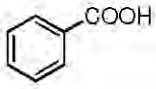
Formatierte Tabelle

Section A2 Identity of Active Substance

Subsection (Annex Point)			Official use only
2.1	Common name (IIA2.1)	Benzoic acid	
2.2	Chemical name (IIA2.2)	Benzoic acid	
2.3	Manufacturer´s development code number(s) (IIA2.3)	Benzoic acid No codes names	
2.4	CAS No and EC numbers (IIA2.4)	Non-entry field	
2.4.1	CAS-No	65-85-0	
2.4.2	EC-No	200-618-2	
2.4.3	Other	CIPAC: 622, RTECS: DG0875000,NIOSH: DG 0875000, E-number (as preservative) E 210	
2.5	Molecular and structural formula, molecular mass (IIA2.5)	Non-entry field	
2.5.1	Molecular formula	$C_7H_6O_2$	
2.5.2	Structural formula		
2.5.3	Molecular mass	122.12 g/mol	
2.6	Method of manufacture of the active substance (IIA2.1)	[redacted]	

Gelöscht: 790

Gelöscht:

Gelöscht: 90

Gelöscht: 99

Section A2.10
Annex Point IIA2.10

Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC

Official use only

Subsection	
2.10.1 Human exposure towards active substance	*Guidance is given in the TNsG on Human Exposure as well as the Technical Guidance Document on Risk Assessment*
2.10.1.1 Production	
i) Description of process	not applicable. Production of active substance outside EU.
ii) Workplace description	not applicable. Production of active substance outside EU.
iii) Inhalation exposure	not applicable. Production of active substance outside EU.
iv) Dermal exposure	not applicable. Production of active substance outside EU.
2.10.1.2 Intended use(s)	
1. Professional Users	
i) Description of application process	Crystalline benzoic acid is dissolved in organic solvents, the remaining ingredients are added and the final product packaged in the sales packaging. Except the first step (pouring benzoic acid into the solvents) all subsequent steps are performed in a closed system. Waste from flushing and cleaning is collected and burned in an incinerator for chemical waste.
ii) Workplace description	Pouring of benzoic acid into the solvents under LEV. Workers are equipped with PPE (safety googles, impermeable gloves, filter mask).
iii) Inhalation exposure	Because of the LEV, the filter mask and the low vapour pressure of benzoic acid negligible. Dust formation is negligible, too. In Document II-B some calculations are given to prove the estimation „negligible“.
iv) Dermal exposure	Because of the LEV, the coverall and the impermeable gloves negligible. Dust formation is negligible, too. In Document II-B some calculations are given to prove the estimation „negligible“.
2. Non-professional Users including the general public	
(i) via inhalational contact	Non professional users including the general public are not exposed because the use (production of biocidal products with the active substance benzoic acid) is performed in enclosed premises. During production no third party is allowed to enter the production room.
(ii) via skin contact	Non professional users including the general public are not exposed because the use (production of biocidal products with the active substance benzoic acid) is performed in enclosed premises. During production no third party is allowed to enter the production room.

(iii) via drinking water	Non professional users including the general public are not exposed because the use (production of biocidal products with the active substance benzoic acid) is performed in enclosed premises. There is no possibility that drinking water may be contaminated.
(iv) via food	Non professional users including the general public are not exposed because the use (production of biocidal products with the active substance benzoic acid) is performed in enclosed premises. There is no possibility that food or feed may be contaminated, because the storage and production is performed in a purpose built building without any stored or handled food or feed.
(v) indirect via environment	Non professional users including the general public are not exposed because the use (production of biocidal products with the active substance benzoic acid) is performed in enclosed premises. There is virtually no emission to the environment during or following production.

2.10.2 Environmental exposure towards active substance

2.10.2.1 Production

(i) Releases into water	not applicable. Production of active substance outside EU.
(ii) Releases into air	not applicable. Production of active substance outside EU.
(iii) Waste disposal	not applicable. Production of active substance outside EU.

2.10.2.2 Intended use(s)

Affected compartment(s):	None. Closed system for the production of biocidal products with the active substance benzoic acid
water	No exposure
sediment	No exposure
air	No exposure
soil	No exposure
Predicted concentration in the affected compartment(s)	Not applicable
water	No exposure
sediment	No exposure
air	No exposure
soil	No exposure

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2010/04/14
Materials and methods	The active substance benzoic acid is not produced in the chemical industry of the EU. Therefore, the exposure during the production of the active substance is not assessed under the requirements of the BPD. Applicant's version is acceptable with the remark, that the cross-reference to documents II-B 10.2 should be indicated.
Conclusion	acceptable
Reliability	Not applicable, because given information are not based on standard tests.
Acceptability	Acceptable
Remarks	No further remarks
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Sample table:

Table A2.10: Workplace exposure / Inhalation exposure (use addtional terminology from the TNsGs on Human exposure

Exposure scenario	Workplace operation	PPE	Year(s) of measurement	Number of measurements	Type of measurements	Exposure concentration
Production	not applicable. Production of active substance outside EU	not applicable. Production of active substance outside EU	not applicable. Production of active substance outside EU	not applicable. Production of active substance outside EU	not applicable. Production of active substance outside EU	not applicable. Production of active substance outside EU
Formulation	*Cleaning*	*Protective coverall*			*area, short-term*	
Application MG../PT..	*Brushing*	*Gloves, goggles*				

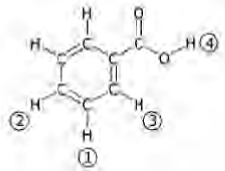
Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density (IIA3.1)								
3.1.1 Melting point		99%	122.4°C		N	1	Williams AE, 1978 (A3.1.1/01) and Maki T, Takeda K, 2002 (A3.1.1/02)	X
3.1.2 Boiling point		99%	249.2°C		N	1	Williams AE, 1978 (A3.1.1/01) and Maki T, Takeda K, 2002 (A3.1.1/02)	X
3.1.3 Relative density		99%	1.321		N	1	Anonymous, 1972 (A3.1.3) and Maki T, Takeda K, 2002 (A3.1.1/02)	X
3.2 Vapour pressure (IIA3.2)		99%	0.0004 – 0.0007 hPa at 20°C		N	1	Anonymous, 1981 (A3.2)	X
3.2.1 Henry´s Law Constant (Pt. I-A3.2)	Calculation		Calculated: 0.0046 – 0.022 Pa x m^3 x mol^{-1}		N	1	Kellner G, 2007a (A3.2.1)	X
3.3 Appearance (IIA3.3)								
3.3.1 Physical state	Visual testing	99%	Crystalline solid substance		N	1	Kellner G, 2007b (A3.3)	X

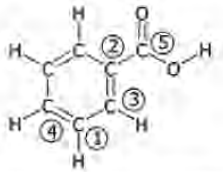
Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.3.2 Colour	Visual testing	99%	White		N	1	Kellner G, 2007b (A3.3)	X
3.3.3 Odour	Sensual testing	99%	Odourless to slightly benzaldehyde-like		N	1	Kellner G, 2007b (A3.3)	X
3.4 Absorption spectra (IIA3.4)								
UV/VIS	UV/VIS	Not given	Molar extinction at 279.0 nm: 729 at 271.5 nm: 893 at 227.5 nm: 11900	Standard Library Spectrum	N	1	Anonymous, 1966 (A3.4.1/01)	
	UV/VIS	100% (by titration)	pH 2 λ_{max} (nm): 230 ε_{max} ($L\ mol^{-1}cm^{-1}$): 15785 $\varepsilon_{290\ nm}$ ($L\ mol^{-1}cm^{-1}$): 184 pH4 λ_{max} (nm): 228 ε_{max} ($L\ mol^{-1}cm^{-1}$): 13544 $\varepsilon_{290\ nm}$ ($L\ mol^{-1}cm^{-1}$): 141 pH7 λ_{max} (nm): 224 ε_{max} ($L\ mol^{-1}cm^{-1}$): 11734 $\varepsilon_{290\ nm}$ ($L\ mol^{-1}cm^{-1}$): 51 pH9 λ_{max} (nm): 224 ε_{max} ($L\ mol^{-1}cm^{-1}$): 11970	Aqueous solution	N	1	Weber L, 2007a (A3.4.1/02)	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
			$\varepsilon_{290\,nm}$ ($L\,mol^{-1}cm^{-1}$): 53					
IR	IR	Not given	IR spectrum in agreement with proposed structure.	Standard Library Spectrum	N	1	Anonymous, 1962 (A3.4.2/01)	
	IR	100% (by titration)	υ (cm^{-1}) / / : 3442.7 / weak, broad / υ(OH); 3072.4 / medium / υ(CH); 3006.8 / medium / υ(CH); 1687.6 / strong / υ(C=O); 1292.2 / strong / υ(C-O); 707.8 / strong / υ (C-H)* * in-phase out-of-plane CH-vibration		N	1	Weber L, 2007b (A3.4.2/02)	X
NMR	NMR	Not given	NMR spectrum in agreement with proposed structure.	Standard Library Spectrum	N	1	Anonymous – (A3.4.3/01)	
	^{1}H-NMR	100% (by titration)	 1: triplet, 7.47, 2 H; 2: triplet, 7.61, 1 H; 3: doublet, 8.11, 2 H; 4: not detectable (J_{HH} 7.6 Hz (ppm))		N	1	Weber L, 2007c (A3.4.3/02)	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
	^{13}C-NMR	100% (by titration)	 1 singlet δ=128.47 ppm 2 singlet δ=129.31 ppm 3 singlet δ=130.21 ppm 4 singlet δ=133.83 ppm 5 singlet δ=172.60 ppm		N	1	Weber L, 2007d (A3.4.3/03)	X
MS	MS	Not given	Mass spectrum in agreement with proposed structure.	Standard Library Spectrum	N	1	Anonymous, 2006 (A3.4.4/01)	
	MS	100% (by titration)	m/z (Intensity) 122.0 (83 %) = $[C_6H_5O_2]^+ = M^+$ 105.0 (100 %) = $[C_6H_5C{=}O]^+$ 77.0 (70 %) = $[C_6H_5]^+$		N	1	Weber L, 2007e (A3.4.4/02)	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.5 Solubility in water (IIA3.5)								
Water solubility 1	EEC A.6 1992	99%	At 20°C pH 5.2: 5.0 g/L pH 9.0: 15.0 g/L	The solution of benzoic acid in distilled water (which is the solvent laid down in the EC guideline) is acidic and not neutral. Therefore, the solubility of benzoic acid in water is not identical with the solubility at neutral pH.	N	1	Kellner G, 1998 (A3.5)	X
Water solubility 2	EEC A.6 1992	99%	At 20°C in distilled water: 2.9 g/L	After dissolution of Benzoic acid in neutral water the pH drops to 2.94 and the solubility to 2.9 g/L	N	1	Kellner G, 1998 (A3.5)	
3.6 Dissociation constant		99%	The dissociation constant at 25°C: $K_a = 6.339 \times 10^{-5}$ at 20°C: $K_a = 6.335 \times 10^{-5}$ Calculated pK_a value is 4.19 Dissociation products of the organic acid: $R\text{-}COO^- + H^+$		N	1	Williams AE, 1978 (A3.1.1/01) and Maki T, Takeda K, 2002 (A3.1.1/02)	X

Section A3 Physical and Chemical Properties of Active Substance

<table>
<tr><th>Subsection (Annex Point)</th><th>Method</th><th>Purity/ Specification</th><th>Results
Give also data on test pressure, temperature, pH and concentration range if necessary</th><th>Remarks/ Justification</th><th>GLP (Y/N)</th><th>Reliability</th><th>Reference</th><th>Official use only</th></tr>
<tr><td>3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA3.1)</td><td></td><td>99%</td><td><table><tr><th>Solvent</th><th>Solubility g/100 g solvent</th></tr><tr><td>Acetone</td><td>55.60</td></tr><tr><td>Benzene</td><td>12.17</td></tr><tr><td>Carbon tetrachloride</td><td>4.14</td></tr><tr><td>Chloroform</td><td>15.02</td></tr><tr><td>Ethanol</td><td>58.40</td></tr><tr><td>Ethyl ether</td><td>40.80</td></tr><tr><td>Hexane (17°C)</td><td>0.94</td></tr><tr><td>Methanol (23°C)</td><td>71.50</td></tr><tr><td>Toluene</td><td>10.60</td></tr></table></td><td></td><td>N</td><td>1</td><td>Williams AE, 1978 (A3.1.1/01) and Maki T, Takeda K, 2002 (A3.1.1/02)</td><td>X</td></tr>
<tr><td>3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA3.2)</td><td></td><td>not applicable</td><td>Benzoic acid is stable in organic solvents.</td><td>Justification for non submission of data submitted</td><td>N</td><td>1</td><td>See Doc IV A3.8</td><td>X</td></tr>
</table>

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.9 Partition coefficient n-octanol/water (IIA3.6)		99%	log P_{ow} = 1.87 (20°C)	The given log P_{ow} 1.87 is for the unionised molecule, which exists in acidic media. For the completely ionised benzoate, which exists in alkaline medium, the log P_{ow} is -2.27. At pH values between 4.5 and 9 there is a gradual decrease from 1.87 to -2.27 because the solubility depends on the pH. Because the log P_{ow} is used for a first estimation of partitioning in the environment, potential bioaccumulation, dermal absorption, a higher log P_{ow} is more alarming than a lower or even negative one. As a conservative base for further estimations and calculations the highest possible value for benzoic acid, namely 1.87, should be used. Benzoic acid is as a reference substance with known log P_{ow} of 1.9 mentioned in the	N	1	Freitag D, Ballhorn L, Geyer H, Korte F, 1985 (A3.9) and Maki T, Takeda K, 2002 (A3.1.1/02)	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
				OECD guideline 117. Additional experimental determinations at intermediate pH values do not add any additional safety and seem to be unnecessary.				
3.10 Thermal stability, identity of relevant breakdown products (IIA3.7)	Literature review	not applicable	Upon heating to 150 °C, some dehydration takes place to form benzoic anhydride. Decarboxylation occurs when benzoic acid is heated above 370 °C or as low as 245 °C in the presence of catalysts . Benzene and a small amount of phenol are formed		N	1	Maki T, Takeda K, 2002 (A3.1.1/02)	
3.11 Flammability, including auto-flammability and identity of combustion products (IIA3.8)	Literature review	not applicable	Benzoic acid is not flammable, however dusts may explode. The explosion limit of the vapour/air mixture is 0.085-0.99g/m^3. Auto-ignition temperature in air: 573°C. Combustion products CO, CO_2. From the structural formula and composition of the substance it can be concluded that benzoic acid is not highly flammable. This conclusion is confirmed by experience in use since more than 150 years. A test according to EC-method A.10. FLAMMABILITY (SOLIDS) is regarded not to be		N N N	1 1 1	Williams AE, 1978 (A3.1.1/01) and Maki T, Takeda K, 2002 (A3.1.1/02)	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
	EEC A10, 1992	99.99% Batch no. 35361068	necessary, but was performed on special request of the German competent authority: **Benzoic acid could not be ignited. Therefore, benzoic acid is considered not to be flammable.** From the structural formula and composition of the substance it can be concluded that benzoic acid does not evolve any flammable gases in contact with water or humid air. From the structural formula and composition of the substance it can be concluded that benzoic acid does not ignite spontaneously after coming into contact with air at room temperature.		Y	1	Fieseler A, 2008a (A3.11/03)	
3.12 Flash-point (IIA3.9)	Literature review	99%	121.1°C	Although the determination of the flash point is generally not required for solids with a melting point above 40 °C, in this special case due to the sublimation at temperatures above 100°C the determination has been performed. The	N	1	Williams AE, 1978 (A3.1.1/01) and Maki T, Takeda K, 2002 (A3.1.1/02)	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
				experimentally determined flash point is below the melting point, which was verified in various tests.				
3.13 Surface tension (IIA3.10)	EEC A5, 1992	99,99% Batch no. 35361068	60.0 mN/m at 20 ± 0.1°C (1 g/L aqueous solution)		Y	1	Fieseler A, 2008b (A3.13)	X
3.14 Viscosity			Only for liquid substances	Justification for non-submission of data submitted			See A3.14	X
3.15 Explosive properties (IIA3.11)	Jusification		No explosive properties	Justification submitted			See A3.15	
3.16 Oxidizing properties (IIA3.12)	Jusification		No oxidising properties	Justification submitted			See A3.16	
3.17 Reactivity towards container material (IIA3.13)	Justification		No reactivity with glass, paper bags, plastic containers and stainless steel.	Justification submitted			See A3.17	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	

Section A4.2.01 **Annex Point IIA4.1/4.2 & IIIA-IV.1**	**Analytical Methods for Detection and Identification** A4.2.01/01 Analytical method for determination of benzoic acid in soil	Official use only
	1 REFERENCE	
	Baziramakenga R, Simard RR, Leroux GD, 1995, Determination of organic acids in soil extracts by ion chromatography Soil Biol. Biochem. 27 (1995), No. 3, pp.349-356 (A4.2.01/01) Jalal MAF, Read DJ, 1983, The organic acid composition of Calluna heathland soil with special reference to phyto- and fungitoxicity Plant and Soil 70 (1983), pp. 273-286 (A4.2.01/02)	
	2 RESULTS	
Baziramakenga R, Simard RR, Leroux GD, 1995 (A4.2a/01)	Extraction of free organic acids from soil with distilled water. Extraction of bound organic acids from soil with 100mM NaOH. Acidification (pH 2.5) of the extracts and partitioning into ethyl acetate. Evaporation to dryness and re-dissolution with water. Determination with Ion chromatography / UV detection Ion chromatograph: Dionex series 4000i (Dionex Corp., Sunnyvale CA) Column: OmniPac Pax-100 (4 x 50) Mobile phase: with A. deionized water, B. 80% acetonitrile, C. 4 M NaCl + 4 mM NaOH, isocratic followed by a gradient step Flow rate 15 mL/min Auto sampler and 50µl value loop injector Degasing module Cell for conductivity detection Wavelength 254 nm UV-Vis detector: Model VDM-II Dionex (Dionex Corp., Sunnyvale CA) and a Dionex 4270 integrator which in turn was connected to an Epson Equity I computer via Labnet Network UV detection at 254 nm of benzoic acid Benzoic and p-hydroxybenzoic acids were found to be the most abundant among aromatic acids. In soil containing decomposed crops a benzoic acid concentration of approximately 4 to 10 mg/kg was determined.	
Jalal MAF, Read DJ, 1983 (A4.2a/02)	Benzoic acid is an ubiquitous occurring substance in soil. o-hydroxybenzoic and benzoic acid are the most abundant aromatics in soil, each reaching levels around 1 mg/100 g soil (= approximately 0.03 mM) in Calluna heathland soil	
	3 CONCLUSION Benzoic acid occurs naturally in soil (0.32-10.8 mg/kg). Regarding the natural occurrence of benzoic acid in plants and - as a consequence - in soil, the biological degradation of this chemical substance (mainly by micro-organisms) is very effective and an accumulation does not occur. The content of benzoic acid in the soil is depending on the intake and subsequent degradation, therefore the found natural concentration levels cover a wide range. Due to the many sources and the ubiquitous occurrence of benzoic acid it is not possible by any method, to distinguish between contamination by using benzoic acid as biocidal product and natural or industrial background levels. By taking account these facts, the evaluation of a separate analytical method for the determination of 0.05 mg/kg benzoic acid in standard soil will not be suitable. Therefore, the described method (Baziramakenga R, Simard RR,	

	Leroux GD, 1995, A4.2a/01) is recommended because a high number of aliphatic and aromatic organic acids can be determined simultaneously with this method.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/03/19
Evaluation of applicant's justification	Because of missing validation data the acceptability of the method of Baziramakenga, Simard and Leroux/1995 cannot be judged. Nevertheless the applicant's justification is adopted. Positive findings of benzoic acid cannot be associated with an application of the biocidal product, as benzoic acid is a ubiquitous occurring substance in soil. Therefore, a method for the determination of benzoic acid in soil is not necessary.
Conclusion	The applicant's conclusion is acceptable. A method for the determination of benzoic acid in soil is not necessary.
Remarks	Some corrections for the description of the method: - size of the used column: 4 x 250 mm instead of "4 x 50"; - description of the mobile phase is not comprehensible, as the composition is missing: for the first 10 min isocratic (70 % eluent A, 25 % eluent B and 5% eluent C) changing in 15 min to 35 % eluent A, 25 % eluent B and 40 % eluent C; - flow: 1 mL/min instead of "15 mL/min" - "cell for conductivity detection" was not used for the detection of the aromatic acids (only for aliphatic acids)
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4.2.02 Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

A4.2.02 Analytical method for determination of benzoic acid in air

Official use only

1 REFERENCE

1.1	**Reference**	Schumacher B, Brockmann A, 2009, Method validation – Determination of benzoic acid in air. SGS Institut Fresenius, Taunusstein, Germany; unpublished report no. VB-09TSAA002-1, November 06, 2009
1.2	**Data protection**	Yes
1.2.1	Data owner	MENNO CHEMIE-VERTRIEB G.M.B.H., Norderstedt, Germany
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline**	Internal SOP_M_1983 Determination of benzoic acid in air 06.11.09 based on NIOSH 7903 Inorganic acids
2.2	**GLP**	No, but quality assurance system DIN EN ISO/IEC 17025
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Benzoic acid as given in section 2
3.1.1	Lot/Batch number	35361068
3.1.1.1	**Description**	As given in section 2
3.1.1.2	**Purity**	99.99%, Certificate of analysis dated 25-October-2004
3.2	**Preliminary treatment**	
3.2.1	Enrichment	ORBO 53 Activated silica gel tube with glass fibre filter, air flow rate 0.5 L/min
3.2.2	Cleanup	No clean up
3.3	**Detection**	
3.3.1	Separation method	Ion chromatography

Ion chromatograph	ICS-3000, Dionex, Idstein With conductivity detector and UV Detector
Pre column	AG 23 Anion-exchange column
Separation Column	AS 23 Anion-exchange column, Dionex, Idstein
Software	Chromeleon 6.80, Dionex, Idstein
Flow rate	1 mL/min

3.3.2	Detector	UV Detector, Dionex, Idstein
3.3.3	Interfering substance(s)	No interferences of the blank samples were observed.
3.4	**Linearity**	
3.4.1	Calibration range	0.1 to 1.0 mg/L and 0.1 to 10 mg/L

Section A4.2.02 Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

A4.2.02 Analytical method for determination of benzoic acid in air

3.4.2 Number of measurements: 10

3.4.3 Linearity: Smooth test according to Mandel

In the range 0.2 to 10 mg/L a linear calibration function was obtained for the chromatographic determination.

For the entire method linearity was proven in the range from 0.2 to 8 mg/m^3

Correlation coefficients:
0.9997 for the calibration range 0.1 to 1.0 mg/L

0.9987 for the calibration range 0.1 to 10 mg/L

3.5 Specifity: interfering substances: Under the described experimental conditions, no interferences have been observed.

3.6 Accuracy / Recovery rates at different levels

Benzoic acid (BA)

BA nominal mg	BA measured [mg/L]	Peak area AU·min	BA measured [mg]	Recovery [%]
0	n.n.	0.000093	n.n.	
0.0062	0.127	0.000952	0.0064	101.9
0.0112	0.229	0.001724	0.0115	102.3
0.0159	0.325	0.002442	0.0163	102.2
0.0212	0.407	0.003057	0.0204	95.8
0.0262	0.502	0.003773	0.0251	95.7
0.0308	0.583	0.004383	0.0292	94.7
0.0345	0.678	0.005099	0.0339	98.3
0.0396	0.762	0.005729	0.0381	96.3
0.0422	0.779	0.005854	0.0389	92.2
0.0477	0.920	0.006917	0.0460	96.4
0.1018	2.06	0.015494	0.1030	101.2
0.2125	4.40	0.033065	0.2200	103.5
0.2990	6.25	0.046961	0.3125	104.5
0.5076	10.47	0.078739	0.5235	103.1
Mean recovery rate			%	99.1
Standard deviation			%	3.95

3.7 Precision

Benzoic acid (BA)

BA nominal mg	Peak area AU·min	BA measured [mg/L]	BA measured [mg]	Recovery [%]
0.0477	0.006917	0.920	0.0460	96.4
0.0487	0.007179	0.955	0.0478	98.0
0.0495	0.006947	0.924	0.0462	93.4
0.0483	0.007256	0.965	0.0483	99.8
0.0495	0.006950	0.924	0.0462	93.4
0.0477	0.007042	0.937	0.0469	98.2
Mean recovery rate			%	96.5
Standard deviation			%	2.68
Confidence region			%	2.81

Section A4.2.02

Annex Point IIA4.1/4.2 & IIIA-IV.1

Analytical Methods for Detection and Identification

A4.2.02 Analytical method for determination of benzoic acid in air

3.8 Robustness

3.8.1 Influence of the flow rate

Benzoic acid (BA)

BA nominal mg	Flow rate L/min	Peak area AU·min	BA Measured [mg/L]	BA Measured [mg]	Recovery [%]
0.0494	0.1	0.006853	0.911	0.0456	92.3
0.0477	0.5	0.006917	0.920	0.0460	96.4
0.0500	0.75	0.006949	0.924	0.0462	92.4
0.0496	1.0	0.006881	0.915	0.0458	92.2

The mean recovery rate was 92.3 %

3.8.2 Influence of the sampling time

Benzoic acid (BA)

BA nominal mg	Sampling time min	Peak area AU·min	BA measured [mg/L]	BA measured [mg]	Recovery [%]
0.0494	60	0.007116	0.946	0.0473	95.7
0.0477	120	0.006917	0.920	0.0477	96.4
0.0500	120	0.006925	0.921	0.0461	92.2
0.0496	300	0.006909	0.919	0.0459	92.6

The mean recovery rate was 94.2 %

3.8.3 Influence of the air humidity

Benzoic acid (BA)

BA nominal mg	Relative air humidity %	Peak area AU·min	BA measured [mg/L]	BA measured [mg]	Recovery [%]
0.0477	45	0.006917	0.920	0.0477	96.4
0.0504	100	0.007518	1.000	0.0500	99.2

The mean recovery rate was 97.8 %

3.8.4 Influence of the storage time and temperature

Benzoic acid (BA)
Storage temperature 20 – 25°C

BA nominal mg	Storage time d	Peak area AU·min	BA measured [mg/L]	BA measured [mg]	Recovery [%]
0.0503	3	0.007661	1.019	0.0509	101.2
0.0504	4	0.007199	0.957	0.0479	95.0
0.0502	7	0.007428	0.988	0.0494	98.4
0.0494	14	0.007178	0.955	0.0477	96.6
0.0495	21	0.007856	1.045	0.0522	105.5
0.0505	28	0.007335	0.976	0.0488	96.6

Storage temperature 4 – 8°C

BA nominal mg	Storage time d	Peak area AU·min	BA measured [mg/L]	BA measured [mg]	Recovery [%]
0.0507	3	0.007635	1.015	0.0508	100.2
0.0503	4	0.007528	1.001	0.0501	99.6
0.0502	7	0.007558	1.005	0.0503	100.2
0.0499	14	0.007196	0.957	0.0478	95.8

Section A4.2.02

Annex Point IIA4.1/4.2 & IIIA-IV.1

Analytical Methods for Detection and Identification

A4.2.02 Analytical method for determination of benzoic acid in air

0.0498	21	0.007268	0.967	0.0483	97.0
0.0497	28	0.007241	0.963	0.0481	96.8

The mean recovery rate (all temperatures) was 99.9 %

3.9	**Limit of determination**	0.03 mg/m^3 Benzoic acid (Limit of determination as defined in DIN 32645)
3.10	**Limit of quantification**	0.10 mg/ m^3 Benzoic acid (Limit of quantification as defined in DIN 32645)
3.10.1	Independent laboratory validation	Not performed

Section A4.2.02

Annex Point IIA4.1/4.2 & IIIA-IV.1

Analytical Methods for Detection and Identification

A4.2.02 Analytical method for determination of benzoic acid in air

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

For determination of benzoic acid as an aerosol or gas in air, a defined air volume was drawn through a silica gel adsorption tube (ORBO 53). The adsorbed benzoic acid was desorbed by a slightly alkaline solution and analysed by ion chromatography with an UV detector at 230 nm.

For a summary of the analytical methods and results in tabular form see Document II A (effects and exposure assessment assessment), Table 1.4.

4.2 Summary

Operating parameters (chromatography)		**Acceptance requirements**
Limit of determination	0.02 mg/m³	0.08
Limit of quantification	0.06 mg/m³	0.25
Method coefficient of variation	1.5 %	-
Operating parameters (entire method)		**Acceptance requirements**
Limit of determination	0.03 mg/m³	0.15
Limit of quantification	0.10 mg/m³	0.5
Method coefficient of variation	2.7 %	-
Accuracy (entire method)		
Mean recovery rate in the range 0.08 – 8 mg/m³	99.1 %	90 - 110
Precision (entire method)		
Mean recovery rate	96.5 %	90 - 110
Relative confidence region of overall mean	2.81 %	≤5
Robustness / Recovery rate (entire method)		
Influence of the flow rate	92.2 – 96.4%	90 - 110
Influence of the sampling time	92.2 – 96.4%	90 - 110
Influence of the air humidity	96.4 – 99.2 %	90 - 110
Influence of the storage time and temperature	95.0 – 105.5%	90 - 110

4.3 Conclusion

The following determination and quantification limits can be obtained for typical sampling scenarios and work place measurements.

Sampling time (min)	**Flow rate (L/min)**	**Sampling volume (L)**	**Determination limit (mg/m³)**	**Quantification limit (mg/m³)**
15	0.5	7.5	0.3	0.8
120	0.5	60	0.03	0.1
240	0.5	120	0.02	0.05
480	0.5	240	0.01	0.03

The method is suitable for work place measurements

4.3.1 Reliability 1

4.3.2 Deficiencies No

Section A4.2.02 Analytical Methods for Detection and Identification

Annex Point IIA4.1/4.2 & IIIA-IV.1

A4.2.02 Analytical method for determination of benzoic acid in air

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2010/01/04
Materials and methods	Exposure at workplaces: The analytical procedure described by the participant is applicable for the determination of workers' exposure at workplaces.
Conclusion	Exposure at workplaces: The analytical procedure described by the participant is applicable for the determination of workers' exposure at workplaces.
Reliability	1
Acceptability	Acceptable
Remarks	No remarks
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4.2.03 Annex Point IIA4.1/4.2 & IIIA-IV.1	Analytical Methods for Detection and Identification A4.2.03 Analytical method for determination of benzoic acid in water	Official use only

Official use only

1 REFERENCE

1.1	**Reference**	Meinerling M, Hermann S, 2004, Validation of an Analytical Method for the Determination of Benzoic Acid in aqueous Samples, IBACON GmbH, Roßdorf, Germany unpublished report no. 21953101; December 21, 2004
1.2	**Data protection**	Yes
1.2.1	Data owner	MENNO CHEMIE-VERTRIEB G.M.B.H., Norderstedt, Germany
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline**	SANCO/825/00 rev.7 guidance document on residue analytical methods, European Directorate General Health and Consumer Protection, March 17, 2004
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**		
3.1.1	Enrichment	No enrichment	
3.1.2	Cleanup	No Cleanup	
3.2	**Detection**		
3.2.1	Separation method	HPLC separation	
		Equipment	LaChrom, Merck L-7100 pump LaChrom, Merck L-7200 autosampler LaChrom, Merck L-7360 column oven
		Column	Ultrasep ES RP 18 250 + 4 mm) with pre column (supplier: SepServ, Berlin)
		Mobile phase	50% methanol / 50% 0.3 mol H_3PO_4 (v/v)
		Flow rate	0.7 mL/min
		Injection volume	50 µl
		Oven temperature	25°C
		Wavelength	245 nm
3.2.2	Detector	LaChrom, Merck L-7420 UV-VIS Detector UV detection of benzoic acid at 245 nm	
3.2.3	Standard(s)	External standard / Benzoic acid pharmaceutical grade (Ph. Eur., BP)	
3.2.4	Interfering substance(s)	No interferences of the blank samples were observed	
3.3	**Linearity**		
3.3.1	Calibration range	0.25 to 2.5 mg/L and 2 to 25 mg/L / a linear regression was obtained	

3.3.2 Number of measurements: 5

3.3.3 Linearity: 0.25 to 2.5 mg/L and 2 – 25 mg/L

Correlation coefficient
for the calibration range 0.25 to 2.5 mg/L at least 0.9974
for the calibration range 2 to 25 mg/L at least 0.9989

3.4 Specifity: interfering substances: Under the described experimental conditions, no interferences have been observed.

3.5 Recovery rates at different levels

The mean recovery rates in the fortified samples (Isomedium)

mg/kg	Recoveries (%)	Mean recovery (%)	Standard deviation (%)
0.75	102, 93, 105, 98, 103	100	5
1	91, 90, 88, 89, 90, 107, 92, 108, 91, 85	93	8
2	80, 82, 85, 83, 85, 78, 82, 91, 82, 83	83	3
12.5	102, 103, 100, 100, 107	102	3
150	103, 104, 102, 101, 106	103	2

The mean recovery rates in the fortified samples (Elendt-Medium)

mg/kg	Recoveries (%)	Mean recovery (%)	Standard deviation (%)
0.75	111, 87, 109, 104, 99	101	10
1	91, 87, 90, 87, 87, 84, 89, 88, 88, 86	88	2
2	80, 79, 87, 81, 76, 87, 87, 87, 95, 91	85	6
12.5	103, 103, 99, 100, 105	102	2
150	105, 106, 104, 104, 103	104	1

3.5.1 Relative standard deviation: The overall relative standard deviation of the recovery rate was ≤ 10 %

3.6 Limit of determination: 0.04 mg/L (Quantification Limit: 0.75 mg/L)

3.7 Precision: The mean recovery rate was in the range of 81 – 104% and the overall relative standard deviation of the recovery rate was ≤ 10 %. Therefore the recovery rates were well within the required range.

3.7.1 Repeatability

Repeatability of injections

Calibration range mg/L	Mean area	Coefficient of variation (%)
0.25	37649	0.6
0.5	65278	0.6
1.5	182192	0.3
2.5	299045	0.6
2	351244	1
10	1243991	1

25	2982933	1.3

The relative standard deviation range from 0.25 to 25 mg/L was ≤ 1.3%

3.7.2 Independent laboratory validation: Not performed

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

Benzoic acid was determined by an external standard isocratic HPLC-method on a reversed stationary phase (Ultrasep ES RP 18) with UV detection of benzoic acid at 245 nm

For a summary of the analytical methods and results in tabular form see Document II-A (effects assessment), Table 1.4.

4.2 Conclusion

The analytical method was valid for analysis of benzoic acid in a concentration range of 0.75 to 150 mg/kg., which covers the expected exposition range for ecotoxicity tests..

The HPLC method can be used for quantification of the test item in aqueous samples.

4.2.1 Reliability: 1

4.2.2 Deficiencies: No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/03/19
Materials and methods	The applicant´s version is acceptable with the following remark: X: The value of 0,04 mg/L refers to the limit of detection..
Conclusion	A validated primary LC-UV method for the determination of benzoic acid in surface water was presented. The LOQ is 0.75 mg/L. A PNEC value is not calculated by the applicant. Therefore, the sensitivity of the method cannot be evaluated. However, based on the NOEC of the known most sensitive aquatic species (Pseudokirchneriella subcapitata) of 7.5 mg/L, method´s sensitivity is sufficient. A validated confirmatory method was not included. The confirmation by DAD spectrum could not be accepted, as only the spectrum of the standard solution was shown. Benzoic acid or their salts are authorized in alcoholic and non-alcoholic beverages at levels of up to 200 mg/L (see Directive 95/2/EC on food additives other than colours and sweeteners). Benzoic acid is an ubiquitously occurring substance and residues arising from the use as biocidal product are not expected. Therefore, a method for the determination of benzoic acid in water is not necessary.
Reliability	1
Acceptability	acceptable
Remarks	In point 3.7. the range of the mean recovery is 83 - 104 % instead of 81 – 104 %; in point 4.2. the dimension unit is mg/L instead of mg/kg.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Gelöscht: Nevertheless positive findings of Benzoic acid in surface and drinking water cannot be associated with an application of the biocidal product, as Benzoic acid is a ubiquitous occurring substance.

Section A6.1.1
Annex Point IIA6.1

Acute Toxicity
A6.1.1 Acute oral toxicity in rat (Determination of LD_{50})

Official use only

1 REFERENCE

1.1	**Reference**	Deuel HJ, Alfin-Slater R, Weil CS, Smyth HF, 1954, Sorbic acid as a fungistatic agent for foods. I. Harmlessness of sorbic acid as a dietary component. Fd. Res., 19, 1-12
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	No data protection / published data

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Study conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	U.S.P Sodium benzoate (Merck)
3.1.1	Lot/Batch number	No lot/batch number available
3.1.2	Specification	U.S.P (Unites states Pharmacopoeia)
3.1.2.1	Description	White crystalline solid substance
3.1.2.2	Purity	>99%
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.

Section A6.1.1 Acute Toxicity

Annex Point IIA6.1 A6.1.1 Acute oral toxicity in rat (Determination of LD_{50})

3.2	**a) Test Animals**	Non-entry field
3.2.1	Species	Rat
3.2.2	Strain	Rattus norvegicus Sherman
3.2.3	Source	A: Department of Biochemistry and Nutritation, University of Southern California, USA B: Mellon Institute of Industrial Research, University of Pittsburgh, USA
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	**Weight** **Laboratory A** Male: 212 - 430 g Female: 163 - 267 g **Laboratory B** Mean weight: 90 - 120 g
3.2.6	Number of animals per group	**Laboratory A** 5 Male and 5 female (total 70 rats) **Laboratory B** 5 Male and 5 female
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Postexposure period	Yes, 14 days
3.3.2	Type	**Oral** **Laboratory A** Gavage previously fastened for 18 hours **Laboratory B** Gavage without fastening
3.3.3	Concentration	0, 1, 2, 4 and 8% (= 0, 0.64, 1.32, 2.62 and 6.29 g/kg day)
3.3.4	Vehicle	Water
3.3.5	Concentration in vehicle	10%
3.3.6	Total volume applied	No data given
3.3.7	Controls	Food only
3.4	**Examinations**	Mortality
3.5	**Method of determination of LD_{50}**	No data given
3.6	**Further remarks**	-

Section A6.1.1 Acute Toxicity

Annex Point IIA6.1 A6.1.1 Acute oral toxicity in rat (Determination of LD_{50})

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	No data given
4.2	**Pathology**	No data given
4.3	**Other**	-
4.4	**LD_{50}**	LD_{50} for sodium benzoate, calculated as benzoic acid **Laboratory A** Previously fastened for 18 hours: LD_{50}: 1700 – 2500 mg/kg bw **Laboratory B** Without fastening: LD_{50}: 3450 – 3740 mg/kg bw

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In this test, the acute oral toxicity of sorbic acid and of sodium benzoate was tested in Sherman rats. The determination of the toxicity of benzoic acid (re calculated from sodium benzoate) was an effective part of this study. The test substance was administered orally by incorporation into the diet. The study was conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid for sodium benzoate and with restriction for benzoic acid.
5.2	**Results and discussion**	LD_{50} for sodium benzoate, calculated as benzoic acid was 1700 – 2500 mg/kg bw (Previously fastened) 3450 – 3740 mg/kg bw (Without fastening)
5.3	**Conclusion**	Non-entry field
5.3.1	Reliability	2
5.3.2	Deficiencies	Sodium benzoate instead of benzoic acid was tested. Obviously, the report was written with the intentition to demonstrate the superiority of sorbic acid compared to benzoic acid and sodium benzoate. It seems that the often cited LD50 for benzoic acid of 1700 mg/kg b.w. originates from this single experimental study, where in one laboratory this result was achieved with sodium benzoate.

Section A6.1.1 Acute Toxicity

Annex Point IIA6.1 A6.1.1 Acute oral toxicity in rat (Determination of LD_{50})

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/11/30
Materials and Methods	3.1.2.3 Not stated in the report 3.2.2 Laboratory A: University of Southern California strain Laboratory B: Sherman strain 3.2.7 Not stated in the report 3.3.2 Laboratory A: Gavage previously fastened for 18 hours Laboratory B: without fastening, gavage not stated in report 3.3.3 Concentrations are not reported. 3.3.4 Laboratory A: unknown vehicle Laboratory B: water 3.3.5 Laboratory A: unknown concentration in vehicle Laboratory B: 10 % 3.3.7 Not stated in the report 5.1 In this test, the acute oral toxicity of sorbic acid and of sodium benzoate was tested in rats of two different strains: University of Southern California strain and Sherman strain.
Results and discussion	Applicant's version is acceptable.
Conclusion	LD_{50} for sodium benzoate, calculated as benzoic acid was 2100 mg/kg bw (1700-2500 mg/kg bw; Laboratory A, fasted rats) 3450 mg/kg bw (3150-3740 mg/kg bw; Laborytory B, unfasted rats)
Reliability	2
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.1 Acute Toxicity

Annex Point IIA6.1 A6.1.1 Acute oral toxicity in rat (Determination of LD_{50})

Table A6_1-1/01. Tables for Acute Toxicity

LD_{50} for sodium benzoate, calculated as benzoic acid was
1700 – 2500 mg/kg bw (Previously fastened)
3450 – 3740 mg/kg bw (Without fastening)

Table A6_1-1/02. Tables for Acute Toxicity / Additional data Benzoic acid and Sodium Benzoate

Mice:

Reference: Abe, S. et al., 1984 (A6.1./02)
Animals: 10 mice per group
Application: Oral, 1206-3000 mg/kg bw benzoic acid
Clinical observations: Sedation, slow respiration, then gasping, lacrimation, ptosis, loss of righting reflex, clonic convulsions, jumping, tail reaction, death within 10-120 0minutes.
LD_{50} = 1940 (1740 – 2170) mg/kg bw

Reference: Caujolle, M. F. & D. Meynier, 1958 (A6.1./07)
Animals: 20 mice per group
Body weight: Ca 20 g
Application: Intraperitoneal, benzoic acid doses near LD_{50} (1460 mg/kg bw)
Clinical observations: Hyperaesthesia, hypothermia, no convulsions.
LD_{50} = 1460 mg/kg bw

Dogs:

Reference: Rost E, Franz F und Weitzel A, 1913 (A6.1.1/13)
Animals: 17 dogs
Initial body weight: 1.8-7.8 kg
Application: Single oral doses (gavage) on different days within 3 weeks with different feeding conditions, 1.8-7.8 g sodium benzoate (ca 1000-2200 mg/kg bw)
Clinical observations: At ca 1000 mg/kg bw: No findings
At ca 1200-2200 mg/kg bw: Vomiting; after re-intake of the vomit: aggressiveness, hallucination and in one animal death
Application: Three oral doses (gavage) to the remaining 3 dogs within 1 hour (non-fasted), 4-6.9 g sodium benzoate (2880 mg/kg bw)
Clinical observations: Vomiting; intermittent convulsions and death in one animal which did not vomit the second and third portion.
Lowest lethal dose = 2000 mg/kg bw

Rabbits:

Reference: Rost E, Franz F und Weitzel A, 1913 (A6.1.1/13)
Animals: Three rabbits
Initial body weight: 2.07-2.65 kg
Application: Three single oral doses (gavage) on different days within 3 weeks to not fasted animals, 4.1-7.2 g sodium benzoate (ca 1700-2440 mg/kg bw as benzoic acid)
Clinical observations: At ca 1700 mg/kg bw: No findings
From ca 2000 mg/kg bw: Refused food intake, convulsions similar to that of dogs, death
Application: Single dose to fasted animals (1 or 2 days), 3.7-7.2 g sodium benzoate (ca 1220-2440 mg/kg bw as benzoic acid)
Clinical observations: At ca 1220 mg/kg bw: No findings
From ca 1500 mg/kg bw: Tremor, side position, convulsions similar to that of dogs, death.
Lowest lethal dose = 1500 mg/kg bw

Guinea pigs:

Reference: Ellinger, A., 1923 (A6.1.1/08)
Application: Intraperitoneal, 1400 mg/kg bw sodium benzoate

Section A6.1.1 Acute Toxicity

Annex Point IIA6.1 A6.1.1 Acute oral toxicity in rat (Determination of LD_{50})

Clinical observations:	Crouched position without movement, without tremor, without loss of muscle tone, piloerection, extreme hypothermia, death by respiratory paralysis within 5-7 hours. Lowest lethal dose = 1400 mg/kg bw
Cats:	
Reference:	Bedford, B. & M.A. Clarke, 1972 (A6.1.1/06)
Rationale:	Suspected poisoning in a cattery, benzoic acid content in the cat meat: 2.39% (Bedford, B. & M.A. Clarke, 1971)
Animal number:	Four cats
Body weight:	1.06-1.7 kg
Application:	Feeding of 120 g meat with 1% benzoic acid (doses consumed: 450-890 mg/kg bw)
Clinical observations:	Onset ca 14 hours after food intake, duration of syndrome 18-176 hours; salivation, aggressiveness, hyperaesthesia, apprehension, hypothermia collapse, death within 32 and 192 hours. One cat, which gradually consumed the food (890 mg/kg bw benzoic acid), exhibited disturbed co-ordination, extreme aggressiveness, hyperaesthesia, hyperthermia, convulsions but recovered thereafter.
Necroscopy:	Gastro-intestinal-tract: Mouth and tongue ulcerated, retention of faeces and urine, no visible abnormalities in stomach and intestines Liver: Pale, centrilobular vacuolation, hepatic and Kupffer cells swollen, cytoplasm foamy and granular, infiltration of inflammatory cells into the portal tract Kidney: Distension of kidney glomeruli with widespread herniation of proximal tubules into Bowman's capsule Lung: oedematous, areas of emphysema and hemorraghia Heart: Myocardial foci of cell infiltration and degeneration
Histopathology:	Central nervous system: no abnormalities
Remark:	The higher toxicity in cats than in other species was attributed by the authors to the lack of capacity in cats for glucuronidation of benzoic acid. Lowest lethal dose = 630 mg/kg bw
Rats:	
Reference:	Hager, G.P. et al., 1942 (A6.1.1/10)
Animals:	Five white rats per group
Body weight:	90-150 g
Application:	Intravenous, 1200-2290 mg/kg bw sodium benzoate
Clinical observations:	Symptoms within few minutes after injection; heightening of CNS reflexes, tremor, clonic and often tetanic convulsions, death during remission, mostly within 30 min and 1 hour. Survivors showed none of the nervous manifestations but salivation, vomiting, diarrhoea and marked diuresis (some animals). LD_{50} = 1714 ± 124 mg/kg bw

Summary: Acute oral toxicity studies with benzoic acid/benzoic salts

Test substance	Result (mg/kg bw)	Reference
Rat		
Benzoic acid	LD_{50}: > 1700	Anonymous, SANCO/1396/2001-Final, 2003, (A6.1.1/05)
Sodium benzoate	LD_{50}: > 2100	
Sodium benzoate / calculated as benzoic acid	LD_{50}: 2100 (fasted)	Deuel HJ, Alfin-Slater R, Weil CS, Smyth HF, 1954 (A6.1.1/01)
	LD_{50}: 3450 (not fasted)	
Benzoic acid	LD_{50}: 2000 – 2500	Anonymous, Bio-Fax, 1973, cited in Anonymous, BUA Report, 1995 (A6.1.1/04)
		Ignatiev AD, 1965 (A6.1.1/11)
	LD_{50}: 1700 – 3040	Fassett DW, Irish DD,1962 (A6.1.1/09)
Sodium benzoate	LD_{50}: 4100 (3720 – 4440)	Smyth HF, Carpenter CP, 1948 (A6.1.1/14)
Mouse		

Section A6.1.1 Acute Toxicity

Annex Point IIA6.1

A6.1.1 Acute oral toxicity in rat (Determination of LD_{50})

	LD_{50}: 1940 (1740-2170)	Abe S, Tsutsui Y Tarumoto Y, Nakane S, 1984 (A6.1.1/02)
Rabbit		
Benzoic acid	lowest lethal dose: ca 1700	Rost E, Franz F und Weitzel A, 1913 (A6.1.1/13)
Dog		
Sodium benzoate	lowest lethal dose: ca 2000	Rost E, Franz F und Weitzel A, 1913 (A6.1.1/13)
Cat		
Benzoic acid / Benzoic salt	lowest lethal dose: ca 2000	Ellinger A, 1923 (A6.1.1/08)
Benzoic acid	lowest lethal dose: ca 630	Bedford B, Clarke MA, 1972 (A6.1.1/06)

Table: Acute toxicity studies with benzoic acid/benzoic salts, other routes

Test substance	Route	Species	Result (mg/kg bw)	Reference
Sodium benzoate	iv	rat	LD_{50}: 1714 ±124	Hager GP, Chapman CW, Starkey EB, 1942 (A6.1.1/10)
Benzoic acid	ip	mouse	LD_{50}: 1460	Caujolle MF, Meynier D, 1958 (A6.1.1/07)
Sodium benzoate	ip	guinea pig	lowest lethal dose: 1400	Ellinger A, 1923 (A6.1.1/08)
Benzoic acid / Benzoic salt	sc	rabbit	lowest lethal dose: ca 2000	Ellinger A, 1923 (A6.1.1/08)

Section A6.1.2 **Annex Point IIA6.1**	**Acute Toxicity** A6.1.2 Acute dermal toxicity in rat (Determination of LD_{50})	Official use only
	1 REFERENCE	
	Anonymous, Review report for the inclusion of benzoic acid in Annex I of Directive 91/414/EEC, SANCO/1396/2001-Final, 2003, (A6.1.1/05) Anonymous, Bio-Fax, 1973, cited in Anonymous, Benzoic acid / sodium benzoate, BUA Report, 1995 (A6.1.1/04) Moreno OM, 1977, Report to RIFM, 22 August 1977, cited in Opdyke DL, 1979, Benzoic acid, Fd Cosmet. Toxicol., 17, 715-722A6.1.2/02)	
	2 RESULTS	
	In a limit test with rabbits, no mortality or signs of intoxication were seen after dermal application of 10.000 mg/kg body weight. The gross autopsy gave no significant findings. (no further information available). Anonymous, Bio-Fax, 1973, cited in Anonymous, BUA report, 1995 (6.1.1/04) The LD_{50} value in rabbits exceeded 5 g/kg body weight. Moreno OM, 1977 (cited in Opdyke DL, 1979, 6.1.2/02)	

Summary

Test substance	Result (mg/kg bw)	Reference
Benzoic acid	LD_{50}: > 5000	Anonymous, SANCO/1396/2001-Final, 2003, (A6.1.1/05)
Benzoic acid	LD_{50}: > 10000	Anonymous, Bio-Fax, 1973, cited in Anonymous, BUA Report, 1995 (A6.1.1/04)
Benzoic acid	LD_{50}: > 5000	Moreno OM, 1977 (cited in Opdyke DL, 1979, A6.1.2/02)

3 Conclusion	Benzoic acid is not toxic after dermal exposure.
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/12/04
Results	The applicant's version is acceptable with the following amendment: In a limit test with rabbits, no mortality or signs of intoxication were seen after dermal application of 10,000 mg/kg body weight.
Conclusion	Benzoic acid is of low toxicity (LD_{50}: > 5000 mg/kg bw) after dermal exposure.
Remarks	None
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*

Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 6.1.3 Acute Toxicity

Annex Point IIA6.1 A6.1.3 Acute inhalation toxicity in rat (Determination of LD_{50})

Official use only

1 REFERENCE

1.1	**Reference**	Rope et. al, 1981, Four Week Subacute Inhalation Toxicity Study of Benzoic Acid in Rats International Research and Development Corporation (IRDC), 1981, Project No. 163-676
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Study cconducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid
2.2	**GLP**	The study was performed prior to GLP regulations but it principally provides the core information necessary to fulfill the objectives of the study and there is no reason to doubt the validity of the results.
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	Benzoic acid, as given in section 2
3.1.1	Lot/Batch number	52930350
3.1.2	Specification	Flake benzoic acid technical
3.1.2.1	Description	White flakes
3.1.2.2	Purity	No data given
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rat
3.2.2	Strain	Sprague Dawley Charles River CD®
3.2.3	Source	Charles River Breeding Laboratories, inc., Michigan, USA
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	Age: 42 days Weight: males: 169-220 g females: 139 -174 g
3.2.6	Number of animals per group	4 groups of 10 male and 10 female rats
3.2.7	Control animals	Yes

Section 6.1.3 Acute Toxicity

Annex Point IIA6.1 A6.1.3 Acute inhalation toxicity in rat (Determination of LD_{50})

3.3	**Administration/ Exposure**	*Inhalation*
3.3.1	Postexposure period	No, test duration 4 weeks
		Inhalation
3.3.2	Concentrations	Desired concentration 0.02 [mg/L] 0.2 [mg/L] 2.0 [mg/L] Nominal concentration 0.3 ± 0.056 [mg/L] 2.2 ± 0.036 [mg/L] 16.5 ± 2.140 [mg/L] Analytical concentration 0.025 ± 0.0076 [mg/L] Group II 0.250 ± 0.110 [mg/L] Group III 1.2 ± 0.35 [mg/L] Group IV
3.3.3	Particle size	During the study, particle size analysis of the exposure atmospheres was performed weekly for each group. The equivalent aerodynamic diameter (EAD) did not vary significantly from group to group nor week to week. Therefore, a representative EAD was calculated for all treatment groups for the full study period. The EAD was found to be 4.7µm.
3.3.4	Type or preparation of particles	The dust aerosol atmosphere was generated utilising the International Research and Development Corporation (IRAD) dust generator.

Group	Desired conc.	Holes		Rotat. rate rpm	Theor. output mg/min	Air flow rate	
		No. of	Diameter (mm)			L/min	L/min
II	0.02	20	2.75	0.96	185	50	7
III	0.2	20	4.70	0.96	960	40	10
IV	2.0	20	6.30	3.90	3200	60	10

Due to the difficulty in achieving de desired concentration for group IV, two IRAD dust generators were used.

3.3.5	Type of exposure	Whole body
3.3.6	Vehicle	No vehicle
3.3.7	Concentration in vehicle	-
3.3.8	Duration of exposure	6 hours per day, five days per week for four weeks
3.3.9	Controls	Sham exposed
3.4	**Examinations**	Mortality
3.5	**Method of determination of LD_{50}**	No data given
3.6	**Further remarks**	The acute LC_{50} for inhalation toxicity can be deduced from the results of the repeated dose inhalation study. Because exposition was 6 h per day (current guideline for acute inhalation toxicity: 4 h) and no mortality and clinical signs after this 6 h exposure period were seen (first clinical signs after 4 days, that is 4 x 6 h exposition)

Section 6.1.3 Acute Toxicity

Annex Point IIA6.1 A6.1.3 Acute inhalation toxicity in rat (Determination of LD_{50})

		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	First clinical signs after 4 days (4 x 6 hours exposition), see section 6.3.3
4.2	**Pathology**	Effects described only after 28 days, see section 6.3.3
4.3	**Other**	-
4.4	**LD_{50}**	The LC_{50} for a single exposure for 6 h is >1.2 mg/l for male and female.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The toxicity of benzoic acid was evaluated when administered to rats as a dust via whole body inhalation exposure 6 hours per day for four consecutive weeks (first exposure during the subacute study). The study was cconducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid
5.2	**Results and discussion**	The acute LC_{50} for inhalation toxicity can be deduced from the results of this study. Because exposition was 6 h per day (current guideline for acute inhalation toxicity: 4 h) and no mortality and clinical signs after this 6 h exposure period were seen (first clinical signs after 4 days, that 4 x 6 h exposition) at the highest concentration of 1.2 mg/l
5.3	**Conclusion**	Non-entry field
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/12/03
Materials and Methods	1.1 Rop, D. A., 1981, Four Week Subacute Inhalation Toxicity Study of Benzoic Acid in Rats 3.1.2.1 Tan flakes 3.1.2.3 Not stated in the report (analysis of test material not mentioned, gravimetric techniques for determination of concentrations).
Results and discussion	Applicant's version is acceptable.
Conclusion	5.3 LC_{50} > 1.2 mg/L x 6 h (highest obtainable concentration)
Reliability	2
Acceptability	Acceptable
Remarks	None

Section 6.1.3 Acute Toxicity

Annex Point IIA6.1 A6.1.3 Acute inhalation toxicity in rat (Determination of LD_{50})

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_1-1. Table for Acute Toxicity (modify if necessary)

Dose [mg/L]	*Number of dead / number of investigated*	*Time of death (range)*	*Observations*
0	0/20	-	-
0.025	0/20	-	-
0.25	0/20	-	-
1.2	2/20	days 6/15	1 female (day 6) and 1 male (day 15)
LD_{50} value	The LC_{50} for a single exposure for 6 h is >1.2 mg/l for male and female.		

Section A6.1.4.02 Acute Eye Irritation

Annex Point IIA6.4b

1 REFERENCE

Anonymous, Acute eye irritation/corrosion study with sodium benzoate in rabbits (unpublished report), RCC Notox B.V., DD 's-Hertogenbosch, NL, cited in Wibbertmann A, 2000, A 6.1.4.01/06

Anonymous, 1973, Benzoic acid, Bio-Fax, Industrial Bio-Test Laboratories, Inc., Northbrook Ill., Data sheet no. 28-4/7, cited in Wibbertmann A, 2000, A 6.1.4.01/06

Anonymous, 1988b, Eye irritation/corrosion study of benzoic acid in the rabbit (unpublished report), RCC Notox B.V., DD 's-Hertogenbosch, NL, cited in Wibbertmann A, 2000, A 6.1.4.01/06

Anonymous, 2003, Review report for the inclusion of benzoic acid in Annex I of Directive 91/414/EEC, SANCO/1396/2001-Final, 2003, (A6.1.1/05)

Anonymous, Bio-Fax, 1973, cited in Anonymous, Benzoic acid / sodium benzoate, BUA Report, 1995 (A6.1.1/04)

Suberg, 1986, Benzoesäure DAB 8, Prüfung auf primär reizende/ätzende Wirkung am Kaninchenauge(unpublished report), Bayer AG data, cited in Wibbertmann A, 2000, A6.1.4.01/06

Wibbertmann A et al., 2000, Concise international chemical assessment document No 26 Benzoic Acid and Sodium Benzoate, IPCS (International Programme on chemical safety), 1. Draft, prepared by Wibbertmann, A., A6.1.4.01/06

2 RESULTS

All acute eye irritation test with Benzoic acid showed irritating effects. Because of animal welfare no additional tests were prepared. It should be noted that several references seem to point to the same experimental study.

Summary

Test substance	Test / Result	Reference
Benzoic acid DAB 8	OECD 405 Within 72 h, the scores for chemosis, reddening of the conjunctivitis, iritis, and keraitis always remained at ≤ 2 Mildly irritating	Suberg, Bayer AG, 1986, cited in Wibbertmann A, 2000, (6.1.4.01/06)
Benzoic acid	Non-standardised experiment Single application of 100 mg dry powder, responses scored at 24, 48 or 72 h. Score 65/100: Irritating	Anonymous, Bio-Fax, 1973, cited in Wibbertmann A, 2000, (6.1.4.01/06)
Benzoic acid	Severely irritating	Anonymous, SANCO/1396/2001-Final, 2003, (A6.1.1/05)
Sodium benzoate	Not irritating	

Section A6.1.4.02 Acute Eye Irritation

Annex Point IIA6.4b

	Benzoic acid (ca 77 mg)	Score 35 according to the scheme of Kay & Calandra, 1962 Severely irritating	Anonymous, RCC Notox, 1988b, cited in Wibbertmann A, 2000, (6.1.4.01/06)
	Sodium benzoate	OECD 405 Score 9.3 according to the scheme of Kay & Calandra, 1962 mildly irritating	Anonymous, RCC Notox, not dated, cited in Wibbertmann A, 2000, (6.1.4.01/06)
3 Conclusion	Benzoic acid is irritating to eyes.		
	Evaluation by Competent Authorities		
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted		
	EVALUATION BY RAPPORTEUR MEMBER STATE		
Date	2008/10/01		

Section A6.1.4.02 Acute Eye Irritation

Annex Point IIA6.4b

Results

No primary data were submitted on eye irritation by benzoic acid. The available data were summarised from comprehensive evaluations prepared by different bodies:

1) BUA Report 145: Benzoic Acid/Sodium Benzoate, GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance, 1993
2) Concise International Chemical Assessment Document No. 26: Benzoic Acid and Sodium Benzoate, IPCS, 2000
3) SANCO/1396/2001-Final, Monograph on Benzoic Acid, EU, 2003
4) OECD SIDS Dossier on Benzoates, UNEP, 2001

The following original studies on eye irritation of benzoic acid were cited in the above reports:

Test substance	Test / Result	Reference
Benzoic acid	Non-standardised experiment; Single application of 100 mg dry powder, responses scored at 24, 48 or 72 h; Score 65/100; **Irritating**	Bio-Fax: Benzoic acid., Data sheet no. 28-4/73, 1973
Benzoic acid	Non-standardised experiment; **Severely irritating**	IRCD: Acute toxicity studies in rats and rabbits. (Unpublished report no. 163-282), 1974
Benzoic acid	Non-standardised experiment; No score given; **Moderately irritating**	Bayer AG: Untersuchung zur Haut- und Schleimhaut-verträglichkeit. (Un-published report) 1978
Benzoic acid	Non-standardised experiment; Eyes not rinsed; **Irritating**	Monsanto Co.: Primary eye irritation of benzoic acid to rabbits. (Unpublished report) 1983
Benzoic acid	OECD 405; Within 72 h, the scores for chemosis, reddening of the conjunctivitis, iritis, and keratitis score ≤ 2; **Mildly irritating**	Bayer AG (Suberg): Benzoesäure DAB8. Prüfung auf primär reizende/ätzende Wirkung am Kaninchenauge. 1986 (unpublished report)
Benzoic acid	OECD 405; Draize's score 35; **Severely irritating**	RCC Notox: Eye irritation/corrosion study of benzoic acid in the rabbit. (Unpublished report no. 0847/1084) 1988

Conclusion

From the data available in published literature it can be concluded that benzoic acid is eye irritating. **Xi, R41 (GHS: Eye Dam. 1, H318)**

Remarks

For this endpoint, a bridging with data on sodium benzoate which is not eye irritating is not possible.

COMMENTS FROM OTHER MEMBER STATE *(specify)*

Date

Give date of comments submitted

Section A6.1.4.02 Acute Eye Irritation

Annex Point IIA6.4b

Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.5 Skin sensitisation
Annex Point IIA6.1.5 (Local Lymph Node Assay – LLNA)

Official use only

		1 REFERENCE
1.1	**Reference**	Gerberick GF, House RV, Fletcher ER, Ryan CA, 1992, Examination of the local lymph node assay for use in contact sensitization risk assessment Fundamental and Applied Toxicology, 19, 438-445
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	No data protection / published data
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No. Study conducted according to prevailing standards and subsequently accepted for international registration. A modification of the method described by Kimber et al. (1989). Results considered to be valid
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	Benzoic acid, as given in section 2
3.1.1	Lot/Batch number	Not available
3.1.2	Specification	As given in section 2
3.1.2.1	Description	White powder
3.1.2.2	Purity	No data given
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.
3.1.2.4	Preparation of test substance for application	Acetone
3.1.2.5	Pretest performed on irritant effects	No

Section A6.1.5		**Skin sensitisation** (Local Lymph Node Assay – LLNA)
Annex Point IIA6.1.5		
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Mice
3.2.2	Strain	CBA/J
3.2.3	Source	Jackson Labs (Bar Harbor, ME)
3.2.4	Sex	
3.2.5	Age/weight at study initiation	6 to 9 weeks
3.2.6	Number of animals per group	5 animals per group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	State study type: Non-Adjuvant
3.3.1	Induction schedule	Not applicable
3.3.2	Way of Induction	Not applicable Not applicable
3.3.3	Concentrations used for induction	Not applicable
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	Not applicable
3.3.5	Challenge schedule	Topical application once daily over four consecutive days. On day five after the first application all mice were sacrificed.
3.3.6	Concentrations used for challenge	5%, 10% and 20% solution, vehicle: acetone
3.3.7	Rechallenge	Not applicable
3.3.8	Scoring schedule	Not applicable
3.3.9	Removal of the test substance	Not applicable
3.3.10	Positive control substance	Yes, 5-Chloro-2-methylisothiazolonone plus 2-methylisothiazolinone (MCI/MI), Rohm & Haas Co. Philadelphia, PA
3.4	**Examinations**	Non-entry field
3.4.1	Pilot study	No
3.5	**Further remarks**	-
		4 RESULTS AND DISCUSSION
4.1	**Results of pilot studies**	-
4.2	**Results of test**	
4.2.1	24h after challenge	-
4.2.2	48h after challenge	-

Section A6.1.5 **Skin sensitisation**
Annex Point IIA6.1.5 (Local Lymph Node Assay – LLNA)

4.2.3	Other findings	A single cell suspension of the lymph node cells for each animal was prepared. The proliferative response of lymph node cells was calculated as the ratio of ^{3}H-methyl thymidine-incorporation into lymph node cells of the test group animals relative to that recorded for control group animals. A chemical was considered to be a sensitizer in the local lymph node assay if two criteria were met. First, exposure to at least one concentration resulted in a 2-fold or greater increase in (^{3}H)TdR expressed as the number of disintegrations per minute (DPM) incorporation compared to the control mice. Second, this mean dpm value was statistically different from vehicle treated mice ($p \leq 0.01$). A chemical in the 2 – 30-fold range was classified as a weakly to moderate sensitizer. Over the 30-fold range, a chemical was classified as a strong sensitizer.
4.3	**Overall result**	Benzoic acid has no sensitising properties.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Benzoic acid was tested for its skin sensitization potential. The test was conducted according a modification of the method described by Kimber et al., 1989, (Local Lymph Node Assay). Measurement of proliferative activity in the draining lymph nodes by quantifying the incorporation of radiolabelled thymidine into the proliferative cells. Proliferative activity correlates with the severity of elicitation reaction. Results considered to be valid
5.2	**Results and discussion**	Benzoic acid has no sensitising properties. See table 6_1_5-1
5.3	**Conclusion**	
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Section A6.1.5 **Skin sensitisation**
Annex Point IIA6.1.5 (Local Lymph Node Assay – LLNA)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/12/06
Materials and Methods	2.2 Not stated in the report, published literature. (GLP was compulsory at the time the study was performed.) 3.2.4 Female 3.3.1 Daily over four consecutive days, on day five after the first application all mice were sacrificed. 3.3.2 Topical application to each side of botch ears 3.3.3 25 µl/ear (12.5 µl/side) of 5 %, 10 % and 20 % benzoic acid solution, dissolved in acetone 3.3.5 N/A 3.3.6 N/A 3.3.8 Negative: < 2-fold increase of test group dpm relative to vehicle group dpm; Weak to moderate: 2-30 fold increase of test group dpm relative to vehicle group dpm; Moderate to strong: >30-fold increase of test group dpm relative to vehicle group dpm
Results and discussion	4.2 Benzoic acid was negative (< 2-fold increase of dpm).
Conclusion	5.3 Benzoic acid was not sensitising in a local lymph node assay.
Reliability	2
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.5 **Skin sensitisation**
Annex Point IIA6.1.5 (Local Lymph Node Assay – LLNA)

Table A6_1_5-1. Detailed information for skin sensitisation test (LLNA)

In *Situ* Measurement of Lymphocyte Proliferation in Murine Local Lymph Node Assay

Test item	Concentration	Mean cell number (x 10^6)	Mean dpm (x 10^6)	dpm-fold increase
Acetone	Acetone	4.91 ± 0.9	5.42 ± 0.8	-
Benzoic acid	5%	3.84 ± 0.3	4.20 ± 0.6	0.8
	10%	3.93 ± 0.4	5.00 ± 0.7	0.9
	20%	3.66 ± 0.6	4.24 ± 0.6	0.8
Acetone	Acetone	5.16 ± 0.5	4.04 ± 1.1	-
MCI/MI	50 ppm	15.7 ± 2.5*	32.8 ± 19.2*	8.1
	500 ppm	29.3 ± 2.6*	112.4 ± 38.5*	27.8
	1000 ppm	23.7 ± 3.3*	194.9 ± 51.4*	48.2

dpm = disintegrations per minute
* = Significant difference from control group ($p \leq 0.01$)

Benzoic acid has no sensitising properties

Table A6_1_5-2. Additional data for skin sensitisation / Benzoic acid

Study	Concentration	Species	Result	Reference
Local lymph node assay, LLNA	5, 10, 20%, four topical inductions	Mouse	not sensitising	Gerberick GF, House RV, Fletcher ER, Ryan CA, 1992, (A6.1.5/01)
Mouse ear swelling test, (MEST)	20%, induction and challenge	Mouse	not sensitising	Gad SC, Dunn BJ, Dobbs DW, Reilly C, Walsh RD, 1986 (A6.1.5/02)
GPMT	20% induction and challenge	Guinea pig	not sensitising	
Buehler test	20% induction and challenge	Guinea pig	not sensitising	
Ear swelling test	0.2-20% topical application onto earlobe	Guinea pig	dose-dependent swelling, within 30-40 min, reversible	Lahti A, Maibach HI, 1984 (A6.1.5/03)

Section A6.2.01 Metabolism studies in mammals

Annex Point IIA-VI.6.2.01 **Human**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Kubota K, Ishizaki T, 1991, Dose-dependent pharmacokinetics of benzoic acid following oral administration of sodium benzoate to humans, Eur. Journal Clin. Pharmacol., 41, 363-368, 1991	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	No data protection / published data	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guidelines available. Study accepted for international registration. Results considered to be valid	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Sodium benzoate	
3.1.1	Lot/Batch number	No batch number given	
3.1.2	Specification	Flake benzoic acid technical	
3.1.2.1	Description	White flakes	
3.1.2.2	Purity	No data given	
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.	
3.1.2.4	Radiolabelling	No labelling	
3.2	**Test persons**		
3.2.1	Human	Healthy male volunteers after a careful interview, physical and laboratory examination, including complete haemogram, urinalysis and measurement of serum creatinine, transaminase, alkaline phosphatase, total serum protein, serum albumin, total serum bilirubin and electrolytes, which were all within normal limits.	
3.2.2	Source	Not applicable	
3.2.3	Sex	Male	
3.2.4	Age/weight at study initiation	Age: 22 – 34 years (mean: 28.8 years) Weight: 54 to 79 kg (mean: 69.9 kg)	
3.2.5	Number of human per group	Six	
3.2.6	Control	Yes, 0 to 1.5 hours or predose baseline	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Number of doses	Three different doses to each volunteer in randomized sequence, at least one week apart.	

Section A6.2.01 **Metabolism studies in mammals**

Annex Point IIA-VI.6.2.01 **Human**

3.3.2	Dose	40, 80 and 160 mg/kg body weight
3.3.3	Vehicle	Water
3.3.4	Administered dose solutions	Dissolved in 300 ml water
3.3.5	Post-exposure period	up to 24 hours
3.3.6	Samples and treatment of samples	**Excreta:** Urinary samples were collected at -1.5 to 0 (baseline), 0 to 1.5, 1.5 to 3, 3 to 4.5, 4.5 to 6, 6 to 9, 9 to 12 and 12 to 24 hours post dose. **Blood and Plasma:** Blood samples were collected at 0 (pre-dose), 0.5, 1, 1.5, 3, 4.5, 6, 9 and 12 hours post dose.
3.3.7	**Analytics**	Benzoic acid and hippuric acid in plasma and urine were determined by HPLC. Peak plasma concentration (C_{max}) and time to C_{max} (t_{max}) for benzoic acid and hippuric acid were read from the observed data. The plasma AUC (area under the plasma concentration curve) for each of the acids was calculated by the trapezoidal method. The urinary excretion rate of hippuric acid biotransformed from the administered sodium benzoate was calculated by subtracting the excretion rate of hippuric acid during the control 0 to 1.5 hours or predose baseline) period on the day of benzoate administration. The renal clearance (CL_R) of hippuric acid was approximated as the amount of hippuric acid excreted urine($Ae_{(\infty)}$) from time 0 to the time at which plasma hippuric acid returned to zero divide by the corresponding AUC value of plasma hippuric acid.
3.3.8	**Pharmacokinetics**	Plasma concentration and urinary excretion time data of conjugate of benzoic acid and hippuric acid are expressed in as benzoic acid equivalents throughout the text. To estimate model-dependent pharmacokinetic parameters, a one-compartment model with first-order rate absorption and Michaelis-Menten elimination for benzoic acid and a one-compartment model with first-order elimination for hippuric acid were employed.

4 RESULTS AND DISCUSSION

4.1 Pharmacokinetic parameters

Model independent parameters of benzoic acid and hippuric acid
Plasma concentrations

	Sodium benzoate administered (mg/kg)		
	40	**80**	**160**
Benzoic acid			
C_{max} (µg/ml)	99.7 (5.6)	202.8 (13.4)	336.5 (28.3)
T_{max} (h)	0.5 (0.0)	0.8 (0.2)	1.8 (0.3)
AUC (µg x h /ml)	104.4 (7.4)	385.4 (16.1)	1257.0 (93.4)
Hippuric acid			
C_{max} (µg/ml)	30.9 (1.5)	35.0 (2.3)	36.9 (2.0)
T_{max} (h)	1.3 (0.1)	2.1 (0.2)	4.1 (0.6)
AUC (µg x h /ml)	53.7 (2.4)	109.3 (5.4)	223.5 (12.2)

The mean AUC's of benzoic acid after the doses of 80 and 160 mg/kg of sodium benzoate were 3.7 – and 12.0 times greater, respectively, than after 40 mg/kg. However the mean AUC of hippuric acid was roughly proportional to the benzoate doses.
The observed data were explained by a one-compartment model with first-order rate absorption and Michaelis-Menten elimination for benzoic acid together with a one-compartment model with first-order elimination for hippuric acid.

Section A6.2.01 Metabolism studies in mammals

Annex Point IIA-VI.6.2.01 **Human**

4.2 Excretion

Model independent parameters of benzoic acid and hippuric acid
% dose of hippuric acid excreted in urine

	Sodium benzoate administered (mg/kg)		
	40	**80**	**160**
Hippuric acid			
$Ae_{(\infty)}$ (of dose)	82.9 (4.7)	90.3 (5.4)	73.1 (2.1)
CL_R (L/ h x kg	0.53 (0.03)	0.57 (0.05)	0.45 (0.02)

The urinary excretion rate of hippuric acid after the administration of sodium benzoate can be interpreted as being near the maximum rate of the metabolism.

Section A6.2.01 **Metabolism studies in mammals**

Annex Point IIA-VI.6.2.01 **Human**

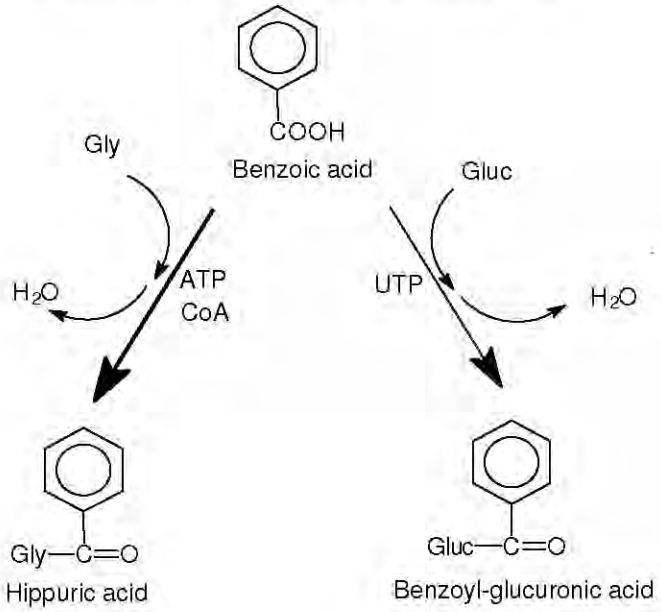
4.3 Metabolism

Although the maximum rate of biotransformation of benzoic acid to hippuric acid varied between 17.2 and 28.8 mg/kg x h. among the six individuals, the mean value (23.0 mg/kg x h) was fairly closed to that provided by daily maximum dose of 500 mg/kg and day recommended in the treatment of hyperammonemia in patients with inborn errors of ureagenesis.

The individual maximum rate of metabolism can be estimated from the urinary excretion rate of hippuric acid 1.5 to 3 hours after the single oral dose of 80 to 160 mg/kg sodium benzoate (approximately 0.7 times the mean V_{max}'/f value).

The main metabolite of benzoic acid/sodium benzoate is hippuric acid (up to 100%, e.g. in humans) followed by benzoyl-glucuronic acid (0% to ca 20%). These metabolites result from conjugation reactions of benzoic acid with glycine or glucuronic acid.

Conjugation reactions of Benzoic acid



Gly : Glycine
Gluc: Glucuronic acid

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.2.01 Metabolism studies in mammals

Annex Point IIA-VI.6.2.01 **Human**

5.1	**Materials and methods**	Plasma concentration-time data for benzoic acid and urinary excretion-time for hippuric acid were analysed simultaneously after oral doses of 40, 80 and 160 mg /kg sodium benzoate administered at least one week apart to 6 healthy subjects (after a careful interview, physical and laboratory examination, including complete haemogram, urinalysis and measurement of serum creatinine, transaminase, alkaline phosphatase, total serum protein, serum albumin, total serum bilirubin and electrolytes, which were all within normal limits).
5.2	**Results and discussion**	The main metabolite of benzoic acid/sodium benzoate is hippuric acid (up to 100%, e.g. in humans) followed by benzoyl-glucuronic acid (0% to ca 20%). These metabolites result from conjugation reactions of benzoic acid with glycine or glucuronic acid. The results showed that the AUC of benzoic acid, but not of hippuric acid, increases disproportionately with increasing oral doses of sodium benzoate from 40 to 160 mg/kg. The respectively AUC's after the 80 and the 160 mg/kg doses were 3.7 – and 12.0 times greater than after 40 mg/kg. However the mean AUC of hippuric acid was roughly proportional to the benzoate doses. This finding indicates, that the biotransformation of benzoic acid to hippuric acid follows saturable or Michaelis-Menten kinetics in humans following oral administration of sodium benzoate. The urinary excretion rate of hippuric acid after the administration of sodium benzoate can be interpreted as being near the maximum rate of the metabolism. The mean value of biotransformation rate (23.0 mg/kg x h) was fairly closed to that provided by daily maximum dose of 500 mg/kg and day recommended in the treatment of hyperammonemia in patients with inborn errors of ureagenesis Additional data see table 6_2_01-2
5.3	**Conclusion**	
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/12/11
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	The main metabolite of benzoic acid/sodium benzoate is hippuric acid (up to 100 %, e.g. in humans) followed by benzoyl-glucuronic acid (0 % to ca 20 %). These metabolites result from conjugation reactions of benzoic acid with glycine or glucuronic acid.
Reliability	2
Acceptability	Acceptable
Remarks	None
	EVALUATION BY RAPPORTEUR MEMBER STATE
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A6.2.01 Metabolism studies in mammals

Annex Point IIA-VI.6.2.01 **Human**

Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_2_01-1. Table for metabolism studies in mammals / human

	Sodium benzoate administered (mg/kg)		
	40	**80**	**160**
Benzoic acid			
C_{max} (µg/ml)	99.7 (5.6)	202.8 (13.4)	336.5 (28.3)
T_{max} (h)	0.5 (0.0)	0.8 (0.2)	1.8 (0.3)
AUC (µg x h /ml)	104.4 (7.4)	385.4 (16.1)	1257.0 (93.4)
Hippuric acid			
C_{max} (µg/ml)	30.9 (1.5)	35.0 (2.3)	36.9 (2.0)
T_{max} (h)	1.3 (0.1)	2.1 (0.2)	4.1 (0.6)
AUC (µg x h /ml)	53.7 (2.4)	109.3 (5.4)	223.5 (12.2)
$Ae_{(\infty)}$ (of dose)	82.9 (4.7)	90.3 (5.4)	73.1 (2.1)
CL_R (L/ h x kg	0.53 (0.03)	0.57 (0.05)	0.45 (0.02)

Table A6_2-01-2. Additional data metabolism studies in mammals

Treatment	Admin.	Result	Reference
Measurement of metabolic rate of benzoic acid to hippuric acid in the Rat	i.v. 2 – 2.2 mg/kg	The urinary excretion of hippuric acid after iv injection of benzoic acid could almost completely be followed by 13C-NMR (10 minutes accumulation time) without any separation steps. Benzoic acid was used as reference substance with known properties to demonstrate the usefulness of the NMR technique.	Akira K, Takagi N, Takeo S, Shindo H, Baba S, 1993, A6.2.01/02

<table>
<tr>
<td>The urinary excretion of benzoic acid in human and 20 species of animals was examined.</td>
<td>oral</td>
<td>
<table>
<tr><th>Species</th><th>B.A. (mg/kg)</th><th>% of excretion</th></tr>
<tr><td colspan="3">Primates</td></tr>
<tr><td>Man (2M)</td><td>1</td><td>99.4, 99.7</td></tr>
<tr><td>Rhesus monkey (3F)</td><td>20</td><td>33-59</td></tr>
<tr><td>Squirrel monkey (2F)</td><td>50</td><td>46, 49</td></tr>
<tr><td>Capuchin (1F)</td><td>50</td><td>57</td></tr>
<tr><td colspan="3">Artiodactyla</td></tr>
<tr><td>Pig (large white) (2F)</td><td>50</td><td>48, 51</td></tr>
<tr><td colspan="3">Lagomorpha</td></tr>
<tr><td>Rabbit
(New zealand white) (3F)</td><td>49
200</td><td>36 – 77
79 - 95</td></tr>
<tr><td colspan="3">Rodents</td></tr>
<tr><td>Rat (Wistar albino) (3F)</td><td>50</td><td>98 - 102</td></tr>
<tr><td>Mouse (LCI) (3 x 10 F)</td><td>56</td><td>43 - 67</td></tr>
<tr><td>Guinea pig (English) (3F)</td><td>49</td><td>63 - 94</td></tr>
<tr><td>Hamster (golden) (3F)</td><td>52</td><td>98 - 101</td></tr>
<tr><td>Lemming (steppe) (3F)</td><td>56</td><td>94 - 102</td></tr>
<tr><td>Gerbil (3F)</td><td>29</td><td>66 - 82</td></tr>
<tr><td colspan="3">Carnivora</td></tr>
<tr><td>Cat (mongrel) (2F)</td><td>51</td><td>29, 86</td></tr>
<tr><td>Dog (mongrel) (2F, 1M)</td><td>51</td><td>87 – 100</td></tr>
<tr><td>Ferret (mongrel) (3F)</td><td>50
198
400</td><td>57 – 90
62 – 88
58 - 74</td></tr>
<tr><td colspan="3">Insectivora</td></tr>
<tr><td>Hedgehog (European) (2M)</td><td>50</td><td>67, 78</td></tr>
<tr><td colspan="3">Chiroptera</td></tr>
<tr><td>Fruit bat (Indian) (2F)</td><td>50</td><td>49, 54</td></tr>
<tr><td colspan="3">Birds</td></tr>
<tr><td>Chicken (Light Sussex) (5F)</td><td>50</td><td>38 - 69</td></tr>
<tr><td>Pigeon (3F)</td><td>50</td><td>84 - 102</td></tr>
<tr><td colspan="3">Reptiles</td></tr>
<tr><td>Turtle (Side-necked) (5F)</td><td>50</td><td>39</td></tr>
<tr><td>Gecko (3F)</td><td>19</td><td>32</td></tr>
</table>
B.A. = Benzoic acid
</td>
<td>Bridges JW, French MR, Smith RL, Williams RT, 1970 A6.2.01/03</td>
</tr>
<tr>
<td>The urinary excretion of hippuric acid after administration of sodium benzoate was examined.
6 F Students</td>
<td>oral
190 mg/kg sodium benzoate</td>
<td>Within 5 hours a percentage of 89% was excreted via urine with a maximal excretion rate in the first hour.</td>
<td>Fujii T, Omori T, Taguchi T, Ogata M, 1991, A6.2.01/04</td>
</tr>
<tr>
<td>Metabolism of benzoic acid in adult marmoset (Callithrix jacchus) and rats</td>
<td>oral</td>
<td>
Metabolites (% of urinary total)(mean)
<table>
<tr><th>Species</th><th>B.A. (mg/kg)</th><th>Benzoyl glucoronide</th><th>Hippuric acid</th><th>Benzoic acid</th></tr>
<tr><td>Marmoset</td><td>1</td><td>6.2</td><td>87.7</td><td>2.7</td></tr>
<tr><td></td><td>40</td><td>26.7</td><td>33.3</td><td>38.8</td></tr>
<tr><td></td><td>100</td><td>38.0</td><td>42.3</td><td>18.8</td></tr>
<tr><td>Rat</td><td>1</td><td>1.8</td><td>97.4</td><td>0.3</td></tr>
<tr><td></td><td>50</td><td>1.0</td><td>99</td><td>tr</td></tr>
<tr><td></td><td>100</td><td>2.2</td><td>94</td><td>3.1</td></tr>
</table>
</td>
<td>Hall BE, James SP AR, 1980, A6.2.01/05</td>
</tr>
</table>

Urinary excretion of separate pairs of rats	oral	The administration of doses of 10 and 100 µg/kg bw, and 1, 10, 100 and 1000 mg/kg bw of 14C-benzoic acid diluted with unlabeled sodium benzoate to rats resulted in an excretion of 80% to 100% with the urine within 24 hours. At the dose of 10 µg/kg bw about 3% were exhaled as $14CO_2$	Jones AR, 1982, A6.2.01/06
Excretion of sodium benzoate in urine, faeces, organs and skin	intraperitoneal	Given ca 250 mg/kg bw 14C-sodium benzoate to rats, practically quantitative excretion occurred in the urine within one to two 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). All radioactivity in the tissues was identified as parent material	Lang H, Lang K, 1956, A6.2.01/07
Urinary excretion after ingestion of benzoic acid / humans	oral 5 g benzoic acid and 3 g glycine	Human volunteers (52, 58 and 77.5 kg) eliminate approximately 80% of a single oral dose of 5 g benzoic acid in six hours via urine	Quick AJ, 1931, A6.2.01/08
Metabolism of sodium benzoate in man	oral	Doses of 6.9, 13.9, 34.7 and 69.3 mMoles (1, 2, 5, 10 g) were given to a healthy human male (60 kg) with at least a two week interval between two successive doses. This resulted in a complete elimination of each dose within 10 to 11 hours.	Schachter D, 1957, A6.2.01/09
Rapidity of drugs from the rat colon	by perfusion	The rapidity of absorption was substantiated by perfusion experiments with colon of the rat. In these experiments, it was also shown that the absorption is based on simple diffusion of the unionized molecule	Schanker LS, 1959, A6.2.01/10
Conjugation of benzoic acid with glycine in human liver and kidney was measured	-	In homogenates of 110 specimens of human liver and 67 specimens of human renal cortex, the rate of conjugation of benzoic acid with glycine was measured. The mean rates of reaction were 254± 90.5 nmol/min per g liver (range: 94.4 to 564 nmol/min) and 321±99.3 nmol/min per g kidney (range: 63.3 to 542 nmol/min).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 A, Mogavero S, Giulianotti PC, Pietrabissa A, Mosca F, Pacifici G M, 1993, A6.2.01/11
Metabolic fate 14C-benzoic acid in protein energy deficient rats	oral 200 mg/kg	Benzoic acid is almost entirely excreted as hippuric acid (99% in 24 h excretion). Under the influence of protein and energy deficiency, rats excreted less hippuric acid (62% to 85%) and more benzoyl-glucuronic acid (14% to 37%)	Thabrew MI, Bababunmi EA, French MR, 1980, A6.2.01/12

Information regarding the absorption, distribution, excretion and metabolism of benzoic acid/sodium benzoate is derived from investigations with very different objectives (e.g. perfusion experiments with different organs, elucidation of basic metabolism principles, special absorption phenomena's). In these investigations, a great variety of animal species has been studied. A comprehensive ADME (absorption, distribution, metabolism, excretion) study based on a current guideline (e.g. EU, OECD or EPA) is not available.

Benzoic acid/sodium benzoate is rapidly and virtually completely absorbed after oral ingestion in many animal species and man. It is rapidly excreted to a high rate via urine after oral, intraperitoneal and subcutaneous administration (80% to 99% within 24 hours). The faecal excretion is only a minor route of elimination.

The main metabolite of benzoic acid/sodium benzoate is hippuric acid (up to 100%, e.g. in humans) followed by benzoyl-glucuronic acid (0% to ca 20%). These metabolites result from conjugation reactions of benzoic acid with glycine or glucuronic acid. Major sites of the conjugation reactions are the liver and the kidney. Marked species differences exist in the rate and extent of benzoate-metabolism in both organs.

Important factors, which affect the tolerance for the benzoates, are the incorporated amount of these substances, the availability of an adequate glycine concentration for the conjugation process and the velocity rate for both the conjugation reactions and the excretory process.

In plants, benzoic acid serves as defensive substance and as basic intermediate for secondary plant products.

Section A6.2.02 Percutaneous absorption (in vivo test)

Annex Point IIA6.2.02 **Rat**

Official use only

		1 REFERENCE
1.1	**Reference**	Rougier A, Dupuis D, Lotte C, Roguet R, Schaefer H, 1983, In vivo correlation between stratum corneum reservoir function and percutaneous absorption The Journal of Investigative Dermatology, 81, 3, 275-278
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2		-
1.2.3	Criteria for data protection	No data protection / published data
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No. No guidelines available. Study accepted for international registration. Results considered to be valid
2.2	**GLP**	No, GLP was not compulsory at that time.
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	Benzoic acid, as given in section 2
3.1.1	Lot/Batch number	No batch number given
3.1.2	Specification	Flake benzoic acid technical
3.1.2.1	Description	White flakes
3.1.2.2	Purity	No data given
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.
3.1.2.4	Radiolabelling	(ring^{14}C) benzoic acid from Radiochemical centre Amersham, UK
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Hairless Sprague-Dawley (OFA)
3.2.3	Source	No data given
3.2.4	Sex	Female
3.2.5	Age/weight at study initiation	Age : 12 weeks Weight: 240 ± 10
3.2.6	Number of animals per group	12 animals
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Dermal

Section A6.2.02 Percutaneous absorption (in vivo test)

Annex Point IIA6.2.02 **Rat**

3.3.1	Preparation of test site	Hairless rat, benzoic acid was applied on 1 cm^2 of dorsal skin. Vehicle was ethanol-water 95/5 (v/v). Removing of the excess product with 2 washings (300µl) with the vehicle solvent following by 2 rinsings (300µl) with distilled water and light drying with cotton wool.
3.3.2	Concentration of test substance	200 nmol/cm^2 and 450 nmol/cm^2
3.3.3	Specific activity of test substance	No data given
3.3.4	Volume applied	No data given
3.3.5	Size of test site	1 cm^2
3.3.6	Exposure period	30 minutes
3.3.7	Sampling time	Urine and faeces: 24h, 48h, 72 and 96 h after initiation of skin contact Epidermis and epidermis area treated, animal body and total penetration: after 96 hours Amounts in stratum corneum treated area 30 minutes after application.
3.3.8	Samples	Urine, faeces, animal body, total penetration, amounts in stratum corneum treated area 30 minutes after application.

4 RESULTS AND DISCUSSION

4.1	**Toxic effects, clinical signs**	No data given
4.2	**Dermal irritation**	No data given
4.3	**Recovery of labelled compound**	No data given
4.4	**Percutaneous absorption**	Benzoic acid is known to be as a very well absorbed. The agreement with literature data is verified. Total penetration of benzoic acid after 30 min exposure and application of 200 nmol/cm^2 13.3% 450 nmol/cm^2 17.6% See tables A6_2_02-1 and A6_2_02-2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Benzoic acid was applied on 1 cm^2 of dorsal skin of hairless rats. The Vehicle was ethanol-water 95/5 (v/v). The excess product was removed with 2 washings (300µl) with the vehicle solvent following by 2 rinsings (300µl) with distilled water and light drying with cotton wool. The animals were divided into 2 equal groups. The stratum corneum of the application area of the animals from the first group was removed 30 minutes after application by 6 stripping using "3M" invisible tape. The amount of substance contained in the stratum corneum was determined by liquid scintillation counting. The animals of the second group were placed individually in metabolism cages for 4 days (food and water ad libitum). Urine and faeces were collected 24h, 48h 72 and 96 h after initiation of skin contact. Epidermis and epidermis area treated, animal body and total penetration were examined after 96 hours. The amount of substance was determined by liquid scintillation counting.

Section A6.2.02 Percutaneous absorption (in vivo test)

Annex Point IIA6.2.02 **Rat**

5.2	**Results and discussion**	Benzoic acid is known to be as a very well absorbed. The agreement with literature data is verified. Total penetration of benzoic acid after 30 min exposure and application of 200 nmol/cm^2 13.3% 450 nmol/cm^2 17.6% The penetration increases with the amount applied. Additional data see table A6_2-02-3 and A6_2-02-4
5.3	**Conclusion**	
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/01/04
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	2
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_2_02-1. Table for Percutaneous absorption (in vivo test)
Total amounts found 96 hours after application (expressed as nmol/cm^2)

	Benzoic acid (nmol/cm^2)				Dexamethasone	
	200	SD*	450	SD*	200 nmol/cm^2	SD*
Urine	24.80	0.50	71.20	2.90	0.16	0.02
Feces	1.32	0.30	6.25	1.65	0.02	0.05

Section A6.2.02 Percutaneous absorption (in vivo test)

Annex Point IIA6.2.02 **Rat**

Epidermis and epidermis area treated	0.50	0.05	1.70	0.40	0.10	0.02
Animal body	0.01	0.004	0.16	0.02	0.04	0.01
Total penetration	26.60	0.70	79.30	3.60	0.60	0.06
Amounts in stratum corneum treated area	17.60	1.50	48.10	5.10	0.41	0.02

Table A6_2_02-2. Table for Percutaneous absorption (in vivo test)
Urinary excretion (expressed as nmol/cm^2)

	Urinary excretion (hours)							
	0-24	**SD**	**24-48**	**SD**	**48-72**	**SD**	**72-96**	**SD**
Benzoic acid								
200 nmol/cm^2	22.95	0.55	0.75	0.06	0.66	0.07	0.43	0.04
400 nmol/cm^2	61.00	2.50	5.60	0.90	2.80	0.45	1.77	0.37
Dexamethasone								
200 nmol/cm^2	0.04	0.004	0.035	0.008	0.035	0.004	0.055	0.010

SD* = Standard deviation (n = 6)

Table A6_2_02-3. Additional data for Percutaneous absorption (in vivo test) benzoic acid

Species	Treatment / Measuring	Result	Reference
Human	4 µg/cm^2 (total area 13 cm^2) were applied on the ventral surface of the forearm of six human volunteers for 24 hours. Urinary excretion was measured.	The total absorption after topical administration was 42.6% (SD 16.5%), measured by urinary excretion over 5 days	Feldmann RJ, Maibach HI, 1970, A6.2.02/05
Human	Application of 4 µg benzoic acid/cm^2 to 7 humans 22-40 years and 8 humans 65-86 years old for 24 hours (total area 2.5 cm^2). The effect of aging on percutaneous absorption of benzoic acid was measured. Urinary excretion was measured.	Percutaneous permeation (% dose absorbed) 36.2 ± 4.6% in the group of 22-40-olds and 19.5 ± 1.6% in the group of 65-86-olds Surface recovery (% dose recoverd) (mean SE) 45.6 (2.8)% in the group of 22-40-olds and 61.4 (2.0)% in the group of 65-86-olds The cumulative percentage dose excreted in the urine (near 100%) following intravenous administration shows no significant differences between the two age groups.	Roskos KV, Maibach HI, Guy R H, 1989, A6.2.02/10
Human and Mexican hairless Dog	Topical application of 4 µg/cm^2 to the neck skin. Urinary excretion, maximum absorption rate and surface counting were measured.	Excretion in the urine following intravenous administration (man and dog): 93.6% after 24 h and 95.6% after 4 days Urinary excretion after topical administration: Man: extensive and rapid, being almost complete by day 3 Dog: less extensive and greatly prolonged. Maximum absorption rate (% of applied dose / Hour) Man: 3.0, Dog: 0.25 The prolonged urinary excretion can be accounted for by persistence in the skin. Surface counting: Man essentially none at 24 h Dog 30% of the initial dase was present at 20 h	Hunziker N, Feldmann RJ, Maibach HI, 1978, A6.2.02/06

<table>
<tr>
<td>Human and rhesus monkeys</td>
<td>Topical application of the ventral forearm
humans (total area 13 cm^2)
rhesus monkeys (total area 6 cm^2)
Percutaneous absorption was quantitated by measuring urinary excretion.</td>
<td>Percutaneous absorption
<table>
<tr><th>Species</th><th colspan="3">Human</th><th colspan="2">Rhesus monkey</th></tr>
<tr><td>Dose ($\mu g/cm^2$)</td><td>3.0 (6)*</td><td>400 (7)</td><td>2000 (7)</td><td>400 (3)</td><td>2000 (3)</td></tr>
<tr><td>Total amount absorbed ($\mu g/cm^2$)</td><td>1.1</td><td>102.8</td><td>288.0</td><td>134.4</td><td>348.0</td></tr>
<tr><td>Total percentage absorbed ($\%/cm^2$)</td><td>37.0</td><td>25.7</td><td>14.4</td><td>33.6</td><td>17.4</td></tr>
</table>
* () number of volunteers or animals</td>
<td>Wester RC, Maibach H I, (1976), A6.2.02/12</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>Single and multiple exposures on four female rhesus monkeys.
Benzoic acid (first and eighth dose 14C-labelled) were applied to exactly the same site on the abdomen every 24 hours for 14 days. The site was not washed between dosing. The absorption rates were compared with the absorption rate after a single application.
The bioavailability following multiple topical exposure was measured.
Urinary excretion was measured.</td>
<td>Excretion in the urine following intravenous administration: 71 ± 7%
In the single dose experiment, the absorption rate was 66±19%.
In the multiple dose experiments, the absorption rate was 85±19% for the initial dose and 89±19% after the eighth dose.
No significant change in the percutaneous absorption was found after multiple dosing.</td>
<td>Bucks DAW, Hinz RS, Sarason R, Maibach HI, Guy RH, 1990, A6.2.02/03</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Application of 4 µg benzoic acid/cm^2 for 24 hours to predmaged skin of guinea pigs
Urinary excretion and percutaneous absorption were measured.</td>
<td>Excretion in the urine following intraperitoneal administration: 90.9% after 1 day and 92.1% after 5 days
Absorption / Intact skin
29.2 % after 1 day, 34.2% after 5 days
Absorption after 5 days (damaged skin)
Stripped 71.4% absorbed
Irritated 73.4% absorbed
Delipidized 94.1% absorbed</td>
<td>Moon KC, Wester RC, Maibach HI, 1990, A6.2.02/07</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>4 µg/cm2 were applied to the back.
Excetion of tracer in urine and faeces and percutaneous absorption were measured.</td>
<td>Excretion in the urine following intraperitoneal administration: 88.1%
Percent of dose absorbed: 31.4%</td>
<td>Andersen KE, Maibach HI, Anjo MD, 1980, A6.2.02/02</td>
</tr>
<tr>
<td>Pigs</td>
<td>Topical application of 40 $\mu g/cm^2$
Urinary excretion, faecal excretion, carcass, border, dosed skin, adjacent skin and total excretion were measured.</td>
<td>Excretion in the urine following intravenous administration: 85.4 ± 9.0%
Percutaneous absorption / corrected (%of dose)
Urinary excretion alone: 23.7% (SD 2.7%).
Total excretion: 25.7% (SD 2.4%).</td>
<td>Carver MP, Riviere JE, 1989, A6.2.02/04</td>
</tr>
</table>

Table A6_2_02-4. Additional data for Percutaneous absorption (in vitro test) benzoic acid

<table>
<tr><th>Species</th><th>Treatment / Measuring</th><th>Result</th><th>Reference</th></tr>
<tr><td>Guinea pig skin</td><td>Application of 2 µg/cm2 in an ethanolvehicle (15 µL/cm2 were applied to the skin.
Skin kept viable for 24 h in a flow-through diffusion cell using a HEPES-bufferd Hank's balanced salt solution (HHBSS).
Skin surface became washed after 24 h and experiment was continued for another 24 h.
Recovery of radioctivity was measured.</td><td>Percentage of applied dose:
<table><tr><th></th><th>HHBSS</th><th>Water</th></tr><tr><td>Receptor fluid</td><td>47.3 ± 2.3</td><td>59.1 ± 5.3</td></tr><tr><td>Skin</td><td>2.2 ± 0.7</td><td>1.0 ± 0.2</td></tr><tr><td>Total</td><td>49.5 ± 3.0</td><td>60.1 ± 5.5</td></tr></table>Metabolism
<table><tr><th></th><th>HHBSS</th><th>Water</th></tr><tr><td>Receptor fluid</td><td></td><td></td></tr><tr><td>Benzoic acid</td><td>73.8 ± 8.0</td><td>92.3 ± 1.7</td></tr><tr><td>Hippuric acid</td><td>6.9 ± 3.4</td><td>0.1 ± 0.1</td></tr><tr><td>Polar</td><td>11.6 ± 4.1</td><td>2.5 ± 1.3</td></tr><tr><td>Skin</td><td></td><td></td></tr><tr><td>Benzoic acid</td><td>83.2 ± 2.9</td><td>84.2 ± 1.7</td></tr><tr><td>Hippuric acid</td><td>0.6 ± 0.6</td><td>0.0 ± 0.0</td></tr><tr><td>Polar</td><td>3.8 ± 0.8</td><td>8.1 ± 1.7</td></tr></table></td><td>Nathan D, Sakr A, Lichtin JL, Bronaugh RL, 1990, A6.2.02/08</td></tr>
<tr><td>Human skin</td><td>Abdominal human female skin 500µm thickness, stored in tissue culture medium.
Permeation of 5.0 mg/ml were measured in isolated stratum corneum, stratum corneum with intact epidermis (heat separated skin) and split-thickness skin in diffusion chambers.</td><td>Permeation
<table><tr><th></th><th>D_m (cm^2/hr) x 10^5</th><th>h (µm)</th><th>P (cm/hr)</th><th>t_{lag} (min)</th></tr><tr><td>isolated stratum corneum</td><td>2.1 ±2,9</td><td>32 ± 41</td><td>0.031 ± 0.060</td><td>4.8 v ± 11</td></tr><tr><td>heat separated skin</td><td>5 ± 10</td><td>70 ± 140</td><td>0.031 ± 0.089</td><td>11.2 39</td></tr><tr><td>split-thickness skin</td><td>8.0 ± 7.8</td><td>110 ± 100</td><td>0.034 ± 0.044</td><td>15.7 ± 24.7</td></tr></table>Skin technics did not alter the barrier function</td><td>Parry GE, Bunge AL, Silcox DG, Pershing LK, Pershing DW, 1990, A6.2.02/09</td></tr>
<tr><td>Human skin and rat skin</td><td>Human skin and rat skin were prepared from frozen skin.
Human skin thickness: 0.3 – 1.8 mm (9 laboratories)
Rat skin (1 laboratory)
Permaetion of 4.0 mg/ml was measured in diffusion chambers</td><td>The mean absorptions rate in human skin membran was 16.54 ± 11.87 µg/cm^2/h, while the amount in the receptor fluid after 24 h was 70.6 ± 17.2% of the dose applied (8 laboratries).
The mean absorptions rate in rat skin membran was 21.21 µg/cm^2/h, while the amount in th receptor fluid after 24 h was 89.8% of the dose applied (8 laboratries).</td><td>van de Sandt JJM, et al., 2004, A6.2.02/11</td></tr>
</table>

Section A6.3.1 Repeated dose toxicity oral

Annex Point IIA6.3.1

Official use only

1 REFERENCE

1.1	**Reference**	Kreis H, Frese K, Wilmes G,1967, Physiologische und morphologische Veränderungen an Ratten nach peroraler Verabreichung von Benzoesäure Fd. Cosmet. Toxicol., 5, 505-511 (published)
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	No data protection / published data

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Study conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	Benzoic acid
3.1.1	Lot/Batch number	No lot/batch number available
3.1.2	Specification	As given in section 2
3.1.2.1	Description	White crystalline solid substance
3.1.2.2	Purity	>99%
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rat
3.2.2	Strain	Royal Wistar
3.2.3	Source	Meyer Arend, Bad Salzuflen, Germany
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Mean weight: 60.0 g
3.2.6	Number of animals per group	

Group	Number of animals	Days of Gavage	Test duration
A I	5	1	1
A II	5	2	2
A III	5	3	3
A IV	8	5	5
B I	10	5	5
B II	15	5	24 / 35

Section A6.3.1 Repeated dose toxicity oral

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		C I	5	7	7
		C II	8	14	14
		C III	10	35	35
		K I	5		3
		K7	II		35
3.2.7	Control animals	Yes			
3.3	**Administration/ Exposure**	Oral			
3.3.1	Duration of treatment	35 days			
3.3.2	Frequency of exposure	Daily			
3.3.3	Postexposure period	No			
3.3.4	**Oral**				
3.3.4.1	Type	In food			
3.3.4.2	Concentration	In food 825 or 2250 mg/kg bw (calculated according to Lehmann AJ, Food Drug Off. Q. Bull. 18, 66 (1954)) Food consumption per day ad libitum			
3.3.4.3	Vehicle	Moistened with food			
3.3.4.4	Concentration in vehicle	1.1% or 3%			
3.3.4.5	Total volume applied	No data given			
3.3.4.6	Controls	Plain diet			
3.4	**Examinations**				
3.4.1	Observations				
3.4.1.1	Clinical signs	Yes, daily			
3.4.1.2	Mortality	Yes, daily			
3.4.2	Body weight	Yes, twice weekly			
3.4.3	Food consumption	No data given			
3.4.4	Water consumption	No data given			
3.4.5	Ophthalmoscopic examination	No			
3.4.6	Haematology	No			
3.4.7	Clinical Chemistry	No			
3.4.8	Urinalysis	No			
3.5	**Sacrifice and pathology**				
3.5.1	Organ Weights	No			
3.5.2	Gross and histopathology	Yes, all animals. Day 1, 2, 3 5, 7, 14 and 35 Organs: Heart, intestine, liver, spleen, kidney and brain			

Section A6.3.1 Repeated dose toxicity oral

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3.5.3	Other examinations	-
3.5.4	Statistics	No data given
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	**Gavage of 3% benzoic acid in food (2250 mg/kg bw)** In rats dosed over 5 days, disorders of the central nervous systems (excitation, ataxia, tonoclonic convulsions). **Gavage of 1.1% benzoic acid in food (825 mg/kg bw)** No clinical signs of intoxication
4.1.2	Mortality	**Gavage of 3% benzoic acid in food (2250 mg/kg bw)** Mortality rate after 5 days: 50% **Gavage of 1.1% benzoic acid in food (825 mg/kg bw)** No treatment related mortality.
4.2	**Body weight gain**	**Gavage of 3% benzoic acid in food (2250 mg/kg bw)** Body weight gain↓ **Gavage of 1.1% benzoic acid in food (825 mg/kg bw)** Body weight gain↓
4.3	**Food consumption and compound intake**	No data given
4.4	**Ophtalmoscopic examination**	-
4.5	**Blood analysis**	-
4.5.1	Haematology	-
4.5.2	Clinical chemistry	-
4.5.3	Urinalysis	-
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No data given
4.6.2	Gross and histopathology	**Gavage of 3% benzoic acid in food (2250 mg/kg bw)** **Macroscopic findings:** Heart, liver, spleen, kidney and brain: no visible changes. **Microscopic findings:** Heart: No treatment related changes Kidney: No treatment related changes Liver: No treatment related changes Intestine: In some cases, bleeding into the gut. Brain: Damage (necrosis of parenchymal cells of the stratum granulosum of

the fascia dentata and the cortex of the lobus piriformis) in most animals dosed over 3-5 days (still present after 35 days)

Brain Damage:

Group	Number of animals	
	with brain damage	tested
A I	0	5
A II	0	5
A III	2	5
A IV	5	8
B I	10	10
B II	13	15
C I	0	5
C II	0	8
C III	0	10
K I	0	5
K7	0	7

Gavage of 1.1% benzoic acid in food (825 mg/kg bw)
Macroscopic findings:
Heart, liver, spleen, kidney and brain: no visible changes.

Microscopic findings:
No difference to the control.

4.7 Other -

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Benzoic acid was tested for its repeated dose toxicity. 6 groups of 5 to 15 male Royal Wistar rats received a daily dose of 3% (2250 mg/kg bw) for 1 to 5 days. 1 group of 15 rats was observed for 19 or 30 days respectively without receiving benzoic acid. 3 groups of 5 to 10 male Royal Wistar rats received a daily dose of 1.1% (825 mg/kg bw) for 7, 14 and 35 days. 2 control groups received food without test substance.

5.2 Results and discussion

Gavage of 3% benzoic acid in food (2250 mg/kg bw)

In some cases, bleeding into the gut. All animals dosed over 5 days: Brain damage (necrosis of parenchymal cells of the stratum granulosum of the fascia dentata and the cortex of the lobus piriformis) in most animals dosed over 3-5 days (still present after 35 days)

Gavage of 1.1% benzoic acid in food (825 mg/kg bw)
No difference to the control.

The discussion reveals, that 1% benzoic acid in the diet (ca 825 mg/kg bw/d) may be the threshold for toxic effects.

5.3 Conclusion

5.3.1 LO(A)EL No data given

5.3.2 NOEL ca 825 mg/kg bw/d

Section A6.3.1 Repeated dose toxicity oral

Annex Point IIA6.3.1

5.3.3	Other	-
5.3.4	Reliability	2
5.3.5	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/01/07
Materials and Methods	Acceptable with corrections as follows: 3.1.2 Specification: not available 3.1.2.1 Description: not available 3.1.2.2 Purity: not specified 3.1.2.3 Stability: not tested. The test substance may be considered stable for the duration of the study. 3.2.6 Number of animals per group: The test substance was not applied by gavage but with the food. The number of animals in group KII was 7. See also Annex 1. 3.3.1 Duration of treatment: 1-35 days 3.3.3 Post-exposure period: Group BII only (19-30 days) 3.3.4.2 Concentration: 0, 11000 or 30000 ppm in food (corr. to 0, 825 or 2250 mg/kg bw/d according to Lehmann AJ, Food Drug Off. Q. Bull. 18, 66 (1954)) 3.3.4.3 Vehicle: not specified 3.3.4.4 Concentration in vehicle: not applicable 3.5.2 Gross and histopathology: All animals at death or sacrifice; Gross pathology: Stomach, Intestine, Liver, Spleen, Kidneys and other unspecified organs; Histopathology: Heart, Liver, Kidneys, Brain
Results and discussion	Acceptable with corrections as follows: 4.1.1 – 4.2, 4.6.2, 5.2: The test item was not applied by gavage but with the diet. See also Annex 1. 5.2 Results and Discussion: The results suggest, that 10000 ppm may be the threshold for toxic effects to the CNS in male juvenile rats. The extent of the examinations performed and the amount and quality of the documentation does not allow to derive conclusions about general toxicity.
Conclusion	CNS toxicity in male juvenile rats **LOAEL:** 30000 ppm (~2250 mg/kg bw/d) **NOAEL:** 11000 ppm (~825 mg/kg bw/d)
Reliability	2, for CNS toxicity, 4, for general subacute toxicity.
Acceptability	Acceptable (with regard to CNS toxicity)
Remarks	Corrections in Annex 1
	COMMENTS FROM ... *(specify)*

Section A6.3.1 Repeated dose toxicity oral

Annex Point IIA6.3.1

Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_3-1. Results of clinical chemistry haematology and urinalysis

No data given

Table A6_3-2. Results of repeated dose toxicity study

Parameter	**Control**		**low dose**		**high dose**		**dose-response +/-**	
	m[a]	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m**	**f**
Number of animals examined	12	-	23	-	48	-		n.a.
Mortality	0	-	0	-	24	-	+	n.a.
Clinical signs / excitation, ataxia, tonoclonic convulsions	0	-	0	-	48	-		n.a.
Body weight		-	↓	-	↓	-		n.a.
Intestine		-		-		-		n.a.
Gross pathology	0	-	0	-	0	-		n.a.
Microscopic pathology / bleeding into the gut	0	-	0	-	some	-		n.a.
Brain		-		-		-		n.a.
Gross pathology	0	-	0	-	0	-		n.a.
Microscopic pathology / Damage (necrosis of parenchymal cells of the stratum granulosum of the fascia dentata and the cortex of the lobus piriformis)	0	-	0	-	most	-		n.a.

Table A6_3-3. Additional data repeated toxicity study benzoic acid and sodium benzoate Benzoic acid

Rat

Reference:	Anonymous, Bio-Fax, 1973 (cited in Wibbertmann, 2000, A6.3.1/10)
Duration:	4 weeks; benzoic acid
Dose:	0-760-3800-7600 ppm (ca 0-65-324-647 mg/kg bw/d)
Application:	Oral (in the diet)
Species/Strain:	Rat (albino)
Animal number:	10 males / group
Initial body weight:	120 g
Control group:	Yes
Parameters:	Clinical signs, body weight change, food consumption, survival rate, autopsy
Findings:	No adverse effect
Conclusion:	The NOEL is at 7600 ppm benzoic acid (ca 647 mg/kg bw/d).
Reference:	Kieckebusch, W. & K. Lang, 1960 (A6.3.1/07)
Duration:	4 weeks / 4 generations, depending on mortality; benzoic acid and sodium benzoate
Dose:	0.5 and 1% (ca 375 and 750 mg/kg bw/d)
Application:	Oral (in the diet)
Species/Strain:	Rat, a strain from Farbwerke Bayer AG, Elberfeld
Animal number:	20 male and 20 female rats
Initial body weight:	40 - 50 g
Control group:	Yes
Parameters:	Body weight change, food consumption, survival rate, urine protein and glucose, gross pathology
Findings:	No adverse effect
Conclusion:	NOEL: 750 mg/kg bw/d

Cat

Reference:	Bedford, B. & M.A. Clarke, 1972 (A6.3.1/03)
Rationale:	Suspected poisoning in a cattery, benzoic acid content of 2.39% in the cat meat (Bedford, B. & M.A. Clarke, 1971) Therefore carrying out the following experiments with 5 heterogeneous groups
1. Duration:	1 x in the food (observation up to 2 months); benzoic acid
Dose:	0-1% (doses consumed: 450-890 mg/kg bw)
Application:	Oral (in the diet)
Species/Strain:	Cat (no more data given)
Animal number:	4/group
Initial body weight:	1.06-1.7 kg, (age: 7 months)
Control group:	Body weight: 3.6-3.8 kg, (age: 16 months)
Parameters:	Clinical signs, survival rate, blood chemistry (urea, SAP, SALAT), pathohistology (including central nervous system)
Findings:	Aggressiveness, hyperaesthesia, convulsions (onset 14-16 h after beginning of the feeding), mortality (2/4 32-192 h after beginning of the feeding); abnormal histological findings in the liver, kidney, lung, mouth and heart muscle One cat recovered (890 mg/kg bw), the fourth was without symptoms (450 mg/kg bw)
Conclusion:	The single dose of 1% benzoic acid in the diet revealed effects in cats.
2. Duration:	3 days (2 cats) and 4 days (2 cats); benzoic acid
Dose:	0-0.5% (daily dose rate: 300-420 mg/kg bw, total doses consumed: 910-1260 mg/kg bw)
Application:	Oral (in the diet)
Species/Strain:	Cat (no more data given)
Animal number:	4/group
Initial body weight:	1.42-2 kg, (age: 7.5-8.5 months)
Control group:	Body weight: 3.6-3.8 kg, (age: 16 months)
Parameters:	Clinical signs, survival rate, blood chemistry (urea, SAP, SALAT), pathohistology (including central nervous system)
Findings:	Hyperaesthesia, apprehension, depression (48-92 hours after the first feeding), mortality (2/4 within 85-120 hours after the first feeding); abnormal biochemical

	and histological findings in the liver and kidney Two cats recovered (300 mg/kg bw/d)
Conclusion:	No NOEL.
3. Duration:	23 days; benzoic acid
Dose:	0-0.25% (daily dose rate: 130-160 mg/kg bw, total doses consumed: 3000-3680 mg/kg bw)
Application:	Oral (in the diet)
Species/Strain:	Cat (no more data given)
Animal number:	4/group
Initial body weight:	3.2-4 kg, (age: 12-18 months)
Control group:	Body weight: 3.6-3.8 kg, age: 16 months
Parameters:	Clinical signs, survival rate, blood chemistry (urea, SAP, SALAT)
Findings:	No adverse effect
Conclusion:	The NOEL is at 0.25% benzoic acid in the diet (130-160 mg/kg bw/d)
4. Duration:	15 days; benzoic acid
Dose:	0-100-200 mg/kg bw/d
Application:	Oral (in the diet)
Species/Strain:	Cat (no more data given)
Animal number:	4/group
Initial body weight:	1.7-2.3 kg, (age: 9-10 months)
Control group:	Body weight: 3.6-3.8 kg, age: 17 months (the same group as above, but 1 month older)
Parameters:	Clinical signs, survival rate, blood chemistry (urea, SAP, SALAT)
Findings:	No adverse effect
Conclusion:	The NOEL for the cat is 200 mg/kg bw/d (benzoic acid); higher dose levels revealed signs of acute (central nervous system) and cumulative toxicity and damage of kidney and liver, but no abnormality of the brain and spinal cord was detected by histological examination. 200 mg/kg bw/d is the lowest relevant NOEL from all available short-term and chronic studies with benzoic acid or sodium benzoate. The reason for the particular sensitivity of cats is a defective glucuronic acid system. In contrast with other species, in the cat only hippuric acid is formed, no reserve detoxification system is available. This investigation (without guideline) performed on one species with a peculiar metabolism is not suitable for deriving a reference dose for humans.

Sodium benzoate

Rat

Reference:	Griffith, W.H., 1929 (A6.3.1/05)
Duration:	40 days (some animals up to 100 days); sodium benzoate
Dose:	0-1.5-2-2.25-2.5-2.75-3-3.25-3.5-3.75% (with and without supplement of glycine)
Application:	Oral (in the diet)
Species/Strain:	White rat
Animal number:	4-19 males
Initial body weight:	45-60 g
Control group:	Yes
Parameters:	Clinical signs, body weight change, growth rate during restricted feeding, food consumption and efficiency, survival rate
Findings:	Decreased growth rate at 2% in the diet, occasionally mortality at 2.5%, above 3% severely depressed growth rate and mortality of more than 50% of the animals with and without restriction of feeding, mortality preceded by restlessness, uncoordinated movement, tremor, convulsions, severe eye inflammation; addition of glycine lowered the toxic effects
Conclusion:	The NOEL is at 1.5% sodium benzoate in the diet (ca 750 mg/kg bw/d).
Reference:	Fujitani, T., 1993 (A6.3.1/04)
Duration:	10 days; sodium benzoate
Dose:	0-1.81-2.09-2.4% (ca 0-1358-1568-1800 mg/kg bw/d)
Application:	Oral (in the diet)
Species/Strain:	Rat, F344/Ducrj
Animal number:	6/sex/group
Initial body weight:	Not reported, (age: 5 weeks)

Control group:	Yes
Parameters:	Clinical signs, body weight change, survival rate, organ weights (liver, heart, kidney, spleen, lung, thymus), clinical chemistry (broad spectrum), histological examination of liver and kidney
Findings:	Mainly at 2.4% in the diet convulsions and mortality in 1/6 males; depressed body weight gain; higher serum levels of albumin, total protein and gamma-glutamyl-transpeptidase, lower levels of cholesterol and cholinesterase; increased relative kidney and liver weights; liver: eosinophilic foci around portal vein, enlarged hepatocytes with glassy cytoplasm; some findings evident only in males
Conclusion:	The NOEL is at 1.81% sodium benzoate in the diet (ca 1358 mg/kg bw/d).
Reference:	Harshbarger, K.E., 1942 (A6.3.1/06)
Duration:	4-5 weeks; sodium benzoate (with and without supplement of glycine)
Dose:	0-1-3% (ca 0-500-1500 mg/kg bw/d)
Application:	Oral (in the diet)
Species/Strain:	White rat
Animal number:	Only inexact information
Initial body weight:	Not reported, (age: about 4 weeks)
Control group:	Yes
Parameters:	Clinical signs, body weight change, food consumption, survival rate
Findings:	At 3% in the diet clinical signs of toxicity (increased irritability, uncoordinated movement, convulsions), mortality (2/8 rats, within 7-20 days), decreased body weight gain; addition of glycine reduced the toxicity Development of tolerance to sodium benzoate (tested with 3% in the diet), when animals previously received 1% or 3% in the diet
Conclusion:	The NOEL is at 1% sodium benzoate in the diet (ca 500 mg/kg bw/d).
Reference:	Smyth, H.F. & Ch.P. Carpenter, 1948 (A6.3.1/08)
Duration:	30 days; sodium benzoate
Dose:	Range from 16 to 1090 mg/kg bw/d
Application:	Oral (in the diet)
Species/Strain:	Rat, Sherman
Animal number:	5/sex/group
Initial body weight:	Ca 120 g
Control group:	Yes
Parameters:	Body weight change, food consumption, survival rate, histology (adrenal, upper intestine, kidney, liver, spleen, testis)
Findings:	No adverse effect
Conclusion:	No effect up to 1090 mg/kg bw/d.
Reference:	Sodemoto, Y. & M. Enomoto, 1980 (A6.3.1/09) Preliminary study for the carcinogenicity study
Duration:	6 weeks; sodium benzoate
Dose:	0-0.5-1-2-4-8% (ca 0-250-500-1000-2000-4000 mg/kg bw/d)
Application:	Oral (in the diet)
Species/Strain:	Rat, Fischer 344
Animal number:	10/sex/group
Initial body weight:	110-150 g, (age: 4-5 weeks)
Control group:	10/sex
Parameters:	Clinical signs, body weight change, survival rate, pathohistology (autopsy and histology of various organs)
Findings:	At 4% and 8% sodium benzoate in the diet 100% mortality within 4 weeks, significantly reduced body weights; hypersensitivity; histological examination revealed atrophy of the spleen and lymph nodes At 0.5, 1 and 2% in the diet 3, 2 and 1 males respectively died of pneumonia (not compound or dose related)
Conclusion:	According to the authors, the MTD is at 2% sodium benzoate in the diet (ca 1000 mg/kg bw/d), the highest dose level for the carcinogenicity study. For the occurrence of hypersensitivity no dose-relationship is given. No other adverse effects were described.

Mouse oral

Reference:	Fujitani, T., 1993 (A6.3.1/04)
Duration:	10 days; sodium benzoate
Dose:	0-2.08-2.5-3% (ca 0-3012-3750-4500 mg/kg bw/d)
Application:	Oral (in the diet)
Species/Strain:	Mouse, B6C3F1
Animal number:	5 males and 4-5 females/group
Initial body weight:	Not reported, (age: 5 weeks)
Control group:	Yes
Parameters:	Clinical signs, body weight change, survival rate, organ weights (liver, heart, kidney, spleen, lung), clinical chemistry (broad spectrum), histological examination of liver and kidney
Findings:	Mainly at 3% in the diet hypersensitivity and convulsions, mortality (2/5 females), higher serum levels of cholesterol and phospholipids, increased kidney and liver weights; hepatocytes: eosinophilic cytoplasm, enlargement, vacuolation and necrosis Most findings evident only in males
Conclusion:	NOEL at 2.08% sodium benzoate in the diet (ca 3012 mg/kg bw/d).

Summary

Studies with benzoic acid

Treatment	Result	Reference
Rat		
Not applicable	NOEL: ca 500 mg/kg bw/d	Anonymous, SANCO/1396/2001-Final, 2003, (A6.3.1/02)
4 weeks 0-760-3800-7600 ppm (ca 0-65-324-647 mg/kg bw/d)	NOEL: 7600 ppm benzoic acid (ca 647 mg/kg bw/d)	Anonymous, Bio-Fax, 1973, cited in Wibbertmann, 2003 (A6,3.1/10)
0.5 and 1% (ca 375 and 750 mg/kg bw/d)	No adverse effect NOEL: 750 mg/kg bw/d	Kieckebusch W, Lang K, 1960 (A6.3.1/07)
Cat		
0-1% (doses consumed: 450-890 mg/kg bw)	The single dose of 1% benzoic acid in the diet revealed effects in cats	Bedford B, Clarke MA, 1972 (A6.3.1/03)
0-0.25% (daily dose rate: 130-160 mg/kg bw, total doses consumed: 3000-3680 mg/kg bw) 0-0.5% (daily dose rate: 300-420 mg/kg bw, total doses consumed: 910-1260 mg/kg bw)	NOEL: 0.25% benzoic acid in the diet (130-160 mg/kg bw/d).	
0-100-200 mg/kg bw/d	NOEL: 200 mg/kg bw/d	

Studies with sodium benzoate

Treatment	Result	Reference
Rat		
0-1.5-2-2.25-2.5-2.75-3-3.25-3.5-3.75% (with and without supplement of glycine)	NOEL: 750 mg/kg bw/d	Griffith, W.H., 1929 (A6.3.1/05)
0-1.81-2.09-2.4% (ca 0-1358-1568-1800 mg/kg bw/d)	NOEL: 1358 mg/kg bw/d	Fujitani, T., 1993 (A6.3.1/04)
0-1-3% (ca 0-500-1500 mg/kg bw/d)	NOEL: 500 mg/kg bw/d	Harshbarger, K.E., 1942 (A6.3.1/06)
Range from 16 to 1090 mg/kg bw/d	NOEL: 1090 mg/kg bw/d	Smyth HF, Carpenter CP, 1948 (A6.3.1/08)
0-0.5-1-2-4-8% (ca 0-250-500-1000-2000-4000 mg/kg bw/d)	The MTD is 2% sodium benzoate in the diet (ca 1000 mg/kg bw/d)	Sodemoto Y, Enomoto M, 1980 (A6.3.1/09)
Mouse		
0-2.08-2.5-3% (ca 0-3012-3750-4500 mg/kg bw/d)	NOEL: 3012 mg/kg bw/d	Fujitani, T., 1993 (A6.3.1/04)

The overall NOEL - 1% benzoic acid in the diet corresponding to ca 500 mg/kg bw/d - has to be derived from all relevant short-term and chronic toxicity/carcinogenicity studies on rats together since no single study according to a current test guideline is available. This NOEL can serve for the calculation of ADI and AOEL.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/01/07

Results and discussion

Additional study summaries have been provided in Table A_6_3-3. With exception of Anonymous, Bio-Fax, 1973 (cited in Wibbertmann, 2003), these study summaries relate to original reports. The subacute dose range finding study by Sodemoto and Enomoto (1980) failed to show typical symptoms (tremor, connvulsion, liver toxicity) at lethal doses. The NOAEL and LOAEL values, along with major effects reported in the other studies are summarised below:

Exposure, Species	NOAEL / LOAEL (mg/kg bw /d)	Main effects	Reference
Oral, 10 d, Rat, Sodium benzoate	1360 / 1570	convulsions + mortality, bw↓, liver weight↑, albumin↑, serum protein↑, GGT↑, hepatocyte enlargement, kidney weight↑	Fujitani (1993)
Oral, 30 d Rat, Sodium benzoate	1090 / >1090	No changes in bw, food consumption, adrenals, upper intestine, kidney, liver, spleen; no mortality	Smyth and Carpenter (1948)
Oral, 4 wk Rat Sodium benzoate	500 / 1500	bw↓, irritability, uncoordinated movements, convulsions, mortality	Harshbarger (1942)
Oral, 40 d Rat Sodium benzoate	750 / 1000	bw↓, mortality, tremor, convulsions, restlessness	Griffith (1929)
Oral, 8 wk Rat Benzoic acid	500 / >500	No changes in bw, lifetime survival, organ weight (brain, heart, liver, spleen, kidneys, testes) or liver histology	Kiecke-busch and Lang (1960)
Oral, 10 d Mouse Sodium benzoate	3750 / 4500	hypersensitivity, convulsions, death, liver weight↑, cholesterol↑, phospholipids↑, hepatocyte enlargement, necrosis and vacuolation	Fujitani (1993)
Oral, 15 d Cat Benzoic acid	200 / 340	Mortality, aggressiveness, hyperaesthesia, convulsions, biochemical and histopathological indications for toxicity to liver, kidney and heart	Bedford and Clarke (1972)

Conclusion

Overall, the lowest reported LOAEL for subacute toxicity of sodium benzoate in the rat was 1000 mg/kg bw/d. Correspondingly, 500 mg/kg bw/d is considered the relevant NOAEL for subacute toxicity of sodium benzoate and benzoic in the rat.

Although the cat is considerably more sensitive than the rat, toxicity in this species is not considered relevant to human risk assessment based on fundamental differences in metabolism (glucuronidation deficiency).

Remarks

Not performed and documented according to current guidelines. Non-GLP.

COMMENTS FROM ... *(specify)*

Date

Give date of comments submitted

Materials and Methods

Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.
Discuss if deviating from view of rapporteur member state

Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.3.2 **Annex Point IIA6.3.2**	**Repeated dose toxicity** **Dermal/rat**	Official use only
	1 REFERENCE	
	Anonymous, 2001, OECD-SIDS, 2001 (A6.3.2)	
	2 RESULTS	
	A 21 day dermal study with male/female New Zealand white rabbits dosed with 100, 500, or 2500 mg/kg bw benzoic acid 5 days/week showed no compound related effects in behaviour, body weight organ weights, clinical laboratory tests or survival. Very slight dermal irritation was noted for 1/8 rabbits at the 2500 mg/kg level. (cited in OECD-SIDS, 2001 (A6.3.11)) This study, however, is not available to us. After dermal absorption through skin, the subsequent metabolism pathway is similar to the one after oral administration. Therefore, data from the repeated dose test with oral administration are suitable for the determination of possible effects after dermal administration. Rerunning a repeated dose dermal toxicity test does not add additional information and is regarded not to be necessary for the purpose of this dossier.	
3 Conclusion	Benzoic acid is not toxic after 21 day dermal exposure.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2008/01/04	
Evaluation of applicant's justification	According to the study summary provided in OECD-SIDS, 2001 (A6.3.11), the NOAEL for subacute dermal toxicity of benzoic acid in rabbits is 2500 mg/kg bw/d when applied 5 days per week over 3 weeks (LOAEL > 2500 mg/kg bw/d). Extrapolation of oral toxicity data provided in A6.3.1 assuming a percutaneous absorption of 40 % as suggested by data presented in A6.2.2 results in an expected NOAE and LOAE levels of 2100 and 5600 mg/kg bw/d, respectively, for the rat.	
Conclusion	There is no indication that systemic toxicity of benzoic acid is increased after subacute dermal exposure as compared to oral exposure, nor that the rabbit is significantly more sensitive than the rat.	
Remarks	In absence of the original study data (IRCD project no. 163-675), the provided subacute dermal NOAEL of 2500 mg/kg bw/d should not be used for derivation of limit values.	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	

Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.3.3 Repeated dose toxicity / inhalation

Annex Point IIA6.3.3 6.3.3

Official use only

		1 REFERENCE
1.1	**Reference**	Rope et. al, 1981, Four Week Subacute Inhalation Toxicity Study of Benzoic Acid in Rats International Research and Development Corporation (IRDC), 1981, Project No. 163-676
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No. Study cconducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid
2.2	**GLP**	The study was performed prior to GLP regulations but it principally provides the core information necessary to fulfill the objectives of the study and there is no reason to doubt the validity of the results.
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	Benzoic acid
3.1.1	Lot/Batch number	52930350
3.1.2	Specification	Flake benzoic acid technical
3.1.2.1	Description	White flakes
3.1.2.2	Purity	No data given
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rat
3.2.2	Strain	Sprague Dawley Charles River CD®
3.2.3	Source	Charles River Breeding Laboratories, inc., Michigan, USA
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Age: 42 days Weight: male: 169-220 g female: 139 -174 g
3.2.6	Number of animals per group	4 groups of 10 male and 10 female rats
3.2.7	Control animals	Yes, Group 0
3.3	**Administration/ Exposure**	Inhalation

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3.3.1	Duration of treatment	28 days
3.3.2	Frequency of exposure	5 days per week
3.3.3	Postexposure period	No data given
3.3.4	**Inhalation**	
3.3.5	Concentrations	Desired concentration 0.02 [mg/L] 0.2 [mg/L] 2.0 [mg/L] Nominal concentration 0.3 ± 0.056 [mg/L] 2.2 ± 0.036 [mg/L³] 16.5 ± 2.140 [mg/L] Analytical concentration 0.025 ± 0.0076 [mg/L] Group II 0.250 ± 0.110 [mg/L] Group III 1.2 ± 0.35 [mg/L] Group IV
3.3.5.1	Particle size	During the study, particle size analysis of the exposure atmospheres was performed weekly for each group. The equivalent aerodynamic diameter (EAD) did not vary significantly from group to group nor week to week. Therefore, a representative EAD was calculated for all treatment groups for the full study period. The EAD was found to be 4.7µm.
3.3.5.2	Type or preparation of particles	The dust aerosol atmosphere was generated utilising the International Research and Development Corporation (IRAD) dust generator.

Group	Desired conc.	Holes		Rotat. rate rpm	Theor. output mg/min	Air flow rate	
		No. of	Diameter (mm)			L/min	L/min
II	0.02	20	2.75	0.96	185	50	7
III	0.2	20	4.70	0.96	960	40	10
IV	2.0	20	6.30	3.90	3200	60	10

Due to the difficulty in achieving the desired concentration for group IV, two IRAD dust generators were used.

3.3.5.3	Type of exposure	Whole body
3.3.5.4	Vehicle	No vehicle / air only
3.3.5.5	Concentration in vehicle	-
3.3.5.6	Duration of exposure	6 h
3.3.5.7	Controls	Sham exposed
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, all animals, once each week
3.4.1.2	Mortality	Yes, all animals, twice each day, prior to exposure and again after each exposure
3.4.2	Body weight	Yes, all animals, prior to exposure, after one, two, three and four weeks of exposure
3.4.3	Food consumption	No

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3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	No
3.4.6	Haematology	Yes, after four weeks of exposure on all surviving animals. Parameters: Haematocrit, erythrocyte count, leukocyte count, platelet count, MCV, MCH and MCHC
3.4.7	Clinical Chemistry	Yes, after four weeks of exposure on all surviving animals. Parameters: Blood Urea Nitrogen, Serum Alkaline Phosphatase, Serum Glutamic Pyruvic Transaminase, Serum Glutamic Oxaloacetic Transaminase and Blood Glucose
3.4.8	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes, after four weeks of exposure on all surviving animals. organs: liver, kidneys, spleen, brain, heard and trachea/lungs
3.5.2	Gross and histopathology	Yes After four weeks of exposure all surviving animals were sacrificed. Each animal received a complete post-mortem examination. Haematoxylin and eosin stained paraffin sections of some tissues from all control and high dose group animals were examined. Ogans: adrenals, adipose tissue, abdominal aorta, sternabrae, sternum, brain, cecum, colon, esophagus, eye with optic nerve, fallopian tubes, pituitary, prostate, salivary glands, skeletal muscle, skin, entire head, testis, ovary, heart, kidneys, liver, lungs, lymph node's, mammary gland, pancreas, parathyroid, sciatic nerve, small intestine, spinal cord, spleen, stomach, thymus, thyroid, urinary bladder, uterus
3.5.3	Other examinations	-
3.5.4	Statistics	Body weights, haematological parameters and organ weights were compared by analysis of variances and appropriate t-test as described by Steel and Torrie using Dunnett's multiple comparison tables.
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Exposure to 0.025 mg/L did not produce any clinical signs. At 0.25 and 1.2 mg/L, there was upper respiratory tract irritation (both sexes), as indicated by red material around the nares. At 1.2 mg/L all animals shows exhibited upper respiratory tract irritation.
4.1.2	Mortality	At 1.2 mg/L, one male and one female died,
4.2	**Body weight gain**	At 1.2 mg/L, mean bodyweight gain was decreased in both sexes
4.3	**Food consumption and compound intake**	No data given
4.4	**Ophtalmoscopic examination**	No data given
4.5	**Blood analysis**	

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4.5.1	Haematology	At 1.2 mg/L, decreased platelet count (males and females) All other haematological data are not treatment related and without toxicological significance.
4.5.2	Clinical chemistry	No treatment related changes.
4.5.3	Urinalysis	No data given
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	At 0.25 and 1.2 mg/L, there were decreased absolute and relative kidney weights (female only). At 1.2 mg/L, there were decreased absolute and relative liver weights (males only), decreased trachea/lung weight (females only)
4.6.2	Gross and histopathology	No treatment related macroscopic lesions were observed in any of the rats that ware terminally sacrificed or died during the study. Microscopy revealed a multifocal to generalise pulmonary fibrosis and inflammatory cell infiltrate in animals from the entire dose groups given benzoic acid.
4.7	**Other**	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The subacute inhalation toxicity of benzoic acid was evaluated when administered to rats as a dust via whole body inhalation exposure 6 hours per day for four consecutive weeks. During the study, particle size analysis of the exposure atmospheres was performed weekly for each group. The equivalent aerodynamic diameter (EAD) did not vary significantly from group to group nor week to week. Therefore, a representative EAD was calculated for all treatment groups for the full study period. The EAD was found to be 4.7µm. The study was cconducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid
5.2	**Results and discussion**	Exposure to 0.025 mg/L did not produce any clinical signs and there was no effect on clinical chemistry, haematology or gross pathology, but some histopathological lesions were evident. At 0.25 and 1.2 mg/L, there was upper respiratory tract irritation (both sexes), as indicated by red material around the nares, and decreased absolute and relative kidney weights (female only). At 1.2 mg/L, one male and one female died, and mean bodyweight gain was decreased in both sexes. Also in the top dose group, there were lower bodyweight gain, decreased platelet count (males and females), decreased absolute and relative liver weights (males only), decreased trachea/lung weight (females only) and all animals exhibited upper respiratory tract irritation. Microscopy revealed a multifocal to generalise pulmonary fibrosis and inflammatory cell infiltrate in animals from the entire dose groups given Benzoic acid. The results of this study indicate that levels as low as 0.025 mg/L for four weeks of exposure produce toxic effects as indicated by pulmonary fibrosis and inflammatory cell infiltrate. This level did not produce any other signs of toxicity. It must be noted that the study was performed with aerosol (crystalline benzoic acid), not with benzoic acid vapour. Clinical signs of irritation

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		are due to the crystals, for systemic effects a NOAEL of 0.025 mg/L after 4 weeks can be deducted. This study was performed to get information on possible health effects on workers in the production of benzoic acid. Workers handling benzoic acid containing biocidal products (in closed premises (e.g.stables) are not exposed to crystals, but to benzoic acid vapour.
5.3	**Conclusion**	The results of this study indicate that levels as low as 0.025 mg/L for four weeks of exposure produce toxic effects as indicated by pulmonary fibrosis and inflammatory cell infiltrate. This level did not produce any other signs of toxicity. The acute LC_{50} inhalation toxicity can be deduced from the results of this study. Because exposition was 6 h per day (current guideline for acute inhalation toxicity: 4 h) and no mortality and clinical signs after this 6 h exposure period were seen (first clinical signs after 4 days, that 4 x 6 h exposition) at the highest concentration of 1.2 mg/L, the LC 50 for a single exposure for 6 h is >1.2 mg/L.
5.3.1	LO(A)EL	-
5.3.2	NO(A)EL	0.025 mg/L males and females
5.3.3	Other	-
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/01/04
Materials and Methods	Acceptable with corrections: 1.1 Rop et. al, 1981, Four Week Subacute Inhalation Toxicity Study of Benzoic Acid in Rats 2.1 Guideline study: Similar to OECD 412 3.1.2.1 Description: tan flakes 3.2.5 Age at study initiation: 49 days

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Results and discussion	Acceptable with corrections, additions and clarifications as follows: 4.1.1 Clinical signs: At 0.025 mg/L there was a reddish discharge around the nares after exposure on day 13. This sign was not evident in the control group. At 0.25 and 1.2 mg/L, the reddish discharge appeared throughout the study from day 4 with varying intensity. This discharge was more pronounced at 1.2 than 0.25 mg/L. The discharge might not be due to upper respiratory tract irritation as stated (no histological correlate in the trachea) but rather to stress and/or eye irritation (secretion of porphyrin from the Harderian gland). 4.6.1 Organ weights: At 0.25 mg/L, the decrease in kidney weight amounted to 8 % and was significantly different ($P < 0.05$) from the control at the absolute, but not the relative level. 5.2 Results and Discussion: Exposure to 0.025 mg/L did not produce any clinical signs of systemic toxicity and there was no effect on clinical chemistry, haematology or gross pathology. However, severe histopathological lesions of the lung (inflammation, fibrosis) were evident at terminal sacrifice. It is well known, that micrometer-sized particles of low-solubility and low-toxicity materials can cause activation of loaded alveolar macrophages, resulting in chronic inflammation and fibrosis. Therefore, this specific low dose effect is considered to be dependent on the existance of benzoic acid as respirable dust particles.
Conclusion	Systemic toxicity, based on mortality, bw and histopathology: LOAEC: 1.2 mg/L x 6 h NOAEC: 0.25 mg/L x 6 h Pulmonary toxicity (dusts), based on lung inflammation and fibrosis LOAEC: ≤ 0.025 mg/L fine dusts x 6 h NOAEC: N/A Other conclusions (acute toxicity): $LC_{50} > 1.2$ mg/L x 6 h
Reliability	2
Acceptability	Acceptable
Remarks	Exposure concentrations of 0.025, 0.25 and 1.2 mg/L x 6 h would correspond to inhaled doses of approx. 7, 70 and 320 mg/kg bw/d based on default data provided by the Technical Guidance Document on Risk Assessment in support of 93/67/EEC, 1488/94/EC and 98/8/EC. However, the study was not considered relevant for the evaluation of benzoic acid for biocidal use because benzoic acid was administered as fine dust (median particle diameter: 4.7 µm) which caused lung fibrosis and inflammatory processes in the lung even at the lowest dose tested as a result of the physico-chemical properties of the particles. This scenario is not relevant for the use of benzoic acid dilutions as biocides.

	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

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Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_3-1. Results of clinical chemistry haematology and urinalysis

parameter changed	Unit	Controls 0 mg/m³			low dose 0.025 mg/L			medium dose 0.25 mg/L			high dose 1.2 mg/L		
weeks after start of treatment		2	3	4	2	3	4	2	3	4	2	3	4
Reddish discharge around the nares								all animals					

Table A6_3-2. Results (inhalation) of repeated dose toxicity study

Parameter	**Control**		**low dose**		**medium dose**		**high dose**		**dose-response +/-**	
	m[a]	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m[a]**	**f[a]**	**m**	**f**
Number of animals examined	10	10	10	10	10	10	10	10	n.a.	n.a.
Mortality	0	0	0	0	0	0	1	1	+	+
Clinical signs										
Reddish discharge around the nares	0	0	0	0	10	10	10	10		
Body weight	-	-	-	-	-	-	↓	↓	+	+
Clinical chemistry*	-	-	-	-	-	-	-	-	n.a.	n.a.
Haematology										
Platelet count	-	-	-	-	-	-	↓	↓	+	+
<u>Kidney</u>										
Organ weight	-	-	-	-	-	↓	-	↓	+	+
Gross pathology*	-	-	-	-	-	-	-	-	n.a.	n.a.
Microscopic pathology*	-	-	-	-	-	-	-	-	n.a.	n.a.
<u>Liver</u>										
Organ weight	-	-	-	-	-	-	↓	-	+	+
Gross pathology*	-	-	-	-	-	-	-	-	n.a.	n.a.
Microscopic pathology*	-	-	-	-	-	-	-	-	n.a.	n.a.
<u>Trachea/lungs</u>										
Organ weight	-	-	-	-	-	-	-	↓	+	+
Gross pathology*	-	-	-	-	-	-	-	-	n.a.	n.a.
Microscopic pathology	-	-	-	-	-	-	-	-	n.a.	n.a.
Interstitial inflammatory cell infiltrate	-	-	↑	↑	↑	↑	↑	↑	+	+
Incidence of intensity of interstitial fibrosis	-	-	↑	↑	↑	↑	↑	↑	+	+

Section A6.4 Subchronic toxicity oral

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Official use only

		1 REFERENCE
1.1	**Reference**	Deuel HJ, Alfin-Slater R, Weil CS, Smyth HF, 1954, Sorbic acid as a fungistatic agent for foods. I. Harmlessness of sorbic acid as a dietary component. Fd. Res., 19, 1-12 (published)
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	No data protection / published data
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No. Study conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	U.S.P Sodium benzoate (Merck)
3.1.1	Lot/Batch number	No lot/batch number available
3.1.2	Specification	U.S.P (Unites states Pharmacopoeia)
3.1.2.1	Description	White crystalline solid substance
3.1.2.2	Purity	>99%
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rat
3.2.2	Strain	Rattus norvegicus Sherman
3.2.3	Source	Mellon Institute of Industrial Research, University of Pittsburgh, USA
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Mean weight: 90 - 120 g
3.2.6	Number of animals per group	5 Male and 5 female
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	90 days

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3.3.2	Frequency of exposure	Daily
3.3.3	Postexposure period	No
3.3.4	**Oral**	
3.3.4.1	Type	Gavage without fastening
3.3.4.2	Concentration	0, 1, 2, 4 and 8% (= 0, 0.64, 1.32, 2.62 and 6.29 g/kg/day)
3.3.4.3	Vehicle	Water
3.3.4.4	Concentration in vehicle	10%
3.3.4.5	Total volume applied	No data given
3.3.4.6	Controls	Food only
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes
3.4.1.2	Mortality	Yes
3.4.2	Body weight	Yes
3.4.3	Food consumption	Yes
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	No
3.4.6	Haematology	No
3.4.7	Clinical Chemistry	No
3.4.8	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes, Liver and kidney
3.5.2	Gross and histopathology	Yes, Tissues, liver and kidney
3.5.3	Other examinations	No
3.5.4	Statistics	No data given
3.6	**Further remarks**	

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs — No treatment related signs

% sodium benzoate in Diets	0	1.0	2.0	4.0	8.0
Uninfected rats	9/10	9/10	10/10	8/10	8/10

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Annex Point IIA6.4

4.1.2 Mortality

% sodium benzoate in Diets	0	1.0	2.0	4.0	8.0
Toxic deaths	0/10	0/10	0/10	0/10	4/10
Average number days to death	-	-	-	-	13.0

4 rats of the 8% group died without infections

4.2 Body weight gain

% sodium benzoate in Diets	0	1.0	2.0	4.0	8.0
Average weight gain of surviving rats (g)	156.3	171.7	160.5	150.0	100.2
p for t-test	-	0.58	0.86	0.82	0.06

4.3 Food consumption and compound intake

% sodium benzoate in Diets	0	1.0	2.0	4.0	8.0
Average diet consumed (g/rat/day)	11.97	12.69	12.63	12.30	11.98
p for t-test	-	0.54	0.62	0.75	0.99

4.4 Ophtalmoscopic examination -

4.5 Blood analysis -

4.5.1 Haematology -

4.5.2 Clinical chemistry -

4.5.3 Urinalysis -

4.6 Sacrifice and pathology

4.6.1 Organ weights

% sodium benzoate in Diets	0	1.0	2.0	4.0	8.0
Liver weight (% body weight)	3.17	3.01	2.97	3.12	4.46
p for t-test	-	0.26	0.26	0.89	0.000 5
Kidney weight (% body weight)	0.64	0.64	0.65	0.70	0.90
p for t-test	-	1.0	0.65	0.70	0.000 1

4.6.2 Gross and histopathology

% sodium benzoate in Diets	0	1.0	2.0	4.0	8.0
Sets of tissues examined from uninfected rats	9	9	10	8	7
Sets with major pathology	0	0	0	0	1
Sets with any pathology	0	1	1	1	7

Frequent pathological lesions were noted in the livers and kidneys in the group which received 8 % benzoic acid in food (1198 mg/kg bw).

At the 3 lower levels (4, 2 and 1%), one liver in each case was found with light cloudy swelling among the uninfected survivors of the several groups. Although this degree of pathological lesions was not found in the

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		specific controls of the study, it is not an infrequent finding and probably should be considered as an artifact.
4.7	**Other**	-
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Sodium benzoate acid was tested for its subchronic toxicity. 5 groups of 5 male and 5 female Sherman rats received a daily dose of 0, 1, 2, 4 and 8% (0, 640, 1320, 2620 and 6290 g/kg day) for 90 days.
5.2	**Results and discussion**	**No effect at 4% sodium benzoate in the diet in this study (2620 mg/kg bw/d).** At the 3 lower levels (4, 2 and 1%), one liver in each case was found with light cloudy swelling among the uninfected survivors of the several groups. Although this degree of pathological lesions was not found in the specific controls of the study, it is not an infrequent finding and probably should be considered as an artefact. **Gavage of 8% benzoic acid in food (6290 mg/kg bw)** 4 rats of the 8% group died without infections Frequent pathological lesions were noted in the livers and kidneys in the group which received 8 % benzoic acid in food (6290 mg/kg bw) In comparison with other studies, the dose of 4% in the diet is unlikely to be the NOEL
5.3	**Conclusion**	
5.3.1	LO(A)EL	No data given
5.3.2	NOEL	2620 mg/kg bw/d
5.3.3	Other	-
5.3.4	Reliability	2
5.3.5	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/01/08
Materials and Methods	Acceptable with corrections as follows: 3.3.2 Strain: Sherman 3.3.4.1 Type of Exposure: Feeding (with diet) 3.3.4.3 Vehicle: None 3.3.4.4 Concentration in Vehicle: n/a 3.4.1.1. Clinical signs: not reported 3.4.2 Body weight: average weight gain (collectively for M and F) 3.5.4 Statistics: Students t-test Tables: see Remarks

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Annex Point IIA6.4

Results and discussion	4.1.1 Clinical signs: not reported 5.2 Results and Discussion: At 1-4 % sodium benzoate in the diet, food consumption, survival, body weight, liver and kidney weight remained unaffected. However, liver histopathology only described as "light cloudy swelling" was reported for at least 1 animal of each treatment group but not the controls. Although the authors claim that such lesions are not infrequent in this strain and may be not treatment related, there was no data provided to support this interpretation. Therefore, 10000 ppm (640 mg/kg bw/d) must be considered the LOAEL for subchronic (90 d) sodium benzoate in the rat. At 8 % sodium benzoate, severe toxicity with mortality, major changes in body weight gain and organ weights was reported.
Conclusion	LOAEL: 10000 ppm (640 mg/kg bw/d) based on liver or liver cell swelling NOAEL: n/a
Reliability	3 (see remarks)
Acceptability	with restrictions
Remarks	Incomplete evaluation (e.g. no serum biochemistry, no full histopathology, no clinical signs/observations with exception of bw and food consumption) and inadequate reporting (e.g. no individual animal data, data for M and F pooled, critical liver histopathology ill described, unsuitable statistics). Two different tables with identical description were provided (Table A6_4-1/01. Results of subchronic toxicity oral). The first of these tables is erroneous and contains no valuable information that is not provided in the second table. The first table should therefore be disregarded.
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_4-1/01. Results of subchronic toxicity oral

Parameter	Control		1% (640 mg/kg/day)		2% (1320 mg/kg/day)		4% (2620 mg/kg/day)		8% (6290 mg/kg/day)		Dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Number of animals examined	5	5	5	5	5	5	5	5	5	5	5	5
Mortality	0	0	0	0	0	0	0	0	4		+	
Clinical signs	1		1		0	0	2		2		+	
Body weight	156.3		171.7		160.5		150.0		100.2		+	
Tissues												
Major pathology	0		0		0		0		1		+	
Any pathology	0		1		1		1		7		+	
Liver												
Weight (% bw)	3.17		3.01		2.97		3.12		4.46		+	
Frequent pathological lesions	0		0		0		0		all		+	
Kidney												
Weight (% bw)	0.64		0.64		0.65		0.70		0.90		+	
Frequent pathological lesions	0		0		0		0		all		+	

Table A6_4-1/01. Results of subchronic toxicity oral

Category	% sodium benzoate in Diets				
	0	1.0	2.0	4.0	8.0
Diet consumed (g/rat/day)	11.97	12.69	12.63	12.30	11.98
p for t-test	-	0.54	0.62	0.75	0.99
Average weight gain of surviving rats (g)	156.3	170.7	160.5	150.0	100.2
p for t-test	-	0.58	0.86	0.82	0.06
Liver weight (% body weight)	3.17	3.01	2.97	3.12	4.46
p for t-test	-	0.26	0.26	0.89	0.0005
Kidney weight (% body weight)	0.64	0.64	0.65	0.70	0.90
p for t-test	-	1.0	0.65	0.70	0.0001
Uninfected rats	9/10	9/10	10/10	8/10	8/10
Toxic deaths	0/10	0/10	0/10	0/10	4/10
Average number days to death	-	-	-	-	13.0
Sets of tissues examined from uninfected rats	9	9	10	8	7
Sets with major pathology	0	0	0	0	1
Sets with any pathology	0	1	1	1	7

Section A6.5 Chronic toxicity / Oral / Rat

Annex Point IIA6.5

Official use only

		1 REFERENCE
1.1	**Reference**	Kieckebusch W, Lang K, 1960, Die Verträglichkeit der Benzoesäure im chronischen Fütterungsversuch Arzneim. Forsch., 10, 1001-1003 (published)
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	No data protection / published data
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No. Study conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	Benzoic acid
3.1.1	Lot/Batch number	No lot/batch number available
3.1.2	Specification	As given in section 2
3.1.2.1	Description	White crystalline solid substance
3.1.2.2	Purity	>99%
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rat
3.2.2	Strain	Strain from Farbwerke Bayer AG, Elberfeld
3.2.3	Source	Farbwerke Bayer AG, Elberfeld, Germany
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Mean weight: 40 - 50 g
3.2.6	Number of animals per group	20 male and 20 female
3.2.7	Control animals	Yes, 20 male and 20 female
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	Generation 1 and 2: lifelong Generation 3: 16 weeks (sacrifice) Generation 4: until breeding

Section A6.5 Chronic toxicity / Oral / Rat

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3.3.2	Frequency of exposure	Daily
3.3.3	Postexposure period	No
3.3.4	**Oral**	
3.3.4.1	Type	In food
3.3.4.2	Concentration	0, 275 or 550 mg/kg bw/d (0.5% = 0.225mmol/ 100g1% = 0.45 mmol/100g) First 8 weeks: paired feed-Technic, then food and water consumption ad libitum
3.3.4.3	Vehicle	Moistened with food
3.3.4.4	Concentration in vehicle	0% 0.5% = 0.225mmol/100g 1% = 0.45 mmol/100g
3.3.4.5	Total volume applied	No data given
3.3.4.6	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, no further data given
3.4.1.2	Mortality	Yes, no further data given
3.4.2	Body weight	Yes, first 8 weeks weekly, then monthly
3.4.3	Food consumption	Yes, no further data given
3.4.4	Water consumption	No data given
3.4.5	Ophthalmoscopic examination	No
3.4.6	Haematology	No
3.4.7	Clinical Chemistry	No
3.4.8	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes, brain, heart, liver, spleen, kidney and testes
3.5.2	Gross and histopathology	Yes, animals of third generation after 16 weeks Organs: brain, heart, liver, spleen, kidney and testes
3.5.3	Other examinations	-
3.5.4	Statistics	No data given
3.6	**Further remarks**	

Section A6.5 Chronic toxicity / Oral / Rat

Annex Point IIA6.5

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs — No treatment related signs

4.1.2 Mortality — Influence of benzoic acid on lifetime of rats

	% benzoic acid in food		
	0	**0.5**	**1.0**
Number of animals	**78**	**79**	**80**
Mean age (days)	**785 ± 20**	**899 ± 28**	**827 ± 25**
Age < 600 days	13	8	13
Age 601 - 800 days	23	18	11
Age 801 - 1000 days	32	27	42
Age > 1000 days	10	26	14

No treatment related mortality.

Feeding of 0.5% benzoic acid in food led to prolongation of lifetime. The oldest rat has been 1346 days old and taken 200 g benzoic acid (1% in food) during its life.

4.2 Body weight gain

Body weight gain

			Benzoic acid			
	Control		0.5%		1%	
	m	f	m	f	m	f
I. Generation after						
4 weeks	70 ± 5.0	67 ± 4.1	69 ± 2.7	64 ± 2.4	75 ± 1.4	61 ± 2.2
8 weeks	159 ± 6.6	124 ± 5.2	160 ± 5.0	135 ± 5.4	147 ± 2.8	133 ± 2.4
12 weeks	219 ± 6.3	-	221 ± 5.6	-	237 ± 6.0	-
II. Generation after						
4 weeks	69 ± 2.3	57 ± 3.3	71 ± 2.2	60 ± 6.7	73 ± 3.3	70 ± 2.4
8 weeks	167 ± 2.0	122 ± 3.6	161 ± 2.6	131 ± 3.5	158 ± 4.6	123 ± 5.3
12 weeks	243 ± 5.5	-	238 ± 3.3	-	231 ± 5.6	-
III. Generation after						
4 weeks	73 ± 3.0	60 ± 3.5	68 ± 3.8	71 ± 2.4	72 ± 3.5	67 ± 3.7
8 weeks	157 ± 4.5	134 ± 4.1	158 ± 5.3	137 ± 3.9	168 ± 2.0	136 ± 4.5
12 weeks	240 ± 4.6	-	226 ± 5.8	-	233 ± 3.8	-
IV. Generation after						
4 weeks	93 ± 1.7	76 ± 4.8	84 ± 2.9	70 ± 3.1	73 ± 5.3	72 ± 3.5
8 weeks	189 ± 5.3	137 ± 4.3	168 ± 3.7	143 ± 2.4	159 ± 6.2	134 ± 4.5
12 weeks	255 ± 4.6	-	245 ± 4.4	-	240 ± 4.4	-

No effects on growth

Section A6.5 Chronic toxicity / Oral / Rat

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4.3	**Food consumption and compound intake**	No effects
4.4	**Ophtalmoscopic examination**	-
4.5	**Blood analysis**	-
4.5.1	Haematology	-
4.5.2	Clinical chemistry	-
4.5.3	Urinalysis	-
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Organ weights (g) as % of body weight / rats of third generation after 16 weeks

			Benzoic acid			
	Control		0.5%		1%	
	m	f	m	f	m	f
Body weight	236 ± 5	262 ± 5	329 ± 5	276 ± 5	316 ± 3	248 ± 6
Brain	0.57 ± 0.01	0.69 ± 0.02	0.58 ± 0.01	0.68 ± 0.01	0.61 ± 0.003	0.75 ± 0.02
Heart	0.34 ± 0.02	0.35 ± 0.01	0.35 ± 0.01	0.39 ± 0.01	0.33 ± 0.01	0.35 ± 0.01
Liver	3.49 ± 0.1	5.32 ± 0.3	3.34 ± 0.1	5.19 ± 0.2	3.49 ± 0.1	5.40 ± 0.2
Spleen	0.21 ± 0.00	0.25 ± 0.04	0.21 ± 0.04	0.23 ± 0.01	0.22 ± 0.01	0.25 ± 0.01
Kidneys	0.72 ± 0.02	0.80 ± 0.03	0.70 ± 0.04	0.79 ± 0.01	0.74 ± 0.02	0.78 ± 0.02
Testes	0.85 ± 0.03	-	0.83 ± 0.04	-	0.92 ± 0.01	-

No treatment related changes.

The higher liver weights of female rats related in higher liver weight in case of lactation. The differences in liver weight related in different litters.

4.6.2	Gross and histopathology	Brain, heart, liver, spleen, kidney and testes: No treatment related changes
4.7	**Other**	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Benzoic acid was tested for its chronic toxicity. 3 groups of 20 male and 20 female rats received a daily dose of 0, 0.5 or 1% benzoic acid (0, 275 or 550 mg/kg bw) in food. Generation 1 and 2: lifelong Generation 3: 16 weeks Generation 4: until breeding

Section A6.5 Chronic toxicity / Oral / Rat

Annex Point IIA6.5

		Rats of third generation were scarified after 16 weeks and brain, heart, liver, spleen, kidney and testes were examined.
5.2	**Results and discussion**	No treatment related mortality. Feeding of 0.5% benzoic acid in food led to prolongation of lifetime. No treatment related changes in brain, heart, liver, spleen, kidney and testes. The NOEL is 1% benzoic acid in the diet (550 mg/kg bw/d)
5.3	**Conclusion**	
5.3.1	LO(A)EL	No data given
5.3.2	NOEL	1% benzoic acid in the diet (550 mg/kg bw/d)
5.3.3	Other	-
5.3.4	Reliability	2
5.3.5	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/01/08
Materials and Methods	According to data provided in the study as well as default assumptions, 0, 5000 and 10000 ppm benzoic acid in the food would correspond to approximate average doses of 0, 250 and 500 mg/kg bw/d.
Results and discussion	No reduction in survival at 10000 ppm. Other relevant endpoints were not reported after chronic exposure.
Conclusion	NOAEL: 10000 ppm (500 mg/kg bw/d) LOAEL: > 10000 ppm
Reliability	3 (see below)
Acceptability	Not acceptable as key study for assessment of chronic toxicity. Although the test item was applied chronically, body weight was only reported up to wk 12 (M) or 8 (F), and organ weights and histopathology was from wk 16 of exposure. Individual data and information on further essential endpoints was not provided. See also Tables A6_5-1 and A6_5-2.

Section A6.5 Chronic toxicity / Oral / Rat

Annex Point IIA6.5

Remarks

Additional study summaries have been provided in Table A6_5-3. The NOAEL and LOAEL values, along with major effects reported in the corresponding studies are summarised below:

Exposure, Species	NOAEL / LOAEL (mg/kg bw /d)	Main effects	Reference
Oral, 18 mo, Rat, Benzoic acid	< 750 / ~750 (15000 ppm)	mortality↑, bw and bw gain↓, food consumption↓, no behavioral changes	Marquardt (1960)
Oral, 18 mo, Rat, Benzoic acid	n/a	Not suitable for risk assessment. no relevant changes reported at the tested dose of 40 mg/kg bw/d	Shtenberg and Ignatev (1970)
Oral, 17 mo, Mouse, Benzoic acid	n/a	Not suitable for risk assessment. mortality↑, weight loss↑ in response to food withdrawal in treatment group (40 mg/kg bw/d)	Shtenberg and Ignatev (1970)

COMMENTS FROM ... *(specify)*

Date *Give date of comments submitted*

Materials and Methods *Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*
Discuss if deviating from view of rapporteur member state

Results and discussion *Discuss if deviating from view of rapporteur member state*

Conclusion *Discuss if deviating from view of rapporteur member state*

Reliability *Discuss if deviating from view of rapporteur member state*

Acceptability *Discuss if deviating from view of rapporteur member state*

Remarks

Table A6_5-1. Results of clinical chemistry haematology and urinalysis

No data given

Table A6_5-2. Results of chronic toxicity study

Parameter	Control		low dose 0.5 % in food (275 mg/kg/day)		high dose 1% in food (275 mg/kg/day)		dose-response +/-	
	m	f	m	f	m	f	m	f
Number of animals examined	No data given / Rats of third generation						n.a.	n.a.
Clinical signs	-	-	-	-	-	-	-	-
Body weight	-	-	-	-	-	-	-	-
Brain	-	-	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-	-	-
Weight	-	-	-	-	-	-	-	-
Heart	-	-	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-	-	-
Weight	-	-	-	-	-	-	-	-
Liver	-	-	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-	-	-
Weight	-	↑	-	↑	-	↑	-	-
Spleen	-	-	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-	-	-
Weight	-	-	-	-	-	-	-	-
Kidney	-	-	-	-	-	-	-	-
Gross pathology	-	-	-	-	-	-	-	-
Weight	-	-	-	-	-	-	-	-
Testes	-	n.a.	-	n.a.	-	n.a.	-	n.a.
Gross pathology	-	n.a.	-	n.a.	-	n.a.	-	n.a.
Weight	-	n.a.	-	n.a.	-	n.a.	-	n.a.

Table A6_3-3. Additional data repeated toxicity study benzoic acid and sodium benzoate

Oral long-term toxicity studies, rat

Reference: Marquardt, P., 1960 (A6.5/03)
Duration: 72 weeks; benzoic acid
Dose: 0-1.5% (ca 0-1125 mg/kg bw/d)
Application: Oral (in the diet)
Species/Strain: Rat, Wistar and Osborne-Mendel
Animal number: Wistar: 30 and 20 males (2 strains), 20 females (1 strain); Osborne-Mendel: 20 males
Initial body weight: 50-60 g, (age: ca 5 weeks), (only data for Wistar)
Control group: Wistar: 13 and 10 males (2 strains), 12 females (1 strain); Osborne-Mendel: 10 males
Parameters: Clinical signs, body weight change, food consumption, survival rate
Findings: Mortality (only in one Wistar strain): 15/50 at 1.5% benzoic acid in the diet, control group: 3/25; decreased body weight change and food consumption in all the strains
Conclusion: No NOEL.

Reference: Shtenberg, A.J. & A.D. Ignatev, 1970 (A6.5/04)

Duration: 72 weeks; benzoic acid
Dose: 0 or 40 mg/kg bw/d
Application: Oral (via an undefined paste prior to feeding)
Species/Strain: Rat, Wistar
Animal number: 10/sex
Initial body weight: 100-120 g
Control group: Yes, but no number of animals given
Parameters: Clinical signs, food and water consumption, survival rate, several blood parameters, tolerance to one lethal dose of sodium benzoate given terminally
Findings: Development of tolerance to benzoic acid (mortality rate in control animals given one lethal dose of 3600 mg/kg bw sodium benzoate at termination: 100 %, mortality rate in the animals fed the test compound for 72 weeks prior one lethal dose of 4000 mg/kg bw sodium benzoate: 25 %)
Conclusion: Available data are not sufficient to justify conclusions from this study.

Oral long term toxicity study, mouse

Reference: Shtenberg, A.J. & A.D. Ignatev, 1970 (A6.5/04)
Duration: 68 weeks; benzoic acid
Dose: 0 or 40 mg/kg bw/d
Application: Oral (via an undefined paste prior to feeding)
Species/Strain: White mouse, cross-bred
Animal number: 25/sex
Initial body weight: 10-20 g
Control group: Yes, but no number of animals given
Parameters: Clinical signs, body weight change, food and water consumption, survival rate, several blood parameters, susceptibility to stress (different parameters, e.g. tolerance to food restriction at the end of the exposure period), tumour rates
Findings: Reduced survival rate in connection with food restriction, reduced body weight gain; increased tumour rate after application of the combination benzoic acid/sodium bisulphite (this leads to the Ehrlich ascites mouse carcinoma test, performed by Dinerman, A.A. & A.D. Ignatev, 1966)
Conclusion: Available data are not sufficient to justify conclusions from this study.

Treatment	Result	Reference
Rat		
Not applicable	No target identified NOEL: ca 500 mg/kg bw/d	Anonymous, SANCO/1396/2001-Final, 2003, (A6.5/02)
72 weeks 0-1.5% (ca 0-1125 mg/kg bw/d)	Mortality (only in one Wistar strain): 15/50 at 1.5% benzoic acid in the diet, control group: 3/25; decreased body weight change and food consumption in all the strains. No NOEL	Marquardt, P., 1960 (A6.5/03)
72 weeks 0 or 40 mg/kg bw/d	Development of tolerance to benzoic acid (mortality rate in control animals given one lethal dose of 3600 mg/kg bw sodium benzoate at termination: 100 %, mortality rate in the animals fed the test compound for 72 weeks prior one lethal dose of 4000 mg/kg bw sodium benzoate: 25 %). No NOEL	Shtenberg AJ, Ignatev AD, 1970 (A6.5/04)
Mouse		
68 weeks 0 or 40 mg/kg bw/d	Reduced survival rate in connection with food restriction, reduced body weight gain; increased tumour rate after application of the combination benzoic acid/sodium bisulphite. No NOEL	Shtenberg AJ, Ignatev AD, 1970 (A6.5/04)

Section A6.6.1 **Genotoxicity in vitro**

Annex Point IIA6.6.1 **Bacterial reverse mutation test**

Official use only

1 REFERENCE

1.1	**Reference**	McCann J, Choi E, Yamasaki E, Ames BN, 1975, Detection of carcinogens as mutagens in the salmonella/microsome test: Assay of 300 chemicals Proc. Nat. Acad. Sci., 72, 12, 1975, 5135-5139
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	No data protection / published data

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Study conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid. Study was carried out following Ames BN, 1971, in Chemical Mutagens: Principles and Methods for their Detection, ed. Hollaender A (Plenum Press, New York), Vol. 1 pp 267-282 and Ames BN, McCann J & Yamasaki, 1975, Mutat Res., 31 (1975) 347-364). Obviously the principles of the later OECD 471 were followed.
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	Benzoic acid (from Aldrich)
3.1.1	Lot/Batch number	No lot/batch number available
3.1.2	Specification	As given in section 2
3.1.2.1	Description	White crystalline solid substance
3.1.2.2	Purity	>99% (purest grade)
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.
3.2	**Study Type**	Bacterial reverse mutation test
3.2.1	Organism/cell type	*S. typhimurium*: TA 1535, TA 1537, TA 98, TA 100
3.2.2	Deficiencies / Proficiencies	Histidine deficiencies
3.2.3	Metabolic activation system	With and without S9 mix Liver homogenate of rats (9000 x g supernatant of rat liver) Induction: yes Aroclor 1254. Rats received a single intraperitoneal injection of Aroclor 1254 for enzyme induction. The livers were homogenised and centrifuged.
3.2.4	Positive control	300 chemicals are tested, a lot of them are mutagen

Section A6.6.1	Genotoxicity in vitro
Annex Point IIA6.6.1	Bacterial reverse mutation test

3.3	**Administration / Exposure; Application of test substance**	Non-entry field
3.3.1	Concentrations	10 - 1000 µg/plate with and without S9-mix
3.3.2	Way of application	No data given
3.3.3	Pre-incubation time	-
3.3.4	Other modifications	-
3.4	**Examinations**	Benzoic acid is not mutagen Number of revertant colonies: < 70 (per microgram benzoic acid) / <0.009 nmol Not mutagen
3.4.1	Number of cells evaluated	No data given
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	Non-entry field
4.1.1	without metabolic activation	No
4.1.2	with metabolic activation	No
4.2	**Cytotoxicity**	No
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Benzoic acid was assessed for its mutagenic potential in vitro in the Ames-test without metabolic activation and with metabolic activation. No guideline was given but obviously the principles of the later OECD 471 was followed
5.2	**Results and discussion**	Benzoic acid is not mutagenic Number of revertant colonies: < 70 (per microgram benzoic acid) / <0.009 nmol
5.3	**Conclusion**	
5.3.1	Reliability	2
5.3.2	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/01/08
Materials and Methods	3.3.2 N/A 3.4.1 N/A Otherwise the applicant's version is acceptable.

Section A6.6.1 **Genotoxicity in vitro**

Annex Point IIA6.6.1 **Bacterial reverse mutation test**

Results and discussion	4.2 Cytotoxicity was not assessed. Otherwise the applicant's version is acceptable.
Conclusion	5.3 Benzoic acid was not mutagenic in the Ames test with and without metabolic activation.
Reliability	2
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM .
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_6_1-1. Table for Gene Mutation Assay (modify if necessary)

Concentration [µg/plate]	Number of revertant colonies			Comments
	—S9	+ S9		
1000	No data given	< 70 (per microgram benzoic acid)	<0.009 nmol	Not mutagen

Table A6_6_1-2. Additional data / Ames test

Test System	Test object	Conc./Dose	Remarks	Result	Reference
Reverse mutation assay	*S. typh.*TA 98, 100, 1535, 1537	Benzoic acid 0.04 –2.5 mg/plate	BA activ. syst.	neg.	Anderson D, Styles JA, 1978 (A6.6.1/02)
Reverse mutation assay	*S. typh.*TA 98, 100, 1535, 1536, 1537, 1538	up to 3.6 µg/plate, ozonation of Benzoic acid	BA activ. syst.	neg.	Cotruvo JA, Simmon VF, Spanggord RJ, 1977 (A6.6.1/03)
Reverse mutation assay	*S. typh.*TA 92, 94, 98, 100, 1535, 1537	Benzoic acid up to 10 mg/plate	BA activ. syst.	neg.	Ishidate Jr M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, Matsuoka A, 1984 (A6.6.1/04)
Reverse mutation assay	*S. typh.*TA 92, 94, 98, 100, 1535, 1537	Sodium benzoate up to 3 mg/plate	SB activ. syst.	neg.	

Section A6.1.4.01 Acute Dermal Irritation

Annex Point IIA6.4

Official use only

		1 REFERENCE
1.1	**Reference**	Stol M, Cifkova I, Brynda E, 1988, Irritation effects of residual products derived from poly (2-hydroxyethyl methacrylate) gels Biomaterials, 9, 273-276
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No. Study cconducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid
2.2	**GLP**	The study was performed prior to GLP regulations but it principally provides the core information necessary to fulfill the objectives of the study and there is no reason to doubt the validity of the results.
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	Sodium benzoate
3.1.1	Lot/Batch number	No data given
3.1.2	Specification	Reagent grade (LACHEMA, Brno)
3.1.2.1	Description	White flakes
3.1.2.2	Purity	99%
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.
3.2	**Test Animals**	Non-entry field
3.2.1	Species	Rat
3.2.2	Strain	Wistar
3.2.3	Source	(VELAZ), Prague
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	180 g
3.2.6	Number of animals per group	10
3.2.7	Control animals	No, intact area served as control
3.3	**Administration/ Exposure**	Dermal
3.3.1	Application	Non entry field

Section A6.1.4.01 Acute Dermal Irritation

Annex Point IIA6.4

3.3.1.1	Preparation of test substance	Test substance was prepared by mixing 500 mg of test substance with 0.25 ml of Isotonic saline.
3.3.1.2	Test site and Preparation of Test Site	Intradermal injection
3.3.2	Occlusion	Semiocclusive
3.3.3	Vehicle	Saline solution
3.3.4	Concentration in vehicle	1, 3, 5, 7.5, 10 and 20%
3.3.5	Total volume applied	0.25 ml
3.3.6	Removal of test substance	Not applicable
3.3.7	Duration of exposure	No data given
3.3.8	Postexposure period	No data given
3.3.9	Controls	Saline solution
3.4	**Examinations**	
3.4.1	**Clinical signs**	No
3.4.2	Dermal examination	Inflammation
3.4.2.1	Scoring system	Radioactive indicator (^{113m}In) was used for the quantitative evaluation of irritation [7-10]. A volume of 0.1 ml (1.11 MBq) was administered by intradermal injection. After application, radioactive indicator is bond *in vivo* with blood plasma proteins. In the case of inflammation, permeability of blood capillaries is markedly increased and the labelled proteins freely pass through, reaching the site of irritation by chemotaxis. Radioactivities of the irritated area of skin, treated with the test compound (A_t) and saline (A_s) were compared with the intact area (A_c). The ratios were calculated from: $R_t = A_t/A_c$ and $R_s = A_s/A_c$ The irritation was evaluated using the intradermal irritation index (IdII) – the difderence between R_t and R_s. The average IdII is defined by: av IdII = $R_t - R_s$
3.4.2.2	Examination time points	No data given
3.4.3	Other examinations	No other examinations
3.5	**Further remarks**	-
		4 RESULTS AND DISCUSSION
4.1	**Average score**	Non-entry field

4.1.1 Intradermal irritation index IdII

IdII caused by sodium benzoate in saline solutions

Conc. (%)	mean R_t	mean R_s	av. IdII
1.0	1.67 ± 0.29	1.45 ± 0.23	0.22
3.0	2.28 ± 0.60	1.70 ± 0.49	0.58
5.0	3.27 ± 0.46	2.01 ± 0.40	1.26
7.5	5.80 ± 0.88	1.88 ± 0.27	3.91
10.0	7.92 ± 1.31	1.69 ± 0.29	6.23
20.0	11.76 ± 3.64	1.79 ± 0.33	9.97

$p < 0.01$

4.2 Reversibility

No data given

4.3 Other examinations

No other examinations

4.4 Overall result

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Benzoic acid was tested for intradermal irritation in rats. Radioactive indicator (^{113m}In) was used to quantify this biological response.

The study was cconducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid

5.2 Results and discussion

At low concentrations (up to 1%) only a slight irritation was recorded while at higher levels (5% or more) a significant adverse reaction developed. The degree of irritation was dose dependent. In the concentration range 0 – 10%, the response was exponential.

5.3 Conclusion

Non-entry field

5.3.1 Reliability

2

5.3.2 Deficiencies

Intradermal application was applied instead of topical as recommended by current guideline. Sodium benzoate was tested instead of benzoic acid. Irritating effects may be overestimated. by this study design

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 2007/12/03

Materials and Methods Applicant's version is acceptable.

Results and discussion 4.4 After intradermal injection, sodium benzoate was irritating in concentrations of ≥ 3 % in saline under the conditions tested.

Otherwise the applicant's version is acceptable.

Conclusion 5.3 After intradermal injection, sodium benzoate was irritating in concentrations of ≥ 3 % in saline under the conditions tested.

Reliability 2

Acceptability Acceptable

Remarks None

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_1-4S-1. Table for skin irritation study (additional data dermal irritation)

Table: Skin irritation studies with benzoic acid/benzoic salts

Study	Test substance	Result	Reference
Rat			
Intradermal	Sodium benzoate (1-20%)	Dose-dependent irritating (at 1% response not significant)	Stol M, Cifkova I, Brynda E, 1988(A6.1.4.01/01)
Rabbit			
(moistened with 0.25 ml Milli-RO water, for 4 h semi occlusive)	Benzoic acid 500 mg	Minimally irritating / primary skin irritation index: 0.5	Anonymous, RCC Notox, 1988a, cited in Anonymous, BUA Report, 1995 (A6.1.1/04)
No current guideline	Benzoic acid (500 mg dry powder)	Score: 1.66/8 irritating Response scored at 24 h and 48 h	Anonymous, Bio-Fax, 1973, cited in Anonymous, BUA Report, 1995 (A6.1.1/04)
Skin irritation (OECD 404)	Sodium benzoate	Not irritating / score: 0	RCC Notox, not dated, cited in Wibbertmann A, 2000, (A6.1.4.01/06)
Non-standardized experiment	Sodium benzoate (dry powder)	Not irritating	Suberg, Bayer AG, 1986, cited in Wibbertmann A, 2000, (A6.1.4.01/06)

Test substance	Result	Reference
Benzoic acid	Not irritating	Anonymous, SANCO/1396/2001-Final, 2003, (A6.1.1/05)
Sodium benzoate	Not irritating	

Formatvorlagendefinition: Standard

Section A6.6.2.01 **Genotoxicity in vitro**

Annex Point IIA6.6.2-01 **In vitro sister chromatid exchange assay in mammalian cells**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Tohda H, Horaguchi K, Takahashi K, Oikawa A, Matsushima T, 1980, Epstein-barr virus-transformed human lymphoblastoid cells for study of sister chromatid exchange and their evaluation as a test system Cancer Research, 40, 4775-4780	
1.2	**Data protection**		
1.2.1	Data owner	Not applicable	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	No data protection / published data	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No. Study conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid	
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Benzoic acid (from Kanto Chemical Co. Tokyo, Japan)	
3.1.1	Lot/Batch number	No lot/batch number available	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	White crystalline solid substance	
3.1.2.2	Purity	>99%	
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.	
3.2	**Study Type**	In Vitro sister chromatid exchange assay in mammalian cells	
3.2.1	Organism/cell type	Mammalian cell lines: Human lymphoblastoid cells (NL2, NL3 and NL4 which were established by Epstein-Barr Virus transformation of peripheral B-lymphocytes from normal healthy donors)	

Formatvorlagendefinition: Standard

Section A6.6.2.01		**Genotoxicity in vitro**
Annex Point IIA6.6.2-01		**In vitro sister chromatid exchange assay in mammalian cells**
3.2.2	Deficiencies / Proficiencies	-
3.2.3	Metabolic activation system	S9 mix From the livers of male Sprague-Dawley rats that had been treated with polychlorinated biphenyl (Kanechlor 500, 54% chlorinated). Modified S9 mix, consisted of: S9 (2.5% as liver homogenate) Culture medium 8 mM $MgCl_2$ 33 mM KCl 8 m D-glucose-6-phosphate 4 mM NADH 4 mM NADPH 0.1 M Sodium phosphate buffer
3.2.4	Positive control	13 typical mutagens and 5 nonmutagens Mitomycin as example for chemicals requiring no metabolic activation Aflatoxin B1 as example for chemicals requiring metabolic activation Benzoic acid as example for nonmutagen chemicals
3.3	**Administration / Exposure; Application of test substance**	Non-entry field
3.3.1	Concentrations	1 – 30 mMol / Litre

Formatvorlagendefinition: Standard

Section A6.6.2.01 **Genotoxicity in vitro**

Annex Point IIA6.6.2-01 **In vitro sister chromatid exchange assay in mammalian cells**

3.3.2	Way of application	Benzoic acid and Aflatoxin B1: The test chemical was dissolved in DMSO (the final concentration of DMSO was 5% as well as in the control) immediately before use and added with the S9 (final concentration , 10% by volume) mix to cultures after one cell cycle. The cultures were incubated for 2 hours at 37°C, washed once with culture medium*, resuspended in fresh medium containing 5-Bromodeoxyuridine, and incubated for an one cell cycle Mitomycin: The medium* was renewed after one cell cycle, the test chemical was dissolved in distilled water immediately before use and added and the cultures were incubated for an additional cell cycle. Colcemid was added and 4 hours later the cells were collected, treated with 0.05 M KCl for 10 minutes, and then fixed in methanol:acetic acid (3:1, v/v). Cells were spread on slides, air dried, and stained by the fluorescence plus Giemsa technique for differential staining of sister chromatids. SCE-inducing Activity Cells were exposed to a series of concentrations (mainly 10-fold dilutions) of the test chemical. The SCE's in more than 30 metaphases were scored for each concentration. The induced SCE was defined as the number of SCE's per cell in the test culture minus that in the control. SCE-inducing activity was estimated graphically from 2 concentrations of a chemical at which the induced SCE's were less and more than 5, respectively, and expressed as $(\mu g/ml)_{5S}$ or pM_{5S} Culture medium: Roswell Park Memorial Institute Medium1640 (Nissui Seiyaku Co., Tokyo, Japan) supplemented with 20% fetal bovine serum (Grand Island Co. Grand Island, N.Y.), mM L-Glutamine, and 50 µg kanamycin per ml used throughout.
3.3.3	Pre-incubation time	Aflatoxin B1: Midterm 2 hours treatment
3.3.4	Other modifications	-
3.4	**Examinations**	*see tables in appendix for examinations and results*
3.4.1	Number of cells evaluated	3 -4 x 10^5
		4 RESULTS AND DISCUSSION.
4.1	**Genotoxicity**	Non-entry field
4.1.1	without metabolic activation	No
4.1.2	with metabolic activation	Not applicable
4.2	**Cytotoxicity**	Yes, at 30 mMol / Litre

Formatvorlagendefinition: Standard

Section A6.6.2.01 **Genotoxicity in vitro**

Annex Point IIA6.6.2-01 **In vitro sister chromatid exchange assay in mammalian cells**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The possible mutagenic clastogenic properties of 13 typical mutagens and 5 nonmutagens were determined with in vitro sister chromatid exchange using human lymphoblastoid cells (NL2, NL3 and NL4 which were established by Epstein-Barr Virus transformation of peripheral B-lymphocytes from normal healthy donors).

After incubation, Colcemid was added and 4 hours later the cells were collected, treated with 0.05 M KCl for 10 minutes, and then fixed in methanol:acetic acid (3:1, v/v). Cells were spread on slides, air dried, and stained by the fluorescence plus Giemsa technique for differential staining of sister chromatids.

Cells were exposed to a series of concentrations (mainly 10-fold dilutions) of the test chemical. The SCE's in more than 30 metaphases were scored for each concentration. The induced SCE was defined as the number of SCE's per cell in the test culture minus that in the control.

SCE-inducing activity was estimated graphically from 2 concentrations of a chemical at which the induced SCE's were less and more than 5, respectively, and expressed as $(\mu g/ml)_{5S}$ or pM_{5S}

No guidelines are given. The study was conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid

Formatvorlagendefinition: Standard

Section A6.6.2.01 **Genotoxicity in vitro**

Annex Point IIA6.6.2-01 **In vitro sister chromatid exchange assay in mammalian cells**

5.2 Results and discussion

The positive reference mutagens induced sister chromatid exchange dose dependently, while nonmutagens did not.

SCE induction

Cemical (M)	Without S9 Mix		With S9 Mix	
	SCE (Mean ± S.E.)	Range	SCE (Mean ± S.E.)	Range
Mitomycin				
0	7.2 ± 0.5	2 – 13	Not determined	
1 x 10^{-10}	8.3 ± 0.8	2 – 19		
1 x 10^{-9}	9.1 ± 0.7	3 – 18		
1 x 10^{-8}	14.6 ± 0.9	4 – 32		
1 x 10^{-7}	33.2 ± 2.0	17 – 73		
Aflatoxin B1				
0	8.2 ± 0.6	3 – 20		
1 x 10^{-8}	-	-	8.9 ± 0.6	4 – 20
1 x 10^{-7}	-	-	10.5 ± 1.2	3 – 33
1 x 10^{-6}	9.3 ± 1.0	5 – 28	15.6 ± 1.4	5 – 50
1 x 10^{-5}	12.4 ± 1.2	4 – 38	20.7 ± 1.9	10 – 64
1 x 10^{-4}	15.7 ± 1.1	8 – 32	No mitosis	
Benzoic acid				
0	9.7 ± 0.6	3 – 23	Not determined	
1 x 10^{-3}	10.7 ± 0.5	5 – 20		
3 x 10^{-3}	10.5 ± 0.5	4 – 19		
1 x 10^{-2}	11.6 ± 0.65	3 – 24		
3 x 10^{-2}	No mitosis	-		

SCE-inducing activity (without S9 Mix)

Chemical	$(\mu g/ml)_{5S}$	pM_{5S}	Mutagenicity	Carcinogenicity
Mitomycin	0.0013	8.4	+	+
Aflatoxin B1	0.013	7.4	+	+
Benzoic acid	No induction		-	-

5.3 Conclusion Benzoic acid is not mutagen.

5.3.1 Reliability 2

5.3.2 Deficiencies Not applicable

Evaluation by Competent Authorities
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
EVALUATION BY RAPPORTEUR MEMBER STATE

Formatvorlagendefinition: Standard

Section A6.6.2.01 **Genotoxicity in vitro**

Annex Point IIA6.6.2-01 **In vitro sister chromatid exchange assay in mammalian cells**

Date	2012/02/13 (Gelöscht: 2008/01/04)
Materials and Methods	3.2 Benzoic acid was not tested with metabolic activation. Otherwise applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	3 (see remarks) (Gelöscht: 2)
Acceptability	Benzyl acetate: not acceptable Benzoic acid: with restrictions (Gelöscht: Acceptable)
Remarks	It has been described previously in an expert panel review that benzyl acetate reacts with the plastic of the culture vessels and may thus produce artefacts (Mitchell et al. 1997). Therefore, the results regarding benzyl acetate are not reliable. Benzoic acid was not tested with metabolic activation (Gelöscht: None)
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_6_2-1/01. **Table for Gene Mutation Assay (modify if necessary)**

Concentration [M]	Number of mutant cells		Comments
	— S9	+ S9	
0	0	Not determined	
1×10^{-3}	0	Not determined	
3×10^{-3}	0	Not determined	
1×10^{-2}	0	Not determined	
3×10^{-2}	0	Not determined	No mitosis / Cytotoxic

Table A6_6_2-2./01 **Table for Cytogenetic In-Vitro-Test: Chromosomal Analysis**

Formatvorlagendefinition: Standard

Section A6.6.2.01 **Genotoxicity in vitro**

Annex Point IIA6.6.2-01 **In vitro sister chromatid exchange assay in mammalian cells**

Cemical (M)	Without S9 Mix		With S9 Mix	
	SCE (Mean ± S.E.)	Range	SCE (Mean ± S.E.)	Range
Mitomycin				
0	7.2 ± 0.5	2 – 13	Not determined	
1×10^{-10}	8.3 ± 0.8	2 – 19		
1×10^{-9}	9.1 ± 0.7	3 – 18		
1×10^{-8}	14.6 ± 0.9	4 – 32		
1×10^{-7}	33.2 ± 2.0	17 – 73		
Aflatoxin B1				
0	8.2 ± 0.6	3 – 20		
1×10^{-8}	-	-	8.9 ± 0.6	4 – 20
1×10^{-7}	-	-	10.5 ± 1.2	3 – 33
1×10^{-6}	9.3 ± 1.0	5 – 28	15.6 ± 1.4	5 – 50
1×10^{-5}	12.4 ± 1.2	4 – 38	20.7 ± 1.9	10 – 64
1×10^{-4}	15.7 ± 1.1	8 – 32	No mitosis	
Benzoic acid				
0	9.7 ± 0.6	3 – 23	Not determined	
1×10^{-3}	10.7 ± 0.5	5 – 20		
3×10^{-3}	10.5 ± 0.5	4 – 19		
1×10^{-2}	11.6 ± 0.65	3 – 24		
3×10^{-2}	No mitosis	-		

SCE-inducing activity (without S9 Mix)

Chemical	$(\mu g/ml)_{SS}$	pM_{SS}	Mutagenicity	Carcinogenicity
Mitomycin	0.0013	8.4	+	+
Aflatoxin B1	0.013	7.4	+	+
Benzoic acid	No induction		-	-

Table A6_6_2-3./01 **Additional data / *in vitro* mutagenicity studies: Chromosome aberrations (SCE)**

Test object	Conc./Dose	Remarks	Result	Reference
Chinese hamster cells (CHO)	1-10 mmol/l	Benzoic acid	neg.	Oikawa A, Tohda H, Kanai M, Miwa M, Sugimura T, 1980 (A6.6.2.01/02)
human lymphocytes	up to 2 mmol/l	Benzoic acid	neg.	Jansson T, Curvall M, Hedin A, Enzell CR, 1988 (A6.6.2.01/03)

Section A6.6.2.02 **Genotoxicity in vitro**

Annex Point IIA6.6.2-02 **In vitro mammalian chromosome aberration test**

Official use only

		1 REFERENCE
1.1	**Reference**	Ishidate M, 1988, Data Book of Chromosomal aberration test in vitro Amsterdam Elsevier, p 9 – 20 and 23, 40, 373 Cancer Research, 40, 4775-4780
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	No data protection / published data
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No. Study conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid. (Similar to method B.10, 2000/32/EC)
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	Benzoic acid and Sodium benzoate
3.1.1	Lot/Batch number	No lot/batch number available
3.1.2	Specification	As given in section 2
3.1.2.1	Description	White crystalline solid substance
3.1.2.2	Purity	>99%
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.
3.2	**Study Type**	In vitro mammalian chromosome aberration test
3.2.1	Organism/cell type	mammalian cell lines: Chinese hamster Ovary (CHO)

Section A6.6.2.02 **Genotoxicity in vitro**

Annex Point IIA6.6.2-02 **In vitro mammalian chromosome aberration test**

3.2.2	Deficiencies / Proficiencies	-
3.2.3	Metabolic activation system	S9 mix From the livers of male Wistar or Fisher rats were intraperitoneal injected with 500 mg/kg of polychlorinated biphenyl and on the 5th day, the 0.000 x g fraction (S9) was prepared from liver homogenate and stored at -80°C. The S9 mix had the following composition: S9 3 ml 20 mM HEPES buffer solution (pH 7.2) 20 ml 50 mM $MgCl_2$ 1 ml 330 mM KCl 1 ml 50 m Glucose-6-phosphate 1 ml 40 mM NADH 1 ml Distilled water 1 ml
3.2.4	Positive control	Benzo(a)pyrene (requiring metabolic activation) 0.01, 0.02, 0.04 mg/ml
3.3	**Administration / Exposure; Application of test substance**	Non-entry field
3.3.1	Concentrations	Benzoic acid 0.5, 1.5 and 1.5 mg/ml Sodium benzoate 0.5, 1.0 and 2.0 mg/ml
3.3.2	Way of application	Benzoic acid (dissolved in DMSO) and Sodium benzoate (dissolved in saline): Cells were seeded in plastic Petri plates with 5 ml of the culture medium. The test agent was added to 3 day old cultures and remained until chromosome preparations were made after 24 and 48 h (100 well spread metaphases per plate). Benzo(a)pyrene: With S9 mix Cells were grown as a monolayer under the same conditions as in the direct method and simultaneous treated with S9 mix (5% final concentration). Cells were then washed with physiological saline and suspended into fresh medium. Chromosome preparations were made after additional culture for 18 hours (100 well spread metaphases per plate). Culture medium: Eagle's MEM (GIBCO) to which 10% calf serum is added.
3.3.3	Pre-incubation time	Benzo(a)pyrene: Midterm 6 hours treatment
3.3.4	Other modifications	-
3.4	**Examinations**	*see tables in appendix for examinations and results*
3.4.1	Number of cells evaluated	4 x 10^3 / ml

4 RESULTS AND DISCUSSION.

4.1	**Genotoxicity**	Non-entry field

Section A6.6.2.02 **Genotoxicity in vitro**

Annex Point IIA6.6.2-02 **In vitro mammalian chromosome aberration test**

4.1.1	without metabolic activation	Benzoic acid after 24 hours: Benzoic acid after 48 hours: Sodium benzoate after24 hours: Sodium benzoate 48 hours:	Inconclusive at 1.5 mg/ml Inconclusive at 1.0 mg/ml Positive at 2.0 mg/ml Positive at 2.0 mg/ml
4.1.2	with metabolic activation	Not applicable	
4.2	**Cytotoxicity**	No data given	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Benzoic acid and sodium benzoate were tested for its clastogenic potential. Chromosomal aberrations assays were performed with Chinese hamster ovary cell cultures. Benzo(a)pyrene No guidelines are given. The study was conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid
5.2	**Results and discussion**	Benzoic acid showed inconclusive results at 1.5 mg/ml after 24 hours and 1.0 mg/ml after 48 hours. Sodium benzoate showed inconclusive results at 1.0 mg/ml after 48 hours and positive results at 2.0 mg/ml after 24 and 48 hours. The positive reference substance benzo(a)pyrene showed positive results at 0.01 mg/ml after metabolic activation
5.3	**Conclusion**	A large number of genotoxicity studies with benzoic acid or sodium benzoate, including the main end points, are available. Although there are some positive results *in vitro*, most of the *in vitro* and all the *in vivo* tests were negative. The following conclusion is drawn: From the available literature, no mutagenic properties of benzoic acid or sodium benzoate are evident.
5.3.1	Reliability	2	
5.3.2	Deficiencies	Not applicable	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/01/04
Materials and Methods	3.2.3 Benzoic acid and sodium benzoate were not tested with metabolic activation. On the 5th day, the *9.000* x g fraction (S9) was prepared from liver homogenate and stored at -80°C. 3.3.1 Benzoic acid 0.5, *1.0* and 1.5 mg/ml Otherwise the applicant's version is acceptable.

Section A6.6.2.02 **Genotoxicity in vitro**

Annex Point IIA6.6.2-02 **In vitro mammalian chromosome aberration test**

Results and discussion	4.4.1 Benzoic acid after 24 hours: Negative at 0.5 and 1.0 mg/ml Inconclusive at 1.5 mg/ml Benzoic acid after 48 hours: Negative at 0.5 mg/ml Inconclusive at 1.0 and 1.5 mg/ml Sodium benzoate after24 hours: Negative at 0.5 and 1.0 mg/ml Positive at 2.0 mg/ml Sodium benzoate after 48 hours: Negative at 0.5 mg/ml Inconclusive at 1.0 mg/ml Positive at 2.0 mg/ml 4.1.2 Benzoic acid and sodium benzoate were not tested with metabolic activation. Otherwise the applicant's version is acceptable.
Conclusion	Benzoic acid showed inconclusive results at higher concentrations, while sodium benzoate showed positive results at the highest concentration tested. In the absence of data on cytotoxicity in this assay, this equivocal results do not allow a conclusion on the in vitro mutagenicity of benzoic acid.
Reliability	2
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_6_2-1/02. **Table for Gene Mutation Assay (modify if necessary)**

Not applicable

Table A6_6_2-2./02 **Table for Cytogenetic In-Vitro-Test: Chromosomal Analysis**

Benzoic acid (mg/ml)		**control 0**		**low dose 0.5**		**mid dose 1.0**		**high dose 1.5**	
		24h	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
Chromatid aberrations (%)	Gaps	2	0	2	2	1	2	4	3
	Breaks	1	1	1	3	0	3	3	4
	Exchanges	0	1	0	0	0	2	0	1
	Fragmentations	0	0	0	0	0	0	0	0
Chromosome	Breaks	0	0	0	0	0	0	0	0

Section A6.6.2.02 **Genotoxicity in vitro**

Annex Point IIA6.6.2-02 **In vitro mammalian chromosome aberration test**

aberrations (%)	Exchanges	0	0	0	0	0	0	0	0
Total (%)		**3**	**2**	**3**	**3**	**1**	**6**	**7**	**8**
polyploidy (%)		0	2	2	0	0	2	0	1
Judge				-	-	-	±	±	±
Sodium benzoate (mg/ml)		**control 0**		**low dose 0.5**		**mid dose 1.0**		**high dose 2.0**	
		24h	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
Chromatid aberrations (%)	Gaps	-	1	0	1	0	5	5	28
	Breaks	-	0	1	0	0	1	5	10
	Exchanges	-	0	0	0	0	0	0	1
	Fragmentations	-	0	0	0	0	0	0	1
Chromosome aberrations (%)	Breaks	-	0	0	0	0	0	0	0
	Exchanges	-	0	0	0	0	0	0	0
Total (%)		**-**	**1**	**1**	**1**	**0**	**5**	**10**	**38**
polyploidy (%)		-	0	1	3	1	1	0	1
Judge				-	-	-	±	+	+

Table A6_6_2-3./02 **Additional data / *in vitro* mutagenicity studies: Chromosome aberrations**

Test object	Conc./Dose	Remarks	Result	Reference
Human lymphocytes	0.001-0.1 mg/ml	Benzoic acid	neg.	Zhurkov VS, 1975 (A6.6.2.02/02)

Section A6.6.3 **Genotoxicity in vitro**

Annex Point IIA6.6.3 **UMU test**

Official use only

1 REFERENCE

1.1	**Reference**	Nakamura S, Oda Y, Shimada T, Oki I, Sugimoto K, 1987, SOS-inducing activity of chemical carcinogens and mutagens in salmonella typhimurium TA 1535/pSK 1002: Examination with 151 chemicals Mutation Research, 192, 1987, 239-246
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	No data protection / published data

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Study conducted according to prevailing standards and subsequently accepted for international registration. Results considered to be valid
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	Benzoic acid from Katayama chemical, Japan
3.1.1	Lot/Batch number	No lot/batch number available
3.1.2	Specification	As given in section 2
3.1.2.1	Description	White crystalline solid substance
3.1.2.2	Purity	>99% / Highest qualities commercial available
3.1.2.3	Stability	The test substance was considered to be stable for the duration of the study.
3.2	**Study Type**	DNA damage / Umu gene expression
3.2.1	Organism/cell type	*S. typhimurium* TA 1535/pSK 1002
3.2.2	Deficiencies / Proficiencies	-
3.2.3	Metabolic activation system	S9 mix Liver S9 fraction prepared from rats pretreated with Phenobarbital and 5,6-benzoflavone.
3.2.4	Positive control	151 chemicals are tested, a lot of them was mutagen
3.3	**Administration / Exposure; Application of test substance**	Non-entry field
3.3.1	Concentrations	With and without S9 mix: up to 1670 µg/ml

Section A6.6.3		**Genotoxicity in vitro**
Annex Point IIA6.6.3		**UMU test**
3.3.2	Way of application	The overnight culture of the bacteria strain was diluted 50-fold with fresh TGA medium and incubated at 37°C until the bacteria density of 600 nm was reached 0.25 – 0.30. The standard incubation mixture (final volume 2.5 ml) contained: 0.1 ml of the test compound dissolved in DMSO, 2 ml of the bacterial suspension described above and 0.4 ml of either 0.1 M phosphate buffer (pH 7.4) or S9 mixture containing 40 µl of S9 fraction. The reaction was run at 37°C for 2 hours with shaking, and terminated by cooling in an ice-water bath. The ß-galactosidase activity was determined.
3.3.3	Pre-incubation time	-
3.3.4	Other modifications	-
3.4	**Examinations**	Benzoic acid is a non genotoxic compound in the *umu* test up to concentrations of 1670 µg/ml (with and without S9 mix).
3.4.1	Number of cells evaluated	-
		4 RESULTS AND DISCUSSION.
4.1	**Genotoxicity**	Non-entry field
4.1.1	without metabolic activation	No
4.1.2	with metabolic activation	No
4.2	**Cytotoxicity**	No data given
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Benzoic acid was assessed for its mutagenic potential using Umu test. This system is based on the ability of a number of chemicals to induce SOS response (*umu* gene expression) in a new tester strain *S. typhimurium* TA 1535/pSK 1002. The background level of *umu* gene depression was 70 – 100 units for water control and 100 – 150 units for the DMSO control as determined by ß-galactosidase activity. Therefore the test compound is found to be genotoxic when the *umu* gene depression was increased 2-fold over the background level.
5.2	**Results and discussion**	Benzoic acid is a non genotoxic compound in the *umu* test up to concentrations of 1670 µg/ml (with and without S9 mix).
5.3	**Conclusion**	Benzoic acid has no genotoxic potential
5.3.1	Reliability	2
5.3.2	Deficiencies	Not applicable

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/01/04

Section A6.6.3	**Genotoxicity in vitro**
Annex Point IIA6.6.3	**UMU test**

Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	5.3 Benzoic acid was not genotoxic under the conditions tested.
Reliability	2
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	