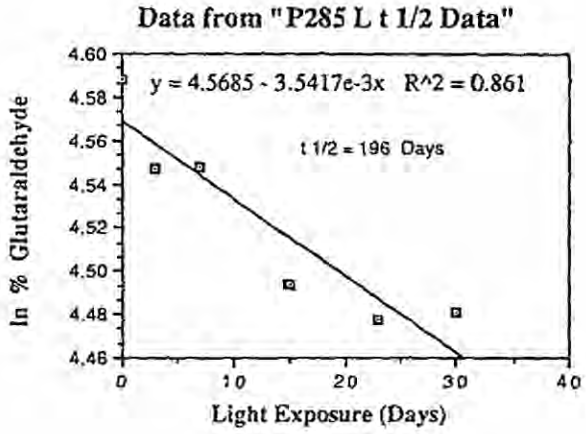
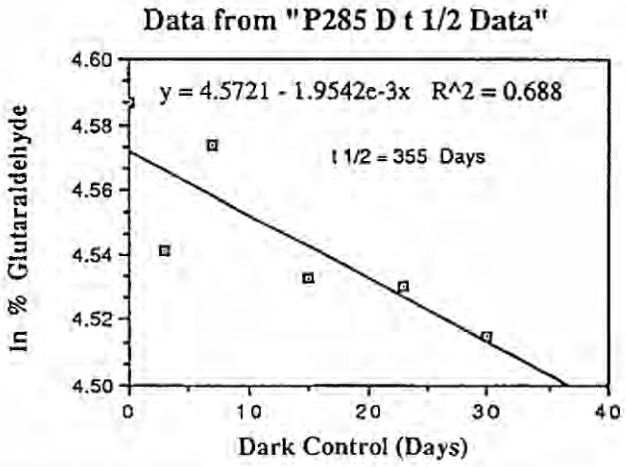
Section A7.1.1.1.2 Annex Point IIA, II.7.6.2.2 IUCLID 3.1.1/01		Phototransformation in water including identity of transformation products	
		W.min / cm^2.	
3.4.4	Temperature	Temperature of the water bath was 24.7 ± 2.6 °C.	
3.4.5	pH	Measurement method not specified.	
3.4.6	Duration of the test	30 days of exposure, approximately 11-12 hours of irradiation by sunlight per day.	
3.4.7	Number of replicates	2 per sampling interval	
3.4.8	Sampling	0, 3, 7, 15, 23 and 30 days	
3.4.9	Analytical methods	Samples were not extracted prior to LSC and HPLC analysis; they were analyzed immediately. At the designated time points, gas traps were also assayed. Liquid scintillation counting was used to quantify the radioactivity. HPLC was used to identify the parent and degradates. HPLC recoveries averaged 92.7 +/- 4.9%. Chromatographic methods were validated with authentic analytical standards. Products with yields as low as 0.2% could be reliably quantified and 0.1% yields detected.	
3.5	**Transformation products**	HPLC was used to identify the parent and degradates at 0, 3, 7, 15, 23 and 30 days.	
		4 RESULTS	
4.1	**Screening test**	Results were not reported. A pilot study was conducted to estimate the half-life of degradation of 1,5-^{14}C-glutaraldehyde (11.9 ppm) in pH 5 buffer solution. Samples were analyzed at 0 and 5 days.	
4.2	**Actinometer data**	Not applicable	
4.3	**Controls**	**Table A7.1.1.1.2/01-3** Glutaraldehyde dark controls did not degrade appreciably, as evidenced by the slow degradation rates observed (83.5% remained after 30 days irradiation) and lack of product formation at greater than 10% yield.	
4.4	**Photolysis data**		
4.4.1	% of applied material remaining after irradiation	Glutaraldehyde did not degrade appreciably under sunlight, as evidenced by the slow degradation rates observed (75.6% remained after 30 days irradiation) and lack of product formation at greater than 10% yield. **Table A7.1.1.1.2/01-3**	
4.4.2	Mass balance	94.4±2.8% (overall material balance) The average % recovery of the radioactivity was 93.2 ± 3.5 %. **Table A7.1.1.1.2/01-3 and 4**	
4.4.3	k^e_p	4.09 x 10^{-3} [1 s^{-1} x 10^5]	
4.4.4	Kinetic order	The half-life was calculated based on pseudo first-order kinetics.	
4.4.5	k^e_p / k^a_p	Not determined	
4.4.6	Reaction quantum yield (ϕ^e_E)	Not determined	

Section A7.1.1.1.2 **Annex Point IIA, II.7.6.2.2** **IUCLID 3.1.1/01**		**Phototransformation in water including identity of transformation products**	
4.4.7	kpE	Not determined	
4.4.8	Half-life ($t_{1/2E}$)	The extrapolated half-life in the light-exposed samples was 196 days (R^2 = 0.861), and in the dark was 355 days (R^2 = 0.688).   	
4.5	**Specification of the transformation products**	There were no products formed at greater than 10% yield.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test was carried out in natural sunlight in Richmond California. The latitude was 37.45N, longitude 122.26W. Light intensity ranged from 4,435 to 19947 µW/cm2 with a mean intensity of 17252 µW/cm2. Total mean light energy (W.min/cm2) was 5.68. The temperature range during the test was 24.7 +/- 2.6°C. The test was performed in duplicate and a dark control was used.	

Section A7.1.1.1.2 **Annex Point IIA, II.7.6.2.2** **IUCLID 3.1.1/01**		**Phototransformation in water including identity of transformation products**	
		Water used to make the buffer solutions was purified to meet ASTM-D1193 standards for reagent grade water. There was no co-solvent used. Buffer solutions were sterilized prior to use and an application solution of 7.3 mL aqueous radiolabeled glutaraldehyde containing 817,123,348 dpm (2707 ug) was added to 253 mL sterilized buffer. The final concentration of the radiolabeled glutaraldehyde was 10.4 ppm. All samples (except the controls) were irradiated in duplicate at time 0 and five subsequent time points. The sampling intervals were 0, 3, 7, 15, 23 and 30 days. Sodium bisulfite and sodium hydroxide traps were used to trap volatiles and CO_2, respectively. Samples were not extracted prior to LSC and HPLC analysis; they were analyzed immediately. Liquid scintillation counting was used to determine radiocarbon. HPLC was used to identify the parent and degradates. Chromatographic methods were validated with authentic analytical standards. Products with yields as low as 0.2% could be reliably quantified and 0.1% yields detected. All samples were stored in freezers until analyzed. Light and dark samples of Day 30 solutions were reanalyzed by HPLC after 4 weeks. No change was observed in the radio-chromatograms. The photodegradation rate constant and half-lives of glutaraldehyde were calculated assuming pseudo first-order kinetics. The degradation rate constants were calculated from a standard equation.	
5.2	**Results and discussion**	The overall mass balance was 94.4%. No radiocarbon was detected in soduim bisulfite traps at levels greater than 3.9%, or in sodium hydroxide traps at levels greater than 2.5%. Cultures of the buffer solution (including light and dark control samples) were evaluated, and no growth was observed in any of the samples. Glutaraldehyde did not degrade appreciably under natural sunlight and in the dark, as evidenced by the slow degradation rates observed and lack of product formation at greater than 10% yield. The extrapolated half-life in the light-exposed samples was 196 days (R^2 = 0.861), and in the dark was 355 days (R^2 = 0.688). Due to the limited extent of degradation, no degradates were identified.	
5.2.1	k^e_p	4.09×10^{-3} [$1\ s^{-1} \times 10^5$]	
5.2.2	K_{pE}	Not determined	
5.2.3	ϕ^e_E	Not determined	
5.2.4	$t_{1/2E}$	Not determined	
5.3	**Conclusion**	1,5-^{14}C-glutaraldehyde did not degrade appreciably in pH 5 buffer systems during a 30-day irradiation period. Extrapolated half-life of exposed and dark samples was 196 days and 355 days, respectively. Results indicate that photochemical processes are not a significant factor in the degradation of glutaraldehyde in aqueous environments.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	
		Evaluation by Competent Authorities	

Section A7.1.1.1.2 Annex Point IIA, II.7.6.2.2 IUCLID 3.1.1/01	**Phototransformation in water including identity of transformation products**	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	3.9.2008	
Materials and Methods	Applicant's version is mostly correct, a few minor deviations to the original test report are given below. 4.4.2: The average % recovery of the radioactivity was 93.2 ± 3.8 %. 4.4.3, 5.2.1: k^e_p of 4.09 x 10^{-3} [1 s^{-1} x 10^5] is not reported in the original study report. 5.1: Light intensity ranged from 61 to 19947 µW/cm^2. 5.2: No radiocarbon was detected in sodium hydroxide traps at levels greater than 1.92%.	
Results and discussion	The photolytic half-life of ^{14}C-glutaraldehyde was 196 days. The corresponding half-live in the dark control was 355 days. No transformation products ≥ 10% were detected. The UV/VIS adsorption maxima was 283 nm.	
Conclusion	Glutaraldehyde is photolytically stable at pH 5.	
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 A7.1.1.1.2/01-1 Description of test solution and controls

Criteria	Details
Purity of water	Sterilized, distilled
Preparation of test solutions and test concentrations (ppm)	The pH 5 buffer system was prepared with 146 mL of 0.10M acetic acid solution, added to 100 mL of 0.10M NaOH. The final volume was brought to 1 litre with distilled water. An application solution of 7.3 mL aqueous radiolabeled glutaraldehyde containing 817,123,348 dpm (2707 µg) to 253 mL sterilized buffer. The final concentration of the radiolabeled glutaraldehyde was 10.4 ppm.
Temperature (°C)	Temperature of the water bath was 24.7 ± 2.6°C.
Preparation of actinometer solution	Not applicable
Controls	Dark control samples were prepared in the same manner as exposed samples. They were placed in pyrex tubes and wrapped in foil to prevent irradiation.
Identity and concentration of co-solvent	None required.

Table A7.1.1.1.2/01-2 Description of test system

Criteria	Details
Laboratory equipment	Solutions were made and irradiated in quartz sample tubes; dark controls were in pyrex tubes, wrapped in foil during the study period. All samples (except the controls) were irradiated in duplicate at time 0 and five subsequent time points. A commercial LSC, UV, and HPLC were required for conduct of the study.
Properties of artificial light source	Not applicable. Natural sunlight was used.
Nature of light source	Not applicable.
Emission wavelength spectrum	Not applicable.
Light intensity	Not applicable.

Table A7.1.1.1.2/01-3 Average Material Balance and Product Distribution for [1,5-^{14}C]-Glutaraldehyde Expressed as Percent of Applied Radiocarbon

Sample		% Recovery	% Glutaraldehyde	% Unknowns
Day 0	Light	98.3	94.1	4.2
	Dark	98.2	94.1	4.2
Day 3	Light	94.3	89.9	4.4
	Dark	93.8	87.6	6.3
Day 7	Light	94.4	88.7	5.7
	Dark	96.9	91.5	5.4
Day 15	Light	89.5	82.1	7.5
	Dark	93.0	89.0	4.0
Day 23	Light	88.0	78.1	9.9
	Dark	92.8	88.3	4.6
Day 30	Light	88.3	75.6	12.7
	Dark	91.4	83.5	7.9
		93.2 ± 3.5 %		

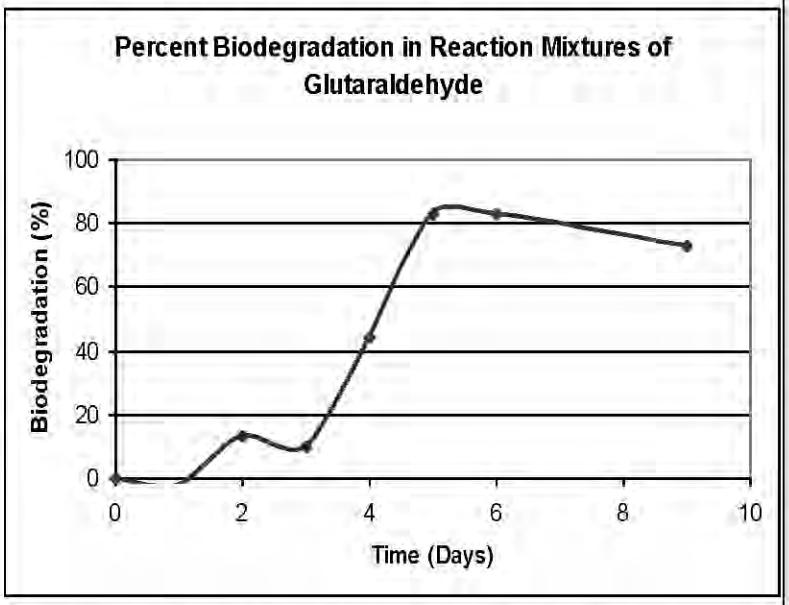
Table A7.1.1.1.2/01-4 Material Balance and Volatile Trapping Following Application of [1, 5-^{14}C]-Glutaraldehyde and Irradiation with Natural Sunlight.

Sample/ Replicate	Nominal Applied		Radiocarbon In Solution		Volatiles NaHCO3		Volatiles KOH		Recovered	
	DPM	PPM	DPM	PPM	DPM	%	DPM	%	DPM	Percent
Day 0										
Light Exposed 1	31391600	10.4	30933257	10.2	-			-	30933257	98.5
Light Exposed 2	31391600	10.4	30778701	10.2	-	-	-	-	30778701	98.0
Dark Control 1	31391600	10.4	30776645	10.2	-	-	-		30776645	98.0
Dark Control 2	31391600	10.4	30887009	10.2		-	-	-	[illegible]	98.4
Day 3										
Light Exposed 1	31391600	10.4	29506976	9.4	29591	0.1	25602	0.08	29650561	94.5
Light Exposed 2	31391600	10.4	29591075	9.8	29583	0.1	25602	0.08	29644[illegible]	94.4
Dark Control 1	31391600	10.4	28519978	9.8	2476	0.01	5114	0.02	29527368	94.1
Dark Control 2	31391600	10.4	29391391	9.7	2476	0.01	5114	0.02	29296981	93.6
Day 7										
Light Exposed 1	31391600	10.4	29580090	9.8	145909	0.5	44812	0.14	29770411	94.8
Light Exposed 2	31391600	10.4	29562003	9.8	145909	0.5	44811	0.14	29751684	95.0
Dark Control 1	31391600	10.4	30430008	10.1	4068	0.01	5490	0.02	30440396	97.0
Dark Control 2	31391600	10.4	30549800	10.1	4068	0.01	5490	0.02	30561168	97.4
Day 15										
Light Exposed 1	31391600	10.4	27830899	9.1	461155	1.5	333384	1.06	28626001	91.2
Light Exposed 2	31391600	10.4	28934857	9.4	461155	1.5	333384	1.06	29129860	92.8
Dark Control 1	31391600	10.4	29968384	9.5	7759	0.02	11991	0.04	28937334	93.3
Dark Control 2	31391600	10.4	29381050	9.7	7759	0.02	11991	0.04	29400100	93.6
Day 23										
Light Exposed 1	31391600	10.4	27772765	9.5	785895	2.5	534868	1.7	29092568	92.7
Light Exposed 2	31391600	10.4	27459003	9.1	785895	2.5	533998	1.7	28758896	91.6
Dark Control 1	31391600	10.4	29249240	9.3	11445	0.04	18126	0.06	29278811	93.3
Dark Control 2	31391600	10.4	29017713	9.5	11445	0.04	18126	0.06	29046984	92.5
Day 30										
Light Exposed 1	31391600	10.4	27983025	9.3	1225956	3.9	601802	1.92	29810783	95.0
Light Exposed 2	31391600	10.4	27481037	9.1	1225956	3.9	601802	1.92	29308795	93.4
Dark Control 1	31391600	10.4	30427300	10.4	12350	0.04	159882	0.51	30600782	97.5
Dark Control 2	31391600	10.4	26974955	8.5	12350	0.04	159882	0.51	27146187	86.5

Average = [illegible]

Section A7.1.1.2.1 Annex Point IIA, VII.7.6.1.1 **IUCLID 3.5/01**		**A7.1.1.2.1 Ready Biodegradability** [redacted] **Ready biodegradability by the dissolved organic carbon die-away test method**	
		1 REFERENCE (A7.1.1.2.1/01)	**Official use only**
1.1	**Reference**	[redacted] (2000) [redacted]: Ready biodegradability by the dissolved organic carbon die-away test method. [redacted] Unpublished, 23 March 2000	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD guideline 301A	
2.2	**GLP**	Yes	
2.3	**Deviations**	The test was terminated after 9 days due to substantial biodegradation, and the reference substance purity and stability was not characterized under GLP's.	x
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde, 50% [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Not given	
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	None	x
3.1.5	Composition of Product	3.1.3	
3.1.6	TS inhibitory to microorganisms	No	x
3.2	**Reference substance**	Sodium benzoate	
3.2.1	Initial concentration of reference substance	15 mg/L	x
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	Details of the inoculum are provided in **Table A7.1.1.2.1/01-2**.	
3.3.2	Test system	Details on test system, laboratory equipment, etc., are provided in **Table A7.1.1.2.1/01-3**.	
3.3.3	Test conditions	Relevant test conditions are provided in **Table A7.1.1.2.1/01-4**.	

Section A7.1.1.2.1 Annex Point IIA, VII.7.6.1.1 **IUCLID 3.5/01**		**A7.1.1.2.1 Ready Biodegradability** [redacted] **Ready biodegradability by the dissolved organic carbon die-away test method**	
3.3.4	Method of preparation of test solution	Portions of the aqueous stock solution containing glutaraldehyde were added to the inoculated mineral medium in separate flasks (darkened 2-liter Erlenmeyer flasks) to give nominal concentrations of 15 mg/L.	
3.3.5	Initial TS concentration	15 mg/L	x
3.3.6	Duration of test	9 days	
3.3.7	Analytical parameter	DOC	
3.3.8	Sampling	Duplicate samples from the 3 reaction mixtures (3 each for inoculum blank, reference substance, and test material) were analyzed for DOC on days 0, 1, 2, 3, 4, 5, 6, 9 using a Shimadzu TOC analyzer or equivalent. The percent degradation based on removal of DOC was determined.	
3.3.9	Intermediates/ degradation products	Not identified as part of this study.	
3.3.10	Nitrate/nitrite measurement	No	
3.3.11	Controls	Yes Positive control and toxicity controls	x
3.3.12	Statistics	**Calculation of Percent Degradation at Time (t)** $D_t = [1-((C_t-C_B/ (C_A-C_{BA}))] * 100$ *where,* D_t = % degradation at time t C_A = mean starting concentration of DOC in test mixtures C_t = mean concentration of DOC in the test mixtures at time t C_{BA} = mean starting concentration of DOC in the solution control C_B = mean concentration of DOC in the inoculum control at time t	
		4 RESULTS	
4.1	**Degradation of test substance**	After a short lag time, the degradation of the test material was observed. An average of 13% DOC removal was observed on day 2. By day 5, DOC removal had reached an average of 83%. Sampling was continued only 2 intervals beyond day 5 since DOC removal had plateaued and the ready biodegradability pass level had been achieved. **The average DOC removal on days 6 and 9 were 83% and 73% respectively.**	

Section A7.1.1.2.1 Annex Point IIA, VII.7.6.1.1 **IUCLID 3.5/01**	**A7.1.1.2.1 Ready Biodegradability** [redacted] **Ready biodegradability by the dissolved organic carbon die-away test method**	
4.1.1 Graph		
4.1.2 Degradation	The average DOC removal on days 6 and 9 were 83% and 73% respectively.	
4.1.3 Other observations	See below.	
4.1.4 Degradation of TS in abiotic control	There were no killed (abiotic) controls.	
4.1.5 Degradation of reference substance	The positive control, sodium benzoate, reached 96% DOC removal in less than 9 days.	
4.1.6 Intermediates/ degradation products	Not identified as part of this study.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The test system is described in 3.3; the guideline is given in 2.1. No relevant deviations from the guideline occurred. Criteria for the biodegradability testing are found in **Table A7.1.1.2.1/01-1**.	
5.2 Results and discussion	The temperature range of the test was 18-21°C, and within the protocol range throughout the test. Measured cell densities and suspended solids concentration of the inocula are reported to be within the levels specified by the testing guidelines. The measured TOC of the test and reference stock solutions used to dose the test media were 224.5 and 421.3 mg C/L respectively. The measured pH of the test solutions prior to the addition of the inoculum ranged from 7.1-7.2. The viability of the inoculum and validity of the test were supported by the results of the reference substance (sodium benzoate) which degraded approximately 96% based on DOC removal. Since the average percent biodegradation of the reference substance was greater than 60% which was achieved prior to day 14, the criteria of a valid test were fulfilled. After a short lag time, the degradation of the test material was observed.	x

Section A7.1.1.2.1 Annex Point IIA, VII.7.6.1.1 **IUCLID 3.5/01**	**A7.1.1.2.1 Ready Biodegradability** [redacted] **Ready biodegradability by the dissolved organic carbon die-away test method**	
	An average of 13% DOC removal was observed on day 2. By day 5, DOC removal had reached an average of 83%. Sampling was continued only 2 intervals beyond day 5 since DOC removal had plateaued and the ready biodegradability pass level had been achieved. The average DOC removal on days 6 and 9 were 83% and 73% respectively.	
5.3 Conclusion	Glutaraldehyde meets the strict criteria (**Table 7.1.1.2.1/01-6**) of a "readily biodegradable" classification according to the current guidelines of the OECD 301 A test, indicating glutaraldehyde will not persist in the environment.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	30.1.2008	
Materials and Methods	3.1.4 The chosen method, OECD 301A, is considered suitable for glutaraldehyde since it is very water soluble (≥ 513 g/l) and it does not volatilize easily from water (Henry's law constant 5.68 x 10^{-2} Pa m^3 mol^{-1}). 3.1.6 The test substance is an antimicrobial agent, EC50 > 51 mg/l in the activated sludge respiration inhibition test, OECD 209. 3.2.1 The concentration of the reference substance is 15 mg TOC/l. 3.3.5 The concentration of test substance is 15 mg TOC/l. According to the test guideline the concentration of the test substance should be 10-40 mg DOC/l. 3.3.11 According to OECD 301A terminology the controls are called inoculum blank and procedure control (control with the reference substance). 5.2 The average percentage of biodegradation required from the reference substance is 70% removal of DOC, not 60%.	
Results and discussion	73% removal of DOC was achieved by day 9. The degradation took place in 10-day window. Glutaraldehyde was considered as readily biodegradable.	
Conclusion	Glutaraldehyde is readily biodegradable.	
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*	

Section A7.1.1.2.1 **Annex Point IIA, VII.7.6.1.1** **IUCLID 3.5/01**	**A7.1.1.2.1 Ready Biodegradability** [redacted] **Ready biodegradability by the dissolved organic carbon die-away test method**	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A7.1.1.2.1/01-1 Guideline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
DOC Die-Away-Test	C.4-A	301A	ready
CO_2 Evolution-Test (Modified Sturm Test)	C.4-C	301B	ready
Modified OECD-Screening-Test	C.4-B	301E	ready
Manometric Respirometry	C.4-D	301F	ready
MITI-I-Test	C.4-F	301C	ready
Closed-Bottle-Test	C.4-E	301D	ready
Zahn-Wellens-test	C.9	302B	Inherent
Modified MITI-Test (II)	-	302C	Inherent
Modified SCAS-Test	C.12	302A	Inherent
Simulation Test with activated Sewage (Coupled Units-Test)	C.10	302A	Simulation Test[1)]

[1)] Test for the determination of the ultimate degradation of test material under conditions which simulate the treatment in an activated sludge plant

Table A7.1.1.2.1/01-2 Inoculum / Test organism

Criteria	Details
Nature	Municipal activated sludge inoculum
Species	a mixture
Strain	a mixture
Source	[redacted]
Laboratory culture	No
Method of cultivation	collected from a wastewater treatment plant
Preparation of inoculum for exposure	A sample of the activated sludge was collected from [redacted] Sludge was screened and filtered, and maintained in a porous pot bioreactor for 20 days prior to use. The inoculum was homogenized in a blender, and allowed to settle for 30 minutes. Due to a high level of unsettled solids, centrifugation was necessary to prepare an acceptable inoculum. The supernatant was removed, and aerated for 4 hours prior to use. Initial DOC and total suspended solids was measured, and a standard plate count was performed on the inoculum. Plates were incubated for 48 hours at 20 ± 3 °C.
Pre-treatment	None
Initial cell concentration	307 mg solids/L (3.3×10^6 CFU/L)

Table A7.1.1.2.1/01-3 Test system

Criteria	Details
Number of culture flasks/concentration	3 vessels/concentration
Measuring equipment	DOC concentrations in aqueous samples were determined using a Shimadzu analyzer or equivalent. Reported DOC concentrations represent the average for duplicate analyses of each sample.
Test performed in closed vessels	No

Table A7.1.1.2.1/01-4 Test conditions

Criteria	Details
Composition of medium	An aqueous mineral salts medium provided essential mineral nutrients and trace elements. The mineral salts medium was prepared by addition of reagent grade salts to Nanopure water. Compound — Concentration in Sample (per Liter of water) Calcium chloride 1 mL Ferric chloride 1 mL Magnesium sulfate 1 mL phosphate buffer (pH 7.2) 10 mL
Additional substrate	No
Test temperature	18-21 °C
pH	7.1-7.2
Aeration of dilution water	Yes, with humidified air
Suspended solids concentration	The final concentration of total suspended solids was 20.6 mg solids/L (2.21×10^5 CFU/mL).
Other relevant criteria	stirred / mixed on 15-minute on-off cycle.

Table 7.1.1.2.1/01-5 Biodegradation of Glutaraldehyde in DOC Die-Away Study (OECD 301A)

	DOC (mgC/L)*							
	Day 0	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Day 6*	*Day 9*
Control	3.411	3.167	3.311	3.556	3.544	3.267	3.200	2.433
Sodium benzoate	19.811	4.560	3.544	3.722	4.233	3.411	3.133	3.100
Glutaraldehyde	18.989	19.044	16.822	17.600	12.222	5.911	5.800	6.567

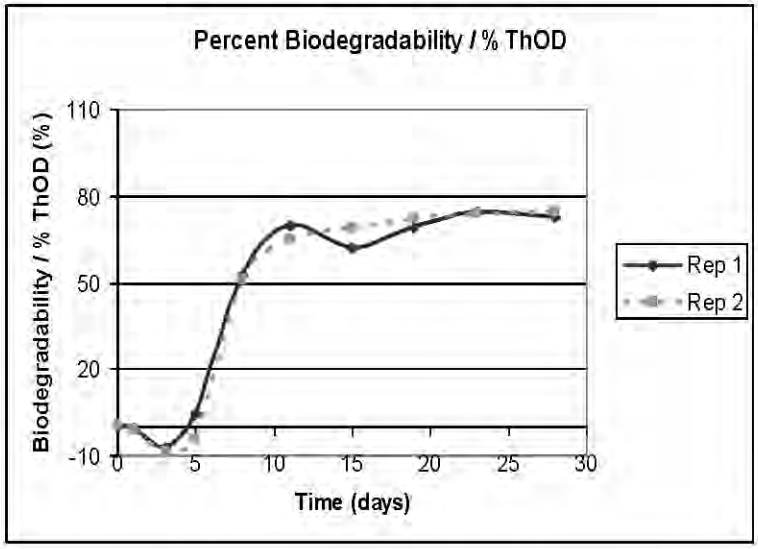
* average of duplicate samples from 3 reaction vessels for each treatment group

Table A7.1.1.2.1/01-6 Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$	X	
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test	X	
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	X	
Percentage of removal of reference substance reaches pass level by day 14	X	

Section A7.1.1.2.3 Annex Point IIA, VII.7.6.2.3 IUCLID 3.5/03	**A7.1.1.2.3 Biodegradation in Seawater** **Biodegradability in Seawater Study- Closed Bottle Method**	
	1 REFERENCE (A7.1.1.2.3/01)	**Official use only**
1.1 Reference	[redacted] (2000) Biodegradability in Seawater Study- Closed Bottle Method, Amended Final Report Including Page 3a, [redacted], Unpublished, 28 March 2000	
1.2 Data protection	Yes	
1.2.1 Data owner	The Dow Chemical Company	
1.2.2 Companies with letter of access	[redacted]	
1.2.3 Criteria for data protection	Data on new a.s. for first entry to Annex I	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes OECD guideline 306	
2.2 GLP	Yes	
2.3 Deviations	No	
	3 MATERIALS AND METHODS	
3.1 Test material	Glutaraldehyde, 50% [redacted]	
3.1.1 Lot/Batch number	[redacted]	
3.1.2 Specification	Not reported	
3.1.3 Purity	[redacted]	
3.1.4 Further relevant properties	None	
3.1.5 Composition of Product	3.1.3	
3.1.6 TS inhibitory to microorganisms	No	
3.2 Reference substance	Sodium benzoate	
3.2.1 Initial concentration of reference substance	A 1000 mg/L (583.44 mgC/L) solution was used to make up the test solution of **2.0 mg/L sodium benzoate**.	
3.3 Testing procedure		
3.3.1 Inoculum / test species	Details of the inoculum are provided in **Table A7.1.1.2.3/01-2**.	
3.3.2 Test system	Details on test system, laboratory equipment, etc., are provided in Table **A7.1.1.2.3/01-3**.	

Section A7.1.1.2.3 Annex Point IIA, VII.7.6.2.3 **IUCLID 3.5/03**	**A7.1.1.2.3 Biodegradation in Seawater** **Biodegradability in Seawater Study- Closed Bottle Method**	
3.3.3 Test conditions	Relevant test conditions are provided in **Tables A7.1.1.2.3/01-4**.	
3.3.4 Method of preparation of test solution	A 1000 mg glutaraldehyde/L (613 mgC/L) stock solution was made by adding the test material to deionized water. The solution was used to make a **3 mg/L test solution**.	
3.3.5 Initial TS concentration	3 mg/L test solution	
3.3.6 Duration of test	28 days	
3.3.7 Analytical parameter	Dissolved oxygen concentration (depletion over time, BOD). The extent of biodegradation was calculated as biological oxygen demand as a percent of theoretical oxygen demand (%ThOD).	
3.3.8 Sampling	Oxygen concentration was measured on days 0, 1, 3, 5, 8, 11, 15, 19, 23, 28. The pH was measured initially and on day 28.	
3.3.9 Intermediates/ degradation products	Not identified as part of this study.	
3.3.10 Nitrate/nitrite measurement	Not applicable.	
3.3.11 Controls	Yes Inoculum blank (natural seawater test medium only) Procedural control (natural seawater medium with the control substance sodium benzoate) Toxicity control (natural seawater medium with the test substance, and with sodium benzoate)	
3.3.12 Statistics	**Percentage degradation of the test substance** BOD/ThOD x 100 where, BOD = biological oxygen demand ThOD = theoretical oxygen demand	
	4 RESULTS	
4.1 Degradation of test substance	Yes	

Section A7.1.1.2.3 Annex Point IIA, VII.7.6.2.3 **IUCLID 3.5/03**	**A7.1.1.2.3 Biodegradation in Seawater** **Biodegradability in Seawater Study- Closed Bottle Method**	
4.1.1 Graph	 data shown above for 2 replicate test systems of glutaraldehyde	
4.1.2 Degradation	72-75 %ThOD after 28 day(s) **Table A7.1.1.2.3/01-5, 6**	
4.1.3 Other observations	None	
4.1.4 Degradation of TS in abiotic control	Not applicable	
4.1.5 Degradation of reference substance	Test data (>89 %ThOD) for the procedural control indicated that the inoculum was viable and able to degrade a soft carbon source like sodium benzoate. **Table A7.1.1.2.3/01-5, 6**	
4.1.6 Intermediates/ degradation products	Not identified as part of this study.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The test method followed that of OECD 306, with no guideline deviations stated in the report or protocol. Duplicate test vessels were prepared for the test substance, the procedural blank, and the toxicity controls at each of 10 sampling points in the following manner: Required volumes of the respective test substance stock solution were added to the treatment bottles to achieve a concentration of 3.0 mg/L for both the test suspension and the toxicity control. Sodium benzoate was used as the reference control at 2.0 mg/L. An inoculum blank was prepared containing only the test medium (seawater amended with mineral salts medium) to monitor background dissolved oxygen depletion. No additional inoculum other than those microorganisms already in the natural seawater was added to the treatment bottles. The seawater had salinity of 30.1ppt, specific conductance of 46,341 uS/cm, dissolved oxygen of 8.36 mg/L, and pH of 7.65 when it was sampled	

Section A7.1.1.2.3 Annex Point IIA, VII.7.6.2.3 **IUCLID 3.5/03**	**A7.1.1.2.3 Biodegradation in Seawater** **Biodegradability in Seawater Study- Closed Bottle Method**	
	from the collection point. Each BOD bottle was partially filled with test medium. Volumes of the test and/or reference substances were added to the bottles as appropriate. BOD bottles were filled to volume. Test vessels were sealed, placed on a platform table, and left at 16-18C for the duration of the study. The dissolved oxygen was determined in the BOD bottles on days 0, 1, 3, 5, 8, 11, 15, 19, 23, and 28.	
5.2 Results and discussion	Test data (high %ThOD) for the procedural control indicated that the inoculum was viable and able to degrade a soft carbon source like sodium benzoate. The test substance met the acceptability criteria for a material that has potential for biodegradation in the marine environment (>60% ThOD). The dissolved oxygen concentrations in the toxicity controls decreased to below 1 mg/L within 11 days, attributed to the large quantity of carbon added to the reaction mixtures. Thus, the extent of degradation in the toxicity controls could not be reliably quantified. However, due to extensive degradation observed in both test and procedural control treatments, inhibition of the microbial inoculum by the concentration of glutaraldehyde added was not a factor in the study. **Table A7.1.1.2.3/01-5, 6** The test substance reached a percent biodegradability of greater than 60% by day 11 and an overall biodegradability of 73.4% by day 28, indicating that it has substantial potential for biodegradation in the marine environment. **Table A7.1.1.2.3/01-5, 6**	
5.3 Conclusion	Since biodegradation of the test material exceeded 60% of ThOD within 28 days, biodegradation in seawater is expected.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	3.2.2009	
Materials and Methods	3.1.6 The test substance is an antimicrobial agent, EC50 > 51 mg/l in the activated sludge respiration inhibition test, OECD 209. Table A7.1.1.2.3/01-7 The validity criteria do not correspond to the validity criteria in the OECD 306.	
Results and discussion	73% biodegradability (BOD/ThOD) was achieved by day 28. There was a five day lag period and 51.78% degradation was achieved by day 8. The validity criteria listed in the OECD 306 were fulfilled. The blank respiration did not exceed 30% of the oxygen in the test bottle. The reference substance met the degradation criteria obtained in the ring test. According to the OECD 306 the test substance can be considered inhibitory to bacteria as the BOD of the toxicity control was less than the sum of BOD from test and procedure control. In the test report the low degradation in the toxicity control was explained by the excess carbon that was added to the test vessels.	
Conclusion	Glutaraldehyde has a potential to biodegrade in the marine environment.	

Section A7.1.1.2.3 **Annex Point IIA, VII.7.6.2.3** **IUCLID 3.5/03**	**A7.1.1.2.3 Biodegradation in Seawater** **Biodegradability in Seawater Study- Closed Bottle Method**	
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 A7.1.1.2.3/01-1 Guideline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
DOC Die-Away-Test	C.4-A	301A	ready
CO_2 Evolution-Test (Modified Sturm Test)	C.4-C	301B	ready
Modified OECD-Screening-Test	C.4-B	301E	ready
Manometric Respirometry	C.4-D	301F	ready
MITI-I-Test	C.4-F	301C	ready
Closed-Bottle-Test	C.4-E	301D	ready
Zahn-Wellens-test	C.9	302B	Inherent
Modified MITI-Test (II)	-	302C	Inherent
Modified SCAS-Test	C.12	302A	Inherent
Simulation Test with activated Sewage (Coupled Units-Test)	C.10	302A	Simulation Test[1)]

[1)] Test for the determination of the ultimate degradation of test material under conditions which simulate the treatment in an activated sludge plant

Table A7.1.1.2.3/01-2 Test organisms and Water

Criteria	Details
Nature	Natural Seawater
Species	a mixture
Strain	a mixture
Source	[redacted]
Laboratory culture	No
Method of cultivation	Natural seawater sample
Preparation of Inoculum	A subsample of the seawater was sent to [redacted] for a marine agar plate count. The result of the plate count was 1.34×10^3 CFU/mL. No additional inoculum other than those microorganisms already in the natural seawater was added to the treatment bottles.

Table A7.1.1.2.3/01-3 Test system

Criteria	Details
Number of culture flasks/concentration	2 vessels/concentration
Test System	Each BOD bottle was partially filled with test medium. Volumes of the test and/or reference substances were added to the bottles as appropriate. BOD bottles were filled to volume. Test vessels were sealed, placed on a platform table, and left at 16-19°C for the duration of the study.
Test performed in closed vessels	Yes

Table A7.1.1.2.3/01-4 Test conditions

Criteria	Details
Additional substrate	No
Test temperature	16-19°C (17.0 ± 1 °C for throughout the study for over 94% of the time)
pH	7.65
Salinity	30.1 ppt
Specific Conductance	46,341 uS/cm
Dissolved Oxygen	8.36 mg/L
Aeration of dilution water	No

Table A7.1.1.2.3/01-5 Biological Oxygen Demand

Test Condition	Biological Oxygen Demand Values (mgO_2 / mg)									
Test Day	0	1	3	5	8	11	15	19	23	28
Glutaraldehyde-Replicate 1	0.00	-0.007	-0.135	0.083	1.013	1.340	1.192	1.323	1.428	1.397
Glutaraldehyde-Replicate 2	0.00	-0.023	-0.172	-0.080	0.973	1.247	1.315	1.383	1.418	1.420
Procedural Control-Replicate 1	0.00	0.940	0.998	1.270	1.380	1.420	1.413	1.470	1.573	1.530
Procedural Control-Replicate 2	0.00	0.930	0.993	1.255	1.320	1.475	1.323	1.465	1.403	1.485
Inoculum Blank-Replicate 1	0.00	0.150	0.900	0.790	0.880	0.950	1.270	1.140	1.250	1.330
Inoculum Blank-Replicate 2	0.00	0.150	0.870	0.830	0.920	0.930	1.160	1.140	1.280	1.390
Toxicity Control-Replicate 1	0.00	0.010	0.319	0.606	1.054	1.246	1.211	1.244	1.217	1.194
Toxicity Control-Replicate 2	0.00	0.010	0.315	0.586	1.012	1.216	1.219	1.250	1.215	1.198

Table A7.1.1.2.3/01-6 Percent Biodegradability / % ThOD

Test Condition	Percent Biodegradability / % ThOD									
Test Day	0	1	3	5	8	11	15	19	23	28
Glutaraldehyde-Replicate 1	0.0	-0.36	-7.04	4.33	52.62	69.87	62.15	68.98	74.46	72.84
Glutaraldehyde-Replicate 2	0.0	-1.20	-8.97	-4.17	50.73	65.02	68.56	72.11	73.94	74.04
Glutaraldehyde-MEAN	0.0	-0.78	-8.01	0.08	51.78	67.45	65.36	70.55	74.20	73.44
Procedural Control-Replicate 1	0.0	56.44	59.92	76.25	82.86	85.26	84.84	88.26	94.45	91.86
Procedural Control-Replicate 2	0.0	55.84	59.62	75.35	79.25	88.56	79.44	87.96	84.24	89.16
Procedural Control-MEAN	0.0	56.14	59.77	75.80	81.06	86.91	82.14	88.11	89.35	90.51
Toxicity Control-Replicate 1	0.0	0.28	8.90	16.91	29.41	34.77	33.79	34.72	33.96	33.32
Toxicity Control-Replicate 2	0.0	0.28	8.79	16.35	28.24	33.93	34.02	34.88	33.91	33.43
Toxicity Control-MEAN	0.0	0.28	8.85	16.63	28.83	34.35	33.91	34.80	33.94	33.38

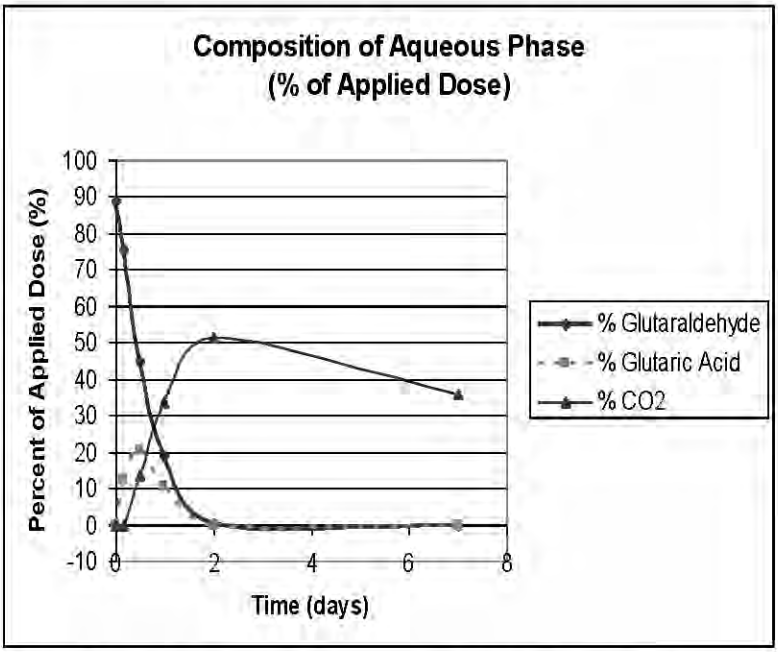
Table A7.1.1.2.3/01-7 Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$	X	
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test	n/a	n/a
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	X	
Percentage of removal of reference substance reaches pass level by day 14	X	

Section A7.1.2.2.2(1) Annex Point IIIA, XII.2.1 IUCLID 3.5/05		Water/Sediment Degradation Study Aerobic Aquatic Metabolism of ^{14}C- Glutaraldehyde in River Water and Sediment	
		1 REFERENCE (A7.1.2.2.2/01)	Official use only
1.1	**Reference**	[redacted] (1994a) Aerobic Aquatic Metabolism of ^{14}C- Glutaraldehyde in River Water and Sediment, [redacted], GLP, Unpublished, 25 May 1994	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company (Dow) [redacted]	
1.2.2	Companies with letter of access	[redacted] Dow	
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 Pesticide Assessment N 162-4	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	1,5-^{14}C-carbonyl-glutaraldehyde	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	 [1,5-^{14}C]-Glutaraldehyde * denotes position of the radiolabel, ^{14}C	
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	Glutaraldehyde specific properties: Vapour pressure: 15.33 hPa @ 20 °C Water solubility: 100% (vol) @ 20 °C Adsorption potential (log P_{ow}): -0.33	
3.1.5	Composition of Product	active substance used	
3.1.6	TS inhibitory to microorganisms	No (**Ref A7.1.2.2.2/01**)	
3.1.7	Specific chemical analysis	Radioactivity in volatile traps and water was monitored by liquid scintillation counting (LSC). Post-extraction sediments analysed by oxidative combustion/LSC. Radiochemical purity was determined by HPLC with radiochemical detection. Thin-layer chromatography was used to confirm the identity of 14C-glutaraldehyde and glutaric acid in water, with confirmatory analysis of the metabolites by HPLC.	

Section A7.1.2.2.2(1) Annex Point IIIA, XII.2.1 IUCLID 3.5/05		Water/Sediment Degradation Study Aerobic Aquatic Metabolism of ^{14}C- Glutaraldehyde in River Water and Sediment	
3.2	**Reference substance**	No	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	The details of the test sediment are presented in **Table A7.1.2.2.2/01-1.**	
3.3.2	Test system	The details on test type and laboratory equipment etc. are presented in **Table A7.1.2.2.2/01-2.**	
3.3.3	Test conditions	The relevant test conditions are presented in **Table A7.1.2.2.2/01-3**.	
3.3.4	Method of preparation of test solution	An isotopically diluted solution in acetonitrile was prepared for 50 flasks containing 4.5 mg ^{14}C-glutaraldehyde (1.357×10^9 dpm) and 48.9 mg non-labeled glutaraldehyde (96 mg of a 50.9% solution). The solution was diluted to a total volume of 53 mL deionized water. The dosing solution contained 25,552,800 dpm/mL. The equivalent of 20 grams dry weight of sediment (26.4 grams) and 100 mL of river water were added to each flask. Aliquots of the dosing solution (1 mL) were then added to the flasks, and swirled to mix. Flasks were stoppered and maintained at an average temperature of 25.0 $\pm$ 0.1°C.	
3.3.5	Initial TS concentration	The final aqueous phase concentration was 9.45 ug/mL (50.2 ug/g oven dry sediment).	
3.3.6	Duration of test	30 days	
3.3.7	Analytical parameter	The ^{14}C content was measured in each compartment: (ie, the volatile traps, water, and sediment extracts).	
3.3.8	Sampling	The aqueous phase was monitored at 0, 4, 12, 24 and 48 hours and 7, 14 and 30 days for pH (6.3-7.7) and oxygen content (4.9-7.7 ppm). Radioactivity was quantified at the same sampling intervals.	
3.3.9	Intermediates/ degradation products	Thin-layer chromatography was used to confirm the identity of 14C-glutaraldehyde and glutaric acid in water, with confirmatory analysis of the metabolites by HPLC. **Table A7.1.2.2.2/01-9**	
3.3.10	Controls	No	
3.3.11	Statistics	**Sediment Combustion (total DPM in sample)** $\frac{\text{Raw DPM} - \text{Background DPM}}{\text{Oxidizer Efficiency}} * \frac{\text{Total Sediment}}{\text{Aliquot Weight}}$ **Liquid Traps & Foam Plug Extracts** $\frac{\text{Raw DPM} - \text{Background DPM}}{\text{Aliquot Weight}} * \text{Total Volume}$ **Sediment Extracts & Pond Water** $\text{Raw DPM} - \text{Background DPM} * \text{Total Volume}$	

Section A7.1.2.2.2(1) Annex Point IIIA, XII.2.1 IUCLID 3.5/05	Water/Sediment Degradation Study Aerobic Aquatic Metabolism of ^{14}C- Glutaraldehyde in River Water and Sediment	
	Aliquot Weight **Percent Recovery (Mass Balance)** Total DPM Recovered * 100 Total DPM Applied **Percent Yields of Parent / Products Based on Applied Dose** Percent of product (determined by HPLC) in water * percent of dose in water / 100 **Rate Constant and Half-Life of ^{14}C-Glutaraldehyde** Assuming pseudo first-order kinetics, the rate constant is: $\ln C = kt + \ln C_o$ y=mx+b where, k= rate constant c = chemical concentration t = time C_o = initial chemical concentration (t=0) The half life is: $t_{1/2} = \ln 2/k = 0.693/k$	
	4 RESULTS	
4.1 Degradation of test substance	In the beginning of the study, glutaraldehyde was found mainly in the river water (86.9-90.8% of the dose within the first 12-hours). Glutaraldehyde decreased rapidly in the water and was completely metabolized within 48 hours (**Table A7.1.2.2.2/01-5**). The major metabolite was $^{14}CO_2$ (total of 80% in the headspace and the water at the end of the study). Glutaric acid was formed as an intermediate in the water phase (18.9-21.5% of the dose at 12 hours), but was itself completely metabolized within 48 hours.	

Section A7.1.2.2.2(1) Annex Point IIIA, XII.2.1 IUCLID 3.5/05	Water/Sediment Degradation Study Aerobic Aquatic Metabolism of ^{14}C- Glutaraldehyde in River Water and Sediment	
4.1.1 Graph		
4.1.2 Other observations	Glutaraldehyde or its metabolites were adsorbed to the sediment (maximum of 21-25.3% at 48 hours) and could not be extracted completely, even through a series of reflux methods (**Table A7.1.2.2.2/01-6**). Overall material mass balance was 93.3 +/- 9.8% (**Table A7.1.2.2.2/01-4**).	
4.1.3 Other observations	Not applicable	
4.1.4 Intermediates/ degradation products	The major metabolite was $^{14}CO_2$ (total of 80% in the headspace and the water at the end of the study). Glutaric acid was formed as an intermediate in the water phase (18.9-21.5% of the dose at 12 hours), but was itself completely metabolized within 48 hours.	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The study to determine the degradation of glutaraldehyde in an aerobic water / sediment system followed US EPA guideline N 162-4 as provided in Section 2.1. No relevant deviations from the guideline occurred.	
5.2 Results and discussion	In the beginning of the study, glutaraldehyde was found mainly in the river water (86.9-90.8% of the dose within the first 12-hours). Glutaraldehyde decreased rapidly in the water and was completely metabolized within 48 hours (**Table A7.1.2.2.2/01-5**). The major metabolite was $^{14}CO_2$ (total of 80% in the headspace and the water at the end of the study). Glutaric acid was formed as an intermediate in the water phase (18.9-21.5% of the dose at 12 hours), but was itself completely metabolized within 48 hours. Radioactivity from glutaraldehyde or its metabolites was incorporated into biomass or adsorbed to the sediment (maximum of 21-25.3% at 48 hours) and could not be extracted completely, even through a series of reflux methods (**Table A7.1.2.2.2/01-6**). Overall material mass balance was 93.3 +/- 9.8%.	

Section A7.1.2.2.2(1) **Annex Point IIIA, XII.2.1** **IUCLID 3.5/05**	**Water/Sediment Degradation Study** **Aerobic Aquatic Metabolism of ^{14}C- Glutaraldehyde in River Water and Sediment**	
5.3 Half-life	The calculated pseudo-first order half life of glutaraldehyde in water under aerobic conditions was 10.6 hours with a correlation coefficient of 0.995.	
5.4 Conclusion	On the basis of these findings glutaraldehyde is not considered persistent, having a half-life in an aerobic water/sediment system of approximately 10.6 hours.	
5.4.1 Reliability	1	
5.4.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	20.3.2009	
Materials and Methods	The test is performed according to the US EPA guidance before adoption of the OECD 308. The test is well designed and carefully performed and reported. The requirements of the used test protocol were less than that of the OECD 308: - only one water-sediment combination used, two required in the OECD 308, the sediment represented a sediment type with a low organic carbon content and a coarse structure. - the amount of sediment was 20 g dw, 50 g dw required in the OECD 308 - the water:sediment ratio was 5:1, 3:1 or 4:1 recommended in the OECD 308 - The sediment and water were stored at room temperature almost one year before the start of the test, while a maximum of four weeks storage period at 4 °C is allowed in the OECD 308. - transformation products have not been identified and quantified in the sediment - transformation rate in the sediment has not been determined - transformation rate for the whole system has not been derived - mineralisation rate has not been derived The deviations are not regarded to invalidate the test results.	
Results and discussion	The applicant's version is correct.	
Conclusion	Glutaraldehyde dissipated within 48 hours in the water phase. It formed a transient intermediate glutaric acid which was further transformed to CO_2 or dissipated to sediment. The cumulative production of CO_2 was 67.85% of applied activity by day 30 indicating a significant extent of mineralization. The pseudo first-order degradation rate was 10.6 hours. Glutaraldehyde or its metabolites were adsorbed to the sediment (maximum of ca. 20% at 48 hours). Major part of radioactivity was bound to the sediment.	
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*	

Section A7.1.2.2.2(1) Annex Point IIIA, XII.2.1 IUCLID 3.5/05	Water/Sediment Degradation Study Aerobic Aquatic Metabolism of ^{14}C- Glutaraldehyde in River Water and Sediment	
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 A7.1.2.2.2/01-1 Sediment and Water Analysis

Criteria	Sediment
SEDIMENT	
Source	River sediment
Sampling site	[redacted]
pH	8.1
% Organic matter	0.9%
% Sand	93%
% Silt	7%
% Clay	0%
Sediment texture	Sand
1/3 bar moisture	5.46%
CEC (meq/100 g)	4.30 meq / 100 g
WATER	
Alkalinity	86 mg $CaCO_3$ / L
pH	7.7
Conductivity	810 umho/cm
Suspended Solids	160 mg/L
Hardness	140 mg $CaCO_3$ / L

Table A7.1.2.2.2/01-2 Test system

Criteria	Details
Culturing apparatus	500 mL Erlenmeyer flasks (covered with aluminium foil) Each flask (500 mL Erlenmeyer, covered with aluminium foil) was equipped with a ground-glass stopper and glass stopcock inlet and outlet tubes (used to remove volatile metabolites and CO2 while providing replacement air). Test samples were maintained in incubators during the study (25°C).
Number of replicates	2 each sampling time
Measuring equipment	LSC, HPLC-UV, TLC
Aeration	Yes

Table A7.1.2.2.2/01-3 Test conditions

Criteria	Details
Pre-incubation	Yes, approximately 11 months prior to study start
Application rate / concentration	The equivalent of 20 grams dry weight of sediment (26.4 grams) and 100 mL of river water were added to each flask. Aliquots of the dosing solution (1 mL) were then added to the flasks, and swirled to mix. The final aqueous phase concentration was 9.45 ug/mL (50.2 ug/g oven dry sediment).
Additional substrate	No
Solvent	Water
Application volume	1 mL of dosing solution
Test temperature	25°C
Dark	yes
Sampling time points	hours 0, 4, 12, 24, 48 and days 7, 14, and 30

Table A7.1.2.2.2/01-4 Radiocarbon Material Balance (Expressed as Percent of Applied Dose)

Sampling Time & Replicate		^{14}C in Sediment		^{14}C in Water		$^{14}CO_2$		Total
		Percent	ppm	Percent	ppm	Percent	ppm	Percent
0-hour	A	8.4	0.79	93.4	8.83	--	--	101.8
	B	6.8	0.64	93.7	8.85	--	--	100.5
4-hour	A	9.0	0.85	97.3	9.19	0.1	<0.01	106.4
	B	8.1	0.76	90.6	8.57	0.0	<0.01	98.7
12-hour	A	15.7	1.49	84.1	7.95	0.6	0.06	100.4
	B	17.6	1.67	85.0	8.03	0.4	0.04	103.0
24-hour	A	22.2	2.10	63.3	5.99	0.6	0.06	86.2
	B	18.6	1.75	71.2	6.73	0.4	0.04	90.2
48-hour	A*							
	B	25.3	2.39	49.8	4.71	10.3	0.97	85.3
7-day	A	20.0	1.89	38.6	3.64	20.4	1.93	78.9
	B	23.7	2.24	26.1	3.32	19.5	1.84	78.3
14-day	A*							
	B	17.1	1.62	18.6	1.75	48.1	4.54	83.8
30-day	A	11.9	1.12	11.1	1.05	69.4	6.56	92.4
	B	16.1	1.52	13.6	1.29	66.3	6.27	96.0
							Average	93.3 ± 9.8

* Measurements for replicate A are not included. These samples revealed serious losses of radioactive material upon storage probably due to $^{14}CO_2$ formation with only 0 % (48 hr-A) and 2.6 % (14 day-A) recovered.

Table A7.1.2.2.2/01-5 Composition of Aqueous Phase (HPLC)

Sampling Time & Replicate		ppm in Water	Products Detected as Percent of Dose (ppm)					
			% Glutaraldehyde (10.9 min)		% Glutaric Acid (9.5 min)		% CO_2 (12.3 min)	
0-hour	A	8.83	90.8	(8.58)	0.0	(0.00)	0.0	(0.00)
	B	8.85	86.9	(8.22)	0.0	(0.00)	0.0	(0.00)
4-hour	A	9.19	82.0	(7.75)	12.9	(1.22)	0.0	(0.00)
	B	8.57	69.4	(6.56)	11.7	(1.11)	0.0	(0.00)
12-hour	A	7.95	43.4	(4.10)	21.5	(2.03)	13.0	(1.23)
	B	8.03	45.9	(4.34)	18.9	(1.79)	14.4	(1.36)
24-hour	A	5.99	13.9	(1.31)	11.0	(1.04)	32.6	(3.08)
	B	6.73	24.0	(2.26)	10.1	(0.96)	35.0	(3.31)
48-hour	A	5.05	0.3	(0.03)	0.0	(0.00)	52.9	(5.00)
	B	4.71	0.0	(0.00)	0.0	(0.00)	49.8	(4.71)
7-day	A	3.64	0.0	(0.00)	0.0	(0.00)	37.1	(3.51)
	B	3.32	0.0	(0.00)	0.0	(0.00)	34.4	(3.25)

Table A7.1.2.2.2/01-6 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	8.4	0.79	36.0	0.28	48.2	0.38
	B	6.8	0.64	43.0	0.28	69.6	0.45
4-hour	A	9.0	0.85	42.0	0.36	64.3	0.55
	B	8.1	0.76	46.8	0.36	70.7	0.54
12-hour	A	15.7	1.49	22.4	0.33	50.6	0.75
	B	17.6	1.67	23.1	0.39	34.6	0.58
24-hour	A	22.2	2.10	5.6	0.12	11.8	0.72
	B	18.6	1.75	12.3	0.21	23.0	0.90
48-hour	A	21.0	1.98	12.4	0.25	54.6	1.08
	B	25.3	2.39	8.2	0.20	50.7	1.21
7-day	A	20.0	1.89	7.8	0.15	74.5	1.41
	B	23.7	2.24	9.0	0.20	98.4	2.20
14-day	A	15.2	1.44	13.5	0.19	129.0	1.86
	B	17.1	1.62	7.4	0.12	92.6	1.50
30-day	A	11.9	1.12	17.3	0.19	87.7	0.98
	B	16.1	1.52	8.8	0.13	91.9	1.18

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

Figure A7.1.2.2.2/01-1 Proposed metabolic pathway for glutaraldehyde under aerobic conditions

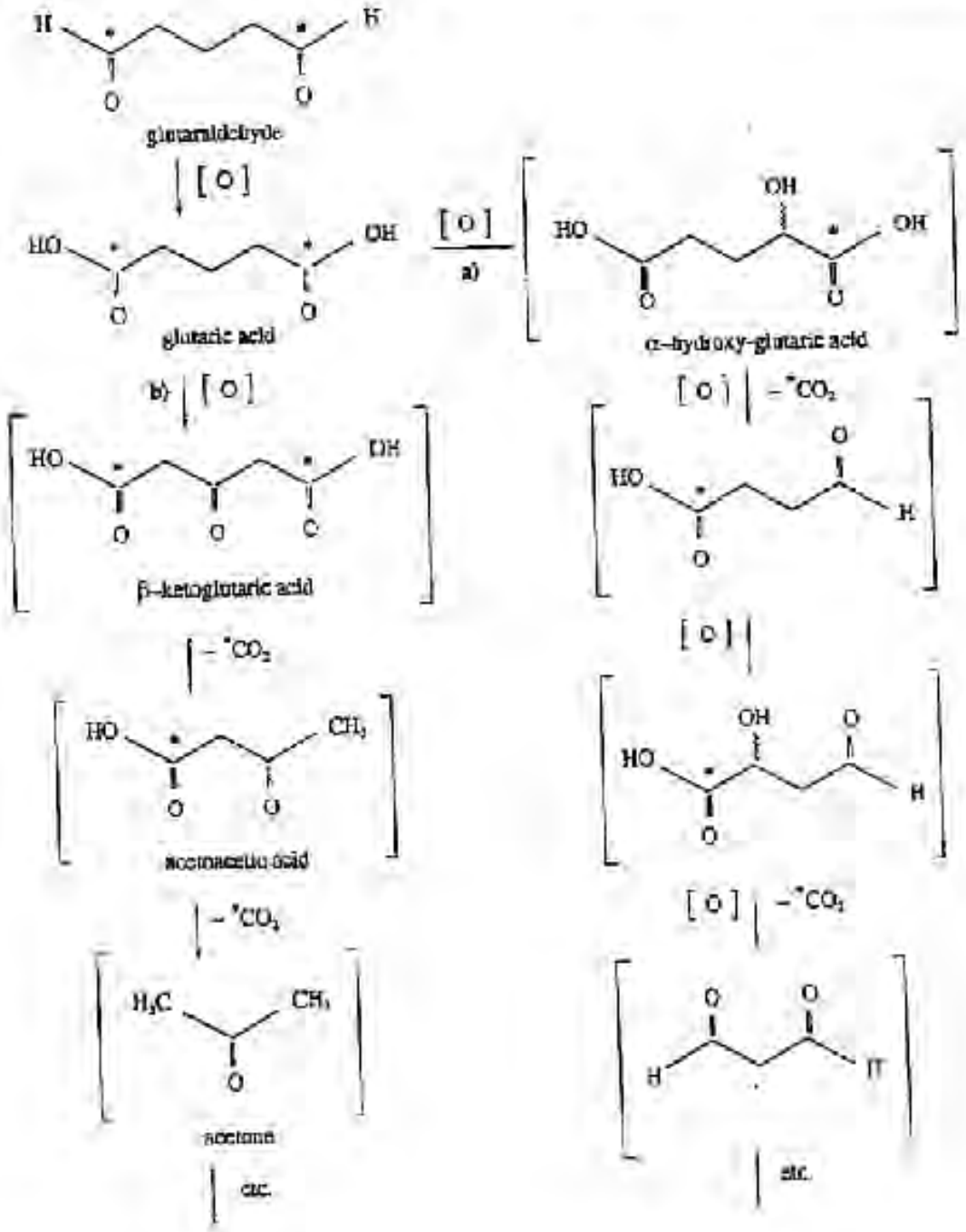
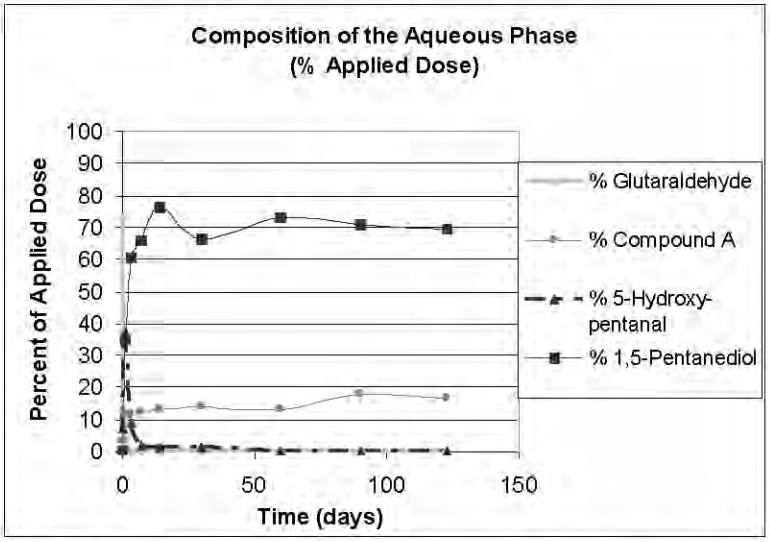


Figure 29. Proposed Metabolic Pathway for [^{14}C]-Glutaraldehyde.

Section A7.1.2.2.2(2) **Annex Point IIIA, XII.2.1** **IUCLID 3.5/06**		**Water/Sediment Degradation Study** **Anaerobic Aquatic Metabolism of ^{14}C-Glutaraldehyde**	
		1 REFERENCE (A7.1.2.2.2/02)	**Official use only**
1.1	**Reference**	[redacted] (1994b) Anaerobic Aquatic Metabolism of ^{14}C-Glutaraldehyde, [redacted], GLP, Unpublished, 2 June 1994	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company (Dow) [redacted]	
1.2.2	Companies with letter of access	[redacted] Dow	
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 Pesticide Assessment N 162-3	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes 1,5-pentanediol was treated as a reagent rather than a reference substance with respect to distribution. The reference and control substances purchased from Aldrich Chemical Co. and Lancaster Synthesis Ltd may not have been characterized under GLP.	
		3 METHOD	
3.1	**Test material**	1,5-^{14}C-carbonyl-glutaraldehyde	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	 [1,5-^{14}C]-Glutaraldehyde * denotes position of the radiolabel, ^{14}C	
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	Glutaraldehyde specific properties: Vapour pressure: 15.33 hPa @ 20 °C Water solubility: 100% (vol) @ 20 °C Adsorption potential (log P_{ow}): -0.33 @ 25 °C	x
3.1.5	Composition of Product	active substance used	
3.1.6	TS inhibitory to microorganisms	No (**Ref A7.1.2.2.2/01**)	
3.1.7	Specific chemical analysis	Radioactivity in volatile traps and water was monitored by liquid scintillation counting (LSC). Post-extraction sediments analysed by LSC. Radiochemical purity was determined by HPLC with	

Section A7.1.2.2.2(2) Annex Point IIIA, XII.2.1 IUCLID 3.5/06		Water/Sediment Degradation Study Anaerobic Aquatic Metabolism of ^{14}C-Glutaraldehyde	
		radiochemical detection. Thin-layer chromatography was used to confirm the identity of ^{14}C-glutaraldehyde and glutaric acid in water, with confirmational analysis of the metabolites by HPLC.	
3.2	**Reference substance**	No	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	The details of the test sediment and water are presented in **Table A7.1.2.2.2/02-1**.	
3.3.2	Test system	The details on test type and laboratory equipment etc. are presented in **Table A7.1.2.2.2/02-2**.	
3.3.3	Test conditions	The relevant test conditions are presented in **Table A7.1.2.2.2/02-3**.	
3.3.4	Method of preparation of test solution	An isotopically diluted solution in acetonitrile was prepared for 50 flasks containing 4.5 mg ^{14}C-glutaraldehyde (1.357×10^9 dpm) and 48.9 mg non-labeled glutaraldehyde (96 mg of a 50.9% solution). The solution was diluted to a total volume of 53 mL deionized water. The dosing solution contained 25,552,800 dpm/mL. The equivalent of 20 grams dry weight of sediment (26.4 grams) and 100 mL of river water were added to each flask. Aliquots of the dosing solution (1 mL) were then added to the flasks, and swirled to mix. Flasks were stoppered and maintained at an average temperature of 25.0 $\pm$ 0.3°C.	
3.3.5	Initial TS concentration	The final aqueous phase concentration was 9.45 ug/mL (50.2 ug/g oven dry sediment).	
3.3.6	Duration of test	123 days	
3.3.7	Analytical parameter	The ^{14}C content was measured in each compartment: (ie, the volatile traps, water, and sediment extracts).	
3.3.8	Sampling	The aqueous phase was monitored on days 0, 1, 3, 7, 14, 30, 60, 90, 123 for pH (3.90-4.94) and oxygen content (0.22-0.55 ppm). Radiocarbon was quantified at the same sampling intervals.	X
3.3.9	Intermediates/ degradation products	Thin-layer chromatography was used to confirm the identity of ^{14}C-glutaraldehyde and glutaric acid in water, with confirmatory analysis of the metabolites by HPLC. **Table A7.1.2.2.2/01-9**	
3.3.10	Controls	No	
3.3.11	Statistics	**Sediment Combustion (total DPM in sample)** $\frac{\text{Raw DPM} - \text{Background DPM}}{\text{Oxidizer Efficiency}} * \frac{\text{Total Sediment}}{\text{Aliquot Weight}}$ **Liquid Traps & Foam Plug Extracts** $\frac{\text{Raw DPM} - \text{Background DPM}}{\text{Aliquot Weight}} * \text{Total Volume}$ **Sediment Extracts & Pond Water** $\frac{\text{Raw DPM} - \text{Background DPM}}{\text{Aliquot Weight}} * \text{Total Volume}$	

Section A7.1.2.2.2(2) **Annex Point IIIA, XII.2.1** **IUCLID 3.5/06**	**Water/Sediment Degradation Study** **Anaerobic Aquatic Metabolism of ^{14}C-Glutaraldehyde**	
	Percent Recovery (Mass Balance) Total DPM Recovered * 100 Total DPM Applied **Percent Yields of Parent / Products Based on Applied Dose** Percent of product (determined by HPLC) in water * percent of dose in water / 100 **Rate Constant and Half-Life of ^{14}C-Glutaraldehyde** Assuming pseudo first-order kinetics, the rate constant is: $\ln C = kt + \ln C_o$ y=mx+b where, k= rate constant c = chemical concentration t = time C_o = initial chemical concentration (t=0) The half life is: $t_{1/2} = \ln 2/k = 0.693/k$	
	4 RESULTS	
4.1 Degradation of test substance		
4.1.1 Degradation	Glutaraldehyde was the major component of the radiocarbon (67.6-78.6%) in the aqueous phase. The concentration, however, dropped to 0.1% after 72 hours. Concurrently, 5-hydroxypentanal reached 35.1-39.0% of the dose at 24 hours, and declined to <1.5% by 30 days. Pentanediol reached 74.3-77.9% of dose at 14 days, and an oligomer of glutaraldehyde (Compound A) reached a yield of 12.6-22.9% at 90 days.	
4.1.2 Graph	**Table A7.1.2.2.2/02-5**	

Section A7.1.2.2.2(2) **Annex Point IIIA, XII.2.1** **IUCLID 3.5/06**		**Water/Sediment Degradation Study** **Anaerobic Aquatic Metabolism of ^{14}C-Glutaraldehyde**	
			
4.1.3	Other observations	Radiocarbon recovery was >87% in the aqueous phase, and 5.4-8.9% incorporated/adsorbed on the sediment (**Table A7.1.2.2.2/02-4**). No significant organic volatiles were detected, and no significant amount of $^{14}CO_2$ was formed. After 123 days, >87% of the radioactivity was still in the aqueous phase, and sediment levels were 7.6-9.2%. The overall material balance for radioactivity was 98.7 +/- 2.5%.	x
4.1.4	Degradation of reference substance	Not applicable	
4.1.5	Intermediates/ degradation products	5-hydroxypentanal and pentanediol (see 4.1.1)	x
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The study to determine the degradation of glutaraldehyde in water/sediment systems followed EPA guideline 162-3. No relevant deviations from the guideline occurred. Anaerobic conditions were initiated by displacing the air in the river water/sediment system with N_2. Twelve days before dosing, anhydrous D-glucose was added to the vessel. Radiolabeled glutaraldehyde was added to the vessels to a total aqueous phase concentration of 9.45 ug/mL. Flasks (0 hour) were measured for pH, oxygen, and further processing. Volatile metabolites and $^{14}CO_2$, as well as dissolved O_2 and pH, were measured bi-weekly (CO_2 trapping efficiency was determined previously to average 100.4%). Sediment weight was determined after centrifugation. Sediment was homogenized, combusted, and radioassayed. Water was radioassayed by liquid scintillation counting. The aqueous phase was monitored on days 0, 1, 3, 7, 14, 30, 60, 90, 123 for pH and oxygen content. Radiocarbon was quantified at the same sampling intervals. Radiochemical purity, and metabolite identification and quantification were done by HPLC and/or TLC.	
5.2	**Results and discussion**	The pH of the system was 7.7 at collection, and ranged between 3.9 and 5.3 during the experiment. Dissolved O_2 was 0.11-0.56ppm, but up to 7 days was below 0.3ppm. Radiocarbon recovery was >87% in the	

Section A7.1.2.2.2(2) Annex Point IIIA, XII.2.1 IUCLID 3.5/06	Water/Sediment Degradation Study Anaerobic Aquatic Metabolism of ^{14}C-Glutaraldehyde	
	aqueous phase, and 5.4-8.9% adsorbed on the sediment (**Table A7.1.2.2.2/02-4**). No significant organic volatiles were detected, and no significant amount of $^{14}CO_2$ was formed. After 123 days, >87% of the radioactivity was still in the aqueous phase, and sediment levels were 7.6-9.2%. The overall material balance of radioactivity was 98.7 +/- 2.5%. **Aqueous Phase (Table A7.1.2.2.2/02-5)** Glutaraldehyde was the major component of the radioactivity (67.6-78.6%). The concentration, however, dropped to 0.1% after 72 hours. Concurrently, 5-hydroxypentanal reached 35.1-39.0% of the dose at 24 hours, and declined to <1.5% by 30 days. Pentanediol reached 74.3-77.9% of dose at 14 days, and an oligomer of glutaraldehyde (Compound A) reached a yield of 12.6-22.9% at 90 days. **Sediment (Table A7.1.2.2.2/02-6)** Adsorption of glutaraldehyde to sediment was <10% of the applied dose, reaching a maximum at 123 days (7.6-9.2%). HPLC analysis of the 90-day extract revealed the same product distribution as in the corresponding water phase.	
5.3 Half-life	The calculated pseudo-first order half-life of glutaraldehyde in water was 7.7 hours with a correlation coefficient of 0.990.	
5.4 Conclusion	Results indicate that anaerobic metabolism is a significant route for dissipation of glutaraldehyde in aquatic environments.	
5.4.1 Reliability	1	
5.4.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	23.3.2009	
Materials and Methods	The test is performed according to the US EPA guidance before adoption of the OECD 308. The test is well designed and carefully performed and reported. The requirements of the used test protocol were less than that of the OECD 308: - only one water-sediment combination used, two required in the OECD 308, the sediment represented a sediment type with a low organic carbon content and a coarse structure. - the amount of sediment was 20 g dw, 50 g dw required in the OECD 308 - the water:sediment ratio was 5:1, 3:1 or 4:1 recommended in the OECD 308 - The sediment and water were stored at room temperature about four months before the start of the test, while a maximum of four weeks storage period at 4 °C is allowed in the OECD 308. - transformation products have not been identified and quantified in the sediment - transformation rate in the sediment has not been determined - transformation rate for the whole system has not been derived - mineralisation rate has not been derived The deviations are not regarded to invalidate the test results. Other remarks: 3.1.4 Vapour pressure in the LOEP is 44 Pa at 20 °C. 3.3.8 pH ranged from 3.9 to 5.3 and oxygen content ranged from 0.11 to 0.56 ppm during the experiment.	

Section A7.1.2.2.2(2) Annex Point IIIA, XII.2.1 IUCLID 3.5/06	**Water/Sediment Degradation Study** **Anaerobic Aquatic Metabolism of ^{14}C-Glutaraldehyde**	
	Table A7.1.2.2.2/02-3 D-glucose is considered as an additional substrate as it was added to enhance anaerobic microbial activity.	
Results and discussion	The applicant's version is correct apart from minor deviations given below: 4.1.3 Radioactivity in water was >91% after 123 days. The minimum amount of radioactivity measured during the test was > 87%. 4.1.5 Pentanediol and Compound A, an oligomer of glutaraldehyde, were stable transformation products that were still present >10% at the end of the test. 5-hydroxypentanal was an intermediate metabolite that disappeared from water within one week.	
Conclusion	Glutaraldehyde and its metabolites were predominantly associated in the aqueous phase. The parent compound was rapidly metabolized with the first-order half-life being 7.7 hours. Glutaraldehyde was transformed to 5-hydroxypentanal which accounted ca. 37% of applied radioactivity on day 1, after that it declined below 10% and after 30 days it was not detected at all. Glutaraldehyde was transformed to Compound A via Aldol condensation, cyclization and dehydration. Compound A accounted about 10-20% of total radioactivity from day 1 on. The second stable transformation product was 1,5-pentanediol which accounted 35% of radioactivity on day 1, peaked on day 3 to 76% after two weeks and accounted 70% of radioactivity at the end of the test. Less than 10% of radioactivity was detected in the sediment. Insignificant amounts of CO_2 were produced during the experiment. No organic volatiles were formed.	
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 A7.1.2.2.2/02-1 Sediment and Water Analysis

Criteria	Sediment
SEDIMENT	
Source	River sediment
Sampling site	[redacted]
pH	8.1
% Organic matter	0.9%
% Sand	93%
% Silt	7%
% Clay	0%
Soil texture	Sand
1/3 bar moisture	5.46%
CEC (meq/100 g)	4.30 meq/100 g
WATER	
Alkalinity	86 mg $CaCO_3$/L
pH	7.7
Conductivity	810 umho/cm
Suspended Solids	160 mg/L
Hardness	140 mg $CaCO_3$/L

Table A7.1.2.2.2/02-2 Test system

Criteria	Details
Culturing apparatus	500 mL Erlenmeyer flasks (covered with aluminium foil). Each flask (500 mL Erlenmeyer, covered with aluminium foil) was equipped with a ground-glass stopper and glass stopcock inlet and outlet tubes (used to remove volatile metabolites and CO_2 while providing replacement nitrogen). Test samples were maintained in incubators during the study (25°C).
Number of replicates	2 each sampling time
Measuring equipment	LSC, HPLC-UV, TLC
Aeration	No, periodic nitrogen flush to maintain anaerobic conditions

Table A7.1.2.2.2/02-3 Test conditions

Criteria	Details
Pre-incubation	56 days
Application rate / concentration	The equivalent of 20 grams dry weight of sediment (26.4 grams) and 100 mL of river water were added to each flask. Aliquots of the dosing solution (1 mL) were then added to the flasks, and swirled to mix. The final aqueous phase concentration was 9.45 ug/mL (50.2 ug/g oven dry sediment).
Additional substrate	No
Solvent	Water
Application volume	1 mL of dosing solution
Test temperature	25°C
dark	yes
Sampling time points	days 0, 1, 3, 7, 14, 30, 60, 90, 123

Table A7.1.2.2.2/02-4 Radiocarbon Material Balance (Expressed as Percent of Applied Dose)

Sampling Time & Replicate		^{14}C in Soil		^{14}C in Water		$^{14}CO_2$		Total
		Percent	ppm	Percent	ppm	Percent	ppm	Percent
0-hour	A	5.4	0.51	92.4	8.73	--	--	97.8
	B	6.0	0.57	91.3	8.63	--	--	97.3
1-day	A	6.1	0.58	95.6	9.03	0.1	0.01	101.8
	B	6.5	0.62	94.5	8.93	0.1	0.01	101.1
3-day	A	6.1	0.58	90.7	857	0.2	0.02	97.0
	B	6.0	0.57	88.5	8.36	0.3	0.03	94.8
7-day	A	7.9	0.75	88.2	8.34	0.0	0.00	96.1
	B	6.4	0.61	89.7	8.48	0.0	0.00	96.1
14-day	A	6.9	0.65	94.0	8.88	0.1	0.01	101.0
	B	7.1	0.67	95.1	8.97	0.1	0.01	102.3
30-day	A	8.9	0.84	86.8	8.20	0.1	0.01	95.8
	B	7.6	0.72	87.1	8.23	0.1	0.01	94.8
60-day	A	7.8	0.74	90.8	8.58	0.2	0.02	98.8
	B	6.6	0.62	92.3	8.72	0.2	0.02	99.1
90-day	A	7.4	0.70	94.3	8.91	0.2	0.02	101.9
	B	7.4	0.70	92.5	8.74	0.3	0.03	100.2
123-day	A	9.2	0.87	91.1	8.61	0.3	0.03	100.6
	B	7.6	0.72	91.7	8.67	0.3	0.03	99.6
							Average	98.7 ± 2.5

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

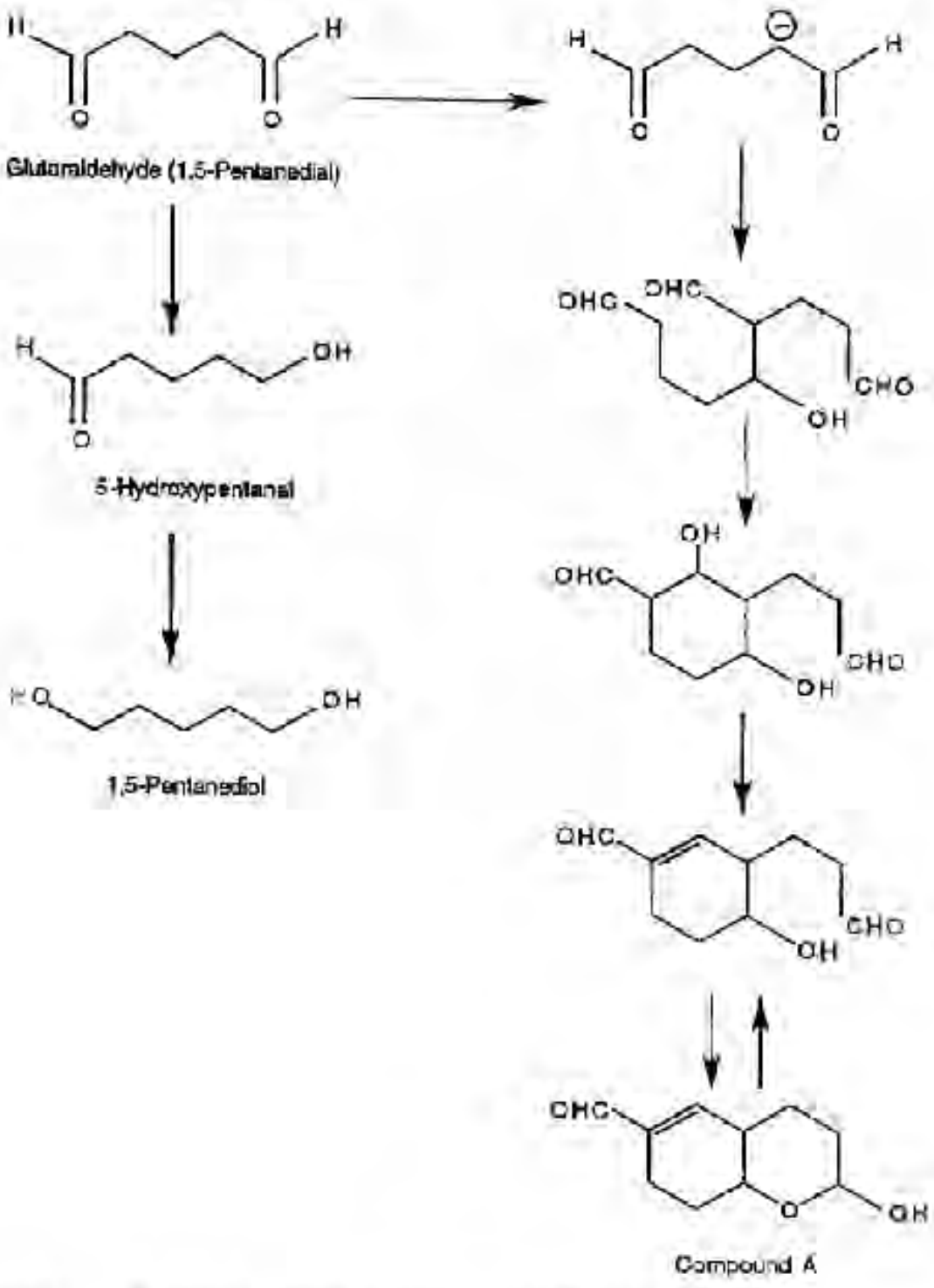


Figure 37 Proposed Metabolic Pathway for [1,5-^{14}C]-Glutaraldehyde.

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 (Dow) [redacted]	
1.2.2	Companies with letter of access	[redacted] Dow	
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, 50%	
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*** Agilent Technologies GC Model 6890A and Model 7683 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

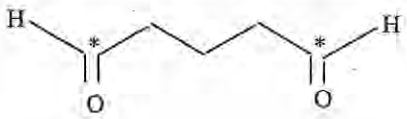
Number of Reaction Vessels	Purpose	Description
2	***Blanks:*** Determine possible background interferences for analysis of test and reference materials	Activated sludge in mineral medium
2	***Reference Mixtures:*** Measure adsorption of reference material on activated sludge to confirm proper operation 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 A7.2.3.1 **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 (Dow) [redacted]	
1.2.2	Companies with letter of access	[redacted] Dow	
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. [redacted]	
		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	[redacted]	
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
[redacted] 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 [redacted] (textural class is sand).

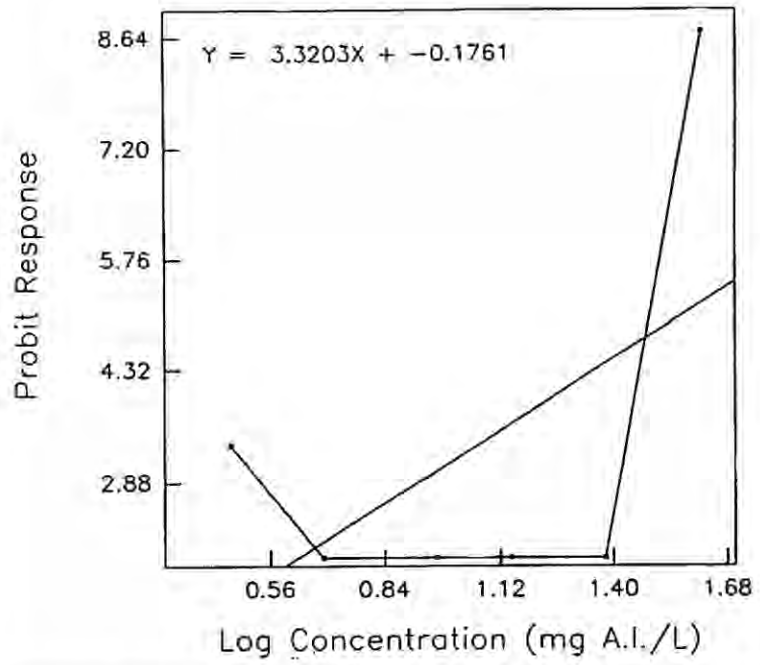
Section A7.3.1 Annex Point IIIA, VII.5		Fate and Behaviour in Air	
		1 REFERENCE	**Official use only**
1.1	**Reference**	U.S. EPA. 2004. EPI Suite software, version v3.12. United States Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, D. C. Available at: http://www.epa.gov/oppt/exposure/docs/episuitedl.htm	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No This study does not meet the requirements of 40 CFR Part 160.	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Structure-Activity Relationship (SAR) based on compound structure only, no lot/batch number was used.	
3.1.2	Specification	Not applicable	
3.1.3	Purity	Assumes 100% purity	
3.1.4	Further relevant properties	The vapour pressure of glutaraldehyde is 49 Pa at 25 °C	x
3.1.5	Method of analysis	Not applicable	
3.2	**Testing procedure**		
3.2.1	Test system	The Atmospheric Oxidation Program (AOP, version 1.91 Syracuse Research Corp., Syracuse, New York, USA). This SAR estimates the rate constant for the atmospheric, gas-phase reaction between photochemically-produced hydroxyl radicals and organic chemicals. It also estimates the rate constant for the gas-phase reaction between ozone and olefinic and acetylenic compounds.	
3.2.2	Test methods	The methods used by the AOP are based upon the structure-activity relationship methods developed by Atkinson and co-workers (5 references are provided in the report), and estimations of global average hydroxyl radical concentrations.	
3.3	**Test performance**		
3.3.1	Photo-oxidative degradation	The reaction rates of glutaraldehyde were estimated for hydrogen abstraction, reaction with N, S, and –OH, addition to triple and olefinic bonds, and addition to aromatic and fused rings. These were combined to give an overall rate constant which was used to determine the	

Section A7.3.1 Annex Point IIIA, VII.5		Fate and Behaviour in Air	
		atmospheric half-life of glutaraldehyde.	
3.3.2	Calculations	All data were processed within the AOP program based on the molecular structure of glutaraldehyde. No further calculations were required.	
		4 RESULTS	
4.1	**Rate constants**	The AOP estimated rate constants for glutaraldehyde for the following reaction paths.	
4.1.1	Hydrogen abstraction	46.9 x 10^{-12} cm^3/molecule second (there will be no reaction)	
4.1.2	Reaction with N, S, and –OH	0.00 x 10^{-12} cm^3/molecule second (there will be no reaction)	
4.1.3	Addition to triple bonds	0.00 x 10^{-12} cm^3/molecule second (there will be no reaction)	
4.1.4	Addition to olefinic bonds	0.00 x 10^{-12} cm^3/molecule second (there will be no reaction)	
4.1.5	Addition to aromatic rings	0.00 x 10^{-12} cm^3/molecule second	
4.1.6	Addition to fused rings	0.00 x 10^{-12} cm^3/molecule second (there will be no reaction)	
4.1.7	Sum of rate constants	46.9 x 10^{-12} cm^3/molecule second	
4.2	**Half-life**	Based on the overall rate constant of 46.9 x 10^{-12} cm^3/molecule second and the assumed hydroxyl radical concentration in the atmosphere of 1.5 x 10^6 radicals/cm^3, a 2.7-hour half-life was calculated. Assuming 12 hours of sunlight per day, the half-life of 2.7 hours is equivalent to 0.23 days.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The Atmospheric Oxidation Program, Syracuse Research Corp., was used to estimate the atmospheric half-life of glutaraldehyde.	
5.2	**Results and discussion**	The following overall degradation rate constant and half-life were calculated. Due to the use pattern and the low vapour pressure of glutaraldehyde (44 Pa at 20°C), it is unlikely to enter the atmosphere.	
5.2.1	Overall degradation rate constant	46.9 x 10^{-12} cm^3/molecule-sec	
5.2.2	Half-life	2.7 hours, assuming a hydroxyl radical concentration of 1.5 x 10^6 radicals/cm^3. For a 12-hour daylight period, the half-life is 0.23 days.	
5.3	**Conclusion**	Due to the short atmospheric half-life and use patterns, a low concentration of glutaraldehyde in the air is predicted. Any glutaraldehyde that does enter the air compartment will be rapidly degraded, therefore, there is negligible risk associated with global warming potential. For the same reasons there is negligible risk of stratospheric ozone depletion, low potential for tropospheric ozone formation, and low potential for acidification.	

Section A7.3.1 Annex Point IIIA, VII.5	Fate and Behaviour in Air	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	
	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. 3.1.4 Vapour pressure in the LOEP is 44 PA at 20 °C. This value is also given in 5.2.	
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 potodegraded in air.	
Reliability	Not relevant, a calculation method.	
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		

Section A7.4.1.1(1) **Annex Point IIA, VII.7.1** **IUCLID 4.1/01**		**Acute toxicity to fish** **Acute Toxicity to [redacted]** **Under Flow-Through Conditions**	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted] 1993a Glutaraldehyde - Acute Toxicity to [redacted] Under Flow-Through Conditions, [redacted], Unpublished, 13 April 1993	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, US EPA FIFRA 72-3	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde, 50%	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	3.1.3	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Samples containing glutaraldehyde were derivatised with 2,4-DNPH and then processed by liquid/liquid extraction using 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 Mobile phase: 80:20:0.7 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 10.0 min.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference**	No reference substance reported.	

Section A7.4.1.1(1) Annex Point IIA, VII.7.1 IUCLID 4.1/01		Acute toxicity to fish Acute Toxicity to [redacted] Under Flow-Through Conditions	
	substance		
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	**Table A7.4.1.1(1)-2**	
3.4.2	Test organisms	**Table A7.4.1.1(1)-3**	
3.4.3	Test system	**Table A7.4.1.1(1)-4**	
3.4.4	Test conditions	**Table A7.4.1.1(1)-5**	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality	
3.4.7	Sampling	All aquaria were examined after 0, 24, 48, 72, and 96 hours of exposure to determine fish behaviour, fish mortalities and physical characteristics of the test solutions. The dilutor system was inspected at least twice daily.	
3.4.8	Monitoring of TS concentration	During the in-life phase of the definitive study, water samples were removed from both replicate test vessels and each treatment control at 0 and 96 hours of exposure for analysis of Glutaraldehyde concentration using HPLC.	
3.4.9	Statistics	The mean measured concentrations tested and the corresponding data derived from the definitive toxicity test were used to estimate the LC50 and 95% confidence interval at each 24 hour interval of exposure period. If at least one test concentration caused mortality ≥ 50% a computer program calculated these statistics. The computer programs included moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence intervals calculated by binomial probability. Selection criteria for computer method used included establishment of a concentration-effect relationship, number of concentrations causing partial responses, and the span of the responses bracketing the LC50 value.	
		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	Nominally, the target concentrations 3.1, 5.2, 8.6, 14, 24, 40 mg a.i./L. The initial mean analytical concentrations were 2.85, 4.8, 9.45, 13, 25,	

Section A7.4.1.1(1) Annex Point IIA, VII.7.1 IUCLID 4.1/01		Acute toxicity to fish Acute Toxicity to [redacted] Under Flow-Through Conditions	
	test substance	and 40.5 mg a.i./L, respectively.	
4.2.2	Actual concentrations of test substance	Mean analytical concentrations were 2.9, 4.9, 9.2, 14, 24, and 41 mg a.i./L.	
4.2.3	Effect data (Mortality)	**Table A7.4.1.1/01-6 and 7** Mortality: 20 test organisms (100%) at the 40 mg a.i./L dose level after 24 hours. Mortality: 1 test organism (5%) at the 3.1 mg a.i./L dose level at 24 hours.	
4.2.4	Concentration / response curve		
4.2.5	Other effects	None noted.	
4.3	**Results of controls**	*Non-entry field*	
4.3.1	Number/ percentage of animals showing adverse effects	1 control animal (5%) died during the exposure period.	
4.3.2	Nature of adverse effects	Mortality	
4.4	**Test with reference substance**	*Non-entry field*	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	

Section A7.4.1.1(1) **Annex Point IIA, VII.7.1** **IUCLID 4.1/01**	**Acute toxicity to fish** **Acute Toxicity to [redacted]** **Under Flow-Through Conditions**	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The toxicity test was conducted using a flow-through proportional diluter with a 60% dilution factor, and temperature-controlled water bath. All treatment levels and the controls were maintained in duplicate. The flow-through system was set for 6.5 volume replacements per vessel in 24 hours. Test solutions were not aerated during the exposure period. The test conditions (photoperiod, temperature) were the same as during the acclimation period. Maximum loading was 0.043g of biomass per liter of flowing test solution per day. A syringe pump was used to inject the glutaraldehyde directly into the diluter system's chemical mixing chamber at appropriate dose volumes for the target concentrations. The mixing chamber was stirred continuously to insure uniform delivery of the test material. Aquaria were examined at study start, 24 hours, 48 hours, 72 hours, and 96 hours for mortalities (dead fish were removed) and observations on the test material and survivors. Water quality measurements (DO, pH, salinity, and temperature) were recorded once daily, and temperature was monitored continuously. Analytical monitoring of glutaraldehyde concentrations was conducted using HPLC. Mean measured concentrations tested and the mortality data derived were used to estimate the median lethal concentrations and the 95% confidence interval at each 24-hour interval.	
5.2 Results and discussion	Water quality parameters were unaffected by the additions of glutaraldehyde, and remained within acceptable ranges for the minnow's survival. Analyses of the test concentrations in the exposure solutions were consistent between replicate samples and the delivery apparatus generally maintained the expected concentration gradient. Analyses reported concentrations averaging >99% of the nominal dose.	
5.2.1 LC_0	24 mg a.i./L	
5.2.2 LC_{50}	The LC_{50} for all time points was 32 mg a.i./L.	
5.2.3 LC_{100}	41 mg/L	
5.3 Conclusion	Based on the results and the criteria established by the U.S. EPA (1985), Glutaraldehyde is considered to be slightly toxic to [redacted]	
5.3.1 Other Conclusions	None	
5.3.2 Reliability	1	
5.3.3 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	30.4.2008	
Materials and Methods	Applicant's version is correct.	

Section A7.4.1.1(1) **Annex Point IIA, VII.7.1** **IUCLID 4.1/01**	**Acute toxicity to fish** **Acute Toxicity to [redacted]** **Under Flow-Through Conditions**	
Results and discussion	Applicant's version is correct. LC50 96 h 32 mg a.i./l based on the mean measured concentrations. 95% confidence interval 24-42 mg a.i./l.	
Conclusion	Glutaraldehyde is harmful to the marine fish, [redacted]	
Reliability	1	
Acceptability	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

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

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

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	[redacted]
Salinity	32‰
Alkalinity	Not reported
Hardness	Not reported
pH	Approximately 8
Oxygen content	83-94% saturation
Conductance	Not reported
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	[redacted]
Source	[redacted]
Wild caught	No
Age/size	Immature individuals with mean wet weight 0.31 (0.14-0.51) g and mean length 24 (19-28) mm
Kind of food	Zeigler Brothers Prime flakes
Amount of food	*Ad libitum*
Feeding frequency	Daily
Pretreatment	Fish were acclimated to the test conditions for a minimum of 14 days. Fish were not fed 48 h before initiation of the test
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	The flow rate was 50 ml/min which provided approximately 6.5 volume replacements per aquarium every 24 h
Volume of test vessels	19.5 L
Volume/animal	0.9 L
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	21-23°C
Dissolved oxygen	5.8-7.1 mg/L (81-100% saturation)
pH	7.9-8.2
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	323-430 lux (30-40 footcandles[1])
Photoperiod	16 h light, 8 h dark

[1] Footcandle=10.76391 lux

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (mean measured) [mg/l]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	1	1	1	1	5	5	5	5
2.9	1	1	1	1	5	5	5	5
4.9	0	0	0	0	0	0	0	0
9.2	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
41	20	20	20	20	100	100	100	100
Temperature [°C]	22	22	21-22	22				
Salinity ‰	31-32	32	32	32				
pH	7.9-8.1	8.2	8.2	7.9-8.0				
Oxygen [mg/l]	5.8-7.1	6.4-6.8	6.7-7.1	6.8-7.1				

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				
LC_{50}	32 (m)	24-42 (m)	32 (m)	24-42 (m)
LC_{100}				

[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 fulfilled
Mortality of control animals <10%	x	
Concentration of dissolved oxygen in all test vessels > 60% saturation	x	
Concentration of test substance ≥80% of initial concentration during test	x	

Criteria for poorly soluble test substances	NA	

Section A7.4.1.2(1) Annex Point IIA, VII.7.2 IUCLID 4.2/01		Acute toxicity to invertebrates Acute Toxicity to [redacted] Under Flow-Through Conditions	
		1 REFERENCE (A7.4.1.2/01)	Official use only
1.1	Reference	[redacted] (2006) Glutaraldehyde - Acute Toxicity to [redacted] Under Flow-Through Conditions, [redacted], Unpublished, 27 February 2006.	
1.2	Data protection	Yes	
1.2.1	Data owner	The Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	Yes, EPA OPPTS 850.1045	
2.2	GLP	Yes	
2.3	Deviations	No	
		3 MATERIALS AND METHODS	
3.1	Test material	Glutaraldehyde, 50%	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	3.1.3	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Recovery samples containing glutaraldehyde were processed by liquid/liquid extraction and derivatisation with 2,4-DNPH. Following derivatisation each sample was extracted with toluene. Final extracts were analyzed by HPLC with UV detection using a Hewlett-Packard 1100 Series HPLC and the following conditions: Analytical Column: Waters Spherisorb CN, 5µm, 150mm x 4.6mm Mobile phase A: 85:15 v/v hexane:methylene chloride Mobile phase B: 100% methanol Gradient: Time (min.) / Solvent A / Solvent B 0 / 97 / 3 3 / 97 / 3 15 / 90 / 10 16 / 90 / 10 17 / 97 / 3	

Section A7.4.1.2(1) **Annex Point IIA, VII.7.2** **IUCLID 4.2/01**		**Acute toxicity to invertebrates** **Acute Toxicity to [redacted] Under Flow-Through Conditions**	
		Column temperature: ambient Flow rate: 1.5 mL/minute Wavelength: 350nm Injection Volume: 50 µl Retention time: approx. 11.6 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(1)-2**	
3.4.2	Test organisms	**Table A7.4.1.2(1)-3 -** [redacted]	
3.4.3	Test system	**Table A7.4.1.2(1)-4** - The toxicity test was conducted using an intermittent-flow proportional diluter and 14 exposure vessels (6 concentrations of the test material and one diluter water control, in duplicate).	
3.4.4	Test conditions	**Table A7.4.1.2(1)-5**	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality and sublethal effects	
3.4.7	Sampling	Examinations/observations were carried out at 0, 3, 6, 12, 24, 48, 72, and 96 hours; sampling for analysis was at 0 and 96 hours only.	
3.4.8	Monitoring of TS concentration	Yes	
3.4.9	Statistics	The mean measured concentrations tested and the corresponding mortality data were used to estimate the 24, 48, 72, and 96-hour LC_{50}'s with 95% confidence intervals (CI). If at least one concentration produced >50% mortality, a computer program was used for calculations. Three statistical methods were available for use: moving average angle analysis, probit analysis, and binomial probability. The method for calculation of LC_{50} depended on the mortality data generated during the study (number of concentrations with partial mortality, span of responses bracketing the LC_{50}, and the establishment of a dose-response). The NOEC was also determined (p=0.05), defined as the highest concentration at and below which there was no test material-related mortality or physical and behavioural abnormalities with respect to the control organisms.	
		4 RESULTS	
4.1	**Limit Test**	No	
4.1.1	Concentration	Not applicable	

Section A7.4.1.2(1) **Annex Point IIA, VII.7.2** **IUCLID 4.2/01**		**Acute toxicity to invertebrates** **Acute Toxicity to [redacted] Under Flow-Through Conditions**	
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**		
4.2.1	Initial concentrations of test substance	0, 3.8, 7.5, 15, 30, 60, and 120 mg a.i. per liter of water	
4.2.2	Actual concentrations of test substance	0, 4.4, 6.2, 18, 31, 61, and 120 mg a.i. per liter of water	
4.2.3	Effect data (Mortality)	**Table A7.4.1.2(1)-7**	
4.2.4	Concentration / response curve	**Figure A7.4.1.2(1)-1**	
4.2.5	Other effects	Lethargy at 31 and 120 mg a.i./L	
4.3	**Results of controls**	No mortality and no sublethal effects noted.	
4.4	**Test with reference substance**	Not applicable	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Juvenile [redacted] shrimp were selected as the test organism. They were obtained from [redacted] Prior to testing, the were held in 500-liter fiberglass tanks with a closed-loop recirculating system under a photoperiod of 16 hours light and 8 hours darkness per 24-hour period. Salinity ranged from 17/22‰. The pH was 7.5-7.8, dissolved oxygen was 73-86% of saturation, and the temperature was 17-23°C. Shrimp were offered *Artemia salina*, live brine shrimp *nauplii*, and flaked fish food *ad libitum*. Shrimp were fasted during the 96-hour exposure period. There was no mortality observed in the 48 hours immediately preceeding exposure to the test material; 30 shrimp were chosen at random to be weighed (0.019 grams wet) and measured (13mm mean length). Filtered natural seawater was used as dilution water [redacted] Salinity was diluted to 20-21‰ at the diluter headboard with laboratory water, and had a pH of 8.0. Samples of the dilution water were reported to have a total organic carbon (TOC) concentration of <2.0 mg/L. The toxicity test was conducted using an intermittent-flow proportional	

Section A7.4.1.2(1) **Annex Point IIA, VII.7.2** **IUCLID 4.2/01**	**Acute toxicity to invertebrates** **Acute Toxicity to [redacted] Under Flow-Through Conditions**	
	diluter and 14 exposure vessels (6 concentrations of the test material and one diluter water control, in duplicate). The photoperiod was maintained at 12 hours light and 12 hours dark per 24-hour period. The test was conducted at 22-24°C. Based on the results of a preliminary test, the definitive test concentrations were 0, 3.8, 7.5, 15, 30, 60, and 120 mg a.i. per liter of water. No stock solution was needed; the material was used as received to fill the delivery syringe. The Harvard syringe pump was calibrated to deliver 0.4151 mg/cycle of glutaraldehyde (567 mg/L a.i.) to the diluter system's mixing chamber containing 1.96 liters of dilution water. The solution in the mixing chamber was mixed with a magnetic stir plate and bar. The concentration of glutaraldehyde in the mixing chamber was equivalent to the highest concentration tested. Dilution achieved the lower target concentrations. A flow-splitting chamber was used to ensure that the flow to duplicate test vessels was identical. Flow of exposure solution achieved 6.4 volume replacements per day (90% replacement of the test solution in 9 hours). Study was initiated when 10 shrimp were selected at random (placed in each vessel 2 at a time until 10 were assigned to each). Aquaria were examined for mortalities (dead shrimp removed), biological observations including sublethal effects, and physical characteristics of the test solutions at 0, 3, 6, 12, 24, 48, 72, and 96 hours. Dissolved oxygen, temperature, salinity, and pH were measured daily in all aquaria. During the definitive study, one sample from one replicate at each treatment level was collected and analyzed for glutaraldehyde concentration at 0 and 96 hours. QC samples were used to monitor the precision of the analytical process. Samples were analyzed by HPLC with UV detection, with a method validated prior to study initiation.	
5.2 Results and discussion	The definitive study was conducted three times. The first study was repeated due to an inability to establish a NOEC during the first experiment. Following 96 hours of exposure, 20 and 65% mortality were reported in shrimp exposed to 15 and 30 mg ai/L, respectively. No mortality or adverse effects were observed among shrimp exposed to the remaining treatment levels tested (0.94, 1.9, 3.8, 7.5 mg ai/L) or the control. At test termination, it was discovered that the recertification for the test material lot was exceeded, and the test was considered invalid. A third attempt was made to determine the acute toxicity of glutaraldehyde to [redacted] shrimp. <u>Results of the Third Repeat</u> Water quality parameters (pH, dissolved oxygen, temperature) were unaffected by concentration of glutaraldehyde. Temperature was 23°C, and salinity ranged from 20-21‰. The diluter functioned properly, and analysis of the test solutions established that the concentrations of the material were within expectations for the test conditions. Mean measured exposure concentrations ranged from 83-120% of nominal, and defined the treatment levels as 0, 4.4, 6.2, 18, 31, 61, and 120 mg a.i./L. Recovery for the material in the analytical samples was 96.2-105%. Following 96 hours of exposure, 25, 50, and 70% mortality was observed in shrimp exposed to 31, 61, and 120 mg ai/L. There was no mortality or adverse effects noted in the 0, 4.4, 6.2, or 18 mg a.i./L vessels.	

Section A7.4.1.2(1) **Annex Point IIA, VII.7.2** **IUCLID 4.2/01**	**Acute toxicity to invertebrates** **Acute Toxicity to [redacted] Under Flow-Through Conditions**	
5.2.1 EC_0	The no observed effect concentration was 18 mg a.i./L	
5.2.2 EC_{50}	96-hour LC_{50} = 68 (52-96) mg a.i./L (probit analysis) 72-hour LC_{50} = >120 mg a.i./L 48-hour LC_{50} = >120 mg a.i./L 24-hour LC_{50} = >120 mg a.i./L	
5.2.3 EC_{100}	> 120 mg a.i./L	
5.3 Conclusion	Based on the results and on labeling criteria established by EEC, Glutaraldehyde would be classified as not harmful to [redacted] shrimp.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	5.5.2008	
Materials and Methods	Applicant's version is correct.	
Results and discussion	Applicant's version is correct. Table A7_4_1_2(1)_8 The validity criteria refer to Daphnia test and may not be appropriate to [redacted] shrimp. LC50 96 h 68 mg a.i./l based on the mean measured concentrations. 95% confidence interval 52-96 mg a.i./l.	
Conclusion	Glutaraldehyde is harmful to marine [redacted]	
Reliability	1	
Acceptability	Acceptable	
Remarks	According to the test guideline the test species should be [redacted]	
	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)-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(1)-2: Dilution water

Criteria	Details
Source	[redacted]
Alkalinity	Not reported
Hardness	Not reported
pH	8.0
Ca / Mg ratio	Not reported
Na / K ratio	Not reported
Oxygen content	7.3-7.9 mg/L
Conductance	Not reported
Holding water different from dilution water	No

Table A7_4_1_2(1)-3: Test organisms

Criteria	Details
Strain	[redacted]
Source	[redacted]
Age	Juvenille
Breeding method	Not reported
Kind of food	*Artemia salina*, live brine shrimp *nauplii*, and flaked fish food
Amount of food	*ad libitum*
Feeding frequency	Daily
Pretreatment	Acclimatisation for at least 14 days
Feeding of animals during test	No

Table A7_4_1_2(1)-4: Test system

Criteria	Details
Renewal of test solution	Renewal: a calibrated diluter system was used which had been calibrated prior to use on the study. The flow provided 6.4 solution volume replacements per day in order to provide a 90% test solution replacement rate of approximately 9 hours.
Volume of test vessels	Volume maintained at 11 L with the use of an over-flow drain 14.5 cm from the bottom of the aquarium. Each aquarium measured 39 (length) x 20 (width) x 25 (height) cm.
Volume/animal	1.1 L
Number of animals/vessel	10
Number of vessels/ concentration	2 vessels at each concentration including control.
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2(1)-5: Test conditions

Criteria	Details
Test temperature	Temperature was maintained at 23°C throughout the study.
Dissolved oxygen	The range recorded throughout the study was 7.3 to 7.9 mg/L (96-104% saturation)
Salinity	20-21‰
pH	The pH was maintained at 8.0 throughout the study.
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Fluorescent bulbs at an intensity of 33 to 77 footcandles (360 to 830 lux).
Photoperiod	12 h photoperiod daily

Table A7.4.1.2(1)-6 Cumulative Percent Mortality

Mean Measured Concentration (mg a.i./L)	Replicate	Cumulative Percent Mortality (Number Dead Shrimp)[a]							
		Observation Interval (hours)							
		0	3	6	12	24	48	72	96
Control	A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	B	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean	0	0	0	0	0	0	0	0
4.4	A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	B	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean	0	0	0	0	0	0	0	0
6.2	A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	B	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean	0	0	0	0	0	0	0	0
18	A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	B	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean	0	0	0	0	0	0	0	0
31	A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0(0)	20(2)
	B	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10(1)	20(2)	30(3)
	Mean	0	0	0	0	0	5[c]	10	25
61	A	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	20(2)	40(4)	50(5)
	B	0 (0)	0 (0)	0 (0)	0 (0)	10(1)	10(1)	20(2)	50(5)
	Mean	0	0	0	0	5	15	30	50
120	A	0 (0)	0 (0)	0 (0)	0 (0)	10(1)	10(1)	20(2)	60(6)
	B	0 (0)	0 (0)	0 (0)	0 (0)	10(1)	40(4)	50(5)	80(8)
	Mean	0	0	0	0[b]	10	25	35	70

[a] actual number of mortalities

[b] Four surviving shrimp were observed to be lethargic

[c] One surviving shrimp was observed to be lethargic

Table A7_4_1_2(1)-7: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg/l]	>120 mg a.i./L (m)	NA	18 mg a.i./L (m)	>120 mg a.i./L (m)
48 h [mg/l]	>120 mg a.i./L (m)	NA	18 mg a.i./L (m)	>120 mg a.i./L (m)

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

The EC50 at 96h was calculated to be 68 mg a.i./L

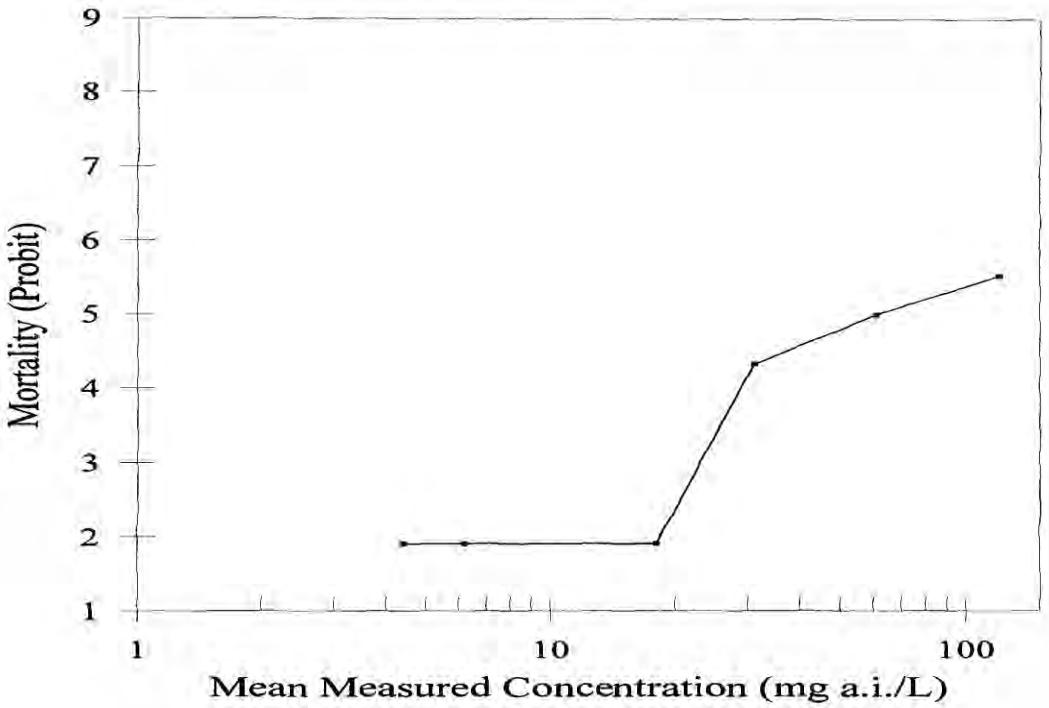
Table A7_4_1_2(1)-8: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202*

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

Criteria for poorly soluble test substances	NA	

*Study conducted according to EPA OPPTS 850.1045

Figure A7.4.1.2(1)-1 Concentration / Response (Mortality) Curve for [redacted] Exposed to Glutaraldehyde



Section A7.4.1.2(2) **Annex Point IIA, VII.7.2** **IUCLID 4.2/02**		**Acute toxicity to invertebrates** **Acute Toxicity to [redacted] Under Flow-Through Conditions**	
		1 REFERENCE (A7.4.1.2/02)	**Official use only**
1.1	**Reference**	[redacted] (1993b) Glutaraldehyde - Acute Toxicity to [redacted] Under Flow-Through Conditions, [redacted], Unpublished, 7 September 1993	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, 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 by Lancaster Laboratories, a separate contract testing facility).	x
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde, 50%	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2.	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	3.1.3	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Samples 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 Hewlett-Packard 1100 Series HPLC and the following conditions: Analytical Column: Phenomenex Spherisorb Cyano, 5µm, 150mm x 4.6mm	

Section A7.4.1.2(2) **Annex Point IIA, VII.7.2** **IUCLID 4.2/02**		**Acute toxicity to invertebrates** **Acute Toxicity to [redacted] Under Flow-Through Conditions**	
		Mobile phase: 80:20:0.7 v/v/v hexane:methylene chloride:methanol Gradient: Time (min.) Solvent A Solvent B 0 97 3 3 97 3 15 90 10 16 90 10 17 97 3 Column temperature: ambient Flow rate: 2.2 mL/minute Wavelength: 350nm Injection Volume: 15 µl Retention time: approx. 9.9 to 10.0 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(2)-2**	
3.4.2	Test organisms	**Table A7.4.1.2(2)-3** [redacted]	
3.4.3	Test system	**Table A7.4.1.2(2)-4 (**The test was conducted using an exposure system consisting of a constant-flow serial diluter with a 60% dilution factor, a temperature controlled water bath, and a set of 14 exposure aquaria (19.5L))	x
3.4.4	Test conditions	**Table A7.4.1.2(2)-5**	x
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality and sublethal effects	
3.4.7	Sampling	Mortality and sublethal effects were observed at the initiation of the test and at every subsequent 24 hour interval during the exposure period. Dissolved oxygen, temperature, salinity, and pH were measured once daily in both replicate vessels of each treatment level and controls throughout exposure period. Water samples were removed from both replicate test solutions of each treatment level and the controls on test days 0 and 4 for analyses of glutaraldehyde concentration.	
3.4.8	Monitoring of TS concentration	Yes	

Section A7.4.1.2(2) **Annex Point IIA, VII.7.2** **IUCLID 4.2/02**		**Acute toxicity to invertebrates** **Acute Toxicity to [redacted] Under Flow-Through Conditions**	
3.4.9	Statistics	The mean concentrations tested and corresponding mortality data derived from the definitive toxicity test were used to estimate the median lethal concentration and 95% confidence interval for each 24 hour interval of the exposure period. If at least one test concentration caused mortality greater than or equal to 50% of the test population, then a computer program (moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence intervals calculated by binomial probability) was used to calculate the LC_{50} and 95% confidence intervals.	
		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 of 0.78, 1.3, 2.2, 3.6, 6.0, 10 mg a.i./L were chosen.	
4.2.2	Actual concentrations of test substance	Mean measured concentrations were 0.78, 1.5, 2.5, 3.9, 6.8, and 12 mg a.i./L.	
4.2.3	Effect data (Immobilisation)	**Table A7.4.1.2(2)-6** - Mortalities were 95% and 35% at 12 mg a.i./L and 6.8 mg a.i./L, respectively. Mortalities of 5% were recorded among [redacted] at the 3.9 and 2.5 mg a.i./L treatment levels. Sublethal effects were noted at 1.5 mg a.i./L and higher and included lethargy and loss of equilibrium.	

Section A7.4.1.2(2) **Annex Point IIA, VII.7.2** **IUCLID 4.2/02**		**Acute toxicity to invertebrates** **Acute Toxicity to [redacted] Under Flow-Through Conditions**	
4.2.4	Concentration / response curve	Refer to **Figure A7.4.1.2(2)-1**	
4.2.5	Other effects	Lethargy, loss of equilibrium, erratic behaviour	
4.3	**Results of controls**	No mortality or sublethal effects noted.	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Shrimp were obtained from a commercial supplier, and held in a holding tank with natural seawater, with a temperature range of 24-26°C. They received a regulated photocycle (16 hours light / 8 hours dark), and were fed live brine shrimp twice daily (tested for pesticides prior to being offered as food) including during exposures. Filtered natural seawater was used as dilution water [redacted]. Dilution water was from the same source as the culture water. The water was analyzed per USEPA and ASTM methods and deemed suitable for use. The test system was designed to provide six treatment levels and a dilution water control. All treatment levels plus the control were maintained in duplicate. Each aquaria housed 10 [redacted] shrimp divided between two retention chambers, with a maximum biomass loading of 0.000139 g/L water. The diluter provided 6.5 volume replacements per 24-hour period. Test solutions were not aerated during exposures. The test material was delivered via syringe pump directly into the chemical mixing chamber, stirred continuously during exposures. Mortality, biological observations, and observations on the physical characteristics of the test material solutions were recorded initially and every 24 hours during the test. Dead organisms were removed at each observation interval. Dissolved O_2, temperature, salinity, and pH were measured once daily in all vessels throughout the study. Concentrations of glutaraldehyde in the vessels were measured on test days 0 and 4 for all dose levels by HPLC.	
5.2	**Results and discussion**	Water quality parameters measured remained constant throughout the exposure period, were unaffected by glutaraldehyde addition, and remained within acceptable limits for the species. Continuous measurement of temperature established that the test vessels' temperature were 24-26°C during the exposure. Measured concentrations of glutaraldehyde averaged 112% of the nominal concentrations. Exposures achieved were sufficient to produce a concentration-related response. Mortalities were 95% and 35% at 12 mg a.i./L and 6.8 mg a.i./L. Mortalities of 5% were recorded among mysids at the 3.9 and 2.5 mg	

Section A7.4.1.2(2) **Annex Point IIA, VII.7.2** **IUCLID 4.2/02**	**Acute toxicity to invertebrates** **Acute Toxicity to [redacted] Under Flow-Through Conditions**	
	a.i./L treatment levels. Sublethal effects were noted at 1.5 mg a.i./L and higher, and included lethargy and loss of equilibrium. The LC_{50} was established at 7.1 mg a.i./L, and the NOEC was reported to be 0.78 mg a.i./L.	
5.2.1 EC_0	The no observed effect concentration was 0.78 mg a.i./L	
5.2.2 EC_{50}	The 96 hr LC_{50} was 7.1 mg a.i./L	
5.2.3 EC_{100}	>12 mg a.i./L	
5.3 Conclusion	Based on the results and on criteria established by U.S. EPA (1985), Glutaraldehyde would be classified as moderately toxic to [redacted].	
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	30.4.2008	
Materials and Methods	2.3 These deviations are not mentioned in the test report, instead two other insignificant protocol deviations are mentioned. 3.4.3 Table A7_4_1_2(2)-4: Total number of test vessels was 14, because six concentrations and a control were included in the test and each treatment was duplicated. 3.4.4 Table A7_4_1_2(2)-5: Salinity range was 31-32‰ which is higher than 20±3‰ recommended in OPPTS 850.1035. 3.4.4 Table A7_4_1_2(2)-5: Photoperiod was 16 h light and 8 h dark, 14 h light and 10 h dark with 15 to 30 min transition period is given in OPPTS 850.1035.	
Results and discussion	Applicant's version is correct. Table A7_4_1_2(3)_8 The validity criteria refer to Daphnia test and may not be appropriate to [redacted]. LC50 96 h 7.1 mg a.i./l based on mean measured concentrations. 95% confidence interval 6.0-8.6 mg a.i./l.	
Conclusion	Glutaraldehyde is toxic to marine [redacted]	
Reliability	1	
Acceptability	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	

Section A7.4.1.2(2) **Annex Point IIA, VII.7.2** **IUCLID 4.2/02**	**Acute toxicity to invertebrates** **Acute Toxicity to [redacted] Under Flow-Through Conditions**	
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(2)-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_1_2(2)-2: Dilution water

Criteria	Details
Source	[redacted]
Alkalinity	Not reported
Hardness	Not reported
pH	8.0 to 8.2
Ca / Mg ratio	Not reported
Na / K ratio	Not reported
Oxygen content	6.9 to 7.3 mg/L
Conductance	Not reported
Holding water different from dilution water	No

Table A7_4_1_2(2)-3: Test organisms

Criteria	Details
Strain	[redacted]
Source	[redacted]
Age	Juvenille
Breeding method	Not reported
Kind of food	Live brine shrimp nauplii (*Artemia salina*)
Amount of food	Not reported
Feeding frequency	Twice daily
Pretreatment	Not reported
Feeding of animals during test	Yes, as detailed above.

Table A7_4_1_2(2)-4: Test system

Criteria	Details
Renewal of test solution	The diluter provided 6.5 volume replacements per 24-hour period. The test material was delivered via syringe pump directly into the chemical mixing chamber, stirred continuously during exposures.
Volume of test vessels	19.5 L glass aquaria measuring 39 x 20 x 25 cm; volume maintained between 7 and 11 L.
Volume/animal	0.7 to 1.1 L
Number of animals/vessel	10; 5 in each retention chamber (glass Petri dishes 100mm diameter and 20mm in height).
Number of vessels/ concentration	2 vessels at each of 5 concentrations and 2 control vessels, therefore a total of 12 vessels.
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2(2)-5: Test conditions

Criteria	Details
Test temperature	Range measured during the study was 24 to 26°C
Dissolved oxygen	Range measured during the study was 6.9 to 7.3 mg/L
Salinity	Range measured during the study was 31-32‰
pH	Range measured during the study was 8.0 to 8.2
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Fluorescent bulbs at an intensity of 320-1100 lux (30-100 footcandles)
Photoperiod	12 h photoperiod daily

Table A7_4_1_2(2)-6: Cumulative Percent Mortality

Test-Substance Concentration (measured) [mg/l]	Dead Shrimp								Oxygen [mg/l]	pH	Tempera-ture [°C]
	Number				Percentage						
	24h	48 h	72h	96h	24h	48 h	72h	96h	96h	96h	96h
12	3	12	15	19	15[a]	60[b,c]	75[e,f,g]	95[g]	7.1 to 7.2	8.2	25 to 26
6.8	0	0	1	7	0[a]	0[a]	5[b,c]	35[b,c,h]	7.1 to 7.2	8.2	25 to 26
3.9	1	1	1	1	5[b]	5[d]	5	5[d]	7.1 to 7.2	8.2	25 to 26
2.5	1	1	1	1	5	5	5	5[i]	7.1 to 7.2	8.2	25 to 26
1.5	0	0	0	0	0[e]	0[e]	0[e]	0[e]	7.1 to 7.2	8.2	25 to 26
0.78	0	0	0	0	0	0	0	0	7.1 to 7.2	8.2	25 to 26
Control	0	0	0	0	0	0	0	0	7.1 to 7.2	8.2	25 to 26

[a] all surviving [redacted] were observed to be lethargic
[b] several surviving [redacted] were observed to be lethargic
[c] several surviving [redacted] were observed with a partial loss of equilibrium
[d] several surviving [redacted] were observed as erratic
[e] one surviving [redacted] was observed with partial loss of equilibrium
[f] two surviving [redacted] were observed to be lethargic
[g] one surviving [redacted] was observed with complete loss of equilibrium
[h] several surviving [redacted] were observed with complete loss of equilibrium
[i] two surviving [redacted] were observed as erratic

Table A7_4_1_2(2)-7: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg/l]				
48 h [mg/l]				
72 h [mg/l]				
96 h [mg/l]	7.1 mg a.i./L	6.0 to 8.6	0.78 mg a.i./L	>12 mg a.i./L

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

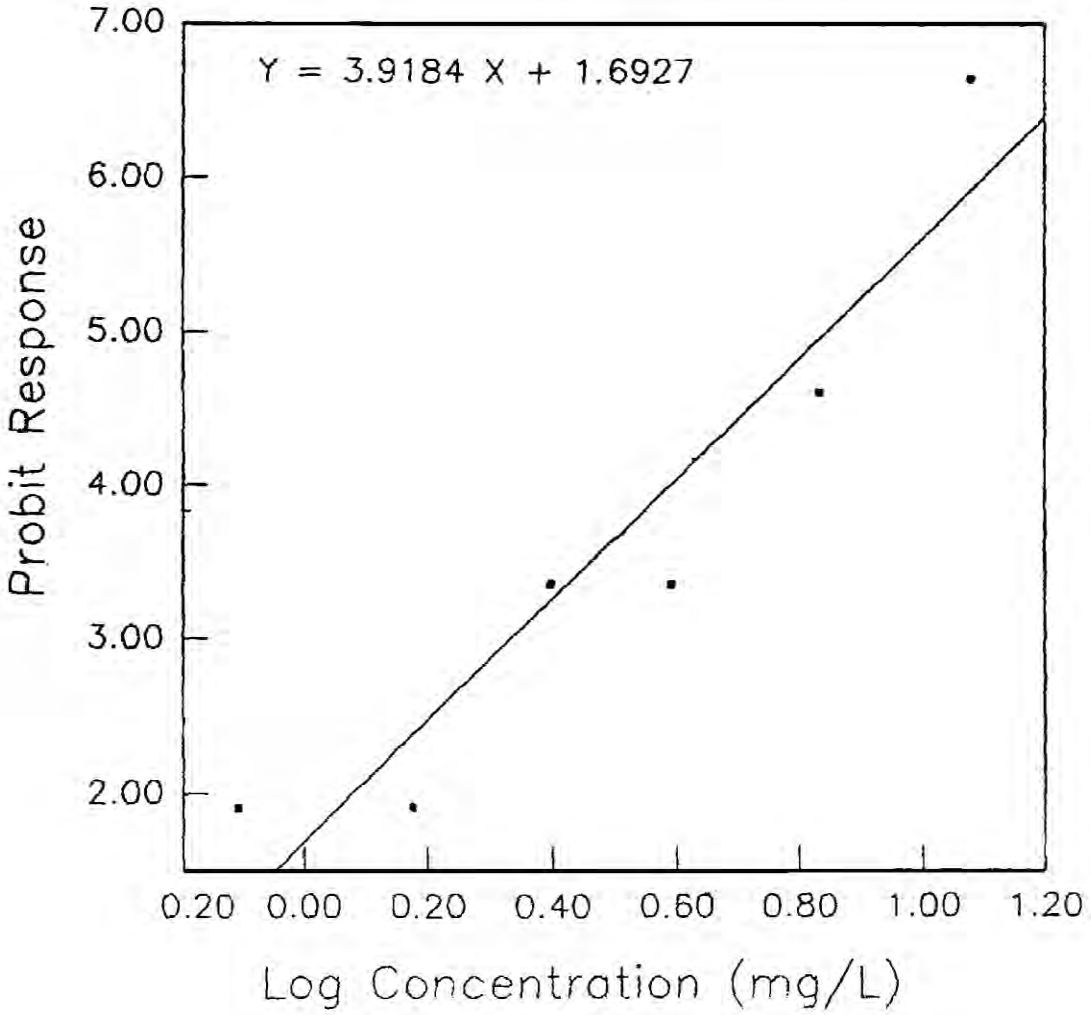
Table A7_4_1_2(2)-8: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202*

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

Criteria for poorly soluble test substances ergänzen		

*Study conducted according to US EPA FIFRA 72-3

Figure A7.4.1.2(2)-1 The 96-hour Concentration-Response (mortality) Curve for [redacted] Exposed to Glutaraldehyde



Section A7.4.1.2(3) **Annex Point IIA, VII.7.2** **IUCLID 4.2/03**		**Acute toxicity to invertebrates** **Acute Toxicity to [redacted] Under Flow-Through Conditions**	
		1 REFERENCE (A7.4.1.2/03)	**Official use only**
1.1	**Reference**	[redacted] (1993) Glutaraldehyde - Acute Toxicity to [redacted] Under Flow-Through Conditions, [redacted], Unpublished, 7 September 1993	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company (Dow) [redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, 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 by Lancaster Laboratories, a separate contract testing facility).	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde, 50%	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	3.1.3	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Samples 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 [redacted] 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** [redacted]	
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 [redacted] 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 ± 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 [redacted] Under Flow-Through Conditions**	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Forty [redacted] from a commercial supplier were allocated to each of 5 dose levels (20 per replicate) randomly after a 14-day acclimation. [redacted] were fed a supplementary diet of algal cells during the testing period. [redacted] 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 [redacted] were removed by grinding the shell to a blunt edge using a grinding wheel. [redacted] 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. [redacted] 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 [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 [redacted] 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 [redacted] 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 [redacted] 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 [redacted] 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 [redacted]. The 96 hour EC_{50} for [redacted] is 0.78 mg a.i./L based on measured concentrations.	
Conclusion	Glutaraldehyde is very toxic to [redacted]	
Reliability	1	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A7_4_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	[redacted]
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	

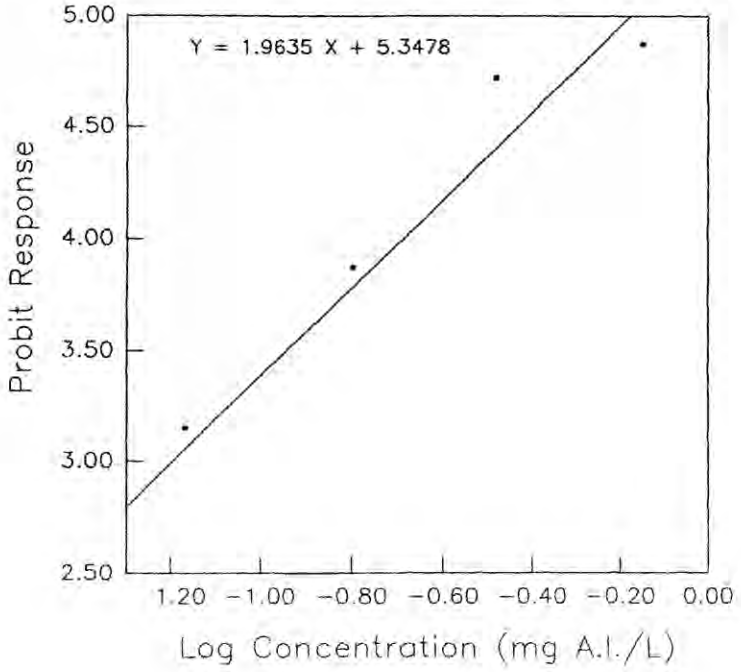
a = test carried out according to FIFRA 72-3
b = measured values were in the range 50 to 79% of target, the decrease was attributed to absorption of the test material by the high number of test organisms and supplemental food.

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 [redacted]



Section A7.4.1.2(4) **Annex Point IIA, VII.7.2** **IUCLID 4.2/04**		**Acute toxicity to invertebrates** **Acute Toxicity to** [redacted]	
		1 REFERENCE (A7.4.1.2/04)	**Official use only**
1.1	**Reference**	[redacted] (1997) [redacted] Acute Toxicity to [redacted], Unpublished, 24 April 1997	
1.2	**Data protection**	Yes	
1.2.1	Data owner	The Dow Chemical Company (Dow) [redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes UK Proposal to ISO TC147/SC5/W92	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde, 50% [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	3.1.3	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	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** [redacted]	
		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** [redacted]	
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 [redacted] 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** [redacted]	
		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

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Acute Toxicity to [redacted]

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

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

Section A7.4.1.2(4) **Annex Point IIA, VII.7.2** **IUCLID 4.2/04**	**Acute toxicity to invertebrates** **Acute Toxicity to [redacted]**	
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A7_4_1_2(4)-1: Preparation of TS solution for poorly soluble or volatile test substances

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

Table A7_4_1_2(4)-2: Dilution water

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

Table A7_4_1_2(4)-3: Test organisms

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

Table A7_4_1_2(4)-4: Test system

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

Table A7_4_1_2(4)-5: Test conditions

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

Table A7_4_1_2(4)-6: Mortality data

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

Table A7_4_1_2(4)-7: Effect data

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

effect data are based on nominal (n) concentrations

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

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

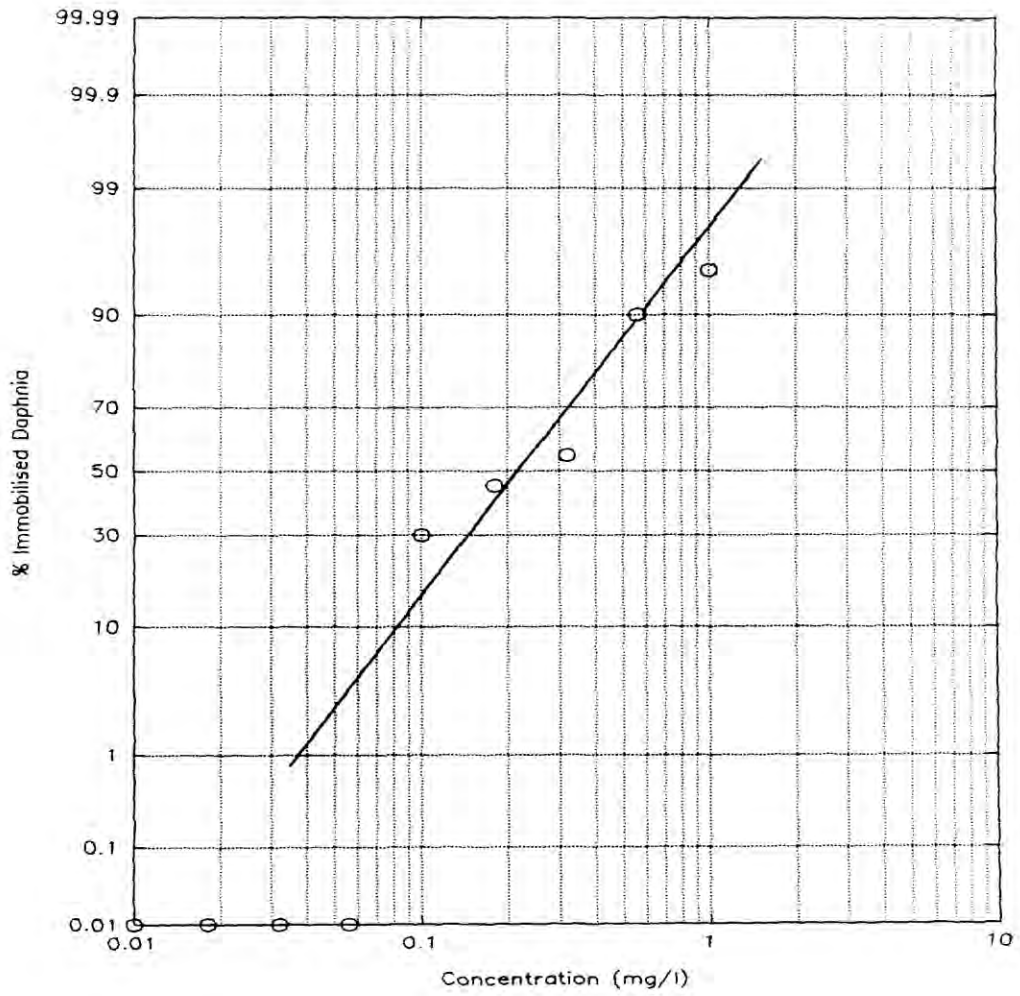
[1] Calculation of geometric mean was done in Microsoft® Office Excel 2003.
[2] na = not applicable
[3] ---- = Analysis of day 0 analysis of concentrations were not conducted on these exposure solutions but concentrations were determined on at all exposure levels at test termination (48 hours). Recoveries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

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

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

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

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



-Section A7.4.1.3(1) **Annex Point IIA, VII.7.3** **IUCLID 4.3/01**		**Growth inhibition test on algae** **Fresh water algal growth inhibition test with glutaraldehyde 50%**	
		1 REFERENCE (A7.4.1.3/01)	**Official use only**
1.1	**Reference**	[redacted] (2001) Fresh water algal growth inhibition test with glutaraldehyde 50%, [redacted], Unpublished, 19 June 2001	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dow Chemical Company	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 201	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Glutaraldehyde, 50%	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	3.1.3	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Samples containing glutaraldehyde were derivatised with 2,4-DNPH and orthophosphoric acid in acetonitrile. Final extracts were analyzed by HPLC with UV detection using a suitable HPLC and the following conditions: Analytical Column: Zorbax 5 CN, 5µm, 250mm x 4.6mm Mobile phase: 75:25:0.1 v/v/v acetonitrile:milli-Q water:orthophosphoric acid Flow rate: 1 mL/minute Wavelength: 365nm Injection Volume: 100 µl Run time: 10 min.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	