Annex VI Report

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: **Benzoic acid**

EC Number: **200-618-2**

CAS Number: **65-85-0**

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PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Benzoic acid

EC Number: EINECS 200-618-2

CAS number: 65-85-0

Registration number (s): -

Purity: > 990 g/kg (pharmaceutic quality)

Impurities:

Cinnamic acid: max. 0.1 %, Sum of heavy metals: max. 0.001 %, Ash: max 0.1 %, Organic and inorganic chloride: not detectable.

(as defined by the German pharmacopoeia)

Proposed classification based on Directive 67/548/EEC:

	Classification	Wording
Hazard Symbols, Indications of danger	Xi	Irritant
R-phrases	R38 R41	Irritating to skin Risk of serious damage to eyes

Proposed classification based on Regulation (EC) No 1272/2008:

	Classification (Codes)	Wording
Hazard classes, Hazard categories	Skin Irrit. 2 Eye Dam. 1	
Hazard statements	H315 H318	Causes skin irritation Causes serious eye damage

Proposed labelling based on Directive 67/548/EEC:

	Labelling	Wording
Hazard Symbols, Indications of danger	Xi	Irritant
R-phrases	R38 R41	Irritating to skin Risk of serious damage to eyes
S-phrases	(S2) S24 S26 S37/39	Keep out of the reach of children Avoid contact with skin In case of contact with eyes, rinse immediately with plenty of water and seek medical advice Wear suitable gloves and eye/face protection

Proposed labelling based on Regulation (EC) No 1272/2008:

	Labelling (Codes)	Wording
Pictograms	 GHS05	
Signal Word	Danger	
Hazard statements	H315 H318	Causes skin irritation, Causes serious eye damage
Suppl. Hazard statements	----	
Precautionary statements	(P102) P280 P302 + P352 P333 + P313 P305 + P351 + P338 P310	Keep out of reach of children Wear protective gloves/eye protection/face protection IF ON SKIN: Wash with plenty of soap and water If skin irritation or rash occurs: Get medical advice/attention IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing Immediately call a POISON CENTER or doctor/physician

JUSTIFICATION

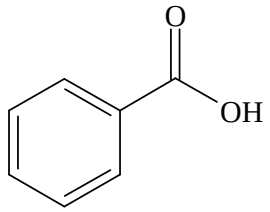
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name: benzoic acid
EC Name: benzoic acid
CAS Number: 65-85-0
IUPAC Name: benzoic acid

1.2 Composition of the substance

Chemical Name: benzoic acid
EC Number: EINECS 200-618-2
CAS Number: 65-85-0
IUPAC Name: benzoic acid
Molecular Formula: $C_7H_6O_2$
Structural Formula:



Molecular Weight: 122.12
Typical concentration (% w/w): 99.0
Concentration range (% w/w): 99.0 – 100.5 %

1.3 Physico-chemical properties

Table 1-1 Summary of physico- chemical properties

REACH ref Annex, §	Property	IUCLID section	Value
VII, 7.1	Physical state at 20°C and 101.3 kPa	3.1	crystalline solid
VII, 7.2	Melting/freezing point	3.2	122.4 °C
VII, 7.3	Boiling point	3.3	249.2 °C
VII, 7.4	Relative density	3.4 density	1.321 g/cm^3
VII, 7.5	Vapour pressure	3.6	0.11 – 0.53 Pa at 20 °C
VII, 7.6	Surface tension	3.10	31 ± 1.3 mN/m at 130 °C
VII, 7.7	Water solubility	3.8	2.9 g/L at pH 2.9 5 g/L at pH 5 15 g/L at pH 9, all at 20 °C
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	log p $_{o/w}$ = 1.87
VII, 7.9	Flash point	3.11	121 – 131 °C
VII, 7.10	Flammability	3.13	not flammable
VII, 7.11	Explosive properties	3.14	not explosive (based on the chemical structure)
VII, 7.12	Self-ignition temperature		ignition temperature in air: 573 °C
VII, 7.13	Oxidising properties	3.15	not expected (based on the chemical structure)
VII, 7.14	Granulometry	3.5	not available
XI, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17	not available
XI, 7.16	Dissociation constant	3.21	pK_a = 4.2
XI, 7.17,	Viscosity	3.22	not determined
	Auto flammability	3.12	Due to the chemical structure and the vapour pressure no auto-flammability properties are expected.
	Reactivity towards container material	3.18	not determined
	Thermal stability	3.19	sublimation starts at > 100 °C; formation of anhydride: at app. 150 °C; decarboxylation: at app. 370 °C

2 MANUFACTURE AND USES

2.1 Manufacture

There are different manufacturers of benzoic acid.

2.2 Identified uses

Benzoic acid is used as bactericide, viricide and fungicide.

Disinfection of materials (deposit areas, fleece mats, ebb/flood benches, culture vessels, knives, gardening equipment).

2.3 REACH Registrations (15.06.2011)

- 2119455536-33-0000
- 2119455536-33-0001

3 CLASSIFICATION AND LABELLING

3.1 Current classification based on Directive 67/548/EEC

Benzoic acid is currently not included in Annex VI of Regulation (EC) No 1272/2008

3.2 Current labelling based on Directive 67/548/EEC

Benzoic acid is currently not included in Annex VI of Regulation (EC) No 1272/2008

3.3 Current classification based on Regulation (EC) No 1272/2008

Benzoic acid is currently not included in Annex VI of Regulation (EC) No 1272/2008

3.4 Current labelling based on Regulation (EC) No 1272/2008

Benzoic acid is currently not included in Annex VI of Regulation (EC) No 1272/2008

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation

4.1.1 Stability

Corresponds to IUCLID 4.1

Hydrolysis

- Draft Assessment Report 2000, Vol. 3, B.8.4, p. 156

No studies were submitted. Taking account of the chemical structure (aromatic monocarbon acid) hydrolysis at pH 5–9 will not occur. Bencoic acid is hydrolytically stable.

Photolysis in water

- Draft Assessment Report 2000, Vol. 1, B.2.1, p. 14

No absorption occurs at $\lambda > 290$ nm. Therefore, no further studies are required. Bencoic acid is photolytically stable in the aquatic environment.

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

4.1.2.2 Screening tests

Readily biodegradability

- Draft Assessment Report 2000, Vol. 3, B.8.4, p. 156

Benzoic acid can be metabolised by microorganisms via pyrocatechol or 3,4-dihydroxy benzoic acid (Schlegel, 1992). Low concentrations of 14C-benzoic acid (0.059 µg/L and 59 µg/L, resp.) were found to be mineralized to 94.5–98.6 % in water samples from two lakes and 99.4–99.5 % in sewage within 7 days at 29 °C (Rubin et al., 1982). The active substance can be regarded as readily biodegradable: 85 % degradation within 14 d was observed in the MITI test at a concentration of 100 mg benzoic acid/L. 84 % degradation within 10 d was observed in the modified MITI test at a concentration of 100 mg sodium benzoate/L. Easy degradation is reported from several studies some of them using special conditions like degradation under anaerobic conditions, degradation in sea water or rain water (GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance 1993). Due to this property sodium benzoate can be used as reference compound in order to check the activity of the inoculum for the investigation of ready biodegradability of chemical substances according to OECD guideline 301. Using adapted activated sludge at 20 °C with benzoic acid as sole carbon source, 99 % COD removal at 88.5 mg COD/g dry inoculum/h is reported (Verschueren, 1977). Testing the inherent biological degradability through the batch method using non-adapted activated sludge (1 g dry matter/L) benzoic acid (COD 1000 mg/l) was found to be degraded to > 90 % within 2 days based on COD measurement (Zahn and Wellens, 1980).

Benzoic acid is readily biodegradable in aquatic systems.

4.1.2.3 Simulation tests

Biodegradation in water/sediment systems

- Draft Assessment Report 2000, Vol. 3, B.8.4, p. 156

No data available.

With regard to toxicity to human beings and ecotoxicology there are no specific concerns arising from the fate and behaviour of benzoic acid in water following application according to the GAP. It is therefore deemed acceptable that studies addressing route and rate of degradation in aquatic systems were not submitted and that the data requirements with regard to fate and behaviour of the active substance in water are waived.

4.1.3 Summary and discussion of persistence

Biodegradation in water

Benzoic acid was found to be readily biodegradable in water. No further data are available about the degradation of benzoic acid in water/sediment systems.

4.2 Environmental distribution

4.2.1 Adsorption/desorption

Corresponds to IUCLID 4.4.1

4.2.2 Volatilisation

Corresponds to IUCLID 4.4.2

4.2.3 Distribution modelling

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

Benzoic acid has a log K_{ow} of 1.87.

An approximate estimation of the bioconcentration factor BCF_{fish} on basis of log K_{ow} = 1.87 was conducted using the standard equation (74) given in the EU Technical Guidance Document (TGD) on Risk Assessment (2003), Part II, 3.8.3.2:

$\text{Log } BCF_{fish} = 0.85 \cdot \log K_{ow} - 0.70$

$\text{Log } BCF_{fish} = 0.85 \cdot (1.87) - 0.70$

$\text{Log } BCF_{fish} = 0.8895$

$BCF_{fish} = 7.754$ (on wet weight basis)

The result of the calculation suggests that benzoic acid would not bioaccumulate in aquatic organisms.

4.3.1.2 Measured bioaccumulation data

No data available.

4.3.2 Terrestrial bioaccumulation

No data available.

4.3.3 Summary and discussion of bioaccumulation

Benzoic acid has a log Pow of 1.87. As it is below 3, a BCF study was not required. The result of the calculation using the standard equation (74), BCF_{fish} = 7.754 (on wet weight basis), is regarded as a worst case for the estimation of bioconcentration in fish.

It is assumed that there is no risk of bioaccumulation in aquatic organisms.

4.4 Secondary poisoning

Assessment of the potential for secondary poisoning

5 HUMAN HEALTH HAZARD ASSESSMENT

Benzoic acid and its salts are natural compounds widely spread in the environment. They are generated in plant and animal metabolism and therefore, are constituents in many foodstuffs: up to ca. 40 mg/kg are found in milk products and up to 100 mg/kg in honey (IPCS, 1999; BUA Report, 1993).

Benzoic acid and benzoates are used as therapeutic substances in human and veterinary medicine. Because of its antimicrobial activity, benzoic acid is commonly used as preservative in food, cosmetics and pharmaceuticals.

The worldwide industrial production volume is about 700 000 tons per year (OECD SIDS dossier, 2005).

Bridging concept

Although the application for Annex I Inclusion under Directive 98/8/EC is restricted to benzoic acid, benzoates are included in the toxicological evaluation because a considerable part of data was generated with these substances. Particularly sodium benzoate has been used in many experiments because of the low water solubility of benzoic acid at neutral pH. Equilibria, depending on the pH in an aqueous environment, are established between the benzoate anion and undissociated benzoic acid. For example, after ingestion of sodium benzoate the acidic pH of the stomach moves the equilibrium to the undissociated benzoic acid molecule (pKa: 4.19). Additionally, for some studies it was not definitely stated whether benzoic acid or benzoates were used. Regarding the great similarity of the toxicological profile, benzoic acid and sodium benzoate can in general be considered together.

Quality of data

The toxicological evaluation of the a.s. benzoic acid is based on literature which is very heterogeneous. In several studies – especially in short-term and long-term studies - only one or two doses and only few parameters were tested. Results were often insufficiently reported.

As stated in the OECD SIDS dossier, *"several of the toxicological studies on [...] benzoic acid and its salts were carried out some years ago and do not always fulfill for 100% present-day guidelines. However, well-known research groups and/or test laboratories ran the studies according to scientific standards and/or accepted protocols at that time. They did appear to be acceptable studies for evaluation. Also, all were peer-reviewed and published in high quality scientific literature. Most of them have been reviewed and accepted by other fora like FDA, JECFA, and IPCS as acceptable studies. In addition, there is good consistency in the individual data for a substance in the group as well as between members of the group [...]. Therefore, taken as a whole, the available studies give a robust database for hazard assessment and hazard evaluation of these compounds and further studies are not indicated."*

Regarding the overall conclusions on the toxicological properties of benzoic acid/benzoates in this report, they resemble those drawn in other evaluations (see Table 1). The US FDA accepted benzoic acid as a GRAS (Generally Recognized As Safe) direct food substance in 1972.

Table 5-1 Evaluation of benzoic acid and/or benzoates

Evaluated by	Year	Reference
US FDA	1972	GRAS (Generally recognized as safe) food ingredients: benzoic acid and sodium benzoate, NTIS, US FDA
JECFA	1973 1983 1996	17th JECFA, published in FNP 4 (1978) 27th JECFA 49th JECFA, published in FNP 52 Add 4
MAK-Commission	1985 1995	Henschler D (ed) Toxikologisch arbeitsmedizinische Begründung von MAK-Werten. Benzoesäure. VCH VerlagsGmbH, Weinheim (1985) DFG: MAK- und BAT-Werte Liste 1995, Wiley-VCH
BIBRA	1989	BIBRA Report Toxicity Profile - Benzoic Acid and its common salts, TNO BIBRA Toxicology International Ltd.
BUA	1993	BUA Report 145: Benzoic Acid/Sodium Benzoate, GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance
IPCS	2000	*Concise International Chemical Assessment Document No. 26: Benzoic Acid and Sodium Benzoate*
OECD	2001	OECD SIDS Dossier on Benzoates, UNEP
EU	2003 2005	SANCO/1396/2001-Final, Monograph on Benzoic Acid *SCCP/0891/05-Opinion on Benzoic Acid and Sodium Benzoate*
FEMA	2005	The FEMA GRAS assessment of benzyl derivatives used as flavor ingredients, Fd Chem Toxicol 43:1207-1240

Taking into account the natural occurrence of benzoic acid and benzoates in the environment and the long and extensive experience with the use of these substances, e.g. as food preservative and as medication, further testing is considered not necessary. The RMS concludes that an exemption from the requirement of toxicological studies for the active ingredient according to current standard test guidelines is justified. No further studies have to be submitted.

Two REACH registration dossiers (2119455536-33-0000, 2119455536-33-0001) were made available and have been taken into consideration. Generally, the data presented there seem to be in line with the current CLH proposal. In some cases the citations (e.g. author or year) used in this report and used in the registration dossiers (for a single, identical study) were different. Whenever possible, the same study was summarized into one entry, however this is not specifically indicated. It is pointed out, that studies mentioned in the dossiers were not available and therefore, the validity of the information provided in the dossier could not be checked.

In the registration dossiers, following classification for toxicological properties are given: R41-R37 and H318-H335

Changed/new criteria in *Commission Regulation (EU) No 286/2011 of 10 March 2011 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures* were taken into account, when preparing the toxicological part of this CLH dossier.

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Extensive information regarding the absorption, distribution, metabolism and excretion (ADME) of benzoic acid/sodium benzoate was derived from the published literature covering investigations with very different objectives (e.g. perfusion experiments with different organs, elucidation of basic metabolism principles, special absorption phenomena). In these investigations, a great variety of animal species, including monkey, rat, and rabbit, as well as humans were studied. A comprehensive ADME study based on a current guideline (e.g. EU, OECD or EPA) is not available.

Absorption

After oral ingestion of benzoic acid/sodium benzoate, the gastrointestinal absorption is rapid and virtually complete in humans, rats, dogs, and hamsters (Bridges et al., 1970, Hall and James, 1980, Jones, 1982). In humans, the peak plasma concentration is reached within 1 to 2 hours (Kubota & Ishizaki, 1991). The rapidity of absorption was substantiated by perfusion experiments with colon of the rat (Schanker et al., 1959). In these experiments, it was also shown that absorption is based on diffusion of the unionized molecule and is dependent on pH.

Excretion

The excretion of benzoic acid/sodium benzoate with urine is rapid and virtually complete in humans, rats, hamsters, and dogs (Lang & Lang, 1956, Schachter, 1957, Bridges et al., 1970, Hall & James, 1980, Akira et al., 1993) but seems less effective in other species such as ferrets and subhuman primates (Bridges et al., 1970). Faecal and respiratory excretion appear to be minor routes of elimination.

Because of the high rate and extent of elimination in most of the species investigated, no accumulation is to be expected.

Metabolism

Hippuric acid and benzoyl glucuronide are the two main metabolites of benzoic acid/sodium benzoate in mammals (Fujii et al., 1991 a.o.). These metabolites result from conjugation reactions of benzoic acid with glycine or glucuronate (figure 1).

From the maximum excretion rate, it was calculated that the elimination capacity via glucuronate and glycine conjugation is approx. 20 g benzoic acid per day in humans.

The maximum rate of biotransformation of benzoic acid to hippuric acid has a mean value of 23.0 mg/kg bw/h which is close to the daily maximum dose of 500 mg/kg bw (21 mg/kg bw/h) recommended for the treatment of hyperammonaemia (see chapter 3.10: Medical data).

Like in humans, benzoic acid is almost entirely excreted as hippuric acid in rabbits, rats, and pigs (e.g. Thabrew et al., 1980), whereas other species excrete also considerable quantities of benzoyl glucuronide; e.g. marmosets up to 38 % (Hall & James, 1980), dogs: 75 % (Barnes, 1959) and ferrets: 20 % (Bridges et al., 1970).

In neonatal and protein-deficient rats, the proportion excreted as hippuric acid appeared to be reduced, approx. 20 % of the urinary radiolabel were identified as benzoyl glucuronide.

In cats, which lack the metabolic pathway of glucuronic acid conjugation, benzoic acid will build up to toxic levels when glycine conjugation to hippuric acid is saturated (Bedford & Clarke, 1972).

The major sites of conversion of benzoic acid to hippuric acid and benzoyl-glucuronic acid in humans are the liver and the kidneys. While the conjugation rate was greater in the renal cortex than

in the liver, the larger mass and strategic anatomical position of the liver were considered to make it quantitatively the more important organ with respect to glycine conjugation (Temellini et al., 1993). *In vitro* experiments indicated that after percutaneous absorption of benzoic acid a small amount can also be converted to hippuric acid in the skin (Nathan et al., 1990).

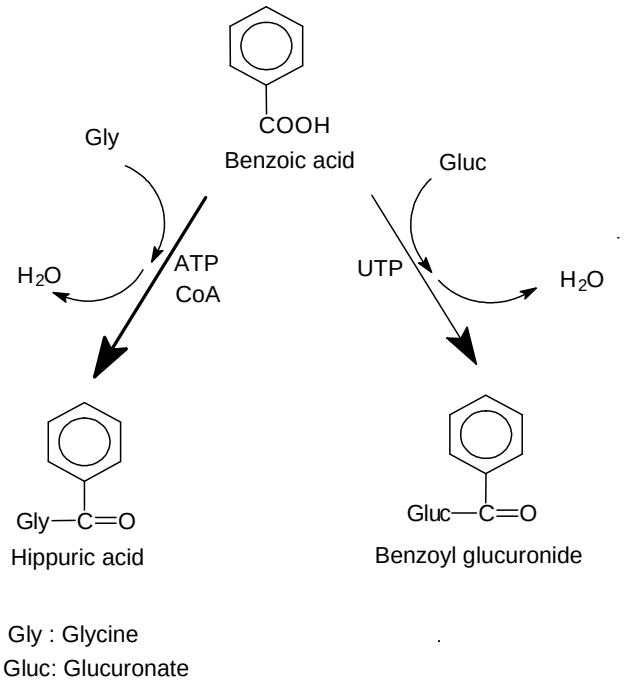


Figure 5-1 Metabolism of benzoic acid in rat and man

Table 5-2 Summary of toxicokinetic studies

Method/ Guideline	**Route**	**Species, Strain, Sex, No/group**	**Dose levels, Duration of exposure**	**Results (excretion via respiration, urine, faeces, bile, half-life time plasma, residues in tissue)**	**Remarks**	**Reference**
Pharmaco-kinetics in humans no guideline, non-GLP	Oral	Human, 6 M	40-80-160 mg/kg bw	Oral absorption: 100 %, peak plasma concentration within 1-2 hours, Urinary excretion: > 99 % within 24 h	Sodium benzoate, dissolved in water	Kubota K., Ishizaki T., Eur. J Clin. Pharmacol . 41:363-368 (1991)

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels, Duration of exposure	Results (excretion via respiration, urine, faeces, bile, half-life time plasma, residues in tissue)	Remarks	Reference
Urinary excretion no guideline, non-GLP	Oral	Human, 2 M; rhesus monkey, 3 F; squirrel monkey, 2 F, capuchin, 1 F, pig, 2 F, rabbit, 3 F, rat, 3 F, mouse, 3 x 10 F, guinea pig, 3 F, hamster, 3 F, lemming, 3 F, gerbil, 3 F, cat, 2 F, dog, 2 F + 1 M, ferret, 3 F	mg/kg bw: 1 20 50 50 50 49-200 50 56 49 52 56 29 51 51 50	Excretion nearly completely urinary in man, rat and rabbit: conjugation with glycine to hippuric acid (80-100 %), conjugation with glucuronate (0-20 %), excretion of parent compound: < 1 %, excretion in man 97 % within 4 h, 100 % within 12 h, entirely as hippuric acid	Sodium ^{14}C benzoate, dissolved in water, gavage (rodents, rabbits); dietary (dogs, pigs, monkeys); dissolved in milk (cats and ferrets)	Bridges, J. W., French, M. R., Smith, R. L., Williams, R. T., Biochem. J. 118:47-51 (1970)
Urinary excretion of hippuric acid, no guideline, non-GLP	Oral	Human, 3 M + 6 F	M: 20 mg/kg bw F: 33/66 mg/person	Within 5 h, 89 % were excreted via urine with a maximal excretion rate in the first hour.	Sodium benzoate, dissolved in soft drink (F), or in water (M)	Fujii, T., Omori, T., Taguchi, T., Ogata, M., J. Food Hyg Soc Japan 32(3):177-182 (1991)
Urinary excretion, no guideline, non GLP	Oral	Human, 3 M	1-2-3-5 g/person	Elimination of approx. 80 % of a single oral dose of 5 g benzoic acid in 6 h via urine, main metabolite: hippuric acid, minor metabolite: benzoyl glucuronide.	Sodium benzoate, dissolved in water	Quick, A. J., J Biol Chem 92(1):65-85 (1931)
Metabolism of sodium benzoate, no guideline, non GLP	Oral	Human, 1 M	1-2-5-10 g/person	Complete elimination of each dose level within 10 to 11 h	Sodium benzoate, dissolved in water	Schachter, D., J. Clin. Invest. 36:297-302 (1957)

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels, Duration of exposure	Results (excretion via respiration, urine, faeces, bile, half-life time plasma, residues in tissue)	Remarks	Reference
Metabolism	Oral	Human, 5 (sex not specified)	21-42 g/person	Urinary excretion of high doses completely within a few hours	Sodium benzoate, dissolved in water	Bignami, G., Biochim. Ter. Sper. 11:383-393 (1924) Italian, no translation submitted
Metabolism	Oral	Human, 5 M	3-10 g	Decrease of urea and uric acid in urine and an increase of uric acid (but not of urea) in blood and plasma. No symptoms were reported	Benzoic acid, vehicle not specified	Swanson, W. W., J. Biol. Chem. 62:565-673 (1925)
Conjugation of benzoic acid with glycine in liver and kidney *in vitro* no guideline, non-GLP	N/A	*In vitro*, samples of human liver and kidney tissue, 110 liver samples, 67 kidney samples	2.5 mg/ml	Rate of conjugation of benzoic acid with glycine: liver: 254 ± 90.5 nmol/min /g (range: 94.4 to 564 nmol/min/g); kidney: 321 ± 99.3 nmol/min/g (range: 63.3 to 542 nmol/min/g)	^{14}C-labelled benzoic acid, dissolved in ethanol/ water (2:8)	Temellini, A., Mogavero, S., Giulianotti, P. C., Pietrabissa, A., Mosca, F., Pacifici, G. M., Xenobiotica 23(12):1427-1433 (1993)
Measurement of metabolic rate of benzoic acid to hippuric acid, no guideline, non GLP	i.v.	Rat, Wistar, 5 M	2 – 2.2 mg/kg	Urinary excretion of ^{13}C-hippuric acid over 2 h: 85-99 % of the injected dose.	^{13}C-labelled benzoic acid, dissolved in saline	Akira, K., Takagi, N., Takeo, S., Shindo, H., Baba, S., Analyt. Biochem. 210:86-90 (1993)

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels, Duration of exposure	Results (excretion via respiration, urine, faeces, bile, half-life time plasma, residues in tissue)	Remarks	Reference
Metabolism, no guideline, non GLP	Oral	Marmoset, Rat, Birming-ham Wistar	 <u>Marmoset:</u> 1 mg/kg bw 40 mg/kg bw 100 mg/kg bw <u>Rat:</u> 1 mg/kg bw 50 mg/kg bw 100 mg/kg bw	Urinary metabolites: 87.7 % HA 6.2 % BG 2.7 % BA 33.3 % HA 26.7 % BG 38.8 % BA 42.3 % HA 38 % BG 18.8 % BA 97.4 % HA 1.8 % BG 0.3 % BA 99 % HA 1 % BG 0.3 % BA 94 % HA 3.1 % BG 2.2 % BA BG: benzoyl glucuronide, HA: hippuric acid BA: Benzoic acid	Benzoic acid, dissolved in blackcur-rent syrup or milk	Hall, B. E., James, S. P., Xenobio-tica 10(6):421-434 (1980)
Urinary excretion of benzoic acid, no guideline, non GLP	Oral	Rat, Sprague-Dawley, 2 M	0.01-0.1-1-10-100-1000 mg/kg bw	Urinary excretion: 80-100 % within 24 h, at 0.01 mg/kg bw: ~3 % exhaled as $^{14}CO_2$	^{14}C-labelled benzoic acid, dissolved in water	Jones, A. R., Xeno-biotica 12(6):387-395 (1982)
Excretion of sodium benzoate in urine, faeces, organs and skin, no guideline, non GLP	Intra-perito-neal	Rat, strain, sex and number not specified	50 mg/animal	Nearly quantitative excretion in the urine within 1-2 days, less than 4 % of the radioactivity appeared in the faeces, up to 0.009 % in the organs and 0.3 % in the skin including fat (ether extract).	^{14}C-labelled benzoic acid, dissolved in water	Lang H., Lang K., Arch. exper. Path. u. Pharma-kol. 229:505-512 (1956), German
Absorption of benzoic acid from the rat colon	Colon perfu-sion	Rat, Sprague-Dawley, 2-4 M	1-10 mmol/L	Absorption (19 % at pH6.8-7.2; 50 % at pH3.6-4.0) by diffusion of the unionized molecule	Benzoic acid, dissolved in isotonic buffer + aspartic acid (20 mmol/L)	Schanker, L. S., J. Pharmacol . Exp. Toxicol. Ther. 126:283-290 (1959)

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels, Duration of exposure	Results (excretion via respiration, urine, faeces, bile, half-life time plasma, residues in tissue)	Remarks	Reference
Metabolism in low-protein fed rats	Intra-perito-neal	Rat, Wistar, 8 M	200 mg/kg bw	Benzoic acid almost entirely excreted as hippuric acid (99 % excretion in 24 h); On a low-protein diet excretion of less hippuric acid (62 % to 85 %) and more benzoyl glucuronide (14 % to 37 %)	^{14}C-labelled benzoic acid, dissolved in water	Thabrew, M. I., Bababun-mi, E. A., French, M. R., Toxicol. Letters 5:363-367 (1980)

Dermal absorption

Because of the immense use of benzoic acid/sodium benzoate as preservative in cosmetics and the suitability of benzoic acid as reference substance, many investigations dealt with the percutaneous absorption in several animal species *in vivo*, e.g. rat, rhesus monkey, dog, pig, guinea pig and in humans as well as in human and rat skin *in vitro*.

Overall, the percutaneous absorption in humans accounts for approximately 40 % *in vivo* (14 – 42.6 %) and 70 % (53 - 99 %) *in vitro*. However, in the absence of data for the biocidal product and due to the heterogeneous results in human dermal absorption studies, a dermal absorption of 100 % is assumed.

Table 5-3 Summary of dermal absorption studies

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels, Duration of exposure	Results	Remarks	Reference
In vivo tests					
In vivo dermal absorption, no guideline, non GLP	Human, 6 (sex not specified)	4 µg/cm², 24 h, non-occlusive	Percutaneous absorption: 42.6 % (SD 16.5 %), measured by urinary excretion over 5 days	Benzoic acid, dissolved in acetone	Feldmann, R. J., Maibach, H. I., J. Invest. Dermat. 54:399-404 (1970)
In vivo dermal absorption, no guideline, non GLP	Human, 7 (age 22-40, sex not specified); 8 (age 65-86, sex not specified)	4 µg/cm², 24 h, non-occlusive	Percutaneous absorption: 36.2 ± 4.6 % (age 22-40) 19.5 ± 1.6 % (age 65-86) Surface recovery 45.6 ± 2.8 % (age 22-40) 61.4 ± 2.0 % (age 65-86)	Benzoic acid, dissolved in acetone	Roskos, K. V., Maibach H. I., Guy, R. H., J. Pharma-cokinetics Biopharmac eutics 17(6):617-630 (1989)
In vivo dermal absorption,	Human, 6 (sex not specified);	4 µg/cm², 24 h, non-occlusive	Maximum absorption rate: *Man:* 3.0 %/h; *Dog:* 0.25 %/h	Benzoic acid, dissolved	Hunziker, N., Feld-mann, R. J.,

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels, Duration of exposure	Results	Remarks	Reference
no guideline, non GLP	Dog, Mexican hairless; 2 (sex not specified)		Persistance on/in skin: *Man:* essentially none (24 h) *Dog:* 30 % of initial dose (20 h) Urinary excretion : *Man:* extensive and rapid, being almost complete by day 3 *Dog:* less extensive and greatly prolonged.	in acetone	Maibach, H. I., Derma-tologica 156:79-88 (1978)
In vivo dermal absorption, no guideline, non GLP	Human, 6-7 M; Rhesus monkey, 3 F	3 (man only)-400-2000 µg/cm², 24 h, non-occlusive	Dermal absorption: Man: 37 ± 16.3 % at 3 µg/cm²; 25.7 ± 9.9 % at 400 µg/cm², 14.4 ± 3.8 % at 2000 µg/cm², Rhesus monkey: 33.6 ± 5.1 % at 400 µg/cm², 17.4 ± 1.2 % at 2000 µg/cm²	Benzoic acid, dissolved in methanol	Wester, R. C., Maibach H. I., J. Invest. Dermatol. 67:518-520 (1976)
In vivo dermal absorption, no guideline, non GLP	Rat, hairless Sprague-Dawley, 12 F	200/450 nmol/cm², 30 min	Percutaneous absorption: 200 nmol/cm²: 13.3 % 450 nmol/cm²: 17.6 %	^{14}C-benzoic acid, dissolved in ethanol/ water (95/5)	Rougier, A., Dupuis, D., Lotte, C., Roguet, R., Schaefer, H., J. Invest. Dermat. 81:275-278 (1983)
In vivo dermal absorption, no guideline, non GLP	Rhesus monkey, 4 F	4 mg/cm², Single dose: 24 h, non-occlusive, multiple dose: 8 x 24 h without washing between doses	Dermal absorption (urinary excretion within 5 days in comparison to intravenous admin.): *single dose:* 66 ± 19 %. *multiple dose:* 85 ± 19 % (initial dose), 89 ± 19 % (8th dose); No significant change in the percutaneous absorption was found after multiple dosing.	Benzoic acid, dissolved in acetone	Bucks, D. A. W., Hinz, R. S., Sara-son, R., Maibach, H. I., Guy, R. H., Fd Chem. Toxicol. 28(2):129-132 (1990)
In vivo dermal absorption, no guideline, non GLP	Guinea pig, hairless, 3-5 F	4 µg/cm², single dose	Dermal absorption (urinary excretion within 5 days in comparison to intraperitoneal admin.): *Normal skin:* 34.2 ± 9.4 %, Tape-stripped skin: 71.1 ±19.8 %, *Irritated skin (2 % SDS):* 73.4 ±14.6 %, delipidized skin (chloroform/methanol (2/1)): 94.1 ±4.8 %	Benzoic acid, dissolved in acetone	Moon, K. C., Wester, R. C., Maibach, H. I., Dermato-logica 180:8-12 (1990)
In vivo dermal absorption, no guideline,	Guinea pig, Hartley, 4 animals	4 µg/cm², single dose	Dermal absorption (urinary excretion within 5 days in comparison to intraperitoneal admin.):	Benzoic acid, dissolved in acetone	Andersen, K. E., Maibach, H. I., Anjo M.

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels, Duration of exposure	Results	Remarks	Reference
non GLP	(sex not specified)		31.4 %		D., Br. J. Dermatol. 102:447-453 (1980)
In vivo dermal absorption, no guideline, non GLP	Pig, Yorkshire swine, 4 (sex not specified)	40 µg/cm², single dose	Dermal absorption (urinary and faecal excretion within 6 days in comparison to intravenous admin.): 25.7 %	Benzoic acid, dissolved in ethanol	Carver, M. P., Riviere, J. E., Fund. Appl. Toxicol. 13:714-722 (1989)
In vitro tests					
In vitro dermal absorption, OECD 428, in part GLP (2/10 participating laboratories)	Human and rat skin	100 µg/cm², 24 h	Mean absorption rate: *Human skin (8 laboratories):* 16.54 ± 11.87 µg/cm²/h Rat skin (1 laboratory): 21.21 µg/cm²/h Total absorption: *Human skin:* 70.6 ± 17.2 % (24 h) Rat skin: 89.8 % (24 h) Total recovery: 53.6-98.5 % (7 laboratories)	Benzoic acid, dissolved in ethanol/ water (1:1) Multi-centre study	van de Sandt J. J. M., Burg-steden, J. A., Cage, S., Carmichael, P. L., Dick, I., Kenyon, S., Korinth, G., et al., Reg. Toxicol. Pharmacol. 39:271-281(2004)
In vitro dermal absorption, no guideline, non GLP	Human skin, F (abdo-minal)	No information available	Physicochemical parameters are investigated (partition coefficient, effective thickness, effective permeability). Those parameters do not allow to derive a percentual dermal absorption.	Scope of the study was to obtain parameters like diffusivity and partition coeffici-ents to calculate and predict *in vivo* permea-tion.	Parry, G. E., Bunge, A. L., Silcox, D. G., Per-shing, L. K., Pershing, D. W., Pharma-ceutical Res. 7(3):230-236 (1990)
In vitro dermal absorption, no guideline, non GLP	Skin of hairless guinea pigs (F)	2 µg/cm², single dose, 24 h exposure	Dermal absorption 49.5 % (receptor fluid: isotonic buffer) - 60.1 % (receptor fluid: water); Skin metabolism: 6.9 % of the applied dose was metabolised to hippuric aicd	Benzoic acid, dissolved in ethanol	Nathan, D., Sakr, A., Lichtin, J. L., Bro-naugh, R. L., Pharma-ceutical Res. 7(11): 1147-1151 (1990)

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

Benzoic acid is of low acute oral toxicity in rat, mouse, rabbit and dog, and of moderate acute oral toxicity in cat. The oral LD_{50} of benzoic acid in the rat is in the range of 2000 to 3040 mg/kg bw.

Table 5-4 Summary of acute oral toxicity studies

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels	Value LD50/LC50 Main effects	Remarks	Reference
Oral toxicity						
Pre-guideline, non-GLP	Oral	Rat, no further information available	No further information available	2000-2500 mg/kg bw	Benzoic acid, vehicle not specified	Ignatiev, A. D., Vop. Pitan. 24(3):61-68 (1965), Russian, abstract in English, no translation submitted
Non-guideline, non-GLP	Oral	Rat, no further information available	No information available	3040 mg/kg bw	Benzoic acid, vehicle not specified	Bio-Fax, data sheet no. 28-4/73 (1973), not submitted, cited in 1), 2), 3)
84/449/EEC, non-GLP	Oral	Rat, no further information available.	No information available	2565 mg/kg bw	Benzoic acid, vehicle not specified	IRDC, Report no. 163-282 (1974), not submitted, cited in 4), 6)
Pre-guideline, non-GLP	Oral	Rat, no further information available	No information available	1700 mg/kg bw	Benzoic acid, compilation of toxicology data, source of the LD_{50} value for benzoic acid not available	Fassett, D. W., Irish, D. D., Industrial Hygiene and Toxicology. Vol 2: Toxicology. Patty, F. A. (ed) Interscience Publishers Exist. York 1838-1839 (1962)
Pre-guideline, non-GLP	Oral, a: gavage, b: not reported	Rat, University of California strain (a), 5 M + 5 F and Sherman (b), 5M + 5 F	No information available	a: 2100 mg/kg bw b: 3450 mg/kg bw	Sodium benzoate (LD_{50} corrected for benzoic acid) a: fasted rats, vehicle unknown; b: unfasted rats, vehicle water	Deuel, H. J., Alfin-Slater, R., Weil, C. S., Smyth, H. F., Fd. Res., 19:1-12 (1954)
Pre-guideline,	Oral	Rat, Sherman,	No information	4070 mg/kg bw	Sodium benzoate,	Smyth, H. F. & Carpenter, C. P.,

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels	Value LD50/LC50 Main effects	Remarks	Reference
non-GLP		10 (sex not specified)	available		vehicle not specified	J. Industr. Hyg. Toxicol., 30:63-68 (1948)
84/449/EEC, non-GLP	Oral	Rat, no further information available.	No information available	3140 mg/kg bw	Sodium benzoate, vehicle not specified	Loeser, E., Bayer AG (Unpublished report, 1977), not submitted, cited in 2), 3)
Non-guideline, non-GLP	Oral	Mouse, no further information available.	No information available	2370 mg/kg bw	Benzoic acid, vehicle not specified	McCormick, G. C., Speaker T. J., Tox. Appl. Pharmacol. 25:478 (1973)
Non-guideline, non-GLP	Oral	Mouse, no further information available.	No information available	2250 mg/kg bw	Benzoic acid, vehicle not specified	BRL, Report no. 9348 (1979), not submitted, cited in 4), 6)
Non-guideline, non-GLP	Oral	Mouse, 10 animals (sex not reported)	1206-1447-1736-2038-2500-3000 mg/kg bw	1940 mg/kg bw	Benzoic acid, vehicle not specified in the English part of the text	Abe, S., Tsutsui, Y., Tarumoto, Y., Nakane, S., Iyakuhin Kenkyu 15 (3):359-370 (1984), Japanese, abstract and tables in English, no translation submitted
Pre-guideline, non-GLP	Oral, gavage	Rabbit, no further information available	1220-1520-1830 mg/kg bw	Lowest lethal dose: ca. 1500 mg/kg bw	Sodium benzoate, dissolved in water	Rost, E., Franz, F., Weitzel, A., Arbeit aus dem kaiserlichen Gesundheitsamte Bd. XLV 425-497 (1913), German, no translation submitted
Pre-guideline, non-GLP	Oral, gavage	Dog, Fox terrier	Range: 100 – 2880 mg/kg bw	Lowest lethal dose: ca. 2000 mg/kg bw	Sodium benzoate, dissolved in water	Rost, E., Franz, F., Weitzel, A., Arbeit aus dem kaiserlichen Gesundheitsamte Bd. XLV 425-497 (1913), German, no translation submitted
Pre-guideline, non-GLP	Oral	Cat, dog, rabbit no further information available.	No information available	Ca. 2000 mg/kg bw	Benzoic acid/benzoic salt, vehicle not specified	Ellinger, A., Handbuch der experimentellen Pharmakologie, Heffter, A.,

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels	Value LD50/LC50 Main effects	Remarks	Reference
						Springer Verlag Berlin, 1923, German, no translation submitted
Non-guideline, non-GLP	Oral, dietary	Cat, 4 M	0-2500-5000-10000 ppm (0-140-340-710 mg/kg bw)	Lowest lethal dose: 630 mg/kg bw Main effects: neurotoxic effects, subnormal temperature, lung, liver, kidney abnormalities	Benzoic acid	Bedford, P. G. C., Clarke, E. G. C., Vet. Rec. 90:53-58 (1972)

1) Anonymous (1995) Benzoic acid / sodium benzoate. BUA report 145. Ed.: GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance, 1995
2) IPCS (International Programme on chemical safety) (2000). Concise international chemical assessment document No 26 Benzoic Acid and Sodium Benzoate
3) Anonymous (2003). Review report for the inclusion of benzoic acid in Annex I of Directive 91/414/EEC, SANCO/1396/2001-Final
4) Anonymous, OECD-SIDS (2001). Benzoates, Review Report OECD SIDS
5) Opdyke, D. L., Fragrance raw materials monographs: Benzoic acid. Fd Cosmet. Toxicol. 17:715-722, 1979
6) REACH registration dossiers 2119455536-33-0000, 2119455536-33-0001 and the CSR attached to them

5.2.2 Acute toxicity: inhalation

Benzoic acid is of low acute inhalative toxicity in the rat with an LC_{50} of >1.2 mg/L x 6 h, the highest obtainable concentration of benzoic acid dust.

Table 5-5 Summary of acute inhalative toxicity studies

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels	Value LD50/LC50 Main effects	Remarks	Reference
OECD Non-GLP	Inhalative, 6 h	Rat, Sprague-Dawley CD, 10 M + 10 F	0-0.025-0.25-1.2 mg/L x 6 h	> 1.2 mg/L x 6 h	Benzoic acid (dust)	Rop, D. A., IRDC Report no: 163-676 (1981)
Non-guideline, non-GLP	Inhalative, 1 h	Rat, no further information available.	No information available	> 0.026	Benzoic acid, vehicle not specified	Bio-Fax, data sheet no. 28-4/73 (1973), not submitted, cited in 1), 2), 3)
Non-guideline, non-GLP	Inhalative, 4 h	Rat, Spartan	No information available	> 12.2 mg/L air	Benzoic acid (dust)	Goldenthal (1974), not submitted, cited in 4)

1) Anonymous (1995) Benzoic acid / sodium benzoate. BUA report 145. Ed.: GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance, 1995
2) IPCS (International Programme on chemical safety) (2000). Concise international chemical assessment document No 26 Benzoic Acid and Sodium Benzoate
3) Anonymous (2003). Review report for the inclusion of benzoic acid in Annex I of Directive 91/414/EEC, SANCO/1396/2001-Final
4) REACH registration dossiers 2119455536-33-0000, 2119455536-33-0001 and the CSR attached to them

5.2.3 Acute toxicity: dermal

Benzoic acid is of low acute dermal toxicity in the rabbit with LD_{50} values of up to 10000 mg/kg bw.

Table 5-6 Summary of acute dermal toxicity studies

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels	Value LD50/LC50 Main effects	Remarks	Reference
Non-guideline, non-GLP	Dermal	Rabbit, no further information available.	No information available	> 10000 mg/kg bw	Benzoic acid	Bio-Fax, data sheet no. 28-4/73 (1973), not submitted, cited in 1), 2), 3)
Non-guideline, non-GLP	Dermal	Rabbit, no further information available.	No information available	> 5000 mg/kg bw	Benzoic acid, vehicle not specified	Moreno, O. M., Report to RIFM (22.08.1977), not submitted, cited in 3), 4)
Non-guideline, non-GLP	Dermal	Rabbit, New Zealand White 4 animals	2000 mg/kg bw, 24 h	> 2000 mg/kg bw (male/female)	Benzoic acid, vehicle not specified, observation for mortality during 24 h	Goldenthal (1974), not submitted, cited in 5)

1) Anonymous (1995) Benzoic acid / sodium benzoate. BUA report 145. Ed.: GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance, 1995
2) IPCS (International Programme on chemical safety) (2000). Concise international chemical assessment document No 26 Benzoic Acid and Sodium Benzoate
3) Anonymous (2003). Review report for the inclusion of benzoic acid in Annex I of Directive 91/414/EEC, SANCO/1396/2001-Final
4) Opdyke, D. L., Fragrance raw materials monographs: Benzoic acid. Fd Cosmet. Toxicol. 17:715-722, 1979
5) REACH registration dossiers 2119455536-33-0000, 2119455536-33-0001 and the CSR attached to them

5.2.4 Acute toxicity: other routes

The LD_{50} values of benzoic acid after intravenous and intraperitoneal administration in the rat are 1712 mg/kg bw and 1460 mg/kg bw, respectively.

Table 5-7 Summary of acute toxicity - other routs

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels	Value LD50/LC50 Main effects	Remarks	Reference
Pre-guideline, non-GLP	Intravenous	Rat, 5 animals (sex and strain not reported)	Range: 1200-2290 mg/kg bw	1714 mg/kg bw	Sodium benzoate, dissolved in water	Hager, G. P., Chapman, C. W., Starkey, E. B., Journal of the American Pharmacology Association 31:253-255 (1942)
Pre-guideline, non-GLP	Intraperitoneal	Mouse,	No information available	1460 mg/kg bw	Benzoic acid, dissolved in isotonic saline	Caujolle, M. F., Meynier, D. C. R., Academie des Sciences 246:851-852 (1958), French, no translation submitted

5.2.5 Summary and discussion of acute toxicity

The low acute toxicity of benzoic acid in most species does not warrant classification and labelling for this endpoint. The cat represents the most sensitive mammalian species tested. This sensitivity is thought to be related to species-specific deficiencies in benzoate metabolism (no glucuronidation pathway). Therefore, the moderate acute oral toxicity observed in cats is not considered relevant for human health risk assessment and/or classification and labelling.

5.3 Irritation

5.3.1 Skin

Benzoic acid and sodium benzoate are not irritating to the skin (below classification threshold) in standard animal irritation studies (cf. Table 5-8). However, benzoic acid dissolved in ethanol (at concentrations of 1-20 %) was shown to elicit a concentration-dependent reaction in the guinea pig ear swelling test (Lahti & Maibach, 1984). By contrast, no response was noted in the rat or mouse ear for a concentration of 20 % (Lahti & Maibach, 1985).
In the literature, skin effects have frequently been reported for humans (cf. table 5-9 for selected publications).

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

Per definition, non-immunologic immediate contact reactions are considered irritant reactions (Lahti & Basketter, 2006) in contrast to immunogenic (allergic) contact reactions. NIICR of the skin are dose-dependent inflammatory reactions that occur within a short time of contact with the eliciting substance and do not require previous sensitisation. Susceptibility of the exposed individuals may vary widely. Contact reactions may involve development of erythema, oedema or appearance of wheals (Lahti & Basketter, 2006).

In an investigation with unselected volunteers (200), double-blind skin tests were run with benzoic acid (Basketter and Wilhelm, 1996). Benzoic acid was applied in chambers onto the skin for 20 min and assessed 30 min after the initiation of the application. In this study, a considerable proportion of volunteers (73.5 %) reacted to benzoic acid at 125 mM (ca. 1.5 % dilution) with erythema, while 12.5 % developed oedema at this test concentration. At 500 mM (ca. 6 % dilution), 78.5 % reacted with erythema and 18 % with oedema. The mean erythema severity scores (applying a grading of 0-8) were 1.81 at 125 mM and 2.12 at 500 mM, respectively, while the oedema scores after 30 min were 0.16 at 125 mM and 0.22 at 500 mM.

Frosch & Kligman (1976) investigated a new method, the chamber-scarification test for skin irritancy, as an alternative for the conventional patch test. Benzoic acid was applied once daily for 3 days in chambers on scarified and unscarified skin on the forearm of 5-10 healthy volunteers and rated 24 h after the last exposure. The irritant threshold concentration of benzoic acid on unscarified skin was 30 %, on scarified skin 7.5 %. Applying a five-point grading system of 0-4, a moderate response (score between 1.5-2.4) was observed at 7.5 % on scarified skin. A marked response, leading to erosions, was observed at 15 % (score between 2.5-4).

Regarding skin testing of benzoates, closed patch tests in patients with an atopic predisposition, asthma or urticaria have been reported (Baer et al., 1955: 4 % positive reactions; Broeckx et al., 1987 (0.7 %), Brasch et al., 1993 (0.2 %), among others). The vehicle used for dissolving benzoic acid or sodium benzoate is considered to be of great influence on the outcome of the test (e.g. Ylipieti & Lahti, 1989). A large amount of positive oral provocation tests with benzoates including skin reactions are also provided in the comprehensive literature (Warin & Smith, 1982 (11 %), Genton et al., 1985 (18 %), Schaubschläger et al., 1991 (14 %), among others).

Further characterisation of benzoic acid skin effects

In a human maximisation test, benzoic acid and related compounds did not show any sensitisation potential in volunteers (Leyden & Kligman, 1977). Investigations of Lahti (1980) indicated that NIICRs due to benzoates are not mediated by histamine. The reactions may be mediated by other vasoactive substances. An alteration in arachidonic acid metabolism or in abundance of eicosanoids has been proposed. In particular, prostaglandin D_2 (PGD_2) has been suspected to act as a mediator of nonimmune contact reactions, as it has been shown to be dose-dependently released in human skin after application of eliciting substances such as benzoic acid. In addition, the NIICR has been reported to be inhibited by oral or topical application of non-steroidal anti-inflammatory drugs that lead to inhibition of prostaglandin synthesis (reviewed by Lahti & Basketter, 2006). Ultraviolet irradiation appears to reduce and infra-red irradiation to increase the NIICRs induced by benzoic acid (Larmi 1989; Larmi et al., 1989a).

Table 5-8 Summary of skin irritation studies in animals

Method/ Guideline	Species, Strain, Sex, No/group	Results	Reversibility yes/no	Remarks	Reference
Pre-guideline, Non-GLP	Rabbit, New Zealand albino, 3 F	**Not irritating (below classification threshold)**; Primary score 0.5; slight erythema, slight oedema (1/3)	Yes	Benzoic acid, 500 mg, moistened	RCC Notox, study no. 0847/1083, 1988, not submitted, cited in 1), 3), 5), 7)

Method/ Guideline	Species, Strain, Sex, No/group	Results	Reversibility yes/no	Remarks	Reference
Pre-guideline, Non-GLP	Rabbit, strain, sex and number not specified	Not irritating	Not specified	Benzoic acid	IRDC, Report no. 163-282 (1974), not submitted, cited in 4), 7)
Pre-guideline, Non-GLP	Rabbit, strain, sex and number not specified	Not irritating (below classification threshold); Score 1.66/8	Not specified	Benzoic acid, 500 mg, dry powder	Bio-Fax, data sheet no. 28-4/73 (1973), not submitted, cited in 1), 2), 3)
OECD 404	Rabbit strain, sex and number not specified	Not irritating	Not specified	Sodium benzoate	RCC Notox, study no. 014658 (1989), not submitted, cited in 2), 3)
Pre-guideline, Non-GLP	Rabbit, strain, sex and number not specified	Not irritating	Not specified	Sodium benzoate, dry powder	Loeser, Bayer AG, 1977, not submitted, cited in 3)
Ear swelling test	Guinea pig, Hartley, 5 F	5/5 showed **erythema and swelling of the ear lobe** at concentrations of ≥ 1 %	Yes	Benzoic acid, dissolved in ethanol	Lahti, A., Maibach, H. I., Toxicol. Appl. Pharmacol. 76:219-224 (1984)
Ear swelling test	Guinea pig, Hartley, 10 F Rat, Sprague-Dawley, 10 F Mouse, ICR, 10 F	**Irritating** (erythema and ear swelling) in guinea pig Not irritating in rat Not irritating in mouse	Yes (Not applicable) (Not applicable)	Benzoic acid, 20 %, dissolved in ethanol Negative results in rat and mouse	Lahti, A., Maibach, H. I., Journal of the American Academy of Dermatology 13: 66-69 (1985)
Pre-guideline, Non-GLP 1, 3, 5, 7.5, 10, 20 %	Rat, Wistar, 10 M	≥ 3 % sodium benzoate: **Moderate to severe irritation**. Exponential dose response between 1 – 10 %	Not specified	Sodium benzoate in saline **Intradermal injection**	Stol, M., Cifkova, I., Brynda, E., Biomaterials 9:273-276 (1988)

1) Anonymous (1995) Benzoic acid / sodium benzoate. BUA report 145. Ed.: GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance, 1995
2) IPCS (International Programme on chemical safety) (2000). Concise international chemical assessment document No 26 Benzoic Acid and Sodium Benzoate
3) Anonymous (2003). Review report for the inclusion of benzoic acid in Annex I of Directive 91/414/EEC, SANCO/1396/2001-Final
4) Anonymous, OECD-SIDS (2001). Benzoates, Review Report OECD SIDS
5) Opdyke, D. L., Fragrance raw materials monographs: Benzoic acid. Fd Cosmet. Toxicol. 17:715-722, 1979
6) EU, Scientific Committee on Consumer Products: Opinion on Benzoic Acid and Sodium Benzoate. SCCP/0891/05 (2005)
7) REACH registration dossiers 2119455536-33-0000, 2119455536-33-0001 and the CSR attached to them

Table 5-9 Observations in humans

Kind of study (e.g. case reports)	Examination methods, number of individuals examined	Results	References
Skin test on non-immune immediate contact reactions, double-blind	45 M + 155 F, 125 mM and 500 mM benzoic acid in petrolatum, in petrolatum;, patch application for 20 min	Erythema: Mean score (0-8) 30 min after initiation: at 125 mM: 1.81 at 500 mM: 2.12; Positive reactions: at 125 mM: 147/200 (73.5% of volunteers) at 500 mM: 157/200 (78.5%) Oedema: Mean score (0-8) *30 min after initiation:* 125 mM: 0.16 500 mM: 0.22; Positive reactions: at 125 mM: 25/200 (12.5 %) at 500 mM: 36/200 (18 %)	Basketter, D. A., Wilhelm, K. P., Contact Dermat. 35:237-240 (1996) Key study
Chamber scarification test for irritancy	5-10 volunteers, 7.5/15/30 % benzoic acid in ethanol	30 % benzoic acid: threshold concentration in unscarified skin, 7.5 % benzoic acid: Threshold concentration in scarified skin, moderate response 15 % benzoic acid: Marked irritation with erosions in scarified skin	Frosch, PJ, Kligman, AM, Contact Dermat. 2:314-324 (1976) Key study
Open application test	13 healthy volunteers, 4-8-16 mM benzoic acid (10 µL) in petrolatum	Positive reactions (erythema after 40 min.): 16 mM benzoic acid: 12/13: cheek; 6/13: forehead, neck; upper back	Larmi, E., Lahti, A., Hannuksela, M., Contact Dermat. 20:38-40 (1989)
Open application test	12 healthy volunteers, 0, 15, 31, 62, 125, 250 mM benzoic acid in 2-propyl alcohol and 1,2-propylene gylcol (75%/25%)	Positive reactions in all tested subjects	Lahti, A., Pylvänen, V., Hannuksela, M., Contact Dermatitis 33:177-182 (1995)
Patch tests in humans	113 patients with dermatoses; 5 % benzoic acid in petrolatum	5 slight reactions ("definite erythema")	Baer, R. L., Serri, F. A., Weissenbach-Vial, C., AMA Arch. Dermatol. Syphil. 71:19-23 (1955)
Patch tests in humans, multicenter study	2045 patients with dermatoses (766 M + 1279 F), 5 % sodium benzoate in petrolatum	Slight reaction: 4/2045 (0.2 %) moderate reaction: 1/2045 (0.05 %)	Brasch, J., Henseler, T., Frosch, P., Dermatosen 41(2):71-76 (1993)

Kind of study (e.g. case reports)	Examination methods, number of individuals examined	Results	References
Patch tests in humans for contact dermatitis to cosmetics	5202 patients with contact dermatitis (1872 M + 3330 F), benzoic acid	Positive reactions: 34/5202 (0.7 %)	Broeckx, W., Blondeel, A., Dooms-Goossens, A., Achten, G., Contact Dermat. 16:189-194 (1987)
Patch tests for contact dermatitis, multicenter study	465 patients with dermatitis, benzoic acid	Positive reactions: 10/465 (2.1 %)	Meynadier, J. M., Meynadier, J., Colmas, A., Castelain, P. Y., Ducombs, G. et al., Ann. Dermatol. Venereol. 109:1017-1023 (1982), French publication, no translation submitted
Chamber method with 20 min occlusion	110 dermatology patients (incl. 36 atopic patients), 5 % benzoic acid in petrolatum	Positive reactions Atopic patients: 10/36 (28 %), urticaria patients: 9/23 (39 %), non-atopic dermatitis patients 14/26 (54 %), healthy controls: 10/25 (40 %) total: 43/110 (39 %)	Lahti, A., Acta Derm. Venerol. 60 (suppl. 91):1-49 (1980)
Patch test	10 volunteers allergic to benzoyl peroxide, 5 % benzoic acid in petrolatum	Positive reactions: 0/10	Leyden, J. J., Kligman, A. M., Contact Dermat. 3:273-275 (1977)
Patch test	111 patients with urticaria, 50, 500 mg sodium benzoate	Positive reactions: 12/111 (11 %)	Warin, R. P., Smith, R. J., Br. J. Dermatol. 94:401-406 (1976)
Patch test	11 healthy volunteers, 3 patients with dermatoses; 50-100-250-500-1000 mM benzoic acid (10 µL) in different vehicles	NIICRs were stronger for formulations based on alcohol-water mixtures and stronger for formulations in 2-propyl alcohol than for ethanol-based formulations	Ylipieti, S., Lahti, A., Contact Dermat. 21:105-106 (1989)
Open application test	5 M + 11 F (healthy volunteers), 125 mM + 500 mM benzoic acid in different solvents	Addition of water or propylene glycol to alcoholic vehicles enhanced non-immunologic contact reactions.	Lahti, A., Poutiainen, A. M., Hannuksela, M., Contact Dermat. 29:22-25 (1993)
Open application test	10-12 healthy volunteers/group, 31-62-125-250 mM benzoic acid (10 µL) in petrolatum	UVA irradiation and UVB irradiation diminished NIICR to benzoic acid.	Larmi, E., Acta Derm Venerol 69:296-301 (1989)
Open application test	10-12 volunteers/group, 31-62-125-250 mM benzoic acid (10 µL) in petrolatum	Infra-red irradiation deteriorated NIICR to benzoic acid	Larmi, E., Lahti, A., Hannuksela, M., Dermatosen 37(6):210-214 (1989)

5.3.2 Eye

Benzoic acid is highly irritating to the eyes. The effects observed on the eyes comprised corneal opacity (severe in 1/3 animals and not reversible in 2/3 animals), no reaction to light up to d 21 (1/3 animals), non-reversible chemosis (2/3 animals), iridial injection and conjunctival redness (RCC Notox study no. 0847/1084, 1988, as reported in the SCCP report (SCCP/0891/05-Opinion on Benzoic Acid and Sodium Benzoate, EU, 2005). Original data and scores are not available.

Information on the eye irritating potential of sodium benzoate is scarce. In a study (RCC NOTOX study no. 014669, 1988) that has been cited in several expert review documents (cf. table 5-8), sodium benzoate was reported to be slightly irritating. The pH resulting from instillation of the test substance is tentatively assumed to play a role in the apparent differences in eye irritating potential between benzoic acid and sodium benzoate.

Table 5-10 Summary of eye irritation studies

Method/ Guideline	Species, Strain, Sex, No/group	Results	Reversibility yes/no	Remarks	Reference
Sim. to OECD 405	Rabbit, New Zealand albino, 3 F	**Highly irritating;** Translucent corneal opacity, iridial injection, moderate chemosis, slight conjunctival redness, which increased after 2 days to severe with a white/grey discoloration.	No (corneal opacity: 2/3 animals; No reaction to light: 1 animal; Chemosis: 2/3 ani-mals)	Benzoic acid, 77 mg fine powder	RCC Notox, study no. 0847/1084, 1988, not submitted, cited in 1), 3), 5), 6), 7)
OECD 405	Rabbit, New Zealand albino, 3 M	Not irritating (below classification threshold)	No data	Benzoic acid	Suberg, Bayer AG, 1986, not submitted, cited in 2)
No guideline, non-GLP	Rabbit	Highly irritating; Irritation score 65.0/110	No data	Benzoic acid, 100 mg fine powder	Bio-Fax, data sheet no. 28-4/73 (1973), not submitted, cited in 1), 2), 3)
No guideline, non-GLP	Rabbit	Highly irritating	No data	Benzoic acid	IRDC #163-282, 1974, not submitted, cited in 4), 7)
Sim. to OECD 405	Rabbit	Not irritating	No data	Sodium benzoate, 50 mg	Loeser, E., Bayer AG data, 1977, not submitted, cited by 1), 2), 4), 6)
OECD 405	Rabbit	Slightly irritating Score 9.3 according to scheme of Kay & Calandra, 1962	Reversed within 14 d	Sodium benzoate	RCC NOTOX study no. 014669, 1988, not submitted, cited in 1), 2), 4), 6)

1) Anonymous (1995) Benzoic acid / sodium benzoate. BUA report 145. Ed.: GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance, 1995
2) IPCS (International Programme on chemical safety) (2000). Concise international chemical assessment document No 26 Benzoic Acid and Sodium Benzoate
3) Anonymous (2003). Review report for the inclusion of benzoic acid in Annex I of Directive 91/414/EEC, SANCO/1396/2001-Final
4) Anonymous, OECD-SIDS (2001). Benzoates, Review Report OECD SIDS
5) Opdyke, D. L., Fragrance raw materials monographs: Benzoic acid. Fd Cosmet. Toxicol. 17:715-722, 1979
6) EU, Scientific Committee on Consumer Products: Opinion on Benzoic Acid and Sodium Benzoate. SCCP/0891/05 (2005)
7) REACH registration dossiers 2119455536-33-0000, 2119455536-33-0001 and the CSR attached to them

5.3.3 Respiratory tract

Regarding observations in animal studies, respiratory tract irritation was observed in a rat study (cf. chapter 5.6.2). This effect was attributed to the physico-chemical properties of fine low-solubility particles (dust study) and not to benzoic acid-inherent properties.

In humans, cases of respiratory reactions such as rhinitis and asthma have been reported for susceptible persons following oral, dermal or inhalation exposure to benzoic acid and sodium benzoate (Hannuksela & Haahtela, 1987; IPCS, 2000).

5.3.4 Summary and discussion of irritation

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

Based on irreversible eye damage reported in several EU assessments (original data are not available), benzoic acid has to be labelled with **R41 resp. H318**.

5.4 Corrosivity

Irritation studies revealed no corrosive potential of benzoic acid.

5.5 Sensitisation

5.5.1 Skin

Several sensitisation studies including LLNA, GPMT and Buehler tests revealed no sensitising potential of benzoic acid.

Table 5-11 Summary of skin sensitisation studies

Method/ Guideline	Species, Strain, Sex, No/group	Number of animals sensitised/ total number of animals	Results	Remarks	Reference
LLNA; Non-GLP, sim. to OECD 429	Mouse, CBA/J, 5 F	0/5	Negative, not sensitising	Benzoic acid, dissolved in acetone	Gerberick, G. F., House, R. V., Fletcher E. R., Ryan, C. A., Fund. Appl Toxicol 19:438-445 (1992), also cited in 1)
GPMT (M+K), Non-GLP, sim. to OECD 406	Guinea pig, Hartley test group: 15 control group: 6	0/15	Negative, not sensitising	Benzoic acid, dissolved in water	Gad, S. C., Dunn, B. J., Dobbs, D. W., Reilly, C., Walsh, R. D., Toxicol. Appl. Pharmacol. 84:93-114 (1986)
Buehler, Non-GLP, no guideline	Guinea pig, strain, number and sex not specified	0 %	Negative, not sensitising	Benzoic acid, dissolved in water	Gad, S. C., Dunn, B. J., Dobbs, D. W., Reilly, C., Walsh, R. D., Toxicol. Appl. Pharmacol. 84:93-114 (1986)
Mouse ear swelling test, Non-GLP, no guideline	Mouse, CF-1, test group: 10-15 F, control group: 5-10 F	0 %	Negative, not sensitising	Benzoic acid, dissolved in acetone	Gad, S. C., Dunn, B. J., Dobbs, D. W., Reilly, C., Walsh, R. D., Toxicol. Appl. Pharmacol. 84:93-114 (1986), also cited in 1)
Buehler Non-GLP, no guideline	Guinea pig, Hartley, male, 10	0/10	Negative, not sensitising	Benzoic acid, solvent not reported	Bier (1979), not submitted, cited in 1)
Patch-Test	human male/female		occasional positive result	Test material (Common name): Benzoic acid	Rademaker & Forsyth (1988), not submitted, cited in 1)
Patch-Test	human male/female		occasional positive result	Test material (Common name): Balsam of Peru	Forsbeck & Skog (1977), not submitted, cited in 1)

1) REACH registration dossiers 2119455536-33-0000, 2119455536-33-0001 and the CSR attached to them

In the registration dossiers following information on the patch tests in humans are given; “Positive skin reactions were seen after application with benzoic acid in patch tests performed in a hospital with 125 children. Since no details of the study were available, no conclusion on the skin sensitizing properties of benzoic acid could be drawn based on this study.

In a patch tests with balsam of Peru (containing a. o. 5% benzoic acid), gave rise to nine immediate reactions among 121 patients with different dermatoses and to 10 reactions amoung 57 patients with chronic urticaria. Urticarial reactions were noted in 3 of 5 patients after dermal exposure to 5% benzoic acid. No conclusion can be drawn on the sensitizing potential of benzoic acid, due to the limited reporting of the study.”

5.5.2 Respiratory system

There is no evidence for respiratory sensitisation.

5.5.3 Summary and discussion of sensitisation

Benzoic acids and its salts are not considered to be skin or respiratory sensitisers but they cause pseudoallergic, non-immunogenic skin and respiratory reactions (cf. chapter 5.3.1). Thus, no classification and labelling regarding sensitisation is required.

5.6 Repeated dose toxicity

5.6.1 Repeated dose toxicity: oral

Subacute/subchronic studies

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

Typical signs caused by benzoic acid in the rat were similar to those from acute studies and included ataxia, aggressiveness, tremor, convulsions, reduction of food consumption and bw gain, and mortality. With few exceptions, histopathological evaluation was not performed or included only few organs. A 35-d CNS toxicity study reported regional CNS necrosis from daily doses of 2250 mg/kg in the rat (Kreis et al., 1967). The kidney weight was increased in some studies (Deuel et al., 1954; Fujitani 1993). Generally, LOAEL values for the rat were between 1200 and 2250 mg/kg bw/d for subacute and subchronic exposure without strict correlation to exposure time. A reliable NOAEL for derivation of threshold limit values may be based on a multigeneration study (Kieckebusch and Lang, 1960). In this study, no changes in bw gain (data supplied only for up to 12/8 weeks for M/F) and histopathology of key organs performed after wk 16 were reported at the top dose of 500 mg/kg bw/d benzoic acid. Survival at this dose remained unaffected. NOAELs reported in other studies were between 500 and 1360 mg/kg bw/d. Mortalities and CNS effects observed at doses ≥ 1200 mg/kg bw/d are considered to be related to metabolic acidosis after saturation of glycine and glucuronate conjugation pathways (Praphanphoj et al., J. Inherit. Metab. Dis. 23:129-136, 2000; Kalbag & Palekar, Biochem. Med. Metabol. Biol. 40:133-142, 1988). Thus, this effect is not regarded to deteriorate from subchronic to chronic exposure.

The submitted data (NOAEL/LOAEL = 600/800 mg/kg bw/d, 25-d oral) further supports the conclusion, that dogs are of similar sensitivity to subacute benzoate toxicity as rats and show similar

symptoms (Rost et al., 1913). In contrast, results by Fujitani (1993) suggest that the mouse is less sensitive (NOAEL/LOAEL = 3750/4500 mg/kg bw/d, 10-d oral), but typical adverse effects were similar to that observed in the rat and included tremor/convulsions as well as liver toxicity.

The cat represents the most sensitive mammalian species tested. This sensitivity is thought to be related to species-specific deficiencies in benzoate metabolism paired with a cat-typical feeding behaviour (Bedford and Clarke, 1972). Therefore, the subacute NOAEL/LOAEL derived for cats (200/340 mg/kg bw/d, 15-d feeding) is not considered relevant for human risk assessment.

Chronic studies

Four studies that aimed to assess the chronic toxicity of benzoic acid in rat and mice were submitted. Of these, only two rat studies are suitable for risk assessment, although these two studies do neither meet current EU nor OECD standards for chronic toxicity testing (Kieckebusch and Lang, 1960; Marquardt, 1960). Both did not go beyond assessment of survival and clinical signs. Gross and histopathology in one study (Kieckebusch and Lang, 1960) was performed after subchronic exposure and body weight was reported for an initial 12/8 wks (M/F) only. In this study, the top dose of 10,000 ppm, corresponding to approx. 500 mg/kg bw/d, neither reduced lifetime survival nor produced overt clinical symptoms of benzoic acid poisoning. Feeding of 15,000 ppm (~750 mg/kg bw/d) benzoic acid over 18 months increased mortality and reduced body weight and body weight gain in comparison to control rats (Marquardt, 1960). Data obtained at later time points and haematological, biochemical or histopathological reports were not available for this study. In summary, it may be concluded that a dose of 500 mg/kg bw/d, as identified as safe in rodents for subchronic exposure, neither affected survival nor produced clinical signs of benzoic acid poisoning when exposure time was extended. However, the available chronic toxicity data is not suitable to establish a NOAEL for target organ (liver, kidneys, brain) toxicity.

Mortalities observed at 750 mg/kg bw/d in the study by Marquardt and in subacute/subchronic studies at 1200/1500 mg/kg bw/d are considered to be related to metabolic acidosis after saturation of glycine and glucuronic acid conjugation pathways (Praphanphoj et al., J. Inherit. Metab. Dis. 23:129-136, 2000; Kalbag & Palekar, Biochem. Med. Metabol. Biol. 40:133-142, 1988). Metabolic acidosis was also observed in acute studies and human case reports when high doses of benzoic acid/benzoate were administered repeatedly within 10-24 h. Thus, this effect is not regarded to aggravate from subacute/subchronic to chronic exposure.

Table 5-12 Summary of oral repeated dose studies

Method/ Guideline	Route of exposure, Duration	Species, Strain, Sex, No/group	Dose levels	NO(A)EL ppm (mg/kg bw /d)	LO(A)EL ppm (mg/kg bw /d)	Results Main effects/ Target organs	Remarks	Reference
Oral, rat								
Subacute toxicity, non-GLP	Oral (feeding), 10 d	Rat, F344, 6 M + 6 F	0-18,100-20,900-24,000 ppm (~0-900-1050-1200 mg/kg bw/d)	20,900 ppm (~1050 mg/kg bw/d)	24,000 ppm (~1200 mg/kg bw/d)	24,000 ppm: Convulsions + mortality (1 M), bw↓, rel. liver weight ↑, GGT↑ (M), hepatocyte enlargement (M), kidney weight↑	Sodium benzoate	Fujitani, T., Toxicol. Letters 69:171-179 (1993)
Sub-chronic toxicity, non-GLP	Oral (feeding), 16 wk	Rat, 20 M + 20 F	0-5,000-10,000 ppm (~0-250-500 mg/kg bw/d)	10,000 ppm (~500 mg/kg bw/d)	> 10,000 ppm (> 500 mg/kg bw/d)	No changes in bw gain and bw, survival, organ weight (brain, heart, liver, spleen, kidneys, testes) or liver histology	Benzoic acid and sodium benzoate, Doc III A6.8.2 Reporting of bw (F) until wk 8, bw (M) also at wk 12, organ weight and liver histology at wk 16	Kieckebusch, W., Lang, K., Arzneim. Forsch. 10:1001-1003 (1960), also cited in 1) German
Subacute range-finding study, non-GLP	Oral (feeding), 30 d	Rat, Sherman, 5 M + 5 F	0-16-1090 mg/kg bw/d	1090 mg/kg bw/d	> 1090 mg/kg bw/d	No changes in bw, food consumption, adrenals, upper intestine, kidney, liver, spleen; no mortality	Sodium benzoate	Smyth, H. F., Carpenter, C. P., J.Industr. Hyg. 30:63-68 (1948)
Subacute clinical toxicity, non-GLP	Oral (feeding), 4(-5) wk	Rat, Albino, 8 M	0-10,000-30,000 ppm (~0-500-1500 mg/kg bw/d)	10,000 ppm (~500 mg/kg bw/d)	30,000 ppm (~1500 mg/kg bw/d)	Irritability (increased reaction to touch or disturbance), aggressiveness, uncoordinated movements, convulsions, bw↓, mortality (2/8)	Sodium benzoate, based on clinical observations and bw only	Harshbarger, K. E., J. Dairy Sci. 25:169-174 (1942)
Subacute clinical and CNS toxicity, non-GLP	Oral (feeding), 35 d	Rat, Juvenile Wistar, 5-15 M	0-11,000-30,000 ppm (~0-825-2250 mg/kg bw/d)	11,000 ppm (~825 mg/kg bw/d)	30,000 ppm (~2250 mg/kg bw/d)	Necrosis of the cortex piriformis and the stratum granulosum of the fascia dentata; from d 4: ataxia, tremor, excitation, aggressive behavior, tonoclonic convulsions	Benzoic acid	Kreis, H., Frese, K., Wilmes, G., Fd. Cosmet. Toxicol., 5:505-511 (1967), also cited in 1) German

Method/ Guideline	Route of exposure, Duration	Species, Strain, Sex, No/group	Dose levels	NO(A)EL ppm (mg/kg bw /d)	LO(A)EL ppm (mg/kg bw /d)	Results Main effects/ Target organs	Remarks	Reference
Subacute range-finding study, non-GLP	Oral (feeding), 6 wk	Rat F344, 10 M + 10 F	0-5,000-10,000-20,000-40,000-80,000 ppm (~0-250-500-1000-2000-4000 mg/kg bw/d)	20,000 ppm (~1000 mg/kg bw/d)	40,000 ppm (~2000 mg/kg bw/d)	≥ 40,000 ppm: Mortality (10/10 M), bw↓ 80,000 ppm: Mortality (20/20)	Sodium benzoate, based on clinical observations and bw only; "hypersensitivity" observed in all benzoate-treated rats, was not considered adverse due to reporting deficiencies	Sodemoto, Y., Enomoto, M., J. Environ. Pathol. Toxicol. 4:87-95 (1980)
Subacute clinical toxicity, non-GLP	Oral (feeding), 40 d	Rat, Albino, 10(-30) M	0-15,000-20,000-25,000-30,000-32,500-35,000-37,500 ppm (~0-750-1000-1250-1500-1625-1750-1825 mg/kg bw/d)	15000 ppm (~750 mg/kg bw/d)	20000 ppm (~1000 mg/kg bw/d)	≥ 20,000ppm: Bw↓ ≥ 25,000 ppm: Mortality, tremor, convulsions, restlessness	Sodium benzoate; based on clinical observations and bw only	Griffith, W. H., J. Biol. Chem. 82:415-427 (1929)
Sub-chronic toxicity, non-GLP	Oral (feeding), 90 d	Rat, Sherman, 4 M + 4 F	0-10,000-20,000-40,000-80,000 ppm (0-640-1320-2620-6290 mg/kg bw/d)	40,000 ppm (2620 mg/kg bw/d)	10,000 ppm (6290 mg/kg bw/d)	6290 mg/kg bw/d: Liver weight↑, kidney weight↑, bw gain↓, mortality (4/8), pathological kidney and liver lesions	Sodium benzoate, No individual data reported	Deuel, H. J., Alfin-Slater, R., Weil, C. S., Smyth, H. F., Fd. Res., 19:1-12 (1954)
Oral, other species								
Subacute toxicity, non-GLP	Oral (feeding), 10 d	Mouse, B6C3F1, 5 M + 4-5 F	0-20,800-25,000-30,000 ppm (~0-3010-3750-4500 mg/kg bw/d)	25,000 ppm (~3750 mg/kg bw/d)	30,000 ppm (~4500 mg/kg bw/d)	Irritability, convulsions, death (F), liver weight↑, cholesterol↑ (M), phospholipids↑ (M), hepatocyte enlargement, necrosis and vacuolation (M)	Sodium benzoate	Fujitani, T., Toxicol. Letters 69:171-179 (1993)
Sub-	Oral	Mouse,	0-80 mg/kg bw/d	N/A	N/A	N/A	Benzoic acid,	Shtenberg, A. J.,

Method/ Guideline	Route of exposure, Duration	Species, Strain, Sex, No/group	Dose levels	NO(A)EL ppm (mg/kg bw /d)	LO(A)EL ppm (mg/kg bw /d)	Results Main effects/ Target organs	Remarks	Reference
chronic toxicity, non-GLP	(gavage), 90 d	Albino, 50 M + 50 F					Not suitable for risk assessment	Ignat'ev, A. D., Fd Cosmetic Toxicol. 8:369-380 (1970)
Subacute dose-escalation, non-GLP	Oral (feeding), ~25 d	Dog, Fox terrier 4	Escalating from ~40 to ~1700 mg/kg bw/d	~600 mg/kg bw/d	~860 mg/kg bw/d	Tremor, convulsions, ataxia, mortality	Sodium benzoate, clinical signs recorded only	Rost, E., Franz, F., Weitzel, A., Arbeit aus dem kaiserlichen Gesundheitsamte Bd. XLV 425-497 (1913), German, No translation submitted
Subacute clinical toxicity	Oral (feeding), 15 d	Cat, 4 M	0-100-200-340-710 mg/kg bw/d	200 mg/kg bw/d	340 mg/kg bw/d	Clinical findings: Mortality, aggressiveness, hyperaesthesia, convulsions, salivation, constipation, urine retention Histopathological findings: Liver (foamy granular cytoplasm of hepatocytes, macrophage infiltration, vacuolation), Kidney (swollen tubules, tubule herniation), Lung (oedema, haemorrhages), Heart (myocardial foci of cellular infiltration, degeneration)	Benzoic acid	Bedford, P. G. C., Clarke, E. G. C., The Veterinary Record 90(3):53-38 (1972)

1) REACH registration dossiers 2119455536-33-0000, 2119455536-33-0001 and the CSR attached to them

Chronic studies

Method/ Guideline	Route of exposure, duration	Species, Strain, Sex, No/group	Dose levels	NO(A)EL ppm (mg/kg bw/d)	LO(A)EL ppm (mg/kg bw/d)	Results Main effects/ Target organs/ Tumours	Remarks	Reference
Chronic survival, non-GLP	Oral (feeding), Lifetime 3-generation	Rat, 20 M + 20 F	0-5,000-10,000 ppm (~0-250-500 mg/kg bw/d)	10,000 ppm (500 mg/kg bw/d)	> 10,000 ppm (> 500 mg/kg bw/d)	No reduction in survival, all other endpoints not reported after chronic exposure	Benzoic acid and sodium benzoate, histopathology at wk 16, bw up to wk 8 (F) or 12 (M)	Kieckebusch, W., Lang, K., Arzneim. Forsch. 10:1001-1003 (1960), German
Chronic toxicity, non-GLP	Oral (feeding), 18 mo	Rat, Wistar, 30 M + 20 F (control: 13 M + 12 F)	0-15,000 ppm (~0-750 mg/kg bw/d)	< 15,000 ppm (< 750 mg/kg bw/d)	15,000 ppm (~750 mg/kg bw/d)	Mortality↑ (30 vs. 15 %), bw and bw gain↓, food consumption↓, no behavioural changes	Benzoic acid, interim report, no other endpoints reported	Marquardt, P., Arzneim. Forsch. 10:1033 (1960), German
Chronic toxicity	Oral (feeding), 18 mo	Rat, Wistar, 10 M + 10 F	0-40 mg/kg bw/d	N/A	N/A	No relevant changes reported	Benzoic acid, not suitable for risk assessment	Shtenberg, A. J., Ignat'ev, A. D., Fd Cosmetic Toxicol. 8:369-380 (1970)
Chronic toxicity	Oral (feeding), 17 mo	Mouse, Albino, 25 M + 25 F	0-40 mg/kg bw/d	N/A	N/A	In response to food withdrawal (40 mg/kg bw/d): mortality↑, weight loss↑	Benzoic acid, not suitable for risk assessment	Shtenberg, A.J., Ignat'ev, A.D., Fd Cosmetic Toxicol. 8:369-380 (1970)

5.6.2 Repeated dose toxicity: inhalation

Inhalation toxicity of benzoic acid was evaluated in one rat study using fine benzoic acid dust with a mean aerodynamic particle diameter of 4.7 µm (Rop, 1981). Evaluation of survival, body weight and organ weight demonstrated significant systemic toxicity including 2/20 mortalities at the top dose of 1.2 mg/L. A reddish discharge around the nares was observed at doses ≥ 0.25 mg/L. In addition to systemic toxicity, compound-related microscopic lesions consisting of multifocal to generalised inflammatory cell infiltrates and interstitial fibrosis of the lung were observed. This was reported for all groups of benzoic acid treated animals with a concentration-dependent increase in intensity and incidence. It is widely accepted, that particles of low-solubility materials retained in the lower lung are cleared by phagocytic cells, mostly alveolar macrophages. Excessive activation of these cells is linked to the release of ROS (reactive oxygen species) as well as inflammatory and fibrogenic mediators initiating lung remodelling well described for silica pneumoconiosis (Kim et al., 2000; Wallace et al., 2007). The systemic effects observed at higher concentrations differed from those observed after oral exposure at higher concentrations (organ weight decrease, platelet decrease) and are thought to be secondary to local lung toxicity. Because the toxic effects observed in this study are attributed to the physico-chemical properties of these fine low-solubility particles, the study is not considered relevant for the evaluation of human health effects after repeated exposure to fluid benzoic acid formulations used in biocidal applications.

Table 5-13 Summary of inhalative repeated dose studies

Method/ Guideline	Route of exposure, Duration	Species, Strain, Sex, No/group	Dose levels	NO(A)EL ppm (mg/kg bw /d)	LO(A)EL ppm (mg/kg bw /d)	Results Main effects/ Target organs	Remarks	Reference
Sim. to OECD 412, non-GLP	Inhalative, 4 wk (5 d/wk, 6 h/d)	Rat, Sprague-Dawley CD, 10 M + 10 F	0-0.025-0.25-1.2 mg/L (corr. to 0-7-70-320 mg/kg bw/d)	Pulm.: N/A Systemic: 0.25 mg/L (~70 mg/kg bw/d)	Pulm.: ≤ 0.025 mg/L Systemic: 1.2 mg/L (~320 mg/kg bw/d)	Pulmonary: Interstitial inflammation, lung fibrosis Systemic: Mortality (2/20), bw↓, liver weight↓ (M), kidney weight↓ (F), platelet count↓	Benzoic acid, fine dust, mean aerodynamic diameter: 4.7 µm	Rop, D. A., IRDC Report no: 163-676 (1981), also cited in 1)

1) REACH registration dossiers 2119455536-33-0000, 2119455536-33-0001 and the CSR attached to them

5.6.3 Repeated dose toxicity: dermal

The NOAEL for subacute dermal toxicity of benzoic acid in rabbits is 2500 mg/kg bw/d (the highest dose tested) when applied 5 days per week over 3 weeks (OECD SIDS Dossier on Benzoates, 2001). No effects were observed at this dose level.

Table 5-14 Summary of dermal repeated dose toxicity

Method/ Guideline	Route of exposure, Duration	Species, Strain, Sex, No/group	Dose levels	NO(A)EL ppm (mg/kg bw /d)	LO(A)EL ppm (mg/kg bw /d)	Results Main effects/ Target organs	Remarks	Reference
Unknown	Dermal, 3 wk (5 d/wk)	Rabbit, New Zealand White, 8 (sex unknown)	0-100-500-2500 mg/kg bw/d	2500 mg/kg bw/d	> 2500 mg/kg bw/d	No effects observed	Benzoic acid (formulation unspecified)	IRCD Project no. 163-675 (1981), not submitted, cited in 1), 2)

1) REACH registration dossiers 2119455536-33-0000, 2119455536-33-0001 and the CSR attached to them

2) Anonymous, OECD-SIDS (2001). Benzoates, Review Report OECD SIDS

5.6.4 Other relevant information

No other relevant information is available.

RAC rapporteur mentioned following observation in the outcome of the accordance check:

> There is evidence for pulmonary toxicity by benzoic acid dust inhalation. This effect is considered not relevant, as it does not apply to dissolved preparation (as used in biocides). However, this may be relevant for workers exposed to solid benzoic acid and/or benzoate, a point that should be addressed. If considered relevant, the CLP labelling STOT RE (pulmonary, inhalation), Category 1 would be applicable. Depending on the interpretation of data, this labelling could be downgraded to Category 2

The dossier submitter is not convinced that STOT-RE would be the appropriate classification for effects in lung, which occurred after repeated inhalation of benzoic acid. This compound induced serious eye damage and was irritating to skin. Hence it might be speculated, that these lung effects were more related to local irritation than a manifestation of systemic effects.

5.6.5 Summary and discussion of repeated dose toxicity:

No classification and labelling regarding repeated dose toxicity or chronic toxicity is necessary.

5.7 Mutagenicity

5.7.1 In vitro data

While the reverse mutation assays with *Salmonella typhimurium* and sister chromosome exchange assays (except one equivocal result) with benzoic acid, sodium benzoate and the metabolite hippuric acid were negative, weak genotoxic effects or equivocal results were observed in most of the chromosome aberration assays in mammalian cell lines and two of the recombination assays in *Bacillus subtilis* with benzoic acid and sodium benzoate.

Table 5-15 Summary of *in vitro* mutagenetic studies

Method/ Guideline	Test system (Organism, strain)	Concentra-tions tested (give range)	Results		Remarks give information on cytotoxicity and other	Reference
			+ S9	- S9		
Reverse mutation assays in bacteria (all studies are non-GLP)						
Reverse mutation assay, sim. to OECD 471	*S. typh.* TA 98, 100, 1535, 1537	0.01-1 mg/plate	Negative	Negative	Benzoic acid	McCann, J., Choi, E., Yamasaki, E., Ames B., N. Proc. Nat. Acad. Sci. USA 72 (12):5135-5139 (1975)
Reverse mutation assay, sim. to OECD 471	*S. typh.* TA 92, 94, 98, 100, 1535, 1537	Benzoic acid: up to 10 mg/plate Sodium benzoate: up to 3 mg/plate	Negative	Negative	Benzoic acid, dissolved in DMSO Soidum benzoate, dissolved in water	Ishidate, M., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., Matsuoka, A., Fd Chem. Toxicol., 22 (8):623-636 (1984)
Reverse mutation assay, sim. to OECD 471	*S. typh.* TA 98, 100, 1535, 1537, 1538	0.1 mg/plate	Negative	Negative	Benzoic acid, hippuric acid	Milvy, P., Garro, A. J., Mut. Res. 40:15-18 (1976)
Reverse mutation assay, sim. to OECD 471	*S. typh.* TA 1535, 1536, 1537, 1538	0.001-0.01-0.1 mg/ plate	Negative	Negative	Benzoic acid, dissolved in DMSO	Commoner, B., Report EPA-600/1-76-022 Office of Research and Development US EPA, Washington D.C. (1976)
Reverse mutation assay, sim. to OECD 471	*S. typh.* TA 98, 100, 1535, 1537	0.04 –2.5 mg/plate	Negative	Negative	Benzoic acid	Anderson, D., Styles, J. A., Appendix II to article of Purchase, I.F.H., Longstaff, E., Ashby, J., Styles, J. A., Anderson, D., Br. J. Cancer 37:924-930 (1978)

Method/ Guideline	Test system (Organism, strain)	Concentra-tions tested (give range)	Results		Remarks give information on cytotoxicity and other	Reference
			+ S9	- S9		
Reverse mutation assay	*S. typh.* TA 100	0.0001-1 mg/plate	N/A	Negative	Benzoic acid	Rapson, W. H., Nazar, M. A., Butsky, V. V., Bull. Environm. Contam. Toxicol. 24:590-596 (1980)
Reverse mutation assay	*S. typh.*, strains not specified	No information given	N/A	Negative	Benzoic acid	Rideg, K., Mut. Res. 97:217 (1982)
Reverse mutation assay, sim. to OECD 471	*S. typh.* TA 97, 98, 100, 1535, 1537	0.033-10 mg/plate	Negative	Negative	Benzoic acid	Zeiger, E., Anderson, B., Haworth, S., Lawelor, T., Mortel-mans, K. S., Environ-m. Mol. Mutagen. 11 (suppl. 12):1-158 (1988)
Reverse mutation assay	*S. typh.* TA 98, 100, 1537	No information given	Negative	Negative	Sodium benzoate	Ishidate, M., Sofuni, T., Yoshikawa, K., Arch. Toxicol. (suppl.) 4:41-44 (1980)
Reverse mutation assay	*S. typh.* TA 98, 100	No information given	Negative	Negative	Sodium benzoate	Kawachi, T., Koma-tsu, T., Kada, T., Ishidate, M., Sasaki, M., Sugiyama, T., Tazima Y., In: Wil-liams et al. (eds.) Elsevier/ North-Holland Biomedical Press: 253-267
Reverse mutation assay, sim. to OECD 471	*S. typh.* TA 98, 100, 1535, 1537, 1538; *E.coli* WP2	0.033-10 mg/plate	Negative	Negative	Sodium benzoate	Prival, M. J., Simmon, V. F., Mortelmans, K. E., Mut. Res. 260:321-329 (1991)
Reverse mutation assay	*S. typh.* TA 98, 1535	0.1-5 µmol/plate	Negative	Negative	Hippuric acid	Wiessler, M., Romruen, K., Pool, B. L., Carcinogenesis 4:867-871 (1983)
Reverse mutation assay	*S. typh.* TA 1538	20-2000 µg/plate	Negative	Negative	Benzoic aicd	Calo (1978), not

Method/ Guideline	Test system (Organism, strain)	Concentra-tions tested (give range)	Results		Remarks give information on cytotoxicity and other	Reference
			+ S9	- S9		
	(contradictory information whether more strains were used)					submitted, cited in 1)
Recombination assays in becteria and yeast (all studies are non-GLP and non-guideline studies)						
Recombination assay	*B. subtilis* H17, M45	1 %	Negative	Negative	Benzoic aicd	Khoudokormoff, B., Gist-Brocades, N. V., Mutat. Res. 53:208 (abstract) (1978)
Recombination assay	B. subtilis H17, M45	No information given	No information given	**Positive**	Benzoic aicd	Nonaka, M., Environ. Mol. Mutagen. 14, (suppl. 15):143 (abstract) (1989)
DNA damage (Umu test)	S. typh. TA 1535 /pSK 1002	up to 1.67 mg/mL	Negative	Negative	Benzoic aicd	Nakamura, S., Oda, Y., Shimada, T., Oki, I., Sugimoto, K., Mut. Res. 192:239-246 (1987)
Lambda prophage induction	*E. coli* WP2s (lambda, microscreen)	max. 0.106 mg/well	N/A	Negative	Benzoic acid	Rossman, T. G., Molina, M., Meyer, L., Boone, P., Klein, C. B., Wang, Z., Li, F., Lin, W. C., Kinney, P. L., Mut. Res. 260: 349-367 (1991)
Recombination assay	*B. subtilis*, strain not specified	No information given	**Positive**	**Positive**	Sodium benzoate	Kawachi, T., Komatsu, T., Kada, T., Ishidate, M., Sasaki, M., Sugiyama, T., Tazima, Y., In: Williams et al. (eds.) Elsevier/ North-Holland Biomedical Press: 253-267

Method/ Guideline	**Test system (Organism, strain)**	**Concentra-tions tested (give range)**	**Results**		**Remarks give information on cytotoxicity and other**	**Reference**
			+ S9	- S9		
Recombination assay	*B. subtilis*, strain not specified	No information given	N/A	Negative	Sodium benzoate	Morita, K. et al. J. Soc. Cosmet. Chem., 15:243 (1981)
Recombination assay	*B. subtilis* H17, M45	- S9: 20 mg/plate + S9: 16 mg/plate	Equivocal	Equivocal	Sodium benzoate	Ishizaki, M., Ueno, S., J. Fd. Hyg. Soc. Jpn. 30:447-451 (1989)
Recombination assay	B. subtilis H17, M45	No information given	N/A	**Positive**	Sodium benzoate	Nonaka, M., Environ. Mol. Mutagen. 14, (suppl. 15):143 (abstract) (1989)
Chromosome aberration tests in mammalian cells *in vitro* (all studies are non-GLP)						
Chromosome aberration test, sim. to OECD 473	Chinese hamster cells (CHL)	Benzoic acid: up to 1.5 mg/mL; Sodium benzoate: up to 2 mg/mL	N/A	**Positive**	Benzoic acid, sodium benzoate	Ishidate, M., Data book of chromosomal aberration test in vivo, Elsevier (Amsterdam):9-20;23;40;373 (1988)
Chromosome aberration test, sim. to OECD 473	Chinese hamster cells (CHL)	Benzoic acid: up to 1.5 mg/mL; Sodium benzoate: up to 2 mg/mL	N/A	Benzoic acid: equivocal sodium benzo-ate: **positive**	Benzoic acid, sodium benzoate	Ishidate, M., Mutat. Res. 195:151-213 (1988)
Chromosome aberration test	Human lymphocytes	0.001-0.1 mg/mL	N/A	Negative	Benzoic acid	Zhurkov, V. S., Sov. Genet. 11:528-530 (1975)
Chromosome aberration test	Chinese hamster cells (CHL)	No clear information given	N/A	Ambiguous	Benzoic acid	Ishidate et al. (1984), not submitted, cited in 1)
Cytogenetic assay (anaphase preparat.)	Human embryonic lung cells (WI-38)	0.01-1 mg/mL	N/A	Negative	Sodium benzoate	Litton Bionetics, Inc., FDA, Washington D.C. PB 245453 (1974)
Chromosome aberration test	Chinese hamster fibroblasts (DON)	1–10 mmol/L (0.1-1 mg/mL)	N/A	**Positive**	Sodium benzoate	Abe, S., Sasaki, M., J. Natl. Cancer Inst. 58 (6):1635-1641 (1977)
Chromosome aberration test	Chinese hamster cells (CHL)	139 mg/mL	N/A	**Positive**	Sodium benzoate	Ishidate, M., Odashima, S., Mut. Res. 48:337-354

Method/ Guideline	Test system (Organism, strain)	Concentra-tions tested (give range)	Results		Remarks give information on cytotoxicity and other	Reference
			+ S9	- S9		
						(1977)
Chromosome aberration test	Chinese hamster cells (CHL)	10 mg/mL	N/A	**Positive**	Sodium benzoate	Ishidate, M., Sofuni, T., Yoshikawa, K., Arch. Toxicol. (suppl.) 4:41-44 (1980)
Chromosome aberration test	Hamster lung fibroblast cells	no data given	N/A	**Positive**	Sodium benzoate	Kawachi, T., Komatsu, T., Kada, T., Ishidate, M., Sasaki, M., Sugiyama, T., Tazima, Y., In: Williams et al. (eds.) Elsevier/ North-Holland Biomedical Press: 253-267
Sister chromosome exchange assays in mammalian cells (all studies are non-GLP)						
SCE, sim. to OECD 479	Human lymphoblastoid cells (transformed by Epstein-Barr virus)	1-30 mmol/L (0.1-3.7 mg/ml)	N/A	Negative	Benzoic acid	Tohda, H., Horaguchi, K., Takahashi, K., Oikawa, A., Matsushima, T., Cancer Res. 40:4775-4780 (1980)
SCE, sim. to OECD 479	Chinese hamster cells (CHO)	1-10 mmol/L (0.1-1 mg/mL)	N/A	Negative	Benzoic acid	Oikawa, A., Tohda, H., Kanai, M., Miwa, M., Sugimura, T., Biochem. Biophys. Res. Comm. 97 (4):1311-1316 (1980)
SCE, sim. to OECD 479	Human lymphocytes	up to 2 mmol/L (-0.2 mg/mL)	N/A	Negative	Benzoic acid	Jansson, T., Curvall, M., Hedin, A., Enzell, C. R., Mut. Res. 206:17-24 (1980)
SCE	Chinese hamster fibroblasts (DON)	1–10 mmol/L (0.1-1 mg/mL)	N/A	Equivocal	Sodium benzoate	Abe, S., Sasaki, M. J., Natl. Cancer Inst. 58 (6):1635-1641 (1977)
SCE	Hamster lung fibroblast cells	No information given	N/A	Negative	Sodium benzoate	Kawachi, T., Komatsu, T., Kada, T.,

Method/ Guideline	**Test system (Organism, strain)**	**Concentra-tions tested (give range)**	**Results**		**Remarks give information on cytotoxicity and other**	**Reference**
			+ S9	- S9		
						Ishidate, M., Sasaki, M., Sugiyama, T., Tazima Y., In: Williams et al. (eds.) Elsevier/ North-Holland Biomedical Press: 253-267
SCE	Human lymphocytes	10 mmol/L (1 mg/mL)	N/A	Negative	Sodium benzoate	Xing, W., Zhang, Z., Mut. Res. 241:109-113 (1990)

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5.7.2 In vivo data

All the *in vivo* genotoxicity tests were negative at somatic or germ cell level.

On this basis and the negative results obtained in two carcinogenicity studies in rats and mice for sodium benzoate, notwithstanding some limitations, it is very unlikely that benzoic acid would interfere with chromosomes *in vivo*. This evaluation is in line with the judgment of the Scientific Committee on Food of the EU (Opinion of the Scientific Committee on Food on Benzoic acid and its Salts, SCF/CS/ ADD/CONS/48 Final, 2002).

Table 5-16 Summary of *in vivo* mutagenetic studies

Method/ Guideline	Species, Strain, Sex, No/group	Route and Frequency of application	Sampling times	Dose levels	Results give dose, sampling time and result +/-/+	Remarks	Reference
Bone marrow chromo-some ab-erration test	Rat, Sprague Dawley, 5 M	Gavage a) single dose b) 5 d	a) 6-48 h b) 6 h	0-50-500-5000 mg/ kg bw	Negative	Sodium benzoate	Litton Bionetics, Inc., FDA, Washing-ton D.C. PB 245453 (1974)
Host-mediated assay (*S. typh.*TA 1530)	Mouse, ICR, 8-10 M	Gavage a) single dose b) 5 d	No informa-tion given	0-50-500-5000 mg/ kg bw	Negative	Sodium benzoate	Litton Bionetics, Inc., FDA, Washing-ton D.C. PB 245453 (1974), not submitted, cited in 1) and 2)
Host-mediated assay (*S. typh.*G 46; *S. cerevisiae* D3)	Mouse, ICR, 8-10 M	Gavage a) single dose b) 5 d	No informa-tion given	0-50-500-5000 mg/ kg bw	Negative	Sodium benzoate	Litton Bionetics, Inc., FDA, Washing-ton D.C. PB 245453 (1974), not submitted, cited in 1)
Bone marrow chromo-some ab-erration test	Rat, strain, number/ group and sex not specified	No information given	No informa-tion given	No infor ma-tion given	Negative	Sodium benzoate	Kawachi, T., Koma-tsu, T., Kada, T., Ishidate, M., Sasa-ki, M., Sugiyama, T., Tazi-ma, Y., In: Wil-liams et

Method/ Guideline	Species, Strain, Sex, No/group	Route and Frequency of application	Sampling times	Dose levels	Results give dose, sampling time and result +/-/+	Remarks	Reference
							al. (eds.) Elsevier/ North-Holland Biomedi-cal Press: 253-267
Dominant lethal assay	Rat, random-bred, 5 M	Gavage a) single dose b) 5 d	No informa-tion given	0-50-500-5000 mg/ kg bw	Negative	Sodium benzoate	Litton Bionetics, Inc., FDA, Washing-ton D.C. PB 245453 (1974), not submitted, cited in 1) and 2)

1) Anonymous (1995) Benzoic acid / sodium benzoate. BUA report 145. Ed.: GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance, 1995

2) Anonymous (2003). Review report for the inclusion of benzoic acid in Annex I of Directive 91/414/EEC, SANCO/1396/2001-Final

5.7.3 Human data

No data are available.

5.7.4 Other relevant information

No other relevant information is available.

5.7.5 Summary and discussion of mutagenicity

In the absence of a genotoxic potential in *in vivo* studies and negative results in carcinogenicity studies, no classification and labelling regarding mutagenicity is required.

5.8 Carcinogenicity

5.8.1 Carcinogenicity: oral

Carcinogenicity of sodium benzoate was assessed in one 2-year study in rats and one lifetime study in mice. Neither of these meets current EU or OECD requirements for carcinogenicity testing or data reporting and analysis. In the rat study, an unspecified number of interim sacrifices was performed and terminal sacrifices started at month 18, reducing the effective exposure time. This

may provide one possible explanation for the low incidence of tumours reported for all treatment groups which were substantially below historical control data cited by the authors. Nevertheless, direct comparison of rats treated with up to 20000 ppm with untreated controls did not indicate carcinogenicity under these conditions. When sodium benzoate was added to the drinking water of mice at the same concentration of 20000 ppm - which according to consumption data provided in the report should correspond to 3000 mg/kg bw/d – no increase in the lifetime tumour incidence, clinical abnormalities or histopathological changes were observed by Toth (1984).

Table 5-17 Summary of chronic and carcinogenicity studies

Method/ Guideline	Route of exposure, duration	Species, Strain, Sex, No/group	Dose levels	Results Main effects/ Target organs/ Tumours	NO(A)EL ppm (mg/kg bw/d)	LO(A)EL ppm (mg/kg bw/d)	Remarks	Reference
Chronic survival, non-GLP	Oral (feeding), Lifetime 3-generation	Rat, 20 M + 20 F	0-5,000-10,000 ppm (~0-250-500 mg/kg bw/d)	No reduction in survival, all other endpoints not reported after chronic exposure	10,000 ppm (500 mg/kg bw/d)	> 10,000 ppm (> 500 mg/kg bw/d)	Benzoic acid and sodium benzoate, histopathology at wk 16, bw up to wk 8 (F) or 12 (M)	Kieckebusch, W., Lang, K., Arzneim. Forsch. 10:1001-1003 (1960), German
Chronic toxicity, non-GLP	Oral (feeding), 18 mo	Rat, Wistar, 30 M + 20 F (control: 13 M + 12 F)	0-15,000 ppm (~0-750 mg/kg bw/d)	Mortality↑ (30 vs. 15 %), bw and bw gain↓, food consumption↓, no behavioural changes	< 15,000 ppm (< 750 mg/kg bw/d)	15,000 ppm (~750 mg/kg bw/d)	Benzoic acid, interim report, no other endpoints reported	Marquardt, P., Arzneim. Forsch. 10:1033 (1960), German
Chronic toxicity	Oral (feeding), 18 mo	Rat, Wistar, 10 M + 10 F	0-40 mg/kg bw/d	No relevant changes reported	N/A	N/A	Benzoic acid, not suitable for risk assessment	Shtenberg, A. J., Ignat'ev, A. D., Fd Cosmetic Toxicol. 8:369-380 (1970)
Chronic toxicity	Oral (feeding), 17 mo	Mouse, Albino, 25 M + 25 F	0-40 mg/kg bw/d	In response to food withdrawal (40 mg/kg bw/d): mortality↑, weight loss↑	N/A	N/A	Benzoic acid, not suitable for risk assessment	Shtenberg, A.J., Ignat'ev, A.D., Fd Cosmetic Toxicol. 8:369-380 (1970)
Carcinogenicity, non-guideline, non-GLP	Oral (feeding), 2 yr	Rat, F344, 50 M + 52 F (control: 25 M + 43 F)	0-10,000-20,000 ppm (~0-500-1000 mg/kg bw/d)	No effects on mortality, bw gain, food consumption, number of tumours in comparison to controls	20,000 ppm	> 20,000 ppm	Sodium benzoate, interim sacrifice (unspecified), final sacrifice from mo 18-25, no. of detected tumours below historical controls	Sodemoto, Y., Enomoto, M., J. Environ. Pathol. Toxicol. 4:87-95 (1980)
Carcinoge-nicity, non-guideline,	Oral (drinking water), 2.5 yr	Mouse, Swiss Albino, 50 M + 50 F	0-20,000 ppm (~0-3000 mg/kg bw/d)	No effects on mortality and tumor incidence	20,000 ppm	> 20,000 ppm	Sodium benzoate	Toth, B., Fund. Appl. Toxicol. 4:494-496 (1984)

Method/ Guideline	Route of exposure, duration	Species, Strain, Sex, No/group	Dose levels	Results Main effects/ Target organs/ Tumours	NO(A)EL ppm (mg/kg bw/d)	LO(A)EL ppm (mg/kg bw/d)	Remarks	Reference
non-GLP		(control: 99 M + 99 F)						

5.8.2 Carcinogenicity: inhalation

No data are available.

5.8.3 Carcinogenicity: dermal

No data are available.

5.8.4 Carcinogenicity: human data

No data are available.

5.8.5 Other relevant information

No other information is available.

5.8.6 Summary and discussion of carcinogenicity

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

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

Reproductive toxicity was assessed in a non-guideline 3-generation study performed in rats with 0, 0.5 and 1% benzoic acid added to the diet, corresponding to approximate doses of 0, 250 and 500 mg/kg bw/d (Kieckebusch and Lang, 1960). These doses failed to produce detectable toxic effects on parental and offspring generations or reproductive capacity.

Table 5-18 Summary of reproduction toxicity studies

Method/ Guideline	Route of exposure	Species, Strain, Sex, No/group	Dose levels	Critical effect Parental, Offspring (F1, F2)	NO(A)EL Parental toxicity	NO(A)EL reproductive toxicity	Remarks	Reference
3-generation-reproductive toxicity, non-GLP	Oral (feeding), lifetime	Rat, 20 M + 20 F	0-5,000-10,000 ppm (~0-250-500 mg/kg bw/d)	Parental and Offspring: No increase in mortality, no changes in bw and bw gain, no clinical signs, no changes in organ weight (brain, heart, liver, spleen, kidneys, testes) or histopathology Reproductive: No effect on no. of pregnancies and offspring, offspring survival and bw development, testes weight	10000 ppm (~500 mg/kg bw/d)	10000 ppm (≥ 500 mg/kg bw/d)	Benzoic acid	Kieckebusch, W., Lang, K., Arzneim. Forsch. 10:1001-1003 (1960). German Also cited in 1)

1) REACH registration dossiers 2119455536-33-0000, 2119455536-33-0001 and the CSR attached to them

5.9.2 Developmental toxicity

Embryotoxicity and teratogenicity of benzoic acid were evaluated (Kimmel et al., 1971) after administration of a single dose of 510 mg/kg bw/d on day 9 of gestation to 7 pregnant rats. Under these experimental conditions, there were no indications for embryotoxic or teratogenic activity of benzoic acid. Interestingly, it was noted that high doses of benzoic acid may increase the teratogenicity of other substances, presumably via toxicokinetic interactions by delaying the elimination of these. Nevertheless, this study can not be regarded suitable as the basis for assessment of developmental toxicity (single dose on day 9 only, low no. of animals).

In a developmental neurotoxicity study, sodium benzoate was administered to rats at a similar top dose of 500 mg/kg bw/d sodium benzoate from day 5 of gestation through lactation and after weaning (Crane & Lachane, 1985). Mortality and bw of parental animals, mortality of embryos and

foetuses as well as survival, activity and bw development of offspring remained unaffected. No teratogenicity was reported, but this endpoint was not addressed specifically.

A more comprehensive teratogenicity study with higher doses of sodium benzoate in rats was reported by Onodera et al. (1978) No increase in offspring variations and malformations was observed up to doses of ~1330 mg/kg bw/d (corr. to ~1130 mg/kg bw/d benzoic acid) administered with the food during the whole gestational period. However, a slight reduction (4.5 %, mortality: 1/22) in offspring survival was noted at this dose level. Higher doses led to reduced maternal survival, maternal bw loss, reduction in the number and weight of viable foetuses, reduced perinatal survival and a more than tenfold increase in skeletal and soft tissue malformations. A NOAEL of 1130 mg/kg bw/d benzoic acid equivalents for maternal and developmental effects and a more conservative NOAEL of 600 mg/kg bw/d for other effects on offspring is concluded.

The report by Onodera et al. (1978) as well as a data review (BUA report 145, 1995) further cites earlier developmental toxicity studies with sodium benzoate in other species including mouse, hamster and rabbit. The highest selected doses were 175 mg/kg bw/d administered from gestational day 6 to 15 in mice, 300 mg/kg bw/d given from day 6 to 10 of pregnant hamsters, and 250 mg/kg bw/d from day 6 to 18 of gestation in rabbits. In all cases, the selected doses were reported to be insufficient to cause detectable maternal toxicity, fetotoxicity or teratogenicity, supporting the conclusions made above.

Table 5-19 Summary of teratogenicity studies

Method/ Guideline	Route of exposure, Duration	Species, Strain, No/group	Dose levels	Critical effects 1) dams 2) foetuses	NO(A)EL Maternal toxicity	NO(A)EL Teratogenicity Embryotoxicity	Remarks	Reference
Terato-genicity/ embryotoxicity, non-GLP	Oral, dietary, from d 1 to 20 of gestation	Rat, Wistar, 12-18 F	0-10,000 - 20,000 - 40,000 - 80,000 ppm (~0-600-1130-1610-820 mg/kg bw/d benz-oic acid eq.)	1) 1610 mg/kg: Survival↓, food consump-tion↓, bw↓ *Offspring:* 1130 mg/kg: 8-wk offspring survival↓ 2) 1610 mg/kg: Viable foetuses↓, foetal weight↓, perinatal mortality↑, skeletal and soft tissue malformations↑	1130 mg/kg bw/d benzoic acid eq. O*ff-spring:* 600 mg/kg bw/d benzoic acid eq.	1130 mg/kg bw/d benzoic acid eq.	Sodium benzoate, doses con-verted in benzoic acid equi-valents	Onodera, H., Ogiu, T., Mat-suoka, C., Furu-ta, K., Takeu-chi, M., et al., Eis. Shik. Hok. 96:47-54 (1978)
Single-dose	Oral (gavage),	Rat, Wistar,	0-510 mg/kg	1) No clinical	510 mg/kg	510 mg/kg bw	Benzoic acid,	Kimmel, C. A.,

Method/ Guideline	Route of exposure, Duration	Species, Strain, No/group	Dose levels	Critical effects 1) dams 2) foetuses	NO(A)EL Maternal toxicity	NO(A)EL Teratogenicity Embryotoxicity	Remarks	Reference
terato-genicity/ embryo-toxicity, non-GLP	single dose on d 9 of gestation	7 F (control: 6 F)	bw	symptoms 2) No embryoto-xicity, no increase in abnormali-ties	bw		poor reliability (single dose, low no. of animals)	Wilson, J. G., Schuma-cher, H. J., Terato-logy 4:15-24 (1971), also cited in 1)
Develop-mental neurotoxi city, non-GLP	Oral (feeding), from d 5 of gestation, during lactation to d 45	Rat, Wistar, 10 F (8 pups per litter group)	0-1000-5,000-10,000 ppm (~0-50-250-500 mg/kg bw/d)	1 and 2) no effects on mortality, bw and food consumption	500 mg/kg bw/d	500 mg/kg bw/d	Sodium benzoate	Crane, S. C., Lachane, P. A., Nutrition Reports Internati-onal 32:169-177 (1985)

1) REACH registration dossiers 2119455536-33-0000, 2119455536-33-0001 and the CSR attached to them

5.9.3 Human data

Human data are not available.

5.9.4 Other relevant information

Other information is not available.

5.9.5 Summary and discussion of reproductive toxicity

Overall, there is no indication for developmental or reproductive toxicity of benzoic acid in rats below the high dose that revealed severe maternal toxicity (1610 mg/kg bw/d). Thus, no classification and labelling for reproductive or developmental toxicity is required

The slight reduction of 4.5 % in 8-wk-survival cannot clearly be attributed to substance uptake via milk. This mortality could possibly be caused by direct food contact. Therefore, in accordance with the Guidance to Regulation (EC) No. 1272/2008, no classification for lactation effects is justified.

5.10 Other effects

5.10.1 Neurotoxicity

Subacute (delayed) neurotoxicity and CNS toxicity was assessed in juvenile rats. Benzoic acid was added to the diet for up to 35 days at 0, 1.1, and 3 %, corresponding to approximate doses of 0, 825,

and 2250 mg/kg bw/d. At day 4, clinical observation showed signs of neurotoxicity in most animals of the top dose group including ataxia, tremor, excitation, aggressive behaviour and tonoclonic convulsions. 50 % of the animals receiving 2250 mg/kg bw/d died or were sacrificed between day 1 and day 5. For a total of 8 top dose animals surviving day 5, treatment was discontinued and sacrifice was performed on day 24-35. Histological evaluation of the brain showed prominent pathological changes in 2/5 animals treated for 3 days, 18/18 animals treated for 5 days and 13/15 animals with 3 wk of recovery following the 5 days of exposure. These changes included preferentially ischaemic necrosis of ganglial cells in the stratum granulosum of the fascia dentata and the cortex of the lobus piriformis. No histopathology of the brain or clinical abnormality was noted in any of the animals of the low dose group receiving approx. 825 mg/kg bw/d over 7, 14, or 35 days.

Acute neuroexcitation by benzoic acid was studied mechanistically in rats at a dose of approx. 500 mg/kg bw administered intravenously by infusion over 60 min (Mattsson et a., 1989). At this dose, neither change in the somatosensory evoked potential (SEP) nor SEP oscillations in the frequency band of 30-120 Hz were observed. Other endpoints were not reported.

In another developmental neurotoxicity study, Crane and Lachane (1985) evaluated the effects of sodium benzoate on activity and behaviour in neonatal and juvenile rats. Treatment with 0, 0.1, 0.5, and 1.0 % benzoate in the diet commenced on day 5 of gestation of the parental animals and continued through lactation and after weaning. Offspring activity was measured every 3 days pre-weaning and continuously thereafter. Observations were comparable for all treatment groups. On day 9, 15, and 21, one animal per litter was sacrificed for measurement of brain region weight and serotonin, dopamine and noradrenalin levels. The remaining 5 animals per litter were subjected to the same analysis on day 45. There were no treatment-related changes in weight or neurotransmitter content for any of 5 analysed brain regions. Consequently, the NOAEL for developmental neurotoxicity in rats was considered as 1 % in the diet, corresponding to approx. 500 mg/kg bw/d.

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

Table 5-20 Summary of neurotoxicity studies

Method/ Guideline	Route of exposure, Duration	Species, Strain, Sex, No/group	Dose levels ppm (mg/kg bw /d)	NO(A)EL ppm (mg/kg bw /d)	LO(A)EL ppm (mg/kg bw /d)	Results Main effects/ Target organs	Remarks	Reference
CNS toxicity, non-guideline, non-GLP	Oral, 1-35 d	Rat, Juvenile Wistar, 5-15 M	0-11,000-30,000 ppm (approx. 0-825-2250 mg/kg bw/d)	11000 ppm (~825 mg/kg bw/d)	30000 ppm (~2250 mg/kg bw/d)	From d 3: necrosis in stratum granu-losum of fascia dentata, lobus piriformis cortex; from d 4: ataxia, tremor, excitation,	Benzoic acid (Doc III submitted A6.3.1-01)	Kreis, H., Frese, K., Wilmes, G., Fd. Cosmet. Toxicol., 5:505-511 (1967), German

Method/ Guideline	Route of exposure, Duration	Species, Strain, Sex, No/group	Dose levels ppm (mg/kg bw /d)	NO(A)EL ppm (mg/kg bw /d)	LO(A)EL ppm (mg/kg bw /d)	Results Main effects/ Target organs	Remarks	Reference
						aggressive behavior, tonoclonic convul-sions		
Mechanis-tic study, acute neuroexci-tation, non-GLP	Parenteral, i.v.; 1 h	Rat, F344, 2 M (potential), 6 M (oscillati-ons)	Approx. 500 mg/kg bw	Approx. 500 mg/kg bw	> 500 mg/kg bw	No changes in somato-sensory evoked potential and potential oscillation s.	Benzoic acid, Non-guideline	Mattsson, J. L., Albee, R. R., Gor-zinski, S. J., Neuro-toxicol. Teratol. 11:71-75 (1989)
Develop-mental neuro-toxicity, non-GLP	Oral (feeding), from d 5 of gestation, during lactation to d 45	Rat, Wistar, 25 M + 25 F + 10 interim at d 9, 15, 21	0-1000-5000-10000 ppm (~0-50-250-500 mg/kg bw/d)	500 mg/kg bw/d	> 500 mg/kg bw/d	No effects on offspring activity, brain region weight or neurotrans mitter content	Sodium benzoate	Crane, S. C., La-chane, P.A., Nutrition Reports Internatio-nal 32: 169-177 (1985)

5.10.2 Medical use of benzoic acid: case studies and reviews

Direct observations

Several studies from the 19th and the beginning of the 20th century regarding the oral exposure of humans to benzoic acid or sodium benzoate are described. However, owing to the low number of individuals exposed, the validity of these studies is limited. No adverse effects were reported after a single oral dose of 10,000 mg benzoic acid or up to 1000 mg/d over a period of up to 90 days (Gerlach, 1909). Chittenden et al. (1909) found no abnormalities in haematology, urine composition, nitrogen balance, or well-being of six men given 300-400 mg/d in the diet for up to 62 days. In another study with volunteers given 1000 mg/d for 5 days and subsequently increasing the dose in increments of 500 mg/d every 5 days to 2500 mg/d, signs of discomfort (nausea, headache, weakness, burning and irritation of oesophagus) were reported (Wiley & Bigelow, 1908).

Medical use

Sodium benzoate is used in the treatment of patients with urea cycle enzymopathies (i.e., hyperammonaemia due to inborn defects of urea synthesis) in order to facilitate an alternative pathway of nitrogen excretion. The therapeutic dose for maintenance of low blood ammonia levels, which might be given over several years, is 125-500 mg/kg bw/d for neonates, children and adults. Clinical signs of toxicity at the maintenance dose of 125-500 mg/kg bw/d are rare and comprise headache, nausea and vomiting. In hyperammonaemia crises and in neonates bolus doses up to 1750 mg/kg bw/d sodium benzoate are reported with some fatalities that might be due to metabolic acidosis (Enns et al., 2007; Praphanphoj et al., 2000). The adverse effects described for benzoic acid overdoses are similar to those observed in salicylate poisoning. Intoxication by both substances is

thought to occur via a similar mechanism (inhibition of mitochondrial respiration after saturation of the glycine and glucuronate conjugation): mild symptoms comprise lethargy, nausea/vomiting, tinnitus, dizziness and burning in mouth, throat and oesophagus. Moderate symptoms include hyperpnea, tachypnoea, restlessness, loss of coordination while severe symptoms include coma/convulsions, pulmonary oedema, and encephalopathy.
In nine patients on penicillin treatment given 12,000 mg benzoic acid divided into eight doses over 5-days in eight subjects and over 14 days in one subject, no adverse effects on blood urea nitrogen or creatinine clearance were reported (Waldo et al., 1949).
Senator at al. (1879) compiled 27 case reports of patients with acute rheumatic arthritis which were treated with 4 to 25 g/d benzoic acid without adverse effects.

Epidemiologic studies (exposure estimates)
The Concise International Chemical Assessment Document No. 26 (Benzoic Acid and Sodium Benzoate, IPCS, 2000) reports intake data of benzoates based on an assessment of JECFA in 1999 including information provided by Australia, China, Finland, France, Japan, New Zealand, Spain, United Kingdom, and the USA. Soft drinks and soy sauce contributed most to the benzoate intake. The national mean intake was 0.18 mg/kg bw/d in Japan and 2.3 mg/kg bw/d in the USA. High consumers of benzoates have an estimated daily intake of 7.3 mg/kg bw/d in the USA and 14 mg/kg bw/d in China.

Table 5-21 Summary case studies and reviews

Kind of study (e.g. case reports)	Examination methods, number of individuals examined	Results	References
Direct observations			
Acute toxicity	1 M, self-experiment 10 g benzoic acid, clinical signs 0.5 and 1 g benzoic acid or sodium benzoate, effects on digestive tract	No effects observed on respiration, body temperature and pulse No effect on total acidity of gastric juice, free HCl and digestion	Gerlach, V., In: Gerlach, V (ed.): Physiologische Wirkungen der Benzosäure und des benzoesauren Natrons. p90-92. Verlag Heinrich Staadt, Wiesbaden, Germany (1909). German
Subacute/subchronic toxicity and metabolism	1 M 1g benzoic acid or 1.5 g sodium benzoate for 6 d 0.5-2 g benzoic acid or 1.5or 2 g sodium benzoate for 44 d 1g benzoic acid for 90 d	No effect observed on total protein, on the utilization of nitrogen and the utilization of lipid components of the food; No symptoms and no influence observed on body weight, body temperature, respiration and pulse; No adverse effects observed	Gerlach, V., In: Gerlach, V (ed.): Physiologische Wirkungen der Benzosäure und des benzoesauren Natrons. p90-92. Verlag Heinrich Staadt, Wiesbaden, Germany (1909). German
Subacute	12 individuals (sex not specified)	Symptoms: discomfort, nausea, headache,	Wiley, H. M., Bigelow, W. D., Bulletin 84, Pt IV,

Kind of study (e.g. case reports)	Examination methods, number of individuals examined	Results	References
	35 g benzoic acid in total over 20 d: 1 g for 5 days, 1.5 g for 5 days, 2 g for 5 days and 2.5 g for 5 days	weakness, burning and irritation of the oesophagus, hunger and indigestion. Only 3 volunteers took the intended entire dose of 35 g. Dose at which onset of symptoms was observed was not specified.	Bureau of Chemistry, US Dep. of Agriculture (1908). Original not submitted, cited in GRAS food ingredients: Benzoic acid and sodium benzoate. PB-221208, NTIS, US FDA (1972) and SANCO/1396/2001-Final, Monograph on Benzoic Acid, EU, 2003
Medical use of benzoic acid: case studies and reviews			
Case report, treatment of ornithine carbamoyl transferase deficiency (hyperammonaemia)	1 M (newborn), 360 mg sodium benzoate per day for 13 months	No adverse effects of sodium benzoate observed, ammonium levels within normal range	Batshaw, M. L., Painter, M. J., Sproul, G. T., Schafer, I. A., Thomas, G. H., Brusilow, S., The John Hopkins Medical Journal 148:34-40 (1981)
Case reports, treatment of ornithine carbamoyl transferase deficiency (hyperammonaemia)	2 M (newborn + 9 mo), 1F (6 yr), sodium benzoate i.v.	No adverse effects of sodium benzoate observed, blood ammonium levels normalised	Brusilow, S. W., Maestri, N. E., Adv. Pediatr. 43:127-170 (1996)
Case report, treatment of ornithine carbamoyl transferase deficiency (hyperammonaemia)	1 F (child) 200 mg sodium benzoate per day for 13 months	No evidence of toxicity in clinical and laboratory examinations	Takeda, E., Kuroda, Y., Toshima, K., Watanabe, T., Naito, E., Miyao, M., Clin. Pediatr. 22(3):206-208 (1983)
Case report, treatment of neonatal hyperammonaemia	3 M (newborn), 1 F (newborn) 125 mg/kg sodium benzoate i.v. every 6 h	No adverse effects observed, serum ammonia concentrations were reduced, while serum glycine concentrations remained normal during treatment. Benzoic acid was completely excreted as benzoic acid and hippuric acid.	Green, T. P., Marchessault, R. P., Freese, D. K., J. Pediatr. 102:785-790 (1983)
Review of alternative pathways treatment for nitrogen excretion including adverse effects	N/A	*Adverse effects observed in animal studies:* Decrease in ATP and Acetyl-CoA, Impairment of mitochondrial pathways, depletion of hepatic glycine, increase of tryptophan uptake in the brain *Adverse effects observed in man:* Decreased carnitine	Feillet, F., Leonard, J. V., J. Inher. Metab. Dis. 21(suppl. 1):101-111 (1998)

Kind of study (e.g. case reports)	Examination methods, number of individuals examined	Results	References
		concentrations, nausea, vomiting, tinnitus, visual disturbances	
Review of 25 year, uncontrolled study in patients with urea-cycle enzyme defects	299 patients, acute bolus injections: 250-2310 mg/kg bw, maintenance dose: 250 mg/kg bw/d	In 13/49 mortalities: overdose of benzoate and sodium phenylacetate, vomiting and seizures frequently observed (might be due to underlying disease)	Enns, G. M., Berry, S. A., Berry, G. T., Rhead, W. J., Brusilow, S. W., Hamosh, A., New Engl. J. Med. 356(22):2282-2292 (2007)
Case reports on sodium benzoate and sodium phenylacetate toxicity	3 patients, 2 M + 1 F (aged 3-6 yr), benzoic acid i.v.: 915 mg/kg bw/12 h, 1750 mg/kg bw/18h, 750 mg/kg bw/10 h	2 mortalities, signs of metabolic acidosis: anion gap, cerebral oedema, somnolence, Kussmaul respiration	Praphanphoj, V., Boyadjiev, S. A., Waber, L. J., Brusilow, S. W., Geraghty, M. T., J. Inherit. Metab. Dis. 23:129-136 (2000)
Case reports, treatment of rheumatic arthritis	20 M + 7 F 4 to 25 g/d benzoic acid	No adverse effects of benzoic acid were observed (gastric effects in combination with salicylic acid).	Senator, H., Zeitschrift f. klin. Medizin 1(2):243-264 (1879). German
Study on renal effects of benzoic acid (attempt to increase blood penicillin levels)	9 (sex not specified) 12 g/d benzoic acid	No changes in creatinine clearance, uninalysis, or blood urea levels. Mild anorexia was reported in 2 patients.	Waldo, J. F., Masson, J. M., Lu, W., Tollstrup, J., J. Amer. J. Med. Sci. 117:563-568 (1948)
Epidemiological studies			
Exposure estimations	Not available	Japan: 0.18 mg/kg bw/d USA: up to 7.3 mg/kg bw/d (mean: 2.3) China: up to 14 mg/kg bw/d; Main source: soya sauce, soft drinks	Concise International Chemical Assessment Document No. 26: Benzoic Acid and Sodium Benzoate, IPCS, 2000

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1 Explosivity

According to the chemical structure explosive properties are not expected.

6.2 Flammability

Ignition temperature in air is 573 °C.

6.3 Oxidising potential

According to the chemical structure oxidising properties are not expected.

7 ENVIRONMENTAL HAZARD ASSESSMENT

Two available REACH registration dossiers (2119455536-33-0000, 2119455536-33-0001) have been considered. The presented data seems plausible and is in line with the current CLH proposal.

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

The used active substance has pharmaceutic quality (99 - 100.5%). The purity as well as the limits of the impurities are defined by the German pharmacopoeia (current revision DAB 10, 1991)

7.1.1.1 Fish

Short-term toxicity to fish

The acute toxicity of benzoic acid to fish is summarised in Table 7-1.

Table 7-1 Acute toxicity of benzoic acid to fish

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (h)	Endpoint	Value (mg/L)	
OECD 203	*Oncorhynchus mykiss*	Semi-static[1]	96	LC_{50}	>120 nom.[2]	Jonas, 1998

[1] Daily renewal of the test solution.
[2] Overall average of measured concentrations of the test substance was 101% of nominal.

Long-term toxicity to fish

The long-term toxicity of benzoic acid to fish is summarised in Table 7-2.

Table 7-2 Long-term toxicity of benzoic acid to fish

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration	Endpoint	Value (mg/L)	
OECD 204 OECD 215	*Oncorhynchus mykiss*	Semi-static[3]	28 days	EC_{50} NOEC	> 120 nom.[4] ≥ 120 nom.[4]	Pawlowski & Wydra, 2004

[3] Renewal of the test solution every 2 - 3 days.
[4] The overall average of measured concentrations of the test substance in the test solutions was 94 -103 % of nominal.

7.1.1.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

The acute toxicity of benzoic acid to invertebrates is summarised in Table 7-3.

Table 7-3 Acute toxicity of benzoic acid to invertebrates

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (h)	Endpoint	Value (mg/L)	
OECD 202 part I (1984)	*Daphnia magna*	Semi-static[5]	48	EC_{50} NOEC	>120 nom.[6] 55 nom.[6]	Jonas, 1998

[5] Daily renewal of the test solution.
[6] The overall average of measured concentrations of the test substance in the test solutions was 100 % of nominal.

Long-term toxicity to aquatic invertebrates

The long-term toxicity of benzoic acid to invertebrates is summarised in Table 7-4.

Table 7-4 Long-term toxicity of benzoic acid to invertebrates

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration	Endpoint	Value (mg/L)	
OECD 211	*Daphnia magna*	Semi-static[7]	21 days	EC_{50} NOEC	> 25 nom.[8] ≥ 25 nom.[8]	Pawlowski & Wydra, 2004

[7] Renewal of the test solution every 2 - 3 days.
[8] The overall average of measured concentrations of the test substance in the test solutions was 90 - 99 % of nominal.

7.1.1.3 Algae and aquatic plants

The toxicity of benzoic acid to algae and aquatic plants is summarised in Table 7-5.

Table 7-5 Toxicity of benzoic acid to algae and aquatic plants

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (h)	Endpoint	Value (mg/L)	
OECD 201	*Pseudokirchneriella subcapitata*	static	72	E_rC_{50} E_bC_{50} NOEC	72 nom.[9] 33 nom.[9] 7,5 nom.[9]	Jonas, 1998

[9] The overall average of measured concentrations of the test substance in the test solutions was 98 % of nominal.

The study with algae can be regarded as the key study for the aquatic toxicity of benzoic acid and hence for classification and labeling. Therefore the study is presented in more detail below:

Reference: Jonas, W., 1998, NA 98 9408/1, WAT98-50277

Test guideline: OECD 201 (1984)

GLP compliance: yes

Materials and methods: Effects of benzoic acid on growth inhibition of *Pseudokirchneriella subcapitata* were examined according to OECD guideline 201. Algae were exposed to 3.75, 7.5, 15, 30, 60 and 120 mg/L benzoic acid and a negative control. Algal concentrations were measured turbidimetrically after 24, 48 and 72 h of incubation. Test conditions and actual concentrations of the test compound were checked at the beginning and the end of the test.

Findings: The test was considered to be valid. The overall average of measured concentrations of the test substance in the test solutions was 98 % of nominal. The buffering capacity of the test medium was surpassed in the test concentrations of 60 and 120 mg/l. Effects therefore might also be attributed to the low pH-value observed at these concentrations.
Assessment: NOEC (72 h): 7.5 mg/L; E_bC_{50} (72 h): 33 mg/L; E_rC_{50} (24-72 h): 72 mg/L.

7.1.1.4 Sediment organisms

No data available.

7.1.1.5 Other aquatic organisms

No data available.

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant for this type of dossier.

7.2 Terrestrial compartment

Not relevant for this type of dossier.

7.2.1 Toxicity test results

7.2.1.1 Toxicity to soil macro organisms

7.2.1.2 Toxicity to terrestrial plants

7.2.1.3 Toxicity to soil micro-organisms

7.2.1.4 Toxicity to other terrestrial organisms

Toxicity to birds

Toxicity to other above ground organisms

7.2.2 Calculation of Predicted No Effect Concentration (PNEC_soil)

Not relevant for this type of dossier.

7.3 Atmospheric compartment

Not relevant for this type of dossier.

7.4 Microbiological activity in sewage treatment systems

7.4.1 Toxicity to aquatic micro-organisms

No data available.

7.4.2 PNEC for sewage treatment plant

Not relevant for this type of dossier.

7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC_oral)

Not relevant for this type of dossier.

7.6 Conclusion on the environmental classification and labelling

Benzoic acid is hydrolytically stable. Benzoic acid was found to be readily biodegradable in aquatic systems.

Benzoic acid has a log Kow of 1.87, therefore a bioconcentration in aquatic organisms is unlikely. The result of the calculation of BCF using the standard equation (74), BCF_{fish} = 7.754 (on wet weight basis), is regarded as a worst case for the estimation of bioconcentration in fish. It is assumed that there is no risk of bioaccumulation in aquatic organisms.

The acute toxicity of benzoic acid to fish and invertebrates is low (above 100 mg/L) with a toxicity of LC_{50} > 120 mg/L to fish and of EC_{50} > 120 mg/L to aquatic invertebrates. The toxicity to algae is ErC_{50} = 72 mg/L and NOEC = 7.5 mg/L.

The long- term toxicity of benzoic acid for aquatic invertebrates was observed at NOEC = 25 mg/L.

Conclusion of environmental classification according to Directive 67/548/EEC

In aquatic toxicity studies, only ErC_{50} value for algae was obtained at benzoic acid concentrations < 100 mg/L, LC_{50} value for fish and EC_{50} value for aquatic invertebrates were obtained at benzoic acid concentrations > 100 mg/L.
The long-term toxicity values for algae und invertebrates were obtained at benzoic acid concentrations > 1 mg/L.
Benzoic acid was found to be readily biodegradable in aquatic systems.
Benzoic acid has a log Kow of 1.87. From the calculation of BCF using the standard equation a BCF_{fish} = 7.754 (on wet weight basis) was determined.

Benzoic acid fulfills no criteria for environmental classification and labelling.

Conclusion of environmental classification according to Regulation EC 1272/2008

In aquatic toxicity studies, only ErC_{50} value for algae was obtained at benzoic acid concentrations < 100 mg/L, LC_{50} value for fish and EC_{50} value for aquatic invertebrates were obtained at benzoic acid concentrations > 100 mg/L.
The long-term toxicity values for algae und invertebrates were obtained at benzoic acid concentrations > 1 mg/L.
Benzoic acid was found to be readily biodegradable in aquatic systems.
Benzoic acid has a log Kow of 1.87. From the calculation of BCF a BCF_{fish} = 7.754 (on wet weight basis) was determined.

Benzoic acid fulfils no criteria for environmental classification and labelling.

Conclusion of environmental classification according to Regulation EC 286/ 2011 (2^{nd} ATP)

The criteria of the 2^{nd} ATP to the CLP Regulation have been considered. The available long-term ecotoxicological data does not change the proposed classification and labelling for environmental hazards.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Benzoic acid is an active substance in the meaning of Biocidal Products Directive (98/8/EC) and of Plant Protection Products Directive (91/414/EEC).
In accordance with Article 36(2) of EC Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures, benzoic acid should now be considered for harmonised classification and labelling.

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