Section A7.4.1.3(3)	**Growth inhibition test on algae**
Annex Point IIA7.3	**Ethylenethiourea: Determination of the Inhibitory Effect on the Cell Multiplication of Unicellular Green Algae**
IUCLID 4.3/04	

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		1 REFERENCE
1.1	**Reference**	Reuschenbach Dr. (2000) Determination of the Inhibitory Effect on the Cell Multiplication of Unicellular Green Algae. BASF Experimental Toxicology and Ecology Laboratory, Project No. 00/0533/60/1 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2		
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/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD 201 "Algal Growth Inhibition Test"
2.2	**GLP**	Yes
2.3	**Deviations**	Yes, the procedure for suspending the algae has not been reported.
		3 MATERIALS AND METHODS
3.1	**Test material**	BF222-ETU (ethylenethiourea)
3.1.1	Lot/Batch number	L33-99
3.1.2	Specification	Deviating from specification given in section 2 as follows:
3.1.3	Description	Beige powder
3.1.4	Purity	99.6%
3.1.5	Composition of Product	Not applicable
3.1.6	Further relevant properties	Reported as being insoluble in water.
3.1.7	Method of analysis	Samples were analysed using HPLC with UV detection. Typical instrumental conditions were: Column: Nucleosil 120 5 C18 (250 mm x 4.0 mm, 5µ) at ambient temperature Column flow: 1.0 mL/minute Injection size: 100 µL Mobile phase: water + 0.05% THF Retention time: 6 minutes Flow program: Isocratic. Detection: UV at 233nm The limit of quantification of the method is approximately 0.2 mg/l.

Section A7.4.1.3(3)	Growth inhibition test on algae
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		The samples were quantified by external calibration of appropriate single standard solutions which were calculated by linear regression. Standard solutions of each calibration sequence were injected at least once. Each sample was injected twice. The accuracy of the standard solution was checked by a separate second weight. Fortified samples were analysed. Recoveries of 103.8% at a nominal concentration of 0.4 mg/l and of 99.1% at a nominal concentration of c. 2 mg/l prove the suitability of the parameters used for the quantitative analysis in the carrier. No interference from the matrix with the test substance could be observed.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_3-1
3.3	**Reference substance**	Yes, potassium dichromate
3.3.1	Method of analysis for reference substance	Not reported.
3.4	**Testing procedure**	
3.4.1	Culture medium	The test medium was prepared according to EEC directive 92/69/EEC, Annex V, PartC.3. Algal inhibition test and OECD Guideline for Testing of Chemicals, No. 201 Algal growth inhibition test . See table A7_4_1_3-5
3.4.2	Test organisms	see table A7_4_1_3-2
3.4.3	Test system	see table A7_4_1_3-3
3.4.4	Test conditions	see table A7_4_1_3-4
3.4.5	Duration of the test	72 hours
3.4.6	Test parameter	Cell multiplication inhibition
3.4.7	Sampling	Fluorescence (*in vivo* chlorophyll-a-fluorescence) was measured at 0, 24, 48 and 72 hours. Cell counting was performed at 72h on replicate 2 of the inoculated control.
3.4.8	Monitoring of TS concentration	Yes, at test initiation (0h) and test termination (72h)
3.4.9	Statistics	Determination of the mean fluorescence after 0, 24, 48 and 72h. Calculation of the integral of biomass growth over the total duration of the test for each concentration level and comparison of values of treated samples in relation to untreated samples. Calculation of the growth rate over the total duration of the study for each concentration level and comparison of values of treated samples in relation to untreated samples. The EC values are calculated (linear regression analysis) from the concentration-response relationship.

Section A7.4.1.3(3) **Growth inhibition test on algae**

Annex Point IIA7.3 **Ethylenethiourea: Determination of the Inhibitory Effect on the Cell Multiplication of Unicellular Green Algae**

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		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal concentrations of 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, and 100 mg/L
4.2.2	Actual concentrations of test substance	Measured concentrations at study initiation were 0.40, 0.78, 1.56, 3.15, 6.36, 10.62, 24.24, 53.67 and 105.37 mg/L. Measured concentrations at study termination were 0.46, 0.86, 1.56, 3.15, 6.19, 12.72, 25.05, 50.00 and 97.15 mg/L.
4.2.3	Growth curves	See Figure 7_4_1_3_1
4.2.4	Concentration / response curve	See Figure 7_4_1_3_1
4.2.5	Cell concentration data	See table A7_4_1_3-6
4.2.6	Effect data (cell multiplication inhibition)	E_bC_{50} (72h) = 23.7 mg/l E_rC_{50} (72h) = 93.8 mg/l
4.2.7	Other observed effects	None
4.3	**Results of controls**	See table A7_4_1_3-6
4.4	**Test with reference substance**	Reference is made to an earlier study.
4.4.1	Concentrations	Not reported.
4.4.2	Results	EC_{50} (72h) = 0.72 mg/l
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The test was performed in accordance with OECD 201 and GLP. The test was performed under static conditions with nine concentrations of test substance and a water control at 23 ± 2°C. Nominal concentrations of ethylenethiourea were 0 mg/L (control), 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L. Analyses of test substance concentrations were carried out at study initiation and study termination. Three replicate test chambers were prepared at each concentration plus 5 replicates for the negative control. Fluorescence measurements were

Section A7.4.1.3(3)	**Growth inhibition test on algae**
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		carried out at 0, 24, 48 and 72h.
5.2	**Results and discussion**	Exposure of *Pseudokirchneriella subcapitata* to ethylenethiourea resulted in 72 hour EC_{50} values for growth rate and biomass of 93.8 mg/L and 23.7 mg/L.
5.2.1	NOE_rC	Not reported.
5.2.2	ErC50	93.8 mg/L (72 hours, nominal).
5.2.3	E_bC_{50}	23.7 mg/L (72 hours, nominal).
5.3	**Conclusion**	The 72 median hour effective concentrations (EC_{50}s) and the no-observed adverse effect concentration (NOAEC) of ethylenethiourea on the freshwater alga, *Pseudokirchneriella subcapitata*, exposed under static conditions were determined. The 72 EC_{50} values determined for the growth rate and the biomass are 93.8mg/L and 23.7 mg/L. The validity criteria for the test 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	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	*Adopt applicant's version or include revised version*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A7.4.1.3(3)	**Growth inhibition test on algae**
Annex Point IIA7.3	**Ethylenethiourea: Determination of the Inhibitory Effect on the Cell Multiplication of Unicellular Green Algae**
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Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes. The test substance was stirred in demineralised water for about 20 hours at approximately 20°C.
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	No
Other procedures	None

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	*Pseudokirchneriella subcapitata*
Strain	CHODAT SAG 61.81
Source	SAG (Collection of algal cultures in Gottingen)
Laboratory culture	Yes
Method of cultivation	A seed culture was incubated for 7 days at 23±2°C with a final cell density of 5.30×10^6 cells/ml. The seed culture was taken to inoculate a pre-culture. The initial cell density of the pre-culture was 1×10^4 cells/ml. The pre-culture was then incubated for 3 days at 23±2°C giving a final cell density of 0.47×10^6 cells.
Pretreatment	For each definitive test and control solution, aliquots (100 ml) were placed into separate 250 ml Erlenmeyer flasks plugged with gas permeable siliconsponge caps. To achieve the desired nominal concentration of approximately 10000 *Pseudokirchneriella subcapitata* cells/ml at test initiation, a volume of algal inoculum from a logarithmically growing stock culture was transferred to each, excluding the abiotic control, flask (test unit).
Initial cell concentration	1×10^4 cells/ml

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	250 mL
Culturing apparatus	The flasks were placed in a climate chamber maintained at 23±2°C.
Light quality	Artificial light, type universal white (e.g. Osram L25)
Procedure for suspending algae	Not reported
Number of vessels/ concentration	3 replicates at each concentration level and 5 replicates for the control group.

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

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	Nominal temperature range 23±2°C (measured continuously; no deviations from the range were reported)
pH	pH 8.2 at the start and 8.0-8.2 at the end of the test.
Aeration of dilution water	Not reported.
Light intensity	Approximately 60-120 µE/(m^2.s) at a wavelength of 400-700 nm
Photoperiod	Continuous illumination.

Table A7_4_1_3-5: Culture Medium

Nutrient	Concentration in the stock solution	Final concentration in the test solution
Stock solution 1: macro-nutrients		
NH_4Cl	1,5 g/l	15 mg/l
$MgCl_2.6H_2O$	1,2 g/l	12mg/l
$CaCl_2.2H_2O$	1,8 g/l	18 mg/l
$MgSO_4.7H_2O$	1,5 g/l	15 mg/l
KH_2PO_4	0,16 g/l	1,6 mg/l
Stock solution 2: Fe-EDTA		
$FeCl_3.6H_2O$	80 mg/l	0,08 mg/l
$Na_2EDTA.2H_2O$	100 mg/l	0,1 mg/l
Stock solution 3: trace elements		
H_3BO_3	185 mg/l	0,185 mg/l
$MnCl_2.4H_2O$	415 mg/l	0,415 mg/l
$ZnCl_2$	3 mg/l	3×10^{-3} mg/l
$CoCl_2.6H_2O$	1,5 mg/l	$1,5 \times 10^{-3}$ mg/l
$CuCl_2.2H_2O$	0,01 mg/l	10^{-5} mg/l
$Na_2MoO_4.2H_2O$	7 mg/l	7×10^{-3} mg/l
Stock solution 4: $NaHCO_3$		
$NaHCO_3$	50 g/l	50 mg/l

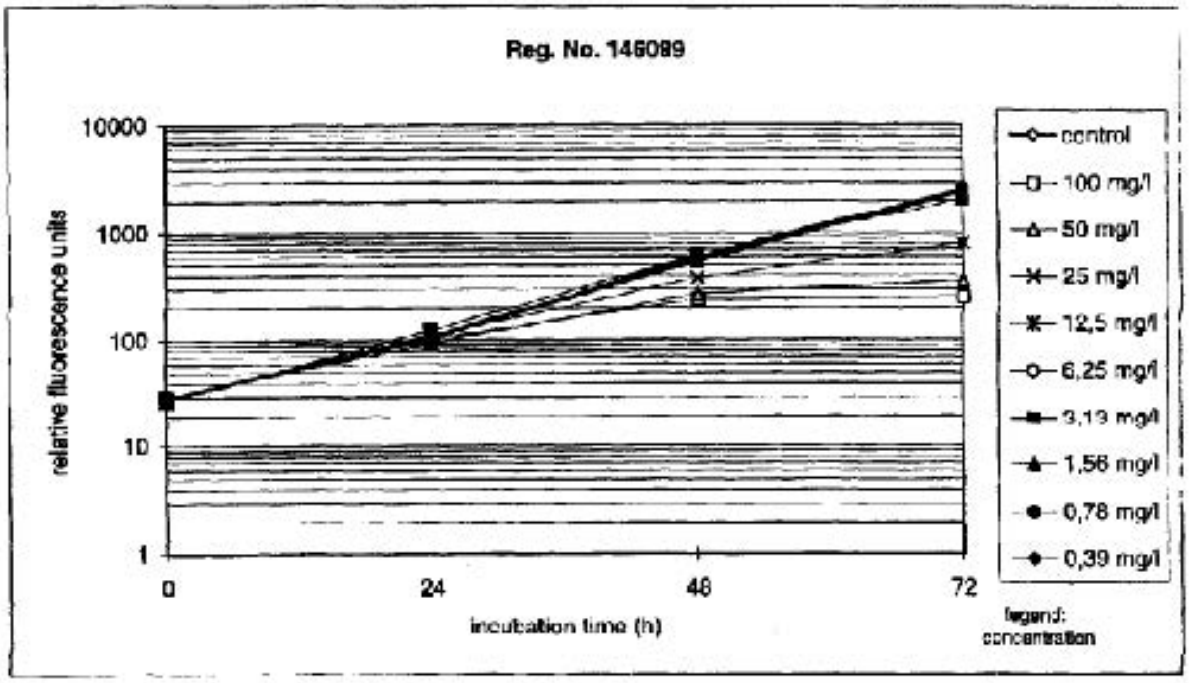
Table A7_4_1_3-6: Cell density data

Test-Substance Concentration (nominal) [mg/l]	Cell density (mean values) [relative units]							
	measured				Percent of control			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
0	30	107	540	2435	100	100	100	100
0.39	30	111	580	2527	100	104	107	103.8
0.78	28	103	582	2534	96	96	108	104.1
1.56	30	102	554	2457	100	95	103	100.9
3.13	28	124	616	2103	93	115	114	86.37
6.25	30	104	608	2617	100	97	113	107
12.5	30	105	550	2477	100	98	102	102
25	29	110	378	800	98	103	70	33
50	30	94	268	355	100	87	50	15
100	29	102	240	252	97	95	44	10
Temperature [°C]	23±2	23±2	23±2	23±2				
pH	8.0-8.2	NM	NM	7.9-8.2				

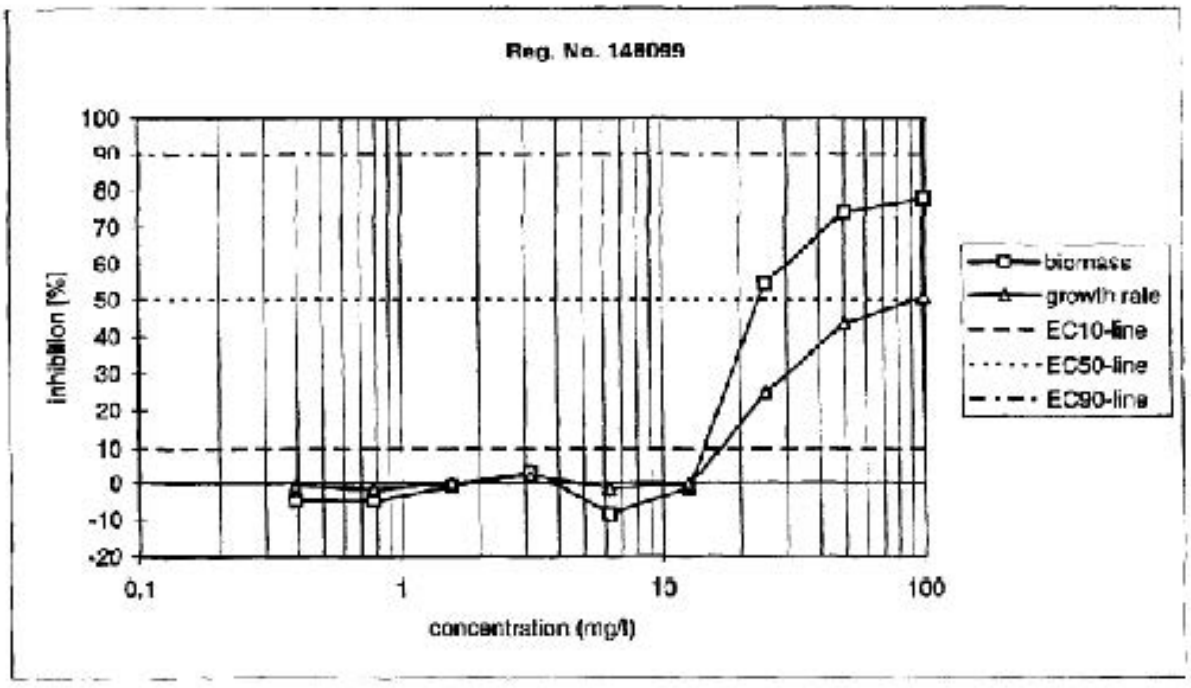
NM = not measured

Figure 7_4_1_3_1 Growth Curves and Percentage Inhibition

Growth curves of *Pseudokirchneriella subcapitata* at different test substance concentrations (relative fluorescence units)



Percentage inhibition of the algal biomass and growth rates at different test substance concentrations after 72 h



3. Tables for Applicant's Summary and Conclusion

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

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

Criteria for poorly soluble test substances	NA	

NA = Not applicable

Section A7.4.1.3(4) Growth inhibition test on algae

Annex Point IIA7.3 **Ethylene Urea: A 96-Hour Toxicity Test with the Freshwater Alga (Selenastrum capricornutum)**

IUCLID 4.3/06

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1 REFERENCE

1.1	**Reference**	Palmer SJ, Kendall TZ, Krueger HO (2001c) Ethylene Urea: A 96-Hour Toxicity Test with the Freshwater Alga (Selenastrum capricornutum). Wildlife International Ltd., Project No. 299A-116 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	EBDC/ETU Task force: BASF/Elf Atochem/Griffin/Rohm & Haas
1.2.2		
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/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD 201 "Algal Growth Inhibition Test"
2.2	**GLP**	Yes
2.3	**Deviations**	Yes The strain number for the test organism was not reported. This is not consider to affect the integrity of the study or the results obtained.

3 MATERIALS AND METHODS

3.1	**Test material**	Ethylene urea
3.1.1	Lot/Batch number	Lot No. 01743-141
3.1.2	Specification	Deviating from specification given in section 2 as follows:
3.1.3	Purity	90.8%
3.1.4	Composition of Product	Not applicable.
3.1.5	Further relevant properties	None
3.1.6	Method of analysis	The samples were analysed by HPLC using a Hewlett-Packard Model 1090 High Performance Liquid Chromatograph with a Jasco Model 975 Variable Wavelength detector with the following conditions: Column: Phenomenex LUNA C18 (250 mm x 4.6 mm, 5µm) column at 40°C Flow: 1.0 mL/minute Total run time: 13 minutes Injection size: 50 µL Mobile phase A: Water (99.7%) Mobile phase B: Acetonitrile (0.3%) Gradient:

Time (mins)	%A	%B	Flow (ml/min)
0.01	99.7	0.3	1.0
4.00	99.7	0.3	1.5

Section A7.4.1.3(4) **Growth inhibition test on algae**

Annex Point IIA7.3 **Ethylene Urea: A 96-Hour Toxicity Test with the Freshwater Alga (Selenastrum capricornutum)**

IUCLID 4.3/06

5.00	99.7	0.3	1.5
5.10	10	90	1.5
8.00	10	90	1.5
8.10	99.7	0.3	1.5
13.00	99.7	0.3	1.5

Retention time: 4.0 minutes

Detection: UV at 200nm

3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_3-1
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Culture medium	As per ASTM Standard Guide 1218-90E. 1990. *Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae*. American Society for Testing and Materials. Philadelphia, Pennsylvania. See Table 7_4_1_3_5
3.4.2	Test organisms	see table A7_4_1_3-2
3.4.3	Test system	see table A7_4_1_3-3
3.4.4	Test conditions	see table A7_4_1_3-4
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Cell multiplication inhibition
3.4.7	Sampling	24, 48, 72 and 96 hours
3.4.8	Monitoring of TS concentration	Yes, at 0, 72 and 96 hours
3.4.9	Statistics	Cell densities, areas under the growth curve, growth rates and percent inhibition values were calculated using "The SAS System for Windows", Release 6.12 (5)

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
4.2	**Results test substance**	

Section A7.4.1.3(4) **Growth inhibition test on algae**

Annex Point IIA7.3 **Ethylene Urea: A 96-Hour Toxicity Test with the Freshwater Alga (Selenastrum capricornutum)**

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4.2.1	Initial concentrations of test substance	Nominal concentrations 7.5, 15, 30, 60 and 120 mg/L
4.2.2	Actual concentrations of test substance	Mean measured concentrations from 0, 72 and 96h samples were 7.5, 15, 29, 58, 119 (121 for abiotic samples) mg/L
4.2.3	Growth curves	Refer to Figure A7_4_1_3-1
4.2.4	Concentration / response curve	Refer to Figure A7_4_1_3-1
4.2.5	Cell concentration data	Refer to Table A7_4_1_3-6
4.2.6	Effect data (cell multiplication inhibition)	72h EC_{50} for cell density, growth rate and biomass is >119 mg/mL
4.2.7	Other observed effects	None
4.3	**Results of controls**	Refer to Table A7_4_1_3-6
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not applicable
4.4.2	Results	Not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The test was performed in accordance with OECD 201 and GLP. The test was performed under static conditions with five concentrations of test substance and a water control at 24 ± 2°C. Nominal concentrations of ethylene urea were 0 mg/L (control), 7.5, 15, 30, 60, and 120 mg/L. Analysis of test substance concentrations were carried out at 0, 72 and 96 hours. Three replicate test chambers were prepared at each concentration plus 3 replicates for the negative control. Two abiotic samples were also prepared at the highest test concentration. Samples were taken from each replicate at 24 hour intervals to determine the cell density.
5.2	**Results and discussion**	Exposure of *Selenastrum capricornutum* to ethylene urea resulted in 72 and 96 hour EC_{50} values for cell density, growth rate and biomass all determined as >119 mg/L (95% confidence interval not calculable). The 72 and 96 hour NOAEC is 119 mg/L for the cell density, growth rate and biomass.
5.2.1	NOE_rC	>119 mg/L
5.2.2	E_rC_{50}	>119 mg/L
5.2.3	E_bC_{50}	>119 mg/L
5.3	**Conclusion**	The 72 median hour effective concentrations (EC_{50}s) and the no-

Section A7.4.1.3(4) **Growth inhibition test on algae**

Annex Point IIA7.3 **Ethylene Urea: A 96-Hour Toxicity Test with the Freshwater Alga (Selenastrum capricornutum)**

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		observed adverse effect concentration (NOAEC) of ethylene urea on the freshwater alga, *Selenastrum capricornutum,* exposed under static conditions were determined. The 72 and 96 hour EC_{50} values determined with the cell density, the growth rate, and the biomass are >119 mg/L (95% confidence interval not calculable). The 72 and 96 hour NOAEC is 119 mg/L. The validity criteria for the test 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	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	*Adopt applicant's version or include revised version*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	A primary stock solution was prepared by dissolving the test substance in freshwater algal medium.
Vehicle	No
Concentration of vehicle	Not applicable.
Vehicle control performed	No
Other procedures	None

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	*Selenastrum capricornutum*
Strain	Not reported.
Source	Original cultures were obtained from the University of Toronto Culture Collection and were maintained at Wildlife International Ltd., Easton, Maryland.
Laboratory culture	Yes
Method of cultivation	Algal cells used in this test were obtained from Wildlife International Ltd. Cultures that had been actively growing in culture medium for at least 2 weeks prior to test initiation.
Pretreatment	An inoculum of the algal cells was prepared in freshwater algal medium at a concentration of approximately 1.0×10^6 cells/mL
Initial cell concentration	10000 cells/mL

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	250 ml
Culturing apparatus	Flasks were placed on a shaker table at 100rpm in an environmental chamber at a target temperature of 24±2°C
Light quality	Cool-white fluorescent light
Procedure for suspending algae	Shaking
Number of vessels/ concentration	3 replicates at each of 5 test concentrations and 3 replicates for the control group plus one additional flask for each group used for analyis of test concentrations. 2 abiotic replicates at the highest test concentration were also included to monitor the stability of the test substance.
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	Target temperature range 24±2°C, measured range 22.4-23.6°C.
pH	pH at test initiation was 7.3 and ranged from 7.5-8.7 at test termination
Aeration of dilution water	No
Light intensity	Target light intensity 4300±10% lux, measured values were in the range 3880-4690 lux.
Photoperiod	Continuous illumination

Table A7_4_1_3-5: Culture Medium

Appendix 1

Freshwater Algal Medium

Compound	Nominal Concentration [1]	
$MgCl_2 \cdot 5H_2O$	12.16	mg/L
$CaCl_2 \cdot 2H_2O$	4.40	mg/L
H_3BO_3	0.1856	mg/L
$MnCl_2 \cdot 4H_2O$	0.416	mg/L
$ZnCl_2$	3.28	µg/L
$FeCl_3 \cdot 6H_2O$	0.1598	mg/L
$CoCl_2 \cdot 6H_2O$	1.428	µg/L
$Na_2MoO_4 \cdot 2H_2O$	7.26	µg/L
$CuCl_2 \cdot 2H_2O$	0.012	µg/L
$Na_2EDTA \cdot 2H_2O$	0.300	mg/L
$NaNO_3$	25.50	mg/L
$MgSO_4 \cdot 7H_2O$	14.70	mg/L
K_2HPO_4	1.044	mg/L
$NaHCO_3$	15.0	mg/L

[1] The pH was 7.5 ± 0.1.

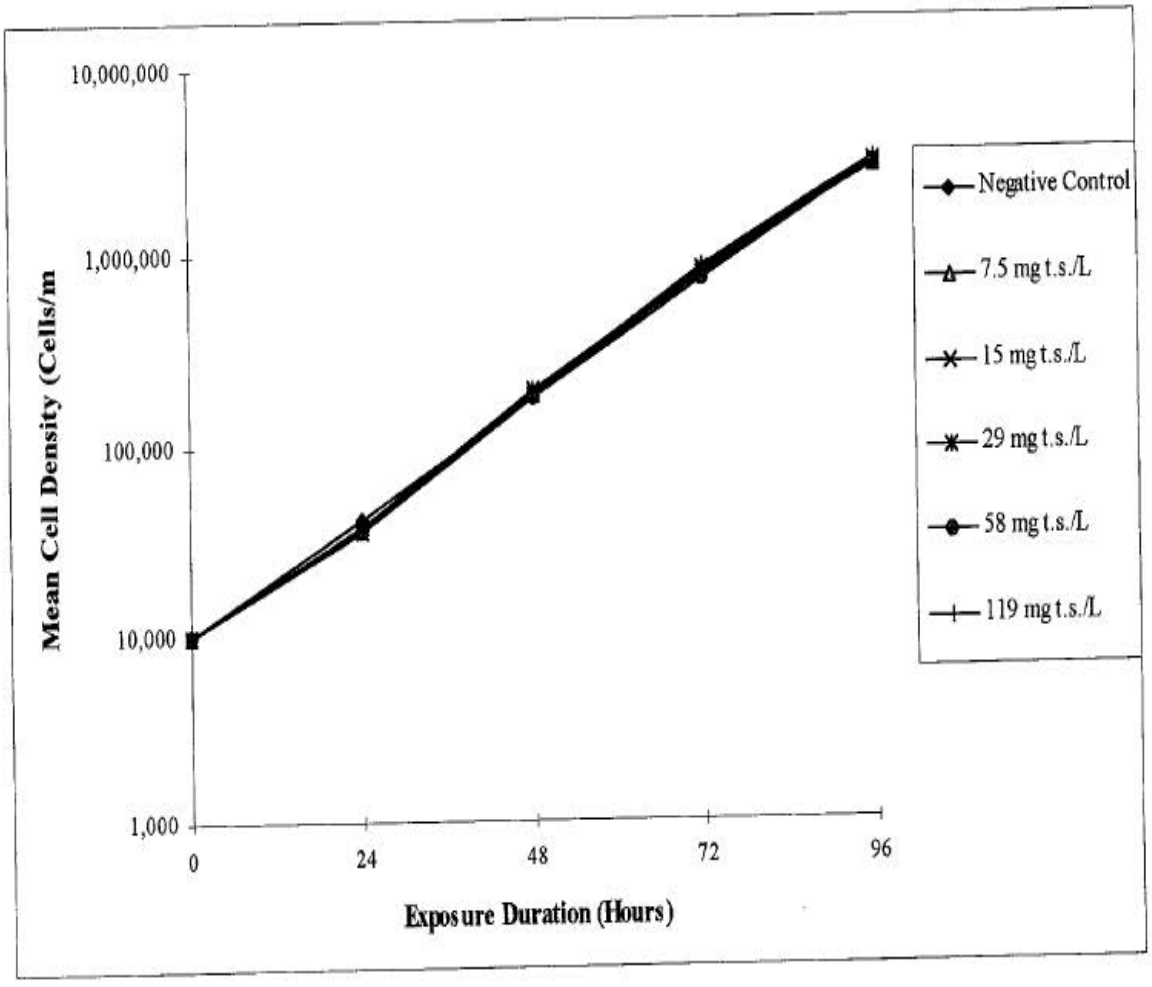
Table A7_4_1_3-6: Cell concentration data

Initial Measured Concentration of EU (mg/l)	Number of Cells per millimeter				
	Hours of Exposure				
	0	24	48	72	96
Negative control	10000	39477	179466	801618	2889469
7.5	10000	35868	186858	775944	2845920
15	10000	34485	182591	813150	2984622
29	10000	36382	195069	813267	2927294
58	10000	34420	175060	736230	2887264
119	10000	35411	190532	851130	3043906

Initial Measured Concentration of EU (mg/l)	Percent of Control				
	Hours of Exposure				
	0	24	48	72	96
Negative control	-	-	-	-	-
7.5	-	9.1	-4.1	3.2	1.5
15	-	13	-1.7	-1.4	-3.3
29	-	7.8	-8.7	-1.5	-1.3
58	-	13	2.5	8.2	0.076
119	-	10	-6.2	-6.2	-5.3

Figure A7_4_1_3_1

Figure 2. Concentration-response curve for *Selenastrum capricornutum* exposed to ethylene urea for 96 hours.



3. Tables for Applicant's Summary and Conclusion

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

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

Criteria for poorly soluble test substances	NA	

NA = not applicable

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4 **Activated Sludge Respiration Inhibition Test with Zineb (Contact Time:30 minutes)**

IUCLID 4.4/01

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		1 REFERENCE
1.1	**Reference**	Desmares-Koopmans, M.J.E. (2005), Activated Sludge Respiration Inhibition Test with Zineb (Contact Time:30 minutes), NOTOX B.V., Hambakenwetering 7, 5231 DD 's-Hertogenbosch, The Netherlands, Project No. 447018, 23 December 2005
1.2	**Data protection**	Yes
1.2.1	Data owner	Cerexagri B.V.
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD Guideline 209
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2 Zineb
3.1.1	Lot/Batch number	Batch No. 9031710096
3.1.2	Specification	As given in section 2
3.1.3	Purity	96.4%
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	Solubility in water is *ca* 0.07 mg/l
3.1.6	Method of analysis	Not applicable. No analysis was conducted
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Refer to Table A7_4_1_4-1
3.3	**Reference substance**	Yes 3,5-Dichlorophenol; Batch No. 15809KI-392; Purity 97%
3.3.1	Method of analysis for reference substance	No analysis was conducted.
3.4	**Testing procedure**	

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**

Annex Point IIA7.4 **Activated Sludge Respiration Inhibition Test with Zineb (Contact Time:30 minutes)**

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3.4.1	Culture medium	Refer to Table A7_4_1_4-2
3.4.2	Inoculum / test organism	Refer to Table A7_4_1_4-2
3.4.3	Test system	Refer to Table A7_4_1_4-3
3.4.4	Test conditions	Refer to Table A7_4_1_4-4
3.4.5	Duration of the test	30 minutes
3.4.6	Test parameter	Oxygen consumption/ respiration inhibition
3.4.7	Analytical parameter	Oxygen measurement
3.4.8	Sampling	Oxygen consumption was measured over a *ca* 10 minute period.
3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	Controls without test material were prepared.
3.4.11	Statistics	The respiration rate from each vessel, in mg 0_2/l/hr, was calculated from the linear part of the respiration curve, which was generally between 2.5 and 6.5 mg 0_2/l. The inhibitory effect (percentage inhibition) at a concentration was calculated as:- % inhibition = [1 – (2 x Rt / Rc (start test series) + Rc (end test series)]x 100 % in which Rc and At were respiration rates of controls and test/reference substance, respectively (in mg 0_2/l/hr). A figure of more than 10% inhibition was considered significant.
		4 RESULTS
4.1	**Preliminary test**	Performed
4.1.1	Concentration	100 mg/l
4.1.2	Effect data	Significant inhibition of respiration of the sludge was recorded.
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 100, 180, 320, 560 and 1000 mg/l
4.2.2	Actual concentrations of test substance	Not measured.
4.2.3	Growth curves	Not applicable
4.2.4	Cell concentration data	Not applicable
4.2.5	Concentration/ response curve	No concentration related increase of inhibition was observed at concentrations of 320 mg/l and higher. It is not therefore possible to

Section A7.4.1.4 **Inhibition to microbial activity (aquatic)**

Annex Point IIA7.4 **Activated Sludge Respiration Inhibition Test with Zineb (Contact Time:30 minutes)**

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		create a concentration/response curve.
4.2.6	Effect data	The EC_{50} was determined to be >1000 mg/l.
4.2.7	Other observed effects	The inhibitory effect of Zineb on aerobic waste water (activated sludge) bacteria increased with increasing concentration, ranging from 18% inhibition at 100 mg Zineb per litre, which confirmed the result of the first measurement, to 39% inhibition at and above 320 mg Zineb per litre.
4.3	**Results of controls**	Refer to Table A7_4_1_4-5, Table A7_4_1_4-6 and Table A7_4_1_4-7.
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	3.2, 10 and 32 mg/l.
4.4.2	Results	The EC_{50} of 3,5-dichlorophenol was in the accepted range of 5-30 mg/l (7.1 mg/l).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was conducted to comply with OECD Guideline 209.

The synthetic sewage feed (16 ml) and an adequate amount of the test solution were mixed and made up to 300 ml with Milli-RO water. Activated sludge (200 ml) was added to provide a final volume of 500 ml. The mixture was then aerated in a 1 litre bottle during the contact time (30 min), using a pipette as an aeration device.

Then a well mixed sample of the contents was poured into a 300 ml oxygen bottle, and the flask was sealed with an oxygen electrode connected to a recorder, forcing the air out of the vessel. Oxygen consumption was measured and recorded for approximately 10 minutes. During measurement, the sample was not aerated but continuously stirred on a magnetic stirrer. The pH and temperature were determined in the remaining part of the reaction mixture.

This procedure was repeated for all concentrations of the test substance. Two controls without test substance were tested in each test series, one at the start and one at the end.

The batch of activated sludge was checked for sensitivity by testing the reference substance 3,5-dichlorophenol.

5.2 Results and discussion

Since significant inhibition (16%) of respiration rate of the sludge was recorded during the range finding test, at 100 mg Zineb per litre, an additional concentration range of 100 to 1000 mg/l, forming a geometric progression with a factor 1.8, was tested.

The inhibitory effect of Zineb on aerobic waste water (activated sludge) bacteria increased with increasing concentration, ranging from 18% inhibition at 100 mg Zineb per litre, which confirmed the result of the first measurement, to 39% inhibition at and above 320 mg Zineb per litre. Hence, no concentration related increase of inhibition was observed at concentrations of 320 mg/l and higher. Consequently, no EC_{50} could be determined and no further testing was needed.

Section A7.4.1.4	**Inhibition to microbial activity (aquatic)**
Annex Point IIA7.4	**Activated Sludge Respiration Inhibition Test with Zineb (Contact Time:30 minutes)**
IUCLID 4.4/01	

		Since all criteria for acceptability of the test were met, this study was considered to be valid. In conclusion, under the conditions of this present test, a significant inhibition of respiration rate of the sludge was recorded at and above a nominal concentration of 100 mg Zineb per litre. The EC_{50}, based on nominal concentrations, was above the highest concentration tested (EC_{50}> 1000 mg/l).
5.2.1	EC_{20}	
5.2.2	EC_{50}	>1000 mg/l
5.2.3	EC_{80}	
5.3	**Conclusion**	The validity criteria can be considered to have been fulfilled. From the results obtained, whilst there was an inhibition effect observed at 100 mg/l, there was no concentration related increase of inhibition observed at concentrations of 320 mg/l and higher. Consequently, no EC_{50} could be determined.
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	*Give date of action*
Materials and Methods	*State if the applicants version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	*Adopt applicant's version or include revised version*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A7.4.1.4	**Inhibition to microbial activity (aquatic)**
Annex Point IIA7.4	**Activated Sludge Respiration Inhibition Test with Zineb (Contact Time:30 minutes)**
IUCLID 4.4/01	

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

Table A7_4_1_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes Optimal contact between the test substance and test medium was ensured by applying continuous aeration and stirring.
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	No - Not applicable *(If yes, specify)*
Other procedures	None other procedures were employed.

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Not stated
Strain	Not stated
Source	Municipal sewage treatment plant, receiving predominantly domestic sewage.
Sampling site	Waterschap de Maaskant, 's-Hertogenbosch, The Netherlands
Laboratory culture	No; obtained from a domestic sewage treatment plant.
Method of cultivation	The batch of sludge was used one day after collection; therefore 50 ml of synthetic sewage feed was added per litre of activated sludge at the end of the collection day. The sludge was kept aerated at test temperature until use.
Preparation of inoculum for exposure	The sludge was coarsely sieved, washed and diluted with tap-water. No mention was made of a centrifugation step.
Pretreatment	50 ml of synthetic sewage feed was added per litre of activated sludge at the end of the collection day. Synthetic sewage feed contains:- 16 g peptone 11 g meat extract 3 g urea 0.7 g NaCl 0.4 g $CaCl_2.2H_20$ 0.2 g $MgSO_4.7H_20$ 2.8 g K_2HPO_4 Dissolved in Milli-Q water, made up to 1 litre and filtered. The pH was 6.8 and adjusted with 1 M NaOH (Merck, Darmstadt, Germany) to 7.1.

Initial cell concentration	Number of micro-organisms was determined as the amount of Mixed Liquor Suspended Solids (MLSS) per litre test medium. 3.5 g/l of sludge were used for the test.

Table A7_4_1_4-3: Test system

Criteria	Details
Culturing apparatus	All glass, approximately 300 ml oxygen bottles and 1 litre test bottles.
Number of culture flasks/concentration	The test substance was tested singly at each concentration level, with the exception of the 100 mg/l level which was tested during the range finding test. Two controls were tested on each occasion, one at the start and one at the end.
Aeration device	A pipette was used pre sample measurement.
Measuring equipment	Oxygen consumption was measured using an oxygen electrode. Oxygen meter:- WTW inolab Oxi Level 2 supplied with a WTW CellOx 325 oxygen electrode, electrolyte type ELY/C. Recorder:- Flatbed recorder SE 102 (Kipp & Zonen).
Test performed in closed vessels due to significant volatility of TS	No Closed vessels were however used during the measurement phase of the study to eliminate any oxygen entering the test vessel.

Table A7_4_1_4-4: Test conditions

Criteria	Details
Test temperature	The temperature of the test medium was between 19.9 and 21.9°C. Individual measurements are not provided.
pH	Refer to Tables A7_4_1_4-5 to A7_4_1_4-7
Aeration of dilution water	Yes The mixture was aerated during contact time using a pipette as an aeration device. The flow rate of the air is not recorded.
Suspended solids concentration	3.5g/l sludge was added.

Table A7_4_1_4-5: Toxicity of the reference substance (3,5-dichlorophenol)

Controls (C) and reference substance (R; 3,5-dichlorophenol): pH, oxygen concentration, oxygen consumption and percentage inhibition

Flask	Concentration (mg/l)	Oxygen Conc. At the Start (mg O_2/l)	Oxygen Consumption (mg O_2/l)	Mean Oxygen Consumption (mg O_2/l)	% Inhibition	pH
C1	0	7.9	36	39 (±13)	-	7.9
C2	0	7.6	41		-	7.8
R1	3.2	7.9	25		35	8.0
R2	10	7.9	17		56	7.9
R3	32	8.6	8		79	7.9

Table A7_4_1_4-6: Toxicity of Zineb - Range Finding

Controls (C) and Zineb (T): pH, oxygen concentration, oxygen consumption and percentage inhibition

Flask	Concentration (mg/l)	Oxygen Conc. At the Start (mg O_2/l)	Oxygen Consumption (mg O_2/l)	Mean Oxygen Consumption (mg O_2/l)	% Inhibition	pH
C3	0	7.9	40	42 (±7)	-	7.7
C4	0	6.9	43		-	7.6
T1	100	7.3	35		16	7.8

Table A7_4_1_4-7: Toxicity of Zineb - Definitive Test

Controls (C) and Zineb (T), additional test series: pH, oxygen concentration, oxygen consumption and percentage inhibition

Flask	Concentration (mg/l)	Oxygen Conc. At the Start (mg O_2/l)	Oxygen Consumption (mg O_2/l)	Mean Oxygen Consumption (mg O_2/l)	% Inhibition	pH
C4	0	6.9	43	43 (±2)	-	7.6
C5	0	6.7	42		-	7.2
T1 a	100	7.7	35		18	7.5
T2 a	180	6.7	33		22	7.5
T3 a	320	7.6	26		39	7.6
T4 a	560	7.3	26		39	7.5
T5 a	1000	7.1	26		39	7.5

Section A7.4.2 Annex Point IIA, VII.7.5	Bioconcentration	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure** [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ] **Other justification** [X]	
Detailed justification:	A theoretical bioconcentration factor (BCF) of 1.41 has been calculated for Zineb using the EUSES 2.0.3 computer programme, suggesting that Zineb will not be subject to bioaccumulation. This conclusion is consistent with the results of a BCF study conducted in fish (refer to TNG summary A7.4.3.3.1).	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	
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 A7.4.3.1 **Annex Point IIIA, XIII.2.1**	**Prolonged toxicity to an appropriate species of fish**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	According to the Technical Notes for Guidance on Data Requirements for Active Substances and Biocidal Products, Chapter 3, Specific data set/Part A, page 111, this test is not usually required as it does not provide information needed in the risk assessment. Furthermore, it is noted that the existing test guidelines are inadequate. Consequently, no tests have been submitted to address this specific data point. The requirement to consider the potential for zineb to cause adverse effects in fish as a result of chronic exposure is, however, adequately addressed by the data summarized in Section A7_4_3_2 (Effects on reproduction and the growth rate on an appropriate species of fish).	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	
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*	

Section A7.4.3.1 Annex Point IIIA, XIII.2.1	Prolonged toxicity to an appropriate species of fish
Remarks	

Section 7.4.3.2 Effects on reproduction and growth rate of fish

Annex Point IIIA XIII 2.2

IUCLID 4.5.1/01,02,03,04

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		1 REFERENCE
1.1	**Reference**	Van Leeuwen CJ, Espeldoorn A, Mol F (1986a) Aquatic toxicological aspects of dithiocarbamates and related compounds. III. Embryolarval studies with rainbow trout (Salmo gairdneri). Aquatic Toxicology 9:129-145.
1.2	**Data protection**	No. Public Domain
1.2.1	Data owner	Not applicable
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No. No guidelines available at the time, but methods used are consistent with current testing requirements under OECD 210.
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	Not a guideline study.
		3 METHOD
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Not reported
3.1.2	Specification	As given in section 2
3.1.3	Purity	Zineb ≥95% DIDT ≥ 98% ETU ≥ 99% EU ≥ 97%
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	Zineb is rapidly degraded to its constituent EBCD and degradates ETU, EU and DIDT. This affects the stability of the test substance in the test solutions.
3.1.6	Method of analysis	Not described.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable.
3.3	**Reference substance**	No.
3.3.1	Method of analysis for reference	Not applicable.

Section 7.4.3.2 Effects on reproduction and growth rate of fish

Annex Point IIIA XIII 2.2

IUCLID 4.5.1/01,02,03,04

	substance	
3.4	**Testing procedure**	
3.4.1	Dilution water	Refer to Table A7_4_3_2-2
3.4.2	Test organisms	Refer to Table A7_4_3_2-3
3.4.3	Handling of embryos and larvae (OECD 210/212)	The embryolarval tests were initiated with freshly, artificially spawned eggs obtained from a hatchery. Within 3 hours of fertilization, egg samples (100 count) were introduced into the aquaria in Petri dishes on the bottom of the tanks. The embryolarval stages were exposed continuously for 60 days to 5-7 concentrations, a solvent control and a blank control. During embryogenesis the rooms were kept as dark as possible. After hatching, a photoperiod of 12 hours light:12 hours dark was maintained. Dead specimens were removed when observed, and unfertilized eggs were removed from the tanks after 28 days. Animals were not fed during the tests. Surviving fish were examined for malformations and a few juveniles (5-10) per concentration were examined histopathologically. Wet weight and length was determined at the end of the tests.
3.4.4	Test system	Refer to Table A7_4_3_2-4
3.4.5	Test conditions	Refer to Table A7_4_3_2-5
3.4.6	Duration of the test	60 days
3.4.7	Test parameter(s)	Survival, length, weight, and teratogenicity
3.4.8	Examination / Sampling	Refer to Section 3.4.3.
3.4.9	Monitoring of TS concentration	No
3.4.10	Statistics	The LC_{50}, EC_{50} and 95% confidence intervals were determined according to Kooyman 1981. The chi-square test was used to test differences in mean survival and normal juvenile development. Differences in mean length and weight were tested using Williams 1971, 1972. Prior to applying the Williams' test, the data were tested for homogeneity of variances using the Bartlett test. The lowest concentration that was significantly different from the control was termed the Lowest Rejected Concentration Tested (LRCT) [equivalent to the Lowest Observable Effect Concentration (LOEC).] Differences were considered significant at $\alpha < 0.01$.
		4 RESULTS
4.1	**Range finding test**	Not performed
4.1.1	Concentrations	Not applicable.
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable.
4.1.3	Nature of adverse effects	Not applicable.

Section 7.4.3.2 Effects on reproduction and growth rate of fish

Annex Point IIIA XIII 2.2

IUCLID 4.5.1/01,02,03,04

4.2 Results test substance

4.2.1 Initial concentrations of test substance

Nominal concentrations of zineb were 0, 32, 56, 100, 180 and 320 µg/l. Test concentrations were not reported for DIDT, ETU and EU.

4.2.2 Actual concentrations of test substance

Not reported

4.2.3 Effect data

Results obtained for zineb are shown in the table below:

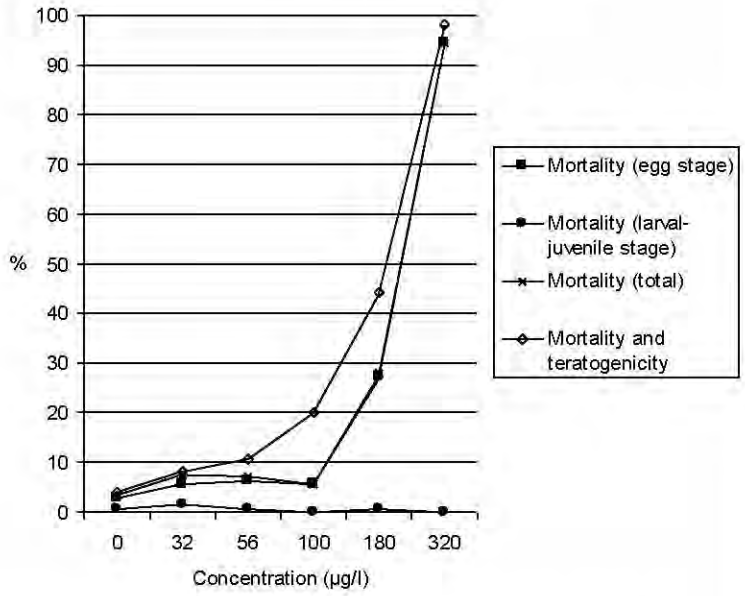
Conc. (µg/l)	Mortality %			Mortality & teratogenicity after 60 days (%)	Mean length & 95% c.i. (mm)	Mean weight & 95% c.i. (mg)
	Egg stage	Larval-juvenile stage	Total after 60 days			
0	2.8	0.6	3.4	4.0	24.4 (24.2-24.6)	131.9 (128.0-135.8)
32	5.7	1.7	7.4	8.0	23.5 (23.3-23.7)	124.9 (121.3-128.5)
56	6.4	0.7	7.1	10.7	22.8 (22.5-23.1)	118.5 (114.1-122.9)
100	5.7	0	5.7	20.0	21.4 (21.0-21.8)	108.9 (104.8-113.0)
180	27.4	0.6	28.0	44.1	21.8 (21.4-22.2)	119.1 (115.1-123.1)
320	94.5	0	94.5	98.0	--	--

Section 7.4.3.2 Effects on reproduction and growth rate of fish

Annex Point IIIA XIII 2.2

IUCLID 4.5.1/01,02,03,04

4.2.4	Concentration / response curve	The concentration mortality curve for Zineb is as follows:



4.2.5	Other effects	Mortality was highest during the egg stages, especially during late gastrulation and early organogenesis. No appreciable mortality occurred during the larval and juvenile stages for zineb. Dithiocarbamate compounds were shown to have teratogenic properties. The most pronounced effects were severe spinal and vertebral abnormalities including flexures (scoliosis), ventral curvatures (lordosis), dorsal curvatures (kyphosis) and irregular dwarfed structures of the trunk.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	Refer to section 4.2.3
4.3.2	Nature of adverse effects	Refer to section 4.2.3
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not applicable.
4.4.2	Results	Not applicable.

Section 7.4.3.2 **Effects on reproduction and growth rate of fish**

Annex Point IIIA XIII 2.2

IUCLID 4.5.1/01,02,03,04

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Artificially spawned rainbow trout eggs, within 3 hours of fertilization, were exposed to zineb, DIDT, ETU and EU for a total of 60 days. Dead specimens were removed as observed, and unfertilized eggs were removed from the tanks after 28 days. Animals were not fed during the tests. Surviving fish were examined for malformations and a few juveniles (5-10) per concentration were examined histopathologically. Wet weight and length were determined at the end of the tests.

5.2 Results and discussion

LC_{50}, EC_{50}, and LOEC values and corresponding 95% confidence limits are reported for zineb and its significant degradates DIDT, ETU and EU. The results suggest that ETU and EU are significantly less toxic than the parent zineb, while DIDT is more toxic. The 60-day LC_{50} and EC_{50} values are reported in the following table:

Compound	LC_{50} (95% c.i.) (mg/l)	EC_{50} (95% c.i.)[a] (mg/l)
Zineb	0.211 (0.200-0.22)	0.188 (0.179-0.199)
DIDT	0.014 (0.012-0.016)	0.007 (0.0057-0.0085)
ETU	1800 (1000-3200)	1000 (600-3200)
EU	10,000 (9000-11,000)	--

[a] mortality and teratogenicity

5.2.1 NOEC

The NOEC for zineb after 60 days based on mortality and teratogenicity was 0.056 mg/l.

An unbounded NOEC of < 0.032 mg/l was obtained on the basis of growth effects.

The level of information presented in the report was insufficient to allow confident determination of NOEC values for DIDT, ETU and EU.

5.2.2 LOEC

Expressed as LRCT for different endpoints as shown in the table:

Compound	LRCT (mg/l)			
	Mortality	Total embryo-toxicity	Length	Weight
Zineb	0.180	0.100	≤0.032	≤0.032
DIDT	0.0032	0.0032	0.010	≤0.00032
ETU	3.2	100	100	3.2
EU	--	--	--	--

5.3 Conclusion

Based on conduct of the studies following acceptable laboratory procedures, the studies are considered to fulfill the validity criteria even though much of the information is not reported in the summary data compilation. The polymeric zineb rapidly degrades to its EBDC

Section 7.4.3.2 **Effects on reproduction and growth rate of fish**

Annex Point IIIA XIII 2.2

IUCLID 4.5.1/01,02,03,04

		constituents and several degradates, which are also tested independently. Based on lack of detail reported the reliability of the study is assigned a 2.
5.3.1	Other Conclusions	This study should be considered fit for the purpose of describing the acute aquatic toxicity of zineb to freshwater fish.
5.3.2	Reliability	2
5.3.3	Deficiencies	Yes. This report comprises a compilation of several studies conducted in the same laboratory and thus does not include the level of detailed reporting that would normally be consistent with GLP studies.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicant's version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	*Adopt applicant's version or include revised version*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / non acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator; if unacceptable, give reasons for unacceptability of study; discuss the relevance of the deficiencies. Indicate if repeat is necessary)*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_3_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes/No
Vehicle	Yes/No
Concentration of vehicle	*Give the concentration (% v/v)*
Vehicle control performed	Yes/No
Other procedures	*e.g. test in completely filled closed vessels for testing volatile test substance*

Table A7_4_3_2-2: Dilution water

Criteria	Details
Source	Reconstituted laboratory water
Salinity	Not applicable
Hardness	50 mg/l as $CaCO_3$
pH	7.2 ± 0.2
Oxygen content	Not reported
Conductance	Not reported
Holding water different from dilution water	No

Table A7_4_3_2-3: Test organisms

Criteria	Details
Species/strain	Rainbow trout (*Salmo gairdneri*, now *Oncorhynchus mykiss*)
Source	Artificially spawned eggs were obtained from a fish hatchery at Vaassen (Gelderland)
Wild caught	No
Age/size	Eggs
Kind of food	None
Amount of food	Not applicable
Feeding frequency	Not applicable
Post-hatch transfer time	Not applicable
Time to first feeding	Not applicable
Feeding of animals during test	No
Treatment for disease within 2 weeks preceeding test	None

Table A7_4_3_2-4: Test system

Criteria	Details
Test type	Semistatic

Renewal of test solution	Test solutions were renewed three times a week
Volume of test vessels	15 litres
Volume/animal	Not applicable for a embryolarval test
Number of animals/vessel	100 eggs per vessel
Number of vessels/ concentration	Duplicate chambers per concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2-5: Test conditions

Criteria	**Details**
Test temperature	10 ± 1 °C in a constant temperature room
Dissolved oxygen	Not reported
pH	7.7 ± 0.2
Adjustment of pH	No
Aeration of dilution water	Yes. Continuous aeration
Intensity of irradiation	Not reported
Photoperiod	Dark during the embryogenesis phase. Photoperiod of 12 hours light: 12 hours dark after hatching.

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

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

Table A7_4_3_2-7: Validity criteria for fish test according to OECD Guideline 215

	fulfilled	**Not fullfilled**
Concentration of dissolved oxygen in all test vessels > 60% saturation		
Difference of water temperature < 1° C between test chambers at any time during test; temperature within a range of 2° C of the temperature for specific test species		
Mortality of control animals <10%		
Increase of fish weight sufficient for detection of the minum variation of growth rate considered as significant		
Criteria for poorly soluble test substances		

Section A7.4.3.3.1 Bioconcentration in aquatic organisms

Annex Point IIIA XIII.2.3

IUCLID 3.7/01

Uptake Distribution and Retention of Zineb and Ziram in Rainbow Trout (Salmo Gairdneri)

Official use only

1 REFERENCE

1.1	**Reference**	Van Leeuwen C.J., Van Hameren P., Bogers M., Griffioen P.S. (1986b), Uptake Distribution and Retention of Zineb and Ziram in Rainbow Trout (*Salmo Gairdneri*). Toxicology, 42:33-46, Elsevier Scientific Publishers Ireland Ltd.
1.2	**Data protection**	No
1.2.1	Data owner	Public Domain Literature
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No This study does not claim compliance with any guideline. However it does meet some of the requirements of OECD Guideline 305. The deviations are listed in Section 2.3.
2.2	**GLP**	No. This paper describes a research project which does not claim GLP compliance. Furthermore, GLP was not compulsory at the time this study was performed.
2.3	**Deviations**	Yes The study was a static study rather than a flow through or semi-static study. However given that zineb degrades rapidly in water, a static design provides a more realistic model for bioconcentration. The lipid content has not been determined, however the quantification techniques involved will not result in removal of the lipid fraction and therefore a requirement to correct the results obtained. One concentration level was tested. The type and characteristics of illumination used were not reported.

3 MATERIALS AND METHODS

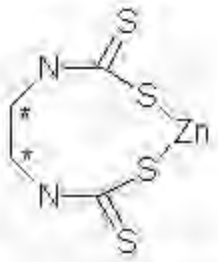
3.1	**Test material**	As given in section 2 Zineb Note:- Ziram, as mentioned in the title of this paper, will not be evaluated.
3.1.1	Lot/Batch number	Not stated
3.1.2	Specification	As given in section 2
3.1.3	Purity	Not stated

Section A7.4.3.3.1 **Bioconcentration in aquatic organisms**

Annex Point IIIA XIII 2.3

IUCLID 3.7/01 **Uptake Distribution and Retention of Zineb and Ziram in Rainbow Trout (Salmo Gairdneri)**

3.1.4	Further relevant properties	No substance specific properties affecting test performance/applicability of the method were reported.
3.1.5	Radiolabelling	[Ethylene-^{14}C]zineb (spec. act.: 11.7 µCi/mg) was obtained from Amersham Radiochemical Centre (England). Dimethylsulphoxide (DMSO) was used as the solvent.



3.1.6	Method of analysis	Liquid Scintillation Counting In the whole-body accumulation experiments, fish were incinerated in a sample oxidizer (Packard, Tri Carb model B 306). CO_2 was trapped in Carbo-sorb and Perma-Fluor V (Packard) and radioactivity was measured by liquid scintillation counting (LKB/Wallac Rackbêta 1215). In the other experiments samples were dissolved in Lumasolve (Lumac; 1 ml/100 mg tissue) and placed in a stove at 40°C for 24 h. Subsequently, scintillator 299 (Packard) was added at a maximum of 5 ml/vial. Before scintillation counting, vials were stored for approximately 6 h in complete darkness. Data were corrected for chemical quenching and background radiation. Measurements were carried out in 3-5 replicates. Whole Body Autoradiography Transverse sections for autoradiography were cut with a whole-body microtome. Sections of 30, 40 and 50 µm thickness were freeze-dried at -20°C for approximately 18 h before being pressed against autoradiography films (Kodak X-OMAT AR). Films were exposed in a light-tight box for 26 days at -20°C and developed in Kodak-LX 24 for 5 min. Next they were rinsed in an acetic acid stop-bath for 30 s. Fixation took place in Kodak AL-4 Röntgenfix for 4 min.
3.2	**Reference substance**	No
3.2.1	Method of analysis for reference substance	Not applicable
3.3	**Testing/estimation procedure**	
3.3.1	Test system/	Test animals and standard water

Section A7.4.3.3.1 **Bioconcentration in aquatic organisms**

Annex Point IIIA XIII.2.3

IUCLID 3.7/01 **Uptake Distribution and Retention of Zineb and Ziram in Rainbow Trout (Salmo Gairdneri)**

performance

Rainbow trout (*Salmo gairdneri*) were obtained from Fijge Trout Farm at Vaassen (The Netherlands). Standard water for the experiments was prepared according to Alabaster and Abram. The pH, hardness and temperature of the water was 8.0 ± 0.1, 50 mg/l (as $CaCO_3$) and 10 ± 1°C, respectively. A 12-h photoperiod was imposed upon the fish. During acclimatisation and elimination, trout were fed, with Trouvit pellets (Trouw & Co. N.V., The Netherlands).

Accumulation studies

Whole-body static accumulation studies were performed in 5 L test vessels to which 4 L well-aerated standard water was added. The weight of the fish was (mean ± S.E.) 0.42 ± 0.13 g. In order to study the distribution of zineb, trout with weights of 3.4 ± 0.4 g, were exposed in 25 L all-glass fish tanks, housed in a water bath. They were fasted for 48 h prior to and during the exposure. In order to keep the NH_3 concentration below 0.025 mg/L, the mass of fish in each tank never exceeded 4 g/l. In the short- term whole-body accumulation studies, however, the loading was approximately 8 g/l, but no detrimental effects were observed. The test solutions were aerated continuously and not renewed. Fish and water were sampled after 6, 24, 48 and 96 h of exposure.

Elimination studies

Following 96 h of exposure to experimentally contaminated water, rainbow trout (weight 7.2 ± 1.7 g) were transferred to toxicant-free water and sampled after 0, 4 and 16 days to measure depuration of radioactivity. Radioactivity in water was measured at regular intervals; a concentration of 1% of the initial ^{14}C-activity during the accumulation period was taken as a maximum, above which water was renewed. During depuration fish were fed once every 4 days.

Collection of organs and tissues

Fish were anesthetised with $NaHCO_3$ buffered tricaine methane sulphonate (MS 222, Sandoz, Basel). After removal of adhering water by blotting on filter paper, the fry were weighed. The following organs and tissues were dissected: eyes, gills, stomach, intestine, liver, gall bladder, head kidney, trunk kidney, brains, heart and spleen. Samples were taken from the vertebral column and muscles. Blood samples were collected from the ventral aorta. Rest fractions were homogenised with an Ultra-turrax mixer. Samples were weighed on a microbalance with a precision of 10 µg.

Data analysis

The results of the liquid scintillation countings (dpm, per kg fish and per litre water) were converted to µg/kg and µg/l respectively, dividing them by the specific activity of the compound. In the whole-body accumulation experiments the rate constants were estimated from a kinetic model. The set of equations, however, had to be extended in order to comprise biotransformation processes. The following set of

Section A7.4.3.3.1 **Bioconcentration in aquatic organisms**

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IUCLID 3.7/01 **Uptake Distribution and Retention of Zineb and Ziram in Rainbow Trout (Salmo Gairdneri)**

equations was used:

$$\frac{d}{dt} C_f(t) = k_1 C_w(t) - k_2 C_f(t) - k_3 C_f(t) \quad (1)$$

$$\frac{d}{dt} C_w(t) = -\frac{N(t)w}{V} k_1 C_w(t) + k_2 \frac{N(t)w}{V} C_f(t) \quad (2)$$

$$\frac{d}{dt} C'_f(t) = k'_1 C'_w(t) - k'_2 C'_f(t) + k_3 C_f(t) \quad (3)$$

$$\frac{d}{dt} C'_w(t) = -\frac{N(t)w}{V} k'_1 C'_w(t) + k'_2 \frac{N(t)w}{V} C'_f(t) \quad (4)$$

The following symbols were used: t: time (h); C_f: concetration of the parent compound in one organism (µg/kg); C_w: concentration of the parent compound in water (µg/l); *w*: weight of one organism (kg); *V*: water volume (litre); N: number of organisms; k_1: uptake rate constant of the parent compound (1/kg per h); k_2: clearance rate constant of the parent compound (1/h); and k_3 biotransformation rate constant (1/h). The prime denotes the concentrations and rate constants of the metabolite.

The biotransformation process is assumed to be first order in the concentration of the parent compound in the organism. For compounds which are slowly biotransformed, k_3 approximates zero and only equations 1 and 2 are used. In this case, the steady-state bioconcentration factor (BCF: 1/kg) equals k_1/k_2.

BCFs for tissues and organs were calculated from the mean total ^{14}C concentrations in fish and water, respectively. Differences in the BCFs were tested with the Student's *t*-test.

3.3.2 Estimation of bioconcentration — Data not provided.

4 RESULTS

4.1 Experimental data

4.1.1 Mortality/behaviour — No fish were found to have died.

4.1.2 Lipid content — Not stated.

4.1.3 Concentrations of test material during test

Whole-Body Accumulation

The whole-body accumulation experiments revealed that radioactivity in early juvenile trout exposed to zineb (Refer to Figure A7_4_3_3_1-1) reached an apparent steady state within approximately 24 h of exposure. The k_1 and k_2 values (means ± S.E.) were 2.41 ± 0.18 and 0.07 ± 0.01; the BCF (k_1/k_2) was 34.

Section A7.4.3.3.1 **Bioconcentration in aquatic organisms**

Annex Point IIIA XIII.2.3

IUCLID 3.7/01 **Uptake Distribution and Retention of Zineb and Ziram in Rainbow Trout (Salmo Gairdneri)**

		Distribution Studies The results of the distribution studies are shown in Figures A7_4_3_3_1-2 and 3. Radioactivity was rapidly disseminated through the tissues. The lowest levels of ^{14}C-activity were found in muscle, heart, brain and vertebral column. Liver and digestive tract contained the highest. Zineb derived radioactivity accumulated in liver, accounted for about 60% of total radioactivity after 2 and 4 days. Radioactivity in the skin accounted for approximately 8% in treated fish. Elimination Experiments The results of the elimination experiments are shown in Figure A7_4_3_3_1-4. In these studies, radioactivity in liver and gall bladder (contents included) were determined separately. The measurements revealed that the gall bladder was the major distribution site for the radiolabelled compounds and/or their degradation products. Whole-body elimination of zineb and/or its degradation product(s) was rapid during the first few days. After 4 days, only 25% of the initial residue was retained by the fish and further clearance was negligible. Autoradiographic Studies The autoradiographic studies revealed the same results. A high labelling of the liver, gall bladder, intestinal lumen and pigmented tissues was observed. Detailed examination revealed that radioactivity was localized at distinct spots which coincided with pigment, in melanophores. Upon transferring the fish to clean water, these spots retained their radioactivity for considerable periods of time. After 16 days of depuration, radioactivity was almost entirely confined to the pigmented tissues. Moderate to high levels of activity were also recorded in distinct spots in the subpharyngeal area, which corresponds to the location of thyroid follicles. High activity at these locations was still found after a 16-day elimination period, in treated fish. In all other tissues, radioactivity was rapidly lost.
4.1.4	Bioconcentration factor (BCF)	The k_1 and k_2 values (means ± S.E.) were 2.41 ± 0.18 and 0.07 ± 0.01; the BCF (k_1/k_2) was 34.
4.1.5	Uptake and depuration rate constants	The uptake rate constant was 2.41 ± 0.18 and depuration rate constant was 0.07 ± 0.01.
4.1.6	Depuration time	75% elimination after 16 days depuration. DT_{50} values have not been calculated.
4.1.7	Metabolites	No metabolites were identified or reported during this study.
4.1.8	Other Observations	Pigmented tissues appeared to be major distribution sites. This may be related to the affinity of the compounds and/or their degradation products to melanin or to complexation with phenoloxidase, a copper-containing enzyme involved in melanin synthesis. Autoradiography also revealed a high labelling of thyroid follicles.

Section A7.4.3.3.1	**Bioconcentration in aquatic organisms**
Annex Point IIIA XIII.2.3	
IUCLID 3.7/01	**Uptake Distribution and Retention of Zineb and Ziram in Rainbow Trout (Salmo Gairdneri)**

4.2	**Estimation of bioconcentration**	Whole-body accumulation was low, with bioconcentration factors < 100. Whole-body elimination was rapid with 25% of the initial radioactivity from zineb being retained by the end of the 16 day depuration period.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	A short term static bioaccumulation study was performed using [ethylene-^{14}C]zineb, in rainbow trout (*Salmo Gairdneri*). The exposure period was 96h followed by a 16 day depuration period.
5.2	**Results and discussion**	The whole-body accumulation experiments revealed that radioactivity in early juvenile trout exposed to zineb reached an apparent steady state within approximately 24 h of exposure. The uptake rate constant was 2.41 ± 0.18 and depuration rate constant was 0.07 ± 0.01. The BCF (k_1/k_2) was 34. Zineb is not expected to accumulate as the whole-body accumulation was low, with bioconcentration factors < 100 and the whole-body elimination was rapid with 25% of the initial radioactivity from zineb being retained by the end of the 16 day depuration period.
5.3	**Conclusion**	The study can be considered to be valid when compared to OECD Guideline 305. Zineb is not expected to accumulate as the whole-body accumulation was low, with bioconcentration factors < 100 and the whole-body elimination was rapid with 25% of the initial radioactivity from zineb being retained by the end of the 16 day depuration period.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes The study was not conducted to GLP and has therefore been assigned a reliability of 2. Whilst no guidelines were stated in the paper, the study does comply with OECD Guideline 305 with minor deviations which are not considered to adversely affect the results from the study.

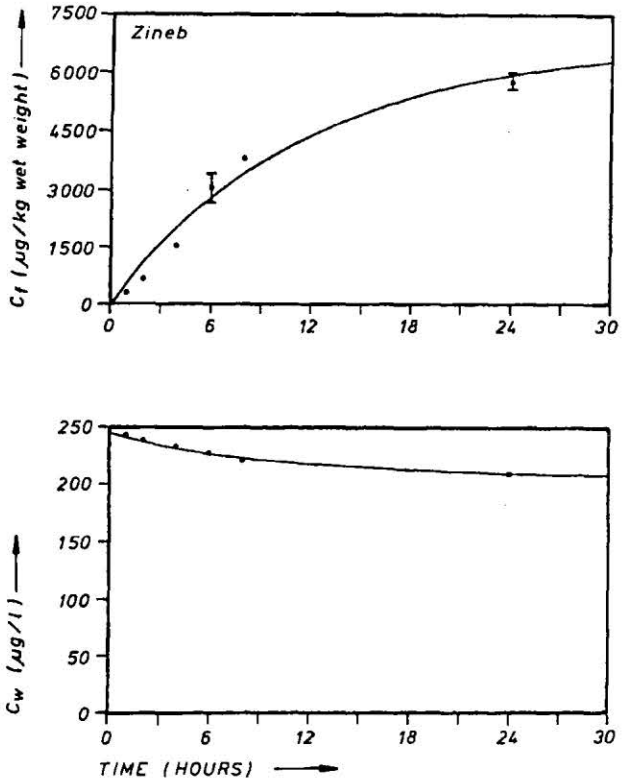
Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicant's version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*

Section A7.4.3.3.1	**Bioconcentration in aquatic organisms**
Annex Point IIIA XIII.2.3	
IUCLID 3.7/01	**Uptake Distribution and Retention of Zineb and Ziram in Rainbow Trout (Salmo Gairdneri)**

Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	*Adopt applicant's version or include revised version*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	

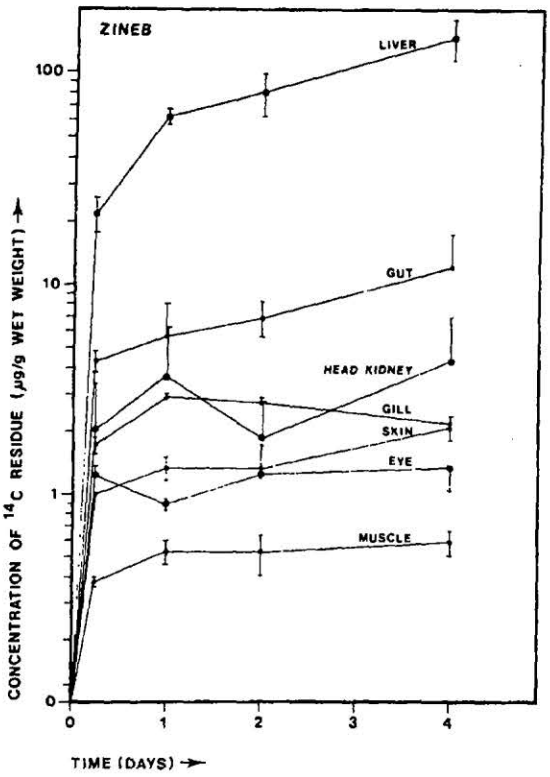
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from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

Figure A7_4_3_3_1-1 Whole-body accumulation of zineb (C*w* = 245 µg/l) in early juvenile rainbow trout (*Salmo gairdneri*)



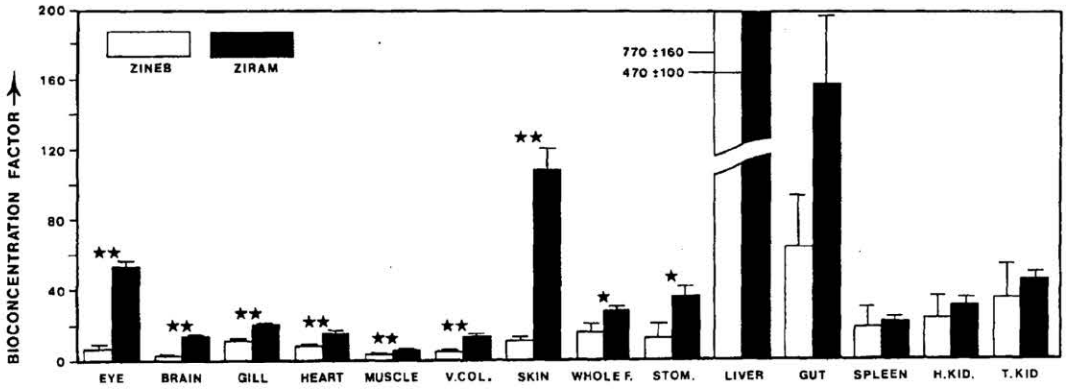
Points and associated vertical lines represent means ± SE. of 5 samples. The experiments were started with 75 fish. Lines are expected values based on the model calculations.

Figure A7_4_3_3_1-2 Uptake of zineb in various tissues of *Salmo gairdneri*.



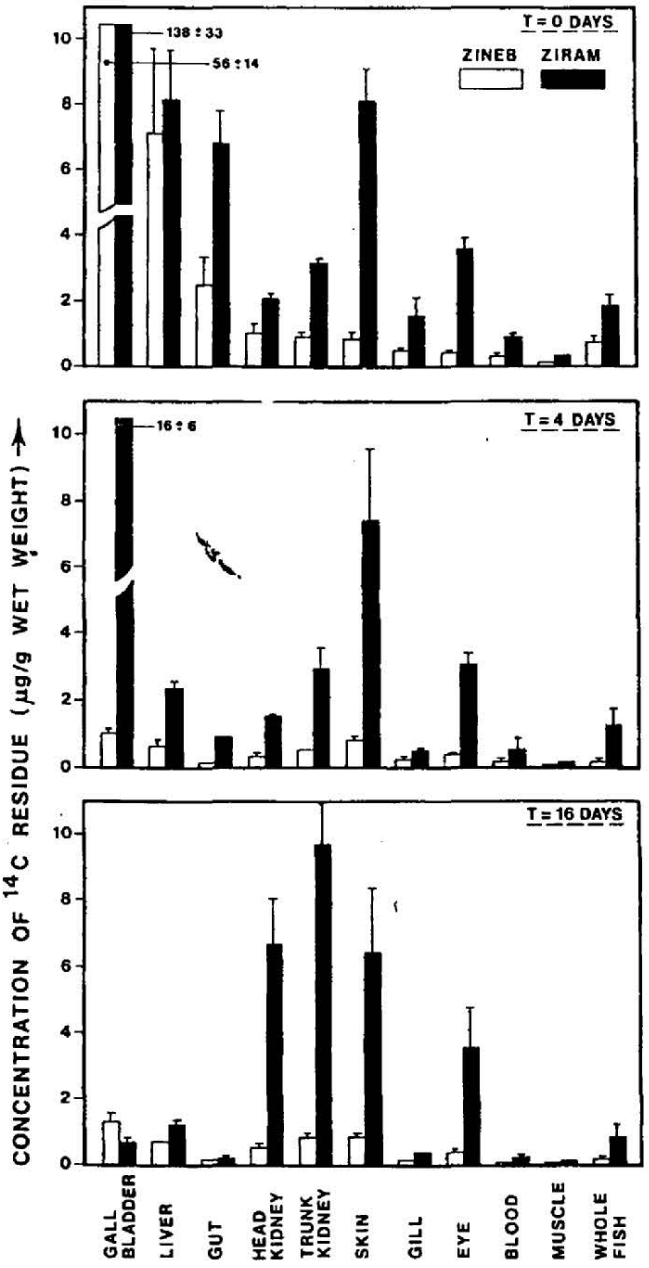
Points and associated vertical lines represent means ± S.E.M. of 3-5 fish. The initial concentration of zineb was 225 µg/l. At the end of the 96-h accumulation period this concentration was 191 µg/l.

Figure A7_4_3_3_1-3 Bioconcentration factors of ^{14}C-residues in whole fish and various tissues of *Salmo gairdneri* after 96 h exposure to 225 µg/l zineb and 138 µg/l ziram



Values and associated vertical lines represent means ± S.E.M. of 3-5 fish. Asterisks denote differences in BCFs at$P < 0.05$ (5) and$P < 0.01$ (*5), respectively.

Figure A7_4_3_3_1-4 Elimination of ^{14}C-residues in whole fish and various tissues of *Salmo gairdneri* after 96 h exposure to 105 and 118 µg/l zineb and ziram, respectively.



Values represent means ± S.E.M. of 3-5 fish.

Section 7.4.3.3.2 **Annex Point IIIA, XIII.2.3**	**Bio-accumulation in an appropriate invertebrate species**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The very low modelled BCF of 1.41 (refer to section A7.4.2) suggests that there is no significant potential for zineb to undergo bioaccumulation in aquatic organisms. This conclusion is supported by the low whole body BCF of 34 that has been derived experimentally in fish using ^{14}C-labelled test material (refer to TNG Summary A7.4.3.3.1). In view of these findings, it is considered to have been demonstrated adequately that there is no risk of bioaccumulation that would lead to secondary poisoning. Consequently, the need for bioaccumulation testing in an aquatic invertebrate is obviated on the basis of available data.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	
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*	

Section 7.4.3.3.2 Annex Point IIIA, XIII.2.3	Bio-accumulation in an appropriate invertebrate species
Remarks	

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species

Annex Point IIIA XIII 2.4

IUCLID 4.5.2/01,02,03,04

Official use only

		1 REFERENCE
1.1	**Reference**	Van Leeuwen CJ, Moberts F, Niebeek G (1985b) Aquatic toxicological aspects of dithiocarbamates and related compounds. II. Effects on survival, reproduction and growth of Daphnia magna. Aquatic Toxicology 7:165-175.
1.2	**Data protection**	No
1.2.1	Data owner	Not applicable
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	No. No guidelines available at the time, but methods consistent with current testing requirements under OECD 211.
2.2	**GLP**	No
2.3	**Deviations**	Not a guideline study
		3 METHOD
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Not reported
3.1.2	Specification	As given in section 2
3.1.3	Purity	Zineb ≥95% DIDT ≥ 98% ETU ≥ 99% EU ≥ 97%
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	Zineb is rapidly degraded to its constituent EBCD and degradates ETU, EU and DIDT. This affects the stability of the test substance in the test solutions.
3.1.6	Method of analysis	Not described
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable
3.3	**Reference substance**	No
3.3.1	Method of analysis	Not applicable

	for reference substance	
3.4	**Testing procedure**	
3.4.1	Dilution water	Refer to Table A7_4_3_4-2
3.4.2	Test organisms	Refer to Table A7_4_3_4-3
3.4.3	Handling of offspring	The number of surviving females and the number of neonates produced were recorded daily, and the new neonates removed from the test vessels following counting.
3.4.4	Test system	Refer to Table A7_4_3_4-4
3.4.5	Test conditions	Refer to Table A7_4_3_4-5
3.4.6	Duration of the test	21 days
3.4.7	Test parameter	Survival, population growth (i.e., reproduction), and carapace length.
3.4.8	Examination / Sampling	Refer to 3.4.3 and 5.1
3.4.9	Monitoring of TS concentration	No
3.4.10	Statistics	Differences in mean survival, intrinsic rate of natural increase (r_m, a measure of population growth), and carapace length between treatments and controls were tested using the procedure described by Williams (1971, 1972). The Bartlett test for homogeneity of variances was applied before using the Williams test. Normality was verified by means of the Shapiro-Wilk test. The LC_{50} values and their 95% confidence limits were determined according to Kooyman (1981).
		4 RESULTS
4.1	**Range finding test**	Not performed
4.1.1	Concentrations	Not applicable
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
4.1.3	Nature of adverse effects	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal concentrations tested for zineb were 0.010, 0.018, 0.032, 0.056, 0.100, 0.180, 0.320, and 0.560 mg/l and controls. Concentrations tested with DIDT, ETU and EU were not reported.
4.2.2	Actual concentrations of test substance	Not measured

Section 7.4.3.4
Annex Point IIIA XIII 2.4
IUCLID 4.5.2/01,02,03,04

Effects on reproduction and growth rate with an invertebrate species

4.2.3 Effect data

The effect data for zineb is shown in the following table. The data for DIDT, ETU and EU were not reported.

Concentration (mg/l)	Percent surviving to day 21	Mean r_m ± SE (per day)	Mean carapace length ± SE (mm)
Control	100	0.354 ± 0.006	4.04 ± 0.11
0.010	100	0.345 ± 0.005	4.02 ± 0.11
0.018	100	0.335 ± 0.007[a]	4.04 ± 0.13
0.032	96	0.339 ± 0.011	4.12 ± 0.17
0.056	62	0.299 ± 0.018 [b]	4.33 ± 0.26
0.100	30	0.197 ± 0.022	--
0.180	34	0.083 ± 0.033	--
0.320	4	--	--
0.560	0	--	--

[a] Author's lowest rejected concentration tested (α <0.01), based upon a statistically observed difference in natural population increase (r_m)..

[b] Biologically relevant Lowest Rejected Concentration Tested

Lowest Rejected Concentration Tested (LRCT) is considered to be equivalent to a Lowest Observed Effect Concentration (LOEC).

4.2.4 Concentration / response curve

The following graph plots % parental survival over the course of the study against the concentration of zineb (mg/l).

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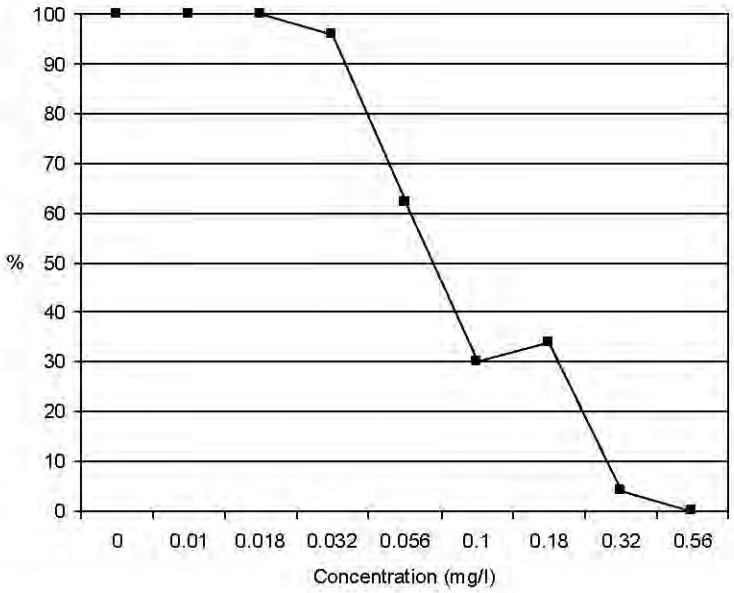
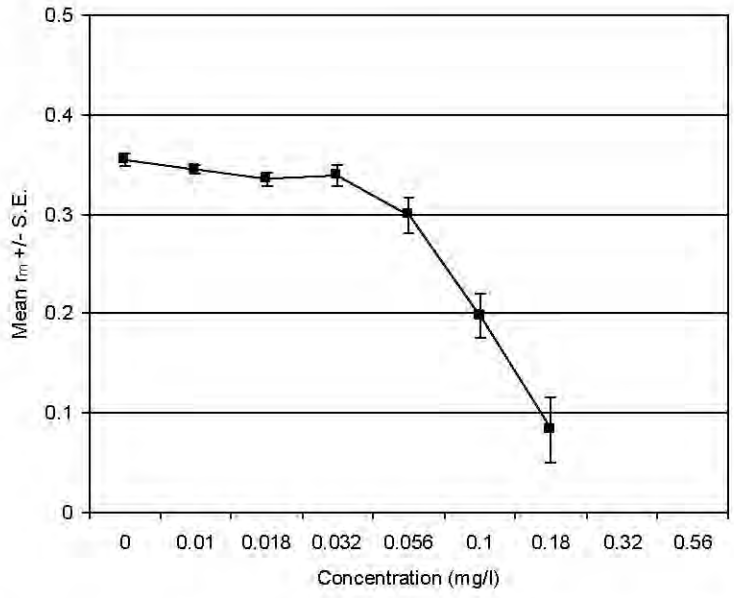


The following graph plots Mean r_m ± S.E. over the course of the study against the concentration of zineb (mg/l).



The following graph plots mean carapace length (mm) ± S.E. over the course of the study against the concentration of zineb (mg/l).

Section 7.4.3.4
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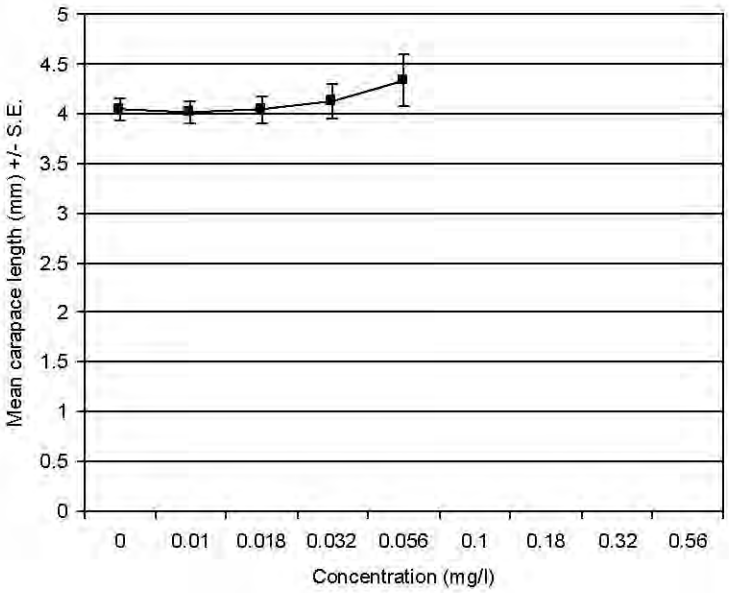
Effects on reproduction and growth rate with an invertebrate species



4.2.5	Other effects	The following LC_{50} values were determined over the course of the 21 day study (with 95% confidence limits): Zineb: 0.089 (0.078 – 0.102) mg/l; DIDT: 0.073 (0.067 – 0.081) mg/l; ETU: 18 (10 – 32) mg/l; EU: 3200 (1800 – 5600) mg/l.
4.3	**Results of controls**	See 4.2.3
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	Not applicable
4.4.2	Results	Not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The experiments were carried out with *Daphnia magna* in a constant temperature room. Tests were conducted in 800-ml vessels containing 500-ml of test solution or control water. Stock solutions were prepared in 50µm filtered and UV-sterilized Lake Ijssel water. The test solutions were renewed three times per week throughout the 21-day study. Oxygen concentration and pH were measured at regular intervals. Eight nominal concentrations (0.010, 0.018, 0.032, 0.056, 0.100, 0.180, 0.320, and 0.560 mg/l) plus a control were tested for zineb. DIDT, ETU and EU were also tested, although the concentrations utilised were not reported. Less than 24-hour old daphnids from laboratory cultures were

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distributed in cohorts of 10 animals each to five replicates of the test concentrations. The number of surviving females and the number of neonates produced were recorded daily. At the end of the experiments carapace length was determined from the anterior margin of the head to the base of the caudal spine using an ocular micrometer. When daphnids appeared to be males, these animals were excluded from fecundity, survival and growth analysis.

The results of this study were reported in terms of differences in mean survival, intrinsic rate of natural increase (r_m, a measure of population growth), and carapace length between treatments and controls.

5.2 Results and discussion

The authors reported a statistically significant difference in the r_m for zineb at 0.018 mg/l and identified this as the LRTC (Equivalent to a LOEC; see the comment in section 4.2.3). However, investigation of the response at the next experimental level indicates that there is no significant difference between this level and the control response. This indicates that although the response seen at 0.018 mg/l was statistically significant, it cannot be considered to be biologically significant. It is therefore concluded that the biological LRTC was 0.056 mg/l and that the resulting NOEC was 0.032 mg/l (nominal).

5.2.1 NOEC

The NOEC for zineb, based upon the discussion in section 5.2, is 0.032 mg/l (nominal).

NOECs for DIDT, ETU and EU were not reported and could not be confidently derived in the absence of sufficient information on the test concentrations used and the effects seen in this study.

5.2.2 LOEC

LOEC values for the different test compounds (expressed in terms of r_m values and mean carapace length) are presented in the following table:

Compound	LOEC (mg/l)	
	r_m	carapace length
Zineb	0.056	>0.056
DIDT	0.056	0.056
ETU	≤1	10
EU	--	180

All concentrations are nominal. The LOEC given for zineb is based on biological significance, as discussed in section 5.2.

5.2.3 EC_{50} (EC_x)

The following LC_{50} values were determined for the different test compounds over the course of the 21 day study (with 95% confidence limits):

Zineb: 0.089 (0.078 – 0.102) mg/l;

DIDT: 0.073 (0.067 – 0.081) mg/l;

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		ETU: 18 (10 – 32) mg/l; EU: 3200 (1800 – 5600) mg/l. All concentrations are nominal.
5.3	**Conclusion**	While not specifically a guideline study, the study was conducted in general accordance with current OECD guidelines and all validity criteria appear to have been met. Based on the non-GLP status of the reports and the relative lack of detail, the reliability of the study is assigned a 2.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes. This journal article is a compilation of many studies conducted in the same laboratory and thus does not include the level of detailed reporting that would normally be consistent with GLP studies.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if the applicant's version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	*Adopt applicant's version or include revised version*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / non acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator; if unacceptable, give reasons for unacceptability of study; discuss the relevance of the deficiencies. Indicate if repeat is necessary)*
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*

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species

Annex Point IIIA XIII 2.4

IUCLID 4.5.2/01,02,03,04

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

Table A7_4_3_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes/No
Vehicle	Yes/No
Concentration of vehicle	*Give the concentration (% v/v)*
Vehicle control performed	Yes/No
Other procedures	*e.g. test in completely filled closed vessels for testing volatile test substance*

Table A7_4_3_4-2: Dilution water

Criteria	Details
Source	UV-sterilized Lake Ijssel water
Salinity	Not applicable
Hardness	225 mg/L as $CaCO_3$
pH	8.1
Ca / Mg ratio	Not reported
Na / K ratio	Not reported
Oxygen content	Measured but not reported
Conductance	Not reported
TOC	Not reported
Holding water different from dilution water	No

Table A7_4_3_4-3: Test organisms

Criteria	Details
Strain / Clone	*Daphnia magna*
Source	In-house cultures
Age	<24-hours at test initiation
Breeding method	In-house cultures
Kind of food	*Chlorella pyrenoidosa*
Amount of food	3 x 10^8 cells/L
Feeding frequency	Daily
Pretreatment	None
Feeding of animals during test	Yes. Daily as above.

Table A7_4_3_4-4: Test system

Criteria	Details
Test type	Semistatic
Renewal of test solution	Renewed three times per week and were prepared fresh at each renewal.
Volume of test vessels	800 mls
Volume/animal	80 mls
Number of animals/vessel	10/vessel
Number of vessels/ concentration	5 vessels/concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_4-5: Test conditions

Criteria	Details
Test temperature	20 ± 0.5°C in a constant temperature room
Dissolved oxygen	Measured but not reported
pH	8.1
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Fluorescent lights
Photoperiod	12 hour light:dark

Table A7_4_3_4-6: Validity criteria for invertebrate reproduction test according to OECD Guideline 211

	fulfilled	Not fullfilled
Mortality of parent animals < 20% at test termination	x	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	x	

Criteria for poorly soluble test substances ergänzen	n.a.	

Section A7.4.3.5.1 **Annex Point IIIA, XIII.3.4**	**Effects on sediment-dwelling organisms**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Ethylenebisdithiocarbamate (EBDC) compounds such as zineb contain bivalent transition metal ions and form polymeric 'salts' of varying chain-length and 3-D arrangement that are essentially insoluble in water. Zineb slowly 'dissolves' in water by terminal cleavage of individual EBDC-Zn units, which dissociate in water to give Zn^{2+} and $EBDC^{2-}$ units. The results of available studies confirm the rapid degradation and removal of zineb and its metabolites from water/sediment systems (refer to TNG Summary A7.1.2.2.2). In view of the above, it is considered that the low potential for exposure of sediment dwelling organisms does not give rise to the need to carry out testing in sediment-dwelling organisms. Furthermore, using the EUSES 2.0.3 computer programme, it has been possible to estimate the toxicity of zineb and its metabolites to these organisms using the equilibrium partitioning method (as set out in the Technical Guidance Documents for Risk Assessment of New and Existing Substances). This method is considered adequate for the purpose of assessing the degree of risk to the sediment compartment in the first instance.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	

Section A7.4.3.5.1 **Annex Point IIIA, XIII.3.4**	**Effects on sediment-dwelling organisms**
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
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 A7.4.3.5.2 **Annex Point IIIA, XIII.3.4**	**Aquatic plant toxicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The available aquatic toxicity testing is considered to be adequate for the assessment of the overall risk posed by the proposed use of zineb to the aquatic environment. In view of this, and the fact that aquatic higher plant toxicity testing is not identified as either a core or a product type-specific data requirement for active ingredients in product type 21, it is concluded that there is no requirement for further testing of this type.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*	
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*	
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 A7.5.1.1(1) Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

Effect of BF 222-ETU on Carbon Transformation of the Soil Microflora

IUCLID 4.4/02

Official use only

		1 REFERENCE
1.1	**Reference**	Kreig, W., (2001a) Effect of BF 222-ETU on Carbon Transformation of the Soil Microflora, BASF Aktiengesellschaft, BASF Agricultural Centre Limburgerhof, Crop Protection Division, Ecology and Environmental Analytics, P.O. Box 120, 67114 Limburgerhof, Germany, Report No. 97479, 9 February 2001.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD-Guideline 217. Soil Microorganisms: Carbon Transformation Test
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Due to technical difficulties with the carbon transformation test on day 28 of the study, the final timepoint reported was Day 29. Soil characterisation was not adequately detailed in the submitted report with respect to sampling site (location, use pattern and sampling depth) and % sand composition. These are considered minor deviations with no impact on the conclusions of the study.
		3 MATERIALS AND METHODS
3.1	**Test material**	BF 222-ETU
3.1.1	Lot/Batch number	01743-165 (PCP06001)
3.1.2	Specification	Deviating from specification given in section 2 as follows:- The test substance is a metabolite of BAS 222 F
3.1.3	Purity	99.9%
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	None reported.
3.1.6	Method of analysis	Not applicable
3.2	**Reference substance**	No concurrent test with reference substance was performed
3.2.1	Method of analysis for reference	Not applicable

	substance	
3.3	**Testing procedure**	
3.3.1	Soil sample / inoculum / test organism	A loamy sand soil was used (see table A7_5_1_1-1).
3.3.2	Test system	See table A7_5_1_1-3.
3.3.3	Application of TS	TS was applied in distilled water. See table A7_5_1_1-4.
3.3.4	Test conditions	Aerobic at 20±2°C with soil containing water at 45% WHC at the start of the study. See table A7_5_1_1-5.
3.3.5	Test parameter	Carbon transformation
3.3.6	Analytical parameter	Glucose induced O_2 consumption
3.3.7	Duration of the test	29 Days
3.3.8	Sampling	Days 0, 7, 14 and 29
3.3.9	Monitoring of TS concentration	No
3.3.10	Controls	Controls were prepared to contain soil and water
3.3.11	Statistics	No formal statistical calculations were applied. The mean glucose induced oxygen consumption was calculated from 4 replicates in each group at each timepoint. Results from treated soil were expressed as % deviation from the untreated control soil. The standard deviation for each set of data was calculated to ensure the variation between replicates was less than 15%.
		4 RESULTS
4.1	**Range finding test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Effect data	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0.56 mg BF 222-ETU/kg dry test soil, equivalent to 0.42 kg BF 222-ETU/hectare 5.6 mg BF 222-ETU/kg dry test soil, equivalent to 4.2 kg BF 222-ETU/hectare
4.2.2	Actual concentrations of test substance	Not measured
4.2.3	Growth curves	Not applicable
4.2.4	Cell concentration data	Not applicable
4.2.5	Concentration/ response curve	Not applicable

Section A7.5.1.1(1) **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4 **Effect of BF 222-ETU on Carbon Transformation of the Soil Microflora**

IUCLID 4.4/02

4.2.6	Effect data	Refer to Table A7_5_1_1-6 for results of the glucose-induced oxygen consumption tests and effects of BF 222-ETU relative to control over 29 days and to Table A7_5_1_1-7 for a summary of the study. The test substance was found to have a NOEC of 5.6 mg/kg dry soil with respect to carbon transformation by soil microflora. This rate was based on the maximum rate of production of BF 222-ETU from the active substance BAS 222 F if applied at l0x the maximum field rate of 2.80 kg a.i./hectare).
4.2.7	Other observed effects	None
4.3	**Results of controls**	Refer to Table A7_5_1_1-6
4.4	**Test with reference substance**	No concurrent test with reference substance was performed
4.4.1	Concentrations	Not applicable
4.4.2	Results	Not applicable
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The effects of BF 222-ETU, a metabolite of BAS 222 F, on carbon transformation in soil were examined in a typical agricultural soil during a 29 day exposure in a laboratory study. The soil was a loamy sand of pH 6.2, containing 0.93% organic carbon of which 1.96% was microbial biomass. The soil was divided into 3 groups: control soil, soil treated with 0.56 mg BF 222-ETU/kg (corresponding to an application rate of 2.80 kg a.i./hectare for the active substance BAS 222 F) and soil treated with 5.6 mg BF 222-ETU/kg (corresponding to an application rate of 28.0 kg a.i./hectare for the active substance BAS 222 F). Carbon transformation was determined in 4 replicates per soil group at a series of timepoints through measurement of glucose-induced oxygen consumption.
5.2	**Results and discussion**	The test substance was applied to soil at a rate of 0.56 mg/kg (corresponding to the maximum transformation rate from the active substance BAS 222 F if applied at the maximum field rate of 2.80 kg a.i./hectare) and 5.6 mg/kg soil (corresponding to the maximum transformation rate from the active substance BAS 222 F if applied at l0x the maximum field rate of 2.80 kg a.i./hectare). Neither application rate had a significant effect on carbon transformation rates in the soil relative to the study control over 29 days as judged by measurement of glucose-induced oxygen consumption.
5.2.1	NOEC	5.6 mg BF 222-ETU/kg dry soil
5.2.2	EC_{10}	Not applicable
5.2.3	EC_{50}	Not applicable
5.3	**Conclusion**	BF 222-ETU applied at rates anticipated as arising from application of lx and l0x the maximum application rates of the a.i. BAS 222 F had no significant effect on carbon transformation by soil microflora.

Section A7.5.1.1(1) Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4 **Effect of BF 222-ETU on Carbon Transformation of the Soil Microflora**

IUCLID 4.4/02

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	*Give month and year of evaluation or comments*
Materials and Methods	*State if the applicant's version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers*
Conclusion	*Adopt applicant's version or include revised version*
Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator*
Acceptability	acceptable / not acceptable *(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_5_1_1-1: Microbial sample / Inoculum (*if applicable; include separate table for different samples*)

<table>
<tr><th>Criteria</th><th>Details</th></tr>
<tr><td>Nature</td><td>Soil sample</td></tr>
<tr><td>Sampling site:</td><td>Not stated</td></tr>
<tr><td>Geographical reference on the sampling site</td><td>Not stated</td></tr>
<tr><td>Data on the history of the site</td><td>Site not treated with plant protection products or fertilizers</td></tr>
<tr><td>Use pattern</td><td>Not stated</td></tr>
<tr><td>Depth of sampling [cm]</td><td>Not stated</td></tr>
<tr><td>Sand / Silt / Clay content [% dry weight]</td><td>Loamy Sand, composition detailed below:-
<table>
<tr><th colspan="2">Particle Size (%)</th></tr>
<tr><td>2000 to ≥ 630 µm</td><td>5.4</td></tr>
<tr><td>630 to ≥ 200 µm</td><td>43.0</td></tr>
<tr><td>200 to ≥ 63 µm</td><td>25.6</td></tr>
<tr><td>63 to ≥ 20 µm</td><td>10.3</td></tr>
<tr><td>20 to ≥ 6.3 µm</td><td>6.5</td></tr>
<tr><td>6.3 to ≥ 2 µm</td><td>4.1</td></tr>
<tr><td><2 µm</td><td>5.3</td></tr>
<tr><td>Soil Type (DIN 4220)</td><td>SI Loamy Sand
Lehmiger Sand</td></tr>
</table></td></tr>
<tr><td>pH</td><td>pH 6.2</td></tr>
<tr><td>Organic carbon content [% dry weight]</td><td>0.93%</td></tr>
<tr><td>Nitrogen content [% dry weight]</td><td>0.07%</td></tr>
<tr><td>Cation exchange capacity [mmol/kg]</td><td>6.6 mval Ba/100g dry weight</td></tr>
<tr><td>Initial microbial biomass</td><td>1.96% (Calculated from reported data)</td></tr>
<tr><td>Reference of methods</td><td>Respiratory activity in the soil was determined by the indirect calculation of oxygen consumed in the course of the test. Carbon dioxide produced in the course of respiration is bound by a CO absorber, meaning that there is a negative pressure change in the vessel related to the consumption of oxygen. Reduction in pressure causes the closure of a contact which results in the electrolytic generation of oxygen until pressure is restored. The device used (BSB digi) measures the amount of electricity consumed over the course of the test and using the Faraday law relates this to the quantity of oxygen required to maintain equilibrium in the system. This equates to eh amount of oxygen consumed in by respiration in the system.</td></tr>
<tr><td>Collection / storage of samples</td><td>Following mixing, each soil treatment group was distributed into sufficient 1.5 L glass jars to allow 4 replicates at each time-point. 4 replicates of each group were used to generate the day 0 carbon transformation values. The remainder of the samples</td></tr>
</table>

	were then stored in a temperature controlled incubator at 20±2°C until sampling at 7, 14 and 29 days. Measurements of glucose-induced O_2 consumption were made over a period of 12 hours at 20±2°C using a BSB digi on samples taken at days 0, 7, 14 and 29.
Preparation of inoculum for exposure	Not applicable
Pretreatment	Water was added to bring the moisture content of each soil group to 45% of it's maximum water holding capacity. Control: 102.9 g water were added to and mixed with 3329.6 g of soil Treated: 92.9 g water plus 10 g water containing BF 222-ETU were added to and mixed with 3329.6 g of soil

Table A7_5_1_1-2: Test organism (*if applicable*)

Criteria	Details
Species	Not applicable
Strain	Not applicable
Source	Not applicable
Sampling site	Not applicable
Laboratory culture	Not applicable
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Not applicable
Pretreatment	Not applicable
Initial cell concentration	Not applicable