The MAK Collection for Occupational Health and Safety

Benzoic acid and alkali benzoates

MAK Value Documentation, addendum – Translation of the German version from 2017

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Keywords: benzoic acid; alkali benzoates; MAK value; maximum workplace concentration; peak limitation; developmental toxicity; skin absorption; irritation; toxicity

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Benzoic acid[1),2)] and alkali benzoates[2)]

MAK Value Documentation

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated benzoic acid and alkali benzoates [65-85-0; 532-32-1; 582-25-2] to derive a maximum concentration at the workplace (MAK value), considering all toxicity endpoints. Available study reports and publications are described in detail.

Based on the NOAEC for lung toxicity of 12.6 mg/m³ in a 4-week inhalation study in rats, a MAK value of 0.5 mg/m³ for the strongly irritating benzoic acid has been derived. Alkali benzoates are only slightly irritating. In consideration of the systemic NOAEC of 250 mg/m³ in another 4-week inhalation study in rats exposed to benzoic acid, a MAK value for alkali benzoates of 10 mg/m³ (inhalable fraction), calculated as benzoate, has been derived. Benzoic acid and alkali benzoates are assigned to Peak Limitation Category II, because systemic effects are critical, and excursion factors of 2 for alkali benzoates and of 4 for benzoic acid are set.

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

Benzoic acid and alkali benzoates are not regarded as genotoxic. Long-term studies with sodium benzoate in rats and mice were of limited validity and do not point to a carcinogenic potential. Sensitization is not expected as benzoic acid and alkali benzoates are not contact sensitizers in animal studies. Immediate contact reactions observed in animals and humans are based on a non-immunologic mechanism. Skin contact is expected to contribute significantly to the systemic toxicity. Therefore, benzoic acid and alkali benzoates are designated with an "H".

Keywords

benzoic acid; benzenecarboxylic acid; phenylcarboxylic acid; sodium benzoate; potassium benzoate; mechanism of action; toxicokinetics; metabolism; (sub)acute toxicity; (sub)chronic toxicity; irritation; allergenic effects; reproductive toxicity; fertility; developmental toxicity; genotoxicity; carcinogenicity; peak limitation; prenatal toxicity; germ cell mutagenicity; absorption through the skin; sensitization; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

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1) The substance can occur simultaneously as vapour and aerosol.

2) causes pseudoallergic reactions, see Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten (21st issue 1995)

Benzoic acid[1),2)] and alkali benzoates[2)]

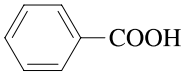
[65-85-0]
[532-32-1]
[582-25-2]

Supplement 2017

MAK value (2016)	
Benzoic acid	**0.1 ml/m³ (ppm) ≙ 0.5 mg/m³ R (respirable fraction)**
Alkali benzoates	**10 mg/m³ I (inhalable fraction, as benzoate)**
Peak limitation (2016)	
Benzoic acid	**Category II, excursion factor 4**
Alkali benzoates	**Category II, excursion factor 2**
Absorption through the skin (2016)	**H**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (2016)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	benzenecarboxylic acid phenylcarboxylic acid
Chemical name	benzoic acid

1) The substance can occur simultaneously as vapour and aerosol.

2) causes pseudoallergic reactions, see Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten (21st issue 1995)

CAS number	*65-85-0* (benzoic acid) *532-32-*1 (sodium benzoate) *582-25-*2 (potassium benzoate)
Structural formula	
Molecular formula	$C_7H_6O_2$ (benzoic acid) $C_7H_5O_2Na$ (sodium benzoate) $C_7H_5O_2K$ (potassium benzoate)
Molar mass	122.12 g/mol (benzoic acid) 144.11 g/mol (sodium benzoate) 160.21 g/mol (potassium benzoate)
Melting point	122.4 °C (benzoic acid) (OECD 2004) 330.6 °C (sodium benzoate) (OECD 2004) no data (potassium benzoate)
Boiling point at 1013 hPa	249.2 °C (benzoic acid) (OECD 2004) 464.9 °C (sodium benzoate) (OECD 2004) no data (potassium benzoate)
Vapour pressure at 25 °C	0.0009 hPa (benzoic acid) (SRC 2014 a) (vapour saturation concentration 4.4 mg/m³) < 0.001 hPa (sodium benzoate) (SRC 2014 b) no data (potassium benzoate)
log K_{OW}[3)]	1.87 (benzoic acid) (SRC 2014 a) –2.27 (calculated) (sodium benzoate) (SRC 2014 b) no data (potassium benzoate)
Solubility at 25 °C	3.4 g/l water (benzoic acid) (SRC 2014 a) 556 g/l water (sodium benzoate) (SRC 2014 b) no data (potassium benzoate)
pKs value	4.19 (benzoic acid) (OECD 2004)
1 ml/m³ (ppm) ≙ 5.067 mg/m³	**1 mg/m³ ≙ 0.197 ml/m³ (ppm)**
Stability	benzoic acid: anhydride formation at about 150 °C, decarboxylation at about 370 °C (ECHA 2011); sodium and potassium benzoate: no data

3) octanol/water partition coefficient.

Production	benzoic acid: almost exclusively by cobalt-catalyzed liquid-phase air oxidation of toluene (NLM 2016); sodium benzoate: neutralization of benzoic acid with sodiumbicarbonate, sodium carbonate or sodium hydroxide (CIR 2011); potassium benzoate: reaction of methylbenzoate with potassium thioacetate (CIR 2011)
Purity	benzoic acid: > 990 g/kg (pharmaceutical quality) (ECHA 2011); sodium and potassium benzoate: no data
Impurities	benzoic acid: according to the German pharmacopoeia (Deutsches Arzneibuch): cinnamic acid max. 0.1%, heavy metals max. 0.001%, ash max. 0.1%, organic and inorganic chlorides not detectable (ECHA 2011); sodium and potassium benzoate: no data
Uses	benzoic acid: production of phenol, sodium benzoate and for the synthesis of other substances (OECD 2004), used as a bactericide, fungicide and viricide, and for the disinfection of materials (ECHA 2011); sodium and potassium benzoate: as preservatives (OECD 2004)

For benzoic acid there is documentation available from 1986 (documentation "Benzoesäure" 1986, available in German only) and a supplement from 1995 (supplement "Benzoesäure" 1995, available in German only) on its allergenic effects. The present supplement is based on new data.

The alkali benzoates have been included in this supplement as in aqueous solution, depending on the pH, the benzoates are present in equilibrium with benzoic acid.

1 Toxic Effects and Mode of Action

Benzoic acid is strongly irritating to the eyes and causes irreversible damage to the cornea in some cases, whereas it is only mildly irritating to the skin. Sodium benzoate is, at most, slightly irritating to the eyes, and not irritating to the skin. Benzoic acid and its salts can induce immediate, non-immunological erythematous or urticarial reactions. Urticarial swelling occurs usually within 15 to 30 minutes after the exposure, and subsides as a rule after 2 to 3 hours. After oral administration of a total of 35 g within 20 days, benzoic acid caused symptoms such as nausea, headaches, weakness, burning and irritation in the oesophagus and digestive disorders in volunteers. Among other effects, reduced body weight gains, increased liver and

kidney weights, ataxia, tremor, excitation, aggressive behaviour, convulsions and mortality were observed in rats after repeated oral administration of benzoic acid or sodium benzoate doses of more than 2000 mg/kg body weight and day. Histopathological changes were diagnosed in the brain, the liver and the kidneys. In a 4-week inhalation study with rats, benzoic acid caused interstitial inflammation of the lungs with subsequent fibrosis at concentrations of 25 mg/m^3 and above.

In a study of the toxic effects on prenatal development in Wistar rats, foetotoxic effects, such as a reduced number of live foetuses, reduced foetal weights, reduced ossification and an increase in the incidences of skeletal, external and internal variations and malformations, were found, with simultaneous maternal toxicity, at sodium benzoate dose levels of about 1850 mg/kg body weight and day and above. In prenatal toxicity studies with mice, rabbits and hamsters, no developmental or maternal toxicity occurred up to the highest doses tested of 175 mg/kg body weight (mouse), 250 mg/kg body weight (rabbit) and 300 mg/kg body weight (hamster).

In vitro genotoxicity studies indicate benzoic acid and its salts have clastogenic effects at high doses, although in vivo studies yielded negative results for chromosomal aberrations in rats.

Long-term studies with rats and mice yielded no evidence of a carcinogenic effect of benzoic acid or sodium benzoate.

2 Mechanism of Action

The effect of benzoic acid on the eyes and mucosa is based on its acidity. The systemic toxicity of benzoic acid is similar to that of intoxication with salicylic acid (*o*-hydroxybenzoic acid). For both substances, a similar mechanism is assumed: the inhibition of mitochondrial respiration after saturation of the conjugation of glycine and glucuronic acid (ECHA 2011).

In isolated rat hepatocytes, benzoic acid (1 mM) causes the inhibition of urea synthesis by about 50% and leads to an 80% reduction in cellular aspartate concentrations. In isolated mitochondria, benzoic acid (1 mM) was shown to inhibit pyruvate carboxylase (Cyr and Tremblay 1989).

Benzoic acid and its salts can induce an immediate, non-immunological response or non-immunological contact urticaria, for which no specific IgE antibodies are detectable. These reactions, which are to be classified as irritant reactions, already occur on first contact, and therefore do not require any previous sensitization phase. The reactions are restricted as a rule to the area of contact, and demonstrate a clear dependency on the amount or concentration of the active substance (see also supplement "Benzoesäure" 1995, available in German only). Prostaglandins or histamine are possibly mediators for the reactions. As regards sensitivity to benzoic acid, there are marked differences between animal species (Lahti 1987; Lahti and Maibach 1985; Lahti et al. 1983, 1987).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

There are no data available for absorption following inhalation exposure.

After ingestion of benzoic acid or sodium benzoate, absorption is rapid and virtually complete in humans, rats, dogs and hamsters (ECHA 2011).

In humans, the peak plasma concentrations are reached within 1 to 2 hours after ingestion (ECHA 2011).

The rapid absorption of benzoic acid was confirmed in perfusion studies with rat colon. It was also shown that absorption is based on diffusion of the undissociated molecule and is dependent on the pH (ECHA 2011).

There are a few studies available for percutaneous absorption in various species such as the rat, rhesus monkey, dog, pig, guinea pig and humans as well as in vitro tests with human and rat skin. The individual studies are shown in Table 1. To summarize, percutaneous absorption in humans in vivo is about 40% in most studies (the individual results are in the range of 14% to 42.6%), and for human skin in vitro it is in the range of 30% to 70%.

In vitro studies with benzoic acid in aqueous solutions or ethanol/water solutions with human and rat skin indicate maximum flux rates of up to 10 to 20 μg/cm^2 and hour for low benzoic acid concentrations (for example 4 mg/ml) and of up to 166 ± 59 μg/cm^2 and hour for higher concentrations (40 mg/ml) (van de Sandt et al. 2004; Nielsen and Nielsen 2006; Nielsen et al. 2009; Nielsen and Sørensen 2012).

Similar flow rates can be estimated from an experimental in vivo study with humans, in which a larger amount of substance was applied: the percentage of benzoic acid absorbed after 24-hour non-occlusive exposure to 2000 μg/cm^2 was 13.6%. This yields an absorbed amount of 272 μg/cm^2 for the 24-hour observation period. Assuming for simplicity that the penetration rate is uniform, the average flux is 11 μg/cm^2 and hour (Wester and Maibach 1976). A limiting factor–and this applies also for the other studies with humans listed in Table 1–is, however, the fact that the course of dermal penetration over time was not investigated. Therefore, the substance fluxes can be given only in the form of averaged values over extended periods of time, and there is a degree of uncertainty, which possibly results in an underestimation of the fluxes.

The elimination of benzoic acid or sodium benzoate (see Section 3.2) with the urine in humans, rats, hamsters and dogs, is rapid and virtually complete (94%–100% over 24 hours; Bridges et al. 1970), especially in the form of metabolites. In other species such as ferrets and non-human primates on the other hand, it seems to be less effective. Faecal excretion and elimination with the exhaled air appear to be minor routes of elimination (ECHA 2011).

Because of the effective elimination in most of the species investigated, no accumulation is to be expected (ECHA 2011).

Table 1 Results of studies of the dermal absorption of benzoic acid

Species, strain, sex, number per group	Dose, area, exposure duration	Result	Remarks	References
in vivo				
humans, 6, no other data	4 µg/cm^2, 13 cm^2, 24 hours, non-occlusive	percutaneous absorption: 42.6% ± 16.5%, calculated from urinary elimination within 5 days; maximum absorption rate 3% of the dose per hour; residues on/in the skin: virtually none (after 24 hours)	^{14}C-labelled benzoic acid, dissolved in acetone	Feldmann and Maibach 1970; Hunziker et al. 1978
humans, ♂ and ♀, 7 (age: 22–40), 8 (age: 65–86)	4 µg/cm^2, 2.5 cm^2, 24 hours, non-occlusive	percutaneous absorption calculated from urinary elimination within 7 days: 36.2% ± 4.6% (age 22–40); 19.5% ± 1.6% (age 65–86); recovery on the surface (after 24 hours): 45.6% ± 2.8% (age 22–40); 61.4% ± 2.0% (age 65–86)	^{14}C-labelled benzoic acid, dissolved in acetone	Roskos et al. 1989
humans, 6–7 ♂	3, 400, 2000 µg/cm^2, 13 cm^2, 24 hours, non-occlusive	percutaneous absorption calculated from urinary elimination within 5 days: 37.0% ± 16.3%, 25.7% ± 9.9%, 14.4% ± 3.8%	^{14}C-labelled benzoic acid, dissolved in methanol	Wester and Maibach 1976
rat, Osborne-Mendel, ♀, no other data	4 µg/cm^2, 2 cm^2, 5 days, non-occlusive	percutaneous absorption calculated from urinary elimination within 5 days: 37.1%	^{14}C-labelled benzoic acid in petrolatum	Bronaugh et al. 1982
rat, Sprague Dawley, hairless, 12 ♀	200, 450 nmol/cm^2 (24.4; 55.0 µg/cm^2), 1 cm^2, 30 minutes, non-occlusive	percutaneous absorption (after 96 hours): 26.6% ± 0.7% or 79.3% ± 3.6%; amount in stratum corneum after 30 minutes: 17.6% ± 1.5% or 48.1% ± 5.1%	^{14}C-labelled benzoic acid, dissolved in ethanol/water (95/5)	Rougier et al. 1983

Table 1 (continued)

Species, strain, sex, number per group	Dose, area, exposure duration	Result	Remarks	References
dog, hairless Mexican, 2, no other data	4 µg/cm^2, area not specified, 24 hours, non-occlusive	percutaneous absorption calculated from urinary elimination within 10 days: maximum absorption rate: 0.25% per hour; residues on/in the skin: 30% (after 90 hours)	^{14}C-labelled benzoic acid, dissolved in acetone	Hunziker et al. 1978
Rhesus monkey, 3 ♀	40 (400)[a)], 2000 µg/cm^2, 6 cm^2, 24 hours, non-occlusive	percutaneous absorption calculated from urinary elimination within 5 days: 33.6% ± 5.1%, 17.4% ± 1.2%	^{14}C-labelled benzoic acid, dissolved in methanol	Wester and Maibach 1976
Rhesus monkey, 4 ♀	4 mg/cm^2, area not specified, 24 hours, non-occlusive, with rinsing after 24 hours	percutaneous absorption calculated from urinary elimination within 7 days: 66% ± 19%	^{14}C-labelled benzoic acid, dissolved in acetone	Bucks et al. 1990
Rhesus monkey, 4 ♀	4 µg/cm^2, area not specified, 14 × 24 hours, non-occlusive, without rinsing	percutaneous absorption calculated from urinary elimination within 7 days: 85% ± 19% (repeated application, 1st dose); 89% ± 19% (repeated application, 8th dose)	^{14}C-labelled benzoic acid, dissolved in acetone (only 1st and 8th application, substance otherwise not labelled)	Bucks et al. 1990
guinea pig, hairless, 3–5 ♀	4 µg/cm^2, 1 cm^2, 24 hours, non-occlusive	percutaneous absorption calculated from urinary elimination within 5 days compared with i.p. administration): 34.2% ± 9.4% (normal skin); 71.1% ± 19.8% (tape-stripped skin); 73.4% ± 14.6% (irritated skin, 2% SDS); 94.1% ± 4.8% (defatted skin)	^{14}C-labelled benzoic acid, dissolved in acetone	Moon et al. 1990

Table 1 (continued)

Species, strain, sex, number per group	Dose, area, exposure duration	Result	Remarks	References
guinea pig, Hartley, 3, no other data	4 µg/cm^2, 1.75 cm^2, 24 hours, non-occlusive	percutaneous absorption (calculated from urinary elimination within 5 days compared with i.p. administration): 31.4% ± 9.1%	^{14}C-labelled benzoic acid, dissolved in acetone	Andersen et al. 1980
pig, Yorkshire, 3 ♀	40 µg/cm^2, 5 cm^2, 6 days, non-occlusive	percutaneous absorption (calculated from urinary and faecal elimination within 6 days compared with i.v. administration): 25.7% ± 2.4%	^{14}C-labelled benzoic acid, dissolved in ethanol	Carver and Riveiere 1989
pig, Yorkshire, ♀, no other data	4 µg/cm^2, up to 32 cm^2, 24 hours, non-occlusive	percutaneous penetration: 9.2% (in urine); recovery: 78% ± 8%	^{14}C-labelled benzoic acid, dissolved in ethanol	Reifenrath et al. 1991
in vitro				
human skin	4 or 40 µg/cm^2, 1 or 2.5 cm^2, 2 days	percutaneous penetration: 44.9%	^{14}C-labelled benzoic acid, dissolved in acetone	Franz 1975
human skin	0.2, 0.3 µg/cm^2, 0.32 cm^2, 72 hours, occlusive	total absorption: 60.5% ± 1.8% or 65.5% ± 7.1% (72 hours); recovery: 85.7%–90.3%	^{14}C-labelled benzoic acid, dissolved in ethanol	Hotchkiss et al. 1992
human skin	0.2, 0.3 µg/cm^2, 0.32 cm^2, 72 hours, non-occlusive	total absorption: 30.9% ± 1.2% or 37.8% ± 6.9% (72 hours); recovery: 80.2%–84.5%	^{14}C-labelled benzoic acid, dissolved in ethanol	Hotchkiss et al. 1992

Table 1 (continued)

Species, strain, sex, number per group	Dose, area, exposure duration	Result	Remarks	References
human skin	100 µg/cm^2, 0.32–3.14 cm^2, 24 hours	mean maximum absorption rate: 16.54 ± 11.87 µg/cm^2/hour; total absorption: 70.6% ± 17.2% (24 hours, 8 laboratories); recovery: 53.6%–98.5% (7 laboratories)	benzoic acid (in some cases ^{14}C-labelled), dissolved in ethanol/water (1/1); multicentric study (9 laboratories), in accordance with OECD Test Guideline 428	van de Sandt et al. 2004
human skin	dose not specified, 2.12 cm^2, 48 hours	maximum flux: 12.8 ± 1.4 µg/cm^2/hour; recovery: 98.2%	^{14}C-labelled benzoic acid, dissolved in water (0.9% NaCl, 1% Tween)	Nielsen and Nielsen 2006
human skin	4 mg/ml (424 µg) 2.12 cm^2, 48 hours	maximum flux: 20 µg/cm^2/hour; recovery: 99.0% ± 0.6%	^{14}C-labelled benzoic acid, dissolved in water (0.9% NaCl, 2% ethanol)	Nielsen et al. 2009
human skin	4, 40 mg/ml (424, 4240 µg) 2.12 cm^2, 48 hours	maximum flux: 13.1 ± 1.8 or 166.4 ± 58.5 µg/cm^2/hour	^{14}C-labelled benzoic acid, dissolved in 45% ethanol in water	Nielsen and Sørensen 2012
rat skin, Osborne-Mendel, ♀	4.2 µg/cm^2, 1.1 cm^2, 5 days	percutaneous absorption: 49.1%, permeability constant: 3.5 × 10^{-4} cm/hour	^{14}C-labelled benzoic acid in petrolatum	Bronaugh et al. 1982
rat skin, F344, ♂	4.6 µg/cm^2, 2 cm^2, 6 hours	percutaneous penetration: 53.0% ± 4.6%; recovery: 73.9%	^{14}C-labelled benzoic acid, dissolved in acetone	Frantz et al. 1990

Table 1 (continued)

Species, strain, sex, number per group	Dose, area, exposure duration	Result	Remarks	References
rat skin, F344, ♂	0.2, 2.2 µg/cm^2, 0.32 cm^2, 72 hours, occlusive	maximum flux: 3.4 or 36.5 ng/cm^2/hour; total absorption: 51.8% ± 3.3% or 51.1% ± 6.6% (72 hours); recovery: 92%–108%	^{14}C-labelled benzoic acid, dissolved in ethanol	Hotchkiss et al. 1992
rat skin, F344, ♂	0.2, 2.2 µg/cm^2, 0.32 cm^2, 72 hours, non-occlusive	maximum flux: 3.4 or 36.5 ng/cm^2/hour; total absorption: 48.3% ± 1.2% or 53.3% ± 7.6% (72 hours); recovery: 69%–88.5%	^{14}C-labelled benzoic acid, dissolved in ethanol	Hotchkiss et al. 1992
rat skin, Sprague Dawley,	100 µg/cm^2, 1.76 cm^2, 24 hours	mean maximum absorption rate: 21.21 µg/cm^2/hour; total absorption: 89.8% ± 4.3% (24 hours); recovery: 98.5% ± 1.1%	^{14}C-labelled benzoic acid, dissolved in ethanol/water (1/1); in accordance with OECD Test Guideline 428	van de Sandt et al. 2004
mouse skin, HRS/J hairless, ♂	4.6 µg/cm^2, 2 cm^2, 6 hours	percutaneous penetration: 73.6%–75.5%; recovery: 78.6%–81.6%	^{14}C-labelled benzoic acid, dissolved in acetone	Frantz et al. 1990
guinea pig skin, hairless, ♀	2 µg/cm^2, rinsing after 24 hours, determined after a further 24 hours	percutaneous absorption: 49.5% ± 2.3% (isotonic buffer as receptor fluid), 60.1% ± 5.5% (water as receptor fluid)	^{14}C-labelled benzoic acid, dissolved in ethanol	Nathan et al. 1990
guinea pig skin, hairless, ♀	12, 40, 120 µg/cm^2, 0.64 cm^2, 24 hours	maximum percutaneous absorption: 1.5%, 2.25% or 5.5% after 3–6 hours	^{14}C-labelled benzoic acid, dissolved in acetone	MacPherson et al. 1996

Table 1 (continued)

Species, strain, sex, number per group	Dose, area, exposure duration	Result	Remarks	References
pig skin, Yorkshire, ♀	4 µg/cm^2, 0.8 cm^2, up to 24 hours	percutaneous penetration: 5% ± 2% (after 24 hours); recovery: 65%–83%	^{14}C-labelled benzoic acid, dissolved in ethanol	Reifenrath et al. 1991
Phenion® FT model (bioartificial human skin), Franz' diffusion cell	284.1 µg/cm^2, 0.358 cm^2, up to 26 hours	permeation (6 hours): 127.1 ± 6.14 µg/cm^2, 44.8% ± 2.16%, P_{app}: 6.93 ± 0.39 × 10^{-6} cm/s	^{14}C-labelled benzoic acid in phosphate-buffered saline solution	Ackermann et al. 2010

[a)] contradictory data in publication; P_{app}: apparent permeability coefficient (cm/s); SDS: sodium dodecylsulfate; FT: full thickness (skin)

3.2 Metabolism

In mammals, the main metabolites of benzoic acid are hippuric acid formed from conjugation with glycine, and benzoyl glucuronide resulting from glucuronidation (see Figure 1; ECHA 2011).

From the maximum elimination rate, it was calculated that the elimination capacity via glucuronate and glycine conjugation is about 20 g benzoic acid per day in humans (ECHA 2011).

The maximum rate of biotransformation of benzoic acid to hippuric acid in humans is on average 23.0 mg/kg body weight/hour which is close to the maximum daily dose of 500 mg/kg body weight (21 mg/kg body weight and hour) recommended for the treatment of hyperammonaemia (see Section 4.2) (ECHA 2011).

In humans, rabbits, rats and pigs, benzoic acid is eliminated almost entirely in the form of hippuric acid. In other species, larger amounts of benzoyl glucuronide are also eliminated, for example up to 38% in marmosets, 75% in dogs and 20% in ferrets (ECHA 2011).

In neonatal rats and rats with protein deficiency, the amount eliminated as hippuric acid was reduced, and about 20% of the radioactively labelled substance in the urine was identified as benzoyl glucuronide (ECHA 2011).

As cats are capable of glucuronidation to a limited extent only, they react in a particularly sensitive way at and above the concentration that leads to saturation of glycine conjugation to hippuric acid (ECHA 2011).

In humans, the most important organs in which the metabolism of benzoic acid takes place are the liver and the kidneys. Although the conjugation rate in the renal cortex is higher than in the liver, the liver is nevertheless considered to be the quantitatively most important organ for glycine conjugation due to its greater mass and central anatomical location (ECHA 2011).

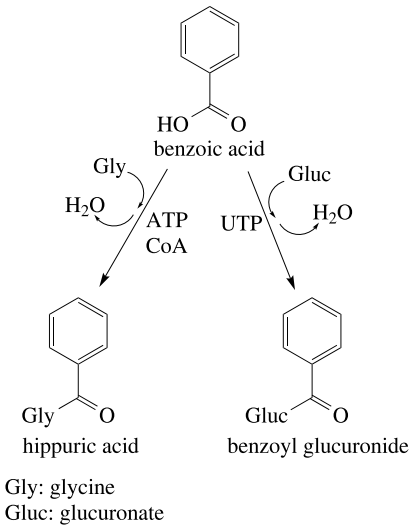


Figure 1 Metabolism of benzoic acid in rats and humans (according to ECHA 2011)

In vitro experiments suggest that after percutaneous uptake of benzoic acid a small amount is also transformed to hippuric acid in the skin (ECHA 2011).

4 Effects in Humans

4.1 Single exposures

Single oral doses of 1 to 1.5 g **benzoic acid** produced stomach disorders, nausea and vomiting (documentation "Benzoesäure" 1986, available in German only).

Single doses of 2000 to 3000 mg **sodium benzoate** resulted in nausea, headaches, weakness, and burning and irritation of the oesophagus (WHO 2000).

In a person weighing 60 kg, the ingestion of 33 g **sodium benzoate** caused pallor, a weak and irregular pulse, general indisposition, headaches and nausea. Similar effects were described also after the intake of 50 g sodium benzoate over a period of five hours (Nair 2001).

The induction of an asthmatic reaction after oral provocation with **sodium benzoate** has been described (for example by Petrus et al. 1996). Oral provocation with an undocumented amount of benzoic acid produced an asthmatic reaction, rhinitis or urticaria in 47 of 100 asthma patients (Rosenhall and Zetterström 1975; see also documentation "Benzoesäure" 1986, available in German only); 86 of these 100 patients reacted to acetyl salicylic acid.

4.2 Repeated exposure

After daily ingestion of 300 to 400 mg benzoic acid with the diet for up to 62 days, no changes in the blood picture, urine composition, nitrogen balance or in well-being were found in 6 men (WHO 2000).

In humans, daily oral doses of less than 500 mg benzoic acid did not produce any symptoms (documentation "Benzoesäure" 1986, available in German only).

An earlier study reports that the daily uptake of up to 1000 mg benzoic acid over a period of up to 92 days did not produce adverse effects (WHO 2000).

In a volunteer study, the participants received oral doses of benzoic acid of 1000 mg per day for 5 days. The dose was increased to 1500, 2000 and 2500 mg/day every 5 days. Three of the 12 participants received the complete dose of 35 g within 20 days. In these volunteers marked symptoms, such as nausea, headaches, weakness, burning and irritation in the oesophagus, and digestive disorders were observed (Nair 2001).

In 9 patients undergoing treatment with penicillin who were given a total dose of 12 000 mg benzoic acid (divided into 8 doses over 5 days in 8 subjects and 8 doses over 14 days in one subject), no adverse effects on blood urea nitrogen or creatinine clearance were reported (WHO 2000).

In humans, sodium benzoate is used as a therapeutic for hyperammonaemia (congenital disorder of the urea cycle metabolism) and reduces the ammonia by conjugating with glycine to form hippuric acid, which is eliminated (BUA 1993). Therapeutic doses are in the range of 250 to 500 mg/kg body weight and day, and only

rarely result in clinical signs of toxicity, which are mostly limited to anorexia and vomiting, especially after intravenous bolus administration (WHO 2000).

Skin reactions after ingestion

In the case of a patient with recurrent urticarial skin reactions over many years and suspected occupational and non-occupational exposure to benzoic acid, oral provocation with 500 mg sodium benzoate led to a severe reaction, which was evaluated by the authors as allergic anaphylactic shock (Pevny et al. 1981). Despite the extensive documentation, the actual cause of this reaction and the previous occupational exposure are not clear.

In addition to this, especially in persons with chronic or recurrent urticaria/prurigo and suspected intolerance to food additives, urticarial or pruriginous reactions after oral provocation with sodium benzoate have been reported (Asero 2006; Juhlin 1981; Malanin and Kalimo 1989; Michils et al. 1991; Nettis et al. 2004; Supramaniam and Warner 1986). After induction by oral provocation with sodium benzoate, the (re-)occurrence of nasal symptoms in patients with chronic or recurrent rhinitis as the result of a corresponding intolerance (Asero 2001, 2002; Pacor et al. 2004) has also been described.

Finally, a "fixed drug eruption" to sodium benzoate has also been reported. A female patient aged 64 years developed pruritis on her ring finger several days after a throat infection. Two days later there was infiltrated erythema on the finger, which exacerbated to a pustulous reaction after a further 4 days. During the following 2 years, similar episodes occurred repeatedly, which the patient attributed to the intake of a syrup containing sodium benzoate for the treatment of airway infections. In a patch test, the patient produced a 1+ reaction to 5% sodium benzoate in petrolatum after 2 and 4 days. Approximately 15 hours after oral provocation with 750 mg benzoic acid in 100 ml water, she reported skin changes on the ring finger and in the interdigital region. Two days later a blister had formed on the area of skin originally affected (Vilaplana and Romaguera 2003).

Recurrent symptoms of the oral mucosa, similar to those of erythema multiforme (for example crust formation or ulcers on the lips, tongue or oral mucosa), and of the skin (papulo-maculous changes on the hands and forearms) in 7 and 5 of 7 patients, respectively, were attributed to intolerance to benzoic acid as a food additive. During a benzoic acid and benzoate-free diet adhered to for between 6 and 30 months, 4 of the 7 patients were free of symptoms, but in the 3 patients who were not able to adhere to an exclusion diet one or two recurrent mucosal or skin changes occurred over 12 to 29 months. All 7 patients reacted in the patch tests to 5% benzoic acid in petrolatum (no data for time of reaction), 3 also reacted to nickel, 3 to cinnamon, 1 to local anaesthetics (Cain-Mix) and 1 to rubber (no other data). During patch testing, the oral symptoms (no other data) recurred in varying degrees of severity in some of them. Herpes simplex viruses or an increased antibody titre were not found in any of the patients and, according to the authors, there was no evidence that pharmaceuticals, ongoing infections or systemic diseases may have been the cause (Lewis et al. 1989).

4.3 Local effects on skin and mucous membranes

(Non-immunological) immediate reactions

Benzoic acid and its salts can induce non-immunological immediate erythematous or urticarial reactions. The clinical picture with oedema or infiltrates in the upper dermis occurs frequently in combination with itching or burning. Purely erythematous reactions are, however, also possible.

In 3 employees engaged in the dispensing or mixing of sodium benzoate, burning and itching or erythematous skin reactions in the area of contact, especially during phases of more pronounced sweating, occurred about 15 to 45 minutes after contact with sodium benzoate, with a latency period of months to several years. The skin changes were reversible between 15 to 30 minutes and a few hours after the end of the exposure. The workers were subjected to patch tests with 0.5% sodium benzoate in water (non-occlusive), 0.5% sodium benzoate in saline (20 minutes occlusive) and 10% sodium benzoate in petrolatum (non-occlusive) as well as with 0.25% benzoic acid in water (non-occlusive) and 5% benzoic acid in petrolatum (20 minutes occlusive). Only 1 of the 3 reacted to occlusive testing with 0.5% sodium benzoate, but all 3 to both benzoic acid preparations. Two of 3 control persons reacted to 0.5% sodium benzoate (occlusive) and the other to 10% sodium benzoate; all three reacted to 0.25% benzoic acid, but not to 5% benzoic acid (Nethercott et al. 1984).

Non-immunological immediate reactions of the skin to benzoic acid are more frequently reported in case reports of patients with non-occupational exposure. These reports involve, for example, patients with perioral urticarial reactions or cheilitis as a result of an assumed intolerance to sodium benzoate in toothpaste (Aguirre et al. 1993; Munoz et al. 1996) and children with perioral immediate reactions to salad dressing containing benzoic acid (Clemmensen and Hjorth 1982). Some of the cases, however, were also evaluated as late reactions to patch testing.

Urticarial reactions to benzoic acid occurred in a 20-minute or 30-minute patch test also in 14 of 40 children in one study (Rademaker and Forsyth 1989), and in a few of the patients tested extensively as a result of an intolerance to balsam of Peru (Forsbeck and Skog 1977), and could be suppressed by previous administration of an antihistaminic agent in two cases (Forsbeck and Skog 1977).

Studies of urticarial reactions in various collectives of volunteers or patients are summarized in Table 2.

4.4 Allergenic effects

Sensitizing effects on the skin

In the documentation from 1986 (documentation "Benzoesäure" 1986, available in German only), merely one case report was cited in which benzoic acid is suspected of causing an allergic reaction. Data from clinical epidemiological studies were not presented.

Table 2 Studies of urticarial reactions to benzoic acid in humans

Study method, application, duration	Collective, concentration, vehicle, applied amount	Reading, assessment scale, results	References
Test for non-immunological immediate reactions, occlusive (double blind, 11 mm Finn Chamber® (FC)), 20 minutes	45 ♂ and 155 ♀, 125 and 500 mM, petrolatum, 20 µl	reading after 30 minutes, scale for erythema and oedema: 0 and 1 (marginal erythema or oedema) to 8 (severe, diffuse erythema or severe oedema with blisters, occasionally diffuse), 125 mM: erythema in 147/200 (mean score 1.81), oedema in 25/200 (0.16); 500 mM: erythema in 157/200 (2.12), oedema in 36/200 (0.22)	Basketter and Wilhelm 1996
Chamber scarification test	5–10 volunteers, 7.5%, 15% and 30%, ethanol	30%: threshold concentration for irritation of intact skin, 15%: marked irritation with erosion of scarified skin, 7.5%: threshold concentration for irritation of scarified skin	Frosch and Kligman 1976
Chamber test, occlusive, 20 minutes	85 volunteers (36× atopy, 23× urticaria, 26× non-atopic patients) and 25 control persons with healthy skin, 5%, petrolatum	reactions in 10/36 atopic patients, 9/23 patients with urticaria, 14/26 non-atopic patients and 10/25 control persons (in total: 43/110, 39%)	Lahti 1980
Chamber test, occlusive (FC on Scanpor®), 20 minutes	23 volunteers with healthy skin (8 stingers, 15 non-stingers), 0.5% and 1%, isopropanol/water (1:1), 15 µl	reading after 10 minutes, scale for erythema: 0 and 1 to 5 (pronounced erythema with pustules or blisters), scale for oedema: 0 and 1 to 4 (clearly recognizable oedema), average score for erythema in stingers (stinging/burning of the skin on testing with 5% lactic acid in water) 1.5 or 2.4 and in non-stingers 0.7 or 0.9 (oedema: 0.3 or 0.5 and 0 or 0.1, respectively)	Lammintausta et al. 1988
Chamber test, occlusive, 10 minutes	12 volunteers 0.5% and 5%, isopropanol/water (1:1) or water, 10 µl	readings after 5 and 15 minutes, average score for formation of wheals 1.3 (0.5%) and 2.3 (5%); score of 0.5 for 5% sodium benzoate in isopropanol/water (1:1)	Gollhausen and Kligman 1985

Table 2 (continued)

Study method, application, duration	Collective, concentration, vehicle, applied amount	Reading, assessment scale, results	References
Chamber test, occlusive (AL test on Scanpor®, 1 cm^2), 45 minutes	7 patients (♂ and ♀) with birch pollen sensitization, 5%, petrolatum, 10 µl	in all 7 persons erythema, in 1/7 with infiltration	Wallengren and Larsson 2001
Chamber test, occlusive (FC on Scanpor®), 20 minutes	86 volunteers with healthy skin (♂ and ♀; 44 stingers, 42 non-stingers), 1 M, petrolatum	reading after 10 minutes, scale for erythema: 0 and + (marginal erythema) to 6+ (intensive, diffuse erythema), average score for erythema in stingers (stinging/burning on the skin on testing with 10% lactic acid in water) 3.1 and in non-stingers 2.4	Coverly et al. 1998
Chamber test, occlusive (FC on Scanpor®), 20 minutes	41 volunteers with healthy skin (♂ and ♀), 0.1%, 1%, 5% and 10%, petrolatum	reading after 20 minutes, 0.1%: erythema (flare) in 14/41, wheals in 0/41; 1% and 5%: erythema in 26/41, wheals in 2/41; 10%: erythema in 26/41, wheals in 3/41	Clemmensen and Hjorth 1982
Chamber test, occlusive (12 mm FC on tape), 20 minutes	58 healthy volunteers, 1 M, petrolatum, 50 µl	reading after 10 minutes and after 1, 2 and 4 hours, erythematous reaction in 39/58	Marriott et al. 2005
Open application test, non-occlusive	13 healthy volunteers, 4, 8 and 16 mM, petrolatum, 10 µl	reading after 40 minutes, 16 mM: erythema in 12/13 (cheek), 6/13 (forehead, neck, upper back)	Larmi et al. 1989 a
Open application test, non-occlusive	12 healthy volunteers, 0, 15, 31, 62, 125 and 250 mM, isopropyl alcohol/propylene glycol (75/25)	reading after 40 minutes, scale for erythema and oedema: 0 and 1+ to 3+, mild reactions in all tested persons at 62 mM and above (no other data); previous 2× daily washing of test area increases the skin reactions at all concentrations	Lahti et al. 1995

Table 2 (continued)

Study method, application, duration	Collective, concentration, vehicle, applied amount	Reading, assessment scale, results	References
Open application test, non-occlusive	5 ♂ and 11 ♀ healthy volunteers, 125 and 500 mM, different vehicles, 10 µl	reading after 20, 40 and 60 minutes, scale for erythema and oedema: 0 and 1+ to 3+, the addition of water to propylene glycol (PG), ethanol or isopropanol vehicles as well as the addition of 25% PG to ethanol or isopropanol vehicles increases the reaction; the addition of 50% PG reduces the extent of the reaction	Lahti et al. 1993
Open application test, non-occlusive	12 healthy volunteers, 31, 62, 125, 250 or 500 mM, petrolatum, 10 µl	reading after 40 minutes, mild reactions at 31 mM and above; 2-day pretreatment three times daily with 20% sorbitan sesquioleate in petrolatum produced stronger reactions to 125 and 250 mM, but not to 31, 62 and 500 mM	Larmi et al. 1988
Open application test, non-occlusive	15 healthy volunteers, 31, 62, 125 and 250 mM, isopropanol, 10 µl	readings after 20, 40 and 60 minutes, (Laser Doppler Flow (LDF), chromameter), mild reactions at 31 mM and above; the addition of 1%, 2%, 5%, 10% or 25% PG enhances the reactions to 125 and 250 mM, but not to 31 and 62 mM	Lahti and Noponen 1998
Open application test, non-occlusive	groups of 10–12 volunteers, 31, 62, 125 and 250 mM, petrolatum, 10 µl	reading after 40 minutes, (LDF), IR irradiation enhances urticarial reactions to benzoic acid, UVA and UVB irradiation reduce such reactions	Larmi 1989; Larmi et al. 1989 b
Open application test, non-occlusive	in total 17 dermatological patients or healthy volunteers, 50, 100, 250 and 500 mM, ethanol, 10 µl	readings after 10, 20, 30, 40, 50, 60, 75, 90, 105 and 120 minutes, 0 (no or only slight erythema/oedema) and + (moderate to marked erythema/oedema), reaction in 0/17, 2/17, 4/17 and 14/17 volunteers, respectively; acetyl salicylic acid markedly reduces the reaction	Lahti et al. 1987

Table 2 (continued)

Study method, application, duration	Collective, concentration, vehicle, applied amount	Reading, assessment scale, results	References
Open application test, non-occlusive	in total 20 dermatological patients or healthy volunteers, 50, 100, 250 and 500 mM, petrolatum, 10 µl	readings after 20, 40 and 60 minutes, 0 (no or only slight erythema/oedema) and + (moderate to marked erythema/oedema), reactions in 8/20, 12/20, 16/20 and 19/20 volunteers, respectively; previous (4 hours) peroral administration of 120 mg terfinadine had no influence	Lahti 1987
Open application test, non-occlusive, 15 minutes	11 healthy volunteers and 3 patients with skin diseases, 50, 100, 250, 500 and 1000 mM, different vehicle, 10 µl	more marked reaction when using unguent, petrolatum or isopropanol/water (1:2) than when using ethanol or a synthetic lanolin substitute	Ylipieti and Lahti 1989

FC: Finn Chamber®; LDF: Laser Doppler Flow; PG: propylene glycol

Recent clinical findings

In view of the relatively frequent non-specific urticarial reactions to free benzoic acid, a 5% preparation of sodium benzoate in petrolatum is recommended for testing by the German Contact Dermatitis Research Group (DKG). The reaction index (RI)[4] of –0.23 and the positivity ratio (PR)[5] of about 91% to 92% (Brasch and Uter 2011; Schnuch et al. 2008, 2011), however, indicate that this test preparation is problematical.

In a comparative evaluation, only a low number (0.2%) of questionable erythematous reactions to sodium benzoate occurred in 441 patients with a reaction to sodium lauryl sulfate (SLS) tested as an irritant control in the clinics of the Information Network of Departments of Dermatology (IVDK). In the 641 patients who did not react to SLS there were no erythematous reactions, but 0.2% were evaluated as 1+ reactions (Geier et al. 2003 b).

In the available studies, when 5% test preparations of benzoic acid or sodium benzoate were used, usually less than 1% of those tested reacted in collectives of up to 79 046 patients.

A higher number (about 1.3%) was found in a Dutch study with a collective of 627 patients tested consecutively with 5% benzoic acid (in petrolatum) from January to April 1985 (De Groot et al. 1986).

A higher percentage of reactions (1.9%) was found also to 5% sodium benzoate in an earlier multicentric study with 465 patients from 7 French dermatological clinics. Benzoic acid produced a reaction that was evaluated as positive in 2.1% of those tested. Reactions occurred especially in elderly patients, and were associated with (chronic) dermatitis on the leg in about half of the cases (Meynadier et al. 1982).

In a study of intolerance to cosmetics, the medical history and patch test findings from 5202 patients were evaluated. Allergic and irritative contact dermatitis were diagnosed in 2567 and 1529 patients, respectively. An intolerance to cosmetics, sometimes associated with other causes of the skin symptoms and irrespective of the genesis, was found in 606 patients (including 420 of an allergic origin), in 309 of which (156 of allergic origin) the symptoms were caused by cosmetics alone. In the group with an allergic reaction to cosmetics (probably 63 patients), a positive reaction to benzoic acid was found in one of the tested persons. In addition, there were 34 reactions in total in the tested patients; the total number of tested patients is unclear (Broeckx et al. 1987).

In a Belgian study, 25 (0.3%) positive reactions occurred in the 8521 patients tested between 1985 and 1997 (Goossens et al. 1998).

The evaluation of the patch test results of 2059 patients from 8 clinics of the IVDK with the ingredients of a provisional series of preservative agents, revealed four 1+ and one 2+ reactions as well as 22 questionable reactions (in total 0.25% and 1.1%, respectively) among the 2045 patients tested also with sodium benzoate. In this study, the reaction index for the test preparation was only –0.66 (Brasch et al. 1993).

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4) The reaction index is defined as the ratio: (a – d – i) / (a + d + i); with: a = number of allergic reactions, d = number of questionable reactions, i = number of irritant reactions (Brasch and Henseler 1992).

5) The positivity ratio is defined as the percentage of 1+ reactions out of the total positive reactions (Geier et al. 2003 a).

In the subsequent period between 1990 and 1994, 11 349 of 11 485 patients were tested also with sodium benzoate in a series in which preservative agents were investigated in the 24 clinics of the IVDK at that time; 45 positive reactions and 127 questionable or irritant reactions occurred. The reaction index was –0.5 (Schnuch et al. 1998).

Between 1995 and 2008, 34 631 patients were subjected to patch tests with 11 preservative agents, including sodium benzoate, in 34 clinics of the IVDK. Positive reactions occurred in 0.8% of the patients and questionable, follicular or irritant reactions in about 1.3% of the patients. The reaction index (RI) and the positivity ratio (PR) were –0.23 and 91.7%, respectively (Brasch and Uter 2011).

Later evaluation of the patch test findings recorded in 51 clinics of the IVDK between 1996 and 2009 yielded a positive result in 512 cases (0.65%) of the 79 046 tested patients. The reaction index was –0.23 and the positivity ratio 92.2%. At 0.89%, the number of reactions was somewhat higher in men than in women (0.58%, both standardized for age and sex). The authors attribute the difference to the higher sensitivity of men to irritants (Schnuch et al. 2011).

In the period between 1995 and 1998, 2273 patients were tested in the Buxtehude Dermatological Centre with the balsam of Peru test preparation included in the standard series. There were 445 positive reactions. Of these patients, 102 were subsequently tested with 20 potential ingredients of balsam of Peru, including 5% benzoic acid in petrolatum; 20 positive reactions (14× 1+, 5× 2+, 1× 3+) were produced. No data for irritant reactions were given (Hausen 2001).

In a multicentre study of patch test reactions from 7 clinics of the Finnish Contact Dermatitis Group, the findings obtained between 1994 and 1998 with an extended series of dental materials were evaluated. Benzoic acid was tested in one of the clinics (test concentration not specified) and produced a reaction regarded as allergic or irritant in 18 and 21 of 417 tested persons, respectively. The authors concluded, however, that in the reactions classified as allergic, an irritant cause may have been present (Kanerva et al. 2001).

Patch tests with 5% benzoic acid in petrolatum produced a positive reaction in 3 of 285 patients with perioral symptoms suspected to be of allergic origin, which was considered relevant by the authors. However, no reactions to 5% sodium benzoate were observed (Torgerson et al. 2007).

The evaluation of patch test findings in 5226 patients with and 14 818 patients without atopic eczema documented between 1995 and 1999 in the clinics of the IVDK, revealed only slight differences between these collectives, with reactions in 0.36% and 0.25% of the patients, respectively. Patients with current leg ulcers or chronic dermatitis on the leg were not included in this study (Jappe et al. 2003).

A 48-hour patch test with 5% sodium benzoate in 45 bakers with a history of eczema on the hand resulted neither in delayed nor immediate reactions, and only in one case in slight erythema (Meding et al. 2003).

Experimental findings

A maximization test carried out with five 48-hour applications of a 2% benzoic acid preparation in petrolatum at one-day intervals (occlusive application of 2.5% sodium dodecyl sulfate during this period) did not lead to sensitization in any of the 25 volunteers. Provocation was carried out using the same test preparation after a 10-

day interval in the treatment and one-hour pretreatment with 5% to 10% sodium dodecyl sulfate (Nair 2001; Opdyke 1979).

After pretreatment with 5% and 10% dibenzoylperoxide for induction in a maximization test, 10 volunteers who had reacted to the preparation did not react to challenge treatment with 5% benzoic acid in petrolatum (Leyden and Kligman 1977).

4.5 Reproductive and developmental toxicity

There are no data available.

4.6 Genotoxicity

There are no data available.

4.7 Carcinogenicity

There are no data available.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

After one-hour inhalation exposure of rats to benzoic acid (as vapour), an LC_{50} of more than 26 mg/m³ was reported; there were no deaths (BUA 1993).

In rats, the LC_{50} for benzoic acid (as dust) after exposure for 4 hours was more than 12 200 mg/m³; again there were no deaths (OECD 2004).

In Sprague Dawley rats exposed by inhalation to benzoic acid (as dust) for 6 hours, an LC_{50} of more than 1200 mg/m³ was obtained (ECHA 2011).

5.1.2 Oral administration

For benzoic acid, oral LD_{50} values of 2565 mg/kg body weight were given for rats and of 2250 mg/kg body weight for mice (OECD 2004).

In another study, an LD_{50} of 3790 mg/kg body weight was obtained after oral administration of benzoic acid to male and female Sprague Dawley rats. The symptoms were exhaustion and convulsions (Monsanto 1981 c).

In cats, 630 mg benzoic acid/kg body weight was determined to be the lowest lethal dose. Neurotoxic effects (aggressiveness, hyperaesthesia), subnormal body temperature and changes in the lungs, liver and kidneys occurred (ECHA 2011).

In the case of sodium benzoate, the oral LD_{50} for rats was 3140 mg/kg body weight and, in another study, 4070 mg/kg body weight (OECD 2004).

The LD_{50} for potassium benzoate in rats, mice and guinea pigs after oral administration was more than 10 000 mg/kg body weight (OECD 2004).

5.1.3 Dermal application

In rabbits, a dermal LD_{50} of more than 2000 mg/kg body weight was reported for benzoic acid (OECD 2004).

In groups of 5 male and 5 female New Zealand White rabbits, 24-hour occlusive application of 5000 mg benzoic acid/kg body weight (moistened with saline) on the abraded skin of the back produced neither deaths nor clinical signs. The LD_{50} is therefore greater than 5000 mg/kg body weight. At necropsy, pale kidneys were found in one animal (Monsanto 1981 d).

In another study with rabbits, a dermal LD_{50} of more than 10 000 mg benzoic acid/kg body weight was determined (ECHA 2011).

5.1.4 Intraperitoneal and intravenous injection

The LD_{50} for benzoic acid in mice was 1460 mg/kg body weight after intraperitoneal injection, and that for sodium benzoate in rats after intravenous administration was 1714 mg/kg body weight (ECHA 2011).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

In a 4-week inhalation study with rats (see Table 3), benzoic acid caused interstitial inflammation and fibrosis of the lungs even at the lowest concentration investigated of 25 mg/m^3; the effects increased in intensity in a concentration-dependent manner (see Table 4). In the animals exposed to 250 mg/m^3 and more, a red nasal discharge was observed. This concentration was the systemic NOAEC (no observed adverse effect concentration) (Velsicol Chemical Company 1981).

In a study carried out according to OECD Test Guideline 412, a NOAEC of 12.6 mg benzoic acid/m^3 was obtained. In the respiratory tract, at this concentration the incidences were only low and statistically not significant, and the severity of the findings obtained was only minimal to slight (see Table 3) (The Personal Care Products Council 2010). In this study, the aerosol is not characterized in detail. It can nevertheless be used for the evaluation, as the results appear plausible when both inhalation studies are considered together.

From the two 4-week inhalation studies with benzoic acid in rats, a NOAEC for local effects of 12.6 mg/m^3 is obtained. Interstitial inflammation and fibrosis occurred in the lungs at concentrations of 25 mg/m^3 and above. The systemic NOAEC is 250 mg/m^3.

Table 3 Studies of the toxicity of benzoic acid after repeated inhalation

Species, strain, number per group	Exposure	Findings	References
rat, CD (SD), 10 ♂, 10 ♀	**4 weeks**, 0, 25, 250, 1200 mg/m³, 6 hours/day, 5 days/week, whole-body, MMAD: 4.4–5.2 µm, GSD: 2.1–3.1, purity: technical-grade (no other data)	**25 mg/m³ and above**: lungs: interstitial inflammation (multifocal to generalized inflammatory cell infiltrates), interstitial fibrosis; concentration-dependent increase in incidence and severity (see Table 4); only lungs examined histopathologically; **250 mg/m³ and above**: red nasal discharge (from day 4); ♀: absolute kidney weights ↓ (8%); only lungs examined histopathologically; **1200 mg/m³**: mortality (1/10 ♂, 1/10 ♀); body weight gains ↓; thrombocyte count ↓; ♂: absolute and relative[a)] liver weights ↓; ♀: absolute and relative[a)] kidney weights ↓, absolute and relative[a)] trachea/lung weights ↓; scope of the study: clinical observations, body weights, haematology, clinical chemistry, organ weights (heart, kidneys, lung/trachea, brain, liver, spleen), gross-pathological and histopathological examinations (adrenals, nasal turbinates, brain, pancreas, colon, pituitary gland, oesophagus, prostate/uterus, eyes with optic nerve, mandibular salivary gland, testes, ovaries, jejunum, Harder's glands, spleen, heart, sternum, kidneys, stomach, liver, thymus, lungs, thyroid gland, bronchial lymph nodes, bladder, mammary gland)	Velsicol Chemical Company 1981

Table 3 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, CD (SD), 10 ♂, 10 ♀	**4 weeks**, 0, 2.5, 12.6 mg/m³, 6 hours/day, 5 days/week, nose-only, MMAD: 2.1–2.5 µm, GSD: 3.03–3.66, purity: 99.6%	**2.5 mg/m³**: no findings, no histopathological examination; **12.6 mg/m³**: statistical NOAEC; larynx: infiltration of mononuclear cells (1/10 ♂, slight; controls 0/10); lymph nodes, mandibular: hyperplasia (2/10 ♂, minimal; controls 0/10); lungs: infiltration of mononuclear cells (2/10 ♂, 2/10 ♀, minimal; controls 0/10), eosinophilic (2/10 ♀, slight; controls 0/10); pharynx: infiltration of mononuclear cells (1/10 ♂, minimal; controls 0/10); scope of the study: clinical observations, body weights, food consumption, ophthalmology, blood parameters, organ weights, gross-pathological and histopathological examinations; study carried out according to OECD Test Guideline 412	The Personal Care Products Council 2010

[a)] organ weight relative to brain weight; GSD: geometric standard deviation; MMAD: mass median aerodynamic diameter

Table 4 Histopathological findings in the lungs after 4-week exposure of rats to benzoic acid (Velsicol Chemical Company 1981)

Concentration (mg/m³)	0		25		250		1200	
Animals investigated	10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀	10 ♂	10 ♀
Interstitial infiltration of inflammatory cells								
focal								
very slight	5	8		1				
slight	3			1				
multifocal								
very slight	1	2	1	1				
slight	1		6	7	6	5	2	1
generalized								
very slight			1			1		2
slight			2		4	4	8	7
Interstitial fibrosis								
focal								
very slight	1	1						
slight					1	2	1	2
multifocal								
very slight		1	1					
slight			4	5	2	3	1	
generalized								
very slight								
slight					2	1	3	1

5.2.2 Oral administration

The studies of the toxicity of benzoic acid and sodium benzoate after oral administration are shown in Table 5. As many of the earlier studies have a limited scope and insufficient documentation, they can be included in the evaluation only with reservations.

In rats, after oral administration of benzoic acid or sodium benzoate for between 10 days and 2 years, the NOAELs (no observed adverse effect levels) are within the range of about 500 to 1000 mg/kg body weight and day. At the LOAEL (lowest observed adverse effect level), the effects observed included reduced body weight gains, increased liver and kidney weights, ataxia, tremor, excitation, aggressive behaviour, convulsions and deaths. Histopathological changes were found in the brain, the liver and the kidneys (see Table 5).

One study provided evidence of increased anxiety and motor impairment in rats after oral administration of 200 mg sodium benzoate/kg body weight and day for 4 weeks (Noorafshan et al. 2014). As only one dose was investigated, it is not possible to assess whether the effects were actually substance-related.

In mice, NOAELs of about 4160 and 3000 mg/kg body weight and day were obtained after oral administration of sodium benzoate for 10 and 35 days, respectively. In the 10-day study, increased enzyme activity was observed in the serum at about 5000 mg/kg body weight and day and above, and at dose levels of about 6000 mg/kg body weight and day and above, irritability, convulsions and increased liver weights were found. In addition, in the males, enlarged, necrotic hepatocytes with vacuolization were observed, and in the females, mortality occurred (Fujitani 1993). Reduced body weights and increased mortality were found also in the 35-day study at about 6000 mg/kg body weight and day and above (Toth 1984). In a lifetime study, the NOAEL in mice was about 2400 mg/kg body weight and day after the ingestion of sodium benzoate with the drinking water for about 2.5 years (Toth 1984). In view of the limited study design and shortcomings in the documentation, other studies with mice, in which benzoic acid was administered for 3 or 17 months, cannot be included in the evaluation.

Cats were the most sensitive species with a NOAEL of 200 mg benzoic acid/kg body weight and day. Mortality occurred even at doses of 340 mg/kg body weight and day (Bedford and Clarke 1972). As their sensitivity is due to metabolic peculiarities (see Section 3.2), studies with cats are not used for the evaluation.

5.2.3 Dermal application

Benzoic acid doses of 0, 100, 500 or 2500 mg/kg body weight and day were applied to the skin of 8 New Zealand White rabbits per group on 5 days a week for 3 weeks. No effects were found up to the highest dose tested, so that the NOAEL in this study was 2500 mg/kg body weight and day. No details of the type of application and the formulation of the test substance are available (ECHA 2011).

Table 5 Effects of benzoic acid/sodium benzoate after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 5–15 ♂	**1–5 days**, 0, 30 000 mg **benzoic acid**/kg diet (about 0, 3600 mg/kg body weight and day[a)]), recovery period 19–30 days in 15 animals	**about 3600 mg/kg body weight**: from day 4 onwards: ataxia, tremor, excitation, aggressive behaviour, convulsions; about 50% mortality after 5 days; intestine: haemorrhage; brain: necrosis of the cortex piriformis and the stratum granulosum of the fascia dentata; no treatment-related histological changes in the heart, liver, kidneys	Kreis et al. 1967
rat, F344, 6 ♂, 6 ♀	**10 days**, 0, 18 100, 20 900, 24 000 mg **sodium benzoate**/kg diet (about 0, 2170, 2500, 2880 mg/kg body weight and day[a)])	**about 2170 mg/kg body weight**: ♀: serum: cholesterol ↓; **about 2500 mg/kg body weight**: ♂: relative liver weights ↑, serum: albumin, total protein and albumin/globulin ratio ↑; **about 2880 mg/kg body weight**: body weights ↓; relative liver weights ↑; relative kidney weights ↑; serum: albumin ↑; ♂: convulsions and mortality (1/6), absolute spleen and thymus weights ↓, serum: total protein and albumin/globulin ratio ↑, cholesterol ↓, GGT ↑; liver: eosinophilic foci and enlarged hepatocytes with glassy cytoplasm; ♀: absolute and relative thymus weights ↓; serum: cholinesterase ↓	Fujitani 1993
rat, not specified, 28	**3 weeks**, 0, 50 000 mg **sodium benzoate**/kg diet (about 0, 6000 mg/kg body weight and day[a)])	**about 6000 mg/kg body weight**: mortality (19/28 within 2 weeks; remaining 9/28 in week 3), food consumption ↓, diarrhoea, haemorrhage in the intestine and encrusted blood in the nose	Kieckebusch and Lang 1960
rat, Sherman, 6 ♂, 6 ♀	**28 days**, 0, 20 000, 50 000 mg **sodium benzoate**/kg diet (♂: 0, 2357, 5686; ♀: 0, 2396, 7780 mg/kg body weight and day)	**2357 mg/kg body weight**: ♂: body weights ↓; **5686/7780 mg/kg body weight**: mortality 100% after 13 days	Fanelli and Halliday 1963

Table 5 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Sherman, 5 ♂, 5 ♀	**30 days**, 0, 16 to 1090 mg **sodium benzoate**/kg body weight and day, with the diet	**about 1090 mg/kg body weight**: NOAEL (body weights, food consumption, survival, intestine, kidneys, adrenals, liver, spleen); study from 1948	ECHA 2011; Nair 2001
rat, Sprague Dawley, 10 ♂	**4 weeks**, 0, 200 mg **sodium benzoate**/kg body weight and day, gavage	**200 mg/kg body weight**: elevated plus maze test: time in open areas ↓, entering the open areas ↓; rotarod: riding time ↓; the authors see the effects as an indication of increased anxiety and motor impairment; only behaviour tests (elevated plus maze, rotarod)	Noorafshan et al. 2014
rat, not specified, 8 ♂	**4–5 weeks**, 0, 10 000, 30 000 mg **sodium benzoate**/kg diet (about 0, 1200, 3600 mg/kg body weight and day[a])	**about 1200 mg/kg body weight**: NOAEL (only clinical observations and body weights); **about 3600 mg/kg body weight**: irritability, aggressiveness, uncoordinated movements, convulsions, body weights ↓, mortality (2/8); only clinical observations and body weights, study from 1942	ECHA 2011
rat, Wistar, 5–10 ♂	**7, 14 or 35 days**, 0, 11 000 mg **benzoic acid**/kg diet (about 0, 1320 mg/kg body weight and day[a])	**about 1320 mg/kg body weight**: body weight gains ↓ (after 35 days); no signs of neurotoxicity and no pathological changes in the brain	Kreis et al. 1967
rat, not specified, 10–30 ♂	**35 days**, 0, 15 000, 20 000, 25 000, 30 000, 32 500, 35 000, 37 500 mg **sodium benzoate**/kg diet (about 0, 1350, 1800, 2250, 2700, 2925, 3150, 3375 mg/kg body weight and day[b])	**about 1350 mg/kg body weight**: NOAEL; **about 1800 mg/kg body weight and above**: body weights ↓; **about 2250 mg/kg body weight and above**: mortality, tremor, convulsions, restlessness; only clinical observations and body weights, study from 1929	ECHA 2011

Table 5 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, F344, 10 ♂, 10 ♀	**6 weeks**, 0, 5000, 10 000, 20 000, 40 000, 80 000 mg **sodium benzoate**/kg diet (about 0, 450, 900, 1800, 3600, 7200 mg/kg body weight and day[b])	**about 450 mg/kg body weight**: mortality (3/10 ♂, 0/10 ♀), caused by pneumonia with abscess, possibly infection-related; **about 900 mg/kg body weight**: mortality (2/10 ♂, 0/10 ♀), caused by pneumonia with abscess, possibly infection-related; **about 1800 mg/kg body weight**: mortality (1/10 ♂, 0/10 ♀), caused by pneumonia with abscess, possibly infection-related; **about 3600 mg/kg body weight**: body weight ↓; mortality (9/10 ♂, 10/10 ♀); spleen: atrophy; lymph nodes: atrophy; **about 7200 mg/kg body weight**: body weights ↓; mortality (20/20); spleen: atrophy; lymph nodes: atrophy; scope of the study: survival, growth, food consumption, clinical observations, morphological and histopathological examinations	Sodemoto and Enomoto 1980
rat, Sherman, 5 ♂, 5 ♀	**90 days**, 0, 10 000, 20 000, 40 000, 80 000 mg **sodium benzoate**/kg diet (0, 640, 1320, 2620, 6290 mg/kg body weight and day)	**2620 mg/kg body weight**: NOAEL; **6290 mg/kg body weight**: relative liver weights ↑, relative kidney weights ↑, body weight gains ↓, mortality (4/8), kidney and liver lesions; limited study design and documentation	Deuel et al. 1954
rat, not specified, 20 ♂, 20 ♀	**16 weeks**, 0, 5000, 10 000 mg **benzoic acid**/kg diet (about 0, 450, 900 mg/kg body weight and day[b])	**about 900 mg/kg body weight**: NOAEL (body weights, food utilization, organ weights, survival, histology); limited study design and documentation	Kieckebusch and Lang 1960
rat, Sprague Dawley, 20 ♂, 20 ♀	**1 year**, 0, 5000, 20 000 mg **benzoic acid**/kg diet (about 0, 250, 1000 mg/kg body weight and day[c])	**about 250 mg/kg body weight**: NOAEL; **about 1000 mg/kg body weight**: body weight gains ↓; study poorly described, Japanese original from 1978 not obtainable	Nair 2001

Table 5 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, not specified	**3, 8 or 18 months**, 0, 40, 80 mg **benzoic acid**/kg body weight and day, with the diet	**80 mg/kg body weight**: body weights and survival not affected, no changes in parenchymal organs, tolerance to lethal doses of benzoic acid increased; study poorly described, Russian original from 1965	Nair 2001
rat, Wistar, 30 ♂, 20 ♀; controls: 13 ♂, 12 ♀	**18 months**, 0 to 15 000 mg **benzoic acid**/kg diet (about 0 to 750 mg/kg body weight and day[c])	**about 750 mg/kg body weight**: mortality ↑ (30%, controls: 12%), body weights and body weight gains ↓, food consumption ↓, no changes in behaviour; limited study design and documentation	Marquardt 1960
rat, Wistar, 10 ♂, 10 ♀	**18 months**, 0, 40 mg **benzoic acid**/kg body weight and day, with the diet, followed by 13-day gavage administration while otherwise fasting	**40 mg/kg body weight**: ♂: food and water intake ↓; limited study design and documentation; study design invalid	Shtenberg and Ignatev 1970
rat, F344, 50 ♂, 52 ♀, controls: 25 ♂, 43 ♀	**18–24 months**, 0, 10 000, 20 000 mg **sodium benzoate**/kg diet (about 0, 500, 1000 mg/kg body weight and day[c]); interim killing of several animals (no other data) half way through study period	**about 1000 mg/kg body weight**: NOAEL (see also Section 5.7.2); mortality after 16 months ↑ in all groups due to infection (mycoplasma and sialodacryoadenitis virus); scope of the study: body weights, food consumption, clinical observations, morphological and histological examination of all animals and various (no other data) organs; due to the occurrence of infection and the insufficient documentation the study is of limited validity for the evaluation	Sodemoto and Enomoto 1980

Table 5 (continued)

Species, strain, number per group	Exposure	Findings	References
mouse, B6C3F1, 5 ♂, 4–5 ♀	**10 days,** 0, 20 800, 25 000, 30 000 mg **sodium benzoate**/kg diet (about 0, 4160, 5000, 6000 mg/kg body weight and day[d])	**about 4160 mg/kg body weight**: NOAEL; **about 5000 mg/kg body weight**: ♂: serum: cholinesterase ↑; ♀: serum: GGT ↑; **about 6000 mg/kg body weight**: irritability, convulsions (1/5 ♂, 2/5 ♀), absolute and relative liver weights ↑; ♂: serum: cholinesterase, cholesterol and phospholipids ↑, hepatocytes: enlargement, necrosis, vacuolization (5/5); ♀: mortality (2/5), relative kidney weights ↑	Fujitani 1993
mouse, Bulb/C, 3 ♂	**10 days,** 0, 25, 30, 40, 50 g **sodium benzoate**/l drinking water (about 0, 4500, 5400, 7200, 9000 mg/kg body weight and day[e])	**about 4500 mg/kg body weight**: body weights unaffected; liver: no degenerative changes; **about 5400 mg/kg body weight and above**: body weights significantly ↓; liver: necrotic and cirrhotic changes; **up to 7200 mg/kg body weight**: liver: weights and activities of catalase, SOD and GPX unaffected; **about 9000 mg/kg body weight**: mortality 100% (day 8); only liver examined	Kaboglu and Aktac 2002
mouse, Bulb/C, 3 ♂	**10 days,** 0, 100, 200 mg **benzoic acid**/l drinking water (about 0, 18, 36 mg/kg body weight and days[e])	**about 18 mg/kg body weight and above**: liver: uricase activity ↑, enlarged hepatocytes with eosinophilic cytoplasm, occasional single cell necrosis and vacuolization, chromatin in the cell nucleus ↓, collagen fibres ↑; only liver examined	Aktac et al. 2003

Table 5 (continued)

Species, strain, number per group	Exposure	Findings	References
mouse, Swiss, 4 ♂, 4 ♀	**35 days**, 0, 5000, 10 000, 20 000, 40 000, 80 000 mg **sodium benzoate**/l drinking water (about 750, 1500, 3000, 6000, 12 000 mg/kg body weight and day[f]), purity: 99%	**about 3000 mg/kg body weight**: NOAEL; **about 6000 mg/kg body weight**: body weights ↓, mortality (6/8); **about 12 000 mg/kg body weight**: mortality (8/8 within 3 weeks); scope of the study: survival, body weights, food consumption, histological changes	Toth 1984
mouse, not specified, 50 ♂, 50 ♀	**3 months**, 0, 80 mg **benzoic acid**/kg body weight and day, gavage, followed by 5-day gavage administration while otherwise fasting	**80 mg/kg body weight**: body weight gains ↓; scope of the study: clinical observations, food consumption, body weight gains; limited study design and documentation	Shtenberg and Ignatev 1970
mouse, not specified, 50 ♂, 50 ♀	**17 months**, 0, 40 mg **benzoic acid**/kg body weight and day, with the diet, followed by 5-day gavage administration while otherwise fasting	**40 mg/kg body weight**: mortality after discontinuation of feed ↑; limited study design and documentation; study design invalid	Shtenberg and Ignatev 1970
mouse, not specified	**3, 8 or 18 months**, 0, 40, 80 mg **benzoic acid**/kg body weight and day, with the diet	**40 mg/kg body weight and above**: body weights ↓, mortality ↑, liver weights ↑, spleen, ovaries, lungs enlarged; study poorly described, Russian original from 1965	Nair 2001

Table 5 (continued)

Species, strain, number per group	Exposure	Findings	References
mouse, Swiss, 50 ♂, 50 ♀, controls: 100 ♂, 100 ♀	**about 2.5 years**, 0, 20 000 mg **sodium benzoate**/l drinking water (about 0, 1800 mg/kg body weight and day[b]), purity: 99%	**about 1800 mg/kg body weight**: NOAEL (see also Section 5.7.2); scope of the study: clinical observations, body weights, pathological and histopathological examinations (liver, spleen, kidneys, bladder, thyroid gland, heart, pancreas, testes, ovaries, brain, nasal turbinates, at least four pulmonary lobes and all conspicuous organs)	Toth 1984
dog, Fox Terrier, 4	**about 25 days**, increasing doses of about 40 to 1700 mg **sodium benzoate**/kg body weight and day, with the diet	**about 600 mg/kg body weight**: NOAEL; **about 860 mg/kg body weight and above**: tremor, convulsions, ataxia, mortality; only clinical observations reported, study from 1913	ECHA 2011
cat, 4 ♂	**3–23 days**, 0, 100, 200 (15 days) or 0, 130–160 (23 days) or 0, 300–420 (3–4 days) mg **benzoic acid**/kg body weight and day, with the diet	**up to 160 mg/kg body weight** (23 days): NOAEL; **200 mg/kg body weight** (15 days): NOAEL; **300–420 mg/kg body weight** (3–4 days): mortality (2/4), hyperaesthesia, salivation, weakness; liver: infiltration of macrophages and fibroblasts; hepatocytes: foamy granular cytoplasm; renal tubuli: swollen; stomach: ulceration; no findings in the brain and spinal cord	Bedford and Clarke 1972

[a] conversion factor 0.12 according to EFSA (2012); [b] conversion factor 0.09 according to EFSA (2012); [c] conversion factor 0.05 according to EFSA (2012); [d] conversion factor 0.2 according to EFSA (2012); [e] conversion factor 0.18 according to EFSA (2012); [f] conversion factor 0.15 according to EFSA (2012); GGT: γ-glutamyl transferase; GPX: glutathione peroxidase; SOD: superoxide dismutase

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

The available studies of the irritant effects of benzoic acid and sodium benzoate on the skin are summarized in Table 6.

On the skin of rabbits, benzoic acid was found to be non-irritating or only minimally irritating in several studies (BUA 1993; ECHA 2011; Monsanto 1981 b).

In two studies, sodium benzoate was found to be non-irritating on the skin of rabbits (ECHA 2011). No other data are available for the studies.

In a test for non-immunological reactions with guinea pigs, marked reactions of the ears occurred in an ear swelling test about 30 to 40 minutes after non-occlusive application of 20% benzoic acid in ethanol, but not when testing was carried out on the animals' back, abdomen or flanks. The effects of preparations containing 0.2%, 1% and 5% benzoic acid were less severe and concentration-dependent (Lahti and Maibach 1984). Rats and mice, unlike guinea pigs, were found to have no or only very slight sensitivity to 20% benzoic acid (Lahti and Maibach 1985).

5.3.2 Eyes

The available studies for the irritating effects of benzoic acid and sodium benzoate in the eye are summarized in Table 7.

In most of the studies, benzoic acid was shown to be highly irritating to the eyes of rabbits (ECHA 2011). In several studies, damage to the cornea occurred which in some cases was irreversible (BUA 1993; ECHA 2011; Monsanto 1981 a, 1983).

In one study, sodium benzoate was not found to be irritating to the rabbit eye (ECHA 2011), whereas slight irritation of the eye was reported in another study (ECHA 2011).

5.4 Allergenic effects

In experimental studies with benzoic acid, negative results were obtained in the local lymph node assay with CBA/J mice using 5%, 10% or 20% benzoic acid in acetone (Gerberick et al. 1992), in the Buehler test with 20% benzoic acid in water (Gad et al. 1986) and with undiluted benzoic acid (ECHA 2011) and in the Draize test (no other data; OECD 2004). Negative results were likewise obtained in a maximization test with Hartley guinea pigs with 10% (intradermal induction) and 20% (topical induction and challenge) benzoic acid (Gad et al. 1986) and a mouse ear swelling test with CF-1 mice with 20% benzoic acid (induction and challenge) (Gad et al. 1986).

Benzoic acid was also investigated in vitro in a screening test in cultures of hepa1C1C7 mouse hepatoma cells by determining the luciferase activity after activation of the antioxidant response element without metabolic activation (KeratinoSens Assay) and in a modified procedure for recognizing potential prohaptens by adding an S9 fraction from Aroclor-induced rat liver. The results of both tests were negative (Natsch and Emter 2008; Natsch and Haupt 2013). Likewise, a similar in vitro experiment with a human skin cell line (HaCaT) and studies of chemical reactivity determined by means of GSH depletion, yielded negative results for benzoic acid (McKim et al. 2010).

Table 6 Studies of the irritating effects on the skin of benzoic acid and sodium benzoate

Substance	Species	Test conditions	Findings	References
benzoic acid	rabbits, New Zealand White, 3 ♀	500 mg, moistened with water, 4 hours semi-occlusive	primary score: 0.5 of 8; slight erythema and slight oedema (in 1/3); assessed as minimally irritating	BUA 1993; ECHA 2011
benzoic acid	rabbits, no other data	no data	assessed as not irritating	ECHA 2011
benzoic acid	rabbits, no other data	500 mg, dry powder, examination after 24 and 72 hours, no other data	score 1.66 of 8; assessed as not irritating	BUA 1993; ECHA 2011
benzoic acid	rabbits, 2	inner side of ear, 500 mg, 24 hours, no other data	assessed as not irritating	BUA 1993
benzoic acid	rabbits, New Zealand White, 3 ♂, 3 ♀	500 mg, moistened with saline, 24 hours occlusive, examination after 24 and 72 hours	primary irritation index: 0.1 of 8 (intact and abraded skin); maximum scores: intact skin 0, abraded skin 1, reversible after 7 days	Monsanto 1981 b
sodium benzoate	rabbits, no other data	according to OECD Test Guideline 404; no data	assessed as not irritating	BUA 1993; ECHA 2011
sodium benzoate	rabbits, no other data	500 mg, dry powder for 24 hours, examination after 24 and 48 hours, no other data	assessed as not irritating	BUA 1993; ECHA 2011

Table 7 Studies of the irritating effects in the eye of benzoic acid and sodium benzoate

Substance	Species	Test conditions	Findings	References
benzoic acid	rabbits, New Zealand White, 3 ♀	similar to OECD Test Guideline 405; 77 mg fine powder	total score 35 of 110; corneal opacity (marked in 1/3; not reversible in 2/3); no reaction to light within 21 days (in 1/3); moderate chemosis (not reversible in 2/3); slight reddening of conjunctiva, after 2 days marked with whitish-grey discoloration, iris: vascularization (iridial injection)	BUA 1993; ECHA 2011
benzoic acid	rabbits, New Zealand White, 3 ♂	according to OECD Test Guideline 405; 0.1 ml benzoic acid, mixed to a paste with water, examination after 1, 24, 48, 72 hours, recovery period up to 21 days	individual animal scores (24–72 hours): corneal opacity 1.0–1.3 of 4; iris 0.0–0.7 of 2; erythema conjunctiva 1.7–2.0 of 3; chemosis 1.0–2.0 of 4; effects reversible in 2/3 after 7 days, in 1/3 corneal opacity up to day 21 (score 1 of 4) with vascularization; assessed as slightly irritating	Bayer AG 1986
benzoic acid	rabbits, no other data	100 mg fine powder	total score 65.0 of 110; assessed as highly irritating	ECHA 2011
benzoic acid	rabbits, New Zealand White, 3 ♂, 3 ♀	similar to OECD Test Guideline 405; 42 mg as powder, examination after 24, 48, 72 hours, recovery period 21 days	total score 26.7 of 110; irreversible effect on cornea in 2/6 after 21 days	Monsanto 1981 a

Table 7 (continued)

Substance	Species	Test conditions	Findings	References
benzoic acid (purity 99.73%)	rabbits, New Zealand White, 3 ♂, 3 ♀	similar to OECD Test Guideline 405; 36 mg as powder, examination after 24, 48, 72 hours, recovery period up to 35 days	average scores of 28 or 32 in 2/6 after 24–72 hours; damage reversible after up to 29 days; no total score calculated due to persisting damage to the cornea after 35 days in 3/6; maximum score 50 of 110 after 1 hour; pustule formation on the conjunctival tissue up to day 4 in 6/6; neovascularization of the cornea in 5/6	Monsanto 1983
benzoic acid (purity 99.73%)	rabbits, New Zealand White, 3 ♀	similar to OECD Test Guideline 405; 36 mg as powder, with rinsing after 25 seconds, examination after 24, 48, 72 hours, recovery period up to 28 days	average scores of 13.7 or 8.3 in 2/3 after 24–72 hours; damage reversible after 4 days; no total score calculated due to persisting damage in 1/3 after 24 days	Monsanto 1983
benzoic acid	rabbits, no other data	no data	assessed as highly irritating	ECHA 2011
sodium benzoate	rabbits, no other data	similar to OECD Test Guideline 405; 50 mg for 24 hours, examination after 24, 48, 72 hours, recovery period: 7 days	assessed as not irritating	BUA 1993; ECHA 2011
sodium benzoate	rabbits, no other data	according to OECD Test Guideline 405; no other data	total score 9.3 of 110, reversible within 14 days; assessed as slightly irritating	ECHA 2011

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

In a 4-generation study with rats, groups of 20 male and 20 female animals were given benzoic acid doses of 5000 or 10 000 mg/kg diet (about 450 or 900 mg/kg body weight and day, conversion factor 0.09 according to EFSA (2012); see also Section 5.2.2). No adverse effects on reproduction (sterility, delayed sexual maturity, litter size, total number of offspring, survival of the offspring) and no toxic effects on the parents or offspring (clinical observations, body weights, organ weights, histopathological examination) were found (Kieckebusch and Lang 1960). Due to shortcomings in the study design and documentation, the usefulness of this study is limited.

The results of a dominant lethal test in male rats given oral doses of sodium benzoate of up to 5000 mg/kg body weight as a single dose or repeat doses on 5 days, were negative (FAS 1974).

Benzoic acid was not found to be uterotrophic in rats and mice and inactive in a human oestrogen receptor (expression of ERα) yeast oestrogenicity assay (Ashby et al. 1997).

5.5.2 Developmental toxicity

Studies of the developmental toxicity of benzoic acid and sodium benzoate are shown in Table 8.

In Wistar rats, gavage administration of 510 mg benzoic acid/kg body weight on gestation day 9 had no effects in either the dams or the foetuses (Kimmel et al. 1971). In the same strain, gavage administration of doses of 25 mg/kg body weight and day and more from gestation days 6 to 15 resulted in increased resorptions (Nair 2001). It is not specified whether these were early or late resorptions. This is also the case in a study by the same research group with hamsters, in which increased resorptions were found at dose levels of 30 mg/kg body weight and day and above (Nair 2001).

In a feeding study of the toxic effects on prenatal development in Wistar rats given sodium benzoate from gestation days 1 to 20, foetotoxic effects such as a reduced number of live foetuses, reduced foetal weights, reduced ossification and increased incidences of skeletal, external and internal variations and malformations were found at about 1850 mg/kg body weight and day and above. At the same time, food consumption and body weight gains were reduced in the dams. The NOAEL for developmental and maternal toxicity was about 1340 mg sodium benzoate/kg body weight and day (BUA 1993; Onodera et al. 1978).

In studies of the toxic effects on prenatal development with rats, mice, rabbits and hamsters, no developmental or maternal toxicity occurred in any of these animals up to the highest sodium benzoate doses tested of 175 mg/kg body weight (rat, mouse), 250 mg/kg body weight (rabbits) and 300 mg/kg body weight (hamster) (FDA 1972).

Two studies with intraperitoneal administration of sodium benzoate (Minor and Becker 1971) are not included in the evaluation, as direct effects on the embryos cannot be excluded due to the route of administration used.

Table 8 Studies of the developmental toxicity of benzoic acid and sodium benzoate

Species, strain, number per group	Exposure	Findings	References
Toxic effects on prenatal development			
rat, Wistar, 12–18 ♀	**GD 1–20**, 0, 10 000, 20 000, 40 000, 80 000 mg **sodium benzoate**/kg diet (according to BUA 1993): about 0, 670, 1340, 1850, 870 mg/kg body weight and day); in high dose group food consumption considerably reduced by about 75%, consequently actual dose only 870 mg/kg body weight and day; examination: GD 20	**about 1340 mg/kg body weight: NOAEL for toxic effects on prenatal development, maternal toxicity**; **about 1850 mg/kg body weight**: dams: food consumption ↓, body weight gains ↓ (GD 0–GD 5 body weight loss, subsequent very low body weight gains of about 30 g up to GD 20, controls: about 100 g from GD 0–GD 20); survival ↓; foetuses: number of live foetuses ↓, foetal weights ↓, resorptions ↑ (no data as to whether early or late resorptions), skeleton: ossification ↓, skeletal variations and malformations ↑ (for example malformations: missing cervical centre: 1, fused ribs: 1); external and internal variations and malformations ↑ (12/36 foetuses, unilateral microphthalmia: 5, bilateral microphthalmia: 1, unilateral anophthalmia: 2, hydrocephalus: 3, bilateral pyelectasis: 2, unilateral renal hypoplasia: 1); **about 870 mg/kg body weight**: dams: food consumption ↓ (about 75%); body weight loss (from about 260 g on GD 0 to about 210 g on GD 20); foetuses: number of live foetuses ↓, foetal weights ↓, resorptions ↑, skeleton: ossification ↓, variations and malformations ↑; external and internal malformations ↑ (11/26 foetuses, unilateral microphthalmia: 6, unilateral anophthalmia: 1, hydrocephalus: 3, cerebral hypoplasia: 1, bilateral pyelectasis: 2)	BUA 1993; Onodera et al. 1978

Table 8 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 7 ♀, controls 6 ♀	**GD 9**, 0, 510 mg **benzoic acid**/kg body weight, gavage, examination: GD 20	**510 mg/kg body weight**: dams: no clinical signs; foetuses: no foetotoxicity, no increase in incidences of malformations or resorptions	Kimmel et al. 1971
rat, Sprague Dawley, ♀	**GD 9–11**, 0, 100, 315, 1000 mg **sodium benzoate**/kg body weight and day, controls: 90 or 600 mg sodium chloride/kg body weight and day, i.p., examination: no data	**100 mg/kg body weight**: dams: no data; foetuses: no significant effects; **315 mg/kg body weight**: dams: no data; foetuses: no significant effects; **1000 mg/kg body weight**: dams: no data; foetuses: foetal weights ↓, mortality in utero ↑, anomalies (gross anomalies) ↑	Minor and Becker 1971
rat, Sprague Dawley, ♀	**GD 12–14**, 0, 100, 315, 1000 mg **sodium benzoate**/kg body weight and day, controls: 90 or 600 mg sodium chloride/kg body weight and day, i.p., examination: no data	**100 mg/kg body weight**: dams: no data; foetuses: no significant effects; **315 mg/kg body weight**: dams: no data; foetuses: no significant effects; **1000 mg/kg body weight**: dams: no data; foetuses: foetal weights ↓, mortality in utero ↑	Minor and Becker 1971

Table 8 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 20 ♀	**GD 6–15**, 0, 5, 25, 50, 500 mg **benzoic acid**/kg body weight and day, gavage, examination: GD 21	**25 mg/kg body weight and above**: dams: no significant effects; foetuses: resorptions ↑ (no data as to whether early or late resorptions)	Nair 2001
rat, Wistar, 23–24 ♀	**GD 6–15**, 0, 1.75, 8, 38, 175 mg **sodium benzoate**/kg body weight and day, gavage, examination: GD 20	**175 mg/kg body weight: NOAEL maternal toxicity, developmental toxicity**; **up to 175 mg/kg body weight**: dams: no significant effects; foetuses: no significant effects	FDA 1972
mouse, CD 1, 20–21 ♀	**GD 6–15**, 0, 1.75, 8, 38, 175 mg **sodium benzoate**/kg body weight and day, gavage, examination: GD 17	**175 mg/kg body weight: NOAEL maternal toxicity, developmental toxicity**; **up to 175 mg/kg body weight**: dams: no significant effects; foetuses: no significant effects	FDA 1972
rabbit, Dutch belted, 10–12 ♀	**GD 6–18**, 0, 2.5, 12, 54, 250 mg **sodium benzoate**/kg body weight and day, gavage, examination: GD 29	**250 mg/kg body weight: NOAEL maternal toxicity, developmental toxicity**; **up to 250 mg/kg body weight**: dams: no significant effects; foetuses: no significant effects	FDA 1972

Table 8 (continued)

Species, strain, number per group	Exposure	Findings	References
hamster, golden, 21–22 ♀	**GD 6–10**, 0, 3, 14, 65, 300 mg **sodium benzoate**/kg body weight and day, gavage, examination: GD 14	**300 mg/kg body weight: NOAEL maternal toxicity, developmental toxicity**; **up to 300 mg/kg body weight**: dams: no significant effects; foetuses: no significant effects	FDA 1972
hamster, golden, 21–24 ♀	**GD 6–10**, 0, 6, 30, 60, 600 mg **benzoic acid**/kg body weight and day, gavage, examination: GD 16	**30 mg/kg body weight and above**: dams: no significant effects; foetuses: resorptions ↑ (no data as to whether early or late resorptions); **600 mg/kg body weight**: dams: no significant effects; foetuses: malformations ↑ (no other data)	Nair 2001
Toxic effects on prenatal and postnatal development			
rat, Wistar, 4–5 ♀	**GD 1–20**, 0, 10 000, 20 000, 40 000, 80 000 mg **sodium benzoate**/kg diet (according to BUA 1993): about 0, 670, 1340, 1850, 870 mg/kg body weight and day); examination: up to PND 56	**about 670 mg/kg body weight: NOEL for toxic effects on postnatal development**; **about 1340 mg/kg body weight**: **NOAEL maternal toxicity**; offspring: survival after 8 weeks: 95.5% (controls 100%), delayed eye opening in 4 animals from 2 litters; **about 1850 mg/kg body weight**: dams: food consumption ↓, body weight gains ↓, survival ↓; offspring: perinatal mortality 100%, index of living offspring at birth 50% (controls 75%); **about 870 mg/kg body weight**: dams: food consumption ↓, body weights ↓; offspring: perinatal mortality 100%, index of live offspring at birth 8% (controls 75%)	BUA 1993; Onodera et al. 1978

Table 8 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 10 ♀	**GD 5–PND 45**, 0, 1000, 5000, 10 000 mg **sodium benzoate**/kg diet (about 0, 90, 450, 900 mg/kg body weight and day[a)]); examination: PND 6–21	**up to about 900 mg/kg body weight**: dams: NOAEL (mortality, body weights and food consumption unaffected); offspring: NOAEL (mortality, body weights, food consumption, motor activity unaffected, brain: monoamines (serotonin, dopamine, noradrenaline) and weights in cerebral areas unaffected)	Crane and Lachance 1985
rat, Sprague Dawley, 10 ♀	**12 weeks** (after weaning up to mating and entire gestation period), 0, 1000, 5000 mg **sodium benzoate**/kg diet (about 0, 90, 450 mg/kg body weight and day[a)]), examination: 1st day after birth	**about 90 mg/kg body weight and above**: dams: serum: urea and uric acid ↑, AP ↑; offspring: body weights ↓ (13.6%, not at about 450 mg/kg body weight); **about 450 mg/kg body weight**: **NOAEL developmental toxicity**; dams: food consumption ↓, serum: AST and ALT ↑; offspring: no significant effects	Mowafy et al. 2001

[a)] conversion factor 0.09 according to EFSA (2012); ALT: alanine aminotransferase; AP: alkaline phosphatase; AST: aspartate aminotransferase; GD: gestation day; Hb: haemoglobin; i.p.: intraperitoneal; PND: postnatal day

In a feeding study with sodium benzoate in Wistar rats from gestation days 1 to 20 and examination of the animals up to postnatal day 56, eye opening was delayed in the offspring at about 1340 mg/kg body weight and day and above. The NOAEL for postnatal development was about 670 mg/kg body weight and day. At about 1850 mg/kg body weight and day and above, food consumption, body weight gains and survival were reduced in the dams. The NOAEL for maternal toxicity was about 1340 mg/kg body weight and day (BUA 1993; Onodera et al. 1978).

A study with prenatal and postnatal administration of sodium benzoate to Wistar rats, did not yield maternal or foetotoxicity up to the high dose of about 900 mg/kg body weight and day (Crane and Lachance 1985).

In Sprague Dawley rats, sodium benzoate doses of about 450 mg/kg body weight and day administered with the diet from weaning up to mating and during the entire gestation period led to reduced food consumption and an increase in liver enzyme activities in the dams. The NOAEL was about 90 mg/kg body weight and day. Changes in the body weights of the offspring were not dose-dependent. Therefore, a NOAEL for toxic effects on postnatal development of about 450 mg/kg body weight and day was obtained (Mowafy et al. 2001).

5.6 Genotoxicity

5.6.1 In vitro

Data for the in vitro genotoxicity of benzoic acid and alkali benzoates are given in Table 9.

In several tests for differential killing with Bacillus subtilis, positive and negative results were obtained with benzoic acid or the alkali salts. Data for cytotoxicity are lacking in all studies, one study is available only as an abstract, some studies are poorly documented.

In several tests for induction of the SOS response in Escherichia coli and Salmonella typhimurium as well as in a large number of bacterial mutagenicity tests with and without the addition of metabolic activation, however, only negative results were obtained (see Table 9).

In the comet assay with human lymphocytes, DNA strand breaks were induced in one study with benzoic acid only at the highest concentration tested of 5 mM and in another study at concentrations of 0.4 mM and above (Demir et al. 2010; Yilmaz et al. 2014). Data for cytotoxicity are not available in either study.

Another comet assay yielded positive results for sodium benzoate at the lowest concentration tested of 6.25 µg/ml (0.04 mM) and above, whereas the results were negative for potassium benzoate up to the highest concentration tested of 1000 µg/ml (Zengin et al. 2011). Data for cytotoxicity are not available.

In several studies with benzoic acid or sodium benzoate, the incidences of sister chromatid exchange were not significantly increased in human and hamster cells whereas an increase in the incidence of sister chromatid exchange occurred at concentrations of 2 mM and above in a study with sodium benzoate in hamster cells (Abe and Sasaki 1977). An increase was likewise found at 0.4 mM benzoic acid and above in a study with human lymphocytes (Yilmaz et al. 2009). In studies with human lymphocytes, the incidences of sister chromatid exchange were increased only

at high concentrations of 8 or 10 mM sodium benzoate (Mpountoukas et al. 2008; Xing and Zhang 1990). In other studies with sodium benzoate and potassium benzoate, increased incidences of sister chromatid exchange were found at 6.25 and 62.5 µg/ml (0.04 and 0.4 mM) and above, respectively; however, compared with the increase in the positive control group (0.2 µg mitomycin C/ml: 10.2-fold increase compared with the incidence in controls) the increase was only slight (sodium benzoate: 2.6-fold increase at 100 µg/ml compared with the incidence in controls ; potassium benzoate: 2.2-fold increase at 1000 µg/ml compared with the incidence in controls) (Zengin et al. 2011).

In the concentration range up to 0.1 mg/ml (0.8 mM), benzoic acid did not induce chromosomal aberrations in CHL cells (a cell line derived from Chinese hamster lung) (ECHA 2011), whereas no unequivocal results were obtained in the higher concentration range at 1 mg/ml (8.2 mM) and above (Ishidate 1988; Ishidate et al. 1984, 1988). An increase was found at concentrations of 0.4 mM benzoic acid and above in a study with human lymphocytes (Yilmaz et al. 2009).

Negative results were obtained for sodium benzoate in concentrations of up to 0.2 mg/ml (1.4 mM) in a chromosomal aberration test with a human embryonal lung cell line (WI-38) (FDA 1974).

In four other studies, an increase in chromosomal aberrations was induced by sodium benzoate in hamster cells, in one study at concentrations of 2 mM and above. The mitotic index was not reduced up to 10 mM (Abe and Sasaki 1977; Ishidate et al. 1988). In another study, a positive result was found for sodium benzoate only at the high concentration of 2 mg/ml (13.9 mM); data for cytotoxicity were not given (Ishidate 1988; Ishidate and Odashima 1977; Ishidate et al. 1984, 1988). The two other studies which reported positive results are poorly documented; for example, no data was given for the concentrations tested and for cytotoxicity (Ishidate and Yoshikawa 1980; Kawachi et al. 1980).

In human lymphocytes, the incidence of chromosomal aberrations was increased at sodium benzoate concentrations of 6.25 µg/ml (0.04 mM) and above and at potassium benzoate concentrations of 62.5 µg/ml (0.4 mM) and above (Zengin et al. 2011). The authors reported that the pH of the medium was unchanged.

In a micronucleus test in human lymphocytes without metabolic activation, micronuclei were induced by benzoic acid concentrations of 1.6 mM and above (Yilmaz et al. 2009), by sodium benzoate concentrations of 25 µg/ml (0.17 mM) and above, and by potassium benzoate concentrations of 125 µg/ml (0.8 mM) and above (Zengin et al. 2011). These concentrations had led to a significantly reduced mitotic index. The authors reported that the pH of the medium was unchanged.

Table 9 Genotoxicity of benzoic acid and alkali benzoates in vitro

End point	Test system	Substance	Concentration	Effective concentration	Result		Remarks	References
					−m. a	+m. a		
Bacteria								
DNA repair Rec assay	B. subtilis M45(rec-), H17(rec+)	benzoic acid	1%	no data	–	–	cytotoxicity: no data	ECHA 2011
	B. subtilis M45(rec-), H17(rec+)	benzoic acid	no data	no data	+	n.i.	cytotoxicity: no data; study poorly documented	Nonaka 1989
	B. subtilis (no other data)	sodium benzoate	no data	no data	+	n.i.	cytotoxicity: no data; study poorly documented	Kawachi et al. 1980
	B. subtilis (no other data)	sodium benzoate	no data	–	–	n.i.	cytotoxicity: no data	ECHA 2011
	B. subtilis M45(rec-), H17(rec+)	sodium benzoate	no data	no data	+	n.i.	cytotoxicity: no data; study poorly documented	Nonaka 1989
	B. subtilis M45(rec-), H17(rec+)	sodium benzoate	−m. a: 20 mg/plate; +m. a: 16 mg/plate	−m. a: 20 mg/plate; +m. a: 16 mg/plate	+	+	cytotoxicity: no data	Ishizaki and Ueno 1989
	B. subtilis M45(rec-), H17(rec+)	potassium benzoate	−m. a: 20 mg/plate; +m. a: 15 mg/plate	−m. a: 20 mg/plate; +m. a: 15 mg/plate	+	+	cytotoxicity: no data	Ishizaki and Ueno 1989
DNA damage SOS chromo-test	E. coli PQ37	benzoic acid	up to 400 µg/ml	–	–	n.i.	cytotoxicity: no data	Adams et al. 2005

Table 9 (continued)

End point	Test system	Substance	Concentration	Effective concentration	Result		Remarks	References
					−m. a	+m. a		
DNA damage SOS response (umu test)	S. typhimurium TA1535/pSK 1002	benzoic acid	up to 1.67 mg/ml	–	–	–	cytotoxicity: no data	ECHA 2011
DNA damage SOS response (lambda prophage induction)	E. coli WP2s	benzoic acid	up to 0.106 mg/well	–	–	n.i.	cytotoxicity: no data	ECHA 2011
gene mutation	S. typhimurium TA98, TA100, TA1535, TA1537	benzoic acid	0.01–1 mg/plate	–	–	–	cytotoxicity: no data	ECHA 2011
	S. typhimurium TA1535, TA1537, TA1538	benzoic acid	0.5%	–	–	–	cytotoxicity: no data	Adams et al. 2005
	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	benzoic acid and hippuric acid	0.1 mg/plate	–	–	–	cytotoxicity: no data	Milvy and Garro 1976
	S. typhimurium TA1535, TA1536, TA1537, TA1538	benzoic acid (in DMSO)	0.001–0.1 mg/plate	–	–	–	cytotoxicity: no data	ECHA 2011
	S. typhimurium TA98, TA100, TA1535, TA1536	benzoic acid	up to 3.6 µg/plate	–	–	–	cytotoxicity: no data	Adams et al. 2005

Table 9 (continued)

End point	Test system	Substance	Concentration	Effective concentration	Result		Remarks	References
					−m. a	+m. a		
	no data	benzoic acid (99.5%)	no data	–	–	–	cytotoxicity: no data	OECD 2004
	S. typhimurium TA1535	benzoic acid	20–2000 µg/plate	–	–	–	cytotoxicity: no data	ECHA 2011
	S. typhimurium TA98, TA100, TA1535, TA1538	benzoic acid	0.004–2.5 mg/plate	–	–	–	cytotoxicity: no data	Anderson and Styles 1978
	S. typhimurium TA100	benzoic acid	0.0001–1 mg/plate	–	–	n.i.	cytotoxicity: no data	ECHA 2011
	S. typhimurium (no other data)	benzoic acid	no data	–	–	n.i.	cytotoxicity: no data	ECHA 2011
	S. typhimurium TA98, TA1535	hippuric acid	0.1–5 µmol/plate	–	–	–	cytotoxicity: no data	ECHA 2011
	S. typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537	benzoic acid (in DMSO; purity: 99.6%)	up to 10 mg/plate	–	–	–	cytotoxicity: no data	Ishidate et al. 1984
	S. typhimurium TA97, TA102	benzoic acid	0.1–10 mg/plate	–	–	–	cytotoxicity: no data	NLM 2014
	S. typhimurium TA97, TA98, TA100, TA1535, TA1537	benzoic acid (purity > 99%, in DMSO)	0.033–10 mg/plate	–	–	–	cytotoxicity: no data	Zeiger et al. 1988
	S. typhimurium TA98, TA100	benzoic acid (in DMSO)	0.1–10 mg/plate	–	–	–	cytotoxicity: no data	NLM 2014
	S. typhimurium TA98, TA100	benzoic acid (in DMSO)	0.1–5 mg/plate	–	–	–	cytotoxicity: no data	Fall et al. 2007

Table 9 (continued)

End point	Test system	Substance	Concentration	Effective concentration	Result		Remarks	References
					−m. a	+m. a		
	S. typhimurium TA98, TA100, TA1537	sodium benzoate	no data	–	–	–	cytotoxicity: no data; study poorly documented	Ishidate and Yoshikawa 1980
	S. typhimurium TA98, TA100	sodium benzoate	no data	–	–	–	cytotoxicity: no data; study poorly documented	Kawachi et al. 1980
	S. typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537	sodium benzoate (in water; purity: 99.0%)	up to 3 mg/plate	–	–	–	cytotoxicity: no data	Ishidate et al. 1984
	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538; E. coli WP2	sodium benzoate	0.033–10 mg/plate	–	–	–	cytotoxicity: no data	ECHA 2011
Mammalian cells								
DNA strand breaks (alkaline comet assay)	human lymphocytes	benzoic acid	0, 0.05, 0.1, 0.5, 1, 5 mM	5 mM	+ (in 5 mM)	n.i.	cytotoxicity: no data; no data for pH	Demir et al. 2010
	human lymphocytes	benzoic acid	50, 100, 200, 500 µg/ml (1 hour)	50 µg/ml (0.4 mM) and above	+	n.i.	cytotoxicity: no data; no data for pH	Yilmaz et al. 2014
	human lymphocytes	sodium benzoate	6.25–100 µg/ml (1 hour)	6.25 µg/ml (0.04 mM) and above	+	n.i.	cytotoxicity: no data	Zengin et al. 2011
	human lymphocytes	potassium benzoate	62.5–1000 µg/ml (1 hour)	–	–	n.i.	cytotoxicity: no data	Zengin et al. 2011

Table 9 (continued)

End point	Test system	Substance	Concentration	Effective concentration	Result		Remarks	References
					−m. a	+m. a		
SCE	human lymphoblastoid cells (transformed Epstein-Barr virus)	benzoic acid	1–30 mmol/l (0.1–3.7 mg/ml)	–	–	n.i.	cytotoxicity: no data	ECHA 2011
	CHO cells	benzoic acid	1–10 mmol/l (0.1–1 mg/ml)	–	–	n.i.	cytotoxicity: no data	ECHA 2011
	human lymphocytes	benzoic acid	up to 2 mmol/l (0.2 mg/ml)	–	–	n.i.	cytotoxicity: no data	ECHA 2011
	human lymphocytes	benzoic acid	50, 100, 200, 500 µg/ml (24 hours/48 hours)	50 µg/ml (0.4 mM) and above	(+) maximum 3-fold increase at 500 µg/ml compared with incidence in controls	n.i.	cytotoxicity: at 100 µg/ml and above (48 hours) or 500 µg/ml (24 hours) (MI); pH of the medium unchanged	Yilmaz et al. 2009
	Chinese hamster cells (Don)	sodium benzoate	1, 2, 5, 10 mmol/l	2 mM and above	+ (2 mM and above)	n.i.	cytotoxicity: > 10 mM (MI); no data for pH	Abe and Sasaki 1977
	CHL cells	sodium benzoate	no data	–	–	n.i.	cytotoxicity: no data; study poorly documented	Kawachi et al. 1980
	human lymphocytes	sodium benzoate	10 mmol/l	10 mM	+	n.i.	cytotoxicity: no data; no data for pH	Xing and Zhang 1990

Table 9 (continued)

End point	Test system	Substance	Concentration	Effective concentration	Result		Remarks	References
					−m. a	+m. a		
	human lymphocytes	sodium benzoate	0.02, 0.2, 2, 4, 8 mM	8 mM	+ (8 mM)	n.i.	cytotoxicity: > 8 mM (MI), at 4 mM and above (PRI); no data for pH	Mpountoukas et al. 2008
	human lymphocytes	sodium benzoate	6.25–100 µg/ml (24 hours/48 hours)	6.25 µg/ml (0.04 mM) and above	(+) 2.6-fold increase at 100 µg/ml compared with incidence in the controls	n.i.	cytotoxicity: at 12.5 µg/ml and above (24 hours); at 6.25 µg/ml and above (48 hours) (MI); pH of the medium unchanged	Zengin et al. 2011
	human lymphocytes	potassium benzoate	62.5–1000 µg/ml (24 hours/48 hours)	62.5 µg/ml (0.4 mM) and above	(+) 2.2-fold increase at 1000 µg/ml compared with incidence in the controls	n.i.	cytotoxicity: at 250 µg/ml and above (24 hours); at 62.5 µg/ml and above (48 hours) (MI); pH of the medium unchanged	Zengin et al. 2011

Table 9 (continued)

End point	Test system	Substance	Concentration	Effective concentration	Result		Remarks	References
					−m. a	+m. a		
CA	CHL cells	benzoic acid	0.001–0.1 mg/ml (up to 0.8 mM)	–	–	n.i.	cytotoxicity: no data	ECHA 2011
	CHL cells	benzoic acid (in DMSO; purity: 99.6%)	0.5, 1.0, 1.5 mg/ml	1.5 mg/ml (12.3 mM; 24 hours); 1.0 mg/ml (8.2 mM) and above; (48 hours)	+/–	n.i.	cytotoxicity: no data; no data for pH	Ishidate 1988; Ishidate et al. 1984, 1988
	human lymphocytes	benzoic acid	50, 100, 200, 500 µg/ml (24 hours/48 hours)	50 µg/ml (0.4 mM) and above	+	n.i.	cytotoxicity: at 100 µg/ml (48 hours) or 500 µg/ml (24 hours) and above (MI); pH of the medium unchanged	Yilmaz et al. 2009
	human embryonal lung cells (WI-38)	sodium benzoate	0.002–0.2 mg/ml	–	–	n.i.	cytotoxicity: at 1 mg/ml (MI) and above; at 0.5 mg/ml and above (CPE)	FDA 1974
	Chinese hamster cells (Don)	sodium benzoate	1, 2, 5, 10 mM	2 mM (0.3 mg/ml) and above	+ (2 mM and above)	n.i.	cytotoxicity: > 10 mM (MI); no data for pH	Abe and Sasaki 1977; Ishidate et al. 1988
	CHL cells	sodium benzoate	no data	no data	+	n.i.	cytotoxicity: no data; study poorly documented	Kawachi et al. 1980

Table 9 (continued)

End point	Test system	Substance	Concentration	Effective concentration	Result		Remarks	References
					−m. a	+m. a		
	CHL cells	sodium benzoate	10 mg/ml (69.4 mM)	no data	+	n.i.	cytotoxicity: no data; study poorly documented	Ishidate and Yoshikawa 1980
	CHL cells	sodium benzoate (in saline; purity: 99.0%)	0.5, 1.0, 2.0 mg/ml	2.0 mg/ml (13.9 mM; 24 hours/48 hours)	+ (13.9 mM)	n.i.	cytotoxicity: no data; no data for pH	Ishidate 1988; Ishidate and Odashima 1977; Ishidate et al. 1984, 1988
	human lymphocytes	sodium benzoate	6.25–100 µg/ml (24 hours/48 hours)	6.25 µg/ml (0.04 mM) and above	+	n.i.	cytotoxicity: at 12.5 µg/ml and above (24 hours); at 6.25 µg/ml and above (48 hours) (MI); pH of the medium unchanged	Zengin et al. 2011
	human lymphocytes	potassium benzoate	62.5–1000 µg/ml (24 hours/48 hours)	62.5 µg/ml (0.4 mM) and above	+	n.i.	cytotoxicity: at 250 µg/ml and above (24 hours); at 62.5 µg/ml and above (48 hours) (MI); pH of the medium unchanged	Zengin et al. 2011

Table 9 (continued)

End point	Test system	Substance	Concentration	Effective concentration	Result		Remarks	References
					−m. a	+m. a		
MN	mouse lymphoma cells (L5178Y $TK^{+/-}$)	benzoic acid (in DMSO, purity: > 99%)	0, 250, 500, 1000, 2000 µg/ml (+m. a: 4 hours; −m. a: 24 hours)	–	–	–	cytotoxicity: at 1000 µg/ml and above (24 hours, MTT test); precipitation at 2000 µg/ml	Nesslany and Marzin 1999
	human lymphocytes	benzoic acid	50, 100, 200, 500 µg/ml (48 hours)	200 µg/ml (1.6 mM) and above	+	n.i.	cytotoxicity: at 100 µg/ml and above (48 hours) (MI); pH of the medium unchanged	Yilmaz et al. 2009
	human lymphocytes	sodium benzoate	6.25–100 µg/ml (48 hours)	25 µg/ml (0.17 mM) and above	+	n.i.	cytotoxicity: at 6.25 µg/ml and above (48 hours) (MI); pH of the medium unchanged	Zengin et al. 2011
	human lymphocytes	potassium benzoate	62.5–1000 µg/ml (48 hours)	125 µg/ml (0.8 mM) and above	+	n.i.	cytotoxicity: at 62.5 µg/ml and above (48 hours) (MI); pH of the medium unchanged	Zengin et al. 2011

+: positive; −: negative; +/−: equivocal;
B. subtilis: Bacillus subtilis; CA: test for chromosomal aberrations; CHL: Chinese hamster lung; CHO: Chinese hamster ovary; CPE: cytopathic effect; DMSO: dimethylsulfoxide; E. coli: Escherichia coli; MI: mitotic index; MN: micronucleus test; n.i.: not investigated; PRI: proliferation rate index; S. typhimurium: Salmonella typhimurium; SCE: sister chromatid exchange

5.6.2 In vivo

The data for the in vivo genotoxicity of benzoic acid and sodium benzoate are given in Table 10.

In a wing mosaic test with Drosophila melanogaster, a positive result was obtained with benzoic acid only at the highest concentration tested of 50 mM. The lower concentrations produced negative or equivocal results. The effect of the test substance was less pronounced than that of the positive control (1 mM EMS) (Demir et al. 2008).

Host mediated assays with sodium benzoate are available in which male ICR mice were treated once or on 5 days with up to 5000 mg/kg body weight by gavage. The mutation frequency was not increased (FDA 1974).

Male ddY mice received single oral doses of benzoic acid or sodium benzoate. After 3 and 24 hours, cells from eight different organs were investigated using the comet assay. In all cases, no DNA strand breaks were induced (Sasaki et al. 2002).

A chromosomal aberration test in the bone marrow of male rats given single oral doses or five daily oral doses of up to 5000 mg sodium benzoate/kg body weight yielded negative results (FDA 1974).

In a poorly documented study which is available only in the form of a table, a negative result was reported in the chromosomal aberration test with sodium benzoate in rats (Kawachi et al. 1980).

In a dominant lethal test, male rats received single oral doses or daily oral doses on five days of up to 5000 mg sodium benzoate/kg body weight. In comparison with the study controls and the historical controls, no dominant lethal mutations were induced (FDA 1974).

Summary:

Benzoic acid and sodium benzoate are not mutagenic in the Salmonella mutagenicity test in vitro. The clastogenic effects in mammalian cells found in vitro were not confirmed in vivo in tests for chromosomal aberrations in rats. Likewise, in the host mediated assay and comet assay in mice, and the dominant lethal test in rats, benzoic acid and sodium benzoate were not genotoxic.

Table 10 Genotoxicity of benzoic acid and sodium benzoate in vivo

Test system		Dose	Results	Remarks	References
wing mosaic test	Drosophila melanogaster	0.1, 0.5, 1, 10, 25, 50 mM benzoic acid (purity 99.5%); positive control: 1 mM EMS	+ (at 50 mM)		Demir et al. 2008
host-mediated assay (S. typhimurium TA1530)	mouse, ICR, 8–10 ♂	0, 50, 500, 5000 mg sodium benzoate/kg body weight, single, gavage	–	increased mutation frequency at 500 mg/kg body weight	FDA 1974
	mouse, ICR, 8–10 ♂	0, 50, 500, 5000 mg sodium benzoate/kg body weight, 5 days, 1×/day, gavage	–		FDA 1974
host mediated assay (S. typhimurium G46; S. cerevisiae D3)	mouse, ICR, 8–10 ♂	0, 50, 500, 5000 mg sodium benzoate/kg body weight, single, gavage	–		FDA 1974
	mouse, ICR, 8–10 ♂	0, 50, 500, 5000 mg sodium benzoate/kg body weight, 5 days, 1×/day, gavage	–		FDA 1974
comet assay (glandular stomach, colon, liver, kidneys, bladder, lungs, brain, bone marrow)	mouse, ddY, 4 ♂	0, 1000 mg benzoic acid/kg body weight (in olive oil, purity 99.5%), single, oral, examination after 3 and 24 hours	–		Sasaki et al. 2002
	mouse, ddY, 4 ♂	0, 1000 mg sodium benzoate/kg body weight (in saline, purity > 98%), single, oral, examination after 3 and 24 hours	–		Sasaki et al. 2002

Table 10 (continued)

Test system		Dose	Results	Remarks	References
CA, bone marrow	rat, Sprague Dawley (CD), 3–5 ♂	0, 50, 500, 5000 mg sodium benzoate/kg body weight, single, gavage, examination after 6, 24, 48 hours	–	mitotic index unchanged	FDA 1974
	rat, Sprague Dawley (CD), 3–5 ♂	0, 50, 500, 5000 mg sodium benzoate/kg body weight, 5 days, 1×/day, gavage, examination after 6 hours	–	mitotic index unchanged	FDA 1974
	rat, no other data	sodium benzoate (no other data)	–	study inadequately reported	Kawachi et al. 1980
DLT	rat, no other data, 5 ♂	0, 50, 500, 5000 mg sodium benzoate/kg body weight, single, gavage	–		FDA 1974
	rat, no other data, 5 ♂	0, 50, 500, 5000 mg sodium benzoate/kg body weight, 5 days, 1×/day, gavage	–		FDA 1974

CA: test for structural chromosomal aberrations; DLT: dominant lethal test; S. typhimurium: Salmonella typhimurium: S. cerevisiae: Saccharomyces cerevisiae

5.7 Carcinogenicity

In a carcinogenicity study (see also Section 5.2.2), groups of 50 male and 52 female F344 rats were given **sodium benzoate** (purity: not specified) for 18 to 24 months with the diet in concentrations of 0%, 1% or 2% (about 0, 500 or 1000 mg/kg body weight and day, conversion factor 0.05 according to EFSA 2012). The control groups consisted of 25 male and 43 female animals. The food consumption was controlled to prevent any excesses; the drinking water was available ad libitum. Due to intercurrent infections (mycoplasms and sialodacryoadenitis virus), the survival in several groups was very low. All surviving animals were killed after 18 to 25 months and examined. There were no unusual clinical effects, and only slight differences in mean body weights and mortality between the treated animals and the control groups were found. The tumours observed in the treated animals were not significantly different from those found in the controls as regards type, incidence and time to occurrence (Sodemoto and Enomoto 1980). Due to the infection-related, low survival in several groups and the limited documentation, the usefulness of the study for the evaluation is also only limited.

In a lifetime study, groups of 50 male and 50 female Swiss mice were given 2% **sodium benzoate** (purity: 99%) with the drinking water (see also Section 5.2.2). The control group consisted of 100 male and 100 female animals. Clinical observations and the determination of body weights as well as gross-pathological and histopathological examinations were carried out (liver, spleen, kidneys, bladder, thyroid gland, heart, pancreas, testes, ovaries, brain, nasal turbinates, at least four pulmonary lobes and all conspicuous organs) in all animals. The average daily intake of sodium benzoate was 1800 mg/kg body weight and day (conversion factor 0.09 according to EFSA 2012). No treatment-related effects on survival or tumour incidences could be found (Toth 1984). In view of the sufficiently high number of animals and the detailed histopathological examination, this study is considered to be of relevance (OECD 2004). However, the study is to be criticized due to the fact that only one dose was tested.

5.8 Other effects

In rabbits, daily intravenous injections of **sodium benzoate** (40 mg/kg body weight) resulted in damage to the retina within three days. Histopathological examination revealed exudative detachment of the retinal neuroepithelium from the pigment epithelium. The toxic effect was especially noticeable in the layer of the rod and cone cells (Grant and Schuman 1993).

6 Manifesto (MAK value/classification)

The critical effects of benzoic acid are the strong irritation of the eyes and the pulmonary toxicity after inhalation exposure. In the case of alkali benzoates, systemic toxicity is the main effect, as sodium benzoate is only slightly irritating to the eyes.

MAK value. No suitable data with humans are available for the derivation of a MAK value.

As regards the **alkali benzoates**, which are at best slightly irritating to the eyes, the systemic toxicity is the main effect, so that the MAK value can be derived from this. After repeated ingestion of benzoic acid or sodium benzoate by rats, relatively high NOAELs in the range of 500 to 1000 mg/kg body weight are obtained. The following toxicokinetic data are taken into consideration for the extrapolation of the NOAEL of 500 mg/kg body weight to a concentration in workplace air: the daily exposure of the animals in comparison with the 5 days per week exposure at the workplace (7:5), the corresponding species-specific correction value (1:4) for the rat, the oral absorption (100%, see Section 3.1), the body weight (70 kg) and respiratory volume (10 m^3) of the person, and the assumed 100% absorption by inhalation. The concentration calculated from this is 1225 mg/m^3. However, the systemic NOAEC of 250 mg benzoic acid/m^3 resulting from the 4-week inhalation study with rats (Velsicol Chemical Company 1981) is lower, so that the MAK value is derived from this. Assuming an increase in effects over time from the short-term study (1:6), the increased respiratory volume at the workplace (1:2) and the extrapolation of the data from experimental studies with animals to humans (1:2), a MAK value of 10 mg/m^3 I (inhalable fraction, as benzoate) has been established for alkali benzoates.

For the strongly irritating **benzoic acid**, the MAK value is derived on the basis of its local effects. In the 4-week inhalation study with rats (Velsicol Chemical Company 1981), benzoic acid caused local pulmonary toxicity in the form of interstitial inflammation and fibrosis at concentrations of 25 mg/m^3 and above. From another 4-week inhalation study with benzoic acid in rats, a NOAEC of 12.6 mg/m^3 for local pulmonary toxicity was calculated (The Personal Care Products Council 2010). Assuming an increase in effects over time from the short-term study (1:6), the increased respiratory volume at the workplace (1:2) and the extrapolation of the data from experimental studies with animals to humans (1:2), the MAK value is derived from this NOAEC of 12.6 mg/m^3. As the vapour saturation concentration of benzoic acid is about 4.4 mg/m^3, at this MAK value the substance can be present in vapour form, and the MAK value is given in ml/m^3. For benzoic acid, therefore, a MAK value of 0.1 ml/m^3 has been established. This value corresponds to 0.5 mg/m^3 for the R (respirable) fraction, as the lungs are the target organ.

Peak limitation. As far as the **alkali benzoates** are concerned, the systemic toxicity is the main effect. They are therefore classified in Peak Limitation Category II with the standard excursion factor of 2, as no data for their half-lives are available.

As the interstitial inflammation in the lungs with fibrosis is the critical effect for determining the threshold limit value and this effect does not occur immediately, but requires a longer exposure period before it manifests itself, **benzoic acid** is classified in Peak Limitation Category II. As in the 4-week inhalation study with rats no irritation occurred at a concentration of 12.6 mg benzoic acid/m^3, an excursion factor of 4 can be set.

Prenatal toxicity. In a study of the toxic effects on prenatal development in Wistar rats, foetotoxic effects such as a reduced number of live foetuses, reduced foetal weights, reduced ossification and increased incidences of skeletal, external and internal variations and malformations were found at dose levels of about 1850 mg **sodium**

benzoate/kg body weight and day and above. At the same time, food consumption and body weight gains were reduced in the dams. The NOAEL for developmental and maternal toxicity is about 1340 mg sodium benzoate/kg body weight and day (BUA 1993; Onodera et al. 1978). In studies of the toxic effects on prenatal development with mice, rabbits and hamsters, no developmental or maternal toxicity occurred up to the highest doses tested of 175 mg/kg body weight (mouse), 250 mg/kg body weight (rabbit) and 300 mg/kg body weight (hamster) (FDA 1972). As these were the highest doses in each case, the true NAEL (no adverse effect level) is higher. In a study with prenatal and postnatal administration to Wistar rats, no maternal toxicity or foetotoxicity was found up to the highest dose tested of about 900 mg/kg body weight and day (Crane and Lachance 1985). The following toxicokinetic data are taken into consideration for the extrapolation of the NOAELs of about 1340 mg/kg body weight and day for rats, of 175 mg/kg body weight and day for mice, of 250 mg/kg body weight and day for rabbits and of 300 mg/kg body weight and day for hamsters to a concentration in workplace air: the corresponding species-specific correction values for the rat, the mouse, the rabbit and the hamster (1:4; 1:7; 1:2.4; 1:5.5), the demonstrated oral absorption (100%), the body weight (70 kg) and respiratory volume (10 m^3) of the person, and the assumed 100% absorption by inhalation. The concentrations calculated from this are 2345, 175, 730, and 382 mg/m^3 air (corresponding to 1970, 147, 613, and 321 mg/m^3 air for the benzoate), respectively. The differences between these values and the MAK value of 10 mg/m^3 are thus 197-fold (rat), 14-fold (mouse), 61-fold (rabbit) and 32-fold (hamster), respectively. As the differences between these values and the MAK value of 10 mg/m^3 are sufficiently large, sodium benzoate is classified in Pregnancy Risk Group C. This applies also to other alkali benzoates, as the effect is not mediated by the alkali content.

For **benzoic acid**, only three studies are available (Kimmel et al. 1971; Nair 2001). These were not carried out according to valid guidelines and have serious shortcomings, such as the use of only one dose and treatment on only one gestation day or the lack of information about early or late resorptions. The systemic effects of benzoic acid, however, are expected to be similar to those of its salts, as these effects are most likely mediated via the benzoate formed endogenously. In addition, the MAK value for benzoic acid of 0.5 mg/m^3 is lower than that for the benzoates, so that the difference between this and the NOAEL for the developmental toxicity of the benzoate is greater by a factor of 20. In view of this greater difference, benzoic acid is likewise classified in Pregnancy Risk Group C.

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

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

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

Sensitization. Although in numerous clinical epidemiological studies patch test reactions to sodium benzoate have been reported, these are almost always only weakly positive and are to be regarded as possibly irritant reactions and thus as falsely positive. The results from animal experiments for contact sensitization are all negative, and the immediate reactions to benzoic acid and sodium benzoate found in humans and guinea pigs are to be regarded as non-immunological reactions. Therefore, in view of the findings detailed in this supplement, there is still no necessity for designating the substance with "Sh" (for substances which cause sensitization of the skin). As no findings are available for respiratory sensitization, benzoic acid and alkali benzoates are not designated with "Sa" (for substances which cause sensitization of the airways).

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