Table A7.1.2.2.2/02-5 Composition of Aqueous Phase (HPLC)

Sampling Time & Replicate		ppm in Water	Products Detected as Percent of Dose (ppm)							
			% Glutaraldehyde (13.2-13.6 min)		% Compound A (16.4-17.1 min)		% 5-Hydroxy-pentanal (18.5-19.9 min)		% 1,5-Pentanediol (22.5-23.0 min)	
0-hour	A	8.73	78.6	(7.43)	2.89	(0.27)	5.22	(0.49)	0.00	(0.00)
	B	8.63	67.6	(6.39)	3.64	(0.34)	9.30	(0.88)	0.00	(0.00)
1-day	A	9.03	4.9	(0.46)	10.50	(0.99)	38.97	(3.68)	34.75	(3.28)
	B	8.93	4.0	(0.38)	13.24	(1.25)	35.11	(3.32)	34.79	(3.29)
3-day	A	8.57	0.0	(0.00)	8.59	(0.81)	7.02	(0.66)	67.16	(6.35)
	B	8.36	0.2	(0.02)	14.64	(1.38)	10.58	(1.00)	54.33	(5.13)
7-day	A	8.34	0.0	(0.00)	12.46	(1.18)	0.79	(0.08)	69.25	(6.54)
	B	8.48	0.0	(0.00)	11.75	(1.11)	2.92	(0.28)	62.98	(5.95)
14-day	A	8.88	0.0	(0.00)	12.76	(1.21)	0.38	(0.04)	77.86	(7.36)
	B	8.99	0.0	(0.00)	13.33	(1.26)	2.18	(0.21)	74.34	(7.02)
30-day	A	8.20	0.0	(0.00)	16.47	(1.56)	0.84	(0.08)	62.17	(5.87)
	B	8.23	0.0	(0.00)	11.49	(1.09)	1.46	(0.14)	70.14	(6.63)
60-day	A	8.58	0.0	(0.00)	15.14	(1.43)	0.0	(0.00)	71.17	(6.73)
	B	8.72	0.0	(0.00)	10.49	(0.99)	0.0	(0.00)	74.78	(7.07)
90-day	A	8.91	0.0	(0.00)	22.86	(2.16)	0.0	(0.00)	66.74	(6.31)
	B	8.74	0.0	(0.00)	12.62	(1.19)	0.0	(0.00)	75.39	(7.12)
123-day	A	8.61	0.0	(0.00)	18.35	(1.43)	0.0	(0.00)	67.51	(6.38)
	B	8.67	0.0	(0.00)	14.81	(1.40)	0.0	(0.00)	71.64	(6.77)

Table A7.1.2.2.2/02-6 Extractability of Radiocarbon from Sediment*

Sampling Time & Replicate		Radiocarbon Available		Extracted Radiocarbon		Residual Radiocarbon	
		Percent of Dose	ppm	Percent of Available	ppm	Percent of Available	ppm
0-hour	A	5.4	0.51	63.9	0.33	21.1	0.11
	B	6.0	0.57	67.6	0.39	13.4	0.08
1-day	A	6.1	0.58	82.6	0.48	33.3	0.19
	B	6.5	0.62	69.4	0.43	15.8	0.10
3-day	A	6.1	0.58	74.4	0.43	35.8	0.21
	B	6.0	0.57	62.1	0.35	38.4	0.22
7-day	A	7.7	0.72	64.1	0.46	20.6	0.15
	B	6.4	0.61	66.3	0.40	30.6	0.19
14-day	A	6.9	0.65	59.4	0.39	25.4	0.17
	B	7.1	0.67	66.2	0.44	29.2	0.20
30-day	A	8.9	0.84	49.9	0.41	37.9	0.32
	B	7.6	0.72	75.5	0.56	23.6	0.17
60-day	A	7.8	0.74	53.1	0.39	38.0	0.28
	B	6.6	0.62	64.6	0.40	33.6	0.21
90-day	A	7.4	0.70	53.5	0.40	36.5	0.26
	B	7.4	0.70	48.9	0.34	29.5	0.21
123-day	A	9.2	0.87	53.9	0.47	27.8	0.24
	B	7.6	0.72	67.7	0.49	35.8	0.26

* Total dose applied was 9.45 ppm based on glutaraldehyde concentration in 106.4 mL water.

Figure A7.1.2.2.2/02-1 Proposed metabolic pathway for glutaraldehyde under anaerobic conditions

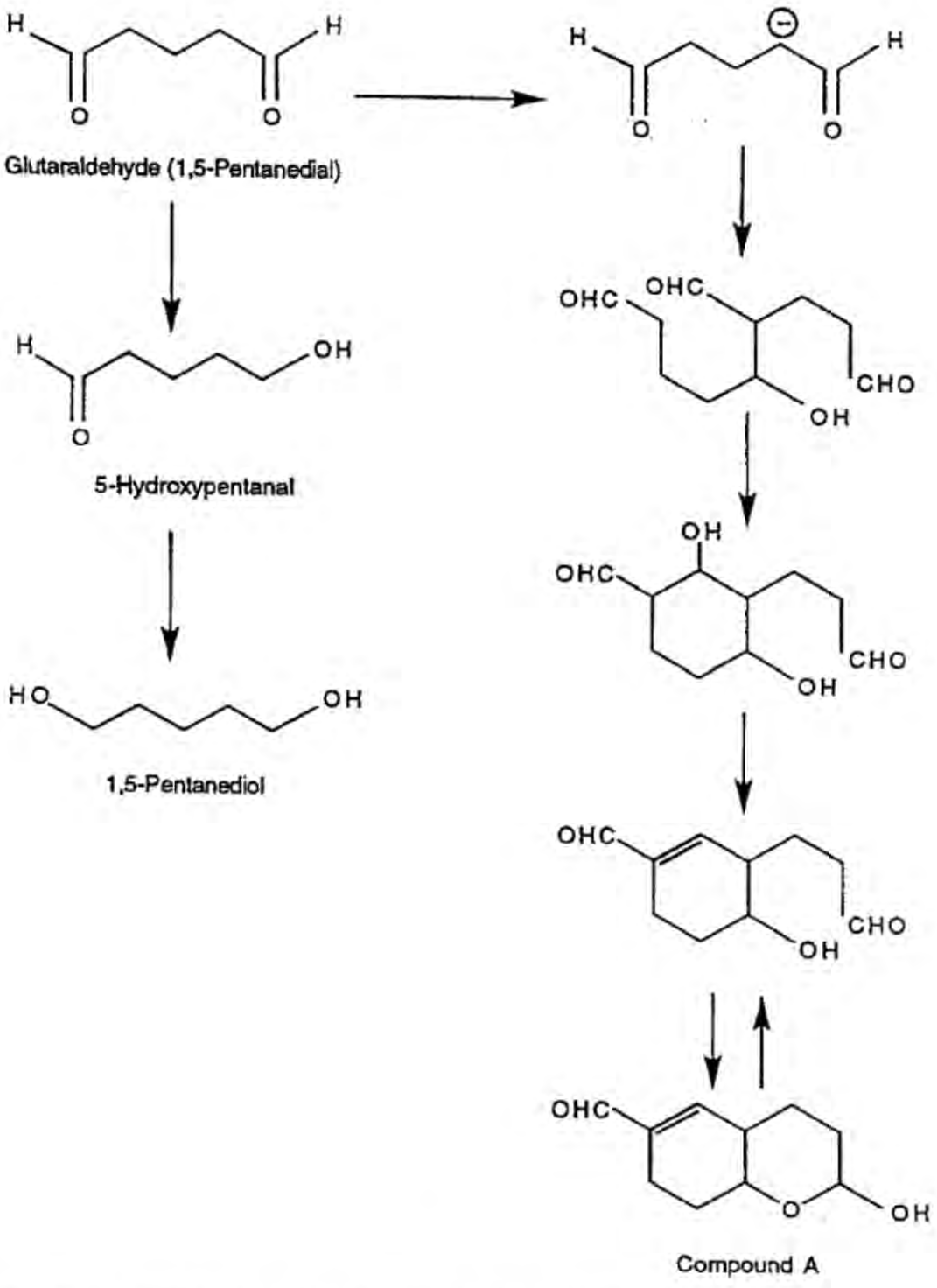

Figure 37. Proposed Metabolic Pathway for [1,5-^{14}C]-Glutaraldehyde.

		Total C-14	Avergae	Extractab	Average	Non-extrac	Average
0-hour	A	5,4	5,7	63,9	65,75	21,1	17,25
	B	6		67,6		13,4	
1-day	A	6,1	6,3	82,6	76	33,3	24,55
	B	6,5		69,4		15,8	
3-day	A	6,1	6,05	74,4	68,25	35,8	37,1
	B	6		62,1		38,4	
7-day	A	7,7	7,05	64,1	65,2	20,6	25,6
	B	6,4		66,3		30,6	
14-day	A	6,9	7	59,4	62,8	25,4	27,3
	B	7,1		66,2		29,2	
30-day	A	8,9	8,25	49,9	62,7	37,9	30,75
	B	7,6		75,5		23,6	
60-day	A	7,8	7,2	53,1	58,85	38	35,8
	B	6,6		64,6		33,6	
90-day	A	7,4	7,4	53,5	51,2	36,5	33
	B	7,4		48,9		29,5	
123-day	A	9,2	8,4	53,9	60,8	27,8	31,8
	B	7,6		67,7		35,8	

Extractab	Average	Non-extr	average
3,45	3,75	1,14	0,97
4,06		0,80	
5,04	4,77	2,03	1,53
4,51		1,03	
4,54	4,13	2,18	2,24
3,73		2,30	
4,94	4,59	1,59	1,77
4,24		1,96	
4,10	4,40	1,75	1,91
4,70		2,07	
4,44	5,09	3,37	2,58
5,74		1,79	
4,14	4,20	2,96	2,59
4,26		2,22	
3,96	3,79	2,70	2,44
3,62		2,18	
4,96	5,05	2,56	2,64
5,15		2,72	

		Total C-14	Avergae	Extractab	Average	Non-extr	Average
0-hour	A	5,40	5,70	3,45	3,75	1,14	0,97
	B	6,00		4,06		0,80	
1-day	A	6,10	6,30	5,04	4,77	2,03	1,53
	B	6,50		4,51		1,03	
3-day	A	6,10	6,05	4,54	4,13	2,18	2,24
	B	6,00		3,73		2,30	
7-day	A	7,70	7,05	4,94	4,59	1,59	1,77
	B	6,40		4,24		1,96	
14-day	A	6,90	7,00	4,10	4,40	1,75	1,91
	B	7,10		4,70		2,07	
30-day	A	8,90	8,25	4,44	5,09	3,37	2,58
	B	7,60		5,74		1,79	
60-day	A	7,80	7,20	4,14	4,20	2,96	2,59
	B	6,60		4,26		2,22	
90-day	A	7,40	7,40	3,96	3,79	2,70	2,44
	B	7,40		3,62		2,18	
123-day	A	9,20	8,40	4,96	5,05	2,56	2,64
	B	7,60		5,15		2,72	

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			Official use only
		1 REFERENCE	
Reference		[redacted] (1985) Determination of adsorption/desorption constants of ^{14}C-Glutaraldehyde. [redacted] (Unpublished), BPD ID A7.01.3_01	
Data protection		Yes	
1.1.1	Data owner	BASF	
1.1.2	Companies with letter of access	[redacted]	
1.1.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
Guideline study		Yes, Guideline 163-1 (EPA "Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate" for Leaching and Adsorption/Desorption Studies No. 163-1)	x
GLP		Yes	
Deviations		No	
		3 MATERIALS AND METHODS	
Test material		^{14}C-glutaraldehyde standard obtained from [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Specific radioactivity 3.40 mCi/mM	
3.1.3	Purity	Radiochemical purity [redacted]%	
3.1.4	Further relevant properties	Not indicated	
3.1.5	Method of analysis	Liquid Scintillation Counting Analysis (LSC) Gas Chromatography (GC)	x
Degradation products		Degradation products tested: No	
3.1.6	Method of analysis for degradation products	Not relevant	
Reference substance		No	
3.1.7	Method of analysis for reference substance	Not relevant	

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Soil types Four types of soil were tested, for details see table A7_1 _3-1 x

Testing procedure

3.1.8 Test system

The soil samples were placed in culture tubes. The soil suspensions were shaken on a mechanical shaker ([redacted]). An IEC clinical model centrifuge was used for centrifugation of the suspensions. Filtration of the samples was performed using a Whatman GF/A glass fiber filter paper. 10 ml mixing cylinders were used for collection of the filtrates. Measurements of radioactivity were performed by means of a Beckman Liquid Scintillation System, Model 3801 bench top microprocessor-controlled spectrometer for radionuclide measuring. Combustion of soil samples for LSC analysis was done in a Packard 306B Tri-Carb sample oxidizer.

3.1.9 Test solution and Test conditions

All the test soils were air-dried for 24 hours.

All test water used for the study was deionized water, boiled for at least 30 minutes to remove CO_2 and sterilized by filtration through a 0.22 µ filter. The test water served for the preparation of a 0.01 M Ca^{++} solution using Ca Cl_2.

A stock solution of the radioactive test substance was prepared by adding the test material to water adjusted to pH 6.5 with HCl to a volume of 100 ml. The concentration of the stock solution was determined to be 1.166 mg/ml and the radiochemical purity was 99%.

The [redacted] loam, the [redacted] silt loam and the [redacted] clay loam were respectively dosed at a level of 10 ppm by addition of 429 µl of the stock solution to 50 g of soil; the [redacted] loamy sand was dosed at 10 ppm by adding 4.29 ml of the stock solution to 500g soil. The soils kept in sealed vessels for one hour to achieve homogeneity. The soils were then aerobically aged for 30 days at 25 +/-1 °C (environmentally controlled chamber). This was followed by a series of extractions on 1 g and 0,5 g subsamples of the soil samples for determination of the best solvent system for extraction of ^{14}C-labelled glutaraldehyde. Extraction was done with 80:20 methanol in water. For each soil extract 3 concentrations of aqueous test solutions were prepared in a 0.01 M Ca^{++} solution; these concentrations were determined by means of LSC analysis and were as follows: x

Soil type	Solution	Concentration*
Loamy Sand	I	0.0366 µg/ml
	II	0.0184 µg/ml
	III	0.0121 µg/ml
Silt Loam	I	0.0389 µg/ml
	II	0.0191 µg/ml
	III	0.0131 µg/ml
Loam	I	0.0521 µg/ml
	II	0.0258 µg/ml
	III	0.0174 µg/ml
Clay Loam	I	0.0537 µg/ml

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	II	0.0266 µg/ml
	III	0.0176 µg/ml

*, Average of duplicate LSC analysis

Twenty gram-samples of each soil typ were autoclaved at 121.11 °C and 15 psi for one hour and then were oven dried over night.

The aqueous solution prepared from each soil extract was equilibrated with the same soil type.

The 0.01 M calcium ion solution as such served for control.

Test performance

3.1.10 Preliminary test

According to (a)"OECD 106": No (study was performed prior 1999)

A preliminary test was performed in order to determine (1) the soil to water ratio to be selected for the main test, and (2) the equilibration time for the aerobically aged residues of 14C-glutaraldehyde and the four soil types.

For this purpose, two half gram-samples of each soil types were placed in culture tubes. Aliquots (5 ml) of the high concentration of the aqueous test solution prepared from the soil extracts were added to the soil samples corresponding to the soil type extracted. Following shaking in darkness the contents of the tubes were sampled sequentially after 4 and 28 hours. For the [redacted] and [redacted] soils, 1 gram samples were placed on culture tubes and 10 ml aliquots of the respectively corresponding aqueous test solutions were added. Sampling here was performed after 48 hours. Duplicate 0.5 ml aliquots of the aqueous phase were taken for LSC analysis at each sampling interval. The percent of test material adsorbed was determined by the change in concentration of the aqueous phase. A blank was prepared from the high test concentration of the aqueous test solution prepared from the [redacted] soil extract. No changes in concentrations were observed after 48 hours, indicating that there was no adsorption of ^{14}C-glutaraldehyde residues from the soil extracts to the walls of the glass vessel.

3.1.11 Screening test: Adsorption

According to (a)"OECD 106": No (study was performed prior 1999)

Duplicate 3 g portions of each soil type were placed in culture tubes. The soil samples in the culture tubes were supplemented with 6 ml aliquots of the 3 aqueous test solutions. The soil suspensions were shaken in darkness on a mechanical shaker ([redacted]) for a least 24 hours at 25 +/- 1 °C (environmentally controlled chamber). This was followed by centrifugation (20 minutes, 1500 rpm), filtration of the supernatants, collection of the filtrates in mixing cylinders and recording of the respective volumes. Duplicate 1 ml aliquots were then taken and subjected to LSC analysis.

3.1.12 Screening test: Desorption

According to (a)"OECD 106": No (study was performed prior 1999)

Appropriate aliquots of 0.01 M Ca^{++} solution were added to each sample according to the volume removed after adsorption phase. The suspensions were shaken in darkness for at lest 48 hours at 25 +/- 1 °C (environmentally controlled chamber). This was followed by centrifugation (20 minutes, 1500 rpm), filtration and collection of the filtrates in mixing cylinders. Duplicate 1 ml aliquots were then taken and subjected to LSC analysis.

The soil was air-dried and combusted in triplicate for radioanalysis to

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determine ^{14}C-mass balance.

3.1.13 HPLC-method: According to (a)" OECD-HPLC-method"[1]: No

3.1.14 Parameters: Kd: adsorption and desorption coefficients
Koc : adsorption and desorption constants
n: a constant

4 RESULTS

Preliminary test

See table A7_1 _3-2

Adsorption of test compound in all 4 soil types was less than 20%; therefore a soil to water ratio of 1:2 was selected for the main study.

Screening test: Adsorption

See table A7_1 _3-3

The percentages referring to the adsorption are mean values for the 3 tested solutions per soil type and as duplicate.

Screening test: Desorption

See table A7_1 _3-4

The percentages referring to the adsorption are mean values for the 3 tested solutions per soil type and as duplicate.

Calculations

4.1.1 Kd

Soil Type	% Org M	% Org C	Adsorption		Desorption	
			Kd	n	Kd	n
Loamy Sand	0.7	0.304	6.3	0.653	0.867	1.22
Loam	1.9	0.826	0.183	1.33	0.278	1.68
Silt Loam	4.1	1.78	0.336	1.26	1.41	1.1
Clay Loam	8.4	3.65	0.186	1.36	1.55	1.03

% Org M, % organic matter

% Org C, % organic carbon = % Org M/2.3

4.1.2 Koc: Koc = Kd x100/ % Organic Carbon

Soil Type	Koc (adsorption)	Koc (desorption)
Loamy Sand	2070	285
Loam	22.2	33.7
Silt Loam	18.9	79.2
Clay Loam	5.1	42.5

[1] OECD (1999) OECD-Guidelines for the Testing of Chemicals. Proposal for a new guideline 121: Estimation of the adsorption coefficient (K_{OC}) on soil and on sewage sludge using High Performance Liquid Chromatography (HPLC), Draft Document (August 1999).

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Degradation product(s) No data

5 APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

The aim of the present study was to determine the adsorption and desorption coefficients/ constants for ^{14}C-labelled glutaraldehyde incorporated onto four different soil types after an incubation period of 30 days at 25 +/1 °C.

^{14}C-glutaraldehyde standard obtained from [redacted], [redacted] radiochemical purity [redacted]%, specific radioactivity 3.40 mCi/mM.

Guideline 163-1 (EPA "Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate" for Leaching and Adsorption/Desorption Studies No. 163-1), GLP

Principal method of analysis: Liquid Scintillation Counting Analysis (LSC)

Soil types: [redacted] Loamy Sand, [redacted] Loam, Nebraska Silt Loam and [redacted] Clay Loam.

A stock solution of the radioactive test substance in water was prepared, which had a concentration of 1.166 mg/ml and a radiochemical purity of [redacted]%.

Samples of the different soil types were respectively dosed at a level of 10 ppm by addition of 429 µl of the stock solution to 50 g of soil (Loamy sand: 4.29 ml of the stock solution to 500g soil). The soils were kept in sealed vessels for one hour to achieve homogeneity and were then aerobically aged for 30 days at 25 +/-1 °C (environmentally controlled chamber). This was followed by a series of extractions with 80:20 methanol in water. For each soil extract 3 concentrations of aqueous test solutions were prepared in a 0.01 M Ca^{++} solution; these concentrations were determined by LSC analysis and were as follows:

Soil type	Solution	Concentration*
Loamy Sand	I	0.0366 µg/ml
	II	0.0184 µg/ml
	III	0.0121 µg/ml
Silt Loam	I	0.0389 µg/ml
	II	0.0191 µg/ml
	III	0.0131 µg/ml
Loam	I	0.0521 µg/ml
	II	0.0258 µg/ml
	III	0.0174 µg/ml
Clay Loam	I	0.0537 µg/ml
	II	0.0266 µg/ml
	III	0.0176 µg/ml

A preliminary test was performed in order to determine (1) the soil to water ratio to be selected for the main test, and (2) the equilibration time for the aerobically aged residues of 14C-glutaraldehyde and the four soil

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types.

For the adsorption test duplicate portions of each soil type were mixed with aliquots of the 3 aqueous test solutions. Following shaking (at least 24 hours at 25 +/- 1 °C in an environmentally controlled chamber), centrifugation, filtration of the supernatants, collection of the filtrates and recording of the respective volumes, duplicate aliquots were subjected to LSC analysis.

For the desorption test appropriate aliquots of 0.01 M Ca^{++} solution were added to each sample according to the volume removed after adsorption phase. Following shaking (at least 24 hours at 25 +/- 1 °C in an environmentally controlled chamber), centrifugation, filtration of the supernatants, collection of the filtrates and recording of the respective volumes, duplicate aliquots were subjected to LSC analysis.

The soil was air-dried and combusted in triplicate for radioanalysis to determine ^{14}C-mass balance.

Results and discussion

5.1.1 Adsorbed a.s. [%]

Soil Type	Adsorption (%)
[redacted] Loamy Sand	25.4
[redacted] Loam	18.9
[redacted] Silt Loam	28.8
[redacted] Clay Loam	20.3

5.1.2 Percentage of organic carbon

Soil Type	Organic Carbon (%)
[redacted] Loamy Sand	0.3
[redacted] Loam	0.8
[redacted] Silt Loam	1.8
[redacted] Clay Loam	3.6

5.1.3 Adsorption , K_d

Soil Type	K_d
Loamy Sand	6.3
Loam	0.183
Silt Loam	0.336
Clay Loam	0.186

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5.1.4 Desorption, K_d

Soil Type	K_d
Loamy Sand	0.867
Loam	0.278
Silt Loam	1.41
Clay Loam	1.55

5.1.5 Kd (adsorption)/Kd (desorption)

Soil Type	Kd (a)	Kd (d)	Kd (a) / Kd (d)
Loamy Sand	6.3	0.867	7.27
Loam	0.183	0.278	0.66
Silt Loam	0.336	1.41	0.24
Clay Loam	0.186	1.55	0.12

5.1.6 Koc (adsorption)

Soil Type	Koc	Log Koc
Loamy Sand	2070	3.3
Loam	22.2	1.4
Silt Loam	18.9	1.3
Clay Loam	5.1	0.7

5.1.7 Koc (desorption)

Soil Type	Koc
Loamy Sand	285
Loam	33.7
Silt Loam	79.2
Clay Loam	42.5

5.1.8 Degradation products (% of a.s.) No data

Conclusion The values reported above indicate that only the Loamy Sand showed an increased potential of adsorption to solid soil particles whereas for the remaining soil types (Loam, Silt Loam, Clay Loam) the adsorption potential was low to moderate. Considering the leaching potential, the test substance was classified medium to slightly mobile in the Loam Sand, and highly to very highly mobile in the Loam, Silt Loam and Clay Loam by the authors.

5.1.9 Reliability **1**

5.1.10 Deficiencies No

5.1.11

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Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	31.3.2009
Materials and Methods	The stability of glutaraldehyde in the test conditions was not determined. Due to rapid transformation of glutaraldehyde (half-life in soil 1.7 days, Doc IIIA.2.1) and a long incubation period (30 days) it is unlikely that any significant amount of the parent compound was present in the soils in the start of the experiment. No specific analytical method was applied after the liquid scintillation counting in order to analyse what substances the radioactivity represented. Other remarks: 2.1 Test guideline not mentioned in the test report. 3.3 LSC is given as the analytical method. GC is not mentioned in the test report. 3.4 Four soils included in the test of which two corresponded approximately to soil types described in the OECD 106.
Results and discussion	The results are assumed to describe the adsorption of transformation products.
Conclusion	The transformation products of glutaraldehyde seem to be mobile in three of four studied soils. In loamy sand the transformation products seem to be less mobile, which is peculiar as both the organic carbon content and clay content of this soil are low.
Reliability	3
Acceptability	Not acceptable because it is unknown to what substances the radioactivity represented.
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_1 _3-1: Classification and physico-chemical properties of soils used as adsorbents

	Soil 1	Soil 2	Soil 3	Soil 4
Soil sample code	Sandy Loam	NY Loam	Silt Loam	Clay Loam
Classification /Soil type	Loamy Sand	Loam	Silt Loam	Clay Loam
Location	[redacted]	[redacted]	[redacted]	[redacted]
Sand [%]	82%	50.8%	12.8%	20.8%
Silt [%]	14%	36%	70%	40%
Clay [%]	4%	13.2%	17.2%	39.2%
Organic matter [%]	0.7%	1.9%	4.1%	8.4%
Carbonate as $CaCO_3$				
Insoluble carbonates [%]				
pH (1:1 H_2O)	8.0	5.4	5.3	6.2
Cation exchange capacity (MEQ/100 g)	7.7	11.2	25.7	54.2
Field Capacity σ 1/3 Bar	5.44	18.1	34.4	45.5
Extractable cations (MEQ/100 g)	-	-	-	-
Ca	-	-	-	-
Mg	-	-	-	-
Na	-	-	-	-
K	-	-	-	-
H	-	-	-	-
Special chemical/mineralogical features	-	-	-	-
Clay fraction mineralogy	-	-	-	-

Table A7_1 _3-2: Results of preliminary test:

Test substance	^{14}C-glutaraldehyde
Sample purity	99%
Weighed soil	0.5 g (all soil types, sampling after 4 and 28 hours) 1 g ([redacted] Highview soil, sampling after 48 hours)
Volume of $CaCl_2$ solution	5 ml (all soil types, sampling after 4 and 28 hours) 10 ml ([redacted] soil, sampling after 48 hours)
Details of the analytical method used:	
Method	-
Recovery rate	-
Detection limit	-

Table A7_1 _3-3: Results of screening test - adsorption:

	Soil 1					
	[redacted] Loamy Sand					
	Sol. I (1)	Sol.I (2)	Sol. II (1)	Sol.II (2)	Sol. III (1)	Sol.III (2)
µg at Initiation	0.22	0.22	0.11	0.11	0.0726	0.0726
µg in Solution after Adsorption Phase	0.157	0.147	0.0792	0.0864	0.0566	0.0587
µg Adsorbed in Soil	0.063	0.073	0.0308	0.0236	0.016	0.0139
% Adsorbed	28.6	33.2	28	21.5	22	19.1
Mean % Adsorbed	25.4%					
Temperature [°C]	25 °C					

	Soil 2					
	[redacted] Loam					
	Sol. I (1)	Sol.I (2)	Sol. II (1)	Sol.II (2)	Sol. III (1)	Sol.III (2)
µg at Initiation	0.313	0.313	0.155	0.155	0.104	0.104
µg in Solution after Adsorption Phase	0.26	0.258	0.128	0.128	0.0792	0.0828
µg Adsorbed in Soil	0.053	0.055	0.027	0.027	0.0248	0.0212
% Adsorbed	16.9	17.6	17.4	17.4	23.8	20.4
Mean % Adsorbed	18.9%					
Temperature [°C]	25 °C					

	Soil 3

	[redacted] Silt Loam					
	Sol. I (1)	Sol.I (2)	Sol. II (1)	Sol.II (2)	Sol. III (1)	Sol.III (2)
µg at Initiation	0.233	0.233	0.115	0.115	0.0786	0.0786
µg in Solution after Adsorption Phase	0.172	0.169	0.0834	0.084	0.0549	0.0518
µg Adsorbed in Soil	0.061	0.064	0.0316	0.031	0.0237	0.0268
% Adsorbed	26.2	27.5	27.5	27	30.2	34.1
Mean % Adsorbed	28.8%					
Temperature [°C]	25 °C					

	Soil 4					
	[redacted] Clay Loam					
	Sol. I (1)	Sol.I (2)	Sol. II (1)	Sol.II (2)	Sol. III (1)	Sol.III (2)
µg at Initiation	0.322	0.322	0.16	0.16	0.106	0.106
µg in Solution after Adsorption Phase	0.268	0.267	0.115	0.129	0.081	0.0882
µg Adsorbed in Soil	0.054	0.055	0.045	0.031	0.025	0.0178
% Adsorbed	16.8	17.1	28.1	19.4	23.6	16.8
Mean % Adsorbed	20.3%					
Temperature [°C]	25 °C					

Table A7_1_3-4: Results of screening test - desorption:

	Soil 1					
	Missouri Loamy Sand					
	Sol. I (1)	Sol.I (2)	Sol. II (1)	Sol.II (2)	Sol. III (1)	Sol.III (2)
µg in Solution after Desorption Phase	0.06	0.0311	0.0323	0.0308	0.015	0.0154
% Desorbed	95.2	42.6	105	131	93.8	111
Mean % Adsorbed	96.4%					
Temperature [°C]	25 °C					

	Soil 2					
	[redacted] Loam					
	Sol. I (1)	Sol.I (2)	Sol. II (1)	Sol.II (2)	Sol. III (1)	Sol.III (2)

µg in Solution after Desorption Phase	0.0864	0.081	0.0322	0.0425	0.021	0.0173
% Desorbed	163	147	119	157	84.7	81.6
Mean % Adsorbed	125%					
Temperature [°C]	25 °C					

	Soil 3					
	[redacted] Silt Loam					
	Sol. I (1)	Sol.I (2)	Sol. II (1)	Sol.II (2)	Sol. III (1)	Sol.III (2)
µg in Solution after Desorption Phase	0.0503	0.0624	0.0272	0.028	0.0207	0.0181
% Desorbed	82.5	97.5	86.1	90.3	87.3	67.5
Mean % Adsorbed	85.2%					
Temperature [°C]	25 °C					

	Soil 4					
	[redacted] Clay Loam					
	Sol. I (1)	Sol.I (2)	Sol. II (1)	Sol.II (2)	Sol. III (1)	Sol.III (2)
µg in Solution after Desorption Phase	0.096	0.09	0.0462	0.044	0.0312	0.0299
% Desorbed	178	164	103	142	125	168
Mean % Adsorbed	147%					
Temperature [°C]	25 °C					

Section A7.1.3 Annex Point XII.2.2 IUCLID 3.4/01		Adsorption/Desorption in Water / Sediment Systems Determination of the Adsorption of Glutaraldehyde to Activated Sludge	
		1 REFERENCE (A7.1.3/01)	Official use only
1.1	Reference	[redacted], (2001) Determination of the Adsorption of Glutaraldehyde to Activated Sludge Using the ISO/CD 18749 Batch Adsorption Test, [redacted] GLP, Unpublished, 28 September 2001	
1.2	Data protection	Yes	
1.2.1	Data owner	The Dow Chemical Company and BASF SE	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data on an existing a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	Yes ISO/CD 18749 Batch Adsorption Test	
2.2	GLP	Yes	
2.3	Deviations	Yes The test material characterization (identity and purity) was performed in a laboratory that does not operate under GLP guidelines.	
		3 MATERIALS AND METHODS	
3.1	Test material	Glutaraldehyde, [redacted]%	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Not reported	
3.1.3	Purity	[redacted]%	
3.1.4	Further relevant properties	None	
3.1.5	Method of analysis	Gas chromatography with flame ionization detection	
3.2	Reference substance	Acid Red 88 Dye (purity 75%) Ethanol (purity 99.5%)	
3.3	Testing procedure		
3.3.1	Test system	Activated sludge was obtained from the municipal waste water treatment plant, and had distinct flocs by visual inspection. Sludge volume index met the requirement of less than 150 mL/g. Sludge was washed, and the mixed liquor suspended solids (MLSS) concentration of the activated sludge was determined to be 1.26 g/L. MLSS was adjusted to a final concentration of 5 g/L.	
3.3.2	Test solution and Test conditions	**Table A7.1.3/01** Seven reaction mixtures were prepared in duplicate, including a blank control mixture. A 3 mg/L concentration of glutaraldehyde was created and added to samples of activated sludge. Reaction mixtures were incubated at 20-25°C. The pH (6.5-7.5) and dissolved O_2 was measured	x

Section A7.1.3 Annex Point XII.2.2 IUCLID 3.4/01	Adsorption/Desorption in Water / Sediment Systems **Determination of the Adsorption of Glutaraldehyde to Activated Sludge**	
	at the beginning and periodically throughout the study. Samples were analyzed for glutaraldehyde at 0.1, 0.25, 0.5, 1, 2, 3, 6, and 24 hours by gas chromatography. Samples were collected from reference mixtures at 0, 3, and 24 hours, and biological inhibition controls were sampled at 3 and 24 hours.	
3.4 Test performance		
3.4.1 Preliminary and screening tests	A preliminary test was conducted to identify appropriate conditions for biological inhibition of the activated sludge which had minimal effect on the adsorption of the glutaraldehyde to the sludge, as well as maintaining the physical characteristics of the activated sludge. Methods included chemical treatment with mercuric chloride, pasteurization, sparging with inert gas, and treatment with UV light.	
3.4.2 Analyses	Activated sludge was extracted at the study conclusion (24 hours) to determine how much glutaraldehyde was adsorbed to the solids. Gas chromatography with flame ionization detection was used to create an adsorption curve for the test material in the reaction mixtures as a function of time. Compound half-life was calculated assuming the removal processes follow pseudo-first order kinetics.	
3.4.3 GC-method	***GC with flame ionization detection*** [redacted] Injector with the following conditions: Carrier gas: helium@ 16psi column head pressure Injection volume: 5uL splitless injection Column; 6 feet x 1/8 inch ID AT-steel column packed with 80/100 mesh Porapak PS Injection Port: 250°C Oven: 210°C Detector: 250°C The glutaraldehyde eluted at approximately 6.3 minutes. Detection limits were 0.2 mg/L in aqueous solution, and 0.1 mg/L in ethyl acetate.	
3.4.4 Statistics	***Calculation of Degree of Adsorption*** The adsorption curve was determined as a function of time. The percentage removal (A_t) of test and reference materials from the aqueous phase for each sampling time were calculated as: $\mathbf{A_t = [1-(C_w-C_{BW})/(f*(C_{W0}-C_{BW0}))]*100}$ *where,* C_w = test / reference material concentration in water at time t_x in test mixture C_{BW} = test / reference material concentration in water at time t_x in blank mixture C_{W0} = test / reference material concentration in water at time t_0 in test mixture C_{BW0} = test / reference material concentration in water at time t_0 in blank	

Section A7.1.3 Annex Point XII.2.2 IUCLID 3.4/01		Adsorption/Desorption in Water / Sediment Systems Determination of the Adsorption of Glutaraldehyde to Activated Sludge	
		mixture F = dilution factor	
		4 RESULTS	
4.1	**Biologically inhibited controls**	The half-life of glutaraldehyde in sludge sparged with inert gas (biologically inhibited) was 0.66 hours.	
4.2	**Sludge adsorption**	Minimal losses of the compound were observed in reaction mixtures prepared without activated sludge, indicating that hydrolysis, volatilization, or adsorption to the test vessels did not account for the losses observed in the test mixtures. Extensive extraction (24 hours) of the activated sludge samples with ethyl acrylate did not recover any detectable quantities of glutaraldehyde.	
4.3	**Half life**	Sludge mixtures 0.14 hours Sludge sparged with inert gas 0.66 hours	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Study was performed according to the ISO/CD 18749 method and per GLP's. Reaction mixtures were treated using mercuric chloride for 3 hours, and the sludge allowed to settle. Supernatant was replaced twice to wash the sludge. Mixtures were pasteurized, and allowed to cool. Mixtures were UV-treated and sparged with inert gas for one hour prior to glutaraldehyde addition. Reaction mixtures were incubated at 20-25°C. The pH and dissolved O_2 was measured at the beginning and periodically throughout the study. Samples were analyzed for glutaraldehyde at 0.1, 0.25, 0.5, 1, 2, 3, 6, and 24 hours by gas chromatography. Activated sludge was extracted at the study conclusion to determine how much glutaraldehyde was adsorbed to the solids. An adsorption curve was determined for the test material in the reaction mixtures as a function of time. Compound half life was calculated assuming the removal processes followed pseudo-first order kinetics. Description of reaction mixtures is found in ***Table 7.1.3/01-2.***	
5.2	**Results and discussion**	***Table 7.1.3/01-3*** Glutaraldehyde was rapidly removed from sludge mixtures, reaching non-detectable levels within one hour. The rate of removal corresponded to a half-life of 0.14 hours. Slightly slower removal of the compound (half-life 0.66 hours) was observed in activated sludge sparged with the inert gas argon to minimize biological activity. Minimal losses of the compound were observed in reaction mixtures prepared without activated sludge, indicating that hydrolysis, volatilization, or adsorption to the test vessels did not account for the losses observed in the test mixtures. Extensive extraction (24 hours) of the activated sludge samples with ethyl acetate did not recover any detectable quantities of glutaraldehyde.	

Section A7.1.3 **Annex Point XII.2.2** **IUCLID 3.4/01**	**Adsorption/Desorption in Water / Sediment Systems** **Determination of the Adsorption of Glutaraldehyde to Activated Sludge**	
5.3 Conclusion	Results indicate that glutaraldehyde is removed by a combination of biodegradation and irreversible binding (covalent binding) to the activated sludge.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	3.4.2009	
Materials and Methods	The applicant's version is correct. 3.3.2, Table A7.1.3/01: The reported concentration of Na_2HPO_4 * $7H_2O$ was 50.4 g/L, in the ISO 18749 the concentration is 33.4 g/L.	
Results and discussion	Glutaraldehyde disappeared from the activated sludge with a half-life of 0.14 hours . In activated sludge sparged with argon to minimize biological activity the half-life was 0.66 hours. It was not possible to extract glutaraldehyde from the sludge. The test is considered valid if the percentage adsorption of the reference substance is greater than 90% after 24 hours. This criterion was not fulfilled, the removal of the reference substance, Acid Red 88, was 71%. The reference substance was different than Basic Violet 4 given in the ISO guideline. The other criterion is that mass balance shall exceed 80%. Mass balance was not determined in this study.	
Conclusion	Glutaraldehyde was rapidly removed in the sludge as a result of biodegradation and irreversible adsorption. The rapid removal was explained to be due to reactivity of glutaraldehyde with amine functional groups that are present in proteins. This assumption is supported by the fact that removal was rapid also in the biologically inactivated test systems.	
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 A7.1.3/01 Mineral Medium

COMPOUND	STOCK CONCENTRATION (g/L)
Solution A	
KH_2PO_4	8.5
K_2HPO_4	21.75
$Na_2HPO_4*7H_2O$	20.4
Solution B	
$MgSO_4*7H_2O$	12.3
Solution C	
$CaCl_2*2H_2O$	29.4
Solution D	
$NaHCO_3$	22.4

Table A7.1.3/01-2 Description of Reaction Mixtures

Number of Reaction Vessels	Purpose	Description
2	***Blanks:*** Determine possible background interferences for analysis of test and reference materials	Activated sludge in mineral medium
2	***Reference Mixtures:*** Measure adsorption of reference material on activated sludge to confirm proper operation of the test system	Activated sludge in mineral medium + Acid Red 88 Dye
2	***Test Mixtures:*** Measure adsorption of test material on activated sludge	Activated sludge in mineral medium + Glutaraldehyde
2	***Abiotic Controls:*** Determine loss of test material by air stripping, degradation, or adsorption to test vessel	Mineral medium + Glutaraldehyde
2	***Biologically Inhibited Blanks***: Determine possible background interferences for analysis of test and reference materials	Activated sludge in mineral medium + Inhibitory treatment[a]
2	***Biologically Inhibited Reference Mixtures:*** Measure adsorption of reference material on treated activated sludge to confirm proper operation of test system	Activated sludge in mineral medium + Inhibitory treatment[a] + Acid Red 88 Dye
2	***Biologically Inhibited Controls:*** Measure adsorption of test material on activated sludge which was treated to inhibit biodegradation	Activated sludge in mineral medium + Inhibitory treatment[a] + Glutaraldehyde

[a] sparging with inert gas (argon) to minimize biological activity

Table A7.1.3/01-3 Measured Glutaraldehyde Concentrations in Reaction Mixtures for Activated Sludge Adsorption Test

Time (hours)	Viable Test (mg/L)	Biologically Inhibited Controls (mg/L)	Abiotic Controls (mg/L)
0	2.99 ± 0.15	not measured	2.83 ± 0.10
0.1	1.70 ± 0.62	2.83	2.27 ± 0.25
0.25	0.37 ± 0.08	1.77 ± 0.36	2.79 ± 0.01
0.5	0.28 ± 0.11	1.94 ± 0.42	3.16 ± 0.20
1	<0.2 mg/L	0.97 ± 0.48	2.83 ± 0.11
2	<0.2 mg/L	1.01 ± 1.14	2.91 ± 0.14
3	<0.2 mg/L	<0.2 mg/L	2.97 ± 0.44
6	a	<0.2 mg/L	2.80 ± 0.26
24	<0.2 mg/L	<0.2 mg/L	2.68 ± 0.53

a inadvertently not analyzed.

Section A7.2.1 _ 01 **Aerobic degradation in soil**

Aerobic soil metabolism of ^{14}C-glutaraldehyde (loamy sand)

Annex Point IIA7.1

			Official use only
		1 REFERENCE	
Reference		[redacted] (1986) Aerobic soil metabolism of ^{14}C-glutaraldehyde. [redacted] (Unpublished), BPD ID A7.02.1_01	
Data protection		Yes	
1.1.1	Data owner	BASF	
1.1.2	Companies with letter of access	[redacted]	
1.1.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
Guideline study		Yes, US EPA Guideline subdivision N 162-1	x
GLP		Yes	
Deviations		No	
		3 MATERIALS AND METHODS	
Test material		^{14}C-glutaraldehyde standard	
3.1.1	Lot/Batch number	Not given	
3.1.2	Specification	Specific radioactivity 3.40 mCi/mM	
3.1.3	Purity	Radiochemical purity > [redacted]%	
3.1.4	Further relevant properties	The test substance was an amber viscous liquid.	
3.1.5	Method of analysis	Liquid Scintillation Counting Analysis (LSC) Gas Liquid Chromatography (GLC)	
Degradation products		Not tested	
3.1.6	Method of analysis for degradation products	Not relevant	
Reference substance		No	
3.1.7	Method of analysis for reference	Not relevant	

Section A7.2.1 _ 01 **Aerobic degradation in soil**

Annex Point IIA7.1 **Aerobic soil metabolism of ^{14}C-glutaraldehyde (loamy sand)**

substance

Soil types

One soil type was used, which was characterized as follows:

Parameters	Value
Organic matter	0.3%
pH	7.8
Cation exchange capacity (C.E.C, meq/100 g)	5.9
Sand	83.6%
Silt	9.2%
Clay	7.2%
Texture	Loamy sand
1/3 Bar Moisture percentage	0.9

Testing procedure

3.1.8 Test system

The test system consisted of a 3000 ml metabolism vessel, with ground glass joints treated with sealing wax to preserve the integrity of the closed system. The test system was operated under positive pressure. The metabolism vessel was connected to an ethylene glycol trap, a 1N H2SO4 trap and two successive 1N KOH traps.

500 g of soil (loamy sand) were placed into the metabolism vessel; the sediment was dosed at 10 ppm to get 5.0 mg of ^{14}C-glutaraldehyde. The vessel was placed within an environmental control chamber maintained under dark conditions at an average temperature of 25 +/-1°C.

Samples for analysis were collected over a period of 120 days at following time point: day 0, 1, 3, 7, 14, 21, 30, 60, 91and 120.

3.1.9 Samples analysis

Radioactivity: The measurements of radioactivity were based on liquid scintillation counting (LSC).

Oxidation: For oxidation, samples weighing about 2 g were combusted in a Packard Model 306 Tri-Crab oxidizer using Carbosorb™ as a trapping agent and Permafluor V™ as scintillator.

Sample extraction: Soil samples weighing about 2 g +/- 0.001 g were used. The moisture level was determined at extraction time for calculation of the dry weight of each soil sample. The samples were extracted with methanol and the extract was transferred to an amber vial and was overlaid with argon gas after taking aliquots for LSC. The extracted samples were stored at –20 °C. For the purpose of characterisation, the soil extracts were applied directly to silica gel TLC plates.

Trapping solutions: About 200 ml of trapping solution was added to each ethylene glycol, H_2SO_4 and KOH trap. At each sampling interval, the solutions were brought up to 250 ml final volume. The ethylene glycol and the H_2SO_4 solutions were transferred to amber glass bottles; the KOH solutions were transferred to polyethylene bottles. Duplicate samples were subjected to LSC analysis. For capture of ^{14}C-volatiles, silica gel Sep-Packs were placed between the metabolism vessel and the ethylene glycol trap. The Sep-Packs were extracted with methanol for LSC.

Thin-layer chromatography: For activation, the prepared silica gel TLC plates were placed for 20 minutes into and oven at 120 °C. Diluted glutaraldehyde primary stock (purity > 98%) was applied to each plate to provide markers and

percentage of recovery for each plate. Developement of the plates was based on pre-equilibration in a TLC tank containing hexane:ethyl acetate:methanol (55:25:20), with ascending chromatography under argon gas. After development, the plates were air-dried and covered with a Kodak x-ray film. After a defined exposure period, the film was developed and radioactive areas on the plates were marked by means of a modified photo slide transillumination box. The radioactive spots were scraped from the plates and mixed to 2ml methanol; 15 ml Hydrocount™ was added and the radioactivity was determined by LSC.

Gas Liquid chromatography: GLC was performed on a Bendix model gas chromatograph with an FID detector. The identification and integration of the peaks was performed on a Hewlett-Packard model 3380A flat bed strip chart recorder or a HP 3390 integrator.

Calculations

3.1.10 Soil extracts

One ml aliquots were taken for LSC. The average of controls (XC) was substracted from the test samples average (XT) and the resultant number was divided by the specific activity (SA):

ppm = XT – XC / SA = dpm – dpm/dpm/µg = µg/ml

3.1.11 Combustions

The post extracted soil samples were dry weight when combusted

ppm = dpm/sample wt /SA = dpm/g/dpm/µg = µg/g

The pre-extracted soil samples were corrected for moisture:

ppm = dpm/sample wt x correction factor /SA = dpm/g/dpm/µg = µg/g

3.1.12 Trapping solutions duplicate

One ml aliquots were taken for LSC. The average of controls (XC) was substracted from the test samples average (XT) and the resultant number was divided by the specific activity (SA):

ppm = XT – XC / SA = dpm – dpm/dpm/µg = µg/ml

3.1.13 Sep-packs

For the 3 first time points the sep-packs were extracted with methanol. One ml aliquots were taken for LSC:

ppm = dpm/1 m/ SA = dpm/ml/dpm/µg = µg/ml

3.1.14 Thin layer chromatography

Standards were applied to each plate to provide a reference Rf for the parent compound. Same size sample aliquots were counted in duplicate by LSC as were applied to plates to give percentage of recovery:

dpm recovered/total dpm applied = % recovery

Individual spots on the TLC plates were counted by LSC after scraping:

dpm spot/total dpm recoverrd = % of the spot sample

3.1.15 First Order Rate Law

The first order rate law is $\ln C_T = \ln C_0 - kt$, with C_T being the percentage of total ^{14}C -residues in terms of parent compound equivalents.

Setting the initial concentrations = 1, at $t_{1/2}$ $C_T = 1 - 0.5 = 0.5$, the equation given above becomes: $\ln C_0/C_T = kt$. Therefore, $t_{1/2} = 1/k \ln 1/0.5 = 1/k \ln 2 = 0.693/k$, with k being the slope (first derivative) of the line generated from the linear regression analysis of the plot of ln % of initial concentration vs days.

3.1.16 Liquid scintillation counting

The control dpm values were manually substracted from test dpms. LSC data sheets indicate automatic LSC substraction in the heading dpm = cpm/efficiency.

4 RESULTS

Section A7.2.1 _ 01 Aerobic degradation in soil

Aerobic soil metabolism of ^{14}C-glutaraldehyde (loamy sand)

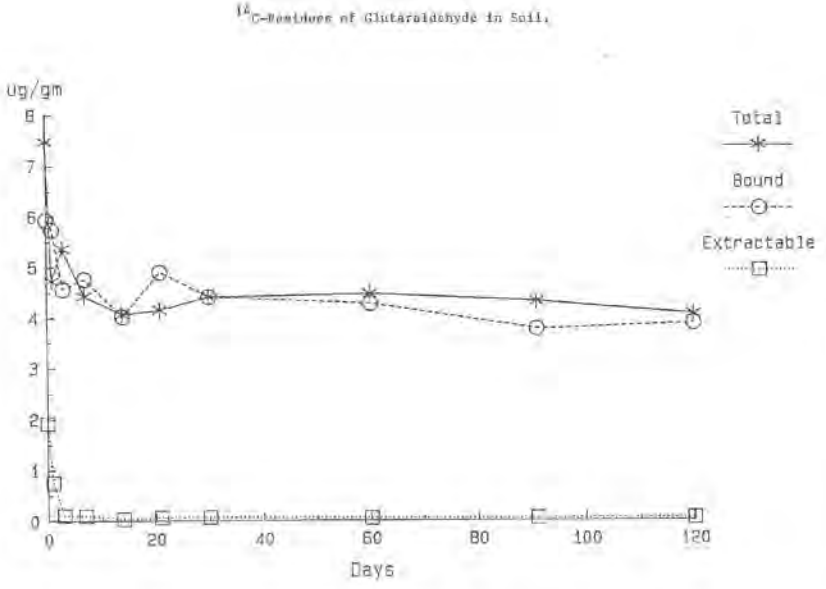
Annex Point IIA7.1

^{14}C residues of glutaraldehyde in soil

^{14}C-Residues of Glutaraldehyde in Soil (µg/gm).

				Percent of Total		
Day	Total	Extractable	Bound	Extractable	Bound	Ext. & Bound
0	7.48	1.92	5.93	25.7	9.3	105
1	4.72	0.760	5.74	16.1	122	138
3	5.36	0.112	4.56	2.09	85.1	87.2
7	4.42	0.103	4.76	2.33	108	110
14	4.06	0.0431	4.02[1]	1.06	99.0	100
21	4.15	0.0746	4.90	1.80	118	120
30	4.41	0.0732	4.41	1.66	100	102
60	4.47	0.0531	4.28	1.19	95.7	96.9
91	4.32	0.0540	3.78	1.25	87.5	88.8
120	4.06	0.0542	3.89	1.33	95.8	97.1

[1]Value obtained by subtraction of extractable from total. Raw data value of 16.1 µg/gm is obviously a contaminated sample or combustion.



The total ^{14}C-residues of glutaraldehyde in soil decreased exponentially from day 0 to day 21, reaching 40% of the initial dose (i.e ca. 4.2 µg/g); the residues percentage remained constant at 40% from day 21 to the end of the experimental period. The extractable ^{14}C-residues were 25% of the initial dose on day 0 and decreased to 2% by day 3 to 7; thereafter, these residues remained < 2% until the end of the experimental period. The bound ^{14}C-residues ranged from 76 to 121% of total 14C-residues throughout the experimental period.

Section A7.2.1 _ 01

Aerobic degradation in soil

Aerobic soil metabolism of ^{14}C-glutaraldehyde (loamy sand)

Annex Point IIA7.1

Capture of volatiles by trapping solutions

^{14}C-Residues of Glutaraldehyde in Trapping Solutions[1].

Day	Ethylene Glycol	H_2SO_4	KOH_1	KOH_2
1	6.50	0.266	140	0.053
3	70.1	0.580	570	*
7	5.17	0.672	62.8	0.173
14	1.03	0.161	204	0.020
21	0.605	*	33.0	0.135
30	0.331	0.021	68.3	1.40
37	0.180	*	30.0	0.779
44	0.058	*	21.6	0.779
51	0.056	*	17.0	0.229
60	0.138	0.020	16.4	0.011
67	0.067	0.005	10.6	*
74	0.049	0.004	9.07	0.082
84	0.014	0.057	9.10	0.029
91	0.050	0.004	6.97	*
98	0.032	0.004	5.76	0.011
105	*	0.021	4.48	*
113	*	0.039	4.48	*
120	0.050	0.004	4.05	*
127	*	*	3.38	0.082
134	*	0.057	3.13	0.028
141	*	0.093	2.85	0.028
Σ	84.4	2.01	1227	3.84

[1]Duplicate 1 ml samples taken for LSC. Values are total µg ^{14}C-Glutaraldehyde equivalents in the trapping solution.

*Denotes values that are at or below background values.

Capture of volatiles by the trapping soluitions accounted for ca. 26% of the initial dose of radioactivity; therefrom, about 25% were trapped in the KOH traps, representing $^{14}CO_2$ evolution. Peaks in 14C activity were seen after 3 days.

Silica gel sep-packs

^{14}C-Residues of Glutaraldehyde in Sep-packs.

Day	Total µg[1]	% of Initial Dose
3	0.26	<0.01
7	0.022	<0.01
14	<0.01	<0.01
21	<0.01	<0.01
30	<0.01	<0.01
37	<0.01	<0.01
44	<0.01	<0.01
51	<0.01	<0.01
60	<0.01	<0.01
67	<0.01	<0.01
74	<0.01	<0.01
84	<0.01	<0.01
91	<0.01	<0.01
98	<0.01	<0.01
105	<0.01	<0.01
113	<0.01	<0.01
120	<0.01	<0.01
127	<0.01	<0.01
134	<0.01	<0.01
141	<0.01	<0.01

[1]Values are total µg ^{14}C-Glutaraldehyde equivalents in the Sep-packs.

No significant amounts of radioactivity were trapped by the silica gel sep-packs placed between the test vessel and the ethylene glycol trap; in fact, a maximum of 0.26 µg glutaraldehyde equivalents were reported for day 3, corresponding to

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Aerobic degradation in soil
Aerobic soil metabolism of ^{14}C-glutaraldehyde (loamy sand)

less than 0.01% of the initial dose of radioactivity.

^{14}C recovery during TLC

^{14}C-Recovery During TLC of Extractable ^{14}C-Residues of Glutaraldehyde in Soil [1].

Day	µl Applied	x DPM Applied[2]	DPM Recovered	Percent Recovery
0	200	7220	4786	66.3
1	600	6672	5406	81.0
3	350	672	1759	38.9
7	350	588	346	58.8
14	450	621	228	36.7

[1] TLC set No. 2 only.

[2] Determined by LSC counting of triplicate aliquots.

14C-recoveries in thin layer chromatography ranged between ca. 37 and 82% of the applied radioactivity.

Total ^{14}C-labelled GA mass balance

^{14}C-Residue Mass Balance Accountability.

	Sediment					Sep-Packs	Trapping Solutions	Total
Day	gm Sediment in System	µg in Sample	µg in System	Total µg Accountability	% of Initial Dose	% of Initial Dose	% of Initial Dose	% of Initial Dose
0	496.00	107.712	3710	3710	74	<0.01	---	74
1	481.60	40.889	2273	2581	48	<0.01	2.9	51
3	472.94	50.706	2535	2684	54	<0.01	1.3	67
7	463.48	42.370	2049	2248	45	<0.01	1.4	46
14	453.89	38.306	1843	2084	42	<0.01	4.1	46
21	444.46	39.837	1845	2124	42	<0.01	0.47	43
30	434.96	275.396	1918	2237	45	<0.01	1.4	46
60	304.34	368.033	1360	1955	39	<0.01	1.7	40
91	240.49	282.973	1039	2002	40	<0.01	0.73	41
120	186.42	184.235	757	2003	40	<0.01	0.39	40

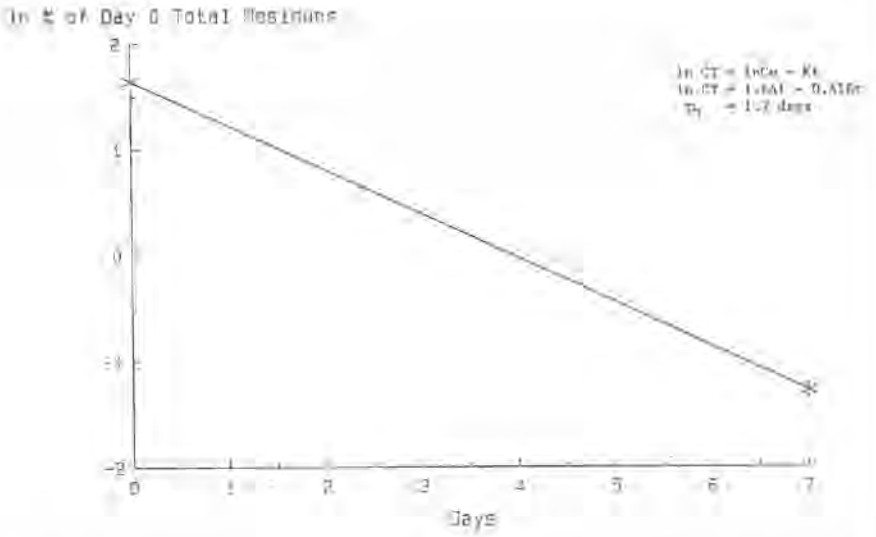
Total accountability declined steadily over the time course to 40% of the initial dose by day 120. The loss of accountability was due to unavoidable volatiles losses during the normal sample collection and processing. This was supported by the fact, that during sample combustions, spiked samples combusted immediately resulted in 100% recovery whereas samples combusted after a delay period of 15 minutes showed 15% loss of radioactivity by volatilisation.

Section A7.2.1_01 Aerobic degradation in soil

Aerobic soil metabolism of ^{14}C-glutaraldehyde (loamy sand)

Annex Point IIA7.1

Half-life calculation



Thin layer chromatography revealed that about 20% of the extracted radioactivity at day 0 was due to the parent compound glutaraldehyde. Up to day 3 and, no parent compound could be identified; however, on day 7, TLC again showed that about 20% of extracted radioactivity was due to the parent compound. A half-life of 1.7 days was calculated on the basis of glutaraldehyde recovery on day 0 and day 7.

Section A7.2.1_01 Aerobic degradation in soil

Aerobic soil metabolism of ^{14}C-glutaraldehyde (loamy sand)

Annex Point IIA7.1

Degradation products

TLC of ^{14}C-Residues of Glutaraldehyde in Soil as Percent of Recovered DPM [1] (Re-Analysis).

Degradation Product	O	G	A	B	
TLC Zone	Origin	I[2]	II	IV	Remainder
R_f	(0)	(0.39)	(0.07)	(0.082)	Remainder
Day-0	25.6	20.1	30.1		24.3
Day-1	76.5				23.5
Day-3	85.7				14.3
Day-7	29.8	20.2		26.6	23.4
Day-14	69.3				30.7
Standard	33.2	41.8	12.3		18.8

TLC of ^{14}C-Residues of Glutaraldehyde Soil as % of Total Residues on Day -0 [3] (Re-Analysis).

	Degradation Products				
	O	G[2]	A	B	Remainder
Day - 0	6.57	5.16	7.73		6.24
Day - 1	7.77				2.39
Day - 3	1.28				0.21
Day - 7	0.41	0.28		0.37	0.32
Day - 14	0.40				0.18

[1] TLC set No. 2 only. $\bar{x}$ of all plates containing the zone.

[2] ^{14}C-Glutaraldehyde.

[3] Percent of total residues at Day -0 = $\frac{\left(\text{Extractable Residues, ppm}\right)\left(\text{Percent Recovered}\right)}{(7.48 \text{ ppm at Day -0})}$

TLC characterisation of ^{14}C residues of glutaraldehyde revealed following 4 degradation products:

(1) Degradation product O: immobile residues of ^{14}C-GA, accounted for 0.4 to 7.77% of the total residues present on day 0.

(2) Degradation product A: second major degradation product, accounted for 7.73% of the total residues present on day 0.

(3) Product G: parent compound on day 0.

(4) Degradation product B: final degradation product, accounted for 0.37% of the total residues present on day 0.

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A7.2.1 _ 01 Aerobic degradation in soil

Aerobic soil metabolism of ^{14}C-glutaraldehyde (loamy sand)

Annex Point IIA7.1

Materials and methods

The aim of the present study was to investigate the comportment of glutaraldehyde in soil under aerobic conditions.

Test substance: ^{14}C-glutaraldehyde standard, radiochemical purity > [redacted]% (primary stock solution), specific radioactivity 3.40 mCi/mM.

Guideline: US EPA 162-1,GLP

One soil type, defined as loamy sand, was used for the present aerobic soil metabolism study, and showed following characteristics: 0.3% organic matter content, ca. 84% sand content, ca. 9% silt content, ca. 7% clay content, pH 7.8, C.E.C. 5.9 meq/100g, and 1/3 Bar moisture percentage of 0.9.

The test system consisted of a 3000 ml metabolism vessel, with ground glass joints treated with sealing wax to preserve the integrity of the closed system. The test system was operated under positive pressure. The metabolism vessel was connected to an ethylene glycol trap, a 1N H2SO4 trap and two successive 1N KOH traps. For capture of ^{14}C-volatiles, silica gel Sep-Packs were placed between the metabolism vessel and the ethylene glycol trap. 500 g of sediment (loamy sand) were placed into the metabolism vessel; the sediment was dosed at 10 ppm to get 5.0 mg of ^{14}C-glutaraldehyde. The vessel was placed within an environmental control chamber maintained under dark conditions at an average temperature of 25 +/-1°C.

Samples for analysis were collected over a period of 120 days at following time point: day 0, 1, 3, 7, 14, 21, 30, 60, 91and 120.

The measurements of radioactivity were based on liquid scintillation counting (LSC), thin layer chromatography (TLC) gas liquid chromatography (GLC).

Results and discussion

The main results of the present study can be summarized as follows: x

The total ^{14}C-residues of glutaraldehyde in soil decreased exponentially from day 0 to day 21, reaching 40% of the initial dose; the residues percentage remained constant at 40% from day 21 to the end of the experimental period. The extractable ^{14}C-residues were 25% of the initial dose on day 0 and decreased to 2% by day 3 to 7; thereafter, these residues remained < 2% until the end of the experimental period. The bound ^{14}C-residues ranged from 76 to 121% of total 14C-residues throughout the experimental period.

Thin layer chromatography revealed that about 20% of the extracted radioactivity at day 0 was due to the parent compound glutaraldehyde. Up to day 3 and, no parent compound could be identified; however, on day 7, TLC again showed that about 20% of extracted radioactivity was due to the parent compound. A half-life of 1.7 days was calculated.

TLC characterisation of ^{14}C residues of glutaraldehyde revealed following 4 degradation products:

(1) Degradation product O: immobile residues of ^{14}C-GA, accounted for 0.4 to 7.77% of the total residues present on day 0.

(2) Degradation product A: second major degradation product, accounted for 7.73% of the total residues present on day 0.

(3) Product G: parent compound on day 0.

(4) Degradation product B: final degradation product, accounted for 0.37% of the total residues present on day 0.

Conclusion

Within the present aerobic soil metabolism study with glutaraldehyde, a DT_{50} of 1.7 days was calculated, indicating that glutaraldehyde is rapidly degraded in soil by microbial biotransformation. Moreover, glutaraldehyde was shown to be readily biodegradable within several studies (see references below). Therefore a persistence of glutaraldehyde in soil is unlikely.

(1) [redacted] (1993) Determination of the Biodegradability or the Elimination of

Section A7.2.1 _ 01 **Aerobic degradation in soil**

Aerobic soil metabolism of ^{14}C-glutaraldehyde (loamy sand)

Annex Point IIA7.1

[redacted] in the DOC Die Away Test. [redacted] (unpublished)

(2) [redacted] (1993) Determination of the Biodegradability [redacted] in the Activated Sludge Simulation Test according to GLP, EN 45001 and ISO 9002. [redacted] (unpublished)

(3) Zoellner H, Kramer A, Youssef P, Youssed U, Adrian V (1995) Preliminary investigations on the biodegradability of selected microbicidal agents. Hyg. Med. 20 (9): 401-407 (published)

5.1.1 Reliability **1**

5.1.2 Deficiencies No

5.1.3

Section A7.2.1 _ 01 **Aerobic degradation in soil**

Aerobic soil metabolism of ^{14}C-glutaraldehyde (loamy sand)

Annex Point IIA7.1

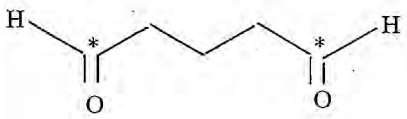
Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	25.3.2009
Materials and Methods	The study is old and does not fulfil the current requirements of the OECD 307. There are several deficiencies: - organic matter content in soil is 0.3%, 0.5-2.5% organic carbon content required - only one soil used, four required for transformation rate - origin of soil not given - microbial biomass in the soil not quantified - repeatability and sensitivity of analytical methods have not been reported - nothing is said about an acclimation period, 2-28 d incubation period recommended - mass balance was 40-74% which is outside the acceptable range for labelled substances - mineralization rate was not determined Other remarks: 2.1 Test guideline not given in the test report.
Results and discussion	Radioactivity in soil declined reaching 45% of initial dose on day 7 and 40% on day 120. Glutaraldehyde accounted for 5.16% of total radioactivity on day 0 and 0.28% on day 7, it was not detected on any other days. The half-life of 1.7 days was calculated according to first order kinetics. Three transformation products were isolated, but not identified. All of them accounted less than 10% of total radioactivity. The bound residues ranged from 85% to 122% of total ^{14}C-residues in soil throughout the experiment. The extractable residues were 25.7% on day 0 and declined to ca. 2% by day 3-7 and remained <2% until the end of the test. CO_2 accounted at max 4.1% of radioactivity.
Conclusion	Glutaraldehyde degraded rapidly to transformation products of which majority were bound to soil. Only small proportion of radioactivity was trapped as CO_2 indicating negligible mineralization. Recovery of radioactivity was low due to 15% losses during the normal sample collection and processing.
Reliability	3
Acceptability	Not acceptable because the material balance was 40-74%.

Section A7.2.1 _ 01 Aerobic degradation in soil

Aerobic soil metabolism of ^{14}C-glutaraldehyde (loamy sand)

Annex Point IIA7.1

Remarks	Please explain how it is possible that glutaraldehyde formed 20% of radioactivity on day 0 and 7 while it was not detected on day 1 and 3. Applicant's response According to the author, since LSC data for DPM applied to TLC plates was lost at some time points during the study, it was necessary to regenerate a repeat set of TLC plates at a later date, despite low radioactivity. The 20% radioactivity value for day 7 refers to this repeat set of data. This value was suspected to be due to the extremely low amounts of radioactivity present. The discrepancy between the day 0 values of percent parent recovered in the two sets of data was considered by the author to probably be due to the volatility of the compound as the 4.5 month time differential and the repeated opening of the sample vials resulted in substantial losses of test material. Having reviewed the study we agree with the CA's position that the present study shows some deficiencies. Particularly the method referring to the regenerated repeat set of TLC plates appear critical. Here, the author reported that it was necessary to regenerate a repeat set of TLC plates at a later date, despite of low radioactivity because of the loss of LSC data at different time points of the study. Discrepancies in terms of radioactivity recovery were observed between the two data sets which, according to the author, probably were related to volatility of the compound as the 4.5 month time differential and the repeated opening of the sample vials resulted in substantial losses of test material. However, according to the TGD on data requirements, a soil study is required if a direct application or emission of the biocide onto/into soil is expected. This is not intended for [redacted]. Furthermore, according to the TGD, such a study should be performed in case of non ready biodegradability or not inherently biodegradability of the substance, when a refinement of the degradation rate and route is needed. Nevertheless, since the substance has been shown to be readily biodegradable (see section A7.1.1.2.1_01) and since a simulation test also had been conducted, which gave no hint on adsorption or other abiotic elimination processes and which confirmed that glutaraldehyde is biodegradable (A7.1.2.1.1_01), a soil simulation test is not required.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.2.3.1 **Annex Point IIIA, XII 1.3** **IUCLID 3.3.1/01**		**Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106 and, where relevant , adsorption and desorption of metabolites and degradation products**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] (1994), Soil adsorption/desorption of [14C] glutaraldehyde by the batch equilibrium method, [redacted], Unpublished, 29 March 1994	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company and BASF SE	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on an existing a.s. for the purpose of its entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes US EPA FIFRA 163-1	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes At the suggestion of the Quality Assurance Unit of the testing facility, retrospective entries were made to data books to clarify data. Data for [redacted] soil and sediment characterizations were not reviewed for GLP compliance.	
		3 METHOD	
3.1	**Test material**	^{14}C-Glutaraldehyde	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	 [1,5-^{14}C]-Glutaraldehyde * denotes the position of the radiolabel, ^{14}C.	
3.1.3	Purity	Radiochemical purity [redacted]% Specific activity 13.6 uCi/umol	
3.1.4	Further relevant properties	None	
3.1.5	Composition of Product	active substance used	
3.1.6	Specific chemical analysis	The radioactivity of samples was determined by LS 6000IC or LS 5000 liquid scintillation spectrometers. Computer-constructed quench curves	

Section A7.2.3.1 Annex Point IIIA, XII 1.3 IUCLID 3.3.1/01	Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106 and, where relevant , adsorption and desorption of metabolites and degradation products	
	automatically converted cpm to dpm. Typical parameters were as follows: counting efficiency 96%, background 45 dpm, counting time 1 min. HPLC analysis was performed on the supernatant for the two highest concentrations (5.0 and 10.3 ppm) for sandy loam, silty clay loam, silty loam and sediment to determine the stability of [14C] glutaraldehyde under the conditions of the study. For loamy sand, the 5.0 ppm samples (combined replicates) were analyzed. Desorption was carried out by resuspending each of the soil pellets with fresh 0.01 M $CaCl_2$ solution, then the tubes were incubated for an additional 24 hours, centrifugated and the aliquots from the solution were analyzed as described for the adsorption phase of the study. Soils were air dried and combusted, $^{14}CO_2$ was trapped for subsequent quantitation by LSC.	
3.2 Reference substance	Yes, analytical reference standards glutaraldehyde glutaric acid	
3.3 Testing procedures		
3.3.1 Test soil	The details of the test soils are presented in **Table A7.2.3.1/01-1**. The test soils were chosen to represent a variety of chemical and physical properties. Soils were air dried at room temperature (25°C) and passed through a 2 mm screen prior to use.	
3.3.2 Preliminary trial	Preliminary testing was conducted to determine the appropriate soil to water ratio and the time to equilibrium. Five sets of duplicate Teflon® centrifuge tubes were prepared for each soil. 1, 5, 10, 15 and 20 g of each soil and 30 ml of glutaraldehyde solution with nominal concentration of 10 ppm were used. Two control tubes containing only 10 ppm glutaraldehyde and no soil were prepared to demonstrate any adsorption of glutaraldehyde to the Teflon® tubes. The tubes were incubated in a shaking water bath in the dark at 25 °C. Triplicate aliquots were taken at 3.5, 20, 24, 44 and 64 hours. At each sampling time, tubes were centrifuged at 2500 rpm for 10 min and the supernatants were removed and radioassayed immediately. The soil in the tubes was then re-suspended into the adsorption solution and the tubes were returned to the water bath. At the last sampling, soils were air dried, then combusted for subsequent radioassay.	x
3.3.3 Definitive assay		
3.3.4 Test system	Teflon® centrifuge tubes were filled with 10 g of sandy loam, 5 g of silty clay loam, 5 g of silty loam, 20 g of loamy sand or 20 g of sediment. To each tube, 30 ml of the appropriate solution was added. The test concentrations were 0.51, 1.0, 2.5, 5.0 and 10.3 ppm and two replicates (tubes) per concentration were prepared. Samples were placed in a shaking water bath and maintained in dark conditions at 25°C.	
3.3.5 Preparation of test solution	A 10.3 ppm stock solution of ^{14}C-glutaraldehyde was prepared by fortifying 116,506,290 dpm of ^{14}C-glutaraldehyde with 7330 ug of	

Section A7.2.3.1 Annex Point IIIA, XII 1.3 IUCLID 3.3.1/01		Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106 and, where relevant , adsorption and desorption of metabolites and degradation products	
		unlabeled glutaraldehyde. The mixture was added to 750 mL of sterile 0.01M $CaCl_2$ solution. Final solutions were prepared from this stock solution (0.51, 1.0, 2.5, or 5 ppm). A control sample of sterile 0.01M $CaCl_2$ was prepared.	
3.3.6	Initial TS concentration	0.51, 1.0, 2.5, 5.0 and 10.3 ppm	
3.3.7	Duration of test	48 hours	x
3.3.8	Analytical parameter	The ^{14}C content was measured in the supernatant decanted off of the vials containing the soil samples. Radioactivity in the soil samples was determined following combustion and trapping of $^{14}CO_2$.	
3.3.9	Sampling	The tubes were agitated for 24 hours in the dark at 25 °C. After incubation, tubes were centrifuged at 2500 rpm for 10 minutes, and the radioactivity of samples was determined by liquid scintillation spectrometers. HPLC was performed on adsorption supernatants. Desorption was carried out by resuspending each of the soil pellets in 0.01N $CaCl_2$ solution, shaken, and incubated for an additional 24 hours. After centrifugation, aliquots from the solution were analyzed as described for the adsorption. Soils were air-dried and combusted, $^{14}CO_2$ was trapped for subsequent quantitation by LSC. Temperature of the water bath was recorded daily during the 48-hour experimental period.	
3.3.10	Intermediates/ degradation products	Identified by HPLC using chromatographic standards.	
3.3.11	Controls	^{14}C-Glutaraldehyde was placed in vials under identical conditions, without any soil.	
3.3.12	Statistics	***Soil Combustion (total dpm in soil)*** Raw dpm – background dpm x total soil weight Oxidizer efficiency (aliquot weight) ***DPM in Adsorption Solution after 24 hours*** Concentration of adsorption solution x 30 mL	
		4 RESULTS	
4.1	**Mass balance**	The overall mean mass balance for all four soil types and sediment was 74.1 +/- 10% of the applied radiocarbon. **Table A7.2.3.1/01-2**	
4.2	**Transformation products**	Glutaric acid was the only identified transformation product. It was found in the range of 10.2-59.4% from the combined replicates. In individual replicates the proportion of glutaric acid was only 0.0-4.8%. In addition there were at least 8 other transformation products but these were not identified. In the combined replicates the proportion of transformation products was lower than 10%, but in the individual replicates the proportion of some metabolites was more than 10%.	
4.3	**Adsorption**	**Table A7.2.3.2/01-3** Data indicates that a non-linear relationship exists between	

Section A7.2.3.1 Annex Point IIIA, XII 1.3 IUCLID 3.3.1/01	Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106 and, where relevant , adsorption and desorption of metabolites and degradation products	
	concentrations in solution and adsorption. The Freundlich equation best describes adsorption, and the linear isotherms were not used. **The Freundlich adsorption coefficients for glutaraldehyde were determined to be (Table A7.2.3.1/01-4):** 2.06 for sandy loam 4.94 for silty clay loam 4.83 for silt loam 1.10 for loamy sand 0.59 for sediment **The K_{oc}'s were determined to be:** 210 for sandy loam 500 for silty clay loam 340 for silt loam 460 for loamy sand 120 for sediment	
4.4 Desorption	**Table A7.2.3.1/01-3** Due to rapid degradation of the compound verified by HPLC, glutaraldehyde could not be measured in solution during the desorption phase. Thus, desorption isotherms could not be obtained.	
4.5 Stability	The half-life of ^{14}C-glutaraldehyde in aerobic aquatic systems was shown to be less than 24 hours in previous studies; the major end products being $^{14}CO_2$, explaining the poor recoveries (mass balance) in the current investigation.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Following a preliminary test, Teflon® centrifuge tubes were filled with 10 g of sandy loam, 5 g of silty clay loam, 5 g of silty loam, 20 g of loamy sand and 20 g of sediment. To each tube, 30 ml of the appropriate solution was added. The test concentrations were 0.51, 1.0, 2.5, 5.0 and 10.3 ppm and two replicates (tubes) per concentration were prepared. Control tubes (duplicate) contained 5 g of each soil type and 30 ml 0.01 M $CaCl_2$ solution. Two tubes, containing only 30 ml of 10.3 ppm glutaraldehyde were used to measure the extent of adsorption of [^{14}C]-glutaraldehyde to the Teflon® tubes. The tubes were agitated 24 hours in the dark at 25 °C. After incubation tubes were centrifuged at 2500 rpm for 10 minutes, the supernatants were decanted and triplicate 0.5 ml aliquots were immediately radioassayed for determination of adsorption isotherms. The radioactivity of samples was determined by liquid scintillation spectrometers. HPLC analysis was performed for the two highest concentrations (5.0 and 10.3 ppm) for sandy loam, silty clay loam, silty loam and sediment to determine the stability of [^{14}C]-glutaraldehyde under the conditions of the study. For loamy sand, the 5.0 ppm samples (combined replicates) were analyzed.	

Section A7.2.3.1 **Annex Point IIIA, XII 1.3** **IUCLID 3.3.1/01**	**Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106 and, where relevant , adsorption and desorption of metabolites and degradation products**	
	Desorption was carried out by resuspending each of the soil pellets with fresh 0.01 M $CaCl_2$ solution, then the tubes were incubated for an additional 24 hours, centrifugated and the aliquots from the solution were analyzed as described for the adsorption. Soils were air dried and combusted, $^{14}CO_2$ was trapped for subsequent quantitation by LSC.	
5.2 Results and discussion	The overall mean mass balance for all four soil types and sediment was 74.1 +/- 10% of the applied radiocarbon. Data indicates that a non-linear relationship exists between concentrations in solution and adsorption. The Freundlich equation best describes adsorption, and the linear isotherms were not used. Glutaric acid was the only identified transformation product. In addition there were at least 8 other transformation products but these were not identified.	
5.3 Conclusion	Adsorption / desorption isotherms with ^{14}C-glutaraldehyde were determined using four soil types and a sediment. Adsorption constants (K_{oc} values) of 210, 500, 340, and 460 in sandy loam, silty clay loam, silt loam, and loamy sand, respectively, predict glutaraldehyde to be moderately mobile in all four soils. The K_{oc} value for glutaraldehyde in sediment was 120. The rapid degradation of glutaraldehyde that was observed during the desorption phase of the study indicates that the compound will not persist in the soil environment. Potential movement/leaching of glutaraldehyde in soil (e.g. as a result of a spill), will be minimized by rapid degradation of the compound.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2.4.2009	
Materials and Methods	The applicant's version is correct. The test was performed according to US EPA FIFRA 163-1 guideline, but seems to follow quite accurately to the OECD 106. Five different soil types were included, but none of them had high organic carbon content. The highest organic carbon content was 1.42% in silt loam. None of soil was strongly acidic with pH less than 5.5. 3.3.2 A blank treatment, duplicate soil samples without test substance, was also included in the preliminary study as recommended by the OECD 106. 3.3.7 The duration of shaking period was 24 hours both in adsorption and desorption phase.	

Section A7.2.3.1 Annex Point IIIA, XII 1.3 IUCLID 3.3.1/01	**Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106 and, where relevant , adsorption and desorption of metabolites and degradation products**	
Results and discussion	The applicant's version is correct. Due to rapid degradation desorption isotherms could not be determined. After 24 hours adsorption phase glutaraldehyde formed 11.8 - 69.2% and glutaric acid 10.2-59.4% of radioactivity in the four soils and sediment. At least eight other metabolites were formed but they accounted less than 10% of radioactivity apart from one metabolite which accounted 10.5% in one soil.	
Conclusion	K_{oc} values of 210, 500, 340, and 460 were determined for sandy loam, silty clay loam, silt loam, and loamy sand, respectively, predicting glutaraldehyde or its metabolites to be moderately mobile in all four soils. Koc in sediment was 120.	
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		
Acceptability		
Remarks		

Table A7.2.3.1/01-1 Test Soils

Soil	Source	pH	Cation Exchange Capability	Field Capacity	% Organic Matter	% Organic Carbon	% Sand	% Silt	% Clay
Sandy Loam	[redacted]	6.8	5.5	15.1	1.70	1.0	67	23	10
Silty Clay Loam		5.7	19.7	30.0	1.68	0.99	16	55	29
Silt Loam		6.7	16.8	29.0	2.41	1.42	17	62	21
Loamy Sand		5.8	2.9	5.09	0.4	0.24	83	17	0
River Sediment		8.1	4.3	5.46	0.9	0.5	93	7	0

Table A7.2.3.1/01-2 Definitive Phase: Summary of Accountability of [^{14}C]-Residues for Four Soils and a Sediment (Replicate Average Percent Recovery of Applied DPM)

	Initial Glutaraldehyde Concentration (µg/g)				
Soil Type	**0.51**	**1.0**	**2.5**	**5.0**	**10.3**
Sandy Loam	61.4%	74.4%	73.1%	80.9%	86.9%
Silty Clay Loam	60.5%	66.7%	72.0%	73.9%	79.2%
Silt Loam	63.7%	69.4%	73.1%	79.8%	84.3%
Loamy Sand	59.8%	60.4%	67.8%	72.1%	78.1%
Sediment	62.0%	79.8%	83.8%	93.4%	96.3%
Mean ± SD [a]	61.5% ± 1.5	70.1% ± 7.4	74.0% ± 5.9	80.0% ± 8.4	85.0% ± 7.3

[a] The overall mean recovery ± SD for all soils and dose rates was 74.1 ± 10.

Table A7.2.3.1/01-3 **Adsorption and Desorption of ^{14}C-Glutaraldehyde by Four Soils and a Sediment.**
Concentrations of ^{14}C-Glutaraldehyde on Soil and in Solution After 24 Hours of Adsorption and 24 Hours of Desorption

Soil	Initial Glutaraldehyde Concentration µg/g				
	0.51	**1.0**	**2.5**	**5.0**	**10.3**
Sandy Loam					
adsorption					
µg of glutaraldehyde/g of soil	0.834	1.266	3.118	4.744	6.801
µg of glutaraldehyde/g of solution	0.230	0.591	1.488	3.417	7.644
desorption					
µg of glutaraldehyde/g of soil	0.220	0.438	1.072	2.045	3.308
µg of glutaraldehyde/g of solution	0.008	0.016	0.015	0.000	0.000
Silty Clay Loam					
adsorption					
µg of glutaraldehyde/g of soil	1.881	3.086	5.976	10.446	17.398
µg of glutaraldehyde/g of solution	0.194	0.499	1.531	3.258	7.011
desorption					
µg of glutaraldehyde/g of soil	0.687	1.083	2.058	3.704	6.339
µg of glutaraldehyde/g of solution	0.000	0.000	0.000	0.000	0.000
Silt Loam					
adsorption					
µg of glutaraldehyde/g of soil	1.765	3.118	6.338	10.239	16.841
µg of glutaraldehyde/g of solution	0.214	0.494	1.471	3.292	7.104
desorption					
µg of glutaraldehyde/g of soil	0.627	1.142	2.261	4.244	7.291
µg of glutaraldehyde/g of solution	0.006	0.019	0.002	0.002	0.000
Loam Sand					
adsorption					
µg of glutaraldehyde/g of soil	0.401	0.758	1.480	2.455	4.007
µg of glutaraldehyde/g of solution	0.241	0.508	1.541	3.362	7.239
desorption					
µg of glutaraldehyde/g of soil	0.089	0.128	0.355	0.640	1.115
µg of glutaraldehyde/g of solution	0.004	0.016	0.000	0.000	0.000
Sediment					
adsorption					
µg of glutaraldehyde/g of soil	0.359	0.409	0.674	1.385	2.167
µg of glutaraldehyde/g of solution	0.268	0.740	2.077	4.075	8.466
desorption					
µg of glutaraldehyde/g of soil	0.108	1.206	3.146	6.879	13.675
µg of glutaraldehyde/g of solution	0.000	0.000	0.000	0.000	0.059

Table A7.2.3.1/01-4 Freundlich Equation K, K_{oc} and 1/n Values for Adsorption of ^{14}C-Glutaraldehyde onto Four Soils and a Sediment

Soil Type	K	K_{oc}	1/n	r
Sandy Loam	2.06	210	0.63	0.990
Silty Clay Loam	4.94	500	0.62	0.998
Silt Loam	4.83	340	0.64	1.000
Loamy Sand	1.10	460	0.66	0.998
Sediment	0.59	120	0.55	0.961
Average of Four Soils [a]	3.23	380	0.64	

[a] Averages do not include Sediment, an aquatic sediment from California (textural class is sand).

Section A7.3.1 _ 01 **Annex Point IIA7.1**		**Phototransformation in air (estimation method), including identification of breakdown products**	**Official use only**
		1 REFERENCE	
1.1	**Reference**	[redacted] (2005) Glutaraldehyde, SRC calculations. [redacted] (Unpublished), BPD ID A3.02.1_01	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	None	
2.2	**GLP**	No	
2.3	**Deviations**	The data refer to an acknowledged calculation program: the EPIWIN program, which was developed by the Syracuse Research Corporation, NY.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde	
3.1.1	Lot/Batch number	Not relevant	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	Not relevant	
3.2	**Reference substances**	None	
3.3	**Test solution**	None	
3.4	**Testing procedure**	Not relevant	
3.5	**Calculation**	Program: SRC AOP v1.91 Data used for calculation: SMILES: O=CCCCC=O CHEM.: Pentanedial MOL. FORMULA: C5 H8 O2 MOL. WEIGHT: 100.12 g/Mol Sensitizer: OH (concentration: 1500000 molecule/cm³)	
		4 RESULTS	

4.1	**Photolysis data**	Overall OH Rate Constant: 46.8857 E-12 cm3/molecule-sec Half-life in days: 0.228 days (12-hr day; 1.5E6 OH/cm^3) Half-life in hours: 2.738 hrs.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	By means of the EPIWIN AOP version 1.91 program developed by the Syracuse Research Corporation (NY) the photodegradation of glutaraldehyde in the air was estimated under sensitized conditions (OH).
5.2	**Results and discussion**	A half-life value in days of 0.228 days (12-hr day) was reported, which corresponded to 2.738 hrs.
5.3	**Conclusion**	The estimated half-life for glutaraldehyde in the air was found to be < 24 hours, indicating that after evaporation or exposure to the air, the substance is subjected to rapid photodegradation.
5.3.1	Reliability	**2**
5.3.2	Deficiencies	No; the used calculation method is acknowledged and scientifically acceptable.

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13.3.2009
Materials and Methods	The applicant's description of the calculation method is correct. The applicant has used defaults used by the EPIWIN AOP program, i.e. 12-hr day and an OH concentration of 1.5E6 molecules/m^3. The U.S. EPA uses a 12-hr day because OH radicals exist only during sunlight hours. The 12-hr period is an average daylight time for a whole year. The U.S. EPA uses 5E6 molecules/m^3 which is an average concentration for daylight hours only. The U.S. EPA previously used a 24-hr day and a concentration of 5E5. The 5E5 value is a 24-hr average that includes night-time (when OH conc. is zero). The 1.5E6 value for daylight hours is based on recent experimental observations. The 24-hr day and 5E6 molecules/m^3 are defaults given in the TGD.
Results and discussion	8.2 h (24 h day, 5E5 OH/cm^3) TGD defaults 2.7 h (12-h day, 1.5E6 OH/cm^3) AOP defaults The value calculated according to the TGD defaults will be used for the risk assessment.
Conclusion	Glutaraldehyde is rapidly photodegraded in air.
Reliability	Not relevant, a calculation method.
Acceptability	Acceptable
Remarks	

Section A7.3.1 _ 01 **Phototransformation in air (estimation method), including identification of breakdown products**

Annex Point IIA7.1

	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	

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1981), Acute toxicity of Glutaraldehyde 50% to Rainbow trout (Salmo gairdneri). [redacted] (Unpublished), BPD ID A7.04.1.1_01
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, according to "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians", U.S. EPA, 1975
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	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	[redacted]% active ingredient
3.1.5	Further relevant properties	No data
3.1.6	Method of analysis	No data
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not relevant
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_1-2
3.4.2	Test organisms	See table A7_4_1_1-3
3.4.3	Test system	See table A7_4_1_1-4
3.4.4	Test conditions	See table A7_4_1_1-5
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Mortality, clinical symptoms of toxicity
3.4.7	Sampling	The biological parameters (mortality, symptoms) were recorded after 24, 48, 72 and 96 hours. The pH- and dissolved oxygen measurements were performed at test initiation and after 24, 48 and 96 hours. The temperature was measured at test initiation and after 24, 48, 72 and 96

hours.

3.4.8	Monitoring of TS concentration	Not performed
3.4.9	Statistics	The estimation of the LC50 was based on a computer program using one of three statistical methods in the following order of preference: (1) Moving average angle analysis (2) Probit analysis (3) Binomial probability

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 1.7, 2.8, 4.6, 7.8, 13 and 22 mg a.i./l
4.2.2	Actual concentrations of test substance	No analytical monitoring performed
4.2.3	Effect data (Mortality)	See table A7_4_1_1-6 and table A7_4_1_1-7
4.2.4	Concentration / response curve	None
4.2.5	Other effects	Following symptoms were reported:

Test Conc.	24 h	48 h	72 h	96 h
0 mg/l	none	none	none	none
1.7 mg/l	none	none	Accelerated respiration	Accelerated respiration
2.8 mg/l	none	Accelerated respiration	Accelerated respiration Lethargy	Accelerated respiration
4.6 mg/l	none	Accelerated respiration	Accelerated respiration Lethargy	Accelerated respiration
7.8 mg/l	Accelerated respiration	Accelerated respiration Lethargy	Accelerated respiration Lethargy Fish at the surface	Accelerated respiration

13 mg/l	Accelerated respiration	Accelerated respiration Lethargy Partial to complete loss of equilibrium	Accelerated respiration Lethargy	No survivors
22 mg/l	No survivors	No survivors	No survivors	No survivors

4.3 Results of controls

4.3.1 Number/ percentage of animals showing adverse effects — No adverse effects observed

4.3.2 Nature of adverse effects — Not relevant

4.4 Test with reference substance — Not performed

4.4.1 Concentrations — Not relevant

4.4.2 Results — Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the acute toxicity of glutaraldehyde [redacted]% to Rainbow trout (Salmo gairdneri).

Test substance: Glutaraldehyde [redacted]%, [redacted]

Guideline according to "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians", U.S. EPA" (1975), no GLP

Fish were purchased from a commercial supplier in [redacted]. They were acclimated over a period of at least 14 days. At test initiation they had a mean weight of 0.14 g and a mean length of 28 mm.

The acute toxicity of the test substance to 10 Rainbow trout (Salmo gairdneri) per concentration was determined in a static test system at following concentrations: 0, 1.7, 2.8, 4.6, 7.8, 13 and 22 mg a.i./l. Each test concentration was prepared starting from a stock solution of 15 mg a.i./ml obtained by adding 3 g of test substance (glutararldehyde [redacted]%) to distilled water to a final volume of 100 ml. The appropriate amount of stock solution was added to the test water of each test vessel to get the wanted test concentration. The dilution water was reconstituted water as recommended by the U.S. guideline. The fish were randomly distributed to each test vessel within 15 minutes following preparation of the test solutions. Control fish were placed in a vessel containing test water without test substance. The fish were checked for mortality and symptoms of toxicity after 24, 48, 72 and 96 hours. The estimation of LC 50 values was based on a computer program using one of 3 statistical methods in the following order of preference: Moving average angle analysis, Probit analysis and Binomial probability. The pH- and dissolved oxygen measurements were performed at test initiation and after 24, 48 and 96 hours. The temperature was measured at test initiation and after 24, 48, 72 and 96 hours.

5.2 Results and discussion

Mortality: no mortality was reported for the control group. In the treated groups, no mortality occurred at test concentrations ranging from 1.7 to 4.6 mg a.i./l. At 7.8 mg a.i./l mortality reached 10% after 24 hours and remained as such after 96 hours. At 13 mg a.i./l 60% of the fish died after 48 hours and mortality reached 100% after 96 hours. At the highest test concentration of 22 mg a.i./l 100% mortality already was reached after 24 hours.

Symptoms of toxicity: symptoms of toxicity were observed in all treated groups; the control group remained free of such symptoms. The main

		symptom that was reported was an accelerated respiration. Further symptoms included lethargy, partial to complete loss of equilibrium and swimming at the surface. The NOEC was < 1.7 mg a.i./l **Temperature, dissolved oxygen and pH:** The temperature of the test solutions was 12 °C and was constant over the whole testing period. The concentration of dissolved oxygen in all test vessels and over the whole testing period ranged between 8 and 10.4 mg/l, corresponding to 74 - 96% of saturation. The pH value ranged between 7.0 and 7.5.
5.2.1	LC_0	4.6 mg a.i. /l (96 h)
5.2.2	LC_{50}	10 mg a.i./ l (96 h); 95% confidence interval 7.8 – 13 mg/l
5.2.3	LC_{100}	13 mg a.i./l (96 h)
5.3	**Conclusion**	The testing of the acute toxicity of glutaraldehyde to Rainbow trout resulted in a LC50 after 96 hours of 10 mg a.i./l. Symptoms of toxicity already occurred from the lowest tested concentration of 1.7 mg a.i./l, resulting in a NOEC < 1.7 mg a.i./l.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 28th, 2008
Materials and Methods	The concentrations have not been measured. The test was conducted under static conditions.
Results and discussion	The nominal LC50 96 h is 5 mg a.i./L and 95% confidence interval 3.9-7.5 mg/L. The result recalculated according to TWA[1] is 0.8 mg a.i./L
Conclusion	Glutaraldehyde is toxic to rainbow trout.
Reliability	2
Acceptability	Not acceptable because test concentrations were not analytically verified
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_1-1: **Preparation of TS solution for poorly soluble or volatile test substances**

[1] CA-May08-Doc.6.5: TNsG Assessment of environmental effects of biocidal active substances that rapidly degrade in environmental compartments of concern.

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

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Soft water reconstituted from deionized water according to procedures recommended by the U.S EPA (1975)
Alkalinity	32 mg/l ($CaCO_3$)
Hardness	40 mg/l ($CaCO_3$)
pH	7.5
Oxygen content	10 to 10.4 mg/l (corresponding to 93 to 96% saturation at a temperature of 12 °C)
Conductance	110 µmhos/cm
Holding water different from dilution water	Yes, holding water was a well-water which flowed into the maintenance tank of the fish. This water had an alkalinity of 20 – 22 mg/l $CaCO_3$ and the hardness was about 22 to 28 mg/l $CaCO_3$. The pH ranged between 6.3 and 6.5 and dissolved oxygen ranged between 86 and 94% of saturation. The specific conductance was about 70 to 90 µmhos/cm. The flow rate was 7 to 17 tank volume replacements per day. The temperature in the holding tank was 11 to 13 °C.

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Rainbow trout (Salmo gairdneri)
Source	Commercial fish supplier in [redacted]
Wild caught	No
Age/size	Mean weight of 0.14 g Mean total length of 28 mm
Kind of food	Dry pelleted food
Amount of food	Ad libitum
Feeding frequency	Daily excepted for the 48 hours preceding the test
Pretreatment	Fish were held in a 500 liter fiber glass tank for an acclimation period of at least 14 days at a temperature of 11 to 13 °C. The photoperiod was 16 hours light and 8 hours darkness.
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	No
Volume of test vessels	19.6 liter glass jars were used as vessels
Volume/animal	Each vessel contained 15 liters of test solution
Number of animals/vessel	Ten animals were used per test concentration and vessel
Number of vessels/ concentration	One vessel per test concentration was used
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	Maintained at 12 +/- 1 °C for all test solutions
Dissolved oxygen	Concentration (nominal) mg a.i/l / Oxygen content (mg/l): Initial / 24 h / 48 h / 96 h 0.00 (control): 10.4 (96%)* / 8.8 (81%) / 8.4 (78%) / 8.0 (74%) 1.7: 10 (93%) / 9.6 (89%) / 9.0 (83%) / 8.4 (78%) 7.8: 10.1 (94%) / 10 (93%) / 9.8 (91%) / 8.9 (82%) 22: 10.2 (94%) / 9.8 (91%) / 9.6 (89%) / 10.2 (94%) *, Percentage of saturation at 12 °C
pH	Concentration (nominal) mg a.i/l / pH: Initial / 24 h / 48 h / 96 h 0.00 (control): 7.5 / 7.2 / 7.1 / 7.0 1.7: 7.4 / 7.2 / 7.1 / 7.0 7.8: 7.4 / 7.2 / 7.1 / 7.0 22: 7.4 / 7.2 / 7.2 / 7.1
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	Not specified
Photoperiod	16 hours light / 8 hours dark

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (nominal) [mg a.i./l]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0.00 (control)	0	0	0	0	0	0	0	0
1.7	0	0	0	0	0	0	0	0
2.8	0	0	0	0	0	0	0	0
4.6	0	0	0	0	0	0	0	0
7.8	1	1	1	1	10	10	10	10
13	0	6	9	10	0	60	90	100
22	10	10	10	10	100	100	100	100
Temperature [°C]	12 °C	12 °C	12 °C	12 °C				
pH	7.2	7.1 – 7.2		7.0 – 7.1				
Oxygen [mg/l]	9.6 - 10	9.0 – 9.8		8.4 – 10.2				

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	95 % c.l.	96 h [mg/l][1]	95 % c.l.
LC_0	4.6 mg a.i./l (n)		4.6 mg a.i./l (n)	
LC_{50}	12 mg a.i./l (n)	9.4 – 14 mg/l	10 mg a.i./l (n)	7.8 – 13 mg/l
LC_{100}	22 mg a.i./l (n)		13 mg a.i./l (n)	

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

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	**yes**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**No analytical monitoring performed**	

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

Section A7.4.1.1 _ 02 **Acute toxicity to fish**

Annex Point IIA7.4 **Bluegill (Lepomis macrochirus)**

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1981), Acute toxicity of Glutaraldehyde [redacted]% to Bluegill (Lepomis macrochirus). [redacted] (Unpublished), BPD ID A7.04.1.1_02
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, according to "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians", U.S. EPA, 1975
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	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	[redacted]% active ingredient
3.1.5	Further relevant properties	No data
3.1.6	Method of analysis	No data
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not relevant
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_1-2
3.4.2	Test organisms	See table A7_4_1_1-3
3.4.3	Test system	See table A7_4_1_1-4
3.4.4	Test conditions	See table A7_4_1_1-5

3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Mortality, clinical symptoms of toxicity
3.4.7	Sampling	The biological parameters (mortality, symptoms) were recorded after 24, 48, 72 and 96 hours. The pH- and dissolved oxygen measurements were performed at test initiation and after 24, 48 and 96 hours. The temperature was measured at test initiation and after 24, 48, 72 and 96 hours.
3.4.8	Monitoring of TS concentration	Not performed
3.4.9	Statistics	The estimation of the LC50 was based on a computer program using one of three statistical methods in the following order of preference: (1) Moving average angle analysis (2) Probit analysis (3) Binomial probability

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 7.8, 13, 22, 36, 60 and 100 mg a.i./l
4.2.2	Actual concentrations of test substance	No analytical monitoring performed
4.2.3	Effect data (Mortality)	See table A7_4_1_1-6 and table A7_4_1_1-7
4.2.4	Concentration / response curve	None
4.2.5	Other effects	At a test concentration of 13 mg a.i. /l one fish showed darkened pigmentation after 24 hours. After 48 hours one case of fish seen at the surface and being lethargic was reported. At a test concentration of 22 mg a.i. /l and after 24 hours, two fish showed lightened pigmentation.

4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects observed
4.3.2	Nature of adverse effects	Not relevant
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the acute toxicity of glutaraldehyde [redacted]% to Bluegill (Lepomis macrochirus).

Test substance: Glutaraldehyde [redacted]%, batch Nr [redacted]

Guideline according to "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians", U.S. EPA" (1975), no GLP

Fish were purchased from a commercial supplier in [redacted]. They were acclimated over a period of at least 14 days. At test initiation they had a mean weight of 0.65 g and a mean length of 39 mm.

The acute toxicity of the test substance to 10 Bluegill fish (Lepomis macrochirus) per concentration was determined in a static test system at following concentrations: 0, 7.8, 13, 22, 36, 60 and 100 mg a.i./l. Each test concentration was prepared starting from a stock solution of 15 mg a.i./ml obtained by adding 15 g of test substance (glutararldehyde [redacted]%) to distilled water to a final volume of 500 ml. The appropriate amount of stock solution was added to the test water of each test vessel to get the wanted test concentration. The dilution water was reconstituted water as recommended by the U.S. guideline. The fish were randomly distributed to each test vessel within 15 minutes following preparation of the test solutions. Control fish were placed in a vessel containing test water without test substance. The fish were checked for mortality and symptoms of toxicity after 24, 48, 72 and 96 hours. The estimation of LC 50 values was based on a computer program using one of 3 statistical methods in the following order of preference: Moving average angle analysis, Probit analysis and Binomial probability. The pH- and dissolved oxygen measurements were performed at test initiation and after 24, 48 and 96 hours. The temperature was measured at test initiation and after 24, 48, 72 and 96 hours.

5.2 Results and discussion

Mortality: no mortality was reported for the control group. In the treated groups, no mortality occurred at the lowest tested concentration of 7.8 mg a.i./l. At 13 mg a.i./l mortality was 20% after 24 hours and increased to 60% after 72 hours; after 96 hours mortality still was 60%. At 22 mg a.i. /l 50% of the fish already died within the first 24 hours and 100% mortality was reached after 48 hours. At the higher test concentrations (from 36 to 100 mg a.i./l) 100% mortality already was reached after 24 hours.

Symptoms of toxicity: symptoms of toxicity were observed at 13 and

		22 mg a.i./l. In fact at 13 mg a.i./l, one case of fish exhibiting darkened pigmentation was reported after 24 hours; after 48 hours one case of fish showing lethargy and swimming at the surface was reported. At 22 mg a.i./l two cases of fish exhibiting lightened pigmentation were reported. The NOEC was 7.8 mg a.i./l **Temperature, dissolved oxygen and pH:** The temperature of the test solutions was 22 °C and was constant over the whole testing period. The concentration of dissolved oxygen in all test vessels and over the whole testing period ranged between 5.2 and 9 mg/l, corresponding to 59 - >100% of saturation. The pH value ranged between 6.5 and 7.5.
5.2.1	LC_0	7.8 mg a.i. /l (96 h)
5.2.2	LC_{50}	13 mg a.i./ l (96 h); 95% confidence interval 7.8 – 22 mg/l
5.2.3	LC_{100}	22 mg a.i./l (96 h)
5.3	**Conclusion**	The testing of the acute toxicity of glutaraldehyde to Bluegill resulted in a LC50 after 96 hours of 13 mg a.i./l. Symptoms of toxicity occurred from 13 mg a.i./l; the NOEC was 7.8 mg a.i./l.
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	April 3rd, 2009
Materials and Methods	Guideline according to "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians", U.S. EPA" (1975), no GLP
Results and discussion	LC_{50} 6.5 mg a.i./ l (96 h); 95% confidence interval 3.9 – 11 mg a.i./l, based on nominal concentrations and expressed as 100 % glutaraldehyde. The result recalculated according to TWA[1] is 1.0 mg a.i./L.
Conclusion	Glutaraldehyde is toxic to *Lepomis macrochirus.*
Reliability	2
Acceptability	Not acceptable because test concentrations were not analytically verified
Remarks	Only one of the validity criteria of OECD guideline 203 was fulfilled. Glutaraldehyde degrades rapidly hence the study should have been semi-static or flow-through. Dissolved oxygen concentration was under 60 % in two samples. The concentrations of the test substances were not measured. The report should have been more detailed.
	COMMENTS FROM ...
Date	*Give date of comments submitted*

[1] CA-May08-Doc.6.5: TNsG Assessment of environmental effects of biocidal active substances that rapidly degrade in environmental compartments of concern.

Section A7.4.1.1 _ 02 **Acute toxicity to fish**

Annex Point IIA7.4 **Bluegill (Lepomis macrochirus)**

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_1-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Soft water reconstituted from deionized water according to procedures recommended by the U.S EPA (1975)
Alkalinity	30 mg/l ($CaCO_3$)
Hardness	42 mg/l ($CaCO_3$)
pH	7.6
Oxygen content	Not specified
Conductance	120 µmhos/cm
Holding water different from dilution water	Yes, holding water was a well-water which flowed into the maintenance tank of the fish. This water had an alkalinity of 22 – 24 mg/l $CaCO_3$ and the hardness was about 24 to 28 mg/l $CaCO_3$. The pH ranged between 6.4 and 6.6 and dissolved oxygen ranged between 92 and 97% of saturation. The specific conductance was about 80 to 90 µmhos/cm. The flow rate was 8 to 15 tank volume replacements per day. The temperature in the holding tank was 20 to 23 °C.

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Bluegill (Lepomis macrochirus)
Source	Commercial fish supplier in [redacted]
Wild caught	No
Age/size	Mean weight of 0.65 g Mean total length of 39 mm
Kind of food	Dry pelleted food
Amount of food	Ad libitum
Feeding frequency	Daily excepted for the 48 hours preceding the test
Pretreatment	Fish were held in a 500-liter fiber glass tank for an acclimation period of at least 14 days at a temperature of 20 to 23 °C. The photoperiod was 16 hours light and 8 hours darkness.
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	No
Volume of test vessels	19.6 liter glass jars were used as vessels
Volume/animal	Each vessel contained 15 liters of test solution
Number of animals/vessel	Ten animals were used per test concentration and vessel
Number of vessels/ concentration	One vessel per test concentration was used
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	Maintained at 22 +/- 1 °C for all test solutions
Dissolved oxygen	<table><tr><th rowspan="2">Concentration (nominal) mg a.i/l</th><th colspan="4">Oxygen content (mg/l)</th></tr><tr><th>Initial</th><th>24 h</th><th>48 h</th><th>96 h</th></tr><tr><td>0.00 (control)</td><td>9.0 (>100%)</td><td>7.1 (81%)</td><td>6.5 (74%)</td><td>5.5 (52%)</td></tr><tr><td>7.8</td><td>9.1 (>100%)</td><td>6.9 (78%)</td><td>6.5 (74%)</td><td>5.2 (59%)</td></tr><tr><td>36</td><td>9.0 (>100%)</td><td>7.8 (89%)</td><td>8.0 (91%)</td><td>6.0 (68%)</td></tr><tr><td>100</td><td>8.9 (>100%)</td><td>7.5 (85%)</td><td>7.8 (89%)</td><td>8.8 (100%)</td></tr></table> *, Percentage of saturation at 22 °C
pH	<table><tr><th rowspan="2">Concentration (nominal) mg a.i/l</th><th colspan="4">pH</th></tr><tr><th>Initial</th><th>24 h</th><th>48 h</th><th>96 h</th></tr><tr><td>0.00 (control)</td><td>7.5</td><td>7.4</td><td>7.3</td><td>6.7</td></tr><tr><td>7.8</td><td>7.4</td><td>7.3</td><td>7.1</td><td>6.5</td></tr><tr><td>36</td><td>7.4</td><td>7.2</td><td>7.3</td><td>6.7</td></tr><tr><td>100</td><td>7.4</td><td>7.3</td><td>7.3</td><td>7.3</td></tr></table>
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	Not specified
Photoperiod	16 hours light / 8 hours dark

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration	Mortality

(nominal) [mg a.i./l]	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0.00 (control)	0	0	0	0	0	0	0	0
7.8	0	0	0	0	0	0	0	0
13	2	3	6	6	20	30	60	60
22	5	10	10	10	50	100	100	100
36	10	10	10	10	100	100	100	100
60	10	10	10	10	100	100	100	100
100	10	10	10	10	100	100	100	100
Temperature [°C]	22 °C	22 °C	22 °C	22 °C				
pH	7.2 – 7.4	7.1 – 7.3		6.5 – 7.3				
Oxygen [mg/l]	6.9 – 7.8	6.5 – 8.0		5.2 – 8.8				

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	95 % c.l.	96 h [mg/l][1]	95 % c.l.
LC_0	7.8 mg a.i./l (n)		7.8 mg a.i./l (n)	
LC_{50}	13 mg a.i./l (n)	7.8 – 22 mg/l	13 mg a.i./l (n)	7.8 –22 mg/l
LC_{100}	22 mg a.i./l (n)		22 mg a.i./l (n)	

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

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	**yes**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**Yes (however in one case the concentration of DO was 59% of saturation**	
Concentration of test substance ≥80% of initial concentration during test	**No analytical monitoring performed**	

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

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1981), Acute toxicity of Glutaraldehyde to Sheepshead minnows (Cyprinodon variegatus). [redacted] (Unpublished), BPD ID A7.04.1.1_03	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, according to the Static acute marine fish toxicity test (BMRL's Test Protocol for Fishes; 1980)	
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	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	[redacted]% active ingredient	
3.1.5	Further relevant properties	No data	
3.1.6	Method of analysis	No data	x
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	Not relevant	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_1-2	
3.4.2	Test organisms	See table A7_4_1_1-3	
3.4.3	Test system	See table A7_4_1_1-4	
3.4.4	Test conditions	See table A7_4_1_1-5	
3.4.5	Duration of the test	96 hours	

3.4.6	Test parameter	Mortality, clinical symptoms of toxicity
3.4.7	Sampling	The biological parameters (mortality, symptoms) were recorded after 24, 48, 72 and 96 hours.
3.4.8	Monitoring of TS concentration	Not performed
3.4.9	Statistics	The estimation of the LC50 was based on a computer program using one of three statistical methods in the following order of preference: (1) Moving average angle analysis (2) Probit analysis (3) Binomial probability

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 13, 22, 36, 60 and 100 mg a.i./l
4.2.2	Actual concentrations of test substance	No analytical monitoring performed
4.2.3	Effect data (Mortality)	See table A7_4_1_1-6 and table A7_4_1_1-7
4.2.4	Concentration / response curve	None
4.2.5	Other effects	Symptoms indicative of toxicity were observed at a test concentration of 36 mg/l and were as follows: After 48 h, one case of mouth distention was reported. After 72 hours, two cases of mouth distention were seen. After 96 hours one case of swimming at the surface and two cases of muscle spasm were reported.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects observed
4.3.2	Nature of adverse	Not relevant

	effects	
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the acute toxicity of glutaraldehyde [redacted]% to a marine fish species, the Sheepshead minnows (Cyprinodon variegatus).

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

Guideline according to the Static acute marine fish toxicity test (BMRL's Test Protocol for Fishes; 1980), no GLP

Fish were hatched and reared at the [redacted]. They were acclimated over a period of at 96 hours. At test initiation they had a mean weight of 4.1 mg and a mean length of 6 mm.

The acute toxicity of the test substance to 10 Sheepshead minnows (Cyprinodon variegatus) per concentration was determined in a static test system at following concentrations: 0, 13, 22, 36, 60 and 100 mg a.i./l. Each test concentration was prepared starting from a stock solution obtained by adding 6 g of test substance to deionized water to a final volume of 100 ml. The appropriate amount of stock solution was added to the test water of each test vessel to get the wanted test concentration. The dilution water was natural seawater pumped from the [redacted]. The fish were randomly distributed to each test vessel following preparation of the test solutions. Control fish were placed in a vessel containing test water without test substance. The fish were checked for mortality and symptoms of toxicity after 24, 48, 72 and 96 hours. The estimation of LC 50 values was based on a computer program using one of 3 statistical methods in the following order of preference: Moving average angle analysis, Probit analysis and Binomial probability. The dissolved oxygen measurements were performed at test initiation and after 24, 48, 72 and 96 hours; the pH was measured at test initiation and after 96 h. The test temperature was 21 to 22 °C and the salinity was 19 – 20 parts per thousand.

5.2 Results and discussion

Mortality: no mortality was reported for the control group. In the treated groups, no mortality occurred at the lowest tested concentrations of 13 and 22 mg a.i./l. At 36 mg a.i./l mortality was 10% after 48 hours and increased to 30% after 72 hours; after 96 hours mortality still was 30%. At 60 and 100 mg a.i./l, 100% mortality was already reached within 24 hours following test initiation.

Symptoms of toxicity: symptoms of toxicity were observed at 36 mg a.i./l. In fact after 48 h, one case of mouth distention was reported. After 72 hours, two cases of mouth distention were seen. After 96 hours one case of swimming at the surface and two cases of muscle spasm were reported. The NOEC was 22 mg a.i./l

Temperature, dissolved oxygen and pH: The temperature of the test solutions was 21 - 22 °C and was constant over the whole testing period. The concentration of dissolved oxygen in all test vessels and over the whole testing period ranged between 6.8 and 7.8 mg/l and was therefore >= 88% of saturation. The pH value ranged between 8.2 at test initiation and 7.7 at test ending.

5.2.1	LC_0	22 mg a.i. /l (96 h)

5.2.2	LC_{50}	39 mg a.i./ l (96 h); 95% confidence interval 29 – 48 mg/l
5.2.3	LC_{100}	60 mg a.i./l (96 h)
5.3	**Conclusion**	The testing of the acute toxicity of glutaraldehyde to a marine fish species, Sheepshead minnows (Cyprinodon variegatus), resulted in a LC50 after 96 hours of 39 mg a.i./l. Symptoms of toxicity occurred at 36 mg a.i./l; the NOEC was 22 mg a.i./l.
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	May 5th, 2008
Materials and Methods	The concentrations have not been measured. The test was conducted under static conditions.
Results and discussion	The LC value is based on nominal concentrations and it is expressed as 100 % glutaraldehyde. LC_{50} 96 h 39 mg/l a.i.; 95% confidence interval 29-48 mg/l The result recalculated according to TWA[1] is 6.2 mg a.i./L.
Conclusion	Glutaraldehyde is harmful to sheepshead minnow.
Reliability	2
Acceptability	Not acceptable because test concentrations were not analytically verified
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	

[1] CA-May08-Doc.6.5: TNsG Assessment of environmental effects of biocidal active substances that rapidly degrade in environmental compartments of concern.

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Natural seawater pumped from the [redacted]. The pump intake was about 80 meters offshore at a depth of ca. 3 meters. The water was pumped via a 316 stainless steel pump through hard PVC pipes, through sand-filled fiver-glass filters and through 10 µm pore size polypropylene core filters into an elevated fiber glass reservoir. Prior reaching the test chambers, the water was filtered through a 5 µm pore size polypropylene core filter.
Alkalinity	No data
Hardness	No data
pH	8.2
Oxygen content	>= 100% of saturation
Conductance	No data
Holding water different from dilution water	No, holding and test water were similar (natural seawater pumped from [redacted]).

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Sheepshead minnows (Cyprinodon variegatus)
Source	[redacted]
Wild caught	No
Age/size	Juveniles Mean weight of 4.1 mg Mean total length of 6 mm
Kind of food	Not specified
Amount of food	Not specified
Feeding frequency	Not specified
Pretreatment	The fish were subjected to an acclimatization period of 96 hours prior initiation of the test. During this period the salinity of the holding water was 17parts per thousand and the temperature was 20 – 23 °C.

Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	No
Volume of test vessels	3.8 liter glass jars were used as vessels
Volume/animal	Each vessel contained 3 liters of test solution
Number of animals/vessel	Ten animals were used per test concentration and vessel, corresponding to a fish loading of ca. 14 mg fish per liter test solution
Number of vessels/ concentration	One vessel per test concentration was used
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	21 – 22 °C
Dissolved oxygen	

Concentration (nominal) mg a.i/l	Oxygen content (mg/l)				
	Initial	24 h	48 h	72 h	96 h
0.00 (Control)	7.7	7.1	7.2	6.8	6.9
13	7.8	7.2	7.4	7.0	7.4
22	7.8	7.3	7.4	7.2	7.3
36	7.8	7.3	7.5	7.2	7.4
60	7.8	7.4	-	-	-
100	7.8	7.4	-	-	-

Salinity	19 – 20 parts per thousand
PH	

Concentration (nominal) mg a.i/l	pH	
	Initial	96 h
0.00 (Control)	8.2	7.6
13	8.2	7.7
22	8.2	7.7
36	8.2	7.7

	60	8.2	-	
	100	8.2	-	
Adjustment of pH	No			
Aeration of dilution water	No			
Intensity of irradiation	Not specified			
Photoperiod	Not specified			

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (nominal) [mg/l]	**Mortality**							
	Number				**Percentage**			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
0.00 (control)	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
36	0	1	3	3	0	10	30	30
60	10	10	10	10	100	100	100	100
100	10	10	10	10	100	100	100	100
Temperature [°C]	21 - 22 °C							
pH	7.6 – 7.7							
Oxygen [mg/l]	>= 88% of saturation							

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	**95 % c.l.**	**96 h [mg/l][1]**	**95 % c.l.**
LC_0	22 mg/l (n)		22 mg/l (n)	
LC_{50}	42 mg/l (n)	29 – 48 mg/l	39 mg/l (n)	29 – 48 mg/l
LC_{100}	60 mg/l (n)		60 mg/l (n)	

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

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	**Not fullfilled**
Mortality of control animals <10%	**yes**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**yes**	

Concentration of test substance ≥80% of initial concentration during test	**No analytical monitoring performed**	

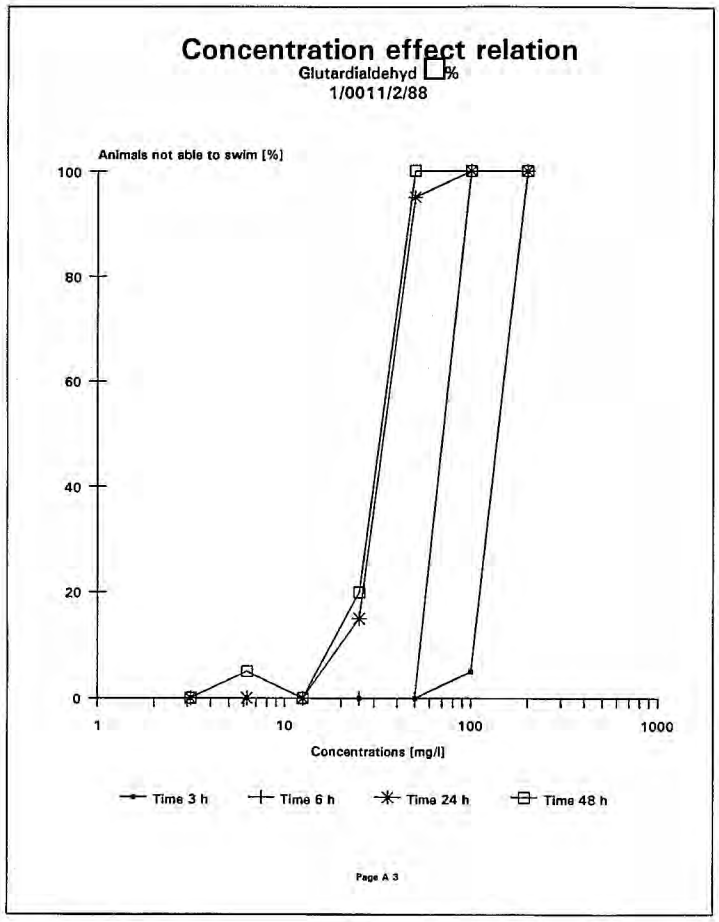
Criteria for poorly soluble test substances	**Not relevant**	

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1988) Determination of the acute toxicity of Glutardialdehyd [redacted] % to the waterflea Daphnia magna Straus. [redacted] (Unpublished), BPD ID A7.04.1.2_01
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, according to Directive 79/831/EEC, C.2
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Glutardialdehyd [redacted]%
3.1.1	Lot/Batch number	Not specified
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]% (aqueous solution)
3.1.4	Composition of Product	Not relevant
3.1.5	Further relevant properties	Solubility in water > 500 mg/l at ca. 20 °C
3.1.6	Method of analysis	No data
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	Not relevant
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_2-2
3.4.2	Test organisms	See table A7_4_1_2-3
3.4.3	Test system	See table A7_4_1_2-4
3.4.4	Test conditions	See table A7_4_1_2-5
3.4.5	Duration of the test	48 hours
3.4.6	Test parameter	Unability to swim
3.4.7	Sampling	The test parameter was checked after 3, 6, 24 and 48 hours. The oxygen and pH measurements were performed at test initiation and after 48 hours.

3.4.8	Monitoring of TS concentration	No
3.4.9	Statistics	The determination of the EC50 values was based on the Spearman-Kaerber method (Sachs, Lothar: Angewandete Statistik, Springer Verlag, Berlin, Heidelberg, New York, 4. Auflage, 1974)
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 3.125, 6.25, 12.5, 25, 50, 100 and 200 mg/l
4.2.2	Actual concentrations of test substance	No analytical monitoring performed.
4.2.3	Effect data (Immobilisation)	See table A7_4_1_2-6 and table A7_4_1_2-7

4.2.4 Concentration / response curve



4.2.5	Other effects	None
4.3	**Results of controls**	See table A7_4_1_2-6 All animals of the control group remained able to swim
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the acute toxicity of glutardialdehyde [redacted]% to aquatic invertebrates.

Test substance: Glutardialdehyde [redacted]%, purity [redacted] (aqueous solution)

Guideline according to Directive 79/831/EEC, C.2, no GLP

The acute toxicity of to the freshwater crustacean Daphnia magna was assessed over a 48 hour-exposure period under static conditions. Following nominal concentrations were tested: 0, 3.125, 6.25, 12.5, 25, 50, 100 and 200 mg/l. These concentrations were prepared starting from a 200 mg/l stock solution of the test substance. The dilution/test water was chlorine free, filtered tap water, which was supplemented (1) with sulphuric acid for reduction of the buffering capacity of the carbonic acid system, and (2) with deionized water for reduction of the total hardness. Reagent tubes with flat bottom were used as test vessels and were respectively filled with 10 ml of test solution. Four vessels were used per test concentration, and five 2 to 24 hours old Daphnia were placed in each vessel. Therefore, a total of twenty Daphnia was used per test con-

centration. The test was performed at a temperature of ca. 19 °C. The Daphnia were examined for their swimming ability at following time points: 0, 3, 6, 24 and 48 hours. Oxygen and pH measurements were performed at test initiation and after 48 hours. The EC values were determined by means of the Spearman-Kaerber method.

5.2 Results and discussion

Swimming unability of Daphnia, dissolved oxygen and pH:

Test-Substance Concentration (nominal) [mg/l]	Daphnia unable to swim		Oxygen [mg/l]	pH
	Percentage		48 h	48 h
	24 h	48 h		
0	0	0	8.45	7.72
3.125	0	0	8.46	7.80
6.25	0	5*	8.43	7.84
12.5	0	0	8.42	7.81
25	15	20	8.29	7.79
50	95	100	8.59	7.90
100	100	100	8.62	7.94
200	100	100	8.66	7.92

Temperature: about 19 °C

*, The 5% swimming unability observed after 48 hours at 6.25 mg/l was not considered to be substance-related.

5.2.1 EC_0 12.5 mg/l (nominal)

5.2.2 EC_{50} 29.73 mg/l (nominal)

5.2.3 EC_{100} 50 mg/l (nominal)

5.3 Conclusion The testing of the acute toxicity of glutardialdehyde [redacted]% to the freshwater crustacean Daphnia magna resulted in a LC50 value (48 h) of 29,73 mg/l, referring to the test material as such.

5.3.1 Reliability **1**

5.3.2 Deficiencies No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date May 21st, 2008

Materials and Methods Agree with the applicant.
3.1.6 No data on method of analysis.

Results and discussion The EC values are based on nominal concentrations. Results are expressed as 100 % glutaraldehyde.
EC_{50} 48 h 14.9 mg/l (95% Confidence limits 12.9-17.1)
The result recalculated according to TWA[1] is 4.6 mg a.i./L

[1] CA-May08-Doc.6.5: TNsG Assessment of environmental effects of biocidal active substances that rapidly degrade in environmental compartments of concern.

Conclusion	Glutaraldehyde is acutely slightly toxic to *Daphnia magna*.
Reliability	2
Acceptability	Not acceptable because test concentrations were not analytically verified
Remarks	The report should have been more detailed.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Tap water purified by charcoal to remove chlorine, and filtered through a 6 um filter. Sulfuric acid was added to reduce the buffering capacity of the carbonic acid system. Deionized water was added to reduce the total hardness of the water.
Acid capacity (Ks) up to pH 4.3	0.80 +/- 0.1 mmol/l
Hardness	2.7 +/- 0.5 mmol/l
pH	7.7 – 8.3
Ca / Mg ratio	4:1
Na / K ratio	10:1
Oxygen content	Aeration of the test water with oil free air until saturation, followed by stabilization over 24 hours preceding test initiation
Conductance	550 – 650 µSiemens/cm
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	Daphnia magna
Source	Originally obtained from the [redacted]
Age	2 to 24 hours old
Breeding method	Not specified
Kind of food	Brewer´s yeast after each water change, washed green algae once a day
Amount of food	See above
Feeding frequency	See above
Pretreatment	No particularities
Feeding of animals during test	Not specified

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	None
Volume of test vessels	10 ml
Volume/animal	2 ml/animal
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	Ca. 19 to 21 °C

Dissolved oxygen

Test Concentration (mg/l)	Oxygen-value after	
	0 h	48 h
0	8.94	8.45
3.125	8.96	8.46
6.250	8.86	8.43
12.5	8.81	8.42
25	8.81	8.29
50	8.83	8.59
100	8.84	8.62
200	8.87	8.66

pH

Test Concentration (mg/l)	pH	
	0 h	48 h
0	8.01	7.72
3.125	8.00	7.80
6.250	8.01	7.84
12.5	8.00	7.81

	25	8.00	7.79	
	50	7.99	7.90	
	100	7.97	7.94	
	200	7.97	7.92	
Adjustment of pH	No			
Aeration of dilution water	No			
Quality/Intensity of irradiation	Light intensity ca. 5 µEinstein/(m*m*s) in the wave-lengthrange of 400 to 750 nm			
Photoperiod	16:8 hours day/night			

Table A7_4_1_2-6: Immobilisation data

Test-Substance Concentration (nominal) [mg/l]	**Daphnia unable to swim**						
	Number		**Percentage**		**Oxygen [mg/l]**	**pH**	**Tempera-ture [°C]**
	24 h	**48 h**	**24 h**	**48 h**	**48 h**	**48 h**	**48 h**
0	0	0	0	0	8.45	7.72	19
3.125	0	0	0	0	8.46	7.80	19
6.25	0	1	0	5	8.43	7.84	19
12.5	0	0	0	0	8.42	7.81	19
25	3	4	15	20	8.29	7.79	19
50	19	20	95	100	8.59	7.90	19
100	20	20	100	100	8.62	7.94	19
200	20	20	100	100	8.66	7.92	19

Table A7_4_1_2-7: Effect data

	EC_{50}[1]	**95 % c.l.**	**EC_0[1]**	**EC_{100}[1]**
24 h [mg/l]	32.99 (n)	28.96 – 37.58	12.5 (n)	100 (n)
48 h [mg/l]	29.73 (n)	25.80 – 34.27	12.5 (n)	50 (n)

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

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202

	fulfilled	**Not fullfilled**
Immobilisation of control animals <10%	**yes**	
Control animals not staying at the surface		
Concentration of dissolved oxygen in all test vessels >3 mg/l	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**No analytical monitoring performed.**	

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

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1981), Acute toxicity of Glutaraldehyde [redacted]% to the Water Flea (Daphnia magna). [redacted] (Unpublished), BPD ID A7.04.1.2_02
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, according to "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians", U.S. EPA, 1975
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	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	[redacted]% active ingredient
3.1.5	Further relevant properties	No data
3.1.6	Method of analysis	No data
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not relevant
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_1-2
3.4.2	Test organisms	See table A7_4_1_1-3
3.4.3	Test system	See table A7_4_1_1-4
3.4.4	Test conditions	See table A7_4_1_1-5
3.4.5	Duration of the test	48 h
3.4.6	Test parameter	Mortality, biological observations
3.4.7	Sampling	The test parameters were checked after 24 and 48 hours. The oxygen and pH measurements were performed at test initiation and after 48

		hours.
3.4.8	Monitoring of TS concentration	No
3.4.9	Statistics	The estimation of the LC50 was based on a computer program using one of three statistical methods in the following order of preference: (1) Moving average angle analysis (2) Probit analysis (3) Binomial probability

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	Nor relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 4.0, 6.6, 11, 18, 30 and 50 mg/l (nominal)
4.2.2	Actual concentrations of test substance	No analytical monitoring performed.
4.2.3	Effect data (Immobilisation)	See table A7_4_1_2-6 and table A7_4_1_2-7
4.2.4	Concentration / response curve	No curve available
4.2.5	Other effects	

Test-Concentration at which effects were seen (nominal)[1] [mg/l]	Additional effects	
	24 h	48 h
11	0	One water flea was trapped on the surface of the test solution.
18	Several water fleas had flared carapace. Several water fleas were lethargic.	One water flea was lethargic.
30	Several water fleas were trapped on the surface of the test solution. Several water fleas had flared carapace.	
50	Several water fleas were trapped on the surface of the test solution. Several water fleas had flared carapace.	

4.3	**Results of controls**	No adverse effects observed
4.4	**Test with refer-**	None

	ence substance	
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the acute toxicity of glutardialdehyde [redacted]% to aquatic invertebrates.

Test substance: Glutardialdehyde [redacted]

Guideline according to "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians", U.S. EPA, 1975; no GLP

The acute toxicity of to the freshwater crustacean Daphnia magna was assessed over a 48 hour-exposure period under static conditions. Following nominal concentrations were tested: 0, 4.0, 6.6, 11, 18, 30 and 50 mg/l. These concentrations were prepared starting from a 5 mg active ingredient /ml working stock solution. This stock solution was prepared by dissolving 1 g of test substance in distilled water and diluting up to 100 ml. The dilution water was reconstituted water; the holding water was prepared by fortifying well water according to U.S. EPA procedures (1975) and filtering it through an Amberlite XAD-7 resin column. Both, the dilution and the holding water had the same quality. Three replicates (i.e. three beakers with 150 ml test solution each) were used per test concentration, with 5 Daphnia per replicate (15 Daphnia per test concentration, <= 24 h old). The test was performed at a temperature of ca. 21+/-1 °C. The Daphnia were examined for mortality and biological effects at following time points: 24 and 48 hours. Oxygen and pH measurements were performed at test initiation and after 48 hours. The estimation of the EC50 value was based on a computer program using one of 3 statistical methods in the following order of preference: Moving average angle analysis, Probit analysis and Binomial probability.

5.2 Results and discussion

Mortality:

Test-Substance Concentration (nominal)[1] [mg/l]	Immobile Daphnia (% of mortality)	
	24 h	48 h
0	0	0
4.0	0	0
6.6	0	0
11	0	0
18	47	93
30	100	100
50	100	100

Further effects:

Test-Concentration at which effects were seen (nominal)[1] [mg/l]	Additional effects	
	24 h	48 h
11	0	One water flea was trapped on the surface of the test solution.
18	Several water fleas had flared carapace. Several water fleas were lethargic.	One water flea was lethargic.

30	Several water fleas were trapped on the surface of the test solution. Several water fleas had flared carapace.	
50	Several water fleas were trapped on the surface of the test solution. Several water fleas had flared carapace.	

5.2.1	EC_0	11 mg/l (nominal ; NOEC = 6.6 mg/l)
5.2.2	EC_{50}	14mg/l (nominal)
5.2.3	EC_{100}	30 mg/l (nominal)
5.3	**Conclusion**	The testing of the acute toxicity of glutardialdehyde 50% to the freshwater crustacean Daphnia magna resulted in a LC50 value (48 h) of 14 mg/l, referring to the active ingredient contained in the test substance.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	May 21st, 2008
Materials and Methods	5.1 In the test 15 animals were used per each concentration. OECD 202 recommends that at least 20 animals should be used.
Results and discussion	The EC values are based on nominal concentrations and they are reported as 100 % glutaraldehyde per litre of test solution. EC_{50} 48 h 7 mg a.i./l The result recalculated according to TWA[1] is 2.1 mg a.i./L.
Conclusion	Glutaraldehyde is acutely slightly toxic to *Daphnia magna.*
Reliability	2
Acceptability	Not acceptable because test concentrations were not analytically verified
Remarks	The test report should have been more detailed.

COMMENTS FROM ...

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

[1] CA-May08-Doc.6.5: TNsG Assessment of environmental effects of biocidal active substances that rapidly degrade in environmental compartments of concern.

Section A7.4.1.2 _ 02 **Acute toxicity to invertebrates**
Annex Point IIA7.2 **Daphnia magna**

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

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Reconstituted water ([redacted]) according to procedures recommended by the U.S EPA (1975)
Alkalinity	120 +/- 10 mg/l ($CaCO_3$)
Hardness	165 +/- 15 mg/l ($CaCO_3$)
pH	7.9 – 8.3
Ca / Mg ratio	
Na / K ratio	
Oxygen content	> 5.4 mg/l (60% of saturation)
Conductance	400 – 600 µmhos/cm
Holding water different from dilution water	The holding water was prepared from well water according to procedures recommended by the U.S EPA (1975); it was filtered through an Amberlite XAD-resin column for removal of potential organic contaminants. The characteristics of the holding water were similar to those of the dilution water (see above).

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	Daphnia magna
Source	[redacted]
Age	<= 24 hours old
Breeding method	Not specified
Kind of food	Not specified
Amount of food	Not specified
Feeding frequency	Not specified
Pretreatment	No particularities
Feeding of animals during test	Not specified

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	None
Volume of test vessels	150 ml
Volume/animal	30 ml/animal
Number of animals/vessel	5
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	Not specified

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	21 +/- 1 °C
Dissolved oxygen	Test Concentration (mg/l) / Oxygen-value after: 0 h / 48 h 0 / 8.7 (99%)* / 8.1 (92%) 4.0 / 8.7 (99%) / 8.0 (91%) 18 / 8.7 (99%) / 8.1 (92%) 50 / 8.6 (98%) / 8.1 (92%) *, Percentage of saturation at 22°C
pH	Test Concentration (mg/l) / Oxygen-value after: 0 h / 48 h 0 / 8.1 / 8.1 4.0 / 8.0 / 8.1 18 / 8.1 / 8.1 50 / 8.1 / 8.1
Adjustment of pH	No
Aeration of dilution water	No

Quality/Intensity of irradiation	Durotest (Optima) fluorescent lights Intensity: 50 to 70 footcandles
Photoperiod	Not specified

Table A7_4_1_2-6: Immobilisation data

Test-Substance Concentration (nominal)[1] [mg/l]	Immobile Daphnia				Oxygen [mg/l]	pH	Tempera-ture [°C]
	Number		Percentage				
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
0	0	0	0	0	8.1 (92%*f)	8.1	22°C
4.0	0	0	0	0	8.0 (91%)	8.1	22°C
6.6	0	0	0	0	-	-	-
11	0	0	0	0*e	-	-	-
18	7	14	47*b,c	93*d	8.1 (92%)	8.1	22 °C
30	15	15	100*a,b	100	-	-	-
50	15	15	100*a,b	100	8.1 (92%)	8.1	22 °C

*, a: several water fleas were trapped on the surface of the test solution; b: several water fleas had flared carapace; c: several water fleas were lethargic, d: one water flea was lethargic; e: one water flea was trapped on the surface of the test solution; f: percentage of saturation.

Table A7_4_1_2-7: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg/l]	18 (n)	11 – 30	11 (n)	30
48 h [mg/l]	14 (n)	11 - 18	11 (n)	30

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

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	Fulfilled	Not fullfilled
Immobilisation of control animals <10%	**Yes**	
Control animals not staying at the surface	**Yes**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**Yes**	
Concentration of test substance ≥80% of initial concentration during test	**No analytical monitoring performed.**	

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

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1981a) Glutaraldehyde ecological fate and effects studies [redacted] (Unpublished), BPD ID A7.04.1.2_03
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, according to the procedures recommended by the EPA Committee on Methods for Toxicity Tests with Aquatic Organisms, EPA-660/3-75-009, 1975
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed
2.3	**Deviations**	Replicate concentrations were not routinely used.
		3 MATERIALS AND METHODS
3.1	**Test material**	Glutaraldehyde [redacted]% aqueous solutions, ([redacted])
3.1.1	Lot/Batch number	No data
3.1.2	Specification	As given in section 2
3.1.3	Purity	[redacted]% in water
3.1.4	Composition of Product	[redacted]% active ingredient
3.1.5	Further relevant properties	No data
3.1.6	Method of analysis	No data
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not relevant
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_1-2
3.4.2	Test organisms	See table A7_4_1_1-3
3.4.3	Test system	See table A7_4_1_1-4
3.4.4	Test conditions	See table A7_4_1_1-5
3.4.5	Duration of the test	48 h
3.4.6	Test parameter	Mortality, biological observations

3.4.7	Sampling	The test parameters were checked after 24 and 48 hours. The oxygen and pH measurements were performed at test initiation and after 48 hours.
3.4.8	Monitoring of TS concentration	No
3.4.9	Statistics	The estimation of the LC50 was usually based on the Spearman-Karber estimator (Finney, 1971).

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	Nor relevant
4.1.2	Number/ percentage of animals showing adverse effects	Not relevant
4.1.3	Nature of adverse effects	Not relevant
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 0.63, 1.25, 2.50, 5, 10, 20, 40 and 80 mg/l (nominal)
4.2.2	Actual concentrations of test substance	No analytical monitoring performed.
4.2.3	Effect data (Mortality)	See table A7_4_1_2-6 and table A7_4_1_2-7
4.2.4	Concentration / response curve	No curve available
4.2.5	Other effects	None described
4.3	**Results of controls**	On case of death in the control group was reported for the testing series with glutaraldehyde [redacted]%
4.4	**Test with reference substance**	None
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The aim of the present study was to investigate the acute toxicity of glutardialdehyde [redacted]% to aquatic invertebrates. Test substances: Glutardialdehyde [redacted]% aqueous solutions, ([redacted]). Glutaraldehyde [redacted] Glutaraldehyde [redacted] Guideline according to the EPA Committee on Methods for Toxicity Tests with Aquatic Organisms, EPA-660/3-75-009, 1975; no GLP The acute toxicity of to the freshwater crustacean Daphnia magna was

assessed over a 48 hour-exposure period under static conditions. Following nominal concentrations were tested for both test substances, GA [redacted]% and GA [redacted]%: 0, 0.63, 1.25, 2.50, 5, 10, 20, 40 mg/l. Additionally, GA [redacted]% was tested at 80 mg/l. The testing of GA [redacted]0% was conducted in one single run whereas for GA [redacted]%, two separate test runs were performed. The dilution water was [redacted] River water obtained from the [redacted] The test was conducted in 250 ml beakers containing 200 ml of test solution; 10 daphnia (< 2 days old) were added to each test vessel. The test was performed at a temperature of ca. 19 to 21 °C. The Daphnia were examined for mortality and biological effects at following time points: 24 and 48 hours. Oxygen and pH measurements were performed at test initiation and after 48 hours. The estimation of the EC50 value was usually based on on the Spearman-Karber estimator (Finney, 1971).

5.2 Results and discussion

Mortality:

Daphnia mortalities after 24 hours (%)				
Test-Substance Concentration (nominal) [mg/l]	Glutaraldehyde [redacted]%	Glutaraldehyde [redacted]%		
	Test run 1	Test run 1	Test run 2	Test runs 1 &2 combined
0	0	0	0	0
0.63	0	0	0	0
1.25	0	0	10	5
2.5	0	0	20	10
5	0	0	0	0
10	10	0	0	0
20	70	10	0	5
40	100	90	90	90
80	-	100	100	100

Daphnia mortalities after 48 hours (%)				
Test-Substance Concentration (nominal) [mg/l]	Glutaraldehyde [redacted]%	Glutaraldehyde [redacted]%		
	Test run 1	Test run 1	Test run 2	Test runs 1 &2 combined (N = 20)
0	0	0	10	5
0.63	0	0	0	0
1.25	0	0	10	10
2.5	0	0	20	10
5	0	0	0	0
10	30	10	20	15
20	100	40	40	40
40	100	100	100	100
80	-	100	100	100

5.2.1 EC_0
5.2.2 EC_{50}
5.2.3 EC_{100}

Glutaraldehyde [redacted]% (test runs 1&2 combined)				
	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg/l]	26.5 (n)	22.5 – 31.1	-	80
48 h [mg/l]	**16.9 (n)**	13.4 – 21.2	-	40
Glutaraldehyde [redacted]%				
	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg/l]	16.3 (n)	12.7 - 20.8	5 (n)	40
48 h [mg/l]	**11.5 (n)**	9.4 - 14.2	5 (n)	20

1, nominal values

5.3 Conclusion

Referring to the test material as such, the testing of the acute toxicity of glutardialdehyde [redacted]% to the freshwater crustacean Daphnia magna resulted in a LC50 value (48 h) of 11.5 mg/l whereas a LC50 (48 h) of ca. 17 mg/l was reported for glutaraldehyde [redacted]%.

5.3.1 Reliability

2

5.3.2 Deficiencies

The testing of glutaraldehyde [redacted]% was conducted as a single run whereas for the testing of glutaraldehyde [redacted]% two separate tests were conducted; no explanation was provided. According to the authors, the pH and the dissolved oxygen were monitored; but no data were presented in the report.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

May 21st, 2008

Materials and Methods

The test conditions were not reported in detail.

5.1 The Daphnias should have been up to 24 h old, in this study they were up to 48 h old. Only 10 instead of 20 animals were used per test concentration. Only one test run was used for [redacted] % glutaraldehyde. The hardness of the water should have been 140-250 mg/l $CaCO_3$, in this study it was 55 mg/l. The dissolved oxygen concentration and pH at the end of the test were not mentioned. It was not mentioned if the animals were fed during the test.

Results and discussion

The actual concentrations have not been measured. Results are expressed as 100 % glutaraldehyde.

EC_{50} 24 h 8.5 mg/l (Cl 95% 6.7-10.6)

EC_{50} 48 h 5.8 mg/l (Cl 95% 4.7-7.1)

Conclusion

Glutaraldehyde is toxic to *Daphnia magna*.

Reliability

3

Acceptability

Not acceptable

The validity criteria for *Daphnia* sp. Acute Immobilisation test according to OECD Guideline 202 were only partially fulfilled.

Remarks

COMMENTS FROM ...

Date

Give date of comments submitted

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

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	[redacted] River water obtained from the [redacted]
Alkalinity	36 mg/l ($CaCO_3$)
Hardness	55 mg/l ($CaCO_3$)
pH	6.7
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	-
Conductance	250µmhos/cm
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	Daphnia magna
Source	[redacted]
Age	<= 2 days old
Breeding method	The young daphnia were obtained from 20 to 50 gravid females, which were isolated for 1 to 2 days.
Kind of food	Laboratory-prepared food consisting of trout food, yeast and alfalfa powder.

Amount of food	-
Feeding frequency	3 times a week
Pretreatment	No particularities
Feeding of animals during test	Not specified

Table A7_4_1_2-4: Test system

Criteria	**Details**
Renewal of test solution	None
Volume of test vessels	250 ml beakers filled with 200 ml test solution
Volume/animal	20 ml/animal
Number of animals/vessel	10
Number of vessels/ concentration	For the testing of GA [redacted]%: one vessel per test concentration For the testing of GA [redacted]%: two vessels per test concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	**Details**
Test temperature	19 to 21 °C
Dissolved oxygen	Data not provided in the document
pH	Data not provided in the document
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Not specified
Photoperiod	Not specified

Table A7_4_1_2-6: Mortality data

Daphnia mortalities after 24 hours			
Test-Substance	**Glutaraldehyde [redacted]%**	**Glutaraldehyde [redacted]%**	

Concentration (nominal) [mg/l]	Test run 1		Test run 1		Test run 2		Test runs 1 &2 combined	
	N	%	N	%	N	%	N	%
0	0	0	0	0	0	0	0	0
0.63	0	0	0	0	0	0	0	0
1.25	0	0	0	0	1	10	1	5
2.5	0	0	0	0	2	20	2	10
5	0	0	0	0	0	0	0	0
10	1	10	0	0	0	0	0	0
20	7	70	1	10	0	0	1	5
40	10	100	9	90	9	90	18	90
80*	-	-	10	100	10	100	20	100

*, 80 mg/l tested for Glutaraldehyde [redacted]% only

Daphnia mortalities after 48 hours								
Test-Substance Concentration (nominal) [mg/l]	Glutaraldehyde [redacted]% Test run 1		Glutaraldehyde [redacted]%* Test run 1		Test run 2		Test runs 1 &2 combined	
	N	%	N	%	N	%	N	%
0	0	0	0	0	1	10	1	5
0.63	0	0	0	0	0	0	0	0
1.25	0	0	0	0	1	10	2	10
2.5	0	0	0	0	2	20	2	10
5	0	0	0	0	0	0	0	0
10	3	30	1	10	2	20	3	15
20	10	100	4	40	4	40	8	40
40	10	100	10	100	10	100	20	100
80	-	-	10	100	10	100	20	100

Table A7_4_1_2-7: Effect data

Glutaraldehyde [redacted]%				
	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg/l]	16.3 (n)	12.7 - 20.8	5 (n)	40
48 h [mg/l]	11.5 (n)	9.4 - 14.2	5 (n)	20

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

Glutaraldehyde [redacted]% (test runs 1&2 combined)				
	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg/l]	26.5 (n)	22.5 – 31.1	-	80
48 h [mg/l]	16.9 (n)	13.4 – 21.2	-	40

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	Not fullfilled
Immobilisation of control animals <10%	**yes**	
Control animals not staying at the surface	**yes**	
Concentration of dissolved oxygen in all test vessels >3 mg/l		**No data**
Concentration of test substance ≥80% of initial concentration during test		**No analytical monitoring performed.**

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

Section A7.4.1.2 _ 04 **Acute toxicity to invertebrates**

Annex Point IIA7.2 **Marine species Mysid Shrimp (Mysidopsis bahia)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1995) Glutaraldehyde - Acute toxicity to Mysids (Mysidopsis bahia) under flow-through conditions. [redacted] (Unpublished), BPD ID A7.04.1.2_04	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, according to FIFRA Guideline No: 72-3, US EPA (1985)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde, from [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]% active ingredient	
3.1.4	Composition of Product	[redacted]% active ingredient	
3.1.5	Further relevant properties	Clear viscous liquid	
3.1.6	Method of analysis	No data	x
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	Not relevant	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_2-2	
3.4.2	Test organisms	See table A7_4_1_2-3	
3.4.3	Test system	See table A7_4_1_2-4	
3.4.4	Test conditions	See table A7_4_1_2-5	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality and biological observations (i.e. check for absence of mobility and absence of response to gentle prodding) as well as physical characteristics of the test solutions (i.e. pH, dissolved oxygen, salinity and temperature)	

3.4.7	Sampling	The test parameter was checked at test initiation and after every following 24 hours interval.
3.4.8	Monitoring of TS concentration	Yes, an analytical monitoring of the test concentrations was performed during a pretest (two replicates). In fact, one water sample from each replicate of the high-, middle- and low-test concentrations as well as from the control were removed and subjected to analysis for the glutaraldehyde concentration. The pretest analysis was conducted 13 days prior test initiation, and the results allowed to establish that sufficient quantities of test substance in the appropriate gradient were delivered to the test vessels. The analytical monitoring was based on HPLC-UV procedure. During the main test the glutaraldehyde concentrations also were analytically monitored at test initiation (0 hour) and at test ending (96 hour); this time, monitoring was performed for all nominal tested concentrations.
3.4.9	Statistics	The estimation of the LC50 was based on a computer program using one of three statistical methods: (1) Moving average angle analysis (2) Probit analysis (3) Binomial probability

4 RESULTS

4.1	**Limit Test**	A preliminary range-finding test was performed under folw-through conditions using 10 mysids per test concentration.
4.1.1	Concentration	0, 0.78, 1.3, 2.2, 3.6, 6.0 and 10 mg a.i. /l
4.1.2	Number/ percentage of animals showing adverse effects	100% mortality was recorded after 96 hours at 10 mg a.i. /l 90% mortality was recorded after 96 hours at 6.0 mg/a.i./l 60% mortality was recorded after 96 hours at 3.6 mg a.i./l 10% mortality was recorded after 96 hours at 2.2 mg a.i./l No mortality was observed at 0.78 and 1.3 mg a.i./l. All surviving mysids of the 2.2, 3.6 and 6.0 mg a.i/l groups and one mysid of the 1.3 mg a.i./l group showed sublethal effects.
4.1.3	Nature of adverse effects	The sublethal effects included e.g. erratic swimming behavior
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0.62, 1.0, 1.7, 2.9, 4.8 and 8.0 mg a.i./l
4.2.2	Actual concentrations of test substance	Results of the analytical monitoring within the pretest:

Nominal concentration (mg a.i./l)	Mean measured concentration* (mg a.i./l)	Percent of nominal concentration
Control	< 0.055	Not applicable
0.62	0.29	47%
2.90	1.90	65.5%
8.00	6.60	82.5%

*, Mean of two replicates; the concentrations of glutaraldehyde were consistent between replicates.

Results of the analytical monitoring within the main test:

Nominal Conc. (mg a.i./l)	Mean measured conc.* at test initiation (mg a.i./l)	Mean measured conc.* at test ending (96 h) (mg a.i./l)	Mean measured conc. over the test period (mg a.i./l)	Percent of nominal Conc.
Control	< 0.041	< 0.030	Not applicable	-
0.62	0.35	0.42	0.38	62%
1.0	0.72	0.70	0.71	71%
1.7	1.15	1.10	1.10	66%
2.9	2.55	2.30	2.40	84%
4.8	4.60	4.45	4.50	94%
8.0	7.75	7.30 **	7.60	95%
Average measured concentration of glutaraldehyde				79%

*, Mean of two replicates; the concentrations of glutaraldehyde were consistent between replicates.

**, In one of the two replicates a concentration < 0.37 mg a.i./l was measured whereas in the second one, 7.30 mg a.i./l glutaraldehyde were found. The first was not considered to be representative of the exposure conditions and was therefore not taken into consideration.

On the basis of the results reported above, following measured concentrations were defined:

Nominal Concentration (mg a.i./l)	Measured concentration (mg a.i./l)
Control	0
0.62	0.38
1.0	0.71
1.7	1.1
2.9	2.4
4.8	4.5
8.0	7.6

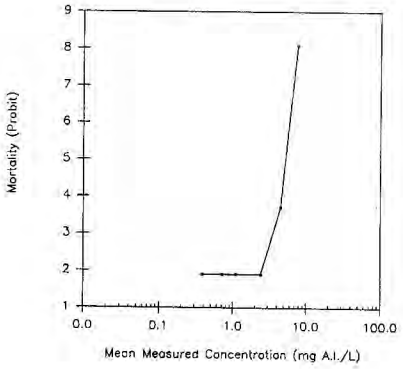
The coefficients of variation for all mean measured concentrations averaged 17%.

4.2.3 Effect data

See table A7_4_1_2-6 and table A7_4_1_2-7

4.2.4 Concentration / response curve

Figure 2. Concentration-response (mortality) curve for the 96-hour flow-through study exposing mysids (*Mysidopsis bahia*) to glutaraldehyde.



4.2.5	Other effects	For sublethal effects, see table A7_4_1_2-6
4.3	**Results of controls**	Inconspicuous, see table A7_4_1_2-6
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not relevant
4.4.2	Results	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the acute toxicity of glutardialdehyde [redacted]% to marine invertebrates (Mysids, Mysidopsis bahia).

Test substance: Glutaraldehyde, from [redacted] %

Test method according to FIFRA Guideline No: 72-3, US EPA (1985)

The Mysids were obtained from Laboratory cultures, [redacted]. They were maintained in artificial seawater with a salinity of 26 to 30 parts/thousand and a temperature of 25 °C. The artifical water was obtained by addition of a commercially prepared salt (i.e. hw-marine mix) to [redacted]., soft freshwater. They were fed brine shrimp nauplii (Artemia salina) twice daily, also during the testing period. Mysids <= 24 hours old were used for the test. Based on the results of a preliminary range-finding test, following nominal concentrations referring to the active ingredient glutaraldehyde were tested: 0.62, 1.0, 1.7, 2.9, 4.8 and 8.0 mg a.i./l. Dilution water was natural seawater from [redacted] with a salinity of 31 to 32 part per thousand. The test concentrations were subjected to an analytical monitoring based on HPLC-UV; analytical monitoring was performed within a pretest and within the main test.

		The test system consisted of glass aquaria with a volume of 19.5 liters. Each test aquarium contained two mysids retention chambers. Each retention chamber consisted of a glass Petri dish with a diameter of 10 cm, to which a 15 cm high screen collar was attached (363 µm opening). Each retention chamber contained 10 mysids. Renewal of the test solution was ensured by the delivery of 50 ml of test solution per minute to each replicate test vessel, corresponding to ca. 10 volume replacements every 24 hours. The test temperature was maintained at 25 +/- 1 °C, and the animals were subjected to a 16:8 hours light/dark photoperiod. The mysids were observed for mortality and sublethal effects indicative of toxicity at 24 hour-intervals. Oxygen, pH, salinity and temperature measurements also were performed at 24 hour-intervals. The statistical assessment of the findings was based on a computer program using one of three statistical methods: Moving average angle analysis, Probit analysis and Binomial probability.
5.2	**Results and discussion**	Analytical monitoring: Within the pretest, recovery of glutaraldehyde was about 47 to 83% (after 96 hours); within the main test, recovery of glutaraldehyde was about 62 to 95%. An average measured concentration of glutaraldehyde of 79% was retained, and the measured concentrations were as follows: 0, 0.38, 0.71, 1.10, 2.40, 4.50 and 7.60 mg a.i./l. Mortality (%): Mortality was neither observed in the control group, nor in the 0.38, 0.71, 1.1 and the 2.4 mg a.i./l group. At 4.5 mg a.i./l, 10% mortality was recorded after 72 hours. At the highest tested concentration of 7.6 mg a.i./l, mortality was 45% after 48 hours and reached 100% after 96 h. Sublethal effects: From 1.1 mg a.i./l, sublethal effects were seen, which included: lethargy, erratic swimming behaviour, darkened pigmentation and partial to complete loss of equilibrium. Physical parameters: During the test period the dissolved oxygen as percentage of saturation ranged from 89 to 102 %. The pH values ranged from 7.75 to 7.9. The temperature was constant, with a value of 25 °C, and the salinity was about 31 to 32 parts/thousand.
5.2.1	NOEC	0.71 mg a.i./l (measured)
5.2.2	LC_{50}	5.5 mg a.i/l (measured)
5.2.3	LC_{100}	7.6 mg a.i./l (measured)
5.3	**Conclusion**	The testing of the acute toxicity of glutardialdehyde to the marine Mysid Mysidopsis bahia using a flow-through system resulted in a LC50 value (96 h) of 5.5 mg/l, referring to the active ingredient; the NOEC was 0.71 mg a.i/l.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 4th, 2008

Materials and Methods	The applicant's version is acceptable. 3.1.6 No data is filled on method of analysis. It can be found from page 60-73 in the test report. The LOQ of 0.0483 mg/L is above the LOQ of 0.05 µg/L in Doc IIIA4.2(c).
Results and discussion	LC_{50} 5.5 mg a.i./l (95% confidence limits 4.5-7.6 mg/l) based on the measured concentrations
Conclusion	Glutaraldehyde is toxic to *Mysidopsis bahia*.
Reliability	1
Acceptability	Acceptable
Remarks	

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

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Natural seawater from [redacted]
Salinity	31 to 32 part per thousand
Alkalinity	-
Hardness	-
pH	7.9
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	-

Conductance	-
Holding water different from dilution water	Yes, the mysids were maintained in artificial seawater with a salinity of 26 to 30 parts/thousand and a temperature of 25 °C. The artifical water was obtained by addition of a commercially prepared salt (i.e. hw-marine mix) to S[redacted] soft freshwater.

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	Mysidopsis bahia
Source	Laboratory cultures, [redacted] The original stocks were obtained from a commercial supplier ([redacted]
Age	<= 24 hours
Breeding method	The young mysids were obtained according to the method of described by Reitsema LA and Neff JM (Estuaries 3(4): 321-323, 1980)
Kind of food	Brine shrimp nauplii (Artemia salina)
Amount of food	-
Feeding frequency	Twice daily
Pretreatment	No particular pretreatment
Feeding of animals during test	Yes, as above

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	Yes, 50 ml of test solution per minute were delivered to each replicate test vessels, corresponding to ca. 10 volume replacements every 24 hours.
Volume of test vessels	The test system consisted of glass aquaria with a volume of 19.5 liters (39 x 20 x 8.5 cm).
Volume/animal	Each test aquarium contained two mysids retention chambers. Each retention chamber consisted of a glass Petri dish with a diameter of 10 cm, to which a 15 cm high screen collar was attached (363 µm opening). Each retention chamber contained 10 mysids. The maximum organism loading concentration was 0.00014 g of biomass per liter of flowing test solution per day.
Number of animals/vessel	
Number of vessels/ concentration	Two replicates per test concentration
Test performed in closed vessels due to significant volatility of TS	-

Table A7_4_1_2-5: Test conditions

Criteria	Details

Test temperature: 25 +/- 1 °C

Dissolved oxygen

Nominal Test Conc. (mg a.i./l)	Dissolved Oxygen mg/l*				
	0 h	24 h	48 h	72 h	96 h
0	7.05	6.80	6.60	6.60	7.00
0.62	7.05	6.80	6.60	6.60	7.05
1.00	7.05	6.85	6.65	6.55	6.70
1.70	7.05	6.80	6.60	6.45	6.40
2.90	7.00	6.75	6.55	6.40	6.40
4.80	6.95	6.70	6.50	6.35	6.30
8.00	7.00	6.75	6.55	6.35	6.15

*, Mean of two replicates; the dissolved oxygen values were consistent between the two replicates.

Nominal Test Conc. (mg a.i./l)	Dissolved Oxygen as % of saturation				
	0 h	24 h	48 h	72 h	96 h
0	102	99	96	96	101
0.62	102	99	96	96	102
1.00	102	99.5	96.5	95	97
1.70	102	99	96	93.5	93
2.90	101.5	98	95	93	92.5
4.80	100.5	97	94	92	91
8.00	101.5	98	95	92	89

pH

Nominal Test Conc. (mg a.i./l)	pH*				
	0 h	24 h	48 h	72 h	96 h
0	7.9	7.9	7.9	7.9	7.9
0.62	7.9	7.85	7.9	7.9	7.9
1.00	7.9	7.85	7.9	7.9	7.85
1.70	7.9	7.85	7.9	7.9	7.9
2.90	7.9	7.85	7.9	7.9	7.75
4.80	7.9	7.85	7.9	7.9	7.8
8.00	7.9	7.85	7.9	7.9	7.8

*, Mean of two replicates; the dissolved oxygen values were consistent between the two replicates.

Temperature (°C)

Temperature (°C)*				
0 h	24 h	48 h	72 h	96 h
25	25	25	25	25

*, Range of daily temperature measured in all test concentrations and the control at the stated time point.

Salinity (parts/thousand)

Salinity*				
0 h	24 h	48 h	72 h	96 h
31	32	32	31	32

Adjustment of pH	No
Aeration of dilution water	No aeration during the exposure period
Quality/Intensity of irradiation	Light intensity of 410 Lux (38 footcandles), provided by Duor-test Vita-Lite fluorescent bulbs.
Photoperiod	16:8 hours light/dark

Table A7_4_1_2-6: Mortality data and sublethal effects

Test-Substance Conc. (measured) [mg a.i./l]	Cumulative mortality (%)											
	24 h			48 h			72 h			96 h		
	R1*	R2	Mean	R1*	R2	Mean	R1*	R2	Mean	R1*	R2	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
0.38	0	0	0	0	0	0	0	0	0	0	0	0
0.71	0	0	0	0	0	0	0	0	0	0	0	0
1.1	0	0	0	0	0	0	0	0	0	0	0	0
2.4	0	0	0	0	0	0	0	0	0	0	0	0
4.5	0	0	0	0	0	0	10	10	10	10	10	10
7.6	0	0	0	40	50	45	100	90	95	100	100	100

Test-Conc. (measured) [mg a.i./l]	Sublethal effects*			
	24 h	48 h	72 h	96 h
0	None	None	None	None
0.38	None	None	None	None
0.71	None	None	None	None
1.1	None	Lethargy (1)	Lethargy (1)	None
2.4	Erratic Swimming (2)* Lethargy (2) Darkened Pigmentation (1)	Darkened Pigmentation (1) Erratic Swimming (> 2) Lethargy (> 2)	Darkened Pigmentation (1) Erratic Swimming (> 2) Lethargy (> 2)	Erratic Swimming (2) Darkened Pigmentation (1) Lethargy (1)
4.5	Lethargy (2) Darkened Pigmentation (>2) Erratic Swimming (1)	Lethargy (> 2) Partial Loss Equilibrium (> 2) Darkened Pigmentation/Complete Loss of Equilibrium (2)	Erratic Swimming (> 2) Lethargy (> 2) Partial Loss Equilibrium (> 2) Erratic Swimming at the surface (1) Darkened Pigmentation/ Partial Loss Equilibrium (2)	Erratic Swimming (> 2) Lethargy (> 2) Partial Loss Equilibrium (> 2) Darkened Pigmentation / Partial Loss Equilibrium (2)
7.6	Lethargy (2) Darkened Pigmentation (>2) Erratic Swimming (> 2)	Lethargy (2) Complete Loss Equilibrium (> 2) Partial Loss Equilibrium (2)	Erratic Swimming / Darkened Pigmentation (1)	No survivors

*, Number of cases observed.

Table A7_4_1_2-7: Effect data

	LC50[1] [mg a.i./l]	95 % c.l.	NOEC [mg a.i./l]

24 h	> 7.6 (m)		-
48 h	> 7.6 (m)		-
72 h	5.7 (m)	5.1 – 6.4	-
96 h	5.5 (m)	4.5 – 7.6	0.71 (m)

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

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202

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

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

Section A7.4.1.2(3) Annex Point IIA, VII.7.2 IUCLID 4.2/03		Acute toxicity to invertebrates Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions	
		1 REFERENCE (A7.4.1.2/03)	**Official use only**
1.1	**Reference**	[redacted] (1993) Glutaraldehyde - Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions, [redacted] Unpublished, 7 September 1993	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company and BASF SE	
1.2.2	Companies with letter of access	None	
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, US EPA FIFRA 72-3	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes The study director had no knowledge of the procedures to characterize the test material. Analyses for stability and homogeneity of glutaraldehyde in the exposure solutions were not conducted. All remaining test material was returned to the study sponsor at study termination. The retainer sample was made the responsibility of the sponsor. Routine water and food contaminant assays were not conducted under GLP (they were conducted [redacted], a separate contract testing facility).	
		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]% (remainder water)	
3.1.4	Composition of Product	3.1.3	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Samples were analysed using a validated method. Recovery samples containing glutaraldehyde were processed by liquid/liquid extraction and derivatisation with 2,4-DNPH. Following derivatisation each sample was extracted with methylene chloride. Final extracts were analyzed by HPLC with UV detection using a Waters HPLC and the following conditions: Analytical Column: Phenomenex Spherisorb Cyano, 5µm, 150mm x 4.6mm	

Section A7.4.1.2(3) **Annex Point IIA, VII.7.2** **IUCLID 4.2/03**		**Acute toxicity to invertebrates** **Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions**	
		Mobile phase: 80-85:15-20:0.7 v/v/v hexane:methylene chloride:methanol Flow rate: 2.2 mL/minute Wavelength: 350nm Injection Volume: 15 µl Retention time: approx. 9.9 to 15.5 min.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Substance is soluble in water. Volatilization is not expected to be significant.	
3.3	**Reference substance**	Not applicable	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**	*Non-entry field*	
3.4.1	Dilution water	**Table A7.4.1.2(3)-2**	
3.4.2	Test organisms	**Table A7.4.1.2(3)-3** (Eastern oysters, *Crassostrea virginica*)	
3.4.3	Test system	**Table A7.4.1.2(3)-4** The diluter system was calibrated to deliver approximately 150 mL/min of exposure solution to each replicate test vessel. The function of the diluter system was monitored daily and a visual check was performed twice daily. Test vessels consisted of glass aquaria measuring 49.5 x 25.5 x 29 cm with a 14-cm overflow drain. The temperature ranged from 19-21°C and a 16 hr light/8 hr dark photoperiod was maintained throughout the study.	
3.4.4	Test conditions	**Table A7.4.1.2(3)/03-5**	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Shell growth and sublethal effects	
3.4.7	Sampling	Test temperature, dissolved O_2, salinity, and pH were measured daily during the test. The vessels were not aerated. Biological observations (visible abnormalities, excessive mucus production or a failure to siphon and feed, lack of feces, etc.) were noted at test initiation and at subsequent 24-hour intervals. Sublethal effects were noted after comparisons to the control organisms. After 96 hours of exposure, the oysters were removed from the vessels and new shell growth was measured microscopically to the nearest 0.1mm using a calibrated micrometer. During the in-life portion of the definitive study, water samples were removed from each replicate treatment level solution and the control at 0 hours and 96 hours for Glutaraldehyde concentration.	
3.4.8	Monitoring of TS concentration	Yes	
3.4.9	Statistics	The 96 hour EC_{50} value and 95% confidence limits were determined by fitting the untransformed and transformed data to a best fit linear regression curve based on least squares. Thus, a total of four linear regression curves was computed. The regression line which provided the best fit of the untransformed or transformed data was selected based on the highest associated coefficient of determination. This regression	

Section A7.4.1.2(3) **Annex Point IIA, VII.7.2** **IUCLID 4.2/03**		**Acute toxicity to invertebrates** **Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions**	
		equation was then applied to calculate EC50 and 95% confidence limits, using the methods of inverse prediction. A computer program assisted in these calculations.	
		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	Nominal concentrations were 0.12, 0.19, 0.32, 0.54, and 0.90 mg a.i./L	
4.2.2	Actual concentrations of test substance	Mean measured concentrations were 0.068, 0.11, 0.16, 0.33, 0.71 mg a.i./L respectively	
4.2.3	Effect data	**Table 7.4.1.2(3)-6 and 7** **Shell Growth** Concentration / Mean shell disposition (mm) (96 hours) / Mean % reduction as compared to control (96 hours) 0.90 mg a.i./L: 1.7: 45* 0.54 mg a.i./L: 1.9: 39* 0.32 mg a.i./L: 2.7: 13 0.19 mg a.i./L: 3.0: 3.2 0.12 mg a.i./L: 3.0: 3.2 control: 3.1: N/A * = significantly different from control values	
4.2.4	Concentration / response curve	Refer to **Figure A7.4.1.2(3)-1**	
4.2.5	Other effects	None noted	
4.3	**Results of controls**	Mean shell deposition was 3.1 $\pm$ 1.3 mm in dilution water controls.	
4.4	**Test with reference substance**	Not applicable	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	

Section A7.4.1.2(3) **Annex Point IIA, VII.7.2** **IUCLID 4.2/03**	**Acute toxicity to invertebrates** **Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions**	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Forty oysters from a commercial supplier were allocated to each of 5 dose levels (20 per replicate) randomly after a 14-day acclimation. Oysters were fed a supplementary diet of algal cells during the testing period. Oysters were determined to be reproductively immature and of a similar age with a mean valve height of 28 mm. Each replicate solution was sampled and analyzed for glutaraldehyde concentration at test initiation and 96 hours. The test material was delivered *via* the dilution camber to the test vessels by a syringe pump. Temperature was kept at 18-22 °C, and a photoperiod (16 hours light) was maintained throughout the test. Prior to testing, 3-5mm of the new peripheral shell growth of each oyster were removed by grinding the shell to a blunt edge using a grinding wheel. Oysters were evaluated for stress for 24 hours following grinding. Organisms that did not show stress had the outer shell edge buffed by hand to remove any newly-deposited shell immediately prior to testing. Oysters were spaced equidistant from one another with their valve inflow openings facing the flow of the water. Filtered natural seawater was used as dilution water from [redacted] The water was analyzed per the US EPA and ASTM methods and deemed suitable for use. Test temperature, dissolved O_2, salinity, and pH were measured periodically during the test. The vessels were not aerated. Biological observations (visible abnormalities, excessive mucus production or a failure to siphon and feed, lack of feces, etc.) were noted daily. Sublethal effects were noted after comparisons to the control organisms. After 96 hours of exposure, the oysters were removed from the vessels and new shell growth was measured microscopically to the nearest 0.1mm using a calibrated micrometer. Individual measurements are presented in **Table 7.4.1.2(3)-9**. They were exposed to target concentrations of 0.90, 0.54, 0.32, 0.19, and 0.12 mg a.i./L for 96 hours.	
5.2 Results and discussion	Measured concentrations were consistent between replicate samples but decreased by an average of 35% between 0 and 96 hours. This decrease was attributed to absorption of the test material by the high number of test organisms and supplemental food. The mean measured concentrations were determined to be 0, 0.068, 0.11, 0.16, 0.33, and 0.71 mg a.i./L. At test termination, no mortality was observed among oysters at any dose level, and no sublethal effects were noted at dose levels below 0.33 mg a.i./L. Shell growth was reduced at the 2 highest dose levels, and was statistically different than the controls. Based on mean measured concentrations and the biological responses, the LC_{50} was calculated by linear regression analysis to be 0.78 mg a.i./L. The NOEC was determined to be 0.16 mg a.i./L.	
5.2.1 EC_0	The no observed effect concentration was determined to be 0.16 mg a.i./L in this study.	

Section A7.4.1.2(3) **Annex Point IIA, VII.7.2** **IUCLID 4.2/03**	**Acute toxicity to invertebrates** **Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions**	
5.2.2 EC_{50}	The 96 hour EC_{50} was calculated at 0.78 mg a.i./L.	
5.2.3 EC_{100}	Not reported	
5.3 Conclusion	Glutaraldehyde is considered highly toxic to *Crassostrea virginica* based on the results and criteria established by the US EPA.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	Jan 15th, 2009	
Materials and Methods	The applicant's version is acceptable. Measured values were in the range 50 to 79% of the nominal concentrations.	
Results and discussion	Table A7_4_1_2(3)_8 The validity criteria are modified to eastern oyster. The 96 hour EC_{50} for *Crassostrea virginica* is 0.78 mg a.i./L based on measured concentrations.	
Conclusion	Glutaraldehyde is very toxic to Eastern Oyster *Crassostrea virginica.*	
Reliability	1	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A7_4_1_2(3)-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_1_2(3)-2: Dilution water

Criteria	Details
Source	[redacted]
Salinity	31 ‰
Alkalinity	Not reported
Hardness	Not reported
pH	7.7 to 8.0
Ca / Mg ratio	Not reported
Na / K ratio	Not reported
Oxygen content	7.2 to 7.8 mg/L
Conductance	Not reported
Holding water different from dilution water	No

Table A7_4_1_2(3)-3: Test organisms

Criteria	Details
Strain	Eastern oysters, *Crassostrea virginica*
Source	[redacted]
Age	Reproductively immature, with a mean valve height of 28mm
Breeding method	Not reported
Kind of food	Algal diet of Isochrysis galbana and Tetraselmis maculata
Amount of food	180 mL of algal suspension containing 10^7 cells/mL maintaining 10^5 cells/mL
Feeding frequency	3 times per day
Pretreatment	14 days acclimatisation
Feeding of animals during test	Yes, algal diet as described above

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	A constant-flow serial diluter was used and had been calibrated to deliver 150 L/min of exposure solution.
Volume of test vessels	Volume maintained at approximately 18 L in a glass aquaria measuring 49.5 x 25.5 x 29 cm with a 14cm overflow drain.
Volume/animal	0.9 L
Number of animals/vessel	20
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	The temperature was maintained at 19°C throughout the study.
Dissolved oxygen	Range measured during the study was 7.2 to 7.8 mg/L
pH	Range measured during the study was 7.7 to 8.0
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Durotest Vita-Lite® fluorescent lights
Photoperiod	16h light and 8 h dark

Table A7_4_1_2(3)-6: Mean Shell Deposition Data

Test-Substance Concentration (measured) [mg/l]	Mean Shell deposition (mm)					
	Growth (mm)[a] (96 h)		Mean Percent reduction[b]	Oxygen [mg/l] 96 h	pH 96 h	Tempera-ture [°C] 96 h
	Mean	SD				
0.71	1.7	0.8	45[c]	7.6	7.8	19
0.33	1.9	1.2	39[c]	7.5	7.8	19
0.16	2.7	1.0	13	7.5	7.7	19
0.11	3.0	1.5	3.2	7.5	7.7	19
0.068	3.0	1.2	3.2	7.5	7.7	19
Control	3.1	1.3	NA	7.5	7.8	19

a = mean of 40 oysters
b = as compared to the control
c = significantly different from control values

Table A7_4_1_2-7: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
96 h [mg/l]	0.78	-	0.16	>0.78

effect data are based on measured (m) concentrations

Table A7_4_1_2(3)-8: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202[a]

	fulfilled	Not fulfilled
Control growth > 2mm	X	
Mortality in the controls should not exceed 10% at the end of the test	X	
Concentration of dissolved oxygen in all test vessels >3 mg/l (≥ 60% of saturation)	X	
Concentration of test substance ≥80% of initial concentration during test[b]		X
Criteria for poorly soluble test substances ergänzen	NA	

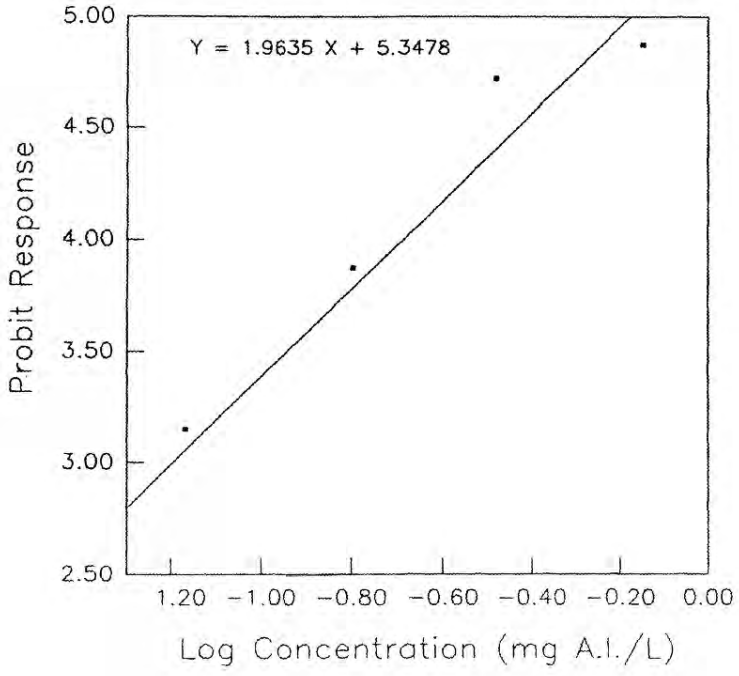
a = test carried out according to FIFRA 72-3
b = measured values were in the range 50 to 79% of target, the decrease was attributed to absorption of the test material by the high number of test organisms and supplemental food.

Table A7.4.1.2(3)-9 Shell Growth Data (mm)

Oyster number	0.71 mg a.i./L		0.33 mg a.i./L		0.16 mg a.i./L		0.11 mg a.i./L		0.068 mg a.i./L		control	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B
1	1.6	2.5	0.8	0.8	2.5	2.8	5.3	2.0	2.8	1.4	3.4	1.3
2	1.7	0.9	1.3	2.0	3.5	1.6	4.2	1.7	2.8	6.0	3.5	1.5
3	1.4	1.7	0.8	2.8	2.6	4.0	1.3	5.9	1.3	2.5	5.6	3.6
4	2.4	1.7	1.9	0.5	3.4	3.1	4.7	1.8	3.7	1.8	2.5	3.7
5	1.7	0.9	1.7	1.0	3.8	3.2	3.2	3.6	3.2	2.7	2.7	2.2
6	0.7	0.8	1.2	2.2	2.8	1.8	4.1	2.5	2.2	3.7	2.3	4.3
7	4.2	3.3	1.3	0.0	2.2	1.3	6.0	2.0	6.1	2.4	2.9	5.6
8	1.3	2.4	2.8	1.0	4.5	0.7	2.1	0.5	3.1	1.4	1.4	3.0
9	1.2	1.2	4.6	3.6	3.6	2.4	2.2	1.8	3.5	2.0	4.8	2.3
10	1.2	2.0	1.8	1.0	3.0	1.9	2.4	2.2	4.2	3.3	4.6	1.6
11	2.3	2.2	3.9	0.2	4.2	2.8	3.0	4.6	2.1	4.5	2.1	4.9
12	1.6	1.1	1.0	0.8	3.2	3.4	2.2	3.7	5.7	2.8	3.5	4.0
13	0.7	0.7	2.5	2.9	1.5	3.3	5.2	2.6	4.6	2.7	2.4	3.3
14	1.7	0.8	1.4	4.1	1.7	2.5	2.2	4.0	3.2	2.6	3.1	2.0
15	2.0	0.8	3.3	3.2	0.9	1.6	2.9	0.7	2.2	1.9	1.2	4.0
16	1.6	1.8	1.4	3.4	2.8	2.5	1.7	4.1	3.5	2.4	2.5	1.5
17	1.7	0.6	0.5	1.6	2.5	5.7	4.5	5.6	4.2	2.2	6.6	4.3
18	1.7	3.1	0.4	2.4	1.9	2.7	1.0	3.0	3.2	1.4	0.8	1.8
19	1.3	2.6	3.3	1.5	4.1	1.3	2.6	1.7	3.0	2.9	3.4	1.7
20	0.3	2.6	1.6	1.7	2.4	2.8	1.5	3.1	1.7	1.8	3.1	3.0
Replicate Mean	1.6	1.6	1.9	1.8	2.9	2.6	3.1	2.9	3.3	2.6	3.1	3.0
Treatment Mean	1.7		1.9		2.7		3.0		3.0		3.1	

(Concentrations are measured values)

Figure A7.4.1.2(3)-1 Concentration-Response (shell reduction) Curve for the 96-hour Shell Deposition Study in Eastern Oysters



Section A7.4.1.2(4) **Annex Point IIA, VII.7.2** **IUCLID 4.2/04**		**Acute toxicity to invertebrates** **Acute Toxicity to *Acartia Tonsa***	
		1 REFERENCE (A7.4.1.2/04)	**Official use only**
1.1	**Reference**	[redacted] (1997) [redacted]: Acute Toxicity to *Acartia Tonsa*, [redacted], Unpublished, 24 April 1997	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company and BASF SE	
1.2.2	Companies with letter of access	None	
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 UK Proposal to ISO TC147/SC5/W92	
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]% (remainder water)	
3.1.4	Composition of Product	3.1.3	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Recovery samples containing glutaraldehyde were processed by derivatisation with 2,4-DNPH. Final extracts were analyzed by HPLC with UV detection using an HPLC and the following conditions: Analytical Column: Lichrosorb RP18, 250mm x 4.6mm Mobile phase A: 90:10 v/v water:acetonitrile Mobile phase B: 100% acetonitrile	

Section A7.4.1.2(4) **Annex Point IIA, VII.7.2** **IUCLID 4.2/04**		**Acute toxicity to invertebrates** **Acute Toxicity to *Acartia Tonsa*** Gradient: Time (min.) Solvent A Solvent B 0 60 40 2 60 40 15 0 100 Flow rate: 2 mL/minute Wavelength: 368nm Injection Volume: 100 µl	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Substance is soluble in water. Volatilization is not expected to be significant.	
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	Dilution water	**Table A7.4.1.2(4)-2**	
3.4.2	Test organisms	**Table A7.4.1.2(4)-3** (*Acartia tonsa*)	
3.4.3	Test system	**Table A7.4.1.2(4)-4** 250 ml glass jars containing 100 ml of test solution were used. At the start of the study 5 *Acartia* were placed in each test and control vessel at random, in prepared test solutions. The water temperature was maintained at 20-22°C, and no treatment-related differences for oxygen concentration or pH were observed. Any deaths or adverse reactions were recorded at 24 and 48 hours after start of exposure.	
3.4.4	Test conditions	**Table A7.4.1.2(4)-5**	
3.4.5	Duration of the test	48 hours	
3.4.6	Test parameter	Mortality and sublethal effects	
3.4.7	Sampling	Water temperature was recorded daily throughout the study. Dissolved oxygen and pH were recorded at the start and termination of the study. Water samples were taken from control and each treatment vessel at 0 hours and at 48 hours for quantitative analysis.	
3.4.8	Monitoring of TS concentration	Yes	
3.4.9	Statistics	The LC_{50} values and associated confidence limits were calculated by the	

Section A7.4.1.2(4) **Annex Point IIA, VII.7.2** **IUCLID 4.2/04**		**Acute toxicity to invertebrates** **Acute Toxicity to *Acartia Tonsa***	
		moving average method of Thompson.	
		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	The initial nominal concentrations were 0.01, 0.018, 0.032, 0.056, 0.1, 0.18, 0.32, 0.56, and 1.0 mg /L.	
4.2.2	Actual concentrations of test substance	The mean measured concentrations were 0.0048, 0.0092, 0.017, 0.030, 0.045, 0.071, 0.12, 0.21, and 0.60 mg/L respectively.	
4.2.3	Effect data	**Table A7.4.1.2(4)-6 and 7**	

Nominal concentration (mg/L)	Cumulative mortalities (initial population: 5 per replicate)											
	24 hours						48 hours					
	R1	R2	R3	R4	Total	%	R1	R2	R3	R4	Total	%
control	0	0	0	0	0	0	0	0	0	0	0	0
0.01	0	0	0	0	0	0	0	0	0	0	0	0
0.018	0	0	0	0	0	0	0	0	0	0	0	0
0.032	0	0	0	0	0	0	0	0	0	0	0	0
0.056	0	0	0	0	0	0	0	0	0	0	0	0
0.1	0	0	0	0	0	0	1	2	1	2	6	30
0.18	0	0	0	0	0	0	3	2	2	2	9	45
0.32	0	0	0	0	0	0	3	1	3	4	11	55
0.56	0	0	0	0	0	0	4	4	5	5	18	90
1	0	2	3	1	6	30	4	5	5	5	19	95

Section A7.4.1.2(4) **Acute toxicity to invertebrates**

Annex Point IIA, VII.7.2

IUCLID 4.2/04

Acute Toxicity to *Acartia Tonsa*

4.2.4 Concentration / response curve

Refer to **Figure A7.4.1.2(4)-1**

4.2.5 Other effects

None noted

4.3 Results of controls

There was no mortality in the control groups at any time point.

4.4 Test with reference substance

4.4.1 Concentrations

1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56, and 100 mg/L potassium dichromate.

4.4.2 Results

Nominal Conc. (mg/L)	Cumulative mortalities (initial population: 5 per replicate)											
	24 hours						*48 hours*					
	R1	R2	R3	R4	Total	%	R1	R2	R3	R4	Total	%
control	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
1.8	0	0	0	0	0	0	0	0	0	0	0	0
3.2	0	0	0	0	0	0	0	0	0	0	0	0
5.6	0	0	0	0	0	0	1	0	1	1	3	15
10	1	0	2	1	4	20	2	2	2	3	9	45
18	3	1	2	3	9	45	5	4	2	2	13	65
32	4	4	4	5	17	85	5	5	4	5	19	95
56	4	5	4	5	18	90	5	5	5	5	20	100
100	5	5	5	5	20	100	5	5	5	5	20	100

The results are consistent with a previously conducted positive control study indicating the test system to be valid.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Doses of 0.010, 0.018, 0.032, 0.056, 0.10, 0.18, 0.32, 0.56, and 1 mg/L were used in static conditions to determine the LC_{50} of glutaraldehyde to marine crustacea. Potassium dichromate was used as the reference (positive control) material, and an additional control group was maintained under identical conditions but not exposed to the test material. The organisms (5 per test vessel) were added to covered test vessels containing the varying concentrations of glutaraldehyde. Test concentrations were determined by HPLC using an external standard method. Water temperature was recorded daily throughout the study. Dissolved O_2 and pH were recorded at the start and termination of the study. The study was conducted with 4 replicates at each test concentration.

The LC_{50} values and associated confidence limits were calculated by the moving average method of Thompson (1947).

5.2 Results and discussion

Based on nominal concentrations of the active ingredient, the results were calculated to be:
LC_{50} (24 hours) =>0.51 mg a.i./L
LC_{50} (48 hours) = 0.11 (0.085-0.14) mg a.i./L

An LC_{50} value was not calculated after 24 hours due to less than 50% mortalities observed at the timepoint. The NOEC based on nominal