Section A7.4.1.2 _ 06 Acute toxicity to invertebrates

A. tonsa

Annex Point IIA7.2

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (2013) Acute toxicity of Protectol GA 50 to the marine species *Acartia tonsa*; [redacted] Study No: 13261/2 (Unpublished), BPD ID A7.4.1.2_06
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	BASF SE
1.2.3	Criteria for data protection	Data on existing a.s. for first entry to Annex I authorisation

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, according to ISO 14669 (1999)
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

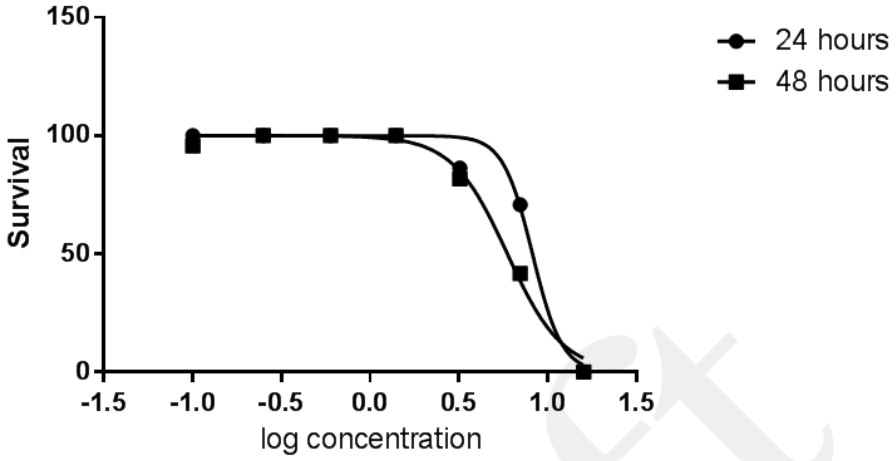
3.1	**Test material**	Protectol GA50; Glutaraldehyd 50%
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	50.8% (aqueous solution)
3.1.4	Composition of Product	Not relevant
3.1.5	Further relevant properties	Solubility in water > 500 mg/l at ca. 20 °C
3.1.6	Method of analysis	The method involved the oximation of glutaraldehyde with O-(2,3,4,5,6-Pentafluorobenzyl)-hydroxylamine hydrochloride (PFBHA) extraction with n-hexane, analysis of the extract was performed with GC/MS (negative chemical ionization) and quantification was performed with glyoxal as the internal standard. A Capillary gas chromatograph (HP 6890, Agilent) equipped with a split/splitless injector and mass spectrometer detector (HP 5973N, Agilent) was used.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant

Section A7.4.1.2 _ 06 Acute toxicity to invertebrates

A. tonsa

Annex Point IIA7.2

3.3	**Reference substance**	3,5-dichlorophenol
3.3.1	Method of analysis for reference substance	Test report does not state whether reference substance has been analysed or not.
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_2-2
3.4.2	Test organisms	See table A7_4_1_2-3
3.4.3	Test system	See table A7_4_1_2-4
3.4.4	Test conditions	See table A7_4_1_2-5
3.4.5	Duration of the test	48 hours
3.4.6	Test parameter	Mortality
3.4.7	Sampling	The oxygen and pH measurements were performed at test initiation, after 24 and 48 hours. Temparature was measured using a max.-min. thermometer.
3.4.8	Monitoring of TS concentration	Yes
3.4.9	Statistics	All mortality and survival data were statistically evaluated with the commercial software programme Graphpad Prism® 6 and a LC_{50} calculated accordingly
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 0.10, 0.25, 0.60, 1.4, 3.2, 7.0 and 16 mg Protectol GA 50/l equal to 0, 0.05, 0.125, 0.30, 0.7, 1.6, 3.5 and 8.0 mg GA/l
4.2.2	Actual	See table A7_4_1_2-6

	concentrations of test substance	
4.2.3	Effect data (Immobilisation)	See table A7_4_1_2-7 and table A7_4_1_2-8
4.2.4	Concentration / response curve	
4.2.5	Other effects	None
4.3	**Results of controls**	See table A7_4_1_2-7 97 % of the animals in the control group remained able to swim
4.4	**Test with reference substance**	
4.4.1	Concentrations	1 g/l
4.4.2	Results	43% mortality after 48 hours

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The aim of the present study was to investigate the acute toxicity of glutardaldehyde 50% to marine crustacean *Acartia tonsa*. Test substance: Protectol GA50 (Glutardaldehyde 50%), purity 50.8% (aqueous solution) GLP and Guideline according to ISO 14669 (1999) and ISO 5667-16 (1998) including analytical verification of the test concentration. The acute toxicity of to the marine crustacean *Acartia tonsa* was assessed over a 48 hour-exposure period under semi-static conditions. The test media was replaced after 24 hours. Following nominal concentrations were tested: 0, 0.10, 0.25, 0.60, 1.4, 3.2, 7.0 and 16 mg Protectol GA 50/l. Test solutions were prepared individually for each concentration by stirring overnight. The dilution/test water was filtered sea water. Test vessels were filled with 40 ml of test solution. Four vessels were used per test

Section A7.4.1.2 _ 06 Acute toxicity to invertebrates

A. tonsa

Annex Point IIA7.2

concentration and six vessels serve as controls. A number of 5 to 7 test organisms with an age of 19 days were placed in each vessel. Therefore, a total of twenty to twenty eight *A. tonsa* was used per test concentration. After 24 hours 40 ml of the test solution was replaced. The test was performed at a temperature of ca. 20 ± 2 °C. The *A. tonsa* were examined for their swimming ability at 24 and 48 hours. Oxygen and pH measurements were performed at test initiation, after replacing of the test solution (24 hours) and after 48 hours.

5.2 Results and discussion

Mortality of Acartia tonsa, dissolved oxygen and pH:

Test-Substance Concentration Protectol GA 50 (nominal) [mg/l]	Acartia tonsa mortality Percentage		Oxygen [mg/l] 48 h	pH 48 h
	24 h	48 h		
0	97	97	6.81	8.02
0.10	100	95	7.06	8.05
0.25	100	100	7.10	8.07
0.60	100	100	7.18	8.06
1.4	100	100	7.21	8.05
3.2	86	82	7.20	8.05
7.0	68	36	7.23	8.06
16	0	0	7.23	8.06

Temperature: 20.2-20.5 °C

5.2.1 EC_0 1.4 mg GA50 %/l (nominal)

5.2.2 EC_{50} 5.8 mg GA50 %/l ; 3.0 mg GA/l (nominal)

5.2.3 EC_{100} 16 mg GA50 %/l (nominal)

5.3 Conclusion The testing of the acute toxicity of glutaraldehyde 50% to the marine crustacean *Acartia tonsa* resulted in a LC50 value (48 h) of 5.8 mg GA50/l, referring to the test material as such. Related to glutaraldehyde itself, the LC_{50} was 3.0 mg/l based on nominal concentrations.

5.3.1 Reliability **1**

5.3.2 Deficiencies No

Section A7.4.1.2 _ 06 Acute toxicity to invertebrates

A. tonsa

Annex Point IIA7.2

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10.10.2013
Materials and Methods	Agree to description of materials and methods by the applicant. 3.4.2 and Table A7_4_1_2-3: The life stage of test organism is not given. According to Annex B of ISO 14669:1999 >14 days old individuals are adults. Adults or stage 5 copepodids should be used in the test. Table A7_4_1_2-6: The nominal concentrations are given as 50% glutaraldehyde and the measured as 100% glutaraldehyde which is confusing. For the nominal concentrations from 1.4-16 mg 50% glutaraldehyde/L the measured concentrations were 94-107% of nominal concentrations which is the range for LC50. For lower concentrations the measured concentrations mostly exceeded 120% of the nominals, but as these don't influence the determination of LC50, the LC50 can be based on the nominal concentrations.
Results and discussion	LC50 3.0 mg a.s./L (95% confidence interval 2.7-3.3). The result is based on the nominal concentrations, but concentrations were measured during the test and maintained within 80-120% of the nominal concentrationsin the concentration range of 0.7-8.1 mg a.s./L that influenced the determination of LC50.
Conclusion	Glutaraldehyde is toxic to *Acartia tonsa*.
Reliability	1
Acceptability	Acceptable
Remarks	-
	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 A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Natural seawater taken from a depth of ca. 60 m in the outer Oslo fjord, Norway and filtered (0.2 µm) prior to use. The salinity of the seawater was 3.2 %.
Acid capacity (Ks) up to pH 4.3	Not relevant
Hardness	Not relevant
pH	7.9 – 8.1
Ca / Mg ratio	Not relevant
Na / K ratio	Not relevant
Oxygen content	6.6 – 7.2 mg/l
Conductance	Not relevant
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Acartia tonsa*
Source	Continuous laboratory cultures
Age	19 days old
Breeding method	Not specified
Kind of food	Not specified
Amount of food	Not specified
Feeding frequency	Not specified
Pretreatment	No particularities
Feeding of animals during test	No feeding throughout the duration of the study

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	After 24 hours
Volume of test vessels	40 ml
Volume/animal	Not specified
Number of animals/vessel	5 to 7
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No, test vessels were sealed with parafilm

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	20 ± 2 °C
Dissolved oxygen	
pH	

Test Concentration Protectol GA50 (mg/l)	Oxygen-value after			
	0h	24h Old	24h New	48h Old
0	6.59	6.92	6.83	6.81
0.10	6.75	6.91	6.92	7.06
0.25	6.74	6.86	6.71	7.10
0.60	6.75	6.87	6.70	7.18
1.4	6.66	6.97	6.68	7.21
3.2	6.66	6.94	6.73	7.20
7.0	6.69	6.98	6.73	7.23
16	6.70	6.92	6.73	7.23

Test	pH

	Concentration Protectol GA50 (mg/l)	0h	24h Old	24h New	48h Old
	0	7.91	8.03	7.94	8.02
	0.10	7.91	8.04	7.91	8.05
	0.25	7.92	8.05	8.01	8.07
	0.60	7.91	8.04	7.96	8.06
	1.4	7.92	8.04	7.95	8.05
	3.2	7.94	8.05	7.96	8.05
	7.0	7.94	8.06	7.96	8.06
	16	7.93	8.02	8.00	8.06
Adjustment of pH	No				
Aeration of dilution water	No				
Quality/Intensity of irradiation	Not specified				
Photoperiod	16:8 hours day/night				

Table A7_4_1_2-6: Measured test concentrations (Glutaraldehyde)

Designation samples	**Test-Substance Concentration (Protectol GA 50; 50.8 % GA ; nominal) [mg/l]***							
	Control	0.10	0.25	0.6	1.4	3.2	7.0	16
	Measured concentration [mg glutaraldehyde/l]							
0h “New”	n.d	0.055	0.189	0.435	0.728	1.61	3.71	8.34
24h “Old”	n.d	<0.050	0.158	0.417	0.703	1.59	3.49	8.04
24h “New”	n.d	0.085	0.200	0.385	0.694	1.84	3.88	8.71
48h “Old”	n.d	<0.050	0.103	0.359	0.669	1.74	3.73	8.56
Geometric Mean	**0**	**0.04**	**0.16**	**0.40**	**0.70**	**1.69**	**3.70**	**8.41**

*The nominal concentrations of Protectol GA50 are based on the formulated product which is 50.8% glutaraldehyde

**0.5 x LOQ (0.05 mg/L) was used for values < 0.050 mg/L for calculation of geometric mean..

n.d not detected

Table A7_4_1_2-7: Immobilisation data

Test-Substance Concentration (Protectol GA 50 ; nominal) [mg/l]	*A. tonsa* unable to swim			
	Number		Percentage	
	24 h	48 h	24 h	48 h
Control	1	1	97	97
0.10	0	1	100	95
0.25	0	0	100	100
0.60	0	0	100	100
1.4	0	0	100	100
3.2	3	4	86	82
7.0	7	14	68	36
16	23	23	0	0

Table A7_4_1_2-8: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
48 h Protectol GA 50 [mg/l]	5.8 (n)	5.3 – 6.5	1.4	Not given
48 h glutaraldehyde [mg/l]	3.0 (n)	Not given	Not given	Not given

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7_4_1_2-9: Validity criteria for determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea) according to ISO 14669:1999

	fulfilled	Not fullfilled
Mortality of control animals ≤10%	**yes**	
Toxicity of reference chemical within the range specified in 8.5 of ISO 14669:1999	**yes**	
Concentration of dissolved oxygen in all test vessels >4 mg/l	**yes**	

Criteria for poorly soluble test substances ergänzen	**Not relevant**	