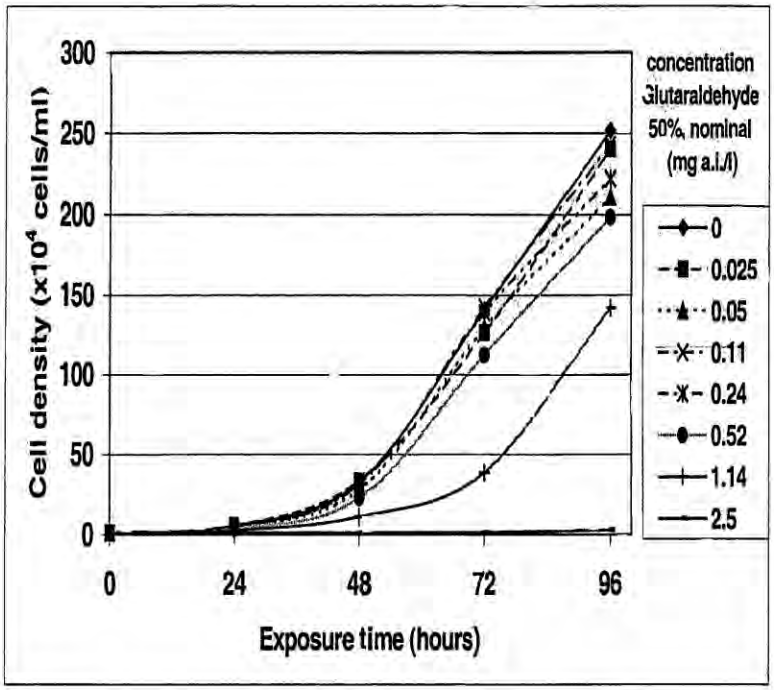
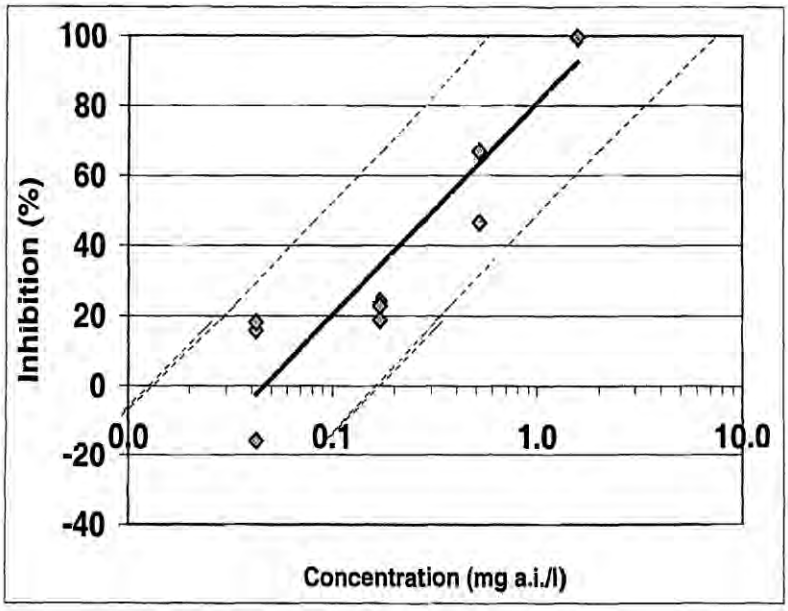
-Section A7.4.1.3(1) **Annex Point IIA, VII.7.3** **IUCLID 4.3/01**		**Growth inhibition test on algae** **Fresh water algal growth inhibition test with glutaraldehyde 50%**	
3.3	**Reference substance**	Potassium dichromate	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Culture medium	Freshwater algal nutrient medium Freshly prepared algal growth medium, without ammonia, formulated under sterile conditions using Milli-Q water preventing precipitation and with the following composition: **Chemical** — **Amt. (mg/L)** $MgCl_2\ 6H_2O$ — 12.16 $CaCl_2\ 2H_2O$ — 4.4 H_3BO_3 — 0.1856 $MnCl_2\ 4H_2O$ — 0.416 $ZnCl_2$ — 0.00328 $FeCl_3\ 6H_2O$ — 0.1598 $CoCl_2\ 6H_2O$ — 0.001428 $Na_2MoO_4\ 2H_2O$ — 0.00726 $CuCl_2\ 2H_2O$ — 0.000012 $Na_2EDTA\ 2H_2O$ — 0.3 $NaNO_3$ — 25.5 $MgSO_4\ 7H_2O$ — 14.7 K_2HPO_4 — 1.044 $NaHCO_3$ — 15 The pH was adjusted to 7.5 +/- 0.1 using 0.1N NaOH and/or 10% HCl.	
3.4.2	Test organisms	[redacted] For culturing conditions, see **Table A7.4.1.3(1)-2**	
3.4.3	Test system	**Table A7.4.1.3(1)-3**	
3.4.4	Test conditions	**Table A7.4.1.3(1)-4**	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Growth (total cell counts per mL), % growth inhibition, and growth rate	
3.4.7	Sampling	Cell density at 24, 48, 72 and 96 h	
3.4.8	Monitoring of TS concentration	Concentrations of glutaraldehyde in the test vessels were determined in 10 mL samples taken at 0, 24, 48, 72, and 96 hours. Control samples were also analyzed at each time period. Samples were analyzed by HPLC.	
3.4.9	Statistics	An effect was considered to be significant if statistical analysis of the data obtained for the concentrations compared with those of the negative control revealed significant reduction of growth or inhibition of growth rate (ANOVA, Dunnett's test, Williams' test). Additionally, the EC10 was determined to meet the recommendations of the OECD. Calculation of	

-Section A7.4.1.3(1) **Annex Point IIA, VII.7.3** **IUCLID 4.3/01**		**Growth inhibition test on algae** **Fresh water algal growth inhibition test with glutaraldehyde 50%**	
		EC_{50} and EC_{10} values were based on linear regression analysis of the percentages of growth inhibition and the percentages of growth rate reduction versus the logarithms of both the corresponding average exposure and nominal loadings of the test substance.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentration	Not applicable	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.2	**Results test substance**	*Non-entry field*	
4.2.1	Initial concentrations of test substance	0.025, 0.05, 0.11, 0.24, 0.52, 1.14, 2.5 mg a.i./L nominally	
4.2.2	Actual concentrations of test substance	Nominal Concentration (mg/L) / 72-hour TWA / 96-hour TWA 0.025 / <LLQ / <LLQ 0.05 / <LLQ / <LLQ 0.11 / <LLQ / <LLQ 0.24 / 0.054 / 0.042 0.52 / 0.23 / 0.17 1.14 / 0.69 / 0.53 2.5 / 1.81 / 1.59	x

-Section A7.4.1.3(1) Annex Point IIA, VII.7.3 IUCLID 4.3/01	**Growth inhibition test on algae** **Fresh water algal growth inhibition test with glutaraldehyde 50%**	
4.2.3 Growth curves		
4.2.4 Concentration / response curve		
4.2.5 Cell concentration data	**Table A7.4.1.3(1)-5**	
4.2.6 Effect data (cell multiplication inhibition)	**Growth Rate (E_rC_{50}) (nominal)** 3-day = 1.32 (0.75-2.3) mg/L (TWA = 0.80 mg a.i./L) 4-day = 1.75 (1.44-2.12) mg/L (TWA = 0.95 mg a.i./L)	

-Section A7.4.1.3(1) **Annex Point IIA, VII.7.3** **IUCLID 4.3/01**		**Growth inhibition test on algae** **Fresh water algal growth inhibition test with glutaraldehyde 50%**	
		Growth Rate (NOEC) (nominal) 3-day = 0.52 mg/L (TWA = 0.23 mg a.i./L) 4-day = 0.52 mg/L (TWA = 0.17 mg a.i./L) **Percent Inhibition (E_bC_{50}) (nominal)** 3-day = 0.78 (0.46-1.32) mg/L (TWA = 0.36 mg a.i./L) 4-day = 0.83 (0.42-1.66) mg/L (TWA = 0.31 mg a.i./L) **Percent Inhibition (NOEC) (nominal)** 3-day = 0.24 mg/L (TWA = 0.054 mg a.i./L) 4-day = 0.24 mg/L (TWA = 0.042 mg a.i./L)	
4.2.7	Other observed effects	None	
4.3	**Results of controls**	**Table A7.4.1.3/01-5**	
4.4	**Test with reference substance**		
4.4.1	Concentrations	0, 0.18, 0.32, 0.56, 1.0, 1.8, 3.2 mg/L	
4.4.2	Results	The EC_{50} for growth inhibition (E_bC_{50}) = 0.59 mg/L for 72 hours The EC_{50} for growth rate reduction (E_rC_{50}) = 1.1 mg/L for 72 hours	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Three days prior to test, cells from a lab-grown algal stock were inoculated in culture medium at a cell density of 2.10^4 cells/mL. Cell density was measured again immediately before use. The test material was dissolved in water 10-15 minutes prior to the test start. Lower test concentrations were made by serial dilutions of the stock in test media. Adequate volumes of an algal suspension were added to each replicate to provide a cell density of 10^4 cells/mL. A range-finding test was performed to determine appropriate levels (0.1-10 mg a.i./L) to use in the definitive testing. Cell densities were measured at 24, 72 and 96h using spectrophotometry at 720 nm using a Varian Cary 50 single beam spectrophotometer. Dose levels of 0.025, 0.05, 0.11, 0.24, 0.52, 1.14, 2.5 mg a.i./L were tested. A control vessel was studied in parallel. Test samples were taken from each vessel at the start of the exposure and daily thereafter for analysis. The pH was measured at the study start and daily thereafter. Cell density was measured at study start using a microscope with counting chamber. Afterward, the density was determined by spectrophotometric measurement of the samples at 720nm. An algal medium was used a blank. Quantification of cell densities was based on a calibration curve.	
5.2	**Results and discussion**	Under the conditions of the present study, glutaraldehyde reduced the growth rate of the fresh water algae significantly at nominally 1.14 mg	

-Section A7.4.1.3(1) **Annex Point IIA, VII.7.3** **IUCLID 4.3/01**	**Growth inhibition test on algae** **Fresh water algal growth inhibition test with glutaraldehyde 50%**	
	a.i./L and higher, corresponding with an analytical time weighted average of 0.53-0.69 mg a.i./L for 72 and 96 hours, respectively.	
5.2.1 NOE_rC	Nominally, 3-day = 0.52 mg a.i./L (TWA = 0.23 mg a.i./L) 4-day = 0.52 mg a.i./L (TWA = 0.17 mg a.i./L) TWA = time weighted average $((C_{t=0} * C_{t=24})^{1/2} + (C_{t=24} * C_{t=48})^{1/2} + (C_{t=48} * C_{t=72})^{1/2})/3$	
5.2.2 E_rC_{50}	3-day = 1.32 (0.75-2.3) mg/L (TWA = 0.80 mg a.i./L) 4-day = 1.75 (1.44-2.12) mg/L (TWA = 0.95 mg a.i./L)	
5.2.3 E_bC_{50}	3-day = 0.78 (0.46-1.32) mg/L (TWA = 0.36 mg a.i./L) 4-day = 0.83 (0.42-1.66) mg/L (TWA = 0.31 mg a.i./L)	
5.3 Conclusion	Glutaraldehyde is considered to be Very Toxic to fresh water algae according to EEC labeling guidelines.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Jan 23rd, 2009	
Materials and Methods	4.2.2 Only two highest concentrations could be measured until 72 hr. Analysis results were not reported for two lowest test concentrations, but obviously they could not be analysed. Limit of determination was 5 µg/L (In Doc IIIA4.2c LOQ is 0.1 µg/L).	
Results and discussion	E_rC_{50} 72 h = 0.82 mg a.i./L with 95% confidence limits 0.76-0.88 mg/L NOEC 72 h = 0.108 mg a.i./L The results are based on the measured geometric mean concentrations. See Tables A7.4.1.3(1)-5, A7.4.1.3(1)-6 and A7.4.1.3(1)-7. The validity criteria are fulfilled. The maintenance of concentrations within the range of ± 20% of the nominal concentrations is not a validity criterion in the current OECD 201 (adopted 23 March 2006). See Tables A7.4.1.3(1)-8 and 3.1.	
Conclusion	Glutaraldehyde is very toxic to [redacted]	
Reliability	2	
Acceptability	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	

-Section A7.4.1.3(1) Annex Point IIA, VII.7.3 IUCLID 4.3/01	**Growth inhibition test on algae** **Fresh water algal growth inhibition test with glutaraldehyde 50%**	
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)-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_3(1)-2: Test organisms

Criteria	Details
Species	[redacted]
Strain	[redacted]
Source	Algae stock culture
Laboratory culture	Yes
Method of cultivation	The stock culture was started by inoculating growth medium with algal cells from a pure culture on agar. The algal suspensions were continuously aerated and exposed to light at a temperature of 23±2°C.
Pretreatment	A pre-culture was started 3 days before the start of the test. Cells from the algal stock culture were inoculated in the culture medium at a cell density of 2.10^4 cells/mL.
Initial cell concentration	2.10^4 cells/mL (pre-culture) and 10^4 cells per mL (exposure phase)

Table A7_4_1_3(1)-3: Test system

Criteria	Details
Volume of culture flasks	100 mL glass containers with 50 mL of test suspension.
Culturing apparatus	Continuous shaking and illumination.
Light quality	TLD-lamps 'Cool-white' 30 Watt (Sylvania) with a light intensity in the range 99-129 $\mu E.m^{-2}.s^{-1}$
Procedure for suspending algae	Shaking
Number of vessels/ concentration	3 replicates at each test concentration (including 3 replicates at 0.11 and 2.5 mg/L without algae, 6 replicates of the blank control and 3 extra replicates at each concentration for sampling purposes and pH measurements.
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3(1)-4: Test conditions

Criteria	Details
Test temperature	Ranged between 22.0 and 23.5°C
pH	0-hour 7.6-7.8 24-hour 7.8-8.1 48-hour 7.8-8.1 72-hour 8.6-8.8 96-hour 8.0-10.3
Aeration of dilution water	No
Light intensity	99-129 $\mu E.m^{-2}.s^{-1}$
Photoperiod	Continuous

Table A7.4.1.3(1)-4 Algal Growth Data

Mean Cell Densities (x10^4 cells/mL)							
			Exposure time (hours)				
Nominal concentration (mg(a.i.)/L)	*TWA 72 h*	*TWA 96 h*	*0*	*24*	*48*	*72*	*96*
control	-	-	1.0	5.3	32.9	140.3	251.6
0.025	-	-	1.0	5.4	33.4	126.4	240.3
0.05	-	-	1.0	4.8	27.9	129.5	210.9
0.11	-	-	1.0	4.8	31.2	142.2	243.0
0.24	0.054	0.042	1.0	4.8	31.6	138.8	222.0
0.52	0.23	0.17	1.0	3.8	22.8	112.4	198.5
1.14	0.69	0.53	1.0	2.7	11.1	38.7	141.8
2.5	1.81	1.59	1.0	1.0	1.0	1.4	2.7

Mean Growth Rate								
			Exposure time (hours)					
Nominal concentration (mg(a.i.)/L)	*TWA 72 h*	*TWA 96 h*	*0-48 hours*	*Reduction (%)*	*0-72 hours*	*Reduction (%)*	*0-96 hours*	*Reduction (%)*
control	-	-	0.07269		0.006857		0.05741	
0.025	-	-	0.07303	-0.5	0.06715	2.1	0.05699	0.7
0.05	-	-	0.06901	5.1	0.06613	3.5	0.05678	1.1
0.11	-	-	0.07152	1.6	0.06854	0	0.05715	0.5
0.24	0.054	0.042	0.07191	1.1	0.06834	0.3	0.05607	2.3
0.52	0.23	0.17	0.06516	10.4	0.06558	4.3	0.05509	4
1.14	0.69	0.53	0.04977	31.5	0.04988	27.2	0.05146	10.4
2.5	1.81	1.59	0.00057	99.2	0.00417	93.9	0.00966	83.2

TWA: time weight average exposure concentration

Table A7.4.1.3(1)-5 Calculation of 72-hour and 96-hour Geometric Mean Measured Concentration Calculations

Nominal Test Conc. mg/L	0-hour Measured Test Conc. mg/L	24-hour Measured Test Conc. mg/L	48-hour Measured Test Conc. mg/L	72-hour Measured Test Conc. mg/L	72-hour Geometric Mean Measured Test Conc. mg/L	Geometric Mean % Nominal	96-hour Measured Test Conc. mg/L	96-hour Geometric Mean Measured Test Conc. mg/L	Geometric Mean % Nominal
0	<LOD	<LOD	<LOD	<LOD	**<LOD**	----	<LOD	**<LOD**	----
0.025	---	---	---	---	**na**	----	---	**na**	----
0.05	---	---	---	---	**na**	----	---	**na**	----
0.11	*0.0825*	---	---	---	**na**	----	<LOD	**na**	----
0.24	0.140	0.129	0.005	0.005	**0.026**	10.8%	0.005	**0.019**	7.8%
0.52	0.3815	0.378	0.186	0.005	**0.108**	20.7%	0.005	**0.058**	11.2%
1.14	0.844	0.927	0.585	0.3295	**0.623**	54.7%	0.005	**0.237**	20.8%
2.50	1.97	2.17	1.68	1.26	**1.73**	69.4%	0.674	**1.44**	57.4%

Note: Day 0 measurements not made on four of nine exposure solutions. Calculation of geometric means not possible. Assume they calculated TWA using nominal values for day 0. Since % nominal on day measurements averaged 100% this is logical conclusion. Thus, the same approach will be used to calculate the geometric mean values.

RMS' note: LOD = 0.005 mg/l.

Table A7.4.1.3(1)-6 Growth Inhibition Test Cell Density Data (area under growth curve and growth rate data calculated from this data)

Nominal Test Conc. mg/L	72-hour Geometric Mean Measured Test Conc. mg/L	96-hour Geometric Mean Measured Test Conc. mg/L	Replicate	CELL DENSITY (x 10^4) 0 hour	24-hour	48-hour	72-hour	96-hour
<LOQ	**<LOQ**	**<LOQ**	1	1.00	5.83	36.1	124.8	185.8
			2	1.00	5.35	36.6	170.2	229.0
			3	1.00	5.59	30.9	149.2	288.7
			4	1.00	4.79	29.6	129.6	308.9
			5	1.00	4.71	30.2	146.3	285.5
			6	1.00	5.59	33.8	121.7	211.5
0.025	**na**	**na**	7	1.00	5.99	35.8	140.2	218.0
			8	1.00	5.43	33.7	111.9	212.0
			9	1.00	4.79	30.7	126.9	290.8
0.05	**na**	**na**	10	1.00	4.16	21.5	112.5	204.9
			11	1.00	5.31	29.9	157.6	246.9
			12	1.00	5.03	32.1	118.3	180.8
0.11	**na**	**na**	13	1.00	4.04	30.1	109.2	207.1
			14	1.00	4.91	27.4	135.2	276.5
			15	1.00	5.47	36.0	182.3	245.4
0.24	**0.026**	**0.019**	16	1.00	5.03	32.1	120.2	198.8
			17	1.00	4.55	29.5	125.5	180.7
			18	1.00	4.71	33.1	170.7	286.7
0.52	**0.108**	**0.058**	19	1.00	3.84	22.2	109.9	190.8
			20	1.00	4.12	24.2	113.2	188.3
			21	1.00	3.48	22.1	114.1	216.4
1.14	**0.623**	**0.237**	22	1.00	3.00	13.8	59.0	176.7
			23	1.00	2.56	9.40	27.1	128.3
			24	1.00	2.64	9.90	29.9	120.4
2.50	**1.73**	**1.436**	25	1.00	1.00	1.10	1.60	3.60
			26	1.00	1.00	1.00	1.30	1.60
			27	1.00	1.00	1.00	1.20	2.80

Table A7.4.1.3(1)-7 Growth Inhibition Test Endpoints Recalculated Based on Geometric Mean Measured Concentrations

Endpoint	Time Point	ErC50 (mg/L)	95% Confidence Interval (mg/L)	NOEC (mg/L)
Growth Rate	72-h	0.82	0.76 - 0.88	0.108
	96-h	0.67	0.48 - 0.86	0.058
		EbC50 (mg/L)	95% Confidence Interval (mg/L)	NOEC (mg/L)
Biomass	72-h	0.52	0.081 - 0.97	0.108
	96-h	0.17	0.13 - 0.22	0.058

Note: Statistical analysis was done using SAS version 9 software

Table A7.4.1.3(1)-8 Determination of Control Validity Criteria

72-HOUR MEAN CONTROL SECTION-BY-SECTION GROWTH RATE

Section-by-Section Growth Rate Calculation

Replicate	Day 1 Cell Count	Growth Rate (hour^{-1})	Day 2 Cell Count	Growth Rate (hour^{-1})	Day 3 Cell Count	Growth Rate (hour^{-1})
1	5.83	0.073	36.1	0.076	124.8	0.052
2	5.35	0.070	36.6	0.080	170.2	0.064
3	5.59	0.072	30.9	0.071	149.2	0.066
4	4.79	0.065	29.6	0.076	129.6	0.062
5	4.71	0.065	30.2	0.077	146.3	0.066
6	5.59	0.072	33.8	0.075	121.7	0.053

Rep	**72-hour Control Culture Growth Rate (per hour)** Mean	SD	CV
1	0.067	0.013	20
2	0.071	0.008	11
3	0.070	0.003	5
4	0.068	0.007	11
5	0.069	0.007	10
6	0.067	0.012	17
		Mean CV[1]	**12**

PASS

[1] Mean Coefficient of Variation (CV) for section-by-section growth rate in control cultures should not exceed 35%.

PASS = ≤ 35 % per hour in 72 hours
FAIL = > 35 % per hour in 72 hours

72-HOUR CONTROL GROWTH RATE

Rep	72-hour Control Growth Rate (per day)	Result
1	1.609	PASS
2	1.71	PASS
3	1.67	PASS
4	1.62	PASS
5	1.66	PASS
6	1.60	PASS
Mean[1]	**1.65**	**PASS**
SD	0.04	
CV[2]	**2.6**	**PASS**

[1] Biomass in control cultures should increase by at least 16X (= to an average specific growth of 0.92 per day) in 72 hours.

PASS = ≥ 0.92 per day in 72 hours

FAIL = < 0.92 per hour in 72 hours

[2] The coefficient of variation of average specific growth rates during the test period must not exceed 7%.

PASS = ≤ 7% in 72 hours (for green algae)

FAIL = > 7% in 72 hours (for green algae)

3. Tables for Applicant's Summary and Conclusion

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

	fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥80% of initial concentration during test		X

Criteria for poorly soluble test substances	NA	

Section A7.4.1.3(2) Annex Point IIA, VII.7.3 IUCLID 4.3/03		Growth inhibition test on algae Marine Algal Inhibition Test	
		1 REFERENCE	Official use only
1.1	**Reference**	[redacted] (1997) [redacted] Marine Algal Inhibition Test, [redacted], Unpublished, 30 April 1997	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, ISO/DIS 10253	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde, 50% [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Not reported	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	3.1.3	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Samples containing glutaraldehyde were derivatised with 2,4-DNPH in concentrated hydrochloric acid:water:acetonitrile (2:5:1, v/v/v). Final extracts were analyzed by HPLC with UV detection using a suitable HPLC and the following conditions: Analytical Column: Lichrosorb 10µODS, 250mm x 4.6mm Mobile phase A: 90:10 v/v water:acetonitrile adjusted to pH2.6 with sulphuric acid Mobile phase B: acetonitrile Flow rate: 2 mL/minute Wavelength: 368 nm Injection Volume: 100 µl Column temperature: 40°C.	x

Section A7.4.1.3(2) Annex Point IIA, VII.7.3 IUCLID 4.3/03		Growth inhibition test on algae Marine Algal Inhibition Test	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	Potassium dichromate	
3.3.1	Method of analysis for reference substance	Not specified	
3.4	**Testing procedure**	*Non-entry field*	
3.4.1	Culture medium	**Stock Solution Concentration (g/L)** Solution 1 $FeCl_3$ $6H_2O$ 0.048 $MnCl_2$ $4H_2O$ 0.144 $ZnSO_4$ $7H_2O$ 0.045 $CuSO_4$ $5H_2O$ 1.57×10^{-4} $CoCl_2$ $6H_2O$ 4.04×10^{-4} H_3BO_3 1.14 Na_2EDTA 1 Solution 2 Thiamin hydrochloride 5.0×10^{-2} Biotin 1.0×10^{-5} Bvitamin B12 1.0×10^{-4} Solution 3 K_3PO_4 3 $NaNO_3$ 50 Na_2SiO_3 $5H_2O$ 14.9 The medium was prepared by mixing 15 mL of concentrated stock solution 1, 0.5 mL of concentrated stock solution 2, and 1.0 mL of concentrated stock solution 3 and then adjusting the volume to 1 litre with natural seawater (sterilised by membrane filtration). The pH was then adjusted to 8.0 ± by the addition of dilute HCl or NaOH.	
3.4.2	Test organisms	**Table A7.4.1.3/03-2** [redacted]	
3.4.3	Test system	**Table A7.4.1.3/03-3**	
3.4.4	Test conditions	**Table A7.4.1.3/03-4**	
3.4.5	Duration of the test	72 hours	
3.4.6	Test parameter	Growth (total cell counts per mL), % growth inhibition, and growth rate	
3.4.7	Sampling	Samples of the solution with algae were taken at 0, 24, 48, and 72 hours, and the cell densities were determined by direct counting with the aid of a haemocytometer.	

Section A7.4.1.3(2) Annex Point IIA, VII.7.3 IUCLID 4.3/03		Growth inhibition test on algae Marine Algal Inhibition Test	
3.4.8	Monitoring of TS concentration	Water samples were taken from the control and each test group at 0 and 72 hours for quantitative analyses. HPLC was the method of analyses.	
3.4.9	Statistics	One way ANOVA was carried out on the area under the growth curve data at 72 hours for the control and all test concentrations to determine any statistically significant differences between test and control groups.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentration	Not applicable	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.2	**Results test substance**	*Non-entry field*	
4.2.1	Initial concentrations of test substance	0.625, 1.25, 2.5, 5.0 and 10 mg/L nominally	x
4.2.2	Actual concentrations of test substance	At 0 hrs actual concentrations were 0.607, 1.28, 2.54, 5.12, and 9.8 mg/L respectively. At 72 hours the actual concentrations were not detectable in the nominal concentrations of 0.625 and 1.25 mg/L. The remaining values were quantified at 0.935, 2.53 and 7.94 respectively.	x
4.2.3	Growth curves	**Refer to Figure A7.4.1.3(2)-1**	
4.2.4	Concentration / response curve	**Refer to Figure A7.4.1.3(2)-2**	
4.2.5	Cell concentration data	**Table A7.4.1.3(3)-5**	
4.2.6	Effect data (cell multiplication inhibition)	E_bC_{50} (72 h) 1.2 mg/L E_rC_{50} (0-24 h) 1.8 mg/L NOEC 0.625 mg/L	
4.2.7	Other observed effects	None	
4.3	**Results of controls**	**Table A7.4.1.3(3)-5**	
4.4	**Test with reference substance**	*Non-entry field*	

Section A7.4.1.3(2) Annex Point IIA, VII.7.3 IUCLID 4.3/03		Growth inhibition test on algae Marine Algal Inhibition Test	
4.4.1	Concentrations	0.625, 1.25, 2.5, 5.0 and 10 mg/L	
4.4.2	Results	E_bC_{50} (72 h) 2.3 mg/L E_rC_{50} (0-24 h) 2.6 mg/L NOEC 0.625 mg/L	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Following a preliminary range-finding study, the algae was exposed to an aqueous dispersion of the test material at concentrations of 0.625, 1.25, 2.5, 5.0, and 10 mg/L (three replicate flasks of 100mL solution per concentration) for 72 hours under constant illumination and shaking at a temperature of 23-25°C. Potassium dichromate was used as the positive control. Samples of the solution with algae were taken at 0, 24, 48, and 72 hours, and the cell densities were determined by direct counting with the aid of a hemocytometer. The pH of each control and test flask was determined at initiation and at 72 hours. The temperature of the incubator was recorded daily. Water samples were taken at 0 and 72 hours (replicates pooled) and evaluated for glutaraldehyde concentration by HPLC with an external standard technique.	
5.2	**Results and discussion**	There were no statistically significant differences between the control and 0.625 mg/L test concentrations, however all other test concentrations were different, and therefore the NOEC was reported to be 0.625 mg/L. **Table A7.4.1.3(3)-5** There were no abnormalities reported when inspected microscopically. The pH ranged 7.8-8.0 at study start, and 8.1-10.4 at termination. The alkaline conditions are assumed to be caused by a large number of cells in log phase, respiring O_2 and producing carbonates and bicarbonates as part of respiration. The increased pH is not considered to have affected the test, as the control cultures satisfied the ISO guideline validation criterion for growth enhancement. Analysis of the 72 hour solutions was markedly lower than initial analyses (less than the limit of quantitation of the analytical method to 79% of nominal). The decline of concentration in solution is considered to be due to adsorption to the algal cells and/or microbial degradation. The EC_{50} is therefore based on nominal concentrations, not analytical.	
5.2.1	NOE_rC	0.33 mg a.i./L (0.625 mg/L)	
5.2.2	E_rC_{50}	0.92 mg a.i./L (0-24 h) (1.8 mg/L)	
5.2.3	E_bC_{50}	0.61 mg a.i./L (72 h) (1.2 mg/L)	
5.3	**Conclusion**	The effect of the test material on the growth of [redacted] has been investigated over a 72 hour period and gave an E_bC_{50} (72 h) value of 1.2 mg/L and a E_rC_{50} (0-24 h) value of 1.8 mg/L. The No Observed Effect Concentration at 72 hours was 0.625 mg/L.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A7.4.1.3(2) **Annex Point IIA, VII.7.3** **IUCLID 4.3/03**	**Growth inhibition test on algae** **Marine Algal Inhibition Test**	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	March 2nd, 2009	
Materials and Methods	3.1.6 The LOQ 0.062 mg/L is above the LOQ of 0.1 µg/L reported in Doc IIIA4.2c. The two lowest concentrations declined below LOQ by the end of the test. 4.2.1 Initial test concentrations are given as a test product, i.e. [redacted] which contains 50.9% glutaraldehyde. 4.2.2 Actual test concentrations are given as a test product, i.e. [redacted] which contains 50.9% glutaraldehyde.	
Results and discussion	E_rC_{50} = 0.61 mg a.i./L (72 h) 95% confidence limit 0.21-1.0 mg/l a.i. NOEC = 0.071 mg a.i./L (72 h) Results are based on the measured geometric mean concentrations. See Tables A7.4.1.3(2)-6, A7.4.1.3(2)-7 and A7.4.1.3(2)-8. The test fulfills the first validity criterion of the current OECD 201 (adopted 23 March 2006): The biomass in the control cultures increased by a factor of at least 16 within the 72-hour test period. However, the growth rate does not seem to be exponential through out the test, see Fig. A7.4.1.3(2)-1. The test does not fulfill the second and third validity criteria. The mean coefficient of variation for section by section specific growth rates is 38% (pass level CV ≤ 35%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures is 10.6% while CV ≤ 10% is expected from other than green algae. However, the validity criteria are created for freshwater algae and may hence not be applicable for the marine alga.	
Conclusion	Glutaraldehyde is very toxic to marine species [redacted]	
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_4_1_3(2)-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_3(2)-2: Test organisms

Criteria	Details
Species	[redacted]
Strain	[redacted]
Source	[redacted]
Laboratory culture	Yes
Method of cultivation	The culture was maintained at 21±1°C under continuous illumination and constant aeration.
Pretreatment	Cultures were maintained by the periodic replacement of culture medium.
Initial cell concentration	The mean cell density at 0h ranged from 1.55×10^4 cells/mL to 2.78×10^4 cells/mL

Table A7_4_1_3(2)-3: Test system

Criteria	Details
Volume of culture flasks	250 mL glass conical flask containing 100 mL of test solution.
Culturing apparatus	Culture flasks were incubated at 24±1°C under continuous illumination and continuous shaking at 100rpm for 72 h.
Light quality	Not specified
Procedure for suspending algae	Shaking at 100 rpm
Number of vessels/ concentration	3 for test and control vessels
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3(2)-4: Test conditions

Criteria	Details
Test temperature	24±1°C
pH	7.8 to 8.0 at 0h; 8.1 to 10.4 at 72h
Aeration of dilution water	No
Light intensity	Approximately 7000 lux
Photoperiod	Continuous

Table A7.4.1.3(2)-5 Cell Density & Growth Inhibition

Nominal concentration (mg/L)	Cell Densities (cells/mL)			
	0 h	24 h	48 h	72 h
control	1.55×10^4	8.94×10^4	3.24×10^5	7.92×10^5
0.625	1.85×10^4	9.58×10^4	3.58×10^5	9.17×10^5
1.25	2.78×10^4	8.65×10^4	1.61×10^5	4.14×10^5
2.5	2.17×10^4	9.58×10^4	4.94×10^4	1.36×10^5
5	2.47×10^4	3.7×10^4	4.01×10^4	7.1×10^4
10	2.16×10^4	1.55×10^4	1.55×10^4	2.78×10^4
	Growth Inhibition (%)			
Nominal concentration (mg/L)	area under curve at 72 h	% inhibition	Growth rate (0-24 h)	% inhibition
control	1.85×10^7	-	0.073	-
0.625	2.08×10^7	[12]	0.068	7
1.25	9.23×10^6	50	0.047	36
2.5	2.48×10^6	87	0.026	64
5	1.22×10^6	93	0.017	77
10	-2.18×10^5	101	-0.014	119

Table A7.4.1.3(2)-6 Calculation of 72-hour Geometric Mean Measured Concentration Calculations

Nominal Test Conc. mg/L	0-hour Measured Test Conc. mg/L	% Nominal	72-hour Measured Test Conc. mg/L	% Nominal	72-hour Geometric Mean Measured Test Conc. mg/L	Geometric Mean % Nominal	Measured A.I. Conc. mg/L
0	<LOQ	na	<LOQ	na	**<LOQ**	NA	<LOQ
0.625	0.61	97%	0.031	na	**0.137**	22%	0.31
1.25	1.28	102%	0.031	na	**0.199**	16%	0.65
2.5	2.5	102%	0.935	37%	**1.54**	62%	1.3
5.0	5.1	102%	2.53	51%	**3.60**	72%	2.6
10.0	9.8	98%	7.94	79%	**8.82**	88%	5.0

Table A7.4.1.3(2)-7 **Growth Inhibition Test Cell Density Data (area under growth curve and growth rate data calculated from this data)**

Nominal Test Conc. mg/L	72-hour Geometric Mean Measured Test Conc. mg/L	Replicate	CELL DENSITY ($x 10^4$) 0 hour	24-hour	48-hour	72-hour
<LOQ	**<LOQ**	1	1.86	5.6	25.0	70.8
		2	0.917	9.3	37.0	79.2
		3	1.86	12.0	35.2	87.5
0.625	**0.137**	4	2.78	4.64	26.9	81.3
		5	1.860	11.1	45.4	97.9
		6	0.917	13.00	35.2	95.8
1.25	**0.199**	7	2.78	7.42	17.6	34.3
		8	2.78	11.1	13.0	39.8
		9	2.78	7.42	17.6	50.0
2.5	**1.54**	10	1.86	4.64	6.47	12.0
		11	1.86	4.64	5.56	16.7
		12	2.78	2.78	2.78	12.0
5.0	**3.60**	13	1.86	3.69	3.69	7.42
		14	2.78	2.78	5.56	6.47
		15	2.78	4.64	2.78	7.42
10.0	**8.82**	16	3.69	1.86	0.917	1.86
		17	0.917	0.917	1.86	2.78
		18	1.86	1.86	1.86	3.69

Table A7.4.1.3(2)-8 **Growth Inhibition Test Endpoints Recalculated Based on Geometric Mean Measured Concentrations**

Endpoint	Time Point	ErC50 (mg/L)	95% Confidence Interval (mg/L)	NOEC (mg/L)
Growth Rate (Test Material)	**72-h**	1.2	0.42 - 2.0	0.14
Growth Rate (Active Ingredient)	**72-h**	0.61	0.21 - 1.0	0.071
		EbC50 (mg/L)	**95% Confidence Interval (mg/L)**	**NOEC (mg/L)**
Biomass (Test Material)	**72-h**	0.37	0.16 - 0.57	0.14
Biomass (Active Ingredient)	**72-h**	0.19	0.081 - 0.29	0.071

Note: The endpoints are presented based on total test material and based on the percentage of active ingredient in the test material (50.9% glutaraldehyde).

Note: Statistical analysis was done using SAS version 9 software.

3. Tables for Applicant's Summary and Conclusion

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	X	
Concentration of test substance ≥80% of initial concentration during test		X

Criteria for poorly soluble test substances	NA	

Figure A7.4.1.3(2)-1 Marine Algal Cell- Mean Cell Densities versus Time

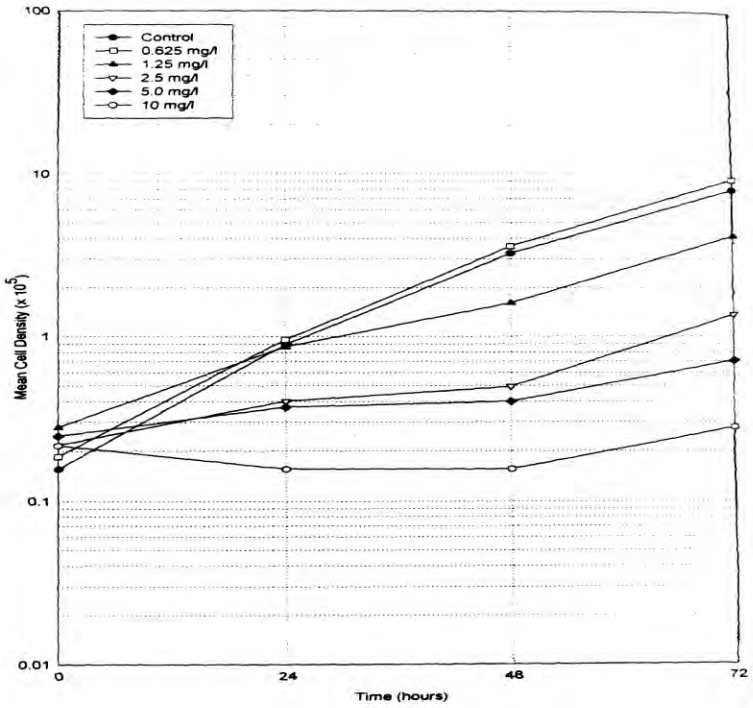
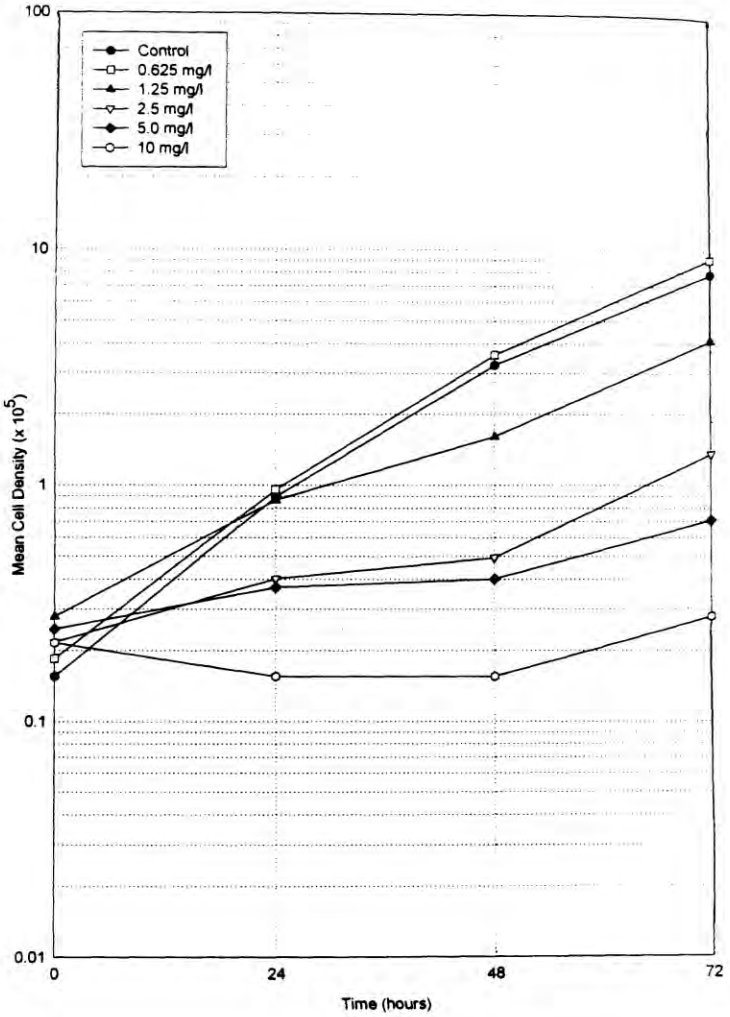


Figure A7.4.1.3(2)-2 Concentration/Dose Response Curve



Section A7.4.1.4 **Annex Point IIA&IIIA, VII.7.4&VII.3** **IUCLID 4.4/01**		**Inhibition to microbial activity (aquatic)** **Assessment of the acute toxicity of [redacted] on aerobic waste water bacteria.**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] (1995) Assessment of the acute toxicity of [redacted] on aerobic waste water bacteria, [redacted], Unpublished, 18 September 1995	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 209	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde, 50% [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	3.1.3	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Analysis was not performed	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable. The material is soluble at the test ranges, and is not significantly volatile.	
3.3	**Reference substance**	3,5-dichlorophenol (97% pure)	
3.3.1	Method of analysis for reference substance	Not applicable. Positive control concentrations were reported as nominal concentrations.	
3.4	**Testing procedure**	*Non-entry field*	
3.4.1	Culture medium	A synthetic sewage feed was made by dissolving the following nominal amounts of substances in one liter of tap water: 16 g peptone; 11 g meat extract; 3 g urea; 0.7 g NaCl; 0.4 g $CaCl_2 2H_2O$; 0.2 g $MgSO_4 7H_2O$; 2.8 g K_2HPO_4. This was added to each test sample (16mL) along with	

Section A7.4.1.4 **Annex Point IIA&IIIA, VII.7.4&VII.3** **IUCLID 4.4/01**		**Inhibition to microbial activity (aquatic)** **Assessment of the acute toxicity of [redacted] on aerobic waste water bacteria.**	
		200mL of activated sludge. The volume was made up to 500mL with water, adjusted for the volume of test solution added.	
3.4.2	Inoculum / test organism	**Table A7.4.1.4(1)-1**	
3.4.3	Test system	**Table A7.4.1.4(1)-2**	
3.4.4	Test conditions	**Table A7.4.1.4(1)-3**	
3.4.5	Duration of the test	30 minutes	
3.4.6	Test parameter	Respiration inhibition	
3.4.7	Analytical parameter	Oxygen consumption rate Dissolved oxygen concentrations in the mixtures were measured over a period of up to 10 minutes with a *WTW OXI 92*. The pH of the test solutions was determined with the *pH 90*.	
3.4.8	Sampling	Up to 10 minutes post exposure	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Inoculum controls (2) were evaluated concurrently.	
3.4.11	Statistics	A percent inhibition value was determined by linear regression.	
		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	Nominally; 3.2, 10, 32, 50, 100 mg/L (based on 51.1% active 1.6, 5.1, 16, 26, 51 mg a.i./L)	
4.2.2	Actual concentrations of test substance	Not measured, nominal values reported.	
4.2.3	Growth curves	Not applicable	
4.2.4	Oxygen consumption	**Table A7.4.1.4(1)-4**	
4.2.5	Concentration/ response curve	**Refer to Figure A7.4.1.4(1)-1**	
4.2.6	Effect data	None reported	
4.2.7	Other observed effects	None	
4.3	**Results of controls**	**See Table A7.4.1.4(1)-4**	

Section A7.4.1.4 **Annex Point IIA&IIIA, VII.7.4&VII.3** **IUCLID 4.4/01**		**Inhibition to microbial activity (aquatic)** **Assessment of the acute toxicity of [redacted] on aerobic waste water bacteria.**	
4.4	**Test with reference substance**	Yes, concurrent to experiments with glutaraldehyde	
4.4.1	Concentrations	1.0, 3.2, 10, 32, 50 mg/L	
4.4.2	Results	**See Table A7.4.1.4(1)-4**	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test was performed with aerobic activated sludge from a domestic waste water treatment plant. The sludge was centrifuged and supernatant decanted. The solid material was resuspended in tap water, and centrifuged again (repeated twice). An aliquot was weighed, dried, and the ratio of wet to dry was determined. Based on the ratio, a volume of washed sludge suspension corresponding to 4g dry material was made per liter of buffer. Synthetic sewage feed was added daily and the sludge was aerated until use. The pH was determined to be 7.7. Test material was weighed into a flask and dissolved in water. 3,5-dichlorophenol was used as the reference compound. The test was conducted in glass flasks aerated at a rate of 0.2L/minute to prevent foam development. The pH was noted, and aeration started. Samples were prepared in 15 minute intervals. After 30 minutes of aeration, the first sample was taken for O_2 measurement over a period of 10 minutes. Thereafter, at 20 minute intervals, the rest of the samples were taken for measurements. The inhibitory effect of the test material on respiration rate (oxygen consumption per minute) was expressed as a percentage of the mean of the two control respiration rates measured at the start and end of oxygen measurements. Validation of the method was performed with the reference compound, and the EC_{50} was expected to be 5-30 mg/L.	
5.2	**Results and discussion**	At concentrations of 3.2, 10, and 32 mg/L, no inhibition of the respiratory rate (-9.5% to -3%) was observed. At test article concentrations of 50 and 100 mg/L, respiratory inhibition rates of 3.5% and 27.1% were observed, respectively. Nevertheless, the EC_{50} (30 minutes) was determined to be greater than 100 mg/L. The EC_{50} for the reference compound 3,5-dichlorophenol was found to be 20.9 mg/L under the same conditions, well within the range of 5-30 mg/L recommended by the testing guidelines.	
5.2.1	EC_{20}	Not calculated.	
5.2.2	EC_{50}	>100 mg/L for 30 minutes (>51 mg a.i./L)	
5.2.3	EC_{80}	Not calculated.	
5.3	**Conclusion**	The test conditions were acceptable, and the EC_{50}, reported to be >100 mg/L, is considered valid. The NOEC is 32 mg/L (16 mg a.i./L). The test is regarded as valid since the variance between the 2 control samples (start and end) was less than 15% (6.7%) and the EC_{50} for the reference compound was at 20.9 mg/L	
5.3.1	Reliability	1	
5.3.2	Deficiencies	Exposure time was 30 minutes.	

Section A7.4.1.4 **Annex Point IIA&IIIA, VII.7.4&VII.3** **IUCLID 4.4/01**	**Inhibition to microbial activity (aquatic)** **Assessment of the acute toxicity of [redacted] on aerobic waste water bacteria.**	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	March 3rd, 2009	
Materials and Methods	Agree with the applicant's version.	
Results and discussion	EC_{50} (30 min) >51 mg a.i./L based on nominal concentrations.	
Conclusion	Glutaraldehyde inhibits microbial activity above 51 mg/l.	
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 A7.4.1.4(1)-1 Inoculum / Test organisms

Criteria	Details
Nature	Activated sludge
Species	Not applicable
Strain	Not applicable
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	Activated sludge was obtained from a domestic waste water treatment plant [redacted]
Laboratory culture	No, obtained from a wastewater treatment plant.
Method of cultivation	Not applicable
Preparation of inoculum for exposure	The sludge was centrifuged and supernatant decanted. The solid material was resuspended in tap water, and centrifuged again (repeated twice). An aliquot was weighed, dried, and the ratio of wet to dry was determined. Based on the ratio, a volume of washed sludge suspension corresponding to 4g dry material was made per liter of buffer.
Pre-treatment	Synthetic sewage feed was added daily and the sludge was aerated until use. The pH was determined to be 7.7.
Initial cell concentration	4g dry material per litre of Soerensen buffer (pH7)

Table A7.4.1.4(1)-2 Test system

Criteria	Details
Culturing apparatus	Test concentrations prepared in bidistilled water in 1000 mL volumetric flasks. The appropriate volume of test solution was added to glass containers along with 16mL of synthetic sewage feed and 200mL of activated sludge. The test samples were made to a final volume of 500mL with water.
Number of culture flasks/concentration	1 for each concentration of test and reference solutions. 2 controls
Aeration	The reaction mixture was vigorously aerated with compressed air at 0.2 liters per minute
Measuring equipment	Dissolved oxygen concentrations in the mixtures were measured over a period of up to 10 minutes with a *WTW OXI 92*. The pH of the test solutions was determined with the *pH 90*.
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.4(1)-3 Test conditions

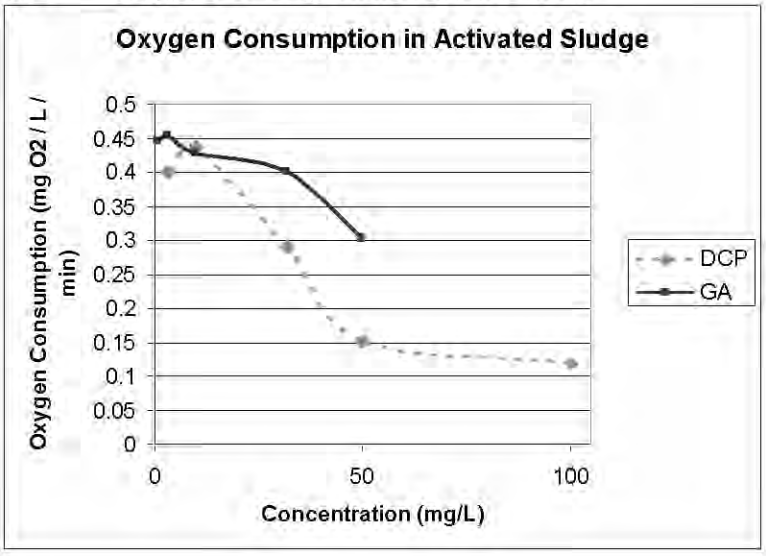
Criteria	Details
Test temperature	21°C
pH (solution of test material)	7.7
Aeration of dilution water	Yes Vigorously aerated with compressed air at a flow rate of 0.2 liters per minute.
Suspended solids concentration	1.6 x 10^{-3} g per sample (500 mL)

Table A7.4.1.4(1)-4 Results for Activated Sludge Inhibition Test [redacted]

	Sample mg/L	Initial pH	Oxygen Consumption (mg O_2 / L / min)	% Inhibition
	Control 1	7.8	0.401	---
	Control 2	7.9	0.428	---
3,5-dichlorophenol	50	7.8	0.118	71.5
	32	7.8	0.152	63.3
	10	7.8	0.290	30
	3.2	7.8	0.436	-5.2
	1	7.8	0.400	3.5
Glutaraldehyde	100 (51)	7.8	0.302	27.1
	50 (26)	7.8	0.400	3.5
	32 (16)	7.8	0.427	-3.0
	10 (5.1)	7.8	0.454	-9.5
	3.2 (1.6)	7.9	0.444	-7.1

Figures in brackets are reported as active ingredient.

Figure A7.4.1.4(1)-1 Oxygen Consumption in Activated Sludge



Section A7.4.3.2(1) Annex Point IIIA, XIII.2.2 IUCLID 4.5.1/01		**Effects on reproduction and the growth rate on an appropriate species of fish** [redacted]: **An early life-stage toxicity test with the Fathead minnow** [redacted]	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] (1999) An early life-stage toxicity test with the Fathead minnow [redacted]. Unpublished, 22 January 1999	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, FIFRA 72-4 and OECD 210	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde [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	3.1.3	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Samples containing glutaraldehyde were derivatised with 2,4-DNPH in orthophophoric acid and acetonitrile. Final extracts were analyzed by HPLC with diode array UV detection using a Hewlett Packard Model 1900 HPLC and the following conditions: Analytical Column: Zorbax Phenyl, 5µm, 250mm x 4.6mm Mobile phase A: 30:70:0.1 v/v/v acetonitrile:water:phosphoric acid Mobile phase A: 95:5:0.1 v/v/v acetonitrile:water:phosphoric acid	x

Section A7.4.3.2(1) **Annex Point IIIA, XIII.2.2** **IUCLID 4.5.1/01**		**Effects on reproduction and the growth rate on an appropriate species of fish** **[redacted]: An early life-stage toxicity test with the Fathead minnow [redacted]**	
		Gradient: Time (min.) Solvent A Solvent B 0.01 100 0 2.00 100 0 10.00 0 100 12.00 0 100 12.10 100 0 20.00 100 0 Flow rate: 1 mL/minute Primary analytical wavelength: 365 nm Oven temperature: 40°C Injection Volume: 50 µl Retention time: approx. 13.6 min.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	No reference substance reported.	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	**Table A7.4.3.2(1)-1**	
3.4.2	Test organisms	**Table A7.4.3.2(1)-2**	
3.4.3	Test system	**Table A7.4.3.2(1)-3**	
3.4.4	Test conditions	**Table A7.4.3.2(1)-4**	
3.4.5	Duration of the test	32 days	
3.4.6	Test parameter	Time to hatch, hatching success, survival and growth	
3.4.7	Sampling	Observations of mortality and other clinical signs were made daily during the test. Time to hatch, hatching success, growth and survival were monitored in each treatment and control group.	
3.4.8	Monitoring of TS concentration	Prior to test initiation, samples of water were collected from each replicate test chamber of the low level and high level treatment groups and analyzed. On days 0, 7, 14, 21, and 28 and at test termination, samples were taken from each treatment group and control and analyzed.	
3.4.9	Statistics	Hatching and survival were analyzed using 2 x 2 contingency tables. Total length, wet body weight and dry body weight were assessed using ANOVA and Dunnett's test. Treatment groups statistically significant for survival were not included in the analysis for effects on weight and	

Section A7.4.3.2(1) Annex Point IIIA, XIII.2.2 IUCLID 4.5.1/01		**Effects on reproduction and the growth rate on an appropriate species of fish** [redacted]: **An early life-stage toxicity test with the Fathead minnow** [redacted]	
		length.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentration	Not applicable	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Results test substance**	*Non-entry field*	
4.2.1	Initial concentrations of test substance	Nominally, the target concentrations were 0.38, 0.75, 1.5, 3.0, and 6.0 mg/L.	
4.2.2	Actual concentrations of test substance	Mean analytical concentrations were 0.29, 0.61, 1.4, 2.9, and 5.9 mg/L.	
4.2.3	Effect data	**Table A7.4.3.2(1)-5** At the 3.0 mg/L dose level there was a 63% survival rate and at the 6.0 mg/L dose level there was a 7% survival rate. Both of these values were statistically significant when compared to the negative control group.	
4.2.4	Concentration / response curve	Not reported.	
4.2.5	Other effects	Total length, wet and dry weight in the 2.9 and 5.9 mg/l a.i. treatment groups were reduced in a dose-dependent manner, but they were not analysed statistically because of a significant effect on survival.	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	**Table A7.4.3.2(1)-5** None	
4.3.2	Nature of adverse effects	Not applicable	
4.4	**Test with reference substance**	*Non-entry field*	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	

Section A7.4.3.2(1) **Annex Point IIIA, XIII.2.2** **IUCLID 4.5.1/01**	**Effects on reproduction and the growth rate on an appropriate species of fish** **[redacted]: An early life-stage toxicity test with the Fathead minnow [redacted]**	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The guidelines followed for the current study included: 1. U.S. EPA Series 72 Pesticide Assessment Guidelines, Subdivision E, Hazard evaluation: Wildlife and aquatic organisms 2. OECD Guideline 210: Fish, Early life stage toxicity 3. U.S. EPA Standard evaluation procedure, fish early life-stage test 4. ASTM Standard E1241-88, Standard guide for conducting early life-stage toxicity tests with fish The study was designed to evaluate the effects of glutaraldehyde on early life-stage development of the fathead minnow. Time to hatch, hatching success, survival, and growth were evaluated during the 32-day study. They were exposed to a geometric series of 5 test concentrations and well water (control) under flow-through conditions. Two replicate chambers were evaluated for each group. Into each test vessel, 40 embryos (less than 24 hours old) per replicate (80 total per dose level) were placed. The exposure period included a 4-day embryo hatching period, and a 28-day post-hatch juvenile growth period. Twice during the first 24 hours, and daily thereafter, all dead embryos and eggs with fungus were counted and removed. After hatching, the larvae were counted and released into the test chambers, where the exposures continued until test termination. Larvae were fed live brine shrimp three times daily for the first seven days post-hatch. Days 8-26, fish were fed brine shrimp three times daily on weekdays, and twice daily on weekends. Fish were not fed for 48 hours prior to study termination. A photoperiod of 16 hour light / 8 hours dark was employed. Samples of water were collected from each treatment group on days 0, 7, 14, 21, 28, and test termination for analysis of test material concentration. Observations of mortality and other clinical signs were made daily during the test. Time to hatch, hatching success, growth, and survival were monitored in each treatment and control group. Data were evaluated to determine the NOEC, the LOEC, and the maximum acceptable toxicant concentration (MATC) when possible. Nominal concentrations: Control, 0.38, 0.75, 1.5, 3.0 and 6.0 mg glutaraldehyde per litre.	
5.2 Results and discussion	**Table A7.4.3.2(1)-5** Hatching percent and percentage post-hatch survival on days 7, 14, 21 and 28 after hatching: ***% Post-hatch survival on Days 0, 7, 14, 21, 28*** mg/l a.i. 0 7 14 21 28 0 89 92 92 92 86 0.29 86 93 93 91 88 0.61 89 94 94 92 79	

Section A7.4.3.2(1) **Annex Point IIIA, XIII.2.2** **IUCLID 4.5.1/01**	**Effects on reproduction and the growth rate on an appropriate species of fish** **[redacted]: An early life-stage toxicity test with the Fathead minnow [redacted]**	

1.4	85	96	96	93	78
2.9	89	85	76	75	62
5.9	85	54	15	12	7

All eggs hatched on day 4 except 1 egg in the control and 2 eggs in the 5.9 mg/l treatment which hatched on day 5. Rest of the eggs that had not hatched on day 5 was determined to be non-viable. Hatching success ranged from 85 to 89%. There were no significant differences in time of hatching or hatching success between the control and any treatment groups.

Total length, wet and dry weight as a mean of two replicates

mg/l a.i.	Total length (mm)	Wet weight (mg)	Dry weight (mg)
0	21.1+0.21	68.9+2.97	14.9+0.71
0.29	20.4+0.50	66.5+6.29	13.9+0.71
0.61	21.1+0.78	66.4+5.23	13.5+1.48
1.4	21.0+0.78	65.1+4.38	12.8+1.06
2.9	19.6+0.21	52.7+3.54	10.2+0.35
5.9	18.1+0.49	42.6+10.3	7.50+1.84

There were no significant differences in fish growth between the control and the 0.29, 0.61 and 1.4 mg a.i./l. treatment groups. Total length, wet and dry weight in the 2.9 and 5.9 mg a.i./l. treatment groups were reduced in a dose-dependent manner, but they were not analyzed statistically because of a significant effect on survival.

	NOEC (mg/l a.i.)	LOEC (mg/l a.i.)
Survival	1.4	2.9
Growth	1.4	2.9

Water pH measurements were consistent over time and showed no obvious differences between control and treatment groups. Dissolved O_2 concentrations were 93% of saturation. Temperature data showed a range of 25 ± 1°C. Results are given as mean measured concentrations. The mean measured concentrations were 0.29, 0.61, 1.4, 2.9, and 5.9 mg a.i./L, representing 76, 81, 93, 97, and 98% of the nominal concentrations respectively.

According to the daily observations, the larvae appeared normal except in the highest glutaraldehyde concentration (5.9 mg a.i./l) where some larvae were lethargic before their death. No other abnormal behaviour or deformations were reported.

5.2.1	NOEC	1.4 mg a.i./L (survival and growth)
5.2.2	LOEC	2.9 mg a.i./L (survival and growth)
5.3	**Conclusion**	Survival was the most sensitive biological factor. The survival of the fathead minnows in the 2.9 and 5.9 mg a.i./L groups was statistically reduced when compared to the negative control group. Post-hatch

Section A7.4.3.2(1) **Annex Point IIIA, XIII.2.2** **IUCLID 4.5.1/01**	**Effects on reproduction and the growth rate on an appropriate species of fish** **[redacted]: An early life-stage toxicity test with the Fathead minnow [redacted]**	
	growth was also reduced in the same groups when compared to the negative controls.	
5.3.1 Other Conclusions	None	
5.3.2 Reliability	1	
5.3.3 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	March 16th, 2009	
Materials and Methods	The applicant's version is acceptable. The LOQ was 0.2 mg/L, being above the LOQ of 0.1 µg/L in Doc IIIA4.2c.	
Results and discussion	NOEC 1.4 mg a.i./L (survival and growth), given as mean measured concentrations. The mean measured concentrations were 81-98% of the nominal concentrations except in the lowest test concentration where the mean measured concentration was 76% of the nominal concentration.	
Conclusion	Glutaraldehyde is slightly toxic to [redacted]	
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 A7.4.3.2(1)-1 Dilution water

Criteria	Details
Source	The water used for culturing and testing was freshwater obtained from a well approximately 40 meter deep [redacted]
Alkalinity	182 mg/L as $CaCO_3$
Hardness	132 mg/L as $CaCO_3$
pH	8.2-8.4
Oxygen content	7.7-8.3 mg/L
Conductance	340 umhos/cm
Holding water different from dilution water	No

Table A7.4.3.2(1)-2 Test Organisms

Criteria	Details
Species Name	[redacted]
Source of Organisms	[redacted]
Wild caught	No
Age/Size	Embryos were less than 24 hours old at the time of initiation of the study.
Feeding (kind of food, amount of food and feeding frequency)	Newly hatched larvae were fed live brine shrimp nauplii 3 times per day during the 1-7 days post hatch. On days 8- 26 post hatch, all fish were fed live brine shrimp nauplii 3 times daily on weekdays and at least 2 times daily on weekends. Fish were not fed at least 48 hours prior to the termination of the study. Rations were adjusted each week to account for losses due to mortality.
Acclimation Period	Embryos collected for use in test were from at least 3 individual spawns and were less than 24 hours old when test was initiated.
Daily Observations	Observations of mortality and other clinical signs were made daily during the test. Time to hatch, hatching success, growth, and survival were monitored in each treatment and control group.

Table A7_4_3_2(1)-3: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	A continuous flow diluter was used to deliver each concentration of test substance and negative control. The diluter was adjusted so that each test chamber received approximately 13 volume additions of test water every 24 hours.
Volume of test vessels	9L glass aquaria filled with approximately 7L of test solution.
Volume/animal	0.175 L
Number of animals/vessel	40
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2(1)-4: Test conditions

Criteria	Details
Test temperature	The range recorded during the test was 24.5 to 26.0°C
Dissolved oxygen	The range recorded during the test was 7.7 to 8.3 mg/L
pH	The range recorded during the test was 8.2 to 8.5
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	260 lux at the surface of the water at test initiation
Photoperiod	16 hours light / 8 hours dark

Table A7.4.3.2(1)-5 Effect Data

Hatching Success of Fathead Minnow Embryos Exposed to the Test Substance

Mean Measured Concentration (mg/L)	Replicate	Number of Eggs Exposed	Total Hatched	Replicate Hatching success	Treatment percent Hatching success
Negative control	A	40	38	95	89
	B	40	33	83	
0.29	A	40	35	88	86
	B	40	34	85	
0.61	A	40	35	88	89
	B	40	36	90	
1.4	A	40	33	83	85
	B	40	35	88	
2.9	A	40	35	88	89
	B	40	36	90	
5.9	A	40	34	85	85
	B	40	34	85	
Percent Hatching Success = (Total hatched/40) x 100					

Survival of Fathead Minnow Larvae Exposed to the Test Substance for 28 Days Post-Hatch

Mean Measured Concentration (mg/L)	Replicate	Initial number of larvae	Live Count Day 28	Replicate Percent Survival	Number of Surviving Larvae	Treatment Percent Survival
Negative control	A	38	32	84	62	87
	B	33	30	91		
0.29	A	35	30	86	60	87
	B	34	30	88		
0.61	A	35	29	83	57	80
	B	36	28	78		
1.4	A	33	28	85	53	78
	B	35	25	71		
2.9	A	35	23	66	45	63*
	B	36	22	61		
5.9	A	34	3	9	5	7*
	B	34	2	6		
Percent Survival = (live count day 28/initial number of larvae) x 100						
* Significantly different from negative control group at $p \leq 0.05$						

Total Length, Wet Weight, and Dry Weight of Fathead Minnow Larvae at the End of the 28-Day Post-Hatch Observation Period

Mean Measured Concentration (mg/L)	Number of Replicates	Total Length (mm)	Wet Weight (mg)	Dry Weight (mg)
Negative control	2	21.1 +/- 0.21	68.9 +/- 2.97	14.9 +/- 0.71
0.29	2	20.4 +/- 0.50	66.5 +/- 6.29	13.9 +/- 0.71
0.61	2	21.1 +/- 0.78	66.4 +/- 5.23	13.5 +/- 1.48
1.4	2	21.0 +/- 0.78	65.1 +/- 4.38	12.8 +/- 1.06
2.9	2	19.6 +/- 0.21	52.7 +/- 3.54	10.2 +/- 0.35
5.9	2	18.1 +/- 0.49	42.6 +/- 10.3	7.5+/- 1.84

Table A7_4_3_2(1)-6: Validity criteria for fish tests according to OECD Guidelines 210/212

	fulfilled	Not fulfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	X	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	X	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	X	
Test substance concentrations maintained within ± 20% of mean measured values		X*
No effect on survival nor any other adverse effect found in solvent control	X	
Further criteria for poorly soluble test substances	NA	

* = at the lowest test concentration the measured concentration was within 24%

Section A7.4.3.4(1) Annex Point IIIA, XIII 2.4 IUCLID 4.5.2/01		Effects on reproduction and growth rate with an invertebrate species Daphnia [redacted], Reproduction Test with Glutaraldehyde 50% (flow-through)	
		1 REFERENCE	Official use only
1.1	Reference	[redacted] (2003) Daphnia [redacted], Reproduction Test with Glutaraldehyde 50% (flow-through), [redacted], Unpublished, 10 April 2003	
1.2	Data protection	Yes	
1.2.1	Data owner	The Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	Yes, OECD 211	
2.2	GLP	Yes	
2.3	Deviations	No	
		3 METHOD	
3.1	Test material	Glutaraldehyde, 50%	
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	3.1.3	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Samples containing glutaraldehyde were derivatised with 2,4-DNPH and orthophosphoric acid in acetonitrile. Final extracts were analyzed by HPLC with UV detection using a suitable HPLC and the following conditions: Analytical Column: Zorbax 5 CN, 250mm x 4.6mm, 5µm Mobile phase: 75:25:0.1 v/v/v acetonitrile:water:phosphoric acid Flow rate: 1 mL/minute Wavelength: 365 nm Injection Volume: 100 µl	x
3.2	Preparation of TS solution for poorly soluble or volatile test substances	The material is soluble in water, and significant volatilization is not expected based on vapour pressure.	
3.3	Reference substance	No	
3.3.1	Method of analysis	Not applicable	

Section A7.4.3.4(1) Annex Point IIIA, XIII 2.4 IUCLID 4.5.2/01		Effects on reproduction and growth rate with an invertebrate species Daphnia [redacted], Reproduction Test with Glutaraldehyde 50% (flow-through)	
	for reference substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	**Table A7.4.3.4/01-1**	
3.4.2	Test organisms	**Table A7.4.3.4/01-2**	
3.4.3	Handling of offspring	Daily, the number of newborn young was counted and the condition of the young recorded. Thereafter the young were removed.	
3.4.4	Test system	**Table A7.4.3.4/01-3**	
3.4.5	Test conditions	**Table A7.4.3.4/01-4**	
3.4.6	Duration of the test	21-days	
3.4.7	Test parameter	Survival, reproduction, and growth (length)	
3.4.8	Examination / Sampling	Daily, the numbers of living, immobile, and dead daphnids were recorded. Dead daphnids were removed when observed. Presence of eggs in the brood pouch was examined daily. Body length was measured at the end of the test. Appearance of the first brood was recorded when first observed, and the number of newborn young was counted daily. The condition of the young was also evaluated, and then the young were removed. The presence of unhatched eggs and incidence of immobility was also recorded when observed. Dissolved oxygen, temperature, and pH were measured and recorded at least twice per week in all test solutions.	
3.4.9	Monitoring of TS concentration	Test concentrations were allowed to stabilize for 43 hours after the start of the dosing, and before introduction of the organisms. Analytical monitoring was done the day before the start of the dosing system from the 2.1 mg/L vessel, and on days 0, 7, 14, and 21 for all test vessels.	
3.4.10	Statistics	For each concentration, the results of reproduction were tested for normality and for homogeneity of variance. Furthermore, these data were statistically tested using an ANOVA test followed by a mean comparison test (Dunnett's test, Bonferroni-T, and Tukey test). The overall threshold level of effect and the overall NOEC were determined on the basis of these statistics. The EC50 for reproduction was calculated.	
		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 applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Results test substance**	*Non-entry field*	

Section A7.4.3.4(1) Annex Point IIIA, XIII 2.4 IUCLID 4.5.2/01		Effects on reproduction and growth rate with an invertebrate species Daphnia [redacted], Reproduction Test with Glutaraldehyde 50% (flow-through)	
4.2.1	Initial concentrations of test substance	Nominal concentrations were 0.9, 1.3, 2.1, 3.3, 4.5 mg a.i./L	
4.2.2	Actual concentrations of test substance	Mean exposure concentrations were 0.26, 0.39, 0.64, 1.28, and 1.89 mg a.i./L respectively.	
4.2.3	Effect data	Five of 40 daphnia died during the test period in the control exposure (meeting criteria of <20% mortality). A clear, dose-related mortality trend was not seen. **Table A7.4.3.4/01-5** Daphnid reproduction decreased with increasing concentration at target concentrations between 0.9-3.3 mg a.i./L (nominal). Reproduction at the highest test concentration was higher than the reproduction a the second highest concentration, with no clear explanation	
4.2.4	Concentration / response curve	Refer to **Figure A7.4.3.4(1)-1**	
4.2.5	Other effects	None	
4.3	**Results of controls**	5 out of 40 parental daphnia died during the test period in the control exposure. Therefore, the parental mortality did not exceed the validity criterion of 20% in the control. The reproduction rates were calculated relative to the controls. Thus, there exists no basis for comparison for the control themselves.	
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**	This study was conducted to GLP and according to OECD Guideline for Testing Chemicals No. 211, *Daphnia* [redacted] Reproduction Test. The study was conducted as a 21-day static renewal test and the data used to calculate LOEC and NOEC values for adults survival and reproduction. Daphnia (10 per vessel), less than 24 hours old, were placed in test vessels held at 18-22°C. They were fed daily with a suspension of fresh water algae. Target concentrations and dosing methods for a definitive study were determined using a feasibility study (determine the frequency of renewal of test solutions) and a semi-static test attempt. The definitive study was performed using a continuous, flow-through system for a 21-day study period. A photoperiod of 16 hours light and 8 hours darkness was used. Stock solutions of milli-Q water were prepared every other day and dosed via a computer-controlled system. Test concentrations were allowed to stabilize for 43 hours after the start of the dosing, and before introduction of the organisms. Analytical monitoring was done the day before the start of the dosing system from the 2.1 a.i. mg/L vessel, and	

Section A7.4.3.4(1) Annex Point IIIA, XIII 2.4 IUCLID 4.5.2/01	**Effects on reproduction and growth rate with an invertebrate species** **Daphnia [redacted], Reproduction Test with Glutaraldehyde 50% (flow-through)**	

on days 0, 7, 14, and 21 for all test vessels.

Daily, the numbers of living, immobile, and dead daphnids were recorded. Dead daphnids were removed when observed. Presence of eggs in the brood pouch was examined daily. Body length was measured at the end of the test. Appearance of the first brood was recorded when first observed, and the number of newborn young was counted daily. The condition of the young was also evaluated, and then the young were removed. The presence of unhatched eggs and incidence of immobility was also recorded when observed. Dissolved oxygen, temperature, and pH were measured and recorded at least twice per week in all test solutions.

For each concentration, the results of reproduction were tested for normality and for homogeneity of variance. Furthermore, these data were statistically tested using an ANOVA test followed by a mean comparison test (Dunnett's test, Bonferroni-T, and Tukey test). The overall threshold level of effect and the overall NOEC were determined on the basis of these statistics. The EC50 for reproduction was calculated.

Adult survival and reproduction was assessed throughout the test.

5.2 Results and discussion

Table A7.4.3.4/01-5

Analysis of samples taken at the beginning of test period indicated the vessels achieved 88-106% of the target concentrations. A rapid decline, however, in test concentrations was evident on days 7, 14, and 21.

Target	Day 0	Day 7	Day 14	Day 21	Mean	TWA[1]	Geom[2]
0.9 mg a.i./L	0.83	0.059	0.086	0.074	0.26	0.12	0.13
1.3 mg a.i./L	1.30	0.097	0.085	0.085	0.39	0.18	0.17
2.1 mg a.i./L	2.00	0.138	0.224	0.209	0.64	0.31	0.34
3.3 mg a.i./L	3.50	0.508	0.536	0.560	1.28	0.80	0.86
4.5 mg a.i./L	4.78	0.985	1.15	0.626	1.89	1.36	1.36

[1]TWA formula used in the test report:
$((Ct=0 \times Ct=7)^{½} \times 7 + (Ct=7 \times Ct=14)^{½} \times 7 + (Ct=14 \times Ct=21)^{½} \times 7)) / 21$
[2]Geometric mean concentrations

Temperatures ranged from 19.6°C to 22.2°C during the 21-day study. The pH ranged from 7.4-8.1, and the dissolved oxygen was reported to be 6.9-8.9.

Five of 40 daphnia died during the test period in the control exposure (meeting criteria of <20% mortality). A clear, dose-related mortality trend was not seen.

Daphnid reproduction decreased with increasing concentration at target concentrations between 0.9-3.3 mg a.i./L (nominal). Reproduction at the highest test concentration was higher than the reproduction at the second highest concentration, with no clear explanation.

The percentages for inhibition in reproductive rate relative to the control

Section A7.4.3.4(1) **Annex Point IIIA, XIII 2.4** **IUCLID 4.5.2/01**	**Effects on reproduction and growth rate with an invertebrate species** **Daphnia [redacted], Reproduction Test with Glutaraldehyde 50% (flow-through)**	
	were calculated. Calculation of the EC_{50} was based on linear regression analysis of the percentages of inhibition in reproduction versus both the corresponding nominal and average measured concentrations of the test material. The EC_{50} (mean) was calculated to be 0.62 mg a.i./L, with 95% confidence limits of 0.45 and 0.87 mg a.i./L (nominal). An EC_{50} (mean body length) could not be calculated, as not more than 24% reduction in average body length was observed at any concentration. The EC_{10} = 0.40 mg a.i./L. Eggs produced by the 0.9 mg a.i./L and 1.3 mg a.i./L parents were green in color, and were produced on day 7 for the 0.9 mg a.i./L group, day 8 for the 2.1 mg a.i./L group, and on day 9 (all immobile) for the 3.3 and 4.5 mg a.i/L groups. No aborted eggs were observed in any of the concentrations tested. The study met the following criteria for acceptability: 1. Mortality of parental control daphnia was not greater than 20%. 2. The average cumulative number of young per female in the controls was >60. 3. pH, dissolved oxygen, and temperature were within recommended ranges for the species at all times. 4. Results were reported as mean and target exposure concentrations.	
5.2.1 NOEC	21-day NOEC for reproduction was 1.3 mg a.i./L based on target concentration (mean measured concentration of 0.26 mg a.i./L). 21-day NOEC for body length was <0.9 mg/L based on target concentration (mean measured concentration of <0.26 mg a.i/L).	x
5.2.2 LOEC	21-day LOEC for reproduction was 1.3 mg a.i./L based on target concentration (mean measured concentration of 0.39 mg a.i./L). 21-day LOEC for body length was 0.9 mg a.i./L based on target concentration (mean measured concentration of 0.26 mg a.i./L).	
5.2.3 EC_{50} (EC_x)	21-day EC_{50} for reproduction was 1.9 mg a.i./L based on target concentration (mean measured concentration of 0.62 mg a.i./L). 21-day EC_{50} for body length was >4.5 mg a.i./L based on target concentration (mean measured concentration of <1.9 mg a.i./L).	
5.3 Conclusion	Daphnid reproduction decreased with increasing concentration at target concentrations between 0.9-3.3 mg a.i./L (nominal). Reproduction at the highest test concentration was higher than the reproduction at the lowest concentration, with no clear explanation. Mean body lengths were statistically significant in regards to length reduction at all test levels.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	

Section A7.4.3.4(1) Annex Point IIIA, XIII 2.4 IUCLID 4.5.2/01	**Effects on reproduction and growth rate with an invertebrate species** **Daphnia [redacted], Reproduction Test with Glutaraldehyde 50% (flow-through)**	
Date	March 23rd, 2009	
Materials and Methods	The applicant's version is acceptable. 3.1.6 No LOQ given. 4.2.2 Despite the continuous flow-through exposure the concentrations dropped significantly below the nominal concentrations, ranging from 7 to 25% of nominal concentrations. Interaction of glutaraldehyde with algae was speculated to be a reason for low recoveries, but this could not be confirmed in the additional experiment. In order to compensate the drop of concentrations the results are expressed as Time Weight Average concentrations calculated with the formula $((Ct=0 \times Ct=7)^{½} \times 7 + (Ct=7 \times Ct=14)^{½} \times 7 + (Ct=14 \times Ct=21)^{½} \times 7)) / 21$. The TWA concentrations are approximately equal to the geometric mean concentrations (see table in section 5.2). A7.4.3.4/01-3 According to the OECD 211, daphnids should be kept individually in the test vessels. In this test ten daphnids were kept in a mesh container within a 1.5 l vessel. One test vessel contained 4 mesh containers. 5.2.1 Target concentration of 1.3 mg a.i./L should be 0.9 mg a.i./L.	
Results and discussion	Due to lack of treatment related mortality NOEC for parental survival could not be determined. It was neither possible to calculate the LC10 for the same reason. In addition, as no treatment related increase of mortality was present the maximum mortality was 35%. Mean number of young per parent decreased with dose dependent manner apart from the highest test concentration. A treatment related delay in production of offspring was observed. Significant numbers of immobile offspring were produced by parent Daphnia exposed to two highest concentrations. NOEC for reproduction was 0.12 mg a.i./L based on the TWA concentrations (see calculation formula above). Mean body length of parental Daphnia decreased with dose dependent manner apart from the highest test concentration. The EC10 of 0.19 mg a.i./L with 95% fiducial limits of 0.09-0.38 was calculated. EC10 can be considered as NOEC. NOEC 0.12 mg a.i./L based on the TWA concentrations will be used for the risk assessment.	
Conclusion	Glutaraldehyde is toxic to *Daphnia* [redacted].	
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*	

Section A7.4.3.4(1) **Annex Point IIIA, XIII 2.4** **IUCLID 4.5.2/01**	**Effects on reproduction and growth rate with an invertebrate species** **Daphnia [redacted], Reproduction Test with Glutaraldehyde 50% (flow-through)**	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A7.4.3.4/01-1 Dilution water

Criteria	Details
Source	Tap water was purified by reversed osmosis and subsequently passed over activated carbon and ion-exchange cartridges: Millipore, Bedford, MA, USA
Salinity	Not reported
Hardness	250 mg/L as $CaCO_3$
pH	8.0 ± 0.2
Ca / Mg ratio	Not reported
Na / K ratio	Not reported.
Oxygen content	> 6 mg/L during the exposure period
Conductance	Not reported
TOC	Not reported
Holding water different from dilution water	No

Table A7.4.3.4/01-2 Test organisms

Criteria	Details
Strain / Clone	[redacted]
Source	[redacted]
Age	Less than 24 hours old
Breeding method	Not applicable (parthenogensis)
Kind of food	A suspension of fresh water algae (*Chlorella pyrenoidosa*)
Amount of food	During first 11 days 20 mL containing 2 x 10^8 cells From day 12 until the end of test amounts of 12.5 mL
Feeding frequency	During first 11 days twice daily From day 12 until the end of the test four times daily
Pretreatment	Test concentrations were allowed to stabilize for 43 hours after the start of the dosing and before introduction of the organisms.
Feeding of animals during test	Yes, see above

Table A7.4.3.4/01-3 Test system

Criteria	Details
Test type	21-day flow-through
Renewal of test solution	Flow rate was 300 mL/h
Volume of test vessels	1.5L
Volume/animal	Not specified
Number of animals/vessel	For each concentration and the controls the system comprised one stainless steel vessel (1.5L) with 4 mesh containers of stainless steel. At the start of the test 10 neonate daphnia were placed in each mesh.
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of test material	Test material considered non-volatile.

Table A7.4.3.4/01-4 Test conditions

Criteria	Details
Test temperature	19.6-22.2°C
Dissolved oxygen	> 6 mg/L during the exposure period
pH	7.4-8.1
Adjustment of pH	No
Aeration of dilution water	yes
Intensity of irradiation	280 lux
Photoperiod	16-hour light/8-hour dark photoperiod daily

Table A7.4.3.4/01-5 Summary of Biological Data

Concentrations of Glutaraldehyde 50% in mg/L							
Target Concentration	**day 1**	**day 0**	**day 7**	**day 14**	**day 21**	**Mean Exposure concentration**	
0.9 mg/L	nm	0.83	0.059	0.086	0.074	0.260 mg/L	
1.3 mg/L	nm	1.30	0.097	0.085	0.085	0.390 mg/L	
2.1 mg/L	1.85	2.00	0.138	0.224	0.209	0.640 mg/L	
3.3 mg/L	nm	3.50	0.508	0.536	0.560	1.280 mg/L	
4.5 mg/L	nm	4.78	0.985	1.150	0.626	1.890 mg/L	
nm: not measured							

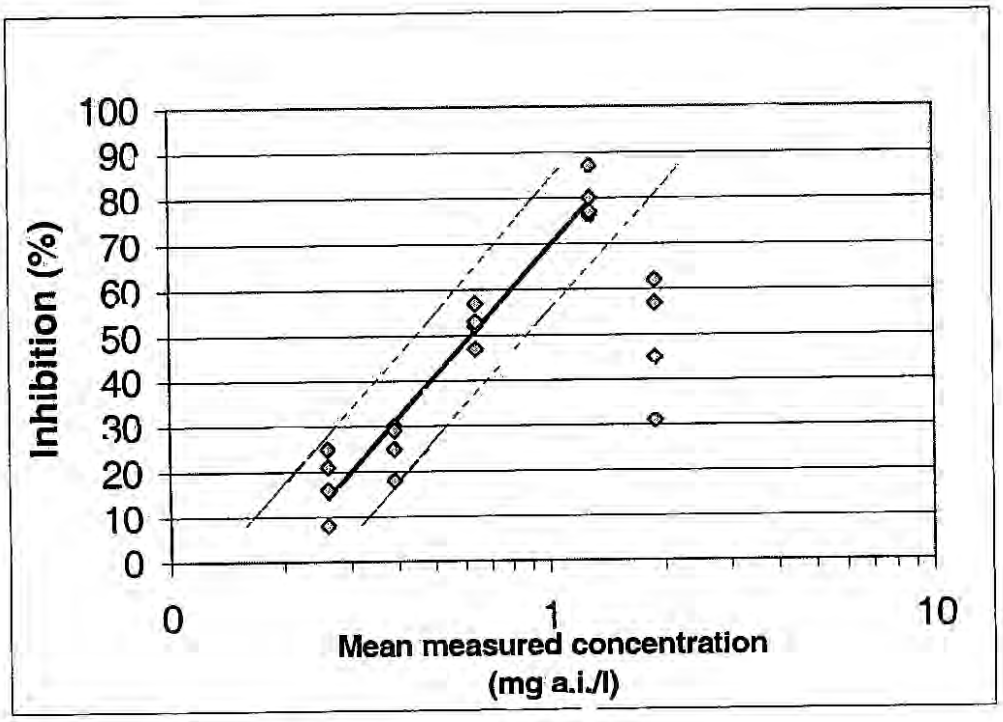
Parameter	Glutaraldehyde 50%	
	Target Concentration	*Mean measured concentration*
observed 21 day EC_{50} for parental survival	> 4.5 mg/L	> 1.9 mg/L
21 day EC_{50} for reproduction	1.9 mg/L	0.62 mg/L
observed 21 day EC_{50} for body length	> 4.5 mg/L	> 1.9 mg/L
observed 21 day for EC_{10} for body length	1.3 mg/L	0.40 mg/L
21 day LOEC for reproduction	1.3 mg/L	0.39 mg/L
21 day NOEC for reproduction	0.9 mg/L	0.26 mg/L
21 day LOEC for body length	0.9 mg/L	0.26 mg/L
21 day NOEC for body length	< 0.9 mg/L	< 0.26 mg/L

Table A7.4.3.4/01-6 Validity criteria for invertebrate reproduction test according to OECD Guideline 211

	fulfilled	**Not fulfilled**
Mortality of parent animals < 20% at test termination	**Yes**	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	**Yes**	
pH, dissolved oxygen, and temperature were within recommended ranges for the species at all times	**Yes**	
Results were reported as mean and target exposure concentrations	**Yes**	

Criteria for poorly soluble test substances	**N/A**	

Figure A7.4.3.4(1)-1 Concentration-Inhibition Curve for Daphnia [redacted] Exposed to Glutaraldehyde for 21 Days



16 January 2012

Project-No.: [redacted]

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 Dipl.-Stat. [redacted]

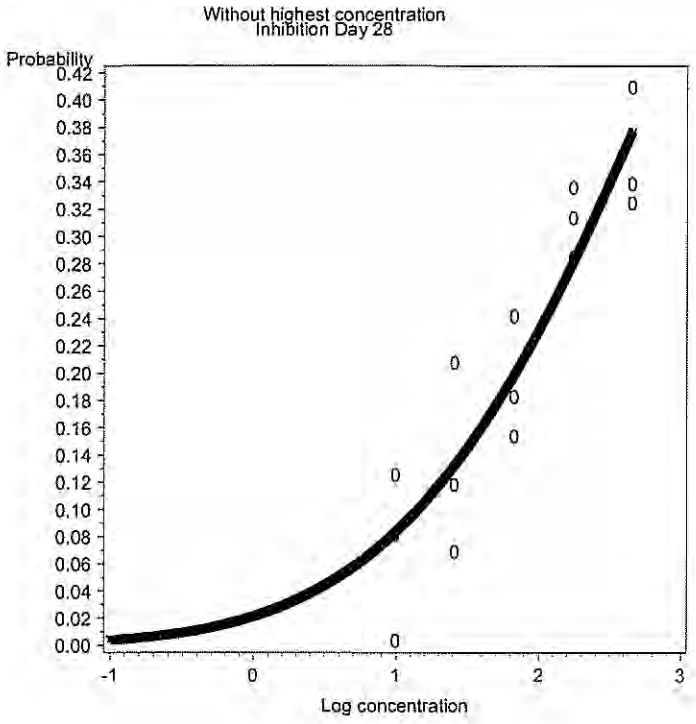
[redacted]

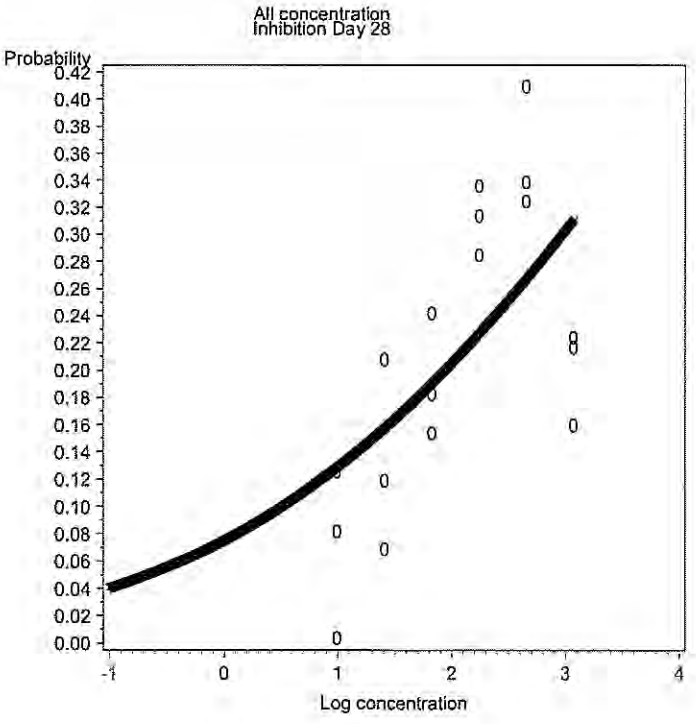
Project No. [redacted]

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) [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted] 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. / 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

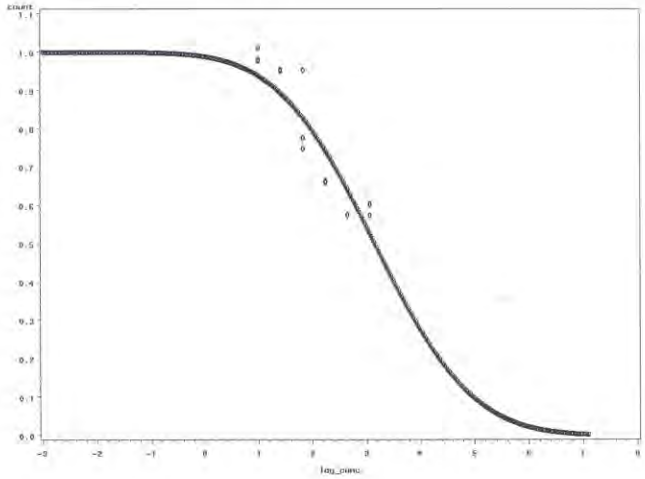
3.3.2	Test system	See table A7_5_1_1-3	
3.3.3	Application of TS	See table A7_5_1_1-4	
3.3.4	Test conditions	See table A7_5_1_1-5	
3.3.5	Test parameter	Inhibition of microbial carbon transformation: The glucose induced respiration rates were measured during 12 consecutive hours for 3 samples of test mixture 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.	

Section A7.5.1.1_01	Inhibition to microbial activity (terrestrial)
Annex Point IIA7.4	Carbon Transformation Test

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	

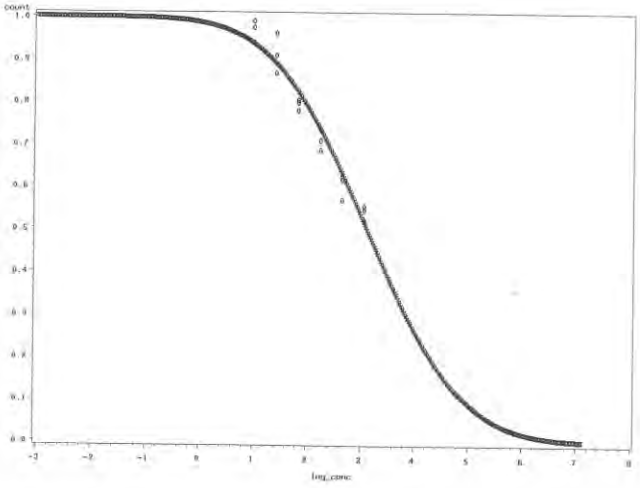
4.2.3.1 At test initiation

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



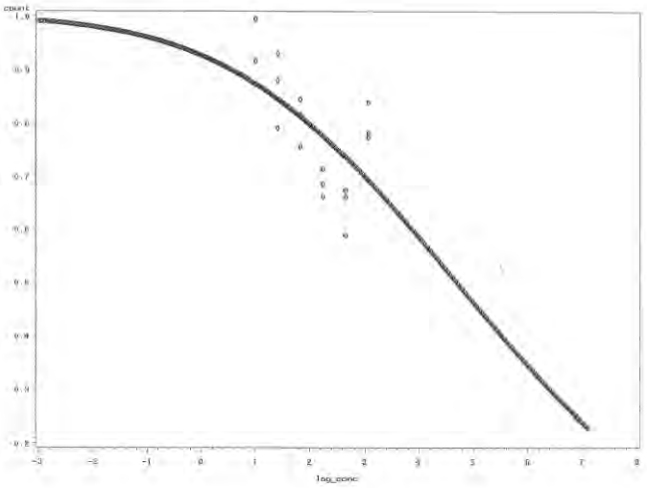
4.2.3.2 At sampling time point 7 days

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

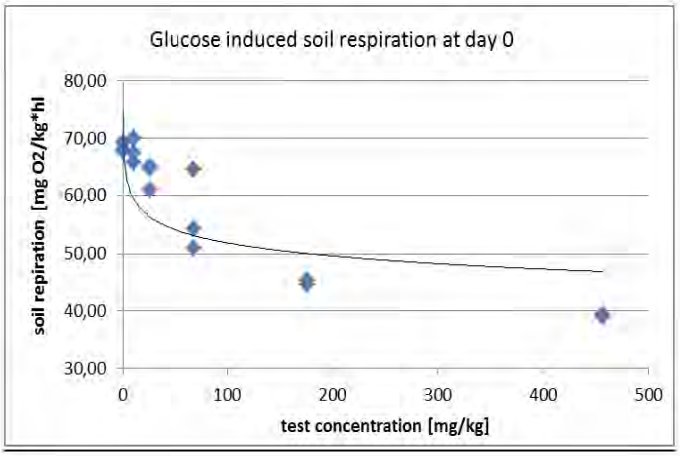


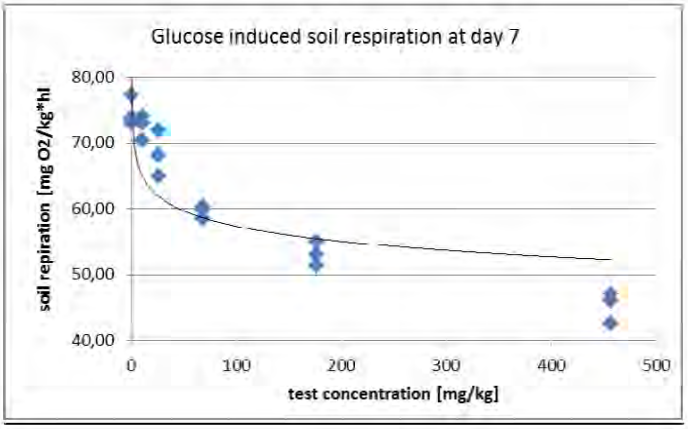
4.2.3.3 At sampling time point 28 days

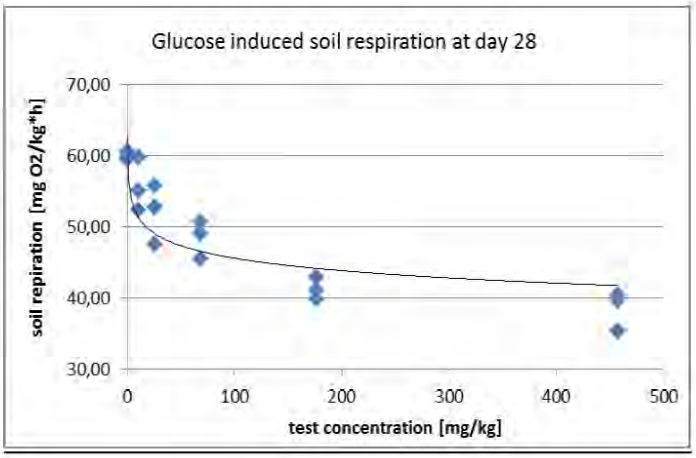
Figure 3: Graphical illustration of the probit analysis of the test substance after an exposure period of 28 days



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]

The test was conducted according to OECD 217, and followed GLP.

About 50 kg of soil were collected [redacted]; the weather conditions were sunshine and 15 °C. [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 [redacted]. 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 (batch no. Sp 5M4205)
Sampling site:	The soil sample was prepared conformely to the specifications of the guideline.
Geographical reference on the sampling site	[redacted]
Data on the history of the site	Sampling date was the 17 October 2005
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 Fa, WTW (Germany): 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)

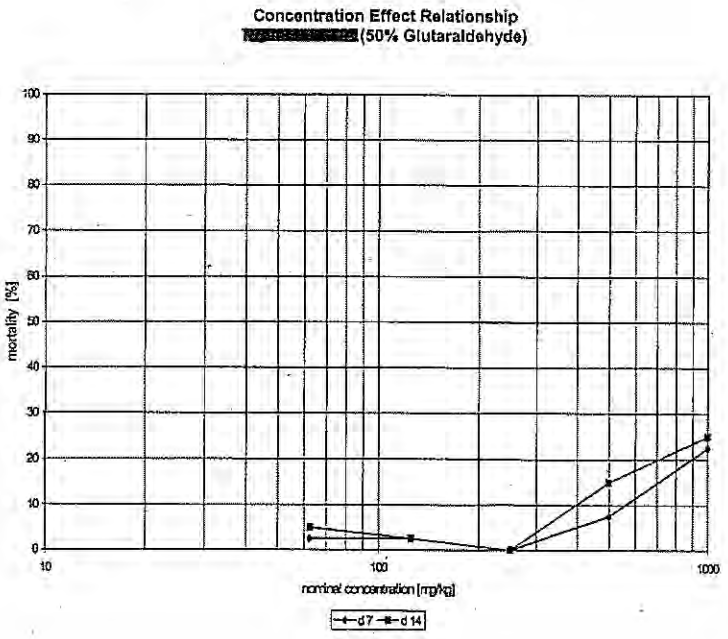
Soil moisture	During the test: 45.5% of the WHC_{max}
Method of soil incubation	The test samples were incubated up to 28 days in the dark; the water content was controlled by weighing of the test samples and water loss was regulated by addition of demineralised water.
Aeration	Aeration was assured by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

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2002) [redacted] (50% Glutaraldehyde) Determination of the acute letal effect of chemicals on the earthworm [redacted]. [redacted] (Unpublished), [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] 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. / 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] (50% Glutaraldehyde)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2 [redacted]	
3.1.3	Purity	[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] (50% 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 50%) to the Earthworm [redacted]. Test substance: [redacted] (50% glutaraldehyde), [redacted] Guideline: OECD 207, 1984; GLP Clitellated adult earthworms ([redacted]; 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] (50% 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 [redacted]
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	[redacted]
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 [redacted]
10% sphagnum-peat [redacted]
0.5% chalk ($CaCO_3$) [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	

Section 7.5.1.3_02 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

			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), [redacted] 28 July 2010	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] the Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
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	48.9 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	[redacted] See table A7_5_1_3-1	
3.4.3	Test system	See table A7_5_1_3-2	

Section 7.5.1.3_02 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

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

Section 7.5.1.3_02 **Terrestrial plant toxicity**
Annex Point IIIA XIII 3.4

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 ([redacted]	80 % after 8 days
Oilseed rape [redacted]	100% after 8 days
Vetch [redacted]	100% after 8 days

4.2 Results test substance

Section 7.5.1.3_02 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

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 [redacted]	Oilseed rape [redacted]	Vetch [redacted]
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 [redacted] 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 [redacted] were observed. At a test concentration of 1000 mg/kg a 31 % inhibition of the plant length of [redacted] was estimated. The plant lengths of [redacted] 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 [redacted] 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 [redacted] and [redacted] were evaluated. At a test concentration of 1000 mg/kg an inhibition of 53% was estimated with [redacted] and 28% inhibition at the test concentration of 250 mg/kg with [redacted].

No statistical significant effects on the shoot dry weight of [redacted] 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 [redacted].

The seed leafs of [redacted] 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].

For detail see table A7_5_1_3-7.

4.2.6 Effect data

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

Parameter	[redacted]	[redacted]	[redacted]
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	[redacted]	[redacted]	[redacted]
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	[redacted]	[redacted]	[redacted]
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	[redacted]	[redacted]	[redacted]
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.

Test substance: [redacted] (ca. 50% glutaraldehyde in water), [redacted]

Seeds of [redacted] 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 [redacted]

Test substance-related effects were reported for the growth rate of [redacted] At a test concentration of 1000 mg/kg a 31 % inhibition of the plant length of [redacted] was estimated. The plant lengths of [redacted] 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 [redacted] were seen.

Test substance-related effects were reported for the dry shoot weight of harvested plants of [redacted] At a test concentration of 1000 mg/kg an inhibition of 53% was estimated with [redacted]s and 28% inhibition at the test concentration of 250 mg/kg with [redacted] No statistical significant effects on the shoot dry weight of [redacted] were reported.

Referring to the morphology of the plants, there was no peculiarity at [redacted] For [redacted], 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	[redacted]	[redacted]	[redacted]
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	[redacted]	[redacted]	[redacted]
Emergence rate	> 1000	> 1000	> 1000
Plant length	> 1000	> 1000	> 1000
Shoot dry weight	> 1000	944	901

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

Parameter	[redacted]	[redacted]	[redacted]
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	[redacted]	[redacted]	[redacted]
Emergence rate	> 1000	> 1000	> 1000
Plant length	> 1000	500**	250*
Shoot dry weight	> 1000	1000**	250**

**, p ≤ 0.01; *, p ≤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 [redacted] to [redacted]

The test material showed no toxic effects to [redacted]

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. 48.9% Glutaraldehyde. The results are based on nominal concentrations. The most sensitive species and endpoint were vetch [redacted] 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)
[redacted]	[redacted]	[redacted]	Oilseed rape	[redacted]
	[redacted]	[redacted]	Vetch	
[redacted]	[redacted]	[redacted]	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**	[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 [redacted] 1.0 cm for [redacted] 1.5 cm for [redacted]
	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 values)	Range of temperature by daily measurement: 59.7 - 60 °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 [%]
[redacted]	0 (control)	10.00	--
	62.5	8.75	12.5* [a]
	125	9.50	5.0
	250	9.50	5.0
	500	9.50	5.0
	1000	9.25	7.5
	0 (control)	10.00	--
	62.5	10.00	0
	125	9.75	2.5
	250	9.75	2.5
	500	10.00	0
	1000	10.00	0
	0 (control)	8.50	--
	62.5	8.50	0
	125	8.25	2.9
	250	9.25	-8.8
	500	8.75	-2.9
	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 [%]
[redacted]	0 (control)	215.5	--
	62.5	213.5	0.9
	125	215.2	0.2
	250	220.1	-2.1
	500	221.9	-3.0
	1000	215.1	0.2
	0 (control)	53.3	--
	62.5	52.7	1.1
	125	53.4	-0.2
	250	52.8	0.9
	500	48.7	8.6
	1000	36.8	31.0**
	0 (control)	368.5	--
	62.5	371.4	-0.8
	125	363.7	1.3
	250	341.8	7.3*
	500	319.6	13.3**
	1000	273.6	25.8**

Statistical significant (Dunnett's test (one-sided)): **p ≤ 0.01, *p ≤ 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 [%]
[redacted]	0 (control)	0.2735	--
	62.5	0.2570	6.0
	125	0.2412	11.8
	250	0.2777	-1.5
	500	0.2794	-2.2
	1000	0.2444	10.7
	0 (control)	0.1549	--
	62.5	0.1533	1.1
	125	0.1744	-12.6
	250	0.1831	-18.2
	500	0.1449	6.5
	1000	0.0723	53.4**
	0 (control)	0.6129	--
	62.5	0.5644	7.9
	125	0.5790	5.5
	250	0.4406	28.1**
	500	0.4028	34.3**
	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.