(1) This study was previously submitted in support of Product Types 2, 3, 4 & 6. This data has therefore not been re-submitted but is cross referenced to the Part C dossier, where it can be found in Document IVA.

Section 5.3: Summary table of experimental data on the effectiveness of the active substance against target organisms at different fields of use envisaged, where applicable

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
Papermill Bacterial Slime	PT 12	Glutaraldehyde	[redacted]	Freshly prepared ground wood pulp suspension (20 g/l solids) was dispensed into sterile bottles (50 g aliquots). The biocide was added to these bottles [redacted] 2 replicates per concentration). The pH was adjusted between 5.0 and 5.5 with a 0,4 % aluminium sulfate or with citrate-phosphate buffer. The bottles were inoculated and incubated [redacted] The number of surviving bacteria were determined using standard plate count methods. Data evaluation: Calculation of % kill. Effectiveness acceptance criteria: The effectiveness for slime control is sufficient, if more than	Biocide test concentrations [redacted] Incubation temperature: 37°C Contact time [redacted] Neutralizer: Phosphate buffered saline containing 1 % glycine	[redacted]	BPD ID A5.3.2_06 [redacted] Key study

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
				99 % kill is observed and the controls meet the common microbiological criteria.			
Papermill Fungal Slime	PT 12	Glutaraldehyde	[redacted]	Freshly prepared groundwood pulp suspension (20 g/l solids) was dispensed into sterile bottles (50 g aliquots). The biocide was added to these bottles [redacted] 2 replicates per concentration). The pH was adjusted between 5.0 and 5.5 with a 0,4 % aluminium sulfate or with citrate-phosphate buffer. The bottles will be inoculated and incubated [redacted] examined for visible growth.	[redacted]	[redacted]	BPD ID A5.3.2_07 [redacted] Supportive information only

Kommentar [KS1]: Information has not been included in Doc II B. No study summary.

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
Papermill Deinking	PT12	Glutaraldehyde	[redacted]	A stock solution of O.6g/l NaOH + 25 ppm Hydrogen Peroxide was made in de-ionised water. For the control at time = 0 100 mls of the stock solution was dosed with Catalase to give a concentration of 115u/ml. The peroxide level was measured over time using the Hydrogen Peroxide test strips. For the test solutions the required level of biocide was added prior to the catalase addition.		[redacted]	BPD ID A5.3.2_08 [redacted] Supportive information only
Oilfield process water	PT12	Glutaraldehyde	[redacted]	Aerobic single strain Aqueous (marine) kill tests Anaerobic single strain aqueous (marine) kill tests	10^5 cfu/ml concentrations of the aerobes [redacted] Glutaraldehyde a.s. and surviving numbers recorded after exposures [redacted] 10^5 cfu/ml concentrations of the anaeraerobe strains [redacted]	[redacted]	BPD ID A5.3.2_10 [redacted] Supportive information only

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference
					[redacted]	[redacted]	

Finnish Safety and Chemicals Agency

16 January 2014

Please refer to Doc IIC, Chapter 15 for Measures to protect man, animals and the environment.

SECTION A9 CLASSIFICATION, PACKAGING AND LABELLING

RMS note, 21 March 2011:	This classification is proposed by the applicant. Please refer to Doc IIA for the detailed proposal by the RMS.

Labelling

Area of use/site of application:

Glutaraldehyde is a 50% w/w liquid product used in industrial processes or professionally formulated into products as a biocide. The general public do not come into contact with Glutaraldehyde in this form.

Contains: Active ingredient: 50 g/kg Glutaraldehyde

Hazard Symbol:

T - toxic

Toxic

N – environmental hazard

Dangerous for the environment

R-Phrases

R23/25	Toxic by inhalation and if swallowed.
R34	Causes burns
R42/43	May cause sensitisation by inhalation and skin contact
R50	Very toxic to aquatic organisms

S-Phrases

S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39	Wear suitable protective clothing, gloves and eye and face protection.
S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
S61	Avoid release to the environment. Refer to special instructions/safety data sheets

Packaging:

Glutaraldehyde is delivered in [redacted] made from high density polypropylene or in [redacted].

Proposal for safety data sheet:

A safety data sheet is attached

Reference:

SECTION A9 CLASSIFICATION, PACKAGING AND LABELLING

Safety Data Sheet [redacted] ([redacted]% glutaraldehyde)
(See Doc V, Ref.Doc I.2 Appendix 7 & 8.)

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1991) Hydrolysis of 14 C-Glutaraldehyde in aqueous solutions buffered at pH 5, 7, and 9 [redacted] (Unpublished), BPD ID A7.01.1.1.1_01
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, according to US EPA Guideline subdivision N 161-1
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	^{14}C-glutaraldehyde [redacted] Non-labelled glutaraldehyde [redacted]
3.1.1	Lot/Batch number	No data
3.1.2	Specification	For the non-labelled glutaraldehyde, as given in section 2
3.1.3	Purity	Radiochemical purity of ^{14}C-glutaraldehyde: ca. [redacted]%
3.1.4	Further relevant properties	Specific activity of ^{14}C-glutaraldehyde as provided by the manufacturer: 43.94 µCi/mg Specific activity of ^{14}C-glutaraldehyde as determined by the CHMR: 40.20 µCi/mg The latter was used for calculations.
3.2	**Reference substance**	-
3.3	**Test solution**	See table A7_1_1_1_1-1 and table A7_1_1_1_1-2
3.4	**Testing procedure**	
3.4.1	Test system	See table A7_1_1_1_1-3
3.4.2	Temperature	Preliminary test: 25 and 80 °C Main test: 25 and 70 °C

3.4.3 pH

Following pH values were considered: 5, 7 and 9.

The pH values of the buffered test solutions remained approximatively stable over the study period, as shown in following table:

Incubation Temperature	Sampling time points				
	0	72 hours	147 hours	11 days	30 days
25 °C	5.00	5.03	5.05	5.08	5.06
	7.02	7.03	7.00	7.00	7.01
	8.98	8.96	9.98	8.96	8.93
70 °C	5.07	5.10	5.30	5.35	5.32
	7.05	7.00	7.19	7.22	7.17
	8.92	8.99	8.97	8.96	8.92

3.4.4 Duration of the test

Preliminary test: 30 days

Main test: 30 days

3.4.5 Number of replicates

Duplicate aliquots for each test solution

3.4.6 Sampling

Concentration of the ^{14}C-glutaraldehyde standard solution	25.9 mg/ml (in water)
Samples	155 µl aliquots of the ^{14}C-glutaraldehyde standard solution were added to individual sterilized glass bottles containing 40 ml aliquots (duplicates) of each of the pH 5, pH 7 and pH 9 buffered solutions. Homogeneity of each mixture was reached by swirling the flask for 5 minutes. Duplicate 20 µl aliquots of each test solution were taken for determination of the intial concentration (Day 0 samples) and subjected to LSC and HPLC.
Initial concentrations of the test solutions (ppm ^{14}C-GA)	(1)- pH 5 (25 °C): 111.47 ppm (2)- pH 7 (25 °C): 109.39 ppm (3)- pH 9 (25 °C): 109.09 ppm (4)- pH 5 (70 °C): 109.77 ppm (5)- pH 7 (70 °C): 108.40 ppm (6)- pH 9 (70 °C): 106.75 ppm
Preparation for incubation	The test solutions were transferred into individual 2 ml sterile borosilicate screw capped vials. In order to avoid volatilization of the test substance in the headspace of each vial, the vials were completely filled with test solution and were immediately sealed.
Incubation	First set of vials: In darkness, at 25 °C (water bath) Second set of vials: In darkness, at 70 °C (water bath)
Additional experiment	Because of the rapid hydrolysis of ^{14}C-GA at 70 °C in pH 9 buffered solutions, an additional experiment was conducted for 7 hours at a test concentration of 142.20 ppm. The samples were analyzed similarly to the other samples; however, in order to achieve a better resolution the flow rate was reduced to 0.4 ml/minute (compare with 3.4.7), resulting in following relative retention times: Parent compound (GA): 55.40 minutes GA-condensation isomers: 53.60 minutes Delta-valerolactone: 67.44 minutes
Sampling	

At each sampling time point duplicate samples of each test solution were taken and were immediately subjected to LSC and HPLC without storage. The sampling time points were as follows: For all tested pH values: 0 hour (initial), 23 hours, 72 hours, 147 hours, 11 days and 30 days. Additional experiment (pH 9, 7 hours): 0 hour (initial), 1 hour, 1.5 hours, 2.5 hours, 4 hours and 7 hours.
Confirmation of the identity of glutaraldehyde and of the hydrolysis products
Aliquots of 8% non-labelled glutaraldehyde were added to two pH 9 buffered solutions in individual vessels at 1000 ppm and 100 ppm, and were subjected to incubation at 70 °C for 72 hours. Aliquots of these solutions were then subjected to GCMS (BASF).

3.4.7 Analytical methods

Liquid scintillation counting (LSC):

Purpose	Determination of the total radioactivity in all samples
Liquid scintillation counter	[redacted]
Scintillation cocktail	[redacted]
Bakground counts	Background counts were estimated to be about 20 cpm and were taken into account for correction of the measured cpm.
Quenching	The degree of quenching was determined by external standardization using radium ^{226}; all values were corrected for quenching and counting efficiency
Disintegration per minute (dpm)	Calculated and reported as ppm and as % of the initially applied radioactivity

High Performance Liquid Chromatography (HPLC):

Purpose	Identification of glutaraldehyde and its hydrolysis products
Detection of eluted peaks	Radioactive flow detection: Radioactive Flow Detector, [redacted] UV detection at 210 nm: UV flow detector; only applicable with cold standards Both detectors were connected in series (UV detectort was proximal) directly from the effluent of the HPLC column
Separation of parent compound and hydrolysis products, chromatographic conditions:	
Instrument	[redacted]
Pre-column	[redacted]
Column	Bio Rad; Aminex HPX-87H (300 x 7.8 mm); [redacted]
Column temperature	30 °C
UV detector	[redacted] Tunable UV Absorbance Detector
Radioactive flow detector	[redacted] Radioactive Flow Detector, Efficiency > 97%
Data acquisition and analyzer	[redacted]

Mobile phase	0.01 N sulphuric acid
Detection limit for ^{14}C	0.87 ppm or 0.80% of the initially applied radioactivity
Solvent flow rate	0.7 ml/minute
Injection volume	100 µl
Relative retention times under the above given conditions (radioflow detector)	Parent compound (GA): 15. 9 to 16.2 minutes GA-condensation isomers: 15.7 minutes Delta-valerolactone: 19.9 minutes
Relative retention times, UV detector	Delta-valerolactone: 18.1 minutes

Gas Chromatograph-Mass Spectrometry (GC-MS):

Purpose	Separation of glutaraldehyde and its hydrolysis products
Samples	Samples from pH 9 buffered solutions treated with the non-labelled glutaraldehyde at 1000 ppm (2 samples) and at 100 ppm (one sample) incubated at 70 °C for 72 hours (GC-MS performed by BASF)
Instruments	[redacted] gas chromatograph
Carrier gas	Helium
Column	DB-5 fused silica capillary column from [redacted], 30 meter, 0.25 mm I.D, 1 µl film thickness
Injection port	Splitless Mode
Injector temperature	250 °C
Injection volume	2 µl for the 1000 ppm samples 5 to 10 µl for the 100 ppm sample
Temperature programming for separation of the samples and standards	Initially: 80 °C during analysis with VG TS250 50 °C for 1 minute Increase in 8°C/minute steps 290 °C for 15 minutes
Mass spectrometer scanning	From 35 to 400 amu ([redacted] mass spectrometers) and 35 to 800 amu (VG TS250) at ca. 1 sec/scan cycle.

3.5 Preliminary test Yes

Concentration of the ^{14}CgGlutaraldehyde standard solution	25.9 mg/ml (in water)

Samples	155 µl aliquots of the ^{14}C-glutaraldehyde standard solution were added to individual sterilized glass bottles containing 40 ml aliquots (duplicates) of each of the pH 5, pH 7 and pH 9 buffered solutions. The final concentration was about 100 ppm. Homogeneity of each mixture was reached by swirling the flask for 5 minutes. Duplicate 100 µl aliquots of each test solution were subjected to LSC and HPLC.
Preparation for incubation	The test solutions were transferred into individual 2 ml sterile borosilicate screw capped vials. In order to avoid volatilization of the test substance in the headspace of each vial, the vials were completely filled with test solution and were immediately sealed.
Incubation	First set of vials: In darkness, at 25 °C (water bath) Second set of vials: In darkness, at ca. 80 °C (water bath)
Sampling	Samples of each test solution (duplicate 100 µl aliquots) were collected at time 0 (immediately after treatment and at various sampling time points up to 30 days (720 hours) post-treatment. These samples were subjected to LSC and HPLC.
Results of the preliminary test	At both tested temperatures 25 and 80 °C, hydrolysis of glutaraldehyde was faster at pH 9 than at pH 5 or 7. Following hydrolysis products were identified: (1) Delta-valerolactone and (2) Glutaraldehyde condensation isomers.

3.6 Rate and half-life calculations

Assuming first-order kinetics, the rate constant (k) was determined by inputting data on concentration of the test substance versus time into a computer linear regression program. The half-life was determined on the basis of following formula:

Half life = Natural log of 2 /k

Log 2 = 0.693

4 RESULTS

4.1 Material balance and hydrolysis

See table A7_1_1_1_1-4 and table A7_1_1_1_1-5

4.2 Hydrolysis rate constant (k_h)

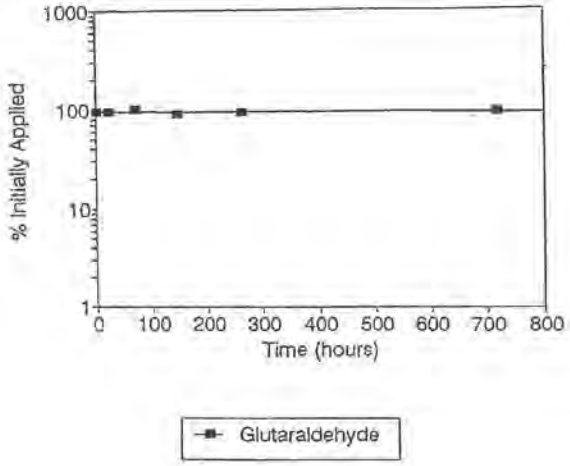
Incubation Temperature	Hydrolysis rate constant ($days^{-1}$)			
	pH 5	pH 7	pH 9	pH 9 additional experiment
25 °C	1.10×10^{-3}	1.76×10^{-3}	1.09×10^{-2}	-
70 °C	1.31×10^{-2}	0.10738	0.124 $hours^{-1}$ (corresponding to 2.99 $days^{-1}$)	

4.3 Dissipation time (DT50) of glutaraldehyde

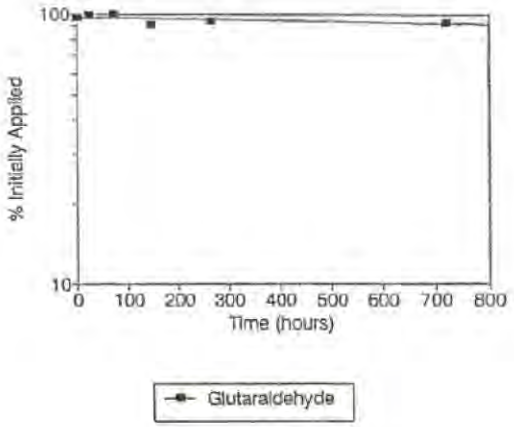
Incubation Temperature	Half-life (days)			
	pH 5	pH 7	pH 9	pH 9 additional experiment
25 °C	628	394	63.8	-
70 °C	53	6.5	5.6 hours (corresponding to 0.232 days)	

4.4 Concentration – time data

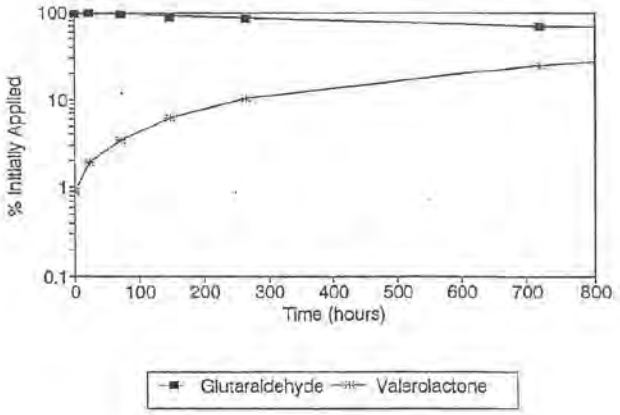
HYDROLYSIS OF GLUTARALDEHYDE AT pH 5 AND 25.05±0.13°C - LOG OF GLUTARALDEHYDE CONCENTRATION VERSUS TIME.



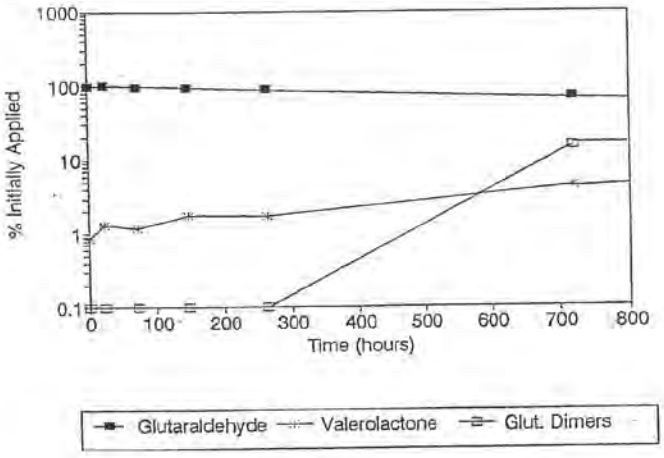
HYDROLYSIS OF GLUTARALDEHYDE AT pH 7 AND 25.05±0.13°C - LOG OF GLUTARALDEHYDE CONCENTRATION VERSUS TIME.



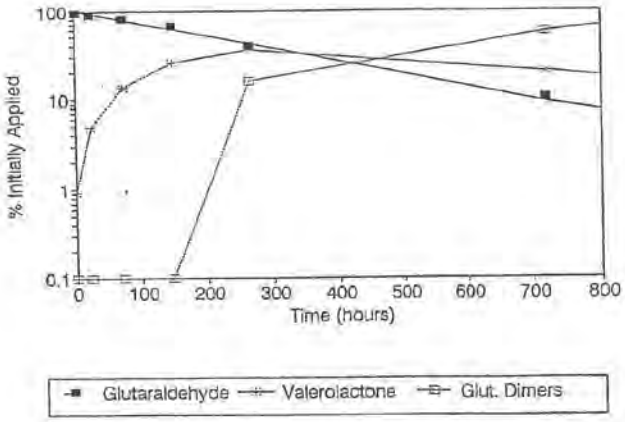
HYDROLYSIS OF GLUTARALDEHYDE AT pH 9 AND 25.05±0.13°C - LOG OF GLUTARALDEHYDE CONCENTRATION VERSUS TIME.



HYDROLYSIS OF GLUTARALDEHYDE AT pH 5 AND 69.54±0.57°C - LOG OF GLUTARALDEHYDE CONCENTRATION VERSUS TIME.

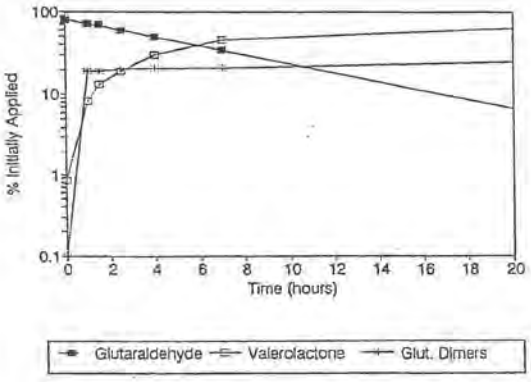


HYDROLYSIS OF GLUTARALDEHYDE AT pH 7 AND 69.54±0.57°C
LOG OF GLUTARALDEHYDE CONCENTRATION VERSUS TIME.

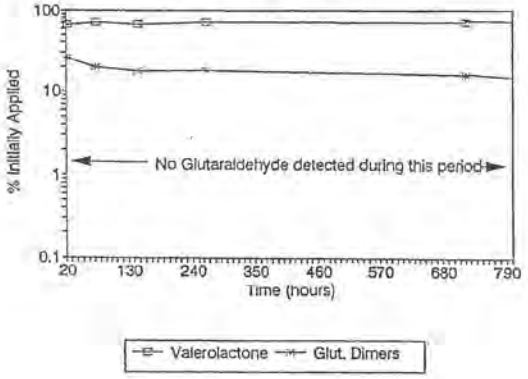


HYDROLYSIS OF GLUTARALDEHYDE AT pH 9 AND 69.54±0.57°C - LOG OF GLUTARALDEHYDE CONCENTRATION VERSUS TIME.

EXPERIMENT 1



EXPERIMENT 2



4.5 Specification of the transformation products

Hydrolysis product identified at 25 °C:

Hydrolysis Product	pH 5	pH 7	pH 9
Delta Valerolactone (CAS No 542-28-9)	No	No	10.6% (11 d) 25% (30 d)
Glutaraldehyde condensation isomers	No	No	

Hydrolysis product identified at 70 °C:

Hydrolysis Product	pH 5	pH 7	pH 9	pH 9 (Additional experiment
Delta Valerolactone (CAS No 542-28-9)	4.3% (30 d)	13.5% (72 h) 26% (147 h) 35.5% (11 d) 20% (30 d)	67.6% (23 h) 71.7% (72 h) 68% (147 h) 72% (11 d) 72% (30 d)	ND* (0 h) 8 % (1 h) 13.3% (1.5 h) 18.8% (2.5 h) 29.5% (4 h) 45% (7 h)
Glutaraldehyde condensation isomers	14.6% (30 d)	16% (11 d) 57.2% (30 d)	25.2% (23 h) 19.5% (72 h) 17.6% (147 h) 18% (11 d) 15.7% (30 d)	19% (0 h) 19.3% (1 h) 19.3% (1.5 h) 20.2% (2.5 h) 20.4% (4 h) 20.7% (7 h)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the abiotic degradation of glutaraldehyde by hydrolysis as function of the pH value and to identify hydrolysis products.

Test substances: (1) ^{14}C-glutaraldehyde [redacted], radiochemical purity of ca. 96.5%, specific activity as determined by the CHMR: 40.20 µCi/mg; (2) non-labelled glutaraldehyde [redacted].

The test was conducted according to US EPA Guideline subdivision N 161-1, with GLP.

Principal methods of analysis: LSC, HPLC, GC-MS

A preliminary test showed that at a temperature of 25 and 80 °C, hydrolysis of glutaraldehyde was faster at pH 9 than at pH 5 or 7; following hydrolysis products were identified: (1) delta-valerolactone and (2) glutaraldehyde condensation isomers. On the basis of these results, the main test was conducted as follows:

155 µl aliquots of the ^{14}C-glutaraldehyde standard solution (25.9 mg/ml in water) were added to individual sterilized glass bottles containing 40 ml aliquots (duplicates) of each of the pH 5, pH 7 and pH 9 buffered solutions. Duplicate 20 µl aliquots of each test solution were taken for determination of the intial concentration (Day 0 samples) and subjected to LSC and HPLC. The initial concentrations of the test solutions were:

1)- pH 5 (25 °C): 111.47 ppm ^{14}C-GA

(2)- pH 7 (25 °C): 109.39 ppm ^{14}C-GA

(3)- pH 9 (25 °C): 109.09 ppm ^{14}C-GA

(4)- pH 5 (70 °C): 109.77 ppm ^{14}C-GA

(5)- pH 7 (70 °C): 108.40 ppm ^{14}C-GA

(6)- pH 9 (70 °C): 106.75 ppm ^{14}C-GA

(7)- pH 9 (70 °C): 142.20 ppm ^{14}C-GA

The test solutions were transferred into individual 2 ml sterile borosilicate screw capped vials, which were completely filled with test solution and immediately sealed. Two sets of vials were subjected to incubation: a first set of vials was incubated in darkness at 25 °C; the second one was incubated in darkness at 70 °C. For the test solutions (1) to (6), the test was run over a period of 30 days. Because of the rapid hydrolysis of ^{14}C-GA at 70 °C in pH 9 buffered solutions, an additional experiment (test solution (7)) was conducted for 7 hours at a test concentration of 142.20 ppm. Sampling for analysis was done as follows:

For all tested pH values: 0 hour, 23 hours, 72 hours, 147 hours, 11 days and 30 days.

Additional experiment (pH 9, 7 hours): 0, 1, 1.5, 2.5, 4 and 7 hours.

At each sampling time point duplicate samples of each test solution were taken and were immediately subjected to LSC and HPLC without storage. All hydrolyse products greater than or egal to 10% were identified.

For confirmation of the identity of glutaraldehyde and of the hydrolysis products, aliquots of 8% non-labelled glutaraldehyde were added to two pH 9 buffered solutions in individual vessels at 1000 ppm and 100 ppm, and were subjected to incubation at 70 °C for 72 hours. Aliquots of these solutions were then subjected to GCMS (BASF).

Assuming first-order kinetics, the rate constant (k) was determined by inputting data on concentration of the test substance versus time into a computer linear regression program. The half-life was determined using following formula:

Half life = Natural log of 2 /k

5.2 Results and discussion

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

Both test parameters, temperature and pH, remained very stable over the study period.

Within the preliminary test and at both tested temperatures 25 and 80 °C, the hydrolysis of glutaraldehyde was faster at pH 9 than at pH 5 or 7. Following hydrolysis products were identified: (1) Delta-valerolactone and (2) Glutaraldehyde condensation isomers.

Within the main test, the material balance for the samples incubated at 25 °C

ranged from 94 to 104% of the initially applied radioactivity at pH 5, from 94 to 103% at pH 7 and from 95 to 102% at pH 9. At a temperature of 70 °C, the material balance at the respective pH values ranged from 89 to 105%, from 93 to 100% and from 94.5 to 101%.

Considering the hydrolysis of the parent compound, glutaraldehyde was found to hydrolyse faster with increasing pH and with increasing temperature. In fact, hydrolysis of glutaraldehyde at 70°C compared to 25 °C was about 12-fold faster at pH 5, 61-fold faster at pH 7 and 277-fold faster at pH 9.

At 25 °C, a relevant hydrolysis product (i.e. >= 10% of applied radioactivity) was identified at pH 9 as delta-valerolactone; no hydrolysis products >= 10% were seen during the 30 day study at pH 5 and pH 7. At 70 °C, hydrolysis relevant products were found at all tested pH values; these products were identified as delta-valerolactone and as two glutaraldehyde condensation isomers. By means of GC-MS, the parent compound glutaraldehyde, delta-valerolactone as well as the two GA-condensation isomers cycloocta 1,5-diene 1,5-dialdehyde and cycloocta 1,4-diene 2,4-dialdehyde were identified in the pH 9 buffered sample s incubated at 70 °C for up to 72 hours.

5.2.1 k_H

At 25 °C the hydrolyse rate constant was as follows:

-At pH 5: 1.10 x 10^{-3} $days^{-1}$

-At pH 7: 1.76 x 10^{-3} $days^{-1}$

-At pH 9: 1.09 x 10^{-2} $days^{-1}$

At 70 °C the hydrolyse rate constant was as follows:

-At pH 5: 1.31 x 10^{-2} $days^{-1}$

-At pH 7: 0.10738 $days^{-1}$

-At pH 9: 0.124 $hours^{-1}$ (corresponding to 2.99 $days^{-1}$)

5.2.2 DT_{50}

At 25 °C the half-life values were as follows:

-At pH 5: 628 days

-At pH 7: 394 days

-At pH 9: 63.8 days

At 70 °C the half-life values were as follows:

-At pH 5: 53 days

-At pH 7: 6.5 days

-At pH 9: 5.6 hours (corresponding to 0.232 days)

5.2.3	r^2	At 25 °C the correlation coefficient was as follows: -At pH 5: 0.0966* -At pH7: 0.3222* -At pH 9: 0.9670 At 70 °C the correlation coefficient was as follows: -At pH 5: 0.9687 -At pH7: 0.9654 -At pH 9: 0.9975 *, Since the correlation coefficients correlate the change in the dependend variable with a corresponding change in the independent variable, it is not a reliable statistic to use for lines with low slopes. Therefore, this correlation coefficient should not be interpreted to mean the data are unreliable.
5.3	**Conclusion**	Glutaraldehyde was shown to be very stable at pH 5 and 7 at 25 °C, with a half-life time > 390 days.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	A discrepancy concerning the initial concentration of the additional pH 9 test solution (i.e. 7 hours test solution) was seen. In fact, on page 20 of 190 of the document, an initial concentration of 106.75 ppm was given for the additional solution, whereas on page 34 of 190, the value given above was attributed to the "main" pH 9 test solution (i.e. the 30 days test solution) whereas the concentration for the additional pH test solution was given as 142.20 ppm. Our opinion is that the data given on page 34 of 190 are the correct data. This discrepancy however does not affect the validity of the present study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29.8.2008
Materials and Methods	The applicant's description of materials and methods is correct. The test method deviates in some details from the current OECD 111. Instead of a tiered approach both the preliminary and definite tests have been conducted at two temperatures, pH 5 has been tested instead of pH 4, bubbling with nitrogen or argon in order to avoid oxygen is not mentioned in the test report, confidence intervals have not been reported for rate constants and half lives.
Results and discussion	The applicant's version is correct. The hydrolytic half-lives of glutaraldehyde were 628 d, 394 d and 63.8 d at pH 5, 7 and 9, respectively. The rate constants calculated from the half lives with eq. $k=\ln 2/t_{½}$ were $1.10E^{-3}$, $1.76E^{-3}$ and $1.09E^{-2}$. Average radioactivity recovery ranged from 94 to 104% at 25 °C fulfilling the quality criteria of 90-110% recovery for labelled chemicals. The analytical method was sufficient to quantify test substance down to less than 10% of initial concentration.
Conclusion	Glutaraldehyde hydrolyses faster with increasing pH and temperature. Results from 25 °C are relevant for the environmental risk assessment and are reported here. Glutaraldehyde is hydrolytically stable in acidic and neutral conditions and no hydrolysis products exceeded 10%. At pH 9 glutaraldehyde hydrolysed and after 30 days 70.08% of radioactivity accounted for glutaraldehyde and 25.05% for delta-valerolactone. The latter was the only hydrolysis product that was formed > 10% of initially applied radioactivity.
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_1-1: Type and composition of buffer solutions (specify kind of water if necessary)

pH	Composition
5	2.56 g potassium hydrogen phthalate 5.65 ml 1 N NaOH 175.85 ml water*
7	1.71 g potassium dihydrogen phosphate 7.25 ml 1 N NaOH 190.25 ml water*
9	1.20 g borax 1.15 ml 1 N HCl 135.35 ml water*

*, The water was filtered-sterilized through a 0.20 µ filter immediately prior use

Table A7_1_1_1_1-2: Description of test solution

Criteria	Details
Purity of water	The water (ASTM Type IIA standards) was filtered-sterilized through a 0.20 µ filter ([redacted]) immediately prior use
Preparation of test medium	155 µl aliquots of the ^{14}C-glutaraldehyde standard solution were added to individual sterilized glass bottles containing 40 ml aliquots (duplicates) of each of the pH 5, pH 7 and pH 9 buffered solutions. Homogeneity of each mixture was reached by swirling the flask for 5 minutes. Duplicate 20 µl aliquots of each test solution were taken for determination of the intial concentration (Day 0 samples) and subjected to LSC and HPLC.
Test concentrations (mg a.i./L)	^{14}C-glutaraldehyde standard solution: 25.9 mg/ml (in water) Initial concentration range of the test solutions: (1)- pH 5 (25 °C): 111.47 ppm ^{14}C-GA (2)- pH 7 (25 °C): 109.39 ppm ^{14}C-GA (3)- pH 9 (25 °C): 109.09 ppm ^{14}C-GA (4)- pH 5 (70 °C): 109.77 ppm ^{14}C-GA (5)- pH 7 (70 °C): 108.40 ppm ^{14}C-GA (6)- pH 9 (70 °C): 106.75 ppm ^{14}C-GA (7)- pH 9 (70 °C, additional): 142.20 ppm ^{14}C-GA
Temperature (°C)	25 and 70 °C
Controls	-
Identity and concentration of co-solvent	None
Replicates	All samples were tested /analysed in duplicates

Table A7_1_1_1_1-3: Description of test system

Glassware	Glass bottles; 2ml- borosilicate screw capped vials
Other equipment	Thermostatically controlled water bath; [redacted] pH meter
Method of sterilization	Sterilization of glassware by exposure to dry heat at 170-190°C for more than 1 hour

Table A7_1_1_1_1-4:

Material balance of the test system at pH 5 and at a test concentration of 111.47 ppm ^{14}C-glutaraldehyde (incubation in the dark at 25 °C; LSC)		
Sampling time point	**Total radioactivity (average of 2 measurements/sampling time point)**	
	As % of initial applied concentration	**As ppm ^{14}C-glutaraldehyde**
0 hour	100	111.47
23 hours	99.31	110.70
72 hours	104.44	116.42
147 hours	93.36	104.07
11 days	94.22	105.03
30 days	95.99	107.00

Material balance of the test system at pH 7 and at a test concentration of 109.39 ppm ^{14}C-glutaraldehyde (incubation in the dark at 25 °C; LSC)		
Sampling time point	**Total radioactivity (average of 2 measurements/sampling time point)**	
	As % of initial applied concentration	**As ppm ^{14}C-glutaraldehyde**
0 hour	100	109.39
23 hours	102.91	112.57
72 hours	102. 73	112.37
147 hours	94.23	103.08
11 days	95.35	104.31
30 days	95.17	104.11

Material balance of the test system at pH 9 and at a test concentration of 109.09 ppm ^{14}C-glutaraldehyde (incubation in the dark at 25 °C; LSC)		
Sampling time point	**Total radioactivity (average of 2 measurements/sampling time point)**	
	As % of initial applied concentration	**As ppm ^{14}C-glutaraldehyde**
0 hour	100	109.09
23 hours	102.25	111.54
72 hours	100.78	109.94
147 hours	95.44	104.11
11 days	95.95	104.67
30 days	96.35	105.11

Material balance of the test system at pH 5 and at a test concentration of 109.77 ppm ^{14}C-glutaraldehyde (incubation in the dark at 70 °C; LSC)		
Sampling time point	**Total radioactivity (average of 2 measurements/sampling time point)**	
	As % of initial applied concentration	**As ppm ^{14}C-glutaraldehyde**
0 hour	100	109.77
23 hours	105.40	115.70
72 hours	98.56	108.19
147 hours	98.65	108.29
11 days	91.96	100.95
30 days*	89.27	97.99

*, One measurement only, due to analytical error

Material balance of the test system at pH 7 and at a test concentration of 108.40 ppm ^{14}C-glutaraldehyde (incubation in the dark at 70 °C; LSC)		
Sampling time point	**Total radioactivity (average of 2 measurements/sampling time point)**	
	As % of initial applied concentration	**As ppm ^{14}C-glutaraldehyde**
0 hour	100	108.4
23 hours	98.39	106.6
72 hours	98.66	106.9
147 hours	96.68	104.8
11 days	93.97	101.9
30 days	92.77	100.6

Material balance of the test system at pH 9 and at a test concentration of 106.75 ppm ^{14}C-glutaraldehyde (incubation in the dark at 70 °C; LSC)		
Sampling time point	**Total radioactivity (average of 2 measurements/sampling time point)**	
	As % of initial applied concentration	**As ppm ^{14}C-glutaraldehyde**
0 hour	100	106.75
23 hours	98.85	105.52
72 hours	100.19	106.95
147 hours	94.47	100.85
11 days	96.54	103.06
30 days	98.46	105.11

Material balance of the test system at pH 9 and at a test concentration of 142.20 ppm ^{14}C-glutaraldehyde (additional experiment, incubation in the dark at 70 °C; LSC)		
Sampling time point	**Total radioactivity (average of 2 measurements/sampling time point)**	
	As % of initial applied concentration	**As ppm ^{14}C-glutaraldehyde**
0 hour	100	142.20
1 hour	99.11	140.93
1.5 hours	101.42	144.21
2.5 hours	98.43	139.96
4 hours	98.56	140.15
7 hours	100.20	142.48

Table A7_1_1_1_1-5:

Hydrolysis of glutaraldehyde at pH 5 and at a test concentration of 111.47 ppm ^{14}C-glutaraldehyde (incubation in the dark at 25 °C; HPLC & LSC)		
Sampling time point	**Percentage of initially applied radioactivity**	
	Glutaraldehyde	**Total**
0 hour	96.60	96.60
23 hours	96.27	96.27
72 hours	101.05	101.05
147 hours	90.05	90.05
11 days	92.62	92.62
30 days	94.23	94.23

Hydrolysis of glutaraldehyde at pH 7 and at a test concentration of 109.39 ppm ^{14}C-glutaraldehyde (incubation in the dark at 25 °C; HPLC & LSC)		
Sampling time point	**Percentage of initially applied radioactivity**	
	Glutaraldehyde	**Total**
0 hour	96.61	96.61
23 hours	99.25	99.25
72 hours	99.71	99.71
147 hours	91.55	91.55
11 days	94.00	94.00
30 days	93.06	93.06

Hydrolysis of glutaraldehyde at pH 9 and at a test concentration of 109.09 ppm ^{14}C-glutaraldehyde (incubation in the dark at 25 °C; HPLC & LSC)			
Sampling time point	**Percentage of initially applied radioactivity**		
	Glutaraldehyde	**Delta-Valerolactone**	**Total**
0 hour	96.29	0.89	97.18
23 hours	97.89	1.91	99.80
72 hours	94.63	3.45	98.08
147 hours	86.60	6.23	92.83
11 days	85.38	10.56	95.94
30 days	70.08	25.05	95.13

Hydrolysis of glutaraldehyde at pH 5 and at a test concentration of 109.77 ppm ^{14}C-glutaraldehyde (incubation in the dark at 70 °C; HPLC & LSC)				
Sampling time point	**Percentage of initially applied radioactivity**			
	Glutaraldehyde	**Delta-Valerolactone**	**Glutaraldehyde Condensation Isomers**	**Total**
0 hour	96.45	0.87	ND*	97.32
23 hours	101.88	1.33	ND	103.21
72 hours	94.61	1.19	ND	95.80
147 hours	94.02	1.79	ND	95.81
11 days	88.56	1.73	ND	90.29
30 days	68.46	4.26	14.62	87.34

*, Detection limit for radioactive flow detector: 0.8% of initial applied concentration

Hydrolysis of glutaraldehyde at pH 7 and at a test concentration of 108.4 ppm ^{14}C-glutaraldehyde (incubation in the dark at 70 °C; HPLC & LSC)				
Sampling time point	**Percentage of initially applied radioactivity**			
	Glutaraldehyde	**Delta-Valerolactone**	**Glutaraldehyde Condensation Isomers**	**Total**
0 hour	96.53	0.92	ND	97.45
23 hours	91.25	4.90	ND	96.15
72 hours	82.51	13.55	ND	95.70
147 hours	67.45	26.10	ND	93.55
11 days	40.12	35.48	15.91	91.51
30 days	10.15	20.00	57.17	87.32

Hydrolysis of glutaraldehyde at pH 9 and at a test concentration of 106.75 ppm ^{14}C-glutaraldehyde (incubation in the dark at 70 °C; HPLC & LSC)**				
Sampling time point	**Percentage of initially applied radioactivity**			
	Glutaraldehyde	**Delta-Valerolactone**	**Glutaraldehyde Condensation Isomers**	**Total**
23 hours	ND	67.64	25.22	92.86
72 hours	ND	71.69	19.53	91.22
147 hours	ND	67.94	17.57	85.85
11 days	ND	72.31	18.08	90.40
30 days	ND	71.69	15.73	87.42

**, At time 0, GA accounted for 96.27% of the initial applied radioactivity and delta valerolactone accounted for 0.78%.

Hydrolysis of glutaraldehyde at pH 9 and at a test concentration of 142.20 ppm ^{14}C-glutaraldehyde (additional experiment, incubation in the dark at 70 °C; HPLC & LSC)				
Sampling time point	**Percentage of initially applied radioactivity**			
	Glutaraldehyde	**Delta-Valerolactone**	**Glutaraldehyde Condensation Isomers**	**Total**
0 hour	80.94	ND	19.07	100.01
1 hour	71.62	8.22	19.26	99.10
1.5 hours	69.97	13.29	19.35	102.61
2.5 hours	59.34	18.85	20.24	98.43
4 hours	49.42	29.56	20.37	99.25
7 hours	33.66	45.11	20.71	99.47

Section A7.1.1.1.2 _ 01 Phototransformation in water including identity of transformation products

Annex Point IIA7.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1986) Determination of the photolysis rate constants and degradation products of Glutaraldehyde. [redacted] (Unpublished), BPD ID A7.01.1.1.2_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new a.s. for first entry to Annex I authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, according to EPA Guideline 161-2	
2.2	**GLP**	Yes	
2.3	**Deviations**	-	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Non-labelled glutaraldehyde 25% — C^{14}-labelled glutaraldehyde	x
3.1.1	Lot/Batch number	No data [redacted]	
3.1.2	Specification	As given in section 2 -	
3.1.3	Purity	Analytical purity — Radiochemical purity [redacted]%.	
3.1.4	Radiolabelling	1,5-pentadial [UL-^{14}C]	
3.2	**Reference substances**	None	
3.3	**Test solution**	(1) Unsensitized solution, dark (2) Unsensitized solution, light (3) Sensitized solution (1% acetone), dark (4) Sensitized solution (1% acetone), light (5) Sensitized solution (1% acetonitrile), dark (6) Sensitized solution (1% acetonitrile), light For details see table A7_1_1_2-1	
3.4	**Testing procedure**		
3.4.1	Test system	See table A7_1_1_2-2	x
3.4.2	Properties of light source	See table A7_1_1_2-2	
3.4.3	Temperature	The recorded temperature over the study period ranged	

		between 19 and 28 °C
3.4.4	Test concentration	250 ppm (or µg/ml)
3.4.5	Duration of the test	20 days, equivalent to 36 days of natural sunlight (12 hours/day) in the sensitized acetone system and to 34 of such days in the sensitized acetonitrile and the unsensitized systems.
3.4.6	Number of replicates	Triplicate per test solution
3.4.7	Sampling	Duplicate 4 ml aliquots of each test solution were transferred into culture tubes (13 x 100 mm), purged with argon and sealed by means of Teflon-lined caps. One tube per test solution was placed in a photochemical turntable. The remaining culture tubes were placed in a box wrapped in a double layer of foil to prevent entry of light. Both light and dark reaction tubes were kept in a fume hood over the study period. <u>Sampling of the sensitized acetone light and dark systems and the sensitized acetonitril dark system:</u> Day 0, 1, 2, 3, 6, 10, 14 and 18 <u>Sampling of the sensitized acetonitril light system and both unsensitized systems (dark and light):</u> Day 0, 1, 2, 3, 7, 10, 14 and 17
3.4.8	Analytical methods	<u>GLC</u>: At each sampling interval triplicate 1 µl aliquots of each test system were analyzed by gas liquid chromatography. <u>TLC</u>: For determination of the total applied activity samples (triplicate, 25 µl) were collected on day 2, 3, 6, 10 and 14 from the sensitized light and dark acetone test systems and from the sensitized dark acetonitrile test system. Further samples were collected on day 3, 7, 10, 14 and 17 from the sensitized light acetonitrile test system and from both unsensitized test systems (dark and light). <u>LSC</u>: After day 2 of the study, triplicate 25 µl aliquots of each system were subjected to liquid scintillation counting. Each sample was counted for 5 minutes or to a Sigma (95%) confidence level using a single label DPM data calculation program. The quench curve was obtained from counting a set of Amersham quenched carbon-14 LSC standards. The amount of quench in a sample is determined by analyzing the position of its Compton spectrum. The DPM program is designed to establish the quench curve and to use it to resolve sample count to DMP values. An unquenched calibration standard of ^{3}H was integrated in the system.

3.4.9	Calculations	Concentration of glutaraldehyde in the aqueous test systems: Determination by GLC analysis and calculation from standard curve equation. Half-life: Estimation on the basis of first order photolysis rate using following equations: (1) $\ln C_T = -kt + \ln C_0$ C_T = total residues of GA (determined by LSC) C_0 = concentration at time 0 (determined by LSC) (2) $t_{1/2} = -\ln 2 / k = -0.693 / k$ Amount of ^{14}C-residues of GA in photolysis solution: Calculation as follows: DPM / (specific Activity DPM/µg)*(Volume for LSC) = µg/ml DPM = Net CPM / Counting Efficiency Parent compound equivalent for TLC characterization: Calculation as above. ^{14}C-residues of GA characterized from TLC plate as % of total ^{14}C-activity recovered: Calculation as follows: % of Total DPM recovered = [(DPM of TLC Zone) / (Total recovered DPM from TLC plate)] * 100
3.5	**Transformation products**	
3.5.1	Method of analysis for transformation products	See 3.4.8

4 RESULTS

4.1 Photolysis data

For results of the GLC analysis, see table A7_1_1_2-3.

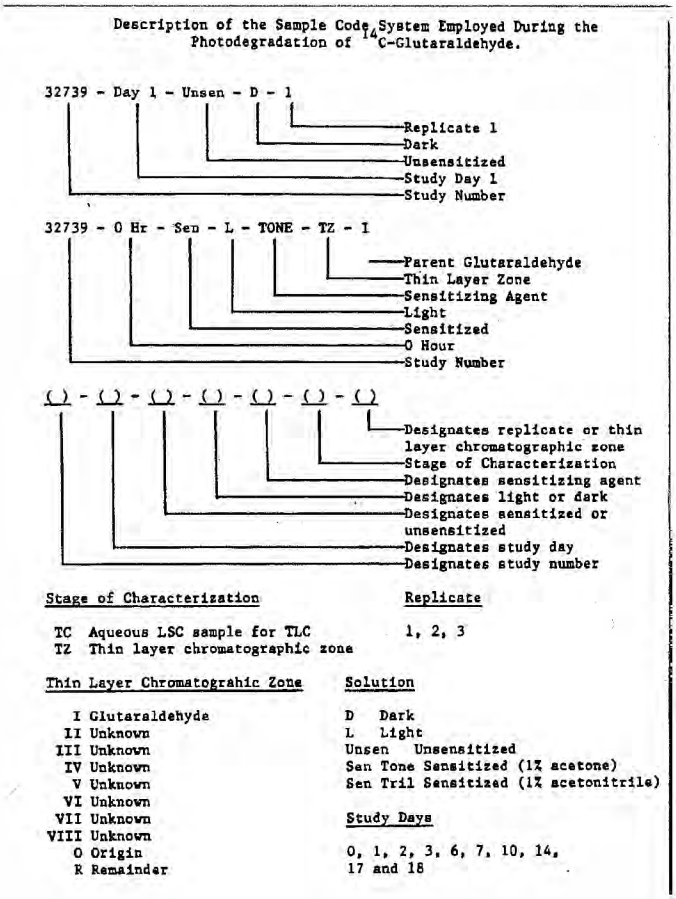
For results of the LSC analysis, see table A7_1_1_2-4.

For data points and line equation for half-life estimation, see table A7_1_1_2-5.

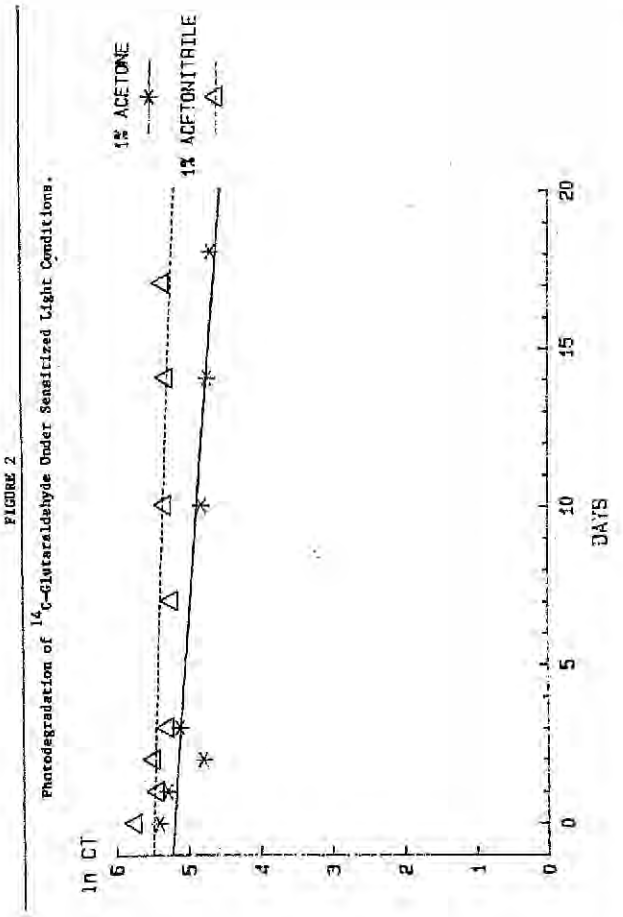
For results of the autoradiography, see A7_1_1_2-6.

For data on recovery of ^{14}C- Activity, see A7_1_1_2-7.

The samples were coded as follows:



4.1.1	Photodegradation of ^{14}C-glutaraldehyde under light conditions	The photolysis half-life of glutaraldehyde in acetone sensitized light system was estimated to be 18 days. The photolysis half-life of glutaraldehyde in sensitized acetonitril light system was estimated to be 49 days.	



4.1.2	Photodegradation of ^{14}C-glutaraldehyde under dark conditions	No apparent photodegradation was observed in the sensitized acetone and the sensitized acetonitril dark systems, as well as in both unsensitized systems (light and dark).	
4.1.3	Kinetic order	First order photolysis rate.	
4.2	**Characterization of the photodegradation products**	For characterization of the photodegradation products, see A7_1_1_2-6. For recovery of ^{14}C- Activity related to the degradation products, see A7_1_1_2-7.	x

The degradation products were assigned following alphabetical notation for identification:

TLC Zone	Corresponding photodegradation product
I (parent; glutaraldehyde)	G
II (unknown)	F
III (unknown)	E
IV (unknown)	A
V (unknown)	B
VI (unknown)	D
VII (unknown)	H
VIII (unknown)	I

Main photodegradation products identified by TLC in the different test systems (>= 10%):

Test System	Photodegradation products	
	Product ID	% (day)
Unsensitized solution, dark	-	
Unsensitized solution, light	B	9.5%(7) 9.8% (17)
	H	12.1%(14)
Sensitized solution (1% acetone), dark	-	
Sensitized solution (1% acetone), light	F	15.3%(2)
	B	9.8%(3) 13.2%(6) 13%(10)
	A	9.1%(6) 10.8%(10)
	H	35.1%(14)
Sensitized solution (1% acetonitrile), dark	-	
Sensitized solution (1% acetonitrile), light	F	9.8%(10)
	B	10.2%(17)
	H	11.5%(14)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the photodegradation of glutaraldehyde in the aquatic environment.

Test substances:

[redacted] C^{14}-glutaraldehyde standard received [redacted]

(2) glutaraldehyde 25[redacted], analytical purity.

The test was conducted according to the EPA Guideline 161-2

Test duration: 20 days, equivalent to 36 days of natural sunlight (12 hours/day) in the sensitized acetone system and to 34 of such days in the sensitized acetonitrile and the unsensitized systems.

The test water used was deionized water boiled for 30 minutes and filtered through a 0.22 micron-filter.

A primary stock solution (PSS) of C^{14}-labelled glutaraldehyde was prepared, with a concentration of 1.186 µg/µl, and the radiochemical purity of 99% as revealed by gas liquid chromatography (GLC) and liquid scintillation counting (LSC). Prior starting the test, further replicates were sampled for analysis and a concentration of 1.174 µg/µl was determined. The non-labelled glutaraldehyde ([redacted]) served for TLC method development and validation, and was used for the preparation of the aqueous test solutions of C^{14} –GA; furthermore it was used for the preparation of the GLC standards. Following aqueous test systems were prepared:

Unsensitized solution:

0.333 ml of C^{14} –GA PSS and 7.5 µl of GA[redacted] were filled up to 10 ml with a pH 5 buffer solution (0.2M acetic acid/0.2 M sodium acetate), resulting in a nominal test concentration of 250 µg/ml.

Sensitized solution (1% acetone):

Prepared as above with additionally 100 µl acetone. Final nominal test concentration: 250 µg/ml.

Sensitized solution (1% acetonitrile):

Prepared as above with additionally 100 µl acetonitrile. Final nominal test concentration of 250 µg/ml.

Duplicate 4 ml aliquots of each test solution were transferred

into culture tubes (13 x 100 mm), purged with argon and sealed by means of Teflon-lined caps. One tube per test solution was placed in a photochemical turntable (450 Watt [redacted] mercury-vapor lamp, located in the center of the turntable in an Ace borosilicate immersion cell with a flow rate of 100 ml H_2O/minute; distance between center of the reaction tube and center of the light well: 9.2 cm). The remaining culture tubes were placed in a box wrapped in a double layer of foil to prevent entry of light.

Both light and dark reaction tubes were kept in a fume hood over the study period. The final series of test solutions was was follows:

(1) Unsensitized solution, dark

(2) Unsensitized solution, light

(3) Sensitized solution (1% acetone), dark

(4) Sensitized solution (1% acetone), light

(5) Sensitized solution (1% acetonitrile), dark

(6) Sensitized solution (1% acetonitrile), light

The test solutions (3), (4) and (5) were sampled on day 0, 1, 2, 3, 6, 10, 14 and 18. The test solutions (1), (2) and (6) were sampled on day 0, 1, 2, 3, 7, 10, 14 and 17.

At each sampling interval triplicate 1 µl aliquots of each test system were analyzed by gas liquid chromatography (GLC). After day 2 of the study, triplicate 25 µl aliquots of each system were subjected to thin layer chromatography (TLC) and further triplicates were subjected to liquid scintillation counting (LSC).

5.2 Results and discussion

The photolysis half-life of glutaraldehyde in acetone sensitized light system was estimated to be 18 days.

The photolysis half-life of glutaraldehyde in sensitized acetonitril light system was estimated to be 49 days.

No apparent photodegradation was observed in the sensitized acetone and the sensitized acetonitril dark systems, as well as in both unsensitized systems (light and dark). Therefore half-lives were not estimated.

5.3 Conclusion

Under sensitized conditions equivalent to 36 days of natural sunlight (12 hours/day; sensitized acetone system) and to 34 of natural sunlight (12 hours/day; sensitized acetonitril system), glutaraldehyde was subjected to photolytic degradation with a half-life of respectively 18 (acetone) and 49 days (acetonitril); no photodegradation was observed under darkness. Under non-sensitized conditions, no photodegradation of glutaraldehyde occurred, i.e. the test substance was stable. These findings were in accordance with the results of a study reported by [redacted] in his review on ecotoxicological glutaraldehyde data ([redacted], Hydrolysis of [1,5-C^{14}]-Glutaraldehyde at pH 5, 7 and 9. [redacted], cited in Leung H-W, Ecotoxicology of Glutaraldehyde: Review of environmental fate and effects studies. Ecotox. Environ. Safety 49: 26-39,

		2001. Within this study the summarized test conditions were as follows: 10 ml aliquots of a 10.4 mg a.i./l solution of glutaraldehyde (14.14 µCi radiocarbon; pH 5) were exposed to natural sunlight at 25 °C for 30 days; the average daily total light energy was 5.68 W.min/cm^2. Under these test conditions, glutaraldehyde was found to slowly degrade with a first-order rate constant of photolysis (K) of 0.0035 day -1 and a half-life of 196 days. Glutaraldehyde therefore is stable to sunlight in an aqueous environment.
5.3.1	Reliability	**2**
5.3.2	Deficiencies	The pH parameter was not taken into consideration. The temperature ranged from 19 to 28 °C over the whole testing period (25 +/-1 °C are required). Under sensitized conditions where photodegradation of glutaraldehyde occurred, the products resulting from degradation were quantified but not identified.

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1.9.2008
Materials and Methods	Applicant's version is correct except that the test guideline EPA Guideline 161-2 is not mentioned in the test report. 3.1 Adsorption spectra UV/VIS λ_{max} 234-283 nm (Doc IIA3.3) is outside the range 290-800 nm where chemicals can undergo a direct photolysis in water. 3.4.1 Material of test tubes was not explained in the test report. 3.4.1 A mercury-vapor lamp has been used while a filtered xenon arc lamp or sunlight irradiation is recommended in the OECD 316. According to the SETAC guidance mercury lamps are not acceptable light sources (Lynch, MR. 1995. Procedures for assessing the environmental fate and ecotoxicity of pesticides, Society of Environmental Toxicology and Chemistry. ISBN 90-5607-002-9). 3.4.3 Temperature ranged between 19 and 28 °C over the study period while a range of 23-27 °C is accepted in the OECD 316. 4 Material balance has not been determined. 4 Photolysis rate constants have not been reported. 4.2 Phototransformation products have not been identified.
Results and discussion	Applicant's version is correct. No photodegradation was observed in non-sensitized conditions and no half-life was determined. Photolytic half-life of glutaraldehyde in light was 18 days in acetone sensitized solution and 49 days in acetonitril sensitized solution.
Conclusion	Glutaraldehyde is photolytically stable under natural conditions.
Reliability	3
Acceptability	Not acceptable due to the mercury lamp.
Remarks	

Section A7.1.1.1.2 _ 01 Phototransformation in water including identity of transformation products

Annex Point IIA7.1

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

Table A7_1_1_2-1: Description of test solution and controls

Criteria	Details
Purity of water	The test water used was deionized water boiled for 30 minutes and filtered through a 0.22 micron filter.
Preparation of test chemical solution	Primary stock solution (PSS): C_{14}-labelled glutaraldehyde (C_{14} –GA) was transferred to 100 ml volumetric with deionized water adjusted to pH 6.5 (HCl). Eight replicates (50 µl each) were sampled and were subjected to LSC; further three replicates (3 µl each) were sampled and were subjected to GLC. The concentration of the stock solution was determined to be 1.186 µg/µl, and the radiochemical purity was found to be 99%. Prior starting the test, further replicates were sampled for GLC analysis: a concentration of 1.174 µg/µl. Use of the non-labelled glutaraldehyde (GA[redacted]): This material served for TLC method development and validation, and was used for the preparation of the aqueous test solutions of C_{14} –GA; furthermore it was used for the preparation of the GLC standards. Preparation of the aqueous test solutions: (1) Unsensitized solution: 0.333 ml of C_{14} –GA PSS and 7.5 µl of GA[redacted] were filled up to 10 ml with a pH 5 buffer solution (0.2M acetic acid/0.2 M sodium acetate), resulting in a nominal test concentration of 250 µg/ml. (2) Sensitized solution (1% acetone): 0.333 ml of C_{14} –GA PSS, 7.5 µl of GA[redacted] and 100 µl acetone were filled up to 10 ml with a pH 5 buffer solution (0.2M acetic acid/0.2 M sodium acetate), resulting in a nominal test concentration of 250 µg/ml. (3) Sensitized solution (1% acetonitrile): 0.333 ml of C_{14} –GA PSS, 7.5 µl of GA[redacted] and 100 µl acetonitrile were filled up to 10 ml with a pH 5 buffer solution (0.2M acetic acid/0.2 M sodium acetate), resulting in a nominal test concentration of 250 µg/ml. Duplicate 100 µl aliquots of each solution were subjected to LSC.
Test concentrations (mg a.s./L)	250 µg/ml
Controls	Controls were kept in the dark

Table A7_1_1_2-2: Description of test system

Criteria	Details
Laboratory equipment	Glass backed pre-coated silica gel 60F-254 TLC plates ([redacted]; activation prior use in a drying oven at 125 °C for 30 minutes). [redacted] Multispotter (for automatical spotting of samples on the plates) Air-tight plastic glove box (for performance of spotting or streaking under an inert atmosphere of Argon) TLC tank (the plates were blanketed with argon prior sealing the tank. The development of the plates was based on ascending chromatography using a 55:25:20 hexane/ethyl acetate/methanol solvent system. Radioactive markings were placed on the plates for identification. The plates were then subjected to autoradiography. The markings were used for correct positioning of the autoradiograph over the TLC plates. Spots corresponding to C^{14}-radioactivity were removed by scraping. To assure C^{14}-radioactivity accountability the remaining area of the TLC plates also was scraped. Desorption of the silica gel scrapings was obtained with 1 ml methanol; the samples then received 9 ml deionized water and 10 ml of [redacted] Liquid scintillation cocktail. The quantification of the C^{14}-radioactivity was achieved by Liquid Scintillation Counting analysis. Known and unknown degradation products were characterized by comparison of Rf values. The parent compound equivalents in each spot were calculated and the percentage of total residue present as each degradate was determined. Autoclave for sterilization of glassware, caps and capliners (one hour at 121 °C and 15 psi)
Test apparatus	<u>Photolysis apparatus and Lamp:</u> - Ace photochemical turntable reactor with a rotation rate of 6 rpm. - 450 Watt [redacted] mercury-vapor lamp, located in the center of the turntable in an Ace borosilicate immersion cell with a flow rate of 100 ml H_2O/minute; distance between center of the reaction tube and center of the light well: 9.2 cm. <u>Gas Liquid Chromatography (GLC):</u> [redacted] Gas Liquid Chromatograph, equipped with a flame ionization detector and a [redacted] recording integrator. The operating parameters were as follows:

Operating parameters	
Column	DB-5 30 m X 0.53 mm fused silica megabore
Flow rate	30 ml/min N_2, 30 ml/min H, 300

<table>
<tr><td></td><td>
<table>
<tr><td></td><td>ml/min Air</td></tr>
<tr><td>Injection Volume</td><td>1 µl</td></tr>
<tr><td>Column Temperature</td><td>95 °C</td></tr>
<tr><td>Injector Temperature</td><td>150 °C</td></tr>
<tr><td>Detector Temperature</td><td>250 °C</td></tr>
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<u>Liquid Scintillation Counting System (LSC)</u>
Bench top microprocessor-controlled spectrometer, Beckman Model 3801 Liquid Scintillation Counting System.
</td></tr>
<tr><td>Properties of artificial light source:</td><td>450 Watt [redacted] mercury-vapor lamp</td></tr>
<tr><td>Nature of light source</td><td>See above</td></tr>
<tr><td>Emission wavelenght spectrum</td><td>_</td></tr>
<tr><td>Light intensity</td><td>
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<tr><th rowspan="2">Lambda</th><th rowspan="2">Radiated Energy of Lamp (W)</th><th colspan="4">Intensity in Microwatts per square cm
(I)</th></tr>
<tr><th>50* cm</th><th>100* cm</th><th>6.0** cm</th><th>9.2 cm</th></tr>
<tr><td>5780</td><td>20</td><td>768
(27.7)</td><td>217
(14.7)</td><td>47524
(218)</td><td>20450
(143)</td></tr>
<tr><td>5461</td><td>24.5</td><td>941
(30.7)</td><td>266
(16.3)</td><td>58564
(242)</td><td>25097
(159)</td></tr>
<tr><td>4358</td><td>20.2</td><td>776
(27.9)</td><td>220
(14.8)</td><td>48400
(220)</td><td>20762
(144)</td></tr>
<tr><td>4045</td><td>11.0</td><td>422
(20.5)</td><td>120
(11.0)</td><td>25600
(160)</td><td>10975
(105)</td></tr>
<tr><td>3660</td><td>25.6</td><td>983
(31.4)</td><td>278
(16.7)</td><td>41378
(203)</td><td>17761
(133)</td></tr>
</table>
*, As supplied by the manufacturer
**, Values determined by interpolation of square root of microwatts at 50 and 100 cm
</td></tr>
<tr><td>Filters</td><td>_</td></tr>
</table>

Table A7 1 1 2-3: Results of the GLC analysis

Concentration of ^{14}C-Glutaraldehyde by GLC Analysis[a]

Sensitized Light (1% acetone)		Sensitized Dark (1% acetone)		Sensitized Light (1% acetonitrile)		Sensitized Dark (1% acetonitrile)		Unsensitized Light		Unsensitized Dark	
Sample Day	Conc. µg/ml	Sample Day	Conc. µg/ml	Sample Day	Conc. µg/ml	Sample Day	Conc. µg/ml	Sample Day	Conc. µg/ml	Sample Day	Conc. µg/ml
Day 0	221	Day 0	238	Day 0	307	Day 0	253	Day 0[b]	244	Day 0[b]	259
Day 1	196	Day 1	202	Day 1	225	Day 1	250	Day 1	227	Day 1	224
Day 3	166	Day 3	222	Day 2	238	Day 2	179	Day 2	216	Day 2	269
Day 10	123	Day 10	236	Day 3	197	Day 3	264	Day 3	163	Day 3	176
Day 14	113	Day 14	246	Day 7	184	Day 10	268	Day 7	173	Day 7	213
Day 18	109	Day 18	223	Day 10	202	Day 14	247	Day 10[b]	158	Day 10[b]	242
				Day 14	199	Day 18	230	Day 14	226	Day 14	230
				Day 17	208			Day 17	211	Day 17	243

[a] Average of triplicate injections.

[b] Average of duplicate injections.

32739-13

Table A7_1_1_2-4: Results of the LSC analysis

Concentration of ^{14}C-Glutaraldehyde by LSC Analysis.

Sensitized (1% acetone)			Sensitized (1% acetonitrile)			Unsensitized		
Sample Day	ppm Light	ppm Dark	Sample Day	ppm Light	ppm Dark	Sample Day	ppm Light	ppm Dark
Day 0	245	245	Day 0	240	240	Day 0	245	246
Day 2	239	248	Day 2	---	253	Day 3	244	247
Day 3	247	250	Day 3	245	253	Day 6	241	---
Day 6	245	254	Day 6	---	249	Day 7	---	255
Day 10	243	255	Day 7	249	---	Day 10	234	253
Day 14	240	238	Day 10	167	253	Day 14	241	252
			Day 14	242	242	Day 17	261	260
			Day 17	251	---			

Table A7_1_1_2-5: Data points and line equation for half-life estimation

Data Points and Line Equation for Half-Life Estimation of ^{14}C-Glutaraldehyde in Sensitized Light (1% acetone) System, by GLC Analysis.

Sample Day	Concentration C_T µg/ml	$\bar{x}$ S.D.	ln C_T
Day 0	226 219 217	221 ±4.7 RSD 2.1%	5.40
Day 1	213 196 179	196 ±17 RSD 8.7%	5.28
Day 2*	120 121 120	120 ±0.58 RSD 0.48%	4.79
Day 3	169 169 160	166 ±5.2 RSD 3.1%	5.11
Day 10	123 123 124	123 ±0.58 RSD 0.47%	4.81
Day 14	114 113 112	113 ±0.58 RSD 0.51%	4.73
Day 18	100 113 113	109 ±7.5 RSD 6.9%	4.69

$r = -0.9612$

$r^2 = 0.9238$

$y = -0.0390x + 5.30$

$t_{\frac{1}{2}} = 18$ days

*Day 2 data point not included in calculations.

Data Points and Line Equation for Half-Life Estimation of ^{14}C-Glutaraldehyde in Sensitized Dark (1% acetone) System, by GLC Analysis.

Sample Day	Concentration C_T µg/ml	$\bar{x}$ S.D.	ln C_T
Day 0	246 231 238	238 ±7.5 RSD 3.1%	5.47
Day 1	201 204 200	202 ±2.1 RSD 1.0%	5.31
Day 2	182 190 184	185 ±4.2 RSD 2.2	5.22
Day 3	214 224 229	222 ±7.6 RSD 3.4%	5.40
Day 10	235 235 237	236 ±1.2 RSD 0.49%	5.46
Day 14	246 250 242	246 ±4.0 RSD 1.6%	5.51
Day 18	235 214 221	223 ±11 RSD 4.8%	5.41

$r = 0.4649$

$r^2 = 0.2161$

$y = 0.00650x + 5.35$

No observable degradation.

Data Points and Line Equation for Half-Life Estimation of ^{14}C-Glutaraldehyde in Sensitized Light (1% acetonitrile) System, by GLC Analysis.

Sample Day	Concentration C_T µg/ml	$\bar{x}$ S.D.	ln C_T
Day 0	304 324 294	307 ±15.3 RSD 5.0%	5.73
Day 1	225 221 230	225 ±4.5 RSD 2.0%	5.42
Day 2	221 239 253	238 ±16 RSD 6.7%	5.47
Day 3	192 203 195	197 ±5.7 RSD 2.9%	5.28
Day 7	193 180 183	185 ±6.8 RSD 3.7%	5.22
Day 10	196 200 210	202 ±7.2 RSD 3.6%	5.31
Day 14	202 194 202	199 ±4.6 RSD 2.3%	5.29
Day 17	213 208 204	208 ±4.5 RSD 2.2	5.34

$r = -0.5529$

$r^2 = 0.3056$

$y = -0.0140x + 5.48$

$t_{\frac{1}{2}} = 49$ days

Data Points and Line Equation for Half-Life Estimation of ^{14}C-Glutaraldehyde in Sensitized Dark (1% acetonitrile) System, by GLC Analysis.

Sample Day	Concentration C_T µg/ml	$\bar{x}$ S.D.	ln C_T
Day 0	225 264 271	253 ±25 RSD 9.8%	5.53
Day 1	263 233 255	250 ±16 RSD 6.2%	5.52
Day 2	182 178 176	179 ±3.1 RSD 1.7%	5.19
Day 3	266 269 256	264 ±6.8 RSD 2.6%	5.58
Day 10	270 264 270	268 ±3.5 RSD 1.3%	5.59
Day 14	245 253 243	247 ±5.1 RSD 2.1%	5.51
Day 18	243 221 225	230 ±12 RSD 5.1%	5.44

$r = 0.0955$

$r^2 = 0.009119$

$y = 0.00184x + 5.47$

No observable degradation.

*Day 6 samples not reported.

Data Points and Line Equation for Half-Life Estimation of ^{14}C-Glutaraldehyde in Unsensitized Light System, by GLC Analysis.

Sample Day	Concentration C_T µg/ml	$\bar{x}$ S.D.	ln C_T
Day 0	235 254	244 ±13.4 RSD 5.5%	5.50
Day 1	235 226 220	227 ±7.5 RSD 3.3%	5.42
Day 2	203 298 247	216 ±27 RSD 12.5%	5.38
Day 3	164 163 163	163 ±0.58 RSD 0.35%	5.09
Day 7	169 175 177	174 ±4.2 RSD 2.4%	5.16
Day 10	155 162	158 ±4.9 RSD 3.1%	5.06
Day 14	236 217 226	226 ±9.5 RSD 4.2%	5.42
Day 17	208 215 210	211 ±3.6 RSD 1.7%	5.35

$r = -0.1418$

$r^2 = 0.02011$

$y = -0.00376x + 5.32$

No observable degradation.

^{14}C-Glutaraldehyde in Unsensitized Dark System, by GLC Analysis.

Sample Day	Concentration C_T ug/ml	$\bar{x}$ S.D.	ln C_T
Day 0	259 259	259 ±0 RSD 0%	5.56
Day 1	233 226 214	224 ±9.6 RSD 4.3%	5.41
Day 2	250 279 278	269 ±16 RSD 6.1%	5.59
Day 3	175 175 178	176 ±1.73 RSD 0.98%	5.17
Day 7	216 214 212	214 ±2.0 RSD 0.93%	5.37
Day 10	223 262	242 ±28 RSD 11.4%	5.49
Day 14	238 231 220	230 ±9.1 RSD 4.0%	5.44
Day 17	252 232 244	243 ±10 RSD 4.1%	5.49

$r = 0.05473$

$r^2 = 0.002995$

$y = 0.00113x + 5.43$

No observable degradation.

Zones Corresponding to ^{14}C-Radioactivity as Located Using Autoradiographs for Sensitized (1% acetone) Solutions.

	Rf Values							
Sample Code	G I	F II	E III	A IV	B V	D VI	H VII	I VIII
Day 2-Sen-L-TONE	0.50	0.43						
Day 3-Sen-L-TONE	0.45	0.36	0.30	0.06	0.15			
Day 6-Sen-L-TONE	0.46	0.39	0.30	0.04	0.16			
Day 10-Sen-L-TONE	0.53	0.45	0.34	0.08	0.19			
Day 14-Sen-L-TONE	0.82		0.69	0.09	0.30		0.93	
Day 2-Sen-D-TONE	0.50			0.05				
Day 3-Sen-D-TONE	0.45	0.36	0.29	0.06	0.15			
Day 6-Sen-D-TONE	0.47	0.39	0.30	0.05	0.16			
Day 10-Sen-D-TONE	0.53	0.43	0.34	0.06	0.19			
Day 14-Sen-D-TONE	0.81		0.69	0.12	0.29		0.91	

Zones Corresponding to ^{14}C-Radioactivity as Located Using Autoradiographs for Sensitized (1% acetonitrile) Solutions.

	Rf Values							
Sample Code	G I	F II	E III	A IV	B V	D VI	H VII	I VIII
Day 3-Sen-L-TRIL	0.53		0.34	0.06				
Day 7-Sen-L-TRIL	0.48	0.39	0.30	0.07	0.17			
Day 10-Sen-L-TRIL	0.54	0.47	0.40	0.06	0.20			
Day 14-Sen-L-TRIL	0.90	0.81	0.76		0.30		0.96	
Day 17-Sen-L-TRIL	0.54	0.47	0.40	0.07	0.20		0.64	0.71
Day 2-Sen-D-TRIL	0.50		0.33	0.05				
Day 3-Sen-D-TRIL	0.45	0.35	0.29	0.06	0.15			
Day 6-Sen-D-TRIL	0.47	0.38	0.29	0.05	0.16			
Day 10-Sen-D-TRIL	0.53	0.44	0.33	0.06	0.19			
Day 14-Sen-D-TRIL	0.82		0.68	0.13	0.30		0.89	

Zones Corresponding to ^{14}C-Radioactivity as Located Using Autoradiographs for Unsensitized Solutions.

	Rf Values							
Sample Code	G I	F II	E III	A IV	B V	D VI	H VII	I VIII
Day 3-Unsen-L	0.51		0.33	0.05				
Day 7-Unsen-L	0.46				0.13			
Day 10-Unsen-L	0.54	0.45	0.38	0.05	0.20	0.32		
Day 14-Unsen-L	0.86	0.79	0.73		0.31		0.94	
Day 17-Unsen-L	0.55	0.47	0.40	0.07	0.20		0.65	0.72
Day 3-Unsen-D	0.52		0.35	0.06				
Day 7-Unsen-D	0.48	0.39	0.29	0.05	0.16			
Day 10-Unsen-D	0.53	0.45	0.35	0.07	0.19			
Day 14-Unsen-D	0.87		0.74		0.33		0.94	
Day 17-Unsen-D	0.55	0.47	0.42	0.08	0.21		0.64	0.71

Table A7_1_1_2-7 Recovery of ^{14}C- Activity

^{14}C-Residues of Glutaraldehyde Characterized From the TLC Plates as Percent of Total ^{14}C-Activity Recovered (Sensitized 1% acetone).

	% of ^{14}C-Activity in Zones								
	Parent	Unknowns							
Sample Code	G I	F II	E III	A IV	B V	D VI	H VII	I VIII	Origin O	Remainder R
Day 2-Sen-L-TONE	51.1	15.3							14.0	19.5
Day 3-Sen-L-TONE	60.8	2.2	2.8	6.4	9.8				15.5	2.5
Day 6-Sen-L-TONE	46.2	4.2	4.2	9.1	13.2				19.4	3.7
Day 10-Sen-L-TONE	44.1	4.7	4.6	10.8	13.0				21.5	1.4
Day 14-Sen-L-TONE	53.7		1.7	0.9	0.9		35.1		3.5	4.2
Day 2-Sen-D-TONE	78.1			5.1					6.8	9.9
Day 3-Sen-D-TONE	85.4	1.6	1.2	2.0	3.1				5.2	1.6
Day 6-Sen-D-TONE	71.8	4.1	2.5	3.7	5.2				10.3	2.5
Day 10-Sen-D-TONE	79.0	3.1	1.7	3.2	4.1				6.7	2.1
Day 14-Sen-D-TONE	85.0		1.7	0.9	0.8		6.8		2.2	2.6

^{14}C-Residues of Glutaraldehyde Characterized From the TLC Plates as Percent of Total ^{14}C-Activity Recovered (Sensitized 1% acetonitrile).

	% of ^{14}C-Activity in Zones								
	Parent	Unknowns							
Sample Code	G I	F II	E III	A IV	B V	D VI	H VII	I VIII	Origin O	Remainder R
Day 3-Sen-L-TRIL	67.9		2.4	2.9					9.9	16.8
Day 7-Sen-L-TRIL	66.7	4.2	2.3	4.9	6.8				11.9	3.2
Day 10-Sen-L-TRIL	63.8	9.8	4.1	4.9	7.6				7.2	2.6
Day 14-Sen-L-TRIL	80.8	2.7	0.9		0.2		11.5		1.1	2.7
Day 17-Sen-L-TRIL	58.3	7.1	3.1	7.8	10.2				9.5	0.7
Day 2-Sen-D-TRIL	78.2		2.2	3.9					7.4	8.3
Day 3-Sen-D-TRIL	82.2	1.7	1.1	2.5	3.2				5.3	4.0
Day 6-Sen-D-TRIL	71.5	4.0	1.7	3.3	5.5				9.6	4.4
Day 10-Sen-D-TRIL	78.0	3.2	1.8	3.4	4.2				7.1	2.3
Day 14-Sen-D-TRIL	83.7		1.6	1.1	0.9		6.8		2.9	3.1

^{14}C-Residues of Glutaraldehyde Characterized From the TLC Plates as Percent of Total ^{14}C-Activity Recovered (Unsensitized).

	% of ^{14}C-Activity in Zones									
	Parent	Unknowns								
Sample Code	G I	F II	E III	A IV	B V	D VI	H VII	I VIII	Origin O	Remainder R
Day 3-Unsen-L	72.9		2.1	3.7					9.4	11.9
Day 7-Unsen-L	63.5				9.5				11.5	15.5
Day 10-Unsen-L	71.2	4.6	1.1	5.4	7.5	1.3			5.6	3.1
Day 14-Unsen-L	81.0	2.9	0.9		0.2		12.1		1.2	1.7
Day 17-Unsen-L	62.2	4.9	2.6	6.4	9.8		1.5	0.4	11.3	0.8
Day 3-Unsen-D	76.7		1.7	2.9					8.3	10.4
Day 7-Unsen-D	71.6	4.4	2.1	3.3	5.5				10.1	3.1
Day 10-Unsen-D	79.8	4.3	1.6	2.6	4.0				5.6	2.0
Day 14-Unsen-D	88.3		1.2		0.2		7.0		0.9	2.5
Day 17-Unsen-D	73.5	4.0	1.7	3.2	7.5		2.7	0.4	6.4	0.6

Section A7.1.1.1.2 Annex Point IIA, VII.7.6.2.2.	Phototransformation in water including identity of transformation products	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [X]	
Limited exposure [ ]	Other justification [ ]	
Detailed justification:	Direct photolysis in water can be a relevant process for removal of light absorbing, hydrolytically or non biodegradable stable organic substances [1]. Glutaraldehyde is readily biodegradable according to OECD criteria [2]. Following the Technical Guidance Document [TGD], a first order rate constant for microbial mineralization in surface water of 4.7×10^{-2} [d^{-1}] is assigned to a substance which is readily biodegradable. Therefore, the removal of glutaraldehyde in surface water is dominated by biodegradation processes. In principle the relevance of direct photodegradation in water can be discussed as well as the photochemical transformation mechanism. Light absorption of organic compounds in the wavelength range between 290 – 600 nm is in most cases associated with the presence of a delocalized л–electron system. Hence, aromatic rings and conjugated double bonds may form a chromophore structural moiety. For this reason, an aldehyde functional group is not a chromophore and adsorbs light in the VIS absorption spectrum in a minor form. This consideration is confirmed by the UV/VIS spectra of glutaraldehyde. The UV/VIS spectra of glutaraldehyde shows in neutral medium two distinguished bands with clear absorption maxima at approx. 234 nm and 282 nm. The spectra in acidic medium shows also the same two bands with absorption maxima at approx. 234 nm and 282 nm [3]. Therefore, photodegradation processes are of less importance under these chemical aspects. In conclusion, glutaraldehyde is readily biodegradable according to OECD criteria. It has no delocalized л–electron system and two absorption maxima at 234 and 282 nm. Glutaraldehyde does not essentially absorb light above 290 nm. For these reasons, it can be assumed that photodegradation processes in water are of low relevance [4]. <u>References:</u> [1] EC (European Commission, 2003) Technical Guidance Document on Risk Assessment, Part III, EUR 20418 EN/3, ECB, Ispra, Italy [2] [redacted] (1993) Determination of the Biodegradability or the	

Section A7.1.1.1.2 **Annex Point IIA, VII.7.6.2.2.**	**Phototransformation in water including identity of transformation products**
	Elimination of [redacted] in the DOC Die Away (ISO 7827)-Test. [redacted],BPD ID A7.01.1.2.1_01 [3] [redacted] (2004) Characterization of "[redacted]" for the notification in the Netherlands. [redacted] BPD ID A3.04.1_01 [4] [redacted] (2007) Justification for non-submission of photodegradation in water. [redacted], unpublished, Nov 2011, BPD ID A7.1.1.1.2_01
Undertaking of intended data submission [ ]	Not relevant
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10.2.2012
Evaluation of applicant's justification	Waiving of photolysis study was accepted at TM III 2012 because Glutaraldehyde has its adsorption max below 290 nm which is the cutoff value for direct photolysis. Due to ready biodegradability photolysis is not considered a relevant degradation pathway for Glutaraldehyde.
Conclusion	Justification for non-submission is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.1.2.1 _ 01 Biodegradability (ready)

Annex Point IIA7.6.1.1

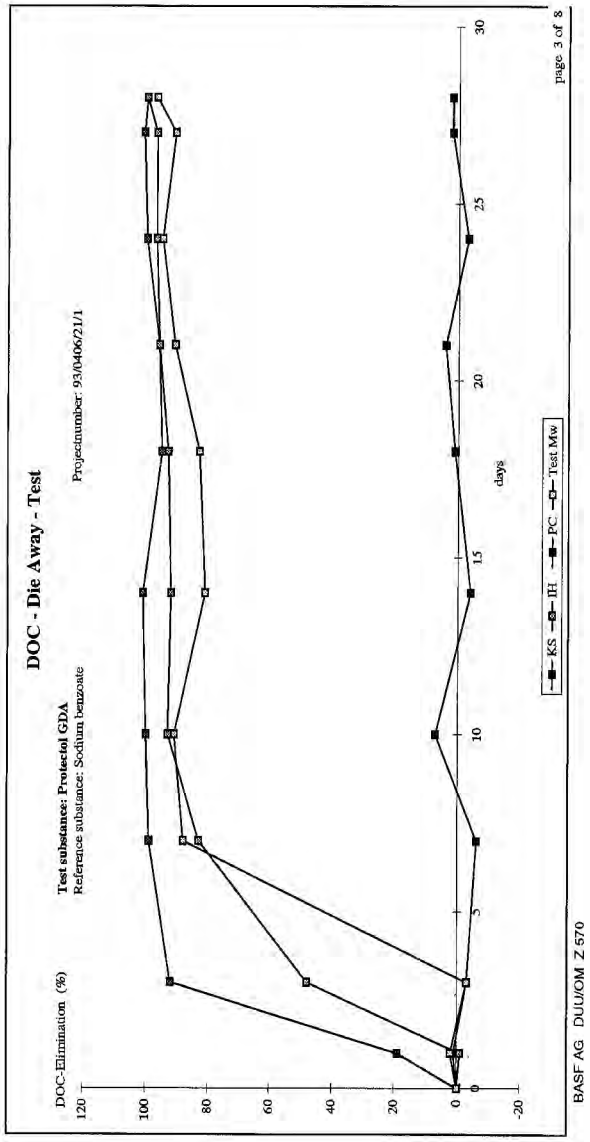
			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1993) Determination of the Biodegradability or the Elimination of [redacted] in the DOC Die Away (ISO 7827)-Test. [redacted] (Unpublished), BPD ID A7.01.1.2.1_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 301 A (new version), 1993	x
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] (1,5-pentandial)	
3.1.1	Lot/Batch number	Not specified	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted] % active substance	
3.1.4	Further relevant properties	Instability against temperature, oxygen, acid and alkali	
3.1.5	Composition of Product	[redacted] % active ingredient	
3.1.6	TS inhibitory to microorganisms	Yes, result of respiration inhibition test EC20 15 mg a.s./L (Doc IIIA7.4.1.4/01)	
3.1.7	Specific chemical analysis	No compound specific analytical technique was applied	
3.2	**Reference substance**	Yes Sodium benzoate	
3.2.1	Initial concentration of reference substance	According to guideline	x
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	For details of inoculum see table A7_1_1_2-2	
3.3.2	Test system	For details on test type, laboratory equipment etc. see table A7_1_1_2-3	
3.3.3	Test conditions	For relevant test conditions see table A7_1_1_2-4	
3.3.4	Method of preparation of test	Not appropriate	

	solution	
3.3.5	Initial TS concentration	20 mg DOC/l
3.3.6	Duration of test	28 days
3.3.7	Analytical parameter	DOC removal
3.3.8	Sampling	0, 1, 3, 7, 10, 14, 18, 21, 24, 27, and 28 days
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	No
3.3.11	Controls	Control without test substance (blank); abiotic controls; toxicity control
3.3.12	Statistics	The percentage degradation at each sampling time was calculated separately for both replicates taking into account the blank control for the respective sampling time. Results refer to the initial concentration (DOC removal).

4 RESULTS

4.1 Degradation of test substance

4.1.1 Graph



KS = reference substance, IH = toxicity control, PC = abiotic control, MW = test substance (mean value)

4.1.2	Degradation	> 80% degradation at plateau (plateau was reached on day 7), degradation 90-100% degradation at the end of incubation and after 10-d window
4.1.3	Other observations	No inhibitory effects were observed.
4.1.4	Degradation of TS in abiotic control	Abiotic de[redacted]adation was in the range of 2-7%
4.1.5	Degradation of reference substance	For graph[redacted]e point 4.1.1
4.1.6	Intermediates/ degradation products	Not appli[redacted]le

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and The aim of the present study was to look for the biodegradability of

	methods	glutaraldehyde in the DOC Die Away Test, which is a static method for the determination of the ultimate aerobic biodegradation of a test substance in the water. Test substance: [redacted] (1,5-pentandial), purity [redacted]% The test was performed according to OECD 301 A (1993), with GLP. The test substance as well as the reference substance sodium benzoate were tested at a concentration of 20 mg DOC/l. Activated sludge of a laboratory waste water treatment plant fed with municipal sewage was used as inoculum. The test or reference substance and the inoculum were mixed together and aerated for up to 28 days at 20-25°C. Samples were taken on day 0, 1, 3, 7, 10, 14, 18, 21, 24, 27, and 28 to measure the DOC concentrations according to DIN 38409 Part 3 (1983). A blank control, a toxicity control and an abiotic control were included in the test. Measurements of the DOC concentration taken from the two replicates per test unit were done separately from each other as percentage degradation.
5.2	**Results and discussion**	90-100% of the initial glutaraldehyde (20 mg/L DOC) was eliminated from water after 28 days. The 10-day window was reached. As neither toxicity nor abiotic degradation was observed in the controls at the concentration tested and further, as the reference substance revealed the validity of the test system, glutaraldehyde can be regarded as readily biodegradable in this test system.
5.3	**Conclusion**	The results of the present study were indicative for the ready biodegradability of glutaraldehyde. Report stated no deviations from test guideline and the validity criteria for the testing of ready biodegradability were fulfilled.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	3.2.2009
Materials and Methods	2.1 The current OECD version is adopted in 17.7.1992. 3.2.1 The concentration of the reference substance was 20 mg/l DOC. Table A7_1_1_2-2 Preparation of inoculum not explained in the test report. Table A7_1_1_2-3 Flask types, sizes or shaking of flasks not mentioned in the test report. Table A7_1_1_2-4 Composition of mineral medium not given in the test report. Table A7_1_1_2-4 Temperature not reported in the test report.
Results and discussion	After three days lag period 88% removal of DOC was observed by day 7. In the end of the test period the DOC removal was 97%. If several measurements had taken place between day 3 and 7, the start of the 10-day window could have been observed more accurately.
Conclusion	Glutaraldehyde is readily biodegradable.
Reliability	2

Section A7.1.1.2.1 _ 01 Biodegradability (ready)

Annex Point IIA7.6.1.1

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_2-1: 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_2-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Source	Laboratory waste water treatment plants, fed with municipal and synthetic sewage
Laboratory culture	Yes
Method of cultivation	Laboratory waste water treatment plant
Preparation of inoculum for exposure	Washing and centrifugation in accordance with guideline
Pretreatment	Not performed
Initial cell concentration	30 mg suspended solids/l

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	Shaken flasks cultured under aerobic conditions and constant temperature; DOC analyser
Number of culture flasks/concentration	2 replicates/concentration
Aeration device	Aerated up to 28 days as prescribed by the guideline
Measuring equipment	DOC-analyzer: Shimadzu 5000
Test performed in closed vessels due to significant volatility of TS	Not indicated due to the low volatility of glutaraldehyde.

Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	In accordance with guideline
Additional substrate	No
Test temperature	In accordance with guideline (20-25°C)
pH	Not specified
Aeration of dilution water	Not specified
Suspended solids concentration	30 mg/L
Other relevant citeria	No

Table A7_1_1_2-5: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC	Yes	
Pass values reached within 10-d window (within 28-d test period)	Yes	
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%	Yes	
Percentage of removal of reference substance reaches pass level by day 14	Yes	

Section A7.1.1.2.3 _ 01 Biodegradability in the marine environment

Annex Point IIA7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2002) [redacted] ([redacted]% Glutaraldehyde), Determination of the Biodegradability in the marine CO2-Evolution Test. [redacted] (Unpublished), BPD ID A7.01.1.2.3_01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, ISO 16221 (2001)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]% glutaraldehyde)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	[redacted]%	
3.1.4	Further relevant properties	Instability against temperature, oxygen, acid and alkali	
3.1.5	Composition of Product	Active ingredient [redacted]%, water [redacted]%, [redacted]%	
3.1.6	TS inhibitory to microorganisms	Yes, result of respiration inhibition test [redacted]: EC_{20} 48 mg/L. See also point 3.3.5 below.	
3.1.7	Specific chemical analysis	No compound specific analytical technique was applied	
3.2	**Reference substance**	Yes, aniline	
3.2.1	Initial concentration of reference substance	According to guideline	x
3.3	**Testing procedure**		

Section A7.1.1.2.3 _ 01 **Biodegradability in the marine environment**

Annex Point IIA7.6.1.1

3.3.1	Inoculum / test species	For details of inoculum see table A7_1_1_2-2	
3.3.2	Test system	For details on test type, laboratory equipment etc. see table A7_1_1_2-3	
3.3.3	Test conditions	For relevant test conditions see table A7_1_1_2-4	
3.3.4	Method of preparation of test solution	Not appropriate	
3.3.5	Initial TS concentration	32 mg TS/l and 100 mg TS/l. However, the inhibition assay showed clear toxic effects at the highest test concentration and only the measurements of the lower test substance concentration were evaluated (32 mg TS/l corresponding to 10 mg TOC/l).	
3.3.6	Duration of test	70 days	x
3.3.7	Analytical parameter	CO_2 evolution	
3.3.8	Sampling	Samples were taken on day 0, 1, 3, 5, 7, 10, 12, 14, 17, 24, 26, 30, 41, 55, 69, 70, and 71 (as the measure values of day 71 were influenced by stripping, they were added to the values of day 70).	
3.3.9	Intermediates/ degradation products	Not identified	
3.3.10	Nitrate/nitrite measurement	No, not applicable	
3.3.11	Controls	Control without test substance (seawater and inorganic medium = blank control; Toxicity control with reference substance and test substance.	
3.3.12	Statistics	For the measurement of the biodegradation the blank control was subtracted and the measured CO_2-evolution in relation to theoretical (calculated) CO_2 value was indicative of the degree for biodegradation. No statistical evaluation was performed.	

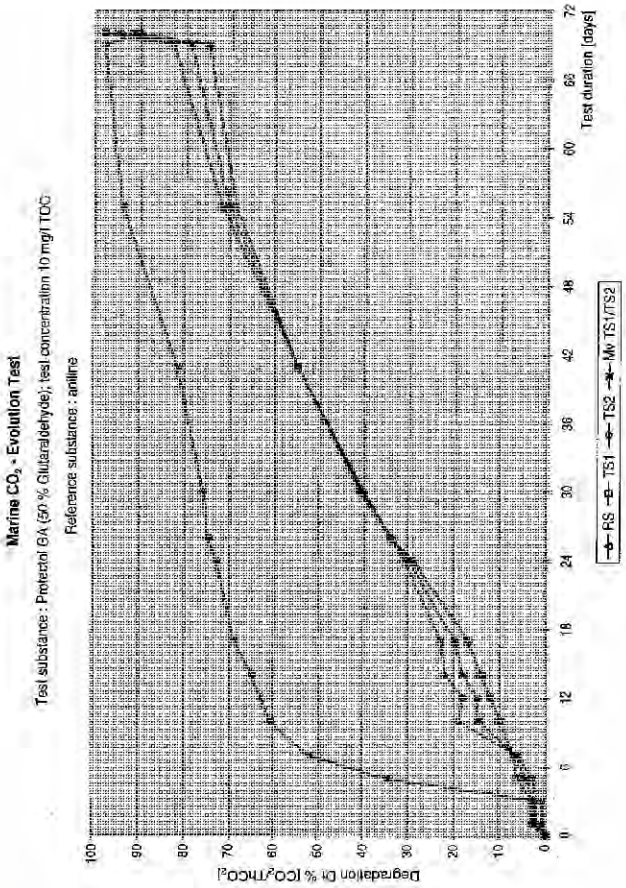
4 RESULTS

4.1 Degradation of test substance

Section A7.1.1.2.3 _ 01 **Biodegradability in the marine environment**

Annex Point IIA7.6.1.1

4.1.1 Graph



RS = reference substance; TS1 and TS2 = test substance (replicate no. 1 and 2, respectively); Mv TS1/TS2 = mean value of test substance replicate 1 and 2

4.1.2	Degradation	Percentage degradation of [redacted] at 10 mg/l TOC was 90-100% at the end of incubation period (70 days).
4.1.3	Other observations	At a test concentration of about 100 mg [redacted] GA/l (equivalent to 30 mg TOC/l) no biodegradation took place due to toxic effects.
4.1.4	Degradation of TS in abiotic control	Not applicable
4.1.5	Degradation of reference substance	See graph above (point 4.1.1): > 60% degradation of the reference substance was measured on day 6. > 90 % degradation after 50 days

Section A7.1.1.2.3 _ 01 **Biodegradability in the marine environment**

Annex Point IIA7.6.1.1

4.1.6	Intermediates/ degradation products	Not identified

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The aim of the present study was to look for the biodegradation ability of glutaraldehyde in Seawater. Test substance: [redacted] (1,5-Pentanedial), [redacted] % (water [redacted] The test was performed according to ISO 16221-2001, with GLP. The biodegradation of [redacted] was evaluated at a concentration of 32 mg test substance/l (corresponding to 10 mg/l TOC). 100 mg TS/l showed clear toxic effects in the inhibition assay, therefore only the measurements of the lowest test substance concentration were evaluated. A blank control (seawater and inorganic medium without test substance), a reference substance (Aniline) and a toxicity control (for both, the reference and the test substance) were considered in this test. Evolved CO_2 was trapped in NaOH absorber flasks. Samples for CO_2 determination were taken on incubation day 0, 1, 3, 5, 7, 10, 12, 14, 17, 24, 26, 30, 41, 55, 69, 70, and 71 (since the measured values of day 71 were influenced by stripping, they were added to the values of day 70). The relation of the determined CO_2-evolution to the calculated theoretical CO_2 value is the measure for the marine biodegradation of [redacted].
5.2	**Results and discussion**	The percentage of degradation of [redacted] GA at 10 mg/l TOC was determined to be 90-100% at the end of incubation (70 days). At a test concentration of about 100 mg/l (equivalent to 30 mg TOC/l) no biodegradation took place due to toxic effects. Biodegradation of the reference substance was > 60% measured on day 6 and > 90 % after 50 days. Since there were no indications for other abiotic elimination processes [redacted] GA can be regarded as biodegradable in this test system.
5.3	**Conclusion**	The test results were indicative of the biodegradability of glutaraldehyde in seawater. The validity criteria for the testing of marine biodegradability were fulfilled.
5.3.1	Reliability	**1**
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	3.2.2009

Section A7.1.1.2.3 _ 01 Biodegradability in the marine environment

Annex Point IIA7.6.1.1

Materials and Methods	3.2.1 Initial concentration of the reference substance was 20 mg/l TOC. 3.3.6 The maximum test duration is 60 d. Table A7_1_1_2-2 The initial microbial concentration was not determined. Table A7_1_1_2-3 Culturing apparatus and measuring equipment not described in the test report. Table A7_1_1_2-4 Composition of the inorganic medium not explained in the test report.
Results and discussion	95% of $ThCO_2$ production was achieved by the end of test (70 d). After 30 d the degree of degradation was 41%. The reference substance aniline achieved the 60% $ThCO_2$ production by day 10. Glutaraldehyde degraded rather steadily until the end of the test. No plateau could be identified.
Conclusion	Glutaraldehyde has a potential to biodegrade in the marine environment.
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_1_2-1: Guideline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test; marine biodegradation

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)]
Biodegradability in the marine CO2-Evolution Test	ISO 16221 (2001)		Marine Biodegradability

[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_2-2: Inoculum / Test organism

Criteria	Details
Nature	Seawater
Source	North Sea
Sampling site	Sampled at [redacted]
Preparation of inoculum for exposure	Seawater was used without further conditioning. After arrival in the laboratory the sample was immediately aerated and kept in a dark room at a temperature of 22 ± 2°C until the start of the test.
Pretreatment	Pre-aerated for about 4 days before the start of the test.
Initial cell concentration	Not applicable (1494 ml sea water/ test vessel)

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	Respirometer, CO_2 evolution
Number of culture flasks/concentration	Two flasks / test units
Aeration device	Not specified
Measuring equipment	Inorganic carbon analyzer
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	Artificial inorganic medium according to guideline
Additional substrate	No
Test temperature	22 ± 2 °C
pH	pH at test begin was 7.9 or 8.1 before correction, then adjusted to pH 7.4. The determined pH values at the end of test were 8.5 and 8.6
Aeration of dilution water	The inoculum was pre-aerated for about four days without artificial mineral medium and one day with artificial mineral medium.
Suspended solids concentration	-
Other relevant criteria	No

Table A7_1_1_2-5: Pass levels and validity criteria for tests on marine biodegradability

	fulfilled	not fulfilled
Pass levels		
60% removal of $ThCO_2$	Yes	
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau < 20%	Yes	
Percentage of removal of reference substance reaches pass level >60% by day 14	Yes	

Section A7.1.2.1.1 _ 01 Aerobic biodegradation
Activated Sludge Simulation Test

Annex Point IIA7.6.1.1

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1 REFERENCE

Reference	[redacted] (1998) Determination of the Biodegradability of [redacted] in the Activated Sludge Simulation Test according to GLP, EN 45001 and ISO 9002. [redacted] (Unpublished), BPD ID A7.01.2.1.1_01
Data protection	Yes
1.1.1 Data owner	BASF AG
1.1.2 Companies with letter of access	[redacted]
1.1.3 Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

Guideline study	Yes, OECD 303A and ISO Standard 11733
GLP	Yes
Deviations	No

3 MATERIALS AND METHODS

Test material	[redacted]
3.1.1 Lot/Batch number	[redacted]
3.1.2 Specification	As given in section 2
3.1.3 Purity	[redacted]%
3.1.4 Further relevant properties	Instability against temperature, oxygen, acid and alkali
3.1.5 Composition of Product	[redacted]% active ingredient
3.1.6 TS inhibitory to microorganisms	Yes, result of respiration inhibition test project no. 93/0406/08/1: EC_{20} 48 mg/L
3.1.7 Specific chemical analysis	No compound specific analytical technique was applied
Reference substance	No, not required
3.1.8 Initial concentration of reference substance	Not relevant

Section A7.1.2.1.1 _ 01 Aerobic biodegradation
Activated Sludge Simulation Test

Annex Point IIA7.6.1.1

Testing procedure

3.1.9	Inoculum / test species	For details of inoculum see table A7_1_1_2-2	
3.1.10	Test system	For details on test type, laboratory equipment etc. see table A7_1_1_2-3	
3.1.11	Test conditions	For relevant test conditions see table A7_1_1_2-4	
3.1.12	Method of preparation of test solution	No data	
3.1.13	Initial TS concentration	64.7 mg TS/ corresponding to 20 mg DOC/l	
3.1.14	Duration of test	73 days	
3.1.15	Analytical parameter	DOC removal	
3.1.16	Sampling	Sampling on day: 0, 1, 3,6, 8, 10, 13, 15, 20, 22, 24, 27, 29,31, 34, 36, 38, 41, 43, 45, 48, 50, 52, 55, 57, 59, 62, 64, 66, 69, 71, 73	
3.1.17	Intermediates/ degradation products	Not identified	
3.1.18	Nitrate/nitrite measurement	Yes, according to guideline	x
3.1.19	Controls	Two continuously operated test units were run in parallel under identical conditions. The test substance was added only to one unit, the second unit was used as control to determine the biodegradation of the organic medium.	
3.1.20	Statistics	The difference of the corresponding influent and effluent DOC values is the measure of the biodegradation. Mean values and standard deviations were determined without considering outlier determined by current statistic methods (confidence interval 95%).	

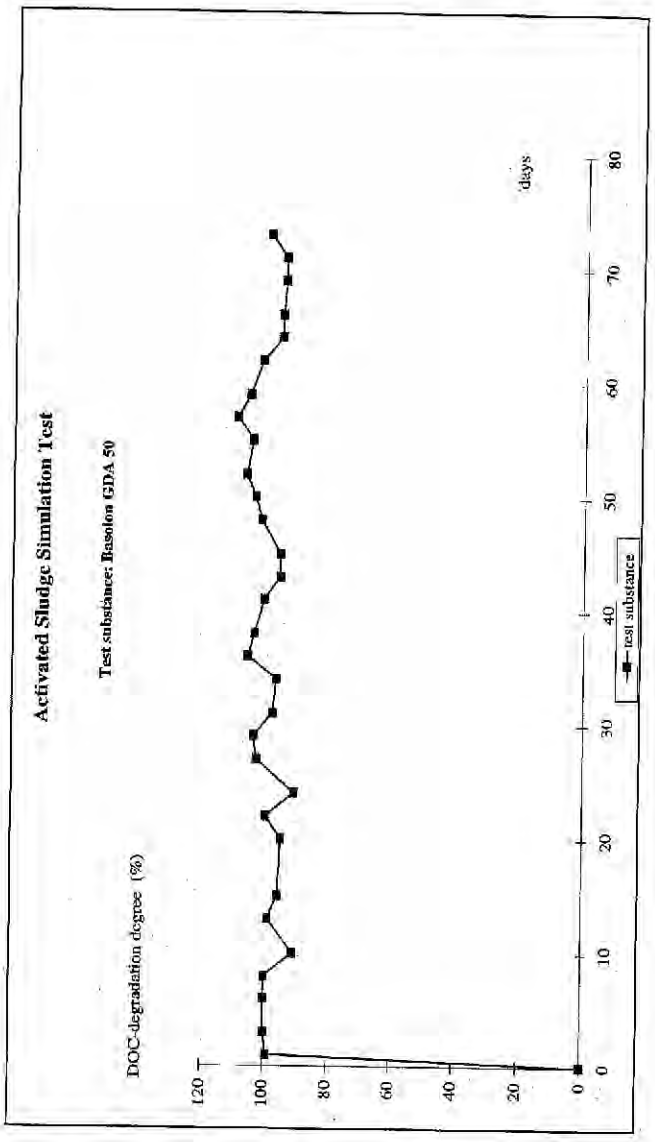
4 RESULTS

Section A7.1.2.1.1 _ 01 Aerobic biodegradation
Activated Sludge Simulation Test

Annex Point IIA7.6.1.1

Degradation of test substance

4.1.1 Graph



4.1.2	Degradation	Duration of the adaptation phase was <1 day. Duration of the plateau phase was 72 days. >80% DOC removal was obtained after 4 weeks. At the end of incubation phase DOC removal was 97 ± 1 % (95% confidence interval).	
4.1.3	Other observations	There were no indications for adsorption or DOC elimination due to other abiotic processes.	x
4.1.4	Degradation of TS in abiotic control	Not relevant (see above)	

Section A7.1.2.1.1 _ 01 Aerobic biodegradation
Activated Sludge Simulation Test

Annex Point IIA7.6.1.1

4.1.5	Degradation of reference substance	Not required
4.1.6	Intermediates/ degradation products	Not required

5 APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

The aim of the present study was to look for the biodegradability of glutaraldehyde in the Activated Sludge Simulation Test.

Test substance: [redacted] (1,5-pentandial), batch Nr [redacted]

The test was performed according to the OECD Guideline 303 A (1993) and ISO Standard 11733, with GLP.

The test system consisted of a test unit and a control unit. The test duration was 73 days, the DOC concentration of the test substance in the influent was 20 mg/l; the nutrient solution was 53 mg/L DOC in the influent, the dry weight of the added inoculum (domestic sludge) was 2.5 g/l, the mean retention period was 6 hours, the volume of the test units were 5 litres, temperature, oxygen content and pH were determined for the aeration chamber. In case of pH increase the pH was adjusted to 6.9 to 7.5.

Samples were taken from the influent and effluent of the two test units. DOC values were measured via a DOC analyser ([redacted]) on day 0, 1, 3,6, 8, 10, 13, 15, 20, 22, 24, 27, 29,31, 34, 36, 38, 41, 43, 45, 48, 50, 52, 55, 57, 59, 62, 64, 66, 69, 71, 73. The difference of the corresponding influent and effluent DOC values is indicative of the biodegradation. Mean values and standard deviations were determined without considering outlier determined by current statistic methods.

Results and discussion

The adaptation phase was < 1 day, followed by a plateau phase of 72 days. The mean value for biodegradation is 97 % after 73 days with a corresponding standard deviation of 1% (95% confidence interval) x

Since there were no hints on adsorption, major loss due to volatility or other abiotic degradation processes, the test substance can be regarded as biodegradable in this test.

Conclusion

The results of the present study gave no hint on adsorption or other abiotic elimination processes; therefore the test substance can be regarded as biodegradable in this test. x

The report stated no deviations from test guideline and the validity criteria for the testing of biodegradability in the activated sludge simulation test were fulfilled.

5.1.1	Reliability	**1**
5.1.2	Deficiencies	No

Section A7.1.2.1.1 _ 01 Aerobic biodegradation
Activated Sludge Simulation Test

Annex Point IIA7.6.1.1

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	16.3.2009
Materials and Methods	The applicant's description of the materials and methods is correct. 3.3.10 Nothing is explained about nitrifying sludge or effects of glutaraldehyde on the nitrification in the test report, but NO2 and NO3 have been measured and are reported. Table A7_1_1_2-4 The measured pH ranged from 7.1 to 8.6.
Results and discussion	4.1.3, 5.2, 5.3 According to the OECD 303A a high elimination of the test substance from the beginning of the test hints for adsorption onto the activated sludge solids. The elimination by adsorption could be verified by analysing the test substance in the sludge, but this has not been done. On the other hand the DOC elimination remained high throughout the test which is not typical for substances eliminated by adsorption. No lag phase was observed in the beginning of the test. The elimination of glutaraldehyde due to reaction with the organic matter has not been discussed. 5.3 The results are assumed to be valid if the degree of DOC elimination in the control unit is >80% after two weeks and for readily biodegradable substances the degree of biodegradation is >90%. These specific criteria are fulfilled, but the elimination by adsorption cannot be ruled out. 16 measurement results were considered as outliers in the test report and the result of mean DOC elimination of 97% is calculated from 15 measurement results. The DOC elimination of the test substance was 99% on day 1 and 100% on day 73 and was maintained high (91-110%) throughout the test including outliers.
Conclusion	The results are not very useful for the risk assessment, because the mechanism of the elimination process is unclear. It's neither possible to derive a degradation rate from this test.
Reliability	2
Acceptability	Acceptable
Remarks	According to the TNsG on data requirements the OECD 303A cannot distinguish between biological degradation and other elimination processes such as adsorption and volatilisation. The test does not fulfil criteria for simulation tests as it does not give a measured rate of primary and ultimate degradation and it does not allow identification and quantification of metabolites.
	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 A7.1.2.1.1 _ 01 Aerobic biodegradation
Activated Sludge Simulation Test

Annex Point IIA7.6.1.1

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_2-1: 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_2-2: Inoculum / Test organism

Criteria	Details
5.1.3 Nature	Activated sludge
5.1.4 Source	From laboratory wastewater treatment plants fed with municipal and/or synthetic sewage.
5.1.5 Laboratory culture	Not applicable
5.1.6 Method of cultivation	Yes
5.1.7 Preparation of inoculum for exposure	Laboratory waste water treatment plants
5.1.8 Pretreatment	Not applicable
5.1.9 Initial cell concentration	No

Table A7_1_1_2-3: Test system

Criteria	Details
5.1.10 Culturing apparatus	Two continuously operating activated sludge plants
5.1.11 Number of culture flasks/concentration	Not applicable; two test units in parallel: one unit was fed with the test substance and organic medium, whereas the other unit served as control (organic medium sole)
5.1.12 Aeration device	Air blast: 1air blast/hour, 20 air blasts/hour from day 38 onwards (nitrite concentration was increased from day 34 to day 45)
5.1.13 Measuring equipment	DOC analyser

5.1.14	Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2-4: Test conditions

Criteria		Details
5.1.15	Composition of medium	Not specified
5.1.16	Additional substrate	No
5.1.17	Test temperature	18.2 – 20.2 °C
5.1.18	pH	Measuring was performed at time of sampling: values were in the range of 7.5 – 8.4. The pH was then adjusted to 6.9 - 7.5.
5.1.19	Aeration of dilution water	No
5.1.20	Suspended solids concentration	2.5 g dry weight activated sludge/l
5.1.21	Other relevant citeria	No

Table A7_1_1_2-5: Pass levels and validity criteria for tests on biodegradability in the activated sludge simulation test

	fulfilled	not fulfilled
Pass levels		
> 80% removal of the test substance	Yes	
Criteria for validity		
Percentage of removal of organic medium reaches pass level (>80%) by day 28	Yes	

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Water/sediment degradation study
Aerobic aquatic metabolism of ^{14}C-glutaraldehyde

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1 REFERENCE

Reference	[redacted] 1986) Aerobic aquatic metabolism of glutaraldehyde. [redacted] (Unpublished), ([redacted]), BPD ID A7.01.2.2.1_01
Data protection	Yes
1.1.1 Data owner	BASF AG
1.1.2 Companies with letter of access	[redacted]
1.1.3 Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

Guideline study	Yes, according to US EPA Guideline subdivision N 162-4
GLP	Yes
Deviations	No

3 METHOD

Test material	14C-Glutaraldehyde
3.1.1 Lot/Batch number	Not specified
3.1.2 Specification	Specific radioactivity 3.40 mCi/mM
3.1.3 Purity	Radiochemical purity > [redacted]% (analysis by GLC and LSC)
3.1.4 Further relevant properties	No data
3.1.5 Composition of Product	See 3.1.3
Testing procedure	
3.1.6 Water and sediment	The sediment (silty clay loam) consisted of ca. 49% silt, 34% clay and 17% sand; The organic matter content was 1.4%, the pH was 6.5, and the percentage of 1/3 bar moisture was about 18%. The cation exchange capacity (C.E.C) was 23.3 meq/100 g soil. The pond water had an initial temperature of 19.5 °C, a pH of 7.95 and a dissolved oxygen content of 8.55 ppm.
3.1.7 Test system	The test system consisted of a 3000 ml metabolism vessel, with ground glass joints treated with sealing wax to preserve the integrity of the closed system. The test system was operated under positive pressure and was kept in the dark

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Aerobic aquatic metabolism of ^{14}C-glutaraldehyde

		at a temperature of 25 +/-1°C within an environmental control chamber. The metabolism vessel was connected to an ethylene glycol trap (for organic volatile compounds), a 1N H_2SO_4 trap (for alkaline volatile compounds) and two successive 1N KOH traps (for CO_2 and other acidic volatiles). Silica gel sep-packs were placed between the metabolism vessel and the ethylene glycol trap.	
3.1.8	Test conditions	125 g of sediment were placed into the test vessel, and 250 ml of pond water, which was dosed at 10 ppm to contain 2.5 mg of ^{14}C-labelled glutaraldehyde, were added.	
3.1.9	Method of preparation of test solution	A stock solution was obtained by mixing the test substance with H_2O (pH 6.5, adjusted with HCl) in a 100 ml volumetric flask. The stock solution was subjected to gas liquid chromatography (GLC) and to liquid scintillation counting (LSC) to confirm that glutaraldehyde content was > [redacted]%. Dilutions were made starting from this stock solution.	
3.1.10	Initial TS concentration	See 3.2.3	
3.1.11	Duration of test	30 days	
3.1.12	Analytical parameter	Measurement of radioactivity: For characterisation of radioactive residues, the sediment was subjected to a radioassay by triplicate combustion and extraction with methanol. The extract was subjected again to a radioassay in duplicate combustion, and depending on the percentage of total residue in the initial sample (i.e. >10% or 0.01 ppm), the radioassay in duplicate combustion was followed by thin-layer chromatography (TLC). The pond water was subjected to a radioassay by duplicate liquid scintillation counting (LSC) and depending on the percentage of total residue in the initial sample (i.e. > 10% or 0.01 ppm), the radioassay in duplicate combustion was followed by thin-layer chromatography (TLC).	
3.1.13	Sampling	Samples for analysis were collected at following time point: day 0, 1, 2, 7, 16, 23 and 30.	
3.1.14	Intermediates/ degradation products	Identified	x
3.1.15	Controls	Yes	
3.1.16	Calculations	Pond water: 1 ml of aliquot was taken for LSC. The average of controls (AvC) was subtracted from the test samples average (AvT) and the resultant number divided by the specific activity (SA): ppm = AvT – AvC / SA = dpm – dpm / dpm/µg 1/1 ml = µg/ml Combustions: Post extracted sediment samples were dry weighed when combusted: ppm = dpm/sample wt./SA = dpm /g/ dpm/µg = µg/g Pre-extracted sediment samples were corrected for moisture: ppm = dpm/sample wt x correction factor/SA = dpm/g/dpm/µg = µg/g Trapping solutions:	

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See pond water.

Sep-packs:

For the first 3 time points, sep-packs were extracted 5 x 1 ml with methanol. For extraction, 1 ml of aliquot was taken for LSC:

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

For the later time points, combustion was done to insure that all radioactivity was being accounted:

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

Thin Layer Chromatography (TLC):

Standards were applied to each plate to provide a reference rf for the parent compound. Same size aliquots of samples were counted in duplicate by LSC and were applied to plates to give % of recovery:

dpm recovered / total dpm applied = % recovery

Individual spots on the TLC plate (visualized by autoradiography) were counted by LSC after scraping:

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

First Order Rate Law:

$\ln C_T = \ln C_o - kt$

C_T = % of total ^{14}C residues in terms of parent compound equivalents.

With an initial concentration of 1, at $t_{1/2}$, $C_T = 1-0.5 = 0.5$. The equation becomes: $\ln C_o/C_T = kt$ and therefore $t_{1/2} = 1/k \ln 1/0.5 = 1/k \ln 2 = 0.693/k$; k is the slope (first derivative) of the line generated from the linear regression analysis of the plot of ln % of initial concentration vs days.

Liquid Scintillation Counting (LSC):

In some cases, the control dpm values are manually subtracted from the test dpms. LSC data sheet indicated automatic LSc subtraction in the heading dpm = cpm/efficiency.

4 RESULTS

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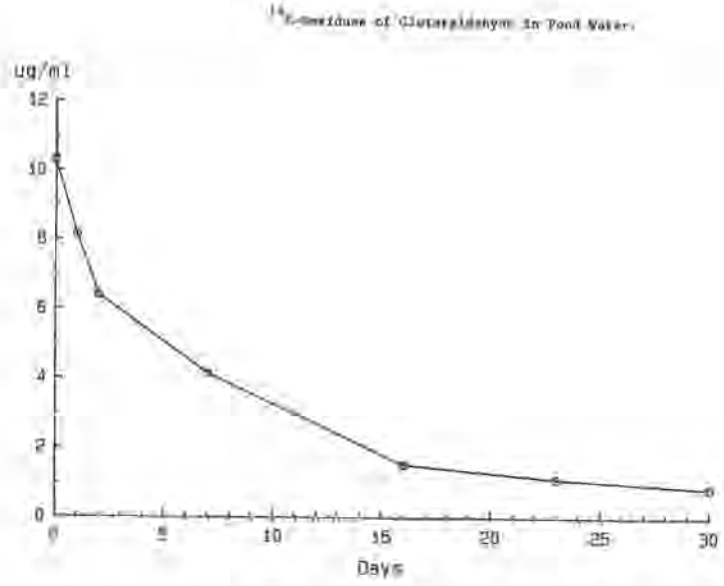
Water/sediment degradation study
Aerobic aquatic metabolism of ^{14}C-glutaraldehyde

Pond water

14C-Residues of glutaraldehyde in Pond water (µg/ml)		
Day	µg/ml (Determination by LSC as glutaraldehyde equivalents; mean values of duplicate samples)	Percentage of system total
0	10.3	103
1	8.13	81
2	6.40	64
7	4.12	41
16	1.54	15
23	1.13	11
30	0.882	8.8



Total ^{14}C residues in the pond water declined in a logarithmic fashion from ca. 103% (day 0) to ca. 9% over the complete period of 30 days.

Sediment

14C-Residues of glutaraldehyde in sediment (µg/g; determination by LSC as glutaraldehyde equivalents; mean values of duplicate samples)					
Day	Total (determined by pre-extraction combustions)	Bound	Percentage of total	Extractable	Percentage of total
0	4.6	3.6	78	0.17	3.3
1	7.5	6.1	81	0.14	1.7
2	7.1	5.4	72	0.14	1.8
7	8.0	5.9	74	0.15	1.8

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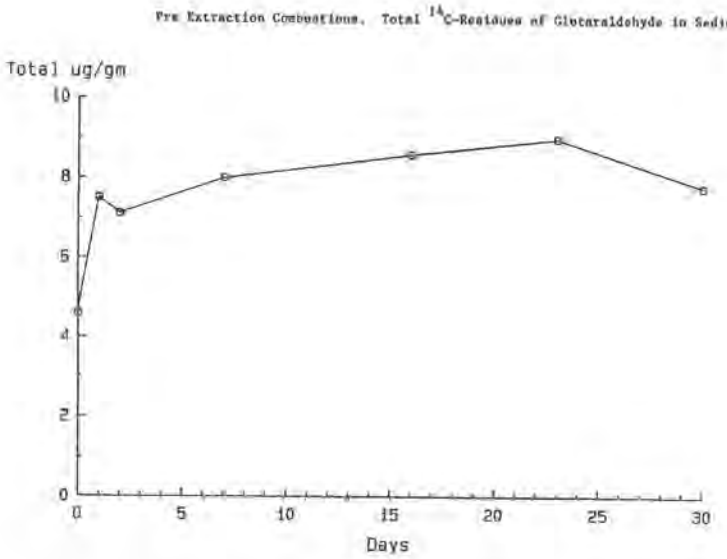
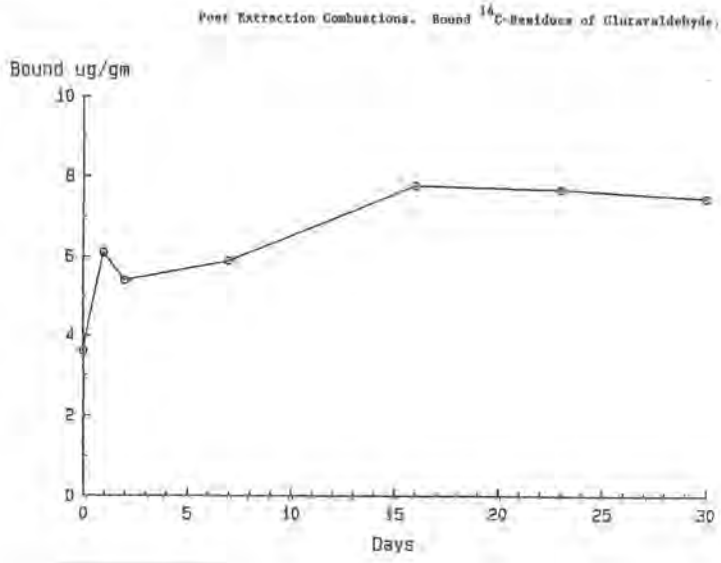
16	8.6	7.8	91	0.13	1.4
23	9.0	7.7	86	0.11	1.2
30	7.8	7.5	96	0.10	1.3





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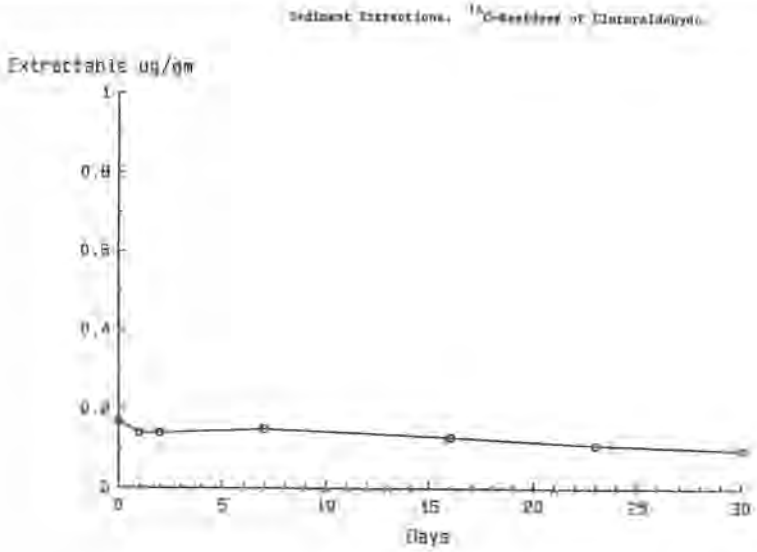

In the sediment, radioactivity increased at the beginning of the experimental period and remained constant thereafter over the whole period of 30 days. Most of the radioactivity in the sediment was bound, extractable ^{14}C residues were < 3%.

Trapping solutions

14C-Residues of glutaraldehyde in trapping solutions (total µg in 250 ml final volume; values as means of duplicate samples)				
Day	Ethylen glycol	H_2SO_4	KOH_1	KOH_2
1	<0.100	< 0.100	< 0.100	< 0.100
2	0.219	< 0.100	2.10	< 0.100
7	1.75	0.125	95.8	10.6
16	1.58	< 0.100	176	< 0.100
23	1.65	< 0.100	27.0	< 0.100
30	0.425 (i.e. 0.22% of initial dose)	< 0.100 (i.e. < 0.01% of initial dose)	35.8 (i.e. 13.5% of initial dose)	< 0.100 (i.e. 0.42% of initial dose)

Main capture of radioactivity was reported for the KOH solutions, especially for the first KOH solution, which refers to $^{14}CO_2$ production; peaks in radioactivity were seen after 7 and 16 days.

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Silica gel sep-packs

14C-Residues of glutaraldehyde in Sep-packs		
Day	Total (µg; determination by LSC as glutaraldehyde equivalents; mean values of duplicate samples)	Percentage of initial dose
1	0.046	< 0.10
2	0.276	< 0.10
7	0.350	< 0.10
16	0.106	< 0.10
23	0.025	< 0.10
30	0.008	< 0.10

No significant amounts of radioactivity were trapped by the silica gel sep-packs.

^{14}C residues masse balance accountability

^{14}C-Residue Mass Balance Accountability[1]

	Sediment					Pond Water					Sep-Packs	Trapping Solutions	Total
Day	kg Sediment in System	µg in Sample	µg in System	Total µg Accountability	% of Initial Dose	kg Water in System	µg in Sample	µg in System	Total µg Accountability	% of Initial Dose	% of Initial Dose	% of Initial Dose	% of Initial Dose
0	120	22.6	575	575	57	450	10.2	2573	2573	103	<0.100	—	160
1	120	29.9	900	922	77	248	5.17	1335	1956	78	<0.100	<0.10	113
2	115	31.8	825	875	73	275	5.80	1666	1491	59	<0.100	<0.10	86
7	113	33.8	900	900	75	214	4.12	882	932	37	<0.100	1.1	84
16	110	15.5	866	1068	42	202	1.96	711	789	11	<0.100	1.1	55
23	108	40.6	554	1065	43	190	1.13	215	279	11	<0.100	1.1	55
30	100	51.2	780	962	38	178	0.883	157	210	7	<0.100	1.4	48

[1] Pond water was dosed at 10 ppm, which is 1500 dpm ^{14}C-glutaraldehyde.

The total masse balance accountability decreased over the complete period, reaching ca. 48% of the initial dose on day 30.

Degradation products

TLC of ^{14}C-Residues of Glutaraldehyde in Pond Water As % of Total Residues on Day -0 [1].

	Degradation Product					
	D	G	F	A	H	Remainder
Day - 0	71	1.9	2.4	21		3.1
Day - 1	42			28	5.4	5.4
Day - 2	26		14	17		4.2
Day - 7	34					6.0
Day - 16	14					1.4
Day - 23	10					1.2

[1] Duplicate plates were run. Samples values are means of duplicate analyses. Standard values are mean of four determinations.

[2] Percent of total residues at day -0 = $\frac{\left(\text{Total Residues (ppm)}\right)\left(\text{Percent Recovered}\right)}{(10.3 \text{ ppm at Day -0})}$

TLC characterisation of ^{14}C residues of glutaraldehyde revealed following 5

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Aerobic aquatic metabolism of ^{14}C-glutaraldehyde

degradation products (i.e TLC zones):

(1) Degradation product O: immobile residues of ^{14}C-GA, accounted for 10 to 71% of the total residues present on day 0.

(2) Degradation product A: accounted for 17 to 26% of the total residues present day 0.

(3) Product G: parent compound, accounted for ca. 2% of the total residues present of day 0.

(4) Degradation product F: final degradation product accounted for 2.4 to 14% of the total residues present on day 0.

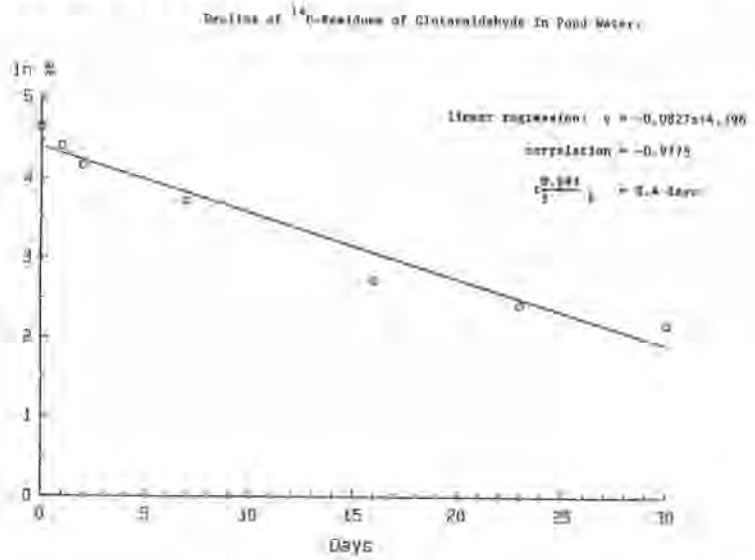
(5) Degradation product H: further final product, accounted for 5.4% of the total residues present on day 0.

Half-life of glutaraldehyde in water under aerobic conditions

Half-life based on the decline of total radioactivity in pond water:

Day	Percentage of initial concentration (glutaraldehyde equivalents; mean values of duplicate samples)	ln %
0	103	4.63
1	81	4.40
2	64	4.16
7	41	3.72
16	15	2.73
23	11	2.42
30	8.8	2.18

A half-life of 8.4 days was calculated.



Half-life based on the degradation of 14C-glutaraldehyde (simple extrapolation):

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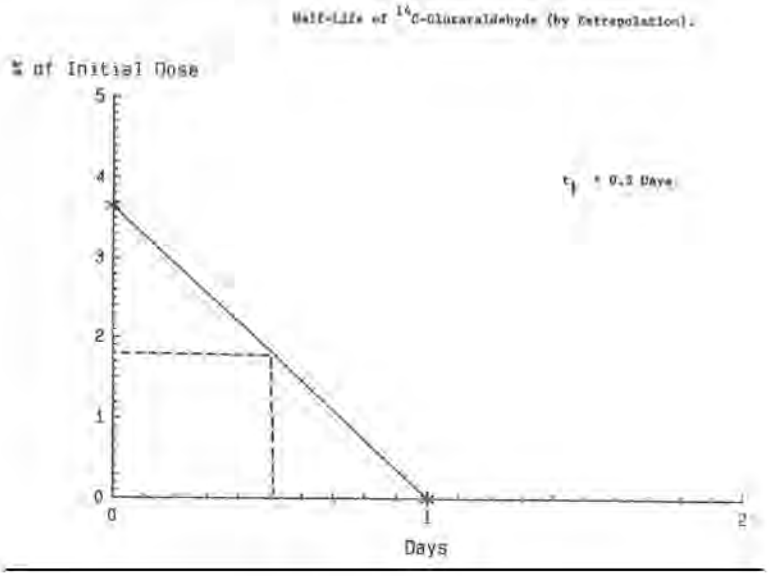

A half-life < 0.5 days was extrapolated.

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Water/sediment degradation study

Aerobic aquatic metabolism of ^{14}C-glutaraldehyde

5 APPLICANT'S SUMMARY AND CONCLUSION

Materials and methods

The aim of the present study was to investigate the environmental fate of glutaraldehyde in pond water and sediment under aerobic conditions.

Test substance: ^{14}C-Glutaraldehyde, Radiochemical purity > [redacted]%, Specific radioactivity 3.40 mCi/mM

The test was performed according to EPA guideline 162-4, with GLP

The sediment (silty clay loam) consisted of ca. 49% silt, 34% clay and 17% sand; The organic matter content was 1.4%, the pH was 6.5, and the percentage of 1/3 bar moisture was about 18%. The cation exchange capacity (C.E.C) was 23.3 meq/100 g soil. The pond water had an initial temperature of 19.5 °C, a pH of 7.95 and a dissolved oxygen content of 8.55 ppm.

The test system consisted of a 3000 ml metabolism vessel, with ground glass joints treated with sealing wax to preserve the integrity of the closed system. The test system was kept in the dark at a temperature of 25 +/-1°C. The metabolism vessel was connected to an ethylene glycol trap, a 1N H_2SO_4 trap and two successive 1N KOH traps. 125 g sediment and 250 ml pond water were placed in the vessel; the test substance was dosed at 10 ppm, corresponding to 2.5 mg of ^{14}C-glutaraldehyde. Samples for analysis were collected over a period of 30 days at following time point: day 0, 1, 2, 7, 16, 23 and 30. For characterisation of radioactive residues, the sediment was subjected to a radioassay by triplicate combustion and extraction with methanol. The extract was subjected again to a radioassay in duplicate combustion, and depending on the percentage of total residue in the initial sample (i.e. >10% or 0.01 ppm), the radioassay in duplicate combustion was followed by thin-layer chromatography (TLC). The pond water was subjected to a radioassay by duplicate liquid scintillation counting (LSC) and depending on the percentage of total residue in the initial sample (i.e. > 10% or 0.01 ppm), the radioassay in duplicate combustion was followed by thin-layer chromatography (TLC).

Results and discussion

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

Total ^{14}C residues in the pond water declined in a logarithmic fashion from ca. 103% (day 0) to ca. 9% over the complete period of 30 days. In the sediment, radioactivity increased at the beginning of the experimental period and remained constant thereafter over the whole period of 30 days. Most of the radioactivity in the sediment was bound; extractable ^{14}C residues were < 3%.

Main capture of radioactivity was reported for the KOH solutions, especially for the first KOH solution, which refers to $^{14}CO_2$ production; peaks in radioactivity were seen after 7 and 16 days..

The total masse balance accountability decreased over the complete period, reaching ca. 48% of the initial dose on day 30.

TLC characterisation of ^{14}C residues of glutaraldehyde revealed following 5 degradation products (i.e TLC zones):

(1) Degradation product O: immobile residues of ^{14}C-GA, accounted for 10 to 71% of the total residues present on day 0.

(2) Degradation product A: accounted for 17 to 26% of the total residues present day 0.

(3) Product G: parent compound, accounted for ca. 2% of the total residues present of day 0.

(4) Degradation product F: final degradation product accounted for 2.4 to 14%

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	of the total residues present on day 0. (5) Degradation product H: further final product, accounted for 5.4% of the total residues present on day 0. Based on the decline of total radioactivity in pond water, a half-life of 8.4 days was calculated; considering the degradation of ^{14}C-glutaraldehyde, a half-life of < 0.5 days was determined by extrapolation.
Conclusion	The test results showed that under aerobic conditions, glutaraldehyde is subjected to rapid aquatic degradation, resulting in five degradation products. These results are in accordance with the fact that glutaraldehyde is readily biodegradable under aerobic conditions ([redacted], Determination of the Biodegradability or the Elimination of [redacted] in the DOC Die Away (ISO 7827)-Test [redacted], 1993).
5.1.1 Reliability	**1**
5.1.2 Deficiencies	No

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Water/sediment degradation study
Aerobic aquatic metabolism of ^{14}C-glutaraldehyde

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	16.3.2009
Materials and Methods	The study is old and does not fulfil the current requirements of the OECD 308. There are several deficiencies: - only one combination of sediment + water tested (two required) - source of water and sediment not given - repeatability and sensitivity of analytical methods have not been reported - nothing is said about an acclimation period - transformation products have not been identified - transformation rate in the sediment was not determined - mineralization rate was not determined, neither the transformation rate for the whole system
Results and discussion	The dissipation half-life of 8.4 days was calculated according to the first-order kinetics based on decline of ^{14}C-activity in pond water. The amount of parent compound in the pond water was very low already at day 0, only 3.64%, and no parent compound was detected during later sampling occasions. A more frequent sampling within the first day might have given more information about degradation of the parent compound. Total radioactivity in the pond water declined in a logarithmic fashion from 103% to 9% at the end of the test (day 30). Four degradation products were formed of which three accounted for more than 10% of radioactivity at one or more sampling occasions. In the sediment, initially measured radioactivity was 23%, maximum radioactivity was 42% (day 16) and in the end of the test radioactivity was 38%. Most of the radioactivity in the sediment was bound, extractable residues formed less than 3% of radioactivity. Main capture of volatile radioactivity was CO_2. The trapping solutions formed at maximum 7.1% of initial radioactivity. Recovery of radioactivity decreased from initial 126% to 48% at the end of the test. Acceptable recoveries for labelled substances range from 90% to 110%. The loss of accountability was explained to be due to volatile losses during normal sample collection and processing. The loss was assumed to be 15% per sampling occasion.
Conclusion	The parent compound transformed rapidly and formed transformation products that disappeared gradually from the water phase. The transformation products were distributed and bound rapidly to the sediment and formed about 20-40% of radioactivity throughout the test. There is no evidence about mineralization as only small amount of applied radioactivity was trapped as CO_2. On the other hand mineralization cannot be ruled out, because the material balance was unacceptably low due to significant losses of radioactivity during processing of water samples.
Reliability	3
Acceptability	Not acceptable because the material balance was less than 50% at the end of the test.
Remarks	

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Water/sediment degradation study
Aerobic aquatic metabolism of ^{14}C-glutaraldehyde

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

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1986) Anaerobic aquatic metabolism of glutaraldehyde. [redacted] (Unpublished), BPD ID A7.01.2.1.2_01
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, according to US EPA Guideline subdivision N 162-3
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 METHOD
3.1	**Test material**	^{14}C-Glutaraldehyde Specific radioactivity 3.40 mCi/mM
3.1.1	Lot/Batch number	
3.1.2	Specification	As given in section 2
3.1.3	Purity	Radiochemical purity > [redacted]% (analysis by GLC and LSC)
3.1.4	Further relevant properties	No data
3.1.5	Composition of Product	Not relevant
3.2	**Test ing procedure**	
3.2.1	Water and sediment	The sediment (silty clay loam) consisted of ca. 49% silt, 34% clay and 17% sand; The organic matter content was 1.4%, the pH was 6.5, and the percentage of 1/3 bar moisture was about 18%. The cation exchange capacity (C.E.C) was 23.3 meq/100 g soil. The pond water had an initial temperature of 19.5 °C, a pH of 7.95 and a dissolved oxygen content of 8.55 ppm.
3.2.2	Test system	The test system consisted of a 3000 ml metabolism vessel, with ground glass joints treated with sealing wax to preserve the integrity of the closed system. The test system was kept in the dark at a temperature of 25 +/-1°C; anaerobic conditions in the test system were obtained by passing a N2 flow (flow rate ca. 100 ml/min) through the system. The metabolism vessel was connected to an ethylene glycol trap (for organic

		volatile compounds), a 1N H_2SO_4 trap (for alkaline volatile compounds) and two successive 1N KOH traps (for CO_2 and other acidic volatiles). Silica gel sep-packs were placed between the metabolism vessel and the ethylene glycol trap.	
3.2.3	Test conditions	125 g sediment and 250 ml pond water were placed in the vessel and the test substance was dosed at 10.5 ppm, corresponding to 2.5 mg of 14C-glutaraldehyde and was added.	
3.2.4	Method of preparation of test solution	A stock solution was obtained by mixing the test substance with H_2O (pH 6.5) in a 100 ml volumetric flask. Dilutions were made starting from this stock solution.	
3.2.5	Initial TS concentration	See 3.2.3	
3.2.6	Duration of test	90 days	
3.2.7	Analytical parameter	Measurement of radioactivity. For characterisation of radioactive residues, the sediment was subjected to a radioassay by triplicate combustion and extraction with methanol. The extract was subjected again to a radioassay in duplicate combustion, and depending on the percentage of total residue in the initial sample (i.e. >10% or 0.01 ppm), the radioassay in duplicate combustion was followed by thin-layer chromatography (TLC). The pond water was subjected to a radioassay by duplicate liquid scintillation counting (LSC) and depending on the percentage of total residue in the initial sample (i.e. > 10% or 0.01 ppm), the radioassay in duplicate combustion was followed by thin-layer chromatography (TLC).	
3.2.8	Sampling	Samples for analysis were collected at following time point: day 0, 1, 3, 7, 14, 21, 30, 37, 44, 54, 61, 68, 75, 83 and 90.	
3.2.9	Intermediates/ degradation products	Identified	x
3.2.10	Controls	Yes	
3.2.11	Calculations	Pond water: 1 ml of aliquot was taken for LSC. The average of controls (AvC) was subtracted from the test samples average (AvT) and the resultant number divided by the specific activity (SA): ppm = AvT – AvC / SA = dpm – dpm / dpm/µg 1/1 ml = µg/ml Combustions: Post extracted sediment samples were dry weighed when combusted: ppm = dpm/sample wt./SA = dpm /g/ dpm/µg = µg/g Pre-extracted sediment samples were corrected for moisture: ppm = dpm/sample wt x correction factor/SA = dpm/g/dpm/µg = µg/g Trapping solutions:	

See pond water.

Sep-packs:

For the first 3 time points, sep-packs were extracted 5 x 1 ml with methanol. For extraction, 1 ml of aliquot was taken for LSC:

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

For the later time points, combustion was done to insure that all radioactivity was being accounted:

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

Thin Layer Chromatography (TLC):

Standards were applied to each plate to provide a reference rf for the parent compound. Same size aliquots of samples were counted in duplicate by LSC and were applied to plates to give % of recovery.

dpm recovered / total dpm applied = % recovery

Individual spots on the TLC plate (visualized by autoradiography) were counted by LSC after scraping:

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

First Order Rate Law:

$\ln C_T = \ln C_o - kt$

C_T = % of total 14 C residues in terms of parent compound equivalents.

With an initial concentration of 1, at $t_{1/2}$, $C_T = 1-0.5 = 0.5$. The equation becomes: $\ln C_o/C_T = kt$ and therefore $t_{1/2} = 1/k \ln 1/0.5 = 1/k \ln 2 = 0.693/k$; k is the slope (first derivative) of the line generated from the linear regression analysis of the plot of ln % of initial concentration vs days.

Liquid Scintillation Counting (LSC):

In some cases, the control dpm values are manually subtracted from the test dpms. LSC data sheet indicated automatic LSc subtraction in the heading dpm = cpm/efficiency.

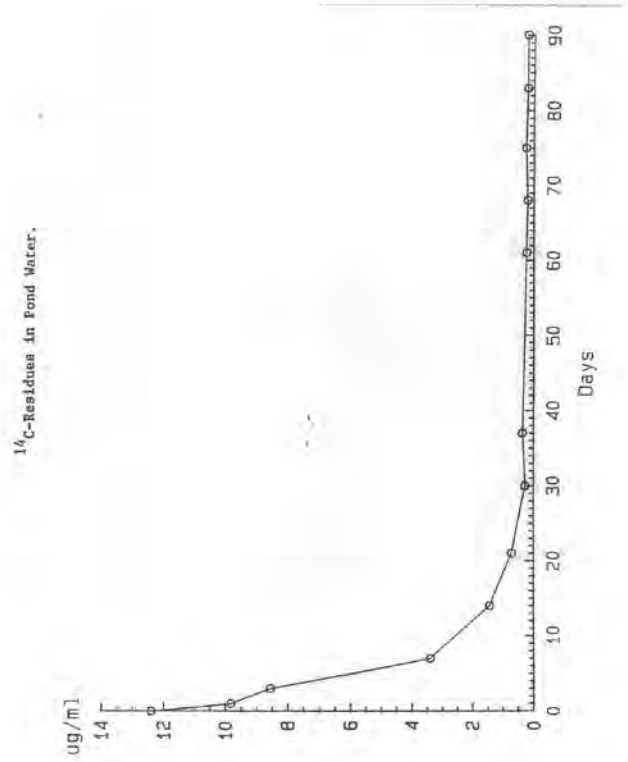
4 RESULTS

4.1 Pond water

^{14}C-Residues in Pond Water (ug/ml).[1]

Day	ug/ml[1]	% of System Total
0	12.4	100
1	9.81	79.1
3	8.56	69.0
7	3.38	27.3
14	1.42	11.4
21	0.714	5.76
30	0.272	2.19
37	0.362	2.92
61	0.235	1.90
68	0.204	1.65
75	0.262	2.11
83	0.208	1.68
90	0.218	1.76

[1]Determined by LSC as ^{14}C-Glutaraldehyde equivalents; values are means of duplicate samples.



Total ^{14}C residues in water declined from ca. 92% to ca. 16% over the complete test period of 90 days. A ^{14}C-residue halflife of 5.6 days was

reported. TLC of pond water samples revealed that the parent compound as such rapidly and already disappeared on day 0.

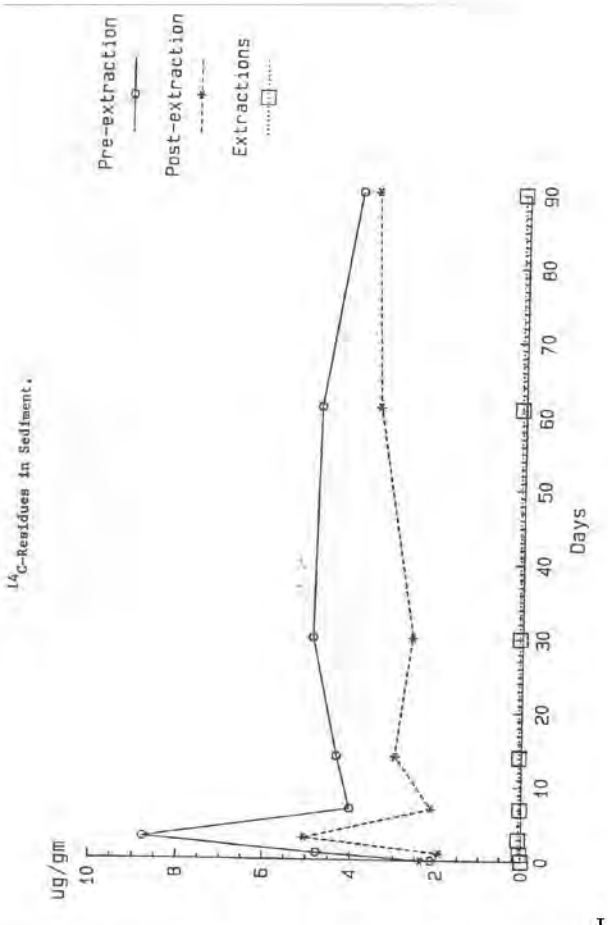
4.2 Sediment

^{14}C-Residues in Sediment (µg/gm).[1]

Day	Total[2]	Bound	% of Total[2]	Extractable[3]	% of Total[2]
0	2.13	2.37	111	0.005	0.230
1	4.76	1.94	40.8	0.054	0.011
3	8.76	5.04	57.5	0.083	0.950
7	4.01	2.13	53.1	0.046	1.15
14	4.31	2.97	68.9	0.065	1,51
30	4.86	2.58	53.1	0.070	1.44
61	4.72	3.40	72.0	0.093	1.97
90	3.88	3.51	90.5	0.117	3.02

[1]Determined by LSC as Glutaraldehyde equivalents; values are means of duplicate samples.

[2]Determined by pre-extraction combustions.



I

In the sediment, an increase in ^{14}C residues was observed during the 3 first days (31%), which was followed by a decrease (13% on day 7).

Most of the radioactivity in the sediment was bound; in fact extractable ^{14}C residues were < 3%.

4.3 Trapping solutions

^{14}C-Residues in Trapping Solutions,[1]

Day	Ethylene Glycol	H_2SO_4	KOH_1	KOH_2
1	<0.250	<0.250	<0.250	<0.250
3	<0.250	<0.250	4.30	<0.250
7	<0.250	0.068	113	<0.250
14	<0.250	0.319	100	<0.250
21	<0.250	<0.250	93.5	<0.250
30	<0.250	<0.250	58.7	<0.250
37	<0.250	<0.250	19.4	<0.250
44	<0.250	<0.250	9.40	0.512
54	<0.250	<0.250	10.1	<0.250
61	<0.250	<0.250	6.85	<0.250
68	<0.250	<0.250	2.65	<0.250
75	<0.250	<0.250	1.62	<0.250
83	<0.250	<0.250	1.81	<0.250
90	<0.250	<0.250	1.36	<0.250
Σ		0.387	209.7	0.512

[1]Total µg in 250 ml final volume. Values are the means of duplicate samples.

A total of 7.12% of the initial ^{14}C dose was recovered in the trapping solutions, with 7.11% having been trapped in the first KOH trap which reveales $^{14}CO_2$ production.

4.4 Silica gel sep-packs

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

Day	Total µg[1]	% of Initial Dose
1	<0.250	<0.01
3	<0.250	<0.01
7	<0.250	<0.01
14	<0.250	<0.01
21	<0.250	<0.01
30	<0.250	<0.01
37	<0.250	<0.01
44	<0.250	<0.01
54	<0.250	<0.01
61	<0.250	<0.01
68	<0.250	<0.01
75	<0.250	<0.01
83	<0.250	<0.01
90	<0.250	<0.01

[1]Determined by LSC as Glutaraldehyde equivalents; values are means of duplicate samples.

No significant amounts of radioactivity were trapped by the silica gel sep-packs.

4.5 ^{14}C residues masse balance accountability

^{14}C-residue Mass Balance Accountability.[1]

	Sediment				Pond Water				Dep-Roche	Trapping Solutions	Total
Day	ng Sediment in System	ug in Sample	ug in System	Total ug Accountability	% of[2] Initial Dose	ul Water in System	ug in Sample	ug in System	Total ug Accountability	% of[2] Initial Dose	% of Initial Dose	% of[2] Initial Dose	% of[2] Initial Dose
0	105,000	1.50	210	210	8.21	230	169	3951	3951	91.7	—	—	100
1	101,524	35.1	515	585	18.0	226	118	2717	2866	74.6	—	—	91.6
3	105,108	59.6	1000	1043	31.1	214	101	1837	1097	68.7	—	0.720	99.5
7	107,309	23.8	430	532	13.4	202	40.6	803	1035	32.7	—	2.51	49.6
14	101,269	31.3	437	353	13.0	190	17.0	330	641	21.2	—	1.19	35.9
30	97,686	31.3	426	383	13.2	118	7.26	178	604	18.8	—	0.163	32.7
61	91,867	30.6	295	461	9.08	118	7.18	70.4	501	15.6	—	1.48	26.1
90	33,021	47.3	128	348	2.98	76	2.38	81.8	508	15.1	—	0.230	18.0

[1] Pond water was dosed at 10 rpm, which is 7500 ng ^{14}C-Glutaraldehyde.
[2] Measured initial dose is ng in sediment + ug in water at day 0 = 3317 ug.
[3] % of all ether points taken between those intervals given in this table.

The total masse balance accountability decreased over the complete period, reaching ca. 27% of the initial dose on day 90.

4.6 Degradation products

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

(1) Degradation product O: immobile residues of ^{14}C-GA, accounted for 10.5 to 69% of the total residues present on day 0.

(2) Degradation product A: accounted for 8 to 27% of the total residues present day 0.

(3) Product G: parent compound.

(4) Degradation product F: final degradation product accounted for about 7% of the total residues present on day 0.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The aim of the present study was to investigate the environmental fate of glutaraldehyde in pond water and sediment under anaerobic conditions.

Test substance: ^{14}C-Glutaraldehyde, Radiochemical purity > [redacted]%,

Specific radioactivity 3.40 mCi/mM

The test was performed according to EPA guideline 162-3, with GLP

The test system consisted of a 3000 ml metabolism vessel, with ground glass joints treated with sealing wax to preserve the integrity of the closed system. The test system was kept in the dark at a temperature of 25 +/-1°C; anaerobic conditions in the test system were obtained by passing a N_2 flow (flow rate ca. 100 ml/min) through the system. The metabolism vessel was connected to an ethylene glycol trap, a 1N H_2SO_4 trap and two successive 1N KOH traps. 125 g sediment and 250 ml pond water were placed in the vessel; the test substance was dosed at 10.5 ppm, corresponding to 2.5 mg of ^{14}C-glutaraldehyde. The sediment (silty clay loam) consisted of ca. 49% silt, 34% clay, 17% sand; The organic matter content was 1.4%, the pH was 6.5, and the percentage of 1/3 Bar moisture was about 18%. The pond water had an initial temperature of 19.5 °C, a pH of 7.95 and a dissolved oxygen content of 8.55 ppm. Samples for analysis were collected over a period of 90 days at following time point: day 0, 1, 3, 7, 14, 21, 30, 37, 44, 54, 61, 68, 75, 83 and 90. For characterisation of radioactive residues, the sediment was subjected to a radioassay by triplicate combustion and extraction with methanol. The extract was subjected again to a radioassay in duplicate combustion, and depending on the percentage of total residue in the initial sample (i.e. >10% or 0.01 ppm), the radioassay in duplicate combustion was followed by thin-layer chromatography (TLC). The pond water was subjected to a radioassay by duplicate liquid scintillation counting (LSC) and depending on the percentage of total residue in the initial sample (i.e. > 10% or 0.01 ppm), the radioassay in duplicate combustion was followed by thin-layer chromatography (TLC).

5.2 Results and discussion

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

Total ^{14}C residues in water declined from ca. 92% to ca. 16% over the complete test period of 90 days. A ^{14}C-residue halflife of 5.6 days was reported. TLC of pond water samples revealed that the parent compound as such rapidly and already disappeared on day 0. In the sediment, an increase in ^{14}C residues was observed during the 3 first days (31%), which was followed by a decrease (13% on day 7). Most of the radioactivity in the sediment was bound; in fact extractable ^{14}C residues were < 3%. A total of 7.12% of the initial ^{14}C dose was recovered in the trapping solutions, with 7.11% having been trapped in the first KOH trap which reveales $^{14}CO_2$ production.

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

(1) Degradation product O: immobile residues of ^{14}C-GA, accounted for 10.5 to 69% of the total residues present on day 0.

(2) Degradation product A: accounted for 8 to 27% of the total residues present day 0.

(3) Product G: parent compound.

(4) Degradation product F: final degradation product accounted for about 7% of the total residues present on day 0.

5.3 Conclusion

The test results indicate that under anaerobic conditions, glutaraldehyde is rapidly biotransformed by microorganisms, and 4 degradation products could be demonstrated.

5.3.1 Reliability **1**

5.3.2 Deficiencies No

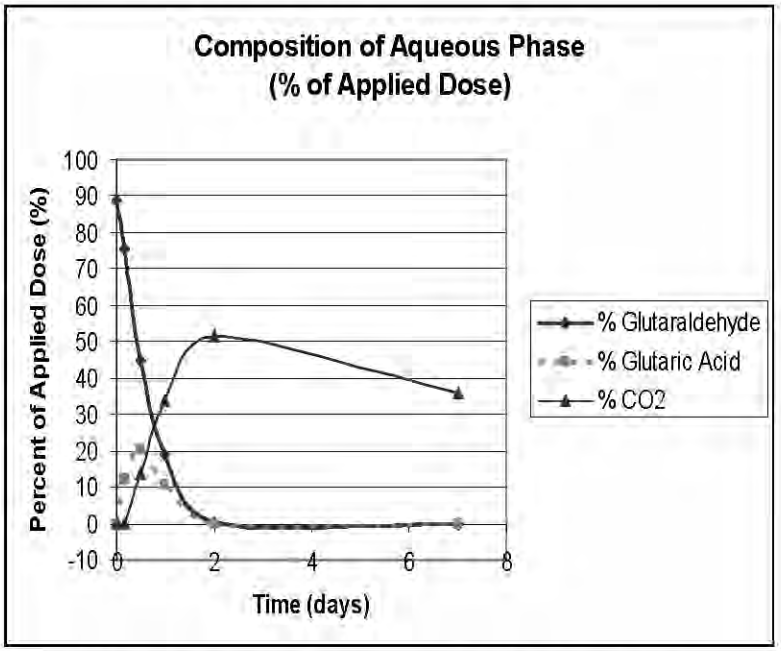
Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20.3.2009
Materials and Methods	The study is old and does not fulfil the current requirements of the OECD 308. There are several deficiencies: - only one combination of sediment + water tested (two required) - source of water and sediment not given - repeatability and sensitivity of analytical methods have not been reported - nothing is said about an acclimation period - no evidence has been provided about anoxic conditions, e.g. oxygen contents have not been reported. - transformation prducts have not been identified - transformation rate in the sediment was not determined - mineralization rate was not determined, neither the transformation rate for the whole system
Results and discussion	The dissipation half-life of 5.6 days was calculated according to the first-order kinetics based on decline of ^{14}C-activity in pond water. Parent compound transformed rapidly and it was not detected at any sampling occassions. Radioactivity in the pond water declined exponentially from 91.7% to 15.7% of initial dose. Three degradation products were formed of which two accounted for more than 10% of radioactivity. Metabolites tended toward greater polarity with time. Radioactivity in the sediment peaked on day 3 accounting 31.1% of initial dose and then declined to the end of the test. Radioactivity accounted 3.98% of initial dose on day 90. Most of the radioactivity was bound, with less than 3% of the ^{14}C-residue being extractable. Main capture of volatile radioactivity was CO_2. The trapping solutions formed at maximum 4.74% of initial radioactivity. Recovery of radioactivity decreased from initial 100% to 19.9% at the end of the test. Acceptable recoveries for labelled substances range from 90% to 110%. The loss of accountability was due to volatile losses during normal sample collection and processing.
Conclusion	The parent compound transformed rapidly and formed transformation products that dissipated within one month from the water phase. The transformation products were distributed and bound to the sediment at the beginning of the experiment and then declined gradually to the end of the experiment. Mineralization is assumed to be only a minor part of transformation as only small amounts of applied radioactivity was trapped as CO_2.
Reliability	3
Acceptability	Not acceptable because the material balance was ca. 20% at the end of the test.
Remarks	
	COMMENTS FROM ... *(specify)*

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

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 and BASF SE	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes US EPA 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	^{14}C-glutaraldehyde 97.8% (radiochemical purity)	
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

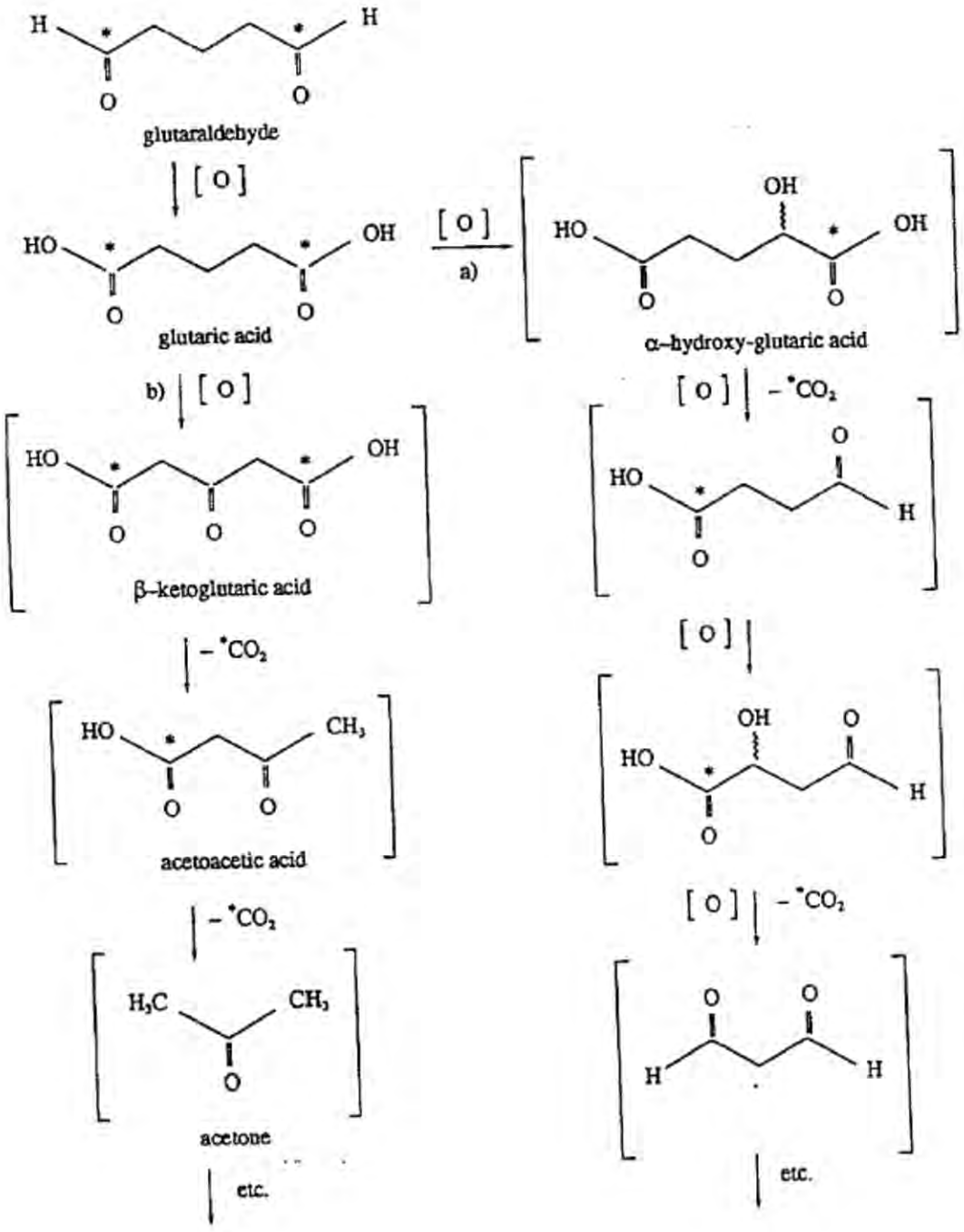
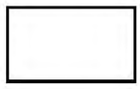


Figure 29. Proposed Metabolic Pathway for [^{14}C]-Glutaraldehyde.

Time	Replicate	C-14 in Sediment (% AR)	Average	Extractable	Not Extractable
0-hour	A	8,4	7,6	36	48,2
	B	6,8		43	69,6
4-hour	A	9	8,6	42	64,3
	B	8,1		46,8	70,7
12-hour	A	15,7	16,7	22,4	50,6
	B	17,6		23,1	34,6
24-hour	A	22,2	20,4	5,6	11,8
	B	18,6		12,3	23
48-hour	A	21	23,2	12,4	54,6
	B	25,3		8,2	50,7
7-day	A	20	21,9	7,8	74,5
	B	23,7		9	98,4
14-day	A	15,2	16,2	13,5	129
	B	17,1		7,4	92,6
30-day	A	11,9	14,0	17,3	87,7
	B	16,1		8,8	91,9

Extractable (% AR)	Average	Not Extractable (% AR)	Average
3,0	3,0	4,0	4,4
2,9		4,7	
3,8	3,8	5,8	5,8
3,8		5,7	
3,5	3,8	7,9	7,0
4,1		6,1	
1,2	1,8	2,6	3,4
2,3		4,3	
2,6	2,3	11,5	12,1
2,1		12,8	
1,6	1,8	14,9	19,1
2,1		23,3	
2,1	1,7	19,6	17,7
1,3		15,8	
2,1	1,7	10,4	12,6
1,4		14,8	

Time	Replicate	C-14 in Sediment (% AR)	Average	Extractable (% AR)	Average	Not Extractable (% AR)	Average
0-hour	A	8,4	7,6	3,0	3,0	4,0	4,4
	B	6,8		2,9		4,7	
4-hour	A	9	8,6	3,8	3,8	5,8	5,8
	B	8,1		3,8		5,7	
12-hour	A	15,7	16,7	3,5	3,8	7,9	7,0
	B	17,6		4,1		6,1	
24-hour	A	22,2	20,4	1,2	1,8	2,6	3,4
	B	18,6		2,3		4,3	
48-hour	A	21	23,2	2,6	2,3	11,5	12,1
	B	25,3		2,1		12,8	
7-day	A	20	21,9	1,6	1,8	14,9	19,1
	B	23,7		2,1		23,3	
14-day	A	15,2	16,2	2,1	1,7	19,6	17,7
	B	17,1		1,3		15,8	
30-day	A	11,9	14,0	2,1	1,7	10,4	12,6
	B	16,1		1,4		14,8	



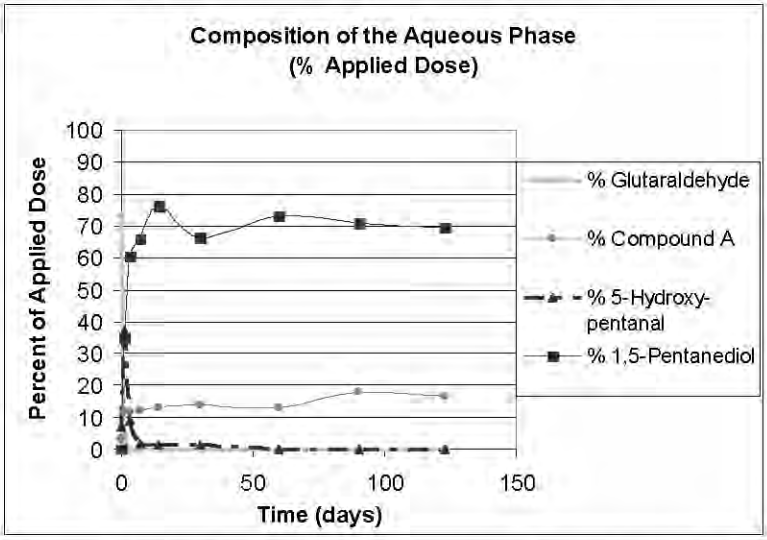
Water phase

		Glut %	Average	Glut acid	Average	CO2	Average
0-hour	A	90,8	88,85	0	0	0	0
	B	86,9		0		0	
4-hour	A	82	75,7	12,9	12,3	0	0
	B	69,4		11,7		0	
12-hour	A	43,4	44,65	21,5	20,2	13	13,7
	B	45,9		18,9		14,4	
24-hour	A	13,9	18,95	11	10,55	32,6	33,8
	B	24		10,1		35	
48-hour	A	0,3	0,15	0	0	52,9	51,35
	B	0		0		49,8	
7-day	A	0	0	0	0	37,1	35,75
	B	0		0		34,4	

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 and BASF SE	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes US EPA 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 [redacted] 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	^{14}C-glutaraldehyde [redacted]% (radiochemical purity)	
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