	saline.
Initial cell concentration	Not applicable

Table A7_4_1_4/02-2: Test system

Criteria	Details
Culturing apparatus	300 ml Erlenmeyer flasks stoppered with cotton-lined plastic caps
Number of replicates/concentration	4
Measuring equipment	Determining of the extinction of monochromatic radiation at 436 nm for a 10mm thin layer of the bacterial suspension by photoelectric measurement.
Oxidation reduction indicator	-

Table A7_4_1_4/02_3: Test conditions

Criteria	Details
Composition of medium	Sterile double distilled water and analytical grade chemicals were used for the preparation of the nutrient medium.

	g/L
$NaNO_3$	1.060
K_2HPO_4,	0.600
KH_2HPO_4	0.300
$MgSO_4$ x 7 H_2O	0.200
$D_{(+)}$ glucose	10.000
Difco Bacto agar	18.000
$FeSO_4$ x 7 H_2O	0.010
Trace element solution	1.5 ml

Trace element solution

	g/L
$Al_2(SO_4)_3$ x 18 H_2O	0.055
KJ	0.028
KBr	0.028
TiO_2	0.055
$SnCl_2$ x 2 H_2O	0.028
LiCl	0.028
$MnCl_2$ x 4 H_2O	0.389
H_3BO_3	0.614
$ZnSO_4$ x 7 H_2O	0.055
$CuSO_4$ x 5 H_2O	0.055
$NiSO_4$ x 6 H_2O	0.059
$Co(NO_3)_2$ x 6 H_2O	0.055

	Saline *0.5 g NaCl in 1000 mL double distilled water*
Additional substrate	Vitamin solution
	Biotin 0.2 mg Nicotinic acid 2.0 mg Thiamine 1.0 mg p-aminobenzoic acid 1.0 mg Panthothenic acid 0.5 mg Pyridoxamine 5 mg Cyanocobalamin 2.0 mg Double distilled water 100 mL
Solvent	Double distilled water
Preparation of medium	Sterilised for 1.5 hours
Test temperature	25°C
pH	Neutralised solutions, not adjusted during test
Suspended solids concentration	-
Other relevant criteria	-

Section 7.4.3.1 Prolonged toxicity - *Oncorhynchus mykiss*

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			Official use only
		1 REFERENCE	
Reference		[redacted] 2004, Toxicity of Benzoic Acid to *Oncorhynchus mykiss* in a Prolonged Semi Static Test over 28 Days, IBACON GmbH, Rossdorf, Germany, report no. 22561221, December 21, 2004	
Data protection		Yes	
1.1.1	Data owner	MENNO CHEMIE-VERTRIEB G.M.B.H., Norderstedt, Germany	
1.1.2	Companies with letter of access	-	
1.1.3	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 study**	Yes, OECD 215 (2000)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Benzoic acid (As given in section 2)	
3.1.1	Lot/Batch number	10400	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	>99,5%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	HPLC-UV according to Meinerling M, Hermann S (2004) Validation of an Analytical Method for the Determination of Benzoic Acid in aqueous Samples (see A4.1 Analytical methods)	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**	*Non-entry field*	
3.4.1	Dilution water	See table A7_4_3_1-2	
3.4.2	Test organisms	Rainbow trout (*Oncorhynchus mykiss*), see table A7_4_3_1-3	
3.4.3	Test system	Semi static, see table A7_4_3_1-4	

Section 7.4.3.1 Prolonged toxicity - *Oncorhynchus mykiss*
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3.4.4	Test conditions	See table A7_4_3_1-5
3.4.5	Duration of the test	28 days
3.4.6	Test parameter	Mortality, symptoms of intoxication and differences in appearance or behaviour of test were observed each working day.
3.4.7	Examination / Sampling	Examination: each working day (1, 2, 3, 4, 7, 8, 9, 10, 11, 14, 15, 16, 17, 18, 21, 22, 23, 24, 25 and 28). Sampling: on days 2, 11, 14, 16, 18, 25 and 28
3.4.8	Monitoring of TS concentration	Yes, in duplicate on day 2, 11, 14, 16, 18, 25 and 28
3.4.9	Statistics	NOEC and LOEC: multivariate William's test, using the Kolmogoroff-Smirnow-Test. Mortality: Fisher Exact Test

4 RESULTS

If appropriate, include tables.

4.1	**Range finding test**	Not performed / concentrations based on acute toxicity test with rainbow trout.
4.1.1	Concentrations	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	**Results test substance**	*Non-entry field*
4.2.1	Initial concentrations of test substance	0, 5.5, 12, 25, 55 and 120 mg/L
4.2.2	Actual concentrations of test substance	Measured/calculated concentrations of benzoic acid

Exposure Day		Concentrations					
Fresh	**Aged**	**0**	**5.5**	**12**	**25**	**55**	**120**
0		LOD	4.812	11.345	24.005	52.676	115.373
0			4.796	11.378	24.007	53.968	116.067
	2	LOD	LOD	LOD	LOQ	51.047	117.963
	2		LOD	LOD	LOQ	51.878	121.560
11		LOD	5.141	11.882	25.139	54.477	122.934
11			5.242	11.868	24.992	58.320	123.317
	14	LOD	LOD	LOD	LOD	LOD	43.297
	14		LOD	LOD	LOD	LOD	44.017
16		LOD	5.496	12.374	26.125	60.295	125.248
16			5.576	12.270	25.910	58.976	128.156
	18	LOD	LOD	LOD	LOD	20.626	85.958
	18		LOD	LOD	LOD	20.404	85.680
25		LOD	5.215	11.905	24.498	57.837	128.875
25			5.218	11.880	24.862	56.573	123.196
	28	LOD	LOD	LOD	LOD	LOD	44.137
	28		LOD	LOD	LOD	LOD	43.894
% of nominal (mean)		n.a.	94 (n=8)*	99 (n=8)*	100 (n=8)*	103 (n=8)	102 (n=8)
SD		n.a.	5	3	3	5	4

Section 7.4.3.1 Prolonged toxicity - *Oncorhynchus mykiss*

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* Aged test media were ignored for the calculation, since benzoic acid is rapidly degraded by the ubiquitous microbial populations.

LOD = Limit of detection 0.02 mg/L

LOQ = Limit of quantification 2. mg/L

4.2.3 Effect data

Mortality

Exposure Day	Nominal concentration (mg/L)					
	Control	5.5	12	25	55	120
0	10	10	10	10	10	10
1	10	10	10	10	10	10
2	10	10	10	10	10	10
3	10	10	10	10	10	10
4	10	10	10	10	10	10
7	10	10	10	10	10	9
8	10	10	10	9	10	9
9	10	10	9	8	10	9
10	10	10	9	8	10	9
11	10	10	9	8	9	9
14	9	10	9	8	9	9
15	9	10	9	8	9	9
16	9	10	9	8	9	8
17	9	10	9	8	9	8
18	9	9	9	8	9	8
21	9	9	9	8	8	8
22	9	9	9	8	8	8
23	9	9	9	8	8	8
24	9	9	9	8	8	8
25	8	9	9	8	8	8
28	8	9	9	8	8	7
% surviving on day 28	80	90	90	70	60	60

Mean body weight at start and end of study

	Nominal concentration (mg/L)					
	0	5.5	12	25	55	120
Start	0.60	0.71	0.77	0.68	0.79	0.72
SD	0.07	0.15	0.15	0.13	0.13	0.18
End	1.88	2.05	1.96	1.97	2.08	1.89
SD	0.20	0.47	0.64	0.44	0.43	0.36
% Growth	213.5	188.0	152.8	190.9	164.3	163.0

Pseudo specific growth rate

Fish No.	Nominal concentration (mg/L)					
	0	5.5	12	25	55	120
1	1.96	1.82	1.00	1.64	1.49	1.45
2	1.89	1.48	1.32	1.80	1.27	1.18
3	1.53	2.03	2.06	1.71	1.70	1.59
4	1.50	1.04	2.01	2.15	1.15	1.75
5	1.93	1.75	1.27	0.81	1.60	1.99
6	1.70	1.96	0.77	1.52	2.06	1.42
7	1.86	1.92	0.88	1.86	1.19	1.26
8	1.70	1.69	1.30	1.64	1.55	-
9	1.89	0.95	1.93	-	-	-

Section 7.4.3.1 **Prolonged toxicity - *Oncorhynchus mykiss***
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10	-	-	-	-	-	-
Mean	1.77	1.63	1.39	1.64	1.50	1.52
SD	0.16	0.37	0.47	0.36	0.28	0.26

The NOEC, the LOEC and the EC_{50} were >120 mg benzoic acid/l

4.2.4	Concentration / response curve	No data given
4.2.5	Other effects	No other effects
4.2.6	Number/ percentage of animals showing adverse effects	Expect to the mortality observed, no symptoms of intoxication could be observed in any fish until the end of thee experiment.
4.2.7	Nature of adverse effects	Not applicable
4.3	**Results of controls**	1 fish died between day 12 and 14. 90% are surviving on day 28
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	-
4.4.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The toxicity of benzoic acid to *Oncorhynchus mykiss* was assessed in a prolonged test over 28 days, following OECD 215 (2000).	
5.2	**Results and discussion**	Mortality: In the control and at 5.5 and 12 mg benzoic acid/L, one fish died until the end of the experiment. At 25 and 55 mg benzoic acid/L respectively two fish died and at 120 mg benzoic acid/ three fish died until the end of the experiment. Signs of intoxication. Except to the mortality observed, no signs of intoxication were observed at the test animals during the test. Growth parameters: The mean body weight increased 213.5% in the control. At the different test item concentrations, mean body weight varied from 152.8 to 190.9%. The NOEC was at least 120 mg benzoic acid/l The LOEC and the LLC were >120 mg benzoic acid/l	
5.2.1	NOEC	120 mg/L	X
5.2.2	LOEC	> 120 mg/L	X
5.2.3	LLC	> 120 mg/L	X
5.3	**Conclusion**	Benzoic acid is not chronic toxic to *Oncorhynchus mykiss*. Validity criteria can be considered as fulfilled, see table A7_4_3_4-6	
5.3.1	Other Conclusions	No other conclusions	
5.3.2	Reliability	1	
5.3.3	Deficiencies	No	

Section 7.4.3.1 Prolonged toxicity - *Oncorhynchus mykiss*
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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/02/15
Materials and Methods	Applicant's version can be adopted with the following comment: 3.4.3 Although a random order of introduction was used, it appears that the smallest fish were introduced into the control basin. This is not considered of relevance for the study results.
Results and discussion	Applicant's version can be adopted with the following comment: 4.2.2: The test substance concentration decreases during the semi-static exposure. At the lower test substance concentrations, benzoic acid was significantly reduced in the 2 or 3 day aged test medium, partly below the LOD. This is probably caused by microbial degradation that was then inhibited at the higher test concentrations. At the highest test concentration of 120 mg/L, the mean recovery was 77 %. 5.2: As the test substance concentration decreases during the exposure period, the effect concentrations have to be related to mean measured concentrations. As no significant effects were observed at the highest test concentration, the mean recovery of 77 % for this concentration can be used to derive a NOEC based on mean measured concentrations of 92.4 mg/L. The LOEC and EC50 are > 92.4 mg/L. 5.3.3 In each of the fish groups exposed to test substance there was at least 1 fish > 25 % heavier than the average. This does not meet the requirements stated in the test guideline, but is not considered of relevance for the study results.
Conclusion	Applicant's version can be adopted.
Reliability	2
Acceptability	acceptable
Remarks	no
	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	

Gelöscht: 1

Table A7_4_3_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	-
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	-

Table A7_4_3_1-2: Dilution water

Criteria	Details
Source	Reconstituted water In deionised water analytical grade were added : $CaCl_2 \times H_2O$ 2.0 mmol/L (= 294 mg/L) $MgSO_4 \times 7\ H_2O$ 0.5 mmol/L (= 123 mg/L) $NaHCO_3$ 0.75 mmol/L (= 65 mg/L) KCL 0.075 mmol/L (= 5.8 mg/L)
Alkalinity	0.8 mmol/L = 80 mg/L as $CaCO_3$
Hardness	2.5 mmol/L = 250 mg/L as $CaCO_3$
pH	6.5 – 8.5
Ca / Mg ratio	4:1 (based on molarity)
Na / K ratio	10:1 (based on molarity)
Oxygen content	9.9 – 9.6 mg/L
Conductance	No data given
Holding water different from dilution water	No

Table A7_4_3_1-3: Test organisms

Criteria	Details
Species/Strain	Rainbow trout (*Oncorhynchus mykiss*)
Source	Forellenzucht Tautenhahn, Trostadt, Germany
Wild caught	No
Age/size	Juveniles Mean body weight: 0.74 ± 0.22 g Mean length: 4.19 ± 0.43 cm
Kind of food	Commercial feed for rainbow trout
Amount of food	Daily 4% dry weight based on the mean initial fish wet weight in each vessel.
Feeding frequency	Two times per day
Pretreatment	Two weeks prior to the start in test water without any medication. No fish died and all fish were healthy.
Feeding of animals during test	Yes, two times per day

Table A7_4_3_1-4: Test system

Criteria	Details
Test type	Semi static
Renewal of test solution	On days 2, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25 and 28 of the exposure period
Volume of test vessels	20 L glass aquaria with 15 L test medium
Volume/animal	1.5 L / animal
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	Not applicable

Table A7_4_3_1-5: Test conditions

Criteria	Details							
pH	Exposure Day		Nominal concentrations					
	Fresh	Aged	0	5.5	12	25	55	120
	0		8.0	8.0	8.0	8.0	8.0	8.0
		2	7.7	7.7	7.6	7.6	7.6	7.6
	2		7.9	7.9	7.9	7.9	7.9	7.9
		4	7.9	7.9	7.9	7.7	7.7	7.7
	4		8.0	8.0	8.0	8.0	8.0	8.0
		7	7.9	8.0	8.0	8.0	8.0	7.8
	7		8.0	8.0	8.0	8.0	8.0	8.0
		9	7.9	7.9	7.9	8.0	7.9	7.7
	9		8.0	8.0	8.0	8.0	8.0	8.0
		11	7.9	7.9	7.9	7.9	7.7	7.7
	11		8.0	8.0	8.0	8.0	8.0	8.0
		14	8.0	7.9	8.0	8.0	8.0	7.8
	14		8.0	8.0	8.0	8.0	8.0	8.0
		16	7.8	7.9	7.9	7.8	7.7	7.6
	16		8.0	8.0	8.0	8.0	8.0	8.0
		18	7.9	7.9	7.9	7.7	7.7	7.6
	18		8.0	8.0	8.0	8.0	8.0	8.0
		21	7.8	7.9	8.0	8.0	8.0	7.8
	21		8.0	8.0	8.0	8.0	8.0	8.0
		23	7.9	7.9	7.9	7.8	7.7	7.7
	23		8.0	8.0	8.0	8.0	8.0	8.0
		25	7.8	7.9	7.9	7.8	7.7	7.7
	25		7.9	7.9	7.9	7.9	7.9	7.9
		28	7.8	7.9	7.9	7.9	7.9	7.7
Dissolved oxygen	Exposure Day		Nominal concentrations					
	Fresh	Aged	0	5.5	12	25	55	120
	0		9.2	9.3	9.6	9.6	9.6	9.6
		2	9.3	9.1	8.7	8.5	8.7	8.3
	2		9.7	10.0	10.0	10.0	10.1	10.0
		4	10.0	10.4	10.4	10.0	9.5	9.5
	4		10.3	10.4	10.4	10.4	10.4	10.4
		7	12.3	12.3	12.2	12.1	11.8	10.8
	7		12.4	12.4	12.3	12.5	12.4	12.5
		9	9.5	9.7	9.7	9.6	8.9	9.1
	9		9.4	9.5	9.5	9.4	9.6	9.5

		11	11.8	11.8	11.2	11.6	10.2	10.0
	11		11.8	12.1	11.8	11.9	12.0	11.9
		14	11.8	11.9	11.7	12.1	11.8	10.7
	14		11.9	12.0	11.9	12.1	12.2	11.9
		16	11.3	11.6	11.6	11.1	10.1	10.1
	16		12.2	12.1	12.4	12.3	12.3	12.4
		18	11.4	12.1	12.1	11.6	11.2	10.8
	18		11.6	12.3	12.3	12.3	12.2	12.2
		21	9.3	9.5	9.5	9.8	9.5	9.0
	21		9.6	9.7	9.7	9.8	10.0	9.7
		23	9.8	10.0	9.8	9.8	9.7	9.5
	23		9.8	10.1	10.0	10.0	9.9	9.9
		25	9.3	9.6	9.6	9.8	9.1	9.2
	25		9.4	9.7	9.7	9.9	9.8	9.7
		28	9.2	9.2	9.1	9.3	9.0	8.7
Test temperature	**Exposure Day**		**Nominal concentrations**					
	Fresh	**Aged**	**0**	**5.5**	**12**	**25**	**55**	**120**
	0		14	14	14	14	14	14
		2	14	14	14	14	14	14
	2		14	14	14	14	14	14
		4	14	14	14	14	14	14
	4		15	15	15	15	15	15
		7	15	15	15	15	15	15
	7		15	15	15	15	15	15
		9	14	14	14	14	14	14
	9		15	15	15	15	15	15
		11	14	14	14	14	14	14
	11		14	14	14	14	14	14
		14	14	14	14	14	14	14
	14		15	15	15	15	15	15
		16	14	14	14	14	14	14
	16		15	15	15	15	15	15
		18	14	14	14	14	14	14
	18		14	14	14	14	14	14
		21	14	14	14	14	14	14
	21		14	14	14	14	14	14
		23	14	14	14	14	14	14
	23		14	14	14	14	14	14
		25	14	14	14	14	14	14
	25		14	14	14	14	14	14
		28	14	14	14	14	14	14
Adjustment of pH	No							
Aeration of dilution water	No							
Quality/Intensity of irradiation	200 - 1200 lux							
Photoperiod	16 h photoperiod daily							

Table A7_4_3_1-7: Validity criteria for fish test according to OECD Guideline 215

	fulfilled	Not fullfilled
Concentration of dissolved oxygen in all test vessels > 60% saturation	X	
Difference of water temperature < 1° C between test chambers at any time during test; temperature within a range of 2° C of the temperature for specific test species	X	
Mortality of control animals <10%	X	
Increase of fish weight sufficient for detection of the minimum variation of growth rate considered as significant	X	

Criteria for poorly soluble test substances		

Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

Official use only

1 REFERENCE

Reference		Pawlowski S, Wydra V, 2004, Influence of Benzoic Acid to *Daphnia magna* in a Reproduction Test, IBACON GmbH, Roßdorf, Germany, report no. 22561221, December 21, 2004
Data protection		Yes
1.1.1	Data owner	MENNO CHEMIE-VERTRIEB G.M.B.H., Norderstedt, Germany
1.1.2	Companies with letter of access	-
1.1.3	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 study**	Yes, OECD 211 (1998)
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 METHOD

3.1	**Test material**	Benzoic acid (As given in section 2)
3.1.1	Lot/Batch number	35361068
3.1.2	Specification	As given in section 2
3.1.3	Purity	>99.5%
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	HPLC-UV according to Meinerling M, Hermann S (2004) Validation of an Analytical Method for the Determination of Benzoic Acid in aqueous Samples (see A4.1 Analytical methods)
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	-
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	-
3.4	**Testing procedure**	*Non-entry field*
3.4.1	Dilution water	See table A7_4_3_4-2
3.4.2	Test organisms	Daphnia magna (Straus), clone 5 see table A7_4_3_4-3
3.4.3	Handling of	

Section 7.4.3.4 Annex Point IIIA XIII 2.4 Effects on reproduction and growth rate with an invertebrate species

	offspring	
3.4.4	Test system	Semi static, see table A7_4_3_4-4
3.4.5	Test conditions	See table A7_4_3_4-5
3.4.6	Duration of the test	21 days
3.4.7	Test parameter	Number of dead and alive offsprings and development of embryos. Mortality, the animals were counted as dead, when they not are able to swim for another 15 seconds after gentle agitation of the test container.
3.4.8	Examination / Sampling	The mortality of the test animals and the number of young were recorded each working day. Dead animals and offspring were removed at the same time. Sampling: Day 0, 2, 5, 7, 9, 12, 14, 16 and 19
3.4.9	Monitoring of TS concentration	Yes, in duplicate on day 5, 7, 12, 14, 16 and 19
3.4.10	Statistics	NOEC and LOEC: Bonferroni-U-test / Kolmogoroff-Smirnow-Test Fisher Exact Test

4 RESULTS

If appropriate, include tables.

4.1	**Range finding test**	Not performed / concentrations based on the results of an acute toxicity test with *Daphnia magna*
4.1.1	Concentrations	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	**Results test substance**	*Non-entry field*
4.2.1	Initial concentrations of test substance	0, 1.2, 2.5, 5.5, 12 and 25 mg/L
4.2.2	Actual concentrations of test substance	Measured concentrations of benzoic acid

Exposure Day		Concentrations					
Fresh	**Aged**	**0**	**1.2**	**2.5**	**5.5**	**12**	**25**
5		0.0	LOQ	2.149	4.624	11.455	24.469
5		-	LOQ	2.115	4.585	11.449	24.161
	7	0.0	LOD	LOD	LOQ	9.334	22.400
	7	-	LOD	LOD	2.101	8.982	22.224
12		0.0	LOQ	2.449	5.843	12.668	26.328
12		-	LOQ	2.482	5.813	12.691	26.652
	14	0.0	LOD	LOD	4.089	11.147	27.030
	14	-	LOD	LOD	4.211	11.515	26.563
16		0.0	LOQ	2.112	5.040	12.109	25.456
16		-	LOQ	2.176	5.137	11.992	25.578
	19	0.0	LOD	LOD	LOQ	10.315	22.976
	19	-	LOD	LOD	2.079	10.357	22.995
% of nominal		n.a.	n.a.	90	94	93	99

Section 7.4.3.4 Annex Point IIIA XIII 2.4 Effects on reproduction and growth rate with an invertebrate species

(mean)			(n=6)*	(n=6)*	(n=12)	(n=12)
SD	n.a.		7	10	10	7

* Aged test media were ignored for the calculation, since benzoic acid is rapidly degraded by the ubiquitous microbial populations.

LOD = Limit of detection 0.02 mg/L

LOQ = Limit of quantification 2. mg/L

4.2.3 Effect data

Survival of Parent Generation Dm682c

Exposure Day	Nominal concentration (mg/L)					
	Control	**1.2**	**2.5**	**5.5**	**12**	**25**
0	10	10	10	10	10	10
1	10	10	10	10	10	10
2	10	10	10	10	10	10
3	10	10	10	10	10	10
4	10	10	10	10	10	10
5	10	10	10	10	10	9
6	10	10	10	10	10	9
7	10	10	10	10	10	7
8	10	10	10	10	10	6
9	10	10	10	10	10	6
10	10	10	10	9	10	6
11	10	10	10	9	10	6
12	10	10	10	9	9	6
13	10	10	10	9	8	6
14	9	10	10	9	8	6
15	9	10	10	9	8	6
16	9	10	9	9	8	6
17	9	10	9	9	8	6
18	9	10	9	9	8	6
19	8	9	9	9	8	6
20	8	9	9	9	7	6
21	8	9	9	9	6	6
% surviving on day 21	80	90	90	70	60	60

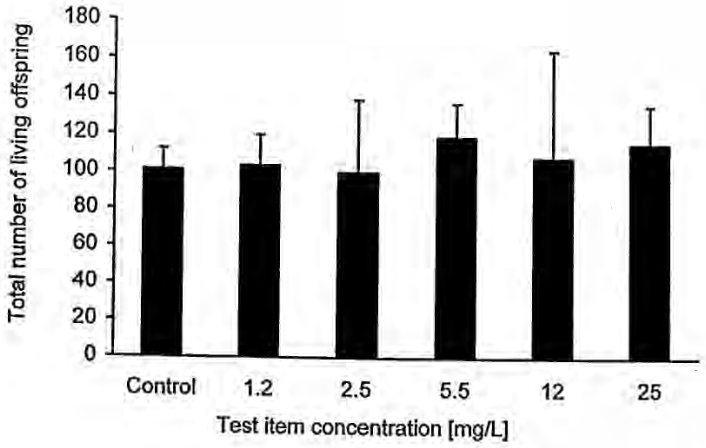
Total number of living offspring per animal on day 21

Replicate No.	Nominal concentration (mg/L)					
	Control	**1.2**	**2.5**	**5.5**	**12**	**25**
1	-	123	111	122	-	114
2	102	77	1	-	-	-
3	93	79	105	127	156	-
4	98	778	126	113	109	113
5	111	111	98	-	145	-
6	105	99	119	105	133	79
7	86	113	123	91	0	139
8	-	101	103	129	-	120
9	121	110	115	-	-	-
10	93	-	-	146	104	127
mean	101.1	103.4	100.1	119.0	107.8	115.3
SD	11.2	16.2	38.3	17.9	56.5	20.2
n	8	9	9	7	6	6
mean in %	100.0	102.3	99.0	117.7	106.6	114.1

The NOEC, the LOEC and the EC_{50} were >25 mg benzoic acid/l

Section 7.4.3.4 Annex Point IIIA XIII 2.4 Effects on reproduction and growth rate with an invertebrate species

4.2.4 Concentration / response curve



4.2.5 Other effects: No other effects

4.3 Results of controls

Survival of Parent Generation

Conc. mg/L	Days after starting								
	0-13	14	15	16	17	18	19	20	21
0	10	9	9	9	9	9	8	8	8

80% surviving on day 21

Total number of living offspring per animal at test termination

	Replicate No.									
	1	2	3	4	5	6	7	8	9	10
Number	-	102	93	98	111	105	86	-	121	93

Mean number of alive offsprings on day 21: 101.1

4.4 Test with reference substance: Not performed

4.4.1 Concentrations: -

4.4.2 Results: -

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The effects of benzoic acid on reproduction and growth rate to Daphnia magna (Waterflea) were shown in a 21-day semi – static test following OECD 211 (1998).

5.2 Results and discussion

Survival of adults:
Mortality of treated animals was not statistically significant different from the control.

Reproduction:
No significant toxic effect of benzoic acid on the mean reproduction rate was determined up to and including the nominal test concentration of 25 mg/L.

Signs of intoxication.
No signs of intoxication were observed at the test animals during the

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA XIII 2.4

		test. The NOEC, the LOEC and the EC_{50} were >25 mg benzoic acid/l
5.2.1	NOEC	>25 mg
5.2.2	LOEC	>25 mg
5.2.3	EC_{50} (EC_x)	>25 mg
5.3	**Conclusion**	Validity criteria can be considered as fulfilled, see validity criteria summarized in table A7_4_3_4-6
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	2008/02/15
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version can be adopted.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
Remarks	No
	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 A7_4_3_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	No
Vehicle control performed	No
Other procedures	No

Table A7_4_3_4-2: Dilution water

Criteria	Details
Source	Reconstituted water (Elendt "M4)
Alkalinity	Elendt M4 medium
Hardness	Elendt M4 medium
pH	8.5 - 7.2
Ca / Mg ratio	Elendt M4 medium (6.6/1)
Na / K ratio	Elendt M4 medium
Oxygen content	> 7 mg/L
Conductance	Elendt M4 medium
TOC	Days 0/1: 0.1 mg/*Daphnia* Days 5-8/12-15/19/20: 0.15 mg/*Daphnia* Days 2/9/16: 0.3 mg/*Daphnia*
Holding water different from dilution water	No No

Table A7_4_3_4-3: Test organisms

Criteria	Details
Strain / Clone	Daphnia magna (Straus), clone 5
Source	Umweltbundesamt, Institut für Wasser-, Boden- und Lufthygiene, Berlin Germany
Age	6 - 24 hours
Breeding method	The daphnids are bred in the laboratories of IBACON under similar temperature and light conditions as in the test, and in the same kind of reconstituted water used in the test.
Kind of food	Green algae (Desmodesmus subspicatus)
Amount of food	Base on the concentration of total organic carbon.
Feeding frequency	Each working day
Pretreatment	No data given
Feeding of animals during test	No data given

Table A7_4_3_4-4: Test system

Criteria	Details
Test type	Semi static
Renewal of test solution	On days 2, 5, 7, 9, 12, 14, 17 and 19 of the exposure period
Volume of test vessels	100 ml glass beakers containing 80 ml test medium
Volume/animal	8 ml / animal
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	Not applicable

Table A7_4_3_4-5: Test conditions

Criteria	Details
Test temperature	20 – 22°C

Dissolved oxygen

Exposure Day		Nominal concentrations					
Fresh	Aged	0	1.2	2.5	5.5	12	25
0		8.9	8.9	8.9	9.0	8.9	8.7
	2	8.6	8.6	8.5	8.5	8.4	8.3
2		8.8	8.6	8.6	8.7	8.6	8.7
	5	8.5	8.4	8.4	8.4	8.3	8.5
5		8.7	8.6	8.5	8.5	8.5	8.6
	7	8.5	8.3	8.2	8.2	7.0	7.8
7		8.7	8.7	8.8	8.7	8.7	8.6
	9	8.5	8.1	8.1	7.9	7.6	7.2
9		8.5	8.6	8.6	8.6	8.6	8.5
	12	8.4	8.2	8.3	8.3	8.2	8.2
12		8.2	8.2	8.2	8.3	8.2	8.0
	14	8.4	8.2	8.0	8.0	8.0	8.0
14		8.9	8.9	8.9	8.9	8.9	8.9
	16	8.0	8.0	8.0	8.0	8.0	8.0
16		7.5	7.5	7.5	7.5	7.5	7.5
	19	7.6	7.6	7.6	7.6	7.6	7.6
19		8.0	8.0	8.0	8.0	8.0	8.0
	21	8.0	8.0	8.0	8.0	8.0	8.0

pH

Exposure Day		Nominal concentrations					
Fresh	Aged	0	1.2	2.5	5.5	12	25
0		8.3	8.4	8.3	8.3	8.4	8.4
	2	8.2	8.2	8.2	8.2	8.1	8.1
2		8.2	8.3	8.3	8.3	8.3	8.3
	5	8.2	8.0	7.9	7.9	7.9	7.9
5		8.2	8.0	8.0	8.0	8.0	8.0
	7	7.2	7.3	7.4	7.4	7.4	7.4
7		7.4	7.7	7.9	8.0	8.1	8.1
	9	7.6	7.6	7.6	7.6	7.6	7.6
9		8.4	8.5	8.5	8.5	8.5	8.5
	12	8.2	8.2	8.3	8.2	8.0	8.0
12		8.0	8.0	8.0	8.1	8.0	8.0
	14	8.2	8.3	8.0	8.0	8.0	8.0
14		8.4	8.4	8.4	8.4	8.4	8.4
	16	8.3	8.3	8.3	8.3	8.3	8.3

	16		7.8	7.8	7.9	7.9	7.9	7.9
		19	7.9	7.8	7.8	7.9	7.9	7.9
	19		8.0	8.0	8.0	8.0	8.0	8.0
		21	8.0	8.0	8.0	8.0	8.0	8.0
Adjustment of pH	No							
Aeration of dilution water	No							
Quality/Intensity of irradiation	210 - 520 lux							
Photoperiod	16 h photoperiod daily							

Table A7_4_3_4-6: Validity criteria for invertebrate reproduction test according to OECD Guideline 211

	fulfilled	Not fullfilled
Mortality of parent animals < 20% at test termination	X	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	X	

Criteria for poorly soluble test substances		

Section A8 Measures necessary to protect man, animals and the environment

Subsection (Annex Point)			Official use only
8.1		**Recommended methods and precautions concerning handling, use, storage, transport or fire (IIA-VIII.8.1)**	
8.1.0	**Methods and precautions concerning placing on the market**	On the basis of available information, benzoic acid is not expected to produce any significant adverse health or environmental effects when the recommended use instructions are followed. No specific precautions have been taken by the producer to reduce emissions.	
8.1.1	**Methods and precautions concerning production, handling and use of the active substance**	The active substance benzoic acid does not impose greater risks to man and environment. Standard personal protection (overall, gloves, safety googles) and standard industrial hygiene (no eating and drinking during use and handling, washing of hands after handling, changing of working cloths at clock out) are sufficient. No further requirement.	*1
8.1.2	**Methods and precautions concerning storage of the active substance**	Tightly closed and dry. Do not store with food or feeding stuff. Keep out of reach of unauthorised persons. Store at temperatures between +5°C and +30°C.	
8.1.3	**Methods and precautions concerning transport of the active substance**	No subject to transport regulations	
8.1.4	**Methods and precautions concerning fire of the active substance**	Use water, CO_2 foam or powder. Combustible. Danger of dust explosion. Vapours heavier than air. Do not stay in the dangerous zone without self-contained breathing apparatus	*2
8.2		**In case of fire, nature of reaction products, combustion gases, etc. (IIA-VIII.8.2)** In the event of fire, combustion products do not impose special risks because the preparation is free of halogen. Decomposition products under fire conditions: CO, CO_2	
8.3		**Emergency measures in case of an accident (IIA8.3)**	

Section A8 Measures necessary to protect man, animals and the environment

			Official use only
8.3.1	**Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment if available**	General information: Remove victims from the danger zone. Remove spoiled or soaked clothing immediately. Wash before re-use. Upon inhalation: Bring accident victims out into the fresh air. Following skin contact: Wash skin immediately with plenty of water. Following eye contact: Rinse immediately with plenty of water for at least 15 minutes. Consult an ophthalmologist. Upon swallowing: Immediately make victim drink plenty of water. Consult an physician	
8.3.2	**Emergency measures to protect the environment**	Clean-up methods: Take up dry. Forward for disposal. Clean up affected area. Avoid generation of dust Do not allow to enter sewage system.	
8.4		**Possibility of destruction or decontamination following release in or on the following: (a) Air; (b) Water, including drinking water; (c) Soil (IIA-VIII.8.4)**	
8.4.1	**Possibility of destruction or decontamination following release in the air**	Not relevant, crystalline substance with low volatility.	
8.4.2	**Possibility of destruction or decontamination following release in water, including drinking water**	No special destruction or decontamination procedures are available. Benzoic acid will undergo microbial degradation in surface water, ground water and in sewage plants as well. The diluted product may be introduced in a public sewer, flushed with surplus of water. Drinking water decontamination is possible with standard filtering (e.g. charcoal) and aeration to enhance microbial activity.	
8.4.3	**Possibility of destruction or decontamination following release in or on soil**	Not special destruction or decontamination procedures are available. Benzoic acid will undergo microbial degradation in soil. Spilled product may be picked up mechanically with shovels, filled into lockable containers and destructed by incineration according to local regulations.	
8.5		**Procedures for waste management of the active substance for industry or professional users e.g. possibility of re-use or recycling, neutralisation, conditions for controlled discharge, and incineration (IIA-VIII.8.5)**	
8.5.1	**Possibility of re-use or recycling**	Re use or recycling of the active substance is not recommended. However, the biocidal product typically is recycled several times. The substance may be disposed of in domestic waste or incinerated according to local regulations.	

Section A8 Measures necessary to protect man, animals and the environment

			Official use only
8.5.2	**Possibility of neutralisation of effects**	Free acid may be neutralized with any kind of alkali. However, this procedure is not recommended for small and large quantities because the surplus of alkali poses at least the same risk than the free acids, which are only slightly acidic. The best way to handle accidental spillage of larger quantities is: Take up dry. Forward for disposal. Clean up affected area. Avoid generation of dust.	
8.5.3	**Conditions for controlled discharge including leachate qualities on disposal**	Packaging must be disposed of in compliance with country specific regulations. If no recycling system available, use for emptied and rinsed packaging waste disposal number 150102 (Plastic)	
8.5.4	**Conditions for controlled incineration**	Substance may be disposed of in domestic waste or incinerated according to local regulations. The substance does not contain halogen.	
8.6		**Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms (IIA-VIII.8.6)** No cases of observations on undesirable or unintended side-effects of benzoic acid are known to the applicant from the use of benzoic acid as biocide.	X
8.7		**Identification of any substances falling within the scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of groundwater against pollution caused by certain dangerous substances (IIA-VIII.8.7)** No substances fall within List II of the Annex to Directive 80/68/EEC.	

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
Evaluation of applicant's justification	The information about PPE is not provided in the necessary quality.
Conclusion	Applicants recommended measures / use instructions for benzoic acid to protect the environment are adopted. Applicant's version about protective measures is acceptable but incomplete. This information is specified by the rapporteur in chapter Doc-II-15.
Remarks	

Section A8 Measures necessary to protect man, animals and the environment

	Official use only
8.1.1 Methods and precautions concerning production, handling and use of the active substance	*1 The information about PPE is not provided in the necessary quality. For this precaution specifications about kind and quality are done by the rapporteur in chapter Doc-II-15.1.2.2 ..
8.1.4 Methods and precautions concerning fire of the active substance	*2 The PPE is described in general terms. Quality standards are not provided by the participant.
	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 A9 Annex Point IIA9	Classification and labelling	Official use only
Hazard symbol	Xi	
Indication of danger	Irritant	
Labelling symbol		
R phrases	41 Risk of serious damage to eyes	
S phrases	25 Avoid contact with eyes 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice 39 Wear eye/face protection	

Evaluation by Competent Authorities
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
EVALUATION BY RAPPORTEUR MEMBER STATE
Date 2010/07/30

Evaluation of applicant's justification	Benzoic acid is not listed in Annex I of Council Directive 67/548/EEC (up to 29th ATP). The classification proposed by the applicant regarding R41(GHS: H318) is in accordance with the proposal under Directive 91/414 EEC (SANCO/1396/2001-Final, Monograph on Benzoic Acid, EU, 2003) However, non-immunologic contact urticaria (transient intense erythema and oedema, also termed pseudoallergy) is evoked by benzoic acid when applied to human skin of sensitive persons as reported in several studies and case reports or to the ear lobes of guinea pigs (Lahti & Maibach, 1984). While not proven in a narrow sense, there is mechanistic evidence, that these skin reactions are mediated by release of vasoactive substances (mainly prostaglandin D_2). An immunological T-cell mediated mechanism is ruled out, also because no systemic reaction has been observed in the respective patients and no sensitisation (induction phase) is required for the reaction. The RMS regards the data sufficient to justify labelling benzoic acid with R38 (Irritating to the skin, GHS: H315). The classification and labelling of the active substance given by the participant in this section is not the same as in the enclosed material safety data sheet (No.15 Regulatory Information: Xn, R22-36, S24). **Environment:** **Classification/labelling for environmental toxicity according to Directive 67/548/EEC:** All acute effect values of the a.s. for aquatic organisms are > 100 mg/l. Benzoic acid was shown to be readily biodegradable. Classification/labelling for environmental toxicity according to Directive 67/548/EEC are not required for benzoic acid.
Conclusion	According to 67/548/EEC: Xi, R38 (Irritating to skin), R41(Risk of serious damage to eyes); According to GHS (COM/2007/0355):Pictogram GHS05; Skin Irrit. 2, H315; Eye Dam. 1, H318 Applicant's version is acceptable but inconsistent with respect to classification and labelling of the active substance. It has to be the same in this section and in section 15 of the material safety data sheet. Applicant's proposal for environmental classification and labeling is acceptable.
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	