl CoA.

4. Toxicological studies

4.1. Acute toxicity

Oral LD₅₀ values have been reported for 30 of the 37 substances in this group (see Table 2). LD₅₀ values in rats are in the range from 1020 mg/kg bw for *p*-isopropylbenzyl alcohol (No. 32) to 12,300 mg/kg bw for hexyl benzoate (No. 22), indicating that the oral acute toxicity of the benzyl derivatives is very low (Graham and Kuizenga, 1945; Draize et al., 1948; Smyth et al., 1951, 1954; Jenner et al., 1964; Taylor et al., 1964; Sporn et al., 1967; Kravets-Bekker and Ivanova, 1970; Owen and Meyer, 1971; Shelanski and Moldovan, 1971; Weir and Wong, 1971; Levenstein, 1973, 1974; Moreno, 1973, 1974, 1977, 1979, 1980; DeGroot et al., 1974; Lewis and Palanker, 1979; Costello and Moore, 1984; Ciba-Geigy, 1991; Proctor and Gamble, 1992). In mice the oral LD₅₀ values are in the range from 1580 mg/kg bw for benzyl alcohol (No. 1) to 9408 mg/kg bw for linalyl benzoate (No. 27) indicating that the acute oral toxicity of benzyl derivatives is very low (Jenner et al., 1964; Hoffmann-LaRoche, 1967; Kravets-Bekker and Ivanova, 1970; Sado, 1973; Shell, 1982; Schafer and Bowles, 1985). In rabbits, guinea pigs and cats the oral LD₅₀ values are in the range from 1000 mg/kg bw for benzaldehyde (No. 13) to greater than 5000 mg/kg bw for linalyl benzoate (No. 27) indicating that the acute oral toxicity of benzyl derivatives is very low (Graham and Kuizenga, 1945; Draize et al., 1948; Jenner et al., 1964; Kravets-Bekker and Ivanova, 1970; Moreno, 1973).

4.2. Short-term and long-term studies of toxicity

Short-term toxicological studies have been reported for nine (9) representative benzyl derivatives. Short-term studies for benzyl alcohol, one benzyl ester, three benzoate esters, and two alkyl-substituted benzaldehyde derivatives are summarized in Table 2 and described in detail below.

4.2.1. Mice

4.2.1.1. Benzyl alcohol (No. 1). Groups of 10 male and 10 female B6C3F₁ mice were administered 0 (control), 50, 100, 200, 400, or 800 mg/kg bw benzyl alcohol daily by gavage 5 days per week for a period of 13 weeks. No compound related histopathology was observed. Deaths occurred in most groups, however, all but one was attributed to the gavage procedure. Mean body weights of males and females were reduced by 5% and 8%, respectively, at the 800 mg/kg bw per day dose level and body weights of females were reduced 5% at

the 400 mg/kg bw per day. At the highest dose level, staggering was seen in both male and female mice during the first 2 weeks of the study. The dose level of 100 mg/kg bw per day produced no adverse effects (NTP, 1989).

4.2.1.2. Benzyl acetate (No. 3). Benzyl acetate was administered to groups of 10 male B6C3F₁ mice in corn oil daily by gavage at doses of 0 (control), 62.5, 125, 250, 500, or 1,000 mg/kg bw 5 days per week for a period of 13 weeks. Groups of 10 female mice were administered benzyl acetate by gavage at doses of 0 (control), 125, 250, 500, 1,000, or 2,000 mg/kg bw on the same schedule. Eight of the 10 female mice that received 2,000 mg/kg bw died; however, one of these deaths was due to gavage error. Compound-related clinical signs, including trembling, inactivity, labored breathing, and depressed body temperature, were reported in the high-dose mice. Gross examination after necropsy and histopathological examination failed to reveal any effects related to administration of the test substance (NTP, 1986). Subsequent microscopic examination revealed hippocampal necrosis in one female at the 1000 mg/kg bw per day dose (NTP, 1993a). No adverse effects were observed at dose levels less than or equal to 500 mg/kg bw per day (NTP, 1986).

For a period of 13 weeks, 5 days per week, groups of 10 male and 10 female B6C3F₁ mice were maintained on diets containing 0, 3130, 6250, 12,500, 25,000, or 50,000 ppm benzyl acetate calculated to provide an average daily intake of 0 (control), 425, 1000, 2000, 3700, or 7900 mg benzyl acetate/kg bw for males and 650, 1280, 2980, 4300, or 9400 mg/kg bw for females (FDA, 1993). At all dose levels, statistically significant ($P < 0.01$) decreases in body weights were reported compared to the control group in both sexes. Absolute and relative organ weights were affected by lower terminal body weights. A non-statistically significant decrease in feed consumption was reported at all dose levels, which may have been due to poor palatability. Hematological examination and clinical chemistry determinations show normal values. In the 50,000 ppm group, brain lesions, consisting of hippocampal necrosis and cerebellar hemorrhage, were reported in 4 mice (1 male, 3 females). Hepatocellular necrosis also occurred in the male mouse with brain lesions (NTP, 1993a).

4.2.1.3. Benzaldehyde (No. 13). Groups of 10 male and 10 female B6C3F₁ mice were administered doses of 0 (control), 75, 150, 300, 600 or 1200 mg benzaldehyde/kg bw by gavage daily in corn oil 5 days per week for a period of 13 weeks. At the 1200 mg/kg bw per day dose, 9/10 males and 1/10 females died during the first week of the study. Mean body weight of males at the 600 mg/kg bw per day level were 9% less than that of controls. Mild to moderate renal tubule degeneration

Table 2
Acute, short- and long-term toxicity studies for benzyl derivatives used as flavor ingredients

Flavoring ingredient	Oral acute studies Oral LD ₅₀ mg/kg bw (Species)	Reference	Short- and long-term studies Species, sex ^a	Time (days)/route	NOAEL (mg/kg bw)	Reference
Benzylalcohol	1040 (Rabbit)	Graham and Kuizenga (1945)	Rat, MF	91	100	NTP (1989)
Benzylalcohol	2979 (Rat)	Ciba-Geigy (1991)	Rat, MF	16	125	NTP (1989)
Benzylalcohol	2080 (Rat)	Graham and Kuizenga (1945)	Rat, MF	721	<200	NTP (1989)
Benzylalcohol	1230 (Rat)	Jenner et al. (1964)	Mouse, MF	91	100	NTP (1989)
Benzylalcohol	1570 (Rat)	Proctor and Gamble (1992)	Mouse, MF	16	250	NTP (1989)
Benzylalcohol	3100 (Rat)	Smyth et al. (1951)	Mouse, MF	721	200	NTP (1989)
Benzylalcohol	1580 (Mouse)	Jenner et al. (1964)	Mouse, MF	16	250	NTP (1989)
Benzyl formate	<5000 (Rat)	Shelanski and Moldovan (1971)	Rat, MF	14	500	NTP (1986)
Benzyl acetate	2640 (Rabbit)	Graham and Kuizenga (1945)	Rat, MF	91	250	NTP (1986)
Benzyl acetate	2490 (Rat)	Jenner et al. (1964)	Rat, MF	91	460	NTP (1993a)
Benzyl acetate	3690 (Rat)	Graham and Kuizenga (1945)	Rat, MF	721	<130	NTP (1993a)
Benzyl acetate			Rat, MF	721	250	NTP (1986)
Benzyl acetate			Mouse, MF	14	1000	NTP (1986)
Benzyl acetate			Mouse, M	91	500	NTP (1986)
Benzyl acetate			Mouse, MF	721	<35	NTP (1993a)
Benzyl acetate			Mouse, MF	721	<500	NTP (1986)
Benzyl propionate	3300 (Rat)	Moreno (1973)				
Benzyl butyrate	1850 (Rat)	Moreno (1973)				
Benzyl butyrate	2330 (Rat)	Jenner et al. (1964)				
Benzyl isobutyrate	2850 (Rat)	Owen and Meyer (1971)				
Benzyl isovalerate	<5000 (Rat)	Moreno (1974)				
Benzyl <i>trans</i> -2-methyl-2-butenate	>5000 (Rat)	Moreno (1979)				
Benzyl benzoate	2240 (Cat)	Graham and Kuizenga (1945)				
Benzyl benzoate	2016 (Rabbit)	Draize et al. (1948)				
Benzyl benzoate	1680 (Rabbit)	Graham and Kuizenga (1945)				
Benzyl benzoate	1120 (Guinea pig)	Draize et al. (1948)				
Benzyl benzoate	1904 (Rat)	Draize et al. (1948)				
Benzyl benzoate	2800 (Rat)	Graham and Kuizenga (1945)				
Benzyl benzoate	1568 (Mouse)	Draize et al. (1948)				
Benzyl phenylacetate	>5000 (Rat)	Owen and Meyer (1971)				
Benzaldehyde	1000 (Guinea pig)	Jenner et al. (1964)	Rat, MF	91	200	Kluwe et al. (1983)
Benzaldehyde	1300 (Rat)	Jenner et al. (1964)	Rat, MF	91	200	NTP (1990a, 1990b)
Benzaldehyde	2850 (Rat)	Sporn et al. (1967)	Rat, MF	189-196	>50	Hagan et al. (1967)
Benzaldehyde	1300 (Rat)	Taylor et al. (1964)	Rat, MF	721	<200	NTP (1990a, 1990b)
Benzaldehyde	1250 (Mouse)	Schafer and Bowles (1985)	Mouse, MF	91	300	Kluwe et al. (1983)
Benzaldehyde			Mouse, MF	91	300	NTP (1990a, 1990b)
Benzaldehyde			Mouse, MF	728	<200	NTP (1990a, 1990b)
Benzaldehyde dimethyl acetal	1220 (Rat)	Moreno (1977)				
Benzaldehyde glyceryl acetal	3749 (Rat)	Levenstein (1974)				

(continued on next page)

Table 2 (continued)

Flavoring ingredient	Oral acute studies Oral LD ₅₀ mg/kg bw (Species)	Reference	Short- and long-term studies Species, sex ^a	Time (days)/route	NOAEL (mg/kg bw)	Reference
Benzaldehyde glyceryl acetal	2750 (Rat)	Moreno (1980)				
Benzaldehyde propylene glycol acetal	3000 (Rat)	Lewis and Palanker (1979)				
Benzoic acid	1250 (Mouse)	Schafer and Bowles (1985)	Rat, MF	540-730	<370	Sodemoto and Enomoto (1980)
Benzoic acid	1996 (Mouse)	Sado (1973)	Mouse, MF	90	80	Shtenberg and Ignat'ev (1970)
Benzoic acid	1950 (Mouse)	Shell (1982)	Mouse, MF	510	40	Shtenberg and Ignat'ev (1970)
Methyl benzoate	2170 (Rabbit)	Graham and Kuizenga (1945)	Rat	183	0.005	Kravets-Bekker and Ivanova (1970)
Methyl benzoate	4100 (Guinea pig)	Kravets-Bekker and Ivanova (1970)				
Methyl benzoate	2170 (Rat)	Graham and Kuizenga (1945)				
Methyl benzoate	1350 (Rat)	Jenner et al. (1964)				
Methyl benzoate	3500 (Rat)	Kravets-Bekker and Ivanova (1970)				
Methyl benzoate	3420 (Rat)	Smyth et al. (1954)				
Methyl benzoate	3330 (Mouse)	Jenner et al. (1964)				
Methyl benzoate	3000 (Mouse)	Kravets-Bekker and Ivanova (1970)				
Ethyl benzoate	2630 (Rabbit)	Graham and Kuizenga (1945)				
Ethyl benzoate	2100 (Rat)	Graham and Kuizenga (1945)				
Ethyl benzoate	6480 (Rat)	Smyth et al. (1954)				
Hexyl benzoate	12,300 (Rat)	Smyth et al. (1951)				
Isopropyl benzoate	3730 (Rat)	Smyth et al. (1951)				
Isobutyl benzoate	3685 (Rat)	Levenstein (1973)				
Isoamyl benzoate	6330 (Rat)	Weir and Wong (1971)				
cis-3-Hexenyl benzoate	>5000 (Rat)	Moreno (1976)				
Linalyl benzoate	>5000 (Rabbit)	Moreno (1973)				
Linalyl benzoate	>5000 (Rat)	Moreno (1973)				
Linalyl benzoate	9408 (Mouse)	Hoffmann-LaRoche (1967)				
Geranyl benzoate	>5000 (Rat)	Moreno (1973)				
Glyceryltribenzoate			Rat, MF	90	604	Carson (1972a)
Propylene glycol dibenzoate			Rat, MF	90	2541	Carson (1972b)
p-Isopropylbenzyl alcohol	1020 (Rat)	Moreno (1973)				
4-Ethylbenzaldehyde	1970 (Rat)	Costello and Moore (1984)				
Tolualdehydes (mixed o, m, p)	2250 (Rat)	Moreno (1973)	Rat, MF	90	36.1	Oser et al. (1965)
Tolualdehydes (mixed o, m, p)			Rat, MF	91	250	Brantom et al. (1972)
Tolualdehyde glyceryl acetal	3400 (Rat)	Moreno (1972)				
Cuminaldehyde	1390 (Rat)	Jenner et al. (1964)				
2,4-Dimethylbenzaldehyde	<5000 (Rat)	DeGroot et al. (1974)	Rat, MF	14	1.75	DeGroot et al. (1974)

^a M = Male; F = Female. If not listed, sex was not specified in the report.

was reported in all males at the 1200 mg/kg bw per day dose level and 1 male at the 600 mg/kg bw per day dose

level. These lesions were not observed in lower dose levels in males or any dose level in females. The no adverse

effect level was reported to be 300 mg/kg bw per day (NTP, 1990a).

4.2.1.4. Benzoic acid (No. 18). Groups of 50 male and 50 female crossbred white mice (strain not specified) were administered 80 mg benzoic acid/kg bw by oral intubation daily for 3 months. Weight gain of the test animals was reduced compared to control animals. This was not due to reduced feed intake, but it may have been due to stress factors such as food restriction, low temperature, swimming test, centrifugation, carbon tetrachloride detoxication test or kidney function testing (Shtenberg and Ignat'ev, 1970). Benzoic acid was administered orally to groups of 25 male and 25 female mice at 40 mg/kg bw daily for 17 months. Survival at 2.5 months was greater for the benzoic acid group (68%) than that for the control group of males (60%) or female rats (62%) (Shtenberg and Ignat'ev, 1970).

4.2.2. Rats

4.2.2.1. Benzyl alcohol (No. 1). Benzyl alcohol was administered to groups of 10 male and 10 female F344/N rats at doses of 0 (control), 50, 100, 200, 400 or 800 mg/kg bw in corn oil daily by gavage five days per week for a period of 13 weeks. After dosing, staggering, lethargy and labored breathing occurred in both sexes at 800 mg/kg bw. Eight (8) males and 2 females at 800 mg/kg bw per day dose died after treatment, with the deaths of 4 males and one female being attributed to gavage errors. Reduction in the relative weight gain of female rats in the 200 and the 400 mg/kg bw per day groups was observed. At the end of the study, body weights of males and females at the 800 mg/kg bw level were reduced by 7% and 5%, respectively. At the 800 mg/kg bw per day level, histopathologic examination revealed necrosis of the dentate gyrus of the hippocampus in all surviving male (9) and female (7) rats, skeletal muscle necrosis in 5 males, kidney nephrosis in 6 males, consisting of degeneration of the tubular epithelium. The renal lesions were non-specific and similar to age related renal disease. No adverse effects were reported at 100 mg/kg bw per day (NTP, 1989).

4.2.2.2. Benzaldehyde (No. 13). Groups of 10 male and 10 female F344/N rats were administered benzaldehyde five days per week for a period of 13 weeks by corn oil gavage at doses of 0 (control), 50, 100, 200, 400 or 800 mg/kg bw. Prior to the end of the study, 6/10 males and 3/10 females at 800 mg/kg bw per day, 1/10 female at the 400 mg/kg bw per day and one control female died. Surviving male rats at 800 mg/kg bw per day had body weights 26% lower than those of controls. At the highest dose, there was necrosis of the cerebellum and neurons of the hippocampus, forestomach hyperplasia and hyperkeratosis, liver degeneration (males only), and kidney tubular epithelial degeneration. No adverse effects

were observed at dose levels of 200 mg/kg (NTP, 1990a,b).

Groups of 5 male and 5 female Osborne–Mendel rats were maintained on a diet calculated to provide an average daily intake of 50 mg benzaldehyde/kg of food for a period of 27–28 weeks (Hagan et al., 1967). Weekly measurement of body weight and food intake revealed no significant difference between test animals and controls. At termination, hematological examinations revealed normal values. At necropsy, no differences were reported between major organ weights of test and control animals. Gross examination and histopathological examinations failed to reveal any changes related to the administration of the test substance. No adverse effects were seen at the dietary level examined (50 mg/kg of food) which was calculated to be 600 µg benzaldehyde/kg bw per day (FDA, 1993).

4.2.2.3. Benzoic acid (No. 18). Male and female Fischer 344 rats were fed 0, 1, or 2% sodium benzoate in the diet (approximately 0, 370, or 735 mg/kg bw per day for males and 0, 445, or 880 mg/kg bw per day for females) for a period of 18–24 months (Sodemoto and Enomoto, 1980). Mortality of all groups was affected by hemorrhagic pneumonia; however, there was no reported difference in mortality, growth, or food intake between treated and control rats. There was also no significant difference in tumor response in treated as compared to control rats.

4.2.2.4. Benzyl acetate (No. 3). Groups of F344 male rats were maintained on diets providing 0 (control), 20,000, 35,000, or 50,000 ppm of benzyl acetate 5 days a week for 103 weeks. These dietary levels correspond to an average daily intakes of 0, 1000, 1750, 2500 mg/kg bw of benzyl acetate (FDA, 1993). No effects on mortality or body weights of rats were reported at dietary concentrations of 20,000 ppm. Compound-related neuronal necrosis was seen in the groups receiving 35,000 or 50,000 ppm benzyl acetate. When a glycine supplement was fed in conjunction with the highest dose, mortality and signs of toxicity were significantly reduced. The authors concluded that adequate levels of glycine were instrumental in reducing the toxicity of benzyl acetate (Abdo et al., 1998).

Groups of 10 male and 10 female F344/N rats were administered 0 (control), 62.5, 125, 250, 500 or 1000 mg benzyl acetate/kg bw in corn oil daily by gavage 5 days per week for a period of 13 weeks. Male and female rats receiving 1000 mg/kg bw per day and females receiving 500 mg/kg bw per day showed signs of ataxia, trembling and sluggishness. On day 86, 2 males and one female died at the highest dose. At 1000 mg/kg bw per day, males showed depressed mean body weight (12%). At necropsy, thickened stomach walls were reported in 2 of 8 surviving males and 4 of 9 surviving

females. No adverse effects were observed at 250 mg/kg bw per day (NTP, 1986). At 1000 mg/kg bw per day, histopathologic reexamination of brains revealed hippocampal necrosis in 8 males and 4 females, the severity being greater in males (NTP, 1993a).

For a period of 13 weeks, groups of 10 male and 10 female F344/N rats were maintained on diets containing 0, 3130, 6250, 12500, 25,000, or 50000 ppm of benzyl acetate. These dietary levels were calculated to provide an average daily intake of 0 (control), 230, 460, 900, 1750, or 3900 mg benzyl acetate/kg bw for males and 0 (control), 240, 480, 930, 1870, or 4500 mg benzyl acetate/kg bw for females (FDA, 1993). At the highest dose level, 9/10 rats of each sex died before completion of the study. Final mean body weights of males and females at the highest dietary level were less than half that of the control group. Males at 1750 mg/kg bw per day showed a 10% decrease in mean body weight compared to that of controls. Food consumption in the males at 1750 and 3900 mg/kg bw and females at 4500 mg/kg bw per day was reduced compared to controls. Serum cholesterol was significantly decreased in females at the three highest dose levels. Substance-related histopathology in males and females at the highest dose level included necrosis of neurons and glial cells in the cerebellum and hippocampus, renal tubule degeneration and regeneration, and degeneration and hyperplasia of the sarcolemmal nuclei in the skeletal muscles of the thigh and in the tongue. Indirect treatment-related effects secondary to the poor condition of the animals included bone marrow depletion, thymus atrophy, degeneration of splenic lymphoid follicles, zymogen granule depletion, and increased basophilia of the pancreatic acinar cells, erosion of the glandular stomach, secretory depletion in of the seminal vesicles and immature/hypoplastic uterus. At termination, examination of females of the control, 1870 and 4500 mg/kg bw per day dose groups showed an increase in the volume, surface, and numerical density of hepatic peroxisomes in treated groups compared to those of controls. At 900 mg/kg bw per day and higher dose levels, testicular changes, including aspermatogenesis and atrophy of the seminiferous tubules were seen. No adverse effects were observed at dosages of 480 mg/kg bw per day (NTP, 1993a).

In an earlier 4-month study, no pancreatic tumors were reported in male F344 and Lewis rats administered 500 mg/kg bw per day of benzyl acetate by gavage for 5 days a week (Longnecker et al., 1986).

4.2.2.5. Benzyl butyrate (No. 5). For a period of 12 weeks, rats (unspecified strain, 12/sex/group) were maintained on a diet containing a mixture of six aromatic esters at levels calculated to provide an average daily target intake of 0 or 100 mg/kg bw per day. Six aromatic esters commonly used in foods (ethyl benzoate,

0.15 ppm; isobutyl benzoate, 25 ppm; benzyl acetate, 18.7 ppm; benzyl butyrate, 25 ppm; ethyl methylphenylglycidate, 25 ppm; and glycidate M-116, 25 ppm) were blended and incorporated in the diet in the ratio of practical use levels in foods. The blend was fed in a nutritionally adequate diet to weanling rats for the 12-week period at a level computed to be 100 times the assumed flavor intake for man (on a per kilogram of body weight basis). Throughout the experimental period the group receiving this aromatic ester blend exhibited normal body weight gain, food consumption, efficiency of food utilization, appearance, and behavior. At the end of the feeding period, blood and urine samples were taken from 3 males and 3 females. There was no significant difference between blood hemoglobin and urine glucose levels in test and control groups. Traces of albumin present in urine specimens from the control as well as the test group are not regarded as significant. At autopsy no abnormalities were observed relating to the test materials and the weights of livers and kidneys were within normal limits for both groups. These experimental data indicate that no toxic effect was manifest in rats as a result of feeding a blend of six aromatic esters in common use as flavoring agents for a 12-week period at a daily dosage level calculated to be 126 mg/kg bw per day (Oser, 1957).

4.2.2.6. Tolualdehydes—mixed o, m, p (No. 34). For a period of 90 days, groups of 15 male and 15 female weanling Sprague-Dawley rats, individually housed, were provided with a diet containing tolualdehyde at levels calculated to provide an average daily intake of 36.1 mg/kg bw. Substances were dissolved in acetone and blended into a basal laboratory diet to yield the required daily intake for the test substance. Control diet was the basal laboratory diet admixed with acetone. Dietary acetone was evaporated before presentation to the animals. Samples of the treatment diet were taken weekly for assessment of stability and concentration of the test material. Daily observations of appearance, behavior, appetite, elimination, gross signs of toxic effects, and mortality were similar among test and control animals. Weekly measurement of body weights and food consumption revealed no significant differences between test and control animals. Hematological examinations, blood chemical determinations, and urine analysis performed during weeks 6 and 12 on 8 males and 8 females revealed normal values. At necropsy, the weights of the liver and kidneys were recorded. Tissues from major organs of 8 male and 8 female rats were subsequently preserved in formalin. In addition, the livers and kidneys of the remaining 7 animals were examined for histopathological changes. There was no difference in absolute or relative organ weights between test and control animals or any evidence of gross pathology or histopathology. The dietary intake of

36.1 mg/kg bw per day produced no changes attributable to tolualdehyde (Oser et al., 1965) is greater than 1,000 times the daily *per capita* intake (“eaters only”) of 19 µg tolualdehydes/kgbw from use as a flavoring agent in USA.

Groups of 15 male and 15 female CFE rats were administered 0 (control), 50, 250, or 500 mg tolualdehyde/kg bw in corn oil daily by oral intubation for a period of 13 weeks. Additional groups of 5 rats per sex were given 0, 250, or 500 mg/kg bw daily for 2 or 6 weeks by the same method. Weekly measurement of body weights and food and water intake revealed that the female group at 500 mg/kg bw per day exhibited significantly lower body weight after 2 weeks. No body weight changes were observed in any other group regardless of the study duration (2, 6, or 13 weeks). Hematological examination, blood chemical determinations, and urine analysis performed at 2, 6, or 13 weeks revealed a transient increase in erythrocyte count, hematocrit, and hemoglobin concentration in males, but only at 2 weeks. At necropsy at week 13, measurement of organ weights revealed a significant decrease (9–18%) in absolute or relative small intestine weight in all treatment groups and decreased relative pituitary weights in females at 500 mg/kg bw per day. The magnitude of the change in mean small intestine weights was not dose related. In a second study, groups of 30 female rats were administered 0 or 500 mg/kg bw in corn oil by gavage daily for 13 weeks. There was no significant difference in absolute or relative mean small intestine weights between the control and test group in the second study or between the control group in the second study and female dosed group in the first 13-week study. The authors noted that, for some unknown reason, the small intestine weights of the control animals in the first 13-week study were abnormally high. The organ weight changes were not associated with any evidence of gross and histopathological abnormalities. The authors reported a no observable adverse effect level of 250 mg/kg bw per day (Brantom et al., 1972).

4.2.2.7. Glyceryl tribenzoate (No. 29) and Propylene glycol dibenzoate (No. 30). For a period of 90 days, 4 groups each consisting of 15 male and 15 female weanling FDRL-weanling rats, individually housed, were maintained on a diet containing glyceryl tribenzoate or propylene glycol dibenzoate at levels calculated to provide an average daily intake of 120, 604 or 2571 mg/kg bw or 126, 633, or 2,541 mg/kg bw, respectively. Daily observations of appearance, behavior, appetite, elimination, gross signs of toxic effects, and mortality were similar among test and control animals. Weekly measurement of body weights and food consumption revealed a depressed growth rate and efficiency of food utilization in high dose males in the glyceryl tribenzoate study. No significant differences in

these parameters were observed in the propylene glycol dibenzoate study. In either study, hematological examinations, blood chemical determinations, and urine analysis performed during weeks 6 and 12 on 8 males and 8 females from each group revealed normal values. At necropsy, the weights of the liver and kidneys were recorded. Tissues from major organs of 8 male and 8 female rats were subsequently preserved in formalin. Histopathologic examinations were made on hematoxylin and eosin-stained sections. In addition, the livers and kidneys of the remaining seven animals were examined for histopathological changes. There was no difference in absolute or relative organ weights between test and control animals or any evidence of gross pathology or histopathology in either study. Based on the depressed weight gain in high dose males, the no observable adverse effect level for glyceryl tribenzoate was reported to be 604 mg/kg bw per day (Carson, 1972a). This dose is greater than 100 times the daily *per capita* intake of 1 µg/kg bw from use of glyceryl tribenzoate as a flavoring substance in the USA. The NOAEL of 2541 mg/kg bw per day is greater than 100,000 times the daily *per capita* intake (“eaters only”) of 0.2 µg propylene glycol dibenzoate/kg bw from use as a flavoring ingredient in the USA.

4.2.2.8. 2,4-Dimethylbenzaldehyde (No. 37). Groups of 5 male and 5 female rats were administered 0 (control), 0.175 or 1.75 mg 2,4-dimethylbenzaldehyde/kg bw by stomach tube daily, 6 days per week for a period of 14 days. At 1.75 mg/kg bw per day, male rats showed an increased relative liver weight. Female rats showed no significant difference in relative liver weight at the same dose level. Histopathological examination of the liver and kidneys revealed no abnormalities associated with the administration of dimethylbenzaldehyde (DeGroot et al., 1974).

4.3. Long-term studies of toxicity and carcinogenicity

The results of six long-term toxicity and carcinogenicity studies with 4 representative benzyl derivatives are summarized in Table 2 and described below.

4.3.1. National toxicology program studies

4.3.1.1. Benzyl alcohol (No. 1), benzaldehyde (No. 13), benzyl acetate (No. 3). The National Toxicology Program (NTP) conducted four long-term toxicity and carcinogenicity studies on three members of this group: benzyl alcohol, benzaldehyde and benzyl acetate (a gavage and a microencapsulation study). Based on extensive metabolic data that benzyl acetate is readily hydrolyzed to benzyl alcohol *in vivo*, and benzyl alcohol is rapidly oxidized to benzaldehyde *in vivo*, the results of all four 2-year bioassay studies are reviewed together.

Each of the three test compounds were administered in corn oil gavage five days per week to groups of 50 F344/N rats and B6C3F₁ mice for a period of two years at two dose levels. An additional study was conducted in which test groups of 60 rats or mice were maintained on diets containing benzyl acetate. Animals were observed once weekly for 12–13 weeks and at least monthly thereafter. All animals were subject to necropsy after death, or at the end of the study. Throughout these studies, mean body weights were comparable among all groups, except for rats and mice maintained on the high dose level of benzyl acetate in the feed, which was reduced (5–16%) due to decreased feed consumption. Increased mortality seen in some of the groups was attributable to the gavage procedure, reflux and aspiration of the gavage material into the lungs, or administration errors resulting in direct disposition of material into the lungs (NTP, 1986, 1989, 1990a,b, 1993b).

4.3.1.2. Results and discussion

4.3.1.2.1. *Benzyl alcohol (No. 1) and benzaldehyde (No. 13)*. According to the NTP, “under conditions of the 2 year gavage study, there was no evidence of carcinogenic activity of benzyl alcohol in male or female F344/N rats or B6C3F₁ mice receiving either 200 or 400 mg/kg bw.”

NTP also concluded “under conditions of the 2 year gavage studies, there was no evidence of carcinogenic activity for male or female F344/N rats receiving 200 or 400 mg/kg bw/d benzaldehyde. However, there was some evidence of carcinogenic activity of benzaldehyde in B6C3F₁ mice at 300 or 600 mg/kg bw, as indicated by increased incidences of squamous cell papillomas and hyperplasia of the forestomach.” (NTP, 1989, 1990a).

The occurrence of squamous cell papillomas and forestomach hyperplasia in rodents is common in NTP bioassay gavage studies in which a high concentration of an irritating material in corn oil is delivered daily by tube into the forestomach for two years. High concentrations of aldehydes (i.e. malonaldehyde, furfural, and benzaldehyde) (NTP, 1988, 1990a,b) and other irritating substances (i.e. dihydrocoumarin and coumarin) (NTP, 1992, 1993b) delivered in corn oil by gavage are consistently associated with these phenomena in the forestomach of rodents. Squamous cell papillomas are benign lesions of surfaces covered with squamous epithelium. A majority of papillomas arise as a result of chronic irritation, or less frequently from infection from some strains of viruses (Smith and Ford, 1993). Additionally, forestomach hyperplasia and papillary proliferation in these studies did not progress to squamous cell carcinomas.

The relevance of the appearance of forestomach tumors in rodents to potential carcinogenic targets in humans has been the subject of much investigation (Grice, 1988; Wester and Kroes, 1988; Clayson et al.,

1990). Although it has been suggested that the mucosa of the rodent forestomach is similar to that of the human esophagus, this is clearly not the case. The rodent forestomach has a storage function and contains mucosa of keratinizing squamous epithelium that is constantly exposed to the strong acid medium of the gastric contents. Conversely, the esophagus has no storage capacity and contains non-keratinizing squamous epithelium that is extremely sensitive to the adverse effects of strong acid medium. The esophagus has no significant contact with food contents in that it is a muscle that exerts a motive action on food contents propelling them from the pharynx to the stomach.

Apparently, the combination of daily introduction of a dosing tube into the forestomach and delivery of high concentrations of an irritating test material in corn oil, which itself is a mild irritant and mitogen, was the likely source of the papillomas in the rodent forestomach. This conclusion is supported by the observation that the occurrence of squamous cell papillomas and forestomach hyperplasia in gavage administration of a test material in corn oil for 2 years (NTP, 1986) disappear when the same substance is administered at similar intake levels in the diet (NTP, 1993a). Therefore, the appearance of these benign lesions in the 2-year rodent bioassay has no relevance to humans, given that human exposure occurs when low levels of benzaldehyde are consumed in the diet.

4.3.1.2.2. *Benzyl acetate (No. 3)*. In the first 2-year bioassay, F344/N rats and B6C3F₁ mice received benzyl acetate in corn oil by gavage daily, five days per week, for a period of 103 weeks at doses of 0 (control), 250, or 500 mg/kg bw and 0 (control), 500 or 1000 mg/kg bw, respectively. The NTP concluded: “Under conditions of the gavage studies, benzyl acetate administration was associated with increased incidence of acinar cell adenoma of the exocrine pancreas in male F344/N rats. No evidence of carcinogenicity was found for female F344/N rats. For male and female B6C3F₁ mice there was evidence of carcinogenicity, in that benzyl acetate caused an increased incidence of hepatocellular neoplasms particularly adenoma, and squamous cell neoplasms of the forestomach.” (NTP, 1986).

In the subsequent benzyl acetate dietary study, F344/N rats and B6C3F₁ mice were maintained on diets containing 0 (control), 3000, 6000, or 12,000 ppm and 0 (control), 330, 1000, or 3000 ppm, respectively, for 103 weeks. This corresponds to 0, 130, 260, or 510 mg/kg bw and 0, 145, 290, or 575 mg/kg bw for male and female rats, respectively, and 0, 35, 110 or 345 mg/kg bw and 0, 40, 130 or 375 mg/kg bw for male and female mice, respectively. In this study NTP concluded: “under conditions of these 2-year feed studies, there was no evidence of carcinogenic activity of benzyl acetate in male or female F344/N rats receiving 3000, 6000, or 12,000 ppm. There was no evidence of carcinogenic

activity of benzyl acetate in male or female B6C3F1 mice receiving 330, 1000, or 3000 ppm.” (NTP, 1993a).

Upon completion of the second study, the NTP concluded that the increased incidence of pancreatic acinar cell neoplasms specific to male rats reported in the earlier study was probably due to the use of corn oil as a vehicle in the gavage study. Administration of high levels of fat to experimental animals has been shown to enhance the development of spontaneous and chemical-induced neoplasms (NTP, 1994a,b). More specifically, administration of corn oil and other high-fat vehicles by gavage daily at doses of 2.5, 5, or 10 ml/kgbw for 2 years was associated with an increased incidence of proliferative lesions of the exocrine pancreas (NTP, 1994a).

The increased incidence of hepatocellular adenomas observed in the gavage study was not observed in the dietary study. The NTP proposed that the difference was due to the different mode of administration and the resulting higher plasma levels of benzyl acetate metabolites (benzoic acid) in the gavage study (NTP, 1993a). In a comparative toxicokinetic study of gavage and dietary mode of administration discussed earlier (Yuan et al., 1995), peak plasma concentrations of benzoic acid were higher in the gavage study than those in the dietary study. Although daily dietary intake level and gavage dose levels were similar, gavage administration saturated the benzoic acid elimination pathway. Hippuric acid plasma concentrations were similar indicating depletion of the glycine pool in the gavage study with concomitant increases in free plasma benzoic acid. The authors suggested that higher plasma levels of benzyl acetate and free benzoic acid in the gavage study may be, in part, associated with the different toxicological outcomes of the gavage and dietary studies. The conclusion that high levels of free benzoic acid is associated with hepatocellular adenomas is highly unlikely, given the fact that chronic administration of high dietary levels of benzoic acid (greater than 1% in the diet) failed to induce hepatocellular neoplasms in lifetime mice studies (Toth, 1984). However, there is evidence that the incidence of hepatocellular adenomas in the gavage study were the result of chronic exposure to high levels of the corn oil vehicle (Haseman et al., 1985).

Statistical analysis of liver adenomas is suspect because the vehicle control animals had an aberrantly low incidence when compared to historical controls. Control values for liver adenomas in NTP bioassays vary from 20% to as high as 42%. In this study, the liver adenoma rates were zero. If these historical controls are adopted for comparison, the statistical significance of the adenoma finding in the high dose animals disappears or becomes marginal ($p = 0.038$ for females, and $p = 0.078$ for males). Procedural concerns include the fact that the diets were not tested for contaminants

and there are interpretive problems with corn oil gavage and tumor occurrence (NTP, 1986; Bernard, 1983).

In conclusion, evidence of carcinogenicity in the gavage benzaldehyde and benzyl acetate studies are associated with the repeated gavage administration of high dose levels of test substance in a corn oil vehicle or a statistical anomaly. The above observations strongly suggest that the tumors found in the gavage NTP studies have no relevance to human carcinogenic risk.

4.4. Genotoxicity studies

Genotoxicity testing has been performed on 13 representative compounds in this group. The vast majority of standardized in vitro genotoxicity assays Ames (AMS), Mouse Lymphoma (MLA), Sister Chromatid Exchange (SCE), Chromosomal Aberration (ABS), and Unscheduled DNA Synthesis (UDS) show no evidence of genotoxicity. Equivocal results have been reported mainly for aromatic aldehydes in the MLA and ABS assays. None of the 13 in vivo assays produced any evidence of genotoxicity for these benzyl derivatives. The results of these tests are presented in Table 3 and are summarized below.

4.4.1. In vitro

The benzyl derivatives were non-mutagenic in all standard plate incorporation and/or pre-incubation Ames assays using *Salmonella typhimurium* strains TA92, TA94, TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA2637, when tested at concentrations ranging up to the level of cytotoxicity or at ICH/OECD-recommended maximum test concentrations, both in the absence and presence of metabolic activation (S9 fraction) (FDA, 1975; McCann et al., 1975; Milvy and Garro, 1976; Cotruvo et al., 1977; Anderson and Styles, 1978; Sasaki and Endo, 1978; Rockwell and Raw, 1979; Florin et al., 1980; Rapson et al., 1980; Kasamaki et al., 1982; Haworth et al., 1983; Ball et al., 1984; Ishidate et al., 1984; Marnett et al., 1985; Nohmi et al., 1985; Mortelmans et al., 1986; Rogan et al., 1986; Schunk et al., 1986; Zeiger et al., 1988; Aeschbacher et al., 1989; Heck et al., 1989; NTP, 1989, 1990a,b; Vamvakas et al., 1989; Dillon et al., 1992; Zeiger et al., 1992). Additional Ames tests on metabolites isolated from the urine of rats administered benzaldehyde (No. 13), isopropylbenzyl alcohol (No. 32), or cuminaldehyde (No. 36) by oral gavage also were negative in *S. typhimurium* strains TA98 and TA100, both with and without metabolic activation (Rockwell and Raw, 1979). A mutation assay in *S. typhimurium* strain TA1535/pSK1002, using *umu* gene expression as an endpoint, produced negative results with benzoic acid (No. 18) (Nakamura et al., 1987). Mutation or DNA repair assays using *Escherichia coli* strains WP2 *uvrA*, PQ37, or Sd-4-73

Table 3

#	Substance name	Test system in vitro	Test object	Maximum concentration of substance	Result	Reference
<i>In vitro genotoxicity studies on benzyl derivatives</i>						
1	Benzyl alcohol	Ames test ¹	<i>Salmonella typhimurium</i> TA92, TA94, TA98, TA100, TA1535, and TA1537	10,000 µg/plate	Negative ²	Ishidate et al. (1984)
1	Benzyl alcohol	Ames test ³	<i>S. typhimurium</i> TA100	1,000 µg/plate	Negative ⁴	Ball et al. (1984)
1	Benzyl alcohol	Ames test ³	<i>S. typhimurium</i> TA98, TA100	Dose not reported	Negative ⁴	Rogan et al. (1986)
1	Benzyl alcohol	Ames test ¹	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	6,666 µg/plate	Negative ²	Mortelmans et al. (1986)
1	Benzyl alcohol	Ames test ³	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	3 µmole/plate	Negative ²	Florin et al. (1980)
1	Benzyl alcohol	Ames test ³	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	50,000 µg/plate	Negative ²	Heck et al. (1989)
1	Benzyl alcohol	Ames test ³	TA1535, TA1537, and TA1538	5 µl/plate	Negative ⁴	Milvy and Garro (1976)
1	Benzyl alcohol	Ames test ³	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	6,666 g/plate	Negative ^{2,5}	NTP (1989)
1	Benzyl alcohol	Mutation	<i>Escherichia coli</i> WP2 uvrA	8.0 mg/plate	Negative ^{6,7}	Yoo (1986)
1	Benzyl alcohol	Rec assay	<i>B. subtilis</i> H17 and M45	21 µg/disk	Negative ⁶	Oda et al. (1979)
1	Benzyl alcohol	Rec assay	<i>B. subtilis</i> H17 and M45	10 µg/disk	Positive ^{6,8}	Kuroda et al. (1984a)
1	Benzyl alcohol	Rec assay	<i>B. subtilis</i> H17 and M45	20 µl/disk	Positive ^{6,7}	Yoo (1986)
1	Benzyl alcohol	Chromosome aberration	Chinese hamster fibroblast cells	1.0 mg/ml	Negative ^{4,9}	Ishidate et al. (1984)
1	Benzyl alcohol	Chromosome aberration	Chinese hamster ovary cells	5,000 µg/ml	Equivocal ^{2,10,11}	Anderson et al. (1990)
1	Benzyl alcohol	Chromosome aberration	Chinese hamster ovary cells	5,000 µg/ml	Positive ^{2,12}	NTP (1989)
1	Benzyl alcohol	Sister chromatid exchange (SCE)	Chinese hamster ovary cells	5,000 µg/ml	Weakly Positive ¹³	NTP (1989)
1	Benzyl alcohol	SCE	Chinese hamster ovary cells	5,000 µg/ml	Weak Positive ^{2,11,14}	Anderson et al. (1990)
1	Benzyl alcohol	Mutation	L5178Y mouse lymphoma cells	5,000 g/ml	Questionable ^{15,11}	McGregor et al. (1988); Myhr et al., 1990
1	Benzyl alcohol	Mutation	L5178Y Mouse Lymphoma Cells	4,500 µg/ml	Positive ^{2,16}	NTP (1989)
1	Benzyl alcohol	Mutation	<i>E. coli</i> WP2 uvrA	Dose not reported	Negative ¹⁷	Kuroda et al. (1984b)
1	Benzyl alcohol	Cytotoxicity	Human alveolar tumor cells	0.5 mM	Negative	Waters et al. (1982)
1	Benzyl alcohol	DNA damage	Human alveolar tumor cells	0.5 mM	Negative	Waters et al. (1982)
1	Benzyl alcohol	DNA damage	Rat hepatocytes	10 mM	Negative ¹⁸	Storer et al. (1996)
1	Benzyl alcohol	DNA damage	<i>E. coli</i> P3478	50 µl/disk	Negative ²	Fluck et al. (1976)
2	Benzyl formate	Rec assay	<i>B. subtilis</i> H17 and M45	20 µl/disk	Positive ^{6,7}	Yoo (1986)
2	Benzyl formate	Mutation	<i>E. coli</i> WP2 uvrA	4.0 mg/plate	Negative ^{6,7}	Yoo (1986)
3	Benzyl acetate	Ames test ¹	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	10 mg/plate	Negative ²	Mortelmans et al. (1986)
3	Benzyl acetate	Ames test ^{1,3}	<i>S. typhimurium</i> TA98, TA100	5,000 µg/plate	Negative ^{2,19}	Schunk et al. (1986)
3	Benzyl acetate	Ames test ²⁰	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	3 µmol/plate	Negative ²	Florin et al. (1980)
3	Benzyl acetate	Rec assay	<i>B. subtilis</i> H17 and M45	21 µg/disk	Negative ^{2,6}	Oda et al. (1979)

3	Benzyl acetate	Rec assay	<i>B. subtilis</i> H17 and M45	20 µl/disk	Positive ^{6,7}	Yoo (1986)
3	Benzyl acetate	Mutation	<i>E. coli</i> WP2 uvrA	2.0 mg/plate	Negative ^{6,7}	Yoo (1986)
3	Benzyl acetate	Mutation	Mouse lymphoma L5178Y cells and Human lymphoblast TK6 cells	Mouse cells = 500 µg/ml Human cells = 1,500 µg/ml	Positive ^{2,12}	Casparly et al. (1988)
3	Benzyl acetate	Mutation	Mouse lymphoma L5178Y cells	1,600 µL/mL	Positive ^{2,18}	McGregor et al. (1988)
3	Benzyl acetate	Mutation	Mouse lymphoma L5178Y cells	Not reported	Positive ^{2,12}	Rudd et al. (1983)
3	Benzyl acetate	Chromosome aberration	Chinese hamster ovary cells	5,000 µg/ml	Negative ²	Galloway et al. (1987)
3	Benzyl acetate	Chromosome aberration	Chinese hamster lung fibroblast cells	2.4 mg/ml	Negative ^{2,18}	Matsouka et al. (1996)
3	Benzyl acetate	SCE	Chinese hamster ovary cells	5,000 µg/ml	Negative ²	Galloway et al. (1987)
3	Benzyl acetate	Unscheduled DNA synthesis	Rat hepatocytes	Not reported	Negative ¹⁷	Mirsalis et al. (1983)
4	Benzyl propionate	Rec assay	<i>B. subtilis</i> H17 and M45	21 µg/disk	Negative ^{4,6}	Oda et al. (1979)
11	Benzyl benzoate	Ames test ²⁰	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	3 µmol/plate	Negative ²	Florin et al. (1980)
11	Benzyl benzoate	Ames test ^{1,3}	<i>S. typhimurium</i> TA98, TA100	5,000 µg/plate	Negative ^{2,19}	Schunk et al. (1986)
13	Benzaldehyde	Ames test ³	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, and TA1538	37,500 µg/plate	Negative ²	Heck et al. (1989)
13	Benzaldehyde	Ames test ³	<i>S. typhimurium</i> TA98, TA100	300 µl/plate	Negative ^{2,21}	Rockwell and Raw (1979)
13	Benzaldehyde	Ames assay ³	<i>S. typhimurium</i> TA98 and TA100	100 µl/plate	Negative ²²	Rockwell and Raw (1979)
13	Benzaldehyde	Ames test ²⁰	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	2.0 mg/plte	Negative ^{2,6}	Nohmi et al. (1985)
13	Benzaldehyde	Ames test ²⁰	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	3.0 µmol/plate	Negative ²	Florin et al. (1980)
13	Benzaldehyde	Ames test ¹	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	1,000 µg/plate	Negative ²	Haworth et al. (1983)
13	Benzaldehyde	Ames test ²⁰	<i>S. typhimurium</i> TA100, TA102 and TA104	3,333 µg/plate	Negative ²	NTP (1990a, 1990b)
13	Benzaldehyde	Ames test ²⁰	<i>S. typhimurium</i> TA100	1.0 mg/plate	Negative ⁷	Rapson et al. (1980)
13	Benzaldehyde	Ames test ¹	<i>S. typhimurium</i> TA98, TA100	Not reported	Negative ²	Sasaki and Endo (1978)
13	Benzaldehyde	Ames test ¹	<i>S. typhimurium</i> TA100, TA102 and TA104	Not reported	Negative ²	Dillon et al. (1992)
13	Benzaldehyde	Ames test ¹	<i>S. typhimurium</i> TA100	2,000 nmol/plate	Negative ²	Vamvakas et al. (1989)
13	Benzaldehyde	Ames test ²⁰	<i>S. typhimurium</i> TA98, TA100	500 µg/plate	Negative ²	Kasamaki et al. (1982)
13	Benzaldehyde	Rec assay	<i>B. subtilis</i> H17 and M45	21 µg/disk	Negative ^{6,7}	Oda et al. (1979)
13	Benzaldehyde	Rec assay	<i>B. subtilis</i> H17 and M45	Not reported	Positive ^{2,12}	Matsui et al. (1989)
13	Benzaldehyde	Unscheduled DNA synthesis (UDS)	Rat hepatocytes	251 µg/ml	Negative ²	Heck et al. (1989)
13	Benzaldehyde	Mutation	Mouse L5178Y lymphoma cells	600 µg/ml	Positive ¹²	Heck et al. (1989)
13	Benzaldehyde	Mutation	Mouse L5178Y lymphoma cells	800 µg/ml	Positive ^{4,23}	McGregor et al. (1991)
13	Benzaldehyde	Chromosomal aberrations	Chinese hamster cells	1.2 mg/ml	Positive ^{6,16,24,25}	Sofuni et al. (1985)

Table 3 (continued)

#	Substance name	Test system in vitro	Test object	Maximum concentration of substance	Result	Reference
<i>In vitro genotoxicity studies on benzyl derivatives</i>						
13	Benzaldehyde	Chromosomal aberrations	Chinese hamster ovary cells	1,600 µg/ml	Negative ²	Galloway et al. (1987)
13	Benzaldehyde	Chromosomal aberrations	Chinese hamster cells	50 nM	Positive ²	Kasamaki et al. (1982)
13	Benzaldehyde	SCE	Chinese hamster ovary cells	1,600 µg/ml	Positive ²	Galloway et al. (1987)
13	Benzaldehyde	SCE	Chinese hamster ovary cells	1,000 µM	Negative ^{2,18}	Sasaki et al. (1989)
13	Benzaldehyde	SCE	Human lymphocytes	2.0 mM	Positive ⁴	Jansson et al. (1988)
18	Benzoic acid	Ames test ³	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1538	2,500 µg/plate	Negative ²	Anderson and Styles (1978)
18	Benzoic acid	Ames test ²⁰	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1536	3.6 µg/plate	Negative ²	Cotruvo et al. (1977)
18	Benzoic acid	Ames test ¹	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535, and TA1537	10 mg/plate	Negative ²	Zeiger et al. (1988)
18	Benzoic acid	Ames test ²⁰	<i>S. typhimurium</i> TA100	1.0 mg/plate	Negative ⁷	Rapson et al. (1980)
18	Benzoic acid	Ames test ³	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	1.0 mg/plate	Negative ²²	McCann et al. (1975)
18	Benzoic acid	Ames test ¹	<i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, and TA1537	10.0 mg/plate	Negative ²	Ishidate et al. (1984)
18	Benzoic acid	Ames test ³	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1538	100 µg/plate	Negative ⁴	Milvy and Garro (1976)
18	Benzoic acid	Ames test ³	<i>S. typhimurium</i> TA1535, TA1537, and TA1538	0.5%	Negative ²	FDA (1975)
18	Benzoic acid	Mutation	<i>S. typhimurium</i> TA1535/psK1002	1.67 mg/ml	Negative ^{2,26}	Nakamura et al. (1987)
18	Benzoic acid	Rec assay	<i>B. subtilis</i> H17 and H45	Not reported	Positive ¹⁷	Nonaka (1989)
18	Benzoic acid	Mutation	Saccharomyces cerevisiae D3	0.18%	Negative ²	Cotruvo et al. (1977)
18	Benzoic acid	Mutation	<i>S. cerevisiae</i> D4	0.15%	Negative ²	FDA (1975)
18	Benzoic acid	Indirect DNA repair	<i>E. coli</i> PQ37	400 µg/ml	Negative ²⁷	Glosnicka and Dziadziuszko (1986)
18	Benzoic acid	Chromosome aberration test	Chinese hamster fibroblast cells	1.5 mg/ml	Weak Positive ^{4,28}	Ishidate et al. (1984)
18	Benzoic acid	SCE	Human lymphocytes	2.0 mM	Negative ⁴	Jansson et al. (1988)
19	Methyl benzoate	Ames test ¹	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535, and TA1537	6,666 µg/plate	Negative ²	Zeiger et al. (1992)
19	Methyl benzoate	Mutation	<i>E. coli</i> Sd-4-73	Dose not reported	Negative ⁴	Szybalski (1958)
25	Isoamyl benzoate	Mutation	<i>E. coli</i> Sd-4-73	Dose not reported	Negative ⁴	Szybalski (1958)
32	Isopropylbenzyl alcohol	Ames assay ³	<i>S. typhimurium</i> TA98 and TA100	100 µl/plate	Negative ²²	Rockwell and Raw (1979)
32	Isopropylbenzyl alcohol	Ames assay ³	<i>S. typhimurium</i> TA98 and TA100	300 µl/plate	Negative ^{2,29}	Rockwell and Raw (1979)
34	Tolualdehydes (mixed <i>o</i> , <i>m</i> , <i>p</i>)	Ames test ¹	<i>S. typhimurium</i> TA104	0.8 µmoles/plate	Negative ²	Marnett et al. (1985)

34	Tolualdehydes (mixed <i>o</i> , <i>m</i> , <i>p</i>)	Ames test ²⁰	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537	3 µmol/plate	Negative ²	Florin et al. (1980)
34	Tolualdehydes (mixed <i>o</i> , <i>m</i> , <i>p</i>)	Ames test ³	<i>S. typhimurium</i> TA98, TA100, TA1535, TA 1537, and TA1538	18,750 µg/plate	Negative ²	Heck et al. (1989)
34	Tolualdehydes (mixed <i>o</i> , <i>m</i> , <i>p</i>)	Ames test ³	<i>S. typhimurium</i> TA98, TA100 and TA102	0.8 mmol/plate	Negative ²	Aeschbacher et al. (1989)
34	Tolualdehydes (mixed <i>o</i> , <i>m</i> , <i>p</i>)	Ames test ¹	<i>S. typhimurium</i> TA97, TA100, TA1535, and TA1537	666 µg/plate	Negative ²	Zeiger et al. (1988)
34	Tolualdehydes (mixed <i>o</i> , <i>m</i> , <i>p</i>)	UDS	Rat hepatocytes	1,000 µg/ml	Negative ²	Heck et al. (1989)
34	Tolualdehydes (mixed <i>o</i> , <i>m</i> , <i>p</i>)	Mouse Lymphoma Mutation Assay	L5178Y mouse lymphoma cells	300 g/ml	Negative ²	Heck et al. (1989)
36	Cuminaldehyde	Ames assay ³	<i>S. typhimurium</i> TA98 and TA100	100 µl/plate	Negative ²²	Rockwell and Raw (1979)
36	Cuminaldehyde	Ames assay ³	<i>S. typhimurium</i> TA98 and TA100	300 µl/plate	Negative ^{2,30}	Rockwell and Raw (1979)
36	Cuminaldehyde	SCE	Chinese hamster ovary cells	333 µM		
<i>In vitro</i> antimutagenicity studies on benzyl derivatives						
1	Benzyl alcohol	Antimutagenesis	<i>E. coli</i> WP2 uvrA	2.5 mg/ml	Positive Antimutagenic Effect ^{6,31}	Yoo (1986)
1	Benzyl alcohol	Antimutagenesis	<i>E. coli</i> WP2 uvrA	1,000 µg/ml	Negative Antimutagenic Effect ^{6,32}	Yoo (1986)
1	Benzyl alcohol	Antimutagenesis	<i>E. coli</i> WP2 uvrA	Dose not reported	Positive Antimutagenic Effect ³¹	Kuroda et al. (1984b)
2	Benzaldehyde	Antimutagenesis	<i>E. coli</i> WP2 uvrA	300 µg/ml	Negative ³³ Antimutagenic Effect	(Ohta et al., 1983)
2	Benzyl formate	Antimutagenesis	<i>E. coli</i> WP2 uvrA	8.0 mg/ml	Negative Antimutagenic Effect ^{6,31}	Yoo (1986)
3	Benzyl acetate	Antimutagenesis	<i>E. coli</i> WP2 uvrA	8.0 mg/plate	Negative Antimutagenic Effect ^{6,31}	Yoo (1986)
13	Benzaldehyde	Antimutagenesis	<i>E. coli</i> PQ37	500 µg/ml	Negative ³⁴ Antimutagenic effect	(Ohta et al., 1986a)
20	Ethyl benzoate	Antimutagenesis	<i>E. coli</i> PQ37	500 µg/ml	Negative Antimutagenic Effect ³⁴	(Ohta et al., 1986a)
20	Ethyl benzoate	Antimutagenesis	<i>S. typhimurium</i> TA98	200 µg/ml	Negative Antimutagenic Effect ³⁵	(Ohta et al., 1986b)
20	Ethyl benzoate	Antimutagenesis	<i>E. coli</i> WP2s	200 µg/ml	Negative antimutagenic effect	(Ohta et al., 1986b)

Table 3 (continued)

#	Substance name	Test system in vitro	Test system in vivo	Test object	Maximum concentration of substance	Result	Reference
<i>In vitro genotoxicity studies on benzyl derivatives</i>							
<i>In vivo genotoxicity studies on benzyl derivatives</i>							
1	Benzyl alcohol	Sex-linked recessive lethal mutations (SLRL)		<i>Drosophila melanogaster</i>	5000 ppm	Negative ³⁶	Fourman et al. (1994)
1	Benzyl alcohol	SLRL		<i>Drosophila melanogaster</i>	8000 ppm	Negative ³⁷	Fourman et al. (1994)
1	Benzyl alcohol	Micronucleus test		Mouse bone marrow cells	200 mg/kg bw	Negative ³⁸	Hayashi et al. (1988)
1	Benzyl alcohol	Replicative DNA synthesis test		Mouse hepatocytes	Not reported	Positive ³⁹	Yoshikawa (1996)
3	Benzyl acetate	SLRL		<i>Drosophila melanogaster</i>	300 ppm	Negative ³⁶	NTP (1993a); Fourman et al. (1994)
3	Benzyl acetate	SLRL		<i>Drosophila melanogaster</i>	20,000 ppm	Negative ³⁷	NTP (1993a); Fourman et al. (1994)
3	Benzyl acetate	SCE		Mouse bone marrow cells	1,700 mg/kg bw	Negative ³⁸	NTP (1993a)
3	Benzyl acetate	Chromosome aberration		Mouse bone marrow cells	1,700 mg/kg bw	Negative ³⁸	NTP (1993a)
3	Benzyl acetate	Micronucleus test		Mouse bone marrow cells	1,250 mg/kg bw	Negative ³⁸	NTP (1993a); Shelby et al. (1993)
3	Benzyl acetate	Micronucleus test		Mouse erythrocytes	50,000 ppm	Negative ³⁸	NTP (1993a)
3	Benzyl acetate	UDS		Rat hepatocytes	Not reported	Negative ^{17,40}	Mirsalis et al. (1983)
3	Benzyl acetate	UDS		Rat hepatocytes	1,000 mg/kg bw	Negative ⁴⁰	Mirsalis et al. (1989)
3	Benzyl acetate	UDS		Rat hepatocytes	1,000 mg/kg bw	Negative ⁴⁰	Steinmetz and Mirsalis (1984)
3	Benzyl acetate	DNA damage		Rat pancreatic cells	1,000 mg/kg bw	Negative ⁴⁰	Longnecker et al. (1990)
3	Benzyl acetate	DNA damage		Rat pancreatic cells	500 mg/kg bw	Negative ³⁶	Longnecker et al. (1990)
13	Benzaldehyde	SLRL		<i>Drosophila melanogaster</i>	0.9%	Negative ³⁶	Woodruff et al. (1985)
13	Benzaldehyde	SLRL		<i>Drosophila melanogaster</i>	1,150 ppm	Negative ³⁶	Woodruff et al. (1985)
13	Benzaldehyde	SLRL		<i>Drosophila melanogaster</i>	2,500 ppm	Negative ³⁷	Woodruff et al. (1985)

¹ Preincubation method.² Assay was performed with and without metabolic activation.³ Plate incorporation method.⁴ Assay was performed without metabolic activation.⁵ Cytotoxicity was reported at the highest concentration tested.⁶ Japanese article. English summary.⁷ Not reported if assay was performed with or without metabolic activation.⁸ Inhibition of growth was reported when assay was performed without metabolic activation.⁹ Cells were exposed for 48 h.²⁹ Assay of urine samples from rats given isopropylbenzyl alcohol by oral gavage.¹⁰ Positive results were not reproducible.¹¹ No dose-response observed.¹² Positive results reported only with metabolic activation.¹³ Dose response at concentrations from 500 to 1250 µg/ml (without metabolic activation).¹⁴ No dose response observed. Increase in SCE at single doses.¹⁵ Positive and negative responses could not be reproduced.¹⁶ Positive result reported only in absence of metabolic activation.¹⁷ Abstract; methods and test concentrations were not reported.¹⁸ Cytotoxicity was observed at the maximum dose tested.¹⁹ Cytotoxicity observed at 3 maximum concentrations.

²⁰ Mutagenesis induced by 2-amino-3-methyl-imidazo[3,4-f]quinoline (IQ), 2-amino-3, 4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-amino-3, 8-dimethylimidazo[4,5-f]quinoxaline HCl (MeIQx), 3-amino-4-dimethyl-5-H-pyrido-[4,3-b]indole (Trp-P-1), 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine HCl (PhIP).

²¹ Assay of urine samples from rats given benzaldehyde by oral gavage.

²² Assay was performed with metabolic activation.

²³ Significant increases in mutant fraction were close to toxic doses.

²⁴ Weak positive results were observed with metabolic activation.

²⁵ Cytotoxicity was observed at the two maximum concentrations tested.

²⁶ Assay measured induction of *umu* gene expression as indicator of genotoxicity.

²⁷ Genotoxicity measured as ability to induce β -galactosidase.

²⁸ Total incidence of cells with aberrations was 5.0–9.0%.

²⁹ Assay of urine samples from rats given isopropylbenzyl alcohol by oral gavage.

³⁰ Assay of urine samples from rats given cuminaldehyde by gavage.

³¹ Mutagenesis induced by furofuramide (AF-2).

³² Mutagenesis induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG).

³³ Mutagenesis induced by 4-nitroquinoline 1-oxide and UV irradiation.

³⁴ Mutagenesis induced by UV irradiation.

³⁵ Mutagenesis induced by 3-amino-1-methyl-5H-pyrido[4,3-b]indole or 2-amino-3-methyl-imidazo[4,5-f]quinoline.

³⁶ Feeding study.

³⁷ Dose administered by injection.

³⁸ Administered by intraperitoneal injection.

³⁹ Route of administration not reported.

⁴⁰ Administered by oral gavage.

with eight different benzyl derivatives (see Table 3) (Szybalski, 1958; Kuroda et al., 1984b; Glosnicka and Dziadziuszko, 1986; Yoo, 1986), and *Saccharomyces cerevisiae* strains D3 or D4 with benzoic acid (FDA, 1975; Cotruvo et al., 1977) also showed no evidence of genotoxicity. In a cytogenetics assay in *E. coli* strain P3478, benzyl alcohol did not show any activity (Fluck et al., 1976).

Equivocal results were obtained with the benzyl derivatives in the Rec DNA repair assay using *Bacillus subtilis* strains H17 and M45 (Oda et al., 1979; Sekizawa and Shibamoto, 1982; Kuroda et al., 1984a; Yoo, 1986; Matsui et al., 1989; Nonaka, 1989). Positive results only were reported for benzyl formate, while negative results were reported for benzyl propionate (No. 4) (Oda et al., 1979; Yoo, 1986). Positive and negative results for Rec assays using the same substance were apparently due to laboratory-specific factors. Yoo (1986) reported only positive findings and Oda et al. (1979) reported only negative results for the same compounds.

In vitro assays in isolated mammalian cells produced both negative and positive results. In addition to benzyl acetate, benzaldehyde exhibited evidence of mutagenicity in the forward mutation assay with L5178Y mouse lymphoma cells (MLA), both with and without metabolic activation (Rudd et al., 1983; Caspary et al., 1988; McGregor et al., 1988, 1991; Heck et al., 1989; Myhr et al., 1990). Questionable results were reported for benzyl alcohol in the MLA (McGregor et al., 1988). Toluolaldehydes (mixed) (No. 34) were negative in the MLA (Heck et al., 1989).

In cytogenetic tests, equivocal results were reported for the benzyl derivatives in the ABS assay. In the ABS assay, performed in Chinese hamster ovary and fibroblast cell lines, both positive and negative results were reported with benzyl alcohol and benzaldehyde, with the positive result usually occurring at the higher culture concentration (Kasamaki et al., 1982; Ishidate et al., 1984; Sofuni et al., 1985; Galloway et al., 1987; Anderson et al., 1990). Negative results in two separate ABS assays were reported for benzyl acetate (Galloway et al., 1987; Matsouka et al., 1996). Weak positive results were reported for benzoic acid in the ABS assay (Ishidate et al., 1984). The positive results in the ABS assays were generally obtained independently of the presence or absence of metabolic activation.

The authors of the MLA and ABS assays (Heck et al., 1989) have emphasized that the positive results in the MLA and ABS assays may be artifacts resulting from changes in culture pH and osmolality. Treatment with high dose levels of substances (e.g., reactive aldehydes and carboxylic acids) with the potential to alter acidity or osmolality may induce a significant increase in mutant frequencies or aberrations in these assays. Often the results are inconsistent with the results of other genotoxicity assays (i.e., AMS and UDS) (Heck et al., 1989).

In the SCE assay, equivocal results were reported for benzyl alcohol and benzaldehyde, in Chinese hamster ovary cell lines and in human lymphocytes (Galloway et al., 1987; Sasaki et al., 1989; Jansson et al., 1988; NTP, 1989; Anderson et al., 1990). Negative results were obtained in this assay for benzyl acetate, benzoic acid, and cuminaldehyde (No. 36) (Galloway et al., 1987; Sasaki et al., 1989; Jansson et al., 1988).

DNA damage was not observed in either rat or human cells exposed to benzyl alcohol (Waters et al., 1982; Storer et al., 1996). No unscheduled DNA synthesis (UDS) was observed in rat hepatocytes exposed to benzaldehyde, benzyl acetate, or mixed tolualdehydes (Mirsalis et al., 1983; Heck et al., 1989).

The standardized in vitro genotoxicity assays {AMS, MLA, SCE, ABS, and UDS} show no evidence of genotoxicity. Equivocal results have been reported for mainly aromatic aldehydes in the MLA and ABS assays and may be due artifacts under experimental conditions.

4.4.2. In vivo

None of the benzyl derivatives showed any evidence of genotoxicity in well-recognized in vivo assays (mouse micronucleus, sex-linked recessive lethal, and in vivo–in vitro UDS assays). In mammals, compounds were administered orally, by gavage, or by ip injection at doses that were significant fractions of the reported lethal dose levels. Benzyl acetate was the most frequently tested compound, producing negative findings in SCE, CA, micronuclei, and UDS in both rats and mice (Mirsalis et al., 1983, 1989; NTP, 1993a,b; Shelby et al., 1993; Steinmetz and Mirsalis, 1984). The micronucleus test also was negative with benzyl alcohol (Hayashi et al., 1988). Positive results were reported for benzyl alcohol in mice (dose not specified) in the Replicative DNA Synthesis Assay (Yoshikawa, 1996).

In the (sub-mammalian) sex-linked recessive lethal mutation assay in fruit flies (*Drosophila melanogaster*), negative results were obtained with benzyl alcohol, benzaldehyde, and benzyl acetate, either after feeding or administration by injection (Woodruff et al., 1985; NTP, 1993a; Foureman et al., 1994).

4.4.3. Conclusion

Based on the mainly negative results in the standardized battery {i.e., AMS (–), UDS (–), SCE (±), ABS (±), and MLA (±)} of in vitro genotoxicity assays and the uniformly negative results in well-recognized in vivo genotoxicity assays, it is concluded that the group of benzyl derivatives is not genotoxic in vivo.

4.5. Other relevant studies

4.5.1. Reproduction

4.5.1.1. *Benzyl alcohol* (No. 1). Fifty female CD-1 mice were administered 550 mg/kg bw per day benzyl

alcohol by gavage on days 6–15 of gestation. A control group of 50 mice received corn oil only. Body weight, clinical observations, and mortality were recorded daily throughout treatment and up to 3 days postpartum. All parameters tested, including gestation index, average number of live pups/litter, postnatal survival, and pup body weight, were statistically similar for the treated and control animals (York et al., 1986).

To examine the reproductive hazard of benzyl alcohol in CD-1 mice, 750 mg/kg bw per day of benzyl alcohol was administered by gavage to 50 mice on gestation days 6–13. A control group of 50 animals were given distilled water only. Maternal body weight gain and mortality, mating, gestation, numbers of live and dead pups per litter, total litter weight on days 1 and 2 postpartum, litter weight change between days 1 and 3 postpartum, and pup survival on days 1 and 3 postpartum were recorded. There was no significant difference in maternal body weight measured on days 4 and 7 of gestation between treated and control animals. However, statistically significant decreases were observed in treated females on gestation day 18 and day 3 postpartum. Maternal body weight gain during days 7–18 of gestation was also significantly lower than that of controls. Significant differences were also observed in pup body weight and weight gain, including mean pup weight per litter, mean litter weight change, and mean pup weight change (between day 1 and 3 postpartum). No differences were observed in the mating or gestation indices, the total number of resorptions, the number of live pups per litter, or in pup survival. Eighteen deaths were reported during the treatment period and they were all attributed to the treatment. One more death was reported the day after treatment was terminated (Hardin et al., 1987). It was reported in a review of this study that clinical signs of maternal toxicity were observed, including hunched posture, tremors, inactivity, prostration, hypothermia, ataxia, dyspnoea, swollen or cyanotic abdomen, and piloerection (York et al., 1986).

4.5.2. *Benzyl acetate* (No. 3)

Sperm morphology and vaginal cytology examinations (SMVCE) were developed by the National Toxicology Program, and used as a screen for reproductive toxicants. These examinations include evaluations of motility, concentration and head morphology of sperm from the caudal epididymis, and male reproductive organ weight data. At the end of the benzyl acetate 13-week dietary study (NTP, 1993a), 10 male and female mice from each dose group (0, 3, 130, 6250, 12,500, 25,000, and 50,000 ppm) were utilized for the SMVCE study. There was no effect on sperm motility, density or percent abnormality in any of the doses tested. There was no statistically significant change in the weights of the epididymis, the caudal epididymis, or the testis. The estrous cycle of the high dose female group was sig-

nificantly increased relative to the control group. There was also a significant decrease in body weight among animals in this group (Morrissey et al., 1988).

Morrissey et al. (1988) performed similar examinations on rats at the end of the benzyl acetate 13-week study (0, 3, 130, 6250, 12,500, 25,000, and 50,000 ppm) (NTP, 1993a). Benzyl acetate had no effect on any of the parameters measured.

Benzaldehyde (No. 13). Benzaldehyde (approximately 5 mg/kgbw) was administered by gavage to 10 breeding age rats every other day for a period of 32 weeks. Ten control animals received only the vehicle oil. Two pregnancies per rat were studied, one at 75 days and one at 180 days. The parameters examined included the number of pregnant females, number of offspring born, pup body weights at days 7 and 21 postpartum, and pup vitality. There was no statistically significant difference between treatment and control groups. It was reported that fewer females in the treated group became pregnant, however, no data or statistical analyses were performed, and the authors concluded that treatment did not cause a significant change in any of the parameters measured (Sporn et al., 1967).

4.6. Teratology

4.6.1. Benzyl acetate (No. 3)

Groups of 20 or more pregnant Wistar rats were administered benzyl acetate by gastric intubation at doses of 0 (control), 10, 100, 500, or 1,000 mg/kg bw from day 6–15 of pregnancy. On day 20 of pregnancy (term), they were terminated and the fetuses were examined for intrauterine death, and internal, external and skeletal malformations. Maternal parameters were comparable for all groups. At the highest dose level, fetal body weight was decreased. In addition, skeletal and internal malformations were observed at 1000 mg/kg bw per day. The authors reported that the skeletal malformations may be related to the significant decrease in fetal body weight. No increase in intrauterine death or external variations was noted at any dose level. No adverse effects were seen at or below 500 mg/kgbw per day. The results of this study show no evidence of teratogenic effects for benzyl acetate (Ishiguro et al., 1993).

4.6.2. Benzyl benzoate (No. 11)

Benzyl benzoate was fed to pregnant Wistar rats at concentrations of 0.4 and 1.0% during gestation days 0–21. These dietary levels correspond to the average daily intake of 200 and 500 mg/kg bw (FDA, 1993). There were no effects reported on the fetus relating to external, skeletal, or visceral anomalies (Morita et al., 1980).

4.6.3. Benzoic acid (No. 18)

Sodium benzoate administered by oral intubation to pregnant rats or rabbits during gestation days 6–15 or

6–18 at doses reaching 175 mg/kgbw per day or 250 mg/kgbw per day, respectively, produced no effects on maternal or fetal rats (Morgareidge, 1972). In another teratology study, sodium benzoate fed during gestation only produced adverse effects on the fetus at maternally toxic concentrations in the diet of 4% (1600 mg/kg bw/d) or higher (Onodera et al., 1978).

5. Recognition of GRASr status

The group of benzyl derivatives discussed here was determined to be generally recognized as safe (GRAS) under conditions of intended use as flavor ingredients by the FEMA Expert Panel in 1965. In 1978, the Panel evaluated the available data and affirmed the GRAS status of these flavor ingredients (GRASa). In 1993, the Panel initiated a comprehensive program to reevaluate the status of all FEMA GRAS flavor ingredients concurrent with a systematic revision of the FEMA Scientific Literature Reviews (SLRs). The group of benzyl derivatives was reaffirmed as GRAS (GRASr) based, in part, on their self-limiting properties as flavoring substances in food; their rapid absorption, metabolic detoxication, and excretion in humans and other animals; their low level of flavor use; the wide margins of safety between the conservative estimates of intake and the no-observed-adverse effect levels determined from subchronic and chronic studies and the lack of significant genotoxic and mutagenic potential. This evidence of safety is supported by the fact that the intake of benzyl derivatives as natural components of traditional foods is greater than their intake as intentionally added flavoring substances.

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Benzoic acid; CASRN 65-85-0

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the [IRIS assessment development process](#). Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the [guidance documents located on the IRIS website](#).

STATUS OF DATA FOR Benzoic acid

File First On-Line 09/07/1988

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	09/07/1988
Inhalation RfC (I.B.)	not evaluated	
Carcinogenicity Assessment (II.)	yes	08/01/1989

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — Benzoic acid

CASRN — 65-85-0

Last Revised — 09/07/1988

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of

information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.A.1. Oral RfD Summary

Critical Effect	Experimental Doses*	UF	MF	RfD
No adverse effects observed	NOAEL: 34 mg/day benzoic acid and 328 mg/day for sodium benzoate (converted to 312 mg/day benzoic acid)	1	1	4E+0 mg/kg/day
Human daily per capita intakes				
FDA, 1973; Selected Committee on Review of the GRAS List	LOAEL: none			

*Conversion Factors -- 328 mg/day sodium benzoate x [122.12 (MW benzoic acid)/144.11 (MW sodium benzoate)] = 278 mg/day benzoic acid. 278 mg/day benzoic acid from sodium benzoate + 34 mg/day benzoic acid = 312 mg/day; assuming adult human body weight of 70 kg, the exposure dose is 312 divided by 70 = 4.4 mg/kg/day.

I.A.2. Principal and Supporting Studies (Oral RfD)

FDA (Food and Drug Administration). 1973. Evaluation of the Health Aspects of Benzoic Acid and Sodium Benzoate as Food Ingredients. DHEW, Washington, DC. Report No. SCOGS-7. NTIS PB-223837/6.

Early studies (Gerlach, 1909) indicate that laboratory animals are inappropriate models for studying the toxicity of benzoic acid in humans (FDRL, 1972) (see Additional Comments). Based on data regarding the amounts of benzoic acid and sodium benzoate produced as a food preservative, FDA (1973) estimated a daily per capita intake of 0.9-34 mg for benzoic acid and 34-328 mg for sodium benzoate. At these levels, there are no reports of toxic effects in humans. These compounds have Generally Recognized as Safe (GRAS) status by FDA. Therefore, the upper ranges can be considered NOAELs for benzoic acid and sodium benzoate. In the stomach, both benzoic acid and sodium benzoate exist in their ionized form, benzoate, which is absorbed

rapidly and completely by the GI tract. Therefore, exposure to sodium benzoate is comparable to exposure to benzoic acid if molecular weight differences are corrected for; here, 328 mg sodium benzoate is equivalent to 278 mg benzoic acid. Adding 278 to the daily intake for benzoic acid of 34 mg yields a total of 312 mg benzoic acid (see Conversion Factors). If no uncertainty factor is used, the RfD is 312 mg/day for a 70 kg human or 4 mg/kg/day.

I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF — An uncertainty factor of 10 for the protection of sensitive subgroups was considered unnecessary; although reactions to benzoate and structurally related compounds do occur, an uncertainty factor of 10 would be of little value to the sensitive individuals.

MF — None

I.A.4. Additional Studies/Comments (Oral RfD)

Sodium benzoate appeared to have no maternal toxicity, fetal toxicity, or teratogenicity in mice, rats, hamsters, or rabbits when given orally (FDRL, 1972). The highest doses tested were 175.0 in mice and rats, 300.0 in hamsters, and 250.0 mg/kg/day in rabbits.

The only chronic oral data available involve administration of benzoic acid to rats and mice (Shtenberg and Ignat'ev, 1970; Ignat'ev, 1965; Marquardt, 1960). A dose of 40 mg/kg/day for 17 months was associated with decreased resistance to stress in mice and possibly with reduced food and water intake in rats after 18 months (Shtenberg and Ignat'ev, 1970). However, another report from this laboratory (Ignat'ev, 1965) indicated that 80 mg/kg/day in rats for 18 months was not associated with adverse effects on body weight, survival, or gross or microscopic pathology. If 40 mg/kg/day in mice in the study by Shtenberg and Ignat'ev (1970) is considered to be the LOAEL, application of an uncertainty factor of 1000 would result in an RfD of 0.04 mg/kg/day or 2.8 mg/day, which is near the lower end of the range of the estimated daily human exposure to benzoic acid (not including exposure to sodium benzoate). The lower RfD based on animal data is not unexpected, however, since application of uncertainty factors is intentionally conservative in the absence of human data. Since human data are available in this case, it is not appropriate to use the animal data for the RfD.

Other long-term dietary studies (Marquardt, 1960) showed decreased food intake and body weight in rats fed 1.5% benzoic acid (750 mg/kg/day); at a dose of 1.0% in the diet (50 mg/kg/day) there were no signs of toxicity or adverse reproductive effects.

Gerlach (1909) reported no externally visible effects in humans ingesting benzoic acid at 0.5-1.0 g/day for 44 consecutive days or for 82/86 or 88/92 days. Assuming a human body weight of 70

kg, this level corresponds to a dose of 14 mg/kg/day. Wiley and Bigelow (1908), however, observed irritation, discomfort, weakness, and malaise in humans given oral bolus doses of less than or equal to 1.75 g/day over a 20-day period (25 mg/kg/day). The RfD (4 mg/kg/day) is well below these doses.

I.A.5. Confidence in the Oral RfD

Study — Medium

Database — Medium

RfD — Medium

Medium confidence is placed in the FDA (1973) estimate of per capita intake. Medium confidence in the database reflects the inappropriateness of using animal data as the basis of the RfD for humans and the lack of reported effects in humans at the estimated intakes. Thus, confidence in the RfD is medium.

I.A.6. EPA Documentation and Review of the Oral RfD

Source Document — U.S. EPA, 1987

Limited peer review and extensive Agency-wide review 1987.

Other EPA Documentation — None

Agency Work Group Review — 09/17/1987

Verification Date — 09/17/1987

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfD for Benzoic acid conducted in August 2003 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or 202-566-1676.

I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — Benzoic acid
CASRN — 65-85-0

Not available at this time.

II. Carcinogenicity Assessment for Lifetime Exposure

Substance Name — Benzoic acid
CASRN — 65-85-0
Last Revised — 08/01/1989

Section II provides information on three aspects of the carcinogenic assessment for the substance in question; the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral exposure and from inhalation exposure. The quantitative risk estimates are presented in three ways. The slope factor is the result of application of a low-dose extrapolation procedure and is presented as the risk per (mg/kg)/day. The unit risk is the quantitative estimate in terms of either risk per ug/L drinking water or risk per ug/cu.m air breathed. The third form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000, 1 in 100,000 or 1 in 1,000,000. The rationale and methods used to develop the carcinogenicity information in IRIS are described in The Risk Assessment Guidelines of 1986 (EPA/600/8-87/045) and in the IRIS Background Document. IRIS summaries developed since the publication of EPA's more recent Proposed Guidelines for Carcinogen Risk Assessment also utilize those Guidelines where indicated (Federal Register 61(79):17960-18011, April 23, 1996). Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

II.A. Evidence for Human Carcinogenicity

II.A.1. Weight-of-Evidence Characterization

Classification — D; not classifiable as to human carcinogenicity

Basis — No human data and inadequate data from animal bioassays.

II.A.2. Human Carcinogenicity Data

None.

II.A.3. Animal Carcinogenicity Data

Inadequate. In a lifetime study, Toth (1984) administered sodium benzoate (of 99% purity) to 50 male and 50 female 5 week-old albino Swiss mice at a level of 2% in the drinking water. Control groups consisted of 100 mice/sex. The dose level was selected based on results of a subchronic study in which levels of 4 and 8% were considered to be too toxic. The 2% level was equivalent to sodium benzoate doses of 4133 mg/kg/day for males and 3973 mg/kg/day for females. Based on average measured daily water consumptions of 6.2 mL for males and 5.9 mL for females and an assumed average body weight of 0.03 kg. The equivalent benzoic acid doses, adjusted for molecular weight differences between sodium benzoate and benzoic acid, are 3502 mg/kg/day and 3367 mg/kg/day for males and females, respectively. Histopathologic examinations of all mice included 11 organs and all gross lesions. The treatment had no apparent effect on survival or tumor incidence.

As part of a 5-generation reproduction study, Shtenberg and Ignat'ev (1970) administered test compounds in a paste in daily doses of 40 mg/kg benzoic acid combined with 80 mg/kg sodium bisulfite in a paste before feeding an otherwise unspecified basic diet to a group of 50 white cross-bred mice/sex for 17 months. Another group received benzoic acid only; no further details were given. An unspecified number of control animals received only basic diet. Malignant tumors (not otherwise specified) occurred in 8/100 treated mice and 1/8 mice in the third generation of the treated group. Tumor incidences were not reported for untreated mice.

II.A.4. Supporting Data for Carcinogenicity

Dinerman and Ignat'ev (1966) reported that a 3-month exposure to 0.2% benzoic acid in the diet increased the susceptibility of mice to the development of carcinomas following intraperitoneal inoculation with Erlich ascites carcinoma cells. Tumors developed in 62/90 (68.8%) of benzoic acid- treated mice and in 16/49 (32.6%) of the control mice.

Benzoic acid and sodium benzoate have been tested for mutagenicity or genotoxicity in prokaryotes (McCann et al., 1975), eukaryotes (Litton Bionetics, Inc., 1974), and several mammalian test systems (Litton Bionetics, Inc., 1974, 1975; Oikawa et al., 1980). No positive results have been reported.

II.B. Quantitative Estimate of Carcinogenic Risk from Oral Exposure

Not available.

II.C. Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

Not available.

II.D. EPA Documentation, Review, and Contacts (Carcinogenicity Assessment)

II.D.1. EPA Documentation

Source Document — U.S. EPA, 1987

The 1987 Health and Environmental Effects Document has received OHEA review.

II.D.2. EPA Review (Carcinogenicity Assessment)

Agency Work Group Review — 03/01/1989

Verification Date — 03/01/1989

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the cancer assessment for Benzoic acid conducted in August 2003 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or 202-566-1676.

II.D.3. EPA Contacts (Carcinogenicity Assessment)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

III. [reserved]

IV. [reserved]

V. [reserved]

VI. Bibliography

Substance Name — Benzoic acid
CASRN — 65-85-0

VI.A. Oral RfD References

FDA (Food and Drug Administration). 1973. Evaluation of the Health Aspects of Benzoic Acid and Sodium Benzoate as Food Ingredients. DHEW, Washington, DC. Report No. SCOGS-7. NTIS PB-223 837/6.

FDRL (Food and Drug Research Labs., Inc.). 1972. Teratologic Evaluation of FDA 71-37 (Sodium Benzoate). p.75-79.

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Wiley, H.M. and W.D. Bigelow. 1908. Influence of benzoic acid and benzoates on digestion and health. Bulletin 84, pt. IV, Bureau of Chemistry, U.S. Dept. Agriculture. (Cited in Informatics, Inc., 1972)

VI.B. Inhalation RfD References

None

VI.C. Carcinogenicity Assessment References

Dinerman, A.A and A.D. Ignat'ev. 1966. Effect of certain food preservatives on the development of tumors in mice. Gig. Sanit. 31(9): 38-42. (Eng. trans.)

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VII. Revision History

Substance Name — Benzoic acid
CASRN — 65-85-0

Date	Section	Description
09/07/1988	I.A.	Oral RfD summary on-line
08/01/1989	II.	Carcinogen summary on-line
10/28/2003	I.A.6., II.D.2.	Screening-Level Literature Review Findings message has been added.

VIII. Synonyms

Substance Name — Benzoic acid
CASRN — 65-85-0
Last Revised — 09/07/1988

- 65-85-0
- benzenecarboxylic acid
- Benzoic acid
- carboxybenzene
- dracylic acid
- phenyl carboxylic acid
- phenylformic acid



Benzoic acid: Human health tier II assessment

28 June 2013

CAS Number: 65-85-0

- Preface
- Chemical Identity
- Import, Manufacture and Use
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- Existing Work Health and Safety Controls
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- Risk Characterisation
- NICNAS Recommendation
- References

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

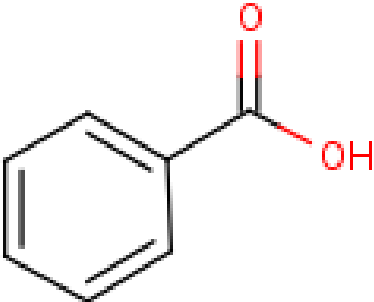
For more detail on this program please visit: www.nicnas.gov.au

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Acronyms & Abbreviations

Chemical Identity

Synonyms	Benzenecarboxylic acid Benzeneformic acid Phenylcarboxylic acid Benzenemethanoic acid Dracrylic acid
Structural Formula	
Molecular Formula	C ₇ H ₆ O ₂
Molecular Weight (g/mol)	122.12
Appearance and Odour (where available)	A white crystalline powder with a pleasant odour.
SMILES	<chem>C(=O)(O)c1ccccc1</chem>

Import, Manufacture and Use

Australian

Under previous NICNAS mandatory and/or voluntary calls for information, the chemical has been identified as having an industrial use in Australia; however, specific uses were not reported.

International

The following international uses have been identified through the European Union Registration, Evaluation and Authorisation of Chemicals (EU REACH) dossiers, the Organisation for Economic Co-operation and Development Screening information Data Set Initial Assessment Report (OECD SIAR), Galleria Chemica, Substances and Preparations in the Nordic Countries (SPIN) database, the European Commission Cosmetic Substances and Ingredients (CosIng) database, and United States (US) Personal Care Products Council International Nomenclature of Cosmetic Ingredients (INCI) directory:

The chemical has reported cosmetic use in:

- bulking and masking agents; and
- fragrance ingredients, preservatives and pH adjusters.

The chemical has reported domestic use including in:

- adhesives, binding agents;
- cleaning/washing agents;
- colouring agents;
- corrosion inhibitors;
- fillers;
- flame retardants and extinguishing agents; and
- paints, lacquers and varnishes.

The chemical has reported commercial use including in:

- fixing agents;
- lubricants and additives;
- reprographic agents;
- softeners; and
- process regulators for polymerisation processes in production of resins, rubbers and polymers.

The chemical has reported site-limited use including as:

- intermediates; and
- laboratory chemicals.

The chemical has reported non-industrial use including in:

- non-agricultural pesticides and preservatives; and

- pharmaceuticals, milk, food/foodstuff flavourings, plant extracts and nutrients.

A 2010 Personal Care Products industry survey reported the following use concentrations: benzoic acid (0.000002–5 %), sodium benzoate (0.000001–1 %), calcium benzoate (0.002–0.004 %) and potassium benzoate (0.002–0.003 %). No uses of magnesium benzoate were reported in the survey (INCI).

Restrictions

Australian

No known restrictions have been identified.

International

The chemical is restricted internationally under the following:

European Union (EU): The use of the chemical in cosmetics is restricted in the EU under Annex V – list of preservatives allowed. It states that benzoic acid and sodium benzoate are safe for use for preservative and non-preservative purposes in cosmetic rinse-off products at a maximum concentration of 2.5 % and in cosmetic oral-care products at a maximum concentration of 1.7 %, and in leave-on products up to 0.5 %.

US Cosmetic Ingredient Review (CIR): The CIR considers the chemical (benzoic acid and its salts) to be safe for use at a maximum allowed concentrations of 5 % (as acid) (INCI).

New Zealand: The chemical is controlled in the New Zealand Cosmetic Products Group Standard – Schedule 7 – list of preservatives allowed in cosmetics. It states that benzoic acid and sodium benzoate are safe for use for preservative and non-preservative purposes in cosmetic rinse-off products at a maximum concentration of 2.5 % and in cosmetic oral-care products at a maximum concentration of 1.7 %, and in leave-on products up to 0.5 %. For other salts of benzoic acid other than listed above, maximum authorised concentrations is 0.5 % (Galleria Chemica).

Existing Work Health and Safety Controls

Hazard Classification

The chemical is not listed on the Hazardous Substances Information System (HSIS) (Safe Work Australia).

Exposure Standards

Australian

No specific exposure standards are available.

International

The following exposure standards are identified (Galleria Chemica):

An exposure limit (TWA) of 5–10 mg/m³ in different countries such as USA (California, Tennessee), Canada and England.

Health Hazard Information

For most part, benzoic acid and its sodium, magnesium, calcium, ammonium and potassium salts are able to be considered together as a group based on the structural similarity and the physico-chemical properties. A considerable proportion of data for benzoic acid was generated using sodium benzoate because of the low water solubility of benzoic acid at neutral pH. Additionally, for some studies it was not definitely stated whether benzoic acid or its salts were used. Benzoic acid (and its salts) will be present as the benzoate ion under physiological conditions. Therefore, it is reasonable to assume that the results of systemic toxicity studies on benzoic acid will apply to the salts of benzoic acid as well. Benzoic acid differs from its salts in that it is likely to have greater local effects (to the skin, eye and respiratory system) than the salts and these have been considered separately from the benzoate salts.

Toxicokinetics

The chemical is rapidly and almost completely absorbed from the gastrointestinal tract after oral dosing. Studies showed that absorption is based on diffusion of the un-ionised molecule and is dependent on pH. The chemical is metabolised in the liver by conjugation with glycine, resulting in the formation of hippuric acid, which is rapidly excreted via the urine. Because of the high extent of metabolism and elimination, accumulation of the chemical or its metabolites in the body is not expected (ECHA, 2011).

Dermal absorption

The percutaneous absorption in humans has been reported as 14–42.6 % in vivo and 53–99 % in vitro studies. However, 100 % skin absorption was assumed due to the variable results seen in the human studies (SCCNFP, 2002).

Acute Toxicity

Oral

The chemical is of low acute toxicity in animal tests following oral exposure. The median lethal dose (LD50) in rats and mice is greater than 2000 mg/kg bw/d.

LD50 in rats ranged from 1700–3040 mg/kg bw/d and in mouse ranged from 1940–2370 mg/kg bw/d. However, the studies that reported the lower LD50s were all pre-guideline studies and no further information was available to critically assess the data. LD50s reported in two reliable studies were 2250 mg/kg bw/d (mice) and 2565 mg/kg bw/d (rats) (OECD, 2004).

Dermal

The chemical exhibits low acute toxicity in animal tests as evidenced by reported dermal LD50 (median lethal concentration) in rats of greater than 2000 mg/kg bw (OECD, 2004).

Inhalation

The chemical exhibits low acute toxicity in animal tests following inhalation exposure. No mortalities or toxic effects were observed in rats and mice with the reported median lethal concentration (LC50) > 12.2 mg/L/4-h (ECHA, 2011; OECD, 2004).

Corrosion / Irritation

Respiratory Irritation

The chemical is not currently classified in HSIS (Safe Work Australia). Available animal and human studies (see **Observation in Humans**) support the classification of the chemical as hazardous with the risk phrase 'Irritating to respiratory system' (Xi; R37) in HSIS (Safe Work Australia).

Inhalation toxicity of the chemical was evaluated in one rat study (0, 0.025, 0.25 and 1.2 mg/L, 6 h/d 5 d/wk over 4 weeks) using fine benzoic acid dust (see **Repeat dose toxicity – Inhalation**). A reddish discharge around the nostrils was seen in the mid and high dose groups. An increased incidence and intensity of interstitial inflammatory cell infiltrate and interstitial fibrosis (indicating upper respiratory tract irritation) was noted at all doses. Observed histopathological changes were most likely due to a persistent irritating effect of the test substance on the lung. No changes in gross pathology were noted (REACH).

Skin Irritation

The chemical is not currently classified in HSIS (Safe Work Australia). Available animal and human data (see **Observation in Humans**) support the classification of the chemical as hazardous with the risk phrase 'Irritating to skin' (Xi; R38) in HSIS (Safe Work Australia).

The chemical was irritating (erythema and swelling of the ear lobe) in the guinea pig ear swelling test at $\geq 1\%$, particularly when dissolved in ethanol, although it was not found irritating in the rabbit (OECD, 2004).

Eye Irritation

The chemical is not currently classified in HSIS. Available animal data support the classification of the chemical as hazardous with the risk phrase 'Risk of serious eye damage' (Xi; R41) in HSIS (Safe Work Australia).

The chemical was highly irritating in rabbit eyes, causing irreversible corneal opacity and chemosis in 2/3 animals, and increasing conjunctival redness severity with white/grey discoloration after 2-day observation. A Draize score of 35 was given based on the effects (REACH). In another rabbit study an irritation score of 65.0/110 was noted. No further details were available from this study (OECD, 2004).

Observation in humans

Several clinical studies have demonstrated the potential of the chemical to cause skin irritation effects in humans.

Positive skin irritant reactions (erythema and oedema) were frequently reported in a number of human studies. The threshold concentrations are strongly dependent on the vehicles (ethanol or petrolatum), skin conditions (scarified/unscarified, dermatitis or urticaria) and application conditions (closed/open patch tests or chamber-scarification tests for skin irritancy) (SCCP, 2005).

Non-immunological contact urticaria has frequently been reported in humans for the chemical. Contact urticarial reactions to the chemical (1 %) were seen in healthy volunteers (SCCP, 2005). Oedematous reaction to the chemical (0.05 %) were also observed in patients during an occlusion test (OECD, 2004).

In humans, cases of respiratory reactions such as rhinitis and asthma have been reported for susceptible persons following oral, dermal or inhalation exposure to benzoic acid (ECHA, 2011).

Sensitisation

Skin Sensitisation

The negative results seen for the chemical from several skin sensitisation animal studies including guinea pig maximisation test (GPMT), Buehler test and local lymph node assay (LLNA) support a conclusion that the chemical is not a skin sensitizer (REACH).

Observation in humans

The chemical did not induce sensitisation in healthy volunteers although some allergic reactions were noted in 34/537 patients with suspected contact dermatitis (at 2 %) (SCCP, 2005) and 9/121 patients with dermatoses and 10/57 patients with chronic urticaria (at 5 %) (ECHA, 2011).

Repeated Dose Toxicity

Oral

Based on the weight of evidence the chemical is not considered to cause serious damage to health by repeated oral exposure (no observed adverse effect level (NOAEL) of 825 mg/kg bw/d). Effects observed at > 1000 mg/kg bw/d included increased mortality, reduced weight gain, and liver and kidney effects (OECD, 2004).

Dermal

Available animal data suggest that the chemical is not likely to cause serious damage to health via repeated dermal exposure.

No treatment-related effects in rabbits at doses of up to 2500 mg/kg bw/d applied 5 d/wk for 3 weeks (OECD, 2004).

Inhalation

Available animal data suggest that the chemical is not likely to cause serious damage to health via repeated inhalation exposure.

The only available rat study for this chemical reported 2/20 mortalities at 1.2 mg/L 6 h/d (5 d/wk over 4 wk). Local reddish discharge around the nostrils and inflammatory cell infiltrates and interstitial fibrosis of the lung secondary to local irritant effects were also observed at ≥ 0.25 mg/L. On the basis of systemic effects, the NOAEC is considered to be > 0.25 mg/L 6 h/d (ECHA, 2011).

Genotoxicity

Based on the weight of the evidence of the in vitro and in vivo genotoxicity data, the chemical is not considered mutagenic or clastogenic.

In vitro data using the reverse mutation assays with various strains of *Salmonella typhimurium* (with and without metabolic activation) and sister chromatid exchange assays (except one equivocal result) were negative. Weak genotoxic effects or equivocal results were observed in most of the chromosome aberration assays in three mammalian cell lines and two of the recombination assays in *Bacillus subtilis* (no further information available, only summary given) (REACH). No genotoxicity was observed in the in vivo cytogenetic, micronucleus, or other assays at either somatic or germ cell level (OECD, 2004).

Carcinogenicity

Based on the available data, the chemical is not considered carcinogenic.

The chemical was not carcinogenic (NOAEL 500 mg/kg bw/d) in a lifetime 3-generation study in rats when given with the diet at doses up to 500 mg/kg bw/d. No increase in the lifetime tumour incidence, clinical abnormalities or histopathological changes were observed (OECD, 2004).

A lifelong study using male/female Swiss Albino mice given the chemical (2 %) continuously in drinking water showed no carcinogenic effect (such as effect on survival or incidence of tumours) (CICAD, 2000).

Reproductive and Developmental Toxicity

No evidence of reproductive or developmental toxicity was observed for the chemical.

A four generation study with benzoic acid was conducted in male and female rats (NOAEL 500 mg/kg bw/d). The first and second generation were fed 0.5 or 1.0 % benzoic acid in the diet (approximately 250 or 500 mg/kg bw/d). The third generation was treated for 16 weeks and generation 4 was treated until breeding. There were no unfavourable side-effects on growth, food utilisation, duration of life, procreation, feeding of the offspring, weight of organs and histological pattern of organs in the 1 % dose group. In the 0.5 % group there was a significant prolongation of lifetime of the rats (OECD, 2004).

In a study to determine the teratologic effects, benzoic acid was administered in a single dose of 510 mg/kg bw/d to Wistar albino rats at day 9 of gestation. The malformations and resorption rates were comparable to those in control animals (NOAEL for maternal toxicity and teratogenicity: 510 mg/kg bw/d) (OECD, 2004).

Other Health Effects

Neurotoxicity

No evidence for neurofunctional abnormalities or histopathological alterations of the central nervous system (CNS) were reported following exposure to doses up to ~500 mg/kg bw/d in rats (ECHA, 2011).

Neurotoxicity and CNS toxicity were assessed in juvenile rats exposed to the diet for up to 35 days at 0, 1.1, and 3 %, corresponding to approximate doses of 0, 825, and 2250 mg/kg bw/d benzoic acid. At day 4, clinical observation showed signs of neurotoxicity in most animals of the highest dose group including ataxia, tremor, excitation, aggressive behaviour and convulsions. However, these changes were deemed secondary to metabolic changes (acidosis, acyl-CoA and ATP depletion). Histological evaluation of the brain showed prominent pathological changes in 2/5 animals treated for 3 days, 18/18 animals treated for 5 days and 13/15 animals with 3 weeks of recovery following the 5 days of exposure. These changes included ischaemic necrosis preferentially of ganglial cells in the stratum granulosum of the fascia dentate and the cortex of the lobus piriformis. No histopathology of the brain or clinical abnormality was noted in any of the animals of the low dose group receiving approximately 825 mg/kg bw/d over 7, 14, or 35 days (ECHA, 2011).

In another developmental neurotoxicity study, the effects of the chemical on activity and behaviour in neonatal and juvenile rats were evaluated. Treatment with 0, 0.1, 0.5, and 1.0 % benzoate in the diet commenced on day 5 of gestation of the parental animals and continued through lactation and after weaning. There were no treatment-related changes in weight or neurotransmitter content for any of 5 analysed brain regions. Consequently, the NOAEL for developmental neurotoxicity in rats was considered as 1 % in the diet, corresponding to approximately 500 mg/kg bw/d (REACH).

Risk Characterisation

Critical Health Effects

The critical health effects associated with the chemical (but not the salts) are skin, eye and respiratory tract irritation. However, no systemic effects were seen with benzoic acid. The salts are expected to exist almost entirely as the benzoate ion under normal physiological conditions and will not have the local irritant properties that arise from the acidity of benzoic acid. Therefore, it is unlikely that any systemic effects will be observed with the salts of benzoic acid.

Public Risk Characterisation

Currently, there are no restrictions on the use of this chemical in Australia. The chemical is reported to be used in cosmetic/domestic products overseas (up to a maximum concentration of 5 % in cosmetics (INCI, 2010), although its use in cosmetic/domestic products in Australia is not known. Taking into account the natural occurrence of benzoic acid (and benzoates) in the environment and the use of these substances in food preservatives and in medications, no concern for the safety of consumers is raised. The likely route of exposure to general public is through dermal use of consumer products containing the chemical. However, in such formulations it is expected that the chemical will be used at very low concentrations and will be buffered to the physiological pH that will mitigate the risk, so no likely harm from the use of cosmetics is expected. Therefore, the risk to the public is considered low.

Occupational Risk Characterisation

Given the critical local health effects, the chemical may pose an unreasonable risk to workers unless adequate control measures to minimise exposure (dermal, ocular and inhalation) to the chemical are implemented. The chemical should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine appropriate controls.

NICNAS Recommendation

Assessment of the chemical is considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Regulatory Control

Public Health

Considering the available information to indicate low public exposure from this chemical, no regulatory controls are recommended.

Work Health and Safety

The chemical (but not its salts) is recommended for classification and labelling under the current Approved Criteria and adopted Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as below. This does not consider classification of physical hazards and environmental hazards.

Hazard	Approved Criteria (HSIS) ^a	GHS Classification (HCIS) ^b
Irritation / Corrosivity	Risk of serious eye damage (Xi; R41) Irritating to skin (Xi; R38) Irritating to respiratory system (Xi; R37)	Causes serious eye damage - Cat. 1 (H318) Causes skin irritation - Cat. 2 (H315) May cause respiratory irritation - Specific target organ tox, single exp Cat. 3 (H335)

^a Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

^b Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

* Existing Hazard Classification. No change recommended to this classification

Advice for consumers

Products containing the chemical should be used according to the instruction on the label.

Advice for industry

Control measures

Control measures to minimise the risk from (dermal, ocular or inhalation) exposure to the chemical should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemical is used. Examples of control measures which may minimise the risk include, but are not limited to:

- using local exhaust ventilation to prevent the chemical from entering the breathing zone of any worker;
- health monitoring for any worker who is at risk of exposure to the chemical if valid techniques are available to monitor the effect on the worker's health;
- air monitoring to ensure control measures in place are working effectively and continue to do so;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

Guidance on managing risks from hazardous chemicals are provided in the *Managing Risks of Hazardous Chemicals in the Workplace—Code of Practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to assist with meeting obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((m)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemical are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (m)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of Safety Data Sheets for Hazardous Chemicals—Code of Practice* and *Labelling of Workplace Hazardous Chemicals—Code of Practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of the chemical has not been undertaken as part of this assessment.

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Last update 28 June 2013

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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
Office of Research and Development
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/m³)	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/m³) 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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EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL
Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

OPINION ON

Benzoic Acid and Sodium Benzoate

Adopted by the SCCP
during the 4th plenary of 21 June 2005

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1. BACKGROUND

1.1. Background

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) was requested to review the data submitted to support the safety of benzoic acid, its salts and esters (COLIPA¹ No. P 2), when used at concentrations other than those laid down in Annex VI to Directive 76/768/EEC as preservatives, for other specific non-preservative purposes apparent from the presentation of the products.

The SCCNFP adopted an opinion on 4 June 2002 on Benzoic acid and Sodium benzoate (SCCNFP/0532/01, final). In its opinion the SCCNFP stated, that *“the SCCNFP can only assess the safety of substances for which appropriate data has been submitted for evaluation. Safety assessment is specific and not generic. Only toxicological data for benzoic acid and its salt sodium benzoate have been made available for review. Therefore, there is no review of other salts of benzoic acid or any of its esters. These will require separate evaluation when the necessary data have been made available.”*

It was stated furthermore, that *“the SCCNFP does not find the submission appropriate for a safety evaluation of benzoic acid and sodium benzoate for the applied “other uses” in cosmetic products”*.

Recently, the European Commission received Submission II on Benzoic acid, its salt and esters.

2. TERMS OF REFERENCE

1. *On the basis of provided data the SCCP is asked to assess the risk to consumers when Benzoic acid, its salts and esters are used for non-preservative purposes in cosmetic rinse-off products at a maximum concentration of 2.5 % and in cosmetic oral-care products at a maximum concentration of 1.7 %, and in leave-on products up to 0.5%.*
2. *Does the SCCP recommend any further restrictions with regard to the use of Benzoic acid, its salts and esters safe when used for non-preservative purposes in cosmetic products?*

¹ COLIPA – European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Benzoic acid (INCI name)

Sodium benzoate (INCI name)

3.1.1.2. Chemical names

Benzoic acid Benzene carboxylic acid; benzene formic acid; carboxybenzene; benzene carboxylic acid; phenylcarboxyl acid; phenylformic acid, E210

Sodium benzoate E211

3.1.1.3. Trade names and abbreviations

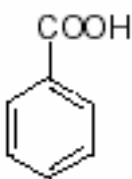
/

3.1.1.4. CAS / EINECS / ELINECS number

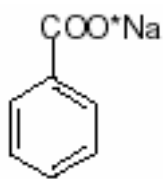
	CAS	EINECS
Benzoic acid :	65-85-0	200-618-2
Sodium benzoate :	532-32-1	208-534-8

3.1.1.5. Structural formula

Benzoic acid



Sodium benzoate



3.1.1.6. Empirical formula

Benzoic acid :	$C_7H_6O_2$
Sodium benzoate :	$C_7H_5O_2Na$

3.1.2. Physical form

Benzoic acid :	solid
Sodium benzoate :	solid

3.1.3. Molecular weight

Benzoic acid : 122.13
Sodium benzoate : 144.11

3.1.4. Purity, composition and substance codes

No data

3.1.5. Impurities / accompanying contaminants

No data

3.1.6. Solubility

Benzoic acid : 2.91 g/l in water at 20°C
Sodium benzoate : 556 g/l in water at 20°C, hygroscopic

3.1.7. Partition coefficient (Log P_{ow})

Benzoic acid : 1.88
Sodium benzoate : -2.269

3.1.8. Additional physical and chemical specifications

Benzoic acid

Organoleptic properties : /
Melting point : 122 °C
Boiling point : 249.2°C
Flash point : /
Vapour pressure : 0.0011hPa
Density : 1.321 g/cm³ at 20°C
Viscosity : /
pKa : -2.269
Refractive index : /
Stability : /

Sodium benzoate

Organoleptic properties :
Melting point : >300.0 °C
Boiling point : 464.9°C
Flash point : /
Vapour pressure : 0.0011hPa
Density :
Viscosity : /
pKa : -2.269
Refractive index : /
Stability : /

3.2. Function and uses

Benzoic acid is a natural ingredient occurring in many foodstuffs and in plant extracts.

Benzoic acid, its salts and esters are used as preservatives in cosmetic products, with a maximum concentration of 0.5 %, (calculated as acid), as regulated by the EU Cosmetics directives 76/768/EEC.

This request is for use for non-preservative purposes in cosmetic rinse-off products, at a maximum concentration of 2.5 %, and in cosmetic oral-care products, at a maximum concentration of 1.7 %, and in leave-on products, up to 0.5%. However these purposes are not specified.

Other uses for benzoic acid and its salts include regulated use as food preservatives, most suitable for foods, fruit juices, and soft drinks in an acidic pH range. In the EU, there are regulations controlling the maximum levels of benzoic acid and its salts for use in foodstuffs ready for consumption and the specific purity criteria of food additives. The levels are expressed as the free acid.

Non alcoholic drinks	150 mg/l
Alcoholic drinks	200 mg/l
Jams & jellies	500 mg/kg
Aspic	500 mg/kg

Ref.: AR 1, AR 2

In the United States, benzoic acid and sodium benzoate are on the FDA list of substances that are generally recognized as safe (GRAS). Both may be used as antimicrobial agents, flavouring agents and as adjuvants with a current maximum level of 0.1% in food. The FDA has not determined whether significantly different conditions of use would be GRAS. The FDA has sought fully up-to-date toxicology information.

Ref.: AR 3

Benzoic acid is used in oral medicines up to 0.15%, in parenteral medicines up to 0.17% and in topical drugs up to 0.2%. Benzoic acid is used as an active ingredient in anti-fungal cream with salicylic acid (3.0%) up to 6%.

Sodium Benzoate, expressed as benzoic acid, is permitted in oral medicines up to 0.5%, in parenterally administered up to 0.5%.

Ref.: AR 4, AR 5

Benzoic acid is also an intermediate in the synthesis of phenol and caprolactam. Other end products include sodium and other benzoates, benzoyl chloride, and diethylene and dipropylene glycol dibenzoate plasticizers. Sodium benzoate is primarily a preservative and corrosion inhibitor (e.g., in technical systems as an additive to automotive engine antifreeze coolants).

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Acute oral toxicity information is based on summary information, no dossiers were provided. The indication is that the substances can be considered to possess low acute oral toxicity. The calculated LD₅₀ values taken from the available experiments on the acute oral toxicity of Benzoic Acid and Sodium Benzoate are listed in the following tables:

Benzoic Acid

Species	LD ₅₀ [mg/kg]	Reference
Rats	1700	
	2530	4
Mice	1940	5
	2370	6

Sodium Benzoate

Species	LD ₅₀ [mg/kg]	Reference
Rats	1714	7
	2100 fasting	8
	3140	9
	3450 not fasted	8
	4070	10

3.3.1.2. Acute dermal toxicity

Benzoic acid

Rabbit	LD ₅₀ > 5 000 mg/kg	
	LD ₅₀ > 10 000 mg/kg	

Ref.: 14, citation only

3.3.1.3. Acute inhalation toxicity

No data submitted with Submission II (2004)

Benzoic acid

Rats	LC ₅₀ > 0.026 mg/l/h	

Ref.: 14, citation only

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Animal studies

Benzoic Acid

Guideline	:	OECD 404 (1981), EEC test method B.4, Annex V of EEC Directive 84/449/EEC (September 1984)
Species/strain	:	New Zealand albino rabbit
Group size	:	3 females
Test substance	:	0.5 g of benzoic acid moistened with 0.25 ml Milli-RO water
Batch	:	/
Purity	:	99.5%
GLP	:	in compliance

A paste, (0.5 g benzoic acid and 0.25 ml water), was applied evenly to 6 cm² Metalline on a permeable tape (Micropore). This was applied to the right flank of the rabbit. A control patch without the test substance was applied to the left flank. These were left in place for 4 hours. The test site was cleaned first with a dry tissue and then by swabbed with a dampened tissue. The skin was examined for erythema, eschar formation and oedema at 1, 24, 48 and 72 hours after removal of the patches.

Results

Two animals showed slight erythema initially. 1 of these also showed slight oedema up to 24 h. This was resolved by 48 h. There was no indication of a systemic effect.

The results of this study indicate that the test item, benzoic acid, was minimally irritating (modified primary irritation index (PII) of 0.5) when applied to the intact rabbit skin under semi-occlusive patch conditions. The test substance does not need to be labelled as a skin irritant.

Ref.: 12

In submission II, three additional animal tests confirmed the low irritation potential of Benzoic Acid were summarized in a table. These were also provided in Submission I, but are only citations or summary information.

<i>Species</i>	<i>Exposure</i>	<i>Skin irritation</i>	<i>Reference</i>
rabbits	neat Benzoic acid/occluded / 24 h	mild	13
rabbits	500 mg dry powder/ 24 and 72h	PII = 1.66/8.00	14
rabbits	500 mg dry powder/ semioclusive / 24 h	not irritating	15

Sodium Benzoate

Guideline	:	OECD 404 (1981)
Species/strain	:	New Zealand albino rabbit
Group size	:	3 females
Test substance	:	0.5 g sodium benzoate moistened with 0.25 ml Milli-RO water
Batch	:	/
Purity	:	> 99.5%

GLP : in compliance

Approximately 24 hours prior to the treatment, the dorsal fur was shaved. A paste, (0.5 g sodium benzoate and 0.25 ml water), was applied evenly to 6 cm² Metalline on a permeable tape (Micropore). The 6 cm² patch was removed four hours after semi-occlusive contact. The skin reactions were assessed approx. 1 hour, 24, 48 and 72 hours after termination of the exposure. Under the conditions of the study, sodium benzoate was neither corrosive nor irritating (PII 0) when applied to the intact rabbit skin under semi-occlusive patch conditions for four hours. The test substance does not need to be labelled as a skin irritant. The animals did not show any symptoms of systemic intoxication.

Ref.: 16

Application of 500 mg sodium benzoate as a dry powder to rabbit for 24 h; responses were scored at end of treatment and after 48 h. It was considered not irritating. There are no details of study.

Ref.: 17

Human Studies

Benzoic Acid

The results, presented only in scientific papers, are from studies both on volunteers and patients from dermatological clinics. 2% benzoic acid in petrolatum over 46h did not irritate intact skin of healthy volunteers. 24h application of 30% benzoic acid in ethanol was found to be the lowest irritating concentration.

Ref. : Gad et al, 1986, Kligman, 1977, Frosch & Kligman, 1976 cited in 13

Chamber test (72 h/occlusive): 0.1 ml of 7.5 % and 15 % benzoic acid in ethanol on scarified skin, 30 % benzoic acid in ethanol on normal skin (6 volunteers).

Results

Scarified skin	: 7.5 % in ethanol, moderate irritant
15.0 % in ethanol	: marked irritant with erosions
Normal skin	: 30 % in ethanol, lowest irritant concentration

Ref.: 19, 21

Chamber test (20 min/occlusive), open test (30 min): 15 µl of 5 % benzoic acid in petrolatum, 15 atopic and 16 non-atopic patients. The atopics showed redness in both the chamber test, (73 %) and the open test, (80 %). Non-atopics showed 80% redness in both the chamber test and in the open test. There was no statistical difference between atopics and non-atopics.

Ref.: 20

8 out of 627 patients (1.3%) from dermatological clinics showed positive reactions to 5 % benzoic acid, in petrolatum under an occlusive dressing for 24 or 48 h. At this concentration, the authors suggest that these results could be interpreted as marginally irritating, rather than allergic.

Ref.: 18

Non-immunologic contact urticaria

Benzoic acid (5.0 % in petrolatum) was tested in an open test on 29 atopic and 74 non-atopic persons.

Results

Contact urticarial reactions to benzoic acid were seen in 27/29 (93 %) of the atopics and in 64/74 (87 %) of the non-atopics.

In the chamber test, 20 min occlusion (recorded 10 min later) 0.1 % benzoic acid in petrolatum and 0.05 % benzoic acid in water elicited reactions. When water was the vehicle, the reactions were oedematous.

Ref.: AR6

Urticaria occurred in 1/10 and 5/12 healthy volunteers with 0.1 and 1.0% benzoic acid, following 20 min covered contact. It was not clear if this was an irritant or immune response.

Ref.: Clemmensen & Hjorth, 1982 cited in 13

Non-immune immediate contact reactions (NIICR), erythema and oedema, have been produced in 78/80 women tested with 2% benzoic acid petrolatum but there was no correlation between the susceptibility to NIICR and age, atopic status or tanning ability. In another study, it was demonstrated that benzoic acid induced non-immune immediate contact reactions in the majority of 200 volunteers with no specific skin condition. Approximately 10% of the volunteers appeared particularly sensitive, reacting fairly strongly. The response, erythema or oedema, was specific to an individual with no significant correlation with age or sex on the degree of NIICR.

Ref.: 54

The Concise International Chemical Assessment Document (CICAD) No. 26 conclusion on benzoic acid and sodium benzoate was: "However, both substances are known to cause non-immunologic immediate contact reactions. This effect is scarce in healthy subjects, while in patients with frequent urticaria or asthma, symptoms or exacerbation of the symptoms were observed".

Ref.: 55

3.3.2.2. Mucous membrane irritation

Guideline	:	EEC test method B.5, Annex V of EEC Directive 84/449/EEC
Species/strain	:	New Zealand albino rabbit
Group size	:	3 females
Test substance	:	Benzoic acid
Purity	:	> 99.5%
Batch no	:	/
Dose	:	77 mg as a fine powder
GLP	:	in compliance

The test substance was instilled in a single application. The product was poured into the right conjunctival sac. After application, the lids of the treated eye were held closed for approximately two seconds. The untreated left eye was used as a control. The degree of eye irritation was

evaluated at immediately after dosing, 1, 24, 48 and 72 hours and 7, 14 and 21 days after treatment.

After 60 min, Animal 1 showed no reaction to light, with translucent corneal opacity and iridial injection. The other 2 also had slight corneal opacity. All 3 had moderate chemosis and slight conjunctival redness.

The corneal opacity noted in the first animal increased to nacreous areas. This persisted for 72 h. The translucent areas of opacity persisted up to Day 21. In Animal 2, the slight corneal opacity also persisted up to Day 21, but was resolved in Animal 3 by Day 7. By Day 14, in Animal 1, the iridial injection was resolved but no reaction to light. Animal 2 showed iridial injection on Day 2. The slight conjunctival redness in all animals increased to severe with a white/grey discolouration. It persisted in Animals 1 and 2 up to Day 21 and in Animal 3 to Day 7. The chemosis decreased slowly. In Animals 1 and 2, it was not completely resolved by Day 21 but it was resolved in Animal 3 by Day 14. The animals did not show any symptoms of systemic intoxication.

The estimated Draize score of 35 (60 min) is classed as severely irritating according to Kay and Calandra. Under the EU criteria, it should be labelled as an eye irritant.

Comment

This experiment was conducted in 1988, but the information was not provided in Submission 1.

Ref.: 22

Guideline	:	OECD 405
Species/strain	:	New Zealand albino rabbit
Group size	:	3 males
Test substance	:	Benzoic acid DAB 8
Batch no	:	/
Dose	:	/
GLP	:	/

The test substance was instilled in a single application. The product was poured into the right conjunctival sac. After application, the lids of the treated eye were held closed for approximately two seconds. The untreated left eye was used as a control. The degree of eye irritation was evaluated at immediately after dosing, 1, 24, 48 and 72 hours and 7, 14 and 21 days after treatment.

The authors considered benzoic acid was considered as a mild irritant, but the data provided is inadequate.

Ref.: 23, cited in BUA (14)

Instillation of 50 mg benzoic acid into the conjunctival sac of 2 rabbits.

Results

Moderate irritation. This is just a summary.

Ref.: 15, cited in BUA (14)

Additional information that was not mentioned but quoted in the BUA reference, was a citation of a single application of 100 mg dry powder to the rabbit eye. The irritation score was 65.0/110. It was scored at 24, 48, and 72 h.

Ref.: cited in BUA (14)

Comment

The data provided is inadequate.

Sodium benzoate

Guideline	:	OECD 405
Species/strain	:	New Zealand albino rabbit
Group size	:	3 females
Test substance	:	Sodium Benzoate
Purity	:	≥ 99.5%
Batch no	:	/
Dose	:	60 mg ground as a fine powder
GLP	:	in compliance

The test substance was instilled in a single application. The product was poured into the right conjunctival sac. After application, the lids of the treated eye were held closed for approximately two seconds. The untreated left eye was used as a control. The degree of eye irritation was evaluated at immediately after dosing, 1, 24, 48 and 72 hours and 7, 14 and 21 days after treatment.

Under the conditions of this study, sodium benzoate produced irritation of the conjunctiva that was reversed within 14 days. There was no effect on the cornea or iris. It was considered mildly irritating (Kay and Calendra score 9.3) and in the EC classification.

Ref.: 24

Application of 50 mg sodium benzoate/rabbit for 24 h; responses were scored at 24 h, 48 h and 72 h; post-exposure observation time: 7 d.

Results: not irritating.

Ref.: 17

3.3.3. Skin sensitisation

Benzoic acid**Animal tests**

The results of two *in vivo* guinea pig assays, a Magnusson-Kligman test and a Buehler test were reported. These were in-house tests with no further details. Benzoic Acid did not induce sensitization in either test and was thus considered to have low skin sensitization potential.

Ref.: 25

Human volunteer studies

Benzoic Acid at 2 % and 5 % in petrolatum did not induce sensitization in two maximization tests.

25 human volunteers were given five 48 h patch tests (over a 10 d period) with 2 % benzoic acid in petrolatum. None gave positive reactions when challenged 10-14 d after the induction phase with a final 48 h closed patch test with 2 % benzoic acid in petrolatum.

This information is as a summary only.

Ref.: 26 and cited in BUA (14)

10 persons allergic to benzoyl peroxide were tested by a 48 h patch test with 5 % benzoic acid in a hydrophilic petrolatum. No reactions at 48, 72 and 96 h.

Ref.: 27

In a cosmetic intolerance assay, 5202 patients with suspected allergic contact dermatitis were patch tested (537 of the patients had a history of “intolerance” or allergy). Patch test conditions were not specified, but allergic reactions was noted in 34 patients (0.7% incidence) to benzoic acid.

Ref.: 28

The OECD SIDS report found both benzoic acid and sodium benzoate were non-sensitizing in animal test but showed a very low incidence in humans (patients) tested by the patch test.

This report included 2 additional human patch test references that were not in the submissions. The results showed that benzoic acid was an occasional or positive sensitizer (Rademaker & Forsyth, 1989; Forsbeck & Skog, 1977)

Ref.: 56

Sodium benzoate**Human**

There were 2 additional human patch test references that were not included with any of the submissions. The first was a large cohort of 2045 patients, 5 gave positive results. These gave occasional positive results (Brasch, J. et al., 1993). In the second test, patch tests gave positive nonimmunologic contact urticaria (Nethercott, J.R., 1984).

Ref.: 56

3.3.4. Dermal / percutaneous absorption

¹⁴C-labelled benzoic acid (4 - 40 µg/cm² dissolved in acetone) was applied to excised human skin in a static diffusion chamber. Samples of penetrated amounts were measured in 1- 2 hr interval over the first 24 hrs and 3-6 hrs interval over the remaining days. No occlusion or washing were applied and the investigation was determined at least in duplicate. A median of 45% of the applied benzoic acid was found in the receptor phase 48 hrs after application.

Ref.: 40

Percutaneous absorption of benzoic acid was studied in the Mexican hairless dog and in man.

¹⁴C-labelled benzoic acid was injected subcutaneously and applied dermally to the neck skin of dogs. Human data were obtained from earlier investigations. Excretion of benzoic acid in man

was rapid, almost complete by day 3. In the dog, excretion was less extensive and greatly prolonged. This was accounted for by the persistence of benzoic acid in the skin.

Maximum absorption rate in man was 3.0 %/h, total absorption was 42.6 % of applied dose. This *in vivo* result is comparable with *in vitro* test results obtained. The total penetration of radio-labelled benzoic acid was found to be approximately 43 % in a 5-day period. In this human study, 6 test subjects received a dose of 4 µg/cm² Benzoic Acid dissolved in acetone. They were allowed to wash their skin 24 hours after application of Benzoic Acid. It should be noted that the exposure of the *in vitro* and *in vivo* studies were extremely long (2 days and 5 days, respectively).

Ref.: 41

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

Oral

Benzoic Acid and sodium benzoate show a low toxic potential in rodents in repeat dose studies.

Benzoic Acid

Guideline	:	Directive 84/449/EEC, B.1 "Acute toxicity (oral)"
Species/strain	:	50 Spartan rat
Group size	:	5 male/ female
Test substance	:	technical grade benzoic acid
Purity	:	/
Batch no	:	/
Dose	:	500, 1250, 1984, 3150, and 5000 mg/kg.
Vehicle	:	corn oil
GLP	:	/

The test compound was suspended in corn oil and administered orally. Volumes of 10 ml/kg bw were administered at all dosage levels.

All surviving rats, males and females, exhibited normal body weight gains during the 14 day observation period. The acute oral LD₅₀ of benzoic acid in male albino rats was calculated to be 2742 mg/kg (2279-3299 mg/kg).

The acute oral LD₅₀ of benzoic acid in female albino rats was calculated to be 2360 mg/kg (2042-2726 mg/kg).

A combined acute oral LD₅₀ for benzoic acid in male and female albino rats was calculated to be 2565 mg/kg (2292-2870 mg/kg).

Ref : IUCLID, Unpublished study (IRDC#163-282). Acute Toxicity Studies in Rats and Rabbits. (1974)

Comment: This study is available on IUCLID but was not submitted.

In rats, 648 mg/kg bw/d in feed for 28 days, has no effects.

Ref.: 14

Sodium benzoate

0.5, 1, 2, 4, and 8 % sodium benzoate in drinking water were administered for 35 days to groups of four female and four male Swiss albino mice.

In the 8 % dose level (approx. 24 g/kg bw/d) all mice died within 3 weeks. In the 4 % dose level (approx. 12 g/kg bw/d) 3 male and 3 female mice died within the 35-day observation period. The bodyweight of the surviving mice was substantially reduced. The 2 % dose level was chosen for a chronic toxicity (carcinogenicity) study.

Ref.: 42

In another study the body weight was slightly decreased at 2200 mg/kg bw/d. Groups of 6 male and 6 female Sherman rats were given 2 % (approx. 2.0 to 2.4 g/kg bw) or 5 % (5.7 g/kg bw for females and 7.8 g/kg bw for males) sodium benzoate in the diet for 28 days. In the 5 % dose group, all female rats died by day 11 and males by day 13. In the 2 % dose group a slight significant body weight depression was observed in male rats.

Ref.: 43

Dose levels from 16 to 1090 mg sodium benzoate/kg bw were given to groups of 10 rats (5 male and 5 female) for 30 days with the diet. No dose related adverse effects were observed.

Ref.: 10

Inhalation

Guideline	:	similar to OECD guideline 403
Species/strain	:	Rat, Sprague-Dawley (Charles River CD).
Group size	:	10 males + 10 females per dose
Test substance	:	benzoic acid
Batch no	:	59230350
Purity	:	/
Dose	:	0, 0.025, 0.25 and 1.2 mg/l
Exposure	:	dust aerosol exposure, (diameter 4.7 µ) 6 h/day for 5 days/week
GLP	:	in compliance

At 1.2 mg/l two animals (out of 20) died, exhibiting irritation of their upper respiratory tract.

These animals did not gain as much weight as controls, exhibited decreased organ weights and multifocal to generalized pulmonary fibrosis and inflammatory cell infiltrate. Exposure to 0.25 mg/l also resulted in an irritation of the upper respiratory tract. All treated animals survived and their body weight gain did not differ from controls. Neither significant effects on organ weights, nor significant effects on hematologic or biochemical parameters in the 0.25, 0.025 or 0 mg/l treatment groups were found. Exposure to the test material at all levels resulted in an increase in the frequency of pulmonary fibrosis and inflammatory cell infiltrate.

No compound related gross lesions were seen in any of the rats from the test groups that were terminally sacrificed or died during the study.

Compound-related microscopic lesions, consisting of an increase in the intensity and extent of inflammatory cell infiltrate and an increase in the incidence, intensity and extent of interstitial fibrosis in lungs of rats from all test groups, were observed.

Ref.: 44

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Benzoic acid

50 male and 50 female mice received 80 mg benzoic acid/kg bw/d by gavage for 90 days. 14 surviving mice were subjected to a restricted dietary intake (90 % restriction) for up to 5 days. 10 mice were tested on their tolerance to CCl₄ (test on detoxifying capacity of liver) by giving 0.1 ml CCl₄ in a single dose by oral intubation at the end of the test.

Reduced body weight gain was observed and the mortality was higher in connection with reduced tolerance to CCl₄ or food restriction than in the control group. The authors did not mention whether this finding was statistically significant. The relevance of the study will be compared to other long-term and carcinogenicity studies.

Data are not sufficient to justify conclusion.

Ref.: 45

Sodium benzoate

Groups of 4-5 male and 4-5 female rats received 0, 1, 2, 4, and 8 % sodium benzoate equivalent to 640, 1320, 2620, and 6290 mg/kg bw/d in the diet for 90 days.

4/8 animals died (average 13 days to death) in the 8 % dose level group, the average weight gain of the surviving rats was reduced and the relative liver and kidney weight was significantly increased with histopathological changes in liver and kidney (7/16).

Data are insufficient to justify a NOAEL of 4 % in the diet (2.6 g/kg bw/d).

Ref.: 8

3.3.5.3. Chronic (> 12 months) toxicity

Benzoic acid

25 male and 25 female mice were given 40 mg/kg bw/d for 17 months. Benzoic acid was fed in a paste prior to the main feed.

The weight of liver, kidney and testes relative to body weight in mice sacrificed at the end of the test period were lower in the group receiving sorbic acid (40 mg/kg bw/d) than in the group treated with benzoic acid. No further details were given.

Ref.: 45

10 male and 10 female rats received 40 mg benzoic acid/kg bw/d for 18 months. Benzoic acid was fed in a paste prior to the main feed.

The rats developed some tolerance to a lethal dose of benzoic acid given terminally (25 % mortality after 4000 mg/kg bw compared to 100 % mortality on the control group given one dose of 3600 mg/kg bw). No further details were given.

Ref.: 45

3.3.6. Mutagenicity / Genotoxicity

Bacterial Reverse Mutation Test**Benzoic acid**

Benzoic acid (up to 10 mg/plate) was tested in the Salmonella/microsome test using *S. typhimurium* TA 92, TA 94, TA 98, TA 100, TA 1535 and TA 1537. No significant increases in the numbers of revertant colonies were detected in any *S. typhimurium* strains at the maximum dose.

Ref.: 29

Benzoic acid (up to 10 mg/plate) was tested in the Salmonella/microsome test using *S. typhimurium* TA 97, TA 98, TA 100, TA 1535, and TA 1537 with and without metabolic activation. Benzoic acid was non-mutagenic in this test.

Ref.: 30

Benzoic acid was tested in the Salmonella/microsome test using *S. typhimurium* TA 98, TA 100, TA 1535, and TA 1537 with and without metabolic activation. Benzoic acid was nonmutagenic in this test (< 0.0099 revertants/nmol).

Ref.: 31

Sodium benzoate

Sodium benzoate (up to 3.0 mg/plate) was tested in the Salmonella/microsome test using *S. typhimurium* TA 92, TA 94, TA 98, TA 100, TA 1535 and TA 1537. No significant increases in the numbers of revertant colonies were detected in any *S. typhimurium* strains at the maximum dose.

Ref.: 29

Sodium benzoate was tested in the *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence and absence of S9 mix. Sodium benzoate was non-mutagenic in this test.

Ref.: 32

***In vitro* mammalian chromosome aberration test in human lymphocytes**

Benzoic acid

Chromosome aberration test was carried out on benzoic acid (up to 1.5 mg/ml) using Chinese hamster lung fibroblasts. No metabolic activation system was applied. 8 % of the cells treated with benzoic acid (1.5 mg/ml) showed structural aberrations at 48 hrs after treatment.

Ref.: 33

Benzoic acid (1.5 mg/ml in DMSO) gave equivocal results using Chinese hamster lung fibroblasts. 8% chromosome aberration and 1% polypoidy were reported.

Ref.: 29

Sodium benzoate

Human embryonic lung cells (WI-38) were used. Dose levels for sodium benzoate were 0, 2, 20, 200 mg/ml. 200 mg/l, previously established as the lowest toxic level. The positive control was 0.1 mg/ml triethylene melamin. The test compound was added to three culture bottles for each dose level, 24 hours after plating. When sufficient mitoses were observed, usually after 24-48 hours, the cells were harvested and prepared for the analysis. 100 anaphases per dose were analysed. For the mitotic index at least 500 cells were counted.

Sodium benzoate produced no significant chromosomal aberrations in human tissue culture cells when tested at any dose level tested. No substance related effects were observed. The chromosome abnormalities as well as the mitotic indices were within normal values.

Ref.: 34

Chromosome aberration test was carried out on sodium benzoate (up to 2.0 mg/ml) using a Chinese hamster fibroblast cell line. No metabolic activation system was applied. 38 % of the cells treated with sodium benzoate showed chromosome aberrations at 48 h.

Ref.: 29, 35,

Chromosome aberration test was carried out on sodium benzoate using a pseudodiploid Chinese hamster cell line (DON). No metabolic activation system was applied.

Concentration above 0.002 mol/l showed twofold background effects of chromosome aberrations; but no increase in the frequency of sister chromatid exchange was observed.

Ref.: 36

Chromosome aberration test was carried out on sodium benzoate using human embryonic lung culture cells. Sodium benzoate produced no significant increase in the aberration frequency in the anaphase chromosomes when tested at the dosage levels 0, 2.0 µg/ml, 20 µg/ml and 200 µg/ml.

Ref.: 33

***In vitro* Sister Chromatid Exchange (SCE)**

Benzoic acid

Benzoic acid, without metabolic activation, was reported to be negative for SCEs *in vitro* using 3 different cell lines. These were literature citations with no other information.

Ref.: 37, 38, 39

***In vivo* Host-Mediated Assay**

0, 50, 500 and 5000 mg sodium benzoate/kg bw was given orally to mice (single dose or once a day for 5 days) in an Host-Mediated Assay. Elevated mutant frequencies were seen with Salmonella TA 1530 in the acute intermediate dose level. The subacute and the other acute dose levels showed no increase in mutant frequencies. Tests with Salmonella G 46 were negative while giving slightly elevated mutant frequencies. Tests with Saccharomyces D 3 produced no increases in recombinant frequencies.

Ref.: 33

***In vivo* chromosome aberration test**

Chromosome aberration of sodium benzoate was investigated *in vivo* in rats. Five rats per group were dosed with 0, 50, 500 and 5000 mg/kg bw by gavage in a single dose or once a day for 5 days. The animals were killed at 6, 24 and 48 h after dosing in the acute study and 6 h after dosing in the subacute study. Bone marrow metaphase chromosomes were checked for aberrations.

A low incidence of treatment related breaks was observed in the acute study but within the control range. In the subacute study, only the mid dose showed breaks (1%) but were not considered to be significant. The mitotic indices were not different from controls. Sodium benzoate did not induce chromosomal aberrations in this test system.

Ref.: 34

***In vivo* dominant lethal assay**

A dominant lethal assay was conducted in rats. Following dosing with sodium benzoate by gavage (0, 50, 500, 5000 mg/kg bw, single dose or once a day for 5 days) treated male rats, 5 per group, were mated with two females per week for 8 weeks (acute study) or 7 weeks (subacute study). Fertility Index, number of implantations, corpora lutea, pre-implantation losses, resorptions/pregnant female and proportions of females with one or more dead, and two and more and overall dead implants were monitored.

In the acute study all three doses showed at week 8 significant, dose-related decreases in corpora lutea, at week 7 significant dose-related increases of average pre-implantation losses. Average resorptions were significant, dose-related increased at the low and high doses of week 2 and the low and intermediate doses of week 7 were significantly increased over the control group. Overall dead implants were significant increased at week 7 for the low and intermediate doses and week 2 for the low dose.

In the subacute study significant increases of average pre-implantation losses were observed at a number of weeks but no significant increases of average resorptions were observed. Overall dead implants were not increased.

The authors considered sodium benzoate to be non-mutagenic in rats in this test system although positive results were obtained.

Ref.: Submission 1, 33

Remark

IPCS CICAD 26 (2000) mentioned this dominant lethal assay as a positive result, however evaluation of the raw data in the original report (by experts of the industry consortium and a recent independent review by Prof. R. Kroes) gives no support for this. In addition the authors of the study clearly conclude negative. FDA also evaluated this study as negative. In addition sodium benzoate doesn't contain a structural alert for genotoxicity.

Ref.: 55

Other recent Evaluations of Genotoxicity

OECD SIDS Initial Assessment Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol

They concluded that Benzoic acid and Sodium benzoate, (also Potassium benzoate and benzyl alcohol) showed no mutagenic activity in *in vitro* Ames tests. Various results were obtained with other *in vitro* genotoxicity assays. Sodium benzoate (and benzyl alcohol) showed no genotoxicity *in vivo*. While some mixed and/or equivocal *in vitro* chromosomal/chromatid responses have been observed, no genotoxicity was observed in the *in vivo* cytogenetic, micronucleus, or other assays. The weight of the evidence of the *in vitro* and *in vivo* genotoxicity data indicates that these chemicals are not mutagenic or clastogenic. They also are not carcinogenic in long-term carcinogenicity studies.

They also remarked since the sodium salt of benzoic acid instantaneously dissociates to the benzoic acid, the studies with sodium benzoate are also representative for benzoic acid and potassium benzoate.

Ref.: 56

JECFA report, 1998

In addition data from *in vivo* genotoxicity studies on benzyl acetate and benzaldehyde are supportive evidence for the non-genotoxicity of benzoic acid and sodium benzoate.

Ref.: 52

Scientific Committee on Food Opinion: Re-evaluation of genotoxicity

In view of the additional genotoxicity data now available, the Committee has compiled its own summary of the studies (Annex 2). The Committee agrees that since benzylacetate,

benzylalcohol and benzylaldehyde are all metabolised to benzoic acid, the results of *in vivo* tests performed with these compounds as well as with sodium benzoate may be applied also to benzoic acid itself. Considering the database as a whole, weak genotoxic effects have been reported mainly at chromosome level in some *in vitro* systems. However, all the *in vivo* genotoxicity tests were negative at somatic or germ cell level. The essentially negative results obtained in three carcinogenicity studies (one in mice, two in rats) on sodium benzoate, notwithstanding some limitations, give further reassurance. On this basis, it is very unlikely that benzoic acid would interfere with chromosomes *in vivo*.

Ref.: 3

3.3.7. Carcinogenicity

Benzoic Acid:

20 male and 20 female rats were fed 1 % Benzoic Acid (approx. 700 mg/kg bw/d) in diet for a maximum of 1000 days. No evidence of carcinogenicity was seen upon microscopic examination of all analysed tissues.

Ref.: 48

Sodium benzoate

Sodium benzoate was given in 2 % in drinking water to 50 female and 50 male Swiss albino mice from weeks 5 on for lifespan. The average daily intake of sodium benzoate was 119.2 mg for a female and 124.0 mg for a male (approx. 5.95 - 6.2 g/kg bw/d).

There was no effect of the survival of the treated mice when compared with the untreated control. There were no significant differences between the tumour distribution in sodium benzoate-treated and untreated control mice.

Ref.: 42

Groups of 50 males and 52 female Fischer 344 rats were fed sodium benzoate at 1 or 2 % in the diet (approx. 0.5 or 1.0 g/kg bw/d) for 18 to 24 months. The control group consisted of 25 males and 43 females.

No clinical signs directly attributed to sodium benzoate were observed in treated animals. Differences in the average body weight between the treated and control groups were negligible. 40 rats died during the first 16 months, except for myeloproliferative disorder developed in one female control rat, all other dead animals showed pneumonia with abscess. Around 100 rats including those of the control group died after 16 months of hemorrhagic pneumonia with oedema.

The poor survival in all groups does limit the value of this study, although the type of tumours was similar between test and control rats of each sex.

Ref.: 42

3.3.8. Reproductive toxicity

3.3.8.1 4-Generation reproductive toxicity

Benzoic acid

A four generation study with benzoic acid was conducted in rats. Males and females of the first and second generation were fed 0.5 or 1.0 % benzoic acid in the diet (approximately 0.25 or 0.5 g/kg bw d). The third generation was treated for 16 weeks and generation 4 was treated until breeding.

There were no unfavourable side-effects on growth, food utilisation, duration of life, procreation, feeding of the offspring, weight of organs and histological pattern of organs in the 1 % dose group. In the 0.5 % group there was a significant prolongation of lifetime.

NOAEL: 500 mg/kg bw.

Ref.: 41

3.3.8.2. Teratogenicity

Benzoic acid

In a study to determine the teratologic effects, benzoic acid was administered in a single dose of 510 mg/kg bw to 7 on pregnant Wistar albino rats at day 9 of gestation. The malformations and resorption rates were comparable to those in control animals. The data was considered inadequate, since only a single dose was used.

Ref.: 46

Sodium benzoate

Sodium benzoate at doses of 0, 1.75, 8, 38 and 175 mg/kg bw was administered by gavage to groups of at least 20 pregnant albino CD outbred mice and White albino rats on gestation day 6 to 15. Groups of 21 to 22 pregnant hamsters were dosed with 0, 3, 14, 65 or 300 mg/kg bw on gestation days 6 to 10. Groups of 10 Dutch-belted rabbits were artificially inseminated and then dosed by oral intubation with 0, 2.5, 12, 54 or 250 mg/kg bw on gestation days 6 to 18. Caesareans were performed on mice, rats, hamsters and rabbits on days 17, 20, 14 and 29, respectively.

There was no clearly discernible effect on nidation or on maternal or foetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.

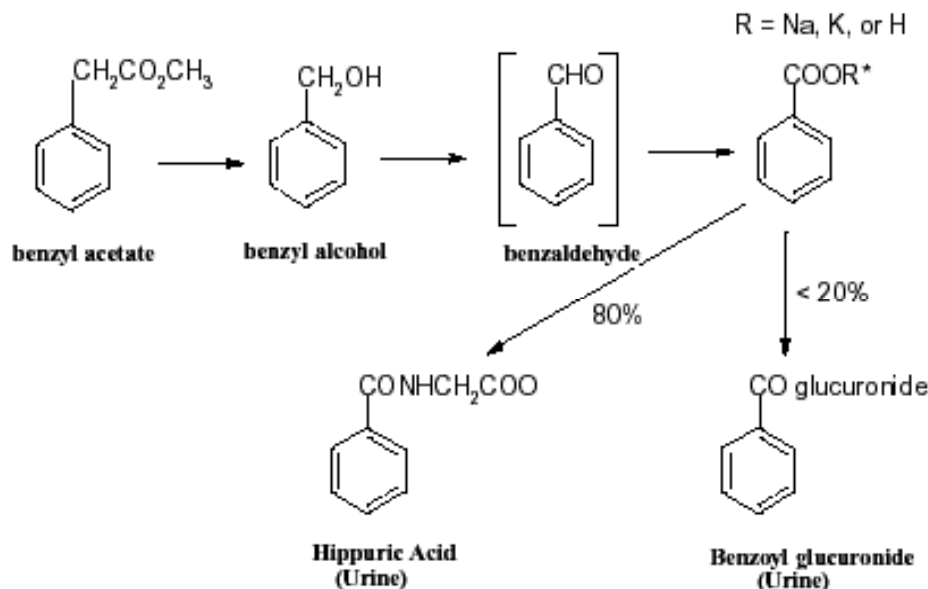
The NOEL in these studies was in all species identical with the highest dose tested:

mice and rats	NOEL : 175 mg/kg bw
hamsters	NOEL : 300 mg/kg bw
rabbits	NOEL : 250 mg/kg bw

Ref.: 47

3.3.9. Toxicokinetics

Benzoic acid and its sodium and potassium salt are considered with benzylacetate, benzylalcohol, benzaldehyde as a single category from the human health view by JECFA, as they are all rapidly metabolized and excreted via a common pathway within 24hrs.



Sodium benzoate is expected to immediately dissociate and form benzoic acid in an aqueous environment.

Ref.: 52

3.3.10. Photo-induced toxicity

No data was submitted.

In the JECFA report, there was a summary of an *in vitro* test by Eberlein-Konig et al, 1993. Suspensions of human erythrocytes were incubated with Benzoic Acid and Sodium Benzoate. Each material was tested at 10^{-5} , 10^{-4} and 10^{-3} mol/l. Erythrocyte-free samples were also incubated with the test material and used as controls. Following incubation, suspensions and samples were exposed to varying amounts of UVA light from one of three sources. Hemolysis was measured as a function of absorbance of 550 nm light.

The substances did not produce significant photohaemolysis.

Ref.: 52

3.3.11. Human data

Included in the appropriate sections

3.3.12. Special investigations**3.3.13. Safety evaluation (including calculation of the MoS)**

100% skin absorption was assumed since the dermal absorption studies were old and not to modern standards.

CALCULATION OF THE MARGIN OF SAFETY

Rinse off-products (ro):

Maximum content of active ingredient	(a. I.) [%]	2.5
Exposure to rinse-off-products	E [g/day]	0.72
Maximum amount of a. I. applied	I _{ro} [mg/day]	18.00

Oral hygiene-products (oh):

Maximum content of active ingredient	(a. I.) [%]	1.7
Exposure to oral hygiene-products	E [g/day]	3.52
Maximum amount of a. I. applied	I _{oh} [mg/day]	59.84

Eye-products (ep):

Maximum content of active ingredient	(a. I.) [%]	0.5
Exposure to eye-products	E [g/day]	0.05
Maximum amount of a. I. applied	I _{ep} [mg/day]	0.25

Non rinse off-products (nro):

Maximum content of active ingredient	(a. I.) [%]	0.5
Exposure to non rinse-off-products	E [g/day]	13.50
Maximum amount of a. I. applied	I _{nro} [mg/day]	67.50

Typical body weight (human)	TBW [kg]	60 kg

Exposure from all product groups:

$$\text{Systemic exposure dose SED [mg/kg bw/d]} = [\text{I}_{oh} + \text{I}_{ro} + \text{I}_{ep} + \text{I}_{nro} \times A] / \text{TBW} = 2.43$$

No observed adverse effect level (NOAEL) from a 4-generation study 500 mg/kg bw/d (selected by SCF)

MARGIN OF SAFETY (MOS):

MOS (NOAEL/SED)	500 mg/kg bw/d / 2.43 mg/kg bw/d	=	206

3.3.14. Discussion

Since the adoption of the previous opinion of the SCCNFP on 4 June 2002 on Benzoic acid and Sodium benzoate (SCCNFP/0532/01, final), there have been 2 major re-evaluations of the data on Benzoic acid and sodium benzoate.

The SIDS report agrees that many toxicological studies on benzoic acid and its salts are old and “do not always fulfil for 100% present-day guidelines”. They did appear to be acceptable studies for evaluation, since ‘well-known’ research groups and/or test laboratories ran the studies according to scientific standards and or accepted protocols at that time. Also, all were peer-reviewed and published in high quality scientific literature. Most of them have been reviewed and accepted by other fora like FDA, JECFA, and IPCS as acceptable studies.

In addition, there is good consistency in the individual data for a substance in the group as well as between members of the group (benzyl acetate and benzaldehyde data inclusive). Therefore, taken as a whole, the available studies give a robust database for hazard assessment and hazard evaluation of these compounds and further studies are not indicated. The JECFA Committee (1997) concluded that the data reviewed for compounds in this group were sufficient to demonstrate lack of teratogenic, reproductive or carcinogenic potential. Consequently, the Committee concluded that further studies were not required’

Ref.: 56

The conclusions of the SCF in 2002 were:

”The database is much more extensive than that considered by the Committee in 1994, both for developmental toxicity and for genotoxicity.

There appear to be sufficient studies to conclude absence of teratogenic potential, with an overall NOAEL for developmental toxicity of 500 mg/kg bw/day, based on effects on foetal weight. The fact that this overall NOAEL takes into account gavage as well as dietary studies gives further reassurance. It is therefore concluded that a further teratogenicity study on benzoic acid should no longer be required.

Similarly for genotoxicity, while some of the *in vitro* tests have been positive or equivocal, all the results from *in vivo* studies have been negative. It is therefore concluded that an *in vivo* study for clastogenic activity on benzoic acid should no longer be required.

On the basis of these data and the other types of study previously evaluated by the Committee, the Committee can establish a full Group ADI of 0 - 5 mg/kg bw for benzoic acid and its salts including benzyl alcohol and related benzyl derivatives used as flavourings.”

Ref.: 3

This consensus of opinion for teratogenic, reproductive or carcinogenic potential of benzoic acid and sodium benzoate is welcome.

Benzoic acid and sodium benzoate rapidly metabolize and excrete via a common pathway within 24hrs. Systemic toxic effects on liver and kidney were observed.

Benzoic acid and sodium benzoate have low acute oral and dermal toxicity with LD₅₀ values >2000 mg/kg bw. The 4 hrs inhalation exposure of benzoic acid at 0.026mg/l/h also show low acute inhalation toxicity.

Benzoic acid is a mild skin irritant, but sodium benzoate was not a skin irritant. Neither benzoic acid nor benzoate gave indication of a sensitizing effect in animals, but occasionally very low positive reactions were recorded with humans in patch tests with benzoic acid. It has been suggested that these positive reactions are a non-immunologic contact urticaria.

In the CICAD report, the conclusion on benzoic acid and sodium benzoate was: “However, both substances are known to cause non-immunologic immediate contact reactions. This effect is scarce in healthy subjects, while in patients with frequent urticaria or asthma, symptoms or exacerbation of the symptoms were observed”. This was considered to be a possible cholinergic mechanism. This should be borne in mind for products used on children.

Benzoic acid should be labelled as an eye irritant under the EU criteria for classification.

The percutaneous absorption study of ¹⁴C-labelled benzoic acid *in vitro* with excised human skin was old, not conforming to modern guidelines.. Total absorption was found to be approximately 45 %. These data were comparable to *in vivo* data in human, in which the total absorption of labelled benzoic acid was approximately 43 %.

Data is only given for sodium benzoate, but not the other salts or esters. It is thought that the loss of acidity due to the salt (sodium, potassium) should decrease toxicity. Since the loss of acidity is not uniform, this could be a problem. No data was provided for the esters.

4. CONCLUSION

Adequate data was provided only for benzoic acid and sodium benzoate, but not the other salts or esters.

On the basis of provided data, the SCCP is of the opinion that benzoic acid and sodium benzoate are safe for use for preservative and non-preservative purposes in cosmetic rinse-off products at a maximum concentration of 2.5 % and in cosmetic oral-care products at a maximum concentration of 1.7 %, and in leave-on products up to 0.5%. The possible non-preservative functions have not been stated.

However, in the interest of consumer safety, generation of data on irritation and sensitisation of the other salts and esters is required if they are to be used in cosmetic products.

Comment: Inclusion of all relevant data should have been made available, particularly if from the grey literature as required by the SCCNFP Notes of Guidance (SCCNFP/0690/03). In the IUCLID database, more modern percutaneous absorption studies were summarised. The study for eye irritation was not provided with the Submission I. These should all have been provided with Submission I.

5. MINORITY OPINION

Not applicable

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7. ACKNOWLEDGEMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion.
The members of the working group are:

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[FOREWORD](#)

[INTRODUCTION](#)

BENZOATES

CAS N°:65-85-0, 532-32-1, 582-25-2, 100-51-6

SIDS Initial Assessment Report for 13th SIAM

(Bern, 7th - 9th November 2001)

Chemical Name: **Benzoates: Benzoic acid, Sodium benzoate,
Potassium benzoate, Benzyl alcohol**

CAS No: **65-85-0, 532-32-1, 582-25-2, 100-51-6**

Sponsor Country: **The Netherlands**

National SIDS Contact Point in Sponsor Country: Mr. Dick Sijm

HISTORY:

In 2001 ICCA asked The Netherlands to be the sponsor country for the benzoates

no testing (X)

testing ()

COMMENTS:

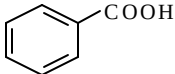
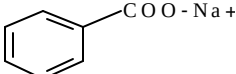
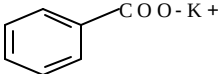
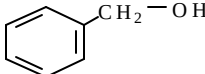
The Benzoates were already discussed in other frameworks such as the WHO. Therefore the original data were not again evaluated. The conclusions of other frameworks are discussed in the SIAR. This SIAR can be considered as a state of the art report on benzoates.

Deadline for circulation:

Date of Circulation:

(To all National SIDS Contact Points and the OECD Secretariat)

SIDS INITIAL ASSESSMENT PROFILE**Benzoates Category**

CAS No.	65-85-0	532-32-1	582-25-2	100-51-6
Chemical Name	Benzoic acid	Sodium benzoate	Potassium benzoate	Benzyl alcohol
Structural Formula				

RECOMMENDATIONS

The chemicals are currently of low priority for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Benzyl alcohol, benzoic acid and its sodium and potassium salt can be considered as a single category regarding human health, as they are all rapidly metabolised and excreted via a common pathway within 24hrs. Systemic toxic effects of similar nature (e.g. liver, kidney) were observed. However with benzoic acid and its salts at higher doses than with benzyl alcohol. For environmental effects the category is less clear, however all are readily biodegradable, non-bioaccumulative and acute toxicity values are similar. For human health all exposure routes are possible, despite benzoic acid and its salts being solids and benzyl alcohol being a liquid. For workers it will mainly be by inhalation and by skin, whereas for consumers it will mainly be by oral and dermal routes.

Human Health

The compounds exhibit low acute toxicity as for the oral and dermal route. The LD50 values are > 2000 mg/kg bw except for benzyl alcohol which needs to be considered as harmful by the oral route in view of an oral LD50 of 1610 mg/kg bw. The 4 hrs inhalation exposure of benzyl alcohol or benzoic acid at 4 and 12 mg/l as aerosol/dust respectively gave no mortality, showing low acute toxicity by inhalation for these compounds.

Benzoic acid and benzyl alcohol are slightly irritating to the skin, while sodium benzoate was not skin irritating. No data are available for potassium benzoate but it is also expected not to be skin irritating. Benzoic acid and benzyl alcohol are irritating to the eye and sodium benzoate was only slightly irritating to the eye. No data are available for potassium benzoate but it is expected also to be only slightly irritating to the eye.

The available studies for benzoic acid gave no indication for a sensitizing effect in animals, however occasionally very low positive reactions were recorded with humans (dermatological patients) in patch tests. The same occurs for sodium benzoate. It has been suggested that the very low positive reactions are non-immunologic contact urticaria. Benzyl alcohol gave positive and negative results in

animals. Benzyl alcohol also demonstrated a maximum incidence of sensitization of only 1% in human patch testing. Over several decades no sensitization with these compounds has been seen among workers.

For benzoic acid repeated dose oral toxicity studies give a NOAEL of 800 mg/kg/day. For the salts values > 1000 mg/kg/day are obtained. At higher doses increased mortality, reduced weight gain, liver and kidney effects were observed.

For benzyl alcohol the long-term studies indicate a NOAEL > 400 mg/kg bw/d for rats and > 200 mg/kg bw/d for mice. At higher doses effects on bodyweights, lesions in the brains, thymus, skeletal muscle and kidney were observed. It should be taken into account that administration in these studies was by gavage route, at which saturation of metabolic pathways is likely to occur.

It can be concluded that benzoic acid and its salts exhibit very low repeated dose toxicity. Benzyl alcohol exhibits low repeated dose toxicity.

All chemicals showed no mutagenic activity in *in vitro* Ames tests. Various results were obtained with other *in vitro* genotoxicity assays. Sodium benzoate and benzyl alcohol showed no genotoxicity *in vivo*. While some mixed and/or equivocal *in vitro* chromosomal/chromatid responses have been observed, no genotoxicity was observed in the *in vivo* cytogenetic, micronucleus, or other assays. The weight of the evidence of the *in vitro* and *in vivo* genotoxicity data indicates that these chemicals are not mutagenic or clastogenic. They also are not carcinogenic in long-term carcinogenicity studies.

In a 4-generation study with benzoic acid no effects on reproduction were seen (NOAEL $\geq$ 750 mg/kg). No compound related effects on reproductive organs (gross and histopathology examination) could be found in the (sub) chronic studies in rats and mice with benzyl acetate, benzyl alcohol, benzaldehyde, sodium benzoate and supports a non-reprotoxic potential of these compounds. In addition, data from reprotoxicity studies on benzyl acetate (NOAEL >2000 mg/kg bw/d; rats and mice) and benzaldehyde (tested only up to 5 mg/kg bw; rats) support the non-reprotoxicity of benzyl alcohol and benzoic acid and its salts.

In rats for sodium benzoate dosed via food during the entire gestation developmental effects occurred only in the presence of marked maternal toxicity (reduced food intake and decreased body weight) (NOAEL = 1400 mg/kg bw). For hamster (NOEL: 300 mg/kg bw), rabbit (NOEL: 250 mg/kg bw) and mice (CD-1 mice, NOEL: 175 mg/kg bw) no higher doses (all by gavage) were tested and no maternal toxicity was observed. For benzyl alcohol: NOAEL = 550 mg/kg bw (gavage; CD-1 mice). LOAEL = 750 mg/kg bw (gavage mice). In this study maternal toxicity was observed e.g. increased mortality, reduced body weight and clinical toxicology. Benzyl acetate: NOEL = 500 mg/kg bw (gavage rats). No maternal toxicity was observed.

Environment

From the data (fish, daphnia, algae, bacteria) it is obvious that neutralization of the pH greatly reduces (up to one order of magnitude) the acute toxicity of benzoic acid. This is also supported by the lower toxicity observed with sodium benzoate. Under environmental relevant conditions therefore the acute toxicity of benzoic acid, sodium benzoate and potassium benzoate for all four trophic levels is > 100 mg/l. Under environmental relevant conditions the acute toxicity of benzyl alcohol for fish, daphnia and bacteria is > 100 mg/l. For algae, an EC 50 3hrs of 95 mg/l is reported. Under environmental relevant conditions, benzoic acid and its salts have very low acute toxicity, whereas benzyl alcohol has low to moderate acute toxicity.

Exposure

Worldwide production capacity of benzoic acid is estimated at 700 kt per year. The major outlet (75%) for benzoic acid is as a chemical intermediate in the production of phenol, which in turn is mainly used to produce caprolactam. The next largest outlet is as a feedstock for sodium benzoate (10%) and chemical synthesis of plasticizers (5%).

Worldwide production capacity of sodium benzoate is estimated at 100 kt per year. The major outlet for sodium benzoate is as preservative in food and beverages (60%). Second most important market is cooling liquids (10%). The main function of sodium benzoate in most applications is as preservative.

Worldwide production capacity of potassium benzoate is estimated at 7 kt per year. It is used as a preservative in nonalcoholic beverages.

Worldwide production capacity of benzyl alcohol is estimated at 50 kt. Major use for benzyl alcohol is as curing agent in epoxy coatings (30%), where it becomes chemically bounded after reaction. Other important uses include the use as a solvent in low concentrations in waterborne coatings (10%) and use in paint strippers (10%) and chemical intermediate for synthesis for benzyl esters that are used in the flavor and fragrance industry (10%). The use in paint strippers is limited to uses in industrial settings.

Benzyl alcohol, benzoic acid and its sodium and potassium salt are also used in pharmaceuticals, cosmetics and/or food. Consumer exposure in these specific applications are controlled by the fact that, for all these applications, specific regulatory frameworks (regional and/or national) with authorization/approval procedures and specific advisory bodies exist (*inter alia*: the US FDA, WHO JECFA, EU SCF, etc), including, on a regular basis, reevaluation of approvals, hazardous properties and factual exposures. According to information from products registers, uses that are not specifically regulated include uses of the substances in different kinds of products e.g. paints, varnishes solvents, cleaning and washing agents, photochemicals and antifreeze agents.

Benzoic acid is a white solid, with a solubility in water of 2.9 g/l and with a vapour pressure of 0.0011 hPa at 20 °C. The log octanol/water partition coefficient was measured to 1.88; the Henry's law constant = 0.0046-0.022 Pa·m³/mol; and the pKa = 4.2. Sodium benzoate and potassium benzoate are white solids, with solubility in water of 556 g/l and with a vapour pressure of <0.0011 hPa at 20 °C. The log octanol/water partition coefficient were measured to -2.269. Benzyl alcohol is a colorless liquid, with a solubility in water of 40 g/l and with a vapour pressure of 0.13 hPa at 20°C.

The log octanol/water partition coefficient was measured to 1.1.

The distribution modeling according to Mackay Level III indicates soil and water to be the favored compartments for the chemicals. However, physical chemical properties and use patterns indicate water to be the main compartment for these substances. None are expected to hydrolyze. All are readily biodegradable. None has bioaccumulative potential.

NATURE OF FURTHER WORK RECOMMENDED

Regarding all the information provided, the substances have low priority for further work.

SIDS Initial Assessment Report (SIAR)

1. IDENTITY

Category name: Benzoates

<u>Chemicals:</u>	<u>CAS#:</u>	<u>Molecular Weight</u>
Benzoic acid	65-85-0	122.12
Sodium benzoate	532-32-1	144.11
Potassium benzoate	582-25-2	160.21
Benzylalcohol	100-51-6	108.4

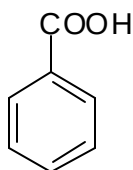
Physico-chemical properties:

Chemical	Appearance	Melting point	Boiling point @ 1013 hPa	Vapor pressure (at 20°C)	octanol/water partition coefficient (LogP)	Water Solubility (at 20°C)	Henry's law constant	pKa
Benzoic acid	White solid	122.4°C	249.2°C	0.0011 hPa	1.88	2.9 g/l	.0046 - .022 Pa*m ³ /mol	4.19
Sodium benzoate	White solid	330.6°C	464.9°C	< 0.001 hPa	-2.269	556 g/l		
Potassium benzoate *	White solid	330.6°C	464.9°C	< 0.001 hPa	-2.269	556 g/l		
Benzyl alcohol	Clear liquid	-15°C	205.3°C	0.13 hPa	1.1	40 g/l		

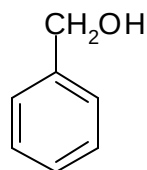
*) No data for Potassium benzoate were available, but they are expected to be the same as for sodium benzoate.

Category Justification:

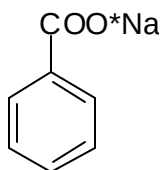
The proposed category of this ICCA HPV Benzoates submission consists of the following chemicals:



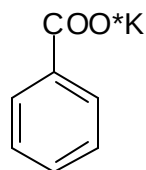
Benzoic Acid
CAS# 65-85-0



Benzyl Alcohol
CAS# 100-51-6

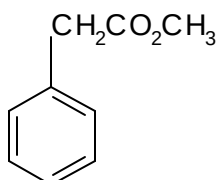


Sodium Benzoate
CAS# 532-32-1

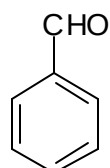


Potassium Benzoate
CAS# 582-25-2

The following chemicals (benzylacetate and benzaldehyde) are being used in this ICCA HPV benzoates submission only for supportive data purposes. They are not as such included in this category submission for reasons stated below:



Benzyl Acetate
CAS# 140-11-4

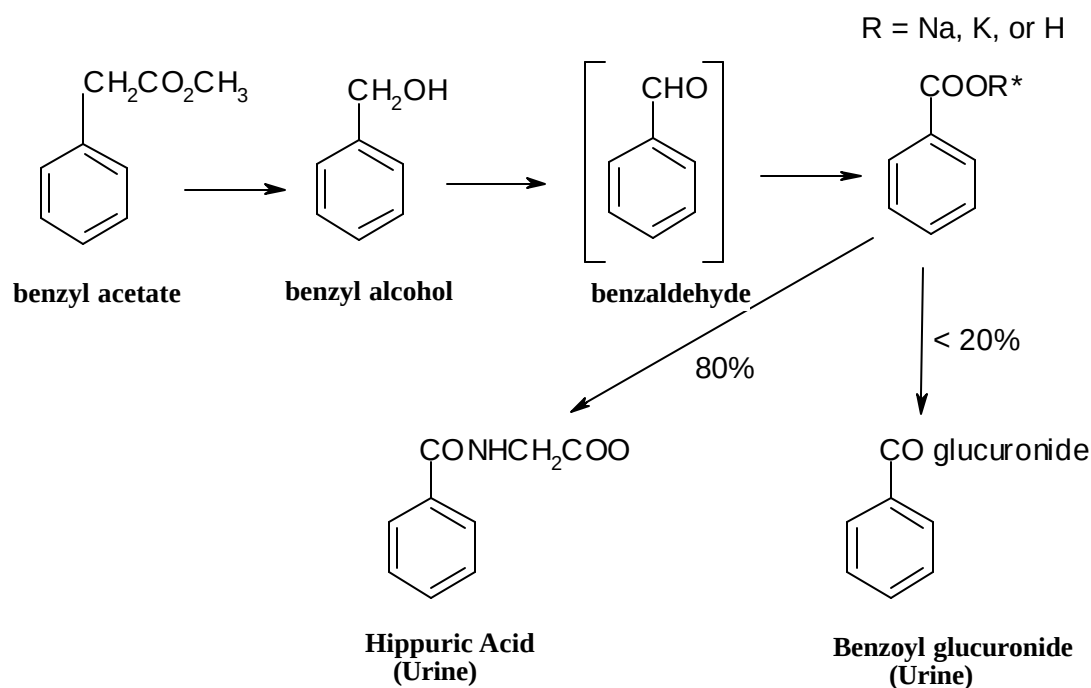


Benzaldehyde
CAS# 100-52-7

Sponsored in the US EPA HPV Program
by the Flavor and Fragrance High Production
Volume Consortia (FFHPVC)

Completed SIDS/SIAR

The common metabolic pathway of all these substances, adapted from JECFA 1997 and the American Conference of Governmental Industrial Hygienists Documentation of the Threshold Limit Values and Biological Exposure Indices, is provided below (ACGIH, 1986):



The sodium and potassium salts of benzoic acid are expected to immediately dissociate and form benzoic acid in an aqueous environment.

The benzylacetate, benzylalcohol, benzaldehyde and benzoic acid and its sodium and potassium salt were considered as a single category regarding human health by JECFA as they are all rapidly metabolized and excreted via a common pathway within 24hrs (JECFA 1997).

Benzyl acetate, the first compound in the metabolic pathway diagram, is very rapidly hydrolyzed by esterases in *several species including man* to benzyl alcohol and acetic acid. The benzylalcohol is then very rapidly metabolized as shown in the above diagram and only at very high dose (> 500 mg/kg/day by oral gavage route) some saturation of metabolic pathways occurs. This is among others very well shown in studies on benzylacetate (see below; from JECFA 1997).

Male B6C3F1 mice and Fischer 344 rats treated either intravenously or orally with ¹⁴C-benzyl acetate. The intravenous dose was equivalent to 10 mg/kg bw for mice and 5 mg/kg bw for rats. For oral administration, benzyl acetate was dissolved in corn oil and administered at doses equivalent to 10, 100, or 1000 mg/kg bw for mice and 5, 50, or 500 mg/kg bw for rats. The compound was readily absorbed from the gastrointestinal tract of both species, and about 90% of the total dose was recovered as urinary metabolites after 24h. A small proportion (0.3-1.3%) of the total dose was excreted in the faeces after both intravenous and oral administration. Elimination of benzyl acetate as carbon dioxide or volatile substances was minimal after intravenous treatment and consequently was not determined after oral treatment. Analysis of tissues of animals sacrificed 24 h after intravenous or oral administration of labelled compound showed no ¹⁴C activity, indicating that elimination of the label was virtually complete by this time. This clearance pattern indicates that benzyl acetate is readily absorbed and excreted after oral administration. The relative amounts of benzyl acetate absorbed, metabolized, and excreted were unaffected by the size or number of doses administered. Repeated treatment of rats with benzyl acetate at 500 mg/kg bw per day for 14 days, followed by a single dose of labelled compound did not change the clearance pattern. More than 90% of the radiolabel in the urine was present as hippuric acid, with minor amounts as benzyl alcohol and benzylmercapturic acid (up to 4%); no unchanged benzyl acetate was found, and the levels of benzoyl glucuronide were not measured.

There was no evidence to suggest saturation or reduction of metabolic capacity in either species over the dose range tested. At much higher dosing the proportion of the dose present as benzoyl glucuronide increased with dose, indicating a limited capacity for glycine conjugation only at extreme high dose levels.

These studies clearly show, that the compound is rapidly absorbed from the gastrointestinal tract of rats and mice, and about 90% of the total dose is recovered as urinary metabolites after 24h. More than 90% of the radio-label in the urine is present as hippuric acid, with minor amounts as benzyl alcohol and benzylmercapturic acid (up to 4%); no unchanged benzyl acetate was found. Only at very high doses, saturation of these pathways will occur.

This clearly shows the rapid pathway of hydrolysis to benzyl alcohol and subsequent oxidation to benzaldehyde to benzoic acid and subsequent conjugation to the hippuric acid.

All supports a very rapid absorption, distribution, biotransformation, and excretion of these substances by the common pathway given above.

Repeated dose toxicity studies (information in this SIAR) reveal only systemic toxic effects (e.g. liver, kidney) of similar nature, at high dose.

For environmental effects the category is less clear, however all are readily biodegradable, non-bioaccumulative and acute toxicity values for water organisms under environmental relevant conditions are similar.

For human health all exposure routes are possible, despite benzoic acid and its salts being solids and benzylalcohol being a liquid. For workers exposure will mainly be by inhalation and by skin, whereas for consumers it will mainly be oral and dermal.

2. GENERAL INFORMATION ON EXPOSURE

Production and use:

Benzoic Acid

Worldwide production capacity is estimated at 700 kt per year. Average operating rate is at max 80% resulting in a production of 560 kt benzoic acid per year. The major outlet (75%) for benzoic acid is in the production of phenol, which in turn is mainly used to produce caprolactam. The next biggest outlet is as a feedstock for sodium benzoate (10%) and chemical synthesis of plasticizers (5%). Benzoic acid is therefore mainly (>80%) used as a chemical intermediate for synthesis of other chemicals, as well as for the production of sodium salt (10%). So it has mainly a controlled use in industrial settings.

Sodium Benzoate

Worldwide production capacity is estimated at 100 kt per year. Average operating rate is at max 75% resulting in a production of 75 kt sodium benzoate per year. The major outlet for sodium benzoate is as a preservative in food and beverages (60%). Second most important market is cooling liquids (10%). The main function of sodium benzoate in most applications is as a preservative.

Potassium Benzoate:

Worldwide production capacity is estimated at 7 kt per year. It is used as a preservative in nonalcoholic beverages.

Benzyl Alcohol

Worldwide production capacity is estimated at 50 kt per year. Average operating rate is at max 80% resulting in a production of 40 kt benzyl alcohol per year. The major use for benzyl alcohol is as a curing agent in epoxy coatings (30%), where it becomes chemically bound after reaction. Other important uses are as a solvent in low concentrations in waterborne coatings (10%), and use in paint strippers (10%) and as chemical intermediate for synthesis of benzyl esters that are used in the Flavor and Fragrance industry (10%). The use in paint strippers is limited to uses in industrial settings.

Benzylalcohol, benzoic acid and its sodium and potassium salt have been used for decades in pharmaceuticals, cosmetics and/or food as preservatives and flavoring/fragrance agents

Information in Product registers:

According to information in Product Registers the substances are used in different kinds of products e.g. paints, varnishes, solvents, cleaning and washing agents, photochemicals and antifreeze agents.

Release into the environment during production and use :

In DSM Geleen The Netherlands, during production, about 650 kg/year of benzylalcohol are emitted into the atmosphere (< 0.01 % of production volume). Based on the amount benzylalcohol discharged to the DSM WWTP, it can be calculated that the influent concentration of the WWTP is at about 1 ug/l. Because of its ready biodegradability and the existing dilution of effluent to the receiving water, the concentration in the receiving water will be < 0.01 ug/l.

In DSM Rotterdam The Netherlands, during production sodium benzoate is emitted to air at < 0.01 % of the production volume. For benzoic acid this is < 0.001 %.

2.1 Environmental Exposure and Fate

Distribution modelling using Mackay Level III (the EPA default: equal releases (10,000 kg/hr) and equal distribution to all compartments was used) indicates water (34.8-50%) and soil (48.4-64.2%) to be the main compartment for all four chemicals. None are expected to volatilize to the atmosphere ($\leq 1.51\%$), nor to adsorb to sediment ($\leq 0.09\%$) (Meylan & Howard, 1999).

However physical chemical properties and use patterns indicate water to be the main compartment for these substances.

Distribution (%) according to Fugacity Level III

Chemical	CAS#	Air	Water	Soil	Sediment
Benzoic acid	65-85-0	0.911	34.8	64.2	0.093
Sodium benzoate	532-32-1	1.45e-007	45.3	54.6	0.0755
Potassium benzoate	582-25-2	1.61 e-007	45.3	54.6	0.0755
Benzyl alcohol	100-51-6	1.51	50.0	48.4	0.0923

Based on structure and organic chemistry rules (e.g. bonding in organic molecules, activation energy, reactivity, transformations, addition, substitution, elimination) no hydrolysis is expected at pH ranges of 4 - 11.

The calculated photodegradation for benzyl alcohol and the benzoates are 50% after 1.3 to 3 days (Meylan and Howard, 1999), and the measured photodegradation for benzoic acid is 90% after 140 minutes (Matthews, 1990).

Biodegradation and Bioaccumulation

All four chemicals are readily biodegradable ($> 90\%$ after 28 days) both aerobically (MITI, 1992; Zahn & Wellens, 1980; Salanitro et al., 1988) and anaerobically (Battersby & Wilson, 1989; Horowitz et al., 1982).

(Benzoic acid is used as positive control in OECD Guideline for ready biodegradability testing). From the results of numerous removal experiments the main elimination pathway for the chemicals is biotic mineralization.

The octanol/water partition coefficient of all compounds indicates a low potential for bioaccumulation. This is also supported by the rapid biotransformation and/or excretion of these compounds in urine in mammals.

2.2 Human Exposure

For human health all exposure routes are possible, despite benzoic acid and its salts being solids and benzyl alcohol being a liquid. For workers exposure will mainly be by inhalation and by skin, whereas for consumers it will mainly be oral and dermal

Consumer exposure:

Benzoic acid, benzylalcohol, sodium benzoate and potassium benzoate are widely used in food, cosmetic and pharmaceutical applications as preservatives and flavoring/fragrance agents. Benzoic acid and benzylalcohol are naturally occurring (Merck Index, 1996). Consumer exposure in these specific applications are controlled by specific regulatory frameworks (regional and/or national) with authorization/approval procedures and specific advisory bodies (among others US FDA, WHO JECFA, EU SCF, etc). A re-evaluation of approvals, hazardous properties and factual exposures (among others compliance to the ADI) inclusive, are performed on a regular basis. According to information in Product Registers the substances are used in different kinds of products e.g. paints, varnishes, solvents, cleaning and washing agents, photo chemicals and antifreeze agents. Benzoic acid and sodium benzoate are under re-evaluation at the EU Scientific Committee for Food. From preliminary information (June 2001) re-approval is expected for these substances. The Joint FAO/WHO Expert Committee on Food Additives (JEFCA) has established a group Acceptable Daily Intake (ADI) for benzoic acid and its salts and benzyl alcohol, benzyl acetate and benzaldehyde of 5 mg benzoic acid equivalent/kg bodyweight. This group ADI is based on the structural similarity and common metabolic fate of these chemicals (WHO, 1997).

Worker exposure:

Companies have provisionally advised exposure limits for benzoic acid and its salts as well as for benzyl alcohol. Also the US WEEL (Workplace Environmental Exposure Limit) Committee of the AIHA has set limits for benzyl alcohol at a value of 10-ppm (44 mg/m³) 8hr TWA.

In the several past decades of production, no cases of health complaints (sensitisation inclusive) have occurred.

Also from companies that use the substances no health complaints (sensitisation inclusive) have ever been reported.

3. HUMAN HEALTH

3.1 Effects on Human Health

In general:

- Benzoate from potassium benzoate and sodium benzoate will change from the ionized form to the undissociated benzoic acid molecule under physiological conditions.
- Benzyl acetate, benzyl alcohol and benzaldehyde are all metabolized to benzoic acid and it is therefore reasonable to assume that the results of studies on members of the group will apply to the others.
- All benzyl compounds are rapidly absorbed, and rapidly and completely excreted in the urine. The main transformation of benzoic acid is the formation of hippuric acid.
- It is considered also that data gaps for one substance can be adequately addressed by the existing data for the other compounds.

Only the results of the critical studies are given, but for most endpoints additional studies exist (see full IUCLID documents), that support the results in the critical studies.

3.1.1 Acute Oral Toxicity

Three of the four compounds were tested according to Guideline methods. All demonstrated very low or low toxicity, especially the benzoate salts. Only benzyl alcohol has a LD50 slightly less than 2000mg/kg bw and should therefore be considered as harmful. Although the studies on potassium benzoate were not Guideline studies, these were accepted because the results showed low toxicity, similar to the sodium salt.

Chemical	Species	Protocol	Result	Reference
Benzoic acid	rat	Directive 84/449/EEC	LD50 =2565 mg/kg	IRDC#163-282, 1974
	mouse	OPPTS 870.1100	LD50 =2250 mg/kg	BRL#9348, 1979
Sodium benzoate	rat	Directive 84/449/EEC	LD50 =3140 mg/kg	Loeser, 1977-A; Deuel et al., 1954
	rat	other	LD50 =4070 mg/kg	Smyth & Carpenter, 1948
Benzyl alcohol	rat	Directive 84/449/EEC	LD50 =1610 mg/kg	Loeser, 1978
	rat	other	LD50 =2080 mg/kg	Graham & Kuizenga, 1945; Opdyke, 1973
	mouse	other	LD50 =1580 mg/kg	Jenner, 1964; Opdyke, 1973
Potassium benzoate	rat	other	LD50 = >10,000 mg/kg	Kravets-Bekker & Ivanova, 1970
	mouse		LD50 = >10,000 mg/kg	Kravets-Bekker & Ivanova, 1970
	guinea pig		LD50 = >10,000 mg/kg	Kravets-Bekker & Ivanova, 1970

3.1.2 Acute Dermal Toxicity

Two of the compounds were tested for acute dermal toxicity. Both demonstrated low toxicity.

Chemical	Species	Protocol	Result	Reference
Benzoic acid	rabbit	EPA OTS 798.1100	LD50= > 2000 mg/kg	IRDC#163-282, 1974; Opdyke, 1973
Benzyl alcohol	Rabbit	Other	LD ₅₀ = 2000 mg/kg	NPIRI,1974
	guinea pig	Other	LD ₅₀ = < 5 ml/kg	Jones, 1967; Opdyke, 1973

3.1.3 Acute Inhalation Toxicity

Two of the compounds were tested for acute inhalation toxicity according to Guideline procedures; both demonstrating very low toxicity.

Chemical	Species	Protocol	Result	Reference
Benzoic acid	rat	EPA OTS 798.1150	LC ₅₀ = >12.2 mg/l/4h. No mortality at 12.2 mg/l as dust.	IRDC#163-282, 1974
Benzyl alcohol	rat	OECD Guide-line 403 and GLP	LC ₅₀ = > 4.178 mg/l/4h. No mortality at 4.178 mg/l as aerosol	Bayer AG, 1990

In conclusion: The compounds exhibit low acute toxicity, except benzylalcohol that has an oral LD₅₀ slightly less than 2000 mg/kg bw and should therefore be considered as harmful by the oral route.

3.1.4 Skin Irritation

Three of the compounds were tested for skin irritation according to Guideline procedures; the potassium salt should be similar to the sodium salt, therefore being non-irritating.

Chemical	Species	Protocol	Result	Reference
Benzoic acid	Rabbit	EPA OTS 798.4470	not irritating	IRDC # 163-282
	rabbit	Directive 84/449/EEC	slightly irritating	RCC NOTOX - study no. 0847/1083, 1988.
Sodium benzoate	Rabbit	OECD Guide-line 404	not irritating	RCC NOTOX - study no. 014658
	rabbit	Directive 84/449/EEC	not irritating	Loeser, E., 1977-B
Benzyl alcohol	rabbit	OECD Guide-line 404	not irritating	Bayer AG data, Report No. 19232, 1990
	rabbit	Other	slightly irritating	Smyth, H. F. et al., 1951; reported in US NTP: TR 343, 1989.

3.1.5 Eye Irritation

Three of the compounds were tested for eye irritation according to Guideline procedures; the potassium salt should be similar to the sodium salt, therefore being non- to slightly irritating.

Chemical	Species	Protocol	Result	Reference
Benzoic acid	Rabbit	Directive 84/449/EEC	highly irritating	RCC NOTOX - study no. 0847/1084, 1988
	rabbit	EPA OTS 798.4500	severely irritating	IRDC #163-282
Sodium benzoate	Rabbit	Directive 84/449/EEC	not irritating	Loeser, E., 1977-B
	rabbit	OECD Guide-line 405	slightly irritating	RCC NOTOX - study no. 014669, 1988
Benzyl alcohol	Rabbit	OECD Guide-line 405	moderately irritating	Bayer AG data, Report No. 19232, 1990
	rabbit	Other: limited data	highly irritating	Smyth, H. F. et al., 1951; reported in US NTP: TR 343, 1989

In conclusion: Benzoic acid and benzylalcohol are slightly irritating to the skin, while sodium and potassium benzoate are not skin irritating. Benzoic acid and benzyl alcohol are irritating to eyes, and sodium and potassium benzoate are only slightly irritating to eyes

3.1.6 Sensitization

The available studies for benzoic acid gave no indication for a sensitizing effect in animals, however some weak positive reactions were recorded with the human patch test. Benzyl alcohol was non-sensitizing in the Draize and Guinea Pig Maximization Tests, but a positive sensitizer in the Freund's Complete Adjuvant Test and the guinea pig Open Cutaneous Test and demonstrated a maximum incidence of sensitization of 1% in clinical human patch testing. A clinical dermatological study showed positive patch test reactions in 0.2% of the patients treated with 5% sodium benzoate in petrolatum. It has been suggested that this very low potential of sodium benzoate to elicit a *non-immunologic* contact urticaria may be due to the formation of benzoic acid at skin contact.

Chemical	Species	Protocol	Result	Reference
Benzoic acid	guinea pig	Draize	not sensitizing	BRL #9347, 1979
	guinea pig	Guinea pig maximization test	not sensitizing	Gad, 1986
	human	Patch test	occasional positive result	Rademaker & Forsyth, 1989; Forsbeck & Skog, 1977
Sodium benzoate	Human	Patch test	5 of 2045 patients positive	Brasch, J. et al., 1993
	human	Patch test	nonimmunologic contact urticaria	Nethercott, J.R., 1984
Benzyl alcohol	guinea pig	Draize Test	not sensitizing	Klecak, G. et al., 1977
	guinea pig	Guinea pig maximization test	not sensitizing	Klecak, G. et al., 1977
	guinea pig	Freund's complete adjuvant test	sensitizing	Klecak, G. et al., 1977
	guinea pig	Open epicutaneous test	sensitizing	Klecak, G. et al., 1977
	human	Patch-Test	sensitizing	Malten, K. E. et al., 1984; Mitchell, J. C. et al., 1982; Nethercott, J. R., 1982

In conclusion: No firm conclusion on the sensitizing potential of benzyl alcohol can be made due to the varied results with the various tests. Both benzoic acid and sodium benzoate were non-sensitizing in animal test but showed a very low incidence in humans (patients) tested by the patch test.

CICAD conclusion on benzoic acid and sodium benzoate was: "However, both substances are known to cause non-immunologic immediate contact reactions. This effect is scarce in healthy subjects, while in patients with frequent urticaria or asthma, symptoms or exacerbation of the symptoms were observed".

3.1.7 Repeat Dose Toxicity

Several short term repeated dose toxicity studies are available (see IUCLID documents) on compounds of the group (as well as benzaldehyde and benzyl acetate) and support the outcome and No Observed Adverse Effect Level (NOAEL) of the longer term studies given below.

In a 4-generation study 20 rats/sex/group were dosed continuously by diet with 375 or 750 mg/kg/day **benzoic acid**. In all 4 generations no influence on growth (weight, weight gain and food efficiency (measured by protein efficiency)) and organ weights was found. The animals of the 3rd generation were killed and examined histopathologically after 16 weeks (after lactation of the pups). No histo-pathological findings were found. In the paper, no information is given on the organs investigated, however the robustness of the total study, the reputation of the investigators, as well as the reputation of the Professor who did the histopathologic investigation, a high scientific quality has to be assumed even though the studies were performed many years ago. From other parameters it can be assumed that as a minimum the brains, heart, liver, kidney, testis and spleen were examined.

Feeding of 375 mg/kg/day led to prolongation of survival compared to controls

NOAEL $\geq$ 750 mg/kg/day

(Kieckebusch & Lang, 1960)

Due to missing hematological and clinical chemistry investigations in all studies only a preliminary NO(A)EL of about 800 mg/kg can be derived for rats which is based on the studies from Kieckbusch & Lang (1960), Kreis et al. (1967) and Bio-Fax (1973) (Details to be found in the IUCLID).

A 21 day dermal study with male/female New Zealand white rabbits dosed with 100, 500, or 2500 mg/kg bw **benzoic acid** 5 days/week showed no compound related effects in behavior, body weight organ weights, clinical laboratory tests or survival. Very slight dermal irritation was noted for 1/8 rabbits at the 2500 mg/kg level.

NOAEL = 2500 mg/kg/day

(IRDC# 163-675, 1981)

Four groups of 10 CD rats/sex/group were exposed to 0, 25, 250 or 1200 mg **benzoic acid** dust aerosol/m³ (analytical concentration; MMAD 4.7 μ m) for 6 hours/day and 5 days/week over 4 weeks. At $\geq$ 25 mg/m³ an increased incidence of interstitial cell infiltrate and interstitial fibrosis in the lungs in treated animals compared with controls was seen. *However, there was no clear dose-dependency*. A concentration of $\geq$ 250 mg/m³ resulted in upper respiratory tract irritation and decreased absolute kidney weights in females. In the highest-dose group one rat/sex died and the body weight gain was decreased in males and females. Other effects included a decrease in platelets (males/females), absolute/relative liver weights (males) and trachea/lung weights (females). LOAEC (local effect) = 25 mg/m³ (However no clear dose-response was observed).

NOAEC (systemic) = 25 mg/m³

(IRDC# 163-676, 1981)

In a 10-day study, rats received **sodium benzoate** in feed. At the lowest tested concentration of 1358 mg/kg changes in serum cholesterol levels occurred in females. At doses of 1568 mg/kg and above changes in further serum parameters and an increased relative liver weight were described. Histopathological changes of the liver, increased relative kidney weights and disorders of the central nervous system were seen after dosing via diet with $\approx$ 1800 mg/kg. (Fujitani, 1993)

A 90-day study with male/female Sherman rats given 640, 1280, 3145, or 6290 mg/kg/day USP **sodium benzoate** continuously in feed showed no adverse effects at $\leq$ 3145 mg/kg bw. There was increased mortality (4/8 died); reduced weight gain; increased weight of livers and kidneys; pathological lesions (not specified) in livers and kidneys at 6290 mg/kg bw.

NOAEL = 3145 mg/kg bw/day

(Deuel, 1954)

For mice the NO(A)EL of **sodium benzoate** is higher. According to a 35 day study (by drinking water) no effects were observed at 3000 mg/kg bw. At this dose level also in a chronic study no toxic effects were found in histopathological examinations (see 3.1.9 paragraph 2, Toth, 1984) (Toth, 1984).

A 13-week study with male/female F344/N rats given 50, 100, 200, 400, or 800 mg/kg/day **benzyl alcohol** by *gavage* showed staggering, respiratory difficulty, and lethargy in rats of the high dose group. Hemorrhages occurred around the mouth and nose, and there were histologic lesions in the brain, thymus, skeletal muscle, and kidney. There were reductions in relative weight gain in male rats dosed with 800 mg/kg and in female rats dosed with 200 mg/kg or more. No notable changes in bw gain or compound-related histopathologic lesions were observed in rats from the lower dose groups.

In the 2-y study (see 3.1.9 paragraph 3), however, no notable changes were found on bw or bw gain at 200 or 400 mg/kg/d. The NOAEL in this 2-y rat study was 400 mg/kg/day, the highest dose tested.

NOAEL = 400 mg/kg/day (based on investigated parameters and taking into account the bw results of the 2-y study)

(US NTP Technical Report No. TR 343, 1989)

A 13-week study with male/female B6C3F1 mice given 50, 100, 200, 400, or 800 mg/kg/day **benzyl alcohol** by *gavage* showed staggering in mice dosed with 800 mg/kg, after dosing during the first 2 weeks of the study. Staggering after dosing occurred during the first 2 w of the study in mice dosed with 800 mg/kg. There were reductions in relative weight gain in male mice dosed with 400 or 800 mg/kg, and in female mice dosed with 200 mg/kg or more. No notable changes in bw gain or compound-related histopathologic lesions were observed in mice from the lower dose groups.

In the 2-y study (see 3.1.9 paragraph 4), however no notable changes were found on bw or bw gain at 200 mg/kg/d. The NOAEL in this 2-y mice study was 200 mg/kg/day the highest dose tested.

NOAEL = 200 mg/kg/day (based on reduction of relative weight gain only and taking into account the bw results of the 2-y study).

(US NTP Technical Report No. TR 343, 1989)

It should be noted: these studies were done by gavage (leading to greater toxicity due to the “bolus effect”). The administration of the benzyl compounds by gavage are likely to reveal changes at lower doses compared to studies where the substances are applied in the diet, leading to a distribution in the body over time.

In conclusion: For benzoic acid repeated dose (long-term inclusive) oral toxicity gives a NOAEL of 800 mg/kg/day. For the salts values > 1000 mg/kg/day are obtained. At higher doses increased mortality, reduced weight gain, liver and kidney effects were observed. For benzyl alcohol taking into account also the results of the long-term studies indicate a NOAEL $\geq$ 400 mg/kg bw/d for rats and $\geq$ 200 mg/kg bw/d for mice, however it should be taken into account that in these studies administration was by gavage, at which bolus dosing occurs and saturation of metabolic pathways is likely to occur. At high doses, effects on bodyweights, lesions in the brains, thymus, skeletal muscle and kidney were observed.

It can be concluded that benzoic acid and its salts exhibit very low repeated dose toxicity. Benzyl alcohol exhibits low repeated dose toxicity.

3.1.8 Genetic Toxicity

3.1.8.1 Genetic Toxicity *in vitro*

Benzoic acid was not mutagenic in Ames tests with and without metabolic activation (EGG# 580-192-1-78, 1978). The Sister Chromatid Exchange assay with human lymphocytes was negative - no metabolic activation was used (Jansson, 1988; Tohda, 1980). A Chromosome Aberration study with CHL cells was ambiguous - no metabolic activation was used (Ishidate, et al., 1984). A recombination assay with *Bacillus subtilis* H17 and M45 was positive (reported with minimal documentation in an abstract, Nonaka, 1989).

Sodium benzoate was not mutagenic in Ames tests with and without metabolic activation (Ishidate, et al., 1984). A cytogenetic assay using anaphase preparations of cultured human embryonic lung cells was negative - no metabolic activation was used (FDA PB 245453, 1974). An *Escherichia coli* reverse mutation assay was negative with and without metabolic activation (Prival, 1991). A cytogenetic assay using CHL cells was positive without metabolic activation (Ishidate, et al., 1984; Ishidate & Odashima, 1977). Sister Chromatid Exchange assays using Chinese hamster cells or human lymphocytes were positive without metabolic activation (Abe & Sasaki, 1977; Xing & Zhang, 1990). A recombination assay with *Bacillus subtilis* H17 and M45 was positive (reported with minimal documentation in an abstract, Nonaka, 1989).

Potassium benzoate tested positive in a recombination assay using *Bacillus subtilis* H17 and M45, with and without metabolic activation (Ishizaki & Ueno, 1989).

Benzyl alcohol was not mutagenic in Ames tests with and without metabolic activation (US NTP Technical Report No. TR 343, 1989). *Escherichia coli* reverse mutation assay was negative with and without metabolic activation (Leifer et al., 1981). A cytogenetic assay using CHO cells was negative without metabolic activation and positive with metabolic activation (Anderson et al., 1990; Zeiger et al., 1990). A Sister Chromatid Exchange assay using CHO cells was ambiguous with and without metabolic activation (US NTP Technical Report No. TR 343, 1989). A recombination assay with *Bacillus subtilis* H17 and M45 was positive (reported with minimal documentation, Kuroda et al., 1984).

Summary of (non-Ames) *in vitro* results:

Species (test system)	End-point	Results		Remarks
		without metabolic activation	with metabolic activation	
Benzoic acid				
Human lymphoblastoid cells (transformed by Epstein-Barr virus)	Sister chromatid exchange	Negative	NT	
Bacillus subtilis H17, M45	Recombination assay			tested positive (no further information available, only summary given)
Chinese hamster cells (CHL)	Chromosome aberration	?	NT	result given as negative in: Ishidate et al. (1984)
Sodium benzoate				
Human embryonic lung cells	Anaphase preparation	Negative	NT	
E.coli WP2	Reverse mutation assay	Negative	Negative	
Bacillus subtilis H17, M45	Recombination assay			tested positive (no further information available, only summary given)
Chinese hamster cells (CHL)	Chromosome aberration	Positive	NT	
Chinese hamster cells (DON)	Sister chromatid exchange	Positive?	NT	slight increase without dosage effect
Human lymphocytes	Sister chromatid exchange	Positive	NT	
Potassium benzoate				
Bacillus subtilis H17, M45	Recombination assay			tested positive (limited data)
Benzyl alcohol				
E.coli	Reverse mutation assay	Negative	Negative	
Chinese hamster cells (CHO)	Cytogenetic assay	Negative	Positive	
Chinese hamster cells (CHO)	Sister chromatid Exchange	?	?	
Bacillus subtilis H17, M45	Recombination assay			tested positive (limited data)

? = ambiguous

NT = not tested

In conclusion: Studies of these chemicals in the Ames point mutation assay do not show evidence of mutagenicity.

However, some have been reported to be positive in the less commonly used *Bacillus subtilis* recombination assay. In a number of cases adverse effects on the chromosome could be noticed, however also negative and/or equivocal results were reported.

However many higher-level *in vivo* tests (clastogenicity inclusive) were negative (see 3.1.8.2).

3.1.8.2 Genetic Toxicity *in vivo*

General remark: Since the sodium salt of benzoic acid instantaneously dissociates to the benzoic acid, the studies with sodium benzoate are also representative for benzoic acid and potassium benzoate.

A cytogenic assay in male rats given single or multiple gavage doses of 50, 500, or 5,000 mg/kg **sodium benzoate** showed no significant increase in chromosomal aberrations in the bone marrow.
(FDA PB 245453, 1974)

A dominant lethal assay using male rats given single or multiple gavage doses of 50, 500, or 5,000 mg/kg **sodium benzoate** was non-mutagenic.
(FDA PB 245453, 1974)

Remark: IPCS CICAD 26 (2000) mentioned this dominant lethal assay as a positive result, however evaluation of the raw data in the original report (by experts of the industry consortium and a recent independent review by Prof. R. Kroes) gives no support for this. In addition the authors of the study clearly conclude negative. FDA also evaluated this study as negative. In addition sodium benzoate doesn't contain a structural alert for genotoxicity.

A host mediated assay using male rats given multiple gavage doses of 50, 500, or 5,000 mg/kg **sodium benzoate** showed no elevation of mutant frequencies in *Salmonella typhimurium* G46; no elevation of mutant frequencies in *Salmonella typhimurium* TA 1530; no increase in recombinant frequencies in *Saccharomyces cerevisiae* D3.
(FDA PB 245453, 1974)

A host mediated assay using male rats given a single gavage dose of 50, 500, or 5,000 mg/kg **sodium benzoate** showed an elevation of mutant frequencies in *Salmonella typhimurium* TA 1530 in the intermediate dose level; the other doses were negative.
(FDA PB 245453, 1974)

A Mouse Micronucleus assay using 50, 100, 200 mg/kg **benzyl alcohol** by i.p. injection was negative at all doses tested.
(Hayashi et al., 1988)

A Replicative DNA Synthesis assay using male Fischer 344 rats given a single dose of 0, 300 or 600 mg/kg bw **benzyl alcohol** by gavage was negative at all doses tested.
(Uno et al., 1994) ;

A Replicative DNA Synthesis assay using male B6C3F1 male mice given a single dose of 0, 400 or 800 mg/kg bw **benzyl alcohol** by gavage was negative at all doses tested.
(Miyagawa et al., 1995)

A *Drosophila melanogaster* SRL assay with **benzylalcohol** 5000 ppm (feed) and 8000 ppm (injection) was negative (Foureman, et al., 1994)

Summary of genetic toxicity *in vivo* results:

Species (test system)	End-point	Results	Remarks
Sodium benzoate			
male Sprague Dawley rats	Cytogenetic Assay (bone marrow)	Negative	
male ICR mice	Host-Mediated Assay (tester strains Salmonella typhimurium TA 1530, G 46 and Saccharomyces cerevisiae D3)	Negative	elevated mutant frequency with T A 1530 in the intermediate single gavage dosing only (clear negative after multiple gavage dosing)
male random bred rats	Dominant Lethal Assay	Negative	
Benzyl alcohol			
male mice	Mouse Micronucleus Assay	Negative	
male Fischer 344 rats	Replicative DNA Synthesis	Negative	
male B6C3F1	Replicative DNA Synthesis	Negative	
Drosophila melanogaster	SLR assay	Negative	

In addition data from *in-vivo* genotoxicity studies on **benzyl acetate** and **benzaldehyde** (JECFA report, 1997) are supportive evidence for the non-genotoxicity of benzyl alcohol and benzoic acid and its salts.

Summary genetic toxicity *in vivo* results:

Species (test system)	End-point	Dose	Results	Remarks
Benzaldehyde				
<i>Drosophila melanogaster</i>	Sex-linked recessive lethal mutation	150 ppm (feed), 2500 ppm (injection)	Negative	Woodruff et al. (1985); US NTP (1990)
Benzyl acetate				
<i>Drosophila melanogaster</i>	Sex-linked recessive lethal mutation	300 ppm (feed), 20,000 ppm (injection)	Negative	US National Toxicology Program (1993)
Mouse bone-marrow cells	Chromosomal aberration	325-1700 mg/kg bw (i.p.)	Negative	US National Toxicology Program (1993)
Mouse bone-marrow cells	Micronucleus formation	312-1250 mg/kg bw (i.p.)	Negative	US National Toxicology Program (1993)
Mouse peripheral blood	Micronucleus formation	3130-50 000 ppm in diet	Negative	US National Toxicology Program (1993)
Mouse bone-marrow cells	Sister chromatid exchange	325-1700 mg/kg bw (i.p.)	Negative	US National Toxicology Program (1993)

In conclusion: The compounds exhibit no genotoxicity in several *in-vivo* assays evaluating different endpoints.

3.1.9 Carcinogenicity

In a 2-year carcinogenicity study, groups of 50 male and 52 female Fischer 344 rats, four to five weeks old, received diets containing 1% (500 mg/kg bw per day) or 2% (1000 mg/kg bw per day) **sodium benzoate** for 18-24 months. Controls, consisting of 25 male and 43 female rats, received basal diet. Food intake was adequately controlled to avoid an excess; tap water was available *ad libitum*. Survival was very poor in all groups, due to intercurrent sialodacryoadenitis and mycoplasma infections. All surviving animals were sacrificed between 18 and 25 months, all were autopsied, and various tissues were examined histopathologically. No adverse clinical signs directly attributable to treatment were observed, and only negligible differences in average body weight and mortality rate were seen between the treated and control groups. Although a variety of tumors occurred among treated and control rats of each sex, they were of similar type and incidence.

(Sodemoto & Enomoto, 1980)

Poor survival in all groups, due to infections, limits the usefulness of this study.

A lifelong study using male/female Swiss Albino mice given 2% **sodium benzoate** continuously in drinking water showed no carcinogenic effect.

In the main study, a 2% solution of sodium benzoate (purity, 99%) was administered in the drinking water to groups of 50 male and 50 female five-week-old mice for their lifetime. Groups of 100 males and 100 females were used as untreated controls. Both treated and control animals were 'carefully checked'; their body weights were measured weekly, and gross pathological changes were recorded. The animals were either allowed to die or were sacrificed when moribund. Complete necropsies were performed on all animals, and the liver, spleen, kidneys, bladder, thyroid, heart, pancreas, testes, ovaries, brain, nasal turbinates, at least four lobes of the lungs, and organs with

gross pathological changes were examined histologically. The average daily intake of sodium benzoate was 124.0 mg for males and 119.2 mg for females on the basis of daily water consumption of 6.2 and 5.9 ml, respectively. The dose of sodium benzoate was equivalent to 6200 mg/kg bw per day for males and 5960 mg/kg bw per day for females. Treatment had no effect on survival or the incidence of tumors.

(Toth, 1984).

This study is sufficiently reliable due to the number of animals and detailed histopathological examinations.

In a 2-year carcinogenicity study, **benzyl alcohol** was administered in corn oil by gavage to groups of 50 Fischer 344/N rats of each sex at a dose of 0, 200, or 400 mg/kg bw per day on five days a week for 103 weeks. The rats were observed twice daily, and body weights were recorded weekly for the first 12 weeks and once a month thereafter. Gross necropsy was performed on all animals and 49 tissues and organs, including brain, kidney, pancreas, and skeletal muscle, from all female rats and from male rats in the vehicle control and high-dose groups and those in the other groups that died before 22 months or which had gross lesions were examined histologically. The mean body weights of treated and control animals were comparable throughout the study. No compound-related clinical signs were observed, although a sialodacryoadenitis viral infection was widespread among the study animals in the third month. The survival of treated females was significantly lower than that of vehicle controls: 70% of controls, 34% of low-dose females, and 34% of high-dose females; this was due to a much higher incidence of accidental deaths related to the gavage process. Survival among the male rats was comparable in all groups: 56% of controls, 54% at the low dose, and 48% at the high dose.

Cataracts and retinal atrophy were observed at increased incidences in rats at the high dose. The authors attributed this effect to the proximity of this group of animals to fluorescent light for most of the study. An increased incidence of hyperplasia of the forestomach epithelium was seen (not statistically significant) in male rats: control, 0/48; low dose, 0/19; high dose, 4/50. Hemorrhage and foreign material in the respiratory tract seen in treated rats that died before the end of the study were suggested by the authors to have been the result of either direct deposition of material into the lung during gavage 'accidents' or the anaesthetic properties of benzyl alcohol resulting in reflux of gavage material and aspiration into the lungs. No pancreatic acinar-cell adenomas were reported, and no other effects of treatment were seen at gross necropsy or histopathological examination.

(US National Toxicology Program, 1989)

In a 2-year carcinogenicity study, **benzyl alcohol** (purity, 99%) was given to groups of 50 B6C3F1 mice of each sex, eight to nine weeks of age, at a dose of 0, 100, or 200 mg/kg bw per day in corn oil by gavage on five days a week for 103 weeks. The doses were selected on the basis of those found to induce neurotoxic effects (lethargy and staggering) in short-term studies. The mice were observed twice daily, and their body weights were recorded weekly for the first 12 weeks and once a month thereafter. Gross necropsy was performed on all animals, and 50 tissues and organs, including brain, liver, kidney, and stomach, from all vehicle controls, animals at the high dose, and animals at the other doses that died before 22 months or had gross lesions were examined histologically.

The mean body weights of treated and control mice were comparable throughout the study. The survival of control females was significantly lower than that of animals at the high dose after week 74, but no other differences in survival were seen: 68% of control, 66% of low-dose, and 70% of high-dose males; and 50% of control, 62% of low-dose, and 72% of high-dose females. No significant treatment-related effects were noted at gross necropsy or histopathological examination.

(US National Toxicology Program, 1989).

In conclusion: The compounds exhibit no carcinogenicity.

3.1.10 Toxicity to Reproduction

In a 4-generation study 20 rats/sex/group were dosed continuously by diet with 375 or 750 mg/kg/day **benzoic acid**. In all 4 generations, no effects on fertility (“Fortpflanzung”) and lactation (“Aufzugt der Jungen”) were found. In addition a so-called “Alters Paarung” after 48 weeks gave no influence on start of menopause.

NOAEL (Parental) $\geq$ 750 mg/kg/day

NOAEL (F1 Offspring) $\geq$ 750 mg/kg/day

NOAEL (F2 Offspring) $\geq$ 750 mg/kg/day
(Kieckebusch & Lang, 1960)

In addition data from reprotoxicty studies on benzyl acetate and benzaldehyde (JECFA report 1997) give supportive evidence for the non-reprotoxicity of benzyl alcohol and benzoic acid and its salts.

The potential reproductive toxicity of **benzyl acetate** was assessed by examining sperm morphology, vaginal cytology, and the weights of male reproductive organs at the end of the 13-week feeding study (US National Toxicology Program, 1993) in mice. Dietary levels of 3130-50 000 ppm benzyl acetate (> 3000 mg/kg bw/d) had no effect on the weights of the epididymis, cauda epididymis, or testis or on sperm motility or density or the percent of abnormal sperm. The mean length of the estrous cycle of mice at the high dose was significantly greater than that of the control group. This effect was associated with a significant decrease in body weight.
(Morrissey et al., 1988)

The potential reproductive toxicity of **benzyl acetate** was assessed by examining sperm morphology, vaginal cytology, and the weights of male reproductive organs at the end of the 13-week feeding study in rats. Dietary levels of 3130-50 000 ppm benzyl acetate (> 2000 mg/kg bw/d) had no effect on the weights of the epididymis, cauda epididymis, or testis, on sperm motility, or on the density or percent of abnormal sperm.

(US National Toxicology Program, 1993)

A single study was conducted to examine the potential reproductive toxicity of **benzaldehyde**, and the report was available as a translation from Romanian. A group of 10 rats of breeding age were given 2 mg benzaldehyde in oil (type not specified) by gavage every other day for 32 weeks, equivalent to about 5 mg/kg bw per day. Ten controls were used. Two pregnancies in each rat, one at 75 days and one at 180 days, were studied. The end-points examined included the number of pregnant females, number of offspring born, pup body weight at days 7 and 21 post partum, and pup viability.

At the end of treatment, the body weights of control and treated rats were similar: 265 g and 260 g, respectively. It was reported that fewer females in the group given **benzaldehyde** than in the control group became pregnant; however, no data or statistical analyses were presented. The authors concluded that treatment did not significantly modify any of the parameters studied. No further details were available.

The NOAEL was about 5 mg/kg bw per day.

(Spom et al., 1967)

In addition no compound related effects on reproductive organs (gross and histopathology examination) could be found in the (sub) chronic studies in rats and mice with benzyl acetate, benzyl alcohol, benzaldehyde, sodium benzoate and supports a non-reprotoxic potential of these compounds (see studies in sections on repeated dose toxicity and carcinogenicity).

In conclusion: According to IPCS CICAD 26 (2000) (only evaluating benzoic acid and sodium benzoate), no clear statement on the reproductive effects can be given on basis of the Kieckebusch & Lang (1960) and Toth (1984) studies only. However, critical evaluation of the original paper of

the Kieckebusch & Lang study gives confidence of an adequately performed study although it was performed many years ago. In addition, reprotoxicity studies on benzaldehyde and benzylacetate and the fact that no compound related effects on reproductive organs were found in the (sub)chronic studies with all the compounds supports the lack of reproductive potential. Therefore the available consistent data on compounds in this group (data on benzyl acetate and benzaldehyde inclusive) taken as a whole are sufficient to demonstrate the lack of reprotoxic potential.

3.1.11 Developmental Toxicity

Pregnant Wistar rats were treated on day 9 of gestation with one dose of 510 mg/kg **benzoic acid** in carboxymethylcellulose. Animals were sacrificed on Day 20 of gestation and the uterus observed in situ for implantation and resorption sites. Live fetuses were removed, examined for gross malformations, weighed, and prepared for histopathological examination. Treatment with benzoic acid resulted in no dead or resorbed implants and 3 % abnormal survivors, rates comparable to the control animals.

NOAEL Maternal toxicity: 510 mg/kg bw
NOAEL Teratogenicity: 510 mg/kg bw
(Kimmel et al., 1971)

A 4-generation study with female rats dosed with 375 or 750 mg/kg/day **benzoic acid** during pregnancy and lactation showed no effects on the dams or on the growth and development of the offspring.

NOAEL Maternal toxicity: $\geq$ 750 mg/kg/day
NOAEL Teratogenicity: $\geq$ 750 mg/kg/day
(Kieckebusch & Lang, 1960)

Studies on the developmental toxicity of sodium benzoate administered by gavage to multiple species (rat, mice, rabbit, hamster) were conducted by Food and Drug Research Labs, Inc. (1972):

A study using pregnant Wistar rats, dosed with 1.75, 8, 38 or 175 mg/kg **sodium benzoate** by gavage on Days 6-15 of gestation showed no effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from controls.

NOAEL Maternal toxicity: 175 mg/kg bw
NOAEL Teratogenicity: 175 mg/kg bw
(FDA PB# 221777, 1972)

A study using pregnant CD-1 mice, dosed with 1.75, 8, 38 or 175 mg/kg **sodium benzoate** by gavage on Days 6-15 of gestation showed no effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from controls.

NOAEL Maternal toxicity: 175 mg/kg bw
NOAEL Teratogenicity: 175 mg/kg bw
(FDA PB# 221777, 1972)

A study using pregnant Dutch-belted rabbits, dosed with 2.5, 12, 54 or 250 mg/kg **sodium benzoate** by gavage on Days 6-18 of gestation showed no effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from controls.

NOAEL Maternal toxicity: 250 mg/kg bw
NOAEL Teratogenicity: 250 mg/kg bw
(FDA PB# 221777, 1972)

A study using pregnant Golden hamsters, dosed with 3, 14, 65 or 300 mg/kg **sodium benzoate** by gavage on Days 6-10 of gestation showed no effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from number in controls.

NOAEL Maternal toxicity: 300 mg/kg bw

NOAEL Teratogenicity: 300 mg/kg bw

(FDA PB# 221777, 1972)

A study using pregnant Wistar rats, dosed with 700, 1400, 2800, 5600 mg/kg **sodium benzoate** in the diet during the entire gestation showed no statistical difference in organ and bone abnormalities of fetuses between experimental groups and controls; growth of treated offsprings was similar to controls in rats dosed with 1400 mg/kg/day; reduced food intake and decreased body weight of the pregnant rats especially in the 5600 mg/kg group; 100% perinatal death rate; organ abnormalities of fetuses involved eye, brain and kidneys, in addition abnormalities of the skeletal system were found in rats dosed with ≥ 2800 mg/kg/day. The authors concluded that the effects on the dams and fetuses at the 2800 and 5600 levels were due to reduced maternal feed intake in these groups, leading to malnutrition,

NOAEL Maternal toxicity: 1400 mg/kg bw

NOAEL Teratogenicity: 1400 mg/kg bw

(Onodera et al., 1978)

Fifty female mice were given **benzyl alcohol** at 550 mg/kg bw per day by gavage on days 6-15 of gestation; a further 50 mice received the corn oil vehicle. All dams were allowed to deliver naturally, and pups and dams were observed until day 3 post partum, when the experiment was terminated. Body weight, clinical observations, and mortality were recorded daily throughout treatment and up to day 3 post partum. Mortality was not significantly increased in animals given benzyl alcohol over that in the control group. One treated mouse showing languid behaviour, laboured breathing, and a rough coat died, but no other deaths or clinical signs were reported. Maternal body weight and body-weight gain during treatment and up to day 3 post partum were virtually identical for treated and control animals. All other parameters examined, including gestation index, average number of live pups per litter, and postnatal survival and pup body weight on days 0 and 3 post partum, were not significantly different from the control values. The authors concluded that, at the predicted LD10, benzyl alcohol had no significant effects on the development of CD-1 mice.

NOAEL = 550 mg/kg bw per day

(York et al., 1986; JECFA, 1997).

Benzyl alcohol dissolved in distilled water was administered by gavage at a dose of 750 mg/kg bw per day to 50 CD-1 mice on days 7-14 of gestation; evidence of copulation was considered the first day of gestation. A control group of 50 animals received distilled water only. All animals were allowed to deliver their litters and nurse their pups for three days, at which time necropsies were performed. Maternal body-weight gain and mortality, mating, gestation, numbers of live and dead pups per litter, total litter weight on days 1 and 2 post partum, litter weight change between days 1 and 3 post partum, and pup survival on days 1 and 3 post partum were recorded. During the treatment period, 18 deaths were reported, all of which were attributed to treatment; a further death was reported on day 15 of gestation, the day after treatment was terminated. Clinical signs of toxicity, including hunched posture, tremors, inactivity, prostration, hypothermia, ataxia, dyspnoea, swollen or cyanotic abdomen, and piloerection, were reported in up to 20 mice during treatment. Piloerection was also reported in some animals up to day 3 post partum, but no other clinical signs were seen after the period of administration. No differences were observed in the mating or gestation indices, the total number of resorptions, the mean length of gestation, or the number of live pups per litter between treated and control groups. Maternal body weight, measured on days 4 and 7 of gestation, was not significantly different from control values; however, statistically

significant reductions were reported on day 18 of gestation ($P < 0.001$) and on day 3 post partum ($P < 0.05$). Maternal body-weight gain during days 7-18 of gestation was significantly lower than that of controls ($P < 0.001$). Significant reductions in pup body weight were reported, including a lower mean pup weight per litter on days 1 ($P < 0.01$) and 3 post partum ($P < 0.001$), a mean litter weight change between day 1 and day 3 post partum ($P < 0.05$), and a mean pup weight change between days 1 and 3 post partum ($P < 0.001$). No differences in pup survival were observed by day 3 post partum. The authors concluded that benzyl alcohol may be a reproductive hazard, apparently on the basis of the reductions in pup body weights, an effect that was observed in conjunction with maternal toxicity evidenced by increased mortality, reduced body weights, and clinical toxicity during the period of administration. As effects were seen on the dams and fetuses at the only dose used in this study, there was no NOAEL.

LOAEL = 750 mg/kg bw per day

(US National Institute of Occupational Safety and Health, 1983; Hardin et al., 1987).

In a developmental toxicity study in rats, **benzyl acetate** given by gavage did not show teratogenic effects and on the basis of fetotoxic effects a NOEL of 500 mg/kg/day could be established.
(Ishiguro et al., 1993)

Many of these studies were done by gavage (leading to greater toxicity due to the “bolus effect”). In these studies NOEL of ≥ 500 mg/kg were found.

Thus, studies on reproductive and/or developmental toxicology performed by the administration of the benzyl compounds by gavage are likely to reveal changes at lower doses compared to studies where the substances are applied in the diet, leading to a distribution in the body over time,

In conclusion: The compounds exhibit no developmental toxicity and a NOEL of 500 mg/kg/day can be established for developmental effects for this group of substances

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

The studies used as the basis for the following data did not always state whether effect values were based on nominal or measured concentrations. However, because of the good water solubility, their insignificant volatility and low adsorption potential, all nominal concentrations of the test substances are expected to correspond to effective concentrations even in tests with open systems and longer exposure durations.

Acute toxicity to fish

Chemical	Species	Protocol	Result	Reference
Benzoic acid	<i>Lepomis macrochirus</i>	EPA-660/3-75-009	LC ₅₀ (96 h) = 44.6 mg/l LC ₀ = 180 mg/l (pH control)	UCES#11506-03-85, 1979
	<i>Salmo gairdneri</i>	EPA-660/3-75-009	LC ₅₀ (96 h) = 47.3 mg/l	Buzzel et al 1968 UCES#11506-03-84, 1979
	<i>Leuciscus idus</i>	other	LC ₅₀ (48 h) = 460 mg/l (pH 7 –8)	Juhnke & Luedemann, 1978
Sodium benzoate	<i>Pimephales promelas</i>	EPA OPP 72-1	LC ₅₀ (96 h) = 484 mg/l (pH 7.4, flow-through, measured concentrations)	Geiger et al., 1985
	<i>Pimephales promelas</i>		LC ₅₀ (96 h) > 100 mg/l	Ewell et al 1986
Benzyl alcohol	<i>Pimephales promelas</i>	EPA OPP 72-1	LC ₅₀ (96 h) = 460 mg/l	Mattson, V.R. et al., EPA-600 /3-76-097, PB-262897, 1976
	<i>Leuciscus idus</i>	DIN 38412 Teil 15	LC ₅₀ (48 h) = 646 mg/l	Knie et al., 1983
Benzyl alcohol		Specific acute spill testing (*)	LC ₅₀ (96 h) 10 and 15 mg/l	Dawson et al 1975/1977

No data for potassium benzoate were identified, but it should be similar to sodium benzoate.

(*) REMARK: For benzylalcohol two valuable guideline studies gave acute toxicity values > 100 mg/l.

Dawson et al, however reported acute toxicity values 10 – 15 mg/l. Their static tests however were directed to simulate acute spill circumstances. The test substances were pipetted or poured undiluted directly into the aquaria with fish.

So without preparing defined concentrations according to guideline. No analytical monitoring was done. Aeration was not used during the first 24 hrs thus allowing chemicals to act in an uninterrupted state at the onset of the test period.

For environmental relevant conditions and for derivation of a PNECaqua a benzylalcohol acute toxicity (LC₅₀ 96 hrs) to fish of > 100 mg/l should therefore be used.

Acute toxicity to aquatic invertebrates

Chemical	Species	Protocol	Result	Reference
Benzoic acid	<i>Daphnia magna</i>	EPA-660/3-75-009	EC ₅₀ (48 h) => 100 mg/l (pH 8.4)	UCES#11506-03-80, 1979
		other	EC ₅₀ (24 h) = 500 mg/l (with neutralization)	Bringmann, & Kuehn, 1982
		other	EC ₅₀ is 102 mg/l (without neutralization)	Bringmann, & Kuehn, 1982
Sodium benzoate Benzyl alcohol	<i>Daphnia magna</i>	other	EC ₅₀ (48 h) => 100 mg/l	Ewell et al., 1986
	<i>Daphnia magna</i>	DIN 38412 Teil 11	EC ₅₀ (24 h) = 400 mg/l	Knie et al., 1983
	<i>Daphnia magna</i>	other	EC ₅₀ (48 h) = 360 mg/l	Bringmann & Kuehn, 1959

No data for potassium benzoate were identified, but it should be similar to sodium benzoate.

Acute toxicity to aquatic plants (algae)

Chemical	Species	Protocol	Result	Reference
Benzoic acid	<i>Scenedesmus quadricauda</i>	other	EC ₅₀ (3 h) = 75 mg/l	Stratton & Corke, 1982
	<i>Scenedesmus quadricauda</i>	cell multiplication inhibition test; static	Inhibition starts at 1630 mg/l (96 hr) (pH = 7)	Bringmann & Kuehn, 1977
	<i>Chlorella pyrenoidosa</i>	other	EC ₅₀ (3 h) = 60 mg/l	Stratton & Corke, 1982
	<i>Anabaena variabilis</i>	other	EC ₅₀ (14d) = >10 mg/l	Stratton & Corke, 1982
Sodium benzoate	Green algae	ECOSAR	EC ₅₀ (96 h) = 478 mg/l	
Benzyl alcohol	<i>Chlorella pyrenoidosa</i>	other	EC ₅₀ (3 h) = 95 mg/l	Stratton & Corke, 1982
	<i>Haematococcus pluvialis</i>	other	EC ₅₀ (4 h) = 2600 mg/l	Knie et al., 1983
	<i>Scenedesmus quadricauda</i>	cell multiplication inhibition test	Inhibition starts at 640 mg/l (96 h)	Bringmann & Kuehn 1959

Remark: The studies are no guideline studies, but despite this shortcoming they indicate a moderate to low acute toxicity. The *Scenedesmus* study of Stratton and Cork was not used because the endpoint is about the inhibition of the photosynthesis and not growth (rate). The blue green algae were left out because they are not directly used for the effect assessment for the aquatic

environment and the endpoint was inhibition of the photosynthesis and not growth (rate). No data for potassium benzoate were identified, but it should be similar to sodium benzoate.

Acute toxicity to micro-organisms (bacteria)

Chemical	Species	Protocol	Result	Reference
Benzoic acid	activated sludge	OECD 209 (respiration inhibition)	EC ₅₀ (3 h) > 1000 mg/l (pH 7.5)	Klecka et al., 1985
	<i>Photobacterium phosphoreum</i>	Static	EC ₅₀ (30 min) = 16.85 mg/l	Kaiser, 1987
	<i>Pseudomonas putida</i>	Static	Inhibition starts at 480 mg/l (16 h) (pH neutral)	Cicad 2000
Sodium benzoate	<i>Achromobacter liquefaciens</i>	other: static	EC ₅₀ (24 h) = > 3000 mg/l	Nikkilae, 1955
	<i>Micrococcus flavus</i>	other: static	EC ₅₀ (24 h) = >500 mg/l	Nikkilae, 1955
Benzyl alcohol	<i>Escherichia coli</i>	cell multiplication inhibition test	EC ₀ (48 h) = 1000 mg/l	Bringmann & Kuhn, 1959
	<i>Pseudomonas putida</i>	cell multiplication inhibition test	EC ₁₀ (16-18 h) = 658 mg/l	Knie et al., 1983

No data for potassium benzoate were identified, but it should be similar to sodium benzoate.

In conclusion:

From the data (fish, daphnia, algae, bacteria) it is obvious that neutralization of the pH greatly reduces (up to one order of magnitude) the acute toxicity of benzoic acid. This is also supported by the lower toxicity observed with the sodium benzoate. Under environmental relevant conditions therefore the acute toxicity of benzoic acid, sodium benzoate and potassium benzoate for all four trophic levels is > 100 mg/l.

Under environmental relevant conditions the acute toxicity of benzylalcohol for fish, daphnia and bacteria is > 100mg/l. For algae an acute EC 50 3hrs of 95 mg/l

Therefore it can be concluded that under environmental relevant conditions, benzoic acid and its salts have very low acute toxicity, whereas benzylalcohol has low to moderate acute toxicity

4.2 Terrestrial Effects

There were no available studies on terrestrial organisms.

IPCS CICAD 26 (2000) concluded for benzoic acid and sodium benzoate: No information on toxic effects of benzoic acid and sodium benzoate on plants, earthworms or other terrestrial organisms or on ecosystems were identified. Only antimicrobial properties were identified preventing bacterial or fungal growth. Based on these data they conclude a low toxicity potential of benzoic acid and sodium benzoate in the terrestrial environment.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Benzylalcohol, benzoic acid and its sodium and potassium salt can be considered as a single category regarding human health, as they are all rapidly metabolised and excreted via a common pathway within 24hrs.

Systemic toxic effects of similar nature (e.g liver, kidney) were observed. However, with benzoic acid and its salts at higher doses than with benzylalcohol. For environmental effects the category is less clear, however all are readily biodegradable, non-bioaccumulative and acute toxicity values are similar.

For human health all exposure routes are possible, despite benzoic acid and its salts being solids and benzylalcohol being a liquid. For workers exposure will mainly be by inhalation and by skin, whereas for consumers it will mainly be by oral and dermal route.

Human Health:

The compounds exhibit low acute toxicity as for the oral and dermal route. The LD50 values are > 2000 mg/kg bw except for benzylalcohol which needs to be considered as harmful by oral route in view of an oral LD50 of 1610 mg/kg bw. The 4 hrs inhalation exposure of benzylalcohol or benzoic acid at 4 and 12 mg/l as aerosol/dust respectively gave no mortality, showing low acute toxicity by inhalation for these compounds.

Benzoic acid and benzyl alcohol are slightly irritating to the skin, while sodium benzoate was not skin irritating. No data are available for potassium benzoate but it is also expected not to be skin irritating. Benzoic acid and benzyl alcohol are irritating to the eye and sodium benzoate was only slightly irritating to the eye. No data are available for potassium benzoate but it is expected also to be only slightly irritating to the eye.

The available studies for benzoic acid gave no indication for a sensitizing effect in animals, however occasionally very low positive reactions were recorded with humans (dermatological patients) in patch tests. The same occurs for sodium benzoate. It has been suggested that the very low positive reactions are a non-immunologic contact urticaria. Benzyl alcohol gave positive and negative results in animals. Benzyl alcohol also demonstrated a maximum incidence of sensitization of only 1% in human patch testing. Over several decades no sensitization with these compounds has been seen among workers.

For benzoic acid repeated dose oral toxicity studies give a NOAEL of 800 mg/kg/day. For the salts values > 1000 mg/kg/day are obtained. At higher doses increased mortality, reduced weight gain, liver and kidney effects were observed.

For benzyl alcohol the long-term studies indicate a NOAEL $\geq$ 400 mg/kg bw/d for rats and $\geq$ 200 mg/kg bw/d for mice. At higher doses effects on bodyweights, lesions in the brains, thymus, skeletal muscle and kidney were observed. It should be taken into account that administration in these studies was by gavage route, at which saturation of metabolic pathways is likely to occur. It can be concluded that benzoic acid and its salts exhibit very low repeated dose toxicity. Benzylalcohol exhibits low repeated dose toxicity.

All chemicals showed no mutagenic activity in *in vitro* Ames tests. Various results were obtained with other *in vitro* genotoxicity assays.

Sodium benzoate and benzyl alcohol showed no genotoxicity *in vivo*.

While some mixed and/or equivocal *in vitro* chromosomal/chromatid responses have been observed, no genotoxicity was observed in the *in vivo* cytogenetic, micronucleus, or other assays. The weight of the evidence of the *in vitro* and *in vivo* genotoxicity data indicates that these chemicals are not mutagenic or clastogenic. They also are not carcinogenic in long-term carcinogenicity studies. In addition data from *in-vivo* genotoxicity studies on benzyl acetate and benzaldehyde (JECFA report, 1997) support the non-genotoxicity of benzylalcohol and benzoic acid and its salts.

Carcinogenicity studies (2-year) with sodium benzoate and benzyl alcohol showed no evidence of carcinogenic activity.

In a 4-generation study with benzoic acid no effects on reproduction were seen (NOAEL $\geq$ 750 mg/kg). No compound related effects on reproductive organs (gross and histopathology examination) could be found in the (sub) chronic studies in rats and mice with benzyl acetate, benzyl alcohol, benzaldehyde, sodium benzoate and supports a non-reprotoxic potential of these compounds. In addition, data from reprotoxicity studies on benzyl acetate (NOAEL >2000 mg/kg bw/d; rats and mice) and benzaldehyde (tested only up to 5 mg/kg bw; rats) support the non-reprotoxicity of benzyl alcohol and benzoic acid and its salts.

In rats for sodium benzoate dosed via food during the entire gestation developmental effects occurred only in the presence of marked maternal toxicity (reduced food intake and decreased body weight) (NOAEL = 1400 mg/kg bw). For hamster (NOEL : 300 mg/kg bw), rabbit (NOEL :250 mg/kg bw) and mice (CD-1 mice, NOEL : 175 mg/kg bw) no higher doses (all by gavage) were tested and no maternal toxicity was observed. For benzyl alcohol: NOAEL= 550 mg/kg bw (gavage; CD-1 mice). LOAEL = 750 mg/kg bw (gavage mice). In this study maternal toxicity was observed e.g. increased mortality, reduced body weight and clinical toxicology. Benzyl acetate: NOEL = 500 mg/kg bw (gavage rats). No maternal toxicity was observed.

Environment:

From the data (fish, daphnia, algae, bacteria) it is obvious that neutralization of the pH greatly reduces (up to one order of magnitude) the acute toxicity of benzoic acid. This is also supported by the lower toxicity observed with sodium benzoate. Under environmental relevant conditions therefore the acute toxicity of benzoic acid, sodium benzoate and potassium benzoate for all four trophic levels is > 100 mg/l.

Under environmental relevant conditions the acute toxicity of benzylalcohol for fish, daphnia and bacteria is > 100mg/l. For algae an acute EC 50 3hrs of 95 mg/l is reported.

Therefore it can be concluded that under environmental relevant conditions benzoic acid and its salts have very low acute toxicity, whereas benzylalcohol has low to moderate acute toxicity.

Exposure:

Worldwide production capacity of benzoic acid is estimated at 700 kt per year. The major outlet (75%) for benzoic acid is as a chemical intermediate in the production of phenol, which in turn is mainly used to produce caprolactam. The next largest outlet is as a feedstock for sodium benzoate (10%) and chemical synthesis of plasticizers (5%).

Worldwide production capacity of sodium benzoate is estimated at 100 kt per year. The major outlet for sodium benzoate is as preservative in food and beverages (60%). Second most important market is cooling liquids (10%). The main function of sodium benzoate in most applications is as preservative.

Worldwide production capacity of potassiumbenzoate is estimated at 7 kt per year. It is used as a preservative in nonalcoholic beverages.

Worldwide production capacity of benzyl alcohol is estimated at 50 kt. Major use for benzylalcohol is as curing agent in epoxy coatings (30%), where it becomes chemically bound after reaction. Other important uses include the use as a solvent in low concentrations in waterborne coatings (10%) and use in paint strippers (10%) and chemical intermediate for synthesis for benzyl esters that are used in the flavor and fragrance industry (10%). The use in paint strippers is limited to uses in industrial settings.

Benzylalcohol, benzoic acid and its sodium and potassium salt are also used in pharmaceuticals, cosmetics and/or food. Consumer exposure in these specific applications are controlled by the fact that for all these applications specific regulatory frameworks (regional and/or national) with authorization/approval procedures and specific advisory bodies exist (among others US FDA, WHO JECFA, EU SCF, etc), with on regular basis reevaluation of approvals, hazardous properties and factual exposures inclusive. According to information from products registers uses that are not specifically regulated includes uses of the substances in different kinds of products e.g. paints, varnishes solvents, cleaning and washing agents, photochemicals and antifreeze agents.

Benzoic acid is a white solid, with solubility in water of 2.9 g/l and with a vapor pressure of 0.0011 hPa at 20 °C. The octanol/water partition coefficient was measured to 1.88; the Henry's law constant = 0.0046-0.022 Pa·m³/mol; and the pKa = 4.2.

Sodium benzoate and potassium benzoate are white solids, with solubility in water of 556 g/l and with a vapor pressure of <0.0011 hPa at 20 °C. The octanol/water partition coefficient were measured to -2.269.

Benzyl alcohol is a colorless liquid, with solubility in water of 40 g/l and with a vapor pressure of 0.13 hPa at 20 °C. The octanol/water partition coefficient was measured to 1.1.

The distribution modeling according to Mackay Level III indicates soil and water to be the favored compartments for the chemicals. None are expected to hydrolyze. All are classified as readily biodegradable. None has bioaccumulative potential.

5.2 Recommendations

Several of the toxicological studies on benzyl alcohol and benzoic acid and its salts were carried out some years ago and do not always fulfill for 100% present-day guidelines. However, well-known research groups and/or test laboratories ran the studies according to scientific standards and or accepted protocols at that time. They did appear to be acceptable studies for evaluation. Also, all were peer-reviewed and published in high quality scientific literature. Most of them have been reviewed and accepted by other fora like FDA, JECFA, and IPCS as acceptable studies. In addition, there is good consistency in the individual data for a substance in the group as well as between members of the group (benzyl acetate and benzaldehyde data inclusive). Therefore, taken as a whole, the available studies give a robust database for hazard assessment and hazard evaluation of these compounds and further studies are not indicated. The JECFA Committee (1997) concluded that the data reviewed for compounds in this group were sufficient to demonstrate lack of teratogenic, reproductive or carcinogenic potential. Consequently, the Committee concluded that further studies were not required.

Taking into account the rapid biodegradability, the low bioaccumulation potential, the low to moderate toxicity to most aquatic species, and the rapid metabolism of these substances, these substances will pose a minimal risk to the aquatic environment.

Taking into account the rapid metabolism and excretion, the non-bioaccumulation, the low toxicity after acute and repeated exposures, the non-reprotoxicity, the non-genotoxicity and the non-carcinogenicity, the low irritating and non- to very low sensitizing properties of these substances, as well as the controlled (industrial settings) and /or regulated (pharma, cosmetics and /or food) uses, these substances will pose a minimal risk to humans (workers and consumers).

Therefore these substances have low priority for further work.

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I U C L I D D a t a S e t

Existing Chemical	ID: 65-85-0
CAS No.	65-85-0
EINECS Name	benzoic acid
EC No.	200-618-2
TSCA Name	Benzoic acid
Molecular Formula	C7H6O2

Producer Related Part
Company: Bayer Corporation
Creation date: 21-OCT-1999

Substance Related Part

Company:	Bayer Corporation
Creation date:	21-OCT-1999

Memo: Bayer Corporation

Printing date: 14-FEB-2002
Revision date:
Date of last Update: 14-FEB-2002

Number of Pages: 82

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Chapter (profile):      Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile): Reliability: without reliability, 1, 2,
                        3, 4
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Flags (profile):
  Flags:      without flag, confidential, non
              confidential, WGK(DE), TA-Luft (DE),
              Material Safety Dataset, Risk Assessment,
              Directive 67/548/EEC, SIDS
```

1. GENERAL INFORMATION

DATE: 14-FEB.-2002
SUBSTANCES ID: 65-85-0**1.0.1 Applicant and Company Information**

Type: lead organisation
Name: American Chemistry Council (formerly Chemical
Manufacturers Association, HPV Benzoates Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States
14-AUG-2001

Type: cooperating company
Name: ATOFINA Chemicals, Inc.
Country: United States
14AUG-2001

Type: cooperating company
Name: Bayer Corporation
Country: United States
14-AUG-2001

Type: cooperating company
Name: DSM Special Products
Country: Netherlands
13-DEC-2000

Type: cooperating company
Name: Noveon, Inc.
Country: United States
14-AUG-2001

Type: cooperating company
Name: Velsicol Chemical Corporation
Country: United States

21-MAY-2001

Type: lead organisation
Name: American Chemistry Council, Benzoates Panel
16-JAN-2001

1.0.2 Location of Production Site, Importer or Formulator**1.0.3 Identity of Recipients****1.0.4 Details on Category/Template**

1.1.0 Substance Identification**1.1.1 General Substance Information****1.1.2 Spectra****1.2 Synonyms and Tradenames****1.3 Impurities****1.4 Additives****1.5 Total Quantity****1.6.1 Labelling****1.6.2 Classification****1.6.3 Packaging****1.7 Use Pattern****1.7.1 Detailed Use Pattern****1.7.2 Methods of Manufacture****1.8 Regulatory Measures****1.8.1 Occupational Exposure Limit Values****1.8.2 Acceptable Residues Levels****1.8.3 Water Pollution****1.8.4 Major Accident Hazards****1.8.5 Air Pollution****1.8.6 Listings e.g. Chemical Inventories**

1.9.1 Degradation/Transformation Products**1.9.2 Components****1.10 Source of Exposure****1.11 Additional Remarks****1.12 Last Literature Search**

Type of Search: Internal and External
Date of Search: 07-SEP-1999

Remark: Only HPV endpoints: TOXLINE data base and
internal studies.

14-AUG-2001

1.13 Reviews

2.1 Melting Point

Value: = 122.4 degree C

Method: other: measured

Test substance: other TS: benzoic acid; purity not noted

Reliability: (2) valid with restrictions

Data from Handbook or collection of data

Flag: Critical study for SIDS endpoint

14-AUG-2001

(1) (2)

Value: = 122 degree C

15-JAN-2001

(3)

Value: = 121.7 degree C

15-JAN-2001

(4)

2.2 Boiling Point

Value: = 249.2 degree C at 1013 hPa

Method: other: measured

Test substance: other TS: benzoic acid; purity not noted

Reliability: (2) valid with restrictions

Data from Handbook or collection of data

Flag: Critical study for SIDS endpoint

14-AUG-2001

(1) (5)

Value: = 250 degree C at 1013 hPa

Reliability: (2) valid with restrictions

15-JAN-2001

(2)

Value: = 249 degree C at 1013 hPa

15-JAN-2001

(4)

2.3 Density

Type: density

Value: = 1.2659 at 15 degree C

Method: other:

Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
14-AUG-2001 (1)

Type: density
Value: = 1.321 g/cm³ at 20 degree C
15-JAN-2001 (6)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: = .0011 hPa at 20 degree C
Method: other (measured): Handbook Value
Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
14-AUG-2001 (7)

Value: = .0053 hPa at 20 degree C
Flag: Critical study for SIDS endpoint
15-JAN-2001 (8)

2.5 Partition Coefficient

log Pow: = 1.88
Method: other (measured): centrifugal distribution chromatography
Year: 1988
Reliability: (2) valid with restrictions
Meets generally accepted scientific method and is described in sufficient detail
Flag: Critical study for SIDS endpoint
14-AUG-2001 (9)

log Pow: = 1.9
Method: other (calculated): CLOGP-3.63 (1991)
Year: 1991

Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
26-JAN-2001 (10)

log Pow: = 1.93

Method: other (measured): gemessen, Schuettelmethode,
spektralphotometrischeKonzentrationsbestimmung
15-JAN-2001 (11)
log Pow: 1.81 - 1.88

Method: other (measured): gemessen, Schuettelmethode,
spektralphotometrischeKonzentrationsbestimmung
14-AUG-2001 (12)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 2.931 g/l at 20 degree C

Method: other: similar to OECD Guideline 105
Test substance: other TS: Research grade benzoic acid (Merck)

Method: According to Pal, A., Maity, S.K., & Lahiri,
S.C. J. Indian Chem. Soc. (1983) 60:475.

Remark: pH-Value: no data
Result: 2.45 g/l at 15 degree C (0.0210 mol/l at 288K)
2.93 g/l at 20 degree C (0.0240 mol/l at 293K)
3.47 g/l at 25 degree C (0.0284 mol/l at 298K)

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
Well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
14-FEB-2002 (13)

Solubility in: Water
Value: = 2.91 g/l at 20 degree C

Remark: pH-value: no data
14-FEB-2002 (14)

2.6.2 Surface Tension

2.7 Flash Point

Value: = 121 degree C

Method: other: no data

Remark: nicht angegeben

15-JAN-2001

(2)

2.8 Auto Flammability

Value: = 574 degree C at 1013 hPa

Method: other

Year: 1990

GLP: no

15-JAN-2001

(15)

2.9 Flammability

Remark: Not applicable.

14-AUG-2001

2.10 Explosive Properties

Remark: Dust explosions possible. LEL 0.95 % and UEL
8.2 %

14-AUG-2001

2.11 Oxidizing Properties

Remark: Not applicable.

14-AUG-2001

2.12 Dissociation Constant

2.13 Viscosity

2.14 Additional Remarks

Remark: Henry-constant (Pa * m3/mol):
0.0046 - 0.022 (calculated as quotient of
vapour pressure and water solubility at 20
degree C)

Flag: Critical study for SIDS endpoint

14-AUG-2001

Remark:	Dissociation-constant (25 degree C): pka = 4.1951
Flag:	Critical study for SIDS endpoint
14-AUG-2001	(16)
Remark:	Dissociation-constant (20 degree C): pKa = 4.21
Flag:	Critical study for SIDS endpoint
14-AUG-2001	(17)
Remark:	Dissociation-constant pKa (25 degree C): 3.99-4.205 (various methods; summarized values)
14-AUG-2001	(18)
Remark:	Begin of sublimation at ca. 100 degree C. At ca. 150 degree C formation of anhydride, at ca. 370 degree C decarboxylation. Volatile with steam.
14-AUG-2001	(2)
Remark:	pH-value: 3,1 at 1 g/l water(roomtemperature)
14-AUG-2001	(19)
Remark:	pH-value: 2.8(saturated solution, 25 degree C)
14-AUG-2001	(2)

3.1.1 Photodegradation

Type: other:mineralization in aqueous TiO₂
Light source: other:20W NEC blacklight blue fluorescent tube
Light spect.: ≤ 350 nm
Conc. of subst.: 50 mg/l at 40 degree C

INDIRECT PHOTOLYSIS

Sensitizer: other: aqueous TiO₂
Conc. of sens.: 40 mg/l
Degradation: 90 % after 140 minute(s)

Method: other(measured):mineralization in aqueous TiO₂
Year: 1990
GLP: no data
Test substance: other TS: benzoic acid, purity not noted

Remark: Photochemical dissociation of benzoic acid by
Irradiation with UV light if fixed on solid
carriers:-90 % mineralization in aqueous
TiO₂- suspension after 2-3 h of irradiation
with sunlight on 1 m² water
surface(concentration 50 mg/l related to test
substance)
This endpoint has been studied several times
by several other investigators/groups and all
support the result of the study mentioned
above.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
Well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
14-FEB-2002 (20)

Type: other: calculated
Light source: Sun light
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1560000 molecule/cm³
Rate constant: ca. .0000000000001242 cm³/(molecule * sec)
Degradation: 50 % after 8.6 day(s)

Method: other (calculated): AOPWin version 1.89
Year: 1999
Test substance: other TS: molecular structure

-
- Reliability: (2) valid with restrictions
Accepted calculation method
- Flag: Critical study for SIDS endpoint
14-AUG-2001 (21)
- Remark: UV-Spectrum lambda max (nm):
227.5 (Methanol; lg epsilon: 4.27)
222 (Methanol/KOH; lg epsilon: 4.07)
15-JAN-2001 (22)
- Remark: photochemical dissociation of benzoic acid by
UV-irradiation if fixed on solid carriers
(SiO₂): -10.2 % mineralization after 17 h
irradiation with light(lambda > 290 nm)(no data
concerning concentration)
15-JAN-2001 (23)
- Remark: photochemical dissociation of benzoic acid by
Irradiation with UV light if fixed on solid
carriers:- 67 % mineralization in aqueous ZnO-
suspension after 24 h of irradiation with
sunlight (concentration 100-200 mg/l
related to DOC)
15-JAN-2001 (24)
- Remark: Formation of a small amount of photochemical
aerosols after irradiation of some cristalls
of benzoic acid with a deuterium lamp (180 <
lamda < 400 nm) in a laboratory reactor.
15-JAN-2001 (25)

3.1.2 Stability in Water

- Result: Based on structure and organic chemistry rules
(e.g. bonding in organic molecules, activation
energy, reactivity, transformations, addition,
substitution, elimination) no hydrolysis will
occur at pH ranges 4 - 11.
26-JAN-2001

3.1.3 Stability in Soil

- Remark: Not available.
14-AUG-2001

3.2.1 Monitoring Data (Environment)

Remark: Not available.
14-AUG-2001

3.2.2 Field Studies**3.3.1 Transport between Environmental Compartments**

Type: adsorption
Media: water - soil
Method: other: see below

Method: 14C-labeled benzoic acid (767MBq mmol⁻¹) of radiochemical purity greater than 98.5% was prepared in 0.01 M calcium nitrate in concentrations of 0.01, 0.1, 1.0, 10 mg/l. The solutions were added to three types of autoclaved, dry soils (2 g) and allowed to equilibrate on a mechanical shaker for 72 hrs at 6C.

The soil types were sandy till, clayey till, and melt water sand.

The suspension was allowed to settle and the supernatant liquid tested for 14C activity. Adsorption constants were determined.

Result: No adsorption was observed for benzoic acid in melt water sand and clayey till; very low adsorption was observed in sandy till (K=0.23).

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (26)

Type: fugacity model level III
Media: other: air - water - soil - sediment

Method: other: EPIWin Modeling Program

Remark: Modeling was performed using equal releases (10,000 kg/hr) and equal distribution to all compartments.

3. ENVIRONMENTAL FATE AND PATHWAYS

DATE: 14-FEB-2002

SUBSTANCES ID: 65-85-0

Result:	Distribution (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	0.911	207	1000	2.3 e-011
Water	34.8	360	1000	6.11e-013
Soil	64.2	360	1000	1.22e-011
Sediment	0.093	1.44e+003	0	4.73e-013

Persistence Time: 421 hr

Reaction Time: 516 hr

Advection Time: 2.28e+003 hr

Percent Reacted: 81.5

Percent Advected

Reliability: (2) valid with restrictions

Accepted calculation method

Flag: Critical study for SIDS endpoint

14-FEB-2002

(21)

3.3.2 Distribution**3.4 Mode of Degradation in Actual Use**

Remark: Benzoic acid is readily biodegradable, and in production and use in chemical industry it is biodegraded in a waste water treatment plant. In many species, benzoic acid is rapidly absorbed, conjugated with glycine and excreted as hippuric acid.

23-OCT-1995

3.5 Biodegradation

Type: aerobic

Inoculum: activated sludge, industrial, non-adapted

Concentration: 1000 mg/l related to COD (Chemical Oxygen Demand)

508 mg/l related to Test substance

Degradation: > 90 % after 2 day(s)

Method: OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"

Year: 1981

GLP: no data

Test substance: other TS: reagent grade benzoic acid

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint
14-FEB-2002 (27)

Type: anaerobic
Inoculum: anaerobic sludge
Concentration: 73 mg/l related to Test substance
Contact time: 28 day(s)
Degradation: 96 - 100 % after 7 day(s)

Method: other: see below
GLP: no data
Test substance: other TS: commercial grade benzoic acid,
purity > 95%

Method: A 10% anaerobic sludge inoculum was transferred to 160 ml serum bottles previously amended with 50 ppm Carbon (related to test substance) using strict anaerobic techniques. Methane production from test bottles vs. controls monitored weekly for 4 weeks or until net production occurred. At that time, the bottles were amended again with the same substrate and methane production monitored to confirm the observation. All data were obtained from duplicate bottles. Methane was measured using a flame ionization detector on a Perkin-Elmer Model 900 GC equipped with a 3-m Tenax-G.C. column

Remark: 96 % mineralisation (CH₄-Production) in 1 week with sludge from Jackson, MI waste-treatment plant 100 % mineralisation (CH₄-Production) in 2 weeks with sludge from Adrian, MI waste-treatment plan

Test condition: The test bottles were incubated at 35 degree C in the dark. Substrates were kept under an atmosphere of 90% N₂ and 10% H₂

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (28)

Type: anaerobic

Concentration: 50 µg/l related to DOC (Dissolved Organic Carbon)
Contact time: 2 month
Degradation: > 75 % after 2 month

Method: other: see below
GLP: no data
Test substance: other TS: benzoic acid, purity not noted

Method: Sludge samples collected from primary and Secondary anaerobic digesters were diluted to 10% and incubated anaerobically with 50 ug Carbon per ml (related to test substance). All compounds were tested in triplicate. Gas production was measured by gas chromatography and by a pressure transducer. Biodegradation was determined by net increase in gas pressure in bottles amended with test chemicals over non-amended controls.

Result: Degradation is expressed as percentage of Theoretical Methane production based on the stoichiometry of degradation.

Test condition: The test bottles were incubated at 35 degree C in the dark.
Substrates were kept under atmospheres of 10% CO₂ and 90% N₂.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, Well documented and acceptable for assessment
Critical study for SIDS endpoint

Flag: 14-AUG-2001 (29)
Type: aerobic
Inoculum: activated sludge, industrial
Degradation: 86.9 % after 5 day(s)

Test substance: other TS: benzoic acid-1-14C (0.026mCi/mg) obtained from NewEngland Nuclear Corporation, Boston, Massachusetts.

Method: Radio-respirometric study using radio-labeled chemicals by activated sludge and in a complex photographic processing effluent using acclimated industrial sludge.
Concentration of test substance was 0.1 or 0.2ml of radioactive substrate(27,000-400,000 dpm). Samples were incubated in the dark at ambient temperature.

3. ENVIRONMENTAL FATE AND PATHWAYS

DATE: 14-FEB-2002

SUBSTANCES ID: 65-85-0

Result: 14C02 recovery without effluent = 68.2% after
5 days 14C02 recovery in presence of effluent
= 86.9% after 5 days

30-JAN-2001

(30)

Inoculum: activated sludge, domestic
Concentration: 10 mg/l related to Test substance
Degradation: 74 % after 5 day(s)

Method: other: BOD test; 20 degree C; pH 7.0; minimal
medium

Remark: Degradation after 20 d: 78 %
t 1/2 for TOC: 1 d
BOD: 2 d
no lag phase

14-FEB-2002

(31)

Inoculum: activated sludge, non-adapted
Concentration: 100 mg/l related to Test substance

Method: other: Respirometer, 20 degree C; pH 7

Remark: Degradation after 65-80 h: 61-69 %; 5-20 h lag
phase

14-FEB-2002

(32)

Inoculum: activated sludge, domestic
Concentration: 500 mg/l related to Test substance
Degradation: after 6 day(s)

Method: other: Warburg-Respirometer, 20 degree C

Remark: Measured O₂-consumption (graphically
determined; considering endogenous
respiration): ca. 525-750 mg/l
= ca. 1050-1500 mg O₂/g substance (ThOD 1967
mg O₂/g substance)

15-JAN-2001

(33)

Inoculum: activated sludge, non-adapted
Concentration: 500 mg/l related to Test substance

Method: other: Warburg-Respirometer; 20 degree C

Remark: Measured O₂-consumption (graphically determined;

	Considering endogenous respiration): after 1 d ca. 410 mg/l = ca. 820 mg O ₂ /g substance (ThOD 1967 mg O ₂ /g substance). Benzoic acid had an initial toxic effect on two of three samples of activated sludge from different communal purification plants, after 24 hours degradation started in these samples, too.
15 - JAN - 2001	(34)
Inoculum:	activated sludge, adapted
Concentration:	200 mg/l related to COD (Chemical Oxygen Demand)
Degradation:	99 % after 5 day(s)
Method:	other: aerobic degradation, 20 degree C
Remark:	Concentration related to 101.7 mg substance/l 20 days adaption, degradation 88.5 mg COD/g.h
14 - FEB - 2002	(35)
Inoculum:	activated sludge, domestic
Concentration:	16 mg/l related to Test substance
Degradation:	100 % after 1 day(s)
Method:	other: aerobic degradation, static, 30 degree C; pH 7.3
Remark:	Substance specific analysis
14 - FEB - 2002	(36)
Inoculum:	activated sludge, domestic
Concentration:	.059 mg/l related to Test substance
Degradation:	99.5 % after 7 day(s)
Method:	other: aerobic degradation; 29 degree C; measurement of radioactivity(C ¹⁴ labelled at the carboxygroup)(CO ₂ -formation)
Remark:	Test with trace concentrations
15 - JAN - 2001	(37)
Inoculum:	activated sludge, industrial
Concentration:	150 mg/l related to Test substance
Degradation:	86 % after 1 day(s)

Method: other: aerobic degradation; semi-continuous;
25-30 degree C; pH 7; parameter: TOC

Remark: 1 day acclimation
15-JAN-2001 (38)

Inoculum: activated sludge, domestic, adapted
Concentration: 1000 mg/l related to COD (Chemical Oxygen
Demand)

Degradation: 97 % after .2 day(s)

Method: other: aerobic degradation; static; test
temperature 30 degree C ; pH 7.2

Remark: Concentration equivalent to 508 mg substance/l
20 days adaptation with glucose as additional
C-source

14-FEB-2002 (39)

Inoculum: other bacteria: obligatory anaerobic species
from sludge of the first purification step

Concentration: 300 mg/l related to Test substance

Degradation: 91 % after 18 day(s)

Method: other: anaerobic degradation, enrichment
culture; 35 degree C; parameter: gas
production

Remark: 8 days lag phase
Degradation after 18 d: 91 +- 7.8 %

14-FEB-2002 (40)

Inoculum: other bacteria: anaerobic sludge, domestic

Concentration: 50 mg/l related to Test substance

Degradation: after 21 day(s)

Method: other: anaerobic degradation, static, 35
degree C, adding of test substance in solid
form; parameter: gas production

Remark: Degradation: 110.5 %

14-FEB-2002 (41)

Inoculum: other bacteria: anaerobic sludge, domestic,
washed

Concentration: 50 mg/l related to Test substance

Degradation: 89.5 % after 35 day(s)

Method: other: anaerobic degradation, static, 35 degree C, adding of test substance in solid form; parameter: gas production

15-JAN-2001 (41)

Inoculum: other bacteria: anaerobic laboratory sludge, adapted

Concentration: 24 mg/l related to Test substance

Degradation: 86 - 93 % after 23 day(s)

Method: other: anaerobic degradation, static, parameter: gas production, 37 degree C

15-JAN-2001 (42)

Inoculum: other bacteria: activated sludge, domestic/industrial sewage

Concentration: .8 mg/l related to Test substance

Degradation: > 71.5 % after 5 day(s)

Method: other: closed bottle-test

15-JAN-2001 (19)

Inoculum: activated sludge, domestic

Concentration: 700 mg/l related to Test substance

Degradation: 76 % after 5 day(s)

Method: other: respirometric determination of BOD; 20 degree C

15-JAN-2001 (43)

3.6 BOD5, COD or BOD5/COD Ratio

Method:

Year:

Method:

Remark: BOD5/COD ratio is 0.72, indicating readily biodegradation.

14-AUG-2001 (15)

3.7 Bioaccumulation

BCF: 3.16

Method: other: BCF Program (v2.13)
Year: 1999
Test substance: other TS: molecular structure

Result: Estimated Log BCF = 0.500 (BCF = 3.162)
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
14-AUG-2001 (21)

Remark: Based on the log P and the fact that many
species absorb benzoic acid rapidly and
rapidly metabolize it to hippuric acid that is
excreted in urine, no bioaccumulation is
indicated.
15-JAN-2001

3.8 Additional Remarks

Remark: Soil sorption coefficient Kd at 50 ug/l
Loamy sand : 0.4 m depth: 1.92
Sand : 18.9 m depth: 0.62
23-OCT-1995 (44)

Remark: Biomagnification factors (modell ecosystem)
(0.01-0.1 ppm; radiolabelled):
Gambusia affinis (mosquito fish) 21
Daphnia magna 1772
Oedogonium cardiacum (green algae) 102
Culex quinquefasciatus (midge, larvae) 138
Physa (snail) 2786
Duration of test: 48 h
Fishes were added after 24 h; no
differentiation between bioaccumulation
and magnification.
There is no evidence whether a plateau was
achieved; the depuration rate is unknown.
23-OCT-1995 (45)

Remark: Bioconcentration factor:
Selenastrum capricornutum (green algae) 7.6
23-OCT-1995 (46)

Remark: Bioconcentration factors:
Leuciscus idus (golden orfe) < 10 (fresh weight) (3 d)
Chlorella fusca (green algae) < 10 (fresh weight) (1 d)
activated sludge 1300 (dry weight) (5 d)
There is no evidence whether a plateau was achieved;
the depuration rate is unknown.
23-OCT-1995 (23)

Remark: Bioconcentration factor (calculated):
Oncorhynchus mykiss (rainbow trout, muscle) 14
23-OCT-1995 (47)

Remark: Degradation in soil:
Half life in soil: 35 d
(Determination of mineralization by
radioactive labelling)
(loamy sand/sand, independent of depth 3-18 m)
23-OCT-1995 (44)

Remark: Degradation in soil:
Inoculum: soil microorganisms ("septic tank
tile fields")
Method: anaerobic degradation, static;
parameter: 14 CO₂;
20 degree C
Concentration: 1 mg/kg related to soil
Half life: 18.2 h
23-OCT-1995 (48)

Remark: Degradation in sea water:
Inoculum: sea water
Method: Determination of BOD

Concentration: 2 mg/l related to test
substance
Degradation after 5 d: 74.9 %
No further information about test conditions
14-AUG-2001 (49)

Remark: Degradation in sea water:
Inoculum: sea water (New York, USA)

	Method: aerobic degradation, static; 29 degree C; measurement of radioactivity of the 14C-labelled substance(at carboxyl group) Concentration: 0.059 mg/l related to test substance Degradation after 7 d: 98.7 % Determination with trace concentrations	
14-AUG-2001		(50)
Remark:	Degradation in marine ecosystems: Benzoic acid can be degraded by different marine yeasts (9 of 12 tested species: <i>Saccharomyces rosei</i> , <i>S. italicus</i> , <i>S. chevaliero</i> , <i>Cryptococcus laurentii</i> , <i>C. luteolus</i> , <i>C. neoformans</i> , <i>Rhodotorulus rubra</i> , <i>R. glutinis</i> , <i>Hansenula anomala</i>). No information about test conditions.	
23-OCT-1995		(50)
Remark:	Elimination in rainwater: Inoculum: rainwater Methode: aerobic degradation; 22 degree C Concentration: 0.001 mg/l related to test substance	
	Degradation after 7 d: 22-40 % Degradation after 45 d: 100 %	
23-OCT-1995		(51)
Remark:	Inoculum: Basische Parabraunerde (ueber p-Hydroxybenzoesaeure isoliertes Inokulum) Method: aerobic degradation, static, room temperature Concentration: 20 mg test substance/kg soil Degradation after 3 d: 40 % Degradation after 7 d: 44 % Degradation after 70 d: 63 % related to the release of labelled CO ₂ in % applied radioactivity (labelled benzene ring)	
23-OCT-1995		(52)
Remark:	Inoculum: soil microorganisms (loamy sand) Method: aerobic degradation, static, 30 degree C, pH = 7.3 Concentration: 16 mg/l related to test substance Degradation after 1 d: 100 % substance specific analysis	

23-OCT-1995

(36)

Remark: Inoculum: soil microorganisms (sandy soil in 18.3 m depth)
Method: aerobic degradation, static, 24 degree C
Concentration: 0.05 mg/kg related to test substance
Degradation after 15 d: 40 %
Half life: 35 d (graphically determined)

14-AUG-2001

(44)

Remark: Inoculum: soil microorganisms (loam)
Method: aerobic degradation, static, 25 degree C
Concentration: 25 mg/l related to test substance
Degradation after 1 d: 100 %
The cleavage of the benzene ring was detected by UV adsorption.

14-AUG-2001

(53)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: *Lepomis macrochirus* (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
NOEC: 10
LC50: 44.6

Method: other: Test conducted according to EPA-660/3-75-009 except that replicate concentrations were not used.

Year: 1975

GLP: no data

Test substance: other TS: technical grade benzoic acid

Remark: Higher LC50s were seen with other species.

Result: 24 hr LC50 = >56.0 mg/l; 48 hr LC50 = 46.0 mg/l; 72 hr LC50 = 46.0 mg/l

Test condition: Purified, deionized ater reconstituted to Ph of 7.49, total hardness of 44 mg/l CaCO₃, total alkalinity of 31 mg/l CaCO₃.

Reliability: (2) valid with restrictions
Guideline study with acceptable restrictions

Flag: Critical study for SIDS endpoint

14-FEB-2002 (54)

Type: static
Species: *Salmo gairdneri* (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
NOEC: 10
LC50: 47.3

Year: 1979

GLP: no data

Test substance: other TS: technical grade benzoic acid

Method: Test conducted according to EPA-660/3-75-009 except that replicate concentrations were not used.

Result: 24 hr LC50 = 47.3 mg/l; 48 hr LC50 = 47.3 mg/l; 72 hr LC50 = 47.3 mg/l

Test condition: Purified, deionized water reconstituted to pH of 7.44, total hardness of 36 mg/l CaCO₃, total alkalinity of 27 mg/l CaCO₃.
Reliability: (2) valid with restrictions Guideline study with acceptable restrictions
Flag: Critical study for SIDS endpoint
14-AUG-2001 (55)

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no data
LC0: 400
LC50: 460
LC100: 600

Method: other: Fish test acc. to Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung L15
Year: 1976
GLP: no data
Test substance: other TS: benzoic acid, purity not noted

Remark: pH 7 - 8
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
14-AUG-2001 (56)

Type: static
Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring:
LC0: 180

Method: other: aerated; 19.5-20.5 degree C; pH control
15-JAN-2001 (31)

Species: Carassius auratus (Fish, fresh water)
Unit: mg/l Analytical monitoring:
LC100: 200
Method: other: no data

Remark: exposure period: 7-96 h
15-JAN-2001 (57)

Species: Lepomis humilis (Fish, fresh water)
Exposure period: 1 hour(s)
Unit: mg/l Analytical monitoring:
LC100: 550 - 570

Method: other: no data

15-JAN-2001 (57)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no data
EC0: 260
EC50: 500
EC100: 1000

Method: other: Immobilization test at 20 degree C; pH 8.0

Year: 1982

GLP: no data

Test substance: other TS: benzoic acid, purity not noted

Remark: standardized culture
without neutralization EC0 : 77 mg/l
EC50 : 102 mg/l
EC100: 136 mg/l

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (58)

Type: static

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
NOEC: 100
EC50: > 100

Method: other: EPA-660/3-75-009
Year: 1979
GLP: no data
Test substance: other TS: technical grade benzoic acid
Test condition: The water was vigorously aerated and determined by analysis to have pH of 8.45, total hardness of 250 mg/l CaCO₃, total alkalinity of 141 mg/l CaCO₃.
Reliability: (2) valid with restrictions
Guideline study
Flag: Critical study for SIDS endpoint
14-FEB-2002 (59)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC0: 540
EC50: 1540

Method: other: Immobilization test
(neutralization); 20-22 degree C;
pH 7.6 - 7.7

Remark: wild population
06-JUN-2001 (60)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 300

Method: other: Immobilization test acc. to Bringmann & Kuehn

15-JAN-2001 (61)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus quadricauda (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: 75

Method: other: see below

Year: 1982
GLP: no data
Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Photosynthesis was assayed by following the uptake of (14C)O₂ from NaH(14C)O₂. Plastic culture flasks contained 9.9ml cell suspension (containing 1.0 E+5 algal cells/ml), 0.1ml radioisotope, and 0.1ml of test chemical. The flasks were incubated for 3 hours and photosynthetic activity assayed. Five replicates of five concentrations, ranging from 0 to 100 mg/ml, were used. Per cent inhibition was calculated relative to photosynthetic activity in the controls. EC₅₀ values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed). Analyses for significant differences (p=0.05) were performed using Dunnett's test (Winer BJ. 1971. Stat. Prin. in Exp. Design, 2nd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, Well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-FEB-2002 (62)

Species: Scenedesmus quadricauda (Algae)
Exposure period: 8 day(s)
Unit: mg/l Analytical monitoring:
TGK : 1630

Method: other: static, inhibition of cell multiplication; 27 degree C; pH 7

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, Well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (63)

Species: Scenedesmus quadricauda (Algae)
Endpoint: growth rate
Exposure period: 14 day(s)
Unit: mg/l Analytical monitoring:

EC50: > 10

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Growth was assessed by measuring the absorbance of cultures with time using a Bausch and Lomb Spectronic 20 spectrophotometer. The wavelength employed (420 nm) was determined by the method of Sorokin C. (1973. Handbook of Phycological Methods). Sidearm flasks containing 94.9ml of medium and 0.1 ml of test chemical were inoculated with 5 ml of an active culture (containing 6.5 E+4 cyanobacterial and 1.0 E+5 algal cells per ml) and incubated for 12 - 14 days. Five replicates of five concentrations of test chemical, ranging from 0 to 10 mg/ml, were used. Optical densities of treated cultures were determined daily and per cent inhibition was calculated relative to the controls. Growth rates were determined by Sorokin C (1973) and EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (62)

Species: Chlorella pyrenoidosa (Algae)
Endpoint: other: inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: 60

Method: other: see below
Year: 1982
GLP: no data
Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method:	Photosynthesis was assayed by following the uptake of (14C)O₂ from NaH(14C)O₂. Plastic culture flasks contained 9.9ml cell suspension (containing 1.0 E+5 algal cells/ml), 0.1ml radioisotope, and 0.1ml of test chemical. The flasks were incubated for 3 hours and photosynthetic activity assayed. Five replicates of five concentrations, ranging from 0 to 100 mg/ml, were used. Per cent inhibition was calculated relative to photosynthetic activity in the controls. EC₅₀ values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed). Analyses for significant differences (p=0.05) were performed using Dunnett's test (Winer BJ. 1971. Stat. Prin. in Exp. Design, 2nd ed).		
Test condition:	20 degree C; 12 h light/dark-cycle; light intensity 7000 lux		
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, Well documented and acceptable for assessment		
Flag:	Critical study for SIDS endpoint		
14-AUG-2001			(62)
Species:	Chlorella pyrenoidosa (Algae)		
Endpoint:	growth rate		
Exposure period:	14 day(s)		
Unit:	mg/l	Analytical monitoring:	
EC ₅₀ :	> 10		
Test substance:	other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA		
Method:	Growth was assessed by measuring the absorbance of cultures with time using a Bausch and Lomb Spectronic 20 spectrophotometer. The wavelength employed(420 nm) wasdetermined by the method of Sorokin C. (1973. Handbook of Phycological Methods). Sidearm flasks containing 94.9ml of medium and 0.1 ml of test chemical were inoculated with 5 ml of an active culture (containing 6.5 E+4 cyanobacterial and 1.0 E+5 algal cells per ml) and incubated for 12 - 14 days.		

Five replicates of five concentrations of test chemical, ranging from 0 to 10 mg/ml, were used. Optical densities of treated cultures were determined daily and per cent inhibition was calculated relative to the controls. Growth rates were determined by Sorokin C (1973) and EC50 values were determined by probit (Finney DJ.1971. Probit Analysis, 3rd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (62)

Species: *Anabaena variabilis* (Algae)
Endpoint: growth rate
Exposure period: 14 day(s)
Unit: mg/l Analytical monitoring: no data
EC50: > 10

Method: other: see below
GLP: no data
Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Growth was assessed by measuring the absorbance of cultures with time using a Bausch and Lomb Spectronic 20 spectrophotometer. The wavelength employed (420nm) was determined by the method of Sorokin C. (1973. Handbook of Phycological Methods). Sidearm flasks containing 94.9ml of medium and 0.1 ml of test chemical were inoculated with 5 ml of an active culture (containing 6.5 E+4 cyanobacterial and 1.0 E+5 algal cells per ml) and incubated for 12 - 14 days. Five replicates of five concentrations of test chemical, ranging from 0 to 10 mg/ml, were used. Optical densities of treated cultures were determined daily and per cent inhibition was calculated relative to the controls.

Growth rates were determined by Sorokin C (1973) and EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
Well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (62)

Species: Anabaena cylindrica (Algae)
Endpoint: other: inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 60

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Photosynthesis was assayed by following the uptake of (14C)O2 from NaH(14C)O2. Plastic culture flasks contained 9.9ml cell suspension (containing 1.0 E+5 algal cells/ml), 0.1ml radioisotope, and 0.1ml of test chemical. The flasks were incubated for 3 hours and photosynthetic activity assayed. Five replicates of five concentrations, ranging from 0 to 100 mg/ml, were used. Per cent inhibition was calculated relative to photosynthetic activity in the controls. EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed). Analyses for significant differences (p=0.05) were performed using Dunnett's test (Winer BJ. 1971. Stat. Prin. in Exp. Design, 2nd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions
23-MAY-2001 (62)

Species: Microcystis aeruginosa (Algae, blue, cyanobacteria)
Exposure period: 8 day(s)

Unit: mg/l Analytical monitoring:
TGK : 55

Method: other: inhibition of cell multiplication at 27 degree C; pH 7

15-JAN-2001 (64)

Species: Anabaena inaequalis (Algae)
Endpoint: growth rate
Exposure period: 14 day(s)
Unit: mg/l Analytical monitoring:
EC50: 9

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Growth was assessed by measuring the absorbance of cultures with time using a Bausch and Lomb Spectronic 20 spectrophotometer. The wavelength employed (600 nm) was determined by the method of Sorokin C. (1973. Handbook of Phycological Methods). Sidearm flasks containing 94.9ml of medium and 0.1 ml of test chemical were inoculated with 5 ml of an active culture (containing 6.5 E+4 cyanobacterial and 1.0 E+5 algal cells per ml) and incubated for 12 - 14 days. Five replicates of five concentrations of test chemical, ranging from 0 to 10 mg/ml, were used. Optical densities of treated cultures were determined daily and per cent inhibition was calculated relative to the controls. Growth rates were determined by Sorokin C (1973) and EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions

13-DEC-2000 (62)

Species: Anabaena cylindrica (Algae)
Endpoint: growth rate
Exposure period: 14 day(s)
Unit: mg/l Analytical monitoring:

EC50: > 10

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Growth was assessed by measuring the absorbance of cultures with time using a Bausch and Lomb Spectronic 20 spectrophotometer. The wavelength employed (600 nm) was determined by the method of Sorokin C. (1973. Handbook of Phycological Methods). Sidearm flasks containing 94.9ml of medium and 0.1 ml of test chemical were inoculated with 5 ml of an active culture (containing 6.5 E+4 cyanobacterial and 1.0 E+5 algal cells per ml) and incubated for 12 - 14 days. Five replicates of five concentrations of test chemical, ranging from 0 to 10 mg/ml, were used. Optical densities of treated cultures were determined daily and per cent inhibition was calculated relative to the controls. Growth rates were determined by Sorokin C (1973) and EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions

06-SEP-2000

(62)

Species: Anabaena inaequalis (Algae)

Endpoint: other: Inhibition of photosynthesis

Exposure period: 3 hour(s)

Unit: mg/l

Analytical monitoring:

EC50: 5

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Photosynthesis was assayed by following the uptake of (14C)O2 from NaH(14C)O2. Plastic culture flasks contained 9.9ml cell suspension (containing 1.0 E+5 algal cells/ml), 0.1ml radioisotope, and 0.1ml of test chemical. The flasks were incubated for 3 hours and photosynthetic activity assayed.

Five replicates of five concentrations, ranging from 0 to 100 mg/ml, were used.
Per cent inhibition was calculated relative to photosynthetic activity in the controls. EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed).
Analyses for significant differences ($p=0.05$) were performed using Dunnett's test (Winer BJ. 1971. Stat. Prin. in Exp. Design, 2nd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions
14-AUG-2001 (62)

Species: Anabaena variabilis (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 55

Method: other: inhibition of photosynthesis;
20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Photosynthesis was assayed by following the uptake of $(^{14}\text{C})\text{O}_2$ from $\text{NaH}(^{14}\text{C})\text{O}_2$. Plastic culture flasks contained 9.9ml cell suspension (containing 1.0×10^5 algal cells/ml), 0.1ml radioisotope, and 0.1ml of test chemical. The flasks were incubated for 3 hours and photosynthetic activity assayed.
Five replicates of five concentrations, ranging from 0 to 100 mg/ml, were used.
Per cent inhibition was calculated relative to photosynthetic activity in the controls. EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed).
Analyses for significant differences ($p=0.05$) were performed using Dunnett's test (Winer BJ. 1971. Stat. Prin. in Exp. Design, 2nd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions

14 -AUG-2001

(62)

4.4 Toxicity to Microorganisms e.g. Bacteria

Species: activated sludge
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: > 1000

Method: OECD Guide-line 209 "Activated Sludge,
Respiration Inhibition Test"
Year: 1984
Test substance: other TS: benzoic acid; purity not noted

Remark: pH 7,5
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
14 -AUG-2001 (62)

Species: Photobacterium phosphoreum (Bacteria)
Exposure period: 30 minute(s)
Unit: mg/l Analytical monitoring:
EC50: 16.85

Method: other: static at 15 degree C; Microtox-Test
Test substance: other TS: benzoic acid; purity not noted

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
14 -AUG-2001 (66)

Species: Pseudomonas putida (Bacteria)
Exposure period: 16 hour(s)
Unit: mg/l Analytical monitoring:
TGK : 480

Method: other: static; 25 degree C; pH 7
Test substance: other TS: benzoic acid; purity not noted

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
Well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint

14-AUG-2001 (63)

Species: Pseudomonas fluorescens (Bacteria)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC0: 1000

Method: other: Bestimmung der biologischen
Schadwirkung toxischer Abwaessergegen
Bakterien. DEV, L 8 (1968) modifiziert

14-AUG-2001 (19)

Species: other bacteria: Pseudomonas Stamm Berlin
Exposure period: 1 hour(s)

Unit: mg/l Analytical monitoring:
EC10: 50

Method: other: Oxygen consumption test acc. to Robra,
GWf-Wasser/Abwasser 117,80-86 (1976)

14-AUG-2001 (61)

Species: other bacteria: population of microorganisms
from communal sewage
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
Tlm : 500

Method: other: static, inhibition of cell
multiplication; 37 degree C; pH 6.9

14-AUG-2001 (31)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

Remark: No data available.
14-AUG-2001

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Remark: No data available.
14-AUG-2001

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

Remark: No data available.
14-AUG-2001

4.6.3 Toxicity to Soil Dwelling Organisms

Remark: No data available.
14-AUG-2001

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

Remark: No data available.
14-AUG-2001

4.7 Biological Effects Monitoring

Remark: No data available.
14-AUG-2001

4.8 Biotransformation and Kinetics

Remark: No data available.
14-AUG-2001

4.9 Additional Remarks

Remark: Toxicity to protozoa:
TT (Chilomonas paramecium): 48 h EC5 356 mg/l
(cell multiplication) pH 6,9
23-OCT-1995 (67)

Remark: Toxicity to protozoa:
Entosiphon sulcatum 72 h EC5: 218 mg/l
(cell multiplication)
23-OCT-1995 (68)

Remark: Toxicity to protozoa:
Uronema parduczi 20 h TT: 31 mg/l, pH 6.9
(cell multiplication)

23-OCT-1995 (69)

Remark: Toxicity to yeast: 6 w MIC (pH 3.5; 25 degree C
adapted non-adapted
-Saccharomyces cerevisiae St 1297 170 mg/l 100 mg/l
-Kluveromyces fragilis 173 125
-Kloeckera apiculata 188 125
-Hansenula anomala 223 140
-Candida crusei 440 300
-Saccharomycodes ludwigii 650 300
-Schizosaccharomyces pombe 567 325
-Zygosaccharomyces bailii 1250 600

23-OCT-1995 (70)

Remark: Toxicity to fungi:
Fusarium oxysporum:
Test concentration: 610 mg/l
Growth inhibition at
pH 4.0 : 83.5 %
pH 4.8 : 74.6 %
pH 5.6 : 57.9 %
pH 6.4 : 39.5 %
pH 7.2 : 23.7 %

23-OCT-1995 (71)

Remark: Antimicrobial effects (pH 6):
minimal microbicide minimal inhib.
Conc. (MMC) conc. (MIC)
(serial dilution
test)
-Aspergillus niger 1000 mg/l 500-1000 mg/l
-Candida albicans 1200 500-1000
-Escherichia coli 160 100-200
-Klebsiella pneumoniae 160 100-200
-Penicillium notatum 1000 500-1000
-Pseudomonas aeruginosa 160 200-500
-Pseudomonas cepacia 160
-Pseudomonas fluorescens 160 200-500
-Staphylococcus aureus 20 50-100

23-OCT-1995 (72)

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Sex: male/female
No. of Animals: 50
Vehicle: other: corn oil
Value: 2565 mg/kg bw

Method: Directive 84/449/EEC, B.1 "Acute toxicity (oral)"

GLP: no data
Test substance: other TS: technical grade benzoic acid

Method: 25 male and 25 female Spartan rats weighing 200 to 250 grams were used for this study. The test compound was suspended in corn oil and administered orally at the following dosage levels: 500, 1250, 1984, 3150, and 5000 mg/kg. Five rats of each sex were used at each dosage level. Volumes of 10 ml/kg bw were administered at all dosage levels. All rats were observed for mortality continuously during the first 4 hours after dosing, at 24 hours and once daily thereafter for a total of 14 days. Body weights were recorded initially and at 14 days.

Result: All surviving rats, males and females, exhibited normal body weight gains during the 14 day observation period. The acute oral LD50 of benzoic acid in male albino rats was calculated to be 2742 mg/kg (2279-3299 mg/kg). The acute oral LD50 of benzoic acid in female albino rats was calculated to be 2360 mg/kg (2042-2726 mg/kg). A combined acute oral LD50 for benzoic acid in male and female albino rats was calculated to be 2565 mg/kg (2292-2870 mg/kg).

LD50 calculations were done according to WR Thompson. 1947.Bact. Rev. 11:115-145.

Dose level (mg/kg)	Mortality
500	0/5
1250	0/5
1984	0/5
3150	4/5
5000	5/5

Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint

14-FEB-2002 (73)

Type: LD50
Species: mouse
Sex: male/female
No. of Animals: 60
Vehicle: other: Tween 80 (1.5%)
Value: 2250 mg/kg bw

Method: EPA OPPTS 870.1100
Year: 1979
GLP: no data
Test substance: other TS: Commercial Grade benzoic acid
(Velsicol lot #52829055)

Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint

14-FEB-2002 (74)

Type: LD50
Species: rat
Value: = 1700 mg/kg bw

26-JAN-2001 (75)

Type: LD50
Species: rat
Value: = 3040 mg/kg bw

26-JAN-2001 (4)

5. TOXICITY

DATE: 14-FEB.-2002
SUBSTANCES ID: 65-85-0

Type: LD50
Species: rat
Value: = 2530 mg/kg bw

26-JAN-2001 (76)

Type: LD50
Species: mouse
Value: = 1940 mg/kg bw

26-JAN-2001 (77)

Type: LD50
Species: mouse
Value: = 2370 mg/kg bw

26-JAN-2001 (78)

5.1.2 Acute Inhalation Toxicity

Type: LC50
Species: rat
Sex: male/female
No. of Animals: 10
Exposure time: 4 hour(s)
Value: > 12.2 mg/l

Method: EPA OTS 798.1150
Year: 1974
GLP: no data
Test substance: other TS: technical grade benzoic acid

Method: Ten rats (4 units of 2 or 3 rats/unit to prevent piling) were placed in a sealed 59.1 liter glass chamber and exposed to a dynamic atmosphere containing the dust of the test material. A Wright Dust Feeder controlled addition of The test substance; airflow regulated by a flowmeter. The rats were observed continuously during the 4-hour exposure, and for a period of 14 days following exposure.

Result: All of the rats survived the 4-hour exposure and the 14-day observation period. Signs during the exposure period included occasional increased motor activity and slight erythema. At the conclusion of exposure, 1 rat exhibited salivation.

At 24 hours and through the 14-day observation period, all rats appeared normal and exhibited normal body weight gains.
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
14-FEB-2002 (73)

Type: LC50
Species: rat
Exposure time: 1 hour(s)
Value: > .026 mg/l

Remark: exposure to vapor
generalized inactivity, lacrimation at 0.026 mg/l/1h, no mortality
15-JAN-2001 (4)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Sex: male/female
No. of Animals: 4
Vehicle: other: neat
Value: > 2000 mg/kg bw

Method: EPA OTS 798.1100
Year: 1974
GLP: no data
Test substance: other TS: technical grade benzoic acid

Method: The test compound was applied once only to a shaved area of the back of each rabbit at a dose of 2000 mg/kg bw.
The skin of 1 male and 1 female was abraded with a scalpel blade prior to test application.
The area was wrapped with a gauze bandage and occluded with plastic wrap.
The bandages were removed and the backs washed 24 hours after application.
The rabbits were observed for a period of 14 days.

Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
14-FEB-2002 (73)

Type: LD50

5. TOXICITY

DATE: 14-FEB.-2002
SUBSTANCES ID: 65-85-0

Species: rabbit
Value: > 10000 mg/kg bw

Remark: mortality: 0/5
15-JAN-2001 (4)

Type: LD50
Species: rabbit
Value: > 5000 mg/kg bw

Remark: mortality: no information
15-JAN-2001 (79)

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: = 1460 mg/kg bw

23-MAR-2001 (80)

5.2 Corrosiveness and Irritation**5.2.1 Skin Irritation**

Species: rabbit
Concentration: undiluted
Exposure: Semiocclusive
Exposure Time: 4 hour(s)
No. of Animals: 6
PDII: 0
Result: not irritating
EC classificat.: not irritating

Method: EPA OTS 798.4470
GLP: no data
Test substance: other TS: benzoic acid, technical flakes

Remark: Primary Skin Irritation and Corrosive Hazard
(Title 49, Transportation, Chapter 1)
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
14-AUG-2001 (73)

Species: rabbit
Concentration: undiluted
Exposure: Semiocclusive

Exposure Time:	4 hour(s)
No. of Animals:	3
PDII:	.5
Result:	slightly irritating
EC classificat.:	not irritating
Method:	Directive 84/449/EEC, B.4 "Acute toxicity (skin irritation)"
GLP:	yes
Test substance:	other TS: benzoic acid, purity not noted
Method:	The flank site of 3 albino rabbits was exposed to 0.5 g of the test substance moistened with 0.25 ml Milli-R0 water for 4 hours using semi-occlusive dressings.
Result:	The primary skin irritation index amounted to 0.5; based on these results, the test substance should be considered as minimally irritating to the skin; According to Annex VI of EEC Council Directive 67/548/EEC (amended by Directive 83/467/EEC), the test substance need not be labelled as a skin irritant.
Reliability:	(1) valid without restriction Guideline study
Flag:	Critical study for SIDS endpoint
14-AUG-2001	(81)
Species:	rabbit
Method:	other: see remarks
Remark:	irritation score: 1.66/8.00 single application of 500 mg dry powder (no further information), response scored at 24 h and 72 h
23-MAR-2001	(4)
Species:	rabbit
Concentration:	undiluted
Exposure Time:	24 hour(s)
No. of Animals:	2
Result:	not irritating
Method:	other:
Test substance:	other TS: benzoic acid, purity not noted
Method:	2 animals; application of 500 mg/animal at the inner side of the ear for 24 h
13-MAR-2001	(82)

Species: human

Method: other: see remarks

Remark: Chamber-Scarification-Test
threshold irritating concentration:
1) normal skin: 30 % in ethanol
2) scarified skin: 7.5 % in ethanol: moderate irritations;
application of 15 % in ethanol leads to marked irritation with erosions

23-MAR-2001 (83) (84)

Species: human

Remark: intermittent exposure, total dose applied: 22 mg, duration of exposure: 3 days
irritation classified as moderate

23-MAR-2001 (85)

Species: human

Method: other: see remarks

Remark: 16 mM benzoic acid (in petrolatum) produced an Erythematous reaction in 12 of 13 healthy volunteers on the cheek and in 6 subjects on the forehead, neck and upper back.
8 mM and 4 mM benzoic acid produced only a reaction on cheek.open application method

23-MAR-2001 (86)

Species: human

Method: other: see remarks

Remark: benzoic acid (in 50 % aqueous isopropanol) was applied to the medial cheek of adult volunteers; a 2 % solution led to wheals (11/11), a 0.04 % solution to erythema (11/11) and pruritus (4/11)

23-MAR-2001 (87)

Species: human

Method: other: see remarks

Remark: non-immunologic immediate contact reactions
30-45 min after application skin-test with 10
ul doses of 50, 100, 250, 500 or 1000 mM
benzoic acid in various vehicles (emollient
cream, petrolatum, 2-propyl alcohol/water-
mixture (1:1), abs. ethyl alcohol, synthetic
lanolin substitute), openly applied on the
back of 11 healthy subjects and 3 patients
with psoriasis, eczema, and rosacea resp. for
15 min

23-MAR-2001

(88)

5.2.2 Eye Irritation

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time: 1 hour(s)
Comment: rinsed after (see exposure time)
No. of Animals: 8
Result: corrosive
EC classificat.: risk of serious damage to eyes

Method: EPA OTS 798.4500
GLP: no data
Test substance: other TS: benzoic acid, technical flakes

Remark: Group I, consisting of 5 rabbits, were exposed
to the test compound for 5 minutes; 3 rabbits
in Group II were exposed to the test substance
for 24 hours.
Following the exposure period, the treated
eyes were washed with a gentle continuous
stream of water for 2 minutes.
Eye Irritation Test in Albino Rabbits (21 CFR,
Part 191)

Result: Both Group I (5 minute exposure) and Group II
(24 hrs exposure) - an extremely irritating
and corrosive substance.

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint

14-AUG-2001

(73)

Species: rabbit
Concentration: undiluted
Dose: 77 other: mg
Result: highly irritating
EC classificat.: irritating

Method: Directive 84/449/EEC, B.5 "Acute toxicity (eye irritation)"

GLP: yes

Test substance: other TS: benzoic acid, purity not noted

Remark: Based on Draize score of 35 the test substance should be classified as severely irritating according to the scheme of Kay & Calandra; according to Annex VI of EEC Council Directive 67/548/EEC (amended by Directive 83/467/EEC), the test substance should be labelled as an eye irritant. instillation of approx. 77 mg in the eye

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint

14-AUG-2001 (89)

Species: rabbit

Method: other: see remarks

Remark: irritation score: 65.0/110

23-MAR-2001 single application of 100 mg dry powder, responses scored at 24, 48 or 72 h (4)

Species: rabbit

Result: slightly irritating

Method: other: OECD Guideline 405

23-MAR-2001 (90)

Species: rabbit

Result: moderately irritating

Method: other: see remark

Remark: 2 animals; instillation of 50 mg/animal into The conjunctival sac

23-MAR-2001 (82)

5.3 Sensitization

Type: Draize Test

Species: guinea pig

Concentration 1st: Induction 500 undiluted occlusive epicutaneous

2nd:Challenge 500 undiluted occlusive epicutaneous
 No. of Animals: 10
 Result: not sensitizing
 Classification: not sensitizing

Method: EPA OPP 81-6
 Year: 1959
 GLP: yes
 Test substance: other TS: benzoic acid, purity not noted

Result: During induction and challenge, the grand mean for erythema and edema at 24 and 48 hours was 0. Based on this study, it was concluded that Benzoic acid is neither an irritant nor a sensitizer when applied to guinea pigs.

Reliability: (1) valid without restriction

GLP guideline study

Flag: Critical study for SIDS endpoint

14-AUG-2001

(91)

Type: Guinea pig maximization test
 Species: guinea pig
 Concentration 1st: Induction 10 % intracutaneous
 2nd: Induction 20 % semiocclusive
 3rd: Challenge 20 % semiocclusive
 Result: not sensitizing
 Classification: not sensitizing

Method: OECD Guide-line 406 "Skin Sensitization"
 GLP: no data
 Test substance: other TS: benzoic acid, purity not noted

Remark: test concentrations: intradermal injection
 10 %, topical induction 20 %, challenge 20 %

Reliability: (1) valid without restriction

GLP guideline study

Flag: Critical study for SIDS endpoint

14-AUG-2001

(92)

Type: Buehler Test
 Species: guinea pig
 Result: not sensitizing

Test substance: other TS: benzoic acid; purity not noted

Remark: test concentrations: induction 20 %, challenge 20 %

14-AUG-2001

(92)

Type: Mouse local lymphnode assay

Species: mouse
Result: not sensitizing

Test substance: other TS: benzoic acid; purity not noted

Remark: test concentrations: 5, 10 or 20 %
14-AUG-2001 (93)

Type: Mouse ear swelling test
Species: mouse
Result: not sensitizing

Test substance: other TS: benzoic acid; purity not noted

Remark: test concentrations: induction 20 %, challenge 20 %
14-AUG-2001 (92)

Type: other: see remarks
Species: guinea pig
Result: sensitizing

Method: other: ear swelling test

Remark: groups of five guinea pigs were challenged by applying various concentrations of benzoic acid to both sides of the earlobe. The thickness of the ear was measured at various time intervals. Benzoic acid was positive (concentration-dependent effect).
14-AUG-2001 (94)

Type: other: see remarks
Species: human

Method: other: patch-test
Test substance: other TS: benzoic acid; purity not noted

Remark: 3 workers of a pharmaceutical plant with transient urticaria after exposure to sodium benzoate and 3 previously unexposed healthy control subjects were tested. All subjects reacted to benzoic acid at 0.25 % in aqueous solution under occlusion. 1 worker and 2 controls reacted to sodium benzoate at 0.5 % in saline under occlusion, but none reacted to sodium benzoate at 0.5 % in aqueous solution. All 3 workers reacted in a closed patch test to benzoic acid at 5 % in petrolatum.

The time course of the responses to benzoic acid and sodium benzoate was similar in controls and workers.
The potential of sodium benzoate to elicit Nonimmunologic contact urticaria may be due to the formation of benzoic acid at skin contact.

14-AUG-2001 (95)

Type: other: see remarks
Species: human

Method: other: patch-test

Remark: 3/5 patients with chronic urticaria developed positive skin reactions in a patch test with benzoic acid (5 % in petrolatum).

14-AUG-2001 (96)

Type: other: see remarks
Species: human

Method: other: patch-test
Test substance: other TS: benzoic acid; purity not noted

Remark: In a patch test with benzoic acid (5 % in petrolatum), 108/113 patients showed no reaction and 5/113 patients showed a 1+ reaction.
Benzoic acid was not classified as a sensitizer.

14-AUG-2001 (97)

Type: other: see remarks

Species: human

Method: other: patch-test

Remark: In a study of cosmetic intolerance with patients tested for possible contact dermatitis, 34 (0.7 %) of all patients and 1 (0.6 %) patient with pure allergy to cosmetics reacted positive.

14-AUG-2001 (98)

Type: other: see remark
Species: human

Method: other: patch-test
Test substance: other TS: benzoic acid; purity not noted

Remark: a baker developed dermatitis from flours which contained traces of benzoic acid; patch tests showed contact type eczematous hypersensitivity to benzoic acid (6 % in petrolatum).

14-AUG-2001 (99)

Type: other: see remark
Species: human

Method: other: patch-test
Test substance: other TS: benzoic acid; purity not noted

Remark: 40 children (under 12 years old) were tested for contact urticaria against food additives. 14 of them reacted positive to benzoic acid (no further information).

Reliability: (3) invalid
Documentation insufficient for assessment

14-AUG-2001 (100)

Type: other: see remarks
Species: human

Method: other: skin-prick-test

Remark: 23 out of 91 subjects suffering from chronic or recurrent urticaria were tested in a skin test: 10/23 positive subjects (at least one histamine equivalent skin test reaction) reacted to benzoic acid (5 % in petrolatum).

14-AUG-2001 (101)

Type: other: see remarks
Species: human

Method: other: oral provocation test

Remark: a chemical worker suffered from allergic reactions of increasing intensity while being constantly exposed to benzoic acid during work. After oral exposure to sodium benzoate (500 mg) he suffered a severe anaphylactic shock. He showed similar but milder reaction after consuming food containing benzoic acid.

14-AUG-2001 (102)

Type: other: see remarks
Species: human
Method: other: oral provocation test
Remark: only one out of 7 subjects with a positive skin test for benzoic acid showed a positive response (itching, wealing) after repeated oral exposure

14-AUG-2001 (101)

Type: other: see remarks
Species: human
Method: other: oral provocation test
Remark: to patients suffering from asthma benzoic acid was given orally (no details reported); approx. 50 % of the subjects showed asthmatic hypersensitivity, rhinitis and urticaria.

14-AUG-2001 (103)

Type: other: see remarks
Species: human
Method: other: patch-test
Remark: 7 patients with recurrent episodes of erythema multiforme were found to be sensitive to benzoic acid. Advice on avoidance of benzoic acid resulted in resolution of attacks in 4 patients (3 patients were not able to adhere to an exclusion diet).

14-AUG-2001 (104)

5.4 Repeated Dose Toxicity

Type: Chronic
Species: rat Sex: male/female
Strain: no data
Route of administration: oral feed
Exposure period: generation 1 and 2: lifelong,
generation 3: 16 weeks,
generation 4: until breeding
Frequency of treatment: continuously in diet
Post exposure period: no
Doses: 0.5 or 1 % in diet (approx. 375 or 750 mg/kg/day)

Control Group:	yes	
NOAEL:	750 mg/kg bw	
Year:	1960	
GLP:	no	
Test substance:	other TS: benzoic acid, purity not noted	
Method:	A robust protocol according to standards at That time was used. Taking into account the reputation of the investigators a high quality has to be assumed.	
Remark:	40 rats/group; initial body weight: 40-50 g The mean compound consumption was calculated according to Lehman, A.J., Assoc. Food Drug Off. Q. Bull. 18, 66 (1954).	
Result:	In all 4 generations no influence on growth (weight, weight gain and food efficiency (measured by protein efficiency))and organ weights was found. In all 4 generations, no effects on fertility ("Forzplanzung")and lactation ("Aufzugt der Jungen")was found. The animals of the 3rd generation were killed and examined histopathological after 16 weeks (after lactation of the pups.)No histo-pathological findings were found. In the paper no information is given on the organs investigated, however due to the robustness of the total study, the reputation of the investigators, as well as the reputation of the Professor who did the histopathologic investigation, a high quality has to be assumed. From other parameters it can be assumed that as a minimum the brains, heart, liver, kidney, testis and were examined. Feeding of 0.5 % led to prolongation of survival compared to controls. In addition a so-called "Alters Paarung" after 48 weeks gave no influence on start of menopause.	
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment	
Flag:	Critical study for SIDS endpoint	
14-FEB-2002		(105)
Type:	Sub-chronic	
Species:	rat	Sex: male
Strain:	no data	
Route of administration:	oral feed	
Exposure period:	28 days	

Frequency of treatment: continuously in diet

Post exposure period: no

Doses: 760, 3800 or 7600 ppm (approx. 65, 324.1 or 647.5 mg/kg/day)

Control Group: yes

NOAEL: 647.5 mg/kg bw

Method: other

GLP: no data

Test substance: other TS: benzoic acid; purity not noted

Remark: 10 rats/group; initial body weight: 120 g
mean feed consumption: 85.5; 85.3 or 85.2 g/kg/d

Result: no deaths or signs of intoxication during experiment, no significant gross pathological lesions at autopsy

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
Well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint

14-FEB-2002

(4)

Species: rabbit Sex: male/female

Strain: New Zealand white

Route of administration: dermal

Exposure period: 21 days

Frequency of treatment: 5 days/week for 3 weeks

Doses: 100, 500, 2500 mg/kg bw

Control Group: yes, concurrent vehicle

NOAEL: 2500 mg/kg bw

GLP: yes

Test substance: other TS: benzoic acid, purity not noted

Method: Four male and four female rabbits were used in each treatment group and in the control group. The skin of one-half of the animals was abraded and the others left intact. Benzoic acid was applied 5 days a week for 3 weeks at dosage levels of 100, 500, 2500 mg/kg bw. The rabbits were observed daily for signs of dermal irritation and changes in general behavior and appearance. Individual body weights were recorded weekly. Hematologic and biochemical studies were conducted once in the pretest period and again

at 21 days of the study. Gross and histopathology was performed on liver, kidneys, thyroid/parathyroid, heart, lung, ovaries, testes, adrenals as well as most gastrointestinal tract and neurological organs.

Result: Very slight dermal irritation was noted for one rabbit at the 2500 mg/kg dosage level. No compound-related effects were seen in general behavior and appearance, body weight, clinical laboratory tests, organ weights, or survival.

Reliability: (1) valid without restriction
Meets generally accepted scientific method and is described in sufficient detail

Flag: Critical study for SIDS endpoint
14-AUG-2001 (106)

Type: Sub-chronic
Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 4 weeks
Frequency of treatment: 6 h/d; 5 d/w
Post exposure period: none
Doses: 0, 25, 250, 1200 mg/m³
Control Group: yes
NOAEL: 25 mg/m³
LOAEL: 250 mg/m³

Year: 1981
GLP: yes
Test substance: other TS: technical grade benzoic acid

Method: Four groups of rats (10 animals/sex/group) were exposed to a dust aerosol of benzoic acid at concentrations of 0, 25, 250, 1200 mg/m³, 6 hrs/day, 5 days/week, 4 consecutive weeks. The animals were observed twice daily, pharmacotoxic signs observed weekly, and their body weights recorded prior to exposure and weekly thereafter.

Animals found in a moribund condition were sacrificed. After 4 weeks of exposure, all surviving animals were necropsied and biochemical, hematologic, organ weights and histopathologic evaluations were conducted.

Result: No compound-related gross lesions were seen in any animal from any dose group.
Compound-related microscopic lesions, consisting of an increase of inflammatory cell infiltrate and an increase in the incidence, intensity, and extent of interstitial fibrosis in lungs of rats from all dose groups (but not dose related), were observed.
1200 mg/3: 1 animal/sex died; decreased body weight; decrease in platelets; decreased absolute and relative weights of liver (m) and trachea/lung (f); no significant difference in biochemical parameters.
>= 250 mg/m3: upper respiratory tract irritation, decreased absolute and relative weights of kidney (f).
0 - 250 mg/m3: No deaths; no effects on weight gain; no significant effects on organ weights, biochemical or hematologic parameters.

Test condition: The concentration was generated as a dust aerosol with an IRAD dust generator.
The test material (white flakes) was ground in an Oster blender to produce a more respirable particle. Actual exposure concentration was determined by gravimetric techniques.
Particle size distribution was determined using Andersen 8 stage cascade impactor.
Average particle size was 4.7um.

Reliability: (1) valid without restriction
Meets generally accepted scientific method and is described in sufficient detail

Flag: Critical study for SIDS endpoint
14-FEB-2002 (107)
Species:

mouse Sex: male/female
Strain: other: cross bred white mice
Route of administration: gavage
Exposure period: 12 weeks
Frequency of treatment: once daily
Post exposure period: no
Doses: 80 mg/kg/day
Control Group: yes

Test substance: other TS: analytical grade benzoic acid

Method: 50 mice/sex (initial body weight: 8-10 g) received benzoic acid by oral intubation.
Observations for general condition, behavior, survival, food consumption, and weight gain were recorded daily.

Result: reduced weight gain without reduced food intake; mortality rate at week 10: 32 % in males and females
Reliability: (3) invalid
Flag: No histopathology or clinical chemistry
14-AUG-2001 Critical study for SIDS endpoint (108)

Species: rat Sex: male
Strain: Wistar
Route of administration: oral feed
Exposure period: 5 days
Frequency of treatment: continuously in diet
Post exposure period: 19 or 30 days
Doses: 3 % in diet (approx. 2250 mg/kg/day)
Control Group: yes

Remark: 15 rats; initial body weight: 60 g
the mean compound consumption was calculated according to Lehman, A.J., Assoc. Food Drug Off. Q. Bull. 18, 66 (1954)
Result: growth retardation; histologically demonstrable brain damage (necrosis of parenchymal cells of the stratum granulosum of the fascia dentata and the cortex of the lobus piriformis) still present after 35 days
Flag: Critical study for SIDS endpoint
14-AUG-2001 (109)

Species: rat Sex: male/female
Strain: Wistar
Route of administration: oral feed
Exposure period: 72 weeks
Frequency of treatment: continuously in diet
Post exposure period: no data
Doses: 1.5 % in diet (approx. 1125 mg/kg/day)
Control Group: yes

Remark: 20 m + 30 f (dosed group), 13 m + 12 f (control); initial body weight: 50-60 g
the mean compound consumption was calculated according to Lehman, A.J., Assoc. Food Drug Off. Q. Bull. 18, 66 (1954)
Result: reduced food intake, growth retardation, increased mortality rate (15/50 vs. 3/25 in the control)
14-AUG-2001 (110)

5. TOXICITY

DATE: 14-FEB.-2002

SUBSTANCES ID: 65-85-0

Species: rat Sex: male
Strain: Wistar
Route of administration: oral feed
Exposure period: 7 - 35 days
Frequency of treatment: continuously in diet
Post exposure period: no
Doses: 1.1 % in diet (approx. 825 mg/kg/day)
Control Group: yes

Remark: 5-10 rats/group
the mean compound consumption was calculated
according to Lehman, A.J., Assoc. Food Drug
Off. Q. Bull. 18, 66 (1954)
Result: reduced food intake, growth retardation, no
pathological findings

15-JAN-2001 (109)

Species: rat Sex: male
Strain: Wistar
Route of administration: oral feed
Exposure period: 5 days
Frequency of treatment: continuously in diet
Post exposure period: no
Doses: 3 % in diet (approx. 2250 mg/kg/day)
Control Group: yes

Remark: 5-10 rats/group; initial body weight: approx.
60 g the mean compound consumption was
calculated according to Lehman, A.J., Assoc.
Food Drug Off. Q. Bull. 18, 66 (1954)
Result: after 4-5 days disorders of central nervous
system: excitation, ataxia, tonic-clonic
convulsions; after 3-5 days brain damage was
demonstrable histologically (necrosis of
parenchymal cells of the stratum granulosum of
the fascia dentata and the cortex of the lobus
piriformis)

15-JAN-2001 (109)

Species: rat Sex: male/female
Strain: Wistar
Route of administration: oral unspecified
Exposure period: 72 weeks
Frequency of treatment: once daily
Post exposure period: no
Doses: 40 mg benzoic acid/kg/day and 80 mg
sodium bisulphite/kg/day
Control Group: yes

Test substance: other TS: analytical grade

Remark: 50 rats/sex; initial body weight: 100-120 g;
test

Result: reduced weight gain, kidney function and the
reaction on stress factors were altered (no
further information); the erythrocyte
sedimentation rate was increased

14-AUG-2001

(108)

Species: rat Sex: male/female

Strain: Wistar

Route of administration: oral unspecified

Exposure period: 72 weeks

Frequency of treatment: once daily

Post exposure period: no data

Doses: 40 mg/kg/day

Control Group: yes

Remark: 10 rats/sex; initial body weight: 100-120 g;
test substance: analytical grade

Result: the rats developed some tolerance to a single
add. application of 4000 mg sodium benzoate/kg
given terminally, the mortality rate was 25 %

15-JAN-2001

(108)

Species: mouse Sex: male/female

Strain: no data

Route of administration: gavage

Exposure period: 12 weeks

Frequency of treatment: once daily

Post exposure period: no

Doses: 80 mg benzoic acid/kg/day and 160 mg
sodium bisulphite/kg/day

Control Group: yes

Remark: 100 mice/group; initial body weight: 8-10 g;
test substance: analytical grade

Result: reduced weight gain without reduced food
intake; mortality rate at week 10: 70 % in
males and 62 % in females

15-JAN-2001

(108)

Species: mouse Sex: male/female

Strain: no data

Route of administration: oral unspecified

Exposure period: 68 weeks

5. TOXICITY

DATE: 14-FEB.-2002
SUBSTANCES ID: 65-85-0

Frequency of treatment: once daily
Post exposure period: no data
Doses: 40 mg/kg/day
Control Group: yes

Remark: 25 mice/sex (initial body weight 10-15 g) or
25 mice/sex (initial body weight 16-20 g) were
tested; test substance: analytical grade
Result: no effects were reported

15-JAN-2001 (108)

Species: mouse Sex: male/female
Strain: no data
Route of administration: oral unspecified
Exposure period: 68 weeks
Frequency of treatment: once daily
Post exposure period: no data
Doses: 40 mg benzoic acid/kg/day and 80 mg
sodium
bisulphite/kg/day
Control Group: yes

Remark: 25 mice/sex (initial body weight 10-15 g) or
25 mice/sex (initial body weight 16-20 g) were
tested; test substance: analytical grade

Result: reduced weight gain without reduced food
intake; mortality rate at week 32: 56-65 % in
males and 45-72 % in females

15-JAN-2001 (108)

Species: cat Sex: male
Strain: no data
Route of administration: oral feed
Exposure period: 15 days
Frequency of treatment: continuously in diet
Post exposure period: no data
Doses: 100 or 200 mg/kg/day
Control Group: yes

Remark: 4 cats/group were tested; initial body weight:
1.7-2.27 kg

Result: no effects were observed
15-JAN-2001 (111)

Species: cat Sex: male
Strain: no data

Route of administration: oral feed
Exposure period: 3-4 days
Frequency of treatment: continuously in diet
Post exposure period: no data
Doses: 0.5 % in diet (approx. 300-420 mg/kg/day)
Control Group: yes

Remark: 4 cats were tested; initial body weight:
1.42-2.0 kg
Result: convulsions, hyperaesthesia, apprehension,
swollen hepatocytes with infiltrations of
macrophages and fibroblasts, swollen kidney
tubules, no pathological findings in brain and
spinal cord; mortality: 2/4

15-JAN-2001 (111)

Species: cat Sex: male
Strain: no data
Route of administration: oral feed
Exposure period: 23 days
Frequency of treatment: continuously in diet
Post exposure period: no data

Doses: 0.25 % in diet (approx. 130-160
mg/kg/day)
Control Group: yes

Remark: 4 cats were tested; initial body weight: 3.2-
4.0 kg
Result: no effects were observed

15-JAN-2001 (111)

5.5 Genetic Toxicity 'in Vitro'

Type: Salmonella typhimurium reverse mutation
assay
System of testing: TA 98, TA100, TA 1535, TA1537, TA1538
Concentration: 0, 20, 100, 500, 1000, 2000ug/plate
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 471
Year: 1983
GLP: no data
Test substance: other TS: technical grade benzoic acid

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint
14-FEB-2002 (112)

Type: other: Sister chromatid exchange
System of testing: human lymphocytes
Concentration: 0 to 2.0 mM
Cytotoxic Concentration: no data
Metabolic activation: without
Result: negative

Method: other: similar to OECD Guide-line 479
Year: 1986
Test substance: other TS: benzoic acid, purity = 99%
(estimated by NMR)

Reliability: (2) valid with restrictions
Comparable to Guideline study with acceptable
restrictions

Flag: Critical study for SIDS endpoint

14-AUG-2001 (113)

Type: other: Sister chromatid exchange
System of testing: human lymphoblastoid cells transformed by
Epstein- Barr virus (NL2, NL3, NL4)

Concentration: 0.001, 0.003, 0.01, 0.03 M
Cytotoxic Concentration: 0.03 M
Metabolic activation: without
Result: negative

Method: OECD Guide-line 479
Year: 1986
Test substance: other TS: benzoic acid purchased from Kanto
Chemical Co., Tokyo, Japan

Test condition: Test done only without metabolic activation.

Reliability: (2) valid with restrictions
Guideline study with acceptable restrictions

Flag: Critical study for SIDS endpoint
14-AUG-2001 (114)

Type: other: Chromosomal aberration test
System of testing: Chinese hamster fibroblast cell line
(CHL)
Concentration: up to 10 mg/plate
Metabolic activation: without
Result: ambiguous

Test substance: other TS: benzoic acid, >99% pure

Method: The study was carried out using a Chinese Hamster fibroblast cell line (CHL) which were exposed to the test substance at one of three dose levels for 24 and 48 hr. No metabolic activation systems were applied. Chromosome preparations were made following treatment with Colcemid. A hundred well-spread metaphases were observed per plate and the incidence of polyploid cells and cells with chromosome aberrations was recorded.

Result: At 48 hr, there was an incidence of 1% polyploid cells and 8% cells with structural aberration (incidence between 5-9.9% is considered equivocal).

Reliability: (2) valid with restrictions

Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-FEB-2002 (115)

Type: Bacillus subtilis recombination assay

System of testing: Bacillus subtilis H17, M45
Metabolic activation: no data
Result: positive

Test substance: no data

Method: An overnight culture of B. subtilis, H17 and M45, was mixed with test solutions and incubated for 30 minutes at 37 degree C. After treatment viable cells were counted and the ratio of 50% survival concentrations were calculated.

Result: Benzoic acid showed DNA damaging potential although it had been negative in the Ames test.

Reliability: (4) not assignable
insufficient documentation (abstract only)

Flag: Critical study for SIDS endpoint
06-JUN-2001 (116)

Type: other: Salmonella microsome assay
System of testing: S. typhimurium TA 98, TA 100, TA 1535, TA 1536, TA 1537, TA 1538
Metabolic activation: with and without
Result: negative

Remark: insufficient documentation
12-JAN-2001 (117)

Type:	other: Salmonella microsome assay
System of testing:	S. typhimurium TA 97, TA 98, TA 100, TA 1535, TA 1537
Metabolic activation:	with and without
Result:	negative
12-JAN-2001	(118)
Type:	other: Salmonella microsome assay
System of testing:	S. typhimurium TA 98, TA 100, TA 1535, TA 1537
Metabolic activation:	with and without
Result:	negative
11-JAN-2001	(119)
Type:	other: Mitotic recombination
System of testing:	Saccharomyces cerevisiae D3
Metabolic activation:	with and without
Result:	negative
Remark:	insufficient documentation
11-JAN-2001	(117)
Type:	other: Chromosomal aberration test
System of testing:	Chinese hamster fibroblast cell line (CHL)
Metabolic activation:	without
Result:	ambiguous
11-JAN-2001	(120)
Type:	other: umu test
System of testing:	S. typhimurium TA 1535/pSK1002
Metabolic activation:	with and without
Result:	negative
11-JAN-2001	(121)

5.6 Genetic Toxicity 'in Vivo'

Remark: See IUCLID data set on sodium benzoate (CAS# 532-32-1).
Data on sodium benzoate reveal no in vivo genotoxicity.
Therefore no in vivo genotoxicity study for benzoic acid is indicated.

14-FEB-2002

5.7 Carcinogenicity

Remark: See IUCLID data set on sodium benzoate (CAS# 532-32-1).
Data on sodium benzoate reveal no in vivo genotoxicity.
Therefore no in vivo genotoxicity study for benzoic acid is indicated.

Flag: Critical study for SIDS endpoint
14-FEB-2002

5.8.1 Toxicity to Fertility

Type: other: 4 generation study
Species: rat
Sex: male/female

Strain: no data
Route of administration: other: oral feed (first 8 weeks paired feed technique; afterwards ad libitum)

Exposure Period: generation 1 and 2: lifelong;
generation 3: 16 weeks;
generation 4: until breeding

Frequency of treatment: continuously in diet

Doses: 0.5 or 1 % in diet (approx. 375 or 750 mg/kg/day)

Control Group: yes
NOAEL Parental: ≥ 750 mg/kg bw
NOAEL F1 Offspring: ≥ 750 mg/kg bw
NOAEL F2 Offspring: ≥ 750 mg/kg bw

Year: 1960
GLP: no

Test substance: other TS: benzoic acid, purity not noted

Method: A robust protocol, according to standards at that time, was used. Taking into account the reputation of the investigators a high quality has to be assumed.

Remark: 40 (20 M = 20 F) rats/group; initial body weight: 40-50 g.
The mean compound consumption was calculated according to Lehman, A.J., Assoc. Food Drug Off. Q. Bull. 18, 66 (1954).

Result: In all 4 generations no influence on growth (weight, weight gain and food efficiency (measured by protein efficiency)) and organ

weights was found.

In all 4 generations, no effects on fertility ("Forzplanzung") and lactation ("Aufzugt der Jungen") was found. The animals of the 3rd generation were sacrificed and examined histopathologically after 16 weeks (after lactation of the pups.) No remarkable histopathological findings were found.

In the paper no information is given on the organs investigated, however the robustness of the total study, the reputation of the investigators, as well as the reputation of the Professor who did the histopathologic investigation, a high quality has to be assumed. From other parameters it can be assumed that as a minimum the brains, heart, liver, kidney, testis and were examined.

Feeding of 0.5 % led to prolongation of survival compared to controls. In addition a so-called "Alters Paarung" after 48 weeks gave no influence on start of menopause.

Reliability: (2) valid with restrictions

Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint

14-AUG-2001

(105)

5.8.2 Developmental Toxicity/Teratogenicity

Species:	rat	Sex:
female		
Strain:	Wistar	
Route of administration:	gavage	
Exposure period:	single application	
Frequency of treatment:	at day 9 of gestation	
Duration of test:	20 days	
Doses:	510 mg/kg	
Control Group:	no	
NOAEL Maternal Toxicity:	510 mg/kg bw	
NOAEL Teratogenicity:	510 mg/kg bw	

Method: other: Kimmel et al. (1971)

GLP: no data

Test substance: other TS: benzoic acid, purity not noted

Method: Pregnant Wistar rats were treated on day 9 of gestation with one dose of benzoic acid in carboxymethylcellulose. Animals were sacrificed on day 20 of gestation and the uterus observed in

situ for implantation and resorption sites. Live fetuses were removed, examined for gross malformations, weighed, and prepared for histological examination. Skeletal examination was carried out under low magnification.

Remark: Group I was dosed with 510 mg/kg.
Group II was dosed with 510 mg/kg; then 2 h later: 250 or 500 mg/kg acetylsalicylic acid

Result: Treatment with benzoic acid alone resulted in no dead or resorbed implants and 3 % abnormal survivors, rates comparable to the control animals.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (122)

Species: rat Sex: male/female
Strain: no data
Route of administration: other: oral feed (first 8 weekspaired feed technique;
Exposure period: generation 1 and 2: lifelong;
generation 3: 16 weeks;
Frequency of treatment: continuously in diet
Duration of test: lifelong
Doses: 0.5 or 1 % in diet (approx. 375 or 750 mg/kg/day)
Control Group: yes
NOAEL Maternal Toxicity: >= 750 mg/kg bw
NOAEL Teratogenicity: 750 mg/kg bw

Year: 1960

Test substance: other TS: benzoic acid, purity not noted

Method: A robust protocol, according to standards at that time, was used. Taking into account the reputation of the investigators a high quality has to be assumed.

Remark: The mean compound consumption was calculated according to Lehman, A.J., Assoc. Food Drug Off. Q. Bull. 18, 66 (1954).

Result: The study demonstrated no effects on the dams or on the growth and development of the offspring.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,

Flag: well documented and acceptable for assessment
Critical study for SIDS endpoint
14-FEB-2002 (105)

Remark: See IUCLID data set on sodium benzoate
(CAS# 532-32-1).
Data on sodium benzoate reveal no in vivo
genotoxicity.
Therefore no in vivo genotoxicity study for
benzoic acid is indicated.

14-FEB-2002

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

Remark: Single oral doses of 1-1.5 g resulted in
dyspepsia, Nausea and vomiting.
23-OCT-1995 (123)

Remark: A systemic inhibitory effect of UV light
(UVA and UVB) on non-immunologic immediate
contact reactions to benzoic acid was found
in healthy volunteers.
23-OCT-1995 (124)

Remark: Effects of infra-red and laser irradiation
were studied on non-immunologic immediate
contact reactions to benzoic acid.
The strength of the contact urticaria was
increased.
23-OCT-1995 (125)

Remark: Daily oral doses of benzoic acid of < 0.5 g
or sometimes up to 4 g/d did not induce
adverse effects in man.
23-OCT-1995 (126)

Remark: Metabolism in humans:
Percutaneous absorption of ¹⁴C-labelled
benzoic acid (4 ug/cm²; area: 2.5 cm²) was
lower in aged subjects (> 65 years) than in
young (18-40 years): cumulative dose
absorbed within 7 days was 19.5 vs. 36.2 %.

The diminished surface lipid content of old skin implies a diminished dissolution medium.

23-OCT-1995 (127)

5.11 Additional Remarks

Type: Metabolism

Remark: The transdermal absorption of benzoic acid was studied in excised human skin and compared to absorption in living man. In equivalent time, the total absorption (% of applied dose) was 42.6 % (in vivo) or 44.9 % (in vitro).

15-JAN-2001 (128)

Type: Metabolism

Remark: The percutaneous absorption and the excretion of benzoic acid were tested in female weanling yorkshire swine (approx.20 kg) after topical and intravenous administration. After i.v. injection of 200 ug (10 uCi)/pig 84.5 % of 14C-activity were excreted with urine and 4.6 % in faeces within 6 days; the radiolabel recovery in carcass was 0.1 %. After topical application of the same dose the radiolabel recovery within 6 days (% of applied dose) was in urine 20 %, faeces 2.9 %, carcass 0.8 %, border 40.2 %, dosed skin 12.2 % and adjacent skin 9.1 %.

23-OCT-1995 (129)

Type: Metabolism

Remark: A concentration of 4 ug/cmE+2 of 14C-labelled benzoic acid was applied to the shaved backs of guinea pigs. The percutaneous absorption was determined from urinary and fecal excretion. Absorption of benzoic acid was similar to published human absorption data (no further information). The percutaneous absorption of 14C-labelled benzoic acid was studied in the Mexican hairless dog and compared to human data. Total absorption and maximum absorption rates were greater in humans than in hairless dogs. Surface counting experiments showed that benzoic acid persisted on the dog skin far

longer than on human skin (no further information). The percutaneous absorption of increasing topical doses of benzoic acid was determined in the Rhesus monkey and humans (dosage: 4, 40, 2000 ug/cmE+2; dose absorbed: monkey 59.2 %, 3.6 %, 17.4 %; human 42.6 %, 25.7 %, 14.4 %). In vivo percutaneous absorption was similar, also the dose-response curve was similar in the two species (no further information).

23-OCT-1995 (130)

Type: Metabolism

Remark: Damaging the skin (tape stripping, irritation, delipidization) increased absorption of benzoic acid dissolved in acetone (200 ug/ml, 50 uCi; topical application: 4 ug/cm2) in hairless guinea pigs: 71.1/73.4/94.1 % vs. 34.2 % absorbed in the group with intact skin.

23-OCT-1995 (131)

Type: Metabolism

Remark: The effect of topical application of benzoic acid on the in vivo percutaneous absorption was tested in 4 rhesus monkeys. Daily applications of 4 ug/cmE+2 were given for 14 days, the 1st and the 8th application used 14C-labelled test substance. To quantify absorption, urine was collected and assayed for radioactivity. The penetration results are expressed as the percentage of the applied dose absorbed, i.e. (% of topical dose eliminated in urine / % of i.v. dose eliminated in urine)*100. After 1st dose 85 % and after 8th dose 89 % were found. No significant change in percutaneous absorption from that following the initial dose was observed following the 8th dose of a multidose regimen.

23-OCT-1995 (132)

Type: Metabolism

Remark: In vitro, the permeation of benzoic acid was measured across isolated stratum corneum, stratum corneum and epidermis, and split-thickness skin. The stratum corneum was shown

- to be the rate limiting barrier and the flux was proportional to the concentration of the undissociated compound.
- 23-OCT-1995 (133)
- Type: Metabolism
- Remark: The percutaneous absorption and metabolism of benzoic acid was determined through hairless guinea pig skin in vitro. The absorption within 48 h was greater through nonviable skin (60.1 % of applied dose) than through viable skin(49.5%). 6.9 % of absorbed dose (2 ug/cm2) were conjugated with glycine to form hippuric acid.
- 23-OCT-1995 (134)
- Type: Metabolism
- Remark: After s.c. administration of radiolabelled benzoic acid to maternal rats it was found, that the acidic compound penetrated the placental barrier readily. The fetal t1/2 values were in general lower than those for the corresponding maternal tissues. The fetal blood-brain barrier was penetrated more readily than the adult one for the tested compound.
- 14-AUG-2001 (135)
- Remark: After a single i.p. injection of 410 umol 14C-labelled benzoic acid/kg to female Wistar rats 90 % of the applied 14C-activity was excreted in urine and 1.3 % in bile within 3 hours, mainly as hippuric acid. After 24 hours the excretion was approx. 100 %.
- 23-OCT-1995 (136)
- Remark: Benzoic acid is detoxicated by some mammalian species mainly by conjugation with glycine to form hippuric acid. There is a marked species difference in the efficiency of the process. After an oral dose of 50 mg 14C-benzoic acid most species excreted 50-100 % of radioactivity in the urine within 24 hours. In the turtle and gecko excretion was slower (39 % in 3 days).

In herbivorous and omnivorous species (rhesus, squirrel and capuchin monkeys, pig, rabbit, rat, mouse, guinea pig, hamster, lemming, gerbil) benzoic acid was excreted in the urine almost entirely as hippuric acid, though 10-20 % of the total ¹⁴C-activity appeared as free benzoic acid in pigs and squirrel monkeys within 24 hours, possibly as a result of the decomposition of benzoyl glucuronide. In the 2 men given 1 mg benzoic acid/kg, almost all the urinary metabolite was hippuric acid, with 97 % of the radioactivity excreted within 4 hours and virtually 100 % within 12 hours.

In the carnivorous animals tested (dog, cat, ferret) the main metabolite was hippuric acid, with the dog and ferret excreting also some benzoyl glucuronide. In the hedgehog, an insectivore, a similar excretion occurred. The Indian fruit bat (*Pteroptus gigantus*) excreted 70-80 % of benzoic acid as the glucuronide and the remainder as free acid within 24 hours. The pigeon excreted mainly hippuric acid and in the chick, turtle and gecko the major metabolite was ornithuric acid. When the dose of benzoic acid in the ferret was raised to 200 and 400 mg/kg, the proportion excreted as glucuronide was markedly increased. During the metabolism of benzoic acid, the relative amount of conjugation with glycine and with glucuronic acid varies from species to species and may depend to some extent upon the magnitude of the dose.

14-AUG-2001

(137)

Remark:

In many species, benzoic acid is rapidly absorbed, conjugated with glycine and excreted as hippuric acid. There appears to be no accumulation of benzoic acid at low doses, but one limiting factor in the biosynthesis of hippuric acid is the availability of glycine: once the glycine pool is exhausted (after application of high doses), an additional metabolite, benzoyl glucuronide, is excreted in the urine of some species (no further information available).

14-AUG-2001

(138)

Remark: 5 days after i.p. injection of 1 ml (4 ug) labeled benzoic acid in saline to female hairless guinea pigs, 92.1 % of the administered dose was excreted in urine.

14-AUG-2001 (131)

Remark: In most animals, the conversion of benzoic acid to hippuric acid has been found to occur in kidney, with conversion possible in the liver when kidney malfunction exists. The monkey metabolized benzoic acid only in the liver (no further information available).

14-AUG-2001 (139)

Remark: After a single i.v. injection of 2.0 to 2.2 mg ¹³C-labelled benzoic acid/kg to male Wistar rats 85 - 99 % of the applied ¹³C-activity was excreted as hippuric acid in urine within 120 minutes after application.

14-AUG-2001 (140)

Remark: In an in vitro study, the nitrosation of methylurea to form N-nitrosomethylurea by benzoic acid at a concentration of 10, 50 or 100 mM was not reduced (101, 108 or 102-110 % compared to control).

14-AUG-2001 (141)

Remark: Regional differences in percutaneous absorption of benzoic acid were tested in vitro (face, abdomen, back, forearm, thigh, lower leg, dorsal foot, dorsal hand, palm and sole).
A trend of increasing permeability from truncal to acral sites was observed (exception: palmar/plantar skin).

23-OCT-1995 (142)

Remark: Benzoic acid was positive in the microsomal degranulation assay, if microsomes were prepared at low 'g' force (10000).

In the test with rough endoplasmatic reticulum prepared at high 'g' forces (≥ 105000) it was negative. The degranulation assay tests the ability of a chemical to dissociate polysomes and ribosomes from the endoplasmatic reticulum.

14-AUG-2001 (143)

Remark: Benzoic acid (purity 99,9 %; 2 % solution in phosphate buffered saline) was administered i.v. (jugular catheter) to two male F 344 rats at approx. 2 mg/l for a total dose of 108 mg. The substance caused no neuroexcitation.

14-AUG-2001 (144)

Remark: The application of benzoic acid (1 % in diet [approx. 450-890 mg/kg/d]) for 1 day to 4 male cats (initial body weight: 1.06- 1.70 kg) resulted in convulsions, aggression, hyperaesthesia, swollen hepatic cells with centrilobular vacuolation, infiltration of inflammatory cells, and marked distension of the kidney glomeruli. No pathological findings in brain and spinal cord. Mortality: 1/4 control group: yes

14-AUG-2001 (111)

Remark: Other: In a screening with COMPACT (computer-optimized molecular parametric analysis of chemical toxicity) benzoic acid was predicted as a potential substrate for cytochrome P450 IIE.

14-AUG-2001 (145)

6.1 Analytical Methods**6.2 Detection and Identification**

7.1 Function

7.2 Effects on Organisms to be Controlled

7.3 Organisms to be Protected

7.4 User

7.5 Resistance

8.1 Methods Handling and Storing**8.2 Fire Guidance****8.3 Emergency Measures****8.4 Possib. of Rendering Subst. Harmless****8.5 Waste Management****8.6 Side-effects Detection****8.7 Substance Registered as Dangerous for Ground Water****8.8 Reactivity Towards Container Material**

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10.1 End Point Summary**10.2 Hazard Summary****10.3 Risk Assessment**

I U C L I D D a t a S e t

(SODIUM BENZOATE: CAS N°: 532-32-1)

Existing Chemical ID: 532-32-1
CAS No. 532-32-1
EINECS Name sodium benzoate
EINECS No. 208-534-8
TSCA Name Benzoic acid, sodium salt
Molecular Formula C7H6O2.Na

Producer Related Part

Company: Bayer Corporation
Creation date: 21-OCT-1999

Substance Related Part

Company: Bayer Corporation
Creation date: 21-OCT-1999

Memo: Bayer Corporation

Printing date: 10-AUG-2001
Revision date:
Date of last Update: 10-AUG-2001

Number of Pages: 68

Chapter (profile): Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1. GENERAL INFORMATION

DATE: 10-AUG-2001
SUBSTANCE ID: 532-32-1**1.0.1 OECD and Company Information**

Type: lead organisation
Name: American Chemistry Council (formerly Chemical Manufacturers Association), Benzoates HPV Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States

09-AUG-2001

Type: cooperating company
Name: ATOFINA Chemicals, Inc.
Country: United States

09-AUG-2001

Type: cooperating company
Name: Bayer Corporation
Country: United States

09-AUG-2001

Type: cooperating company
Name: DSM Fine Chemicals
Country: Netherlands

03-JAN-2001

Type: cooperating company
Name: Noveon, Inc.
Country: United States

09-AUG-2001

Type: cooperating company
Name: Velsicol Chemical Corporation
Country: United States

26-MAY-2000

1.0.2 Location of Production Site**1.0.3 Identity of Recipients**

1.1 General Substance Information**1.1.0 Details on Template****1.1.1 Spectra****1.2 Synonyms****1.3 Impurities****1.4 Additives****1.5 Quantity****1.6.1 Labelling****1.6.2 Classification****1.7 Use Pattern****1.7.1 Technology Production/Use****1.8 Occupational Exposure Limit Values****1.9 Source of Exposure****1.10.1 Recommendations/Precautionary Measures****1.10.2 Emergency Measures****1.11 Packaging****1.12 Possib. of Rendering Subst. Harmless****1.13 Statements Concerning Waste**

1.14.1 Water Pollution**1.14.2 Major Accident Hazards**

Legislation:
Substance listed:
10-JUL-2000

1.14.3 Air Pollution

Classified by:
Labelled by:
Number:
Class of danger:
10-JUL-2000

1.15 Additional Remarks**1.16 Last Literature Search**

Type of Search: Internal and External
Date of Search: 07-SEP-1999

Remark: Only HPV endpoints: TOXLINE data base and
internal studies.
09-AUG-2001

1.17 Reviews**1.18 Listings e.g. Chemical Inventories**

2.1 Melting Point

Value: > 300 degree C
Method: other: measured
Remark: Carbonisation at temperature > 500 degree C
Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
09-AUG-2001 (1) (2)

Value: 330.6 degree C
Method: other: (calculated) MPBPWIN (v1.31) Program;
Adapted Joback Method
Year: 1999
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
09-AUG-2001 (3)

Value: 410 - 430 degree C
Method: other
Remark: DSM datasheet.
26-JAN-2001

2.2 Boiling Point

Value: 464.9 degree C
Method: other: (calculated) MPBPWIN (v1.31) Program ;
Adapted Stein and Brown Method
Year: 1999
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
09-AUG-2001 (3)

2.3 Density

Type: relative density
Value: = 1.44 g/cm3
Flag: Critical study for SIDS endpoint
26-JAN-2001 (4) (5)

Type: bulk density
Value: 650 kg/m3
Remark: thickened
Source: DSM Special Products B.V. GeleenECB -
Existing Chemicals Ispra (VA)
26-MAY-2000 (6)

Type: bulk density
Value: 350 kg/m3
Remark: not thickened
Source: DSM Special Products B.V. GeleenECB -
Existing Chemicals Ispra (VA)
26-MAY-2000 (6)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: .00000000489 hPa at 25 degree C
Method: other (calculated): MPBPWIN (v1.31) Program;
Modified Grain Method
Year: 1999
Testsubstance: other TS: molecular structure
Result: 3.67E-009 mm Hg; 4.89E-09 hPa
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
09-AUG-2001 (3)

2.5 Partition Coefficient

log Pow: -2.269
Method: other (calculated): Log Kow(version 1.65
estimate)
Year: 1999
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
09-AUG-2001 (7)

log Pow: = -2.13
Method: other (calculated): CLogP
Year:
Testsubstance: other TS: molecular structure

Remark: Calculated according to C. Hansch et al 1985.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
23-MAR-2001

2.6.1 Water Solubility

Value: 556 g/l at 20 degree C
Method: other
Remark: pH-value: about 8.
Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
09-AUG-2001 (8) (9)

Value: 630 g/l at 20 degree C
pH: 7
26-JAN-2001 (6) (6)

Remark: concentrated solutions react neutral
diluted solutions react weakly alkaline (pH 8)
26-JAN-2001 (10)

2.6.2 Surface Tension

2.7 Flash Point

Value: > 100 degree C
Type: closed cup
Method: other: DIN 51758
Year:
Reliability: (1) valid without restriction
Meets National standards method (AFNOR/DIN)
09-AUG-2001 (6)

2.8 Auto Flammability

2.9 Flammability

2.10 Explosive Properties

Result:
Remark: Can form explosive mixtures with air.
09-AUG-2001

2.11 Oxidizing Properties**2.12 Additional Remarks**

Remark: At a rel. humidity of > 50% the salt is
hygroscopic and it dissolves at r. F.-values >
85 %

23-OCT-1995 (10)

Remark: UV spectrum lambda max (nm):
225 (water; lg epsilon: n.a.)

23-OCT-1995 (11)

Remark: pH value ca. 7.5 at 10 g/l water

23-OCT-1995 (6)

3.1.1 Photodegradation

Type: air
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1560000 molecule/cm3
Rate constant: .00000000000017775 cm3/(molecule * sec)
Degradation: 50 % after 72.2 hour(s)
Method: other (calculated): AOP Program (v1.89)
Year: 1999 GLP:
Test substance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
09-AUG-2001 (3)

Type:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on benzoic acid (CAS# 65-85-0); the photodegradation of the sodium salt should be similar.
09-AUG-2001

3.1.2 Stability in Water

Type:
Method:
Year: GLP:
Test substance:
Remark: Based on structure and organic chemistry rules (e.g. bonding in organic molecules, activation energy, reactivity, transformations, addition, substitution, elimination) no hydrolysis will occur at pH ranges 4 - 11.
Flag: Critical study for SIDS endpoint
26-JAN-2001

3.1.3 Stability in Soil

3.2 Monitoring Data (Environment)

Type of

measurement:

Medium:

Method:

Concentration

Remark:

See IUCLID on benzoic acid (CAS# 65-85-0); the data on the sodium salt should be similar.

09-AUG-2001

3.3.1 Transport between Environmental Compartments

Type:

fugacity model level III

Media:

other: air - water - soil - sediment

Air (Level I):

Water (Level I):

Soil (Level I):

Biota (L.II/III):

Soil (L.II/III):

Method:

other: EPIWin Modeling Program

Year: 1999

Result:

	Distribution (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	1.45e-007	144	1000	4.83e-019
Water	45.3	360	1000	1.38e-020
Soil	54.6	360	1000	6.16e-019
Sediment	0.0755	1.44e+003	0	1.15e-020

Persistence Time: 421 hr

Reaction Time: 520 hr

Advection Time: 2.21e+003 hr

Percent Reacted: 80.9

Percent Advected: 19.1

Reliability:

(2) valid with restrictions

Accepted calculation method

Flag:

Critical study for SIDS endpoint

09-AUG-2001

(12)

3.3.2 Distribution**3.4 Mode of Degradation in Actual Use**

Remark:

In many species benzoic acid sodium salt is rapidly absorbed and rapidly metabolized namely conjugated with glycine and excreted as hippuric acid in the urine.

The substance is readily biodegradable, and is biodegraded within chemical industry via a waste water treatment plant.

09-AUG-2001

3.5 Biodegradation

Type: aerobic
 Inoculum: activated sludge, domestic
 Concentration: 50 mg/l related to Test substance
 Degradation: ca. 90 % after 7 day
 Result: readily biodegradable
 Method: OECD Guide-line 301 B "Ready Biodegradability:
 Modified Sturm

Test (CO₂ evolution)"
 Year: 1981 GLP: no data
 Test substance: other TS: sodium benzoate, purity not noted
 Remark: Sodium benzoate is the recommended "readily biodegradable reference substance" for OECD Guideline studies.
 This endpoint has been studied several times by several other investigators/groups and all support the result of the study mentioned above.

Test condition: 25 degree C
 Reliability: (1) valid without restriction
 Guideline study
 Flag: Critical study for SIDS endpoint

09-AUG-2001

(13) (14)

Type: anaerobic
 Inoculum: other bacteria: anaerobic sewage, domestic and industrial
 Concentration: 50 mg/l related to DOC (Dissolved Organic Carbon)
 Degradation: 93 % 7.5 after 7 day
 Method: other: see below
 Year: GLP: no data
 Test substance: other TS: technical grade sodium benzoate purchased from Aldrich Chemical Co. , UK

Method: 2-3 g sludge plus sodium benzoate (concentration equivalent to 50 mg Carbon/liter or 85 mg substance/l). Controls and tests done in triplicate. Temperature = 35 degree C. Measured gas production (CH₄ + CO₂).

3. ENVIRONMENTAL FATE AND PATHWAYS

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Remark: retard lag 2 d
 Result: Degradation is expressed as percentage of theoretical methane production based on the stoichiometry of degradation.
 Reliability: (2) valid with restrictions
 Meets generally accepted scientific standards, well documented and acceptable for assessment
 Flag: Critical study for SIDS endpoint
 09-AUG-2001 (15)

Type: aerobic
 Inoculum: other: suspension from marine aquarium filters
 Concentration: 10 mg/l related to DOC (Dissolved Organic Carbon)
 Degradation: > 97 % after 28 day
 Result: readily biodegradable
 Testsubstance: 2 day 20 %
 4 day 45 %
 6 day 55 %
 8 day 70 %
 20 day 85 %
 Method: OECD Guide-line 301 B "Ready Biodegradability:
 Modified Sturm Test (CO₂ evolution)"
 Year: 1981 GLP:
 Test substance:
 Method: Guideline adapted to use seawater as test medium and inoculum
 Reliability: (1) valid without restriction
 03-JAN-2001 (16)

Type: anaerobic
 Inoculum: other bacteria: anaerobic sewage, domestic, 2 weeks preincubated
 Concentration: related to Test substance
 Method: other: anaerobic degradation, static, 35 degree C, parameter: gas production
 Year: GLP:
 Test substance:
 Remark: concentration: 50/60/90 mg/l
 degradation : 47/49/28 d = 60.5/82.7/74 %
 26-JAN-2001 (17)

Type: aerobic
 Inoculum: domestic sewage, non-adapted
 Contact time: 28 day
 Degradation: 84 % after 14 day
 Result: readily biodegradable

3. ENVIRONMENTAL FATE AND PATHWAYS

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Test substance:	14 day	84 %
	28 day	92 %
Method:	Directive 84/449/EEC, C.7 "Biotic degradation - modified MITI test"	
Year:	1982	GLP:
Test substance:	other TS: purchased from Sigma Chemicals	
Reliability:	(1) valid without restriction	
19-MAY-2000		(17)

Type:	aerobic	
Inoculum:	other: microorganisms already present in seawater	
Concentration:	11.6 mg/l related to DOC (Dissolved Organic Carbon)	
Contact time:	61 day	
Degradation:	80.5 % after 20 day	
Result:	readily biodegradable	
Testsubstance:	5 day	57.4 %
	10 day	72.8 %
	30 day	83.4 %
	50 day	91.7 %
	61 day	96.4 %

Method: OECD Guide-line 301 A (new version) "Ready
Biodegradability: DOC Die Away Test"
Year:
GLP:
Test substance: no data
09-MAY-2000

Type:	aerobic	
Inoculum:		
Degradation:	100 % after 28 day	
Result:	readily biodegradable	
Method:	OECD Guide-line 301 D	"Ready Biodegradability:
Closed Bottle	Test"	
Year:		GLP:
Test substance:	no data	
09-MAY-2000		(20)

Type:	
Inoculum:	activated sludge, non-adapted
Concentration:	100 mg/l related to Test substance
Degradation:	84 % after 10 day
Method:	Directive 84/449/EEC, C.7 "Biotic degradation - modified MITI test"
Year:	
GLP:	
Test substance:	

3. ENVIRONMENTAL FATE AND PATHWAYS

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Remark: degradation after 10 d: 64 - 98 % (n=14)
 after 28 d: 75 - 111 % (n=14)
 0 d lag phase EG-Ringtest 1981-82
 26-JAN-2001 (21)

Type:
 Inoculum:
 Degradation: 88 % after 28
 Test substance: 60 day 95 %
 Method: OECD Guide-line 301 A (new version) "Ready
 Biodegradability: DOC Die Away Test"
 Year: GLP:
 Test substance: no data
 09-MAY-2000 (20)

Type:
 Inoculum: other bacteria: purification plant outflow
 mixed with a soil suspension
 Concentration: 5 mg/l related to Test substance
 Method: other: Respirometer-Test (Closed Bottle Test)
 Year: GLP:
 Test substance:
 Remark: degradation after 30 d: 75 - 111 % ThSB
 54-89 %: Medium without NH₄ Cl
 71-130%: Medium with NH₄ Cl
 26-JAN-2001 (22)

Type:
 Inoculum: other bacteria: anaerobic laboratory-sewage,
 adapted
 Concentration: 300 mg/l related to Test substance
 Degradation: 98 % after 4 day
 Method: other: anaerobic degradation, static
 Year: GLP:
 Test substance:
 Remark: parameter: gasproduction
 Test condition: 35 degree, enrichment culture
 26-JAN-2001 (23)

Type:
 Inoculum: other bacteria: anaerobic sewage, domestic,
 washed
 Concentration: 50 mg/l related to Test substance
 Degradation: 49.8 % after 61 day
 Method: other: anaerobic degradation, static,
 35 degree C, parameter: gasproduction
 Year: GLP:
 Test substance:

Remark: concentration: 60/60 mg/l
degradation : 35/56 d = 95.3/96.5 %
26-JAN-2001 (17)

Type:
Inoculum: other bacteria: methanogenic sewage laboratory
culture, benzoate-adapted
Concentration: 3000 mg/l related to Test substance
Degradation: ca. 99 % after 5 day
Method: other: anaerobic degradation, static,
37 degree C, analytical control of
concentration, pH 6.7-6.9
Year: GLP:
Test substance:
26-JAN-2001 (24)

Type:
Inoculum: other bacteria: anaerobic sewage from a
purification plant of woodmanufacturing
industry, benzoate-adapted
Concentration: 307 mg/l related to Test substance
Degradation: ca. 99 % after 2 day
Method: other: anaerobic degradation, static,
analytical control of concentration
Year: GLP:
Test substance:
Remark: Original data of concentration: 2.13 mM
Test condition: 37 degree C
26-JAN-2001 (24)

Type:
Inoculum: other bacteria: anaerobic enrichment culture
(fen), adapted
Concentration: 2306 mg/l related to Test substance
Degradation: 100 % after 4 day
Method: other: anaerobic degradation, static,
parameter: gas production by GC, 39degree C,
pH 6.7
Year: GLP:
Test substance:
26-JAN-2001 (25)

3.6 BOD5, COD or BOD5/COD Ratio

Remark: No data.
09-AUG-2001

3.7 Bioaccumulation

Species:
Exposure period:
Concentration:
BCF: 3.16
Elimination:
Method: other: (calculated) BCF Program (v2.13)
Year: 1999 GLP:
Test substance: other TS: molecular structure
Remark: Based on the log P and its rapid
metabolization and excretion in many species
no bioaccumulation is indicated.
Result: Estimated Log BCF = 0.500 (BCF = 3.162)
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
09-AUG-2001 (3)

Species:
Exposure period:
Concentration:
BCF:
Elimination:
Method:
Year: GLP:
Test substance:
Remark: Based on the log P and its rapid
metabolization and excretion in many species
no bioaccumulation is indicated.
09-AUG-2001

3.8 Additional Remarks

Remark: Aerobic degradation in sea water:
Inoculum: sea water; salinity 18,6 %,
20 degree C
Method: Modified OECD Screening Test, OECD
Guideline 301 E adopted 12 May 81, EG-
Richtlinie 84/449/EWG, part C.3 im EG-
Amtsblatt L 251, ISO 7824 (1984)
Concentration: 20 mg/l DOC
degradation after 28d: 100 %
degradation after 12d: 95 %
23-OCT-1995 (26)

Remark: Aerobic degradation in sea water:
 Inoculum: sea water (38.7 o/oo), 20 degree C
 Method: shake flask test - die away-test;
 parameters: DOC initial concentration: 20 mg/l
 related to Test substance; 11.6 mg DOC/l (1)
 40 mg/l resp. 23.2 mg/l related to DOC (2)
 degradation: after 5/20/61 d: 57.4/80.5/96.4 %;
 lag time: 4 d (1)
 after 5/20/61 d: 30.8/72.8/98.0 %;
 lag time: 3 d (2)

23-OCT-1995 (27)

Remark: Anaerobic degradation in lake water:
 Inokulum: sediment (eutrophic lake)
 Method: anaerobic degradation, semistatic;
 28 resp. 37 degree C; pH 7,4 - 7,6
 Concentration: 724 mg/l related to Test
 substance
 Degradation after 20 d: ca. 100 %
 Remark: 50% (w/v) sediment in culture medium
 started after 4 h (only in undiluted
 sediment), complete transformation
 to methane, detection of C14 sodium
 benzoate (ring-labelled); adaption
 to aliphatic fatty acids

23-OCT-1995 (28)

Remark: Anaerobic degradation in laboratory aquifer
 column:
 Method: continuous, room temperature, contents
 of column:
 30 % material of water-bearing soil
 sediment/
 70 % slate-debris
 Concentration: 28.1 mg/l related to Test
 substance
 Degradation: > 95 %
 Remark: Degradation after adaptation phase of
 1 week to m-Xylol; 30 degree C; flow through
 time: 2.6 cm/h, length of column: 25 cm

23-OCT-1995 (29)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
NOEC: > 245
EC50 : 484
Method: EPA OPP 72-1
Year: GLP: no data
Test substance: other TS: sodium benzoate, 99+% purity
Method: pH was adjusted to approximate that of Lake Superior water (pH 7.8) with NaOH or HCL. Compound analyses were done by HPLC: all exposure chambers at 0, 24, 48, 72 and 96 hr. Fathead minnows used in this experiment were cultured at US EPA Environmental Research Laboratory, Duluth, MN and University of Wisconsin - Superior campus. 20 fish/concentration and control. Behavior and toxic signs were noted at 4,24,48,72 and 96 hours and used to calculate EC50.
Remark: Affected fish were hyperactive and lost equilibrium prior to death. No effect data were recorded. Individual lengths and weights of the test fish were not recorded, however the measured mean weight was 230 mg. Alkalinity increased with increasing toxicant concentration. This endpoint had been studied by another investigator and reported results similar to the study mentioned above.
Test condition: temperature =23.9 degree C (+/-0.3); dissolved oxygen = 7.0 mg/l; pH=7.37; hardness = 43.4 mg/l CaCO3; alkalinity = 80.9mg/l CaCO3; tank volume = 7.3 liter; average measured concentrations 101, 163, 245, 400, 680 mg/l
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
09-AUG-2001 (30) (31)

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: > 100
Method: other: see below
Year: GLP: no data
Test substance: other TS: sodium benzoate, reagent-grade
Method: 10 fish/dose were exposed to a solution of the test substance for 96 hours (a total of seven aquatic species were tested simultaneously). Biological observations and determinations of temperature, dissolved oxygen and pH were done daily. Survival, condition, and behavior were recorded.
The LC50 values were estimated by interpolation Method (Stephan, CE, ASTM STP 634, FL Mayer & JL Hamelink (eds.) pps 65-84).
Test condition: 20 degree C; pH 6.5-8.5; 16 hr light/day; size of minnows = 200-500 mg; food was withheld for 24 hr prior to exposure; tests were done in duplicate.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
09-AUG-2001 (32)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: > 100
Method: other: see below
Year: GLP: no data
Test substance: other TS: sodium benzoate, reagent grade
Method: 10 organisms/dose were exposed to a solution of the test substance for 96 hours (a total of seven aquatic species were tested simultaneously). Biological observations and determinations of temperature, dissolved oxygen and pH were done daily.

Survival, condition, and behavior were recorded. The LC50 values were estimated by interpolation method (Stephan, CE, ASTM STP 634, FL Mayer & JL Hamelink (eds.) pps 65-84).

Remark: This endpoint had been studied by another investigator and reported results similar to the study mentioned above.

Test condition: 20 degree C; pH 6.5-8.5; 16 hr light/day; Daphnia were at first and second larval instar; food was withheld for 24 hr prior to exposure; tests were done in duplicate.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
09-AUG-2001 (33) (32)

Type: static

Species: Gammarus fasciatus (Crustacea)

Exposure period: 96 hour(s)

Unit: mg/l Analytical monitoring: no

EC50: > 100

Method: other: see below

Year: GLP: no data

Test substance: other TS: sodium benzoate, reagent grade

Method: 10 organisms/dose were exposed to a solution of the test substance for 96 hours (a total of seven aquatic species were tested simultaneously). Testing concentrations were 0.1, 1.0, 10, and 100 mg/l. Biological observations and determinations of temperature, dissolved oxygen and pH were done daily. Survival, condition, and behavior were recorded. The LC50 values were estimated by interpolation method (Stephan, CE, ASTM STP 634, FL Mayer & JL Hamelink (eds.) pps 65-84).

Test condition: 20 degree C; pH 6.5-8.5; 16 hr light/day; Gammarus weighed approximately 7 mg at testing; food was withheld for 24 hr prior to exposure; tests were done in duplicate.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, Well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
09-AUG-2001 (32)

Type: static
Species: Asellus intermedius (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: > 100
Method: other: see below
Year: GLP: no data
Test substance: other TS: sodium benzoate, reagent grade
Method: 10 organisms/dose were exposed to a solution of the test substance for 96 hours (a total of seven aquatic species were tested simultaneously). Testing concentrations were 0.1, 1.0, 10, and 100 mg/l. Biological observations and determinations of temperature, dissolved oxygen and pH were done daily. Survival, condition, and behavior were recorded. The LC50 values were estimated by interpolation Method (Stephan, CE, ASTM STP 634, FL Mayer & JL Hamelink (eds.) pps 65-84).
Test condition: 20 degree C; pH 6.5-8.5; 16 hr light/day; organisms weighed approximately 12 mg at testing; food was withheld for 24 hr prior to exposure; tests were done in duplicate.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment.
09-AUG-2001 (32)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring:
EC50: < 650
Method: other: no data
Year: GLP:
Test substance:
Test condition: 25 degree C
09-AUG-2001 (34)

Type: static
Species: other aquatic mollusc: Helisoma trivolvis
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: > 100
Method: other: see below

Year:	GLP: no data
Test substance:	other TS: sodium benzoate, reagent grade
Method:	10 organisms/dose were exposed to a solution of the test substance for 96 hours (a total of seven aquatic species were tested simultaneously). Testing concentrations were 0.1, 1.0, 10, and 100 mg/l. Biological observations and determinations of temperature, dissolved oxygen and pH were done daily. Survival, condition, and behavior were recorded. The LC50 values were estimated by interpolation method (Stephan, CE, ASTM STP 634, FL Mayer & JL Hamelink (eds.) pps 65-84).
Test condition:	20 degree C; pH 6.5-8.5; 16 hr light/day; organisms weighed approximately 180 mg at testing; food was withheld for 24 hr prior to exposure; tests were done in duplicate.
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment.
09-AUG-2001	(32)

Type:	static
Species:	other aquatic worm: <i>Dugesia tigrina</i>
Exposure period:	96 hour(s)
Unit:	mg/l
EC50:	> 100
Method:	other: see below
Year:	GLP: no data
Test substance:	other TS: sodium benzoate, reagent grade
Method:	10 organisms/dose were exposed to a solution of the test substance for 96 hours (a total of seven aquatic species were tested simultaneously). Testing concentrations were 0.1, 1.0, 10, and 100 mg/l. Biological observations and determinations of temperature, dissolved oxygen and pH were done daily. Survival, condition, and behavior were recorded. The LC50 values were estimated by interpolation method (Stephan, CE, ASTM STP 634, FL Mayer & JL Hamelink (eds.) pps 65-84).
Test condition:	20 degree C; pH 6.5-8.5; 16 hr light/day; organisms weighed approximately 6 mg at testing; food was withheld for 24 hr prior to exposure; tests were done in duplicate.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment.
09-AUG-2001 (32)

Type: static
Species: other aquatic worm: *Lumbriculus variegatus*
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: > 100
Method: other: see below
Year: GLP: no data
Test substance: other TS: sodium benzoate, reagent grade
Method: 10 organisms/dose were exposed to a solution of
the test substance for 96 hours (a total of seven
aquatic species were tested simultaneously).
Testing concentrations were 0.1, 1.0, 10, and 100
mg/l.
Biological observations and determinations of
temperature, dissolved oxygen and pH were done
daily.
Survival, condition, and behavior were recorded.
The LC50 values were estimated by interpolation
Method (Stephan, CE, ASTM STP 634, FL Mayer & JL
Hamelink (eds.) pps 65-84).
Test condition: 20 degree C; pH 6.5-8.5; 16 hr light/day;
organisms weighed approximately 6 mg at testing;
food was withheld for 24 hr prior to exposure;
tests were done in duplicate.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment
09-AUG-2001 (32)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: green algae
Endpoint:
Exposure period: 96 hour(s)
Unit: g/l Analytical monitoring: no
EC50: 430
Method: other: (calculated) ECOSAR Program (v0.99e)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Result: ECOSAR Class: Neutral Organics Organism: Green Algae
Predicted 96-hr EC50 = 4.3e+005 mg/l
(> saturation)

Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (12)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type:
Species: other bacteria: *Achromobacter liquefaciens*
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: >= 3000
Method: other: static, 22 degree C, pH 7
Year: GLP:
Test substance: other TS
Remark: 7 d-EC0 >= 3000 mg/l
Test substance: sodium benzoate; purity not noted
Flag: Critical study for SIDS endpoint
10-AUG-2001 (35)

Type:
Species: other bacteria: *Micrococcus flavus*
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: > 500
Method: other: static, 22 degree C, pH 7
Year: GLP:
Test substance: other TS
Remark: 7 d-EC0 >= 3000 mg/l
Test substance: sodium benzoate; purity not noted
Flag: Critical study for SIDS endpoint
10-AUG-2001 (35)

Type:
Species: other bacteria: *Sarcina flava*
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: < 100
Method: other: static, 22 degree C, pH 7
Year: GLP:
Test substance: other TS
Remark: 7 d-EC0 >= 3000 mg/l
Test substance: sodium benzoate; purity not noted
Flag: Critical study for SIDS endpoint
10-AUG-2001 (35)

4. ECOTOXICITY

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Type:
Species: other bacteria: *Micrococcus luteus*
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 500
Method: other: static, 22 degree C, pH 7
Year: GLP:
Test substance:
Remark: 7 d-EC0 500 mg/l
10-AUG-2001 (35)

Type:
Species: other bacteria: *Sarcina lutea*
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: < 100
Method: other: static, 22 degree C, pH 7
Year: GLP:
Test substance:
Remark: 7 d-EC0 1000 mg/l
10-AUG-2001 (35)

4.5 Chronic Toxicity to Aquatic Organisms**4.5.1 Chronic Toxicity to Fish**

Species:
Endpoint:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: No data. Based on the low acute toxicity and the readily biodegradation no relevant chronic toxicity is expected.
10-AUG-2001

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species:
Endpoint:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: No data.
10-AUG-2001

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

Type:
Species:
Endpoint:
Exposure period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: No data.
10-AUG-2001

4.6.2 Toxicity to Terrestrial Plants

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: No data.
10-AUG-2001

4.6.3 Toxicity to other Non-Mamm. Terrestrial Species

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: No data.
10-AUG-2001

4.7 Biological Effects Monitoring

Remark: No data.
10-AUG-2001

4.8 Biotransformation and Kinetics

Type:
Remark: Rapid absorbtion and metabolisation and excretion. Conjugation with glycine and excreted in urine as hippuric acid.
10-AUG-2001

4.9 Additional Remarks

Remark: Carcinogenicity in fishes (*Oryzias latipes*): no tumor incidence up to concentration of 80000 mg/kg in food (ca. 8 g sodium benzoate salt/kg fish and day); proliferation of tissue in the bile-duct (observation period 24 weeks) 13/50 fishes died after an exposure period of 12-24 weeks
23-OCT-1995 (36)

Remark: Toxicity to fungi: MIC:
100 mg/l (*Talaromyces flavus*, 35 d, pH 3.5)
> 600 mg/l (*Talaromyces flavus*, 35 d, pH 5.4)
23-OCT-1995 (37)

Remark: Toxicity to fungi: MIC (at room temperature):
100 mg/l (*Byssoschlamys fulva*, 16 d, pH 3.5)
23-OCT-1995 (38)

Remark: Toxicity to fungi: Depending on temperature (21, 30 oder 37 degree C) and concentration of sodium benzoate (0, 200, 300, 400 oder 500 mg/l) production of biomass by *Byssoschlamys nivea* was reduced in apple- and grapefruit juice up to an exposure period of 105 days.
23-OCT-1995 (39) (40)

Remark: Toxicity to fungi:
no visible growth of:

	<i>Saccharomyces cerevisiae</i>	<i>Willia anomala</i>	<i>Penicillium glaucum</i>
pH 2.6	200 mg/l	120 mg/l	600 mg/l
5	2000 mg/l	1000 mg/l	4000 mg/l
7	30000 mg/l	20000 mg/l	60000 mg/l

Method: n.a.
Test duration: n.a.

4. ECOTOXICITY

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

23-OCT-1995

(41)

Remark:

Toxicity to yeast:

no visible growth of:

Saccharomyces ellipsoideus pH 3.5 500 mg/l
5.0 5000 mg/l
6.5 >25000 mg/l

Method: n.a.

Test duration: n.a.

23-OCT-1995

(42)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: no data
Sex: male/female
Number of Animals: 5
Vehicle: water
Value: = 3450 mg/kg bw
Method: other: see below
Year: GLP: no data
Test substance: other TS: USP Sodium benzoate, purchased from Merck
Method: 5 animals/sex/group; animals did not fast prior to treatment; animals observed for 14 days.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
10-AUG-2001 (43)

Type: LD50
Species: rat
Strain: Sherman
Sex: no data
Number of Animals: 6
Vehicle: no data
Value: = 4070 mg/kg bw
Method: other: see below
Year: GLP: no
Test substance: other TS: sodium benzoate, purity not noted
Method: Groups of 6 rats were given single oral doses differing by a factor of 10. Animals were observed for morbidity and mortality.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
10-AUG-2001 (44)

Type: LD50

5. TOXICITY

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Species: rat
Strain:
Sex:
Number of Animals:
Vehicle:
Value: = 3140 mg/kg bw
Method: Directive 84/449/EEC, B.1 "Acute toxicity (oral)"
Year: GLP: no data
Test substance: other TS: sodium benzoate, purity not noted
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
10-AUG-2001 (45)

Type: LD50
Species: rat
Strain:
Sex: male/female
Number of Animals: 70
Vehicle:
Value: = 2100 mg/kg bw
Method:
Year: GLP: no data
Test substance: other TS: USP Sodium benzoate, purchased from Merck
Method: Animals fasted 18 h prior to treatment; dosed by gavage; observed for 5 days.
Reliability: (2) valid with restrictions
30-JAN-2001 (43)

5.1.2 Acute Inhalation Toxicity

Type:
Species:
Strain:
Sex:
Number of Animals:
Vehicle:
Exposure time:
Value:
Method:
Year: GLP:
Test substance:

Remark: See IUCLID dataset on benzoic acid
(CAS# 65-85-0); the loss of acidity due to the
sodium salt should decrease toxicity.

10-AUG-2001

5.1.3 Acute Dermal Toxicity

Type:
Species:
Strain:
Sex:
Number of
Animals:

Vehicle:

Value:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID dataset on benzoic acid
(CAS# 65-85-0); the loss of acidity due to the
sodium salt should decrease toxicity.

10-AUG-2001

5.1.4 Acute Toxicity, other Routes

Type: LD50

Species: rat

Strain:

Sex:

Number of
Animals:

Vehicle:

Route of admin.: i.v.

Value: = 1714 mg/kg bw

Method:

Year:

GLP:

Test substance:

10-AUG-2001

(46)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit

Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: not irritating
EC classificat.:
Method: OECD Guide-line 404 "Acute Dermal
Irritation/Corrosion"
Year: 1981 GLP: yes
Test substance: other TS: sodium benzoate; purity not noted
Reliability: (1) valid without restriction
GLP guideline study
Flag: Critical study for SIDS endpoint
10-AUG-2001 (47)

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: not irritating
EC classificat.:
Method: other: see remarks
Year: GLP:
Test substance: other TS: sodium benzoate; purity not noted
Remark: application of dry powder (500 mg/animal) for
24 h; responses were scored at end of
treatment and after 48 h
Flag: Critical study for SIDS endpoint
10-AUG-2001 (48)

Species: rat
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: irritating
EC classificat.:

5. TOXICITY

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Method: other: intradermal; see remark
 Year: GLP:
 Test substance: other TS: sodium benzoate; purity not noted
 Remark: sodium benzoate (dose 0.1 ml; 0, 10, 20 %
 saline solution) was tested for intradermal
 irritation in male Wistar rats.
 Radioactive indicator was used to quantify the
 biological response (increase of permeability
 of blood capillaries).
 At low concentrations (1 %) little irritation
 and at higher levels (≥ 3 %) significant
 irritation was recorded. The degree of
 irritation was dose-dependent.

10-AUG-2001 (49)

5.2.2 Eye Irritation

Species: rabbit
 Concentration:
 Dose:
 Exposure Time:
 Comment:
 Number of
 Animals:
 Result: slightly irritating
 EC classificat.:
 Method: OECD Guide-line 405 "Acute Eye
 Irritation/Corrosion"
 Year: 1987 GLP: yes
 Test substance: other TS: sodium benzoate; purity not noted
 Remark: according to EEC Directive 84/449/EEC, Annex V
 of the EEC Directive 67/548/EEC no labelling
 as eye irritant
 Draize score: 9.3
 Reliability: (1) valid without restriction
 GLP guideline study
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (50)

Species: rabbit
 Concentration:
 Dose:
 Exposure Time:
 Comment:
 Number of
 Animals:
 Result: not irritating

EC classificat.:

Method: Directive 84/449/EEC, B.5 "Acute toxicity (eye irritation)"

Year:

GLP:

Test substance: other TS: sodium benzoate; purity not noted
 Remark: application of dry powder (50 mg/animal) for 24 h; responses were scored at 24 h, 48 h and 72 h; postexposure observation time: 7 d

Reliability: (1) valid without restriction

Guideline study

Flag: Critical study for SIDS endpoint

10-AUG-2001

(48)

5.3 Sensitization

Type: Patch-Test

Species: human

Number of
Animals:

Vehicle:

Result:

Classification:

Method: other: patch-test

Year:

GLP:

Test substance: other TS: sodium benzoate; purity not noted
 Remark: 5 of 2045 patients of dermatological clinics developed positive reactions to the treatment with 5% sodium benzoate in petrolatum.

Flag: Critical study for SIDS endpoint

10-AUG-2001

(51)

Type: Patch-Test

Species: human

Number of
Animals:

Vehicle:

Result:

Classification:

Method: other: patch-test

Year:

GLP:

Test substance: other TS: sodium benzoate; purity not noted
 Remark: 3 workers of a pharmaceutical plant with transient urticaria after exposition to sodium benzoate and 3 previously unexposed healthy control subjects were tested.

Flag: 10-AUG-2001	All subjects reacted to benzoic acid at 0.25 % in aqueous solution under occlusion. 1 worker and 2 controls reacted to sodium benzoate at 0.5 % in saline under occlusion, but none reacted to sodium benzoate at 0.5 % in aqueous solution. All 3 workers reacted in a closed patch test to benzoic acid at 5 % in petrolatum. The time course of the responses to benzoic acid and sodium benzoate was similar in controls and workers. The potential of sodium benzoate to elicit nonimmunologic contact urticaria may be due to the formation of benzoic acid at skin contact. Critical study for SIDS endpoint
Type: Species: Concentration: Number of Animals: Vehicle: Result: Classification: Method: Year: Test substance: Remark:	other: oral provocation test human Challenge 100 other: mg other: oral 81 not sensitizing not sensitizing other GLP: no other TS: sodium benzoate; purity not noted Oral challenge test: double blind challenge; 81 persons who claimed to suffer from a food-related intolerance. No sensitisation found.
Flag: 10-AUG-2001	Critical study for SIDS endpoint
Type: Species: Concentration: Number of Animals: Vehicle: Result: Classification: Method: Year: Test substance:	other human Challenge 50 other: mg other: oral Challenge 500 other: mg other: oral other: none ambiguous other: oral challenge GLP: no other TS: sodium benzoate; purity not noted

5. TOXICITY

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Remark: Various oral challenge tests; patients suffering from asthma or rhinitis dosed with 50-500 mg benzoic acid sodium salt orally. Result : 15/157; 11/531; 10/46 positive
10-AUG-2001 (55) (56)

Type: other: see remarks
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method: other: double-blind oral challenge test
Year: GLP:
Test substance:
Remark: A patient with Melkersson-Rosenthal syndrome reacted positive to sodium benzoate (50 mg). no further information available
10-AUG-2001 (57)

Type: other: see remarks
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method: other: gastric challenge test
Year: GLP:
Test substance:
Remark: in a double-blind placebo-controlled study 25 children with severe atopic dermatitis were challenged with food and food additives, applied by nasogastric tube. 3/6 patients challenged with sodium benzoate showed a response. Reactions were exacerbations of isolated skin symptoms in all 3 and additionally abdominal pain in association with rash in one child.
10-AUG-2001 (58)

Type: other: see remarks
Species: human
Number of Animals:
Vehicle:

Result:

Classification:

Method: other: oral challenge test

Year: GLP:

Test substance:

Remark: in 21 patients (5-64 years old) with severe atopic eczema oral challenge tests with food additives were performed.
4/19 patients reacted to sodium benzoate (10, 50, 100, 300 mg; administered in gelatine capsules) with exacerbation of symptoms (flare up of atopic eczema, anaphylactoid reactions, generalized pruritus).

10-AUG-2001

(59)

Type: other: see remarks

Species: human

Number of
Animals:

Vehicle:

Result:

Classification:

Method: other: oral provocation test

Year: GLP:

Test substance:

Remark: a chemical worker suffered from allergic reactions of increasing intensity while being constantly exposed to benzoic acid during work. After oral exposure to sodium benzoate (500 mg) he suffered a severe anaphylactic shock. He showed similar but milder reaction after consuming food containing benzoic acid.

10-AUG-2001

(60)

Type: other: see remarks

Species: human

Number of
Animals:

Vehicle:

Result:

Classification:

Method: other: oral provocation test

Year: GLP:

Test substance:

Remark: In a 19-year-old girl with no medical history apart from atopic asthma during infancy, a severe anaphylaxis was observed after eating food which mainly contained sodium benzoate as food additive. The patient remained symptom-free during a sodium benzoate free diet. In the oral provocation test (single oral application of 20 mg sodium benzoate) a localized urticaria (arms) and generalised itching was observed. In a second oral challenge (application of 160 mg sodium benzoate), a higher tolerance level was noted.

10-AUG-2001 (61)

Type: other: see remarks
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method: other: oral provocation test
Year: GLP:
Test substance:
Remark: after a single oral application of 20 mg sodium benzoate, 2/10 patients with asthma and 2/7 patients with atopic dermatitis reacted positive; observed were bronchial obstruction/meteorism, nausea or dermatitis resp.

10-AUG-2001 (62)

5.4 Repeated Dose Toxicity

Species: rat Sex: male/female
Strain: Sherman
Route of admin.: oral feed
Exposure period: 90 d
Frequency of treatment: continuously in diet
Post. obs. period: no
Doses: 1, 2, 4 or 8 % in diet (approx. 640-6290 mg/kg/day)
Control Group: yes
NOAEL: 3145 mg/kg bw

LOAEL:	6290 mg/kg bw	
Method:	other: see below	
Year:		GLP: no data
Test substance:	other TS: USP sodium benzoate purchased from Merck	
Method:	Male rats (weighing 212 -430 grams) and female rats (weighing 163 to 267 grams) were dosed by gavage after being fasted for 18 hours. Animals were observed for 5 days (time interval chosen because all survivors were gaining weight and in "satisfactory nutritional condition").	
Result:	<= 4 % in diet: no adverse effects; 8 % in diet: increased mortality (4/8 died); reduced weight gain; increased weight of livers and kidneys; pathological lesions(not specified) in livers and kidneys	
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(43)
Species:	rat	Sex: male/female
Strain:	Sherman	
Route of admin.:	oral feed	
Exposure period:	30 d	
Frequency of treatment:	continuously in diet	
Post. obs. period:	no data	
Doses:	16-1090 mg/kg/day	
Control Group:	yes	
NOAEL:	> 1090 mg/kg	
Method:	other: see below	
Year:		GLP: no data
Test substance:	other TS: sodium benzoate, purity not noted	
Method:	Groups of 10 rats (5 males, 5 females) were administered doses of sodium benzoate by oral feed for thirty days. Animals were observed for weight gain, appetite, morbidity and mortality. Surviving animals were necropsied. Adrenal, upper intestine, kidney, liver, and spleen were examined.	
Remark:	10 rats/group This endpoint has been studied several times by several other investigators/groups and all reported results similar to the study mentioned above.	

Result: No adverse effects were observed.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
10-AUG-2001 (63) (44)

Species: mouse Sex: male/female
Strain: other: Albino Swiss
Route of admin.: drinking water
Exposure period: 35 days
Frequency of treatment: continuously in drinking water
Post. obs. period: no data
Doses: 0.5; 1; 2; 4 or 8 % in drinking water
Control Group: yes
NOAEL: 2 %
LOAEL: 4 %
Method: other: Toth, B. (1984)
Year: GLP: no data
Test substance: other TS: sodium benzoate, purity not noted
Remark: "By taking into account four parameters
(survival rate, body weight, chemical
consumption, histological changes), the 2%
dose level was found suitable for the
lifelong treatment."

Result: 8 %: 4/4 males and 4/4 females died within 3
weeks; 4 %: 3/4 males and 3/4 females died
during the treatment period and the body
weight of surviving mice was substantially
reduced.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
10-AUG-2001 (64)

Species: rat Sex: male/female
Strain: other: F344/Ducrj
Route of admin.: oral feed
Exposure period: 10 d
Frequency of treatment: continuously in diet
Post. obs. period: no
Doses: 1.81; 2.09 or 2.4 % in diet (approx. 1358,
1568 or 1800 mg/kg/d)
Control Group: yes

LOAEL:	1358 mg/kg bw	
Method:		
Year:		GLP:
Test substance:	other TS: sodium benzoate, purity not noted	
Remark:	The mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954).	
Result:	At the lowest tested concentration of 1358 mg/kg changes in serum chlolesterol levels occurred in females. At doses of 1568 mg/kg and above changes in further serum parameters and an increased relative liver weight were described. Histopathological changes of the liver, increased relative kidney weights and disorders of the central nervous system were seen after dosing via diet with > 1800 mg. 1/6 male rat in the 2.4 %-group, who developed increased sensitivity to stimuli and convulsions, died.	
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment.	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(65)
Species:	mouse	Sex: male/female
Strain:	B6C3F1	
Route of admin.:	oral feed	
Exposure period:	10 d	
Frequency of treatment:	continuously in diet	
Post. obs. period:	no	
Doses:	2.08; 2.5 or 3 % in diet (approx. 3012, 3750 or 4500 mg/kg/d)	
Control Group:	yes	
NOAEL:	3750 mg/kg bw	
LOAEL:	4500 mg/kg bw	
Method:	other: see below	
Year:		
GLP:	no data	
Test substance:	other TS: sodium benzoate (specific grade) purchased from Wako Pure Chemical Ind., Osaka, Japan	
Method:	Sodium benzoate, mixed with the powdered diet, was fed to groups of 12 mice (6 males, 6 females) for 10 days.	

Animals were observed for body weight gain and clinical signs 5 day/ week.
At the end of the experiment, surviving animals were necropsied. Organ weights, clinical chemistry and histological examinations were performed.

Remark: The mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954).

Result: All mice in the 3.0 %-group showed increased sensitivity to stimuli and 1/5 male and 2/5 females showed convulsions; 2/5 females died; liver weights of males and females and kidney weights of females were dose-dependently increased; histopathologic examination showed enlarged hepatocytes, single cell necrosis and vacuolation of hepatocytes in all livers from males; no histopathologic changes of the kidney were described; serum cholesterol, lipid levels and cholinesterase were increased in males.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
10-AUG-2001 (65)

Species: rat Sex: male/female
Strain: Fischer 344
Route of admin.: oral feed
Exposure period: 18-24 months
Frequency of treatment: continuously in diet
Post. obs. period: no
Doses: 1 or 2 % in diet
Control Group: yes
NOAEL: 2 %
Method: other: OECD 451
Year: GLP: no data
Test substance: other TS: sodium benzoate, purity not noted

Remark: Mean compound consumption:
2 % in diet: m: 280 +- 9.8 mg/d
f: 202 +- 10.5 mg/d

Result: No adverse clinical signs in treated rats; no differences in average body weight and mortality in comparison to controls.

Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(66)
Species:	rat	Sex: male/female
Strain:	Sherman	
Route of admin.:	oral feed	
Exposure period:	28 d	
Frequency of treatment:	continuously in diet	
Post. obs. period:		
Doses:	2 or 5 % in diet (see remarks)	
Control Group:	other: no data	
LOAEL:	2002 - 2357 mg/kg bw	
Method:	other: see below	
Year:		GLP: no data
Test substance:	other TS: sodium benzoate, food grade	
Method:	Food grade sodium benzoate was incorporated into the basal diet at concentrations of 2% and 5%. The rats were weighed individually twice a week and were inspected daily for signs of toxicity. Food consumption for each group was recorded weekly and the drug intake as mg/kg bw was calculated using the average body weights for each group. Fisher's T test for small samples was used as a test for significant differences between body weights for the various groups.	
Remark:	6 rats/group; initial body weight: 40-50 g; mean compound consumption: 2 % in diet: m: 2002 - 2357 mg/kg/day f: 2171 - 2396 mg/kg/day 5 % in diet: m: 5686 mg/kg/day f: 7780 mg/kg/day	
Result:	2 %: slight depression of body weight gain only in males 5 %: urine incontinence, convulsions, 100 % mortality after 2nd week	
Reliability:	(3) invalid Significant methodological deficiencies	
10-AUG-2001		(67)
Species:	rat	Sex: male/female
Strain:	Fischer 344	
Route of admin.:	oral feed	
Exposure period:	42 d	

Frequency of treatment: continuously in diet
 Post. obs. period: no data
 Doses: 0.5; 1; 2; 4 or 8 % in diet (approx. 375-6000 mg/kg/day)
 Control Group: yes
 Method: other: see below
 Year: GLP:
 Test substance: other TS: sodium benzoate, purity not noted; supplied by National Institute of Hygienic Sciences pellets in the basal diet
 Method: 10 rats/group; initial body weight: 110-150 g; the mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954); Animals were administered diets containing various concentrations of sodium benzoate for 6 weeks. Survival rate, growth, food intake, behavior and general status were observed during the feeding period. Morphological examinations were carried out.
 Result: 2 % in diet (approx. 1500 mg/kg/day): maximum tolerated dose;
 >= 4 % in diet (approx. >= 3000 mg/kg/day): mortality 10/11 or 10/10; atrophy of the spleen and lymph nodes at autopsy.

10-AUG-2001 (66)

Species: rat Sex: no data
 Strain: no data
 Route of admin.: oral feed
 Exposure period: until death (see below)
 Frequency of treatment: continuously in diet
 Post. obs. period: no
 Doses: 5 % in diet (approx. 3750 mg/kg/day)
 Control Group: yes
 Method:
 Year: GLP:
 Test substance: other TS: benzoic acid
 Remark: the mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954)
 Result: 19/28 young rats (initial body weight: 62-70 g) died during the first 2 weeks; all others died 1 week later; reduced food intake, diarrhea, intestinal haemorrhage and crusted blood in the nose at autopsy.

10-AUG-2001

(68)

Species: rat Sex: no data
Strain: no data
Route of admin.: oral feed
Exposure period: no data
Frequency of treatment: continuously in diet
Post. obs. period: no data
Doses: 5 % in diet (approx. 3750 mg/kg/day)
Control Group: other: no data
Method:
Year: GLP:
Test substance: other TS: benzoic acid
Remark: the mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954)
Result: 4/5 adult rats (initial body weight: 221-232 g) died during 4-5 weeks; body weight was reduced to 161 g

10-AUG-2001

(68)

Species: rat Sex: male
Strain: no data
Route of admin.: oral feed
Exposure period: 23 weeks
Frequency of treatment: continuously in diet
Post. obs. period: no
Doses: 5 % in diet (approx. 3750 mg/kg/d)
Control Group: yes
Method:
Year: GLP:
Test substance: other TS: sodium benzoate, purity not noted
Remark: Basic diet: low casein diet; the study was done to investigate the effect of several xenobiotics on the growth retardation provoked in rats by sodium benzoate; the data presented here are the results of the "long-term" positive control group.
The mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954).
Result: marked growth inhibition, occasionally restlessness, irritability, tremors

10-AUG-2001

(69)

5. TOXICITY

DATE: 10-AUG-2001

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Species:	dog	Sex: male/female
Strain:	other: fox terrier	
Route of admin.:	oral feed	
Exposure period:	<= 250 days	
Frequency of treatment:	once daily	
Post. obs. period:		
Doses:	0.1 - 7 g/animal/day	
Control Group:	other: no data	
Method:		
Year:		GLP:
Test substance:		
Result:	0.1 - < 7 g/animal/day: no toxic effect; 7 g/animal/day (approx. 1 g/kg/day): toxic dose (ataxia, tonoclonic convulsions, vomiting, death)	

26-JAN-2001

(70)

5.5 Genetic Toxicity 'in Vitro'

Type:	Ames test
System of testing:	Salmonella typhimurium TA 92, TA 94, TA 98, TA 100, TA 1535, TA 1537
Concentration:	0-3 mg/plate
Cytotoxic Conc.:	
Metabolic activation:	with and without
Result:	negative
Method:	OECD Guide-line 471 "Genetic Toxicology: Salmonella thyphimurium Reverse Mutation Assay"
Year:	1983
Test substance:	other TS: samples obtained from Japan Food Additives Association; purity = 99% analysed at Ministry of Health and Welfare of Japan
Remark:	This endpoint has been studied by several other investigators/groups and all support the result of the study mentioned above.
Reliability:	(1) valid without restriction Guideline study
Flag:	Critical study for SIDS endpoint
10-AUG-2001	(71) (72)

Type:	Cytogenetic assay
System of testing:	cultured human embryonic lung cells

Concentration: 2.0, 20.0, 200.0 ug/ml
 Cytotoxic Conc.:
 Metabolic
 activation: without
 Result: negative
 Method: other: anaphase preparations
 Year: GLP: no data
 Test substance: other TS: FDA 71-37 supplied by Food and Drug Administration
 Remark: This endpoint has been studied by several other investigators/groups and all support the result of the study mentioned above.
 Reliability: (2) valid with restrictions
 Meets generally accepted scientific standards, well documented and acceptable for assessment
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (73) (74)

Type: other: Chromosomal aberration test
 System of
 testing: Chinese hamster fibroblast cell line (CHL)
 Concentration: 0 - 2 mg/plate
 Cytotoxic Conc.:
 Metabolic
 activation: without
 Result: positive
 Method: other: similar to OECD Guideline 473
 Year: 1983 GLP: no data
 Test substance: other TS: samples obtained from Japan Food Additives Association; purity = 99% analysed at Ministry of Health and Welfare of Japan

Reliability: (2) valid with restrictions
 Comparable to Guideline study with acceptable restrictions
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (75) (72)

Type: other: E. coli reversion mutation assay
 System of
 testing: E. coli WP2
 Concentration: no data
 Cytotoxic Conc.: no data
 Metabolic
 activation: with and without
 Result: negative
 Method: EPA OTS 798.5100
 Year: GLP: no data
 Test substance: other TS: sodium benzoate, purchased from Baker; purity not noted

Reliability:	(1) valid without restriction Guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(76)
Type:	other: Sister chromatid exchange	
System of testing:	Chinese hamster cell line (Don)	
Concentration:	0.001 to 0.01 M / plate	
Cytotoxic Conc.:	no data	
Metabolic activation:	without	
Result:	ambiguous	
Method:	other: see below	
Year:		GLP: no data
Test substance:	other TS: sodium benzoate, supplied by National Institute of Hygienic Sciences, Japan; purity not noted	
Method:	Sodium benzoate was dissolved in Hank's balanced salt solution to desired concentrations. All cultures were kept in complete darkness at 37 degree C for 26 hours (two cell cycles) and 0.25 ug colchicine/ml added for final 2 hours. Cells were collected and stained by acridine orange technique for fluorescence or modified FPG (fluorecence plus Giemsa) for Giemsa.	
Remark:	The number of SCE per cell was determined on the basis of 20-50 intact metaphases without gross chromosome aberrations. slight increase in SCE/cell, but no dosage effect	
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(77)
Type:	other: Sister chromatid exchange	
System of testing:	human lymphocytes	
Concentration:		
Cytotoxic Conc.:		
Metabolic activation:	without	
Result:	positive	
Method:		
Year:		GLP:
Test substance:		
Remark:	only one concentration (10E-2 M) tested	

Reliability: (3) invalid
 Significant methodological deficiencies
 Flag: Critical study for SIDS endpoint
 10-AUG-2001
 (78)

Type: other: Inhibition of DNA synthesis
 System of testing: Vicia faba root meristems
 Concentration:
 Cytotoxic Conc.:
 Metabolic activation: without
 Result: positive
 Method:
 Year: GLP:
 Test substance:
 Remark: other observed effects:
 a. concentration-dependent decrease in mitotic figures;
 b. concentration-dependent increase in anaphase bridges;
 c. premature chromosome condensation heading to pycnotic nuclei; d. chromatin erosion in interphase nuclei

Reliability: (3)invalid
 Unsuitable test system
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (79)

Type: Bacillus subtilis recombination assay
 System of testing: Bacillus subtilis H17, M45
 Concentration:
 Cytotoxic Conc.:
 Metabolic activation: no data
 Result: positive
 Method:
 Year: GLP:
 Test substance: no data
 Method: An overnight culture of B. subtilis, H17 and M45, was mixed with test solutions and incubated for 30 minutes at 37 degree C. After treatment, viable cells were counted and the ratio of 50% survival concentrations were calculated.
 Result: Sodium benzoate showed DNA damaging potential although it had been negative in the Ames test.

Reliability:	(4) not assignable Documentation insufficient for assessment; abstract only	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(80)
Type:	Bacillus subtilis recombination assay	
System of testing:	Bacillus subtilis H17, M45	
Concentration:	6-20 mg/disk, in water	
Cytotoxic Conc.:		
Metabolic activation:	with and without	
Result:	ambiguous	
Method:		
Year:		GLP:
Test substance:		
10-AUG-2001		(81)
Type:	Ames test	
System of testing:	Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538	
Concentration:		
Cytotoxic Conc.:		
Metabolic activation:	with and without	
Result:	negative	
Method:		
Year:		GLP:
Test substance:		
11-JAN-2001		(76)
Type:	Ames test	
System of testing:	Salmonella typhimurium, TA 98, TA100, TA1537	
Concentration:		
Cytotoxic Conc.:		
Metabolic activation:	with and without	
Result:	negative	
Method:		
Year:		GLP:
Test substance:		
01-SEP-2000		(82)
Type:	other: Chromosomal aberration test	
System of testing:	Chinese hamster cell line (Don)	
Concentration:		

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Cytotoxic Conc.:

Metabolic

activation: without

Result: positive

Method:

Year:

GLP:

Test substance:

11-JAN-2001

(77)

Type:

other: Chromosome aberration test

System of

testing: Chinese hamster fibroblast cell line (CHL)

Concentration:

Cytotoxic Conc.:

Metabolic

activation: with

Result:

positive

Method:

Year:

GLP:

Test substance:

Method:

other TS: purity not given

other: Ishidate M. and Odashima S. Mutation
Res. 48: 337-354(1977) and Matsuoka A. et al.
Mutation Res. 66: 277-290 (1979)

01-SEP-2000

(82)

Type:

other: Sister chromatid exchange

System of

testing: Vicia faba root tip cells

Concentration:

Cytotoxic Conc.:

Metabolic

activation: without

Result:

positive

Method:

Year:

GLP:

Test substance:

Remark:

only one concentration (10E-2 M) tested

11-JAN-2001

(78)

5.6 Genetic Toxicity 'in Vivo'

Type:

Cytogenetic assay

Species:

rat

Sex: male

Strain:

no data

Route of admin.:

gavage

Exposure period:

single application

Doses:

50, 500 or 5000 mg/kg

Result:

negative

Method:	EPA OTS 798.5385	
Year:		GLP: yes
Test substance:	other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration	
Result:	no detectable significant aberrations of the bone marrow metaphase chromosomes	
Reliability:	(1) valid without restriction GLP guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(74)
Type:	Cytogenetic assay	
Species:	rat	Sex: male
Strain:	no data	
Route of admin.:	gavage	
Exposure period:	once daily for 5 consecutive days	
Doses:	50, 500 or 5000 mg/kg	
Result:	negative	
Method:	EPA OPPTS 870.5385	
Year:		GLP: yes
Test substance:	other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration	
Result:	no detectable significant aberrations of the bone marrow chromosomes	
Reliability:	(1) valid without restriction GLP guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(74)
Type:	Dominant lethal assay	
Species:	rat	Sex: male
Strain:	no data	
Route of admin.:	gavage	
Exposure period:	single application	
Doses:	50, 500 or 5000 mg/kg	
Result:	negative	
Method:	EPA OPPTS 870.5450	
Year:		GLP: yes
Test substance:	other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration	
Reliability:	(1) valid without restriction GLP guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(74)

Type:	Dominant lethal assay	
Species:	rat	Sex: male
Strain:	no data	
Route of admin.:	gavage	
Exposure period:	once daily for 5 consecutive days	
Doses:	50, 500 or 5000 mg/kg	
Result:	negative	
Method:	EPA OPPTS 870.5450	
Year:		GLP: yes
Test substance:	other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration	
Reliability:	(1) valid without restriction GLP guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(74)

Type:	other: Host mediated assay	
Species:	mouse	Sex: male
Strain:	no data	
Route of admin.:	gavage	
Exposure period:	single application	
Doses:	50, 500 or 5000 mg/kg	
Result:	negative	
Method:	other: EPA	
Year:		GLP: yes
Test substance:	other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration	
Result:	No elevation of mutant frequencies in Salmonella Typhimurium G46 and no increase in recombinant frequencies in Saccharomyces cerevesiae D3	
Reliability:	(1) valid without restriction GLP guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(74)

Type:	other: Host mediated assay	
Species:	mouse	Sex: male
Strain:	no data	
Route of admin.:	gavage	
Exposure period:	single application	
Doses:	50, 500 or 5000 mg/kg	
Result:	negative	
Method:	other: EPA	
Year:		GLP: yes
Test substance:	other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration	

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Result: elevation of mutant frequencies in *Salmonella typhimurium* TA 1530 in the intermediate dose level; Not dose dependent and negative at multiple dose exposure.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint
10-AUG-2001 (74)

Type: other: Host mediated assay

Species: mouse Sex: male

Strain: no data

Route of admin.: gavage

Exposure period: once daily for 5 consecutive days

Doses: 50, 500 or 5000 mg/kg

Result: negative

Method: other: EPA

Year: GLP: yes

Test substance: other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration

Result: no elevation of mutant frequencies in *Salmonella Typhimurium* G46; no elevation of mutant frequencies in *Salmonella typhimurium* TA 1530; no increase in recombinant frequencies in *Saccharomyces cerevisiae* D3

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint
10-AUG-2001 (74)

5.7 Carcinogenicity

Species: rat Sex: male/female

Strain: Fischer 344

Route of admin.: oral feed

Exposure period: 18-24 months

Frequency of treatment: continuously in diet

Post. obs. period: no

Doses: 1 or 2 % in diet (see remarks)

Result: negative

Control Group: yes

Method: OECD Guide-line 451 "Carcinogenicity Studies"

Year: GLP: no data

Test substance: other TS: sodium benzoate, purity not noted

Method:	Groups of 50 male and 52 female Fischer 344 rats, four to five weeks old, received diets containing 1% (500 mg/kg bw per day) or 2% (1000 mg/kg bw per day) sodium benzoate for 18-24 months. Controls, consisting of 25 male and 43 female rats, received basal diet.	
	Food intake was adequately controlled to avoid an excess; tap water was available ad libitum.	
	Mean compound consumption:	
	1 % in diet: m: 141 +- 9.7 mg/d	
	f: 102 +- 11.8 mg/d	
	2 % in diet: m: 280 +- 9.8 mg/d	
	f: 202 +- 10.5 mg/d	
Remark:	about 40 rats including control animals died during the first 16 months of the experimental period (pneumonia with abscess) about 100 rats including control animals died after 16 months of hemorrhagic pneumonia (infection)	
Result:	Survival was very poor in all groups, due to intercurrent sialodacryoadenitis and mycoplasma infections. All surviving animals were sacrificed between 18 and 25 months, all were autopsied, and various tissues were examined histopathologically. No adverse clinical signs directly attributable to treatment were observed, and only negligible differences in average body weight and mortality rate were seen between the treated and control groups. Although a variety of tumours occurred among treated and control rats of each sex, they were of similar type and incidence; no evidence of carcinogenicity.	
Reliability:	(1) valid without restriction	
Flag:	Guideline study	
10-AUG-2001	Critical study for SIDS endpoint (66)	
Species:	mouse	Sex: male/female
Strain:	other: Albino Swiss	
Route of admin.:	drinking water	
Exposure period:	lifelong	
Frequency of treatment:	continuously in drinking water	
Post. obs. period:	no data	
Doses:	2 % in drinking water	
Result:	negative	
Control Group:	yes	

Method:	other: see below	
Year:		GLP: no data
Test substance:	other TS: sodium benzoate, purity not noted	
Method:	In the main study, a 2% solution of sodium benzoate (purity, 99%) was administered in the drinking-water to groups of 50 male and 50 female five-week-old mice for their lifetime. Groups of 100 males and 100 females were used as untreated controls. Both treated and control animals were 'carefully checked'; their body weights were measured weekly, and gross pathological changes were recorded. The animals were either allowed to die or were sacrificed when moribund. Complete necropsies were performed on all animals, and the liver, spleen, kidneys, bladder, thyroid, heart, pancreas, testes, ovaries, brain, nasal turbinates, at least four lobes of the lungs, and organs with gross pathological changes were examined histologically.	
Remark:	50 males and 50 females were treated; 99 males and 99 females served as controls; average daily intake: 119.2 mg (f) or 124.0 mg (m)	
Result:	The average daily intake of sodium benzoate was 124.0 mg for males and 119.2 mg for females on the basis of daily water consumption of 6.2 and 5.9 ml, respectively. The dose of sodium benzoate was equivalent to 6200 mg/kg bw per day for males and 5960 mg/kg bw per day for females. Treatment had no effect on survival or the incidence of tumours.	
Reliability:	(2) valid with restrictions This study is sufficiently reliable due to a sufficient number of animals and a detailed histopathological examination.	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(64)
Species:	rat	Sex: male
Strain:	Fischer 344	
Route of admin.:		
Exposure period:		
Frequency of treatment:		
Post. obs. period:		
Doses:		
Result:		
Control Group:		
Method:		

Year:	GLP:
Test substance:	
Remark:	DEN-PH model; final, general protocol: Group 1: single i.p. dose of diethylnitrosamine, repeated treatment with the test compound from week 2, hepatectomy at week 3, sacrifice at week 8. Group 2: single i.p. dose of diethylnitrosamine, hepatectomy at week 3, sacrifice at week 8. Group 3: single i.p. dose of saline, repeated treatment with the test compound from week 2, sacrifice at week 8. The enhancing effects of chemicals on induction of preneoplastic form of glutathione S-transferase positive foci was measured by comparing the GST-P positive foci in liver slices of treated and control animals.
Result:	positive
26-JAN-2001	(83)

5.8 Toxicity to Reproduction

Type:	other: 2 year carcinogenicity study
Species:	rat Sex: male/female
Strain:	Fischer 344
Route of admin.:	oral feed
Exposure Period:	18 - 24 months
Frequency of treatment:	continuously in diet
Duration of test:	24 months
Doses:	1 or 2 % in diet
Control Group:	yes
NOAEL Parental:	2 %
Method:	other: OECD 451
Year:	GLP: no data
Test substance:	other TS: sodium benzoate, purity not noted
Result:	No evidence of compound related effects in the testes or ovaries of treated rats.
Reliability:	(2) valid with restrictions In the 2 yr feeding study, reproductive organs were examined macroscopically and histologically.
Flag:	Critical study for SIDS endpoint
10-AUG-2001	(66)

Species: Sex:
Strain:
Route of admin.:
Exposure Period:
Frequency of treatment:
Duration of test:
Doses:
Control Group:
Method:
Year: GLP:
Test substance:
Remark: A 4-generation reprotoxicity test with benzoic acid revealed no reproductive effects. Therefore no indication for reproductive toxicity testing for the benzoic acid sodium salt. See IUCLID on benzoic acid (CAS# 65-85-0); the data on the sodium salt should be similar.
Flag: Critical study for SIDS endpoint
10-AUG-2001

5.9 Developmental Toxicity/Teratogenicity

Species: rat Sex: female
Strain: Wistar
Route of admin.: gavage
Exposure period: Day 6-15 of gestation
Frequency of treatment: once daily
Duration of test:
Doses: 1.75; 8; 38 or 175 mg/kg/d
Control Group: yes
NOAEL Maternalt.: ≥ 175 mg/kg bw
NOAEL Teratogen.: ≥ 175 mg/kg bw
Method: EPA OPPTS 870.3700
Year: GLP: no data
Test substance: other TS: sodium benzoate, purity not noted
Remark: This endpoint has been studied several times by several other investigators/groups and all reported results similar to the study mentioned above.
Result: no effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from number in controls; maternal toxicity was not reported at any dose applied

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint (84) (85)
10-AUG-2001

Species: rat Sex: female
Strain: Wistar
Route of admin.: oral feed
Exposure period: whole gestation period (20 d)
Frequency of treatment: continuously in diet
Duration of test:
Doses: 1, 2, 4 or 8 % in diet (700 to 5600 mg/kg)
Control Group: yes
NOAEL Maternalt.: = 1400 mg/kg bw
NOAEL Teratogen.: = 1400 mg/kg bw
Method: other
Year: GLP: no data
Test substance: other TS: sodium benzoate, purity not noted
Remark: The mean food consumption was calculated from graph:
 <= 2 % in diet: approx. 20 mg/kg/day
 4 % in diet: approx. 12 mg/kg/day
 8 % in diet: approx. 2.5 mg/kg/day
 The mean compound consumption was calculated from graph:
 1 % in diet: approx. 700 mg/kg/day
 2 % in diet: approx. 1400 mg/kg/day
 4 % in diet: approx. 2800 mg/kg/day
 8 % in diet: approx. 5600 mg/kg/day

Result: A study using pregnant Wistar rats, dosed with 700, 1400, 2800, 5600 mg/kg sodium benzoate in the diet during the entire gestation showed no statistical difference in organ and bone abnormalities of fetuses between experimental groups and controls; growth of treated offsprings was similar to controls in rats dosed with 1400 mg/kg/day; reduced food intake and decreased body weight of the pregnant rats especially in the 5600 mg/kg group; 100% perinatal death rate; organ abnormalities of fetuses involved eye, brain and kidneys, in addition abnormalities of the skeletal system were found in rats dosed with >2800 mg/kg/day.

Conclusion: The authors concluded that the effects on the dams and fetuses at the 2800 and 5600 levels were due to reduced maternal feed intake in these groups, leading to malnutrition.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
10-AUG-2001 (86)

Species: mouse Sex: female
Strain: CD-1
Route of admin.: gavage
Exposure period: Day 6-15 of gestation
Frequency of treatment: once daily
Duration of test:
Doses: 1.75; 8; 38 or 175 mg/kg/d
Control Group: yes
NOAEL Maternalt.: ≥ 175 mg/kg bw
NOAEL Teratogen.: ≥ 175 mg/kg bw
Method: EPA OPPTS 870.3700
Year: GLP: no data
Test substance: other TS: sodium benzoate, purity not noted
Result: No effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from controls; maternal toxicity was not reported at any dose applied.

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint
10-AUG-2001 (85)

Species: rabbit Sex: female
Strain: other: Dutch-belted
Route of admin.: gavage
Exposure period: Day 6-18 of gestation
Frequency of treatment: once daily
Duration of test:
Doses: 2.5; 12; 54 or 250 mg/kg/d
Control Group: yes
NOAEL Maternalt.: 250 mg/kg bw
NOAEL Teratogen.: 250 mg/kg bw
Method: EPA OPPTS 870.3700
Year: GLP: no data

5. TOXICITY

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Test substance: other TS: sodium benzoate, purity not noted
 Result: No effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from controls; maternal toxicity was not reported at any dose applied.
 Reliability: (1) valid without restriction
 Guideline study
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (85)

Species: hamster Sex: female
 Strain: other: golden; outbred
 Route of admin.: gavage
 Exposure period: Day 6-10 of gestation
 Frequency of treatment: once daily
 Duration of test:
 Doses: 3, 14, 65 or 300 mg/kg/d
 Control Group: yes
 NOAEL Maternalt.: 300 mg/kg bw
 NOAEL Teratogen.: 300 mg/kg bw
 Method: EPA OPPTS 870.3700
 Year: GLP: no data

Test substance: other TS: sodium benzoate, purity not noted
 Result: No effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from controls; maternal toxicity was not reported at any dose applied.
 Reliability: (1) valid without restriction
 Guideline study
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (85)

Species: rat Sex: female
 Strain: Sprague-Dawley
 Route of admin.: i.p.
 Exposure period: day 9-11 of gestation
 Frequency of treatment: once daily
 Duration of test:
 Doses: 100, 315 or 1000 mg/kg/d
 Control Group: other: sodium chloride 90 or 600 mg/kg/d
 NOAEL Teratogen.: 315 mg/kg bw
 Method:
 Year: GLP:

Test substance:
Remark: no further information available
Result: 1000 mg/kg: increased rate of in utero deaths,
reduced fetal body weight
26-JAN-2001 (87)

Species: rat Sex: female
Strain: Sprague-Dawley
Route of admin.: i.p.
Exposure period: day 12-14 of gestation
Frequency of treatment: once daily
Duration of test:
Doses: 100, 315 or 1000 mg/kg/d
Control Group: other: sodium chloride 90 or 600 mg/kg/d
NOAEL Teratogen.: 315 mg/kg bw
Method:
Year: GLP:

Test substance:
Remark: no further information available
Result: 1000 mg/kg: reduced fetal body weight,
increased rate of in utero deaths, gross
anomalies in fetuses
26-JAN-2001 (87)

Species: hen Sex:
Strain: Leghorn
Route of admin.: other
Exposure period: once
Frequency of treatment: single injection in eggs
Duration of test:
Doses: highest level tested: 5 mg/egg
Control Group: yes
Method:
Year: GLP:
Test substance:
Remark: Fresh fertile eggs were used, 4 test
conditions were used: injection via the air
cell and via the yolk twice, preincubation 0 h
and 96 h; total number of eggs treated:
approx. 100.
Result: LD50 (injection via air cell at 96 h):
4.74 mg/egg; no teratogenic effects in the
developing chicken embryo.
26-JAN-2001 (88)

Species: hen Sex:
 Strain: other: Ross I stock
 Route of admin.: other
 Exposure period: once
 Frequency of treatment: single injection
 Duration of test:
 Doses: highest level tested: 0.1 mg/embryo
 Control Group: yes
 Method: other: Chick Embryotoxicity Screening Test (CHEST)
 Year: GLP:
 Test substance:
 Result: no embryotoxicity was observed at a concentration of 100 ug/embryo
 26-JAN-2001 (89)

Species: other: chick embryo neural retina cells Sex:
 Strain:
 Route of admin.: other: in vitro
 Exposure period: 24 hours
 Frequency of treatment: single treatment
 Duration of test: 7 days
 Doses: up to cytolethal or solubility limit
 Control Group: yes
 Method: other: Chick embryo retina cell (CERC) assay
 Year: GLP:
 Test substance: other TS: purchased from Sigma Chemical
 Method: The chemical was dissolved in Gibco medium 199 or DMSO and adjusted to pH 7.2. At least five concentrations were tested, with six flasks per concentration. 7-10 E+06 cells were dispersed in 3ml culture medium, plus the test chemical, and incubated for 18-24 hours. Cell aggregates were counted and the medium changed to Gibco 199 without the test chemical. The cells were cultured for an additional 6 days. Protein content was measured by the Lowry method and glutamine synthetase activity measured by a spectrophotometric assay. Statistics: pairwise comparisons among treatment groups were done by ANOVA and concentration-response relationships analyzed by general linear methods (SAS, 1987). A chemical was classified as active if there was a significant concentration-dependent decrease in glutamine

synthetase activity, protein content or aggregate size; or increasing trend in aggregate number and at least one concentration group that was significantly different ($p < 0.05$) from the control. Result: Sodium benzoate was classified as inactive in the CERC assay with LOEL at $>34.7\text{mM}$.
19-MAY-2000 (90)

5.10 Other Relevant Information

Type: Metabolism
Remark: The experimental study on the inducibility of the hepatic and renal hippurate-synthesizing system by gradually increasing daily i.p. doses (125-375 mg/kg, given between 17 and 21 days) of sodium benzoate to mice showed no effects. Sodium benzoate did not induce its own metabolizing system.
23-OCT-1995 (91)

Type: Metabolism
Remark: A 15 mM aqueous solution of sodium benzoate was shown to inhibit in vitro the noradrenaline-induced aggregation of platelets from healthy volunteers by blocking the cyclo-oxygenase-thromboxane enzyme system.
23-OCT-1995 (92)

Type: Metabolism
Remark: Six female volunteers (case I) and three male volunteers (case II) were orally given (case I) 33 or 66 mg sodium benzoate in a soft drink or (case II) a sodium benzoate solution at a dosage of 20 mg/kg bw.. In case I, 66 to 86 % of the administered dose was excreted in urine within 3 hours as hippuric acid (maximum at 0 to 30 minutes); in case II, approx. 89 % of the administered dose was excreted in urine within 5 hours as hippuric acid (maximum at 0 to 1 hour). In case I, the concentration of hippuric acid recovered to the predose level after 3 hours, while in case II the concentration of hippuric acid did not recover to the predose level within 5 hours.
23-OCT-1995 (93)

Type: Metabolism

Remark:	After i.p. injection of 2.5 to 10 mmol sodium benzoate/kg bw in male Sprague-Dawley rats, changes in metabolic levels of the liver and in amino acid levels in liver and plasma were noted.
23-OCT-1995	(94)
Type:	Metabolism
Remark:	Sodium benzoate inhibited the dissolution of hydrochlorothiazide (HCT) in vitro. In bioavailability studies with 6 male volunteers, the rate of increase in mean urine volume after intake of HCT-sodium benzoate was 6:1 compared to HCT alone.
23-OCT-1995	(95)
Type:	Metabolism
Remark:	In an in vitro study with gastric mucosa from patients with asthma, atopic eczema and urticaria, the release of histamine and prostaglandin was significantly increased by sodium benzoate at a concentration of 0.4 %. The mucosa of control persons did not react to sodium benzoate.
23-OCT-1995	(62)
Type:	Metabolism
Remark:	In experiments with isolated rat hepatocytes and mitochondria, sodium benzoate at concentrations from 0 to 2.0 mM inhibited gluconeogenesis (max. 67 %) and urea production (max. 52 %) in a dose-dependent manner by depletion of acetyl CoA.
23-OCT-1995	(96)
Type:	
Remark:	Toxicity: I.p. injection of 7.5 mmol/kg ammonium acetate alone produced 10 % mortality in male Swiss albino mice. Subsequent i.p. administration of 7.5 mmol/kg sodium benzoate resulted in 100 % mortality. Pretreatment of mice with carbamyl glutamate (2-20 mmol/kg), a structural analogue of N-acetyl glutamate, reduced mortality to 20 %. The protective effect of carbamyl glutamate is accompanied by an increase in urea production and of carbamyl phosphate synthetase activity.
10-AUG-2001	(97)

Type:

Remark: Effect on ammonia levels:
Male SD-rats received i.p. injections of saline, L-norvaline (1 mmol/kg), L-methionine-SR-sulfoximine (250 umol/kg), sodium benzoate (2.5-10 mmol/kg) in saline, either alone or in combination. L-norvaline and L-methionine-SR-sulfoximine caused an increase in the concentration of ammonia in plasma and in liver (interference with urea and glutamine formation). Subsequent injection of sodium benzoate failed to alleviate ammonia levels, and on the contrary, caused further increase. Sodium benzoate itself resulted in higher levels of ammonia in plasma and liver. Application of glycine did not lower ammonia levels indicating that other factors besides glycine may also be necessary for the removal of sodium benzoate.

10-AUG-2001

(98)

Type:

Remark: Liver perfusion:
In isolated perfused rat liver (livers of male Wistar rats, body weight 120-150 g), addition of sodium benzoate to the perfusion medium led to a rapid and marked stimulation of glutamate release from the liver (maximal glutamate efflux: 0.9 umol/min/g), which was fully reversible. Benzoate concentrations as low as 15 uM were effective to stimulate glutamate release significantly. Simultaneously benzoate inhibits urea and glutamine synthesis and diminishes hepatic ammonia uptake.

10-AUG-2001

(99)

5.11 Experience with Human Exposure

Remark: case-report: A 34 year old man reported in 1985 recurrent swelling of the upper lips and gums associated with the presence of a fissured tongue since he was 10 years old. In 1980 episodes became more frequent and were caused by the ingestion of different foods, including wine, sausages, and "hot foods". Each time, remission occurred spontaneously within 2 weeks. The patient reacted positive in a double-blind challenge test with sodium benzoate (see chapter 4.3).

23-OCT-1995	Upon elimination of sodium benzoate and another food additive, tartrazine, from the usual diet, complete remission of the clinical manifestation occurred.	(57)

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-

7.1 End Point Summary**7.2 Hazard Summary****7.3 Risk Assessment**

I U C L I D D a t a S e t

(POTASSIUM BENZOATE; CAS: 582-25-2)

Existing Chemical	ID: 582-25-2
CAS No.	582-25-2
EINECS Name	potassium benzoate
EINECS No.	209-481-3
Molecular Formula	C7H6O2.K

Producer Related Part
Company: Bayer Corporation
Creation date: 21-OCT-1999

Substance Related Part

Company:	Bayer Corporation
Creation date:	21-OCT-1999

Memo: Bayer Corporation

Printing date: 10-AUG-2001
Revision date:
Date of last Update: 10-AUG-2001

Number of Pages: 21

Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SID

1.0.1 OECD and Company Information

Type: lead organisation
Name: American Chemistry Council (formerly Chemical Manufacturers Association), Benzoates HPV Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States

10-AUG-2001

Type: cooperating company
Name: ATOFINA Chemicals, Inc
Country: United States

10-AUG-2001

Type: cooperating company
Name: Bayer Corporation
Street: 100 Bayer Road
Town: PA 15205-9741 Pittsburgh
Country: United States

06-JUL-2000

Type: cooperating company
Name: DSM Fine Chemicals
Country: Netherlands

06-JUL-2000

Type: cooperating company
Name: Noveon, Inc.
Country: United States

10-AUG-2001

Type: cooperating company
Name: Velsicol Chemical Corporation
Country: United States

06-JUL-2000

1.0.2 Location of Production Site**1.0.3 Identity of Recipients**

1.1 General Substance Information**1.1.0 Details on Template****1.1.1 Spectra****1.2 Synonyms****1.3 Impurities****1.4 Additives****1.5 Quantity****1.6.1 Labelling****1.6.2 Classification****1.7 Use Pattern****1.7.1 Technology Production/Use****1.8 Occupational Exposure Limit Values****1.9 Source of Exposure****1.10.1 Recommendations/Precautionary Measures****1.10.2 Emergency Measures****1.11 Packaging****1.12 Possib. of Rendering Subst. Harmless****1.13 Statements Concerning Waste**

1.14.1 Water Pollution**1.14.2 Major Accident Hazards****1.14.3 Air Pollution****1.15 Additional Remarks****1.16 Last Literature Search**

Type of Search: Internal and External

Date of Search: 07-SEP-1999

Remark: Only HPV endpoints: TOXLINE data base and
internal studies

10-AUG-2001

1.17 Reviews**1.18 Listings e.g. Chemical Inventories**

2.1 Melting Point

Value: 330.6 degree C
Method: other: (calculated) MPBPWIN (v1.31) Program;
Adapted Joback
Method
Year: 1999
GLP: no
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

2.2 Boiling Point

Value: 464.9 degree C
Method: other: (calculated) MPBPWIN (v1.31) Program;
Adapted Stein and Brown Method
Year: 1999
GLP: no
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

2.3 Density

2.3.1 Granulometry

2.4 Vapour Pressure

Value: .00000000489 hPa at 25 degree C
Method: other (calculated): MPBPWIN (v1.31) Program;
Modified Grain Method
Year: 1999
GLP: no
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

2.5 Partition Coefficient

log Pow: -2.269
Method: other (calculated): Log Kow(version 1.65 estimate)
Year: 1999
GLP: no
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

2.6.1 Water Solubility

Value: 556 g/l at 20 degree C
Method: other
Testsubstance: other TS: sodium benzoate
Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
10-AUG-2001 (2)

Value: > 1000 g/l at 25 degree C
Method: other: (calculated) WSKOW v1.36 Program
Year: 1999
GLP: no
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

2.6.2 Surface Tension

2.7 Flash Point

2.8 Auto Flammability

2.9 Flammability

2.10 Explosive Properties**2.11 Oxidizing Properties****2.12 Additional Remarks**

3.1.1 Photodegradation

Type: air
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1560000 molecule/cm3
Rate constant: .0000000000017775 cm3/(molecule * sec)
Degradation: 50 % after 72.2 hour(s)
Method: other (calculated): AOP Program (v1.89)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

3.1.2 Stability in Water

Type:
Method:
Year: GLP:
Test substance:
Remark: Based on structure and organic chemistry rules
(e.g. bonding in organic molecules, activation
energy, reactivity, transformations, addition,
substitution, elimination) no hydrolysis will
occur at pH ranges 4 - 11.
26-JAN-2001

3.1.3 Stability in Soil

3.2 Monitoring Data (Environment)

3.3.1 Transport between Environmental Compartments

Type: fugacity model level III
Media: other: air - water - soil - sediment
Air (Level I):
Water (Level I):
Soil (Level I):
Biota (L.II/III):
Soil (L.II/III):
Method: other: EPIWin Modeling Program

Year:				
Result:	Distribution	Half-Life	Emissions	Fugacity
	(percent)	(hr)	(kg/hr)	(atm)
Air	1.61e-007	144	1000	4.83e-019
Water	45.3	360	1000	1.38e-020
Soil	54.6	360	1000	6.16e-019

Sediment 0.0755 1.44e+003 0

Persistence Time: 421 hr
 Reaction Time: 520 hr
 Advection Time: 2.21e+003 hr
 Percent Reacted: 80.9
 Percent Advected: 19.1

Reliability: (2) valid with restrictions

Accepted calculation method

Flag: Critical study for SIDS endpoint

10-AUG-2001

(1)

3.3.2 Distribution

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Type: aerobic

Inoculum:

Degradation: 80.9 % after 22 day

Method: other: (calculated) Fugacity Level III

Year: 1999 GLP: no

Test substance: other TS: molecular structure

Reliability: (2) valid with restrictions

Accepted calculation method

Flag: Critical study for SIDS endpoint

10-AUG-2001

(1)

Type: aerobic

Inoculum: activated sludge, domestic

Concentration: 50 mg/l related to Test substance

Degradation: ca. 90 % after 7 day

Result: readily biodegradable

Method: OECD Guide-line 301 B "Ready Biodegradability:

Modified Sturm Test (CO₂ evolution)"

Year: 1981 GLP: no data
 Test substance: other TS: sodium benzoate
 Remark: See IUCLID on sodium benzoate (CAS# 532-32-1); the biodegradation of the potassium salt would be similar to the sodium salt.
 Test condition: temperature = 25 degree C
 Reliability: (1) valid without restriction
 Guideline study
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (3)

Type: anaerobic
 Inoculum: other bacteria: anaerobic sewage, domestic and industrial
 Concentration: 50 mg/l related to DOC (Dissolved Organic Carbon)
 Degradation: 93 % after 7 day
 Method: other: see below
 Year: GLP: no data
 Test substance: other TS: sodium benzoate
 Method: 2-3 g sludge plus sodium benzoate (concentration equivalent to 50 mg Carbon/liter or 85 mg substance/l).
 Controls and tests done in triplicate.
 Temperature = 35 degree C.
 Measured gas production (CH₄ + CO₂).
 Remark: See IUCLID on sodium benzoate (CAS# 532-32-1); the biodegradation of the potassium salt would be similar to the sodium salt.
 Result: Degradation is expressed as percentage of theoretical methane production based on the stoichiometry of degradation.
 Reliability: (2) valid with restrictions
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (4)

Type:
 Inoculum:
 Method:
 Year: GLP:
 Test substance:
 Remark: See IUCLID on benzoic acid (CAS# 65-85-0); the potassium salt is expected to immediately dissociate and form benzoic acid in an aqueous environment.
 10-AUG-2001

3.6 BOD5, COD or BOD5/COD Ratio

3.7 Bioaccumulation

Species:
Exposure period:
Concentration:
BCF: 3.16
Elimination:
Method: other: (calculated) BCF Program (v2.13)
Year: GLP: no
Test substance: other TS: molecular structure
Result: Estimated Log BCF = 0.500 (BCF = 3.162)

Log Kow (estimated) : 1.87
Log Kow (experimental): 1.87
Log Kow used by BCF estimates: 1.87

Equation Used to Make BCF estimate:
Log BCF = 0.50 (Ionic; Log Kow dependent)
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

3.8 Additional Remarks

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other: ECOSAR calculations
Species: other: fresh water fish
Exposure period: 96 hour(s)
Unit: g/l Analytical monitoring: no
LC50: > 1000
Method: other: ECOSAR (v 0.99)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Remark: ECOSAR class: Neutral organics. Chemical may
not be soluble enough to measure the predicted
effect.

Result:	ECOSAR Class	Organism	Duration	End Pt	mg/L
=====					
	Neutral Organic SAR:	Fish	14-day	LC50	1.13e+006
		(Baseline Toxicity)			
	Neutral Organics:	Fish	96-hr	LC50	1.23e+006
	Neutral Organics:	Fish	14-day	LC50	1.13e+006
	Neutral Organics:	Fish	30-day	ChV	79360.031
Reliability:	(2) valid with restrictions Accepted calculation method				
Flag:	Critical study for SIDS endpoint				
10-AUG-2001	(1)				

Type:
Species:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.
10-AUG-2001

4.2 Acute Toxicity to Aquatic Invertebrates

Type:
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: g/l Analytical monitoring: no
EC50: 978
Method: other: ECOSAR (v 0.99)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Remark: ECOSAR class: Neutral organics. Chemical may not be soluble enough to measure the predicted effect.

Result: ECOSAR	Class	Organism	Duration	End Pt	mg/L

Neutral Organics: Daphnid	48-hr	LC50	9.78e+005
Neutral Organics: Daphnid	16-day	EC50	7746.435
Neutral Organics: Mysid Shrimp	96-hr	LC50	7.45e+006

Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

Type:
Species:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on sodium benzoate (CAS# 532-32-1); the toxicity of the potassium salt would be similar to the sodium salt.
Flag: Critical study for SIDS endpoint
10-AUG-2001

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green Algae
Endpoint: biomass
Exposure period: 96 hour(s)
Unit: g/l Analytical monitoring: no
EC50: 478
Method: other: ECOSAR (v 0.99)
Year: 1999 GLP: no

Test substance: other TS: molecular structure
Remark: ECOSAR class: Neutral organics.
Result: ECOSAR Class Organism Duration End Pt mg/L

=====
Neutral Organics: Green Algae 96-hr EC50 4.78e+005
Neutral Organics: Green Algae 96-hr ChV 4053.982
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type:
Species:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.
10-AUG-2001

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

4.6.3 Toxicity to other Non-Mamm. Terrestrial Species

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: > 10000 mg/kg bw
Method:
Year: GLP:
Test substance: other TS: potassium benzoate; purity not noted
Reliability: (4) not assignable
Original reference in foreign language
Flag: Critical study for SIDS endpoint
10-AUG-2001 (5)

Type: LD50
Species: mouse
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: > 10000 mg/kg bw
Method:
Year: GLP:
Test substance: other TS: potassium benzoate; purity not noted
Reliability: (4) not assignable
Original reference in foreign language
Flag: Critical study for SIDS endpoint
10-AUG-2001 (5)

Type: LD50
Species: guinea pig
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: > 10000 mg/kg bw
Method:
Year: GLP:

Test substance: other TS: potassium benzoate; purity not noted
Reliability: (4) not assignable
Flag: Original reference in foreign language
10-AUG-2001 Critical study for SIDS endpoint (5)

5.1.2 Acute Inhalation Toxicity

Type:
Species:
Strain:
Sex:
Number of
Animals:
Vehicle:
Exposure time:
Value:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on benzoic acid (CAS# 65-85-0); the
loss of acidity due to the potassium salt
should decrease toxicity.
10-AUG-2001

5.1.3 Acute Dermal Toxicity

Type:
Species:
Strain:
Sex:
Number of
Animals:
Vehicle:
Value:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on benzoic acid (CAS# 65-85-0); the
loss of acidity due to the potassium salt
should decrease toxicity.
10-AUG-2001

5.1.4 Acute Toxicity, other Routes

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species:

Concentration:

Exposure:

Exposure Time:

Number of
Animals:

PDII:

Result:

EC classificat.:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the irritating ability of the potassium salt
would be similar to the sodium salt.

10-AUG-2001

5.2.2 Eye Irritation

Species:

Concentration:

Dose:

Exposure Time:

Comment:

Number of
Animals:

Result:

EC classificat.:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the irritating ability of the potassium salt
would be similar to the sodium salt.

10-AUG-2001

5.3 Sensitization

5.4 Repeated Dose Toxicity

Species: Sex:
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.
10-AUG-2001

5.5 Genetic Toxicity 'in Vitro'

Type: Bacillus subtilis recombination assay
System of testing: Bacillus subtilis H17, M45
Concentration: 1-20 mg/disk; vehicle: water and ethanol
(1:1)
Cytotoxic Conc.:
Metabolic activation: with and without
Result: positive
Method:
Year: GLP:
Test substance: other TS: potassium benzoate; purity not noted
Result: Authors judged results as positive.
Reliability: (3) invalid
Significant methodological deficiencies: one
dose tested
Flag: Critical study for SIDS endpoint
10-AUG-2001 (6)

Type:
System of
testing:
Concentration:

Cytotoxic Conc.:

Metabolic

activation:

Result:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.

10-AUG-2001

5.6 Genetic Toxicity 'in Vivo'

Type: Cytogenetic assay

Species:

Sex:

Strain:

Route of admin.:

Exposure period:

Doses:

Result:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.

10-AUG-2001

5.7 Carcinogenicity

Species:

Sex:

Strain:

Route of admin.:

Exposure period:

Frequency of
treatment:

Post. obs.
period:

Doses:

Result:

Control Group:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.

10-AUG-2001

5.8 Toxicity to Reproduction

Type:

Species:

Sex:

Strain:

Route of admin.:

Exposure Period:

Frequency of

treatment:

Duration of test:

Doses:

Control Group:

Method:

Year:

GLP:

Test substance:

Remark: A 4-generation reprotoxicity test with benzoic
acid revealed no reproductive effects. Therefore
no indication for reprotoxicity for the benzoic
acid potassium salt.
See IUCLID on benzoic acid (CAS# 65-85-0); the
loss of acidity due to the potassium salt should
decrease toxicity.

10-AUG-2001

5.9 Developmental Toxicity/Teratogenicity

Species:

Sex:

Strain:

Route of admin.:

Exposure period:

Frequency of

treatment:

Duration of test:

Doses:

Control Group:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.

10-AUG-2001

5.10 Other Relevant Information

5.11 Experience with Human Exposure

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7.1 End Point Summary

7.2 Hazard Summary

7.3 Risk Assessment

I U C L I D D a t a S e t

(BENZYL ALCOHOL; CAS: 100-51-6)

Existing Chemical ID: 100-51-6
CAS No. 100-51-6
EINECS Name benzyl alcohol
EC No. 202-859-9
TSCA Name Benzenemethanol
Molecular Formula C7H8O

Producer Related Part

Company: Bayer Corporation
Creation date: 15-JUL-1999

Substance Related Part

Company: Bayer Corporation
Creation date: 15-JUL-1999

Memo: Bayer Corporation

Printing date: 14-FEB-2002
Revision date:
Date of last Update: 14-FEB-2002

Number of Pages: 82

Chapter (profile): Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 Applicant and Company Information

Type: lead organisation
Name: American Chemistry Council, Benzoates Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States

14-DEC-2000

Type: cooperating company
Name: B.F. Goodrich
Country: United States

26-MAY-2000

Type: cooperating company
Name: Bayer Corporation
Country: United States

14-DEC-2000

Type: cooperating company
Name: DSM Fine Chemicals
Country: Netherlands

14-DEC-2000

Type: cooperating company
Name: Elf Atochem NA
Country: United States

26-MAY-2000

Type: cooperating company
Name: Velsicol Chemical Corporation
Country: United States

26-MAY-2000

Type: lead organisation
Name: American Chemistry Council, Benzoates Panel

16-JAN-2001

1.0.2 Location of Production Site, Importer or Formulator**1.0.3 Identity of Recipients****1.0.4 Details on Category/Template****1.1.0 Substance Identification****1.1.1 General Substance Information****1.1.2 Spectra****1.2 Synonyms and Tradenames****1.3 Impurities****1.4 Additives****1.5 Total Quantity****1.6.1 Labelling****1.6.2 Classification****1.6.3 Packaging****1.7 Use Pattern****1.7.1 Detailed Use Pattern****1.7.2 Methods of Manufacture****1.8 Regulatory Measures****1.8.1 Occupational Exposure Limit Values****1.8.2 Acceptable Residues Levels**

1.8.3 Water Pollution**1.8.4 Major Accident Hazards****1.8.5 Air Pollution****1.8.6 Listings e.g. Chemical Inventories****1.9.1 Degradation/Transformation Products****1.9.2 Components****1.10 Source of Exposure****1.11 Additional Remarks****1.12 Last Literature Search****1.13 Reviews**

2.1 Melting Point

Value: -15.2 degree C

Method: other: Handbook value

Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Data from Handbook or collection of data

Flag: Critical study for SIDS endpoint
14-FEB-2002 (1)

Value: -15.3 degree C

Test substance: other TS: benzyl alcohol, purity not noted

12-FEB-2002 (2)

2.2 Boiling Point

Value: 205.3 degree C at 1013 hPa

Method: other: Handbook value

Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Data from Handbook or collection of data

Flag: Critical study for SIDS endpoint
14-FEB-2002 (1)

Value: 205.4 degree C at 1013 hPa

19-JAN-2001 (2)

2.3 Density

Type: density

Value: 1.041 g/cm³ at 24 degree C

Method: other: Handbook value

Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Data from Handbook or collection of data

Flag: Critical study for SIDS endpoint
14-FEB-2002 (1)

Type: density
Value: 1.0442 g/cm³ at 22.5 degree C
19-JAN-2001 (2)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: .03 hPa at 20 degree C
Test substance: other TS: benzyl alcohol, purity not noted
Flag: Critical study for SIDS endpoint
12-FEB-2002 (2)

Value: .09 hPa at 30 degree C
Test substance: other TS: benzyl alcohol, purity not noted
Flag: Critical study for SIDS endpoint
12-FEB-2002 (2)

Value: .67 hPa at 50 degree C
Flag: Critical study for SIDS endpoint
19-JAN-2001 (2)

2.5 Partition Coefficient

log Pow: 1.1
Method: other (calculated): Leo, A.: CLOGP-3.54 MedChem Software 1989. Daylight, Chemical Information Systems, Claremont, CA 91711, USA
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint

06 - JUN - 2001 (3)

log Pow: 1.1

Method: other (measured)

Remark: experimentally determined

Flag: Critical study for SIDS endpoint
14 - FEB - 2002 (4)

2.6.1 Solubility in different media

Solubility in: Water
Value: 40 g/l at 20 degree C

Flag: Critical study for SIDS endpoint
14 - FEB - 2002 (5)

Solubility in: Water
Value: 44 g/l at 50 degree C

Flag: Critical study for SIDS endpoint
14 - FEB - 2002 (5)

2.6.2 Surface Tension

2.7 Flash Point

Value: 101 degree C
Type: closed cup

Method: other: DIN 51758

19 - JAN - 2001 (5)

2.8 Auto Flammability

Value:

Remark: ignition temperature: 435 degree C
19 - JAN - 2001 (2)

2.9 Flammability**2.10 Explosive Properties**

Result: other: explosive limits: lower 1.3 % by vol.,
upper 13.0 % by vol. at 170 degree C and 1.013
bar

19-JAN-2001

(2)

2.11 Oxidizing Properties**2.12 Dissociation Constant****2.13 Viscosity****2.14 Additional Remarks**

3.1.1 Photodegradation

Type: air
Light source: Sun light
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1560000
Rate constant: .0000000000082541 cm³/(molecule * sec)
Degradation: 50 % after 1.3 day(s)

Method: other (calculated): AOPWin version 1.89
Year: 1999
GLP: no
Test substance: other TS: molecular structure

Remark: Experimental Database Structure Match:
experimental OH rate constant= 22.9 E-12
cm³/molecule-sec.

Reliability: (2) valid with restrictions
Accepted calculation method

Flag: Critical study for SIDS endpoint
14-FEB-2002 (6)

3.1.2 Stability in Water

Remark: Based on structure and organic chemistry rules
(e.g. bonding in organic molecules, activation
energy, reactivity, transformations, addition,
substitution, elimination) no hydrolysis will
occur at pH ranges 4 - 11.

Flag: Critical study for SIDS endpoint
26-JAN-2001

3.1.3 Stability in Soil**3.2.1 Monitoring Data (Environment)****3.2.2 Field Studies**

3.3.1 Transport between Environmental Compartments

Type: fugacity model level III
 Media: other: other: air - water - soil - sediment
 Method: other: EPIWin Modeling Program

Remark: Modeling was performed using equal releases (10,000 kg/hr) and equal distribution to all compartments.

Result:	Distribution (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	1.51	11.2	1000	2.95e-011
Water	50.0	360	1000	6.71e-012
Soil	48.4	360	1000	1.7 e-010
Sediment	0.0923	1440	0	5.52e-012

Persistence Time: 287 hr
 Reaction Time: 353 hr
 Advection Time: 1.54e+003 hr
 Percent Reacted: 81.3
 Percent Advected:

Reliability: (2) valid with restrictions
 Flag: Critical study for SIDS endpoint
 14-FEB-2002

(6)

3.3.2 Distribution**3.4 Mode of Degradation in Actual Use****3.5 Biodegradation**

Type: aerobic
 Inoculum: activated sludge
 Concentration: 100 mg/l
 Degradation: 92 - 96 % after 28 day(s)

Method: OECD Guide-line 301 C "Ready Biodegradability:
 Modified

MITI Test (I)"

Year: 1981

GLP: no data

Test substance: other TS: benzyl alcohol, purity not noted

Remark: slugde conc.: 30 mg/l

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
14-FEB-2002 (7)

Type: aerobic
Inoculum: predominantly domestic sewage
Degradation: > 90 % after 30 day(s)
Method: OECD Guide-line 301 D "Ready Biodegradability:
Closed Bottle Test"
Year: 1972
GLP: no
Test substance: other TS: benzyl alcohol, purity not noted

Remark: related to BOD
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
29-JAN-2001 (8)

Type: anaerobic
Inoculum: anaerobic sludge
Contact time: 28 day(s)
Degradation: 100 % after 7 day(s)
Result: readily biodegradable

Method: other: see below
Year: 1982
GLP: no data
Test substance: other TS: commercial grade benzyl alcohol,
purity > 95%

Method: A 10% anaerobic sludge inoculum was transferred to 160 ml serum bottles previously amended with 50 ppm carbon (related to test substance) using strict anaerobic techniques. Methane production from test bottles vs. controls was monitored weekly for 4 weeks or until net production occurred. At that time, the bottles were amended again with the same substrate and methane production monitored to confirm the observation. All data were obtained from duplicate bottles. Methane was measured using a flame ionization detector on a Perkin-Elmer Model 900 GC equipped with a 3-m Tenax-G.C. column.

Remark:	100 % mineralisation (CH ₄ -Production) in 1 week with sludge from Jackson, MI waste-treatment plant 100 % mineralisation (CH ₄ -Production) in 2 weeks with sludge from Adrian, MI waste-treatment plant
Test condition:	The test bottles were incubated at 35 degree C in the dark. Substrates were kept under an atmosphere of 90% N ₂ and 10% H ₂
Reliability:	(2) valid with restrictions
Flag:	Critical study for SIDS endpoint
23-MAR-2001	(9)
Type:	anaerobic
Inoculum:	domestic sewage
Concentration:	50 µg/l related to DOC (Dissolved Organic Carbon)
Contact time:	2 month
Degradation:	> 75 % after 2 month
Method:	other: see below
Year:	1984
GLP:	no data
Test substance:	other TS: benzyl alcohol, purity not noted
Method:	Sludge samples collected from primary and secondary anaerobic digesters were diluted to 10 % and incubated anaerobically with 50 ug Carbon per ml (related to test substance). All compounds were tested in triplicate. Gas production was measured by gas chromatography and by a pressure transducer. Biodegradation was determined by net increase in gas pressure in bottles amended with test chemicals over non-amended controls.
Result:	Degradation is expressed as percentage of theoretical methane production based on the stoichiometry of degradation.
Test condition:	The test bottles were incubated at 35 degree C in the dark. Substrates were kept under atmospheres of 10% CO ₂ and 90% N ₂ .
Reliability:	(2) valid with restrictions
Flag:	Critical study for SIDS endpoint
23-MAR-2001	(10)
Type:	aerobic
Degradation:	62 % after 5 day(s)

Method:	OECD Guide-line 301 D	"Ready Biodegradability:
Closed		
GLP:	Bottle Test"	
	no	
Remark:	related to ThOD	
19-JAN-2001		(11)
Type:	aerobic	
Degradation:	77 % after 20 day(s)	
Method:	OECD Guide-line 301 D	"Ready Biodegradability:
Closed		
GLP:	Bottle Test"	
	no	
Remark:	related to ThOD	
19-JAN-2001		(11)
Type:	aerobic	
Inoculum:	activated sludge, adapted	
Degradation:	95 % after 28 day(s)	
Method:	other: Closed bottle test	
Remark:	Test concentration: 2 - 5 mg/l	
	Degradation related to ThOD	
19-JAN-2001		(12)
Type:	aerobic	
Inoculum:	domestic sewage	
Degradation:	89.2 % after 5 day(s)	
Method:	other: respirometric diluting method	
GLP:	no	
Remark:	related to ThOD	
19-JAN-2001		(13)
Type:	aerobic	
Inoculum:	activated sludge, industrial	
Degradation:	88.9 % after 5 day(s)	
Test substance:	other TS	

Method: Radio-respirometric study using radio-labeled chemicals by activated sludge and in a complex photographic processing effluent using acclimated industrial sludge. Concentration of test substance was 0.1 or 0.2ml of radioactive substrate(27,000-400,000 dpm). Samples were incubated in the dark at ambient temperature.

Remark: ¹⁴C02 recovery without effluent = 85.7% after 5 days
¹⁴C02 recovery in presence of effluent = 88.9% after 5 days

Test substance: benzyl-alcohol-7-¹⁴C (carbinol-¹⁴C) obtained from New England Nuclear Corporation, Boston, Massachusetts.

17 - JAN - 2001 (14)

Type: aerobic
Degradation: 85 % after 5 day(s)

GLP: no

Remark: related to ThOD
19 - JAN - 2001 (15)

Remark: The activity of degradation is at a concentration of 100 mg/l not hindered in a model plant (Ascomat)
19 - JAN - 2001 (8)

Remark: Biodegradation characteristics: biodegraded completely in a short time by general microorganisms.
19 - JAN - 2001 (16)

3.6 BOD5, COD or BOD5/COD Ratio

Method:
Year:
Method:
Remark: ThOD: 2515.1 mg/l
19 - JAN - 2001 (13)

3.7 Bioaccumulation

BCF: .31

Method: other: (calculated) BCF Program (v2.13)
Year: 1999
Test substance: other TS: molecular structure

Result: Estimated Log BCF = -0.503 (BCF = 0.3141)
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
14-FEB-2002 (6)

3.8 Additional Remarks

Remark: ThOD 2520 mg/g
COD 2520 mg/g
BOD5 1560 mg/g
Influence on biological purification plants:
adapted 1180 mg/l degradable
27-MAY-1993 (17)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: 460

Method: other: see below
Year: 1976
GLP: no data
Test substance: other TS: reagent grade benzyl alcohol
purchased from Curtin Matheson Scientific,
Inc.

Method: Juvenile fathead minnows were obtained from Environmental Reserach Laboratory, Duluth. All fish used for the test were 4 to 8 weeks of age, 1.1 to 3.1 cm in length, and acclimated for at least 48 hr before testing. Test solutions were prepared by adding a weighed amount of chemical to 4 liters of Lake Superior water (all concentrations are nominal). Water temperature during the test was 18-22 degree C. Range-finding tests were done and definitive tests were conducted with 10 fish per container, 20 fish per concentration. Complete immobilization was considered the biological endpoint and equated with death. Standard graphical procedures were followed to determine LC50 (American Public Health Assn., 1971) Analyses of test water was done for dissolved oxygen and pH at the beginning and 1 or 2 times during the test.

Result: 1 hour LC50 = 770 mg/l
24 hour LC50 = 770 mg/l
48 hour LC50 = 770 mg/l
72 hour LC50 = 480 mg/l
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

23-MAR-2001

(18)

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
LC0: 630
LC50: 646
LC100: 662

Method: other: Bestimmung der Wirkung von
Wasserinhaltsstoffen auf
Fische, DIN 38412 Teil 15
Year: 1983
GLP: no
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
12-FEB-2002 (19)

Type: static
Species: Petromyzon marinus
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: >= 5
GLP: no

Remark: larvae; screening test
17-JAN-2001 (20)

Species: Carassius auratus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
LC0: >= 5
17-JAN-2001 (21)

Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
LC0: 136
GLP: no

Remark: Testing of acute oral toxicity
Unit: mg/kg

17 - JAN - 2001 (22)

Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
LC0: >= 5

17 - JAN - 2001 (21)

Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring:
LC50: 10

Remark: The static test was directed to simulate acute spill circumstances. The test substances were pipetted or poured undiluted directly into the aquaria with fish. There was no preparation of defined concentrations according to guideline. No analytical monitoring was done. Aeration was not used during the first 24 hrs thus allowing chemicals to act in an uninterrupted state at the onset of the test period.

Reliability: (4) not assignable
Significant methodological deficiencies

12 - FEB - 2002 (23)

Species: Menidia beryllina (Fish, estuary, marine)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring:
LC50: 15

Remark: The static test was directed to simulate acute spill circumstances. The test substances were pipetted or poured undiluted directly into the aquaria with fish. There was no preparation of defined concentrations according to guideline. No analytical monitoring was done. Aeration was not used during the first 24 hrs thus allowing chemicals to act in an uninterrupted state at the onset of the test period.

Reliability: (4) not assignable
Significant methodological deficiencies

12 - FEB - 2002 (23)

Species: Salmo trutta (Fish, fresh water, marine)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
LC0: >= 5

17-JAN-2001 (21)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
EC0: 300
EC50: 400
EC100: 500

Method: other: Daphnien-Kurzzeittest, DIN 38412 Teil
11, Bestimmung der Wirkung von
Wasserinhaltsstoffen auf Kleinkrebse

Year: 1983

GLP: no

Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
14-FEB-2002 (19)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
TGK : 360

Method: other: acute immobilisation test
GLP: no

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
06-JUN-2001 (24)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
EC0: 26
EC50: 55
EC100: 100

GLP: no

Reliability: (2) valid with restrictions
16-JAN-2001 (25)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Chlorella pyrenoidosa (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: 95

Method: other: see below
Year: 1982
GLP: no data
Test substance: other TS: benzyl alcohol purchased from
Aldrich Chemical Co. Wisconsin, USA. Purity >
95%

Method: Photosynthesis was assayed by following the uptake of $^{14}\text{C}\text{O}_2$ from $\text{NaH}^{14}\text{CO}_3$ (Amersham/Searle, Ontario, Canada). Plastic culture flasks containing 9.9 ml of cell suspension (1.0×10^5 cells/ml), 0.1 ml radioisotope and 0.01 ml of test chemical were incubated for 3 hours. Five concentrations, ranging from 0 to 100 ppm, were tested and replicated five times. Photosynthetic activity was assayed according to Stratton et al. (1979) Appl. Environ. Microbiol. 38: 537-43. Per cent inhibition values were calculated relative to photosynthetic activity in the solvent controls and EC50 values determined by Probit analysis. Analyses for significant differences were performed using Dunnett's test and Duncan's multiple range test.

Test condition: Cultures were maintained in a liquid nitrogen-free medium at 20 degree C and a light intensity of 7000 lux on a 12 hour light-dark cycle.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

14-FEB-2002

(26)

Species: Haematococcus pluvialis (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 4 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: 2600

Method: other: according to Tuempling v.W. (Fortschritte
Der Wasserchemie. 14 S: 205-213 (1972) using a
Warburg apparatus

GLP: no
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

29-JAN-2001

(19)

Species: Scenedesmus quadricauda (Algae)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring:
TGK : 640

Method: other: cell multiplication inhibition test

Remark: green algae
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

06-JUN-2001

(24)

Species: Anabaena cylindrica (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 90

Remark: blue-green algae

17-JAN-2001

(26)

Species: Anabaena inaequalis (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC0: 30

Remark: blue-green algae (26)
17-JAN-2001

Species: Anabaena variabilis (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 35

Remark: blue-green algae (26)
17-JAN-2001

Species: Scenedesmus quadricauda (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 79

GLP: no

Remark: green algae (26)
17-JAN-2001

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: Escherichia coli (Bacteria)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
EC0: 1000

Method: other: cell multiplication test
GLP: no

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint (24)
06-JUN-2001

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Unit: mg/l Analytical monitoring: no
EC10: 658

Method: other: Test according to Bringmann and Kuehn
(cell multiplication inhibition test)

GLP:	no	
Remark:	Exposure period: 16-18 h	
Reliability:	(2) valid with restrictions	
Flag:	Critical study for SIDS endpoint	
06-JUN-2001		(19)
Type:	aquatic	
Species:	Photobacterium phosphoreum (Bacteria)	
Exposure period:	30 minute(s)	
Unit:	mg/l	Analytical monitoring: no
EC50:	71.42	
Method:	other: Microtox	
GLP:	no	
19-JAN-2001		(27)
Type:	aquatic	
Species:	Photobacterium phosphoreum (Bacteria)	
Exposure period:	5 minute(s)	
Unit:	mg/l	Analytical monitoring: no
EC50:	50	
GLP:	no	
19-JAN-2001		(28)
Type:	aquatic	
Species:	other bacteria: Aerobic heterotrophic	
Exposure period:	49 hour(s)	
Unit:	mg/l	Analytical monitoring:
IC50 :	2100	
GLP:	no	
Remark:	Inhibition of respiration;	
	prolonged incubation compared with ISO 8192	
19-JAN-2001		(29)
Type:	aquatic	
Species:	other bacteria: Nitrosomonas	
Exposure period:	24 hour(s)	
Unit:	mg/l	Analytical monitoring:
IC50 :	390	

Method: other: Inhibition of nitrification, comparable
with ISO/DIS 9509
GLP: no

Remark: Inhibition of N-oxidation
19-JAN-2001 (29)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

4.6.3 Toxicity to Soil Dwelling Organisms

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

Remark:

Aedes aegypti, eggs	(72h)	LD50	160 l/ha	
		LD90	251 l/ha	
Aedes aegypti, larval stage L1	(24h)	LD50	105 l/ha	
		LD90	132 l/ha	Aedes
aegypti, larval stage L3-L4	(24h)	LD50	129 l/ha	
		LD90	184 l/ha	
Aedes scutellaris, eggs	(72h)	LD50	160 l/ha	
		LD90	265 l/ha	
Aedes scutellaris, larval stage L1	(24h)	LD50	110 l/ha	
		LD90	151 l/ha	
Aedes scutellaris, larval stage L3-L4	(24h)	LD50	126 l/ha	
		LD90	172 l/ha	
19-JAN-2001				(30)

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Sex: male
Value: = 1610 mg/kg bw

Method: other
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
12-FEB-2002 (31)

Type: LD50
Species: mouse
Sex: male/female
No. of Animals: 10
Vehicle: other: corn oil
Value: = 1580 mg/kg bw

Method: other: see below
GLP: no data
Test substance: other TS: commercial grade benzyl alcohol

Method: Mice were dosed on full stomachs by intubation.
All animals were observed for toxic signs and
time of death for 2 weeks.
The LD50 was computed by the method of
Litchfield & Wilcoxon(1949).
Remark: Toxic signs: depression, death
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
12-FEB-2002 (32) (33)

Type:	LD50
Species:	rat
Strain:	other: Osborne-Mendel
Sex:	male/female
No. of Animals:	10
Vehicle:	other: neat
Value:	= 1230 mg/kg bw
Test substance:	other TS: commercial grade benzyl alcohol
Method:	Groups of 10 young adult Osborne-Mendel rats, evenly divided by sex were fasted for approximately 18 hrs prior to treatment. Animals were dosed by intubation. All animals were observed for toxic signs and time of death for 2 weeks. The LD50 was computed by the method of Litchfield & Wilcoxon (1949).
Remark:	Toxic signs: depression, excitability, coma, death
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment
12-FEB-2002	(32)
Type:	LD50
Species:	rat
Value:	= 2080 mg/kg bw
Method:	other: no data
GLP:	no data
Test substance:	other TS: benzyl alcohol, purity not noted
Reliability:	(4) not assignable Secondary literature; Original reference not available
Flag:	Critical study for SIDS endpoint
12-FEB-2002	(34) (33)
Type:	LD50
Species:	rabbit
Value:	= 1040 mg/kg bw
12-FEB-2002	(34) (35)
Type:	LD50

Species: rat
Value: = 3100 mg/kg bw

16 - JAN-2001 (36)

Type: LDLo
Species: rat
Value: ca. 1040 - 3120 mg/kg bw

16 - JAN-2001 (37)

Type: LD50
Species: mouse
Value: = 1150 mg/kg bw

16 - JAN-2001 (38)

Type: LDLo
Species: mouse
Value: ca. 1040 mg/kg bw

16 - JAN-2001 (37)

Type: LDLo
Species: guinea pig
Value: ca. 1040 - 2600 mg/kg bw

16 - JAN-2001 (37)

5.1.2 Acute Inhalation Toxicity

Type: LC50
Species: rat
Exposure time: 4 hour(s)
Value: > 4.178 mg/l

Method: other
GLP: no data

Test substance: other TS: benzyl alcohol, purity not noted

Flag: Critical study for SIDS endpoint
12-FEB-2002 (39)

Type: LC50
Species: rat

Exposure time: 4 hour(s)
Value: ca. 8.8 mg/l

Remark: Extrapolation according to Haber`s law: LC50
(8h) = 1000 ppm.
19-JAN-2001 (36)

Type: LC50
Species: rat
Exposure time: 4 hour(s)
Value: > .9 mg/l

Remark: LC33 (4h) = 200 ppm.
19-JAN-2001 (40)

Type: LC50
Species: rat
Sex: no data
No. of Animals: 6
Vehicle: other: neat
Exposure time: 4 hour(s)
Value: 8.9 mg/l

Test substance: no data

Result: Exposure to 2000 ppm kills either 2/6, 3/6 or
4/6 rats.
Therefore benzyl alcohol is considered to be
of moderate toxicity.
07-SEP-2000 (41)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Value: = 2000 mg/kg bw

Method: other
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Flag: Critical study for SIDS endpoint
29-JAN-2001 (42)

Type: LD50
Species: guinea pig
Value: < 5 ml/kg bw

Method: other
GLP: no data

Test substance: other TS: benzyl alcohol, purity not noted

Flag: Critical study for SIDS endpoint
29-JAN-2001 (43) (35)

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: rat
Route of admin.: i.p.
Value: > 400 - 800 mg/kg bw

19-JAN-2001 (44)

Type: LD50
Species: mouse
Strain: CD-1
Sex: male
Route of admin.: i.p.
Value: = 1000 mg/kg bw

Remark: Acute toxicity after 4 h.
14-FEB-2002 (45)

Type: LD50
Species: mouse
Strain: CD-1
Sex: male
Route of admin.: i.p.
Value: = 650 mg/kg bw

Test substance: other TS: benzyl alcohol, purity not noted

Remark: Acute delayed toxicity after 7 d.
14-FEB-2002 (45)

Type: LD50
Species: guinea pig
Route of admin.: i.p.
Value: > 400 - 800 mg/kg bw

19-JAN-2001 (44)

Type: LD50
Species: rat
Route of admin.: s.c.
Value: = 1700 mg/kg bw

Test substance: other TS: benzyl alcohol, purity not noted

14 -FEB-2002 (46)

Type: LD50
Species: mouse
Route of admin.: s.c.
Value: = 950 mg/kg bw

19 -JAN-2001 (38)

Type: other: LDLO
Species: rabbit
Route of admin.: s.c.
Value: ca. 2080 mg/kg bw

19 -JAN-2001 (37)

Type: LD50
Species: rat
Route of admin.: i.v.
Value: = 314 mg/kg bw

19 -JAN-2001 (47)

Type: LD50
Species: rat
Route of admin.: i.v.
Value: = 53 mg/kg bw

Remark: Rapid injection
19 -JAN-2001 (47)

Type: LD50
Species: mouse
Route of admin.: i.v.
Value: = 324 mg/kg bw

19 -JAN-2001 (48)

Type: LD50
Species: mouse
Route of admin.: i.v.
Value: ca. 105 mg/kg bw

Remark: LD50 value depends on speed of injection
19 -JAN-2001 (49)

Type: LD50
Species: mouse
Route of admin.: i.v.
Value: = 1460 mg/kg bw

19 - JAN - 2001 (47)

Type: other: LDLO
Species: mouse
Strain: CD-1
Sex: male
Route of admin.: i.v.
Value: ca. 135 mg/kg bw

14 - FEB - 2002 (50)

Type: other: LDLO
Species: dog
Route of admin.: i.v.
Value: ca. 50 mg/kg bw

19 - JAN - 2001 (47)

Type: LD50
Species: rat
Route of admin.: other
Value: = 410 mg/kg bw

Remark: Application: intra-arterial.
19 - JAN - 2001 (47)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Result: not irritating

Method: OECD Guide-line 404 "Acute Dermal
Irritation/Corrosion"
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
14 - FEB - 2002 (49)

Species: rabbit
Concentration: 10 other: mg
Exposure Time: 24 hour(s)
Result: slightly irritating

Method:	other: see remarks	
GLP:	no data	
Test substance:	other TS: benzyl alcohol, purity not noted	
Flag:	Critical study for SIDS endpoint	
14-FEB-2002		(51) (36)
Species:	rabbit	
Exposure:	Open	
Exposure Time:	24 hour(s)	
Result:	moderately irritating	
Method:	other: see remarks	
Remark:	Exposure time: 24 h, clipped skin, 100	
mg/animal, open,	observation time: 72 h.	
14-FEB-2002		(52)
Species:	rabbit	
Result:	not irritating	
Method:	other: see remarks	
Remark:	Exposure time: 24 h, ear, ca. 500 mg/animal,	
19-JAN-2001	semi-occlusive, observation time: 7 d.	(53)
Species:	guinea pig	
Result:	moderately irritating	
Method:	other: see remarks	
Remark:	Exposure time: 24 h, depilated skin, dose:	
19-JAN-2001	undiluted material, no other data, open,	
	observation time: no data.	(44)
Species:	guinea pig	
Result:	slightly irritating	
Method:	other: see remarks	
Remark:	Exposure time: 24 h, clipped flank,	
19-JAN-2001	dose: 8 mg/animal (30 % in unspecified	
	solvent), open, observation time: no data.	(54)
Species:	guinea pig	
Result:	slightly irritating	

Method: other: see remarks

Remark: Exposure time: 24 h, shaved flanks,
dose: 26 mg/animal (25 % unspecified solvent),
intradermally, observation time: no data.
19-JAN-2001 (55)

Species: guinea pig
Result: not irritating

Method: other: see remarks

Remark: Exposure time: 24 h, clipped skin,
100 mg/animal, open, observation time: 72 h.
19-JAN-2001 (52)

Species: human
Result: irritating

Method: other: Closed Patch Test

Remark: Observation time: 24/48 h, 0.05 % in either
ethanol or a cream base produced irritation in
18 of 614 subjects.
19-JAN-2001 (56)

Species: human
Result: irritating

Method: other: Uncovered Patch Test

Remark: 0.5 % in petrolatum induced contact urticaria
in 7 of 32 volunteers.
19-JAN-2001 (57)

Species: human
Result: slightly irritating

Method: other: Patch Test

Remark: Exposure time: 48 h, ca. 50 mg/person (30 % in
acetone), observation time: up to 120 h.
19-JAN-2001 (52)

Species: other: Male nude mouse
Result: highly irritating

Method: other: see remarks

Remark: Exposure time: 24 h, 10 % in purified water,
occlusive, observation time: no data.
19-JAN-2001 (58)

Species: other: mini-pig
Result: not irritating

Method: other: Patch Test

Remark: Exposure time: 48 h, clipped skin,
50 mg/animal, observation time: no data.
19-JAN-2001 (52)

5.2.2 Eye Irritation

Species: rabbit
Result: moderately irritating

Method: OECD Guide-line 405 "Acute Eye
Irritation/Corrosion"
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
14-FEB-2002 (49)

Species: rabbit
Result: highly irritating

Method: other: see remarks
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Remark: Exposure time: 24 h, dose: 750 microg., no other
data.
Flag: Critical study for SIDS endpoint
14-FEB-2002 (51) (36)

Species: rabbit
Concentration: 4 %
Result: not irritating

Method: other: see remarks
Test substance: other TS: benzyl alcohol, purity not noted

Remark: 4 % aqueous solution, tested for stability, no
other data.
Flag: Critical study for SIDS endpoint
14-FEB-2002 (59)

Species: rabbit
Result: not irritating
Method: other: see remarks
Remark: Exposure time: 4 d, 2 drops of a 0.08 %
aqueous solution, no other data.
19-JAN-2001 (38)

Species: rabbit
Result: moderately irritating
Method: other: see remarks
Remark: ca. 100 mg/animal, observation time: 7 d.
19-JAN-2001 (53)

5.3 Sensitization

Type: Draize Test
Species: guinea pig
Result: not sensitizing
Test substance: other TS: benzyl alcohol, purity not noted
Flag: Critical study for SIDS endpoint
14-FEB-2002 (54)

Type: Guinea pig maximization test
Species: guinea pig
Result: not sensitizing
Test substance: other TS: benzyl alcohol, purity not noted
Flag: Critical study for SIDS endpoint
14-FEB-2002 (54)

Type: Freund's complete adjuvant test
Species: guinea pig
Result: sensitizing
Test substance: other TS: benzyl alcohol, purity not noted
Flag: Critical study for SIDS endpoint
14-FEB-2002
(54)

Type:	Open epicutaneous test
Species:	guinea pig
Result:	sensitizing
Test substance:	other TS: benzyl alcohol, purity not noted
Flag:	Critical study for SIDS endpoint
14-FEB-2002	(54)
Type:	Patch-Test
Species:	human
Result:	sensitizing
Test substance:	other TS: benzyl alcohol, purity not noted
Remark:	Maximum incidence of sensitization: 1 %.
Flag:	Critical study for SIDS endpoint
14-FEB-2002	(60) (61) (62)
Type:	Patch-Test
Species:	human
Result:	sensitizing
Test substance:	other TS: benzyl alcohol, purity not noted
14-FEB-2002	(63) (64)
Type:	Patch-Test
Species:	human
Result:	ambiguous
Test substance:	other TS: benzyl alcohol, purity not noted
14-FEB-2002	(57)
Type:	Patch-Test
Species:	human
Test substance:	other TS: benzyl alcohol, purity not noted
Remark:	Two patients with contact dermatitis were found to be sensitised by benzyl alcohol: 1 per cent in petrolatum
14-FEB-2002	(65)
Type:	Patch-Test
Species:	human
Test substance:	other TS: benzyl alcohol, purity not noted

Remark: A previously to balsam of Peru sensitised patient reacted on patch testing with benzyl alcohol: 0.5 per cent in olive oil.
14-FEB-2002 (66)

Type: other
Species: laboratory animal

Method: other: additional animal studies are reported

Test substance: other TS: benzyl alcohol, purity not noted
14-FEB-2002 (68) (69) (70) (67)

Type: other
Species: human

Method: other: additional data
Test substance: other TS: benzyl alcohol, purity not noted
14-FEB-2002 (71) (72) (73) (74) (75) (76) (77) (78) (79) (80)
(81) (82) (83) (84) (85) (86) (87) (88) (89) (90)
(91) (92) (93) (94) (95) (96)

Type: other: Application to shaved skin
Species: guinea pig
Result: not sensitizing

Test substance: other TS: benzyl alcohol, purity not noted
14-FEB-2002 (38)

Type: other: Intradermal application
Species: guinea pig
Result: not sensitizing

Test substance: other TS: benzyl alcohol, purity not noted
14-FEB-2002 (38)

Type: other: Maximization Test
Species: human
Result: not sensitizing

Test substance: other TS: benzyl alcohol, purity not noted
14-FEB-2002 (97)

5.4 Repeated Dose Toxicity

Type: Sub-chronic
Species: rat Sex: male/female
Strain: other: F344/N
Route of administration: gavage
Exposure period: 13 w

Frequency of treatment: daily
Post exposure period: no
Doses: 50, 100, 200, 400, 800 mg/kg/d
Control Group: yes
NOAEL: 400 mg/kg bw

Year: 1981
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity =99%)

Method: Groups of 10 rats of each sex were administered 0, 50, 100, 200, 400, or 800 mg/kg benzyl alcohol in corn oil by gavage, 5 days/week for 13 weeks (dose volume = 5 ml/kg). Rats were housed five/cage with feed and water available ad libitum. Animals were observed twice daily; moribund animals were sacrificed. Animal weights were recorded weekly. At the end of the study, survivors were sacrificed. A necropsy was performed on all animals; histologic exams performed on all vehicle controls and animals in the 800 mg/kg group. Brains were examined from rats in the 400 mg/kg group.

Remark: Biochemistry and hematology studies were not performed.

Result: 8/10 male rats dosed with 800 mg/kg died during w 7 and 8. Rats of the high dose group exhibited clinical signs indicative of neurotoxicity including staggering, respiratory difficulty, and lethargy. Hemorrhages occurred around the mouth and nose, and there were histologic lesions in the brain, thymus, skeletal muscle, and kidney. There were reductions in relative weight gain in male rats dosed with 800 mg/kg and in female rats dosed with 200 mg/kg or more. No notable changes in bw gain or compound-

related histopathologic lesions were observed in rats from the lower dose groups. In the 2-y study, however, no notable changes were found on bw or bw gain at 200 or 400 mg/kg/d.

NOAEL = 400 mg/kg/day (based on investigated parameters and taking into account the bw results of 2-y study)

Reliability: (1) valid without restriction
GLP, Comparable to Guideline study

Flag: Critical study for SIDS endpoint

14-FEB-2002 (98)

Type: Sub-chronic
Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: gavage
Exposure period: 13 w
Frequency of treatment: daily
Post exposure period: no
Doses: 50, 100, 200, 400, 800 mg/kg/d
Control Group: yes
NOAEL: 200 mg/kg bw

Year: 1981
GLP: yes

Test substance: other TS: technical grade benzyl alcohol (purity =99%)

Method: Groups of 10 mice of each sex were administered 0, 50, 100, 200, 400, or 800 mg/kg benzyl alcohol in corn oil by gavage, 5 days/week for 13 weeks (dose volume = 5 ml/kg). Mice were housed five/cage with feed and water available ad libitum. Animals were observed twice daily; moribund animals were sacrificed. Animal weights were recorded weekly. At the end of the study, survivors were sacrificed. A necropsy was performed on all animals; histologic exams performed on all vehicle controls and animals in the 800 mg/kg group. Brains were examined from mice in the 400 mg/kg group and from all mice dying before the end of the study.

Remark: Biochemistry and hematology studies were not performed.

Result: Staggering after dosing occurred during the first 2 w of the study in mice dosed with 800 mg/kg.

There were reductions in relative weight gain in male mice dosed with 400 or 800 mg/kg, and in female mice dosed with 200 mg/kg or more. No notable changes in bw gain or compound-related histopathologic lesions were observed in mice from the lower dose groups. In the 2-y study, however no notable changes were found on bw or bw gain at 200 mg/kg/d. NOAEL = 200 mg/kg/day (based on investigated parameters and taking into account the bw results of 2-y study)

Reliability: (1) valid without restriction
GLP, Comparable to Guideline study

Flag: Critical study for SIDS endpoint

14-FEB-2002 (98)

Type: Chronic
Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: gavage
Exposure period: 103 weeks
Frequency of treatment: 5 d/w
Post exposure period: no
Doses: 200, 400 mg/kg/d
Control Group: yes
NOAEL: 400 mg/kg bw

Year: 1981
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Method: Groups of 50 rats of each sex were administered 0, 200, or 400 mg/kg benzyl alcohol in corn oil by gavage, 5 days/week for 103 weeks. The rats were placed on the study at 8-9 weeks of age. All animals were observed twice daily and clinical signs recorded at least once per month. Body weights were recorded once per week for the first 12 weeks, then once a month thereafter. Animals found moribund and those surviving to the end of the study were humanely killed. Necropsy was performed on all animals; histological exams performed on all female rats and vehicle controls, and high dose rats that died before month 22, and male rats with gross lesions.

Remark: Biochemistry and hematology studies were not performed.
Result: No effect on bw gain or mortality was observed. No apparent compound-related non-neoplastic responses were observed.
Reliability: (1) valid without restriction
GLP, Comparable to Guideline study
Flag: Critical study for SIDS endpoint
14-FEB-2002 (98)

Type: Chronic
Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: gavage
Exposure period: 103 w
Frequency of treatment: 5 d/w
Post exposure period: no
Doses: 100, 200 mg/kg/d
Control Group: yes
NOAEL: 200 mg/kg bw

Method: other: OECD 451
Year: 1981
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Method: Benzyl alcohol (purity, 99%) was given to groups of 50 B6C3F1 mice of each sex, eight to nine weeks of age, at a dose of 0, 100, or 200 mg/kg bw per day in corn oil by gavage on five days a week for 103 weeks. The doses were selected on the basis of those found to induce neurotoxic effects (lethargy and staggering) in short-term studies.
The mice were observed twice daily, and their body weights were recorded weekly for the first 12 weeks and once a month thereafter. Gross necropsy was performed on all animals, and 50 tissues and organs, including brain, liver, kidney, and stomach, from all vehicle controls, animals at the high dose, and animals at the other doses that died before

22 months or had gross lesions were examined histologically.
Remark: Biochemistry and hematology studies were not performed.

Result: The mean body weights of treated and control mice were comparable throughout the study. The survival of control females was significantly lower than that of animals at the high dose after week 74, but no other differences in survival were seen: 68% of control, 66% of low-dose, and 70% of high-dose males; and 50% of control, 62% of low-dose, and 72% of high-dose females. No significant treatment-related effects were noted at gross necropsy or histopathological examination. No increase was seen in the incidence of hepatocellular or forestomach neoplasia.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint
14-FEB-2002 (98)

Type: Sub-acute
Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: oral feed
Exposure period: 10 d
Frequency of treatment: continuously in diet
Post exposure period: no
Doses: 2.08; 2.5 or 3 % in diet (approx. 3012, 3750 or 4500 mg/kg/d)
Control Group: yes
NOAEL: 3750 mg/kg bw
LOAEL: 4500 mg/kg bw

GLP: no data
Test substance: other TS: sodium benzoate (specific grade)
purchased from Wako

Method: Sodium benzoate, mixed with the powdered diet, was fed to groups of 12 rats (6 males, 6 females) for 10 days. Animals were observed for body weight gain and clinical signs 5 day/ week.

At the end of the experiment, surviving animals were necropsied. Organ weights, clinical chemistry and histological examinations were performed.

Remark: Benzyl alcohol will rapidly be metabolized to benzaldehyde and so to benzoic acid (sodium benzoate is the salt of benzoic acid). Therefore the data of sodium benzoate can also be supportive in the repeat dose endpoint.

Result: the mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954)
All mice in the 3.0 %-group showed increased sensitivity to stimuli and 1/5 male and 2/5 females showed convulsions; 2/5 females died; liver weights of males and females and kidney weights of females were dose-dependently increased; histopathologic examination showed enlarged hepatocytes, single cell necrosis and vacuolation of hepatocytes in all livers from males; no histopathologic changes of the kidney were described; serum cholesterol, lipid levels and cholinesterase were increased in males.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-FEB-2002 (99)

Type: Sub-acute
Species: rat Sex: male/female
Strain: other: F344/N
Route of administration: gavage
Exposure period: 16 d
Frequency of treatment: daily
Post exposure period: no
Doses: 125, 250, 500, 1000, 2000 mg/kg/d
Control Group: no data specified

Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Remark: No. of animals: 5/sex/dose.

Result: All male and female rats dosed with 2000 mg/kg died. 2/5 male and 3/5 female rats dosed with 1000 mg/kg died. Rats in the 2 highest dose groups were lethargic after dosing.
Other toxic responses in these 2 dose groups included blood around the mouth and nose, subcutaneous hemorrhages, and blood in the urinary and gastrointestinal tract. Animals administered lower doses had no compound-related histologic lesions.

14-FEB-2002 (98)

Type: Sub-acute
Species: mouse Sex: male/female

Strain: B6C3F1
Route of administration: gavage
Exposure period: 16 d
Frequency of treatment: daily
Post exposure period: no data specified
Doses: 125, 250, 500, 1000, 2000 mg/kg/d
Control Group: no data specified

Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Remark: No. of animals: 5/sex/dose.
Result: All male and female mice dosed with 2000 mg/kg died. 1/5 male and 2/5 female mice dosed with 1000 mg/kg died. Mice of each sex in the 2 highest dose groups were lethargic after dosing. Other toxic responses in these 2 dose groups included blood around the mouth and nose, subcutaneous hemorrhages, and blood in the urinary and gastrointestinal tract and in the urinary bladder. Animals administered lower doses had no compound-related histologic lesions.
14-FEB-2002 (98)

Species: rat Sex: male
Strain: no data
Route of administration: inhalation
Exposure period: no data
Frequency of treatment: 4 h/d
Post exposure period: no data specified
Doses: 216-270 ppm
Control Group: no data specified

NOAEL: 270 ppm

Test substance: other TS: benzyl alcohol, purity not noted

Remark: No. of animals: 6.
Result: Subacute exposure to male rats for 4 h periods produced no clinical or pathologic signs of toxicity.
14-FEB-2002 (40)

Species: rat Sex: male/female
Strain: no data
Route of administration: gavage
Exposure period: 3 w
Frequency of treatment: 6 d/w
Post exposure period: no
Doses: 50, 150, 500 mg/kg

Control Group: yes

Test substance: other TS: benzyl alcohol, purity not noted

Remark: No. of animals: 5/sex/dose.

Result: The compound was administered in propylene glycol. Increases in weight were the same in all groups, and there were no pathological effects on blood or organs.

14-FEB-2002

(38)

Species: mouse

Sex: no data

Strain: no data

Route of administration: gavage

Exposure period: 8 d

Frequency of treatment: daily

Post exposure period: no data specified

Doses: 325, 645, 1300, 2595 mg/kg/d

Control Group: no data specified

Test substance: other TS: benzyl alcohol, purity not noted

Remark: No. of animals: no data.

Result: Decreased muscle coordination, a "hunched" appearance, depression, and fur changes were reported in mice given 645 mg/kg but not in those receiving 325 mg/kg or below. At 1300 mg/kg, animals additionally suffered breathing difficulties,

discharge from the eyes, and various CNS effects, and death occurred on day 1 in all mice given 2595 mg/kg.

14-FEB-2002

(100)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test

System of testing: S. typhimurium TA 98, TA 100, TA 1535, TA 1537

Concentration: up to 6666 ug/ml

Cytotoxic Concentration: >= 3333 ug/plate

Metabolic activation: with and without

Result: negative

Method: other: similar to OECD Guide-line 471; protocol according to Haworth, et.al. (1983)

Year: 1983

GLP: yes
 Test substance: other TS: technical grade benzyl alcohol
 (purity = 99%)

Method: Separate trials were done using metabolic
 activation with Aroclor 1254-induced S9 from
 male Syrian hamster liver and
 male Sprague-Dawley rat liver.

Reliability: (1) valid without restriction
 GLP guideline study

Flag: Critical study for SIDS endpoint
 14-FEB-2002 (98)

Type: other: Point-mutation
 System of testing: E. coli
 Metabolic activation: with and without
 Result: negative

Test substance: other TS: benzyl alcohol, purity not noted

Flag: Critical study for SIDS endpoint
 14-FEB-2002 (101) (102)

Type: Cytogenetic assay
 System of testing: CHO cells
 Concentration: up to 5000 ug/ml

Cytotoxic Concentration: none noted
 Metabolic activation: without
 Result: negative

Method: other: similar to OECD 473; Galloway S.M. et
 al., Environ. Mutagen. 7, 1-52 (1985)
 Year: 1989
 GLP: yes

Test substance: other TS: technical grade benzyl alcohol
 (purity = 99%)

Result: No significant increase in chromosome
 aberrations was observed after exposure to
 benzyl alcohol in the absence of S9.

Reliability: (1) valid without restriction
 GLP guideline study

Flag: Critical study for SIDS endpoint
 14-FEB-2002 (103) (98) (104)

Type: Cytogenetic assay
 System of testing: CHO cells
 Concentration: up to 5000 ug/ml
 Cytotoxic Concentration: none noted

Metabolic activation: with
Result: positive

Method: other: similar to OECD 473; according to
Galloway S.M. et al. Environm. Mutagen.7, 1-52
(1985)
Year: 1989
GLP: no data
Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Result: A significant increase in chromosome
aberrations was observed after exposure to
benzyl alcohol in the presence of S9.

Reliability: (1) valid without restriction
Similar to Guideline study

Flag: Critical study for SIDS endpoint
14-FEB-2002 (103) (98) (104)

Type: Cytogenetic assay
System of testing: CHO cells
Concentration: 16 -5000 ug/ml
Cytotoxic Concentration: none noted
Metabolic activation: with and without
Result: equivocal

Method: other: similar to guideline study
Year: 1989
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Result: Sister chromatid exchange (SCE) an equivocal
response with and without metabolic
activation.

Reliability: (1) valid without restriction
Similar to Guideline study

Flag: Critical study for SIDS endpoint
14-FEB-2002 (98)

Type: Bacillus subtilis recombination assay
System of testing: B. subtilis M 45, H 17
Result: positive

Remark: limited data
Flag: Critical study for SIDS endpoint
12-FEB-2002 (105)

Type: Mouse lymphoma assay
System of testing: L5178Y cells
Concentration: up to 5000 ug/ml
Cytotoxic Concentration: >/= 3500 ug/ml
Metabolic activation: with and without

Method: other: similar to OECD 476; according to Myhr G.
et al., Prog. Mutat. Res. 5, 555-586 (1985)
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Result: Benzyl alcohol induced an increase in
trifluorothymidine-resistant cells in the
absence, but not in the presence of, S9
activation. The effect was associated with
toxicity.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint

14-FEB-2002 (98)

Type: other: transformation assay
System of testing: BALB/c-3T3 cells
Concentration: 5 to 20 mM
Cytotoxic Concentration: The cytotoxic response (millimolar
LD50) = 17.9.
Metabolic activation: without
Result: positive

Method: other: Matthews E.J., J. Tissue Culture Methods
10, 157-164 (1986), Matthewy E.J. et al.,
Environm. Health Perspect. 101
[Suppl 2], 319-345 (1993)
Year: 1993
GLP: no data
Test substance: other TS: Supplied by Radian Corp. (Houston,
TX); purity not noted

Method: The A31-1-13 clone of BALB/c-3T3 cells was used
to evaluated the transforming potential of
numerous chemicals including benzyl alcohol.
Each transformation assay contained a
standard clonal survival assay, a co-culture
clonal survival assay, and a transformation
assay.
For each test, chemical-induced transformation
was detected using 18-20 vessels per dose
seeded with 3.2x10(e4) cells/vessel.

Remark:

Result:

Each dose was applied to cell cultures for 48 hrs. days 2-4, using standard procedures.
A total of 3 to 6 test chemicals were included in each transformation experiment and each was tested at four treatment doses in at least two independent trials.
The doses covered a range of cytotoxicity responses of approximately 10-100% relative cloning efficiency.
Each test chemical in each experiment was evaluated as sufficiently positive (statistically significant at two or more doses), limited activity (statistically significant at one dose at 99% conf. or two at 95% conf.), sufficiently negative (no statistically significant responses), or limited negative (no cytotoxicity or abnormal positive control). The number of type I-III transformed foci were identified microscopically considering their various different phenotypic properties.
REFERENCES:
Matthews E.J., J. Tissue Culture Methods 10, 157-164 (1986),
Matthews E.J. et al., Environm. Health Perspect. 101 [Suppl 2], 319-345 (1993)
Benzyl alcohol (BA) was tested as a coded sample.
The author noted that BA can be oxidized by air and may have been altered during the treatment period. They state that BA was noncytotoxic to BALB/c-3T3 cells and that the statistical sensitivities for trial 1 and 2 were 2 and 38/110, respectively. BA was evaluated as active in this assay with actual and estimated rank t-statistics both 1.95.
For the purpose of this study benzyl alcohol (BA) was grouped as a noncytotoxic, nonmutagenic, noncarcinogenic chemical. Notations for BA were: reacts with acid, air, acid chlorides and is temperature sensitive. BA's potential to be oxidized by air was noted as a potential confounding factor. It had limited activity in the first test and Was sufficiently positive in the second. It, therefore, was given the overall evaluation of active in the transformation assay. The cytotoxic response (millimolar LD50) 17.9.
In trial 1 BA concentrations ranged 5 to 20mM

	with an increase in transformation only noted at the 10mM concentration (85% coculture clonal survival). RESULT: 7.36foci/vessel - rank order 2 (p<=0.001) -limited active mean t-statistic 2.11 In trial 2 BA concentrations ranged 5 to 20mM with an increase in transformation noted at the 10mM concentration (95% coculture clonal survival;p<=0.001) and 15mM concentration (84% coculture clonal survival;p<= 0.01 to 0.05).
	Fewer foci were observed in the second trial. RESULT: 0.609 foci/vessel - rank order 38 - sufficient positive mean t-statistic 1.79 The positive control B(a)P performed well. The number of foci/vessel for the neg control was 7.36 and 0.609 in Trials 1 and 2, respectively.
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag:	Critical study for SIDS endpoint
14-FEB-2002	(106)
Type:	Ames test
System of testing:	S. typhimurium TA 98, TA 100, TA 1535, TA 1537
Metabolic activation:	with and without
Result:	negative
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment
14-FEB-2002	(107)
Type:	Ames test
System of testing:	S. typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Metabolic activation:	without
Result:	negative
16-JAN-2001	(108)
Type:	Ames test
System of testing:	S. typhimurium TA 98, TA 100, TA 1535, TA 1537

Metabolic activation:	with and without	
Result:	negative	
Remark:	Rat and hamster liver S-9 mix.	
16-JAN-2001		(109) (104)
Type:	Ames test	
System of testing:	S. typhimurium TA 98, TA 100	
Metabolic activation:	without	
Result:	negative	
16-JAN-2001		(110)
Type:	Ames test	
System of testing:	S. typhimurium TA 92, TA 94, TA 98, TA 100, TA 1535, TA 1537	
Metabolic activation:	with	
Result:	negative	
16-JAN-2001		(111)
Type:	Ames test	
System of testing:	S. typhimurium TA 98, TA 1535	
Metabolic activation:	no data	
Result:	negative	
16-JAN-2001		(112)
Type:	other: Point-mutation	
System of testing:	E. coli WP2 uvrA	
Metabolic activation:	no data	
Result:	negative	
16-JAN-2001		(105)
Type:	Bacillus subtilis recombination assay	
System of testing:	B. subtilis M 45, H 17	
Result:	positive	
16-JAN-2001		(113)
Type:	other: Point-mutation	
System of testing:	E. coli WP2 uvrA	
Metabolic activation:	without	
Result:	negative	
16-JAN-2001		(113)
Type:	Cytogenetic assay	

System of testing: CHL cells

Result: negative

16-JAN-2001 (111) (114)

Type: Mouse lymphoma assay
System of testing: L5178Y tk+/tk- cells
Metabolic activation: with and without
Result: ambiguous

16-JAN-2001 (115) (116) (104)
Type:

Ames test
System of testing: S. typhimurium TA 100
Metabolic activation: without
Result: negative

16-JAN-2001 (117)

Type: Sister chromatid exchange assay
System of testing: CHO cells
Metabolic activation: with and without
Result: positive

16-JAN-2001 (104)

Type: other: DNA Double Strand Breaks
System of testing: rat hepatocytes
Concentration: 0, 1, 3, 10 mM in 1 % DMSO
Metabolic activation: no data
Result: ambiguous

Method: other: in vitro alkaline elution assay
Year: 1994
GLP: no data
Test substance: no data

Remark: Positive only in the highest dose.
16-JAN-2001 (118) (119)

5.6 Genetic Toxicity 'in Vivo'

Type: Micronucleus assay
Species: mouse Sex: male
Strain: other: ddY strain, obtained from Shizuoka
Agricultural Cooperative Association for
Laboratory Animals, Shizuoka, Japan

Route of admin.: i.p.
Exposure period: 24 h
Doses: 50, 100, 200 mg/kg
Result: negative

Method: OECD Guide-line 474 "Genetic Toxicology:
Micronucleus Test"
Year: 1983
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Remark: No. of animals: 6/dose.
Result: There was no indication of micronucleus
induction at any dose tested.

	1 Dose (mg/kg)	MNPCE (%)	PCE (%)
Mortality			
0	0.23 +/-0.18	48.8 +/-6.2	0/6
50	0.23 +/-0.15	55.5 +/-4.0	0/6
100	0.27 +/-0.12	51.8 +/-9.5	0/6
200	0.12 +/-0.10	48.7 +/-5.2	0/6
(4 doses)			
100	0.20 +/-0.14	63.1 +/-4.1	0/6
Mitomycin C			
2.0	2.63 +/-0.32*	43.8 +/-1.1	0/6

MNPCE = Micronucleated polychromatic erythrocyte

PCE = polychromatic erythrocyte

* = (P < 0.01)

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint
14-FEB-2002 (120)

Type: other: replicative DNA synthesis
Species: rat Sex: male
Strain: Fischer 344
Route of admin.: gavage
Exposure period: once
Doses: 0, 300, 600 mg/kg bw
Result: negative

Method: other: according to Uno Y. et al., Toxicol.
Lett. 63, 191-199, 201-209 (1992)
Year: 1994
GLP: no data
Test substance: no data

Result: Benzyl alcohol did not induce replicative DNA synthesis in rat hepatocytes following oral treatment.

Flag: Critical study for SIDS endpoint (121)
14-FEB-2002

Type: other: replicative DNA synthesis
Species: mouse Sex: male
Strain: B6C3F1
Route of admin.: gavage
Exposure period: once
Doses: 0, 400, 800 mg/kg bw
Result: negative

Method: other: according to Uno Y. et al.,
Toxicol.Lett.63,191-199,201-209 (1992),
Year: 1995
GLP: no data
Test substance: no data

Result: Benzyl alcohol did not induce replicative DNA synthesis in mice hepatocytes following oral treatment.

Flag: Critical study for SIDS endpoint (122)
23-MAR-2001

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex: male
Strain: other: Canton S
Route of admin.: drinking water
Exposure period: 72 hrs
Doses: 0, 5000 (unit not given) in 5 % succrose solution

Method: other
Year: 1994
GLP: no data
Test substance: other TS: purity: 99.8 %

Result: no evidence for mutagenicity

19-JAN-2001 (123)

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex: male
Strain: other: Canton S
Route of admin.: i.p.
Exposure period: once
Doses: 0, 8000 (unit not given)

Method: other
Year: 1994
GLP: no data
Test substance: other TS: purity.99.8 %

Result: no evidence for mutagenicity
19-JAN-2001 (123)

5.7 Carcinogenicity

Species: rat Sex: male/female
Strain: other: F344/N
Route of administration: gavage
Exposure period: 103 w
Frequency of treatment: 5 d/w
Post exposure period: no
Doses: 200, 400 mg/kg/d
Result: negative
Control Group: yes

Method: OECD Guide-line 451 "Carcinogenicity Studies"
Year: 1981
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity =99%)

Method: Benzyl alcohol was administered in corn oil by gavage to groups of 50 Fischer 344/N rats of each sex at a dose of 0, 200, or 400 mg/kg bw per day on five days a week for 103 weeks. The rats were observed twice daily, and body weights were recorded weekly for the first 12 weeks and once a month thereafter. Gross necropsy was performed on all animals; and 49 tissues and organs, including brain, kidney, pancreas, and skeletal muscle, from all female rats and from male rats in the vehicle control and high-dose groups and those in the other groups that died before 22 months or which had gross lesions were examined histologically.

Remark: Biochemistry and hematolgy studies were not performed.

Result: The mean body weights of treated and control animals were comparable throughout the study. No compound-related clinical signs were observed, although a sialodacryoadenitis viral infection

was widespread among the study animals in the third month. The survival of treated females was significantly lower than that of vehicle controls: 70% of controls, 34% of low-dose females, and 34% of high-dose females; this was due to a much higher incidence of accidental deaths related to the gavage process.

Survival among the male rats was comparable in all groups: 56% of controls, 54% at the low dose, and 48% at the high dose.

Cataracts and retinal atrophy were observed at increased incidences in rats at the high dose.

The authors attributed this effect to the proximity of this group of animals to fluorescent light for most of the study. An increased incidence of hyperplasia of the forestomach epithelium was seen (not statistically significant) in male rats: control, 0/48; low dose, 0/19; high dose, 4/50.

Haemorrhage and foreign material in the respiratory tract seen in treated rats that died before the end of the study were suggested by the authors to have been the result of either direct deposition of material into the lung during gavage 'accidents' or the anaesthetic properties of benzyl alcohol resulting in reflux of gavage material and aspiration into the lungs. No pancreatic acinar-cell adenomas were reported, and no other effects of treatment were seen at gross necropsy or histopathological examination.

Reliability: (1) valid without restriction

GLP guideline study

Flag: Critical study for SIDS endpoint

14-FEB-2002

(124) (98)

Species:	mouse	Sex: male/female
Strain:	B6C3F1	
Route of administration:	gavage	
Exposure period:	103 w	
Frequency of treatment:	5 d/w	
Post exposure period:	no	
Doses:	100, 200 mg/kg/d	
Result:	negative	
Control Group:	yes	

Method: OECD Guide-line 451 "Carcinogenicity Studies"
Year: 1981
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity =99%)

Method: Benzyl alcohol (purity, 99%) was given to groups of 50 B6C3F1 mice of each sex, eight to nine weeks of age, at a dose of 0, 100, or 200 mg/kg bw per day in corn oil by gavage on five days a week for 103 weeks. The doses were selected on the basis of those found to induce neurotoxic effects (lethargy and staggering) in short-term studies. The mice were observed twice daily, and their body weights were recorded weekly for the first 12 weeks and once a month thereafter. Gross necropsy was performed on all animals, and 50 tissues and organs, including brain, liver, kidney, and stomach, from all vehicle controls, animals at the high dose, and animals at the other doses that died before 22 months or had gross lesions were examined histologically.

Remark: Biochemistry and hematology studies were not performed.

Result: The mean body weights of treated and control mice were comparable throughout the study. The survival of control females was significantly lower than that of animals at the high dose after week 74, but no other differences in

survival were seen: 68% of control, 66% of low-dose, and 70% of high-dose males; and 50% of control, 62% of low-dose, and 72% of high-dose females.

No significant treatment-related effects were noted at gross necropsy or histopathological examination. No increase was seen in the incidence of hepatocellular or forestomach neoplasia.

Reliability: (1) valid without restriction

GLP guideline study

Flag: Critical study for SIDS endpoint

14-FEB-2002

(124) (98)

Species: mouse

Sex: male

Strain: B6C3F1

Route of administration: i.p.

Exposure period: 22 d

Frequency of treatment: once on day 1, 8, 15, 22
Post exposure period: up to 1 a
Doses: 3.75 umol (total dose) in trioctanoin
Control Group: yes

Remark: 35 mice received injections prior to weaning.
The mice were weaned at 4 weeks of age. All
surviving mice were killed at 12 months for
enumeration of hepatomas.

Result: Benzyl alcohol had no detectable activity for
the initiation of hepatic tumors on
administration to male mice prior to weaning.

19-JAN-2001 (125)

5.8.1 Toxicity to Fertility

Type: other: 2 year gavage study
Species: rat
Sex: male/female
Strain: Fischer 344
Route of administration: gavage
Exposure Period: 103 weeks
Frequency of treatment: 5d/w
Duration of test: 103 weeks
Doses: 200, 400 mg/kg/d
Control Group: yes
NOAEL Parental: 400 ml/kg bw

Method: other: OECD 451
Year: 1981
GLP: yes

Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Remark: Benzyl alcohol was administered in corn oil.
Result: No evidence of compound related effects in the
testes or ovaries of treated rats.
Changes noted in general in the reproductive
system were inconsequential.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint

14-FEB-2002 (98)

Type: other: 2 year gavage study
Species: mouse
Sex: male/female
Strain: B6C3F1
Route of administration: gavage

Exposure Period:	103 weeks
Frequency of treatment:	5 d/w
Duration of test:	103 weeks
Doses:	100, 200 mg/kg/d
Control Group:	yes
NOAEL Parental:	200 ml/kg bw

Method:	other: OECD 451
GLP:	yes
Test substance:	other TS: technical grade benzyl alcohol (purity =99%)

Remark:	Benzyl alcohol was administered in corn oil.
Result:	No evidence of compound related effects in the testes or ovaries of treated mice. Changes noted in general in the reproductive system were inconsequential.

Reliability:	(1) valid without restriction GLP guideline study

Flag:	Critical study for SIDS endpoint
14-FEB-2002	(98)

Type:	other: 4 generation study
Species:	rat
Strain:	no data
Route of administration:	oral feed
Exposure Period:	generation 1 and 2: lifelong; generation 3: 16 weeks; generation 4: until breeding
Frequency of treatment:	continuously in diet
Doses:	0.5 or 1 % in diet (approx. 375 or 750 mg/kg/day)
Control Group:	yes
NOAEL Parental:	>= 750 ml/kg bw
NOAEL F1 Offspring:	>= 750 ml/kg bw
NOAEL F2 Offspring:	>= 750 ml/kg bw

Test substance:	other TS: benzoic acid

Remark:	See IUCLID data set on benzoic acid (CAS# 65-85-0). Benzyl alcohol will rapidly be metabolized to benzaldehyde and so to benzoic acid. Therefore the data of benzoic acid can also be supportive to state that benzyl alcohol is not a reproductive (fertility and developmental) toxicant.
Result:	No effects on fertility, lactation, growth and survival or the incidence of foetal malformations were observed in a 4 generation

reproduction study with rats (20 m and 20 f) exposed to 0.5% and 1.0% benzoic acid in the diet.
 Flag: Critical study for SIDS endpoint (126)
 06-JUN-2001

Type: Fertility
 Species: rat
 Sex: female
 Route of administration: oral unspecified
 Exposure Period: 32 weeks
 Frequency of treatment: every second day
 Premating Exposure Period
 female: 75 days
 Duration of test: 32 weeks
 Doses: 5 mg/kg
 NOAEL Parental: 5 mg/kg bw

Test substance: other TS: benzaldehyde

Remark: Benzyl alcohol will rapidly be metabolized to benzaldehyde and so to benzoic acid. Therefore the data of benzaldehyde can also be supportive to state that benzyl alcohol is not a reproductive (fertility and developmental) toxin.

Result: No treatment related effects noted.
 Flag: Critical study for SIDS endpoint
 16-JAN-2001 (127) (128)

5.8.2 Developmental Toxicity/Teratogenicity

Species: mouse Sex:
 female
 Strain: CD-1
 Route of administration: gavage
 Exposure period: day 7-14 of gestation
 Frequency of treatment: daily
 Duration of test: until 3 days after pregnancy
 Doses: 750 mg/kg bw/day
 Control Group: yes
 LOAEL Maternal Toxicity : 750 mg/kg bw
 LOAEL Fetotoxicity : 750 mg/kg bw

GLP: no data
 Test substance: other TS: benzyl alcohol, purity not noted

Method: Benzyl alcohol dissolved in distilled water was administered by gavage at a dose of 750 mg/kg bw per day to 50 mice on days 7-14 of

Result:

gestation; evidence of copulation was considered the first day of gestation. A control group of 50 animals received distilled water only. All animals were allowed to deliver their litters and nurse their pups for three days, at which time necropsies were performed. Maternal body-weight gain and mortality, mating, gestation, numbers of live and dead pups per litter, total litter weight on days 1 and 2 post partum, litter weight change between days 1 and 3 post partum, and pup survival on days 1 and 3 post partum were recorded.

During the treatment period, 18 deaths were reported, all

of which were attributed to treatment; a further death was reported on day 15 of gestation, the day after treatment was terminated. Clinical signs of toxicity, including hunched posture, tremors, inactivity, prostration, hypothermia, ataxia, dyspnoea, swollen or cyanotic abdomen, and piloerection, were reported in up to 20 mice during treatment. Piloerection was also reported in some animals up to day 3 post partum, but no other clinical signs were seen after the period of administration. No differences were observed in the mating or gestation indices, the total number of resorptions, the mean length of gestation, or the number of live pups per litter between treated and control groups. Maternal body weight, measured on days 4 and 7 of gestation, was not significantly different from control values; however, statistically significant reductions were reported on day 18 of gestation ($P < 0.001$) and on day 3 post partum ($P < 0.05$). Maternal body-weight gain during days 7-18 of gestation was significantly lower than that of controls ($P < 0.001$). Significant reductions in pup body weight were reported, including a lower mean pup weight per litter on days 1 ($P < 0.01$) and 3 post partum ($P < 0.001$), a mean litter weight change between day 1 and day 3 post partum ($P < 0.05$), and a mean pup weight change between days 1 and 3 post partum ($P < 0.001$). No differences in pup survival

Conclusion: were observed by day 3 post partum.
The authors concluded that benzyl alcohol may be a reproductive hazard, apparently on the basis of the reductions in pup body weights, an effect that was observed in conjunction with maternal toxicity evidenced by increased mortality, reduced body weights, and clinical toxicity during the period of administration. As effects were seen on the dams and fetuses at the only dose used in this study, there was no NOAEL. The LOAEL was 750 mg/kg bw per day.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-FEB-2002 (129) (130) (131)

Species: mouse Sex: female

Route of administration: gavage

Exposure period: days 6-15 of gestation

Frequency of treatment: daily

Duration of test: until day 3 post partum

Doses: 550 mg/kg bw

Control Group: yes, concurrent vehicle

NOAEL Maternal Toxicity: 550 mg/kg bw

NOAEL Teratogenicity: 550 mg/kg bw

GLP: no data

Test substance: other TS: benzyl alcohol; purity not noted

Method: 50 female mice were given benzyl alcohol at 550 mg/kg bw per day by gavage on days 6-15 of gestation; a further 50 mice received the corn oil vehicle. All dams were allowed to deliver naturally, and pups and dams were observed until day 3 post partum, when the experiment was terminated. Body weight, clinical observations, and mortality were recorded daily throughout treatment and up to day 3 post partum.

Remark: abstract only

Result: Mortality was not significantly increased in animals given benzyl alcohol over that in the control group.
One treated mouse showing languid behaviour, laboured breathing, and a rough coat died, but no other deaths or clinical signs were

reported. Maternal body weight and body-weight gain during treatment and up to day 3 post partum were virtually identical for treated and control animals. All other parameters examined, including gestation index, average number of live pups per litter, and postnatal survival and pup body weight on days 0 and 3 post partum, were not significantly different from the control values.

Conclusion: The authors concluded that, at the predicted LD10, benzyl alcohol had no significant effects on the development of CD-1 mice. The NOAEL was 550 mg/kg bw per day.

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint

14-FEB-2002 (132) (133)

Species: rat Sex: male/female

Strain: no data

Route of administration: oral feed

Exposure period: generation 1 and 2: lifelong; generation 3: 16 weeks; generation 4: until breeding

Frequency of treatment: continuously in diet

Duration of test: 4 generations

Doses: 0.5 or 1% in diet (approx. 375 or 750 mg/kg/day)

Control Group: yes

NOAEL Maternal Toxicity: 750 mg/kg bw

NOAEL Teratogenicity: 750 mg/kg bw

Method: other

GLP: no data

Test substance: other TS: benzoic acid

Remark: See IUCLID data set on benzoic acid (CAS# 65-85-0). Benzyl alcohol will rapidly be metabolized to benzaldehyde and so to benzoic acid. Therefore the data of benzoic acid can also be supportive to state that benzyl alcohol is not a reproductive (fertility and development) toxicant.

Result: No effects on the dams or on the growth and development of the offspring were seen when groups of 10 rats were fed diets containing up to 1% benzoic acid during pregnancy and lactation.

Flag: Critical study for SIDS endpoint
06-JUN-2001 (126)

Species: other: chicken embryo Sex:
Route of administration: other
Exposure period: 11 to 18 d

Frequency of treatment: 1 injection before incubation or on
different d after incubation

Doses: 0.01-0.02 ml/egg = 10-20 mg/egg

Control Group: yes

Remark: Injections of benzyl alcohol into the yolks of
fertile eggs, either before incubation, or
from the 1. through the 7. d after the
beginning of their incubation give rise to
meningoceles, limb deformities, beak defects
such as, arched upper beaks, localized blebs
and generalized edema.

no post observation

30-JAN-2001 (134)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

Remark: Benzyl alcohol poisoning can cause the
gasping syndrome in neonates. The infants
had a typical course of gradual neurologic
deterioration, severe metabolic acidosis,
the striking onset of gasping respirations,
thrombocytopenia, hepatic and renal failure,
hypotension, cardiovascular collapse and
death. In every infant, unmetabolized
benzyl alcohol was identified in the urine.

19-JAN-2001 (135) (136) (137) (138) (139) (140) (141) (142)

Remark: Local anaesthesia occurred when neat benzyl
alcohol was applied to the (presumably
uncovered) skin or when 1 % aqueous
solution was injected intradermally.

Source: Bayer AG Leverkusen

20-AUG-1992 (37)

Remark: A methylprednisolone sodium succinate formulation, containing 18 mg / dose of benzyl alcohol, was well tolerated in human volunteers after i.v. injection. No important drug-related side effects were encountered.

Source: Bayer AG Leverkusen (143)
20-AUG-1992

Remark: Cases of allergic contact dermatitis, and even systemic hypersensitivity have been reported in humans.

Source: Bayer AG Leverkusen
15-JUL-1993 (144) (145) (146) (147) (148) (149) (150) (151) (152) (153) (154) (155)

Remark: No contact allergy could be detected in humans treated with a 10 % formulation of benzyl alcohol (no other data).

Source: Bayer AG Leverkusen (156)
20-AUG-1992

Remark: Premature neonates may receive multiple drugs in the neonatal intensive care unit, some of which may contain benzyl alcohol. As there may be no safe lower dose of benzyl alcohol in these patients, it would seem prudent to avoid the use of multiple dose vials containing benzyl alcohol whenever alternatives exist.

Source: Bayer AG Leverkusen (157)
20-AUG-1992

Remark: It also seems prudent to avoid the use of products containing benzyl alcohol to pregnant patients within whom the benzyl alcohol molecule, given its small size, presumably crosses the placental barrier into immature fetal tissues as readily as it crosses the blood-brain barrier.

Source: Bayer AG Leverkusen (158)
22-MAR-1993

Remark: high levels of benzyl alcohol (5-500 ug/10 ml plasma) were found in uremic patients on hemodialysis; benzyl alcohol was not detected in normal controls.

Source: Bayer AG Leverkusen (159)
24-FEB-1998

Remark: In 2 long-term double blind studies on humans comparing benzyl alcohol , placebo and Catalin in the topical treatment of progressive cataract, rapid (2-3 weeks treatment) reversal of incipient cataract was obtained accompanied by a marked improvement of vision and by a significantly lower percentage of eyes requirering surgery after 22 months of treatment with benzyl alcohol than with placebo and Catalin.

Source: Bayer AG Leverkusen (160)
24-FEB-1998

Remark: Study on healthy adult voluteers: Benzyl alcohol is itself an effective anesthetic and can reduce the pain of injection for lidocain without adversely affecting its anesthetic properties.

Source: Bayer AG Leverkusen (161)
24-FEB-1998

Remark: Benzyl alkohol is commonly used as a preservative in many injectable drugs and solutions. A number of neonatal deaths and severe respiratory and metabolic complications in low-birth-weight premature infants have been associated with the use of this agent.

Source: Bayer AG Leverkusen (162) (163) (164) (165) (166) (167) (168)
26-FEB-1998

5.11 Additional Remarks

Type: Metabolism

Remark: Humans, rabbits and rats readily oxidize benzyl alcohol to benzoic acid, which, after conjugation with glycine, is rapidly eliminated as hippuric acid in the urine.

19-JAN-2001 (169) (170) (171) (172) (173) (174) (175)

Type: other

Remark: Bacillus subtilis spore rec-assay can be used as a simple screening test taking the place of animal methods for detection of the allergenicity.

Source: Bayer AG Leverkusen (176)
24-FEB-1998

Type: other

Remark: yeast test: according to the author an alternative to the contemporary mode of acute toxicity testing. In the test, the increase in the cell count after treatment in relationship to the increase in cell count of untreated cells is measured and expressed as "medium inhibitory concentration = IC 50 " : benzyl alcohol IC 50 = 277 mg/l

Source: Bayer AG Leverkusen (177) (178)
24-FEB-1998

Type: other

Remark: Benzylalkohol differentially altered the specific activity of subcellular rat epididymal and testicular aldehyde dehydrogenase activity as well as hepatic aldehyde dehydrogenase activity.

Source: Bayer AG Leverkusen (179) (180) (181) (182)
24-FEB-1998

Type: other

Remark: different concentrations of benzyl alcohol (1,2,5,10 % v/v) in sesame oil were subcutaneously injected to rats. only the 1 % benzyl alcohol produced an insignificant increase in skin fold thickness.

Source: Bayer AG Leverkusen (183)
24-FEB-1998

Type: other

Remark: In vitro, benzyl alcohol relaxes airway smooth muscle, probably through the decrease in intracellular Ca^{2+} release by inhibiting agonist-mediated phosphatidylinositol turnover.

Source: Bayer AG Leverkusen

24-FEB-1998 (184)

Remark: Aseptic meningitis has been observed following intrathecal administration of radiopharmaceuticals that contain benzyl-alcohol as a preservative. Cisterna magna injections of benzylalcohol in concentrations as high as 10 times that normally used did not produce meningitis in adult or immature dogs. With 9 % benzyl alcohol, transient respiratory arrest was observed in adult dogs and death was observed in immature dogs; 7 % and 4.5 % benzyl alcohol produced clonic seizures in puppies.

Source: Bayer AG Leverkusen

15-JUL-1993 (185)

Remark: Injection of benzyl alcohol (700-900 mg/kg, i.p.) caused rapid immobilization of mice. The mice were immobilized within 2 min. and remained unresponsive (no righting reflex, no wink reflex, and no leg reflex) for about 30 min. The immobilizing effect was accompanied by a marked hyperglycemia. Tracer studies indicated that the hyperglycemic effect may have resulted from increased gluconeogenesis.

Source: Bayer AG Leverkusen

27-MAY-1993 (186)

Remark: Benzyl alcohol used as a stabilizer for antibiotics of aminoglycosid structure is the substance responsible for the displacement of bilirubin from albumin. The free, unbound, unconjugated bilirubin tends to diffuse into the lipid of the brain of young Gunn rats with resultant kernicterus.

Source: Bayer AG Leverkusen

15-JUL-1993

(187)

Remark: Duodenal and jejunal brush border membrane vesicle integrity was studied after in vitro treatment of rabbit tissue with benzylalcohol. The effect of the alcohol on gastric parietal cell apical and microsomal membrane vesicle integrity was also studied. Exposure of vesicles to the alcohol caused concentration dependent decreases in enclosed volume. All concentrations tested reduced the enclosed volume of both gastric apical membrane vesicles and gastric microsomes. The alcohol induced disruption of the vesicle membranes appears to result from a fluidising effect. The main effect of the raised fluidity is to increase membrane fragility.

Source: Bayer AG Leverkusen

15-JUL-1993

(188) (189)

Remark: Benzyl alcohol as a fragrance ingredient used in cosmetic and other products is lipophilic and therefore has the potential to be readily absorbed through skin. The percutaneous absorption was determined in vivo in rhesus monkeys. Absorption through occluded skin was high (56-80 %) in 24 h. No correlation was seen between skin penetration and the octanol-water partition coefficient. Under unoccluded conditions skin penetration was reduced (32 %), because of evaporation of the compound.

Source: Bayer AG Leverkusen

27-MAY-1993

(190)

Remark: After i.v. injection in mice, benzyl alcohol was found to inhibit TBPS binding and to stimulate GABA receptor mediated Cl influx into brain vesicles.

Source: Bayer AG Leverkusen

27-MAY-1993

(191)

Remark: Benzyl alcohol can cause hemolysis of human and rabbit erythrocytes in the presence of 0.9 % NaCl.

Source: Bayer AG Leverkusen

15-JUL-1993

(192) (193)

Remark: Benzyl alcohol produced up to 6-fold increases in cAMP concentrations in purified human peripheral blood lymphocytes. Significant but less marked augmentation of cAMP was observed in human platelets, human granulocytes, and rabbit alveolar macrophages. The mechanism of the alcohol-induced cAMP accumulation is probably secondary to membrane perturbation and consequent activation of adenylate cyclase.

Source: Bayer AG Leverkusen

15-JUL-1993

(194)

Remark: Uncoupled sonic submitochondrial particles from beef heart and rat liver were studied for mitochondrial electron transport. Benzyl alcohol was found to inhibit each of the segments of the electron transport chain assayed. NADH oxidase and NADH-cytochrome c oxidoreductase required the lowest concentration for inhibition, and cytochrome c oxidase required the highest concentration. Beef heart submitochondrial particles are less sensitive to inhibition than are rat liver particles.

Source: Bayer AG Leverkusen

27-MAY-1993

(195)

Remark: Lactated Ringer's solution containing 1.5 % benzyl alcohol can cause severe symptoms of toxicity in cats including hyperesthesia leading to depression, coma, and finally death. In the cat, only hippuric acid is formed, as this species lacks adequate glucuronic acid conjugation capacity, resulting in a decreased rate of metabolism.

Source: This results in an accumulation of benzoic acid. Benzoic acid has been shown to be extremely toxic to cats, causing clinical signs similar to those observed.
Bayer AG Leverkusen

15-JUL-1993 (196)

Remark: 50 mM benzyl alcohol fluidized proximal brush-border membranes prepared from human small intestine and increased p-nitrophenyl-phosphatase activity in this membrane. This agent also shifted the phase transition temperature of the membrane and breakpoint temperature of this enzymatic activity.

Source: Bayer AG Leverkusen

15-JUL-1993 (197)

Remark: Microscopic examination revealed local nerve degeneration when 5 % benzyl alcohol was injected into the side of a cat's face. At 10 % local anaesthesia was produced.

Source: Bayer AG Leverkusen

27-MAY-1993 (198)

Remark: Benzyl alcohol displays a pronounced antiarrhythmic-anti- fibrillatory effect, when injected i.v. into dogs and rats with spontaneous or drug-induced arrhythmias. Mechanisms which might be responsible for the antiarrhythmic effect: lengthening of the effective refractory period, local and general anaesthetic effects, changes of osmolality. The i.v. injection of benzyl-alcohol in high doses, produces intravascular haemolysis.

Source: Bayer AG Leverkusen

27-MAY-1993 (199)

Remark: The length of the oestrus cycle was reduced when 0.52-2.1 d (1-4 mg/kg bw) benzyl alcohol was injected into the uterus of each of 48 cows.

Source: Bayer AG Leverkusen

27-MAY-1993 (200)

Remark: The in vitro effect of local anesthetic benzyl alcohol was studied using isolated cells from rat stomach. Lower concentrations of the alcohol increased the basal aminopyrine accumulation and potentiated the secretory response of parietal cells to histamine and dbcAMP.
At higher concentrations the alcohol progressively inhibited both the basal 14-C-aminopyrine accumulation and that stimulated by histamine, dbcAMP or carbachol. While a low concentration increased gastric microsomal (H-K)-ATPase activity, higher concentrations inhibited enzyme activity to about 80 % of those activities found in resting parietal cells.

Source: Bayer AG Leverkusen

15-JUL-1993 (201)

Remark: Benzyl alcohol is a fairly efficient anesthetic for intact mucous membranes, greatly surpassing procain. Its action is not as lasting as that of cocain. It appears that 1 % does not produce satisfactory anesthesia of the tongue, even after 10 min.

contact.

Source: Bayer AG Leverkusen

27-MAY-1993 (202)

Remark: Benzyl alcohol in non-toxic concentrations was found to markedly reduce the hemoglobin minor/hemoglobin major ratio and to moderately reduce the total hemoglobin induced by DMSO or HMBA in mouse erythroleukemia (MEL) cells, while only slightly decreasing the ratio induced by hemin or butyrate.

Source: Bayer AG Leverkusen

27-MAY-1993 (203)

Remark: It was demonstrated that benzyl alcohol, a neutral local anesthetic, inhibits the uptake and degradation of lowdensity lipoprotein and endocytosis of transferrin receptors of guinea-pig leukemic B lymphocytes. This inhibition is very rapid, concentration dependant and reversible by simple washing. Membrane fluidity of the living cells is also modified.

Source: Bayer AG Leverkusen

27-MAY-1993 (204)

Remark: The tissue culture lethal dose (TCLD50) in mouse embryo cells was found to be 0.002 mg/ml.

Source: Bayer AG Leverkusen

27-MAY-1993 (205)

Remark: Benzyl alcohol is more toxic to infant jaundiced (jj) than to non-jaundiced (Jj) Gunn rats. Before excretion as hippuric acid, benzyl alcohol is metabolized to benzoic acid, a potent competitor for bilirubin-albumin binding sites. These pathways are immature in newborns. Therefore the kernicterus in jj pups is probably due to increased levels of unbound bilirubin.

Source: Bayer AG Leverkusen

27-MAY-1993 (206)

Remark: The plasma half-life of benzyl alcohol administered as a 2.5 % solution in saline was found to be approximately 1.5 h in dogs injected i.v. at doses of 52 and 105 mg/kg.

Source: Bayer AG Leverkusen

27-MAY-1993 (47)

Remark: Larger percentages of benzyl alcohol doses were found in urine as benzoic acid in preterm babies, while less hippuric acid appeared in their urine than in term newborns.

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- Source: These results indicate that hippuric acid formation is deficient in preterm neonates.
Bayer AG Leverkusen
- 27-MAY-1993 (207)
- Remark: In vitro studies of human liver alcohol dehydrogenase (ADH) variants revealed that benzyl alcohol is slowly metabolized by beta-2-ADH. Working with this solvent might lead to toxic effects; these could be particularly prominent in individuals possessing the beta-2-ADH if they have a lower capacity to eliminate them, or they could be particularly prominent in those with beta-1-ADH if they quickly convert them into toxic aldehydes.
- Source: Bayer AG Leverkusen
- 27-MAY-1993 (208)
- Remark: Perfusing the anterior chamber of enucleated rabbit eyes with 1.18 % benzyl alcohol, the corneal endothelial cells changed the appearance and the corneas began to swell.
- Source: Bayer AG Leverkusen
- 24-AUG-1993 (209)
- Remark: The invitro effects of benzyl alcohol and benzaldehyde on subcellular rat liver NAD-dependant alcohol and aldehyde dehydrogenase were studied as a function of gender. These effects were compared with those of the primary substrates ethanol and acetaldehyde. the results suggest metabolic competitions between benzyl alcohol and ethyl alcohol for catalysis by alcohol dehydrogenase.
- Source: Bayer AG Leverkusen
- 03-MAR-1998 (210)
- Remark: Acute intravenous toxicity of benzyl alcohol was determined in CD2F1 (0.05-0.2 ml/kg bw), B6D2F1 (0.05-0.4 ml/kg) and C57BL/6 mice.

The lowest dose was a safe dose and the highest one was the dose causing mortality in no more than half the animals of each group. Clinical signs were convulsion, dyspnea and reduced mortality in all strains for 24 hours. The slight decrease in body weight in the first week following treatment returned to normal in the second week.

Source: Bayer AG Leverkusen

03-MAR-1998

(211)

6.1 Analytical Methods

6.2 Detection and Identification

7.1 Function

7.2 Effects on Organisms to be Controlled

7.3 Organisms to be Protected

7.4 User

7.5 Resistance

8.1 Methods Handling and Storing**8.2 Fire Guidance****8.3 Emergency Measures****8.4 Possib. of Rendering Subst. Harmless****8.5 Waste Management****8.6 Side-effects Detection****8.7 Substance Registered as Dangerous for Ground Water****8.8 Reactivity Towards Container Material**

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10.1 End Point Summary**10.2 Hazard Summary****10.3 Risk Assessment**

Evaluation of certain food additives

Ninety-second report of the Joint
FAO/WHO Expert Committee on
Food Additives



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Evaluation of certain food additives

Ninety-second report of the Joint
FAO/WHO Expert Committee on
Food Additives

*This report contains the collective views of an international group of experts and
does not necessarily represent the decisions or the stated policy of the World Health Organization*



**Food and Agriculture
Organization of the
United Nations**



**World Health
Organization**

Evaluation of certain food additives: ninety-second report of the Joint FAO/WHO Expert Committee on Food Additives

(WHO Technical Report Series, No. 1037)

ISBN (WHO) 978-92-4-005464-6 (electronic version)

ISBN (WHO) 978-92-4-005465-3 (print version)

ISBN (FAO) 978-92-5-137190-9

ISSN 0512-3054

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Acknowledgements

FAO and WHO acknowledge the significant contributions of the experts, as well as their institutions (where relevant), to the work of the ninety-second meeting of JECFA. The Committee thanks Ms Elisabeth Heseltine, Saint Léon-sur-Vézère, France, for her assistance in the preparation of the report.

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Ninety-second meeting of the Joint FAO/WHO Expert Committee on Food Additives

Virtual meeting, 7–18 June 2021

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List of abbreviations and acronyms

ADI	acceptable daily intake
bw	body weight
CCFA	Codex Committee on Food Additives
CSAF	chemical-specific adjustment factor
EFSA	European Food Safety Authority
FAD	flavin-adenine dinucleotide
FAO	Food and Agricultural Organization of the United Nations
GSFA	General Standard for Food Additives
INS	International Numbering System
JECFA	Joint Expert Committee on Food Additives
LD ₅₀	median lethal dose
MOE	margin of error
MPL	maximum permitted level
MRUL	maximum reported use level
NOAEL	no-observed-adverse-effect level
TMDI	theoretical maximum daily intake
TOS	total organic solids
WHO	World Health Organization



1. Introduction

The ninety-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was convened by video conference from 7 to 18 June 2021. The meeting was opened on behalf of the Director-General of the Food and Agriculture Organization of the United Nations (FAO) by Dr Markus Lipp (Food Systems and Food Safety Division, FAO) and on behalf of the Director-General of the World Health Organization (WHO) by Mr Kim Petersen (Programme Manager, Department of Nutrition and Food Safety, WHO). Dr Lipp welcomed all meeting participants and stressed that, despite the challenges of the ongoing COVID-19 pandemic, the work of JECFA had progressed, and the Committee continued to provide sound scientific advice to the Codex Alimentarius Commission and Member States. He reminded the participants of their responsibility to impart the least biased, best scientific advice possible and that they had been invited to serve solely in their capacity as experts to provide sound scientific advice and not as representatives of their employer or country. He closed by reiterating his sincere gratitude to all participants for providing their time and expertise to the present JECFA meeting.

Mr Petersen welcomed all meeting participants on behalf of WHO and thanked all experts for their commitment and dedication to the work of JECFA. He underlined the importance of their work in relation to the work of the Codex Alimentarius Commission in developing international food safety standards.

1.1 Procedural matters

Owing to travel restrictions and lockdowns due to the response to the COVID-19 pandemic in many countries, it was not possible to convene a physical meeting and it was instead decided to hold it online by video-conferencing. In view of the time differences in the countries of the invited experts, the only possible time for a video-conference was restricted to a 4-h time slot (12:00–16:00 CET) each day. This allowed only 40% of the usual daily length (8–10 h) of a typical JECFA meeting. Although the experts participated fully, they noted that online meetings do not permit the necessary in-depth, robust scientific discussions that are characteristic of JECFA meetings and are therefore not a suitable substitute for face-to-face meetings. In particular, the experts felt that the online format did not foster the atmosphere of trust, inclusiveness and openness that has marked all physical JECFA meetings. The experts considered that the success of the ninety-second meeting was due mainly to the cohesion among them stemming from the trust built on the relationships they had formed during previous face-to-face meetings. The experts also decried the significant difficulty of holding any

informal meetings outside the scheduled meeting times because of the widely differing time zones. Perhaps the greatest loss due to the virtual meeting format rather than in-person meetings is in efficiency in solving issues that arise shortly before or during the meeting that require immediate input from individuals or small groups of both FAO and WHO representatives. Indeed, this deficiency means that fewer food additives can be evaluated within a two-week meeting.

The experts emphasized further that an invitation to a physical JECFA meeting at FAO or WHO headquarters gives rise to more significant recognition by the expert's employer of the weight, reach, responsibility and workload required for full participation in a JECFA meeting. The same degree of acknowledgement is not granted by employers for online meetings, as the experts remain available locally. This lack of recognition of the workload and significance of participation in a JECFA meeting led to an increase in other demands on the experts, resulting in more distractions and more frequent scheduling conflicts. The experts concluded that, cumulatively, such factors would be counterproductive for participation in future JECFA meetings if FAO and WHO maintained the online-only format.

In recognition of the difficulties and the tremendous efforts made, the Joint FAO/WHO Secretariat expressed its deep gratitude to all the experts for their commitment and flexibility, not least as the scheduled meeting times were exceedingly inconvenient for many.

The meeting report was adopted on 18 June 2021.

1.2 Declarations of interests

The Secretariat informed the Committee that all experts participating in the ninety-second JECFA meeting had completed a declaration of interests form. The declarations were assessed to determine the extent to which any interest could be reasonably expected to influence the experts' judgement. The declared interests were considered unlikely to impair any individual's objectivity or have any significant influence on the impartiality, neutrality and integrity of their work. Neither FAO nor WHO received any public comments in response to the online posting of the names and brief biographies of the individuals considered for participation in the expert meeting. The interests of all participants were disclosed at the beginning of the meeting to all attendees.

1.3 Adoption of the agenda

After discussion between the experts and the sponsor, the name of the enzyme listed on the agenda as "phosphodiesterase" was changed to "ribonuclease P", and

this change was reflected on the agenda and in the subsequent monograph and specifications. Spirulina was removed from the agenda as the sponsor could not provide the information necessary for establishing full specifications before the meeting. The meeting agenda was adopted with no further modification.



2. Toxicological evaluations and exposure assessments

2.1 Benzoic acid, its salts and derivatives

2.1.1 Explanation

The Committee first evaluated benzoic acid and its salt, sodium benzoate, at its sixth meeting ([Annex 1](#), reference 6) in 1962. A group acceptable daily intake (ADI) of 0–5 mg/kg body weight (bw) for benzoic acid and sodium benzoate (expressed as benzoic acid) was established at that meeting. This group ADI was based on the absence of any observed adverse effects in rats over four successive generations, two of which involved lifetime dietary exposure to benzoic acid at a maximal concentration of 1% (equivalent to 500 mg/kg bw per day). The potassium and calcium salts were subsequently included in the group ADI for benzoic acid at the ninth, seventeenth, twenty-seventh and forty-sixth meetings ([Annex 1](#), references 11, 32, 62 and 122). Dietary exposure to benzoic acid and its salts (benzoates) was evaluated by the Committee at its fifty-first and eightieth meetings ([Annex 1](#), references 137, 138, 223 and 224). At its eightieth meeting, in 2015, the Committee estimated dietary exposure to benzoates through consumption of water-based flavoured drinks on the basis of reported average typical use levels up to 209 mg/L. The highest high dietary exposure estimates were for consumers-only of these drinks, which was up to 10.9 mg/kg bw per day for toddlers and young children. After a literature review, the Committee concluded that, in most countries, water-based flavoured drinks contribute most to dietary exposure to benzoates.

Benzoic acid and its salts are used as food preservatives, whereas derivatives such as benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate are used as flavouring agents. Benzyl alcohol and benzyl benzoate are also used as carrier solvents in foods. The Committee has evaluated benzyl derivatives when used as flavouring agents, most recently at its fifty-seventh meeting ([Annex 1](#), references 154 and 155). Benzyl acetate was evaluated at the eleventh, twenty-seventh, twenty-ninth, thirty-first, thirty-fifth, forty-first and forty-sixth meetings ([Annex 1](#), references 14, 62, 70, 77, 88, 107 and 122). Benzaldehyde and benzyl alcohol were evaluated at the eleventh, twenty-third and forty-sixth meetings ([Annex 1](#), references 14, 50 and 122).

As all these structurally related compounds are metabolized along common pathways to benzoate in both rodents and humans, the Committee at its forty-sixth meeting evaluated benzyl acetate, benzyl alcohol, benzaldehyde, benzoic acid and the benzoate salts (calcium, potassium and sodium) together and re-affirmed the group ADI of 0–5 mg/kg bw expressed as benzoic acid equivalents ([Annex 1](#), reference 122).

The current request to re-evaluate benzoic acid and its salts was made by the Codex Committee on Food Additives at its Forty-ninth Session (1). The sponsor provided a report of an extended one-generation reproductive toxicity study according to OECD 443 and two published reports on the use of default uncertainty factors for benzoic acid. In addition, the sponsor provided dietary exposure estimates for benzoates from water-based flavoured drinks for Brazil, Canada, Mexico and the USA based on maximum use levels and market volume-weighted average use levels of benzoates (expressed as benzoic acid).

A comprehensive literature search for toxicological and biochemical data from January 2002 to March 2021 was conducted in PubMed/TOXLINE; 49 publications were considered relevant and further evaluated. As benzoic acid and its salts were evaluated by the European Food Safety Authority (EFSA) from the literature available up to 2016, the Committee decided to use only 20 studies published after 2016 and those published since 2002 that the Committee relevant for this evaluation.

At its eightieth meeting, the Committee reviewed publications on dietary exposure to benzoates from all foods between 2000 and 2015. Therefore, the Committee performed a literature search from January 2015 to March 2021 in PubMed and found four publications that were considered relevant for the evaluation.

2.1.2 Chemical and technical considerations

Benzoic acid ($C_7H_6O_2$; CAS No. 65-85-1; INS 210) occurs naturally in organic tissues and can be generated in fermented products. As benzoic acid has antibacterial and antifungal activities, it has applications in food manufacture.

Benzoic acid is synthesized by liquid-phase oxidation of toluene with oxygen in the presence of a cobalt-containing catalyst (2). During oxidation, several by-products are formed, such as benzaldehyde, benzyl alcohol and benzyl benzoate; small amounts of benzyl formate, benzyl acetate, biphenyl and methyl biphenyls and phthalic acid may also be formed.

For food and pharmaceutical uses, benzoic acid is purified by further processing, including sublimation, recrystallization and neutralization. Treatment with amines and rinsing are required to remove phthalic acid. Benzoic acid has been used as a preservative or flavouring agent in food, cosmetic, hygiene and pharmaceutical products. To extend its application in foods, the following water-soluble salts have been produced by neutralization: sodium benzoate ($C_7H_5NaO_2$; CAS No. 532-32-1; INS 211), potassium benzoate ($C_7H_5KO_2$; CAS No. 582-25-2; INS 212) and calcium benzoate ($C_{14}H_{10}CaO_4$; CAS No. 2090-05-3; INS 213).

2.1.3 Biochemical aspects

The Committee noted previously that benzoic acid is absorbed, primarily metabolized in the liver and completely excreted in the urine as hippuric acid (major metabolite) and benzoyl-glucuronide.

Two pharmacokinetics approaches have been proposed in order to reduce the default uncertainty (safety) factor used to set the current ADI for benzoic acid, its salts and derivatives (e.g., benzyl acetate, benzyl alcohol and benzaldehyde). Zu et al. (3) applied the procedures described in the IPCS Guidance document for use of data in dose/concentration–response assessment (4) to derive a chemical-specific adjustment factor (CSAF) from data on rat and human pharmacokinetics. A reduction of four to two times was proposed for the pharmacokinetics subfactor usually applied to account for inter-species differences. This reduction resulted in an overall uncertainty factor of 50 (i.e., $2 \times 2.5 \times 10$), rather than the default 100 (i.e. $4 \times 2.5 \times 10$). The Committee noted that a CSAF can be set for a food additive only when suitable pharmacokinetics data are available for both the relevant experimental animal species and humans. For deriving a CSAF for benzoic acid, data are available from the use of sodium benzoate at doses as high as 500 mg/kg bw per day in the long-term treatment of patients with inborn errors of urea cycle enzymes that result in hyperammonaemia.

Using a physiologically based pharmacokinetics modelling approach to simulate benzoic acid concentrations in the plasma of rats and humans, Hoffman and Hanneman (5) incorporated a seven-compartment model (i.e., blood, liver, brain, adipose, testes/ovaries and rapidly and poorly perfused tissues) with input from three compartments (blood, liver and the remaining body) for some metabolic precursors (i.e., benzyl acetate, benzyl alcohol and benzaldehyde). After a comparison of simulated benzoic acid concentrations in rat and human plasma, the authors concluded that the pharmacokinetics subfactor used to account for inter-species differences was in the range of 0.3–0.4. Hoffman and Hanneman suggested that this fractional value could be used instead of the conventional interspecies pharmacokinetics factor of 4 to yield an overall uncertainty factor of 7.5–10 (i.e., $0.3\text{--}0.4 \times 2.5 \times 10$). The Committee noted that, although the absorption and disposition of benzoic acid and its precursors is essentially the same, Hoffman and Hanneman provided no comparison of goodness-of-fit to experimental results with fewer compartments, which increases the model uncertainty. Furthermore, as the interspecies pharmacokinetics factor is essentially determined by hepatic clearance in both rats and humans, the low hepatic clearance in rats (i.e., the Michaelian constant for conversion of benzoic acid to hippuric acid) used in this model may be an underestimate, as it is based on the data after a single intravenous dose of 122 mg/kg bw.

2.1.4 Toxicological studies

In studies previously evaluated by the Committee, the oral acute toxicity (median lethal dose, LD₅₀) of benzoic acid ranged from 200 to 1200 mg/kg bw in mice to 2700 mg/kg bw for sodium benzoate in rats and 2000 mg/kg bw in rabbits and dogs.

A large number of short-term studies on benzoic acid, sodium benzoate and its benzyl derivatives have been evaluated previously by the Committee, none of which showed effects at doses up to 1000 mg/kg bw per day.

No new long-term studies were found. The Committee previously reviewed long-term studies in mice and rats on benzyl derivatives, benzyl alcohol, benzaldehyde, benzyl acetate, benzoic acid and sodium benzoate and concluded that the data did not indicate carcinogenic potential.

The Committee previously reviewed studies on genotoxicity, and, although positive results were seen in some in vitro studies, the results of in vivo studies were consistently negative. The Committee concluded that there was no concern about the genotoxicity of benzoic acid, its salts and its derivatives.

The Committee previously evaluated a four-generation reproductive toxicity study in rats, in which the highest dose tested, 10 000 ppm (1%) in the diet equivalent to 500 mg/kg bw day, was not associated with any toxicological effect (6). On the basis of these results, the Committee established a group ADI of 0–5 mg/kg bw for benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate, expressed as benzoic acid equivalents, applying a default uncertainty factor of 100.

In an extended one-generation reproductive toxicity study, conducted according to OECD 443 extended one-generation reproductive toxicity test guideline, doses of 0, 500, 750 or 1000 mg benzoic acid/kg bw per day were given in the diet to rats through F₀, F₁ and F₂ generations (7, 8). The study included offspring cohorts that were assessed for potential developmental immunotoxicity and developmental neurotoxicity. No treatment-related adverse effects were observed on reproductive performance, estrous cycles, parturition, litter viability or survival, pre- or post-weaning developmental landmarks, neurobehaviour, thyroid hormones, clinical pathology, gross necropsy, organ weights, histopathology or sperm parameters. Immunophenotyping and T-cell-dependent antibody responses, organ weights, histopathological examination, neuropathology and brain morphometry in the offspring were not affected by the treatment. The Committee identified a no-observed-adverse effect level (NOAEL) of 1000 mg/kg bw per day, the highest dose tested, for reproductive and developmental toxicity.

2.1.5 Allergenicity

The available human data indicate that benzoic acid and its sodium salt can trigger intolerance and allergic reactions in some individuals when ingested in food.

2.1.6 Assessment of dietary exposure

Benzoic acid and its salts are endorsed for use in 59 food categories at maximum permitted levels (MPLs) ranging from 200 mg/kg up to 5000 mg/kg, as specified in the Codex General Standard for Food Additives (GSFA), all expressed as benzoic acid. At the current meeting, the Committee evaluated estimates of dietary exposure to benzoates from water-based flavoured drinks submitted by the sponsor for Brazil, Canada, Mexico and the USA (9, 10), based on maximum use levels of benzoates (expressed as benzoic acid) of up to 438 mg/kg in regular carbonated soft drinks and market volume-weighted average use levels ranging from 39 to 197 mg/kg. In addition, dietary exposure estimates from the literature were assessed for Europe (11), India (12), the Islamic Republic of Iran (13, 14) and various other countries, as reviewed at the eightieth meeting of the Committee ([Annex 1](#), references 223 and 224). [Table 1](#) gives an overview of the dietary exposure estimates, all expressed as benzoic acid. The estimates of dietary exposure for Brazil, Canada, Mexico and the USA and for Europe are “brand-loyal estimates”, which account for brand loyalty by mapping the consumption of such foods at a maximum reported use level and that of other foods that may contain benzoates at a typical use level or at a market volume-weighted average use level. The estimates of dietary exposure to benzoates for Europe covered 26 of the 32 food categories for which the use of benzoates is authorized in the European Union according to Annex II of Regulation (EC) No. 1333/2008.

Benzoic acid may also be present naturally in foods, such as in berries, but the concentrations are usually not high. In Europe, benzoates may also be present in food due to their use as preservatives in food additives, food enzymes and flavouring preparations according to Annex III to Regulation (EC) No 1333/2008; however, the concentrations of benzoic acid in food are not expected to be high. In view of the wide range of foods in which benzoic acid may occur, naturally and from use of benzoates in food additives, food enzymes and flavouring preparations, however, dietary exposure to benzoic acid may not be negligible.

The most complete assessment of dietary exposure to benzoates from their use as food additives was performed for the European population. Dietary exposure in a brand-loyal scenario could be as high as 7.1 mg/kg bw per day for children aged 3–9 years ([Table 1](#)). The Committee considered that this high estimate of dietary exposure was the most suitable estimate currently available for

Table 1

Overview of estimated dietary exposure of the total population to benzoates (as benzoic acid)^a from their use as food additives

Country/ source	Foods included	Source concentrations	Consumption	Dietary exposure (mg/kg bw per day) ^{b,c}			
				Children ^d		General population ^e	
				Mean	High	Mean	High
Brazil, Canada, Mexico, USA	Water-based flavoured drinks	Maximum and market volume- weighted use levels	Individual food consumption data	0.63 0.15–0.48 1.5–1.6 0.44–0.83	2.7 0.89–2.4 4.8–5.1 2.7–3.2	0.41 0.33 1.2 0.74	1.8 1.7 4.3 2.9
Europe ^f	Whole diet	Maximum and average use; analytical concentrations	Individual food consumption data	0.07–3.2	0.4–7.1	0.07–3.2	0.4–7.1
India	Pickles, sauces, soft and fruit drinks, jellies, jams	Analytical concentrations	Individual food consumption data	0.9–1.3	–	–	4.3
Iran, Islamic Republic of	Orange juice Cake, toast bread, tomato paste, mayonnaise, carbonated soft drinks, <i>olovieh</i> salad	Analytical concentrations	Per capita Mean consumption	– 0.1–1.5	0.94 ^g 0.14	–	2.9
Eightieth meeting literature ^h	Whole diet	Use / analytical concentrations	Individual food consumption data ⁱ	0.1–1.5	0.4–3.9 ^j	0.01–1.5	0.2–3.1

Source: references 9–14, [Annex I](#), reference 244.

^a Exposure estimates for India and the Islamic Republic of Iran refer to dietary exposure to sodium benzoate but converted to benzoic acid according to molecular weight. As no information was provided for the estimates from the literature summarized by the Committee at its eightieth meeting, they were assumed to refer to benzoic acid.

^b High exposure: 95th percentile

^c Dietary exposure for Brazil, Canada, Mexico and the USA and for Europe were calculated for a “brand-loyal” scenario. For more details see the text.

^d Children aged 10–17 years in Brazil, 2–17 years in Canada, 1–17 years in Europe, Mexico, the USA and literature from the eightieth meeting, and 2–19 years for India.

^e The general population of Europe covers people aged from 12 weeks up to > 65 years; for Mexico, the USA and estimates from the literature, people aged ≥ 1 year; for Brazil, ≥ 10 years and for Canada ≥ 2 years. The ages for the general population in the Islamic Republic of Iran were not specified.

^f The European countries were Austria, Belgium, Bulgaria, Czechia, Cyprus, Denmark, France, Germany, Greece, Finland, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden and the United Kingdom.

^g The Committee noted that no information was provided on the consumption level of orange juice, body weight and the actual sodium benzoate concentration used to obtain this dietary exposure estimate.

^h Refers to literature-derived dietary exposure estimates for exposure to benzoates in all foods for the total population as published between 2000 and 2015 and summarized by the Committee at its eightieth meeting. These estimates are for Australia, Austria, Belgium, Brazil, China, Denmark, France, Ireland, Italy, Lebanon, New Zealand, Republic of Korea, Saudi Arabia, Serbia and the United Kingdom.

ⁱ Exposure estimates for Brazil were based on per capita estimates of consumption.

^j High exposure in children is the 97.5th percentile.

evaluating dietary exposure to benzoates expressed as benzoic acid, as it accounts for people who are loyal to brands of foods, such as water-based flavoured drinks, over a long period. In addition, this estimate applies to the majority of foods to which benzoates may be added as additives in the European Union. The estimate was also considered conservative enough to include dietary exposure to benzoic acid from natural sources and from authorized use of benzoates as preservatives in food additives, food enzymes and flavouring preparations in the European

Union. The Committee further noted that this high exposure estimate exceeds the high dietary exposure estimates for consumers only reported by the sponsor.

2.1.7 Benzene as a reaction product in benzoic acid-containing beverages

Benzene is a known human carcinogen after chronic inhalation (15). During the early 2000s, it was found in trace quantities in some soft drinks and other beverages, where it might have been formed during storage by radical-initiated decarboxylation of benzoic acid (16). Studies have indicated higher concentrations of benzene in beverages that contain benzoate and ascorbic acid (17, 18).

Following investigations by a number of national regulatory agencies and the development of mitigation strategies, analyses of reformulated products demonstrated that benzene could not be detected in some samples and that benzene levels were commonly < 5 ng/mL in all others (17–20). The present Committee considered these findings in its assessment of the safety of foods containing benzoic acid and related compounds.

Estimated dietary exposure to benzene from beverages and foods is low. An assessment made as part of an examination of the use of the concept of “margin of exposure” (MOE) presented estimates of dietary exposure to benzene from beverages of 8 ng/kg bw per day and from food of 3–50 ng/kg bw per day. Based on a benchmark dose level with a 10% extra risk of adverse effects (BMDL_{10}) of 17.6 mg/kg bw per day from dose–response modelling of data on experimental animals, the MOEs were calculated to be 2×10^6 for beverages and between 6×10^6 and 0.4×10^6 for food (21).

The Committee noted that food and beverages make a much smaller contribution to human exposure than other sources, such as inhalation, vehicle fuel fumes and cigarette smoking (20–22). WHO has set a guideline for benzene in drinking-water at 10 µg/L (23).

2.1.8 Evaluation

The Committee previously established a group ADI of 0–5 mg/kg bw for benzoic acid, its salts and derivatives expressed as benzoic acid on the basis of a four-generation reproductive toxicity study in rats that showed no toxicological effects at the highest dose tested, 10 000 ppm (1%) in the diet equivalent to 500 mg/kg bw per day. The previous Committee applied a default uncertainty factor of 100 to the dose of 500 mg/kg bw per day.

At its present meeting, the Committee evaluated a new extended one-generation reproductive toxicity study on benzoic acids. This study showed no treatment-related adverse effects, indicating a NOAEL of 1000 mg/kg bw per day, the highest dose tested.

The Committee evaluated two approaches to refining the uncertainty factor to be used in establishing an ADI and concluded that the CSAF approach of Zu et al. (3) was the most appropriate. The Committee therefore applied a CSAF of 2 for interspecies toxicokinetics variation instead of the default factor of 4.0. An overall uncertainty factor of 50 (2 for interspecies toxicokinetic variation $\times$ 2.5 for interspecies toxicodynamic variation $\times$ 10 for interindividual variation) was applied to the NOAEL of 1000 mg/kg bw per day identified in the new one-generation reproductive toxicity study in rats. The Committee established a group ADI of 0–20 mg/kg bw. This group ADI applies to benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate, expressed as benzoic acid equivalents. The Committee withdrew the previous group ADI of 0–5 mg/kg bw.

The Committee noted that the high dietary exposure estimate, expressed as benzoic acid, of 7.1 mg/kg bw per day for children aged 3–9 years does not exceed the group ADI of 0–20 mg/kg bw. The Committee considered this dietary exposure to be of no concern. In addition, the Committee noted that the highest estimates of dietary exposure, expressed as benzoic acid, from water-based flavoured drinks evaluated by the Committee at its eightieth meeting also did not exceed the ADI of 0–20 mg/kg bw ([Annex 1](#), references 223 and 224). These dietary exposure estimates do not include contributions from benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate due to their use as flavouring agents; however, dietary exposure to these derivatives is expected to be low and would be covered by the conservative dietary exposure estimates to benzoic acid and its salts as food additives.

At its previous meeting, the Committee identified reports of human idiosyncratic intolerance and of allergy to benzoate. The Committee noted that intolerance and allergenicity to benzoate may pose a health concern to sensitive individuals.

The Committee noted that benzene can be formed as a reaction product in benzoic acid-containing beverages. On the basis of the available information, the Committee concluded that exposure to benzene from soft drinks and other foods formulated with benzoic acid or its salts is of little concern from a public health perspective.

An addendum to the toxicology and dietary exposure monograph was prepared.

The specifications were revised, and a chemical and technical assessment was prepared.

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2.2 Collagenase from *Streptomyces violaceoruber* expressed in *S. violaceoruber*

2.2.1 Explanation

At the request of the Codex Committee on Food Additives (CCFA) at its Fifty-first Session (1), the Committee evaluated the safety of collagenase (microbial collagenase; IUBMB EC No. 3.4.24.3) from *Streptomyces violaceoruber* pCol, which it had not previously considered.

In this report, the term “collagenase” refers to the collagenase enzyme and its amino acid sequence, the term “enzyme concentrate” to the test material used in the toxicity studies and the term “enzyme preparation” to the formulated product for commercial use.

At its present meeting, the Committee considered the submitted data and also conducted a literature search in Google Scholar with the linked search terms “collagenase” and “*Streptomyces violaceoruber*”, which identified 27 references. One reference (2) was relevant to this toxicological evaluation; however, it was based entirely on the studies in the submitted dossier.

2.2.2 Genetic background

The production organism, *S. violaceoreuber*, also referred to as *S. lividans* or *S. coelicolor*, belongs to the genus *Streptomyces*. *S. violaceoreuber* is non-pathogenic, non-toxicogenic, occurs in nature as a component of soil (3) and has a history in the production of enzymes intended for use in food processing (4).

The *S. violaceoreuber* pCol production strain was obtained by transforming a plasmid containing a promoter sequence obtained from *S. avermitilis* ATCC 31267, the collagenase gene obtained from *S. violaceoruber* NBRC 15146, a terminator sequence obtained from *S. cinnamoneus* NBRC 12852 and a selectable marker. The resulting plasmid was incorporated into the host organism, *S. violaceoruber* 1326, by electroporation. The stability of the introduced sequences was confirmed by cultivating the production strain over three generations and measuring collagenase activity each time. The final enzyme preparations were tested for the absence of an antibiotic resistance gene by polymerase chain reaction. The production strain has been deposited at National Institute of Technological Evaluation in Japan.

2.2.3 Chemical and technical considerations

Collagenase is produced by controlled fermentation of a pure culture of the *S. violaceoruber* production strain. Manufacture of the collagenase enzyme preparation includes fermentation (pre-, seed and main fermentation), recovery and formulation. After fermentation, the broth containing the collagenase enzyme is separated from the biomass by sedimentation; this is followed by three filtration steps. The resulting liquid preparation is formulated with water and glycerol. Two powdered enzyme preparations are produced by further filtering and freeze-drying the liquid filtrate, followed by formulation with dextrin. The entire process is performed in accordance with current good manufacturing practices and with raw materials that are food-grade. The primary sequence of collagenase produced by *S. violaceoruber* consists of 865 amino acids; its molecular weight calculated from the determined amino acid sequence is 92.4 kDa. The enzyme concentrate is tested to ensure that it is free from the production organism and any antibiotic activity.

The activity of collagenase is determined spectrophotometrically by measuring the hydrolysis of a defined peptide substrate at 570 nm; one unit of activity is defined as the quantity of enzyme required to liberate one $\mu\text{mol}/\text{min}$ of glycine under the conditions of the assay. The mean activities of collagenase from three batches each of the liquid and the two powder enzyme preparations were 477 U/g, 122 U/g and 2690 U/g, respectively.

The enzyme catalyses the hydrolysis of peptide bonds in collagen. The collagenase enzyme preparation is intended for use as a processing aid in the

production of meat and sausage casings and in the production of collagen hydrolysates used as ingredients in foods, such as those for special nutritional purposes, sports foods and health foods and in dietary supplements. The collagenase enzyme preparations are used at maximum levels of 1188 mg total organic solids (TOS)/kg raw material (as a liquid) or 36 mg TOS/kg raw material (as a powder) and 1566 mg TOS/kg raw material (as a powder). The TOS includes the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during manufacture.

The collagenase enzyme is inactivated by heat treatment before use of the final foods. If it were present in the finished food it would probably be digested, like most other proteins, although no data were available on its digestibility.

2.2.4 Biological data

Biotransformation

No information was available.

2.2.5 Allergenicity

The enzyme collagenase from *S. violaceoruber* was assessed as a potential allergen according to bioinformatics, consistent with the criteria recommend by FAO/WHO and others (5–7). The amino acid sequence of the enzyme was compared with those of known allergens in two online databases of known allergens. No statistically significant matches were found in either database. The Committee concluded that the enzyme was not anticipated to pose an allergenic risk.

2.2.6 Toxicological studies

A study of acute oral toxicity in rats (8) was conducted with the enzyme concentrate (TOS: 93.6%), which was mixed in water and administered by gavage. The oral LD₅₀ of the enzyme concentrate was > 2000 mg/kg bw enzyme concentrate, equal to 1879.2 mg TOS/kg bw.

In a 13-week study of oral toxicity in rats (9), a powdered enzyme concentrate (TOS: 93.96 %) was mixed in water and administered by gavage at 1000 mg /kg bw per day, equal to 939.6 mg TOS/kg bw per day, for 90 days. Adverse effects included several anomalies in blood chemistry, but these were observed only in one high-dose female. The cause of these changes could not be established; however, the Committee noted that the changes were extreme. The findings were considered not to be due to treatment, as only one animal was affected, and there was no indication of similar effects in any other animal. The results for the one animal were therefore excluded. The Committee identified a NOAEL of 940 mg TOS/kg bw per day (rounded from 939.6), the highest dose tested.

A powdered enzyme concentrate (TOS content, 93.96%) was tested for genotoxicity in a bacterial reverse mutation test, an in vitro mammalian cell gene mutation assay (mouse lymphoma TK assay) and an in vivo micronucleus induction assay in rats (10–13). The results of the bacterial reverse mutation assay were negative, those of the mammalian cell gene mutation assay were equivocal, and those of the in vivo micronucleus induction assay were negative. The Committee had no concern about the genotoxicity of the collagenase enzyme concentrate.

2.2.7 Observations in humans

No information was available.

2.2.8 Assessment of dietary exposure

The Committee evaluated an estimate of the theoretical maximum daily intake (TMDI) of the collagenase enzyme preparation conducted with the budget method. The TMDI was based on the level of TOS in the collagenase enzyme preparation and its maximum proposed use levels (equivalent to ≤ 36.36 mg TOS/kg in solid foods and ≤ 7 mg TOS/kg in non-milk beverages) and an assumption that 12.5% of solid foods and 25% of the non-milk beverages contain the enzyme preparation. The TMDI also included exposure to dietary supplements based on maximum proposed use levels (70 mg TOS/kg) and a daily dose of 24 g/day. The resulting TMDI was 0.43 mg TOS/kg bw per day from solid food, non-milk beverages and dietary supplements. For the dietary exposure assessment, it was assumed that 100% of the enzyme remains in the final food. The Committee noted that the enzyme is inactivated during the processing of food ingredients and will have no function in the final food.

2.2.9 Evaluation

The Committee identified a NOAEL of 940 mg TOS/kg bw per day, the highest dose tested in a 13-week study of oral toxicity in rats. When this NOAEL was compared with the estimated dietary exposure of 0.43 mg TOS/kg bw per day, the MOE was > 2100 . In view of this MOE and the lack of concern about genotoxicity, the Committee established an ADI “not specified”¹ for collagenase from *S. violaceoruber*, when used in the applications specified and in accordance with good manufacturing practice.

A toxicology and dietary exposure monograph was prepared.

New specifications and a chemical and technical assessment were prepared.

¹ The reader is referred to the Technical Report of the 87th JECFA meeting (Annex 1, reference 243) for clarification of the term “ADI not specified”.

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2.3 β -Glucanase from *Streptomyces violaceoruber* expressed in *S. violaceoruber*

2.3.1 Explanation

At the request of the Codex Committee on Food Additives at its fifty-first session (1), the Committee evaluated the safety of β -glucanase (Enzyme Commission No. 3.2.1.39; Chemical Abstract Services No. 9025-37-0) from *Streptomyces violaceoruber* pGlu. The Committee had not evaluated this enzyme preparation previously.

In this report, the term “ β -glucanase” refers to the β -glucanase enzyme and its amino acid sequence, the term “enzyme concentrate” to the test material used in the toxicity studies and the term “enzyme preparation” to the formulated product for commercial use.

At the present meeting, the Committee considered the submitted data and also conducted a literature search in Google Scholar with the linked search terms “ β -glucanase” and “*Streptomyces violaceoruber*”, which identified 25 references; however, none was considered relevant for the toxicological evaluation.

2.3.2 Genetic background

The production organism, *S. violaceoruber*, also referred to as *S. lividans* or *S. coelicolor*, belongs to the genus *Streptomyces*. *S. violaceoruber* is non-pathogenic, non-toxicogenic, occurs in nature as a component of soil (2) and has a history of use in the production of enzymes intended for use in food processing (3).

The *S. violaceoruber* pGlu production strain was obtained by transforming a plasmid containing a promoter sequence obtained from *S. cinamomeus* TH 2, the β -glucanase gene obtained from *S. violaceoruber* NBRC 15146 and a terminator sequence obtained from *S. cinamomeus* NRBC 12852. The stability of the introduced sequences was confirmed by cultivating the production strain for multiple generations and measuring β -glucanase activity each time. The final enzyme preparations were tested for the absence of antibiotic resistance genes by PCR. The production strain has been deposited at the National Institute of Technology and Evaluation in Japan.

2.3.3 Chemical and technical considerations

β -Glucanase is produced by controlled fermentation of a pure culture of the *S. violaceoruber* production strain. The manufacture of the β -glucanase enzyme preparation includes fermentation (seed, pre- and main culture), recovery and formulation. After fermentation, the broth containing the β -glucanase enzyme is separated from the biomass; this is followed by multiple filtration steps and dispersion at controlled temperature, pressure and pH. The resulting precipitate is formulated with glycerol to the final β -glucanase enzyme preparation. A powdered enzyme preparation is produced by further filtering and freeze-drying of the liquid formulation, followed by standardization with sodium chloride. The entire process is performed in accordance with current good manufacturing practices and with food-grade raw materials. The primary sequence of β -glucanase produced by *S. violaceoruber* consists of 453 amino acids; its molecular weight calculated from the determined amino acid sequence is 42.7 kDa. The enzyme concentrate is tested to ensure that it is free of the production organism and any antibiotic activity.

The activity of β -glucanase is determined spectrophotometrically by measuring the hydrolysis of 1,3- β -D-glucan substrate by the enzyme at 490 nm; one unit of activity is defined as the quantity of enzyme required to catalyse the formation of 1 μ mol/min of glucose under the conditions of the assay. The mean activity of β -glucanase from three batches of the liquid and powder enzyme concentrates were 12 897 U/g and 23 041 U/g, respectively.

β -Glucanase catalyses the hydrolysis of the (1 $\rightarrow$ 3)- β -D-glucosidic linkages in (1 $\rightarrow$ 3)- β -D-glucans to produce D-glucose and β -glucans. The enzyme preparation is intended for use as a processing aid in the manufacture of yeast and mushroom extracts for use as ingredients in seasonings and in the production of beer. The enzyme preparation is added to disrupt the cell walls of mushroom and yeast raw material to improve yield, with residual filtration of the extract products; it is used as a clarifying and filtration aid in the production of beer. The β -glucanase enzyme preparation is intended to be used at a maximum level of 151 mg TOS of powdered β -glucanase/kg raw material and 202 mg TOS of liquid β -glucanase/kg raw material. The TOS includes the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during manufacture.

The β -glucanase enzyme is inactivated by heat treatment during processing. It is not expected to have any technological function in the finished foods. If it is present in finished foods, it would probably be digested, like most other proteins, although no data were available on its digestibility.

2.3.4 Biological data

Biotransformation

No information was available.

2.3.5 Assessment of potential allergenicity

β -Glucanase was assessed as a potential allergen according to bioinformatics consistent with the criteria recommend by FAO/WHO (4), Codex Alimentarius (5) and JECFA (6). The amino acid sequence of the enzyme was compared with those of known allergens in two publicly available databases. A search for matches with > 35% identity over a sliding window of 80 amino acids and a search for sequence identity of eight contiguous amino acids produced a small number of matches. Upon examination, however, these matches were considered not significant. In view of the intended use and available information, the Committee did not anticipate that β -glucanase would pose an allergenic risk.

2.3.6 Toxicological studies

A study of acute oral toxicity in rats (7) was conducted with the enzyme concentrate mixed in water and administered as a single gavage dose. The oral LD₅₀ was > 2000 mg/kg bw of the enzyme concentrate, equal to 1906.6 mg TOS/kg bw.

In a 2-week dose range-finding study in rats (8), no significant toxicity was observed when the enzyme concentrate was mixed in water and administered by gavage at doses $\leq$ 1000 mg/kg bw, equal to 953.3 mg TOS/kg bw.

In a 13-week study of oral toxicity in rats (9), the enzyme concentrate was mixed in water and administered at doses $\leq$ 1000 mg/kg bw, equal to 953.3 mg TOS/kg bw, to groups of rats by gavage. The only treatment-related observation was hyperplasia of the forestomach in male and female rats at the high dose. This was considered not to be related to systemic toxicity but rather an artefact of gavage with increasing concentrations of an acidic substance, which resulted in local irritation. The Committee identified a NOAEL of 950 mg TOS/kg bw per day (rounded by the Committee from 953.3), the highest dose tested.

The enzyme concentrate gave negative results in a bacterial reverse mutation assay, an in vitro micronucleus assay and an in vivo micronucleus assay (10–12). The enzyme concentrate gave negative results in an in vitro chromosomal aberration assay without metabolic activation and positive results with metabolic activation, after 6 h of exposure. Under the conditions of the in vitro chromosomal aberration assay with metabolic activation, the enzyme concentrate caused structural aberrations, but it did not induce polyploidy aberrations (13). The Committee had no concern about the genotoxicity of the enzyme concentrate.

2.3.7 Observations in humans

No information was available.

2.3.8 Assessment of dietary exposure

The Committee evaluated an estimate of the TMDI of the β -glucanase enzyme preparation derived with the budget method. The TMDI was based on the level of TOS in the β -glucanase enzyme preparation and its maximum proposed use levels (equivalent to ≤ 8.08 mg TOS/kg in solid foods and ≤ 1.9 mg TOS/kg in non-milk beverages) and on the assumption that 25% of the food supply contains the enzyme preparation. The resulting TMDI was 0.15 mg TOS/kg bw per day (rounded by the Committee from 0.149) from both solid food and non-milk beverages. For the dietary exposure assessment, it was assumed that 100% of the enzyme remains in the final food. The Committee noted that the enzyme will be inactivated during the processing of food ingredients and will have no technical function in the final food.

2.3.9 Evaluation

The Committee identified an NOAEL of 950 mg TOS/kg bw per day, the highest dose tested in the 13-week study of oral toxicity in rats. Comparison of this NOAEL with the estimated dietary exposure of 0.15 mg TOS/kg bw per day gave an MOE > 6300 . On the basis of this MOE and the lack of concern about genotoxicity, the Committee established an ADI “not specified”¹ for β -glucanase from *S. violaceoruber*, for the proposed uses and in accordance with good manufacturing practice.

A toxicology and dietary exposure monograph was prepared.

New specifications and a chemical and technical assessment were prepared.

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2.4 Phospholipase A2 from *Streptomyces violaceoruber* expressed in *S. violaceoruber*

2.4.1 Explanation

At the request of the CCFA at its fifty-first Session (1), the Committee evaluated the safety of phospholipase A2 (Enzyme Commission No. 3.1.1.4) from *Streptomyces violaceoruber* for the first time.

In this report, the term “phospholipase A2” refers to the phospholipase A2 enzyme and its amino acid sequence, the term “enzyme concentrate” refers to the test material used in the toxicity studies, and the term “enzyme preparation” refers to the product formulated for commercial use.

The Committee at its present meeting considered the submitted data and conducted a literature search in the PubMed (all fields), Scopus (title, abstract, keywords) and Embase (title, abstract, keywords) with the linked search terms “phospholipase A2” and “streptomyces” or “violaceoruber”. The search yielded 118 unique references, none of which reported biochemical and/or toxicological studies on phospholipase A2 from *S. violaceoruber*.

2.4.2 Genetic background

The production organism, *S. violaceoruber*, also referred to as *S. lividans* or *S. coelicolor*, belongs to the genus *Streptomyces*. *S. violaceoruber* is non-pathogenic and non-toxigenic and occurs in nature as a component of soil (2). It has a history of use in the production of enzymes intended for use in food processing (3).

The *S. violaceoruber* AS-10 production strain was obtained by transforming a plasmid containing an expression cassette with the phospholipase A2 encoding gene from *S. violaceoruber* NBRC 15146 donor, a suitable promoter and terminator encoding phospholipase D from *S. cinnamomeum* and a selectable marker, ligated with a plasmid obtained from *S. violaceoruber* ATCC 35287. The resulting plasmid was incorporated into the host organism, *S. violaceoruber* 1326, by electroporation. The stability of the introduced sequences was confirmed by cultivating the production strain over three generations and by measuring phospholipase A2 activity each time. The final enzyme preparations were tested for the absence of an antibiotic resistance gene by PCR. The production strain has been deposited at the National Institute of Technology and Evaluation in Japan.

2.4.3 Chemical and technical considerations

Phospholipase A2 is produced by controlled submerged fermentation of a pure culture of the *S. violaceoruber* production strain. Manufacture of the phospholipase A2 enzyme preparation includes fermentation (pre-, seed and main fermentation), recovery and formulation. After fermentation, the broth containing phospholipase A2 enzyme is separated from the biomass by

sedimentation; this is followed by several filtration steps. The resulting liquid filtrate is formulated with water, sorbitol, potassium sorbate and sodium chloride to obtain the liquid phospholipase A2 enzyme preparation. A powdered enzyme preparation is produced by further filtering and freeze-drying the liquid filtrate, followed by formulation with sodium chloride. The entire process is performed in accordance with current good manufacturing practices and with food-grade raw materials. The primary amino acid sequence of phospholipase A2 produced by *S. violaceoruber* consists of 151 amino acids; its molecular weight, calculated from the determined amino acid sequence, is 16.4 kDa. The enzyme preparation is tested for the absence of any of the major food allergens that are present in the fermentation medium. The enzyme concentrate is tested to ensure that it contains neither the production organism nor any antibiotic activity.

The activity of phospholipase A2 is determined spectrophotometrically by measuring the hydrolysis of a phosphatidylcholine substrate by the enzyme at 550 nm; one unit of activity is defined as the quantity of enzyme required to liberate 1 $\mu\text{mol/min}$ of fatty acid from L- α -phosphatidylcholine under the conditions of the assay. The mean activities of phospholipase A2 from three batches of the liquid and the powder enzyme concentrates are 10 400 U/g and 114 200 U/g, respectively.

Phospholipase A2 catalyses the hydrolysis of the sn-2 ester bonds of diacylphospholipids to form 1-acyl-2-lysophospholipids and free fatty acids; when added to food, this improves emulsification. The enzyme preparation is intended for use as a processing aid in the manufacture of enzyme-modified egg yolk, lecithin, cereal flour, dairy products and vegetable oil. The phospholipase A2 enzyme preparation is intended to be used as a processing aid at a maximum level of 105 mg total organic solids (TOS) of powdered phospholipase A2/kg raw material and 459 mg TOS of liquid phospholipase A2/kg raw material. The TOS includes the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during the manufacturing process.

The phospholipase A2 enzyme is inactivated by heat treatment before use of the final foods. If present, it is expected that phospholipase A2 will be digested, as would most other protein occurring in food, but no data were available on its digestibility.

2.4.4 Biotransformation

No information was available.

2.4.5 Assessment of potential allergenicity

Phospholipase A2 from *S. violaceoruber* was evaluated for allergenicity according to the bioinformatics criteria recommended by FAO/WHO (4, 5) and modified at the eightieth meeting of the Committee ([Annex 1](#), reference 223). The amino acid sequence of phospholipase A2 from *S. violaceoruber* was compared with those of known allergens in publicly available databases. A search for matches with > 35% identity in a sliding window of 80 amino acids, a search for sequence identity of 8 contiguous amino acids and a full-length FASTA sequence search produced no matches. Therefore, the Committee considered that dietary exposure to phospholipase A2 from *S. violaceoruber* is not anticipated to pose a risk of allergenicity.

2.4.6 Toxicological data

In a study of oral acute toxicity in rats with powdered phospholipase A2 concentrate, the LD₅₀ was estimated to be > 1912 mg TOS/kg (6).

No treatment-related effects were observed in a range-finding study in which rats were given powdered enzyme concentrate at doses up to 956 mg TOS/kg bw per day by oral gavage for 2 weeks (7).

In a 13-week study of oral toxicity in rats, treatment-related effects were observed on the caecum and the stomach when the powdered phosphodiesterase enzyme concentrate was administered by gavage (8). In the stomach, hyperplasia of the limiting ridge was observed in all animals at the high dose, one female at the mid dose, one male at the low dose and two males in the control group. Diffuse mucosal hyperplasia of the stomach was observed in four males and two females at the high dose, and globule leukocyte infiltration was observed in the stomachs of two males and five females at the high dose, one female at the mid dose and one male in the control group. The effects were classified as minimal or mild. The Committee considered that the effects in the stomach were probably local irritation due to administration of the enzyme concentrate by gavage and were not relevant to the human situation. In the caecum, minimal diffuse mucosal hyperplasia was observed in three males at the high dose, one female at the mid dose and one female at the high dose. The Committee considered that the effects in the caecum were treatment-related adverse effects. In addition, two males at the high dose had extramedullary haematopoiesis in the spleen, although no accompanying effects on haematological parameters were seen. Minimal mineralization of the arterial wall of the lungs occurred in two males and two females at the high dose and in one male in the control group. A statistically significant decrease in grip strength and a statistically significant increase in relative, but not absolute, liver weight were also observed in males at the high dose. Although the effects at the high dose of 956 mg/kg bw per day were small

or occurred at a low incidence, they might have been related to treatment. On this basis, the Committee identified a no-observed-adverse-effect level (NOAEL) of 190 mg/kg per day (rounded by the Committee from 191 mg/kg bw per day).

The powdered enzyme concentrate was not genotoxic in a bacterial reverse mutation assay (9) or in an in-vitro chromosomal aberration assay (10). The Committee had no concerns with respect to the genotoxicity of the phospholipase A2 enzyme preparation.

2.4.7 Assessment of dietary exposure

The Committee evaluated one estimate of dietary exposure for phospholipase A2 from *S. violaceoruber* submitted by the sponsor. The enzyme is used in a broad spectrum of food and beverages, including milk. The estimate was derived with the budget method and was based on maximum use levels of 6.42 mg TOS/kg for solid foods, 4.59 mg TOS/kg for non-milk beverages and 9.17 mg TOS/kg for milk, and on the assumption that 25% of the food supply would contain the enzyme preparation. The theoretical maximum daily intake was estimated to be 0.25 mg TOS/kg bw per day (rounded by the Committee from 0.252 mg TOS/kg bw per day). For the dietary exposure assessment, it was assumed that the enzyme is not removed during final processing of ingredients or foods and that 100% of the enzyme remains in the ingredient and final food. The Committee noted that the enzyme will be inactivated during the processing of food ingredients and has no function in the final food.

2.4.8 Evaluation

The Committee identified a NOAEL of 190 mg TOS/kg bw per day in a 13-week study in rats. A comparison of the estimated dietary exposure of 0.25 mg TOS/kg bw per day with the NOAEL of 190 mg TOS/kg bw per day from the oral toxicity study gives a MOE of 760. On this basis and in the absence of concern about genotoxicity, the Committee established an ADI “not specified”¹ for the phospholipase A2 enzyme preparation from *S. violaceoruber* when used in the applications specified and in accordance with good manufacturing practice.

A toxicology and dietary exposure monograph was prepared.

New specifications and a chemical and technical assessment were prepared.

¹ The reader is referred to the Technical Report of the 87th JECFA meeting (Annex 1, reference 243) for clarification of the term “ADI not specified”.

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2.5 Riboflavin from *Ashbya gossypii*

2.5.1 Explanation

Riboflavin, or 7,8-dimethyl-10-(1'-D-ribityl)isoalloxazine, (7,8-dimethyl-10-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]benzo[g]pteridine-2,4-dione), is commonly

known as vitamin B2. It can be obtained by chemical synthesis or by fermentation. Riboflavin and riboflavin-5'-phosphate are used as food colours.

At its thirteenth meeting, the Committee established an ADI of 0–0.5 mg/kg bw for riboflavin on the basis of the absence of any adverse effects at the only dose tested of 50 mg/kg bw per day in a three-generation study of reproductive toxicity in rats (1). At its twenty-fifth meeting, the Committee included riboflavin and riboflavin-5'-phosphate, expressed as riboflavin, in a newly established group ADI of 0–0.5 mg/kg bw (2). At its fifty-first meeting, the Committee evaluated riboflavin produced by fermentation from *Bacillus subtilis* and included it in the group ADI of 0–0.5 mg/kg bw for riboflavin and riboflavin-5'-phosphate, on the basis of its equivalence to riboflavin (3).

The CCFA at its Fifty-first Session requested the Committee to evaluate riboflavin from *Ashbya gossypii* as an alternative source of riboflavin for colouring purposes and as a nutrient source (4). At its present meeting, the Committee evaluated riboflavin from *A. gossypii* for use as a food colour for the first time. It did not review the nutrient properties of riboflavin but took into account dietary exposure from all sources of riboflavin, including as a nutrient.

A toxicological dossier was received, with relevant study reports and publications. A comprehensive literature search on riboflavin from *A. gossypii* conducted on eight databases identified two additional studies for evaluation, and an additional search on riboflavin from other sources identified four additional studies for evaluation.

2.5.2 Genetic background

The Committee at its present meeting evaluated the information provided by the sponsor on use of the filamentous fungus *A. gossypii* (*Eremothecium gossypii*) in the production of commercial riboflavin.

A. gossypii is a naturally occurring phenotypic riboflavin-overproducing organism (5), which possibly provides protection against ultraviolet radiation (6). Early development of *A. gossypii* strains for commercial production of riboflavin involved classical mutagenesis and strain selection to obtain a high riboflavin titre. The *A. gossypii* strain LU8907 was continuously developed into several commercial strains to further increase riboflavin production. The present production strain, *A. gossypii* LU11439, was constructed from the recipient strain *A. gossypii* LU8907. The recipient was modified by the addition of several genes from the wild-type *A. gossypii* strain under the control of translation elongation factor promoters and antibiotic resistance marker genes. The DNA sequences for transformation were prepared as linear, vector-free fragments and inserted by electroporation, followed by homologous recombination and targeted

integration. The production strain was confirmed to be genetically stable and not to contain any transferable marker genes or sequences derived from vector DNA.

The complete sequence and annotation of the *A. gossypii* genome was published in 2004. It shows a 95% homology and gene synteny to the genome of budding yeast, *Saccharomyces cerevisiae* (7, 8). The complete genome sequences of the sponsor's first self-cloned production strain and of the published *A. gossypii* wild-type strain (ATCC 10895) were analysed for the presence of gene clusters encoding secondary metabolites. No gene clusters of polyketide synthases or non-ribosomal peptide synthases were identified in the genome. The genome of *A. gossypii* has no potential for production of secondary metabolites.

2.5.3 Chemical and technical considerations

Riboflavin is obtained from *A. gossypii* by fermentation under controlled conditions. Several filtration and precipitation or crystallization steps result in a highly purified food-grade dry powder containing not less than 98% riboflavin, free of fermentation medium components and the production organism. The entire process is carried out in accordance with current good manufacturing practices; all raw materials used in the manufacture are food-grade.

Riboflavin was evaluated previously by JECFA (9) as a synthetic product (1987) and as a product of fermentation from *B. subtilis* (1999). Independent of the source, these additives contain not less than 98% and not more than 101% of riboflavin (on a dried basis). Riboflavin is relatively stable during food processing and storage but is very sensitive to light.

2.5.4 Biochemical aspects

Riboflavin is absorbed actively and passively mainly in the proximal small intestine, partly in the large intestine and also in the colon (10–12). Riboflavin is absorbed by two mechanisms – a saturable active component that dominates at near-physiological vitamin concentrations and a passive component that is revealed under conditions of high levels of supplementation with riboflavin. In plasma, some riboflavin is bound to albumin; however, a large portion of riboflavin is associated with immunoglobulins (A or G) for transport (13). When riboflavin is absorbed in high concentrations, little is stored in the body tissues, and the excess is excreted, primarily in the urine (14–16).

The metabolism of riboflavin begins with ATP-dependent phosphorylation to flavin mononucleotide, catalysed by the enzyme flavokinase under hormonal control. Flavin mononucleotide is then complexed with specific apoenzymes to form a variety of flavoproteins or is mainly converted to flavin-adenine dinucleotide (FAD) by FAD synthetase (17). Although FAD was the major

form in plasma, plasma riboflavin and erythrocytes flavin mononucleotide were suggested to represent riboflavin status in humans (18). Lumichrome and lumiflavin have been identified as metabolites of riboflavin in the rat, while hydroxyriboflavin and formylmethylflavin have been identified as metabolites in human plasma (19, 20).

2.5.5 Toxicological studies

The acute oral toxicity of riboflavin from *A. gossypii* is low, with an LD₅₀ of > 2500 mg/kg bw (21, 22).

In a 90-day repeated-dose oral toxicity study in rats (23), riboflavin from *A. gossypii* (purity, 82.3%, feed grade) was fed in the diet at a concentration of 0, 500, 5000 or 50 000 mg/kg diet, equal to 0, 35, 362 or 3659 mg/kg bw in males and 0, 41, 410 or 4325 mg/kg bw in females. Treatment had no effects on bw, bw gain, feed or drinking-water consumption. Foci were detected in the kidneys of two female rats at the highest dose, but were considered not to be toxicologically relevant. When accounting for the purity of the preparation used, the Committee identified a NOAEL of 3011 mg/kg bw per day for males and 3559 mg/kg bw per day for females, the highest doses tested.

In another 90-day oral toxicity study in rats (24), reviewed previously by the Committee (3), riboflavin from *B. subtilis* with a purity of 98% or 96% was fed in the diet at concentrations providing 0, 20, 50, or 200 mg/kg bw per day. The previous Committee identified a NOAEL of 200 mg/kg bw per day (3).

The European Food Safety Authority (EFSA) (25) described a further 90-day oral toxicity study in rats (Bachmann et al., 2005) performed with riboflavin from *B. subtilis* (containing 80.1 % riboflavin, feed grade). The test material was administered in the diet to provide doses of 0, 50, 100 and 200 mg/kg bw per day. Additional groups of rats were treated at the same doses for 13 weeks and then observed for a 4-week recovery period. Eosinophilic granules were observed in the renal tubules of male rats receiving 100 or 200 mg/kg bw per day at the end of the treatment period, but renal morphology returned to normal after the 4-week recovery period. The study authors pointed out that accumulation of hyaline droplets is associated with α -2 μ -globulin and is considered to be a response specific to male rats and therefore not relevant to humans. EFSA (25) concurred with this consideration and concluded that the NOAEL for the test material in this study was 200 mg/kg bw per day, the highest dose tested, corresponding to 160 mg/kg bw per day expressed as riboflavin. The Committee at its present meeting agreed with this evaluation.

No studies of chronic toxicity or carcinogenicity with riboflavin from *A. gossypii* or riboflavin from any other source were available.

Riboflavin from *A. gossypii* (purity, 99% and 80.8%) was tested in two bacterial mutagenicity assays and in an in vitro micronucleus induction assay in human lymphocytes (26–28). In spite of minor limitations, the combination of these tests fulfilled the basic requirements for an assessment of genotoxic potential, and the Committee concluded that there is no concern with respect to the genotoxicity of riboflavin from *A. gossypii*.

No reproductive or developmental toxicity was observed in a multigeneration study in which rats received riboflavin at a daily dose of 0 or 10 mg per rat (equivalent to 0 or approximately 50 mg/kg bw per day) from weaning for three generations (29). The dose of 50 mg/kg bw per day was used as the basis for the ADI of 0–0.5 mg/kg bw per day established by the Committee in 1969 (1). The present Committee noted that the report of the study provided limited experimental data, poor reporting and only one dose level was used.

In a series of intervention studies with oral administration of riboflavin, no adverse effects were reported in populations of children and adults, including healthy individuals and patients suffering from migraine, cardiovascular diseases, colorectal polyp or anaemia (30–40).

2.5.6 Assessment of dietary exposure

The Committee noted that riboflavin is endorsed for use in 71 food categories in the Codex GSFA at MPLs of 30–1000 mg/kg, while riboflavin may be used in amounts consistent with national good manufacturing practice in Australia, New Zealand, the Republic of Korea and the USA and in the European Union. The sponsor provided maximum reported use levels (MRULs) for riboflavin of 10–400 mg/kg as a food colour for the 29 food categories in which it is authorized in the European Union according to Annex II to Regulation (EC) No. 1333/2008.

Estimates of dietary exposure to riboflavin from GSFA MPLs and MRULs, in combination with food consumption data from the Food Additive Intake Model 2.0, by the sponsor were reviewed by the Committee. This model includes food consumption data from various European countries for six age groups. High-level dietary exposure estimates are calculated by adding the 95th percentile of dietary exposure to one food category at the highest dietary exposure to the mean dietary exposure resulting from consumption of all other food categories. Estimated mean and high-level dietary exposure to riboflavin of the six age groups were 2.8–18.3 mg/kg bw per day and 4.8–25.5 mg/kg bw per day with GSFA MPLs and 0.2–2.4 mg/kg bw per day and 0.4–3.6 mg/kg bw per day with MRULs. The Committee noted that the MRULs for riboflavin as a food colour were well below the GSFA MPLs for most food categories.

Estimates of dietary exposure to riboflavin from all sources, including from its use as a food additive, are available from many national dietary surveys.

EFSA (25) estimated dietary exposure to be in the range of 0.05–0.09 mg/kg bw per day for children and 0.02–0.04 mg/kg bw per day for adults. The Committee also noted estimates of dietary exposure to riboflavin from Australia (0.03 mg/kg bw per day for adults and 0.06 mg/kg bw per day for children; 41), New Zealand (0.03 mg/kg bw per day for adults and 0.05 mg/kg bw per day for children; 41), the Republic of Korea (0.02–0.03 mg/kg bw per day for adults; 42) and the USA (from 0.05 mg/kg bw per day for men to 0.14 mg/kg bw per day for children; 43). The group pf milk and dairy products group was the main contributor to dietary exposure to riboflavin in Spain at 32.3% (44), Australia at 27–28% (45) and New Zealand at 23% (46, 47).

The Committee concluded that the highest estimate of high-level dietary exposure to riboflavin of 3.6 mg/kg bw per day for children aged 3–9 years, calculated with the Food Additive Intake Model 2.0 with MRULs, should be considered in the safety assessment of riboflavin.

2.5.7 Evaluation

In its present evaluation of riboflavin from *A. gossypii*, the Committee noted that it has low acute toxicity and did not raise concern for genotoxicity. The NOAEL from a 90-day oral toxicity study in rats on riboflavin from *A. gossypii* was 3000 mg/kg bw per day (rounded by the Committee from 3011 mg/kg bw per day), the highest dose tested.

Comparison of the NOAEL of 3000 mg/kg bw per day with the estimate of dietary exposure of 3.6 mg/kg bw per day, based on maximum reported use levels, resulted in an MOE > 800. The Committee concluded that exposure to riboflavin from all sources does not represent a safety concern.

The NOAEL of 3000 mg/kg bw per day in the present evaluation of riboflavin from *A. gossypii* is considerably higher than the 50 mg/kg bw per day in the multigeneration study with a single dose level that was used by the previous Committee to establish an ADI of 0–0.5 mg/kg bw. The Committee at its present meeting noted that the toxicity database on riboflavin from various sources reviewed previously by the Committee does not indicate any adverse effects. The Committee at its present meeting established a group ADI “not specified”¹ for riboflavin, riboflavin- 5'-phosphate, riboflavin from *B. subtilis* and riboflavin from *A. gossypii* and withdrew the previous group ADI of 0–0.5 mg/kg bw.

A toxicology and a dietary exposure monograph was prepared.

New specifications and a chemical and technical assessment were prepared.

2.5.8 Future work

Regarding the previously established specifications for riboflavin and riboflavin from *B. subtilis*, the Committee proposes to:

- rename “riboflavin” as “riboflavin, synthetic”;
- replace the existing method for determination of lumiflavin in both specifications to avoid the use of chloroform; and
- delete the functional use of “nutrient supplement” from the specifications monograph on riboflavin from *B. subtilis*, as the Codex food additive definition does not include nutrients.

2.5.9 Recommendation

In view of information received at the current meeting which implies that riboflavin is no longer produced synthetically for use as a food additive, the Committee recommends that the CCFA reconsider the requirement for specifications for synthetically produced riboflavin.

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2.6 Ribonuclease P from *Penicillium citrinum*

2.6.1 Explanation

At the request of the CCFA at its Fifty-first Session (1), the Committee evaluated the safety of ribonuclease P (IUBMB EC No. 3.1.26.5) from *Penicillium citrinum*, which has not previously been evaluated.

In this report, the term “ribonuclease P” refers to the ribonuclease P enzyme and its amino acid sequence, the term “powdered enzyme concentrate” to the test material used in the toxicity studies submitted and the term “enzyme preparation” to the product formulated for commercial use.

The Committee at its present meeting considered the submitted data and searched the literature in the PubMed database (all fields), Scopus and Embase (title, abstract, keywords) with the linked search terms “ribonuclease” and (“penicillium” or “citrinum”). In total, 188 unique references were found, of which only two described biochemical and/or toxicological studies with ribonuclease P from *P. citrinum*. Most of the toxicological studies with ribonuclease P from *P. citrinum* described below were therefore submitted by the sponsor.

2.6.2 Genetic background

P. citrinum is a filamentous fungus that is ubiquitous in the environment. It occurs on various plants, including citrus fruits and wheat and other cereal grains (2). *Penicillium* species are recognized for use in food applications (3), including as a source organism in the production of ribonuclease P for use in food processing (4). The taxonomy of the source organism was confirmed from its macroscopic and microscopic characteristics. The *P. citrinum* production strain used in the

manufacture of ribonuclease P was *P. citrinum* AE-RP. The strain was verified as from *P. citrinum* by phylogenetic analysis of the rDNA sequence from the results of a BLAST homology search in the APOLLON DB-FU ver.1.0 database, which includes all sequences in the International Nucleotide Sequence Database (GenBank/DBK/EMBL).

The *P. citrinum* production strain was obtained by conventional mutation with *N*-methyl-*N'*-nitrosoguanidine, ultraviolet light and monospore isolation of the parent strain, *P. citrinum* IAM 7003. The parent strain was originally housed at the Institute of Applied Microbiology Culture Collection; it is presently held at the Japan Collection of Microorganisms under *P. citrinum* JCM 22500.

P. citrinum is known to produce citrinin, a mycotoxin (5); however, citrinin is not produced in the manufacture of ribonuclease P by fermentation of *P. citrinum* AE-RP. *P. citrinum* is an occasional opportunistic human pathogen and has been identified rarely as a cause of pneumonia in immunocompromised individuals (6, 7). No viable *P. citrinum* organisms are present in the enzyme preparation.

2.6.3 Chemical and technical considerations

Ribonuclease P is produced by controlled aerobic submerged batch fermentation of a pure culture of a selected strain of *P. citrinum* AE-RP. Ribonuclease P can also be produced by *P. citrinum* RP-4, but insufficient information was available on the enzyme concentrate produced from this strain, and enzyme preparations manufactured with the RP-4 strain were not included in this evaluation. During fermentation, the enzyme is secreted into the fermentation broth by the microbial cells. Fermentation continues for a predetermined time or until the enzyme production rate decreases below a defined threshold. The enzyme is separated from the fermentation medium in a series of filtration steps. The biomass is pre-treated with flocculants and filtration aids to facilitate removal of cell material. Germ and polish filtration are performed as part of the recovery process to prevent microbial contamination. The liquid enzyme concentrate is spray-dried, and the activity is standardized with dextrin in production of the final powdered enzyme preparation. The entire process is performed in accordance with current good manufacturing practice with food-grade raw materials. The final ribonuclease P enzyme preparation does not contain the production strain. The enzyme preparation conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing. The primary sequence of ribonuclease P produced by *P. citrinum* consists of 342 amino acids; its molecular weight by calculation from the determined amino acid sequence is 35 kDa.

Ribonuclease P catalyses the hydrolysis of RNA to monophosphate nucleotides. Ribonuclease P enzyme preparation is intended for use in processing

yeast products and flavouring substances and preparations with naturally occurring RNA. The degradation of the RNA substrate in raw materials to produce free phosphonucleotides, specifically guanine and adenine, enhances the consistency and organoleptic (flavour) properties of the final food or food ingredient. Ribonuclease P activity is measured spectrophotometrically as the release of phosphate from adenosine 3'-phosphate. One unit is defined as the amount of enzyme that liberates one $\mu\text{mol/min}$ of phosphate under the assay conditions. The mean activity of ribonuclease P from three batches of the powdered enzyme concentrate was 112 600 U/g.

The mean TOS content of the enzyme concentrate is 444 mg/g. The TOS includes the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism during manufacture. Ribonuclease P enzyme preparation is used at concentrations up to 1000 mg TOS/kg raw material. Ribonuclease P is denatured and inactivated by high temperatures ($> 80^\circ\text{C}$) during the production of processed yeast and has no technological effect in the final food. When used in the production of flavouring substances or flavouring preparations, the enzyme is either denatured or removed from the final product. Any carry-over of active ribonuclease P to food is negligible. If present, it is expected that ribonuclease P will be digested, as are most other proteins occurring in food, but no data were available on its digestibility.

2.6.4 Biotransformation

No data were available.

2.6.5 Assessment of allergenicity

Ribonuclease P from *P. citrinum* was evaluated for potential allergenicity according to the bioinformatics criteria recommended by FAO/WHO (8, 9) and modified at the eightieth meeting of the Committee ([Annex 1](#), reference 223). The amino acid sequence of ribonuclease P from *P. citrinum* was compared with those of known allergens in publicly available databases. A search for matches with $> 35\%$ identity in a sliding window of 80 amino acids, a search for sequence identity of eight contiguous amino acids and a full-length FASTA sequence search produced no matches. Therefore, the Committee concluded that dietary exposure to ribonuclease P from *P. citrinum* would not be anticipated to pose a risk of allergenicity.

2.6.6 Toxicological studies

In addition to the studies submitted on ribonuclease P from *P. citrinum* AE-RP, the literature search resulted in toxicity studies with ribonuclease P from *P.*

citrinum RP-4 (10, 11). Manufacture of ribonuclease P from *P. citrinum* RP-4 includes a precipitation step with ethanol. Therefore, the composition of the enzyme concentrates obtained with *P. citrinum* AE-RP is different from those obtained with *P. citrinum* RP-4. The Committee concluded that the studies with ribonuclease from *P. citrinum* RP-4 were not relevant for the current evaluation of ribonuclease P from *P. citrinum* AE-RP and did not include them in their evaluation.

In a 2-week dose range-finding study and a 13-week study of oral toxicity in rats with ribonuclease P from *P. citrinum* AE-RP, no treatment-related adverse effects were seen when the powdered enzyme concentrate was administered by gavage at doses up to 984 mg TOS/kg bw per day, the highest dose tested (12, 13). The Committee identified a NOAEL of 980 mg TOS/kg bw per day (rounded by the Committee from 984 mg TOS/kg bw per day), the highest dose tested.

Powdered ribonuclease P concentrate from *P. citrinum* AE-RP was not genotoxic in a bacterial reverse mutation assay or in an in vitro chromosomal aberration assay (14, 15). The Committee had no concern with respect to the genotoxicity of the preparation of ribonuclease P from *P. citrinum* AE-RP.

2.6.7 Observations in humans

No data were available.

2.6.8 Assessment of dietary exposure

The Committee evaluated an estimate of dietary exposure to ribonuclease P from *P. citrinum* submitted by the sponsor. The estimate was derived with the budget method and was based on maximum use levels of 20 mg TOS/kg for solid foods and for non-milk beverages and 1000 mg TOS/kg for dietary supplements and the assumption that 25% of the food supply would contain the enzyme preparation. It was assumed that the maximum consumption of dietary supplements would be 30 g/day. The theoretical maximum daily intake was estimated to be 1.3 mg TOS/kg bw per day (rounded by the Committee from 1.25 mg TOS/kg bw per day). For the dietary exposure assessment, it was assumed that 100% of the enzyme remains in the ingredient and final food. The enzyme is removed or inactivated by high temperatures during processing of food ingredients and would have no technological function in the final food.

2.6.9 Evaluation

The Committee identified a NOAEL of 980 mg TOS/kg bw per day (the highest dose tested) in a 13-week study in which rats were treated with ribonuclease P concentrate from *P. citrinum* AE-RP by gavage. A comparison of the estimated dietary exposure of 1.3 mg TOS/kg bw per day with the NOAEL of 980 mg TOS/

kg bw per day gives an MOE > 750. On the basis of this MOE and the lack of concern for genotoxicity, the Committee established an ADI “not specified”¹ for the ribonuclease P enzyme preparation from *P. citrinum* AE-RP, used in the applications specified and in accordance with good manufacturing practice.

A toxicology and dietary exposure monograph was prepared.

New specifications and a chemical and technical assessment were prepared.

2.6.10 Recommendations

Ribonuclease P can also be produced by *P. citrinum* RP-4, but insufficient information was available on the enzyme concentrate produced from this strain. To evaluate the safety of ribonuclease P from *P. citrinum* RP-4, toxicological studies with well-characterized enzyme concentrate are required.

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¹ The reader is referred to the Technical Report of the 87th JECFA meeting (Annex 1, reference 243) for clarification of the term “ADI not specified”.

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3. Revision of specifications and analytical methods

3.1 Modified starches

3.1.1 Explanation

The Committee at its eighty-sixth meeting reviewed full specifications for three modified starches, International Numbering System (INS) 1404, 1420 and 1451, tentative specifications for the remaining 13 modified starches (INS 1400, 1401, 1402, 1403, 1405, 1410, 1412, 1413, 1414, 1422, 1440, 1442 and 1450) and data on the method of manufacture, identity and purity of all 16 modified starches. At the same meeting, the Committee drafted a modular specifications monograph entitled “Modified starches”, consisting of an explanatory introduction, “General specifications for modified starches”, applying to all 16 modified starches, and eight annexes with specifications applicable to individual modified starches according to their treatment(s) (Table 2). The general specifications and annexes 1, 2, 3, 5, 7 and 8 were made tentative. Data and the information necessary to remove the tentative status and revise the modular specifications monograph were requested.

At its current meeting, the Committee reviewed the information and data received, revised the modular specifications monograph and removed the tentative status of the “General specifications” and annexes 1, 2, 3, 5, 7 and 8. Each modified starch should fulfil the specification requirements of the “General specifications” and in the applicable annexes.

Table 2
Modified starches considered and applicable annexes

Modified starch	INS	Annex
Dextrin roasted starch	1400	1
Acid treated starch	1401	1
Alkaline treated starch	1402	1
Bleached starch	1403	2
Oxidized starch	1404	5
Enzyme-treated starch	1405	1
Monostarch phosphate	1410	3
Distarch phosphate	1412	3
Phosphated distarch phosphate	1413	3
Acetylated distarch phosphate	1414	3, 4
Starch acetate	1420	4
Acetylated distarch adipate	1422	4, 8
Hydroxypropyl starch	1440	7
Hydroxypropyl distarch phosphate	1442	3, 7

Table 2 (continued)

Modified starch	INS	Annex
Starch sodium octenylsuccinate	1450	6
Acetylated oxidized starch	1451	4, 5

Corrigenda

The following requests for corrections, reported to the JECFA Secretariat, were evaluated by the 92nd JECFA meeting and found to be necessary. These corrections will be made, however, only in the electronic versions and in the online database of specifications.

Food additive	Original text	Revised text	Additional information
Riboflavin INS 101(i)	% <i>Riboflavin</i> = $A \times 5000328 \times W \times 1.367\%$ riboflavin = $A \times 5000328 \times W \times 1.367$	% <i>Riboflavin</i> = $A \times 5000328 \times W\%$ riboflavin = $A \times 5000328 \times W$	Correction to calculation in the method of assay; removal of a wrongly assigned factor
Riboflavin from <i>Bacillus subtilis</i> INS 101(iii)	% <i>Riboflavin</i> = $A \times 5000328 \times W \times 1.367\%$ riboflavin = $A \times 5000328 \times W \times 1.367$	% <i>Riboflavin</i> = $A \times 5000328 \times W\%$ riboflavin = $A \times 5000328 \times W$	Correction to calculation in the method of assay; removal of a wrongly assigned factor
Riboflavin 5'-phosphate sodium INS 101(ii)	CAS number 130-40-5	CAS number 130-40-5 (anhydrous) CAS number 6184-17-4 (dihydrate)	Current specifications provide the formula for the dihydrate but no applicable CAS number
Potassium polyaspartate	Missing "Method of assay"	Add "Method of assay" under "Purity tests" after the test entitled "Molecular weight and molecular weight distribution". Delete the bold text "Potassium polyaspartate", which appears in the test for "Molecular weight and molecular weight distribution", and replace with "Principle" (as the method of assay).	Correct errors in format of specifications monograph
Vol. 4 procedure			
Unsulfonated primary aromatic amines	See printed version of Vol. 4	See revised text below; modified text is in bold.	Correction to the range of the standard curve

Revised text:

Procedure

Preparation of standard aniline solution

Weigh **100 mg** of redistilled aniline into a small beaker, and transfer to a 100-mL volumetric flask, rinsing the beaker several times with water. Add 30 mL of 3 N hydrochloric acid, and dilute to the mark with water at room temperature. Dilute 10.0 mL of this solution to 100 mL with water, and mix well. **Dilute 20.0 mL of this solution to 100 mL with water, and mix well (1 mL of this standard solution is equivalent to 20 µg of aniline).** Measure the following volumes of the standard aniline solution into a series of 100-mL volumetric flasks: 5 mL, 10 mL, 15 mL, 20 mL and 25 mL. Dilute to 100 mL with 1 N hydrochloric acid, and mix well (**100 mL of the resulting working standard solutions contains 100, 200, 300, 400 and 500 µg of aniline, respectively).** Prepare all standard solutions freshly.

Construction of standard curve

Pipette 10 mL of each **working standard** solution into clean, dry test tubes; cool them for 10 min by immersion in a beaker of ice water. To each tube, add 1 mL of the potassium bromide solution and 0.05 mL of the sodium nitrite solution. Mix, and allow the tubes to stand for 10 min in the ice-water bath while the aniline is diazotized. Into each of five 25-mL volumetric flasks, measure 1 mL of the R salt solution and 10 mL of the sodium carbonate solution. Pour each diazotized aniline solution into a separate flask containing R salt solution and sodium carbonate solution; rinse each test tube with a few drops of water. Dilute to the mark with water, stopper the flasks, mix the contents well, and allow them to stand for 15 min in the dark. Measure the absorbance of each coupled solution at 510 nm in 40-mm cells. As a reference solution, use a mixture of 10.0 mL of 1 N hydrochloric acid, 10.0 mL of the sodium carbonate solution and 2.0 mL of the R salt solution, diluted to 25.0 mL with water. **Construct a standard curve of the absorbance versus the weight (g) of aniline in each 100 mL of working standard solution.**

Preparation and evaluation of a test solution

Weigh, to the nearest 0.01 g, about 2.0 g of the colouring matter sample (W) into a separatory funnel containing 100 mL of water, rinse the sides of the funnel with a further 50 mL of water, swirling to dissolve the sample, and add 5 mL of 1 N sodium hydroxide. Extract with two 50-mL portions of toluene, and wash the combined toluene extracts with 10-mL portions of 0.1 N sodium hydroxide to remove traces of colour. Extract the washed toluene with three 10-mL portions of 3 N hydrochloric acid, and dilute the combined extract to 100 mL with water. Mix well. Call this "solution T". **Pipette** 10.0 mL of solution T into a clean, dry test tube, cool for 10 min by immersion in a beaker of iced water, add 1 mL of the potassium bromide solution, and proceed as described above for preparation of the **standard curve**, starting with addition of 0.05 mL of the sodium nitrite solution. Measure the absorbance of the coupled test solution at 510 nm in a 40-mm cell. Use a reference solution prepared from 10.0 mL of solution T, 10 mL of the sodium carbonate solution and 2.0 mL of the R salt solution diluted to 25.0 mL with water. From the **standard curve**, read the weight of aniline (WA) corresponding to the observed absorbance of the test solution.

$$\text{Calculation: \% unsulfonated primary aromatic amine (as aniline)} = \frac{100 \times \text{WA}}{\text{W}}$$

Annex 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. General principles governing the use of food additives (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. Procedures for the testing of intentional food additives to establish their safety for use (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants) (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. Evaluation of the carcinogenic hazards of food additives (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. Evaluation of the toxicity of a number of antimicrobials and antioxidants (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. Specifications for identity and purity and toxicological evaluation of food colours. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.
11. Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases. FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
18. Specifications for the identity and purity of some antibiotics. FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.
19. Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
20. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances. FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.
21. Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives. FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.
22. Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.
23. Toxicological evaluation of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.
24. Specifications for the identity and purity of some extraction solvents and certain other substances. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.
25. A review of the technological efficacy of some antimicrobial agents. FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.
26. Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some

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27. Toxicological evaluation of some enzymes, modified starches, and certain other substances. FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.
 28. Specifications for the identity and purity of some enzymes and certain other substances. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.
 29. A review of the technological efficacy of some antioxidants and synergists. FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.
 30. Evaluation of certain food additives and the contaminants mercury, lead, and cadmium (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.
 31. Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocabamate, and octyl gallate. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
 32. Toxicological evaluation of certain food additives with a review of general principles and of specifications (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
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44. Evaluation of certain food additives (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. Summary of toxicological data of certain food additives. WHO Food Additives Series, No. 12, 1977.
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67. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 19, 1984.
68. Specifications for the identity and purity of food colours. FAO Food and Nutrition Paper, No. 31/1, 1984.
69. Specifications for the identity and purity of food additives. FAO Food and Nutrition Paper, No. 31/2, 1984.
70. Evaluation of certain food additives and contaminants (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.
71. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 34, 1986.
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89. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 26, 1990.
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91. Evaluation of certain veterinary drug residues in food (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
92. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 27, 1991.
93. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/3, 1991.
94. Evaluation of certain food additives and contaminants (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
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187. Evaluation of certain food additives and contaminants (Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 947, 2007.

188. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 59, 2008.
189. Compendium of food additive specifications. FAO JECFA Monographs 4, 2007.
190. Evaluation of certain food additives (Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 952, 2009.
191. Safety evaluation of certain food additives. WHO Food Additives Series, No. 60, 2009.
192. Compendium of food additive specifications. FAO JECFA Monographs 5, 2009.
193. Evaluation of certain veterinary drug residues in food (Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 954, 2009.
194. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 61, 2009.
195. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 6, 2009.
196. Evaluation of certain food additives (Seventy-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 956, 2010.
197. Safety evaluation of certain food additives. WHO Food Additives Series, No. 62, 2010.
198. Compendium of food additive specifications. FAO JECFA Monographs 7, 2009.
199. Evaluation of certain contaminants in food (Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 959, 2011.
200. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 63/FAO JECFA Monographs 8, 2011.
201. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 9, 2010.
202. Evaluation of certain food additives and contaminants (Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 960, 2011.
203. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 64, 2011.
204. Compendium of food additive specifications. FAO JECFA Monographs 10, 2010.
205. Evaluation of certain food additives and contaminants (Seventy-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 966, 2011.
206. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 65, 2011.
207. Compendium of food additive specifications. FAO JECFA Monographs 11, 2011.
208. Evaluation of certain veterinary drug residues in food (Seventy-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 969, 2012.
209. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 66, 2012.
210. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 12, 2012.
211. Evaluation of certain food additives (Seventy-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 974, 2012.
212. Safety evaluation of certain food additives. WHO Food Additives Series, No. 67, 2012.

213. Compendium of food additive specifications. FAO JECFA Monographs 13, 2012.
214. Evaluation of certain food additives and contaminants (Seventy-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 983, 2013.
215. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 68, 2013.
216. Compendium of food additive specifications. FAO JECFA Monographs 14, 2013.
217. Evaluation of certain veterinary drug residues in food (Seventy-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 988, 2014.
218. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 69, 2014.
219. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 15, 2014.
220. Evaluation of certain food additives (Seventy-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 990, 2015.
221. Safety evaluation of certain food additives. WHO Food Additives Series, No. 70, 2015.
222. Compendium of food additive specifications. FAO JECFA Monographs 16, 2014.
223. Evaluation of certain food additives and contaminants (Eightieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 995, 2016.
224. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 71, 2015.
225. Compendium of food additive specifications. FAO JECFA Monographs 17, 2015.
226. Evaluation of certain veterinary drug residues in food (Eighty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 997, 2016.
227. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 72, 2016.
228. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 18, 2016.
229. Safety evaluation of certain food additives and contaminants. Supplement 1: Non-dioxin-like polychlorinated biphenyls. WHO Food Additives Series, No. 71-1, 2016.
230. Evaluation of certain food additives (Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1000, 2016.
231. Compendium of food additive specifications. FAO JECFA Monographs 19, 2016.
232. Safety evaluation of certain food additives. WHO Food Additives Series, No. 73, 2017.
233. Evaluation of certain contaminants in food (Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1002, 2017.
234. Evaluation of certain food additives (Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1007, 2017.
235. Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 74, FAO JECFA Monographs 19 bis, 2018.
236. Compendium of food additive specifications. FAO JECFA Monographs 20, 2017.

237. Safety evaluation of certain food additives. WHO Food Additives Series, No. 75, 2019.
238. Evaluation of certain veterinary drug residues in food (Eighty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1008, 2018.
239. Residue evaluation of certain veterinary drugs. FAO JECFA Monographs 21, 2018.
240. Compendium of food additive specifications. FAO JECFA Monographs 22, 2018.
241. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 76, 2019.
242. Evaluation of certain food additives (Eighty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1014, 2019.
243. Evaluation of certain food additives (Eighty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives) WHO Technical Report Series, No. 1020, 2019.
244. Compendium of food additive specifications. FAO JECFA Monographs 23, 2019.
245. Evaluation of veterinary drug residues in food (Eighty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1023, 2020.
246. Evaluation of certain food additives (Eighty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 1027, 2021.
247. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 79, 2021.

Annex 2

Toxicological and dietary exposure information and information on specifications

Food additives evaluated toxicologically and assessed for dietary exposure

Food additive	Specifications	Acceptable daily intakes (ADIs) and other conclusions on toxicology and dietary exposure
Benzoic acid, its salts and derivatives	N	The Committee evaluated a new extended one-generation reproductive toxicity study on benzoic acid. This study showed no treatment-related adverse effects, indicating a NOAEL of 1000 mg/kg bw per day, the highest dose tested. Applying a chemical specific adjustment factor of 2 for interspecies toxicokinetics variation instead of the default factor of 4.0, the Committee established a group ADI of 0–20 mg/kg bw, which applies to benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate, expressed as benzoic acid equivalents. The Committee withdrew the previous group ADI of 0–5 mg/kg bw. The Committee noted that the high dietary exposure estimate, expressed as benzoic acid, of 7.1 mg/kg bw per day for children aged 3–9 years does not exceed the group ADI of 0–20 mg/kg bw.
Collagenase from <i>Streptomyces violaceoruber</i> expressed in <i>S. violaceoruber</i>	N	Negative results were observed in genotoxicity studies with a powdered enzyme concentrate. The Committee identified a NOAEL of 940 mg TOS/kg bw per day (rounded from 939.6), the highest dose tested in a 13-week study of oral toxicity in rats. The Committee identified a NOAEL of 940 mg TOS/kg bw per day, the highest dose tested in a 13-week study of oral toxicity in rats. Comparison of this NOAEL with the estimated dietary exposure of 0.43 mg TOS/kg bw per day gave a margin of exposure (MOE) of > 2100. In view of this MOE and the lack of concern about genotoxicity, the Committee established an ADI “not specified”¹ for collagenase from <i>S. violaceoruber</i>, when used in the applications specified and in accordance with good manufacturing practice.
β-Glucanase from <i>Streptomyces violaceoruber</i> expressed in <i>S. violaceoruber</i>	N	The Committee noted negative results in studies of genotoxicity and in studies of oral toxicity in rats. The Committee identified a NOAEL of 950 mg TOS/kg bw per day (rounded by the Committee from 953.3), the highest dose tested. Comparison of this NOAEL with the estimated dietary exposure of 0.15 mg TOS/kg bw per day gave an MOE > 6300. On the basis of this MOE and the lack of concern about genotoxicity, the Committee established an ADI “not specified”¹ for β-glucanase from <i>S. violaceoruber</i>, for the proposed uses and in accordance with good manufacturing practice.

¹ The reader is referred to the Technical Report of the 87th JECFA meeting for clarification of the term “ADI not specified”.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other conclusions on toxicology and dietary exposure
Phospholipase A2 from <i>Streptomyces violaceoruber</i> expressed in <i>S. violaceoruber</i>	R	Negative results were obtained in genotoxicity tests. In a 13-week study of oral toxicity in rats, small effects were seen at low incidence at the high dose of 956 mg TOS/kg bw per day, which might have been related to treatment. The Committee therefore identified a NOAEL of 190 mg TOS/kg per day (rounded by the Committee from 191 mg TOS/kg bw per day). A comparison of the estimated dietary exposure of 0.25 mg TOS/kg bw per day with the NOAEL of 190 mg TOS/kg bw per day from the oral toxicity study gives an MOE of 760. On this basis and in the absence of concern about genotoxicity, the Committee established an ADI “not specified”¹ for the phospholipase A2 enzyme preparation from <i>S. violaceoruber</i> when used in the applications specified and in accordance with good manufacturing practice.
Riboflavin from <i>Ashbya gossypii</i>	N	The Committee noted that riboflavin from <i>A. gossypii</i> has low acute toxicity and does not raise concern for genotoxicity. The NOAEL from a 90-day oral toxicity study in rats was 3000 mg/kg bw per day, the highest dose tested. Comparison of this NOAEL with the estimated dietary exposure of 3.6 mg/kg bw per day, based on maximum reported use levels, resulted in an MOE > 800. The Committee established a group ADI “not specified”¹ for riboflavin, riboflavin- 5'-phosphate, riboflavin from <i>B. subtilis</i> and riboflavin from <i>A. gossypii</i>, expressed as riboflavin. The Committee withdrew the previous group ADI of 0–0.5 mg/kg bw.
Ribonuclease P from <i>Penicillium citrinum</i>	N	The Committee identified a NOAEL of 980 mg TOS/kg bw per day (the highest dose tested) in a 13-week study in which rats were treated with ribonuclease P concentrate from <i>P. citrinum</i> AE-RP by gavage. A comparison of the estimated dietary exposure of 1.3 mg TOS/kg bw per day with the NOAEL of 980 mg TOS/kg bw per day gives an MOE > 750. On the basis of this MOE and the lack of concern for genotoxicity, the Committee established an ADI “not specified”¹ for the ribonuclease P enzyme preparation from <i>P. citrinum</i> AE-RP, used in the applications specified and in accordance with good manufacturing practice.

N: new specifications, R: revised specifications

Food additives considered for specifications only

Food additive	Specifications
Modified starches	R

R: revised specifications

¹ The reader is referred to the Technical Report of the 87th JECFA meeting for clarification of the term “ADI not specified”.

Annex 3

Meeting agenda



Food and Agriculture
Organization of the
United Nations



World Health
Organization

**92nd JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)
7–18 June 2021**

Virtual meeting: 12:00–16:00 (CET)

1. Opening
2. Declarations of Interests (information by the Secretariat on any declared interests and discussion, update by experts)
3. Election of Chairperson and Vice-Chairperson, appointment of Rapporteurs
4. Adoption of the agenda
5. Matters of interest arising from previous Sessions of the Codex Committee on Food Additives
6. Critical issues and questions from Working Papers (first brief round of discussion on all subjects to inform the full Committee)
7. Evaluations
 - 7.1. Food additives
 - Benzoic acid and its salts
 - Riboflavin from *Ashbya gossypii*
 - 7.2. Enzymes
 - β -Glucanase
 - Collagenase
 - Phosphodiesterase
 - Phospholipase A2
8. Other matters to be considered (general considerations).

9. Other matters as may be brought forth by the Committee during discussions at the meeting.
10. Adoption of the report.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Evaluation of certain food additives

Eighty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1027, 2020 (106 pages)

Evaluation of certain veterinary drug residues in food

Eighty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1023, 2020 (116 pages)

Evaluation of certain food additives

Eighty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1020, 2019 (109 pages)

Evaluation of certain food additives

Eighty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1014, 2019 (156 pages)

Evaluation of certain veterinary drug residues in food

Eighty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1008, 2017 (150 pages)

Safety evaluation of certain food additives

Eighty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 75, 2018 (244 pages)

Evaluation of certain food additives

Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1007, 2017 (92 pages)

Safety evaluation of certain contaminants in food

Eighty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives
WHO Food Additives Series, No. 74, 2018 (897 pages)

Evaluation of certain contaminants in food

Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series, No. 1002, 2017 (166 pages)

Further information on these and other WHO publications can be obtained from
WHO Press, World Health Organization ■ 1211 Geneva 27, Switzerland ■ www.who.int/bookorders
tel.: +41 22 791 3264; **fax:** +41 22 791 4857; **email:** bookorders@who.int

Evaluation of certain food additives

This report presents the conclusions of a Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met virtually from 7 to 18 June 2021.

The toxicological and dietary exposure monographs in this volume summarize data on the safety of and dietary exposure to specific food additives: benzoic acid, its salts and derivatives; collagenase from *Streptomyces violaceoruber* expressed in *S. violaceoruber*; β -glucanase from *Streptomyces violaceoruber* expressed in *S. violaceoruber*; phospholipase A2 from *Streptomyces violaceoruber* expressed in *S. violaceoruber*; riboflavin from *Ashbya gossypii*; and ribonuclease P from *Penicillium citrinum*.

This volume and others in the WHO Food Additives series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved in controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories and universities.



SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.7

Revision Date 01.03.2024

Print Date 17.03.2025

GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking**1.1 Product identifiers**

Product name : Benzoic acid

Product Number : 242381

Brand : Sigma-Aldrich

Index-No. : 607-705-00-8

REACH No. : 01-2119455536-33-XXXX

CAS-No. : 65-85-0

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Merck Life Science Sp.z.o.o.
Szelągowska 30
PL-61-626 POZNAN

Telephone : +48 61 8290-100

Fax : +48 61 8290-120

E-mail address : TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone # : +(48)-223988029 (CHEMTREC) 112
(numer alarmowy)

SECTION 2: Hazards identification**2.1 Classification of the substance or mixture**

Skin irritation, (Category 2) H315: Causes skin irritation.

Serious eye damage, (Category 1) H318: Causes serious eye damage.

Specific target organ toxicity - repeated exposure, (Category 1), Lungs H372: Causes damage to organs through prolonged or repeated exposure if inhaled.



2.2 Label elements

Labelling according Regulation (EC) No 1272/2008

Pictogram



Signal Word

Danger

Hazard Statements

H315

Causes skin irritation.

H318

Causes serious eye damage.

H372

Causes damage to organs (Lungs) through prolonged or repeated exposure if inhaled.

Precautionary Statements

P260

Do not breathe dust.

P264

Wash skin thoroughly after handling.

P280

Wear protective gloves/ eye protection/ face protection.

P302 + P352

IF ON SKIN: Wash with plenty of water.

P305 + P351 + P338

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P314

Get medical advice/ attention if you feel unwell.

Supplemental Hazard Statements

none

Reduced Labeling (<= 125 ml)

Pictogram



Signal Word

Danger

Hazard Statements

H372

Causes damage to organs through prolonged or repeated exposure if inhaled.

H318

Causes serious eye damage.

Precautionary Statements

P260

Do not breathe dust.

P264

Wash skin thoroughly after handling.

P305 + P351 + P338

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P314

Get medical advice/ attention if you feel unwell.

Supplemental Hazard Statements

none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.



Ecological information:

The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

Toxicological information:

The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Formula	: C ₇ H ₆ O ₂
Molecular weight	: 122,12 g/mol
CAS-No.	: 65-85-0
EC-No.	: 200-618-2
Index-No.	: 607-705-00-8

Component		Classification	Concentration
Benzoic acid			
CAS-No.	65-85-0	Skin Irrit. 2; Eye Dam. 1; STOT RE 1; H315, H318, H372	<= 100 %
EC-No.	200-618-2		
Index-No.	607-705-00-8		

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first-aid measures

General advice

Show this material safety data sheet to the doctor in attendance.

If inhaled

After inhalation: fresh air. Call in physician.

In case of skin contact

In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact

After eye contact: rinse out with plenty of water. Immediately call in ophthalmologist. Remove contact lenses.

If swallowed

After swallowing: immediately make victim drink water (two glasses at most). Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11



4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media

Water Foam Carbon dioxide (CO₂) Dry powder

Unsuitable extinguishing media

For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture

Carbon oxides

Combustible.

Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters

Stay in danger area only with self-contained breathing apparatus. Prevent skin contact by keeping a safe distance or by wearing suitable protective clothing.

5.4 Further information

Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Avoid generation and inhalation of dusts in all circumstances. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

6.2 Environmental precautions

Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up

Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Advice on safe handling

Work under hood. Do not inhale substance/mixture.

Hygiene measures



Immediately change contaminated clothing. Apply preventive skin protection. Wash hands and face after working with substance.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions

Tightly closed. Dry. Keep in a well-ventilated place. Keep locked up or in an area accessible only to qualified or authorized persons.

Storage class

Storage class (TRGS 510): 6.1C: Combustible, acute toxic Cat.3 / toxic compounds or compounds which causing chronic effects

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

Derived No Effect Level (DNEL)

Application Area	Routes of exposure	Health effect	Value
Workers	Inhalation	Long-term local effects	0,1 mg/m ³
Workers	Inhalation	Long-term systemic effects	3 mg/m ³
Workers	Skin contact	Long-term systemic effects	62,5mg/kg BW/d

Predicted No Effect Concentration (PNEC)

Compartment	Value
Soil	0,151 mg/kg
Sea water	0,034 mg/l
Sea sediment	0,175 mg/kg
Fresh water sediment	1,75 mg/kg
Sewage treatment plant	100 mg/l
Aquatic intermittent release	0,331 mg/l

8.2 Exposure controls

Personal protective equipment

Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Tightly fitting safety goggles

Skin protection

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other



substances and under conditions deviating from those stated in EN 16523-1 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).

Full contact

Material: Nitrile rubber

Minimum layer thickness: 0,11 mm

Break through time: 480 min

Material tested: KCL 741 Dermatril® L

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN 16523-1 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).

Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0,11 mm

Break through time: 480 min

Material tested: KCL 741 Dermatril® L

Body Protection

protective clothing

Respiratory protection

required when dusts are generated.

Our recommendations on filtering respiratory protection are based on the following standards: DIN EN 143, DIN 14387 and other accompanying standards relating to the used respiratory protection system.

Recommended Filter type: Filter type P2

The entrepreneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.

Control of environmental exposure

Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a) Physical state	crystalline
b) Color	white
c) Odor	No data available
d) Melting point/freezing point	Melting point/range: 121 - 125 °C - lit.
e) Initial boiling point and boiling range	249 °C - lit.
f) Flammability (solid, gas)	No data available



g) Upper/lower flammability or explosive limits	No data available
h) Flash point	Not applicable
i) Autoignition temperature	No data available
j) Decomposition temperature	No data available
k) pH	2,8 at 25 °C
l) Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
m) Water solubility	No data available
n) Partition coefficient: n-octanol/water	log Pow: 1,88 - Bioaccumulation is not expected.
o) Vapor pressure	No data available
p) Density	1,26 g/cm ³ at 15 °C
Relative density	No data available
q) Relative vapor density	No data available
r) Particle characteristics	No data available
s) Explosive properties	No data available
t) Oxidizing properties	none

9.2 Other safety information

Surface tension	67,5 mN/m at 1g/l at 20 °C - OECD Test Guideline 115
Relative vapor density	4,22 - (Air = 1.0)

SECTION 10: Stability and reactivity

10.1 Reactivity

The following applies in general to flammable organic substances and mixtures: in correspondingly fine distribution, when whirled up a dust explosion potential may generally be assumed.

10.2 Chemical stability

The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions

Risk of ignition or formation of inflammable gases or vapours with:
Fluorine



Exothermic reaction with:
bases
Strong oxidizing agents
Strong bases
nitrites
strong reducing agents

10.4 Conditions to avoid

no information available

10.5 Incompatible materials

No data available

10.6 Hazardous decomposition products

In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity

LD50 Oral - Mouse - male and female - 2.250 mg/kg
(OECD Test Guideline 401)

Symptoms: Nausea, Vomiting, Irritation of mucous membranes

LC50 Inhalation - Rat - male and female - 4 h - > 12,2 mg/l - dust/mist

Remarks: (ECHA)

Symptoms: Cough, Possible damages:, mucosal irritations

LD50 Dermal - Rabbit - male and female - > 2.000 mg/kg

Remarks: (ECHA)

Skin corrosion/irritation

Skin - Guinea pig

Result: Skin irritation - 3 h

Remarks: (ECHA)

Remarks: (Regulation (EC) No 1272/2008, Annex VI)

Serious eye damage/eye irritation

Eyes - Rabbit

Result: Corrosive - 21 Days

(Directive 67/548/EEC, Annex V, B.5.)

Remarks: (Regulation (EC) No 1272/2008, Annex VI)

Respiratory or skin sensitization

Local lymph node assay (LLNA) - Mouse

Result: negative

Remarks: (ECHA)

Germ cell mutagenicity

Test Type: Mutagenicity (mammal cell test): chromosome aberration.

Test system: Chinese hamster fibroblasts

Metabolic activation: without metabolic activation

Result: Positive results were obtained in some in vitro tests.

Remarks: (ECHA)



Test Type: Micronucleus test
Test system: mouse lymphoma cells
Metabolic activation: with and without metabolic activation
Method: OECD Test Guideline 487
Result: negative

Carcinogenicity

No data available

Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

Inhalation - Causes damage to organs through prolonged or repeated exposure.

- Lungs

Remarks: Classified according to Regulation (EU) 1272/2008, Annex VI (Table 3.1/3.2)

Aspiration hazard

No data available

11.2 Additional Information

Endocrine disrupting properties

Product:

Assessment

The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

Repeated dose toxicity - Rat - male and female - inhalation (dust/mist/fume) - 28 Days

Repeated dose toxicity - Rabbit - male and female - Dermal - 21 Days - NOAEL (No observed adverse effect level) - > 2.500 mg/kg

RTECS: DG0875000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish	static test LC50 - Lepomis macrochirus - 44,6 mg/l - 96 h (US-EPA)
Toxicity to daphnia and other aquatic invertebrates	static test LC50 - Daphnia magna (Water flea) - > 100 mg/l - 48 h (US-EPA)



Toxicity to algae	static test ErC50 - Pseudokirchneriella subcapitata - > 33,1 mg/l - 72 h (OECD Test Guideline 201)
Toxicity to bacteria	static test IC50 - activated sludge - > 1.000 mg/l - 3 h (OECD Test Guideline 209) microtox test EC50 - Photobacterium phosphoreum - 17 mg/l - 30 min Remarks: (Lit.)
Toxicity to fish(Chronic toxicity)	semi-static test EC50 - Oncorhynchus mykiss (rainbow trout) - > 120 mg/l - 28 d (OECD Test Guideline 204)
Toxicity to daphnia and other aquatic invertebrates(Chronic toxicity)	semi-static test EC50 - Daphnia magna (Water flea) - > 25 mg/l - 21 d (OECD Test Guideline 211)

12.2 Persistence and degradability

Biodegradability	anaerobic - Exposure time 35 d Result: 89,5 % - Biodegradable (OECD Test Guideline 311)

12.3 Bioaccumulative potential

No data available

12.4 Mobility in soil

No data available

12.5 Results of PBT and vPvB assessment

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties

Product:

Assessment	: The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects

No data available



SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product

See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions.

SECTION 14: Transport information

14.1 UN number

ADR/RID: -

IMDG: -

IATA: -

14.2 UN proper shipping name

ADR/RID: Not dangerous goods

IMDG: Not dangerous goods

IATA: Not dangerous goods

14.3 Transport hazard class(es)

ADR/RID: -

IMDG: -

IATA: -

14.4 Packaging group

ADR/RID: -

IMDG: -

IATA: -

14.5 Environmental hazards

ADR/RID: no

IMDG Marine pollutant: no

IATA: no

14.6 Special precautions for user

No data available

Further information

Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

Authorisations and/or restrictions on use

Other regulations

Take note of Dir 94/33/EC on the protection of young people at work.

15.2 Chemical Safety Assessment

A Chemical Safety Assessment has been carried out for this substance.

SECTION 16: Other information

Full text of H-Statements

H315 Causes skin irritation.

H318 Causes serious eye damage.



H372 Causes damage to organs through prolonged or repeated exposure if inhaled.

Full text of other abbreviations

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

Further information

The information is believed to be correct but is not exhaustive and will be used solely as a guideline, which is based on current knowledge of the chemical substance or mixture and is applicable to appropriate safety precautions for the product. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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