Concise International Chemical Assessment Document 26

BENZOIC ACID AND SODIUM BENZOATE

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Corrigenda published by 12 April 2005 have been incorporated in this file

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The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research, and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

Concise International Chemical Assessment Documents (CICADs) are the latest in a family of publications from the International Programme on Chemical Safety (IPCS) — a cooperative programme of the World Health Organization (WHO), the International Labour Organization (ILO), and the United Nations Environment Programme (UNEP). CICADs join the Environmental Health Criteria documents (EHCs) as authoritative documents on the risk assessment of chemicals.

CICADs are concise documents that provide summaries of the relevant scientific information concerning the potential effects of chemicals upon human health and/or the environment. They are based on selected national or regional evaluation documents or on existing EHCs. Before acceptance for publication as CICADs by IPCS, these documents undergo extensive peer review by internationally selected experts to ensure their completeness, accuracy in the way in which the original data are represented, and the validity of the conclusions drawn.

The primary objective of CICADs is characterization of hazard and dose–response from exposure to a chemical. CICADs are not a summary of all available data on a particular chemical; rather, they include only that information considered critical for characterization of the risk posed by the chemical. The critical studies are, however, presented in sufficient detail to support the conclusions drawn. For additional information, the reader should consult the identified source documents upon which the CICAD has been based.

Risks to human health and the environment will vary considerably depending upon the type and extent of exposure. Responsible authorities are strongly encouraged to characterize risk on the basis of locally measured or predicted exposure scenarios. To assist the reader, examples of exposure estimation and risk characterization are provided in CICADs, whenever possible. These examples cannot be considered as representing all possible exposure situations, but are provided as guidance only. The reader is referred to EHC 170[1] for advice on the derivation of health-based tolerable intakes and guidance values.

[1] International Programme on Chemical Safety (1994) *Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits.* Geneva, World Health Organization (Environmental Health Criteria 170).

While every effort is made to ensure that CICADs represent the current status of knowledge, new information is being developed constantly. Unless otherwise stated, CICADs are based on a search of the scientific literature to the date shown in the executive summary. In the event that a reader becomes aware of new information that would change the conclusions drawn in a CICAD, the reader is requested to contact IPCS to inform it of the new information.

Procedures

The flow chart shows the procedures followed to produce a CICAD. These procedures are designed to take advantage of the expertise that exists around the world — expertise that is required to produce the high-quality evaluations of toxicological, exposure, and other data that are necessary for assessing risks to human health and/or the environment.

The first draft is based on an existing national, regional, or international review. Authors of the first draft are usually, but not necessarily, from the institution that developed the original review. A standard outline has been developed to encourage consistency in form. The first draft undergoes primary review by IPCS to ensure that it meets the specified criteria for CICADs.

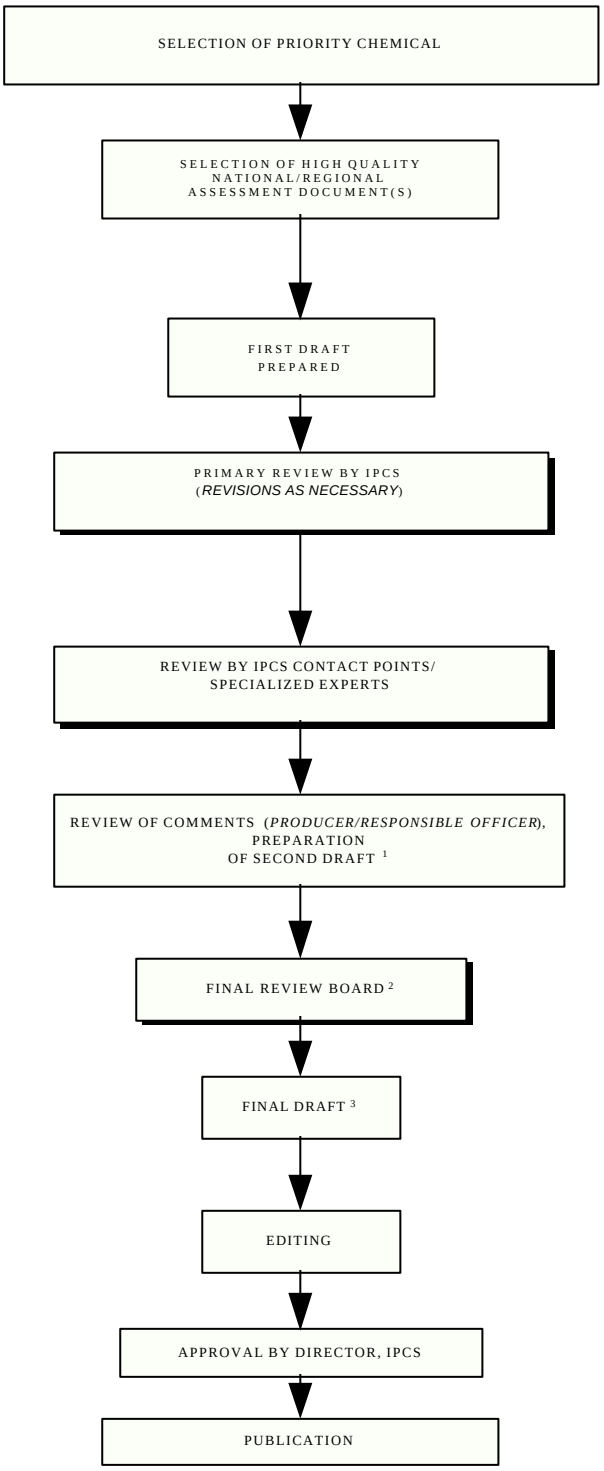
The second stage involves international peer review by scientists known for their particular expertise and by scientists selected from an international roster compiled by IPCS through recommendations from IPCS national Contact Points and from IPCS Participating Institutions. Adequate time is allowed for the selected experts to undertake a thorough review. Authors are required to take reviewers' comments into account and revise their draft, if necessary. The resulting second draft is submitted to a Final Review Board together with the reviewers' comments.

The CICAD Final Review Board has several important functions:

- to ensure that each CICAD has been subjected to an appropriate and thorough peer review;
- to verify that the peer reviewers' comments have been addressed appropriately;
- to provide guidance to those responsible for the preparation of CICADs on how to resolve any remaining issues if, in the opinion of the Board, the author has not adequately addressed all comments of the reviewers; and
- to approve CICADs as international assessments.

Board members serve in their personal capacity, not as representatives of any organization, government, or

CICAD PREPARATION FLOW CHART



1 Taking into account the comments from reviewers.
2 The second draft of documents is submitted to the Final Review Board together with the reviewers' comments.
3 Includes any revisions requested by the Final Review Board.

industry. They are selected because of their expertise in human and environmental toxicology or because of their experience in the regulation of chemicals. Boards are chosen according to the range of expertise required for a meeting and the need for balanced geographic representation.

Board members, authors, reviewers, consultants, and advisers who participate in the preparation of a CICAD are required to declare any real or potential conflict of interest in relation to the subjects under discussion at any stage of the process. Representatives of nongovernmental organizations may be invited to observe the proceedings of the Final Review Board. Observers may participate in Board discussions only at the invitation of the Chairperson, and they may not participate in the final decision-making process.

1. EXECUTIVE SUMMARY

This CICAD on benzoic acid and sodium benzoate was prepared by the Fraunhofer Institute for Toxicology and Aerosol Research, Hanover, Germany. The two compounds are being considered together because it is undissociated benzoic acid that is responsible for its antimicrobial activity. As benzoic acid itself is only slightly soluble in water, sodium benzoate — which, under acid conditions, converts to undissociated benzoic acid — is often used instead.

This CICAD was based on reviews compiled by the German Advisory Committee on Existing Chemicals of Environmental Relevance (BUA, 1995), the US Food and Drug Administration (US FDA, 1972a), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO, 1996) to assess potential effects of benzoic acid and sodium benzoate on the environment and on humans. A comprehensive literature search of relevant databases was conducted in September 1999 to identify any relevant references published subsequent to those incorporated in these reports. Information on the preparation and peer review of the source documents is presented in Appendix 1. Information on the peer review of this CICAD is presented in Appendix 2. This CICAD was approved as an international assessment at a meeting of the Final Review Board, held in Sydney, Australia, on 21–24 November 1999. Participants at the Final Review Board meeting are listed in Appendix 3. The International Chemical Safety Card (ICSC 0103) for benzoic acid, produced by the International Programme on Chemical Safety (IPCS, 1993), has also been reproduced in this document (Appendix 4).

Benzyl acetate, its hydrolysis product, benzyl alcohol, and the oxidation product of this alcohol, benzaldehyde, are extensively metabolized to benzoic acid in experimental animals and humans. Therefore, toxicological data on these precursors were also utilized in the assessment of the potential health effects of benzoic acid.

Benzoic acid (CAS No. 65-85-0) is a white solid that is slightly soluble in water. Sodium benzoate (CAS No. 532-32-1) is about 200 times more soluble in water. Benzoic acid is used as an intermediate in the synthesis of different compounds, primarily phenol (>50% of the amount produced worldwide) and caprolactam. Other end products include sodium and other benzoates, benzoyl chloride, and diethylene and dipropylene glycol dibenzoate plasticizers. Sodium benzoate is primarily used as a preservative and corrosion inhibitor (e.g., in technical systems as an additive to automotive engine antifreeze coolants). 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.

After oral uptake, benzoic acid and sodium benzoate are rapidly absorbed from the gastrointestinal tract and metabolized in the liver by conjugation with glycine, resulting in the formation of hippuric acid, which is rapidly excreted via the urine. To a lesser extent, benzoates applied dermally can penetrate through the skin. Owing to rapid metabolism and excretion, an accumulation of the benzoates or their metabolites is not to be expected.

In rodents, the acute oral toxicity of benzoic acid and sodium benzoate is low (oral LD_{50} values of >1940 mg/kg body weight). In cats, which seem to be more sensitive than rodents, toxic effects and mortality were reported at much lower doses (about 450 mg/kg body weight).

Benzoic acid is slightly irritating to the skin and irritating to the eye, while sodium benzoate is not irritating to the skin and is only a slight eye irritant. For benzoic acid, the available studies gave no indication of a sensitizing effect; for sodium benzoate, no data were identified in the literature.

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. Other effects included reduced weight gain, changes in organ weights, changes in serum parameters, or histopathological changes in the liver. The information concerning long-term oral exposure of experimental animals to benzoic acid is very limited, and there is no study available dealing specifically with possible carcinogenic effects. From a limited four-generation study, only a

preliminary no-observed-(adverse-)effect level (NO(A)EL) of about 500 mg/kg body weight per day can be derived. With sodium benzoate, two long-term studies with rats and mice gave no indication of a carcinogenic effect. However, the documentation of effects is inadequate in most of these studies; therefore, no reliable NO(A)EL values can be derived. Data on its precursors support the notion that benzoic acid is unlikely to be carcinogenic.

Benzoic acid tested negative in several bacterial assays and in tests with mammalian cells, while *in vivo* studies were not identified. Sodium benzoate was also inactive in Ames tests, whereas tests with mammalian cells gave consistently positive results. In one *in vivo* study (dominant lethal assay with rats), a positive result was obtained. At present, a genotoxic activity of sodium benzoate cannot be ruled out entirely.

For benzoic acid, two limited studies gave no indication of adverse reproductive or developmental effects. With sodium benzoate, several studies on different species have been performed, and embryotoxic and fetotoxic effects as well as malformations were seen only at doses that induced severe maternal toxicity. In a dietary study in rats, a NO(A)EL of about 1310 mg/kg body weight was established. Data on its precursors support the notion that benzoic acid is unlikely to have adverse reproductive effects at dose levels not toxic to the mother.

In humans, the acute toxicity of benzoic acid and sodium benzoate is low. However, both substances are known to cause non-immunological contact reactions (pseudoallergy). This effect is scarce in healthy subjects; in patients with frequent urticaria or asthma, symptoms or exacerbation of symptoms was observed. A provisional tolerable intake of 5 mg/kg body weight per day can be derived, although benzoates at lower doses can cause non-immunological contact reactions (pseudo-allergy) in sensitive persons. As there are no adequate studies available on inhalation exposure, a tolerable concentration for exposure by inhalation cannot be calculated.

From their physical/chemical properties, benzoic acid and sodium benzoate emitted to water and soil are not expected to volatilize to the atmosphere or to adsorb to sediment or soil particles. From the results of numerous removal experiments, the main elimination pathway for both chemicals should be biotic mineralization. Data from laboratory tests showed ready biodegradability for both substances under aerobic conditions. Several isolated microorganisms (bacteria, fungi) have been shown to utilize benzoic acid under aerobic or anaerobic conditions. From the experimental data on bioconcentration, a low to moderate potential for bioaccumulation is to be expected.

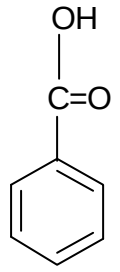
From valid test results available on the toxicity of benzoic acid and sodium benzoate to various aquatic organisms, these compounds appear to exhibit low to moderate toxicity in the aquatic compartment. The lowest EC_{50} value of 9 mg/litre (cell multiplication inhibition) reported in a chronic study was observed in the cyanobacterium *Anabaena inaequalis*. EC_{50}/LC_{50} 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 EC_{50} (102 mg/litre) at acidic pH. For the freshwater fish golden ide (*Leuciscus idus*), a 48-h LC_{50} 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 EC_{50} 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 LC_{50} values of greater than100 mg/litre. A 96-h LC_{50} 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 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.

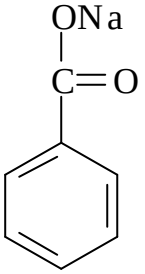
2. IDENTITY AND PHYSICAL/CHEMICAL PROPERTIES

Benzoic acid (CAS No. 65-85-0; $C_7H_6O_2$; C_6H_5COOH; benzenecarboxylic acid, phenyl carboxylic acid [E 210 (EU No. Regulation on Labelling of Foodstuffs)]; molecular weight 122.13) is a white solid that starts to sublime at 100 °C, with a melting point of 122 °C and a boiling point of 249 °C. Its solubility in water is low (2.9 g/litre at 20 °C), and its solution in water is weakly acid (dissociation constant at 25 °C = 6.335×10^{-5}; Maki & Suzuki, 1985; pK_a 4.19). It is soluble in ethanol and very slightly soluble in benzene and acetone. It has an octanol/water partition coefficient (log K_{ow}) of 1.9. Its vapour pressure at 20 °C ranges from 0.11 to 0.53 Pa. Its calculated Henry's law constant at 20 °C was given as 0.0046–0.022 Pa·m^3/mol (BUA, 1995). Additional physical and chemical properties are presented in the International Chemical Safety Card reproduced in this document (Appendix 4).

Sodium benzoate (CAS No. 532-32-1; $C_7H_5O_2Na$; benzoic acid, sodium salt [E 211 (EU No. Regulation on Labelling of Foodstuffs)]; molecular weight 144.11) has a melting point above 300 °C. It is very soluble in water (550–630 g/litre at 20 °C) and is hygroscopic at a relative humidity above 50%. Its pH is about 7.5 at a concentration of 10 g/litre water. It is soluble in ethanol, methanol, and ethylene glycol. Dry sodium benzoate is electrically charged by friction and forms an explosive mixture when its dust is dispersed in air (Maki & Suzuki, 1985).



Benzoic acid



Sodium benzoate

3. ANALYTICAL METHODS

Analytical methods for the determination of benzoic acid include spectrophotometric methods, which need extensive extraction procedures and are not very specific; gas chromatographic (GC) methods, which are more sensitive and specific but need lengthy sample preparation and derivatization prior to determination; and high-performance liquid chromatography (HPLC), which has a high specificity and minimum sample preparation and does not require derivatization.

A direct determination of benzoic acid in air by flash desorption at 240 °C with helium into capillary-GC gave a detection limit of 0.1 ppm (0.5 mg/m^3) in a 20-litre sample (=10 µg benzoic acid). This method has been developed and used for monitoring occupational exposure (Halvorson, 1984).

A method for the determination of benzoic acid in solid food at 0.5–2 g/kg levels involves extraction with ether into aqueous sodium hydroxide and methylene chloride, conversion to trimethylsilyl esters, and detection by GC and flame ionization (Larsson, 1983; AOAC, 1990). For margarine, a method using HPLC and ultraviolet (UV) detection has been described with prior extraction with ammonium acetate/acetic acid/methanol (Arens & Gertz, 1990).

When benzoic acid is used as a preservative in soft drinks and fruit drinks, other additives, colouring agents, and other acids (e.g., sorbate) may interfere with its analysis. Liquid chromatographic methods were developed to overcome this (e.g., Bennett & Petrus, 1977; Puttemans et al., 1984; Tyler, 1984). For the sensitive determination of benzoic acid in fruit-derived products, a clean-up pretreatment with solid-phase extraction followed by liquid chromatography with UV absorbance detection is described (Mandrou et al., 1998). The detection limit is 0.6 mg/kg, with a range of quantification of 2–5 mg/kg. For soft drinks, a simultaneous second-order derivative spectrophotometric determination has been developed (detection limit 1 mg/litre) (Castro et al., 1992). Sodium benzoate was measured in soya sauce, fruit juice, and soft drinks using HPLC with a UV spectrophotometric detector. Before injection, all samples were filtered (Villanueva et al., 1994).

GC determination of low concentrations (down to 10 ng/ml) of benzoic acid in plasma and urine was preceded by diethyl ether extraction and derivatization with pentafluorobenzyl bromide (Sioufi & Pommier, 1980). Detection was by ^{63}Ni electron capture. HPLC methods have been developed for the simultaneous determination of benzoic acid and hippuric acid — the metabolite of sodium benzoate that is eliminated in the urine — that require no extraction step (detection limit for both, 1 µg/ml; Kubota et al., 1988). Hippuric acid and creatinine levels have been determined simultaneously by HPLC, and measured hippuric acid levels corrected for urinary creatinine excretion (Villanueva et al., 1994).

4. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

4.1 Natural sources of benzoic acid

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).

4.2 Anthropogenic sources

4.2.1 Benzoic acid

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 & Umano, 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).

4.2.2 Sodium benzoate

Sodium benzoate is produced by the neutralization of benzoic acid with sodium hydroxide. Worldwide sodium benzoate production in 1997 can be estimated at about 55 000–60 000 tonnes (Srour, 1998). The largest producers are the Netherlands, Estonia, the USA, and China.

4.3 Uses

4.3.1 Benzoic acid

In 1988, of the benzoic acid produced in Europe, about 60% was further processed to phenol and 30% to caprolactam (for nylon fibres). Five per cent was used for the production of sodium and other benzoates, 3% for benzoyl chloride, and the rest for alkyd resins, benzoate esters, such as methyl benzoate, and various other products (Srour, 1989). These percentages are still approximately correct today (Srour, 1998). Caprolactam seems to be produced only by European companies (Srour, 1998).

Benzoic acid is increasingly used in the production of diethylene and dipropylene glycol dibenzoate plasticizers in adhesive formulations (about 40 000 tonnes in 1997). It is also used to improve the properties of alkyd resins for paints and coatings and as a “down hole” drilling mud additive in secondary oil production. Its use as a rubber polymerization retarder is diminishing (Srour, 1998).

Benzoic acid and sodium benzoate (see section 4.3.2) are used as preservatives in beverages, fruit products, chemically leavened baked goods, and condiments, preferably in a pH range below 4.5. A disadvantage is the off-flavour they may impart to foods (Chipley, 1983). Owing to their inhibitory effect on yeast, they cannot be used in yeast-leavened products (Friedman & Greenwald, 1994). Examples of upper concentrations allowed in food are up to 0.1% benzoic acid (USA) and between 0.15% and 0.25% (other countries) (Chipley, 1983). The European Commission limits for benzoic acid and sodium benzoate are 0.015–0.5% (EC, 1995).

Benzoic acid and its salts and esters are found in 11 of 48 (23%) toothpastes (Sainio & Kanerva, 1995) to a maximum of 0.5% (Ishida, 1996) and in mouthwashes and dentifrices. Benzoic acid is also used in cosmetics (in creams and lotions with pH values under 4, up to 0.5%) (Wallhäusser, 1984). Sixteen out of 71 deodorants tested contained benzoic acid (Rastogi et al., 1998).

Benzoic acid is a breakdown product of benzoyl peroxide, which is used as an additive at levels of between 0.015% and 0.075% to bleach flour (Friedman & Greenwald, 1994) and in dermatological antifungal preparations (BMA, 1998). Benzoic acid is reported to leach from denture-base acrylic resins, where benzoyl peroxide is added as a polymerization initiator (Koda et al., 1989, 1990).

Benzoic acid can be used in combination with salicylic acid (Whitfield’s ointment) as a fungicidal treatment for ringworm (BMA, 1998).

4.3.2 Sodium benzoate

Although undissociated benzoic acid is the more effective antimicrobial agent for preservation purposes, sodium benzoate is used preferably, as it is about 200 times more soluble than benzoic acid. About 0.1% is usually sufficient to preserve a product that has been properly prepared and adjusted to pH 4.5 or below (Chipley, 1983).

A major market for sodium benzoate is as a preservative in the soft drink industry, as a result of the demand for high-fructose corn syrup in carbonated beverages. Sodium benzoate is also widely used as a preservative in pickles, sauces, and fruit juices (Srour, 1998). Benzoic acid and sodium benzoate are used as antimicrobial agents in edible coatings (Baldwin et al., 1995).

Sodium benzoate is also used in pharmaceuticals for preservation purposes (up to 1.0% in liquid medicines) and for therapeutic regimens in the treatment of patients with urea cycle enzymopathies (see section 9).

Possibly the largest use of sodium benzoate, accounting for 30–35% of the total demand (about 15 000 tonnes of benzoic acid), is as an anticorrosive, particularly as an additive to automotive engine antifreeze coolants and in other waterborne systems (Scholz & Kortmann, 1991; Srour, 1998). A new use is the formulation of sodium benzoate into plastics such as polypropylene, to improve strength and clarity (BFGoodrich Kalama Inc., 1999). Sodium benzoate is used as a stabilizer in photographic baths/processing (BUA, 1995).

4.4 Estimated global release

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.

5. ENVIRONMENTAL TRANSPORT, DISTRIBUTION, TRANSFORMATION, AND ACCUMULATION

5.1 Transport and distribution between media

5.1.1 Benzoic acid

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.

5.1.2 Sodium benzoate

No information on the environmental transport and distribution of sodium benzoate could be identified. Owing to its use pattern, which is similar to that of benzoic acid, most of the amounts released to the environment are also expected to be emitted to aquatic compartments (e.g., surface waters).

5.2 Transformation

5.2.1 Benzoic acid

The experimental determination of the photodegradation of benzoic acid in aqueous solution (25 °C; λ = 240–300 nm) in terms of quantum yield (average number of photons absorbed) resulted in very low values — in the order of 6×10^{-2} mol/einstein[1] (Oussi et al., 1998). However, benzoic acid adsorbed on silica gel (SiO_2) and irradiated with UV light (λ > 290 nm) for 17 h showed 10.2% photodegradation (Freitag et al., 1985). This may be due to a photocatalytic effect, which was also observed with other oxides, notably zinc oxide (ZnO)

[1] An einstein is a unit of light energy used in photochemistry, equal to Avogadro's number times the energy of one photon of light of the frequency in question.

and titanium dioxide (TiO_2). When benzoic acid was irradiated with sunlight in aqueous suspensions of zinc or titanium dioxide, 67% (after 2–3 h) or 90% (after 24 h) of the applied amount was mineralized (Kinney & Ivanuski, 1969; Matthews, 1990).

Indirect photolysis by reaction with hydroxyl radicals is expected to be low. Hydroxyl radical rate constants (k_{OH}) for benzoic acid and its anion have been estimated to be approximately 0.5×10^{-12} and 2×10^{-12} cm^3/s, respectively (Palm et al., 1998).

Standardized tests on ready (MITI, 1992) or inherent (Zahn & Wellens, 1980) biodegradation showed benzoic acid to be readily biodegraded. The degrees of aerobic degradation were as follows:

MITI I test	85%	(100 mg/litre; 2 weeks; OECD No. 301C)	(MITI, 1992)
Zahn-Wellens test	>90%	(508 mg/litre; 2 days)	(Zahn & Wellens, 1980)

Easy degradation of benzoic acid to methane and carbon dioxide was also observed in different non-standardized experiments using sewage sludge as inoculum (BUA, 1995). Benzoic acid was found to be degraded by adapted anaerobic sewage sludge at 86–93% after 14 days (Nottingham & Hungate, 1969), by aerobic activated sludge (adapted) at >95% after 5–20 days (Pitter, 1976; Lund & Rodriguez, 1984), and by unadapted aerobic activated sludge at 61–69% after 2–3 days with a preceding lag time of 2–20 h (Urano & Kato, 1986). The use of a synthetic sewage inoculated with laboratory bacterial cultures led to complete degradation of benzoic acid after 14 days under anaerobic conditions (Kameya et al., 1995).

A greater variability in degradation (0–100%) was seen in tests using environmental matrices (e.g., rain, lake water, seawater, soil, etc.). It depended mainly on substance concentration and time for acclimation (see Table 1). Test durations exceeding 2 days resulted in removal of $40% when initial concentrations were below 20 mg/litre. A rapid mineralization occurred in groundwater and subsurface soil samples. In groundwater, a half-life of 41 h has been found for benzoic acid (initial concentration 1–100 µg/litre; metabolized to $^{14}CO_2$) under aerobic conditions (Ventullo & Larson, 1985). Half-lives of 7.3 h and 18.2 h, respectively, have been observed for aerobic and anaerobic degradation of benzoic acid (initial concentration 1 mg/kg dry weight; metabolized to $^{14}CO_2$) in subsurface soils of septic tank tile fields (Ward, 1985). Anaerobic degradation of benzoic acid (initial concentration 250 mg carbon/litre) in a methanogenic microcosm (consisting of aquifer solids and groundwater) required 4 weeks of adaptation, followed by nearly complete depletion after 8 weeks of incubation (Suflita & Concannon, 1995).

Several isolated microorganisms have been shown to utilize (and therefore probably degrade) benzoic acid under aerobic or anaerobic conditions. They include, among others, fungal species such as *Rhodotorula glutinis* and other yeast-like fungi (Kocwa-Haluch & Lemek, 1995), the mould *Penicillium frequentans* (Hofrichter & Fritsche, 1996), and bacteria, such as *Alcaligenes denitrificans* (Miguez et al., 1995), *Rhodopseudomonas palustris*, several strains of denitrifying pseudomonads (Fuchs et al., 1993; Elder & Kelly, 1994; Harwood & Gibson, 1997), and *Desulfomicrobium escambiense* (Sharak Genthner et al., 1997).

Although benzoic acid is primarily metabolized to hippuric acid in rats (see section 7), some other species do excrete other metabolites, such as dibenzoylornithine (hen), benzoylglutamic acid (Indian fruit bat), benzoylarginine (tick, insects), or benzoyltaurine (southern flounder, *Paralichthys lethostigma*) (Parke, 1968; Goodwin, 1976; James & Pritchard, 1987).

5.2.2 Sodium benzoate

Experimental data on photodegradation of sodium benzoate are not available. As with benzoic acid, photolysis in aqueous solution is assumed to be unlikely with regard to its known UV spectra (Palm et al., 1998). Indirect photolysis by reaction with hydroxyl radicals plays only a minor role, with estimated and measured hydroxyl rate constants of about 0.33×10^{-11} cm^3/s (Palm et al., 1998).

Sodium benzoate was readily biodegradable under aerobic conditions in several standard test systems:

Modified MITI test	84%	(100 mg/litre; 10 days)	(King & Painter, 1983)
Modified Sturm test	80–90%	(50 mg/litre; 7 days)	(Salanitro et al., 1988)
Closed bottle test	75–111%	(5 mg/litre; 30 days)	(Richterich & Steber, 1989)

Degradation assays using seawater as test medium ("natural water") or as inoculum (marine filter material given into a synthetic marine medium) according to an adapted Organisation for Economic Co-operation and Development (OECD) guideline (301B) resulted in a degradation of 85% and 97%, respectively (10 mg/litre;

Table 1: Removal of benzoic acid in freshwater, marine, and soil matrices.

Matrix	Initial concentration (mg/litre or mg/kg)	Conditions	Duration (days)	Removal (%)	Measured parameter	Reference
Rainwater	0.001	22 °C; shaking once per day; dark	2 7 45	0 40 100	benzoic acid	Kawamura & Kaplan (1990)
Lake water (eutrophic/mesotrophic)	0.059	29 °C; no shaking; dark	7	98.7	^{14}C (in CO_2, biomass)	Rubin et al. (1982)
Seawater (estuary)		20 °C; dark; rotary shaking			^{14}C (in CO_2, biomass)	Shimp & Young (1987)
USA	20 0.005		30 8	<10 70–80		
Canada	20 0.005		16 10	60 >70		
Seawater	2		5	75	BOD[a]	Takemoto et al. (1981)
Soil (grey soil, alkaline)	20	2 mg benzoic acid in 0.1 ml acetone + 100 g soil + 10 ml H_2O	70	63	$^{14}CO_2$	Haider et al. (1974)
Soil (sand; 18.9 m depth)	0.05	24 °C; 20–25% moisture content	15	40	$^{14}CO_2$	Federle (1988)

[a] BOD = biological oxygen demand.

carbon dioxide measurement; 28 days) (Courtes et al., 1995).

Anaerobic mineralization of sodium benzoate (50–90 mg/litre) by domestic sewage sludge varied from 50% to 96.5% (measurement of carbon dioxide and methane; 28–61 days) (Birch et al., 1989). In another study using anaerobic sludge from sewage works receiving a mixture of domestic and industrial wastewaters, 93% mineralization was observed after 1 week of incubation (measurement of carbon dioxide and methane; initial concentration 50 mg carbon/litre) (Battersby & Wilson, 1989). Benzoate-acclimated sludges were reported to be capable of completely degrading benzoate concentrations of 3000 mg/litre within 5–7 days (Kobayashi et al., 1989).

5.3 Accumulation

5.3.1 Benzoic acid

The *n*-octanol/water partition coefficient (log K_{ow}) 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 capricorutum*) (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 quinquifasciatus*), 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.

Geoaccumulation of benzoic acid has also been found to be low. Depending on soil depth, sorption coefficients (K_d) of 0.62 (18.9 m) to 1.92 (0.4 m) have been measured (Federle, 1988). Mobility determinations of ^{14}C-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).

5.3.2 Sodium benzoate

No experimental data on bioaccumulation or geo-accumulation of sodium benzoate have been identified. From the information on benzoic acid, a significant potential for accumulation is not to be expected.

6. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

6.1 Environmental levels

Generally, benzoic acid can occur in almost all environmental compartments. Whether it exists in the undissociated or dissociated form depends on the specific physicochemical conditions. Above pH 6, the benzoate anion prevails (Chipley, 1983).

There is a series of reports on positive qualitative analyses of benzoic acid in various environmental media, such as air (Belgium: Cautreels & van Cauwenberghe, 1978; Germany: Helmig et al., 1989), rain or snow (Norway: Lunde et al., 1977; Germany: Winkeler et al., 1988), surface waters (Norway, river: Schou & Krane, 1981), and soils (United Kingdom, heathland soil: Jalal & Read, 1983; Germany, river terrace soil: Cordt & Kußmaul, 1990), but these do not provide quantitative measurements.

Semiquantitative measurements of concentrations of benzoic acid in urban air in Pasadena, California (USA) were in the range of 0.09–0.38 µg/m^3 (Schuetzle et al., 1975). This was comparable to quantitative measurements performed in 1984 in Los Angeles, California (USA), which resulted in atmospheric concentrations of 0.005–0.13 µg/m^3 (n = 8) (Kawamura et al., 1985). Most of the quantitative data compiled in Table 2 with respect to water samples refer to concentrations of benzoic acid in groundwater, with a maximum of 27.5 mg/litre measured in the vicinity of a point source.

Benzoic acid occurs naturally in free and bound form in many plant and animal species. It is a common metabolite in plants and organisms (Hegnauer, 1992). Appreciable amounts have been found in gum benzoin (around 20%) and most berries (around 0.05%) (Budavari et al., 1996). For example, ripe fruits of several *Vaccinium* species (e.g., cranberry, *V. vitis idaea*; bilberry, *V. macrocarpon*) contain as much as 300–1300 mg free benzoic acid per kg fruit (Hegnauer, 1966). Benzoic acid is also formed in apples after infection with the fungus *Nectria galligena* (Harborne, 1983) or in *Pinus thunbergii* callus inoculated with a pathogenic pine wood nematode (*Bursaphelenchus xylophilus)* (Zhang et al., 1997). Among animals, benzoic acid has been identified primarily in omnivorous or phytophageous species, e.g., in viscera and muscles of the ptarmigan (*Lagopus mutus*) (Hegnauer, 1989) as well as in gland secretions of male muskoxen (*Ovibos moschatus*) (Flood et al., 1989) or Asian bull elephants (*Elephas maximus*) (Rasmussen et al., 1990).

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 *Vaccinium* species)	traces – 14 mg/kg
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).

6.2 Human exposure

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

Table 2: Concentrations of benzoic acid in rain, snow, groundwater, and leachate samples.

Medium	Location; sampling date	Concentration (µg/litre)	Reference
	Los Angeles area, California, USA; 1982–1983	Sum concentrations[a]	Kawamura & Kaplan (1986)
Rain: urban		0.06–10.2 (*n* = 6)	
Rain: semirural		0.02 (*n* = 1)	
Snow: rural		0.04–0.1 (*n* = 3)	
Groundwater	Wyoming, USA (near underground coal gasification site; 15 months after the end of gasification)	16–860 (*n* = 3)	Stuermer et al. (1982)
Groundwater	Florida, USA (near wood treatment facility); 1984	10–27 500 (*n* = 3)	Goerlitz et al. (1985)
Groundwater	Ontario, Canada (near landfill[b]); 1983	traces (*n* = 2)	Barker et al. (1988)
Groundwater	Barcelona area, Spain (near landfill[b])	up to 0.21 (*n* = 3)	Guardiola et al. (1989)
Leachate (from landfill[b])	Ontario, Canada; 1981	<0.1–>1000 (*n* = 5)	Reinhard & Goodman (1984)
Leachate (from landfill[b])	Ontario, Canada; 1983	traces (*n* = 2)	Barker et al. (1988)
Leachate (from foundry wastes)	USA; 1986–1988	200–400[c] (*n* = 3)	Ham et al. (1989)

[a] Including benzoic acid, 3-methyl benzoic acid, and 4-methyl benzoic acid.
[b] Receiving rural, municipal (domestic), and industrial wastes.
[c] Concentrations estimated from gas chromatography/mass spectrometry data.

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/m^3 (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 m^3 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/m^3 (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 m^3 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.

7. COMPARATIVE KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

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 (^{14}C-labelled benzoic acid dissolved in acetone; 4 µg/cm^2; circular area of 13 cm^2; 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/cm^2), the percent absorption decreased from 35% to 14% within 24 h. However, the total uptake per cm^2 increased from 1 to 288 µg (Wester & Maibach, 1976). For sodium benzoate, no data concerning dermal uptake were identified in the literature.

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).

No information is available on absorption via inhalation.

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).

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 (US FDA, 1972a, 1973).

In the acid conditions of the stomach, the equilibrium moves to the undissociated benzoic acid molecule, which should be absorbed rapidly. Benzoate from sodium benzoate would change from the ionized form to the undissociated benzoic acid molecule. As a result, the metabolism and systemic effects of benzoic acid and sodium benzoate can be evaluated together.

7.1 Precursors of benzoic acid

Benzyl acetate, its hydrolysis product, benzyl alcohol, and the oxidation product of this alcohol, benzaldehyde, are precursors of benzoic acid in experimental animals and humans. Benzyl acetate is metabolized to benzoic acid and further to hippuric acid and benzoyl glucuronide to an extent of >90% both in mice and in rats of different strains. Benzyl alcohol was metabolized to benzoic acid and its conjugates in preterm infants. Benzaldehyde is metabolized to benzoic acid and its conjugates in rabbits to an extent of approximately 90% (WHO, 1996).

8. EFFECTS ON LABORATORY MAMMALS AND *IN VITRO* TEST SYSTEMS

8.1 Single exposure

With oral LD_{50} values (administration by gavage) of 3040 mg benzoic acid/kg body weight in rats (Bio-Fax, 1973) and 1940–2263 mg benzoic acid/kg body weight in mice (McCormick, 1974; Abe et al., 1984), the acute toxicity of benzoic acid is low. Clinical signs of intoxication (reported for rats only) included diarrhoea, muscular weakness, tremors, hypoactivity, and emaciation (Bio-Fax, 1973). With oral LD_{50} values of 2100–4070 mg sodium benzoate/kg body weight in rats, the acute toxicity of sodium benzoate is similar to that of benzoic acid, as are the symptoms (Smyth & Carpenter, 1948; Deuel et al., 1954; Bayer AG, 1977).

In four cats given diets containing 0 or 1% benzoic acid (approximately 0 or 450–890 mg/kg body weight), aggression, hyperaesthesia, and collapse starting 14–16 h after feed uptake were seen at a dose level equal to 630 mg/kg body weight. The duration of the syndrome was about 18–176 h, 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 (Bedford & Clarke, 1972). The authors attributed the higher toxicity of benzoic acid in cats compared with other species to the low capacity of cats for glucuronidation (see section 7).

In rats, exposure by inhalation to 26 mg/m^3 over 1 h caused no mortality, but generalized inactivity and lacrimation were noted. The gross autopsy gave no significant findings (no further information available; Bio-Fax, 1973).

In a limit test with rabbits, no mortality or signs of intoxication were seen after dermal application of 10 000 mg/kg body weight. The gross autopsy gave no significant findings (no further information available; Bio-Fax, 1973).

8.2 Irritation and sensitization

8.2.1 Benzoic acid

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).

8.2.2 Sodium benzoate

An acute dermal irritation/corrosion study with rabbits conducted according to OECD Guideline 404 (no data about physical state; score 0: RCC Notox, n.d., a) as well as a non-standardized experiment with the solid substance (score not given: Bayer AG, 1977) gave no indication for skin irritating effects.

In a study performed according to OECD Guideline 405 (no data about physical state; RCC Notox, n.d., b), sodium benzoate was only slightly irritating to the eye (score 9.3, according to the scheme of Kay & Calandra, 1962). The application of the solid substance in a non-standardized experiment caused no irritation (score not given: Bayer AG, 1977).

For sodium benzoate, no data on sensitizing effects were identified in the available literature.

8.3 Short-term exposure

8.3.1 Oral exposure

In general, the database for benzoic acid and sodium benzoate is limited, and there are no studies available performed according to current guidelines. In addition, the documentation of these studies in most cases is insufficient. Detailed information is given in Table 3.

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 (Kreis et al., 1967). In two other studies with rats dosed with approximately 825 mg/kg body weight per day over 7–35 days (Kreis et al., 1967) or with 65–647 mg/kg body weight per day over 28 days (Bio-Fax, 1973), 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 in the study by Kreis et al. (1967). 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 (Bio-Fax, 1973). As given in Table 3, both studies have several limitations (i.e., missing haematological and clinical chemical investigations, incomplete histopathological

Table 3: Toxicity of benzoic acid and sodium benzoate after short-term oral exposure.

Species; strain; number of animals per dose[a]	Treatment	Duration (days)	Organs examined in histopathology, clinical chemistry, haematology	Results[a]	Reference
Benzoic acid					
cat; 4 m	0 or 0.5% in diet (~0 or 300–420 mg/kg body weight)	3–4	liver, kidney, heart, stomach, lung, brain, spinal cord (only animals that died were examined); blood samples were taken from surviving cats	mild hyperaesthesia, apprehension, and depression starting 48–92 h after uptake; duration of the syndrome: about 20–48 h; mortality rate: 50%; degenerative changes in liver, kidneys, and lung, but no pathological findings in brain or spinal cord; surviving cats: urea and serum alanine aminotransferase (S-ALAT) ↑, indicating liver and kidney damage	Bedford & Clarke (1972)
cat; 4 m	a) 100 or 200 mg/kg body weight via diet b) 0 or 0.25% in diet (~0 or 130–160 mg/kg body weight)	a) 15 b) 23	only blood samples were taken	no adverse effects were reported	Bedford & Clarke (1972)
rat; Wistar; 5–15 m	0 or 3% in diet (~0 or 2250 mg/kg body weight)	1–5	heart, liver, spleen, kidney, brain	body weight gain ↓; in rats dosed over 5 days, disorders of the central nervous system (excitation, ataxia, tonoclonic convulsions); mortality rate ~50%; in some cases, bleeding into the gut; brain damage (necrosis of parenchymal cells of the stratum granulosum of the fascia dentata and the cortex of the lobus piriformis) in most animals dosed over 3–5 days (still present after 35 days)	Kreis et al. (1967)
rat; Wistar; 5–10 m	0 or 1.1% in diet (~0 or 825 mg/kg body weight)	7–35	heart, liver, spleen, kidney, brain	body weight gain ↓; no clinical signs of intoxication	Kreis et al. (1967)
rat; albino; 10 m	0, 760, 3800, or 7600 ppm via diet (~0, 65, 324, or 647 mg/kg body weight)	28	liver, kidney, adrenals, testes	no deaths or signs of intoxication 324 mg/kg body weight: relative kidney weights ↑; no further information available	Bio-Fax (1973)
Sodium benzoate					
rat; F344/Ducrj; 6 m/f	0, 1.81, 2.09, or 2.4% in diet (~0, 1358, 1568, or 1800 mg/kg body weight)	10	liver, kidney; standard clinical chemistry	≥1358 mg/kg body weight: changes in serum levels (cholesterol ↑ (f)) ≥1568 mg/kg body weight: relative liver weight ↑ (m); changes in serum levels (albumin ↓ (m), total protein ↓ (m)) 1800 mg/kg body weight: 1/6 males died (hypersensitivity, convulsions); body weight ↓ (m/f); relative liver weight ↑ (f); relative kidney weights ↑ (m/f); absolute weights of spleen and thymus ↓ (m); absolute/relative weights of thymus ↓ (f); changes in serum levels (gamma-glutamyltranspeptidase (GGT) ↑ (m), albumin ↓ (f), cholinesterase ↓ (f)); eosinophilic foci around periportal vein and enlargement of hepatocytes with glassy cytoplasm in the periportal area of the liver (m); no changes in the kidney (m)	Fujitani (1993)
rat; Sherman; 6 m/f	0, 2, or 5% in diet (~0, 2200, or 6700 mg/kg body weight)	28	no data available	2200 mg/kg body weight: slight depression of body weight gain (m) 6700 mg/kg body weight: mortality 100% within 11 days; signs of intoxication included hyperexcitability, urinary incontinence, and convulsions no further information available	Fanelli & Halliday (1963)
rat; 28 (no further data)	0 or 5% in diet (~0 or 3750 mg/kg body weight)	28	no data available	mortality about 100% within 3 weeks; decreased feed intake, diarrhoea, intestinal haemorrhage and crusted blood in the nose; no further information available	Kieckebusch & Lang (1960)
rat; 5 (no further data)	0 or 5% in diet (~0 or 3750 mg/kg body weight)	≥28	no data available	mortality 80% within 4–5 weeks; decreased body weight; no further information available	Kieckebusch & Lang (1960)
rat; F344; 10–11 m/f	0, 0.5, 1, 2, 4, or 8% in diet (~0, 375, 750, 1500, 3000, or 6000 mg/kg	42	histopathology performed, but not further specified	≥375 mg/kg body weight: hypersensitivity after dosing ≥3000 mg/kg body weight: mortality about 100% within 4 weeks; apart from atrophy of the spleen and lymph nodes, no other morphological changes were	Sodemoto & Enomoto (1980)

Table 3 (contd).

Species; strain; number of animals per dose[a]	Treatment	Duration (days)	Organs examined in histopathology, clinical chemistry, haematology	Results[a]	Reference
	body weight)			noted	
rat; Sherman; 5 m/f	0 or 16–1090 mg/kg body weight via diet	30	adrenals, upper intestine, kidney, liver, spleen	no adverse effects were reported; no further information available	Smyth & Carpenter (1948)
mouse; B6C3F_1; 4–5 m/f	0, 2.08, 2.5, or 3% in diet (~0, 3000, 3750, or 4500 mg/kg body weight)	10	liver, kidney; standard clinical chemistry	$3750 mg/kg body weight: changes in serum levels (cholinesterase ↓ (m)) 4500 mg/kg body weight: hypersensitivity in all animals; convulsions 1/5 males and 2/5 females (both females died); absolute/relative liver weight ↑ (m/f); relative kidney weight ↑ (f); changes in serum levels (cholesterol ↑ (m), phospholipids ↓ (m)); enlarged hepatocytes, single cell necrosis and vacuolation of hepatocytes in all livers (m); no changes in the kidney (m/f)	Fujitani (1993)
mouse; albino Swiss; 4 m/f	0, 0.5, 1, 2, 4, or 8% via drinking-water (~0–12 000 mg/kg body weight)	35	survival, chemical consumption, histological changes (not further specified) (prestudy for carcinogenicity study)	3000 mg/kg body weight: “suitable for lifelong treatment” based on four parameters: survival, body weight, chemical consumption, and histology 6000 mg/kg body weight: mortality 75% in m/f; body weight of surviving mice ↓ (m/f) 12 000 mg/kg body weight: mortality 100% within 3 weeks	Toth (1984)

[a] m = male; f = female.

examinations); therefore, both of these studies were inadequate for derivation of a NO(A)EL.

More information on dose–response can be gained from the study of Fujitani (1993), in which rats received sodium benzoate for 10 days in feed. At the lowest tested concentration of 1358 mg/kg body weight per day, changes in serum cholesterol levels occurred in females. At doses of 1568 mg/kg body weight per day 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 (convulsions) were seen after dosing via diet with approximately 1800 mg/kg body weight per day. In several other studies listed in Table 3, adverse effects were seen only at higher doses after feeding sodium benzoate over periods from 10 to 42 days, so that a lowest-observed-(adverse-)effect level (LO(A)EL) of 1358 mg sodium benzoate/kg body weight per day for short-term exposure can be derived.

With cats (Bedford & Clarke, 1972), also described in Table 3, the effect levels with benzoic acid were lower. However, due to the differences in the metabolism of benzoic acid in cats compared with other experimental animals and humans, this study was not taken into further consideration (see section 7).

8.3.2 Inhalation exposure

Ten CD rats per sex per group were exposed to 0, 25, 250, or 1200 mg benzoic acid dust aerosol/m^3 (analytical concentration; mass aerodynamic diameter [MAD]/Fg (standard deviation): 0, 4.6/3.1, 4.4/2.1, 5.2/2.1; mass median aerodynamic diameter [MMAD]: 4.7 µm) for 6 h per day and 5 days per week over 4 weeks. After this time, various serum biochemical, haematological, organ weight, and histopathological examinations were conducted. At $25 mg/$m^3$, 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 $250 mg/$m^3$ 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 (Velsicol Chemical Corp., 1981).

Studies concerning repeated exposure by inhalation to sodium benzoate were not identified in the available literature.

8.3.3 Dermal exposure

Studies concerning repeated dermal exposure to benzoic acid or sodium benzoate were not identified in the available literature.

8.4 Long-term exposure

In general, the database for benzoic acid and sodium benzoate is limited, and there are no studies available performed according to current guidelines. In addition, the documentation in most cases is limited. Detailed information is given in Table 4.

8.4.1 Subchronic exposure

In a 90-day study with rats dosed with 0, 1, 2, 4, or 8% sodium benzoate via diet, the mortality in the highest dose group (~6290 mg/kg body weight per day) was about 50%. Other effects in this group included a reduced weight gain, increased relative weights of liver and kidneys, and pathological changes (not further specified) in these organs (Deuel et al., 1954).

8.4.2 Chronic exposure and 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) (Marquardt, 1960). No further information on these studies is available, as only provisional results were published. 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) (Kieckebusch & Lang, 1960). Only animals of the third generation were autopsied after 16 weeks, but it is not clear if a complete histopathological investigation was performed.

With sodium benzoate, two long-term studies with rats (administration of up to 1400 mg/kg body weight per day via diet over 18–24 months; Sodemoto & Enomoto, 1980) or mice (lifelong application of up to 6200 mg/kg body weight per day via drinking-water; Toth, 1984) are available. The results gave no indication of a carcinogenic effect in the tested animals. Although the study with mice was not performed according to current guidelines, the results seem to be reliable, due to a sufficient number of animals and detailed histopathological

Table 4: Results of studies concerning long-term oral exposure to benzoic acid and sodium benzoate.

Species; strain; number of animals per dose[a]	Treatment	Duration	Examinations; organs in histopathology, clinical chemistry, haematology	Results[a]	Reference
Benzoic acid					
rat; Wistar; dose group: 30 m/20 f; controls: 13 m/12 f	0 or 1.5% in diet (~0 or 750 mg/kg body weight)	18 months	no data available	reduced weight gain with decreased feed intake; increased mortality rate (15/50 vs. 3/25 in controls); no further information available (only provisional results are given)	Marquardt (1960)
rat; Wistar or Osborne-Mendel; dose group: 20 m; controls: 10 m	0 or 1.5% in diet (~0 or 750 mg/kg body weight)	18 months	no data available	reduced weight gain with decreased feed intake; no further information available (only provisional results are given)	Marquardt (1960)
rat; not given; 20 m/f	0, 0.5, or 1% in diet (~0, 250, or 500 mg/kg body weight)	generation 1 and 2: lifelong generation 3: 16 weeks generation 4: until breeding	histopathology in animals of generation 3 (not further specified)	no effects on growth and organ weights; feeding of 0.5% led to prolongation of survival compared with controls; no further information available	Kieckebusch & Lang (1960)
Sodium benzoate					
rat; Sherman; 5 m/f	0, 1, 2, 4, or 8% in diet (~0, 640, 1320, 2620, or 6290 mg/kg body weight)	90 days	histopathology performed, but not further specified	6290 mg/kg body weight: mortality about 50%; weight gain 9; relative weights of liver and kidneys 8; pathological lesions (not further specified) in liver and kidneys	Deuel et al. (1954)
rat; F344; dose group: 50 m/52 f; controls: 25 m/43 f	0, 1, or 2% in diet (m: ~0, 700, or 1400 mg/kg body weight; f: ~0, 290, or 580 mg/kg body weight)	18–24 months	histopathology performed, but not further specified	average mortality rate of all animals during the first 16 months: 14.5% (all dead rats showed pneumonia with abscess); about 100 rats including controls died after 16 months due to haemorrhagic pneumonia (infection); no adverse clinical signs and no differences in average body weight and mortality in dosed animals compared with controls; non-carcinogenic effects not reported	Sodemoto & Enomoto (1980)
mouse; albino Swiss; dose group: 50 m/f; controls: 99 m/f	0 or 2% via drinking-water (~0 or 5960–6200 mg/kg body weight)	lifelong	liver, spleen, kidney, bladder, thyroid, heart, pancreas, testes, ovaries, brain, nasal turbinates, lung	no difference in survival rates in treated animals compared with controls; no pathological or statistical evidence of tumour induction	Toth (1984)

[a] m = male; f = female.

examinations. However, the results from the study with rats are uncertain, due to a very high mortality in animals of all dose groups, including controls (from an "infection" after 16 months), no detailed information about dosing regimen (only mean values given), and the considerable differences in the body weight of male and female rats (the body weight of females was about twice that of males).

8.4.3 Carcinogenicity of benzyl acetate, benzyl alcohol, and benzaldehyde

As benzyl acetate, benzyl alcohol, and benzaldehyde are practically quantitatively metabolized via benzoic acid (see section 7.1), data on their carcinogenicity from 2-year studies may be used as supportive evidence in the assessment of the hazards associated with benzoic acid.

Benzyl acetate was administered in corn oil via gavage to F344/N rats (0, 250, or 500 mg/kg body weight per day) or $B6C3F_1$ mice (0, 500, or 1000 mg/kg body weight per day). In high-dose male rats, the incidence of acinar cell adenomas of the exocrine pancreas was increased, whereas there was no evidence of carcinogenicity in female rats. In high-dose male and female mice, benzyl acetate caused increased incidences of hepatocellular adenomas and squamous cell neoplasms of the forestomach (US NTP, 1986). In contrast to these findings, no such tumours were observed in another study with the same strain of rats and mice when benzyl acetate was administered via diet (rats: #575 mg/kg body weight per day; mice: #375 mg/kg body weight per day) (US NTP, 1993).

With benzyl alcohol, no treatment-related increase in tumours was observed in F344/N rats or $B6C3F_1$ mice after administration of #400 mg/kg body weight per day in rats or #200 mg/kg body weight per day in mice by gavage in corn oil (US NTP, 1989).

In $B6C3F_1$ mice dosed with benzaldehyde in corn oil by gavage (males: 0, 200, or 400 mg/kg body weight per day; females: 0, 300, or 600 mg/kg body weight per day), the incidences of squamous cell papillomas of the forestomach were significantly greater in both exposure groups than in controls. A dose-related increase in the incidence of forestomach hyperplasia was also observed. In F344/N rats dosed with #400 mg/kg body weight per day, there was no evidence of carcinogenic activity (US NTP, 1990).

8.5 Genotoxicity and related end-points

8.5.1 Benzoic acid

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 (McCann et al., 1975; Ishidate et al., 1984; Nakamura et al., 1987; Zeiger et al., 1988). Only in one recombination assay with *Bacillus subtilis* H17 and M45 was a positive result obtained (Nonaka, 1989). However, due to missing experimental details (only results given), the validity of this study cannot be judged. 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 (Oikawa et al., 1980; Tohda et al., 1980; Ishidate et al., 1984; Jansson et al., 1988).

In vivo studies with benzoic acid were not identified in the literature.

8.5.2 Sodium benzoate

Sodium benzoate also gave negative results in some Ames tests and in *Escherichia coli* in the presence or absence of metabolic activation (Ishidate et al., 1984; Prival et al., 1991). As with benzoic acid in recombination assays with *Bacillus subtilis* H17 and M45, positive results were obtained (Ishizaki & Ueno, 1989; Nonaka, 1989). Although sodium benzoate tested negative in a cytogenetic assay with WI-38 cells in the absence of metabolic activation (US FDA, 1974), consistently positive results (in contrast to the negative results of benzoic acid) were obtained in tests on sister chromatid exchange and chromosome aberrations with CHL/CHO and DON cells or human lymphocytes without metabolic activation (Abe & Sasaki, 1977; Ishidate & Odashima, 1977; Ishidate et al., 1984, 1988; Xing & Zhang, 1990). However, from the limited information given in the publications (i.e., only results given), it cannot be judged if these positive results may have been attributable to cytotoxic effects.

In a valid *in vivo* study performed by the US FDA (1974), sodium benzoate tested negative in a cytogenetic assay (bone marrow) in rats after single or multiple oral application of doses up to 5000 mg/kg body weight. In a study with mice (comparable dosing scheme), there was also no indication of mutagenic activity in a host-mediated assay (US FDA, 1974).

However, in a dominant lethal assay with rats (comparable dosing scheme; males were mated with untreated females following 7 or 8 weeks of dosing),

some statistically significant and dose-related findings were reported in week 7: decreased fertility index for both treatment regimens and an increased number of preimplantation losses after single dosing (US FDA, 1974).

In summary, the *in vitro* studies with benzoic acid gave no indications for genotoxic effects, whereas *in vivo* studies were not identified. Sodium benzoate was also inactive in bacterial test systems, whereas tests with mammalian cells gave consistently positive results. In addition, in an *in vivo* study with sodium benzoate (dominant lethal assay in rats), a positive result was obtained. As a result, a genotoxic activity of sodium benzoate cannot be ruled out entirely at present.

Detailed information concerning the genotoxicity of benzoic acid and sodium benzoate *in vitro* is given in Table 5.

8.6 Reproductive and developmental toxicity

8.6.1 Fertility

There are no studies available dealing specifically with the effects of benzoic acid or sodium benzoate on fertility that have been conducted according to current protocols.

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) (see also section 8.4.2; 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 (see also Table 3; Bio-Fax, 1973) or in mice after lifelong application of 6200 mg sodium benzoate/kg body weight per day via drinking-water (see also Table 4; Toth, 1984).

In summary, no clear statement can be given as to the possible effects of benzoic acid or sodium benzoate on fertility.

8.6.2 Developmental 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 (Kimmel et al., 1971).

For sodium benzoate, several teratogenicity studies are available that have been performed with different species. As given in Table 6, no effects were seen in dams or offspring of rats, mice, rabbits, or hamsters given oral doses of up to 300 mg/kg body weight per day (highest dose tested) during gestation (US FDA, 1972b). In a study with rats by Onodera et al. (1978), doses of 4% or 8% via diet (uptake of 1875 or 965 mg/kg body weight per day) induced severe maternal toxicity (no weight gain/loss in body weight, increased mortality) and were associated with embryotoxic and fetotoxic effects as well as malformations. However, the authors suggested that the effects on the dams and fetuses at $4% dietary levels were caused by reduced maternal feed intake, leading to malnutrition. The intake of sodium benzoate in the highest dose group (8%) was lower than that at 2%, where no adverse effects were seen. From this study, a NO(A)EL of about 1310 mg/kg body weight per day can be derived. In a study with rats by Minor & Becker (1971), however, fetotoxic and teratogenic effects occurred at 1000 mg/kg body weight per day. In this study, sodium benzoate was applied by intraperitoneal injection. Therefore, differences in pharmacokinetics between oral and intraperitoneal administration may be the reason for the higher sensitivity.

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).

8.6.3 Reproductive toxicity of benzyl acetate, benzyl alcohol, and benzaldehyde

As benzyl acetate and benzyl alcohol are practically quantitatively metabolized via benzoic acid (see section 7.1), data on their reproductive toxicity may be used as supportive evidence in the assessment of the hazards associated with benzoic acid.

Dietary benzyl acetate (up to 5% in the diet for 13 weeks) had no effect on the weights of the epididymis, cauda epididymis, or testis, on sperm motility or density, or on the percentage of abnormal sperm in mice or rats (US NTP, 1993).

Benzyl acetate (0, 10, 100, 500, or 1000 mg/kg body weight per day by gavage on days 6–15) had no significant effects on maternal health in rats and did not induce changes in the numbers of corpora lutea, implantations, live or dead fetuses, or resorptions, implantation ratio, sex ratio, external or internal malformations, or

Table 5: Genotoxicity of benzoic acid and sodium benzoate *in vitro*.

Species (test system)	End-point	Concentration range	Results[a]		Remarks	Reference
			Without metabolic activation	With metabolic activation		
Benzoic acid						
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537	Reverse mutations	10–1000 µg/plate	!	!		McCann et al. (1975)
Salmonella typhimurium TA 97, TA 98, TA 100, TA 1535, TA 1537	Reverse mutations	33–10 000 µg/plate	!	!	cytotoxic effects at $5000 µg/plate	Zeiger et al. (1988)
Salmonella typhimurium TA 92, TA 94, TA 98, TA 100, TA 1535, TA 1537	Reverse mutations	up to 10 000 µg/plate	!	!	10 000 µg/plate was the highest non-cytotoxic concentration tested	Ishidate et al. (1984)
Salmonella typhimurium TA 1535/pSK 1002	DNA damage (umu test)	up to 1670 µg/ml	!	!	no further information available (only results given)	Nakamura et al. (1987)
Bacillus subtilis H17, M45	Recombination assay	not given			tested positive (no further information available, only summary given)	Nonaka (1989)
Chinese hamster cells (CHL)	Chromosome aberration	up to 1500 µg/ml	?	0	1500 µg/ml was given as maximum effective concentration; result given as negative in Ishidate et al. (1988)	Ishidate et al. (1984)
Human lymphoblastoid cells (transformed by Epstein-Barr virus)	Sister chromatid exchange	1–30 mmol/litre	!	0	cytotoxic effects at 30 mmol/litre	Tohda et al. (1980)
Human lymphocytes	Sister chromatid exchange	up to 2 mmol/litre	!	0		Jansson et al. (1988)
Chinese hamster cells (CHO)	Sister chromatid exchange	up to 10 mmol/litre	!	0		Oikawa et al. (1980)
Sodium benzoate						
Salmonella typhimurium TA 92, TA 94, TA 98, TA 100, TA 1535, TA 1537	Reverse mutations	up to 3000 µg/plate	!	!	3000 µg/plate was the highest non-cytotoxic concentration tested	Ishidate et al. (1984)
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538	Reverse mutations	33–10 000 µg/plate	!	!		Prival et al. (1991)
Escherichia coli WP2	Reverse mutation assay	33–10 000 µg/plate	!	!		Prival et al. (1991)
Bacillus subtilis H17, M45	Recombination assay	not given			tested positive (no further information available, only summary given)	Nonaka (1989)
Bacillus subtilis H17, M45	Recombination assay	!S9: 20 mg/disc +S9: 16 mg/disc	(+)	(+)		Ishizaki & Ueno (1989)
WI-38 cells	Cytogenetic assay	10–1000 µg/ml	!	0	examination of anaphase preparations cytotoxic effects at $500 µg/ml	US FDA (1974)

Table 5 (contd).

Species (test system)	End-point	Concentration range	Results[a]		Remarks	Reference
			Without metabolic activation	With metabolic activation		
Chinese hamster cells (CHL)	Chromosome aberration	up to 2000 µg/ml	+	0	2000 µg/ml was given as maximum effective concentration	Ishidate et al. (1984, 1988)
Chinese hamster cells (CHL)	Chromosome aberration	139 mg/ml	+	0	only maximum effective dose given	Ishidate & Odashima (1977)
Chinese hamster cells (DON)	Chromosome aberration	290 µg/ml	0	0	only minimum effective dose given	Ishidate et al. (1988)
Chinese hamster cells (DON)	Chromosome aberration	1–10 mmol/litre	+	0		Abe & Sasaki (1977)
Chinese hamster cells (DON)	Sister chromatid exchange	1–10 mmol/litre	(+)	0	slight increase without dosage effect	Abe & Sasaki (1977)
Human lymphocytes	Sister chromatid exchange	10 mmol/litre	+	0		Xing & Zhang (1990)

[a] !, negative; +, positive; (+) weakly positive; ?, equivocal; 0, not tested.

Table 6: Results of studies concerning reproductive and developmental toxicity of benzoic acid and sodium benzoate.

Species; strain; number of animals per dose[a]	Application	Duration[b]	Parameters investigated	Results	NO(A)EL (mg/kg body weight)	Reference
Benzoic acid						
rat; Wistar; dose group: 7 f; controls: not given	0 or 510 mg/kg body weight via gavage	gd 9	F_0: implantation and resorption sites F_1: malformations	F_0: resorption rates were given as "comparable with controls" F_1: malformations (not further specified) were given as "comparable with controls" no further information available	510	Kimmel et al. (1971)
rat; not given; 20 f	0, 0.5, or 1% in diet (~0, 250, or 500 mg/kg body weight)	F_0 and F_1: lifelong F_2: 16 weeks F_3: until breeding	fertility and lactation	F_0– F_3: no adverse effects compared with controls were reported no further information available	500	Kieckebusch & Lang (1960)
Sodium benzoate						
rat; Wistar; 20 f	0, 1.75, 8, 38, or 175 mg/kg body weight via gavage	gd 6–15	F_0: numbers of corpora lutea, implantation and resorption sites, examination of the urogenital tract F_1: numbers of live and dead fetuses, body weights, gross examination for external malformations, microscopic visceral and skeletal examination	F_0 and F_1: no adverse effects compared with controls were reported	175	US FDA (1972b)
mouse; CD-1; 25–31 f	0, 1.75, 8, 38, or 175 mg/kg body weight via gavage	gd 6–15	F_0: numbers of corpora lutea, implantation and resorption sites, examination of the urogenital tract F_1: numbers of live and dead fetuses, body weights, gross examination for external malformations, microscopic visceral and skeletal examination	F_0 and F_1: no adverse effects compared with controls were reported	175	US FDA (1972b)
rabbit; Dutch belted; 14–32 f	0, 2.5, 12, 54, or 250 mg/kg body weight via gavage	gd 6–18	F_0: numbers of corpora lutea, implantation and resorption sites, examination of the urogenital tract F_1: numbers of live and dead fetuses, body weights, gross examination for external malformations, microscopic visceral and skeletal examination	F_0 and F_1: no adverse effects compared with controls were reported	250	US FDA (1972b)
hamster; golden; 22 f	0, 3, 14, 65, or 300 mg/kg body weight via gavage	gd 6–10	F_0: numbers of corpora lutea, implantation and resorption sites, examination of the urogenital tract F_1: numbers of live and dead fetuses, body weights, gross examination for external malformations, microscopic visceral and skeletal examination	F_0 and F_1: no adverse effects compared with controls were reported	300	US FDA (1972b)
rat; Sprague-Dawley (no further data)	0, 100, 315, or 1000 mg/kg body weight intraperitoneally	a) gd 9–11 b) gd 12–14	F_0: not specified F_1: body weights, *in utero* deaths, gross anomalies	a) F_0: no data given F_1: 1000 mg/kg body weight: body weights 9; *in utero* deaths 8 (16%); gross anomalies 8 (not further specified) b) F_0: no data given F_1: 1000 mg/kg body weight: body weights 9; *in utero* deaths 8 (12%); gross anomalies : (not further specified) no further information available	315	Minor & Becker (1971)
rat; Wistar; 27–30 f	0, 1, 2, 4, or 8% via diet (~0, 700, 1310, 1875, or 965 mg/kg body weight)	gd 1–20	a) all but five animals in each group were sacrificed on gd 20 (numbers of viable/dead fetuses, early/late resorptions, fetal, placental, and ovarian weights, and abnormalities of maternal organs and fetal appearance were recorded) b) the remaining five dams delivered naturally (number of offspring, survival, body weight, and abnormalities were	a) $4% (1875 or 965 mg/kg body weight): F_0: weight gain :; feed intake 9; mortality 8 (convulsions, depressed motor activity) F_1: number of dead/resorbed fetuses 8; body weight of viable fetuses 9; mild systemic oedema, anophthalmia, microphthalmia, hydrocephalus,	1310	Onodera et al. (1978)

Table 6 (contd).

Species; strain; number of animals per dose[a]	Application	Duration[b]	Parameters investigated	Results	NO(A)EL (mg/kg body weight)	Reference
			recorded); 3 weeks after birth, all surviving pups were weaned and examined for gross abnormalities (one-half of the pups and all dams were necropsied); the remaining pups were necropsied at 8 weeks of age (body weight and food intake were measured weekly until necropsy)	pyelectasis, hydroplasia, cerebral hypoplasia; delayed ossification, lumbar or cervical ribs, and varied sternebrae 8%: F_0: body weight 9 b) F_1: #2% (1310 mg/kg body weight): no adverse effects compared with controls $4% (1875 or 965 mg/kg body weight): delivery rates 9 (50 and 8.2%, respectively); complete loss of litters after parturition		

[a] m = male; f = female.
[b] gd = gestation day.

placental weight. Fetal weights were significantly reduced at the highest dose (Ishiguro et al., 1993).

Benzyl alcohol at 550 mg/kg body weight per day by gavage on days 6–15 of pregnancy had no effect on gestation index, average number of live pups per litter, postnatal survival, or pup body weight on days 0 and 3 in CD-1 mice (York et al., 1986), while 750 mg/kg body weight per day (days 7–14) induced a reduction in the pup weight and maternal weight gain, but no pup mortality or changes in mating or gestation indices, the total number of resorptions, or the number of live pups per litter (Hardin et al., 1987).

9. EFFECTS ON HUMANS

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 (Maibach & Johnson, 1975; Clemmensen & Hjorth, 1982; Larmi et el., 1988; Ring, 1989; Gailhofer et al., 1990; Aberer et al., 1992; Lahti et al., 1995; Anderson, 1996; Bindslev-Jensen, 1998; Coverly et al., 1998).

In the literature, several studies (e.g., oral provocation tests or patch tests) are available, which have been performed with small groups of patients suffering from urticaria, dermatitis, asthma, and Melkersson-Rosenthal syndrome (Juhlin et al., 1972; Freedman, 1977; Østerballe et al., 1979; Lahti & Hannuksela, 1981; Clemmensen & Hjorth, 1982; Ibero et al., 1982; Moneret-Vautrin et al., 1982; Veien et al., 1987; Aguirre et al., 1993; McKenna et al., 1994; BUA, 1995; Munoz et al., 1996; Petrus et al., 1996; Vogt et al., 1999). In most of these studies, atopic individuals have demonstrated reactions to oral and dermal challenge with benzoic acid or sodium benzoate.

The information concerning skin reactions caused by benzoic acid or sodium benzoate in the general population is limited. In a study with 2045 patients of dermatological clinics, only 5 persons (approximately 0.2%) showed a positive reaction in patch tests (Brasch et al., 1993), while 34 of 5202 patients (approximately 0.7%) with contact urticaria reacted positively (Broeckx et al., 1987). From these data, it can be concluded that skin reactions caused by benzoic acid or sodium benzoate in the healthy general population are rare.

In US FDA (1972a) and WHO (1996), several older studies concerning oral exposure to benzoic acid or sodium benzoate are described. However, owing to the limited number of individuals (mostly single case studies), 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 per day over a period of up to 92 days (Gerlach, 1909). In another 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 oesophagus) were reported (Wiley & Bigelow, 1908). Chittenden et al. (1909) found no abnormalities in blood picture, urine composition, nitrogen balance, or well-being in six men given 300–400 mg per day via diet for up to 62 days. 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 (Waldo et al., 1949). A single dose of 2000–3000 mg sodium benzoate caused signs of intoxication similar to those described for benzoic acid by Wiley & Bigelow (1908).

Sodium benzoate is used in the treatment of patients with urea cycle enzymopathies (i.e., hyperammonaemia due to inborn errors of urea synthesis) in order to facilitate an alternative pathway of nitrogen excretion. The therapeutic dose given over several years is in the range of 250–500 mg/kg body weight per day (Batshaw & Brusilow, 1981; Green et al., 1983; Batshaw & Monahan, 1987; O'Connor et al., 1987; Kubota & Ishizaki, 1991; Tremblay & Qureshi, 1993; Feillet & Leonard, 1998). At this dose level, clinical signs of toxicity are rare and in most cases limited to anorexia and vomiting, especially after intravenous bolus infusions.

10. EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD

10.1 Aquatic environment

For the toxicity data mentioned in this section, it is not always stated whether the cited effect values are based on nominal or measured concentrations of benzoic acid or sodium benzoate. However, because of their water solubility, their insignificant volatility, and their low adsorption potential (see sections 2 and 5), all nominal concentrations of the test substances are expected to correspond to effective concentrations, even in tests with open systems and longer exposure durations.

In Table 7, several valid toxicity test results for the most sensitive aquatic species of various taxonomic

Table 7: Aquatic toxicity of benzoic acid.

Most sensitive species (test method/end-point)	Special features	Effective concentration (mg/litre)		Reference
Mixed microbial inoculum				
Activated sludge (respiration inhibition test; OECD Guideline 209)	pH 7.5	3-h EC_{50}	>1000	Klecka et al. (1985)
Bacteria				
Pseudomonas putida (cell multiplication inhibition test) (static)	pH neutral	16-h MIC[a]	480	Bringmann & Kuehn (1977)
Photobacterium phosphoreum (Microtox test: bioluminescence reduction)	–	30-min EC_{50}	16.85	Kaiser et al. (1987)
Cyanobacteria				
Anabaena inaequalis				Stratton & Corke (1982)
(cell multiplication inhibition test) (static)	–	14-day EC_{50}	9	
(photosynthesis reduction)	–	3-h EC_{50}	5	
Algae				
Scenedesmus quadricauda				
(cell multiplication inhibition test) (static)	pH neutral	8-day MIC	1630	Bringmann & Kuehn (1977)
(photosynthesis reduction)	–	3-h EC_{50}	75	Stratton & Corke (1982)
Chlorella pyrenoides (photosynthesis reduction)	–	3-h EC_{50}	60	Stratton & Corke (1982)
Protozoa				
Uronema parduczi (cell multiplication inhibition test)	pH 6.9	20-h MIC	31	Bringmann & Kuehn (1980)
Tetrahymena pyriformis (cell multiplication inhibition test)	–	2-day EC_{50}	252	Schultz et al. (1996)
Invertebrata: Mollusca				
Teredo digensis (marine) (static)	larvae	72-h LC_{50}	100	Vind & Hochman (1960)
Invertebrata: Crustacea				
Daphnia magna (immobilization)	pH neutral	24-h EC_{50}	500	Bringmann & Kuehn (1982)
	pH acid	24-h EC_{50}	102	
Vertebrata: Fish				
Leuciscus idus (lethality, DEV L15)	pH 7–8	48-h LC_{50}	460	Juhnke & Luedemann (1978)
Vertebrata: Amphibia				
Xenopus laevis	embryos			Dawson et al. (1996)
(lethality)	pH 7.2–7.4	96-h LC_{50}	1291	
(malformation)		96-h EC_{50}	433	

[a] MIC = minimum inhibitory concentration.

groups — bacteria, cyanobacteria, green algae, protozoa, invertebrates, and vertebrates — with benzoic acid have been compiled. From the aquatic organisms tested so far, cyanobacteria (*Anabaena inaequalis*) proved to be most sensitive, showing a 14-day EC_{50} of 9 mg/litre in the cell multiplication inhibition test (Stratton & Corke, 1982). EC_{50}/LC_{50} values (24–96 h) for most of the other aquatic species tested (protozoa, molluscs, crustaceans, fish, amphibians) were in the range of 100–1291 mg/litre. 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 (Bringmann & Kuehn, 1980). Developmental toxicity effects seen in frog (*Xenopus*) embryos were craniofacial defects, especially microcephaly, and abnormal gut coiling (Dawson et al., 1996). A recently developed cytotoxicity assay with cultured fathead minnow (*Pimephales promelas*) cells resulted in a PI_{50} (the concentration required to induce a 50% reduction in total protein content) of 1450 mg/litre (Dierickx, 1998).

Ninety-six-hour LC_{50} values of >100 mg sodium benzoate/litre have been found for *Daphnia magna* (first and second larval instar) and *Gammarus fasciatus* (juvenile: 7 mg in size) under static test conditions (multispecies test; pH 6.5–8; 20 °C) (Ewell et al., 1986). The same was true for juveniles of other invertebrates tested simultaneously: *Asellus intermedius* (Arthropoda; 12 mg body weight), *Dugesia tigrina* (Platyhelminthes; 6 mg body weight), *Helisoma trivolvis* (Mollusca; 180 mg body weight), and *Lumbriculus variegatus* (Annelida; 6 mg body weight) (Ewell et al., 1986).

Two different tests with the freshwater fathead minnow (*P. promelas*; juvenile stages) resulted in 96-h LC_{50} values of 484 mg sodium benzoate/litre (measured concentration; flow-through system; pH 7.4; 24 °C) (Geiger et al., 1985) and >100 mg/litre (nominal concentration; static system; pH 6.5–8.5; 20 °C) (Ewell et al., 1986).

10.2 Terrestrial environment

It is undissociated benzoic acid that is responsible for its antimicrobial activity. As benzoic acid itself is only slightly soluble in water, sodium benzoate — which, under acidic conditions, converts to undissociated benzoic acid — is often used instead. Their antimicrobial properties are used for different applications, such as food preservation (Chipley, 1983; see section 4), optimally under acidic conditions.

Minimum microbiocidal concentrations ranged from 20 to 1200 mg benzoic acid/litre in suspension tests (pH 6) with different bacterial or fungal species (Wallhäusser, 1984; Russell & Furr, 1996). Minimum inhibitory concentrations (serial dilution technique) were in the range of 50–1000 mg/litre (Wallhäusser, 1984; Russell & Furr, 1996).

The pH dependence of benzoic acid's antimicrobial activity is shown in several studies. Growth inhibition of the fungus *Fusarium oxysporum* (related to dry weight) measured 5 days after incubation with 610 mg benzoic acid/litre was 23.7% at pH 7.2 and 83.5% at pH 4 (Soni & Bhatia, 1980). There was no visible growth of yeast (*Saccharomyces cerevisiae*, *Willia anomala*) or mould (*Penicillium glaucum*) fungi at sodium benzoate concentrations of 120–600 mg/litre at pH 2.6, 1000–4000 mg/litre at pH 5, or 20 000–60 000 mg/litre at pH 7 (Schelhorn, 1951). Minimum inhibitory concentrations preventing growth of *Talaromyces flavus* on agar plates after 35 days of incubation were 100 mg/litre at pH 3.5 and >600 mg/litre at pH 5.4 (King & Halbrook, 1987).

The minimum inhibitory concentrations of benzoic acid on the growth of several species of yeasts ranged from 170 to 1250 mg/litre in cultures preadapted to benzoic acid and from 100 to 600 mg/litre in unadapted cultures (pH 3.5; 25 °C; 6 weeks of incubation) (Warth, 1988).

No information on the toxic effects of benzoic acid or sodium benzoate on plants, earthworms, or other terrestrial organisms or on ecosystems was identified.

11. EFFECTS EVALUATION

11.1 Evaluation of health effects

11.1.1 Hazard identification and dose–response assessment

After oral ingestion of benzoic acid and sodium benzoate in experimental animals or humans, there is rapid absorption of the undissociated benzoic acid from the gastrointestinal tract. The substances are metabolized in the liver mainly by conjugation with glycine, resulting in the formation of hippuric acid, which is rapidly excreted via the urine. Benzoates applied dermally can penetrate through the skin. Owing to their rapid metabolism and excretion, an accumulation of the benzoates or their metabolites is not to be expected.

With oral LD_{50} values of >1940 mg/kg body weight, the acute toxicity of benzoic acid and sodium benzoate in rodents is low.

Benzoic acid is slightly irritating to the skin and irritating to the eye, whereas sodium benzoate is not irritating to the skin and is only a slight eye irritant. Benzoic acid was not skin sensitizing in several animal models. For sodium benzoate, no data were identified covering this specific end-point.

Studies concerning short-term, subchronic, or chronic oral exposure conducted according to current guidelines are not available for benzoic acid or sodium benzoate. Effects on the central nervous system, weight gain (in several cases without reduced food intake), and liver and kidney were recorded at high concentrations of both compounds. As expected, and as far as it is possible to conclude with the limited database, toxic effects and effect levels seem to be similar for both compounds. A preliminary NO(A)EL of about 500 mg/kg body weight per day (the highest dose tested) may be derived based on a limited four-generation study (Kieckebusch & Lang, 1960; see section 8.4.2 and Table 4). This is supported by two short-term studies in which no adverse effects were observed at the highest tested dose levels of 647–825 mg/kg body weight per day (Kreis et al., 1967;

Bio-Fax, 1973) and by the fact that no serious side-effects have been reported after therapeutic use of sodium benzoate at a dose level of 250–500 mg/kg body weight per day in humans, although occasionally anorexia and vomiting were observed.

In a short-term inhalation study with rats exposed to benzoic acid (0, 25, 250, or 1200 mg dust aerosol/m^3; 6 h per day, 5 days per week, over 4 weeks), indications of fibrosis in the lung were seen even at the lowest concentration. The number of these microscopic lesions was higher in treated animals than in controls, but there was no clear dose dependency for this effect. Therefore, a no-observed-(adverse-)effect concentration (NO(A)EC) value cannot be derived. Long-term inhalation studies with benzoic acid or sodium benzoate were not identified.

Two long-term studies with rats (application of up to 1400 mg/kg body weight per day via diet over 18–24 months; quality of the study questionable) or mice (lifelong application of up to 6200 mg/kg body weight per day via drinking-water) gave no indication of a carcinogenic effect in either species. Studies on the precursors of benzoic acid — benzyl acetate, benzyl alcohol, and benzaldehyde — support the notion that it is unlikely that benzoic acid is carcinogenic.

In several *in vitro* tests on genotoxicity, benzoic acid and sodium benzoate tested negative. For sodium benzoate, in contrast to benzoic acid, consistently positive results were obtained in tests on sister chromatid exchange and chromosome aberrations without metabolic activation. *In vivo* studies for benzoic acid were not identified. For sodium benzoate, negative results were obtained *in vivo* in a cytogenetic assay with rats and a host-mediated assay with single or multiple oral application. However, a dominant lethal assay with rats gave a positive result. Therefore, a possible genotoxic activity of sodium benzoate cannot be ruled out entirely at present.

For benzoic acid, two limited studies gave no indication of adverse reproductive or developmental effects. With sodium benzoate, several studies on different species have been performed. Embryotoxic and fetotoxic effects as well as malformations were seen only at doses that induced severe maternal toxicity. In a dietary study in rats, a NO(A)EL of about 1310 mg/kg body weight per day was established. Studies on the precursors of benzoic acid support the notion that benzoic acid is unlikely to have adverse reproductive effects at dose levels not toxic to the mother.

The acute toxicity of benzoic acid and sodium benzoate in humans is low. However, both substances are known to cause contact dermatitis (pseudoallergy). In patients with urticaria or asthma, an exacerbation of the symptoms was observed after testing (oral provocation test or patch tests), whereas this effect is unusual in healthy subjects.

11.1.2 Criteria for setting tolerable intakes or guidance values for benzoic acid and sodium benzoate

As given in section 11.1.1, the database is insufficient for deriving NO(A)EL values for oral uptake. If the provisional NO(A)EL of about 500 mg/kg body weight per day is applied, and by incorporating an uncertainty factor of 100 (10 for uncertainty of the database, 10 for interspecies variation), a provisional tolerable intake would be 5 mg/kg body weight per day.

Applying this tolerable intake, one has to keep in mind that benzoates at lower doses can cause non-immunological contact reactions (pseudoallergy) in sensitive persons.

There are also no studies available concerning longer-term exposure by inhalation, and the only short-term inhalation toxicity study is not adequate for confidently establishing a NO(A)EC. Therefore, a tolerable concentration for exposure by inhalation cannot be calculated.

11.1.3 Sample risk characterization

As given in section 6.2, workers may be exposed to benzoic acid or sodium benzoate via inhalation or skin contact during production and processing. However, owing to the lack of information on specific working operations and conditions (e.g., duration of exposure) involved, it is impossible to derive a realistic estimate of occupational exposure.

For the general population, the main route of exposure to benzoic acid and sodium benzoate is likely via foodstuffs, which contain the substances naturally or added as antimicrobial agents. As given in section 6.2, the uptake depends on the individual's choice of food to be consumed and the limit values for benzoates in different countries. Therefore, considerable deviations may occur. Recent intake estimations from surveys from several countries gave mean values in the range of 0.18–2.3 mg/kg body weight. Only in high consumers was an intake of up to 14 mg/kg body weight calculated. Benzoates have not been detected in drinking-water. As given in section 6.1, the inhalative uptake via ambient or indoor air may contribute only marginally to exposure of the general population.

For normal consumers, the uptake of benzoates is about 2–28 times lower than the provisional tolerable intake of 5 mg/kg body weight day, and only in high consumers would this value be exceeded 3 times.

Additional information is required in order to evaluate whether sodium benzoate has a possible genotoxic activity.

11.2 Evaluation of environmental effects

Significant releases of benzoic acid and sodium benzoate into the environment are primarily into water and soil from their uses as preservatives in food, mouthwashes, dentifrices, and cosmetics. Benzoic acid occurs naturally in many plants.

From their physical/chemical properties, benzoic acid and sodium benzoate are not expected to volatilize from water and soil to the atmosphere or to adsorb to sediment or soil particles. The main elimination pathway for both chemicals should be biotic mineralization. Because of their ready biodegradability and their low volatility, both substances are not considered to contribute directly to the depletion of the stratospheric ozone layer or to global warming. From experimental data on bioconcentration, a low to moderate potential for bioaccumulation is to be expected.

Benzoic acid and sodium benzoate exhibited low to moderate toxicity to aquatic organisms. The lowest reported EC_{50} value of 9 mg/litre was determined in a chronic study (14 days) for cell multiplication inhibition by benzoic acid in the cyanobacterium *Anabaena inaequalis*. EC_{50}/LC_{50} values for the other aquatic species tested were in the range of 17–1291 mg/litre. Exposure levels of benzoic acid and benzoate in water have been determined only in rain and snow, groundwater, and leachate in the vicinity of point sources. Thus, 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 few available data from the antimicrobial action of benzoic acid and sodium benzoate indicate only a low toxicity potential of both substances in the terrestrial compartment. Due to the lack of measured exposure levels, a sample risk characterization with respect to terrestrial organisms could not be performed.

12. PREVIOUS EVALUATIONS BY INTERNATIONAL BODIES

JECFA (WHO, 1996) has allocated an acceptable daily intake (ADI) for benzoic acid and sodium benzoate of 0–5 mg/kg body weight.

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APPENDIX 1 — SOURCE DOCUMENTS

US FDA (1972a) *GRAS (Generally Recognized As Safe) food ingredients: benzoic acid and sodium benzoate*. Washington, DC, US Food and Drug Administration

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For the BUA review process, the company that is in charge of writing the report (usually the largest producer in Germany) prepares a draft report using literature from an extensive literature search as well as internal company studies. This draft is subject to a peer review in several readings of a working group consisting of representatives from government agencies, the scientific community, and industry.

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The first draft on benzyl acetate, benzyl alcohol, benzaldehyde, and benzoic acid and its salts was prepared by E. Vavasour, Chemical Health Hazard Assessment Division, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Health Canada, Ottawa, Ontario. The meeting of the Joint FAO/WHO Expert Committee on Food Additives was held from 6 to 15 February 1996 in Geneva.

APPENDIX 2 — CICAD PEER REVIEW

The draft CICAD on benzoic acid and sodium benzoate was sent for review to institutions and organizations identified by IPCS after contact with IPCS National Contact Points and Participating Institutions, as well as to identified experts. Comments were received from:

A. Aitio, International Programme on Chemical Safety, World Health Organization, Switzerland

M. Baril, Institut de Recherche en Santé et en Sécurité du Travail du Québec (IRSST), Canada

R. Benson, Drinking Water Program, US Environmental Protection Agency, USA

W.F. ten Berge, WXS, Netherlands

R. Cary, Health and Safety Executive, United Kingdom

R.S. Chhabra, National Institute for Environmental and Health Sciences/National Institutes of Health (NIEHS/NIH), USA

S. Dobson, Institute of Terrestrial Ecology, United Kingdom

P. Edwards, Department of Health, United Kingdom

R. Hertel, Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV), Germany

C. Hiremath, US Environmental Protection Agency, USA

P. Schulte, National Institute for Occupational Safety and Health, USA

D. Willcocks, National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Australia

P. Yao, Chinese Academy of Preventive Medicine, People's Republic of China

K. Ziegler-Skylakakis, Beratergremium für Umweltrelevante Altstoffe (BUA), Germany

APPENDIX 3 — CICAD FINAL REVIEW BOARD

Sydney, Australia, 21–24 November 1999

Members

Dr R. Benson, Drinking Water Program, US Environmental Protection Agency, Region VIII, Denver, CO, USA

Dr T. Berzins, National Chemicals Inspectorate (KEMI), Solna, Sweden

Dr R.M. Bruce, National Center for Environmental Assessment, US Environmental Protection Agency, Cincinnati, OH, USA

Mr R. Cary, Health and Safety Executive, Merseyside, United Kingdom

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Dr H. Gibb, National Center for Environmental Assessment, US Environmental Protection Agency, Washington, DC, USA

Dr R.F. Hertel, Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany

Dr J. Kielhorn, Fraunhofer Institute for Toxicology and Aerosol Research, Hanover, Germany

Dr S. Kristensen, National Occupational Health and Safety Commission (Worksafe), Sydney, NSW, Australia

Mr C. Lee-Steere, Environment Australia, Canberra, ACT, Australia

Ms M. Meek, Environmental Health Directorate, Health Canada, Ottawa, Ontario, Canada

Ms F. Rice, National Institute for Occupational Safety and Health, Cincinnati, OH, USA

Dr J. Sekizawa, National Institute of Health Sciences, Tokyo, Japan

Dr D. Willcocks, National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Sydney, NSW, Australia (*Chairperson*)

Professor P. Yao, Institute of Occupational Medicine, Chinese Academy of Preventive Medicine, Beijing, People's Republic of China

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BENZOIC ACID

0103
October 1999

CAS No: 65-85-0
RTECS No: DG0875000

Benzenecarboxylic acid
Phenyl carboxylic acid
$C_7H_6O_2$ / C_6H_5COOH
Molecular mass: 122.1

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 particles form explosive mixtures in air.	Prevent deposition of dust; closed system, dust explosion-proof electrical equipment and lighting.	In case of fire: keep drums, 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.

SPILLAGE DISPOSAL	PACKAGING & LABELLING
Sweep spilled substance into plastic containers; if appropriate, moisten first to prevent dusting. Use face shield and extra personal protection: protective clothing. Wash away remainder with plenty of water.	

EMERGENCY RESPONSE	STORAGE
NFPA Code: H 2; F 1; R -	

IPCS
International Programme on Chemical Safety

Prepared in the context of cooperation between the International Programme on Chemical Safety and the European Commission

SEE IMPORTANT INFORMATION ON THE BACK.

IMPORTANT DATA

Physical State; Appearance
WHITE CRYSTALS OR POWDER.

Physical dangers
Dust explosion possible if in powder or granular form, mixed with air.

Chemical dangers
The solution in water is a weak acid. Reacts with oxidants.

Occupational exposure limits
TLV not established.

Routes of exposure
The substance can be absorbed into the body by inhalation and by ingestion.

Inhalation risk
No indication can be given about the rate in which a harmful concentration in the air is reached on evaporation of this substance at 20°C.

Effects of short-term exposure
The substance irritates the eyes, the skin and the respiratory tract. The substance may cause a non-allergic rash on contact.

PHYSICAL PROPERTIES

Boiling point: 249°C
Melting point: 122°C (see Notes)
Density: 1.3 g/cm^3
Solubility in water, g/100 ml at 20°C: 0.29
Vapour pressure, Pa at 25°C: 0.1

Relative vapour density (air = 1): 4.2
Relative density of the vapour/air-mixture at 20°C (air = 1): 1
Flash point: 121°C c.c.
Auto-ignition temperature: 570°C
Octanol/water partition coefficient as log Pow: 1.87

ENVIRONMENTAL DATA

NOTES

The substance begins to sublime at 100°C.

ADDITIONAL INFORMATION

RÉSUMÉ D'ORIENTATION

Ce CICAD consacré à l'acide benzoïque et au benzoate de sodium a été préparé par l'Institut Fraunhofer de Toxicologie et d'étude des Aérosols de Hanovre (Allemagne). Ces deux composés sont examinés ensemble car c'est l'acide benzoïque non dissocié qui est responsable de l'activité anti-infectieuse de ces produits. Comme l'acide benzoïque lui-même n'est que peu soluble dans l'eau, c'est le benzoate de sodium – qui en milieu acide redonne l'acide non dissocié – que l'on utilise à sa place.

Ce CICAD est basé sur des études bibliographiques effectuées par le Comité consultatif allemand sur les produits chimiques qui posent des problèmes écologiques (BUA, 1995), la Food and Drug Administration des États-Unis (US FDA, 1972a) et le Comité mixte FAO/OMS d'experts des Additifs alimentaires (JECFA) (WHO/OMS, 1996) dans le but d'évaluer les effets possibles de l'acide benzoïque et du benzoate de sodium sur l'environnement et sur l'organisme humain. Une étude bibliographique exhaustive sur les bases de données appropriées a été effectuée en septembre 1999 afin de rechercher toute référence intéressante qui aurait été publiée postérieurement à celles qui figurent dans ces différents rapports. On trouvera à l'appendice 1 des indications sur la préparation de l'examen par des pairs et sur les sources documentaires utilisées pour cet examen. Les renseignements concernant l'examen du présent CICAD font l'objet de l'appendice 2. Ce CICAD a été approuvé en tant qu'évaluation internationale lors de la réunion du Comité d'évaluation finale qui s'est tenue à Sydney (Australie) du 21 au 24 novembre 1999. La liste des participants à cette réunion figure à l'appendice 3. La fiche d'information internationale sur la sécurité chimique (ICSC 0103) relative à l'acide benzoïque, établie par le Programme international sur la sécurité chimique (IPCS, 1993) est également reproduite dans ce document (appendice 4).

Chez les animaux de laboratoire et chez l'Homme, l'acétate de benzyle, l'alcool benzylique, son produit d'hydrolyse, et le produit qui en résulte par oxydation, c'est-à-dire le benzaldéhyde, sont largement métabolisés en acide benzoïque. On a donc utilisé les données toxicologiques relatives à ces précurseurs dans l'évaluation des effets sanitaires potentiels de l'acide benzoïque.

L'acide benzoïque (No CAS 65-85-0) se présente sous la forme d'un solide blanc légèrement soluble dans l'eau. La solubilité dans l'eau du benzoate de sodium (No CAS 532-32-1) est environ 200 fois plus élevée. On utilise l'acide benzoïque comme intermédiaire dans la synthèse de divers composés, principalement le phénol (plus de 50 % de la production mondiale) et la caprolactame. Il sert également à la préparation de divers sels, dont le sel de sodium, du chlorure de benzoyle ainsi que de plastifiants comme les dibenzoates de diéthylène- et de dipropylène-glycol. Le benzoate de sodium est principalement utilisé comme conservateur et inhibiteur de corrosion (par ex. comme additif à l'antigel des moteurs à explosion). L'acide benzoïque et le benzoate de sodium sont employés comme conservateurs dans l'alimentation et ils conviennent particulièrement bien pour les produits comme les jus de fruits et les boissons non alcoolisées, qui ont un pH acide. Leur utilisation comme conservateurs dans les denrées alimentaires, les boissons, les pâtes dentifrices, les bains de bouche, les cosmétiques et les produits pharmaceutiques est réglementée. On estime que la capacité mondiale de production d'acide benzoïque est d'environ 600 000 tonnes par an. On estime en outre qu'en 1997 la production mondiale de benzoate de sodium a été comprise entre 55 000 et 60 000 tonnes. L'acide benzoïque est naturellement présent dans beaucoup de végétaux et chez un grand nombre d'animaux. C'est donc un constituant normal de nombreux aliments, notamment du lait et des produits laitiers. Les rejets d'acide benzoïque et de benzoate de sodium dans l'environnement qui sont imputables aux activités humaines aboutissent essentiellement dans les eaux et dans le sol et proviennent de leur utilisation comme conservateurs. On constate que dans un certain nombre de denrées alimentaires, la concentration de l'acide benzoïque d'origine naturelle ne dépasse pas 40 mg/kg en moyenne. Les concentrations maximales d'acide benzoïque ou de benzoate de sodium relevées dans des produits alimentaires auxquels ils avaient été ajoutés comme conservateurs sont de l'ordre de 2000 mg/kg.

Après ingestion, l'acide benzoïque et le benzoate de sodium sont rapidement résorbés dans les voies digestives et métabolisés dans le foie par conjugaison avec la glycine pour donner de l'acide hippurique, rapidement excrété dans les urines. Les benzoates appliqués sur la peau sont résorbés dans une moindre proportion par voie transcutanée. En raison de la rapidité du métabolisme et de l'excrétion, il n'y a vraisemblablement pas d'accumulation des benzoates ou de leurs métabolites.

Chez les rongeurs, la toxicité aiguë par voie orale de l'acide benzoïque et du benzoate de sodium est faible (la DL_{50} par voie orale est supérieure à 1940 mg/kg de poids corporel). Chez le chat, qui semble plus sensible que les rongeurs, on a signalé des effets toxiques et une mortalité à des doses beaucoup plus faibles (environ 450 mg/kg p.c.).

L'acide benzoïque est légèrement irritant pour la peau et irritant pour la muqueuse oculaire, tandis que le benzoate de sodium n'irrite pas la peau est n'est que

légèrement irritant pour l'oeil. En ce qui concerne l'acide benzoïque, les données disponibles n'indiquent aucun effet sensibilisateur; dans le cas du benzoate de sodium, aucune donnée n'a été relevée dans la littérature à ce sujet.

Les études à court terme effectuées sur des rats ont révélé la présence de troubles du système nerveux central (acide benzoïque / benzoate de sodium) ainsi que des anomalies histopathologiques dans l'encéphale (acide benzoïque) après administration de doses élevées dans l'alimentation ($1800 mg/kg p.c.) pendant 5 à 10 jours. Les autres effets constatés étaient les suivants : réduction du gain de poids, modification du poids des organes, modification des paramètres sériques ou encore anomalies histopathologiques au niveau du foie. On ne dispose que de données très limitées sur l'exposition de longue durée des animaux de laboratoire à l'acide benzoïque par voie orale et il n'existe pas d'étude qui soit spécialement consacrée à la recherche d'effets cancérogènes éventuels. Une étude limitée, portant sur quatre générations, n'a permis d'obtenir qu'une estimation préliminaire de la dose sans effet (nocif) observable (NO(A)EL), estimation qui est d'environ 500 mg/kg p.c. par jour. En ce qui concerne le benzoate de sodium, les deux études à long terme effectuées sur des rats et des souris n'ont pas mis en évidence d'effet cancérogène. Il faut dire cependant que dans la plupart de ces études, les effets ne sont pas parfaitement attestés, d'où l'impossibilité d'en tirer des valeurs fiables pour la dose sans effet observable et la dose sans effet nocif observable. Les données concernant leurs divers précurseurs corroborent l'hypothèse selon laquelle l'acide benzoïque ne serait pas vraisemblablement pas cancérogène.

L'acide benzoïque a donné des résultats négatifs dans un certain nombre de tests sur bactéries ou cellules mammaliennes, mais on n'a pas trouvé de comptes rendus de tests *in vivo*. Le benzoate de sodium s'est également révélé inactif dans le test d'Ames mais il a donné des résultats systématiquement positifs sur cellules mammaliennes. Dans une étude *in vivo* (test de létalité dominante chez le rat) on a également obtenu un résultat positif. Dans ces conditions, on ne peut pour l'instant exclure que le benzoate de sodium ait une activité génotoxique.

Dans le cas de l'acide benzoïque, on dispose de deux études limitées qui n'indiquent aucun effet indésirable sur la reproduction ou le développement. En ce qui concerne le benzoate de sodium, plusieurs études ont été menées sur un certain nombre d'espèces et si des effets embryotoxiques et foetotoxiques ou même des malformations ont été observés, c'est uniquement à des doses déjà toxiques pour les mères. Une étude d'alimentation sur des rats a permis de fixer à environ 1310 mg/kg p.c. la dose sans effet (nocif) observable. Les données concernant ses divers précurseurs corroborent l'hypothèse selon laquelle l'acide benzoïque n'a vraisemblablement pas d'effets indésirables sur la reproduction aux doses qui ne sont pas toxiques pour la mère.

Chez l'Homme, l'acide benzoïque et le benzoate de sodium sont peu toxiques. On sait cependant que ces deux composés peuvent produire des réactions de contact non immunologiques (pseudoallergie). Cet effet est rare chez les sujets en bonne santé. En revanche, chez les personnes qui souffrent fréquemment d'asthme ou d'urticaire, on a observé une exacerbation des symptômes. On peut établir une dose journalière tolérable provisoire par ingestion de 5 mg/kg de poids corporel, encore que les benzoates soient susceptibles de provoquer chez les sujets sensibles des pseudoallergies de contact à des doses plus faibles. Comme on ne dispose pas d'étude appropriée sur l'exposition par inhalation, on ne peut déterminer la concentration tolérable en cas d'exposition par cette voie.

Compte tenu de leurs propriétés physiques et chimiques, il est exclu que l'acide benzoïque et le benzoate de sodium rejetés dans le sol ou dans l'eau soient à même de s'évaporer dans l'atmosphère ou de s'adsorber aux sédiments ou aux particules du sol. De nombreuses expériences on permis de constater que la principale voie d'élimination de ces deux composés était la minéralisation biologique. Des essais en laboratoire ont montré qu'en aérobiose, les deux composés sont facilement biodégradables. Un certain nombre de microorganismes isolés (bactéries, champignons) se sont révélés capable d'utiliser l'acide benzoïque en aérobiose comme en anaérobiose. Les données fournies par les expériences de bioconcentration montrent que ces produits ont un potentiel de bioaccumulation faible à modéré.

À en juger d'après les tests effectués sur des organismes aquatiques dans de bonnes conditions de validité, l'acide benzoïque et le benzoate de sodium se révèlent peu à modérément toxiques dans ce milieu. La valeur la plus faible de la CE_{50} qui ait été mesurée, soit 9 mg/litre (critère : inhibition de la multiplication cellulaire), a été obtenue dans une étude de toxicité chronique sur une corynébactérie, *Anabaena inaequalis*. Les valeurs de la CE_{50} et de la CL_{50} obtenues sur d'autres espèces aquatiques, se situaient aux alentours de 60-1291 mg/litre. On a montré que l'immobilisation de la daphnie dépendait du pH, la CE_{50} à 24 h étant plus basse (102 mg/litre) pour les pH acides. Dans le cas de l'ide rouge (*Leuciscus idus*), on a trouvé une CL_{50} à 48 h de 460 mg/litre. Des effets ont été constatés sur le développement des embryons de grenouille (*Xenopus*) à la concentration de 433 mg/litre (CE_{50} à 96 h; critère : malformation). Dans le cas du

benzoate de sodium, l'exposition des stades juvéniles d'organismes aquatiques appartenant à plusieurs espèces (*Daphnia magna*, *Gammarus fasciatus*, *Asellus intermedius*, *Dugesia tigrina*, *Helisoma trivolvis* et *Lumbriculus variegatus*) a permis de constater que la CL_{50} à 96 h était supérieure à 100 mg/litre. On a mesuré une CL_{50} à 96 h de 484 mg/litre pour le vairon à grosse tête (*Pimephales promelas*). Compte tenu du caractère limité des données concernant le niveau d'exposition dans l'eau, il n'a pas été possible de quantifier le risque couru par les organismes qui peuplent les eaux de surface. En s'appuyant sur la biodégradation rapide des composés, la valeur faible à modérée de leur potentiel de bioaccumulation, la faible toxicité qu'ils présentent pour la plupart des espèces aquatiques et leur métabolisation rapide, il apparaît que l'acide benzoïque et le benzoate de sodium ne représentent qu'un risque minimum pour les organismes aquatiques, mis à part le cas de déversements accidentels.

Les quelques données disponibles indiquent que l'acide benzoïque et le benzoate de sodium n'ont qu'un faible potentiel toxique dans l'environnement terrestre. À l'exception de l'action antimicrobienne de l'acide benzoïque, qui se caractérise par une concentration microbicide comprise entre 20 et 1200 mg/litre, on ne possède aucune donnée sur les effets toxiques de ce composé sur les organismes terrestres. Dans le cas du benzoate de sodium, il y a inhibition de la croissance bactérienne et fongique pour des concentrations comprises entre 100 et 60 000 mg/litre et cette inhibition dépend du pH. Faute de connaître la valeur des niveaux d'exposition, il n'a pas été possible de caractériser le risque pour les organismes terrestres.

RESUMEN DE ORIENTACIÓN

El presente CICAD sobre el ácido benzoico y el benzoato de sodio se preparó en el Instituto Fraunhofer de Toxicología y de Investigación sobre los Aerosoles de Hannover, Alemania. Se examinan los dos compuestos juntos porque es el ácido benzoico no disociado el responsable de su actividad antimicrobiana. Debido a que el propio ácido benzoico es sólo ligeramente soluble en agua, con frecuencia se utiliza en su lugar el benzoato de sodio, que en condiciones ácidas se convierte en ácido benzoico no disociado.

El presente CICAD se basa en exámenes compilados por el Comité Consultivo Alemán sobre las Sustancias Químicas Importantes para el Medio Ambiente (BUA, 1995), la Administración de Alimentos y Medicamentos de los Estados Unidos (US FDA, 1972a) y el Comité Mixto FAO/OMS de Expertos en Aditivos Alimentarios (JECFA) (WHO/OMS, 1996) para evaluar los efectos potenciales del ácido benzoico y el benzoato de sodio en el medio ambiente y en el ser humano. En septiembre de 1999 se realizó una búsqueda bibliográfica amplia de las bases de datos pertinentes para localizar cualquier referencia de interés publicada después de las incorporadas a estos informes. La información relativa a la preparación de los documentos originales y su examen colegiado figura en el apéndice 1. La información sobre el examen colegiado de este CICAD aparece en el apéndice 2. Este CICAD se aprobó como evaluación internacional en una reunión de la Junta de Evaluación Final celebrada en Sidney, Australia, los días 21-24 de noviembre de 1999. En el apéndice 3 figura la lista de los participantes en esta reunión. La Ficha internacional de seguridad química (ICSC 0103) para el ácido benzoico, preparada por el Programa Internacional de Seguridad de las Sustancias Químicas (IPCS, 1993), también se reproduce en el presente documento (apéndice 4).

El acetato de bencilo, su producto de hidrólisis, el alcohol de bencilo, y el producto de la oxidación de este alcohol, el benzaldehído, se metabolizan ampliamente a ácido benzoico en animales experimentales y en el ser humano. Por consiguiente, también se utilizaron datos toxicológicos sobre estos precursores en la evaluación de los efectos potenciales del ácido benzoico para la salud.

El ácido benzoico (CAS Nº 65-85-0) es una sustancia sólida blanca ligeramente soluble en agua. El benzoato de sodio (CAS Nº 532-32-1) es alrededor de 200 veces más soluble en agua. El ácido benzoico se utiliza como producto intermedio en la síntesis de distintos compuestos, fundamentalmente el fenol (>50 por ciento de la cantidad producida en todo el mundo) y la caprolactama. Otros productos finales son el sodio y otros benzoatos, el cloruro de benzoílo y los agentes plastificantes de dibenzoato de dietilenglicol y dipropilenglicol. El benzoato de sodio se utiliza sobre todo como conservante e inhibidor de la corrosión (por ejemplo, en sistemas técnicos como aditivo de los refrigerantes anticongelantes de los motores de automóviles). El ácido benzoico y el benzoato de sodio se utilizan como conservantes de los alimentos y son los más idóneos para los productos alimenticios, los jugos de frutas y las bebidas no alcohólicas, que por su naturaleza tienen un pH ácido. Su utilización como conservantes en alimentos, bebidas, pastas de dientes, colutorios, dentífricos, cosméticos y productos farmacéuticos está reglamentada. La capacidad de producción mundial estimada de ácido benzoico es de alrededor de 600 000 toneladas al año. La producción mundial de benzoato de sodio en 1997 puede estimarse en unas 55 000-60 000 toneladas. El ácido benzoico está presente de manera natural en muchas plantas y en los animales. Por consiguiente, es un elemento constitutivo natural de numerosos alimentos, entre ellos los productos lácteos. Las liberaciones antropogénicas de ácido benzoico y benzoato de sodio en el medio ambiente son primordialmente emisiones al agua y al suelo a partir de su uso como conservantes. Las concentraciones del ácido benzoico presente de manera natural en varios productos alimenticios no superaron el valor medio de 40 mg/kg de alimentos. Las concentraciones máximas notificadas de ácido benzoico o benzoato de sodio añadido a los productos alimenticios con fines de conservación fueron del orden de 2000 mg/kg de alimentos.

Tras la ingesta oral, el ácido benzoico y el benzoato de sodio se absorben con rapidez del tracto gastrointestinal y se metabolizan en el hígado por conjugación con la glicina, dando lugar a la formación de ácido hipúrico, que se excreta rápidamente a través de la orina. Los benzoatos aplicados por vía cutánea pueden penetrar en menor medida a través de la piel. Debido a la rapidez del metabolismo y de la excreción, no cabe prever una acumulación de benzoatos o sus metabolitos.

En roedores, la toxicidad oral aguda del ácido benzoico y el benzoato de sodio es baja (valores de la DL_{50} por vía oral >1940 mg/kg de peso corporal). En gatos, que parecen ser más sensibles que los roedores, se notificaron efectos tóxicos y mortalidad con dosis mucho menores (unos 450 mg/kg de peso corporal).

El ácido benzoico es ligeramente irritante de la piel e irritante de los ojos, mientras que el benzoato de sodio no irrita la piel y es sólo ligeramente irritante de los ojos. En cuanto al ácido benzoico, en los estudios disponibles no apareció ningún indicio de efecto sensibilizante; para el benzoato de sodio no se encontraron datos en la bibliografía.

En estudios de corta duración con ratas, se observaron trastornos del sistema nervioso central (ácido benzoico/benzoato de sodio), así como cambios histopatológicos en el cerebro (ácido benzoico) después de administrar dosis elevadas ($1800 mg/kg de peso corporal) durante 5-10 días. Otros efectos fueron una reducción del aumento del peso corporal, cambios en el peso de los órganos, cambios en los parámetros del suero o cambios histopatológicos en el hígado. La información relativa a la exposición oral prolongada de animales experimentales al ácido benzoico es muy limitada y no hay ningún estudio disponible que trate expresamente de los posibles efectos carcinogénicos. De un estudio limitado de cuatro generaciones sólo puede derivarse una concentración sin efectos (adversos) observados (NO(A)EL) de carácter preliminar de alrededor de 500 mg/kg de peso corporal al día. Con el benzoato de sodio, en dos estudios de larga duración con ratas y ratones no se obtuvo ningún indicio de efecto carcinogénico. Sin embargo, la documentación de los efectos es insuficiente en la mayoría de estos estudios; por consiguiente, no pueden derivarse valores de la NO(A)EL fidedignos. Los datos sobre sus precursores respaldan la idea de que probablemente el ácido benzoico no es carcinogénico.

El ácido benzoico dio resultados negativos en varias valoraciones bacterianas y en pruebas con células de mamífero, pero no se localizaron estudios *in vivo*. El benzoato de sodio también fue inactivo en pruebas Ames, mientras que las pruebas con células de mamífero dieron sistemáticamente resultados positivos. En un estudio *in vivo* (valoración letal dominante con ratas) se obtuvo un resultado positivo. En la actualidad no se puede excluir totalmente la actividad genotóxica del benzoato de sodio.

En cuanto al ácido benzoico, en dos estudios limitados no se obtuvieron indicios de efectos adversos reproductivos o en el desarrollo. Con el benzoato de sodio se han realizado varios estudios en distintas especies y solamente se observaron efectos embriotóxicos y fetotóxicos, así como malformaciones, con dosis que inducían una toxicidad materna grave. En un estudio de alimentación en ratas se estableció una NO(A)EL de alrededor de 1310 mg/kg de peso corporal. Los datos sobre sus precursores respaldan la idea de que no es probable que el ácido benzoico tenga efectos reproductivos adversos con los niveles de dosis que no son tóxicos para la madre.

En el ser humano, la toxicidad aguda del ácido benzoico y el benzoato de sodio es baja. Sin embargo, se sabe que ambas sustancias provocan reacciones de contacto no inmunológicas (pseudoalergia). Este efecto no es frecuente en personas sanas; en pacientes con ataques frecuentes de urticaria o asma se observaron síntomas o su exacerbación. Puede derivarse una ingesta tolerable provisional de 5 mg/kg de peso corporal al día, aunque dosis menores de benzoatos pueden producir reacciones de contacto no inmunológicas (pseudoalergia) en personas sensibles. Debido a que no hay estudios adecuados disponibles sobre la exposición por inhalación, no se puede calcular una concentración tolerable para este tipo de exposición.

Dadas sus propiedades físicas/químicas, no cabe prever que el ácido benzoico y el benzoato de sodio que pasan al agua y al suelo se volatilicen a la atmósfera o se adsorban sobre los sedimentos o las partículas del suelo. Según los resultados de numerosos experimentos de eliminación, la principal vía que siguen ambos productos químicos debe ser la mineralización biótica. Los datos obtenidos en pruebas de laboratorio pusieron de manifiesto una biodegradabilidad rápida de ambas sustancias en condiciones aerobias. Se ha comprobado que varios microorganismos aislados (bacterias, hongos) utilizaron el ácido benzoico en condiciones aerobias o anaerobias. De acuerdo con los datos experimentales sobre la bioconcentración, cabe prever un potencial de bajo a moderado para la bioacumulación.

Según los resultados de las pruebas válidas disponibles sobre la toxicidad del ácido benzoico y el benzoato de sodio para diversos organismos acuáticos, estos compuestos parecen mostrar una toxicidad de baja a moderada en el compartimento acuático. El valor más bajo de la CE_{50}, de 9 mg/litro (inhibición de la multiplicación celular), notificado en un estudio crónico se observó en la cianobacteria *Anabaena inaequalis*. Los valores de la CE_{50}/CL_{50} para otras especies acuáticas estudiadas fueron del orden de 60-1291 mg/litro. Se ha demostrado que la inmovilización de *Daphnia magna* es dependiente del pH, con una CE_{50} más baja en 24 horas (102 mg/litro) cuando el pH es ácido. Para un pez de agua dulce, el cacho (*Leuciscus idus*), se ha determinado una CL_{50} en 48 horas de 460 mg/litro. Se han encontrado efectos en el desarrollo en embriones de rana (*Xenopus*) con una concentración de 433 mg/litro (CE_{50} en 96 horas para la malformación). Para el benzoato de sodio, en una exposición de estadios juveniles de organismos acuáticos en una prueba multiespecífica (con inclusión de *Daphnia magna*, *Gammarus fasciatus*, *Asellus intermedius*, *Dugesia tigrina*, *Helisoma trivolvis*, y *Lumbriculus variegatus*) se obtuvieron valores de la CL_{50} en 96 horas superiores a 100 mg/litro. En el pez de agua dulce *Pimephales promelas* se ha determinado una CL_{50} en 96 horas de 484 mg/litro. Debido a los limitados datos disponibles sobre los niveles de exposición en agua, no se pudo realizar una caracterización cuantitativa del riesgo con respecto a los organismos acuáticos de aguas superficiales. Teniendo en cuenta la rápida biodegradabilidad, el potencial de bioacumulación entre moderado y bajo, la escasa toxicidad para la mayoría de las especies acuáticas y el rápido metabolismo de estas sustancias, el ácido benzoico y el benzoato de sodio -

con la excepción de vertidos accidentales - representan un riesgo sólo mínimo para los organismos acuáticos.

Los escasos datos disponibles indican que el ácido benzoico y el benzoato de sodio tienen solamente un potencial de toxicidad bajo en el medio terrestre. Salvo la acción antimicrobiana del ácido benzoico, que se caracteriza por concentraciones microbicidas mínimas que oscilan entre 20 y 1200 mg/litro, no se encontraron datos sobre los efectos tóxicos del ácido benzoico en los organismos terrestres. En cuanto al benzoato de sodio, inhibió el crecimiento bacteriano y fúngico de manera dependiente del pH en concentraciones que oscilaban entre 100 y 60 000 mg/litro. Debido a la falta de mediciones de los niveles de exposición, no se pudo realizar un muestreo de la caracterización del riesgo con respecto a los organismos terrestres.