Section A7.2.3.1(3)	**Adsorption / Desorption screening test**
Annex Point IIIA XII.1.2	**(^{14}C)-Ethylene urea, a metabolite of Mancozeb: Adsorption/Desorption in Soil**
IUCLID 3.3.2/03	

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_2 _3_1-1: Classification and physico-chemical properties of soils used as adsorbents

	Soil 1 **SK 961089**	**Soil 2** **SK 179618**	**Soil 3** **SK 566696**	**Soil 4** **SK 920191**
Soil order	Not reported	Not reported	Not reported	Not reported
Soil series	Not reported	Not reported	Not reported	Not reported
Classification	Clay Loam (USDA)	Loam (USDA)	Loamy Sand (USDA)	Clay Loam (USDA)
Location	Chapel Hill Farm, Empingham, Rutland, UK	Kenslow Farm, Middleton, Derbyshire, UK	Grid Ref SK566696, Warsop, Nottinghamshire, UK	Grid Ref SK920191, South Witham Quarry, South Witham, Lincolnshire, UK
Horizon	15-30 cm	5-20 cm	12-20 cm	5-20 cm
Sand [%]	38 (USDA)	34 (USDA)	85 (USDA)	38 (USDA)
Silt [%]	28 (USDA)	46 (USDA)	4 (USDA)	26 (USDA)
Clay [%]	34 (USDA)	20 (USDA)	11 (USDA)	36 (USDA)
Organic carbon [%]	4.6	3.8	0.8	2.1
Carbonate as $CaCO_3$	187.6 mg/kg	Not reported	Not reported	Not reported
insoluble carbonates [%]	Not reported	Not reported	Not reported	Not reported
pH (1:1 H_2O)	8.0	6.0	5.1	8.0
Cation exchange capacity (MEQ/100 g)	38.2	24.9	13.4	23.0
Extractable cations (MEQ/100 g)	-	-	-	-
Ca	Not reported	Not reported	Not reported	Not reported
Mg	Not reported	Not reported	Not reported	Not reported
Na	Not reported	Not reported	Not reported	Not reported
K	Not reported	Not reported	Not reported	Not reported
H	Not reported	Not reported	Not reported	Not reported
Special chemical/mineralogical features	None reported	None reported	None reported	None reported
Clay fraction mineralogy	Not reported	Not reported	Not reported	Not reported

Table A7_2 _3_1-2a: Results of preliminary test for Soil 1 (SK 961089):

Test substance	(^{14}C)-Ethylene urea		
Sample purity	99.5%		
Weighed soil	10	5	1
Volume of $CaCl_2$ solution	10	25	25
Nominal concentration of a.s. final solution	5.0 µg/mL	5.0 µg/mL	5.0 µg/mL
Analytical concentration final of a.s. solution	Not reported	Not reported	Not reported
Concentration of the test solution (show calculation)	Not reported	Not reported	Not reported
Details of the analytical method used:			
Method	Liquid Scintillation Counting (LSC) of Supernatant		
Recovery rate	82.4*	94.1*	100.0*
Detection limit	1.5 x Background radioactivity		

* Determined from reported % of applied radioactivity adsorbed by soil

Table A7_2 _3_1-2b: Results of preliminary test for Soil 2 (SK 179618):

Test substance	(^{14}C)-Ethylene urea		
Sample purity	99.5%		
Weighed soil	10	5	1
Volume of $CaCl_2$ solution	10	25	25
Nominal concentration of a.s. final solution	5.0 µg/mL	5.0 µg/mL	5.0 µg/mL
Analytical concentration final of a.s. solution	Not reported	Not reported	Not reported
Concentration of the test solution (show calculation)	Not reported	Not reported	Not reported
Details of the analytical method used:			
Method	Liquid Scintillation Counting (LSC) of Supernatant		
Recovery rate	89.3*	98.4*	100.0*
Detection limit	1.5 x Background radioactivity		

* Determined from reported % of applied radioactivity adsorbed by soil

Table A7_2 _3_1-2c: Results of preliminary test for Soil 3 (SK 566696):

Test substance	(^{14}C)-Ethylene urea		
Sample purity	99.5%		
Weighed soil	10	5	1
Volume of $CaCl_2$ solution	10	25	25
Nominal concentration of a.s. final solution	5.0 µg/mL	5.0 µg/mL	5.0 µg/mL
Analytical concentration final of a.s. solution	Not reported	Not reported	Not reported
Concentration of the test solution (show calculation)	Not reported	Not reported	Not reported
Details of the analytical method used:			
Method	Liquid Scintillation Counting (LSC) of Supernatant		
Recovery rate	83.4*	98.1*	100.0*
Detection limit	1.5 x Background radioactivity		

* Determined from reported % of applied radioactivity adsorbed by soil

Table A7_2 _3_1-2d: Results of preliminary test for Soil 4 (SK 920191):

Test substance	(^{14}C)-Ethylene urea		
Sample purity	99.5%		
Weighed soil	10	5	1
Volume of $CaCl_2$ solution	10	25	25
Nominal concentration of a.s. final solution	5.0 µg/mL	5.0 µg/mL	5.0 µg/mL
Analytical concentration final of a.s. solution	Not reported	Not reported	Not reported
Concentration of the test solution (show calculation)	Not reported	Not reported	Not reported
Details of the analytical method used:			
Method	Liquid Scintillation Counting (LSC) of Supernatant		
Recovery rate	80.7*	95.6*	100.0*
Detection limit	1.5 x Background radioactivity		

* Determined from reported % of applied radioactivity adsorbed by soil

Table A7_2 _3_1-3a: Results of screening test – adsorption for Soil 1 (SK 961089):

	5.0 µg/mL		2.5 µg/mL		0.5 µg/mL		0.25 µg/mL		0.05 µg/mL	
	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean
Concentration of test material [mg/l]	5.0	-	2.5	-	0.5	-	0.25	-	0.05	-
After contact of....hours with soil	24	-	24	-	24	-	24	-	24	-
Correction for blank with soil	Not applied									
Correction for blank without soil	Not applied									
Final corrected concentration [mg/l]	5.0	-	2.5	-	0.5	-	0.25	-	0.05	-
Initial concentration of test solution [mg/l]	5.0	-	2.5	-	0.5	-	0.25	-	0.05	-
Decrease in concentration [mg/l]	Not reported									
Quantity adsorbed [µg]	9.2787 4.0506*	6.6647	3.8353 4.5748	4.2050	1.0112 0.9270	0.9691	0.3813 0.3576	0.3694	0.0674 0.0836	0.0755
Quantity of soil [g of oven-dried equivalent]	10	-	10	-	10	-	10	-	10	-
Quantity adsorbed [µg] per gram of soil	0.92787 0.40506*	0.66647	0.38353 0.45748	0.42050	0.10112 0.09270	0.09691	0.03813 0.03576	0.03694	0.00674 0.00836	0.00755
Test material adsorbed [%]	18.6 8.1*	13.4	15.3 18.3	16.8	20.6 18.9	19.8	15.3 14.3	14.8	13.5 16.7	15.1
Temperature [°C]	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-
Volume of solution recovered after centrifugation [ml]	Not detailed. Report states that as much of the supernatant solution was removed as was possible									
Volume of solution not recovered [ml]	Not detailed. Report states that as much of the supernatant solution was removed as was possible									
Corresponding quantity of test substance [mg]	Not reported									

* Data flagged as not used in Regression Analysis

Table A7_2 _3_1-3b: Results of screening test – adsorption for Soil 2 (SK 179618):

	5.0 µg/mL		2.5 µg/mL		0.5 µg/mL		0.25 µg/mL		0.05 µg/mL	
	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean
Concentration of test material [mg/l]	5.0	-	2.5	-	0.5	-	0.25	-	0.05	-
After contact of....hours with soil	24	-	24	-	24	-	24	-	24	-
Correction for blank with soil	Not applied									
Correction for blank without soil	Not applied									
Final corrected concentration [mg/l]	5.0	-	2.5	-	0.5	-	0.25	-	0.05	-
Initial concentration of test solution [mg/l]	5.0	-	2.5	-	0.5	-	0.25	-	0.05	-
Decrease in concentration [mg/l]	Not reported									
Quantity adsorbed [µg]	6.7932 5.8705	6.3319	3.0549 2.9584	3.0066	0.5722 0.5228	0.5475	0.2728 0.2190	0.2459	0.0536 0.0762	0.0649
Quantity of soil [g of oven-dried equivalent]	10	-	10	-	10	-	10	-	10	-
Quantity adsorbed [µg] per gram of soil	0.67932 0.58705	0.63319	0.30549 0.29584	0.30066	0.05722 0.05228	0.05475	0.02728 0.02190	0.02459	0.00536 0.00762	0.00649
Test material adsorbed [%]	13.6 11.8	12.7	12.2 11.8	12.0	11.7 10.7	11.2	10.9 8.8	9.8	10.7 15.2	13.0
Temperature [°C]	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-
Volume of solution recovered after centrifugation [ml]	Not detailed. Report states that as much of the supernatant solution was removed as was possible									
Volume of solution not recovered [ml]	Not detailed. Report states that as much of the supernatant solution was removed as was possible									
Corresponding quantity of test substance [mg]	Not reported									

Table A7_2 _3_1-3c: Results of screening test – adsorption for Soil 3 (SK 566696):

	5.0 µg/mL		2.5 µg/mL		0.5 µg/mL		0.25 µg/mL		0.05 µg/mL	
	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean
Concentration of test material [mg/l]	5.0	-	2.5	-	0.5	-	0.25	-	0.05	-
After contact of....hours with soil	24	-	24	-	24	-	24	-	24	-
Correction for blank with soil	Not applied									
Correction for blank without soil	Not applied									
Final corrected concentration [mg/l]	5.0	-	2.5	-	0.5	-	0.25	-	0.05	-
Initial concentration of test solution [mg/l]	5.0	-	2.5	-	0.5	-	0.25	-	0.05	-
Decrease in concentration [mg/l]	Not reported									
Quantity adsorbed [µg]	6.1783 4.1377	5.1580	2.7223 3.8707	3.2965	0.6407 0.7828	0.7118	0.3635 0.4680	0.4157	0.0579 0.0870	0.0725
Quantity of soil [g of oven-dried equivalent]	10	-	10	-	10	-	10	-	10	-
Quantity adsorbed [µg] per gram of soil	0.61783 0.41377	0.51580	0.27223 0.38707	0.32965	0.06407 0.07828	0.07118	0.03635 0.04680	0.04157	0.00579 0.00870	0.00725
Test material adsorbed [%]	12.4 8.3	10.3	10.9 15.5	13.2	13.1 16.0	14.5	14.5 18.7	16.6	11.6 17.4	14.5
Temperature [°C]	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-
Volume of solution recovered after centrifugation [ml]	Not detailed. Report states that as much of the supernatant solution was removed as was possible									
Volume of solution not recovered [ml]	Not detailed. Report states that as much of the supernatant solution was removed as was possible									
Corresponding quantity of test substance [mg]	Not reported									

Table A7_2_3_1-3d: Results of screening test – adsorption for Soil 4 (SK 920191):

	5.0 µg/mL		2.5 µg/mL		0.5 µg/mL		0.25 µg/mL		0.05 µg/mL	
	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean
Concentration of test material [mg/l]	5.0	-	2.5	-	0.5	-	0.25	-	0.05	-
After contact of....hours with soil	24	-	24	-	24	-	24	-	24	-
Correction for blank with soil	Not applied									
Correction for blank without soil	Not applied									
Final corrected concentration [mg/l]	5.0	-	2.5	-	0.5	-	0.25	-	0.05	-
Initial concentration of test solution [mg/l]	5.0	-	2.5	-	0.5	-	0.25	-	0.05	-
Decrease in concentration [mg/l]	Not reported									
Quantity adsorbed [µg]	8.4662 7.7841	8.1252	4.4431 4.4910	4.4670	3.3957* 0.9802	2.1879	0.3849 0.4924	0.4387	0.1085 0.0762	0.0923
Quantity of soil [g of oven-dried equivalent]	10	-	10	-	10	-	10	-	10	-
Quantity adsorbed [µg] per gram of soil	0.84662 0.77841	0.81252	0.44431 0.44910	0.44670	0.33957* 0.09802	0.21879	0.03849 0.04924	0.04387	0.01085 0.00762	0.00923
Test material adsorbed [%]	17.0 15.6	16.3	17.8 18.0	17.9	69.3* 20.9	44.7	15.4 19.7	17.5	21.7 15.2	18.5
Temperature [°C]	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-
Volume of solution recovered after centrifugation [ml]	Not detailed. Report states that as much of the supernatant solution was removed as was possible									
Volume of solution not recovered [ml]	Not detailed. Report states that as much of the supernatant solution was removed as was possible									
Corresponding quantity of test substance [mg]	Not reported									

* Data flagged as not used in Regression Analysis

Table A7_2 _3_1-4a: Results of screening test – desorption for Soil 1 (SK 961089):

	5.0 µg/mL		2.5 µg/mL		0.5 µg/mL		0.25 µg/mL		0.05 µg/mL	
	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean
Temperature [°C]	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-
Concentration in combined washings [mg/l]	0.1833 0.2134	0.1984	0.0976 0.0856	0.0916	0.0167 0.0177	0.0172	0.0095 0.0095	0.0095	0.0018 0.0018	0.0018
Corresponding quantity of test material [mg]	0.001833 0.002134	0.001984	0.000976 0.000856	0.000916	0.0167 0.0177	0.000172	0.000095 0.000095	0.000095	0.000018 0.000018	0.000018
Quantity desorbed [µg][1]	1.6923 2.1585	1.9254	0.8051 0.4002	0.6026	0.0612 0.1599	0.1106	0.0880 0.0667	0.0730	-0.0031 0.0095	0.0032
[%] of adsorbed test material, which is desorbed[2]	18.2 53.3*	35.8	21.0 8.7	14.9	6.1 17.3	11.7	23.1 18.6	20.9	-4.7 11.3	3.3
[%] of adsorbed test material, which is not desorbed[3]	81.8 46.7*	64.2	79.0 91.3	85.1	93.9 82.7	88.3	76.9 81.4	79.1	104.7 88.7	96.7

* Data flagged as not used in Regression Analysis

[1] Reported data for quantity desorbed was calculated from: (µg EU in soil at end of adsorption phase) – (µg EU in soil at end of desorption phase)

[2] Reported data for % EU desorbed was calculated from:

$$\frac{(\mu g \text{ EU in soil at end of adsorption phase} - \mu g \text{ EU in soil at end of desorption phase})}{(\mu g \text{ EU in soil at end of adsorption phase})} \times 100$$

[3] Reported data for % EU not desorbed was calculated from:

$$\frac{(\mu g \text{ EU in soil at end of desorption phase})}{(\mu g \text{ EU in soil at end of adsorption phase})} \times 100$$

Table A7_2 _3_1-4b: Results of screening test – desorption for Soil 2 (SK 179618):

	5.0 µg/mL		2.5 µg/mL		0.5 µg/mL		0.25 µg/mL		0.05 µg/mL	
	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean
Temperature [°C]	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-
Concentration in combined washings [mg/l]	0.1728 0.1809	0.1769	0.0812 0.0853	0.0833	0.0171 0.0177	0.0174	0.0082 0.0088	0.0085	0.0018 0.0016	0.0017
Corresponding quantity of test material [mg]	0.001728 0.001809	0.001769	0.000812 0.000853	0.000833	0.000171 0.000177	0.000174	0.000082 0.000088	0.000085	0.000018 0.000016	0.000017
Quantity desorbed [µg][1]	3.0210 3.3387	3.1799	0.6011 0.6896	0.6453	0.2223 0.3075	0.2649	0.0666 0.1143	0.0905	0.0271 0.0240	0.0256
[%] of adsorbed test material, which is desorbed[2]	44.5 56.9	50.7	19.7 23.3	21.5	38.8 58.8	48.8	24.4 52.2	38.3	50.6 31.6	41.1
[%] of adsorbed test material, which is not desorbed[3]	55.5 43.1	49.3	80.3 76.7	78.5	61.2 41.2	51.2	75.6 47.8	61.7	49.4 68.4	58.9

[1] Reported data for quantity desorbed was calculated from: (µg EU in soil at end of adsorption phase) – (µg EU in soil at end of desorption phase)

[2] Reported data for % EU desorbed was calculated from:

$$\frac{(\text{µg EU in soil at end of adsorption phase} - \text{µg EU in soil at end of desorption phase})}{(\text{µg EU in soil at end of adsorption phase})} \times 100$$

[3] Reported data for % EU not desorbed was calculated from:

$$\frac{(\text{µg EU in soil at end of desorption phase})}{(\text{µg EU in soil at end of adsorption phase})} \times 100$$

Table A7_2 _3_1-4c: Results of screening test – desorption for Soil 3 (SK 566696):

	5.0 µg/mL		2.5 µg/mL		0.5 µg/mL		0.25 µg/mL		0.05 µg/mL	
	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean
Temperature [°C]	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-
Concentration in combined washings [mg/l]	0.1713 0.1751	0.1732	0.0842 0.0860	0.0851	0.0158 0.0163	0.0161	0.0084 0.0082	0.0083	0.0018 0.0016	0.0017
Corresponding quantity of test material [mg]	0.001713 0.001751	0.001732	0.000842 0.000860	0.000851	0.000158 0.000163	0.000161	0.000084 0.000082	0.000083	0.000018 0.000016	0.000017
Quantity desorbed [µg][1]	1.2566 0.8365	1.0465	0.3761 1.0407	0.7084	0.0279 0.169	0.0985	0.0967 0.1027	0.0996	0.0270 0.0158	0.0214
[%] of adsorbed test material, which is desorbed[2]	20.3 20.2	20.3	13.8 26.9	20.4	4.4 21.6	13.0	26.6 21.9	24.3	46.5 18.2	32.4
[%] of adsorbed test material, which is not desorbed[3]	79.7 79.8	79.7	86.2 73.1	79.6	95.6 78.4	87.0	73.4 78.1	75.7	53.5 81.8	67.6

[1] Reported data for quantity desorbed was calculated from: (µg EU in soil at end of adsorption phase) – (µg EU in soil at end of desorption phase)

[2] Reported data for % EU desorbed was calculated from:

$$\frac{(\text{µg EU in soil at end of adsorption phase} - \text{µg EU in soil at end of desorption phase})}{(\text{µg EU in soil at end of adsorption phase})} \times 100$$

[3] Reported data for % EU not desorbed was calculated from:

$$\frac{(\text{µg EU in soil at end of desorption phase})}{(\text{µg EU in soil at end of adsorption phase})} \times 100$$

Table A7_2 _3_1-4d: Results of screening test – desorption for Soil 4 (SK 920191):

	5.0 µg/mL		2.5 µg/mL		0.5 µg/mL		0.25 µg/mL		0.05 µg/mL	
	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean	Replicate 1 and 2	Mean
Temperature [°C]	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-	20±2°C	-
Concentration in combined washings [mg/l]	0.1755 0.1684	0.1720	0.0858 0.0844	0.0851	0.0064 0.0071	0.0068	0.0090 0.0088	0.0089	0.0016 0.0016	0.0016
Corresponding quantity of test material [mg]	0.001755 0.001684	0.001720	0.000858 0.000844	0.000851	0.000064 0.000071	0.000068	0.000090 0.000088	0.000089	0.000016 0.000016	0.000016
Quantity desorbed [µg][1]	1.7314 0.9777	1.3546	0.4122 0.4255	0.4188	0.0499 -0.7721	-0.3612	0.0876 0.1040	0.0959	0.0186 -0.0059	0.0063
[%] of adsorbed test material, which is desorbed[2]	20.5 12.6	16.5	9.3 9.5	9.4	1.5* -78.8*	-38.7	22.8 21.1	21.9	17.2 -7.8	4.7
[%] of adsorbed test material, which is not desorbed[3]	79.5 87.4	83.5	90.7 90.5	90.6	98.5* 178.8*	138.7	77.2 78.9	78.1	82.8 107.8	95.3

* Data flagged as not used in Regression Analysis

[1] Reported data for quantity desorbed was calculated from: (µg EU in soil at end of adsorption phase) – (µg EU in soil at end of desorption phase)

[2] Reported data for % EU desorbed was calculated from:

$$\frac{(\text{µg EU in soil at end of adsorption phase} - \text{µg EU in soil at end of desorption phase})}{(\text{µg EU in soil at end of adsorption phase})} \times 100$$

[3] Reported data for % EU not desorbed was calculated from:

$$\frac{(\text{µg EU in soil at end of desorption phase})}{(\text{µg EU in soil at end of adsorption phase})} \times 100$$

Section A7.2.3.2 Aged Soil Leaching Study

Annex Point IIA7.7 **Leaching characteristics of soil incorporated mancozeb following aerobic aging**

IUCLID 3.3.2/04

Official use only

	1 REFERENCE	
1.1	**Reference**	Daly, D., 1988, Leaching characteristics of soil incorporated mancozeb following aerobic aging. Analytical Bio-Chemistry Laboratories, Inc. Report No.36291.
1.2	**Data protection**	Yes
1.2.1	Data owner	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, US EPA Method No. 163
2.2	**GLP**	Yes
2.3	**Deviations**	No
	3 MATERIALS AND METHODS	
3.1	**Test material**	[^{14}C]-Dithane® (active ingredient = mancozeb)
3.1.1	Lot/Batch number	Lot # 541.0409
3.1.2	Specification	Deviating from specification given in section 2 as follows:
3.1.3	Purity	Radiochemical purity = 87.4% (first analysis) and 88% (second analysis)
3.1.4	Further relevant properties	None reported.
3.1.5	Method of analysis	HPLC using a Varian 2010 HPLC pump, a Varian 2050 variable UV detector and a Gilson 202 fraction collector with the following settings: Flow rate: 1.0 ml/min Wavelength: 220nm Column: Beckman Ultrasphere IP Mobile phase: 56:44 (methanol:Millipore water) at a 0.025M tetrabutyl ammonium hydroxide concentration. TLC using silica gel TLC plates 60F-254 (Merck) and ethanol/ethyl acetate/ammonium hydroxide (60/20/20, v/v/v) as the solvent system. Following development plates were air-dried and visualised under u.v. light, stained with iodine vapours and exposed to Kodak X-R OMAT X-ray film to obtain autoradiograms. Radioactive zones were scraped from the plates desorbed in 2ml of methanol and analysed by LSC. Liquid Scintillation Counting (LSC) This was performed on samples to quantify ^{14}C-radioactivity. Liquid samples (trapping solutions, soil extracts, leachates) were aliquotted by volume and liquid scintillation cocktail was added to each sample. Solid

		samples were combusted prior to analysis. All samples were counted for 5 min. Counts per min (cpm) were converted to disintegration per min (dpm) based on a quench curve. Combustion analysis Solid samples (soil, post-extraction soil) were combusted using a Packard 306D Tri-Carb oxidiser. The gaseous effluent from the oxidiser was collected directly into oxidiser LSC cocktail. Combustion efficiency was determined with each analysis. Combustion efficiencies were 93.6%. Sample dpm were corrected for combustion efficiency. Analysis of Volatiles Trap Contents Total radioactivity collected in the volatile trap solutions was determined by LSC of triplicate aliquots. Leachate Analysis After all the standing water above the soil had drained, the volume of leachate was measured and the leachate was then filtered andanalysed by LSC. Soil extraction Aged soil: Each soil sample (10g wet weight) was extracted with methanol (3 x 30ml) by shaking for 30-60 minutes. The sample was then centrifuged (10 min @ 1500 rpm) and the supernatant decanted. The resulting supernatants were pooled and the volume adjusted to 100ml. Determination of bound residues **Aged soils**: The extracted soil pellet was air dried and analysed by combustion /LSC of triplicate aliquots to determine unextracted (bound) radioactivity. **Leaching column soil**: Following the leaching period the columns were segmented, the soil mixed and aliquots combusted in triplicate.
3.2	**Degradation products**	Degradation products were not identified.
3.2.1	Method of analysis for degradation products	Degradation products were not identified.
3.3	**Reference substance**	No.
3.3.1	Method of analysis for reference substance	Not applicable.

Section A7.2.3.2 Aged Soil Leaching Study

Annex Point IIA7.7 **Leaching characteristics of soil incorporated mancozeb following aerobic aging**

IUCLID 3.3.2/04

3.4	**Soil types**	See Table A7_2_3_2-1 Approximately 80g dry weight of sieved soil was weighed into individual wide-mouthed bottles. The soil moisture of each sample was then adjusted to 75% of the field capacity. Twelve samples were prepared for use in this study. The equilibration period for the soils has not been reported. Soils were sieved (2mm) prior to use in the test. The soils were analysed for microbial activity by conducting bacterial plate count analyses on aerobically aged treated and control soil.
3.5	**Testing procedure**	
3.5.1	Test system	**Soil aging**: wide-mouthed jars containing 80g dry weight soil were treated with the test substance. The vessels were then incubated in the dark at 25±1°C. A stream of humidified air was circulated through the metabolism vessel. The air exiting the vessel was passed through a series of traps (ethylene glycol, sulphuric acid and 5N potassium hydroxide) to trap any volatile degradation products. **Soil leaching**: Soil columns were constructed from 3 inch inner diameter aluminium pipe 36 inches long and sealed with aluminium tape and silicone. Twent mesh stainless steel screens were fused to a 2-inch section of 3 inch inner diameter schedule 40 PVC pipe and attached to the bottom of the aluminium columns with silicone. A 3 inch diameter GFA filter was placed over the screen. The column was filled to a depth of 12 inches with test soil. Aerobically aged soil (10g wet weight) was then applied to the top of the column and covered with 10g (wet weight) of control test soil and a GFA filter. The columns were then filled with a total of 20 acre inch equivalents of deionised water. Volatile traps containing 5N KOH were connected to the top and bottom of each column. The leachate was collected at the bottom of the column.
3.5.2	Test solution and Test conditions	**Preparation of Stock Solution** An aliquot of the ^{14}C-material was transferred to a 100ml volumetric flask and made to volume with methanol to prepare a primary stock solution. This was sonicated for 20 minutes. Five x 100µl aliquots of this primary stock solution were analysed by LSC and a concentration of 57.029µg/ml was determined to be 87.4% (recovery of injected radioactivity was 81.9%) **Soil fortification** A 20g sub-sample (from the 80g test sample) was fortified with 14ml of the dosing solution (*ca* 800µg of the test substance). The carrier solvent was removed by evaporation under a stream of nitrogen. This fortified sample was then tumble mixed with the remaining 60g for 1 hour. The soil moisture content was then adjusted to 75% field capacity.
3.6	**Test performance**	
3.6.1	Definitive test	The soil samples were aged for 24h. After aging a 10g sample was transferred to the leaching column and 2320ml of water added to the column.

Section A7.2.3.2 **Aged Soil Leaching Study**

Annex Point IIA7.7 **Leaching characteristics of soil incorporated mancozeb following aerobic aging**

IUCLID 3.3.2/04

4 RESULTS

4.1 Definitive test

After 24h of aerobic incubation extracable residues were 48.9%, 20.2%, 34.6% and 11.1%. Total recoveries from aged soil were 99.2%, 101%, 101%, and 98.3%.

The ^{14}C-residues of mancozeb demonstrated low mobility in all soil types with 19.1% of the total dpm applied being recovered in the leachate and 77.8% remaining in the soil for the sandy loam 8.7% and 98.9% in the silt loam and 4.2% and 90.2% in the higher organic matter silt loam. Total recoveries for the leaching columns were 96.9%, 107% and 94.3%. Based on the information given in the report the data for the clay loam soil was incomplete.

4.2 Degradation product(s)

The report is incomplete regarding degradation products.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

[^{14}C]-Dithane (radiochemical purity aprroximately 88%) was applied at a nominal concentration of 10 ppm to 80g (dry weight equivalent) samples of four test soils and incubated in darkness under aerobic conditions (with volatiles trapping) for 24h at 25±1°C. A 10g sample was then transferred to the top of a soil leaching column of corresponding soil type, in triplicate. Further 10g samples, from the aged soil, were taken to determine the microbial plate count, extraction and combustion/combustion only for comparison. A head of water was applied to the leaching column and allowed to drain, the leachate was then collected. The draining times were 1 day for the sandy loam and Washington silt loam, 6-8 days for the Lawrenceville silt loam and was still on-going after 25-34 days for the Hollister clay loam, and a final report was not available. The columns were also set up for volatile trapping at the top and bottom of the column.

Aged soil (10g) was extracted with methanol (3 x 30ml). Extracted soils were analysed by combustion/LSC to determine total unextractable radioactivity. Following the leaching period the columns were segmented, the soil mixed and aliquots combusted.

5.2 Results and discussion

After 24h of aerobic incubation extracable residues were 48.9%, 20.2%, 34.6% and 11.1%. Total recoveries from aged soil were 99.2%, 101%, 101%, and 98.3%. Analysis of extracts of aged soil showed that less than 5% parent was present for each soil type.

The ^{14}C-residues of mancozeb demonstrated low mobility in all 3 soil types (clay loam results incomplete) with 19.1% of the total dpm applied being recovered in the leachate and 77.8% remaining in the soil for the sandy loam; 8.7% and 98.9% in the Lawrenceville silt loam and 4.2% and 90.2% in the higher organic matter Washington silt loam. Total recoveries for the leaching columns were 96.9%, 107% and 94.3%. Based on the information given in the report the data for the clay loam soil was incomplete. The majority of the recovered radioactivity was found in the top segment of the soil column, 56.8% in the sandy loam;

Section A7.2.3.2	**Aged Soil Leaching Study**
Annex Point IIA7.7	**Leaching characteristics of soil incorporated mancozeb following aerobic aging**
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		84.2 in the Lawrenceville silt loam and 90.2 in the Washington silt loam. The ^{14}C-residues in the soil column decreased with increasing soil depth. Total recoveries for the column leaching were 96.9% (sandy loam), 107% (Lawrenceville silt loam) and 94.3% (Washington silt loam). The leaching potential of ^{14}C-mancozeb decreased with increasing organic matter in the soil.
5.2.1	Degradation products (% of a.s.)	Degradation products were not characterised.
5.3	**Conclusion**	
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
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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.)*
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Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

Section A7.2.3.2	**Aged Soil Leaching Study**
Annex Point IIA7.7	**Leaching characteristics of soil incorporated mancozeb following aerobic aging**
IUCLID 3.3.2/04	

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

Table A7_2 _3_2-1: Classification and physico-chemical properties of soils used as adsorbents

	Soil 1 Sandy loam	Soil 2 Lawrenceville	Soil 3 Hollister	Soil 4 Washington
Soil order	Not reported	Not reported	Not reported	Not reported
Soil series	Not reported	Not reported	Not reported	Not reported
Classification	Sandy loam	Silt loam	Clay loam	Silt loam
Location	Not reported	Not reported	Not reported	Not reported
Horizon	Not reported	Not reported	Not reported	Not reported
Sand [%]	76	12	26	28
Silt [%]	18	62	46	54
Clay [%]	6	26	28	18
Organic matter [%]	0.4	2.0	3.0	4.5
Carbonate as $CaCO_3$	Not reported	Not reported	Not reported	Not reported
insoluble carbonates [%]	Not reported	Not reported	Not reported	Not reported
pH (1:1 H_2O)	7.8	6.1	6.9	6.2
Cation exchange capacity (MEQ/100 g)	7.5	6.1	16.9	7.9
Extractable cations (MEQ/100 g)	-	-	-	-
Ca	Not reported	Not reported	Not reported	Not reported
Mg	Not reported	Not reported	Not reported	Not reported
Na	Not reported	Not reported	Not reported	Not reported
K	Not reported	Not reported	Not reported	Not reported
H	Not reported	Not reported	Not reported	Not reported
Special chemical/mineralogical features	None reported	None reported	None reported	None reported
Clay fraction mineralogy	Not reported	Not reported	Not reported	Not reported

Table A7_2 _3_2-2: Distribution of ^{14}C-Residues in Aerobically Aged Soils (% of applied radioactivity)

Soil type	Sandy Loam		Lawrenceville silt loam		Hollister clay loam		Washington silt loam	
Timpoint	Day 0	Day 1	Day 0	Day 1	Day 0	Day 1	Day 0	Day 1
Extractable	72.1	48.5	42.2	20.4	52.1	34.8	22.4	10.9
Non-extractable	27.9	50.8	57.9	80.6	48.2	65.7	78.1	87.3
Volatile	-	0.05	-	0.05	-	0.05	-	0.11
Total	100.0	99.2	100.1	101.1	100.3	100.6	100.5	98.3

Table A7_2 _3_2-3: Distribution of ^{14}C-Residues in Aged soil leaching columns (% of applied radioactivity)

Soil type	Sandy loam	Lawrenceville silt loam	Washington silt loam
Leachate	19.1	8.7	4.2
Segments 1-12	77.8	98.9	90.2
Total	96.9	107.6	94.4

Table A7_2 _3_2-4: Distribution of ^{14}C-Residues in Aged soil leaching column segments (% of applied radioactivity)

Soil type/segment number	Sandy loam	Lawrenceville silt loam	Washington silt loam
1	56.8	84.2	83.0
2	6.1	2.3	2.6
3	4.0	1.9	0.8
4	3.0	1.5	0.7
5	2.1	1.1	0.5
6	1.6	1.4	0.6
7	1.3	0.6	0.3
8	0.9	0.5	0.3
9	0.7	3.1	0.4
10	0.6	0.9	0.2
11	0.4	0.6	0.4
12	0.3	0.8	0.4
Total	77.8	98.9	90.2

Segment 1 is the top of the leaching column.

Section 7.3.1 **Annex Point IIIA, VII.5**	**Phototransformation in air (estimation method), including identification of breakdown products**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Zineb has a very low vapour pressure of 7.9×10^{-5} Pa (refer to TNG Summary A3_2). It is therefore considered that there is no potential for significant quantities of zineb to reach the troposphere and that it is not necessary to carry out an experimental determination of phototransformation in air. A theoretical determination of the specific first order degradation rate of zineb with –OH radicals has been carried out using the USEPA EPIWIN v. 3.12 computer program, the results of which are presented in Document IIA.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
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	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*	

Section 7.3.1 Annex Point IIIA, VII.5	**Phototransformation in air (estimation method), including identification of breakdown products**
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 7.3.2 **Annex Point IIIA, XII.3**	**Fate and behaviour in air, further studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	If the active substance is to be used in preparations for fumigants or it causes risk to the atmospheric environment, its degradation behaviour has to be determined experimentally. As zineb will not be used in fumigation products and has a very low vapour pressure, it is considered that there is no need to carry out further studies of its fate and behaviour in air.	
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 A7.4.1.1(1) **Acute toxicity to fish**

Annex Point IIA7.1 **Acute toxicity of Zineb to Plaice (*Pleuronectes platessa*)**

IUCLID 4.1/01

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (2001b) Zineb Nautec: Acute Toxicity to Plaice (*Pleuronectes platessa*). Brixham Environmental Laboratory, Report No. BL7217/B, December 2001 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Cerexagri s.a.
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 203
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Zineb Nautec
3.1.1	Lot/Batch number	Batch Ref 054072
3.1.2	Specification	As given in section 2
3.1.3	Purity	95.4%
3.1.4	Composition of Product	Not applicable.
3.1.5	Further relevant properties	Zineb is rapidly degraded to its constituent EBDC and degradates ETU, EU and DIDT. This affects the stability of the test substance in the test solutions.
3.1.6	Method of analysis	Analysed using the HPLC method under the following conditions: Column: 150 mm x 4.1 mm id Column packing: Hamilton PRP-X100 (10 particle size) Injection volume: 100 µl Eluent: Deionised water containing 8.4 g/l sodium perchlorate and 3.8 g/l ethylenediamine acetic adic tetrasodium salt hydrate Eluent flow rate: 1.0 ml/min Wavelength: 286 nm
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Refer to Table A7_4_1_2-1
3.3	**Reference substance**	No

Section A7.4.1.1(1) **Acute toxicity to fish**

Annex Point IIA7.1 **Acute toxicity of Zineb to Plaice (*Pleuronectes platessa*)**

IUCLID 4.1/01

3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Dilution water	Refer to Table A7_4_1_1-2
3.4.2	Test organisms	Refer to Table A7_4_1_1-3
3.4.3	Test system	Refer to Table A7_4_1_1-4
3.4.4	Test conditions	Refer to Table A7_4_1_1-5
3.4.5	Duration of the test	96-hours
3.4.6	Test parameter	Mortality
3.4.7	Sampling	Samples for analysis of the test substance were taken from the control and test substance concentration (triplicate samples) immediately after addition of the test substance, at the start of the test (Day 0) and when the solutions were renewed on Day 3. The corresponding test solutions were also sampled 24 hours after addition of the test substance, on Day 1 (prior to solution renewal) and on Day 4 (at the end of the test).
3.4.8	Monitoring of TS concentration	Yes, as described in Section 3.4.7
3.4.9	Statistics	No statistical procedures were applied as only 20% mortality occurred in the limit test concentration
		4 RESULTS
4.1	**Limit Test**	Performed
4.1.1	Concentration	32 mg/L nominal, based on preliminary range-finding data
4.1.2	Number/ percentage of animals showing adverse effects	20% (2 of 10 animals in 32 mg/L nominal concentration)
4.1.3	Nature of adverse effects	Mortality of 2 animals occurred between 24 and 48 hours. No further mortality or other symptoms of toxicity were subsequently observed in the surviving fish.
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	32 mg/L

Section A7.4.1.1(1) **Acute toxicity to fish**

Annex Point IIA7.1 **Acute toxicity of Zineb to Plaice (*Pleuronectes platessa*)**

IUCLID 4.1/01

4.2.2 Actual concentrations of test substance

Nominal Conc (mg/L)	Time (hours of test)	New/Old solution	Measured conc (mg/L)	Mean Measured conc (mg/L)	Mean Measured conc as % of nominal
Control	0	New	<0.0038	-	-
	24	Old	<0.0038		
	72	New	<0.0032		
	96	Old	<0.0032		
32	0	New	32 [a]	21	66
	24	Old	12 [b]		
	72	New	32 [c]		
	96	Old	8.1 [d]		

[a] Mean of triplicate analyses: 32, 32, 32 mg/L

[b] Mean of triplicate analyses: 12, 13, 9.5 mg/L

[c] Mean of triplicate analyses: 32, 32, 33 mg/L

[d] Mean of triplicate analyses: 7.9, 8.4, 7.9 mg/L

4.2.3 Effect data (Mortality) — Refer to Tables A7_4_1_1-6 and A7_4_1_1-7

4.2.4 Concentration / response curve — Not applicable - Limit test

4.2.5 Other effects — No other effects

4.3 Results of controls

4.3.1 Number/ percentage of animals showing adverse effects — 0% (0 of 10 animals in control)

4.3.2 Nature of adverse effects — None

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 — Plaice (*Pleuronectes platessa*) were exposed to Zineb for 96-h in a semi-static system with daily test solution renewals in accordance with OECD Guideline No. 203. This was a limit test with one test solution concentration of 32 mg/l nominal (mean measured concentration 21 mg/l (66% of nominal)).

5.2 Results and — No mortality was observed in the controls. Two of ten animals in the

Section A7.4.1.1(1) **Acute toxicity to fish**

Annex Point IIA7.1 **Acute toxicity of Zineb to Plaice (*Pleuronectes platessa*)**

IUCLID 4.1/01

	discussion	test concentration died between 24 and 48 hours, but no other signs of toxicity or stress were observed in the surviving animals. Nor was further mortality observed for the duration of the study. Because Zineb rapidly degrades to its EBDC constituents and other degradates, measured test concentrations showed decreases during the period between test solution renewals.
5.2.1	LC_0	Not determined
5.2.2	LC_{50}	48-h and 96-h: >32 mg/L nominal (>21 mg/l mean measured)
5.2.3	LC_{100}	Not determined
5.3	**Conclusion**	Zineb was not significantly acutely toxic to plaice at a nominal concentration of 32 mg/l (mean measured concentration of 21 mg/l). The <10% control mortality and >60% dissolved oxygen saturation validity criteria were fulfilled. The criterion to show evidence that the concentration of the test substance was maintained is only partially fulfilled. The mean measured concentration was 21 mg/l during the study, which is 66% percent of the initial concentration rather than ≥80%. However, this is to be expected given that the polymeric Zineb rapidly degrades to its EBDC constituents and several other degradates (ETU, EU, DIDT). Test solutions were renewed daily in order to maintain test concentrations of Zineb as stable as possible. The study was conducted under GLP guidelines. However, based on measured concentrations and inherent difficulties in maintaining concentrations of unstable test substances in a static test, the derived EC_{50} can only be viewed as indicative and the reliability of the endpoint is assigned a 2.
5.3.1	Other Conclusions	This study should be considered fit for the purpose of describing the aquatic acute toxicity of Zineb to marine fish. While two fish did die in the test concentration, the lack of other overt signs of stress or other mortality suggests that these deaths may have been incidental and conduct of a full study would likely not contribute further to the understanding of Zineb toxicity.
5.3.2	Reliability	2
5.3.3	Deficiencies	Yes. Baased on measured concentrations and inherent difficulties in maintaining concentrations of unstable test substances in a static test, the derived EC_{50} can only be viewed as indicative and the reliability of the endpoint is assigned a 2.

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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.*

Section A7.4.1.1(1)	**Acute toxicity to fish**
Annex Point IIA7.1	**Acute toxicity of Zineb to Plaice (*Pleuronectes platessa*)**
IUCLID 4.1/01	

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	

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

Criteria	Details
Dispersion	Yes. Test concentrations were prepared by direct addition of the test substance to dilution water followed by thorough mixing.
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	No
Other procedures	None

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Natural seawater from Tor Bay, Devon, UK and filtered to 10 µm
Alkalinity	Not applicable
Hardness	Not applicable
pH	7.96-8.20
Oxygen content	7.85-8.21 mg/l
Conductance	Salinity: 35 ± 1‰
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Plaice (*Pleuronectes platessa*)
Source	Tor Bay, Devon
Wild caught	Yes. Collected locally from Tor Bay, Devon and held at Brixham Environmental Laboratory for at least 24 days before test initiation.
Age/size	Mean weight: 1.7 g (0.95-2.86 g) Mean length: 49 mm (44-59 mm)
Kind of food	Commerical fish diet
Amount of food	Appropriate amounts
Feeding frequency	Daily until 24 hours prior to test initiation

Pretreatment	Held at least 24 days
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Semi-static
Renewal of test solution	Fish were transferred to a second set of vessels containing newly prepared test solutions after 24, 48 and 72 hours
Volume of test vessels	20 litres
Volume/animal	2 litres per animal
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	By thermometer: 14.8-15.6°C By autorecorder: 15 ± 1°C
Dissolved oxygen	7.85-8.21 mg/l
pH	7.96-8.20
Adjustment of pH	No
Aeration of dilution water	Yes, gently aerated throughout the study
Intensity of irradiation	Fluorescent light
Photoperiod	16 hours light, 8 hours dark with 20 minute dawn and dusk transition periods

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (nominal/measured)[1] [mg/l]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control (<0.0098)*	0	0	0	0	0	0	0	0
32 (21)*	0	2	2	2	0	20	20	20

*(#) = measured conc.								
Temperature [°C]	15.2	15.3	15.2	15.3				
pH	8.00	7.96	8.02	7.98				
Oxygen [mg/l]	7.94	8.10	8.15	7.89				

[1] specify, if TS concentrations were nominal or measured

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	**95 % c.l.**	**96 h [mg/l][1]**	**95 % c.l.**
LC_0				
LC_{50}	>32 (n) >21 (m)	n.a.	>32 (n) >21 (m)	n.a.
LC_{100}				

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

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

	fulfilled	**Not fullfilled**
Mortality of control animals <10%	**x**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**x**	
Concentration of test substance ≥80% of initial concentration during test		**x**

Criteria for poorly soluble test substances		

Section A7.4.1.1(2) Acute toxicity to fish

Annex Point IIA7.1

IUCLID 4.1/02,03,05,07

Acute toxicity of Zineb, DIDT, ETU and EU to fish.

Official use only

		1 REFERENCE
1.1	**Reference**	Van Leeuwen CJ, Maas-Diepeveen JL, Niebeek G, Vergouw, WHA, Griffioen PS, Luijken MW (1985a) Aquatic toxicological aspects of dithiocarbamates and related compounds. I. Short-term toxicity tests. Aquatic Toxicology 7:145-164.
1.2	**Data protection**	No
1.2.1	Data owner	Public Domain
1.2.2		
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes. OECD 203
2.2	**GLP**	No. GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Not indicated
3.1.2	Specification	Deviating from specification given in section 2 as follows: Zineb and the metabolites DIDT, ETU and EU were tested.
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**	See table A7_4_1_1-1
3.3	**Reference substance**	No
3.3.1	Method of analysis	Not applicable

Section A7.4.1.1(2) **Acute toxicity to fish**

Annex Point IIA7.1

IUCLID 4.1/02,03,05,07 **Acute toxicity of Zineb, DIDT, ETU and EU to fish.**

	for reference substance	
3.4	**Testing procedure**	
3.4.1	Dilution water	Refer to Table A7_4_1_1-2
3.4.2	Test organisms	Refer to Table A7_4_1_1-3
3.4.3	Test system	Refer to Table A7_4_1_1-4
3.4.4	Test conditions	Refer to Table A7_4_1_1-5
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Mortality
3.4.7	Sampling	Not reported
3.4.8	Monitoring of TS concentration	No
3.4.9	Statistics	LC_{50} values and their 95% confidence intervals were calculated according to the Litchfield and Wilcoxon (1949) method.

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
4.1.3	Nature of adverse effects	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Not reported
4.2.2	Actual concentrations of test substance	Not reported
4.2.3	Effect data (Mortality)	See table A7_4_1_1-7
4.2.4	Concentration / response curve	Not reported
4.2.5	Other effects	Not reported
4.3	**Results of controls**	
4.3.1	Number/ percentage of	Not reported

Section A7.4.1.1(2) **Acute toxicity to fish**

Annex Point IIA7.1

IUCLID 4.1/02,03,05,07 **Acute toxicity of Zineb, DIDT, ETU and EU to fish.**

	animals showing adverse effects	
4.3.2	Nature of adverse effects	Not reported
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**	Guppies (*Poecilia reticulata*) were exposed in separate semi-static tests to Zineb and its degradates (DIDT, ETU and EU) for 96 hours. Test solutions were renewed daily. While some details are omitted from this compilation journal article, the studies were carried out in accordance with OECD Guideline 203 and are considered to be fit for the purpose of describing the acute toxicity of Zineb and its significant degradates to fish.
5.2	**Results and discussion**	LC_{50} values and corresponding 95% confidence limits are reported for zineb and its significant degradates DIDT, ETU and EU. Results indicate that ETU and EU are significantly less toxic than the parent zineb, while DIDT is more toxic.
5.2.1	LC_0	Not reported.
5.2.2	LC_{50}	All values as nominal concentrations (with 95% confidence limits). Zineb: 96 h LC_{50} = 7.2 mg/l (5 – 10.3 mg/l). DIDT: 96 h LC_{50} = 0.49 mg/l (0.32 – 1.0 mg/l). ETU: 96 h LC_{50} = 7500 mg/l (5,600 – 10,000 mg/l). EU: 96 h LC_{50} = 13,000 mg/l (10,000 – 18,000 mg/l).
5.2.3	LC_{100}	Not reported.
5.3	**Conclusion**	Based on conduct of the studies following OECD Guideline 203 and with no suggestion to the contrary, the studies may reasonably be assumed 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 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, DIDT, ETU and EU to freshwater fish. The results demonstrate the relative toxicity of the parent zineb compound and its significant degradates.
5.3.2	Reliability	2
5.3.3	Deficiencies	Yes. This journal article is 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.

Section A7.4.1.1(2) **Acute toxicity to fish**

Annex Point IIA7.1

IUCLID 4.1/02,03,05,07 **Acute toxicity of Zineb, DIDT, ETU and EU to fish.**

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

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

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Laboratory prepared dilution water
Alkalinity	Not reported
Hardness	260 mg/L as $CaCO_3$
pH	8.1 ± 0.2
Oxygen content	Not reported
Conductance	Not reported
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	*Poecilia reticulata* (guppy)
Source	Laboratory cultures
Wild caught	No
Age/size	Not reported
Kind of food	Not fed during study
Amount of food	Not applicable
Feeding frequency	Not applicable
Pretreatment	Not reported
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Semistatic
Renewal of test solution	Renewed daily using stock solutions made fresh daily
Volume of test vessels	Not reported
Volume/animal	Not reported
Number of animals/vessel	Not reported
Number of vessels/ concentration	Duplicate vessels per concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	20°C
Dissolved oxygen	Not reported
pH	8.1 ± 0.2
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	Not reported
Photoperiod	Not reported

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (nominal/measured)[1] [mg/l]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Not reported								
Temperature [°C]								
pH								
Oxygen [mg/l]								

[1] specify, if TS concentrations were nominal or measured

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	95 % c.l.	96 h [mg/l][1]	95 % c.l.
LC_0	*7.5 (n)*			
LC_{50}			All values as nominal concentrations Zineb: 7.2 DIDT: 0.49 ETU: 7500 EU: 13,000	 5.0-10.3 0.32-1.0 5600-10,000 10,000-18,000
LC_{100}				

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

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

	fulfilled	Not fullfilled
Mortality of control animals <10%	x	
Concentration of dissolved oxygen in all test vessels > 60% saturation	x	
Concentration of test substance ≥80% of initial concentration during test		x

Criteria for poorly soluble test substances	n.a.	

Section A7.4.1.1(3) Acute toxicity to fish

Annex Point IIA7.1

Acute Toxicity of ETU to Rainbow Trout (*Oncorhynchus mykiss* WALBAUM 1792) in a Static System (96 hours).

IUCLID 4.1/04

Official use only

1 REFERENCE

1.1 Reference

[redacted] (2001) Acute Toxicity Study in Rainbow Trout (*Oncorhynchus mykiss* WALBAUM 1792) in a Static System (96 hours). BASF Aktiengesellschaft, Experimental Toxicology and Ecology, 67056 Ludwigshafen, Germany, Report No. 12F0533/005042, 15 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/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study Yes

OECD-Guideline 203. Fish Acute Toxicity Test

2.2 GLP Yes

2.3 Deviations No

3 MATERIALS AND METHODS

3.1 Test material Reg. No. 146099, CAS No. 96-45-7

3.1.1 Lot/Batch number: 01743-136

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 stated in report

3.1.6 Method of analysis: Analytical method: CF-A 446 UPLC method and calibration with external standard.

Column: Nucleosil 120 S C18, 250 mm x 4.0 mm.

Mobile phase: Water + 0.05% THF.

Injection volume: 100 µl.

Flow rate: 1.0 ml/min.

Detection: UV at 233 nm.

Oven temperature: Ambient.

The limit of quantification of the analytical method was approximately 0.2 mg/l. The samples were quantified by

			external calibration or 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. No interference from the matrix with the test substance could be observed under the conditions of the study. The identity of the test substance was confirmed by comparison of the mean HPLC retention time of the reference substance with the mean retention time of the corresponding peak of the test substance.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**		See table A7_4_1_1-1
3.3	**Reference substance**		No
	3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**		
	3.4.1	Dilution water	See table A7_4_1_1-2
	3.4.2	Test organisms	See table A7_4_1_1-3
	3.4.3	Test system	See table A7_4_1_1-4
	3.4.4	Test conditions	See table A7_4_1_1-5
	3.4.5	Duration of the test	96 hours
	3.4.6	Test parameter	Adverse symptoms, mortality
	3.4.7	Sampling	Samples were collected for analysis at 1, 48 and 96 hours after commencement of the test and were either analysed immediately or stored at ambient temperature and analysed within 24 hours.
	3.4.8	Monitoring of TS concentration	Yes, at 1, 48 and 96 hours
	3.4.9	Statistics	If possible, the median lethal concentration (LC_{50}) after 1. 4, 24. 48, 72 and 96 hours, based on the nominal concentrations and based on the mean of the analytically determined concentrations is calculated using probit analysis*. If possible the LC 5 and LC 95 are given as well. Symbols of the model for the dose response relationship are as follows:- F(P) A+E*LN(K) K = concentration P = relative frequency of dead animals after exposure with K F = inverse function of the cumulative standard normal distribution

Section A7.4.1.1(3)

Annex Point IIA7.1

IUCLID 4.1/04

Acute toxicity to fish

Acute Toxicity of ETU to Rainbow Trout (*Oncorhynchus mykiss* WALBAUM 1792) in a Static System (96 hours).

LN = natural logarithm

A, B = model parameters

In situations where the data obtained are inadequate for statistical methods of calculation of the LC_{50}, an approximate LC_{50} is calculated as the geometric mean of LC_0 and LC_{100}.

*Finney, D.J., Probit Analysis. Cambr. Univ. Press, 3rd ed., 1971; certain aspects of this method have been modified.

4 RESULTS

4.1	**Limit Test**		Not performed
	4.1.1	Concentration	Not applicable
	4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
	4.1.3	Nature of adverse effects	Not applicable
4.2	**Results test substance**		
	4.2.1	Initial concentrations of test substance	Nominal concentrations of 0 (Control), 22, 50, 100, 220 and 500 mg/L
	4.2.2	Actual concentrations of test substance	See table A7_4_1_1-9 for details of measured concentrations after 1, 48 and 96 h expressed as determined and % of nominal.
	4.2.3	Effect data (Mortality)	Mortality data are summarised in table A7_4_1_1-6 LC_0, LC_{50}, and LC_{100} values for at 48 and 96 h are summarised in table A7_4_1_1-7
	4.2.4	Concentration / response curve	See Figure A7_4_1_1-1
	4.2.5	Other effects	1 Fish at 500 mg/L (nominal) showed tumbling behaviour at 48h.
4.3	**Results of controls**		
	4.3.1	Number/ percentage of animals showing adverse effects	No Control group animals suffered adverse effects or mortality (see table A7_4_1_1-6)
	4.3.2	Nature of adverse effects	None observed
4.4	**Test with reference substance**		Not performed
	4.4.1	Concentrations	Not applicable
	4.4.2	Results	Not applicable

Section A7.4.1.1(3)

Annex Point IIA7.1

IUCLID 4.1/04

Acute toxicity to fish

Acute Toxicity of ETU to Rainbow Trout (*Oncorhynchus mykiss* WALBAUM 1792) in a Static System (96 hours).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

This study was conducted to comply with OECD guideline No. 203 'Fish Acute Toxicity Test' July 1992. No deviations were noted.

The study was designed to assess the acute toxic effects of the test compound on the Rainbow trout (Oncorhynchus nykiss WALBAUM 1792) over a range of concentrations.

Based on the results of a range finding test (LC_{50} after 96 h >500 mg/l) the concentrations, spaced by a factor of about 2.2, were fixed as follows for the definitive test: 0, 0, 22, 22. 50, 50, 100. 100, 220, 220, 500, 500 mg/l. A static system was used.

5.2 Results and discussion

The analytically detected concentrations were within a range of ± 10 % of the theoretical values over the whole study period. The following results were obtained based on mean analytically detected concentrations:

Exposure time	**24h**	**28h**	**72h**	**96h**
LC0 (mg/l)	501.95	218.74	218.74	218.74
LC50 (mg/l)	> 501.95	ca 1390.23	ca 1390.23	ca 1390.23
LC100 (mg/l)	> 501.95	> 501.95	> 501.95	> 501.95

The no observed effect concentration NOEC after 96 hours was 220 mg/l based on nominal concentrations and 218.7 mg/l based on mean analytically detected concentrations.

In conclusion, under the conditions of this test the LC_{50} after 96 hours was > 500 mg/l.

5.2.1 LC_0 220 mg/l (nominal)

5.2.2 LC_{50} >500 mg/l (nominal)

5.2.3 LC_{100} >500 mg/l (nominal)

5.3 Conclusion The validity criteria can be considered to have been fulfilled.

5.3.1 Other Conclusions None

5.3.2 Reliability 1

5.3.3 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

Section A7.4.1.1(3)	**Acute toxicity to fish**
Annex Point IIA7.1	**Acute Toxicity of ETU to Rainbow Trout (*Oncorhynchus mykiss* WALBAUM 1792) in a Static System (96 hours).**
IUCLID 4.1/04	

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

Criteria	Details
Dispersion	Yes The mixture was homogenised with an ultra-turrax stirrer.
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	No- not applicable
Other procedures	Since the dissolvation of the test substance was slow, the test solutions were prepared the day before study initiation.

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Municipal water, city of Frankenthal (non-chlorinated), charcoal filtered and aerated prior to use
Alkalinity	Not stated
Hardness	*ca* 2.5 mmol/L (= ca 250 mg $CaCO_3$/L)
pH	*ca* 8.0-8.6
Oxygen content	>60% of maximum saturation
Conductance	Not stated
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Rainbow Trout (*Oncorhynchus mykiss* WALBAUM 1792)
Source	[redacted]
Wild caught	No
Age/size	*ca* 4 months / 4.7-5.5 cm / 0.8-1.4 g
Kind of food	Growing feed 'Forellenfutter (Zeigler)', NAFAG AG, Gosau, Switzerland, frozen and live brine shrimp (artemia).
Amount of food	Growing feed: Ad libitum Shrimp: Not stated
Feeding frequency	Growing feed: Ad libitum

	Shrimp: Not stated
Pretreatment	14 days acclimatisation in flow through tank in non-chlorinated tap-water, passed through a charcoal filter and aerated with oil-free air. Food was withdrawn 1 day before exposure.
Feeding of animals during test	No.

Table A7_4_1_1-4: Test system

Criteria	**Details**
Test type	Static
Renewal of test solution	Not applicable
Volume of test vessels	84 L containing 25 L of test solution
Volume/animal	2.5 L
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	Not stated

Table A7_4_1_1-5: Test conditions

Criteria	**Details**
Test temperature	See Table A7_4_1_1-10
Dissolved oxygen	See Table A7_4_1_1-11
pH	See Table A7_4_1_1-12
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	Not stated
Photoperiod	16 hour photoperiod daily

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (nominal) [mg/l]	Mortality[1]							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0 (Control)	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0
220	0	0	0	0	0	0	0	0
500	0	2	2	2	0	10	10	10
Temperature [°C]	11-12°C. See table A7_4_1_1-10							
pH	8.2-8.6. See table A7_4_1_1-12							
Oxygen [mg/l]	7.3-10.3. See table A7_4_1_1-11							

[1] Number and % Mortality are taken as a total of the 2 tanks at each concentration

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	95 % c.l.	96 h [mg/l][1]	95 % c.l.
LC_0	220	-	220	-
LC_{50}	>500	-	>500	-
LC_{100}	>500	-	>500	-

[1] data are based on nominal concentrations

- Confidence Intervals could not be calculated from the data

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

	fulfilled	Not fullfilled
Mortality of control animals <10%	X	
Concentration of dissolved oxygen in all test vessels > 60% saturation	X	
Concentration of test substance ≥80% of initial concentration during test	X	

Criteria for poorly soluble test substances	X	

Table A7_4_1_1-9: Determined Concentration of Test Substance over Period of Test

Test-Substance Concentration (nominal) [mg/l]	Determined Concentration of Test Substance after			Mean
	1 h	48 h	96 h	
0	ND	ND	ND	ND
0	ND	ND	ND	ND
22	21.5 (97.7%)	21.5 (97.7%)	21.79 (99.0%)	21.60 (98.2%)
22	21.58 (98.1%)	21.48 (97.6%)	21.88 (99.5%)	21.65 (98.4%)
50	50.D1 (100.0%	49.29 (98.6%)	50.73 (101.5%)	50.01 (100.0%)
50	49.16 (99.5%)	48.98 (98.0%)	50.58 (101.2%)	49.77 (99.5%)
100	102.30 (102.3%)	100.99 (101.0%)	102.27 (102.3%)	101.85 (101.9%)
100	99.96 (100.0%)	98.98 (99.0%)	100.02 (100.0%)	99.65 (99.7%)
220	216.10 (98.2%)	213.18 (96.9%)	225.52 (102.5%)	218.27 (99.2%)
220	220.05 (100.0%)	216.41 (98.4%)	221.17 (100.5%)	219.21 (99.6%)
500	503.92 (100.8%)	484.71 (96.9%}	504.85 (101.0%)	497.83 (99.6%)
500	499.22 (99.8%)	503.62 (100.7%)	515.36 (103.1%)	506.07 (101.2%)

ND = Not detected.

Data are presented as determined concentrations with % of nominal in brackets.

Each result is the mean of 2 measurements.

Table A7_4_1_1-10: Temperatures over Period of Test

Test-Substance Concentration (nominal) [mg/l]	Test Temperature (°C) after				
	1 h	24 h	48 h	72 h	96 h
0	12	12	12	12	12
0	12	12	11	12	11
22	12	12	12	12	12
22	12	12	12	12	12
50	12	11	11	12	11
50	12	11	11	11	11
100	12	11	11	12	12
100	12	12	12	12	12
220	12	12	12	12	12
220	12	12	12	12	12
500	12	12	12	12	12
500	12	12	12	12	12

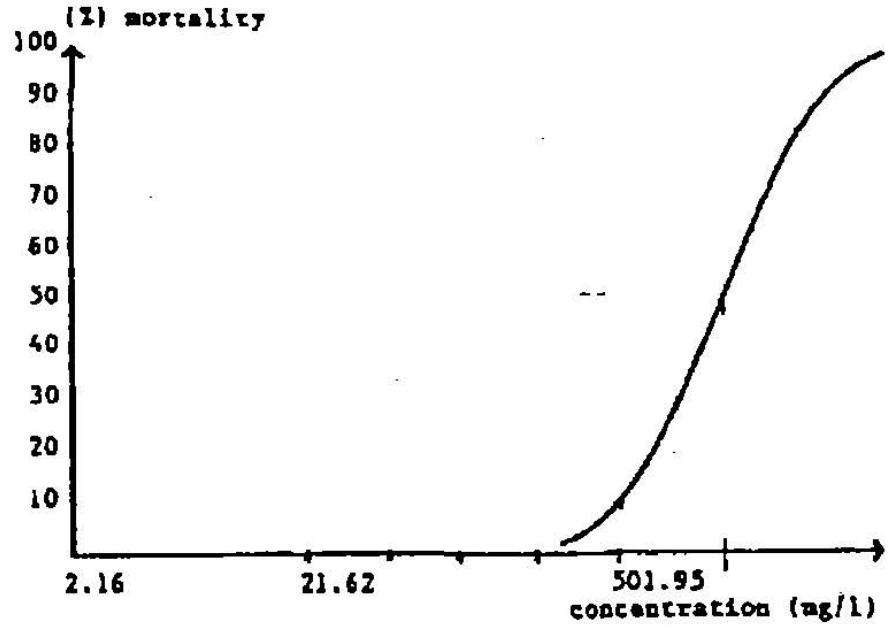
Table A7_4_1_1-11: Dissolved Oxygen over Period of Test

Test-Substance Concentration (nominal) [mg/l]	Oxygen content (mg/l) after				
	1 h	24 h	48 h	72 h	96 h
0	10.2	8.6	8.5	8.2	8.5
0	10.2	8.7	8.3	7.8	8.1
22	10.2	8.5	8.1	7.6	7.8
22	10.2	8.4	7.8	7.3	7.6
50	10.3	8.9	8.5	8.1	8.6
50	10.3	8.8	8.6	8.2	8.4
100	10.3	8.9	8.4	7.8	8.1
100	10.2	8.2	7.9	7.8	8.2
220	10.3	9.1	8.7	8.2	8.5
220	10.2	8.6	8.2	7.7	7.8
500	10.3	8.9	8.8	8.2	8.3
500	10.3	8.9	8.7	8.2	8.4

Table A7_4_1_1-12: pH over Period of Test

Test-Substance Concentration (nominal) [mg/l]	pH After				
	1 h	24 h	48 h	72 h	96 h
0	8.6	8.5	8.4	8.4	8.3
0	8.5	8.4	8.3	8.3	8.2
22	8.5	8.4	8.3	8.3	8.2
22	8.6	8.5	8.4	8.3	8.3
50	8.6	8.5	8.4	8.3	8.3
50	8.6	8.5	8.4	8.3	8.3
100	8.5	8.5	8.4	8.4	8.3
100	8.6	8.4	8.3	8.3	8.3
220	8.6	8.5	8.4	8.4	8.3
220	8.5	8.4	8.3	8.3	8.2
500	8.5	8.4	8.4	8.4	8.4
500	8.6	8.5	8.4	8.4	8.3

Figure A7_4_1_1-1: LC 50 after 96 hour (mean .analytically detected concentrations)



Section A7.4.1.1(4) Acute toxicity to fish

Annex Point IIA7.1

Ethylene Urea: A 96-Hour Static Acute Toxicity Study with Rainbow Trout (*Oncorhynchus mykiss*)

IUCLID 4.1/06

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1 REFERENCE

1.1	**Reference**	[redacted] (2001a) Ethylene Urea: A 96-Hour Static Acute Toxicity Study with the Rainbow Trout (*Oncorhynchus mykiss*). Wildlife International, Ltd., 8598 Commerce Drive, Easton, Maryland 21601, USA. Report No. 299A-115, 29 January 2001.
1.2	**Data protection**	Yes
1.2.1	Data owner	EBDC/ETU Taskforce: 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-Guideline 203. Fish Acute Toxicity Test
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Ethylene Urea
3.1.1	Lot/Batch number	01743-141
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	90.8%
3.1.4	Composition of Product	Not applicable.
3.1.5	Further relevant properties	None stated in report.
3.1.6	Method of analysis	The method used for the analysis of ethylene urea in freshwater was based upon methodology provided by the Sponsor entitled: "HPLC Analytical Method for Ethylene Thiourea in Detergent Solution", Method Number KP-017-00. The analytical method consisted of diluting the samples in freshwater, as necessary, and analysing by direct injection high performance liquid chromatography (HPLC) at 200nm. Concentrations of ethylene urea in the samples were determined by HPLC using a Hewlett Packard Model 1090 High Performance Liquid Chromatograph (HPLC) equipped with a Jasco Model 975 Variable Wavelength Detector. Chromatographic separations were achieved using a Phenomenex LUNA C18 column (250 mm x 4.6 mm, 5 µm particle size) using the following conditions. Flow rate: 1.00 ml/min

Oven temperature: 40°C

Solvent A: H_2O

Solvent B: CH_3CN

Injection volume: 35 µl

Ethylene urea retention time: Approximately 4.1 Minutes

Gradient profile:

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

Calibration standards of ethylene urea, ranging in concentration from 5.00 to 50.0 mg/L, were analysed with each sample set. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The concentrations of ethylene urea in the samples were determined by substituting the peak area responses of the samples into the applicable linear regression equation.

The method limit of quantification (LOQ) for these analyses was defined as 5.00 mg/L, calculated as the product of the concentration of the lowest calibration standard (5.00 mg/L) and the dilution factor of the matrix blank sample (1.00) analysed concurrently with the test samples. Two matrix blank samples were analysed to determine possible interferences.

3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_1-1)
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Dilution water	See table A7_4_1_1-2
3.4.2	Test organisms	See table A7_4_1_1-3

Section A7.4.1.1(4) **Acute toxicity to fish**

Annex Point IIA7.1 **Ethylene Urea: A 96-Hour Static Acute Toxicity Study with Rainbow Trout (*Oncorhynchus mykiss*)**

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3.4.3	Test system	See table A7_4_1_1-4
3.4.4	Test conditions	See table A7_4_1_1-5
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Adverse symptoms, mortality
3.4.7	Sampling	Samples were collected for analysis at 0, 48 and 96 hours after commencement of the test and were analysed immediately.
3.4.8	Monitoring of TS concentration	Yes, at 0, 48 and 96 hours
3.4.9	Statistics	The absence of mortality during the study precluded the statistical calculation of an LC50 value using the appropriate computer program. The program was designed to calculate the LC50 value and the 95% confidence interval by probit analysis, the moving average method, and binomial probability with nonlinear interpolation. Therefore, the 24, 48, 72 and 96-hour LC50 values, as well as the no mortality concentration and the 96-hour NOEC, were determined by visual interpretation of the mortality and observation data.

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
4.1.3	Nature of adverse effects	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal concentrations of 0 (Control), 16, 26, 43, 72 and 120 mg/L after correction for the stated purity.
4.2.2	Actual concentrations of test substance	See table A7_4_1_1-9 for details of measured concentrations after 0, 48 and 96 h expressed as determined and % of nominal.
4.2.3	Effect data (Mortality)	Mortality data are summarised in table A7_4_1_1-6 LC_0, LC_{50}, and LC_{100} values for at 48 and 96 h are summarised in table A7_4_1_1-7
4.2.4	Concentration / response curve	Not applicable as no mortality was observed during the study.
4.2.5	Other effects	No adverse effects of the test substance were observed at any of the

		concentrations studied over the duration of the study.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No Control group animals suffered adverse effects or mortality (see table A7_4_1_1-6
4.3.2	Nature of adverse effects	None observed
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 study was conducted according to OECD Guideline 203 and ASTM Standard E729-88a. The study was conducted in accordance with the principles of GLP. Rainbow trout were exposed to a geometric series of five test concentrations and a negative (well water) control. Two replicate test chambers were maintained in each treatment and control group, with 10 trout in each test chamber for a total of 20 trout per test concentration. Nominal test concentrations were selected in consultation with the Sponsor, and were based upon the results of exploratory rangefinding toxicity tests. Nominal test concentrations selected were 16, 26, 43, 72 and 120 mg tested substance (t.s.)/L, and were adjusted to 100% based on a reported test substance purity of 90.8%. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, at 48 hours and at test termination. Rainbow trout were impartially assigned to exposure chambers at test initiation. Observations of mortality and other signs of toxicity were made approximately 1.5, 24, 48, 72 and 96 hours after test initiation. Cumulative percent mortality observed in the treatment groups was used to estimate or calculate LC50 values at 24, 48, 72 and 96 hours.
5.2	**Results and discussion**	Daily observations of mortality, immobility and other signs of toxicity observed during the test are presented in Table 7_4_1_1-6. Rainbow trout in the negative control group appeared healthy and normal throughout the test. Trout in the 16, 26, 43, 73 and 122 mg t.s.IL treatment groups also appeared normal throughout the test with no mortalities or overt signs of toxicity. LC50 values at 24, 48, 72 and 96 hours were estimated from the mortality data and are presented in Table 7_4_1_1-7.
5.2.1	LC_0	122 mg test substance/L
5.2.2	LC_{50}	>122 mg test substance/L

Section A7.4.1.1(4) Acute toxicity to fish

Annex Point IIA7.1

Ethylene Urea: A 96-Hour Static Acute Toxicity Study with Rainbow Trout (*Oncorhynchus mykiss*)

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5.2.3	LC_{100}	>122 mg test substance/L
5.3	**Conclusion**	The 96-hour LC50 value for rainbow trout, Oncorhynchus mykiss, exposed to ethylene urea was > 122 mg t.s./L, the highest concentration tested. Rainbow trout exposed to ethylene urea at concentrations up to 122 mg t.s./L appeared normal with no mortality or overt signs of toxicity. The no mortality concentration and the 96-hour NOEC were 122 mg t.s./L.
5.3.1	Other Conclusions	None
5.3.2	Reliability	1
5.3.3	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*