Section A7.4.1.2(4) **Annex Point IIA, VII.7.2** **IUCLID 4.2/04**	**Acute toxicity to invertebrates** **Acute Toxicity to *Acartia Tonsa***	
	concentrations of the active after 24 and 48 hours were 0.29 mg a.i./L and 0.029 mg a.i./L, respectively. The water temperature was maintained at 20-22°C, and no treatment-related differences for oxygen concentration or pH were observed. Analysis of the test preparations at time 0 were near the target / nominal concentrations. There was a marked decline in measured test concentrations within 48 hours, with values ranging from 8-33% of the nominal at that sampling time. Because pre-study analysis of the material showed it to be stable over the exposure period, the marked decline in concentrations was attributed to biodegradation in the seawater. Because of the marked decline in test material concentrations, calculations (NOEC, LC_{50}) were based on the time-weighted mean measured test concentrations. They were reported as follows: 24-hour LC_{50} >0.31 mg a.i./L and NOEC of 0.10 mg a.i./L, and the 48-hour LC_{50} = 0.062 (0.048-0.080) mg a.i./L and NOEC of 0.015 mg a.i./L. Results of the positive control study were consistent with previously conducted positive control analyses, indicating that the test system was valid. The 24- and 48-hour LC_{50}'s were 19 and 12 mg a.i./L when the test species was treated with potassium dichromate.	
5.2.1 EC_0	The no observed effect concentration at 48 hours was 0.029 mg a.i./L.	
5.2.2 EC_{50}	The 48 hour LC_{50} value was 0.11mg a.i./L.	
5.2.3 EC_{100}	> 1 mg a.i./L	
5.3 Conclusion	The results based on nominal concentrations of active ingredient gave a 48-hour LC_{50} value of 0.11 mg a.i./L with 95% confidence limits of 0.085 – 0.14 mg a.i./L. The no observed effect concentration was 0.029 mg a.i./L.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Jan 16th, 2009	
Materials and Methods	Applicant's version is correct.	
Results and discussion	Table A7_4_1_2(4)_9 The validity criteria refer to Daphnia test and may not be appropriate to *Acartia tonsa*. LC_{50} (48h) for glutaraldehyde was 0.07 mg a.i./L with 95% confidence limits of 0.058-0.084 mg a.i./L based on the geometric mean measured concentrations. Calculation of the geometric mean concentrations are given in Table A7_4_1_2(4)-8.	
Conclusion	Glutaraldehyde is very toxic to marine copepod *Acartia tonsa*.	
Reliability	2	
Acceptability	acceptable	
Remarks		

Section A7.4.1.2(4) **Annex Point IIA, VII.7.2** **IUCLID 4.2/04**	**Acute toxicity to invertebrates** **Acute Toxicity to *Acartia Tonsa***	
	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(4)-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_1_2(4)-2: Dilution water

Criteria	Details
Source	Synthetic sea water prepared using a commercially available formulation ([redacted]). An amount of formulation (320mg) was dispersed in 10 L of deionised reverse osmosis water. The pH was adjusted to 8.0 using 10 M HCl and the specific gravity was 1.02.
Alkalinity	Not reported
Hardness	Not reported
pH	8.1 to 8.2
Ca / Mg ratio	Not reported
Na / K ratio	Not reported
Oxygen content	7.8 to 8.5 mg/L
Conductance	Not reported
Holding water different from dilution water	Yes, holding water was sea water with a salinity of 33±2‰.

Table A7_4_1_2(4)-3: Test organisms

Criteria	Details
Strain	*Acartia tonsa* (marine copepod)
Source	[redacted]
Age	At least 14 days old
Breeding method	Not reported
Kind of food	Mixed culture of marine algae
Amount of food	Not specified
Feeding frequency	Not specified
Pretreatment	Not specified
Feeding of animals during test	No

Table A7_4_1_2(4)-4: Test system

Criteria	Details
Renewal of test solution	Static system
Volume of test vessels	100 mL (in a 250 mL jar)
Volume/animal	20 mL
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2(4)-5: Test conditions

Criteria	Details
Test temperature	Range recorded during the study was 20 to 21°C
Dissolved oxygen	Range recorded during the study was 7.8 to 8.5 mg/L
pH	Range recorded during the study was 8.1 to 8.2
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Not specified
Photoperiod	16h light and 8h dark

Table A7_4_1_2(4)-6: Mortality data

Test-Substance Concentration (nominal) [mg/l]	Mortalities						
	Number		Percentage		Oxygen [mg/l]	pH	Tempera-ture [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
Control	0	0	0	0	7.8	8.2	20
0.01	0	0	0	0	7.9	8.2	20
0.018	0	0	0	0	7.9	8.2	20
0.032	0	0	0	0	7.8	8.2	20
0.056	0	0	0	0	7.9	8.2	20
0.1	0	6	0	30	7.8	8.2	20
0.18	0	9	0	45	7.9	8.2	20
0.32	0	11	0	55	7.9	8.2	20
0.56	0	18	0	90	7.9	8.2	20
1.0	6	19	30	95	7.8	8.2	20

Table A7_4_1_2(4)-7: Effect data

	EC_{50}	95 % c.l.	EC_0	EC_{100}
24 h [mg a.i./l]	>0.51	-	0.29	>1.0
48 h [mg a.i./l]	0.11	0.085 – 0.14	0.029	>1.0

effect data are based on nominal (n) concentrations

Table A7_4_1_2(4)-8: Calculation of Geometric Mean Measured Concentrations for Glutaraldehyde Concentrations

Nominal Test Conc. mg/L	Day 0 Measured Test Conc. mg/L	% Nominal	Day 4 Measured Test Conc. mg/L	Geometric Mean Measured Test Conc. mg/L[1]	Geometric Mean % Nominal
0	<LOQ	na[2]	<LOQ	<LOQ	----
0.01	0.0113	113%	0.00177	**0.0042**	42.1%
0.018	----[3]	----	0.00383	**0.0083**	46.1%
0.032	0.0306	96%	0.0073	**0.015**	47.8%
0.056	----	----	0.0138	**0.028**	49.6%
0.10	0.0954	95%	0.0147	**0.038**	38.3%
0.18	----	----	0.0184	**0.058**	32.0%
0.32	0.308	96%	0.0267	**0.092**	28.9%
0.56	----	----	0.0461	**0.16**	28.7%
1.0	0.977	98%	0.0327	**0.18**	18.1%

[1] Calculation of geometric mean was done in Microsoft® Office Excel 2003.
[2] na = not applicable
[3] ---- = Analysis of day 0 analysis of concentrations were not conducted on these exposure solutions but concentrations were determined on at all exposure levels at test termination (48 hours). Recoveries on day 0 averaged 99.6%. Based on this, nominal test concentrations were used for day 0 values for calculation of geometric mean measured concentrations.

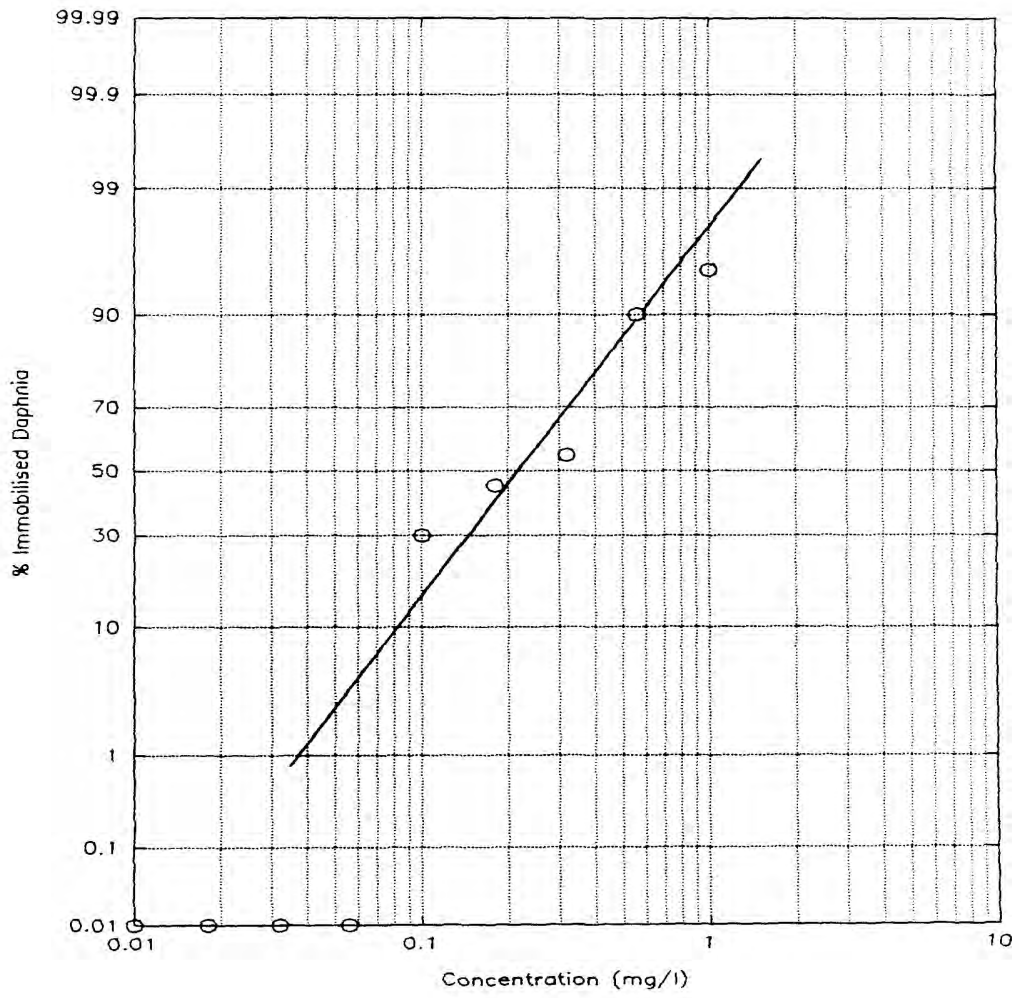
Table A7_4_1_2(4)-9: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202*

	Fulfilled	Not fulfilled
Mortality of control animals <10%	X	
Control animals not staying at the surface	NA	
Concentration of dissolved oxygen in all test vessels >3 mg/l	X	
Concentration of test substance ≥80% of initial concentration during test		X

Criteria for poorly soluble test substances ergänzen	NA	

* = study carried out according to ISO TC147/SC5/W92

Figure A7.4.1.2(4)-1 Concentration-Mortality Curve after 48 hours in the Definitive Study



Section A7.4.1.2 _ 07 Acute toxicity to invertebrates

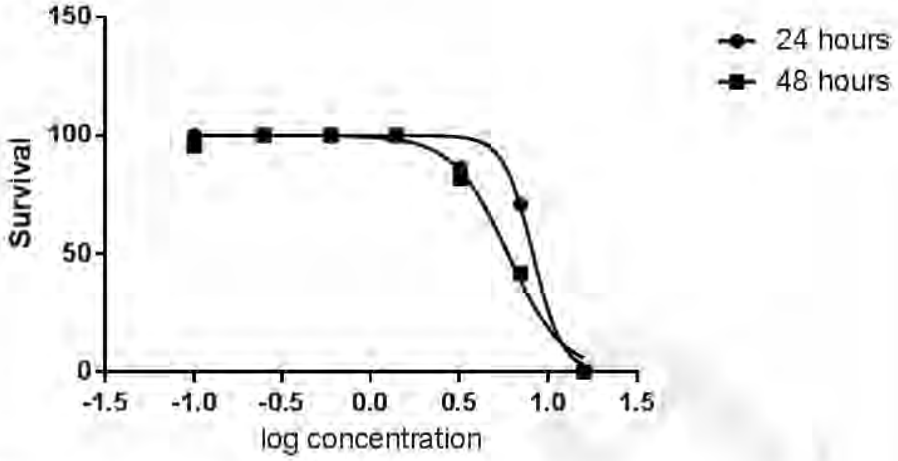
A. tonsa

Annex Point IIA7.2

Official use only

	1 REFERENCE	
1.1	**Reference**	[redacted] (2013) Acute toxicity of [redacted] to the marine species *Acartia tonsa*; [redacted] (Unpublished), BPD ID A7.4.1.2_07
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE and the Dow Chemical Company
1.2.2	Companies with letter of access	[redacted]
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**	[redacted], Glutaraldehyd [redacted]%
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]% (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 ([redacted]) equipped with a split/splitless injector and mass spectrometer detector ([redacted]) was used.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant

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 [redacted]/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 [redacted]% to marine crustacean *Acartia tonsa*.

Test substance: [redacted] (Glutardaldehyde [redacted]%), purity [redacted]% (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 [redacted]/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

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 [redacted] (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 GA[redacted] %/l (nominal)

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

5.2.3 EC_{100} 16 mg GA[redacted] %/l (nominal)

5.3 Conclusion The testing of the acute toxicity of glutardaldehyde [redacted]% to the marine crustacean *Acartia tonsa* resulted in a LC50 value (48 h) of 5.8 mg GA[redacted]/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 _ 07 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 [redacted]% glutaraldehyde and the measured as 100% glutaraldehyde which is confusing. For the nominal concentrations from 1.4-16 mg [redacted]% 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 [redacted] 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	[redacted]
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

Test Concentration [redacted] (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

pH

Test	pH

	Concentration [redacted] (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 ([redacted]; [redacted] % 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 [redacted] are based on the formulated product which is [redacted]% 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 ([redacted]; 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 [redacted] [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

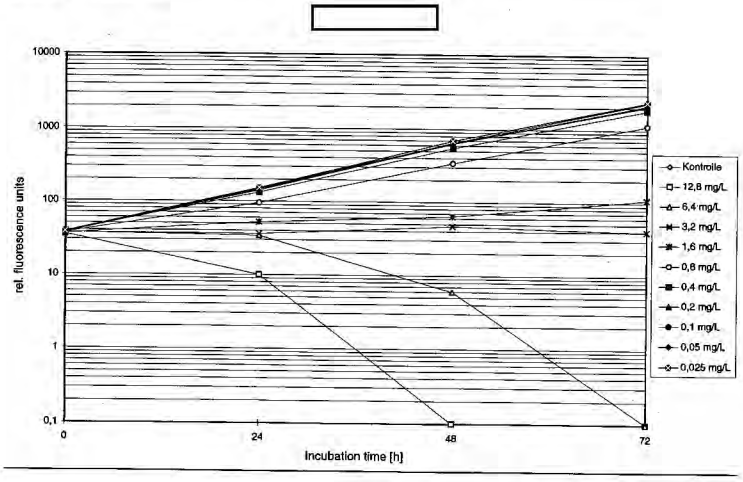
	fulfilled	Not fullfilled
Mortality of control animals ≤10%	**yes**	
Toxicity of reference chemical within the range specified in 8.5 of ISO 14669:1999	**yes**	
Concentration of dissolved oxygen in all test vessels >4 mg/l	**yes**	

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

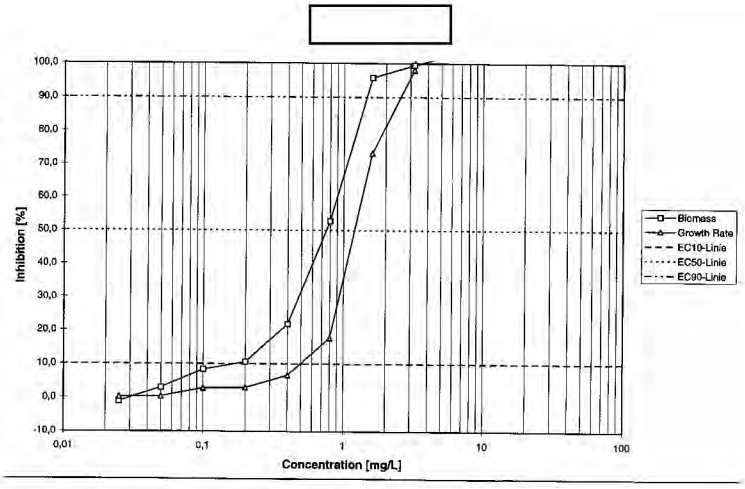
			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1997) Determination of the inhibitory effect of [redacted] on cell multiplication of unicellular green algae. [redacted] (Unpublished), BPD ID A7.04.1.3_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, Directive 92/69/EEC, C.3 (1992)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted] %	
3.1.4	Composition of Product	[redacted] % active ingredient (i.e. glutaraldehyde [redacted] % water, [redacted]	
3.1.5	Further relevant properties	Instability against temperature, oxygen, acid and alkali	
3.1.6	Method of analysis	Reversed phase HPLC with UV/VIS-detection after pre-column derivatization; determination by the method of external standards	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant since the TS is soluble in water up to 10 g/l.	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not relevant	
3.4	**Testing procedure**		
3.4.1	Culture medium	Mineral composition [mg/l]: 15 mg/l NH_4Cl, 12 mg/l $MgCl_2$ * 6 H_2O, 18 mg/l $CaCl_2$ *2 H_2O, 15 mg/l MgSO4 *7 H_2O; 1.6 mg/l KH_2PO_4, 0.08 mg/l $FeCl_3$ * 6 H_2O, 0.1 mg/l Na_2EDTA * 2 H_2O, 50 mg/l $NaHCO_3$, 0.185 mg/l H_3BO_3, 0.415 mg/l $MnCl_2$ * 4 H_2O, 0.003 mg/l $ZnCl_2$,0.0015 mg/l $CoCl_2$ * 6 H_2O, 0.00001 mg/l $CuCl_2$ * 2 H_2O, 0.007 mg/l Na_2MoO_4 * 2 H_2O pH approx. 8	
3.4.2	Test organisms	See table A7_4_1_3-2	x
3.4.3	Test system	See table A7_4_1_3-3	
3.4.4	Test conditions	See table A7_4_1_3-4	

3.4.5	Duration of the test	72 hours	
3.4.6	Test parameter	Cell multiplication inhibition	
3.4.7	Sampling	Fluorescence measurements were performed after 0, 24, 48, and 72 hours.	
3.4.8	Monitoring of TS concentration	Yes, at the start of the test the uninoculated replicates were analyzed, at the end of the test (after 72 h) both the uninoculated and inoculated replicates were analyzed. The analytical monitoring was performed for following nominal concentrations: 0 (control), 0.1, 1.6 and 12.8 mg/l.	
3.4.9	Statistics	Biomass growth: calculation via the integral over the total duration of the test for each concentration. Growth rate: calculated over the total duration of the study for each concentration level and compared to control. The EC values are calculated from the concentration-response relationship. The LOEC was determined by comparing the means of the fluorescence measurement of the various concentration levels with the control. The Duncan multiple range test was performed at a 95% significance level. Every higher tested concentration must have at least the same or stronger effects then the LOEC.	x

4 RESULTS

4.1	**Limit Test**	Not performed. In fact the test concentrations were selected on the basis of a previous algae study, which resulted in an EC50 < 1 mg/l.
4.1.1	Concentration	Not relevant
4.1.2	Effect data	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 mg/l
4.2.2	Actual concentrations of test substance	The analytical monitoring of the nominal concentrations 0, 0.1, 1.6 and 12.8 mg/l revealed following measured concentrations: < 0.1, 0.1, 1.5 and 12.8 mg/l. These measured concentrations indicate a recovery rate greater than 80%; therefore the effect concentrations were based on the nominal values.
4.2.3	Growth curves		

4.2.4 Concentration / response curve



4.2.5	Cell concentration data	See table A7_4_1_3-5	
4.2.6	Effect data (cell multiplication inhibition)	E_bC_{50} (72 h)	0.75 mg/l
		E_rC_{50} (72 h)	1.2 mg/l
		NOEC (72 h)	0.025 mg/l
		LOEC (72 h)	0.05 mg/l
4.2.7	Other observed effects	None	
4.3	**Results of controls**	The cell multiplication factor in the untreated control was after 72 h: 63-fold. For details see table A7_4_1_3-5	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not relevant	
4.4.2	Results	Not relevant	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to look for the inhibitory effect of [redacted] on cell multiplication of the unicellular green algae *Scenedesmus subspicatus* [redacted]

Test substance: [redacted], batch No. [redacted] purity [redacted]% (glutaraldehyde [redacted]%; impurities: [redacted]% water, [redacted])

The test was carried out according to Directive 92/69/EEC, C.3 (1992), with GLP.

Exponentially growing algae (*Scenedesmus subspicatus*) were cultured for several generations. Multiplication of cells was determined under the influence of [redacted] in relation to untreated control. Following concentrations were tested 0.0 (control), 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 mg /l. The initial cell density of *Scenedesmus subspicatus* was 10E+4 cells/ml. Fluorescence measurements were performed after 0, 24, 48, and 72 hours.

An analytical monitoring of the test concentrations was conducted at the start of the test (uninoculated replicates) and at test ending (after 72 h; both uninoculated and inoculated replicates) by means of phase HPLC with UV/VIS-detection. The analytical monitoring was carried out for following nominal concentrations: 0, 0.1, 1.6 and 12.8 mg/l.

5.2 Results and discussion

Analytical monitoring:

The results of the analytical monitoring revealed an overall recovery rate, which was > 80%; therefore the effect concentrations were based on the nominal concentrations.

Cell concentrations:

Test-Substance Concentration (nominal) [mg/l]	Cell concentrations (mean values) [cells/ml] Percent of control 0 h	24 h	48 h	72 h
0	100	100	100	100
12.8	95	7	0	0
6.4	100	23	1	0
3.2	100	25	7	2
1.6	97	35	9	5
0.8	100	65	49	48
0.4	103	90	79	78
0.2	100	96	91	89
0.1	103	99	92	91
0.05	100	100	94	99
0.025	103	103	100	102

5.2.1	NOE_rC	0.025 mg/l	x
5.2.2	ErC50	1.2 mg/l	
5.2.3	E_bC50	0.75 mg/l	
5.3	**Conclusion**	The test resulted in an EC50 for the growth rate of the algal cells of 1.2 mg/l. The NOEC was 0.025 mg/l. As the analytical recovery of the test substance was above 80%, the reported effective concentrations are therefore based on the nominal concentrations of [redacted]; they further refer to the test material as such. The validity criteria for algal growth inhibition test according to OECD Guideline 201 were fulfilled.	
5.3.1	Reliability	**1**	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date April 21st, 2008

Materials and Methods

3.4.2, table 7_4_1_3-2: OECD 201 recommends that there should be 2-5x10^3 cells/ml.

3.4.9 More elaborated statistical analysis is recommended in OECD 201.

Results and discussion

5.2.1 According to the Dunnett's test the highest concentration not deviating significantly from the control is 0.05 mg/l and hence this value is regarded as NOEC.

The EC values are based on nominal concentrations. This is considered acceptable as the measured concentrations were rather close (>80 %) to the nominal concentrations (see 4.2.2). However, there is some uncertainty because the lowest concentration measured was 0.1 mg/l which was also the limit of detection of the analytical method (Test report, p. 12). In Doc IIIA4.2c the LOQ is 0.05 µg/l.

The test fulfils the criteria concerning the coefficient of variation. The mean coefficient of variation for section-by-section specific growth rates is 12.6% (criterion CV ≤ 35%). The coefficient of variation of average specific growth rates during 0-72 hours is 0.3% (Criterion CV ≤ 7% for *Desmodesmus subspicatus*).

E_rC_{50} (72 h) 0.6 mg a.i./L
NOEC (72 h) 0.025 mg a.i./L

Conclusion Glutaraldehyde is very toxic to *Desmodesmus subspicatus.*

Reliability 2

Acceptability	Acceptable
Remarks	The test report should have been more detailed.
	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_3-1: 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	None

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	*Scenedesmus subspicatus* [redacted]
Strain	[redacted]
Source	[redacted]
Laboratory culture	Yes
Method of cultivation	Liquid culture, weekly passage, 23°C, 10000 cells/ml (volume 100 ml)
Pretreatment	Algae were pre cultured 72 h prior to test start. No further pre-treatment was carried out
Initial cell concentration	10^4 cells/ml

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	250 ml
Culturing apparatus	According to guideline
Light quality	Artificial light, OSRAM L25 universal white, permanent illumination (about 120 µE/m² s) in the range of 400 – 700 nm
Procedure for suspending algae	Not specified, probably as prescribed by the guideline

Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No (flasks were plugged with gas permeable silicone sponge caps)

Table A7_4_1_3-4: Test conditions

Criteria	**Details**
Test temperature	23 ± 2 °C
pH	Start of the test, uninoculated: 7.8-7.9 End of test, uninoculated: 7.8-8.0 End of test, inoculated: 7.9 – 9.4 (control, 9.4)
Aeration of dilution water	-
Light intensity	About 120 µE/m²s
Photoperiod	Permanent illumination

Test Concentration	pH
Control	9.4
0.025 mg/l	9.3
0.05 mg/l	9.4
0.1 mg/l	9.2
0.2 mg/l	9.2
0.4 mg/l	9.1
0.8 mg/l	8.8
1.6 mg/l	8.1
3.2 mg/l	8.0
6.4 mg/l	8.0
12.8 mg/l	7.9

Table A7_4_1_3-5: Cell concentration data

Test-Substance Concentration (nominal) [mg/l]	**Cell concentrations (mean values) [cells/ml]**							
	Measured				**Percent of control**			
	0 h	**24 h**	**48 h**	**72 h**	**0 h**	**24 h**	**48 h**	**72 h**
0	37	147	694	2350	100	100	100	100
12.8	35	10	0	0	95	7	0	0
6.4	37	34	6	0	100	23	1	0
3.2	37	36	47	40	100	25	7	2
1.6	36	52	64	109	97	35	9	5
0.8	37	95	341	1128	100	65	49	48
0.4	38	132	551	1833	103	90	79	78
0.2	37	141	632	2080	100	96	91	89
0.1	38	146	638	2149	103	99	92	91
0.05	37	148	651	2317	100	100	94	99
0.025	38	152	693	2390	103	103	100	102
Temperature [°C]	23 ± 2 °C							
pH	7.8 – 7.9	-	-	7.9 – 9.4				

3.1 Validity criteria for algal growth inhibition test according to OECD Guideline 201

	Fulfilled	**Not fullfilled**

Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**Yes**	
Concentration of test substance ≥80% of initial concentration during test	**Yes**	
Criteria for poorly soluble test substances	**Not relevant**	

Section A7.4.1.3 _ 02 Growth inhibition test on algae

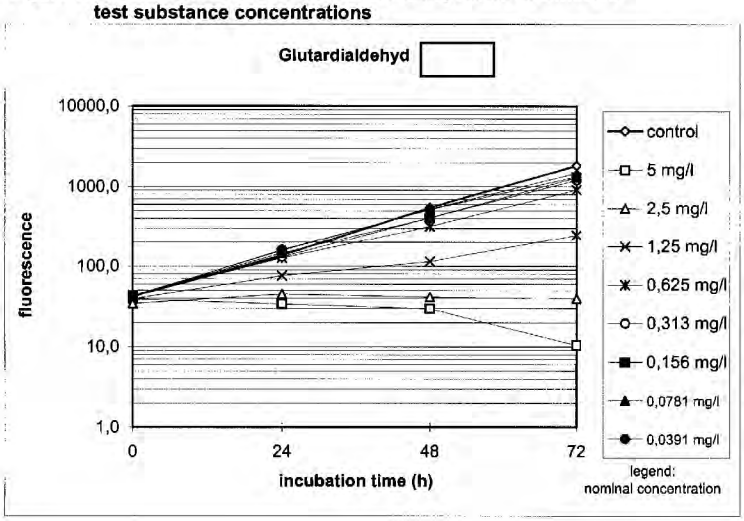
Annex Point IIA7.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1993) Algal growth inhibition test. [redacted] (Unpublished, translation of a German report dated 1988), BPD ID A7.04.1.3_02	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, DIN 38412, part 9	
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutardialdehyde [redacted]%	
3.1.1	Lot/Batch number	Not specified	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	[redacted]% glutardialdehyde in water	
3.1.5	Further relevant properties	Instability against temperature, oxygen, acid and alkali	
3.1.6	Method of analysis	Not applicable	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable since the TS is soluble in water up to 10 g/l	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not relevant	
3.4	**Testing procedure**		
3.4.1	Culture medium	Mineral composition [mg/l]: 15 mg/l NH_4Cl, 12 mg/l $MgCl_2$ * 6 H_2O, 18 mg/l $CaCl_2$ *2 H_2O, 15 mg/l MgSO4 *7 H_2O; 1.6 mg/l KH_2PO_4, 0.08 mg/l $FeCl_3$ * 6 H_2O, 0.1 mg/l Na_2EDTA * 2 H_2O, 50 mg/l $NaHCO_3$, 0.185 mg/l H_3BO_3, 0.415 mg/l $MnCl_2$ * 4 H_2O, 0.003 mg/l $ZnCl_2$,0.0015 mg/l $CoCl_2$ * 6 H_2O, 0.00001 mg/l $CuCl_2$ * 2 H_2O, 0.007 mg/l Na_2MoO_4 * 2 H_2O The pH after aerating was approx. 8. This nutrient solution compared with the	

		algal nutrient solution prescribed by the OECD guideline 201.
3.4.2	Test organisms	See table A7_4_1_3-2
3.4.3	Test system	See table A7_4_1_3-3
3.4.4	Test conditions	See table A7_4_1_3-4
3.4.5	Duration of the test	72 hours
3.4.6	Test parameter	Cell multiplication inhibition
3.4.7	Sampling	Fluorescence measurements were performed after 0, 24, 48, and 72 hours.
3.4.8	Monitoring of TS concentration	No
3.4.9	Statistics	The EC values were calculated by linear regression analysis from the concentration-response relationship. The LOEC was determined by comparing the means of the calculated biomass or growth rate of the various concentration levels with the control. The Dunnett's test (one sided) was carried out at a 95% significance level.
		4 RESULTS
4.1	**Limit Test**	Not performed.
4.1.1	Concentration	Not relevant
4.1.2	Effect data	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0 (control), 0.0391, 0.0781, 0.156, 0.313, 0.625, 1.25, 2.5 and 5 mg/l
4.2.2	Actual concentrations of test substance	No analytical monitoring performed.

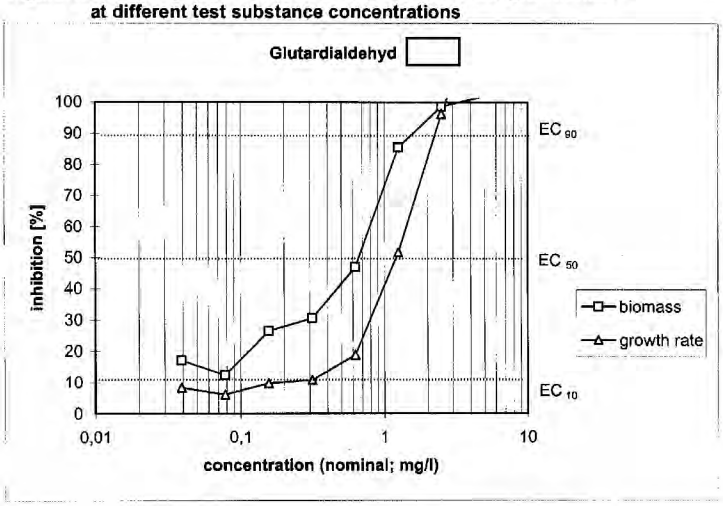
4.2.3 Growth curves

Figure 1: Growth curves of Desmodesmus *subspicatus* at different test substance concentrations



4.2.4 Concentration / response curve

Figure 2: Percentage inhibition of the algal biomass and growth rates at different test substance concentrations



4.2.5 Cell concentration data (72 h)

Inhibition of growth rate after 72 hours:

Test concentrations (Nominal; mg/l)	Inhibition of growth rate (% of control)
0	0
0.0391	8.3
0.0781	6.1
0.156	9.7
0.313	10.8
0.625	18.9
1.25	51.7
2.5	96.3
5	135.3

*, Growth inhibition as percentage of control

Inhibition of biomass after 72 hours:

Test concentrations (Nominal; mg/l)	Inhibition of growth rate (% of control)
0	0
0.0391	17.1
0.0781	12.3
0.156	26.5
0.313	30.6
0.625	46.9
1.25	85.6
2.5	98.6
5	101.9

1.1.1 Effect data (cell multiplication inhibition)

E_rC_{50} (72 h)	1.21 mg/l
E_bC_{50} (72 h)	0.66 mg/l
NOEC (72 h)	< 0.0391 mg/l
LOEC (72 h)	0.0391 mg/l

4.2.6 Other observed None

	effects	
4.3	**Results of controls**	Inconspicuous
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The aim of the present study was to look for the inhibitory effect of glutardialdehyde (purity: 50%) on cell multiplication of the unicellular green algae *Scenedesmus subspicatus* [redacted] Test substance: Glutaraldehyde [redacted]% (in water) The test was carried out according to DIN 38412, part 9; GLP was not compulsory at the time the study was performed. Exponentially growing algae (*Scenedesmus subspicatus*) were cultured for several generations. Multiplication of cells was determined under the influence glutardialdehyd [redacted]% in relation to untreated control. Following concentrations were tested: 0 (control), 0.0391, 0.0781, 0.156, 0.313, 0.625, 1.25, 2.5 and 5 mg/l. Algal exposition was performed in 10 ml tubes with flat bottoms. The initial cell density of *Scenedesmus subspicatus* was 10E4 cells/ml. Fluorescence measurements were performed after 0, 24, 48, and 72 hours (prompt chlorophyll a fluorescence at 685 nm as a criterion for biomass). No analytical monitoring of the test concentrations was conducted. The EC values were calculated by linear regression analysis from the concentration-response relationship. The LOEC was determined by comparing the means of the calculated biomass or growth rate of the various concentration levels with the control. The Dunnett's test (one sided) was carried out at a 95% significance level.
5.2	**Results and discussion**	Algal growth rate and biomass inhibition (72h): An inhibitory effect of algal growth rate already was seen at lowest test concentration of 0.0391 mg/l test substance; at 2.5 mg/l, growth rate inhibition reached 96% and at the highest test concentration of 5 mg/l, inhibition was > 100%. Similar results were seen for the biomass.
5.2.1	NOEC	< 0.0391 mg/l (nominal)
5.2.2	ECr10	0.19 mg/l (nominal)
5.2.3	ECr50	1.21 mg/l (nominal)
5.2.4	ECr90	2.27 mg/l (nominal)
5.3	**Conclusion**	The treatment of the algae with glutaraldehyde [redacted]% had an inhibitory effect on both parameters, the growth rate and the biomass. The determined NOEC and EC values refer to the test material as such. The validity criteria for algal growth inhibition test according to OECD Guideline 201 were fulfilled.
5.3.1	Reliability	**2**
5.3.2	Deficiencies	No analytical monitoring performed. The EC values were recalculated recently.

Section A7.4.1.3 _ 02 Growth inhibition test on algae

Annex Point IIA7.3

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 3rd, 2009
Materials and Methods	The applicant's version is correct. Results of the pH measurements were not reported. The report should have been more detailed.
Results and discussion	The test fulfils the criteria concerning the coefficient of variation. The mean coefficient of variation for section-by-section specific growth rates is 9.7% (criterion CV ≤ 35%). The coefficient of variation of average specific growth rates during 0-72 hours is 1.8% (Criterion CV ≤ 7% for *Desmodesmus subspicatus*). EC_r50 0.61 mg a.i/l based on nominal concentrations NOEC <0.02 mg a.i./l based on nominal concentrations
Conclusion	Glutaraldehyde is very toxic to *Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*).
Reliability	Not acceptable because test concentrations were not analytically verified
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_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	None

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	*Scenedesmus subspicatus* [redacted]
Strain	Not specified
Source	Not specified
Laboratory culture	Yes
Method of cultivation	Liquid culture, weekly passage, 23 ±2 °C, 10000 cells/ml (volume 100 ml)
Pretreatment	Algae were pre cultured 72 h prior to test start. No further pre-treatment was carried out
Initial cell concentration	10^4 cells/ml

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	10 ml
Culturing apparatus	Not specified
Light quality	Artificial light, (about 120 µE/m² s)
Procedure for suspending algae	Not specified, probably as prescribed by the guideline
Number of vessels/ concentration	Not specified
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	23 ± 2 °C
pH	Measurements were performed at study initiation and at the end of the study (72 hours). Results of the pH measurements were not reported
Aeration of dilution water	Yes, no further specification
Light intensity	About 120 µE/m²s
Photoperiod	According to guideline

3.1 Validity criteria for algal growth inhibition test according to OECD Guideline 201

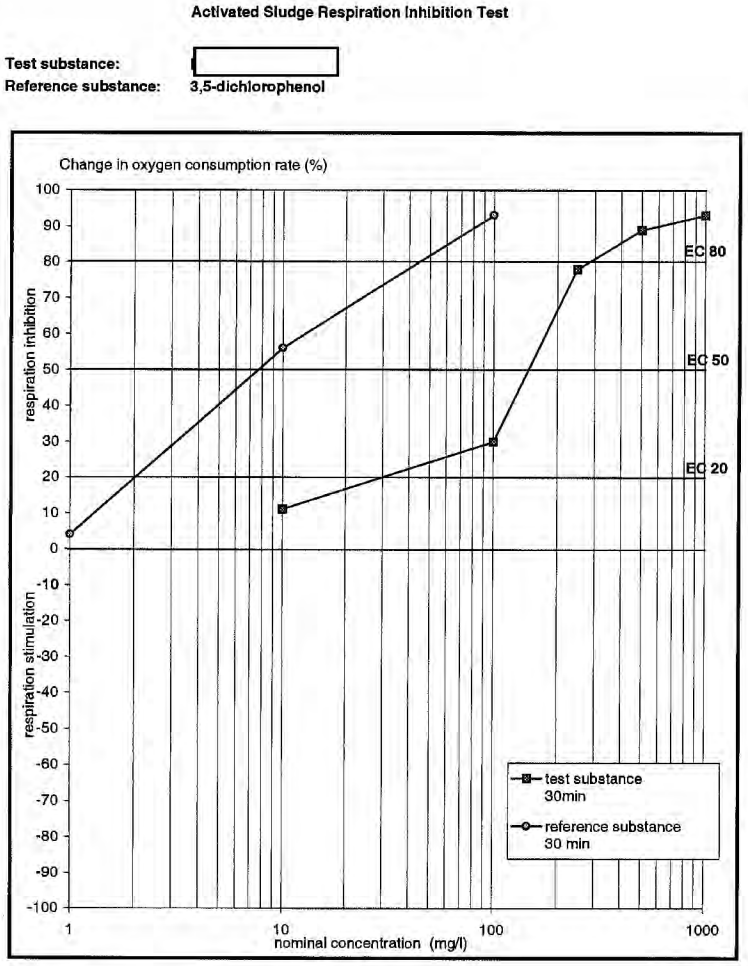
	fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**Yes**	
Concentration of test substance ≥80% of initial concentration during test	**No analytical monitoring performed.**	

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

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1998) Determination of the inhibition of Oxygen Consumption by activated Sludge by [redacted] in the Respiration Inhibition Test. [redacted] (Unpublished), BPD ID A7.04.1.4_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 209 (1993)	x
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] (1,5-Pentandial)	
3.1.1	Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted] %	
3.1.4	Composition of Product	[redacted]% glutaraldehyde, [redacted]% water, [redacted]	
3.1.5	Further relevant properties	Instability against temperature, oxygen, acid and alkali	
3.1.6	Method of analysis	Not performed	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant	
3.3	**Reference substance**	Yes, 3,5-dichlorophenol	
3.3.1	Method of analysis for reference substance	Not relevant	
3.4	**Testing procedure**		
3.4.1	Synthetic medium	8 ml/vessel 100-fold concentrated OECD Medium	
3.4.2	Inoculum / test organism	For details on inoculum see table A7_4_1_4-2	x
3.4.3	Test system	For details on test type, laboratory equipment etc. see table A7_4_1_4-3	
3.4.4	Test conditions	For relevant test conditions see table A7_4_1_4-4	
3.4.5	Duration of the test	30 minutes	
3.4.6	Test parameter	Respiration inhibition (by Oxygen measurement)	
3.4.7	Analytical parame-		

	ter	
3.4.8	Sampling	The oxygen consumption was measured for 6-10 minutes after an incubation time of 30 minutes.
3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	Control without test substance (blank control), reference substance as positive control
3.4.11	Statistics	Statistics was not performed.
		4 RESULTS
4.1	**Preliminary test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Effect data	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	10, 100, 248, 500, and 1000 mg/l
4.2.2	Actual concentrations of test substance	Analysis was not performed, reported values refer to nominal concentrations
4.2.3	Growth curves	Not relevant
4.2.4	Cell concentration data	Not relevant

4.2.5 Concentration/ response curve



4.2.6	Effect data	EC_{50}	ca. 160 mg/l
		EC_{20}	ca. 30 mg/l
		EC_{80}	ca. 280 mg/l
4.2.7	Other observed effects	No other inhibition phenomena were observed	
4.3	**Results of controls**	The blank control (mean value of three replicates):	
		Oxygen consumption rate:	2.7 mg O_2/l*6 min.
		Specific oxygen consumption rate:	27 mg O_2/g*h
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	1, 10, 100 mg/l	
4.4.2	Results	EC_{50}	ca. 7.6 mg/l

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to determine the inhibition of oxygen consumption in activated sludge by Basolon GDA 50.

Test substance: [redacted] (1,5-Pentandial), [redacted], purity [redacted]% glutaraldehyde (impurities [redacted]% water, [redacted])

The test was performed according to the OECD Guideline 209 (1993) with GLP.

[redacted] was tested in Erlenmeyer-vessels (250 ml, 20 ± 2°C) at initial nominal concentrations of 10, 100, 248, 500, and 1000 mg/l. The reference substance 3,5-dichlorophenol was tested at 1, 10 and 100

		mg/l. Activated sludge from a laboratory wastewater plant fed with municipal and synthetic sewage was used as inoculum (washed and aerated for 24 hours, final volume: 1 g/l dry substance). The blank control comprised 3 test vessels, whereas the test and reference substance included 1 vessel/concentration. After an incubation time of 30 minutes the oxygen consumption was measured with an O_2-electrode for 6 minutes. The change in oxygen consumption was the measure for respiration inhibition.
5.2	**Results and discussion**	The specific oxygen consumption rate of the blank control (mean value of three replicates) was determined to be 27 mg O_2/g^*h. The specific oxygen consumption rate of Basolon GDA at 1000, 500, 248, 100 and 10 mg/l was found to be 2, 3, 6, 19, and 24 mg O_2/g^*h, respectively. The corresponding changes in oxygen consumption rate compared to blank control were 93%, 89%, 78%, 30%, and 11%, respectively.
5.2.1	EC_{20}	About 30 mg/l
5.2.2	EC_{50}	About 160 mg/l (versus ca. 7.6 mg/l for the reference substance)
5.2.3	EC_{80}	About 280 mg/l
5.3	**Conclusion**	The test substance showed a clear dose-response relationship: the increase of the test substance concentration was accompanied by an increased inhibition of respiration of the active sludge. The validity criteria for this test system were fulfilled, since the deviations of the blank controls are less then 15%. The EC_{50} of the reference substance 3,5-dichlorophenol is in the range of 5-30 mg/l according to OECD 209.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 21st, 2008
Materials and Methods	2.1: The OECD Guideline 209 was adopted in 1984. 3.4.2, table A7_4_1_4-2: The source of the inoculum is not mentioned in the test report. It is neither known whether the activated sludge is coming from a STP treating predominantly domestic sewage.
Results and discussion	The EC values are based on nominal concentrations and they are expressed as 100 % glutaraldehyde. EC_{50} ca. 80 mg a.i./l EC_{20} ca. 15 mg a.i./l
Conclusion	Glutaraldehyde inhibits microbial activity in activated sludge at and above15 mg/l.
Reliability	2
Acceptability	Acceptable
Remarks	The test report should have been more detailed.
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A7.4.1.4 _ 01 Annex Point IIA7.4	Inhibition to microbial activity in activated sludge (aquatic)
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_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	No other procedures performed

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Not applicable
Strain	Not applicable
Source	Laboratory wastewater plant municipal and synthetic sewage
Sampling site	Laboratory wastewater plant
Laboratory culture	Cultured in the laboratory wastewater plant
Method of cultivation	Laboratory wastewater plant
Preparation of inoculum for exposure/ Pretreatment	The inoculum was washed, brought to a concentration of 5 g/l substance and aerated for 24 hours. 50 ml were added to a test volume of 250 ml to obtain a concentration of 1 g/l dry substance in the test.
Initial cell concentration	1 g/l dry substance

Table A7_4_1_4-3: Test system

Criteria	Details
Culturing apparatus	Erlenmeyer-vessel (250 ml)
Number of culture flasks/concentration	Blank control: 3 vessels test and reference substance: 1 vessel/concentration
Aeration device	According to OECD guideline
Measuring equipment	O_2-electrode
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_4-4: Test conditions

Criteria	Details
Test temperature	20 ± 2°C
pH	Start of test (before adding the inoculum): 6.3 – 6.4 adjusted to 7.4 – 7.9 End of test: pH 7.7
Aeration of dilution water	According to guideline; the oxygen concentration during aeration was > 2.5 mg/l, immediately before measurement the oxygen concentration was 6.5 mg/l
Suspended solids concentration	1 g/l dry weight

Official use only

		1 REFERENCE
1.1	**Reference**	Zok S (2000) [redacted] ([redacted]% Glutaraldehyde) - Early Life-Stage toxicity test on the Rainbow trout (Oncorhynchus mykiss). [redacted] (Unpublished), BPD ID A7.04.3.2_01
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD 210 (1992) and EPA-FIFRA
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 METHOD
3.1	**Test material**	[redacted]% Glutardialdehyde)
3.1.1	Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	[redacted] % of active substance
3.1.5	Further relevant properties	Instability against temperature, oxygen, acid and alkali The storage stability of the test substance over the study period has been verified by analytical characterization
3.1.6	Method of analysis	The concentration of the test substance was measured via spectrophotometry: 10 ml of the sample was treated with 5 ml of a Thiobarbituric acid solution and 1 ml of Fe-III-chloride hexahydrate solution. The mixture was heated to 70°C for 30 minutes. The measuring was performed at a wavelength maximum of ca. 453 nm in quartz cells with the reagent blank as reference. Evaluation was carried out using a calibration graph set up with known concentrations of the test substance.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable, since the test substance is soluble
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_3_2-2
3.4.2	Test organisms	See table A7_4_3_2-3
3.4.3	Handling of embryos and larvae (OECD 210/212)	The embryos did not receive any disinfection or medical treatment. The eggs were placed in the exposure chamber approx. 3 hours after fertilization (day 0) and all embryos appeared to be in good condition at the beginning of the study. On the morning of day 1 unfertilized eggs were

		replaced to unexposed fertilized eggs. The number of replaced eggs in the concentration groups was comparable to the number of replaced eggs in the control group.
3.4.4	Test system	For details on test type, renewal of TS solution, laboratory equipment, loading, replicates etc. see table A7_4_3_2-4
3.4.5	Test conditions	For relevant test conditions see table A7_4_3_2-5
3.4.6	Duration of the test	97 days
3.4.7	Test parameter(s)	Survival, time to hatch and swim-up, toxic signs and abnormalities as well as body weight and length were examined
3.4.8	Examination / Sampling	Mortality was determined daily, for time to hatch and swim-up as well as for toxic signs and abnormalities were determined generally at least on workdays, body weight and length were determined at the end of the study
3.4.9	Monitoring of TS concentration	Yes, samples were taken on day zero and then generally at weekly intervals and generally alternating from all test aquaria generally before the replacement of the stock solutions.
3.4.10	Statistics	For the body weight and the length of the fishes the statistical evaluation was carried out using Dunnett's test for a simultaneous comparison of several doe groups with the control group. The test was performed two-sided. For the embryo, larvae, and fish survival, a pairwise comparison of each group with the control group was carried out via the log-rank test. The test was performed one-sided. The Wilcoxon-test was performed one-sided with the replicate as statistical unit to consider the variability between the replicate.

4 RESULTS

4.1	**Range finding test**	Not performed
4.1.1	Concentrations	Not applicable
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	Flow-through system (for analytically determined concentrations of the test substance in the test water see point 4.2.2 below)
4.2.2	Actual concentrations of test substance	Analytically determined concentrations of the test substance in the test water [mg/l and % of nominal concentration]

	Study group / nominal concentrations				
Day	0 mg/l	0.32 mg/l	1.0 mg/l	3.2 mg/l	10.0 mg/l
0	≤ 0.1	0.29 90.6%	0.84 84%	2.8 87.5%	9.4 94%
7	Approx. 0.1	0.36 112.5%	1.0 100%	3.3 103.1%	9.9 99%
14	≤ 0.1	0.36 112.5%	1.04* 104%	4.0 125%	12.5 125%
21	≤ 0.1	0.31 96.9%	1.1 110%	3.4 106.3%	10.3 103%

28	Approx. 0.15	0.44 137.5%	1.27 127%	3.62 113.1%	9.54 95.4
35	> 0.15	0.19 59.4%	1.25 125%	3.25* 101.6%	11.1 111%
42	> 0.15	0.25 78.1%	1.04 104%	3.3 103.1%	10.8 108%
49	≤ 0.1	Ca. 0.15** 46.9%	0.62 62%	3.39* 105.9%	11.25* 112.5%
56	≤ 0.1	0.18 56.3%	0.59 59%	3.6 112.5%	10.40 104%
63	≤ 0.1	0.25 78.1%	0.6** 60%	3.34 104.4%	--
70	≤ 0.15	Ca. 0.1 31.3%	0.38 38%	3.34 107.2%	--
77	≤ 0.15	≤ 0.15*** ≤ 46.9%	0.3 30%	3.42 106.9%	--
84	> 0.15	0.33 103.1%	0.47 47%	3.78 118.1%	--
86	> 0.25	>0.25*** >78.1%	0.75 75%	3.2 100%	--
91	> 0.15	0.33 103.1%	0.95 95%	3.33 104.1%	--
97	> 0.15	0.35 109.4%	0.4 40%	3.5 109.4%	--
mean		0.28	0.79	3.41	10.58
SD		0.095	0.317	0.265	0.968

SD standard deviation
-- not measured (all animals dead)
* value taken from retain samples
** retain samples confirmed low values and were not considered for calculation of mean
***outlier, value was not included in the calculation of the mean

4.2.3 Effect data

Survival data:

Embryo stages (days 0-30) survival rate:
Nom. Concentration

0.0 mg/l	95%	(88 – 100%)
0.32 mg/l	91%	(80 – 100%)
1.0 mg/l	92%	(84 – 96%)
3.2 mg/l	96%	(92 – 100%)
10.0 mg/l	94%	(88 – 100%)

For the embryo survival at beginning of hatch there was no evidence of any statistically significant concentration dependent effect.

Embryo/Larvae survival rate days 30-35, (from beginning of hatch to termination of hatch) = survivors on day 35 related to survivors on day 30:
Nom. Concentration

0.0 mg/l	96.8%	(92 – 100%)
0.32 mg/l	95.6%	(85 – 100%)
1.0 mg/l	93.5%	(85.7 – 96%)
3.2 mg/l	93.8%	(91.3 – 100%)
10.0 mg/l	10.6%	(4.3 – 25%)

For the embryo/larvae survival there was a statistically significant effect in comparison to the control group in the test group 10.0 mg/l ($p \leq 0.01$ log-rank-test, $p \leq 0.05$ Wilcoxon-test)

Embryo/Larvae survival rate at the termination of hatch days 0-35, = survivors at day 35 related to 100 individuals at the beginning:

Nom. Concentration		
0.0 mg/l	92%	(88 – 96 %)
0.32 mg/l	87%	(68 – 100%)
1.0 mg/l	86%	(72 – 92%)
3.2 mg/l	90%	(84 – 100%)
10.0 mg/l	10%	(4 – 24%)

For the embryo/larvae survival days 0-35 there was a statistically significant effect in comparison to the control group in the test group 10.0 mg/l ($p \leq 0.01$ log-rank-test, $p \leq 0.05$ Wilcoxon-test)

Survival of larvae at termination of hatch days 0-35, = survivors at day 35 related to the number of fertilized eggs calculated from the viability control on day 14 = 97.5%:

Nom. Concentration		
0.0 mg/l	94.4%	(90.3 – 98.5 %)
0.32 mg/l	89.2%	(69.7 – 102.6%)
1.0 mg/l	88.2%	(73.8 – 94.4%)
3.2 mg/l	92.3%	(86.2 – 102.6%)
10.0 mg/l	10.3%	(4.1 – 24.6%)

For the larvae survival days 0-35 there was a statistically significant effect in comparison to the control group in the test group 10.0 mg/l ($p \leq 0.01$ log-rank-test, $p \leq 0.05$ Wilcoxon-test)

Survival of larvae days 35-56 (termination of hatch until end of swim-up), = number of survivors day 56 related to number of hatched larvae day 35

Nom. Concentration		
0.0 mg/l	96.7%	(90.9 – 100%)
0.32 mg/l	96.6%	(90.9 – 100%)
1.0 mg/l	97.7%	(95.7 – 100%)
3.2 mg/l	94.4%	(86.4 – 100%)
10.0 mg/l	0.0%	(0%)

For the larvae survival days 35-56 there was a statistically significant effect in comparison to the control group in the test group 10.0 mg/l ($p \leq 0.01$ log-rank-test, $p \leq 0.05$ Wilcoxon-test)

Survival of larvae at termination of swim-up (days 0-56), = survivors at day 56 related to 100 individuals at the beginnig

Nom. Concentration		
0.0 mg/l	89%	(80 - 92%)
0.32 mg/l	84%	(68 – 100%)
1.0 mg/l	84%	(72 - 88%)
3.2 mg/l	85%	(76 – 100%)
10.0 mg/l	0.0%	(0%)

For the larvae survival days 0-56 there was a statistically significant effect in comparison to the control group in the test group 10.0 mg/l ($p \leq 0.01$ log-rank-test, $p \leq 0.05$ Wilcoxon-test)

Survival of young fish day 56 – 97, = survivors at study and related to day 56 survivors at end of swim-up

Nom. Concentration		
0.0 mg/l	97.8%	(95.7 - 100%)
0.32 mg/l	100%	(100%)
1.0 mg/l	98.8%	(94.4 - 100%)
3.2 mg/l	96.5%	(94.7 – 100%)

For fish survival days 56-97 there was no statistically significant effect in comparison to the control group in the concentration groups with survivors.

Survival of young fish from day 0 to test termination (day 97), = survivors at day 97 related to 100 individuals at the beginning

Nom. Concentration		
0.0 mg/l	87%	(80 - 92%)
0.32 mg/l	83.7%	(68 – 100%)
1.0 mg/l	82.8%	(68 - 88%)
3.2 mg/l	82%	(72 – 96%)
10.0 mg/l	0.0%	(0%)

For the young fish survival days 0-97 there was a statistically significant effect in comparison to the control group in the test group 10.0 mg/l ($p \leq 0.01$ log-rank-test, $p \leq 0.05$ Wilcoxon-test)

Thus, the NOAEC for survival is 3.2 mg/l (nominal concentration) and 3.41 mg/l (based on mean analytically determined concentration) and the LOSAEC is 10 mg/l (nominal) and 10.58 mg/l (based on analytically determined concentration)

Time to start of hatching and end of hatching
start of hatch = day before the first larvae were observed

Nom. Concentration	start (days)	end (days)
0.0 mg/l	30-31	33-35
0.32 mg/l	31	33-34
1.0 mg/l	30-31	33-34
3.2 mg/l	31-32	34-35
10.0 mg/l	33	34-35

In the highest concentration group hatch started slightly later than in the control group. It can not be excluded that this is an effect which was caused by the test substance

Numbers of larvae hatching each day

Nom. Conc.	31 d	32 d	33 d	34 d	35 d
0.0 mg/l	11	55	21	4	1
0.32 mg/l	0	51	27	8	0
1.0 mg/l	2	64	15	4	0
3.2 mg/l	0	2	9	77	2
10.0 mg/l	0	0	0	4	1

Length and weight of surviving animals

Weight

Nom. Conc.	Mean weight [g]	% of control*	Stat. signifi-cance compared to control
0.0 mg/l	1.02	100%	--
0.32 mg/l	1.04	101.8%	--
1.0 mg/l	1.04	101.8%	--
3.2 mg/l	1.02	99.5%	--
10.0 mg/l	-	-	-

- = No data since individuals were dead
* = calculated on the basis of the individual values
-- = Not statistically significant

No significant difference of the mean body weight in comparison to the control group was seen in the survivors of any of the test groups. In conclusion, the NOEC for the development of body weight is 3.2 mg/l (nominal).

Length

Nom. Conc.	Mean length [cm]	% of control*	Stat. signif. comp. to control
0.0 mg/l	4.73	100%	--
0.32 mg/l	4.75	100.4%	--
1.0 mg/l	4.74	100.3%	--
3.2 mg/l	4.72	99.8%	--
10.0 mg/l	-	-	-

- = No data since individuals were dead
* = calculated on the basis of the individual values
-- = Not statistically significant

No significant difference of the mean body length in comparison to the control group was seen in the survivors of any of the test groups. In conclusion, the NOEC for the development of body length is 3.2 mg/l (nominal)

Numbers of deformed larvae and toxic signs

No fish with deformations were seen in any of the test groups at study end. The abundance of deformations was therefore not increased in the concentration groups.

In the control group anomalies in swimming behaviour of single animals were the only observed effects. In the 2 highest concentrations 3.2 and 10.0 mg/l the larvae had difficulties to remove the egg shells. The egg shells of most of the larvae of the 3.2 mg/l group were removed within 1 week after hatch and in the rest of the larvae about 2 weeks after hatch and the animals developed normally, while in the 10 mg/l group most animals died within some days after hatch. Any other effects observed in the concentration groups occurred in single animals and were considered to be substance related effects. No effects were observed in the control group from day 75 on and in the survivors of the concentration groups from day 65 on.

Thus the NOAEC for sublethal effects is 3.2 mg/l (nominal) and 3.41 mg/l (based on the mean analytically determined concentration) and the LOAEC is > 3.2 mg/l (nominal) and > 3.41 mg/l (based on mean analytically determined concentration).

4.2.4 Concentration / response curve

A detailed concentration response evaluation is presented under point 4.2.3.

4.2.5 Other effects

Single fish exposed to a concentration of 1.0 mg/[redacted] swam near the bottom and revealed convulsions similar to the control animals. At a concentration including and above 3.2 mg/l following findings were noted: apathy, narcotic state, shortened and/or crippled body, extended/still existing yolk sac. However these findings were restricted to single animals (one fish per monitoring date) except of the finding "head in egg shell" revealing a maximum of 11 effected fish on day 34-36.

Symptoms observed for fish exposed to [redacted] at a concentration of 10.0 mg/l were: head remaining in egg shell, narcotic state, apathy. However these observations were made for one fish per monitoring date.

4.3 Results of controls

4.3.1 Number/ percentage of survival/animals showing adverse effects

The mean control survival was:

-At beginning of hatch (day 30, related to total of 100 individuals) = 95% (88%-100%)

-At termination of hatch (day 35, related to day 30 survivors) = 96.8% (92.0%-100%)

-At termination of hatch (day 35, related to total of 100 individuals =

92% (88%-96%)

-At termination of hatch (day 35, related to 97.5% fertilized eggs in the viability control on day 14) = 94.4 (90.3%-98.5%)

-At termination of swim-up (day 56, related to day 35 survivors) = 96.7%

-At termination of swim-up (day 56, related to total of 100 individuals) = 89% (80%-92%)

-At test termination (day 97, related to day 56 survivors) = 97.8 (95.7%-100%)

-At test termination (day 97, related to total of 100 individuals) = 87% (80%-92%)

4.3.2 Nature of adverse effects

Control

Replicate	A	B	C	D
Day 41	k1	0	0	0
Day 42	k1	0	0	0
Day 43	k1	0	0	0
Day 44	k1	0	0	0
Day 45	k1	0	0	0
Day 46	k1	0	0	0
Day 47	k1	0	0	0
Day 48	k1	0	0	0
Day 49	k1	0	0	0
Day 50	k1	0	0	0
Day 51	k1	k1	0	0
Day 52	k1	k1	0	0
Day 53	k1	k1	0	0
Day 54	k1	k1	0	0
Day 55	k1	k1	0	0
Day 56	k1	k1	0	0
Day 57	k1	k1	0	0

k1 = convulsion, one fish

Prior to day 40 and from day 57 onwards no findings were noted for the control fish.

4.4 Test with reference substance

Not performed

4.4.1 Concentrations

Not applicable

4.4.2 Results

Not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the toxicity of [redacted] % Glutaraldehyde) to early life-stages of Rainbow trout (Oncorhynchus mykiss).

Test substance: [redacted] (purity: [redacted] %, batch No: [redacted])

The test was performed according to guideline OECD 210 (1992) and EPA-FIFRA, with GLP.

Fertilized eggs of rainbow trout were exposed under flow-though conditions for 97 days. The eggs were placed in the exposure chamber approx. 3 hours after fertilization (day 0) and were then exposed to follow-

ing concentrations of [redacted]: 0, 0.32, 1.0, 3.2, 10.0 mg/l.

The test parameters were survival, time to hatch and swim-up, toxic signs and abnormalities as well as body weight and length. Mortality was determined daily; time to hatch and swim-up as well as toxic signs and abnormalities were determined generally at least on workdays, body weight and length were determined at the end of the study

The test temperature was generally 10°C, dissolved oxygen was maintained in a range between 8.3 and 11.3 mg/l; Until swim-up the embryos and larvae were kept in dark. Thereafter, they were exposed to dim light (ca. 150 Lux) at a light cycle of 16 hours light and 8 hours dark till termination of the study.

For monitoring the test substance concentration samples were taken on day zero and then generally at weekly intervals and generally alternating from all test aquaria generally before the replacement of the stock solutions.

For the body weight and the length of the fishes statistical evaluation was carried out according to Dunnett's test. For the embryo, larvae, and fish survival, a pairwise comparison of each group with the control group was carried out via the log-rank test. The Wilcoxon-test was performed one-sided with the replicate as statistical unit to consider the variability between the replicate.

5.2 Results and discussion

Survival:
Over the whole study period (day 0 - 97) survival in comparison to the control group was statistically significantly decreased in the 10.0 mg/l group. Mortality occurred predominantly shortly after hatch. In conclusion, the NOAEC for survival is 3.2 mg/l (nominal concentration) and 3.41 mg/l (based on the mean analytically determined concentrations), the LOAEC is 10.0 mg/l (nominal concentration) and 10.58 mg/l (based on the mean analytically determined concentrations).

Time to hatch and swim-up:
The first swim-up was observed at day 48 in the replicates of the control group and on days 48 - 50 in the concentration groups. The swim-up was completed at days 55 and 56 in all test groups.
In conclusion, the only possibly substance-related effect on time to hatch and swim-up was a slight delay in the start of hatch in the highest concentration group 10.0 mg/l.

Toxic signs (symptoms) and abnormalities:
Since in spite of the observation in the 3.2 mg/l group, survival and growth in this test group were not affected, the delayed removal of the egg shell is considered not to be an adverse effect.
Thus, the NOAEC for sublethal effects is 3.2 mg/l (nominal concentration) and 3.41 mg/l (based on the mean analytically determined concentrations), the LOAEC is > 3.2 mg/l (nominal concentration) and > 3.41 mg/l (based on the mean analytically determined concentrations).

Body weight and length:
No effects on body weight and length in comparison to the control group were observed in any of the concentration groups with surviving individuals.

5.2.1 NOAEC

In conclusion, under the conditions of this study, the overall no observed adverse effect concentration (NOAEC) was 3.2 mg/l /nominal concentration) and 3.41 mg/l (based on the mean analytically determined concentration)

5.2.2 LOAEC

The lowest concentration with adverse effects (LOAEC) was 10.0 mg/l (nominal concentration) and 10.58 mg/l (based on the mean analytically determined concentration) due to a marked decrease in survival.

5.3 Conclusion

The chronic treatment of early-life-stages of fish [redacted] [redacted]% glutardialdehyde) resulted in mortality and in a slight delay in the start of hatch at a nominal test concentration of 10mg/l test substance;

		the NOAEC therefore was 3.2 mg/l. Referring to the active ingredient glutardialdehyde as such ([redacted] % in the test substance), the NOAEC- and LOAEC values are: NOAEC: 1.6 mg a.i./l LOAEC: 5 mg a.i./l. The validity criteria can be considered as fulfilled (see validity criteria summarized in tables A7_4_3_2-6)
5.3.1	Other Conclusions	-
5.3.2	Reliability	**1**
5.3.3	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 23rd, 2008
Materials and Methods	Agree with the applicant. The detection limit for glutaraldehyde was 0.1-0.25 mg/l, which is above the LOQ 0.05 µg/l in Doc IIIA4.2c.
Results and discussion	Agree with the applicant. NOAEC 1.6 mg a.i./l measured concentrations
Conclusion	Glutaraldehyde is slightly toxic to Rainbow trout in the long-term exposure.
Reliability	1
Acceptability	Acceptable
Remarks	
	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	

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_3_2-2: Dilution water

Criteria	Details
Source	Municipal water works of the city [redacted], purified through a charcoal filter and aerated
Salinity	Not relevant
Hardness	Approx. 2.3 – 2.5 mmol/L (approx. 230 – 250 mg/l $CacO_3$)
pH	7.7 – 8.1
Oxygen content	8.3 – 11.3 mg/l
Conductance	523 – 558 micro Siemens [µS]
Holding water different from dilution water	No

Table A7_4_3_2-3: Test organisms

Criteria	Details
Species/strain	Rainbow trout (Oncorhynchus mykiss [redacted] 1792)
Source	[redacted]
Wild caught	No
Age/size	The eggs were placed in the exposure chambers approx. 3 hours after fertilization (day 0)
Kind of food	First, newly hatched brine shrimp larvae (Artemia naupli) were fed. Additionally commercial high protein trout starter "Kronen Fish Aminostart" was offered when start of eating was observed. After termination of the swim-up the young fish received additionally "Trouvit" trout starter obtained from the trout farm.
Amount of food	Feeding was increased in quantity with the duration of the study and thus with the size of the fish.
Feeding frequency	According to guideline
Post-hatch transfer time	6 days after termination of hatch the larvae were placed into the steel aquaria and the egg cups were removed
Time to first feeding	Beginning at swim-up of the larvae
Feeding of animals during test	Yes, feeding was continued until one day before the termination of the study.
Treatment for disease within 2 weeks proceeding test	Information supplied by the trout farm indicated that the trout-breeding farm is certified disease free by the Governmental Veterinary Office of the local administration. The embryos did not receive any disinfection or medical treatment.

Table A7_4_3_2-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	The flow-rates were 10 l/hour/test group. They were split by an "udder" into 4 equal parts of the 4 replicates of each test group and in two parts for the viability control. Thus, each test aquarium was supplied by 2.5 l test water/h. Considering the size of the aquaria, the theoretical exchange rate of the water contents was approx. 6.7 exchanges per 24 hours. The 10 l/h for the 2 viability controls were split into approx. 2 equal parts (5 l/aquarium; approx. 13 exchanges/24 hours).
Volume of test vessels	Aquaria (inner dimensions: 29 cm long, 21 cm wide, 22 cm high) with an overflow 15 cm above the bottom (covered with gauze). Each aquarium maintained a constant water volume of approx. 9 litres. The fertilized eggs were placed in egg cups (cylinders made of transparent glass, diameter 12 cm, 10 cm high, with a grid 2x2 mm) submerged in each replicate test vessel.
Volume/animal	Aquarium: 90 ml/animal (test concentrations, control)
Number of animals/vessel	25 fertilized eggs in each test vessel (4 aquaria, 100/test group). The viability control consisted of 4 X 50 = 200 eggs, 50/egg cup, 2 egg cups/aquarium

Number of vessels/ concentration	4 replicates for each test concentration and control, and two replicates as viable control for the first 14 days.
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2-5: Test conditions

<table>
<tr><th>Criteria</th><th>Details</th></tr>
<tr><td>Test temperature</td><td>The test temperature was generally 10°C in all aquaria throughout the study (deviation maximum ± 1°C, daily measurement)</td></tr>
<tr><td>Dissolved oxygen</td><td>Dissolved oxygen was maintained in a range between 8.3 and 11.3 mg/l (corresponding to 70 – 100% of the maximum saturation at the test temperature of 10°C.
Dissolved oxygen content [mean in mg/l]
<table>
<tr><th></th><th colspan="6">Test concentration in mg/l</th></tr>
<tr><th>Day</th><th>0</th><th>0.32</th><th>1.0</th><th>3.2</th><th>10.0</th><th>v-C</th></tr>
<tr><td>3</td><td>11.1</td><td>11.1</td><td>11.1</td><td>11.1</td><td>11.1</td><td>11.0</td></tr>
<tr><td>7</td><td>10.7</td><td>10.8</td><td>10.7</td><td>10.7</td><td>10.8</td><td>10.8</td></tr>
<tr><td>10</td><td>10.9</td><td>10.9</td><td>11</td><td>11</td><td>11.1</td><td>11.1</td></tr>
<tr><td>14</td><td>10.7</td><td>10.9</td><td>10.9</td><td>11.0</td><td>11.0</td><td>--</td></tr>
<tr><td>17</td><td>10.9</td><td>11.0</td><td>11.0</td><td>11.1</td><td>11.1</td><td></td></tr>
<tr><td>21</td><td>11.1</td><td>11.2</td><td>11.2</td><td>11.2</td><td>11.2</td><td></td></tr>
<tr><td>24</td><td>11.1</td><td>11.2</td><td>11.2</td><td>11.2</td><td>11.2</td><td></td></tr>
<tr><td>28</td><td>10.8</td><td>10.8</td><td>10.8</td><td>10.8</td><td>10.7</td><td></td></tr>
<tr><td>31</td><td>10.8</td><td>10.9</td><td>10.9</td><td>10.9</td><td>10.9</td><td></td></tr>
<tr><td>35</td><td>10.8</td><td>10.8</td><td>10.8</td><td>10.9</td><td>10.8</td><td></td></tr>
<tr><td>38</td><td>11.1</td><td>11.1</td><td>11.1</td><td>11.1</td><td>11.1</td><td></td></tr>
<tr><td>42</td><td>10.7</td><td>10.7</td><td>10.8</td><td>10.8</td><td>10.9</td><td></td></tr>
<tr><td>45</td><td>10.8</td><td>10.9</td><td>11.0</td><td>11.0</td><td>11.2</td><td></td></tr>
<tr><td>49</td><td>10.8</td><td>10.8</td><td>10.8</td><td>10.9</td><td>11.0</td><td></td></tr>
<tr><td>52</td><td>10.7</td><td>10.7</td><td>10.7</td><td>10.9</td><td>10.9</td><td></td></tr>
<tr><td>56</td><td>10.6</td><td>10.8</td><td>10.7</td><td>10.8</td><td>10.9</td><td></td></tr>
<tr><td>59</td><td>10.7</td><td>10.8</td><td>10.7</td><td>10.9</td><td></td><td></td></tr>
<tr><td>63</td><td>10.5</td><td>10.5</td><td>10.4</td><td>10.8</td><td></td><td></td></tr>
<tr><td>66</td><td>10.1</td><td>10.1</td><td>10.0</td><td>10.1</td><td></td><td></td></tr>
<tr><td>70</td><td>9.9</td><td>9.9</td><td>9.9</td><td>10.2</td><td></td><td></td></tr>
<tr><td>73</td><td>9.7</td><td>10.0</td><td>9.9</td><td>9.8</td><td></td><td></td></tr>
<tr><td>77</td><td>10.1</td><td>10.0</td><td>9.9</td><td>9.9</td><td></td><td></td></tr>
<tr><td>80</td><td>9.8</td><td>9.6</td><td>9.5</td><td>9.9</td><td></td><td></td></tr>
<tr><td>84</td><td>9.3</td><td>9.2</td><td>9.3</td><td>9.3</td><td></td><td></td></tr>
<tr><td>87</td><td>9.5</td><td>9.6</td><td>9.4</td><td>9.4</td><td></td><td></td></tr>
<tr><td>91</td><td>8.7</td><td>8.7</td><td>8.8</td><td>9.2</td><td></td><td></td></tr>
<tr><td>93</td><td>8.4</td><td>8.6</td><td>8.4</td><td>8.6</td><td></td><td></td></tr>
</table>
v-C viability control
-- not measured</td></tr>
<tr><td>pH</td><td>pH values
<table>
<tr><th></th><th colspan="6">Test concentration in mg/l</th></tr>
<tr><th>Day</th><th>0</th><th>0.32</th><th>1.0</th><th>3.2</th><th>10.0</th><th>v-C</th></tr>
<tr><td>3</td><td>8.1</td><td>8.1</td><td>8.1</td><td>8.1</td><td>8.1</td><td>8.1</td></tr>
<tr><td>7</td><td>8.1</td><td>8.1</td><td>8.1</td><td>8.1</td><td>8.1</td><td>8.1</td></tr>
<tr><td>10</td><td>8.0</td><td>8.0</td><td>8.0</td><td>8.0</td><td>8.0</td><td>8.0</td></tr>
<tr><td>14</td><td>8.0</td><td>8.0</td><td>8.0</td><td>8.0</td><td>8.0</td><td>8.0</td></tr>
</table></td></tr>
</table>

	17	8.0	8.0	8.0	8.0	8.0
	21	8.0	8.0	8.1	8.1	8.1
	24	8.0	8.0	8.0	8.0	8.0
	28	8.0	8.0	8.0	8.0	8.0
	31	8.0	8.0	8.0	8.0	8.0
	35	8.0	8.0	8.0	8.0	8.0
	38	8.0	8.0	8.0	8.0	8.0
	42	8.0	8.0	8.0	8.0	8.0
	45	8.0	8.0	8.0	8.0	8.0
	48	8.0	8.0	8.0	8.0	8.0
	52	8.0	7.9	8.0	7.9	8.0
	56	8.0	8.0	8.0	8.0	
	59	8.0	8.0	8.1	8.0	
	63	8.0	8.0	8.0	8.0	
	66	7.9	7.9	7.9	7.9	
	70	7.8	7.8	7.8	7.9	
	73	7.8	7.8	7.8	7.9	
	77	7.8	7.8	7.8	7.9	
	80	7.8	7.8	7.8	7.9	
	84	7.9	7.9	7.9	8.0	
	87	7.8	7.8	7.8	7.9	
	91	7.8	7.8	7.8	7.9	
	93	7.7	7.7	7.8	7.9	
Adjustment of pH	No					
Aeration of dilution water	Yes, aerated in the dilution water storage tanks, nearly saturated with oxygen					
Intensity of irradiation	See below					
Photoperiod/illumination	Until swim-up the embryos and larvae were kept in dark to protect them from light. Thereafter, until to the termination of the study, they were exposed to dim light (about 150 Lux under the lid) at a light cycle of 16 hours light and 8 hours dark.					

Table A7_4_3_2-6: Validity criteria for fish tests according to OECD Guidelines 210/212

	Fulfilled	Not fullfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	**Yes**	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	**Yes**	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species (in this matter: 70%)	**Yes**	

Test substance concentrations maintained within ± 20% of mean measured values	Mean recovery rate of the test concentration of 1.0 mg/l was 79%. All other recovery rates are >87.5%*	
No effect on survival nor any other adverse effect found in solvent control	**Not applicable**	
Further criteria for poorly soluble test substances	**Not applicable**	

*The lower concentrations were near the analytical detection limit. A reliable analytical determination was therefore difficult. The dilution system was check regularly and no technical problems occurred which could explain the deviation from the theoretical concentration values. The relevant concentrations for the determination of the effect concentrations were within the range of ± 20% of the nominal concentrations, with the only exception: in one week values of 125% of the nominal concentrations were determined.

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1993) Determination of the chronic toxicity of [redacted] to Daphnia magna. [redacted] (Unpublished), BPD ID A7.04.3.4_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, according to EEG Directive XI/681/86	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	[redacted] (1,5-Pentadial)	
3.1.1	Lot/Batch number	No batch number available; the test substance was taken [redacted]	x
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]%	
3.1.4	Composition of Product	[redacted]% glutaraldehyde, > [redacted]% water, [redacted]	
3.1.5	Further relevant properties	Stability: 6 months until 30 °C without oxygen Not stable against acids/caustic solution, heat and oxygen Solubility in water: up to 10 g/l	
3.1.6	Method of analysis	Reversed phase HPLC (40% water, 60%acetonitrile; flow: 1 ml/min., injection volume: 20 µl, temp. 40°C) with UV/VIS-detection (370 nm); determination by the method of external standards	x
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable since the TS is soluble in water up to 10 g/l.	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not relevant	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_3_4-2	
3.4.2	Test organisms	See table A7_4_3_4-3	
3.4.3	Handling of offspring	Counting and removing of offspring on each Monday, Thursday, and Friday.	
3.4.4	Test system	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	Mortality and reproduction.
3.4.8	Examination / Sampling	Animals were examined daily for mortality and for reproduction; offspring was removed thrice a week at time of renewal of the test solutions.
3.4.9	Monitoring of TS concentration	Yes, 100 (stock solution), 20, 1.25, 0.156 and 0.0 (control) mg/l [redacted] test solutions were analyzed each week. Besides the freshly prepared test solutions, the solutions with or without Daphnia were analyzed after 72 hours.
3.4.10	Statistics	LOEC and NOEC calculations were performed according to "Duncan's multiple range test" (SAS/STAT guide for personal computers, version 6 edition, Cary, NC, SAS Institute Inc. pp. 1-1028, 1987)
		4 RESULTS
4.1	**Range finding test**	Not performed
4.1.1	Concentrations	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	Stock solution: 100 mg/l (nominal) Test concentrations: 0, 0.039, 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10 and 20 mg/l (nominal)
4.2.2	Actual concentrations of test substance	The following nominal concentrations were subjected to analytical monitoring: 0, 0.156, 1.25, 20 and 100 mg/l. Samples for analysis were taken in the first, the second and the third week of the test. For each concentration, the freshly prepared test solution and the corresponding 48 h or 72 h old test solution (with or without Daphnia) were analysed. The analytical monitoring revealed that for the small concentrations (0.156 and 1.25 mg/l), the measured concentrations were significantly below the expected values. This was probably related to the oxidation sensitivity of the test substance. Since, there were no effects observed on reproduction at the low concentrations, these deviations in the recovery rate will not have any influence on the test results. For the higher concentrations (i.e. 20 and 100 mg/l) the measured concentrations were as expected, with a recovery of 95 to 100% of the nominal values.

4.2.3 Effect data (21 d)

Conc. (mg/l)	Survival of Parents	Live young/live parent		Dead young/live parent	
	%	N	+/- 1 SD	N	+/- 1 SD
0	100	182.6	12.4	0	0.0
0.039	100	176.5	9.6	0	0.0
0.078	100	181.6	5.6	0	0.0
0.156	100	178.8	10.0	0	0.0
0.313	100	175.4	10.1	0	0.0
0.625	100	179.0	9.6	0	0.0
1.25	100	181.6	9.8	0	0.0
2.5	100	184.4	9.6	0	0.0
5	100	182.0	10.4	0	0.0
10	100	180.6	9.3	0	0.0
20	0 (day 2)	--	--	--	--

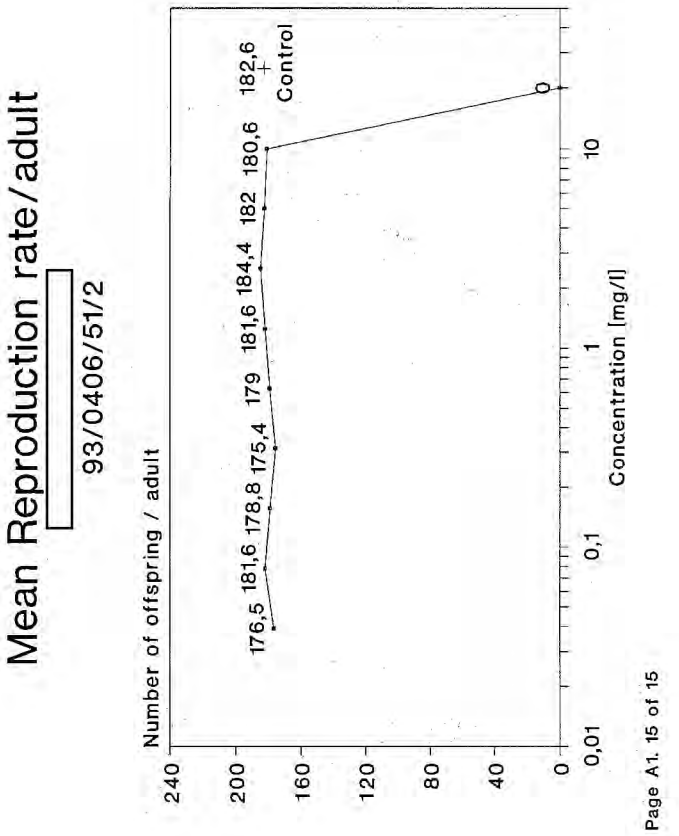
N: Number, mean value; 1 SD: 1 Standard deviation

NOEC and LOEC values for reproduction and for mortality of parent animals:

Up to and including 10 mg/l [redacted] there were no significant effects on neither reproduction nor parent mortality observed.

NOEC	10 mg/l (nominal)
LOEC	20 mg/l (nominal)
LC0	10 mg/l (nominal)

4.2.4 Concentration / response curve



4.2.5	Other effects	None
4.3	**Results of controls**	Controls were inconspicuous (see point 4.2.3)
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to determine the chronic toxicity of [redacted] to Daphnia magna.

Test substance: [redacted] (1,5-pentadial), purity [redacted]% (> [redacted]% water [redacted])

The study was conducted according to the EEC Directive XI/681/86, with GLP.

[redacted] was tested under semi-static conditions for its effect on mortality and reproduction to *Daphnia magna* [redacted]. 10 daphnids (2 – 24 hours old) per concentration were exposed for 21 days to nominal concentrations of 20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, 0.039, and 0 mg/l [redacted]. Animals were examined daily for mortality and for reproduction; offspring was removed three times a week. LOEC and NOEC calculations were performed according to "Duncan's multiple range test".

		Scenedesmus subspicatus served as daily food, during the test the measured oxygen content of the test solutions was in the range of 7.1 to 9.2 mg/l, the pH value was 7.5 – 8.5. Analysis for the content of active ingredient was performed for following test concentrations: 100 (stock solution), 20, 1.25, 0.156, and 0.0 (control) mg/l [redacted]. Besides the freshly prepared test solutions, the solutions with and without *Daphnia* were analyzed after 72 hours.
5.2	**Results and discussion**	Analytical monitoring: The analytical monitoring revealed that for the small concentrations (0.156 and 1.25 mg/l), the measured concentrations were significantly below the expected values. This was probably related to the oxidation sensitivity of the test substance. Since, there were no effects observed on reproduction at the low concentrations, these deviations in the recovery rate will not have any influence on the test results. For the higher concentrations (i.e. 20 and 100 mg/l) the measured concentrations were as expected, with a recovery of 95 to 100% of the nominal values. Effect data (after 21 days): Excepted for the 20 mg/l group, no parental mortality was observed; in the 20 mg/l group 100% mortality (i.e. 0% survival) was reached after 2 days. At the lower tested concentrations of [redacted] there were no significant effects on reproduction or offspring.
5.2.1	NOEC	10 mg/l (nominal)
5.2.2	LOEC	20 mg/l (nominal)
5.2.3	LC0	10 mg/l (nominal)
5.3	**Conclusion**	Up to and including 10 mg/l [redacted] no significant effects on reproduction or parent mortality were observed. Since the mortality rate of the parent control animals was < 20% at test termination and the mean number of live offspring produced per parent control animal surviving at test termination was ≥ 60, the test can be considered as valid. Concerning the concentration – toxicity relationship there was a steep slope between 10 and 20 mg/l [redacted].
5.3.1	Reliability	**1**
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 15th, 2008
Materials and Methods	3.1.1 Batch number should be defined. 3.1.6 No LOQ given. Table A7_4_3_4-3 Daphnia culturing methods are not mentioned in the report.
Results and discussion	Results are expressed as 100 % glutaraldehyde. NOEC (21 d) 5 mg/l measured concentrations
Conclusion	Glutaraldehyde is slightly toxic to *Daphnia magna*.
Reliability	2
Acceptability	Acceptable
Remarks	The report should have been more detailed.
	COMMENTS FROM ... (specify)

Section A7.4.3.4 _ 01 Annex Point IIIA XIII 2.4	Effects on reproduction and growth rate with an invertebrate species
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	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	Not relevant

Table A7_4_3_4-2: Dilution water

Criteria	Details
Source	Synthetic water
Salinity	Not relevant
Hardness	2.20 -3.20 mmol/l
pH	7.5 – 8.5
Ca / Mg ratio	About 4 / 1 (Mol)
Na / K ratio	Not specified
Oxygen content	The test water was aerated nearly to saturation followed by a stabilization phase of 24 hours (without aeration). During the test the measured oxygen content was in the range of 7.1 to 9.2 mg/l.
Conductance	550 – 650 µS/cm
TOC	Not specified
Holding water different from dilution water	No

Table A7_4_3_4-3: Test organisms

Criteria	Details
Strain / Clone	*Daphnia magna* [redacted]
Source	[redacted]
Age	2 – 24 hours
Breeding method	According to laboratory standard methods
Kind of food	*Scenedesmus subspicatus*
Amount of food	**Day** / **Amount of food/ parent animal/day as mg chemical oxygen demand**: 0 – 1: 0.22; 2 – 3: 0.25; 4 – 5: 0.35; 6 – 7: 0.43; 8 - 21: 0.75

Feeding frequency	Daily
Pretreatment	No, the Daphnia was reared under laboratory standard conditions.
Feeding of animals during test	Yes, see above

Table A7_4_3_4-4: Test system

Criteria	Details
Test type	Semi-static
Renewal of test solution	The test solutions were changed 3 times a week (on Monday, Wednesday and Friday)
Volume of test vessels	100 ml
Volume/animal	50 ml/animal
Number of animals/vessel	1 animal/vessel
Number of vessels/ concentration	10 vessels/concentration
Test performed in closed vessels due to significant volatility of TS	Yes

Table A7_4_3_4-5: Test conditions

Criteria	Details
Test temperature	18.7 – 21.2 °C

Dissolved oxygen (mg O_2/l)

Conc. mg/l	[Day]									
	0	2	5	7	9	12	14	16	19	21
0	8.9	9.2	8.3	8.4	8.1	8.3	7.9	7.6	7.7	8.0
0.039	9.1	9.0	8.6	8.6	8.2	8.0	8.6	7.4	7.9	8.1
0.078	9.1	9.0	8.4	8.5	8.2	8.3	8.2	7.4	8.0	8.1
0.156	9.0	8.9	8.3	8.4	8.1	8.3	8.5	7.4	7.7	8.0
0.313	9.0	8.9	8.3	8.5	8.1	8.4	8.3	7.1	8.0	8.0
0.625	8.9	9.0	8.3	8.7	8.2	8.3	8.3	7.3	7.7	8.0
1.25	8.9	9.2	8.4	8.4	8.3	8.4	8.3	7.5	7.8	8.0
2.5	8.8	9.2	8.4	8.4	8.3	8.5	8.2	7.7	8.0	8.1
5	8.7	9.0	8.3	8.4	8.2	8.5	8.2	7.4	7.9	8.2
10	8.7	8.9	8.4	8.4	8.3	8.4	8.2	7.5	7.9	8.1
20	8.6	9.0	*	*	*	*	*	*	*	*

*, No data

pH

Conc. mg/l	[Day]									
	0	2	5	7	9	12	14	16	19	21
0	8.2	8.1	8.2	7.9	7.8	7.7	7.7	7.5	7.6	7.7
0.039	8.2	8.1	8.2	7.9	7.8	7.7	7.8	7.6	7.6	7.7
0.078	8.2	8.1	8.0	7.9	7.8	7.8	7.8	7.6	7.7	7.7
0.156	8.2	8.1	8.0	7.9	7.8	7.8	7.8	7.6	7.7	7.7
0.313	8.2	8.1	8.0	7.9	7.8	7.8	7.8	7.6	7.7	7.7
0.625	8.2	8.1	8.0	7.9	7.8	7.8	7.8	7.6	7.7	7.7
1.25	8.2	8.1	8.0	7.9	7.8	7.8	7.8	7.6	7.7	7.7

	2.5 | 8.2 | 8.1 | 8.0 | 7.9 | 7.8 | 7.8 | 7.8 | 7.7 | 7.7 | 7.7 5 | 8.2 | 8.1 | 8.0 | 7.9 | 7.8 | 7.8 | 7.8 | 7.6 | 7.7 | 7.8 10 | 8.2 | 8.1 | 7.9 | 7.9 | 7.8 | 7.8 | 7.8 | 7.6 | 7.7 | 7.8 20 | 8.2 | 8.1 | * | * | * | * | * | * | * | *
Adjustment of pH	No
Aeration of dilution water	Yes, dilution water was aerated nearly to saturation. Prior to use the water was non-aerated for 24 hours.
Quality/Intensity of irradiation	Fluorescent tube
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 control animals < 20% at test termination	**Yes**	
Mean number of live offspring produced per parent control animal surviving at test termination ≥ 60	**Yes**	

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

16 January 2012

Project-No.:

The statistical evaluation of the data was carried out using the SAS®-System.

A curve was fitted between the concentration and the %inhibition via the probit model.
This curve was used for the estimation and the confidence limits of the EC10 and EC50.

In the first analysis the data of all concentrations was used.

At day 28 the inhibition of the high concentration 1186 mg/kg is lower than the inhibition at the concentrations 176 mg/kg and 457 mg/kg.
The inhibition at the highest test concentration is similar to the concentration of 68 mg/kg. This result is mechanistically arbitrary. Additionally there are two concentrations of 176 mg/kg and 457 mg/kg having a monotone increase and these inhibitions are both higher than the inhibition at the concentration of 1186 mg/kg.
A typical biological dose response curve with increased inhibition by increasing test concentrations is not present using the highest test concentration and it is biologically not feasible.
Furthermore the EC10 is in the range of the two lowest test concentrations. These results should influence the calculation of the EC10. Therefore a better fit of the model in this range should be applied and consequently the highest test concentration was excluded.

The fit of the curve excluding the highest dose concentration was much better than considering all test concentration (see figure 1 and 2).

Results:
The ECx values are given in mg/kg. The 95% percent confidence limits are given in brackets:

```
Inhibition on day 28:   EC10= 14 [7;31]   EC50=1401 [650;3018]
```

The estimation of the EC10 is 14 mg/kg and lies between the concentrations of 10 mg/kg and 26 mg/kg. Looking at the observed inhibitions of these two concentrations the recalculation is more feasible.

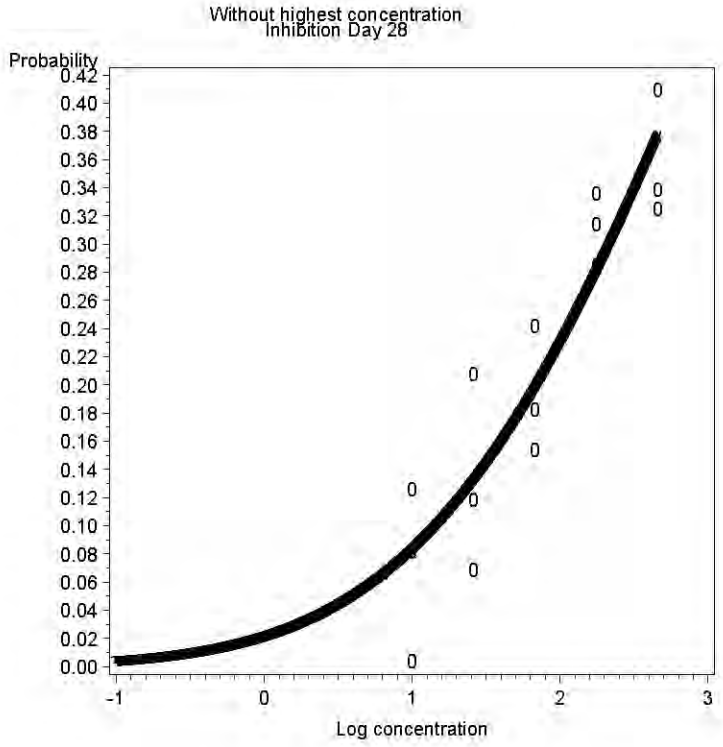
....., ...
Date

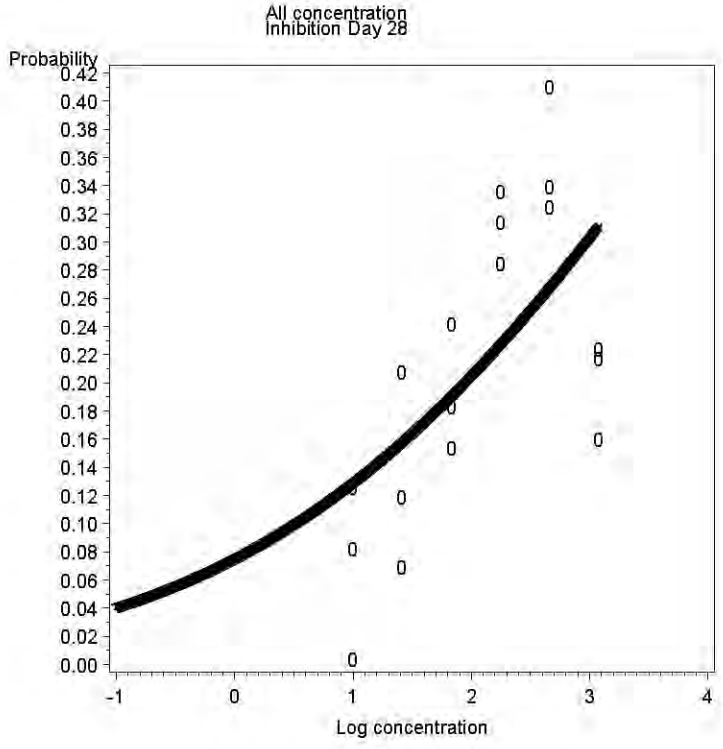
Project No.3

Obs	Concentration	Inhibition Day28
1	10	12.6
2	10	8.2
3	10	0.4
4	26	11.9
5	26	6.9
6	26	20.8
7	68	15.4
8	68	24.2
9	68	18.3
10	176	31.4
11	176	33.6
12	176	28.5
13	457	41.0
14	457	32.5
15	457	33.9

Day 28

Parameter	Estimate	Lower CL(95%)	Upper CL(95%)
EC10	14.458	6.754	30.951
EC50	1401.011	650.427	3017.758


Without highest concentration
Inhibition Day 28
Probability
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-1
0
1
2
3
Log concentration


All concentration
Inhibition Day 28
Probability
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-1
0
1
2
3
4
Log concentration

Section A7.5.1.1_01 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4 Carbon Transformation Test

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (2006[redacted], Determination of the carbon transformation by the glucose induced soil respiration (Carbon Transformation Test). [redacted] (Unpublished), BPD ID A7.5.1.1_01
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD 217
2.2	**GLP**	Yes
2.3	**Deviations**	Soil was stored 4.5 months which exceeds the maximum three months given in the OECD 217.

3 MATERIALS AND METHODS

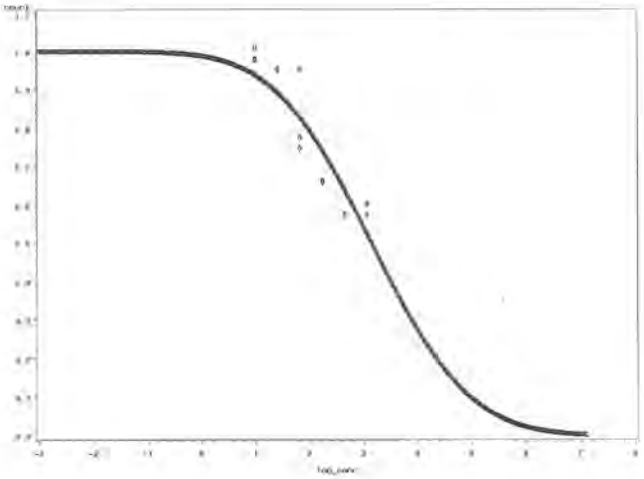
3.1	**Test material**	[redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]%
3.1.4	Composition of Product	Test substance diluted in water
3.1.5	Further relevant properties	The test substance was defined as homogeneous and was described as colourless clear liquid, which was miscible at ca. 20 °C. The stability under storage conditions (refrigerator) over the exposure period was guaranteed.
3.1.6	Method of analysis	Not reported
3.2	**Reference substance**	No
3.2.1	Method of analysis for reference substance	Not relevant as no reference substance was tested.
3.3	**Testing procedure**	
3.3.1	Soil sample / inoculum / test organism	See table A7_5_1_1-1

3.3.2	Test system	See table A7_5_1_1-3	
3.3.3	Application of TS	See table A7_5_1_1-4	
3.3.4	Test conditions	See table A7_5_1_1-5	
3.3.5	Test parameter	Inhibition of microbial carbon transformation: The glucose induced respiration rates were measured during 12 consecutive hours for 3 samples of test mixture per test concentration following addition of glucose (about 400 mg per test sample). Each sample of test mixture weighed about 118 g .	
3.3.6	Analytical parameter	The degradation of glucose in the soil samples was determined by absorption of the CO_2 produced by the glucose; the absorption of CO_2 induced a negative pressure in the test pots, which was detected with the OxiTop pressure heads . The calculation of glucose induced soil respiration (BA) was based on following formula: $BA = M_{O2}/R \mathbf{x} T \quad \mathbf{x}\ V_{fr}/m_{Bt}\ \mathbf{x}\ I\ \Delta P\ I$ BA = glucose-induced soil respiration (mg O_2/kg dry matter soil) M_{O2} = molecular weight of O_2 (31998.8 mg/mol) R = gas constant (8.314 hPa/mol/K) T = test temperature (K) V_{fr} = free gas volume in the test assay (L) m_{Bt} = mass of dry substance soil (Kg) I ΔP I = absolute value of the pressure alternation (hPA) The calculated respiration rate was expressed as mg O_2 consumed / kg dry mater soil/h). The mean respiration rate of 3 single samples of test mixture per test concentration was determined and was compared with the control value; the percent of deviation from control was calculated.	x
3.3.7	Duration of the test	28 days	
3.3.8	Sampling	Samples were taken on day 0, 7 and 28 of incubation and were examined for glucose induced respiration rates. For each test concentration and sampling time point, 3 samples were considered (each about 118 g)	
3.3.9	Monitoring of TS concentration	Not performed as not of importance for the present type of study and not required by the guideline	
3.3.10	Controls	Control without test substance were added to the test series.	
3.3.11	Statistics	The dose-response curve was fitted via the probit model to the inhibition values. The curve was used for estimation of the EC10 and EC50 and the confidence limist (95%).	
		4 RESULTS	
4.1	**Range finding test**	Not performed	
4.1.1	Concentration	Not relevant as no range-finding test was performed.	

4.1.2	Effect data	Not relevant as no range-finding test was performed.
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 10, 26, 68, 176, 457 and 1186 mg/kg matter soil
4.2.2	Actual concentrations of test substance	See 3.3.9
4.2.3	Concentration/ response curve	

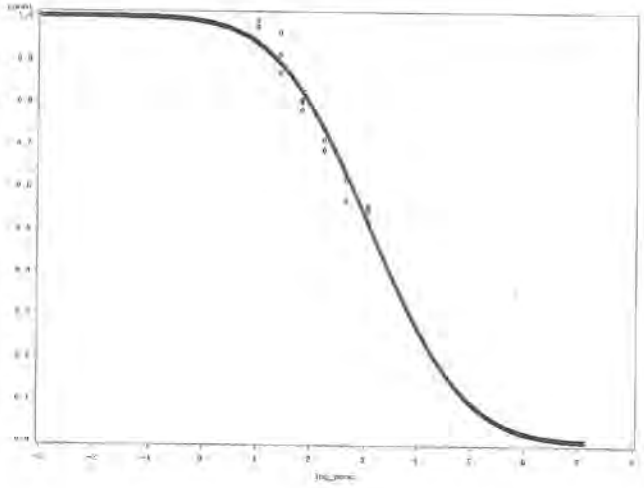
4.2.3.1 At test initiation

Figure 1: Graphical illustration of the probit analysis of the test substance at the begin of exposure period



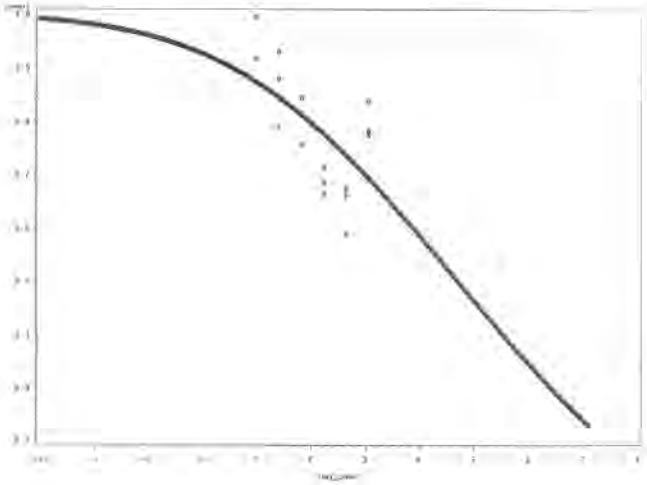
4.2.3.2 At sampling time point 7 days

Figure 2: Graphical illustration of the probit analysis of the test substance after an exposure period of 7 days

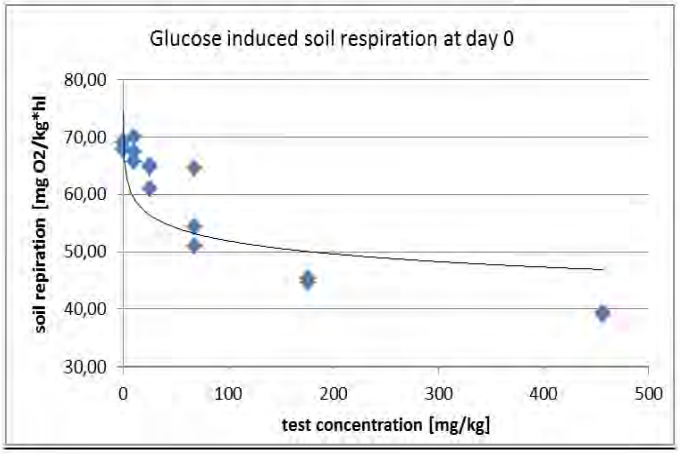


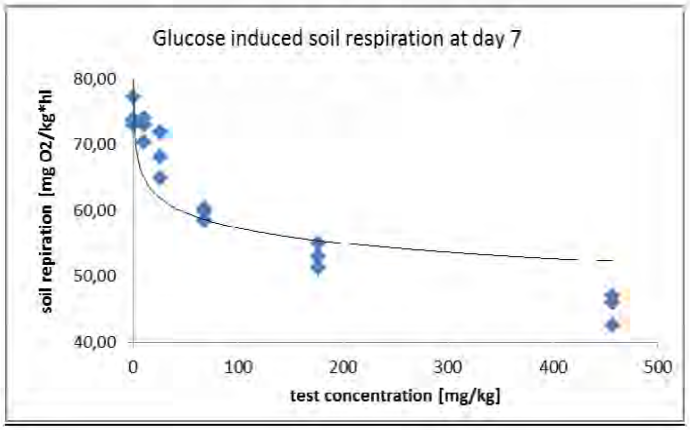
4.2.3.3 At sampling time point 28 days

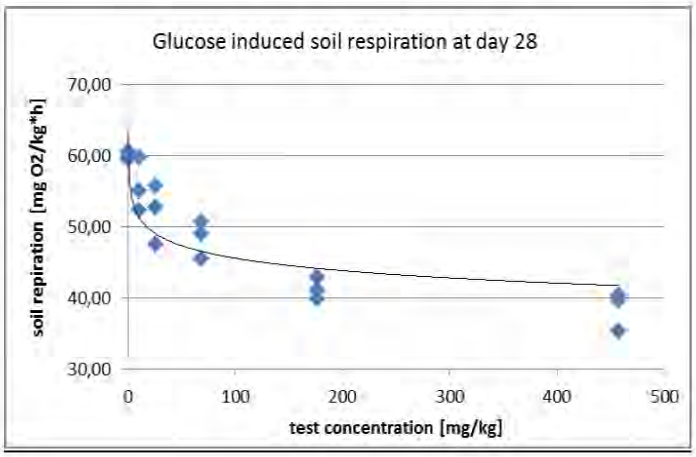
Figure 3: Graphical illustration of the probit analysis of the test substance after an exposure period of 28 days



4.2.4 Effect data







4.2.4.1 Glucose-induced soil respiration

Comparative mean (3 samples per test concentration) glucose induced soil respiration after 12 hours (mg O_2/kg dry matter soil/h):

Mean glucose induced soil respiration after 12 hours (3 samples per test concentration, time points 0, 7 and 28 days)			
Test concentration (nominal)	**At day 0**	**At day 7**	**At day 28**
0 (control)	68.28	74.69	60.00
10 mg/kg dry matter soil	67.72	72.47	55.75
26 mg/kg dry matter soil	63.61	68.33	52.06
68 mg/kg dry matter soil	56.61	59.47	48.42
176 mg/kg dry matter soil	45.14	53.11	41.31
457 mg/kg dry matter soil	39.25	45.22	38.53
1186 mg/kg dry matter soil	40.53	40.50	48.00

4.2.4.2 Soil respiration inhibition

Mean percentage of soil respiration inhibition: x

Inhibition of glucose induced soil respiration after 12 hours (3 samples per test concentration, time points 0, 7 and 28 days)			
Test concentration (nominal)	**At day 0**	**At day 7**	**At day 28**
0 (control)	0%	0%	0%
10 mg/kg dry matter soil	0.8%	3%	7%
26 mg/kg dry matter soil	7%	8.5%	13%
68 mg/kg dry matter soil	17%	20%	19%
176 mg/kg dry matter soil	34%	29%	31%
457 mg/kg dry matter soil	42.5%	39.5%	36%
1186 mg/kg dry matter soil	40.6%	46%	20%

4.2.4.3 Effect concentrations

Summary of the effect concentrations of the test substance:

Time point	EC_{10}	EC_{50}
Day 0	22 mg/kg dry matter soil (CL: 9 – 53)	> 1186 mg/kg dry matter soil
Day 7	21 mg/kg dry matter soil (CL: 14 – 34)	> 1186 mg/kg dry matter soil
Day 28	3 mg/kg dry matter soil (CL: 0.1 – 82)	> 1186 mg/kg dry matter soil

CL: confidence Limits (p=0.95)

4.2.5 Other observed effects: None

4.3 Results of controls: See 4.2.4

4.4 Test with reference substance: Not performed

4.4.1 Concentrations: Not relevant as no test with a reference substance was performed.

4.4.2 Results: Not relevant as no test with a reference substance was performed.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and: The aim of the present study was to investigate the adverse effects of x

	methods	glutaraldehyde on aerobic soil microorganisms by means of the Carbon Transformation Test. Test substance: [redacted], batch Nr. [redacted], purity [redacted]% The test was conducted according to OECD 217, and followed GLP. About 50 kg of soil were collected from [redacted]; the weather conditions were sunshine and 15 °C. The supplier was [redacted]. The soil was defined as silty sand according to German DIN. The soil sample was stored in a closed plastic sack at 4 +/- 2 °C in the dark until test initiation. For testing, the soil sample was dried for one day at room temperature. The sample was then presieved < 10 mm and < 2 mm, and was then moistened to about 28% of the maximal water holding capacity (WHC_{max} = 40.4 +/- 4.3 g/100 g soil); the rest water was about 11% of water content of the delivered soil at test initiation (WC = 10.2 g/100 g dry matter). For preparation of the test mixture, a suitable quantity of soil was placed in a mixer. A defined amount of test substance was added without carrier material, and the mixture was blended. Water was added to 45 +/- 5% of the WHC_{max} and the mixture was mixed again. The test mixtures were incubated up to 28 days in the dark, in test pots closed with a perforated aluminium cap, at a mean temperature of 20.4 °C; the water content was controlled by weighing of the test samples and water loss was regulated by addition of demineralised water. Following concentrations of test substance in soil were tested: 10, 26, 68, 176, 457 and 1186 mg/kg dry matter soil. Sampling time points were day 0, day 7 and day 28. At each time point, 3 samples of 118 g test mixture per test concentration were taken and were supplemented with glucose (about 400 mg/sample). Glucose induced respiration rates were measured for 12 consecutive hours in respiration measurement units OxiTop by measuring the negative pressure resulting from absorbed CO_2 produced by glucose. The calculated respiration rate was expressed as mg O_2 consumed / kg dry mater soil/h). The mean respiration rate of 3 single samples of test mixture per test concentration was determined and was compared with the control value; the percent of deviation from control was calculated. Control consisted of about 118 g of test medium (i.e. soil) without test substance. Statistics: EC_{10} and EC_{50} were determined; confidence limits (p=0.95). The percent effect was determined by means of the Probit analysis according to Finney.
5.2	**Results and discussion**	
5.2.1	NOEC	
5.2.2	EC_{10}	Referring to the test material as such: After 7 days : 21 mg/mg dry matter soil After 28 days : 3 mg/kg dry matter soil Referring to the active ingredient: After 7 days : 10 mg/mg dry matter soil After 28 days : 1.5 mg/kg dry matter soil

5.2.3	EC_{50}	After 7 days > 1186 mg/kg dry matter soil After 28 days > 1186 mg/kg dry matter soil
5.3	**Conclusion**	The carbon transformation test with glutaraldehyde resulted in an EC_{10} after 28 days of 3 mg/kg dry matter soil; the EC_{50} after 28 days was > 1186 mg/kg dry matter soil. The deviation of glucose induced respiration in the blank controls was < 15% at the end of the exposure, confirming the validity of the test.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30.1.2012
Materials and Methods	2.3 Soil was stored 4.5 months which exceeds the maximum three months given in the OECD 217. 4.2.4 Glucose induced soil respiration is plotted after TM III 2011 in order to show the variance in the control data. 4.2.4.2 The soil respiration followed dose response until the second highest concentration. At the highest concentration the inhibition was smaller than in the second and third highest concentrations.
Results and discussion	After TM III 2011 the applicant submitted re-analyses of data where the highest concentration was excluded in order to get a better fit of the response curve (Basf A7.5.1.1App.pdf). The recalculated EC10 and EC50 are 14 and EC50 1401 mg/kg dw. The corresponding values converted to 100% glutaraldehyde are 7 and 700.5 mg a.i./kg dw. The results are converted to organic matter content of 3.4%: 7 x 0.034/0.02278 = 10.45 mg/kg dw 700.5 x 0.034/0.02278 = 1045 mg/kg dw The factor 0.02278 was obtained by multiplying the organic carbon content of the soil with 1.7 in order to get the organic matter content of the soil: 1.34% x 1.7 = 2.278% The results are further converted from dry weight to wet weight soil by dividing with a factor of 1.13: EC_{10} 10.45/1.13 = **9.2 mg a.i./kg ww** EC_{50} 1045/1.13 = **925 mg a.i./kg ww**
Conclusion	Inhibition of carbon transformation was strongest at the start of the test at the higher concentrations. At the two lowest concentrations the inhibition slightly increased with time. Clear inhibitory effects were observed despite anticipated rapid degradation of glutaraldehyde in the soil (half-life 1.7 d in aerobic soil degradation study, Doc IIIA7.2.1).
Reliability	2

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_5_1_1-1: Microbial sample / Inoculum

Criteria	Details
Nature	Silty sand, defined as soil type 5 M [redacted]
Sampling site:	The soil sample was prepared conformely to the specifications of the guideline.
Geographical reference on the sampling site	About 50 kg of soil were collected from [redacted] the weather conditions were sunshine and 15 °C. The supplier was [redacted]
Data on the history of the site	[redacted]
Use pattern	Not specified
Depth of sampling [cm]	About 20 cm
Sand / Silt / Clay content [% dry weight]	Soil defined as silty sand according to German DIN Percentage of sand (i.e. particles > 0.063-2.0 mm) 55.4 +/- 1.1 %
pH	7.1 +- 0.3
Organic carbon content [% dry weight]	1.34 +/- 0.28
Maximal water holding capacity (WHC_{max}; g/100 g)	40.4 +/- 4.3
Nitrogen content [% dry weight]	
Cation exchange capacity [mmol/kg]	12 +/-1
Initial microbial biomass	142.5 mg/kg dry soil matter
Water content of the delivered soil at test initiation (WC; g/100 g dry matter)	10.0
Reference of methods	Determination of the initial microbial biomass: The initial microbiological biomass of the soil was determined by means of the OxiTop according to ISO 14240-1 and to the application report [redacted]): Atmungsaktivität AT4". Determination of the initial water content: According to ISO 11465
Collection / storage of samples	The soil sample was stored in a closed plastic sack at 4 +/- 2 °C in the dark until test initiation
Preparation of inoculum for exposure	The soil sample was dried for one day at room temperature. The sample was then presieved < 10 mm and < 2 mm, and was then moistened to about 28% of the WHC_{max} ; the rest water was about 11% of the water content (WC).
Pretreatment	None

Table A7_5_1_1-2: Test organism

Criteria	Details
Species	Not relevant, see table A7_5_1_1-1

Strain	"
Source	"
Sampling site	"
Laboratory culture	"
Method of cultivation	"
Preparation of inoculum for exposure	"
Pretreatment	"
Initial cell concentration	"

Table A7_5_1_1-3: Test system

Criteria	Details
Culturing apparatus	Test pots closed with a perforated aluminium cap
Number of vessels / concentration	3 samples per test concentration
Aeration device	Aeration was assured by the perforations in the caps.
Measuring equipment	Glucose induced respiration rates were measured for 12 consecutive hours in respiration measurement units OxiTop; in fact, the OxiTop pressure heads measure the negative pressure resulting from absorbed CO_2 produced by glucose.
Test performed in closed vessels	See above

Table A7_5_1_1-4: Application of test substance

Criteria	Details
Application procedure	A suitable quantity of soil was placed in a mixer. A defined amount of test substance was added without carrier material, and the mixture was blended. Water was added to 45 +/- 5% of the WHC_{max} and the mixture was mixed again.
Carrier	None
Concentration of liquid carrier [% v/v]	Not relevant as no carrier was used.
Liquid carrier control	Not relevant as no carrier was used.
Other procedures	None

Table A7_5_1_1-5: Test conditions

Criteria	Details
Organic substrate	For the carbon transformation test, the soil samples were amended with 400 mg glucose per 118 g of test mixture; 1 g quarz sand was used as carrier..
Incubation temperature	20.3 – 20.8 °C (mean: 20.6 °C)

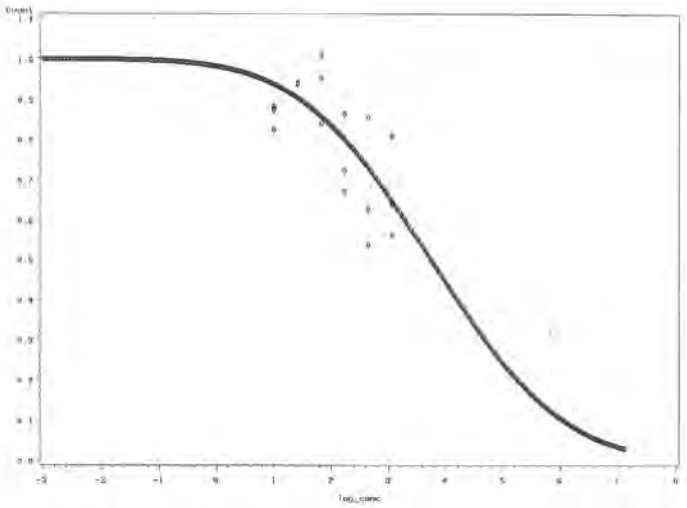
Soil moisture	During the test: 45.5% of the WHC_{max}
Method of soil incubation	The test samples were incubated up to 28 days in the dark; the water content was controlled by weighing of the test samples and water loss was regulated by addition of demineralised water.
Aeration	Aeration was assured by the perforations in the caps.
pH in test mixtures at test initiation	7.3 – 7.4

Section A7.5.1.1 _ 02 Annex Point IIA7.4		Inhibition to microbial activity (terrestrial) Nitrogen Transformation Test	Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2006) [redacted], Determination of the nitrate production in soil (Nitrogen Transformation Test). [redacted] (Unpublished), BPD ID A7.05.1.1_02	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE, the Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 216	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	x
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]%	
3.1.4	Composition of Product	Test substance diluted in water	
3.1.5	Further relevant properties	The test substance was defined as homogeneous and was described as colourless clear liquid, which was miscible at ca. 20 °C. The stability under storage conditions (refrigerator) over the exposure period was guaranteed.	
3.1.6	Method of analysis	Not reported	
3.2	**Reference substance**	No	
3.2.1	Method of analysis for reference substance	Not relevant as no reference substance was tested.	
3.3	**Testing procedure**		
3.3.1	Soil sample / inoculum / test organism	See table A7_5_1_1-1	
3.3.2	Test system	See table A7_5_1_1-3	
3.3.3	Application of TS	See table A7_5_1_1-4	
3.3.4	Test conditions	See table A7_5_1_1-5	

3.3.5	Test parameter	Inhibition of microbial nitrogen transformation Lucerne meal was used as source of nitrogene and was supplied by [redacted]. Lucerne meal contained 42.0 g Carbon /100 g and 3.4 g Nitrogen/100 g; the C/N ratio was 12:1 ([redacted])
3.3.6	Analytical parameter	The soil samples taken at each sampling time point were carefully shaken; distilled water was added and the soil suspensions were shaken again. Following centrifugation, the supernatant of each suspension was stored frozen until nitrate determination. The nitrate determination was based on ion chromatography using an IC system [redacted] apparatus; the reagents were deionized water, a solution of sodium carbonate and sodium hydroxide and a standard solution of nitrate. The analytical monitoring of the nitrate concentrations was performed by [redacted]
3.3.7	Duration of the test	28 days
3.3.8	Sampling	Samples were taken on day 0, 7 and 28 of incubation and were examined for nitrate concentration. For each test concentration and sampling time point, 3 samples were considered (each about 24 g)
3.3.9	Monitoring of TS concentration	Not performed as not of importance for the present type of study and not required by the guideline
3.3.10	Controls	Control without test substance were added to the test series.
3.3.11	Statistics	The dose-response curve was fitted via the probit model to the inhibition values. The curve was used for estimation of the EC10 and EC50 and the confidence limits (95%).
		4 RESULTS
4.1	**Range finding test**	Not performed
4.1.1	Concentration	Not relevant as no range-finding test was performed.
4.1.2	Effect data	Not relevant as no range-finding test was performed.
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 10, 26, 69, 175, 457 and 1186 mg/kg matter soil
4.2.2	Actual concentrations of test substance	See 3.3.9
4.2.3	Concentration/ response curve	
4.2.4	At test initiation	No determination of NO_3 inhibition on day 0 was possible.

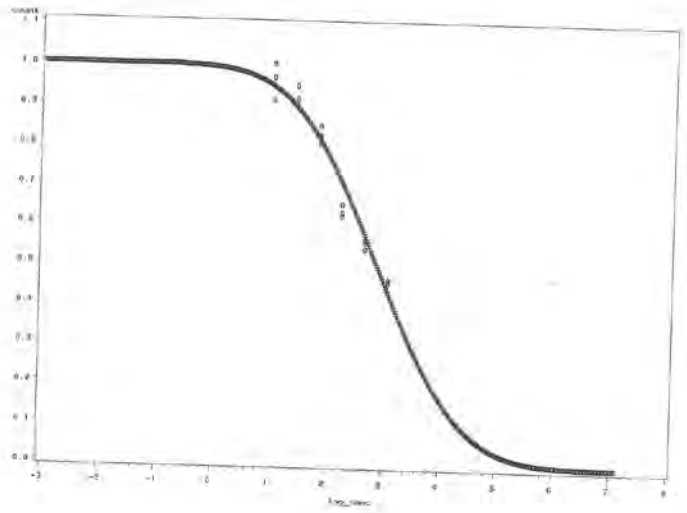
4.2.5 At sampling time point 7 days

Figure 1: Graphical illustration of the probit analysis of the test substance after an exposure period of 7 days:



4.2.6 At sampling time point 28 days

Figure 2: Graphical illustration of the probit analysis of the test substance after an exposure period of 28 days:



4.2.7 Effect data

4.2.8 Nitrate content

Nitrate concentration in test mixture (mg /kg dry matter of soil):

Nitrate concentrations (mg/kg dry matter of soil) for each test mixture, at each sampling time point (3 samples)				
Test concentration (nominal)	**Sample**	**At day 0**	**At day 7**	**At day 28**
0 (control)	1	298	368	422
	2	289	324	369
	3	289	315	365
10 mg/kg dry matter of soil	1	303	297	387
	2	290	278	374
	3	284	294	352
26 mg/kg dry matter of soil	1	309	316	354
	2	299	317	349
	3	297	317	366
69 mg/kg dry matter of soil	1	314	340	311
	2	309	321	319
	3	302	283	328
175 mg/kg dry matter of soil	1	318	291	240
	2	313	244	244
	3	314	227	252
457 mg/kg dry matter of soil	1	318	288	209
	2	314	181	216
	3	309	212	218
1186 mg/kg dry matter of soil	1	315	273	180
	2	313	189	176
	3	313	215	177

4.2.9 Percentage of Inhibition

Mean percentage of inhibition of nitrate production in test mixture:

Inhibition of nitrate production (%) (mean of 3 samples per test concentration and time point)			
Test concentration (nominal)	**At day 0**	**At day 7**	**At day 28**
0 (control)	0	0	0
10 mg/kg dry matter of soil	0	14	4
26 mg/kg dry matter of soil	-3	6	8
69 mg/kg dry matter of soil	-6	6	17
175 mg/kg dry matter of soil	-8	24	36
457 mg/kg dry matter of soil	-7	32	44
1186 mg/kg dry matter of oil	-7	33	54

4.2.10 Summary of the effect concentrations of the test substance

Time point	EC_{10}	EC_{50}
Day 0	Not applicable	Not applicable
Day 7	27 mg/kg dry matter of soil (CL: 5 – 143)	> 1186 mg/kg dry matter of soil (CL: 773 – 37940)
Day 28	23 mg/kg dry matter of soil (CL: 15 – 36)	720 mg/kg dry matter of soil (CL: 571 – 909)

CL: confidence Limits (p=0.95)

4.3 Results of controls See 4.2.6

4.4 Test with reference substance Not performed

4.4.1 Concentrations Not relevant as no test with a reference substance was performed.

4.4.2 Results Not relevant as no test with a reference substance was performed.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the adverse effects of glutaraldehyde on nitrate production by aerobic soil microorganisms using the Nitrate Transformation Test. Lucerne meal as source of nitrogen.

Test substance: [redacted], batch Nr. [redacted], purity [redacted]%

The test was conducted according to OECD 216, and followed GLP.

About 50 kg of soil were collected from [redacted] the weather conditions were sunshine and 15 °C. The supplier was [redacted]. The soil was defined as silty sand according to German DIN. The soil sample was stored in a closed plastic sack at 4 +/- 2 °C in the dark until test initiation.

For testing, the soil sample was dried for one day at room temperature The sample was then presieved < 10 mm and < 2 mm, and was then

		moistened to about 28% of the maximal water holding capacity (WHC_{max} = 40.4 +/- 4.3 g/100 g soil); the rest water was about 11% of water content of the delivered soil at test initiation (WC = 10.2 g/100 g dry matter). For the nitrogen transformation test, amounts of test substance defined to adjust the scheduled test concentrations were added without carrier to suitable quantities of soil; one g of lucerme meal was added as well as 16.4 ml distilled water (to get 45 +/- 5% of the WHC_{max}). The test mixtures were incubated up to 28 days in the dark, in test pots closed with a perforated aluminium cap, at a mean temperature of 20.6 °C. The water content was controlled by weighing the test samples, and water loss was regulated by addition of demineralised water. Control consisted of test medium (i.e. soil) without test substance. Following concentrations of test substance in soil were tested: 10, 26, 69, 175, 457 and 1186 mg/kg dry matter soil. Sampling time points were day 0, day 7 and day 28; at each time point 3 samples per test concentration were considered. The content of nitrate in aqueous soil extracts obtained from the test mixture samples was determined by means of ion chromatography using an IC system [redacted] apparatus. The reagents were deionized water, a solution of sodium carbonate and sodium hydroxide and a standard solution of nitrate. The nitrate contents in the test mixtures were compared to those of the controls, and the percentage of inhibition of the nitrate production in the treated soil samples was calculated.
5.2	**Results and discussion**	The microbial nitrogen transformation process in soil was not affected by glutaraldehyde when applied at a concentration of 10 mg/kg dry matter of soil. The prolonged application of glutaraldehyde at test concentrations above 10 mg/kg dry matter of soil resulted in dose-dependent increased inhibition of nitrate production by soil microorganisms, which resulted in an EC_{50} after 28 days of 720 mg/kg dry matter of soil.
5.2.1	NOEC	10 mg /kg dry soil matter
5.2.2	EC_{10}	After 7 days: 27 mg/mg dry matter of soil After 28 days: 23 mg/kg dry matter of soil
5.2.3	EC_{50}	After 7 days > 1186 mg/kg dry matter of soil After 28 days: 720 mg/kg dry matter of soil
5.3	**Conclusion**	The nitrate transformation test resulted in an EC_{10} after 28 days of 23 mg/kg dry matter soil and an EC_{50} after 28 days of 720 mg/kg dry matter soil. The deviation of formed nitrate in the blank controls was < 15% at the end of the exposure, confirming the validity of the test.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	No

Section A7.5.1.1 _ 02	Inhibition to microbial activity (terrestrial)
Annex Point IIA7.4	Nitrogen Transformation Test

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 9th, 2009
Materials and Methods	2.3 Soil was stored 4.5 months which exceeds the maximum three months given in the OECD 216.
Results and discussion	The EC_{10} and EC_{50} after 28 days were 11.7 and 366 mg/kg dry mass of the soil based on the nominal concentrations. The result is expressed as 100% glutaraldehyde. The results are converted to organic matter content of 3.4%: 11.7 x 0.034/0.02278 = 17.5 mg/kg dw 366 x 0.034/0.02278 = 546 mg/kg dw The results are further converted from dry weight to wet weight soil by dividing with a factor of 1.13: EC_{10} 17.5/1.13 = **15.4 mg a.i./kg ww** EC_{50} 546/1.13 = **483 mg a.i./kg ww**
Conclusion	Glutaraldehyde is assumed to disappear from the soil in a few days (half-life 1.7 d in aerobic soil degradation study, Doc IIIA7.2.1), but it still seems to inhibit nitrogen transformation and the inhibition is increasing towards the end of test.
Reliability	2
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_5_1_1-1: Microbial sample / Inoculum

Criteria	Details
Nature	Silty sand, defined as soil type 5 M ([redacted]
Sampling site:	The soil sample was prepared conformely to the specifications of the guideline.
Geographical reference on the sampling site	About 50 kg of soil were collected from [redacted] the weather conditions were sunshine and 15 °C. The supplier was [redacted]
Data on the history of the site	[redacted]
Use pattern	Not specified
Depth of sampling [cm]	About 20 cm
Sand / Silt / Clay content [% dry weight]	Soil defined as silty sand according to German DIN Percentage of sand (i.e. particles > 0.063-2.0 mm) 55.4 +/- 1.1 %
pH	7.1 +- 0.3
Organic carbon content [% dry weight]	1.34 +/- 0.28
Maximal water holding capacity (WHC_{max}; g/100 g)	40.4 +/- 4.3
Nitrogen content [% dry weight]	
Cation exchange capacity [mmol/kg]	12 +/-1
Initial microbial biomass	142.5 mg/kg dry soil matter
Water content of the delivered soil at test initiation (WC; g/100 g dry matter)	10.2
Reference of methods	Determination of the initial microbial biomass according to ISO 14240-1 Determination of the initial water content according to ISO 11465
Collection / storage of samples	The soil sample was stored in a closed plastic sack at 4 +/- 2 °C in the dark until test initiation
Preparation of inoculum for exposure	The soil sample was dried for one day at room temperature. The sample was then presieved < 10 mm and < 2 mm, and was then moistened to about 28% of the WHC_{max}; the rest water was about 11% of the water content (WC).
Pretreatment	None

Table A7_5_1_1-2: Test organism

Criteria	Details
Species	Not relevant, see table A7_5_1_1-1
Strain	"
Source	"
Sampling site	"
Laboratory culture	"

Method of cultivation	"
Preparation of inoculum for exposure	"
Pretreatment	"
Initial cell concentration	"

Table A7_5_1_1-3: Test system

Criteria	Details
Culturing apparatus	Test pots closed with a perforated aluminium cap
Number of vessels / concentration	3 samples per test concentration
Aeration device	Aeration was assured by the perforations in the caps.
Measuring equipment	Analyse of nitrate contents in aqueous soil extracts: Content of nitrate was determined by means of ion chromatography using an IC system [redacted] apparatus. The reagents were deionized water, a solution of sodium carbonate and sodium hydroxide and a standard solution of nitrate
Test performed in closed vessels	See above

Table A7_5_1_1-4: Application of test substance

Criteria	Details
Application procedure	A suitable quantity of soil was placed in a mixer. A defined amount of test substance was added without carrier material to adjust the scheduled test concentrations. One g of Luzerne meal was added as wells 16.4 ml distilled water (to get 45 +/- 5% of the WHC_{max}).
Carrier	None
Concentration of liquid carrier [% v/v]	Not relevant as no carrier was used.
Liquid carrier control	Not relevant as no carrier was used.
Other procedures	None

Table A7_5_1_1-5: Test conditions

Criteria	Details
Organic substrate	For the nitrogen transformation test, the soil samples were amended with about 1 g lucerne meal a source of nitrogen.
Incubation temperature	20.3 – 20.8 °C (mean: 20.6 °C)
Soil moisture	18.2 g /100 g dry soil matter
Method of soil incubation	The test samples were incubated up to 28 days in the dark. The water content was controlled by weighing the test samples, and water loss was regulated by addition of demineralised water.

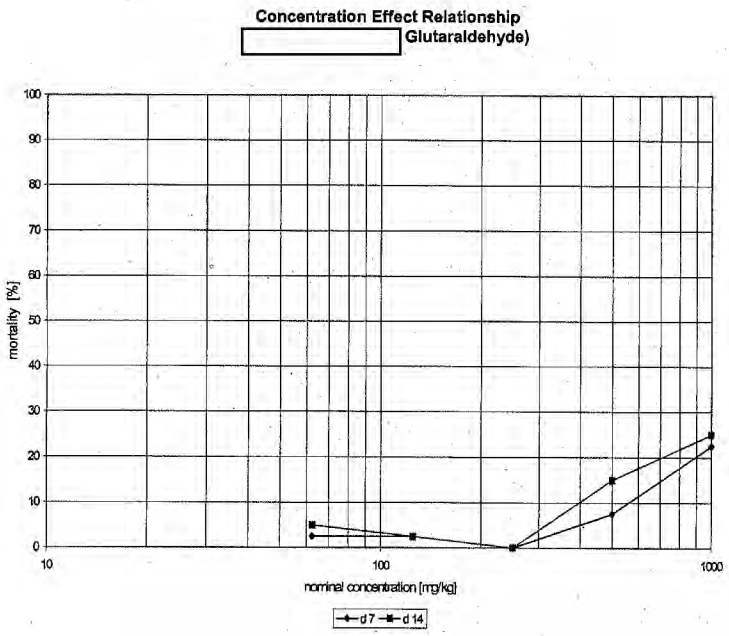
Aeration	Aeration was assured by the perforations in the caps.
pH in test mixtures at test initiation	7.2 – 7.4

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (2002) [redacted]% Glutaraldehyde) Determination of the acute letal effect of chemicals on the earthworm Eisenia foetida. [redacted] (Unpublished), BPD ID A7.05.1.2_01
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD 207, 1984
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 METHOD
3.1	**Test material**	[redacted]% Glutaraldehyde)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 [redacted]
3.1.3	Purity	[redacted]% ([redacted]% water[redacted])
3.1.4	Composition of Product	See above
3.1.5	Further relevant properties	Miscible in water
3.1.6	Method of analysis	No data
3.2	**Reference substance**	Yes, 2-Chloroacetamide
3.2.1	Method of analysis for reference substance	No data
3.3	**Testing procedure**	
3.3.1	Preparation of the test substance	See table A7_5_1_2-1
3.3.2	Application of the test substance	The test substance was mixed to the test substrate as a stock solution prepared with demineralized water, at a ratio of 1000 ml stock solution to 3000 g dry test substrate.
3.3.3	Test organisms	See table A7_5_1_2-2
3.3.4	Test system	See table A7_5_1_2-3

3.3.5	Test conditions	See table A7_5_1_2-4	
3.3.6	Test duration	14 days	
3.3.7	Test parameter	Mortality, body weight	
3.3.8	Examination	Examination was performed after 7 and 14 days	
3.3.9	Monitoring of test substance concentration	No	x
3.3.10	Statistics	No statistical calculations were done	

4 RESULTS

4.1	**Filter paper 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	**Soil test**		
4.2.1	Initial concentrations of test substance	0, 62.5, 125, 250, 500 and 1000 mg/kg dry weight artificial soil	x
4.2.2	Effect data (Mortality)	See table A7_5_1_2-5 and table A7_5_1_2-6	
4.2.3	Concentration / effect curve		

4.2.4 Other effects

Body weight of the earthworms:

Test Concentration (mg/kg)	Mean weight at test initiation (mg/worm)	Mean weight at test ending (mg/worm)
Control	331 (Ni=40*)	336 (Ne=38**)
62.5	295 (Ni= 40)	304 (Ne=38)
125	327 (Ni=40)	324 (Ne=39)
250	324 (Ni=40)	326 (Ne=40)
500	340 (Ni=40)	307 (Ne=34)
1000	326 (Ni=40)	267 (Ne=30)

*, Ni = Number of worms at test initiation.

**, Ne = Number of worms at test ending, i.e. after 14 days of exposure.

Inhibtion of the biomass:

Inhibition (%) = 100 – ((day 14/day 0)*100)

Test Conc. (mg/kg)	Mean value biomass/animal at test initiation (mg)	Mean value biomass/animal at test ending (mg)	Inhibition (%)
Control	331 (Ni=40*)	336 (Ne=38**)	-1.5
62.5	295 (Ni= 40)	304 (Ne=38)	-3.1
125	327 (Ni=40)	324 (Ne=39)	0.9
250	324 (Ni=40)	326 (Ne=40)	-0.6
500	340 (Ni=40)	307 (Ne=34)	9.7
1000	326 (Ni=40)	267 (Ne=30)	18.1
Mean*	322	306	
S.D.*	17	24	
Max val.*	340	326	
Min. val.*	295	267	

*, Mean, Standard Deviation, Maximal and Minimal values refer to the treated samples (i.e. without the control group)

The values of the table above clearly show that the exposure of earthworm to test substrate containing the test substance [redacted] ([redacted]% Glutaraldehyde) had a negative impact on worm biomass, resulting in nearly 20% inhibition at a nominal test concentration of 1000 mg/kg substrat. No further behavioural or morphological effects

were seen.

4.3	**Results of controls**	
4.3.1	Mortality	Two animals died during the exposure period of 14 days; the first case of death was observed on day 7 (See table A7_5_1_2-5). Therefore a mortality of 2.5% was reported for the control group at the end of the experiment.
4.3.2	Number/ percentage of earthworms showing adverse effects	No adverse effect observed
4.3.3	Nature of adverse effects	No adverse effect observed
4.4	**Test with reference substance**	Performed (date of last control experiment: 26 Sep 2002, [redacted], non GLP).
4.4.1	Concentrations	Not specified
4.4.2	Results	LC50 (14 days) = 16.5 mg/kg test substrat.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The aim of the present study was to investigate the toxicity of [redacted] (Glutardialdehyde [redacted]%) to the Earthworm Eisenia foetida. Test substance: [redacted] ([redacted]% glutaraldehyde), batch No [redacted], purity [redacted]% ([redacted]% water[redacted]) Guideline: OECD 207, 1984; GLP Clitellated adult earthworms (Eisenia fetida; less than one year old)) were exposed during 14 days to [redacted]-treated artificial soil.

The test concentrations were 0, 62.5, 125, 250, 500 and 1000 mg/kg dry weight artificial soil. 4 replicates/concentration with 10 worms each were tested. The animals were checked after 7 and after 14 days for mortality. Further observed sublethal parameters were the behaviour and the body weight.

The physico-chemical parameters over the testing period were as follows:

Temperature [°C]	21.8 to 22.7 °C
pH	6.5
Moisture content in the substrat	31.9 g /100 g of dry weight (mean value)

5.2 Results and discussion

Mortality after 14 days was as follows:

Test Substance Concentration (nominal) [mg/kg artificial soil]	Mortality 14 d	
0 (control)	2/40*	5%
62.5	2/40	5%
125	1/40	2.5%
250	0/40	0%
500	6/40	15%
1000	10/40	25%

Test initiation: 40 animals/ test concentration

Body weight and inhibition of biomass after 14 days:

Test Concentration (mg/kg)	Mean weight at test initiation (mg/worm)	Mean weight at test ending (mg/worm)	Inhibition (%)
Control	331 (Ni=40*)	336 (Ne=38**)	-1.5
62.5	295 (Ni= 40)	304 (Ne=38)	-3.1
125	327 (Ni=40)	324 (Ne=39)	0.9
250	324 (Ni=40)	326 (Ne=40)	-0.6
500	340 (Ni=40)	307 (Ne=34)	9.7
1000	326 (Ni=40)	267 (Ne=30)	18.1

*, Ni = Number of worms at test initiation.

**, Ne = Number of worms at test ending, i.e. after 14 days of exposure.

5.2.1 LC_0 250 mg/kg soil (nominal)

5.2.2 LC_{50} > 1000 mg/kg soil (nominal)

5.2.3 LC_{100} > 1000 mg/kg soil (nominal)

5.3 Conclusion

The exposure of earthworm to test substrate containing the test substance [redacted]% glutaraldehyde) had a negative impact on worm biomass, resulting in nearly 20% inhibition at a nominal test concentration of 1000 mg/kg substrat. No further behavioural or morphological effects were seen. Test substance-related mortality was observed at the two highest tested concentrations of 500 (15%) and 1000 mg/kg soil (25%); at 250 mg/kg soil, no mortality was seen. The mortalities of 5 and 2.5% observed respectively at 62.5 and 125 mg/kg

		soil were not test substance-related and were in the range of control-mortality. Mortality within the control group was < 10% and therefore the validity criteria for acute earthworm test according to OECD 207 were fulfilled. The LC50 was > 1000 mg/kg soil.
5.3.1	Other Conclusions	
5.3.2	Reliability	**1**
5.3.3	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	17.4.2009
Materials and Methods	3.3.9 Analytical verification of test concentrations is not required in the OECD 207. It is, however, likely that glutaraldehyde disappeared rapidly from the soil on the basis of the soil metabolism study where a half-life of 1.7 d was determined. 4.2.1 According to the OECD 207 one test concentration should result in total mortality. The highest mortality was 25% in the highest test concentration.
Results and discussion	9.7% and 18.1% weight loss was observed at the two highest test concentrations. LC50: > 500 mg/kg dw soil based on the nominal concentrations and 100% Glutaraldehyde The result is converted to organic matter content of 3.4%. The organic matter content in the test is assumed to be 10% (artificial soil contains 10% sphagnum peat). 500 x 0.034/0.1 = 170 mg/kg dw The result is converted to wet weight by dividing with a factor of 1.13 (EUSES manual) 170/1.13 = 150 mg/kg ww **LC50 150 mg a.i./kg ww**
Conclusion	Glutaraldehyde is slightly toxic to the earthworm *Eisenia foetida.*
Reliability	2
Acceptability	Acceptable
Remarks	
	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_5_1_2-1: Preparation of TS solution

Criteria	**Details**
Type and source of dilution water	Demineralized water
Alkalinity / Salinity	Not specified
Hardness	Not specified
pH	Not specified
Oxygen content	Not specified
Conductance	Not specified
Holding water different from dilution water	Not relevant
In case of the use of an organic solvent	
Dispersion	Not relevant
Vehicle	Not relevant
Concentration of vehicle	Not relevant
Vehicle control performed	Not relevant
Other procedures	None

Table A7_5_1_1-2: Test organisms

Criteria	**Details**
Species/strain	Eisenia foetida
Source of the initial stock	[redacted]
Culturing techniques	Prior being used for the test, the animals were kept in boxes and were fed with horse manure
Age/weight	Between 2 and 12 month old (i.e. adults, less than one year old) Mean weight/animal about 324 mg at test initiation
Pre-treatment	Prior starting the test, the animals were subjected to a 24 h - period of adaptation at test conditions without test substance.

Table A7_5_1_1-3: Test system

Criteria	**Details**
Artificial soil test substrate	Composition of the test substrate:

<table>
<tr><td></td><td>69.6% quartz sand ([redacted]
20 % kaolin clay (from [redacted]
10% sphagnum-peat (from [redacted]
0.5% chalk ($CaCO_3$) (from [redacted]</td></tr>
<tr><td>Test mixture</td><td>
<table>
<tr><th>Amount of artificial soil test substrate</th><th>Added test substance (stock solution)*</th><th>Resulting test concentrations (nominal)</th></tr>
<tr><td>3000 g</td><td>1000 ml of a 3 g/l stock solution (corresponding to 3000 mg TS**)</td><td>1000 mg/kg soil weight</td></tr>
<tr><td>3000 g</td><td>1000 ml of a 1.5 g/l stock solution (corresponding to 1500 mg TS)</td><td>500 mg/kg soil weight</td></tr>
<tr><td>3000 g</td><td>1000 ml of a 0.75 g/l stock solution (corresponding to 750 mg TS)</td><td>250 mg/kg soil weight</td></tr>
<tr><td>3000 g</td><td>1000 ml of a 0.375 g/l stock solution (corresponding to 375 mg TS)</td><td>125 mg/kg soil weight</td></tr>
<tr><td>3000 g</td><td>1000 ml of a 0.187 g/l stock solution (corresponding to 187.5 mg TS)</td><td>62.5mg/kg soil weight</td></tr>
</table>
*, Demineralized water was used for the preparation of the gradual series of stock solutions; **, Mean test substance.
</td></tr>
<tr><td>Size, volume and material of test container</td><td>One-liter glass jars with glass lids</td></tr>
<tr><td>Amount of artificial soil (kg)/ container</td><td>750 g</td></tr>
<tr><td>Nominal levels of test concentrations</td><td>0, 62.5, 125, 250, 500 and 1000 mg/kg soil weight</td></tr>
<tr><td>Number of replicates/concentration</td><td>4</td></tr>
<tr><td>Number of earthworms/test concentration</td><td>40</td></tr>
<tr><td>Number of earthworms/container</td><td>10</td></tr>
<tr><td>Light intensity</td><td>400 to 800 Lux</td></tr>
<tr><td>Test performed in closed vessels due to significant volatility of test substrate</td><td>Glass jars with glass lids were used but it was not specified whether the vessels were closed of open.</td></tr>
</table>

Table A7_5_1_2-4: Test conditions

Criteria	**Details**
Test temperature	Ranging from 21.9 to 22.6 °C during the adaptation period (deviation of the max. and min. values: 0.7 °C) Ranging from 21.8 to 22.7 °C during the exposure period (deviation of the max. and min. values: 0.9 °C)

<table>
<tr><td>Moisture content</td><td>Calculated water content of the test substrate in the adaptation phase:
32.5 g/100 g of dry weight.
Measured water content of the test substrate in the exposure phase:
- At test starting: 32.4 g/100 g of dry weight (measured in rests of the control accretion)
- - At test ending: 31.3 g/100 g of dry weight (measured in a separate vessel close to the test vessels)
- Mean value: 31.9 g/100 g of dry weight
Water content of the dry test substrate:
1.9 g/100 g of dry weight, which was not considered by the moisture during the test.</td></tr>
<tr><td>pH</td><td>6.5 (dry test substrate)</td></tr>
<tr><td>Adjustment of pH</td><td>Yes, 0.5 % chalk ($CaCO_3$) were added to adjust the pH of the artificial soil to the allowed pH range</td></tr>
<tr><td>Light intensity / photoperiod</td><td>Light intensity at the beginning of the adaptation phase: 742 Lux
Light intensity at the beginning of the exposure phase:
<table><tr><td>Localization</td><td>Light Intensity</td></tr><tr><td>Middle</td><td>797</td></tr><tr><td>Left-front</td><td>767</td></tr><tr><td>Left-back</td><td>669</td></tr><tr><td>Right-front</td><td>685</td></tr><tr><td>Right-back</td><td>626</td></tr><tr><td>Mean value</td><td>709</td></tr></table></td></tr>
<tr><td>Relevant degradation products</td><td>No data</td></tr>
</table>

Table A7_5_1_2-5: Mortality data

Nominal Test Substance Concentration (TWA concentrations) [mg/kg artificial soil]	**Mortality**			
	Number		**Percentage**	
	7 d	**14 d**	**7 d**	**14 d**
0 (control)	1/40	2/40	2.5%	5%
62.5 (11)	1/40	2/40	2.5%	5%
125 (22)	1/40	1/40	2.5%	2.5%
250 (44)	0/40	0/40	0%	0%

500 (87)	3/40	6/40	7.5%	15%
1000 (175)	9/40	10/40	22.5%	25%
Temperature [°C]	21.8 to 22.7 °C			
pH	6.5			
Moisture content	31.9 g /100 g of dry weight (mean value)			

Table A7_5_1_2-6: Effect data

	14 d [mg/kg soil][1]	**95 % c.l.**
LC_0	250 (n)	-
LC_{50}	> 1000 (n)	-
LC_{100}	> 1000 (n)	-

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

Table A7_5_1_2-7: Validity criteria for acute earthworm test according to OECD 207

	fulfilled	**Not fulfilled**
Mortality of control animals < 10%	**yes**	

Terrestrial plant toxicity

Acute toxicity glutaraldehyde to Tomato (*Lycopersicon esculentum*) and Radish (*Raphanus sativus*)

			Official use only
		1 REFERENCE	
1.1	**Reference**	Lange JH, Haarmann F, Mago JL, Marchese JR, Rizzo MA, Yuskovic WJ, Murnock JM, Luteran TM (1999) A study of root elongation using tomato and radish seeds: Evaluation of growth, temperature and pH, and toxicity for cacodylic and glutaraldehyde. Fresenius Envir. Bull. 8: 37-44 (Published), BPD ID A7.05.1.3_01	
1.2	**Data protection**	No	
1.2.1	Data owner	Not relevant as published data	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	The study followed the basic requirements of the OPPTS public draft 850.4200.	
2.2	**GLP**	No	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Glutaraldehyde [redacted]	
3.1.1	Lot/Batch number	None provided	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	purity [redacted]% (ca. [redacted]% water, [redacted])	x
3.1.4	Composition of Product	See above	
3.1.5	Further relevant properties	No data	
3.1.6	Method of analysis	No data	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant	
3.3	**Reference substance**	For purpose of comparison, cacodylic acid (reagent grad) obtained from [redacted] was tested.	
3.3.1	Method of analysis for reference substance	No data	
3.4	**Testing procedure**		
3.4.1	Dilution water	Distilled water	
3.4.2	Test plants	The seed of tomato (Lycopersicon esculentum; Solanaceae) and radish (Raphanus sativus; Brassicaceae) were obtained from [redacted]	
3.4.3	Test system	The test was based on a soilless culture, using plastic Petri plates (10	

		cm) containing Whatman No.1 filter paper. For toxicity testing, each experiment consisted of 4 plates with a total of 20 seeds (i.e. 5 seeds/plate); 2 to 3 replicates were done for each experiment. Each plate was filled with 5 ml distilled water (control) or 5 ml of the test solution. The seeds were soaked in distilled water for 15 minutes and thereafter they were transferred to the test plates. The seeds were incubated in the plates in the dark.	
3.4.4	Test conditions	Toxicity testing was performed at 25 °C an pH 7	
3.4.5	Test duration	120 hours (i.e. 5 days)	
3.4.6	Test parameter	Root length: measured to 1mm	
3.4.7	Sampling	See above	
3.4.8	Method of analysis of the plant material	The present study was based on the roots elongation toxicity test methods, presented by Wang W (Wang W, Root elongation method for toxicity testing of organic and inorganic pollutants. Environ. Toxicol. Chem. 6: 409-414, 1987) and Linder G et al. (Linder G, Greene JC, Ratsch H, Nwosu J, Smith S, Wilborn D, Seed germination and root elongation toxicity tests in hazardous waste site evaluation: methods development and aplications. Plants for Toxicity Assessment, ASTM STP 1019, Wang W, Gorsuch JW, Lower WR Eds., American Society for testing and Materials, Philadelphia: 177-187, 1990).	
3.4.9	Quality control	Yes	x
3.4.10	Statistics	The statistical assessment was based on two-fold and confidence limits procedures.	
		4 RESULTS	
4.1	**Results test substance**		
4.1.1	Toxicity of glutaraldehyde to radish	An EC_{50} of 280 ppm was determined on the basis of root length.	
4.1.2	Toxicity of glutaraldehyde to tomato	An EC_{50} of 340 ppm was determined on the basis of root length.	
4.2	**Test with reference substance**		
4.2.1	Toxicity of cacodylic acid to radish	An EC_{50} of 0.797 ppm was determined on the basis of root length.	
4.2.2	Toxicity of cacodylic acid to tomato	An EC_{50} of 7.14 ppm was determined on the basis of root length.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The authors tested two chemicals, glutaraldehyde and cacodylic acid, for their toxicity to terrestrial plants. Test substance: Glutaraldehyde [redacted]% aq. solution (EM grade), from [redacted], purity [redacted]% (ca. [redacted]% water [redacted]) The test method was based on the roots elongation toxicity test methods, presented by Wang W (Wang W, Root elongation method for toxicity	

testing of organic and inorganic pollutants. Environ. Toxicol. Chem. 6: 409-414, 1987) and Linder G et al. (Linder G, Greene JC, Ratsch H, Nwosu J, Smith S, Wilborn D, Seed germination and root elongation toxicity tests in hazardous waste site evaluation: methods development and aplications. Plants for Toxicity Assessment, ASTM STP 1019, Wang W, Gorsuch JW, Lower WR Eds., American Society for testing and Materials, Philadelphia: 177-187, 1990). The test followed the basic requirements of the OPPTS public draft 850.4200. It was not specified whether the test was conducted according to GLP or not.

The seed of tomato (Lycopersicon esculentum; Solanaceae) and radish (Raphanus sativus; Brassicaceae) were obtained from [redacted]

The test was based on a soilless culture, using plastic Petri plates (10 cm) containing Whatman No.1 filter paper. For toxicity testing, each experiment consisted of 4 plates with a total of 20 seeds (i.e. 5 seeds/plate); 2 to 3 replicates were done for each experiment. Each plate was filled with 5 ml distilled water (control) or 5 ml of the test solution. The seeds were soaked in distilled water for 15 minutes and thereafter they were transferred to the test plates. the seeds were incubated in the plates in the dark.

Toxicity testing was performed at 25 °C at pH 7. The test period was 120 h. The root length measured to 1mm was considered as parameter for determination of the effect concentration (EC_{50})

The statistical assessment was based on two-fold and confidence limits procedures.

5.2	**Results and discussion**	
5.2.1	EC_{50} for radish	280 ppm (based on root length)
5.2.2	EC_{50} for tomato	340 ppm. (based on root length)
5.3	**Conclusion**	Glutaraldehyde showed similar toxicitx for both radish and tomato seeds, but was less toxic than cacodylic acid.
5.3.1	Reliability	**2**
5.3.2	Deficiencies	The reported data can be considered as scientifically acceptable as the test method following the basic requirements of the OPPTS public draft 850.4200.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 6th, 2009
Materials and Methods	The report was based on an article in Fresenius Envir. Bull. 8:037-044. The test was insufficiently reported. 3.1.3 Identity of glutaraldehyde was not specified in the article. 3.4.2 No mono-cotyledon species was tested. 3.4.3 Test was conducted in soilless cultures. According to the OECD 208 the test should be conducted in soil. Test concentration was not given. 3.4.9 Quality control was not mentioned in the article.
Results and discussion	The EC50 of 280 mg/l for radish and 340 mg/l for tomato were reported, but it's unclear how these values were derived as only single concentration was tested. Seedling emergence and mean survival of emerged control seeds were not reported.
Conclusion	The results are not regarded reliable due to insufficient reporting and invalid test system.
Reliability	3
Acceptability	Not acceptable
Remarks	
	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	

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Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (2010) [redacted] Determination of the effect of chemicals on the emergence and growth of higher plants. [redacted] (Unpublished), BPD ID A7.5.1.3_02, 28 July 2010
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	Not applicable since supplier is data owner
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes; the study was conducted according to the OECD TG 208
2.2	**GLP**	Yes; with certificate
2.3	**Deviations**	No
		3 METHOD
3.1	**Test material**	[redacted]
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted] %
3.1.4	Composition of Product	[redacted] g a.i. in water
3.1.5	Further relevant properties	Liquid, homogeneous, unlimited solubility in water, storage at room temperature under nitrogen, stability under storage conditions over the testing period ensured.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	No applicable
3.4	**Testing procedure**	
3.4.1	Dilution water	demineralized water
3.4.2	Test plants	*Brassica napus, Vicia sativa, Avena sativa* See table A7_5_1_3-1

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3.4.3 Test system — See table A7_5_1_3-2

3.4.4 Test conditions — see table A7_5_1_3-3

3.4.5 Test concentrations — Nominal test concentrations:

0, 62.5, 125, 250, 500 and 1000 mg/kg dry matter of soil (nominal concentrations based on test substance mass without correction for purity).

Preparation of the test solutions:

Test concentration nominal [mg/kg DM soil]	Test substance stock solution [mL]	Demineralised water [mL]
0	0	98
62.5	5.5	92.5
125	10.9	87.1
250	21.8	76.2
500	44	54
1000	88	10

Analytical verification:

The concentration of the test substance in the initial stock solution was analytically determined by TOC-analysis. A recovery rate of 99 % of the nominal concentration was found. This confirms the correct initial weight for the preparation of the stock solution.

3.4.6 Test duration — 19 days

3.4.7 Test parameter — Emergence rate, growth (shoot length and dry weight)

3.4.8 Sampling — The number of emerged seedlings was documented daily. In case of emergence of more than 5 seedlings in the control pots of the 3 species they were thinned to 5 plants in all test pots.

The total numbers of emerged seedlings during the exposure were documented.

After 14 days when 50 % of the plants of the controls have been emerged, the observation period was terminated and the data of shoot length and dry weight were determined.

3.4.9 Validity criteria — 1. Seedling emergence is at least 70 % in the controls.

2. No visible phytotoxic effects during the exposure in the controls are observed.

3. The mean survival of emerged control seedlings is at least >90% at the end of exposure.

4. The environmental conditions for all test pots are identical.

3.4.10 Statistics

3.4.10.1 NOEC calculation

Statistical analyses for the NOEC calculation were based on following methods:

Parameter	Statistical test used
Dry weight	comparison of each group with the control using Dunnett's Test (one-sided) for the hypothesis of equal means
Shoot length	comparison of each group with the control using Dunnett's Test (one-sided) for the hypothesis of equal means
Emergence	A pair-wise comparison of the dose groups with the control group using the Fisher's exact test (one-sided) for the hypothesis of equal proportions. To consider the variability between the pots a pair-wise comparison of the dose groups with the control group was performed using the Wilcoxon-test (one-sided) for the hypothesis of equal medians.

3.4.10.2 EC_{25} and EC_{50} calculation

For the emergence no monotone concentration response exists. Only one significant result was observed for oats at the lowest concentration while the results of the other concentrations were near to the results of the control group. Therefore no EC was calculated.

For the dry weight and the shoot length of the oats also no monotone concentration response exists. Therefore no EC was calculated.

For the shoot length of the oilseed rape and the vetch the EC_{50} was not determined because it lies outside the experimental range.

4 RESULTS

4.1 Preliminary test

The emergence rates of the seeds were determined in a preliminary test to be as follows:

Plant	Emergence rate as %
Oats (*Avena sativa*)	80 % after 8 days
Oilseed rape (*Brassica napus*)	100% after 8 days
Vetch (*Vicia sativa*)	100% after 8 days

4.2 Results test substance

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4.2.1 Applied initial concentration

Test concentration nominal [mg/kg DM soil]	actual concentrations for each plant species (Based on actual weights and volumes)		
	Oats (*Avena sativa*)	Oilseed rape (*Brassica napus*)	Vetch (*Vicia sativa*)
0			
62.5	62.5 (+0.7%)*	62.5 (+0.7%)	62.5 (+0.7%)
125	125 (-0.2 %)	125 (-0.2%)	125 (-0.2 %)
250	250 (-0.2%)	250 (-0.2%)	250 (-0.2%)
500	500 (+0.7%)	500 (+0.7%)	500 (+0.7%)
1000	1000 (+0.7%)	1000 (-0.5%)	1000 (+0.7%)

*, The numbers in parenthesis show the deviation of the actually prepared test concentrations to the nominal concentrations

The actual concentrations deviated less than 1% from the nominal concentrations.

4.2.2 Emergence rate

[redacted] had no statistical significant effect on the germination capacity of the three plant species (*Avena sativa*, *Brassica napus* and *Vicia sativa*), used in this study. For detail see table A7_5_1_3-4.

4.2.3 Plant length

Clear effects of [redacted] on the growth rate of *Brassica napus* and *Vicia sativa* were observed. At a test concentration of 1000 mg/kg a 31 % inhibition of the plant length of *Brassica napus* was estimated. The plant lengths of *Vicia sativa* were inhibited by 13% at the test concentrations of 500 mg/kg and by 26 % at 1000 mg/kg at the end of exposure, respectively.

No statistical significant effects on the growth rate of *Avena sativa* could be detected.

For detail see table A7_5_1_3-5.

4.2.4 Plant dry weight

The effects of [redacted] on the dry shoot weights of harvested plants of *Brassica napus* and *Vicia sativa* were evaluated. At a test concentration of 1000 mg/kg an inhibition of 53% was estimated with *Brassica napus* and 28% inhibition at the test concentration of 250 mg/kg with *Vicia sativa*.

No statistical significant effects on the shoot dry weight of *Avena sativa* could be detected.

For detail see table A7_5_1_3-6.

4.2.5 Morphological findings

The evaluation of the morphological observation showed that there was no peculiarity at *Avena sativa*.

The seed leafs of *Brassica napus* get yellow and fallen off partly but there was no peculiarity regarding to the content of test substance.

At *Vicia sativa* the plant got more yellowish with increasing test concentrations from 250 mg/kg dry matter which was probably caused by the content of [redacted].

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For detail see table A7_5_1_3-7.

4.2.6 Effect data

4.2.6.1 EC_{25} (mg/kg soil DM; related to dry mass of the soil)

Parameter	*Avena sativa*	*Brassica napus*	*Vicia sativa*
Emergence rate	> 1000	> 1000	> 1000
Plant length	> 1000	896	929
Shoot dry weight	> 1000	667	450

4.2.6.2 EC_{50} (mg/kg soil DM; related to dry mass of the soil)

Parameter	*Avena sativa*	*Brassica napus*	*Vicia sativa*
Emergence rate	> 1000	> 1000	> 1000
Plant length	> 1000	> 1000	> 1000
Shoot dry weight	> 1000	944	901

4.2.6.3 NOEC (mg/kg soil DM; related to dry mass of the soil)

Parameter	*Avena sativa*	*Brassica napus*	*Vicia sativa*
Emergence rate	≥ 1000	≥ 1000	≥ 1000
Plant length	≥ 1000	250	125 (at 250 mg/kg soil DM there was a statistical significance with p ≤ 0.05 at 7.3 % inhibition)
Shoot dry weight	≥ 1000	500	125

4.2.6.4 LOEC (mg/kg soil DM; related to dry mass of the soil)

Parameter	*Avena sativa*	*Brassica napus*	*Vicia sativa*
Emergence rate	> 1000	> 1000	> 1000
Plant length	> 1000	500**	250*
Shoot dry weight	> 1000	1000**	250**

**, $p \leq 0.01$; *, $p \leq 0.05$

4.3 Results of controls

Controls were inconspicuous; for details, see the tables mentioned above.

4.4 Test with reference substance

Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The present study was conducted to assess effects on seedling emergence and early growth of higher plants following exposure of [redacted] in the soil. The test was conducted according to the OECD TG 208 and GLP.

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Test substance: [redacted] (ca. [redacted]% glutaraldehyde in water), batch no. [redacted].

Seeds of *Brassica napus, Vicia sativa* and *Avena sativa* were placed in contact with soil treated with [redacted] and evaluated for effects of following 14 days after 50 % emergence of the seedlings in the control group. The complete test duration was 19 days. The nominal test concentrations of [redacted] were 0, 62.5, 125, 250, 500 and 1000 mg/kg dry matter of soil (nominal concentrations based on test substance mass without correction for purity).The endpoints measured were visual assessment of seedling emergence, dry shoot weight and shoot length, as well as an assessment of visible detrimental effects on different parts of the plant. These measurements and observations are compared to those of untreated plant.

5.2 Results and discussion

A recovery rate of 99 % of the nominal concentration was found. This confirmed the correct initial weight for the preparation of the stock solution.

[redacted] had no statistical significant effect on the germination capacity of *Avena sativa*, *Brassica napus* and *Vicia sativa*.

Test substance-related effects were reported for the growth rate of *Brassica napus* and *Vicia sativa*. At a test concentration of 1000 mg/kg a 31 % inhibition of the plant length of *Brassica napus* was estimated. The plant lengths of *Vicia sativa* were inhibited by 13% at the test concentrations of 500 mg/kg and by 26 % at 1000 mg/kg at the end of exposure, respectively. No statistical significant effects on the growth rate of *Avena sativa* were seen.

Test substance-related effects were reported for the dry shoot weight of harvested plants of *Brassica napus* and *Vicia sativa*. At a test concentration of 1000 mg/kg an inhibition of 53% was estimated with *Brassica napus* and 28% inhibition at the test concentration of 250 mg/kg with *Vicia sativa*. No statistical significant effects on the shoot dry weight of *Avena sativa* were reported.

Referring to the morphology of the plants, there was no peculiarity at *Avena sativa*. For *Brassica napus*, seed leafs get yellow and fallen off partly but there was no peculiarity regarding to the content of test substance. *Vicia sativa* plant got more yellowish with increasing test concentrations from 250 mg/kg dry matter which was probably test substance-related.

5.2.1 EC_{25} (mg/kg soil DM; related to dry mass of the soil)

Parameter	*Avena sativa*	*Brassica napus*	*Vicia sativa*
Emergence rate	> 1000	> 1000	> 1000
Plant length	> 1000	896	929
Shoot dry weight	> 1000	667	450

5.2.2 EC_{50} (mg/kg soil DM; related to dry mass of the soil)

Parameter	*Avena sativa*	*Brassica napus*	*Vicia sativa*
Emergence	> 1000	> 1000	> 1000

	rate			
	Plant length	> 1000	> 1000	> 1000
	Shoot dry weight	> 1000	944	901

5.2.3 NOEC (mg/kg soil DM; related to dry mass of the soil)

Parameter	*Avena sativa*	*Brassica napus*	*Vicia sativa*
Emergence rate	≥ 1000	≥ 1000	≥ 1000
Plant length	≥ 1000	250	125 (at 250 mg/kg soil DM there was a statistical significance with p ≤ 0.05 at 7.3 % inhibition)
Shoot dry weight	≥ 1000	500	125

5.2.4 LOEC (mg/kg soil DM; related to dry mass of the soil)

Parameter	*Avena sativa*	*Brassica napus*	*Vicia sativa*
Emergence rate	> 1000	> 1000	> 1000
Plant length	> 1000	500**	250*
Shoot dry weight	> 1000	1000**	250**

**, $p \leq 0.01$; *, $p \leq 0.05$

5.3 Conclusion

The present study was conducted to assess effects on seedling emergence and early growth of higher plants following exposure of [redacted] in the soil.

The sensitivity of the used plant species to the applied test material concentrations decreased from *Vicia sativa*, *Brassica napus* to *Avena sativa*.

The test material showed no toxic effects to *Avena sativa*.

5.3.1 Reliability

1

5.3.2 Deficiencies

The study was a guideline study conducted according to GLP principles, and showed no deficiencies.

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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	27.9.2010
Materials and Methods	Agree with the applicant's version.
Results and discussion	4.2.6: Test concentrations are given as test material, i.e. [redacted]% Glutaraldehyde. The results are based on nominal concentrations. The most sensitive species and endpoint were vetch (*Vicia sativa*) and shoot dry weight, respectively. The respective EC50 and NOEC were 441 mg/kg dw and 61 mg/kg dw expressed as 100% Glutaraldehyde. The results are converted to organic matter content of 3.4%: EC50 441 x 0.034/0.0123 = 1219 mg/kg dw NOEC 61 x 0.034/0.0123 = 169 mg/kg dw The results are further converted from dry weight to wet weight soil by dividing with a factor of 1.13: EC50 1219/1.13 = **1079 mg a.i./kg ww** NOEC 169/1.13 = **150 mg a.i./kg ww**
Conclusion	Glutaraldehyde does not seem to be very toxic to terrestrial plants, but it should be observed that Glutaraldehyde is expected to rapidly degrade in soil and hence the results may underestimate the toxicity.
Reliability	2
Acceptability	Acceptable
Remarks	
	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_5_1_3-1: Test plants

	Family	Species	Common name	Source (seed/plant)
Dicotyledonae	Brassicaceae (Cruciferae)	*Brassica napus*	Oilseed rape	[redacted]
	Fabaceae (Leguminosae)	*Vicia sativa*	Vetch	
Monocotyledonae	Poaceae (Gramineae)	*Avena sativa*	Oats	

Table A7_5_1_3-2: Test system

Criteria		**Details**
Test Chamber		Ecophyte
Test vessels		PVC plant pots with an upper internal diameter of about 95 mm, covered by plastic dishes until the start of emergence, approx. 250 mL
Soil	**Type and batch**	-From [redacted]
	Sampling site	[redacted]
	Type	- Loamy sand (IS) according to German DIN ISO 19682-2 - Sandy loam according to USDA
	Soil humidity in the exposure phase	40 % (of maximum water holding capacity)
	Storage time	2 weeks
Seeds	**Storage**	In the dark under dry conditions and at room temperature until test start.
	Filling the test vessels	The plant pots were loosely filled with test substrate to about 360 g soil with 35% of the WHCmax
	Loading (plant/pot):	10 dry seeds, not pre-germinated
	Sowing depth:	0.5 cm for *Brassica napus* 1.0 cm for *Vicia sativa* 1.5 cm for *Avena sativa*
	Total number of seeds per concentration	40
	Number of replicates per concentration and control	4
	Watering of the plants	Daily with demineralised water
	Measurement of emergence	Daily, starting with the emergence of the first seedlings after 3 days and ending after 19 days of exposure
	Measurement of plant length	After 19 days of exposure
	Measurement of dry weight	After 19 days of exposure and constant weight
	Conditions for determination of dry substance of the germs (actual	Range of temperature by daily measurement: 59.7 - 60

	values)	°C, for 7 days until constancy weight

Table A7_5_1_3-3: Test conditions

Criteria	**Details**
Light rhythm	day/night: 16 hours light, 8 hours darkness
Measurement of the temperature	Continuous over the exposure period
Temperature over the exposure period; minimum, maximum, mean value	20.7 °C, 21.3 °C, 21.0 °C
Measurement of relative humidity	Continuous over the exposure period
Relative humidity over the exposure period; minimum, maximum value	65.2 %, 78.8 %, 75.3 %
Measurement of the light intensity	Single values measured at 5 points in the test chamber at the start of exposure
Light intensity, mean value	7452 Lux

Table A7_5_1_3-4: Inhibition of germination at the end of exposure, per treatment group

Plant	Test concentration [mg/kg soil] nominal value	Number of germinated seedlings	Inhibition versus control [%]
Avena sativa	0 (control)	10.00	--
Avena sativa	62.5	8.75	12.5* [a]
Avena sativa	125	9.50	5.0
Avena sativa	250	9.50	5.0
Avena sativa	500	9.50	5.0
Avena sativa	1000	9.25	7.5
Brassica napus	0 (control)	10.00	--
Brassica napus	62.5	10.00	0
Brassica napus	125	9.75	2.5
Brassica napus	250	9.75	2.5
Brassica napus	500	10.00	0
Brassica napus	1000	10.00	0
Vicia sativa	0 (control)	8.50	--
Vicia sativa	62.5	8.50	0
Vicia sativa	125	8.25	2.9
Vicia sativa	250	9.25	-8.8
Vicia sativa	500	8.75	-2.9
Vicia sativa	1000	9.25	-8.8

Statistical significant (Wilcoxon-test (one-sided)): *p ≤0.05

[a] not used for evaluation because the following higher test concentration are not statistical significant.

Table A7_5_1_3-5: Inhibition of dry shoot length at the end of exposure, per treatment group

Plant	Test concentration [mg/kg soil] nominal value	Shoot length mean values [mm]	Inhibition versus control [%]
Avena sativa	0 (control)	215.5	--
Avena sativa	62.5	213.5	0.9
Avena sativa	125	215.2	0.2
Avena sativa	250	220.1	-2.1
Avena sativa	500	221.9	-3.0
Avena sativa	1000	215.1	0.2
Brassica napus	0 (control)	53.3	--
Brassica napus	62.5	52.7	1.1
Brassica napus	125	53.4	-0.2
Brassica napus	250	52.8	0.9
Brassica napus	500	48.7	8.6
Brassica napus	1000	36.8	31.0**
Vicia sativa	0 (control)	368.5	--
Vicia sativa	62.5	371.4	-0.8
Vicia sativa	125	363.7	1.3
Vicia sativa	250	341.8	7.3*
Vicia sativa	500	319.6	13.3**
Vicia sativa	1000	273.6	25.8**

Statistical significant (Dunnett's test (one-sided)): **$p \leq 0.01$, *$p \leq 0.05$

Table A7_5_1_3-6: Inhibition of dry shoot weight at the end of exposure, per treatment group

Plant	Test concentration [mg/kg soil] nominal value	Dry shoot weight mean values [g]	Inhibition versus control [%]
Avena sativa	0 (control)	0.2735	--
Avena sativa	62.5	0.2570	6.0
Avena sativa	125	0.2412	11.8
Avena sativa	250	0.2777	-1.5
Avena sativa	500	0.2794	-2.2
Avena sativa	1000	0.2444	10.7
Brassica napus	0 (control)	0.1549	--
Brassica napus	62.5	0.1533	1.1
Brassica napus	125	0.1744	-12.6
Brassica napus	250	0.1831	-18.2
Brassica napus	500	0.1449	6.5
Brassica napus	1000	0.0723	53.4**
Vicia sativa	0 (control)	0.6129	--
Vicia sativa	62.5	0.5644	7.9
Vicia sativa	125	0.5790	5.5
Vicia sativa	250	0.4406	28.1**
Vicia sativa	500	0.4028	34.3**
Vicia sativa	1000	0.2819	54.0**

Statistical significant (Dunnett's test (one-sided)): **$p \leq 0.01$

Table A7_5_1_3-7: Morphological findings

Test vessel no.	Date	Observations
All vessel no. for test concentrations of 1000 mg/kg - *Brassica napus, Vicia sativa*	20 Apr 10	The plants were clearly smaller as the plants in the pots with a test concentration 500 mg/kg DM.
22 *(Avena sativa)*	25 Apr 10	One plant was stunted and had brown leafs
End of the exposure on 04 May 2010: Test vessel no. 22: The plant from the 25 April 2010 was completely brown and stunted. It was separately dried. This value was not used for determination the dry weight in pot 22. *Brassica napus:* The seed leaf in the controls and the test concentrations 62.5, 125, 250, 500 mg/kg DM were yellowish or were fallen off. *Vicia sativa*: With increasing of the test concentration from 250 mg/kg DM the plants get more yellowish compared to the controls. The plants in the other pots were without a statement.		

Section A1 Applicant

Annex Point IIA1

IUCLID 1.0.1

1.1 Applicant

Name: Dow Benelux B.V.
Address: Herbert H Dowweg 5
NL-4530Terneuzen
The Netherlands

[redacted]

1.2 Manufacturer of Active Substance (if different)

Name: The Dow Chemical Company
Address: Route 25
Institute, WV 25112
U.S.A.
Telephone: not available
Fax number: not available

1.3 Manufacturer of Product(s) (if different)

1) Product – [redacted]

As above

Section A2 Identity of Active Substance

Subsection (Annex Point)			Official use only
2.1	**Common name (IIA2.1)**	Glutaraldehyde	
2.2	**Chemical name (IIA2.2)**	1,5-Pentanedial or glutaral	
2.3	**Manufacturer's development code number(s) (IIA2.3)**	Not available please refer to TNG justification for non-submission	
2.4	**CAS No and EC numbers (IIA2.4)**	Non-entry field	
2.4.1	**CAS-No**	111-30-8	
	Isomer 1	No isomers	
2.4.2	**EC-No**	203-856-5	
	Isomer 1	No isomers	
2.4.3	**Other**	Not applicable	
2.5	**Molecular and structural formula, molecular mass (IIA2.5)**	Non-entry field	
2.5.1	**Molecular formula**	$C_5H_8O_2$ (OHC-CH_2-CH_2-CH_2-CHO)	
2.5.2	**Structural formula**		
2.5.3	**Molecular mass**	100.1 g/mol a.s.	
2.6	**Method of manufacture of the active substance (IIA2.1)**	Refer to Confidential Appendix 11 for IIIA TNG Section A2.6	
2.7	**Specification of the purity of the active substance, as appropriate (IIA2.7)**	g/kg: [redacted] % w/w: [redacted] min. theoretical dry weight g/kg: [redacted]	
2.8	**Identity of impurities and additives, as appropriate (IIA2.8)**	Refer to Confidential Appendix 11 for IIIA Section A2.8	
2.8.1	**Isomeric composition**	The active ingredient is not an isomeric substance hence information on this data point is not applicable.	
2.9	**The origin of the natural active substance or the precursor(s) of the active substance**	The active ingredient and its precursors are produced by chemical synthesis. There is no natural source of either the active ingredient or its intermediate precursors hence information on this data point is not applicable.	

Section A2 Identity of Active Substance

(IIA2.9)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 19th, 2011 point 2.7 revised September 18, 2014
Materials and methods	Agree with applicant's version.
Conclusion	Not relevant
Reliability	Not relevant
Acceptability	Acceptable
Remarks	Theoretical dry weight added to point 2.7.
	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 A2 Identity of Active Substance

Subsection (Annex Point)		Official use only
2.1	Common name (IIA2.1)	Glutaraldehyde

Doc III Section A2.10 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

Annex Point IIA2.10

Subsection — Official use only

2.10.1 Human exposure towards active substance

2.10.1.1 Production

i) Description of processes

See Doc III A 2.6 as presented in Document V

ii) Workplace description

Closed system

iii) Inhalation exposure

Engineering controls and the closed system of manufacture means that inhalation exposure is negligible.

The handling of glutaraldehyde should only be carried out in sufficiently well ventilated areas, where this is not possible respirators with type A gas filter (for organic gases and vapours) should be used.

[redacted]

iv) Dermal exposure

By the use of a water-resistant protective barrier cream, chemical resistant gloves and protective clothing (impermeable protective work-wear and footwear) in combination with the closed system, direct contact with [redacted] can be kept very low or excluded.

2.10.1.2.1 Professional Users

i) Description of application processes

PT6 In-can preservation of drilling muds and fluids:

[redacted]

PT11 Hydrotesting.

[redacted]

Doc III 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

PT11 Injection water during oil and gas production

[redacted]

ii) Workplace description

In-can preservation of drilling muds and fluids

[redacted]

Doc III 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
	Hydrotesting. and injection water [redacted]
	All uses Professional workers are expected to wear chemical resistant gloves and protective clothing so that inhalation exposure and dermal exposure can be excluded during the manufacture and use of biocidal products containing glutaraldehyde. Engineering controls in the work place (ventilation equipment, exhaust systems) and the use of protective clothing are employed to reduce exposures.
iii) Inhalation exposure	A combination of engineering controls, workplace ventilation and the use of recommended respiratory equipment should be used to ensure that inhalation exposure is negligible. Full protective equipment including self-contained breathing apparatus should be used for spraying glutaraldehyde products and for dealing with large spillages. Individuals should only enter treated areas after ventilation has removed glutaraldehyde from the workplace.
iv) Dermal exposure	By using the recommended personal protective equipment (chemical resistant gloves, impermeable protective work-wear and footwear), direct contact with [redacted] can be kept very low or excluded.
A2.10.1.2.2 **Non-professional Users including the general public**	[redacted] is not used by the general public and exposure during production or arising from oilfield applications is not expected..

2.10.2 Environmental exposure towards active substance

[redacted]

Doc III 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**
[redacted]	[redacted]

Doc III 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

10.2.2 Intended use(s)

Glutaraldehyde is used in a wide range of oilfield applications within Product Types 6, 11 and 12

In-can preservation of drilling muds and fluids

[redacted]

Hydrotesting.

[redacted]

Injection water

[redacted]

Doc III Section A2.10 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

Annex Point IIA2.10

Affected compartment(s):

water Low

sediment Very low

air Not relevant

soil Excluded

Predicted concentration in the affected compartment(s)

water Very low concentrations may be found in the receiving waters but release to the receiving water will be negligible due to the effects of dilution and ready biodegradability.

Scenario	$PEC_{seawater}$ (µg/l)	$PEC_{marine\ sediment}$ (mg/kg)
Produced water		
Shock dose	0.142	1.3
Routine dose	0.567	5.1
Drilling chemical		
Continuous discharge	0.073	0.21
Batchwise discharge	61	-
Cementing fluids	6	-
Hydrotesting fluids	30	-

sediment Minimal release – see table above

air Not relevant.

soil Release excluded

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 28th, 2012
Materials and methods	2.10.1.2 Intended use(s) Human and environmental exposure assessments were also performed by the RMS - please refer to Doc IIB.
Conclusion	The uses are as described by the applicants. Please see the human and environmental exposure assessments in Doc IIB.
Reliability	Not applicable
Acceptability	Acceptable
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 A2.10(2b)
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

Product Type 2 - Hard Surface Disinfection Hospitals and Industrial Areas (PT2.01)

Subsection			Official use only
2.10.1	**Human exposure towards active substance**		
2.10.1.1	**Production**	**Refer to Justification for non-submission of data TNG Section 2.10.1.1**	
	i) Description of process		
	ii) Workplace description		
	iii) Inhalation exposure		
	iv) Dermal exposure		
2.10.1.2	**Intended use(s)**		
	1. Professional Users	This application is for use in hospitals and industrial areas and is used by professionals only.	
	i) Description of application process	[redacted] Scenario 3 – Hard surface disinfection – mixing and loading This scenario represents the task of handling containers of [redacted] and diluting with water for use as a hard surface cleaner. Scenario 4 – Hard surface disinfection – application This scenario represents the task of mopping floors and wiping surfaces with the diluted (0.3%) Glutaraldehyde solution.	
	ii) Workplace description	[redacted]	

Section A2.10(2b)
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

Product Type 2 - Hard Surface Disinfection Hospitals and Industrial Areas (PT2.01)

iii) Inhalation exposure	Scenario 3 Using ConsExpo Version 4.1:- *Cleaning and washing → Floor, carpet and furniture Products → Floor cleaning liquid → Mixing and loading*, and adapting this model to suit this scenario. The Tier 1 potential inhalation exposure has been estimated to be: 2.16E-05 mg/m^3 (mean event); 1.12E-08 mg/m^3 (daily mean). Scenario 4 Using ConsExpo Version 4.1:- *Cleaning and washing → Floor, carpet and furniture Products → Floor cleaning liquid → Application*, and adapting this model to suit this scenario. The Tier 2 potential inhalation exposure has been estimated to be: 0.0171 mg/m^3 (mean event); 0.0007 mg/m^3 (daily mean)..
iv) Dermal exposure	Scenario 3 Using ConsExpo Version 4.1:- *Cleaning and washing → Floor, carpet and furniture Products → Floor cleaning liquid → Mixing and loading*, and adapting this model to suit this scenario. The Tier 1 potential dermal external dose has been estimated to be: 0.00014 mg/cm^2. Scenario 4 Using ConsExpo Version 4.1:- *Cleaning and washing → Floor, carpet and furniture Products → Floor cleaning liquid → Application,* and adapting this model to suit this scenario. The potential dermal external dose for 10% penetration of PPEhas been estimated to be: 0.003 mgcm^2.
2. Non-professional Users including the general public	Scenario 5 - Indirect exposure could occur if a child was to enter the room following hard surface disinfection and contact wet residues. This is unlikely to occur.
(i) via inhalational contact	Acute short term exposure could occur if a child entered the room immediately after cleaning. The Tier 1 potential inhalation exposure has been estimated to be: 0.0032 mg/m^3 (mean event); 0.0032 mg/m^3 (daily mean).
(ii) via skin contact	Acute short-term exposure could occur immediately post application if a child was to play on a freshly washed floor that had not fully dried.The Tier 1 potential dermal external dose has been estimated to be: 0.007 mg/cm^2.
(iii) via drinking water	Not applicable-there will be no non-professional use
(iv) via food	Not applicable-there will be no non-professional use
(v) indirect via environment	Not applicable-there will be no non-professional use