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

Annex Point IIA-VII.7.6.2.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	Ref.: A 7.1.1.1.2/03 [redacted] (2006): Aqueous photolysis of Cu-HDO [-U-^{14}C]; [redacted] unpublished, report No. 242482, [redacted] 2006/1019626, (June 2006)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	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: Directives 94/37/EEC, and 91/414/EEC SETAC Procedures for assessing the Environmental Fate and Ecotoxicity of Pesticides, SETAC Europe, Brussels, March 1995 OECD Principles of Good Laboratory Practice	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cu-HDO	
3.1.1	Lot/Batch number	751-1023	
3.1.2	Specification	Labelled stock solution, 12.8mg of [U-^{14}C] Cu-HDO in 10 ml Acetonitrile	
3.1.3	Purity	> 95% radiochem. purity	
3.1.4	Radiolabelling	[U-^{14}C] - Batch: 751-1023 - Specific activity: 3.81 MBq/mg - Radiochemical purity: >95%	
3.1.5	UV/VIS absorption spectra and absorbance value	UV/VIS absorption of Cu-HDO; absorption maximum at 292.5 nm. 292.5nm: A = 0.215 AU 300nm: A = 0.177 AU 400nm: A = 0.015 AU 500nm: A = 0.003 AU	
3.1.6	Further relevant properties	Solubility in water: approx. 6mg/L at pH=7 Hydrolytic stability: hydrolytically stable at pH 7	
3.2	**Reference substances**	Yes: Pyridine and p-nitroacetophenone (PNAP) (quantum yields of these test substances were determined for system validation)	
3.3	**Test solution**	A description of the preparation of the test solution is given in tabular form (see enclosed table A7_1_1_1_2_01-1).	

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

Annex Point IIA-VII.7.6.2.2

3.4	**Testing procedure**	
3.4.1	Test system	A detailed description of the test system is given in tabular form (see enclosed table A7_1_1_1_2_01-2).
3.4.2	Properties of light source	A detailed description of the artificial light source is given in tabular form (see enclosed table A7_1_1_1_2_01-2).
3.4.3	Determination of irradiance	Lamp: Xenon lamp Intensity: 3mW/cm² simulating a clear summer day Filter: UV filter to cut off wavelengths<290nm Data for the light intensity of the used irradiation source are provided by the manufacturer. As the same light source was used both for the irradiation of the actinometer solutions and the respective solutions of the test item, a standardisation of the light intensity used during the experiment was not necessary.
3.4.4	Temperature	20±3°C
3.4.5	pH	Buffer solution: pH 7 (Titrisol Merck 1.09887, phosphate buffer, pH=7)
3.4.6	Duration of the test	2 days continuously
3.4.7	Number of replicates	Duplicate sample and 1 control (dark control) replicate per sampling interval
3.4.8	Sampling	Sampling times: 0 h, 2 h, 4 h, 6 h, 24 h and 48 h
3.4.9	Analytical methods	All samples were measured for radioactivity (LSC) and were analysed by HPLC to determine a metabolite pattern. Identification of main metabolites was performed by GC/MS analysis. Radiocarbon determinations procedure (LSC): TRI-CARB –2900 TR (Perkin Elmer, Rodgau, Germany). HPLC: HPLC LC 176, Gynkothek HPLC pump 580, Kontron HPLC/UV detector 535, HPLC radioactivity monitor Berthold LB 509 GC/MS: Finnigan TSQ 7000 GC-MS EI
3.5	**Transformation products**	Yes
3.5.1	Method of analysis for transformation products	Transformation products were measured by HPLC and identified by GC/MS described under 3.4.9
		4 RESULTS
4.1	**Screening test**	Not performed
4.2	**Actinometer data**	Actinometer system acc. to Dulin and Mill: Pyridine and p-nitroacetophenone (PNAP), with $c_{pyridine}$ = 0.78mol/L and $c_{PNAP}=2x10^{-5}$ mol/L Quantum yield actinometer: Φ = 0.013 Dt_{50} [h]: 5 h.
4.3	**Controls**	Yes, incubation in the dark: when incubated in the dark, Cu-HDO was shown to be stable.
4.4	**Photolysis data**	
4.4.1	Concentration values	Photolysis data are defined as % of applied radioactivity

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

Annex Point IIA-VII.7.6.2.2

4.4.2 Mass balance

Photolysis of Cu-HDO at pH 7 – material balance

Time after treatment (hours)	Photolysis					Dark control
	Water (% TAR)	Volatiles			Total (% TAR)	Water (% TAR)
		NaOH trap	Ethylene glycol trap	H_2SO_4 trap		
0	100.0	n.a.	n.a.	n.a.	100.0	100.00
2	100.3	0.00	0.07	0.00	100.4	96.9
4	99.9	0.01	0.18	0.00	100.1	98.6*
6	101.0	0.00	0.03	0.02	101.1	96.6
24	101.2	0.08	1.59	0.02	102.8	98.5
48	99.8	0.11	1.54	0.03	101.5	98.7

**duplicate sample not considered due to poor recovery (<95 5)*

n.a. = not analyzed

TAR = Total applied radioactivity

4.4.3 k_p

Estimated photolysis rate constant k_p.(1/d) for the test substance (SFO kinetic):

pH 7: 0.1185

4.4.4 Kinetic order

First order

4.4.5 k_p / k_a

Estimated photolysis rate constant k_p.(1/d) for the actinometer photolysis (SFO kinetic): 0.1539. The ratio of k_p / k_a was not used in the report.

4.4.6 Reaction quantum yield

The quantum yield of Cu-HDO was calculated to be $\Phi = 0.0276$

4.4.7 k_{pE}

The direct photolysis sunlight rate constant of the test substance in water bodies in the environment is not given in the report, but can be easily calculated by assuming 1st order kinetics and using the equation $t_{1/2} = \ln 2 / k_{pE}$ and the data from 4.4.8

4.4.8 Half-life ($t_{1/2E}$)

The experimental half-life (DT_{50}) of Cu-HDO was calculated to be 5.9 hours.

The experimental DT_{90} of Cu-HDO is calculated to be 19.4 hours.

Estimation of half-lives of Cu-HDO in the top layer of aqueous systems under Central European conditions: (software used: Quantum 301).

Assuming a concentration of 1 mg/L Cu-HDO in the top layer with a thickness of 1cm the DT_{50} is considered to be < 1 hour during the months April-August.

4.5 Specification of the transformation products

See enclosed table A7_1_1_1_2_01-3

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

Annex Point IIA-VII.7.6.2.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was performed in accordance with FAO revised guidelines on Environmental Criteria for Registration of Pesticides cited within 94/37/EEC and SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, SETAC Europe, Brussels, March 1995.

Actinometer system according to Dulin and Mill: Pyridine and p-nitroacetophenone (PNAP), with $c_{pyridine}$ = 0.78mol/L and $c_{PNAP}=2x10^{-5}$ mol/L

5.2 Results and discussion

5.2.1 k_p

Estimated photolysis rate constant k_p.(1/d) for the test substance (SFO kinetic):

pH 7: 0.1185

5.2.2 K_{pE}

The direct photolysis sunlight rate constant of the test substance in water bodies in the environment is not given in the report, but can be easily calculated by assuming 1st order kinetics and using the equation $t_{1/2} = \ln 2/k_{pE}$ and the data from 4.4.8

5.2.3 ϕ

The quantum yield of Cu-HDO was calculated to be

$\Phi = 0.0276$

5.2.4 $t_{1/2E}$

The experimental half-life (DT50) of Cu-HDO was calculated to be 5.9 hours.

The experimental DT90 of Cu-HDO is calculated to be 19.4 hours.

Estimation of half-lives of Cu-HDO in the top layer of aqueous systems under Central European conditions: (software used: Quantum 301)

Assuming a concentration of 1mg/L Cu-HDO in the top layer with a thickness of 1cm the DT50 is considered to be < 1 hour during the months April-August.

5.3 Conclusion

Photolysis of Cu-HDO in water showed rapid degradation of the test item and the formation of [redacted] (45% TAR after 48 hours) and [redacted] (51% TAR after 48 hours), which further degraded to volatile degradation products of low molecular weight, e.g. carbon dioxide. No other metabolite above 5% TAR occurred.

Cu-HDO is readily degraded by aqueous photolysis; the experimental half-life is 6 hours under condition irradiation. In the dark control, no degradation of Cu-HDO was observed. The calculated half-life for the top-layer of aqueous systems considering the quantum yield of Cu-HDO was less than 1 hour.

5.3.1 Reliability: 1

5.3.2 Deficiencies: No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*July 2006*
Materials and Methods	*Applicant's version is acceptable*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	Table A7_1_1_1_2_01-2: Description of test system, Light intensity: 3 mW/cm²

Table A7_1_1_1_2_01-1: Description of test solution and controls.

Criteria	Details
Purity of water	Buffer solution: pH 7 (Titrisol Merck 1.09887, phosphate buffer, pH=7)
Preparation of test chemical solution	For the preparation of the test solution with a concentration of 3mg/L, a volume of 1.2ml (corresponding to 1.5mg) of Cu-HDO were brought to a volume of 500mL with the buffer solution. Subsets of 18mL were used as dark control. For the identification of degradation products, a separate test with a volume of 1.5 L test solution was conducted.
Test concentrations	3mg /L
Temperature	20±3°C
Preparation of a.s. solution	See "Test solutions" above
Controls	Yes, incubations in the dark
Identity and concentration of cosolvent	Buffer solution

Table A7_1_1_1_2_01-2: Description of test system.

Criteria	Details	
Laboratory equipment	The experiment was performed in a SUNTEST device. The glass vessels (volume of 18ml) with a quartz glass covering were situated in rectangular thermostated blocks. Each vessel had an air inlet and and an air outlet. The incoming air was sterilised, moistened and CO_2 was removed. Items volatilised from the test solution were trapped in three different trapping solutions (Ethylenglycol, H_2SO_4, NaOH)	
Test apparatus	Suntest CPS	
Properties of artificial light source:		
Nature of light source	Xenon lamp, 3mW/cm²	
Emission wavelength spectrum	290 – 800nm	
Light intensity	17.2W/m²	X
Filters	UV filter to simulate outdoor sunlight (UV-edge 290nm)	
Properties of natural sunlight:	natural sunlight was not used	
Latitude	n.a.	
Hours of daylight	n.a.	
Time of year	n.a.	
Light intensity	n.a.	
Solar irradiance ($L\lambda$)	n.a.	

n.a. = not applicable

Table A7_1_1_1_2_01-3: Specification and amount of transformation products (expressed as percentage of the applied radioactivity) after 2 day incubation in the light.

CAS-No	Common Name		Amount [%] of parent compound measured after 2 days
			PH=7
312600-89-8	Cu-HDO		1.1%
100-64-1	[redacted]		51.2%
108-94-1	[redacted]		44.5%
-	Others		3.0%

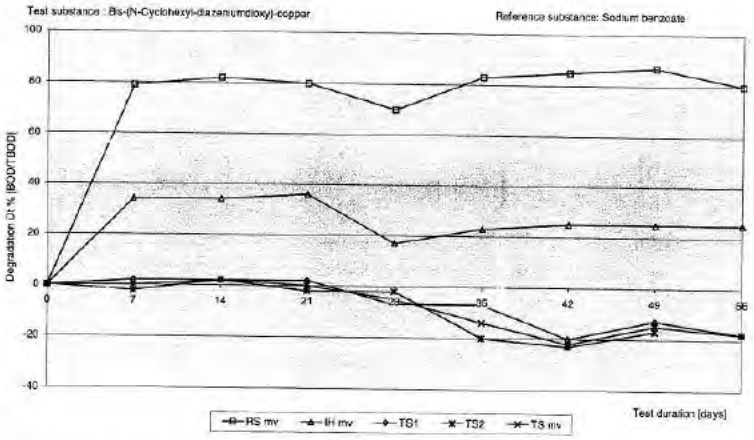
Section A7.1.1.2.1
Annex Point IIA7.6.1.1

Biodegradability (ready)

Official use only

		1 REFERENCE	
1.1	**Reference**	A 7.1.1.2.1 [redacted] (2001) BIS-(N-CYCLOHEXYLDIAZENIUMDIOXY)-COPPER, Determination of the biodegradability in the closed bottle test: 00/0801/23/1, [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	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 301 D "Ready Biodegradability: Closed Bottle Test"	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cu-HDO	
3.1.1	Lot/Batch number	W-86	
3.1.2	Specification	Solid, crystalline, blue	
3.1.3	Purity	99%	
3.1.4	Further relevant properties	Vapour pressure	< 0.0000001 hPa (20°C)
		Water solubility (23°C)	pH=4: 34.6mg/L Cu-HDO pH=7: 6.1mg/L Cu-HDO pH=9: 8.6mg/L Cu-HDO
		Adsorption potential (log Pow)	2.46 (25°C)
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms		
3.1.7	Specific chemical analysis	Test substance has been analyzed by UV/VIS spectroscopy after derivatization with $FeCl_3$ (method is described in Ref. A 4.1)	
3.2	**Reference substance**	Yes, Sodium benzoate	
3.2.1	Initial concentration of reference substance	2mg/l	
3.3	**Testing procedure**	Non-entry field	
3.3.1	Inoculum / test species	Effluent of a laboratory waste water plant	

Section A7.1.1.2.1 **Biodegradability (ready)**
Annex Point IIA7.6.1.1

3.3.2	Test system	Closed bottle test	
3.3.3	Test conditions	See table A7_1_1_2-4	x
3.3.4	Method of preparation of test solution	Test substance was directly added to the test flasks	
3.3.5	Initial TS concentration	2mg/l	
3.3.6	Duration of test	56 days	
3.3.7	Analytical parameter	Biochemical oxygen demand	
3.3.8	Sampling	The measurement of the oxygen consumption was performed on days 0, 7, 14, 21, 28, 35, 42, 49, 56	
3.3.9	Intermediates/ degradation products	Not identified	
3.3.10	Nitrate/nitrite measurement	The analysis of NO_2 and NO_3 was unnecessary. No oxygen consumption was measured in the test substance assays.	
3.3.11	Controls	Control without test substance (blank control), reference substance, inhibition control	
3.3.12	Statistics	Not necessary	
		4 RESULTS	
4.1	**Degradation of test substance**	Non-entry field	
4.1.1	Graph		
4.1.2	Degradation	Biodegradation degree (BOD/THOD) after 28 days:<10% Biodegradation degree (BOD/THOD) after 56 days:<10% Degree of substance specific elimination after 56 days:<10%	
4.1.3	Other observations	Not reported	
4.1.4	Degradation of TS in abiotic control	No abiotic control was performed	
4.1.5	Degradation of	80% -90% after 14 days	x

Section A7.1.1.2.1 **Biodegradability (ready)**
Annex Point IIA7.6.1.1

	reference substance	
4.1.6	Intermediates/ degradation products	Not determined
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	– Annex to EEC-Directive 92/69 EEC of 31 July 1992 Closed Bottle Test (Method C.4-E) Official Journal of the European Communities L383 A, 29 December 1992 – OECD Guidelines for Testing of Chemicals Ready Biodegradability – Closed Bottle Test 301 D, Paris 1993 – International Standard ISO 10707
5.2	**Results and discussion**	Biodegradation degree (BOD/THOD) after 28 days:<10% Biodegradation degree (BOD/THOD) after 56 days:<10% Degree of substance specific elimination after 56 days:<10%
5.3	**Conclusion**	The test substance is in this test poorly biodegradable (not readily biodegradable according to OECD criteria).
5.3.1	Reliability	1
5.3.2	Deficiencies	No

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2006
Materials and Methods	**3.3.3 Test conditions**
	Table A7_1_1_2-4: The test temperature at day 42 was 19.7°C (instead of 29.7°C; typing error)
Results and discussion	**4.1.5 Degradation of reference substance**
	82% (mean value) after 14 days
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	Acceptable
Remarks	

Table A7_1_1_2-1: Guideline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

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

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

Table A7_1_1_2-2: Inoculum / Test organism

Criteria	Details
Nature	*Effluent of a laboratory waste water plant fed with municipal sewage*
Species	*Not applicable*
Strain	*Not applicable*
Source	*Laboratory waste water plant fed with municipal sewage*
Sampling site	*Laboratory plant with municipal waste water*
Laboratory culture	*Cultured in the laboratory waste water plant*
Method of cultivation	*Mixtures of the test substance, a defined inorganic medium and a not pre-adapted inoculum (effluent of a municipal or laboratory waste water treatment plant) are incubated and aerated in closed bottles at room temperature (20±2°C) up to 28 days.*
Preparation of inoculum for exposure	*Inoculum was pre-aerated for one day*
Pretreatment	*No adaptation*
Initial cell concentration	*Not reported*

Table A7_1_1_2-3: Test system

Criteria	**Details**
Culturing apparatus	*Closed bottles*
Number of culture flasks/concentration	*2*
Aeration device	*Yes*
Measuring equipment	*Oxygen electrode*
Test performed in closed vessels due to significant volatility of TS	*The test substance is not volatile; however test is performed in closed bottles to allow the measurement of the oxygen demand.*

Table A7_1_1_2-4: Test conditions

Criteria	**Details**
Composition of medium	*Inorganic medium according to OECD guideline* 301 D *, composition is not reported*
Additional substrate	*No*
Test temperature	*Test day* / *Test temperature (°C)* *0* / *20,0* *7* / *20,9* *14* / *19,7* *21* / *20,0* *28* / *20,0* *35* / *20,0* *42* / *29,7* *49* / *20,1* *56* / *20,0*
pH	*Not reported*
Aeration of dilution water	*Not reported*
Suspended solids concentration	*Not reported*
Other relevant criteria	*Inoculum was pre-aerated for one day.* *Due to the poor water solubility the test substance was directly added to the test flasks*

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

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$		X
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		X
Criteria for validity		
Deviation of the degradation degree of the test substance in the plateau phase>20%	X	
Degradation degree of the reference substance>60% after 14 days	X	
Oxygen demand at the end of the test, blank control<1.5mg/l	X	
Oxygen concentration at the end of the test, test substance>0.5mg/l	X	
Degradation degree in the inhibition control>25% after 14 days	X	

The test is valid

Criteria for poorly soluble test substances	Not applicable	

Table A7_1_1_2-6: Pass levels and validity criteria for inherent biodegradability tests

	fulfilled	not fulfilled
Pass levels		
20% removal (DOC or COD);	Not applicable	
Pass values reached within 10-d window (within 28-d test period)	Not applicable	
Removal of reference substance (DOC or COD)>70% within 14 d	Not applicable	
Criteria for validity		
Percentage of DOC/COD-removal of reference compound≥70% within 14 days (OECD 302 B)	Not applicable	
Percentage of DOC-removal of reference compound≥40% within 7 days and≥65% within 14 days Average residual amount of test compound in blank tests≥40% (OECD 302 C)	Not applicable	
Removal curve of DOC or COD in the test suspension indicative for biodegradation (gradual elimination over days/weeks)	Not applicable	

Criteria for poorly soluble test substances	Not applicable	

Section A7.1.1.2.2 **Biodegradability (inherent)**
Annex Point IIA7.6.1.2

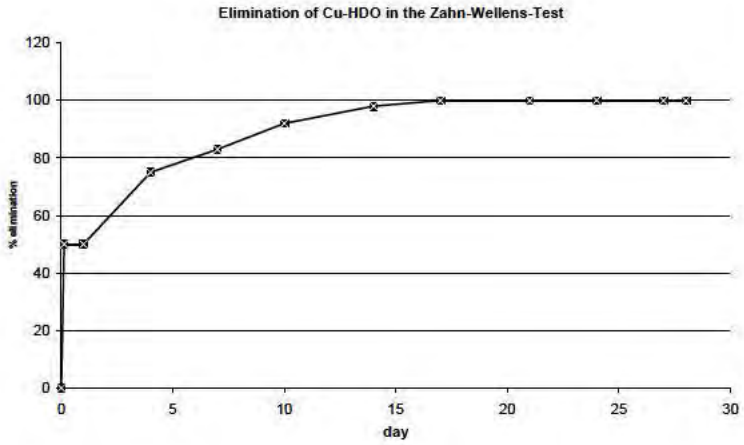
Official use only

		1 REFERENCE
1.1	**Reference**	A 7.1.1.2.2 [redacted] (1993) Determination of the biodegradability or the Elimination of BIS-(N-CYCLOHEXYLDIAZENIUMDIOXY)-KUPFER, Cu-HDO in the Zahn-Wellens-Test: Report 92/1699/10/1, [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	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 Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Cu-HDO
3.1.1	Lot/Batch number	Reu-E 7360 B
3.1.2	Specification	Solid, blue
3.1.3	Purity	99%
3.1.4	Further relevant properties	
3.1.5	Composition of Product	
3.1.6	TS inhibitory to microorganisms	
3.1.7	Specific chemical analysis	
3.2	**Reference substance**	Yes Diethylene glycol
3.2.1	Initial concentration of reference substance	
3.3	**Testing procedure**	Non-entry field
3.3.1	Inoculum / test species	activated sludge from laboratory plants with municipal waste water, no adaptation
3.3.2	Test system	Zahn-Wellens-Test
3.3.3	Test conditions	

Section A7.1.1.2.2 **Biodegradability (inherent)**
Annex Point IIA7.6.1.2

3.3.4	Method of preparation of test solution		
3.3.5	Initial TS concentration	*6mg/l*	x
3.3.6	Duration of test	*28 days*	
3.3.7	Analytical parameter	*DOC concentration*	
3.3.8	Sampling		
3.3.9	Intermediates/ degradation products		
3.3.10	Nitrate/nitrite measurement		
3.3.11	Controls	*Yes*	x
3.3.12	Statistics		

4 RESULTS

4.1	**Degradation of test substance**	Non-entry field	
4.1.1	Graph		
4.1.2	Degradation	90 - 100(±)% after 10 day(s)	x
4.1.3	Other observations		
4.1.4	Degradation of TS in abiotic control		
4.1.5	Degradation of reference substance	100% after 7 days	
4.1.6	Intermediates/ degradation products		

Section A7.1.1.2.2 **Biodegradability (inherent)**
Annex Point IIA7.6.1.2

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	– EU Directive 88/302/EWG – OECD 302 B – ISO 9888	x
5.2	**Results and discussion**	The test results indicate a total elimination of 90-100% after 10 days. Test duration: 28 days Adsorption onto activated sludge (3-h value): 50%	
5.3	**Conclusion**		x
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	x

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	January 2006
Materials and Methods	**3.3.5 Initial TS concentration** The substance was tested at inhibitory concentrations (6mg/l). EC20 = 2.5mg/l in the activated sludge respiration inhibition test. **3.3.11 Controls** A blanc control and a control with a reference substance have been performed.
Results and discussion	**4.1.2 Degradation** An elimination (adsorption and degradation) of 90 – 100% after 10 days was reached.
Conclusion	**5.1 Materials and methods** EC C.9 **5.3 Conclusion** Cu-HDO is rapidly eliminated from water. 50% of the elimination takes place within the first 3 hours and is due to adsorption. Therefore Cu-HDO cannot be regarded as being inherently and/or ultimately biodegradable. **5.3.2 Deficiencies** Cu-HDO has been tested at inhibitory concentrations.
Reliability	1
Acceptability	Acceptable
Remarks	-

Table A7_1_1_2-1: Guideline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

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

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

Table A7_1_1_2-2: Inoculum / Test organism

Criteria	Details
Nature	activated sludge
Species	
Strain	
Source	activated sludge from laboratory plants with municipal waste water
Sampling site	
Laboratory culture	
Method of cultivation	
Preparation of inoculum for exposure	
Pretreatment	No adaptation
Initial cell concentration	

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	DOC analyser
Number of culture flasks/concentration	
Aeration device	
Measuring equipment	
Test performed in closed vessels due to significant volatility of TS	

Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	
Additional substrate	
Test temperature	
pH	
Aeration of dilution water	
Suspended solids concentration	
Other relevant criteria	

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

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

Criteria for poorly soluble test substances		

Table A7_1_1_2-6: Pass levels and validity criteria for inherent biodegradability tests

	fulfilled	**not fulfilled**
Pass levels		
20% removal (DOC or COD);		
Pass values reached within 10-d window (within 28-d test period)		
Removal of reference substance (DOC or COD)>70% within 14 d		
Criteria for validity		
Percentage of DOC/COD-removal of reference compound≥70% within 14 days (OECD 302 B)		
Percentage of DOC-removal of reference compound≥40% within 7 days and≥65% within 14 days Average residual amount of test compound in blank tests≥40% (OECD 302 C)		
Removal curve of DOC or COD in the test suspension indicative for biodegradation (gradual elimination over days/weeks)		

Criteria for poorly soluble test substances		

A.7.1.2.2.2 Water/sediment degradation study

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1 REFERENCE

1.1	**Reference**	[redacted] (2008) Aerobic aquatic metabolism of ^{14}C Cu-HDO 2008/7007202, unpublished.	x
1.2	**Data protection**	Yes	
1.2.1	**Data owner**	*BASF SE*	
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 subdivision N, Section 162-4 (835.4300- study performed before revision of 835.4300 guideline in October 2008) Canada PMRA DACO number 8.2.3.5.4
2.2	**GLP** *(only where required)*	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	^{14}C Cu-HDO
3.1.1	Lot/Batch number	Batch 930-1004
3.1.2	Specification	Specific activity: 223,820 dpm/µg
3.1.3	Description	CAS 312600-89-8
3.1.4	Purity	Radiochemical purity is 100 %
3.2	**Test matrix**	Water and its associated sediment were collected from a pond located in Wabasha Country, Minnesota. The collection was performed by AgResource Inc., Arkansaw, WI. Characterisation of the water and sediment are reported in Table 1.
3.3	**Experimental Set-up**	The samples were prepared in individual 250 mL centrifuge bottles. Each sample contained approx. 120 mL of water and 50 g of associated sediment. The samples were placed in an incubator set at 25°C in complete darkness. A total of 24 samples were prepared to allow removal of duplicate samples for analysis at every sampling interval selected.
3.4	**Application procedure**	Each sample (water/sediment), a total of 18, was treated with 258.3 µg of ^{14}C-Cu-HDO, which resulted in a final concentration of 2.2 mg/L. This concentration corresponds to the EEC and is well below 50 % of

A.7.1.2.2.2 Water/sediment degradation study
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	the water solubility. Three additional water/sediment samples were used for identification of metabolites and further three water/sediment samples were kept untreated and were used for measurement of dissolved oxygen and redox potential.	
3.5 Experimental conditions and monitoring	Interval checks for pH, redox potential, and oxygen content in the water and pH and redox potential in the sediment were performed at each sampling interval using untreated controls. The sampling intervals were performed after 0, 3, 7, 10, 16, 24 and 30 days of incubation. The sampling details are summarized in table 2.	
3.6 Analytical methods	At each sampling interval the water content was separated from the sediment by centrifugation. The radioactivity was determined by liquid scintillation counting (LSC). Water samples were directly analyzed by HPLC. Sediment samples were extracted and the extract was separated and analysed by LSC and HPLC. The sample processing flow chart is attached in figure 1. The extracted samples were further analysed for bound residues The flow chart of the measurement is attached in figure 2. The quantification of residues in solutions was determined by LSC assays. The residues in sediments were estimated by combustion of samples followed by LSC assays. GC-MS was used for identification and characterization of the HPLC fraction collection to isolate HPLC peaks.	
	4 RESULTS	
4.1 Test conditions	The distribution and degradation of Cu-HDO was studied in a natural system of water and sediment. The water/sediment system was taken from a pond located in Wabasha Country, MN, USA. Radiolabelled ^{14}C-Cu-HDO was used and applied to the test system. The specific radioactivity of the active substance was 223,820 dpm/µg with a radiochemical purity of 98.6%. An application rate of 2.2 mg/kg Cu-HDO was used. For the isolation and identification of degradation products, some water/sediment systems were additionally treated. The test vessels were incubated in the dark at a temperature of 25 ± 1 °C for up to 30 days. A trapping system for volatiles was connected to each test vessel.	x x
4.2 Results	**Material Balance:** The mass balance of the applied radioactivity as total applied radioactivity (TAR) is summarized in Table 3. The average material balance for all the sampling intervals, from the total system (water and sediment), was 95.2% TAR. The material balance was 88 % TAR on day 24 and 84 % TAR on day 30. In general the material balance should be assessed within the context of the entire study and not on a single sampling interval. Therefore the averaged mass balance of 95.2 % is in the desired target range of 90 – 110 %. **Distribution of radioactive residues in the water phase:** At time zero, approx. 78.2 % total applied radioactivity (TAR) was found in the water phase of the test system. Immediately after the application, some of the applied radioactivity dispersed into the sediment phase and the dispersing was rapid with time. Three days after the treatment, only 33.8% TAR was found in the water phase. The radioactivity content in the water continued to decrease over time and only about 5.5% TAR was present at 30 DAT (Material balance see	x

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table 3) .

Distribution of Radioactive Residues in the Sediment Phase
Some of the applied radioactivity dispersed into the sediment phase just after application. The majority of the radioactivity associated with sediment at 0 DAT was extractable (16.6% TAR). Approximately 9.3% of the total applied radioactivity at time zero was un-extractable. The total applied radioactivity (extractable plus un-extractable) continued to increase over time and was about 65.5% TAR at 30 DAT. The extractable radioactivity content in the sediment phase increased over time to an average of 45.2% TAR at 10 DAT and then, declined to 21.55% TAR at 30 DAT (table 3). The non-extractable residue increased with time and an average of 44 % was found at day 30.

Quantitative Distribution and Composition of Residues
The distribution of Cu-HDO and its transformation products is shown in Tables 4 and 5. The values given in the tables reflect the individual sample results for all sampling intervals (0 through 30 DAT).
Water Phase
Cu-HDO (parent) was the major component during the entire experimental period and accounted for 74.3% TAR and 76.5% TAR at 0 DAT for rep1 and rep2 respectively. The amount of Cu-HDO in the water declined gradually over time, accounting for 1 resp. 4.5% TAR at 30 DAT (rep1 and rep 2). A number of minor degradation products were observed in water phase and none exceeded 5% TAR at any sampling interval. Among all these minor metabolites cyclohexanone was the only identifiable metabolite and accumulated to a maximum of 4.3% TAR (10 DAT, rep 2).
Sediment Phase
The amount of Cu-HDO initially increased in the sediment over time and accumulated to a maximum of 42.2% TAR and 46.4% TAR at 10 DAT for rep 1 and rep 2 respectively. After 10 DAT, the amount of Cu-HDO gradually declined and was 20.7% TAR and 22.2% TAR at 30 DAT for rep 1 and rep 2 respectively. Several minor metabolites were observed in the sediment extracts and none exceeded 2.8% TAR during the study period. [redacted] was the only identifiable metabolite and was found at a maximum of 2.2% TAR (16 DAT, rep 1).
Total System
The levels of Cu-HDO in the total system were 90.4% TAR and 93.3% TAR at 0 DAT for rep1 and rep 2 respectively. The amount of Cu-HDO slowly decreased to 21.7% TAR and 26.7% TAR at 30 DAT. A number of degradation products were observed at various sampling intervals, but none was major (>5% TAR). [redacted] was the only identifiable metabolite and was found at a maximum of 4.3% TAR at 10 DAT and declined over time.
Mineralisation
Moreover a kinetic analysis of the $^{14}CO_2$ formation arising from mineralisation of ^{14}C Cu-HDO was performed in an addendum. First-order and logistic kinetic models were evaluated using the guidance of FOCUS (2006) as a general basis for conducting the analysis, statistical assessment, and selection of the best fit kinetic model. The $^{14}CO_2$ data used in the kinetic analysis is given in table 6.
Optimization of model parameters was performed using the Solver feature of Excel (Microsoft, 2003). Standard errors of optimized parameters were estimated using the bootstrap method. Selected statistical and graphical analysis, including $\chi 2$ error percentage, t-test, model efficiency r2, and residual plots were conducted as outlined in the

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FOCUS (2006) guidance and implemented using Excel (Microsoft, 2003).
The logistic model was visually and statistically superior for this data set. The resulting time to convert 50%, 75%, and 90% of the potentially mineralizable carbon in ^{14}C Cu-HDO (defined as 100% TAR) to volatile $^{14}CO_2$ was 89.1, 170, and 325 days, respectively. However, since these values greatly exceed the limit of the observed data (30 days), they are considered to be beyond the range of reliable extrapolation and should only be used to give a qualitative indication of mineralization kinetics.

Radioactive residue analysis
Immediately after the treatment, a significant amount of the applied radioactivity dispersed into the sediment phase. At 0 DAT about 16.6% TAR as extractable and 9.3% TAR as non-extractable was found to be associated with sediment. Initially the extractable radioactivity content in the sediment phase increased over time to 45.2% at 10 DAT and then, gradually decreased to 21.5% at final sampling interval (30 DAT). Most all of the extractable radioactivity was parent (Cu-HDO). A number of very minor metabolites were observed in the sediment extracts at several sampling intervals. [redacted] was the only identifiable metabolite.
A significant amount of the volatile radioactivity was produced during the course of the study (13.2% TAR at 30 DAT). The majority of the volatile radioactivity was $^{14}CO_2$ and found in NaOH traps. The $^{14}CO_2$ data is summarized in table 6. A small amount of the volatile radioactivity was found in the ethylene glycol trap and was mainly [redacted] (<2% TAR). The volatile radioactivity present in sulphuric acid trap was insignificant. There were no major transformation products (>5% TAR) for Cu-HDO during this study.
Degradation in the total system was well described using first-order kinetic model (SFO) with DT_{50}, DT_{75}, and DT_{90} values of 14.5, 29.0, and 48.2 days, respectively. Dissipation from the water phase was best described by the biphasic model (FMOC) with DT_{50}, DT_{75}, and DT_{90} values of 2.4, 6.5 days, and 17.1 days, respectively. Dissipation from the sediment phase was best described by using SFO model and the DT_{50}, DT_{75}, and DT_{90} values were 20.3, 40.6, and 67.4 days, respectively.
The results this study indicate that in an aerobic aquatic environment Cu-HDO quickly partitions to sediment phase and sediment associated radioactivity gradually degrades to a number of minor metabolites and $^{14}CO_2$ as a major metabolite.
Cu-HDO is gradually metabolised in sediment, producing a significant amount of CO_2 in a relatively short period. Under these circumstances, there is very little possibility of accumulation of Cu-HDO in an aerobic aquatic water/sediment system.

Identification of transformation products:
The metabolic pathway of Cu-HDO is shown in Figure 3. [redacted] was the only identifiable metabolite (10 DAT: 2.2% TAR). The metabolites [redacted], [redacted] and [redacted] metabolites were also suggested by GC/MS analysis.

A.7.1.2.2.2 Water/sediment degradation study
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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

US-EPA subdivision N, Section 162-4 (835.4300)

Canada PMRA DACO number 8.2.3.5.4

5.2 Conclusion

a) The decline of ^{14}C-Cu-HDO in the total system, water phase, and sediment phase of a natural water/sediment system was assessed. Degradation in the total system was well described using first-order kinetic model (SFO) with DT_{50}, DT_{75}, and DT_{90} values of 14.5, 29.0, and 48.2 days, respectively. Dissipation from the water phase was best described by the biphasic model (FMOC) with DT_{50}, DT_{75}, and DT_{90} values of 2.4, 6.5 days, and 17.1 days, respectively. Dissipation from the sediment phase was best described using SFO model and the DT_{50}, DT_{75}, and DT_{90} values were 20.3, 40.6, and 67.4 days, respectively.

b) Partitioning of Cu-HDO from the water to sediment was the dominate process leading to decline of ^{14}C-Cu-HDO from the water phase.

c) The results this study indicate that in an aerobic aquatic environment Cu-HDO quickly partitions to sediment phase and sediment associated radioactivity gradually degrades to a number of minor metabolites and $^{14}CO_2$ as a major metabolite (approx. 13.2 % after 30 days).

d) Copper HDO is hydrolytically stable around neutral pH at ambient temperature and has a very short photolytic half-life of 6 hours. This suggests that Cu-HDO in water, although in very small amount, will degrade very quickly in the natural environment. Also, Cu-HDO is gradually metabolising in sediment, producing significant amount of CO_2 in a relatively short period. Under these circumstances, there is very little possibility of accumulation of Cu-HDO in an aerobic aquatic water/sediment system.

e) The resulting time to convert 50%, 75%, and 90% of the potentially mineralizable carbon in ^{14}C Cu-HDO (defined as 100% TAR) to volatile $^{14}CO_2$ was 89.1, 170, and 325 days, respectively. x

5.3.1 Reliability *1*

5.3.2 Deficiencies No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date November 2010

Materials and Methods **1.1 Reference**

[redacted] (2010) Addendum to Aerobic Metabolism of ^{14}C Cu-HDO, Study 325744: Kinetic Evaluation - ^{14}C Formation for Cu-HDO (Aerobic Aquatic Metabolism) [redacted] 2010/7003160

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Results and discussion

4.1 Test conditions

Since the study was performed before revision of guideline 835.4300 in October 2008 the following test design, which at that time was in agreement with the still existing guideline, was used:

- Only one water/sediment system was tested
- Temperature was at 25°C
- Test duration was 30 days

4.2 Results

Material balance

Explanation given in the test report for the recovery rates < 90% at day 24 and 30: During air-drying process prior to combustion the un-extracted volatile residues associated with sediment were lost. This observation was confirmed by combusting extracted samples (wet) prior to air-drying. This loss was more visible after day 10, because a significant amount of metabolism was happening in the sediment phase as seen by the rapid increase in the amount of volatile residues found in trapping solutions.

Conclusion

5.2 Conclusion

e) However, since these values greatly exceed the limit of the observed data (30 days), they are considered to be beyond the range of reliable extrapolation and should only be used to give a qualitative indication of mineralization kinetics. Measured mineralisation rate after 30 days was 13.2% of TAR.

In the study it is shown, that the major elimination pathway for Cu-HDO from water is adsorption onto sediment.

Cu-HDO is biodegraded most probably at the water/sediment interface with a DT_{50} of 14.5 days at 25°C for the whole system. The mineralisation rate was determined with 13.2% after 30 days. No other major metabolites than CO_2 were found in the system.

Water phase dissipation DT_{50}: 2.4 days (25°C)

Sediment phase dissipation DT_{50}: 20.3 days (25°C)

Reliability 1

Acceptability Yes

Remarks

COMMENTS FROM

Date *Give date of the 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*

Table 1:

Sediment		
Parameter	**Results/Units**	**Reference**
Geographic Location	Wabasha County, MN USA	NA
Texture Class	Silt Loam	USDA-NRCS Soil Survey Division
Sand Silt Clay	40% 53% 7%	Hydrometer method (NUT.02.06)
pH	6.0	1:1 Soil: Water suspension (NUT.02.05)
Organic Matter	3.1%	Walkley-Black (NUT.02.09)
Organic Carbon % organic matter / 1.724	1.80%	By calculation (Org. Matter/1.724)
Microbial Biomass Carbon	216.1µg/g dry basis	Fumigation Extraction Procedure - E.D. Vance (Mic.02.01).
Cation Exchange Capacity (CEC)	4.9 meq/100 g	Determined by summing the cations with hydrogen (NUT.02.03)
Field Moisture Capacity at 0.33 bar	18.7%	Water remaining when saturated soil was placed under 0.33 bar pressure
Field Moisture Capacity at 15 bar	6.0%	Water remaining when saturated soil was placed under 15 bar pressure (NUT.02.013)
Bulk Density (disturbed)	0.91 g/CC	Weight of known volume of dried and ground soil (NUT.02.10)
Water		
Parameter	**Results/Units**	**Reference**
pH	8.0	pH electrode (NUT.02.17)
Hardness ($CaCO_3$)	25mg equivalent $CaCO_3$/L	Calculated from Ca & Mg content (NUT.02.18)
Electrical conductivity	0.09 mmhos/cm	Conductivity Meter (NUT.02.22)
Oxygen concentration	10.2 mg/L	Oxygen Electrode

Table 2:

Sampling Details

Observations	**Details**
Sampling intervals for the parent/transformation products	0, 3, 7, 10, 16, 24 and 30 days of incubation.
Sampling method	Collect the entire test vessel contents at each sampling point.
Method of sampling volatile compounds	The head space of the test vessel was constantly purged out and bubbled through the trapping solutions. Samples were collected at 3, 7, 10, 16, 24 and 30 days of incubation.
Measurement intervals/times for: pH Redox potential Dissolved oxygen	At dosing, and at every sampling time point for water phase and zero time for sediment. At dosing, and at every sampling time point for both water and sediment. At dosing, and at every sampling time point for water phase only.
Sample storage before analysis	Samples were processed immediately after collection. In most cases the processed samples were analyzed just after processing. In a few cases processed samples were stored in fridge for less than a week before analyses.
Other observations	NA.

Table 3: Material balance of radioactivity applied to the test system.

		%TAR Distribution of Radioactive Residues[a]						
		0 DAT	3 DAT	7 DAT	10 DAT	16 DAT	24 DAT	30 DAT
Volatile Residues	Rep 1	N/A	0.2	1.2	2.0	4.4	9.3	12.6
Cumulative	Rep 2	N/A	0.2	1.1	2.4	5.5	11.8	13.8
	Average	**N/A**	**0.2**	**1.2**	**2.2**	**5.0**	**10.6**	**13.2**
Water Residues	Rep 1	77.3	21.2	26.4	28.1	22.7	6.6	5.0
	Rep 2	79.1	46.3	30.7	21.0	20.3	5.6	6.1
	Average	**78.2**	**33.8**	**28.6**	**24.5**	**21.5**	**6.1**	**5.5**
Sediment Residues	Rep 1	16.4	40.0	40.1	42.9	39.0	31.9	20.7
ERR	Rep 2	16.8	33.5	37.6	47.5	40.6	29.6	22.2
	Average	**16.6**	**36.7**	**38.8**	**45.2**	**39.8**	**30.8**	**21.5**
Sediment	Rep 1	9.4	37.6	33.4	24.0	25.8	41.6	42.3
NER	Rep 2	9.3	19.1	31.1	27.1	27.4	39.5	45.8
	Average	**9.3**	**28.3**	**32.2**	**25.5**	**26.6**	**40.5**	**44.0**
Mass Balance	Rep 1	103.1	99.0	101.1	96.9	91.9	89.4	80.6
	Rep 2	105.2	99.1	100.5	98.0	93.8	86.5	87.9
	Average	**104.2**	**99.0**	**100.8**	**97.4**	**92.9**	**88.0**	**84.2**
Average Mass Balance for Entire Study Duration								**95.2**

ERR = Extractable Radioactive Residues
NER = Non-extractable Radioactive Residues

Table 4: HPLC quantitation of radioactive residues in the water phase.

Table 5: HPLC quantitation of radioactive residues in sediment extracts.
[redacted]

Table 6: Volatile CO_2 arising from ^{14}C Cu-HDO mineralization

Time (days)	$^{14}CO_2$ (% TAR)[1]
0	0.0[2]
0	0.0
3	0.2
3	0.2
7	1.2
7	1.1
10	2.0
10	2.4
16	4.4
16	5.5
24	9.3
24	11.8
30	12.6
30	13.8

1 Reported as "Volatile residues" in the original Table of [redacted] (2008). The volatile residue was shown to be almost exclusively comprised of $^{14}CO_2$.
2 Day 0 values were reported in the original Table of [redacted] (2008) as "N/A", not applicable or not analyzed. These values were assumed to be zero.

Figure 1:

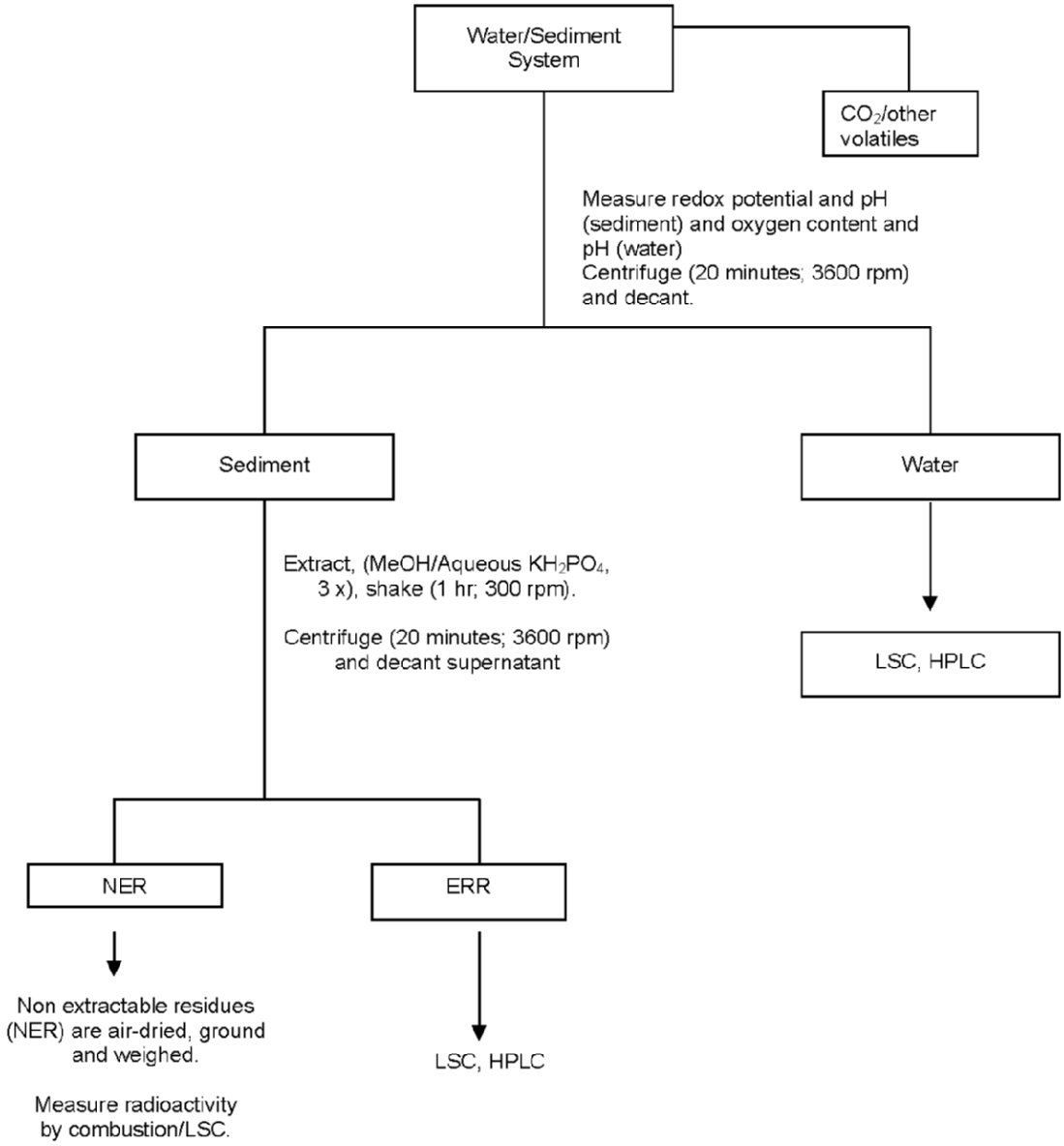
Water/Sediment System
CO_2/other volatiles
Measure redox potential and pH (sediment) and oxygen content and pH (water)
Centrifuge (20 minutes; 3600 rpm) and decant.
Sediment
Water
Extract, (MeOH/Aqueous KH_2PO_4, 3 x), shake (1 hr; 300 rpm).
Centrifuge (20 minutes; 3600 rpm) and decant supernatant
LSC, HPLC
NER
ERR
Non extractable residues (NER) are air-dried, ground and weighed.
Measure radioactivity by combustion/LSC.
LSC, HPLC

Figure 2: Bound residue flow chart

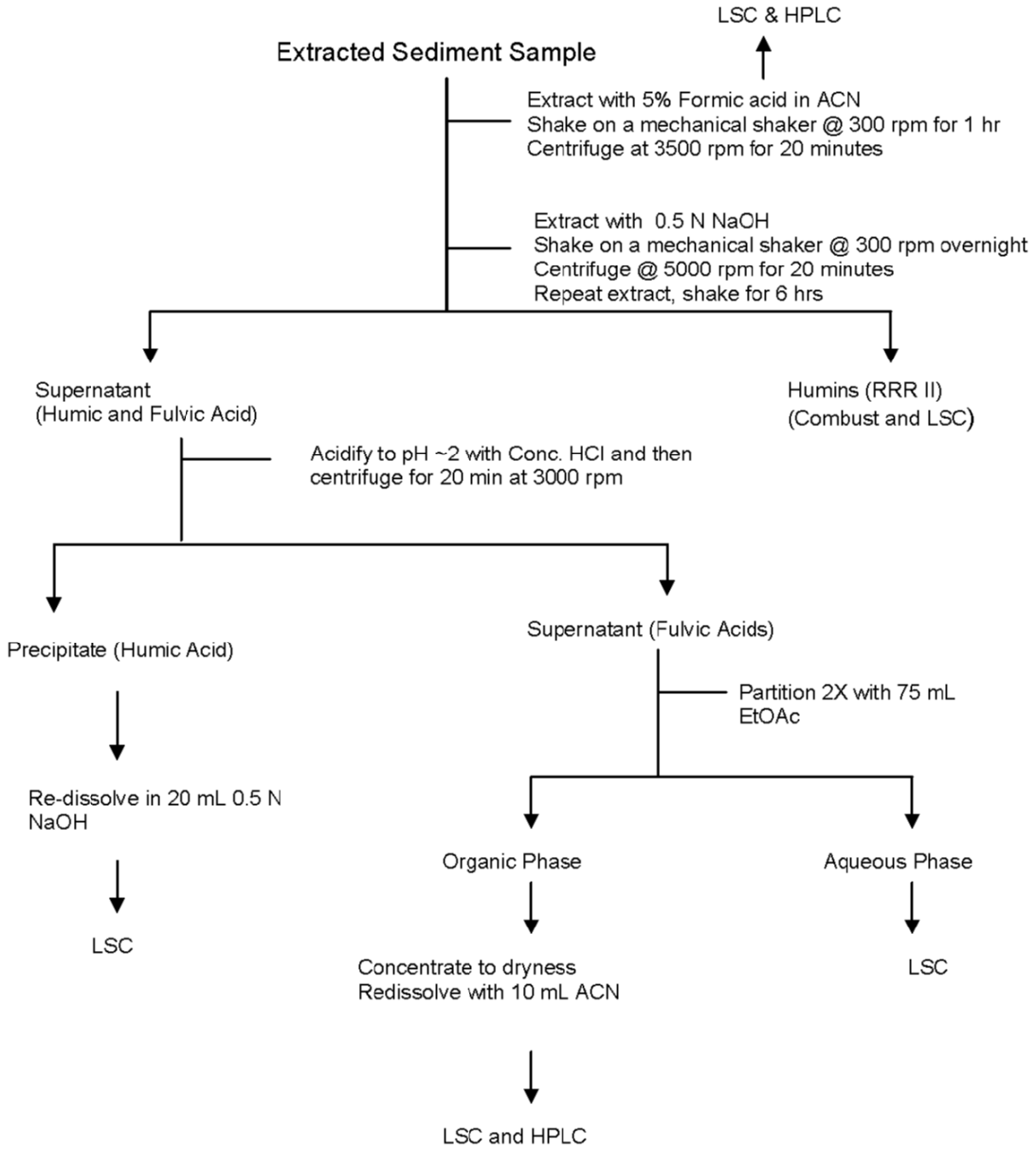

Figure 3: Degradation pathway of Cu-HDO in water/sediment:

Section A7.1.3 Adsorption / Desorption screening test

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			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], 2006, Adsorption/desorption study with Cu-HDO according to OECD 106, [redacted], Report no. 05 10 35 2028, 2006, unpublished, Ref. A 7.1.3	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
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 106	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Bis-(N-cyclohexyldiazenium-dioxy)-copper	
3.1.1	Lot/Batch number	U-9598	
3.1.2	Specification	Blue powder	
3.1.3	Purity	>99% as Bis-(N-cyclohexyldiazenium-dioxy)-copper	
3.1.4	Further relevant properties	Vapor pressure: $1x10^{-6}$ hPa Water solubility (6.1mg/L at pH=7)	
3.1.5	Method of analysis	The determination of the Cu-HDO-copper in aqueous $CaCl_2$ solutions was performed out by analysis of copper using GF-AAS-method (Graphite Furnace-Atomic Adsorption Spectrometry). The determination of copper in soils after aqua regia extraction was performed out with Flame-AAS. The methods were calibrated and verified.	
3.2	**Degradation products**	The recovery rates of the tests identifying loss during filtration, adsorption to container walls and by degradation were calculated to be >95%. Therefore the determination of degradation products was not required.	
3.2.1	Method of analysis for degradation products	Not necessary	
3.3	**Reference substance**	Reference study of K-HDO	x
3.3.1	Method of analysis for reference substance	HPLC-UV	
3.4	**Soil types**	see table A7_1 _3-1	x
3.5	**Testing procedure**		
3.5.1	Test system	The adsorption/desorption behaviour of Cu-HDO was investigated according to the OECD guideline 106, determination of soil adsorption/desorption using a batch equilibrium method. The study was	

Section A7.1.3 Adsorption / Desorption screening test

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performed with five certified soils.

3.5.2 Test solution and Test conditions

Solution	Preparation
Cu-Standard solution	Merck Standard solution 1000mg/L in HNO_3
Blank solution	1.831 g $CaCl_2$ Tetra hydrate (Merck, CertiPur), 1 mL HNO_3 (Suprapur) in 1 L PE-volumetric flask, filled up with water
Stock Standard solution	1 mL of the Cu-Standard solution dissolved with Blank solution in 100 mL PE-volumetric flasks Concentration 10.00 mg/L
Standard solution I	10 mL of the Stock-Standard solution dissolved with Blank solution in 100 mL PE-volumetric flasks Concentration 1000 µg/L
Standard 10 µg/L	1.0 mL of the Standard solution I dissolved with Blank solution in 100 mL PE-volumetric flasks Concentration 10.0 µg/L
0.01 m $CaCl_2$-solution	3.661 g $CaCl_2$ Tetra hydrate (Merck, Certipur) in 2 L water
Test item solution	Saturated solution of the test item in 0.01 m $CaCl_2$-solution: (see chapter 4.1.3 " Determination of the solubility of the test item in 0.01 m $CaCl_2$-extraction solution")
Aqua Regia	21 mL hydrochloric acid, 7 mL nitric acid

3.6 Test performance *Non-entry field*

3.6.1 Preliminary test

According to (a)"OECD 106": Yes

Tier 1 Preliminary test:

The test was performed to assure the applicability of the analytical method and the soils. The Cu-HDO-copper analysis was checked for the $CaCl_2$ soluble Cu-HDO and the blank soil copper, the aqua regia-soluble copper blank and the substance loss during filtration, adsorption and stability.

Two soil types and three soil/solution ratios were used. The soils chosen were LUFA 2.1 and LUFA 6 S. x

The optimal soil/solution ratio was determined and the adsorption equilibration time was estimated.

3.6.2 Screening test: Adsorption

According to (a)"OECD 106": Yes, Tier 2

Three soil types and three soil/solution ratios (nine experiments) were used. The test parameters were based on the results of the preliminary tests. The adsorption test was performed with a maximum agitation time of 8 hours and sampling time of 2, 4, and 8 hours. One control sample without soil and one blank sample without test item solution were subjected to the same procedure. All experiments including controls and blank were performed in duplicate. The distribution coefficient K_d at equilibrium as well as the organic carbon normalized adsorption coefficient K_{OC} was calculated. x

In a tier 3 approach the adsorption isotherms were determined. The five soils with five test item concentrations covering two orders of magnitude were used (2-200mg/kg soil).

3.6.3 Screening test: Desorption

According to (a)"OECD 106": Yes. Tier 3

The determination of desorption kinetics was carried out with all soils. For the determination of desorption the indirect serial analytical method was used. After the adsorption test, the aqueous phase was decanted after centrifugation and replaced by 49.5 mL 0.01 n $CaCl_2$ solution. The extraction bottle was shaked for 2, 4, 8, 24 for all five soils and additional 48 hours for three soils. After each period the bottle was centrifuged and an aliquot of 1mL was discharged and analysed. After sampling an equivalent volume (1 mL) of 0.01 m $CaCl_2$ solution was added and the extraction procedure was continued as described. The K_{des} values were calculated at equilibrium time.

Desorption isotherms were established after adsorption equilibrium was

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

		reached and the aqueous extract was separated. The desorption isotherms were calculated from the analysed Cu-HDO-copper concentrations in the aqueous phase at equilibrium after 8 hours of agitation.	
3.6.4	HPLC-method	OECD 106 method was used and not the OECD 121 method	
3.6.5	Other test	Not applicable	
		4 RESULTS	
4.1	**Preliminary test**	see table A7_1 _3-2	x
		Summary: At a mean temperature of 22.1°C the time dependant adsorption at different soil/test item ratios for two soils was investigated. The adsorption in both soils reached more than 90%. The equilibrium time was fixed to 8 h.	
4.2	**Screening test: Adsorption**	see table A7_1_3-3	
4.3	**Screening test: Desorption**	Desorption isotherms and Desorption kinetics were measured.	x
		Results see table A7_1 _3-4	
4.4	**Calculations**	Non-entry field	
4.4.1	Ka , Kd	The Freundlich adsorption coefficients are in the range from 55.1 to 2103. The averaged Freundlich adsorption coefficient amounts to 764.6. See details given in table A7_1 _3-3	x
		The Freundlich desorption coefficients are in the range from 210 to 22684. The averaged Freundlich desorption coefficient amounts to 7134. See details given in table A7_1 _3-3	x
4.4.2	Ka_{oc} , Kd_{oc}	See details given in table A7_1_3-3 and A7_1_3-4	
4.5	**Degradation product(s)**	No significant amount of degradation products was measured.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The adsorption/desorption behaviour of Cu-HDO was investigated according to the OECD guideline 106, determination of soil adsorption/desorption using a batch equilibrium method. The study was performed with five certified soils.	
5.2	**Results and discussion**		
5.2.1	Adsorbed a.s. [%]	The adsorption in all soils exceeded 95%. The detailed values per soil are given in table A7_1_3-3	
5.2.2	K_a	The Freundlich adsorption coefficients are in the range from 55.1 to 2103. The averaged Freundlich adsorption coefficient amounts to 764.6. See details given in table A7_1_3-3	
5.2.3	K_d	The Freundlich desorption coefficients are in the range from 210 to 22684. The averaged Freundlich desorption coefficient amounts to 7134. The organic carbon normalized Freundlich desorption coefficients are in the range from 33339 to 893081. The averaged organic carbon normalized Freundlich desorption coefficient amounts to 298450. See details given in table A7_1 _3-3	
5.2.4	Ka_{oc}	The organic carbon normalized Freundlich adsorption coefficients are in the range from 8739 to 114910. The averaged organic carbon normalized Freundlich adsorption coefficient amounts to 42471. See details given in	

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

		table A7_1 _3-3.	
5.2.5	Ka/Kd	Not calculated	
5.2.6	Degradation products (% of a.s.)	No significant amount of degradation products was measured.	
5.3	**Conclusion**	At the tested high concentrations of Cu-HDO, adsorption exceeds 95% on the soils. The organic carbon normalized Freundlich adsorption coefficient values are considerable and in the range from 8739 to 114910. (Mean value amounts to 42471). The resulting organic carbon normalized Freundlich desorption coefficient value are in the range from 33339 to 893081 (Mean value amounts to 298450). The test item Cu-HDO is practically irreversibly adsorbed on the soils.	
5.3.1	Reliability	1	x
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**3.3 Reference substance** No reference substance was used in the study. **3.4 Soil types** The five chosen soils match four of the proposed seven soil types of the guideline. **3.6.1 Preliminary test** The chosen soils represent soil types with a) Low C_{org} and low clay content (instead of low C_{org} and high clay content) b) High C_{org} and high clay content (instead of high C_{org} and low clay content) **3.6.2 Screening test: Adsorption** Three soil types were used in addition to those already tested in the preliminary test.
Results and discussion	**4.1 Preliminary test** The high copper blank values (up to 0.45 mg/kg soil) negatively impact the determination of the Freundlich adsorption and desorption isotherms by interfering the spiked Cu-HDO-copper values. The high Aqua Regia copper blank values (up to 176.8 mg/kg soil) make the determination of a mass balance impossible. However the stability of the test item could be proven. The recovery rates of the test identifying loss by adsorption to container walls and by degradation were calculated to be 107.6% and 114.1%. The recovery rates of $CaCl_2$-soluble Cu-HDO-copper were calculated to be 100.2%. **4.3 Screening test: Desorption** The evaluation of the Freundlich equation results for the soil Bruch West is a curved line, so that the evaluation range had to be reduced to the linear range. For the soil LUFA 6S no Freundlich desorption isotherm could be calculated, because the Cu-HDO-copper concentrations in the low concentration range were calculated to be negative under the influence of the high blank copper values. **4.4.1 K_a, K_d** Correction of values in table A7_1_3-3: Organic carbon normalized adsorption coeffidient for LUFA 6S: 17457-186107 Freundlich adsorption coefficient for Brunch West: 817 Details for Freundlich desorption coefficients are given in table A7_1_3-4
Conclusion	**5.2.3 K_d** Details for Freundlich desorption coefficients are given in table A7_1_3-4
Reliability	2
Acceptability	Acceptable
Remarks	-

Evaluation by Competent Authorities

COMMENTS FROM ...

Date

Materials and Methods

Results and discussion

Conclusion

Reliability

Acceptability

Remarks

Table A7_1 _3-1: Classification and physico-chemical properties of soils used as adsorbents

Soil specification		Bruch West	LUFA 2.1 F212905	LUFA 2.2 F222905	Standard soil type LUFA 2.3	Standard soil type LUFA 6 S
BASF soil sample No.		05/060/03	05/735/03	05/736/03		
LUFA soil sample No.					Sp2.3 4105	Sp6S 4505
Total nitrogen	%	0.15	0.06	0.18	-	-
Org.C (%)	%	2.54	0.63	2.11	1.02	1.83
pH-value ($CaCl_2$)		7.2	5.8	5.6	5.8	6.8
Ion exchange capacity	$cmol^+/kg$	12.7	1.8	7.9	9	18
Water holding capacity	g/100g	40.2	30.5	43.4	35	41.9
Bu k density	g/L	1252	1321	1118	1320	1225
Particle size distribution USDA						
Clay < 0.002 mm	%	11.4	2.4	5.6	8.8	42.2
Silt 0.002 - 0.05 mm	%	24.6	6.9	9.7	28.8	36.1
Sand 0.05 - 2.0 mm	%	64.0	90.7	84.7	62.5	21.7
Soil class		Sandy loam	sand	Loamy sand	Sandy loam	clay
Particle size distribution DIN						
Clay < 0.002 mm	%	11.4	2.4	5.6	8.6	39.1
Silt 0.002 - 0.063 mm	%	27.6	7.6	10.5	32.5	39.2
Sand 0.063 - 2.0 mm	%	61.0	89.9	83.9	59.4	21.8
Soil class		loamy sand	sand	Loamy sand	Sandy silt loam	Clayey loam
Granular size DIN						
0.63 - 2.0 mm	%	1.6	2.7	0.6	2.4	3.6
0.2 - 0.63 mm	%	20.7	32.9	42.2	25.9	9.0
0.063 - 0.2 mm	%	38.7	54.3	41.1	31.1	9.2
0.020 - 0.063 mm	%	13.6	3.6	4.8	19.7	15.4
0.006 - 0.020 mm	%	8.7	2.0	3.5	10.3	13.9
0.002 - 0.006 mm	%	5.3	2.0	2.2	2.5	9.9
< 0.002 mm	%	11.4	2.4	5.6	8.6	39.1

Table A7_1 _3-2: Results of preliminary test:

Test substance	Cu-HDO
Sample purity	>99% as Bis-(N-cyclohexyldiazenium-dioxy)-copper
Weighed soil	2g soil
Volume of $CaCl_2$ solution	30-50mL $CaCl_2$ extraction solution
Nominal concentration of a.s. final solution	Analytically verified concentrations used (41-206-825µg/L Cu)
Analytical concentration final of a.s. solution	Was determined for each solution and used for calculation
Concentration of the test solution (show calculation)	Analytically verified concentrations used
Details of the analytical method used:	Determination of the Cu-HDO content was performed by analysis of Cu using Graphite Furnace-Atomic Adsorption Spectrometry. The method was calibrated in the range from 2-10µg/L Cu by automatically dilution by the AAS-autosampler.
Method	GF-AAS
Recovery rate ($CaCl_2$-soluble Cu-HDO recovery rate)	100.2%+/-5.1%
Detection limit	0.06µg/L Cu $CaCl_2$ extract

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

	Bruch West	LUFA 2.1	LUFA 2.2	LUFA 2.3	LUFA 6S
Cu-concentration of test material [mg/L]	0.015-1.5	0.015-1.5	0.015-1.5	0.015-1.5	0.015-1.5
Final corrected concentration [mg/L]	0.0004-0.017	0.00049-0.219	0.00017-0.0416	0.00018-0.065	0.00095-0.037
Initial Cu concentration of test solution [mg/L]	0.015-1.5	0.015-1.5	0.015-1.5	0.015-1.5	0.015-1.5
Decrease in Cu concentration [mg/L]	0.0146-1.483	0.0145-1.281	0.01483-1.46	0.01482-1.435	0.014-1.463
Quantity Cu adsorbed [µg]	0.73-73.8	0.72-63.68	0.74-72.6	0.645-62.14	0.6-63.5
Quantity of soil [g of oven-dried equivalent]	2	2	2	2	2
Quantity Cu adsorbed [µg] per gram of soil	0.36-36.9	0.36-31.8	0.37-36.3	0.32-31.1	0.303-31.8
Test material Cu adsorbed [%]	97.3-99.4	85.3-97.7	97.2-99.3	95.0-98.8	92.7-99.3
Temperature [°C]	22.3	22.3	22.3	22.3	22.3
Distribution coefficient at adsorption equilibrium Kd	909-4268	145-1071	871-3467	477-2130	319-3406
Organic carbon normalized adsorption coefficient	35801-168030	23053-169949	41297-164310	46771-208819	46723-186107
Freundlich adsorption coefficient	8170	55.1	525.1	322.9	2102.9
Organic carbon normalized Freundlich adsorption coefficient	32167	8739	24884	31655	114910
PH-value of the aqueous phase at adsorption equilibrium	5.3-5.4	5.3-5.4	5.3-5.4	5.9-6.3	6.2-6.6

Table A7_1 _3-4: Results of test - desorption:

	Bruch West	LUFA 2.1	LUFA 2.2	LUFA 2.3	LUFA 6S
Temperature [°C]	21.6	21.6	21.6	21.6	21.6
Evaluated concentration Cu range [µg/g]	0.37-37.3	0.37-37.3	0.37-37.3	0.33-32.7	0.37-32.7
Freundlich desorption coefficient	22684	210	2825	2816	-
Organic carbon normalized Freundlich desorption coefficient	893081	33339	133902	133479	-
[%] of desorbed test material	0.15-1.3	0.87-5.7	0.7-1.3	0.45-0.87	-
Correlation coefficient R^2	0.945	0.981	0.976	0.979	-

Section A 7.2.1 Annex Point IIIA, VII.4., Annex Point IIIA, XII.1.1 Aerobic degradation in soil, initial study

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.2.1 [redacted], 1994, Examinations concerning the degradation of HDO in soil, performing laboratory: [redacted]	
1.2	**Data protection**	Yes	
1.2.1	**Data owner**	Dr. Wolman GmbH	
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, according to BBA (Biologische Bundesanstalt für Land- und Forstwirtschaft – Bundesrepublik Deutschland) guideline 4.1 "Destination of pesticides in the ground – degradation, transmutation, metabolism" (yore BBA leaflet No. 36 and No. 56)	x
2.2	**GLP** *(only where required)*	No Study was performed prior GLP requirement was mandatory.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	List lot/batch number where relevant	
3.1.2	Specification	Technical formulation	
3.1.3	Description	Formulation Wolmanit CX-S (see description of Wolmanit CX-S in confidential chapter)	
3.1.4	Purity	blue liquid	
3.1.5	Stability	end-use formulation containing 6,1% Cu-HDO	
3.2	**Analytical method**	The determination of the active ingredient content in soil was carried out by chemical separation of NO and its detection by a Thermal Energy Analyzer (TEA). The Diazenium compounds in the sample were separated equivalently to NO by the mixture of Na(I) / acetic acid / sulfonic acid (temp. 90°C) in the reactor. Together with the He-gas flow NO gets into the TEA, where it can be determined through chemoluminiszens, which appears after transformation of NO with ozone. The LOD for this method at that time has been estimated to be below 0,1mg HDO/kg soil. The estimated LOQ is therefore between 0.1 and 0.2mg HDO/kg soil.	
3.3	**Testing procedure**	Non-entry field	

Section A 7.2.1 Annex Point IIIA, VII.4., Annex Point IIIA, XII.1.1 Aerobic degradation in soil, initial study

3.3.1 Test system

Standard Soil (slightly loamy sand):

Content of Clay: 3.5%
Content of Silt: 9.1%
Content of Sand: 87.4%
Content of Carbon: 0.7%

The soil has been obtained from the Agricultural Analysis and Research Institution in Speyer (Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer), Germany

3.3.2 Test conditions

The aqueous Wolmanit CX-S solution has been added to the soil to a calculated active ingredient concentration of 5mg HDO per kg soil. The ratio soil to aqueous solution was 5 : 1. The storage times were 0, 2, 4, 8, 16, 32, 64 and 100 days. Storage occurred in airtight phial glasses at room temperature in the darkness. x

3.3.3 Duration of the test

100 days

3.3.4 Test parameter

HDO content in soil

3.3.5 Sample preparation

After termination of the storage time, 5 ml Dichloromethane were added to approximately 5g soil (weighted exactly). The soil was then extracted on a shaker for 30 minutes. Subsequently the overlaying solution was decanted. The soil has then been washed 2 times with 5 ml Dichloromethane in each case. The unified extracts have then been concentrated to a volume of 1 ml and dried over sodium sulphate. Determination of the HDO content occurred via TEA. The blind value has been determined twice and is below 0.1mg/kg.

4 RESULTS

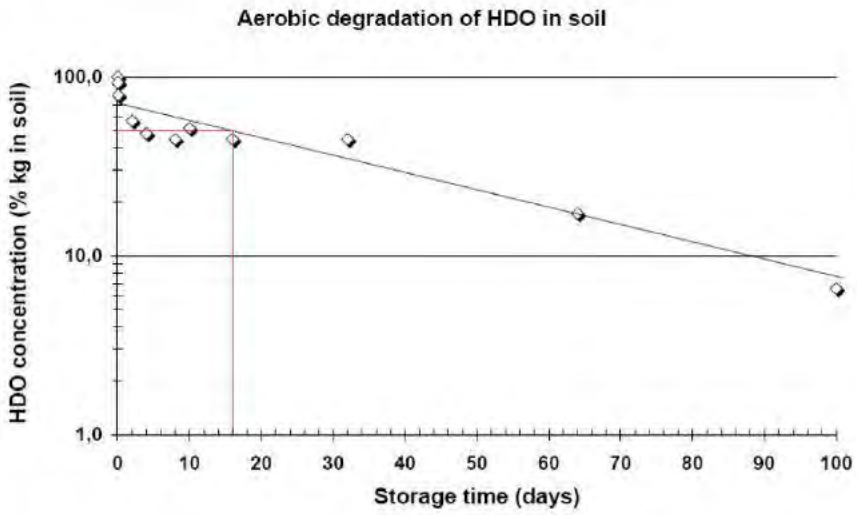
4.1 Aerobic Degradation in soil x

Storage time (hours)	Storage time (days)	1. Measurement mg HDO/kg soil	2. Measurement mg HDO/kg soil	Mean value
0	0	2.9	2.9	2.9*
1	0.042	2.5	2.9	2.7
2	0.083	2.5	2.1	2.3
48	2	1.6	1.7	1.65
96	4	1.5	1.3	1.4
192	8	1.2	1.4	1.3
240	10	1.3	1.7	1.5
384	16	1.	1.2	1,3
768	32	1.2	1.4	1.3
1536	64	0.5	0.5	0.5
2400	100	0.17	0.21	0.19

* based on a calculated start concentration of 5mg/kg the used extraction method has a recovery of approx. 60%. It seems that the used matrix causes the unsatisfactory recovery. Other experiments at this time with equal sample preparation have shown clearly better recoveries of 70% to 80%.

Section A 7.2.1 Annex Point IIIA, VII.4., Annex Point IIIA, XII.1.1 **Aerobic degradation in soil, initial study**

4.2 Concentration/ response curve



4.2.1 DT-50: The Disappearance time after which 50% of the start concentration is disappeared is about16 days (graphical evaluation).

4.2.2 DT-90 The Disappearance time after which 90% of the start concentration is disappeared is about 88 days (graphical evaluation).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The test was performed according to the guideline 4.1 "Destination of pesticides in the ground – degradation, transmutation, metabolism" – of the "Biological Federal Agency for Agriculture and Forestry" May 1990 (yore BBA leaflet No. 36 and No. 56). The storage times of the soil were 0, 2, 4, 8, 16, 32, 64 and 100 days. Storage occurred in airtight phial glasses at room temperature in the darkness.

The soil was prepared with an aqueous solution of Wolmanit CX-S (end-use formulation containing 6.1% Cu-HDO). The amount of Wolmanit CX-S was calculated in order to obtain a concentration of the active ingredient of 5mg per kg soil. The ratio soil/solution was 5:1. The degradation of HDO was measured during storage in regular intervals after extraction with Dichloromethane. The determination of the content was carried out by chemical separation of NO and its detection by a Thermal Energy Analyser.

The used test substance was the technical formulation Wolmanit CX-S, which contains the active ingredient in a solved formulation. The used soil was slightly loamy sand.

5.2 Results and discussion

The DT values were determined by plotting the measured HDO concentrations (mean value of 2 measurements) versus the storage time of the soil sample. Metering the time after which 50% and 90% of the start concentration disappeared gives the DT-50 and the DT-90 value, respectively.

The DT-50 value of HDO is about 16 days

The DT-90 value of HDO is about 88 days

5.3 Conclusion

The DT-50 value is below 30 days and the DT-90 value is below 100 days. Because x

Section A 7.2.1 Annex Point IIIA, VII.4., Annex Point IIIA, XII.1.1 Aerobic degradation in soil, initial study

		of this minor permanence, adverse effects on the natural environment are not expected.
5.1.1	Reliability	2
5.1.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	January 2006
Materials and Methods	**2.1 Guideline study** The cited BBA guideline 4.1 has inter alia been used to develop OECD test-guideline 307. **3.3.2 Test conditions** Room temperature was assumed to be 22°C
Results and discussion	**4.1 Aerobic degradation in soil** The recovery rate was approximately 60%. In the BBA guideline 4.1 no limit for the recovery rate is given.
Conclusion	**5.3 Conclusion** The study exhibits many deficiencies, like a rate of recovery of 60%, no mass balance, no degradation and mineralisation rates, missing identification of transformation products, etc. Therefore the study was not accepted and the applicant was asked to perform an aerobic soil study according to OECD test guideline 307.
Reliability	3
Acceptability	Not acceptable
Remarks	-

A.7.0.2.3.3 **Annex Point/TNsG**	Aerobic degradation study in soil	
	1 REFERENCE	Official use only
1.1 Reference	[redacted], (2012) Transformation in Soil under aerobic conditions with radio labelled test substance [redacted]. 19G0225/10G013, unpublished.	x
1.2 Data protection	Yes	
1.2.1 Data owner	*BASF SE*	
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 307, Commission Regulation (EC) No 440/2008 C. 23	
2.2 GLP *(only where required)*	Yes	
2.3 Deviations	No	x
	3 MATERIALS AND METHODS	
3.1 Test material	Bis-(N-Cyclohexyldiazeniumdioxy)-copper , ^{14}C Cu-HDO	
3.1.1 Lot/Batch number	Batch 930-2006	
3.1.2 Specification	Specific activity: 3.88 MBq/mg	
3.1.3 Description	CAS 312600-89-8	
3.1.4 Purity	Radiochemical purity is 98.3 % Chemical purity is 99.3 %	x
3.2 Test matrix	Four different natural soils from „Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer, Obere Langgasse 40, 67346 Speyer, Germany (Batch F 2.2 1311, F 2.3 1211, F 2.4 1311, F 5M 1211)“ were used in this study. Characterisations of the different soils are reported in Table 1.	
3.3 Test design	120 days exposure of the test substance in treated soil blends according to OECD 307 and Commission Regulation (EC) No 440/2008 C.23 was added to the test vessels in one concentration; 4 treatment groups with 18 test vessels with test substance and 2 controls per soil blend; 2 replicates test vessels with test substance were treated per sampling day. Assessment of the biodegradability of the test substance in soils under aerobic conditions in the dark.	
3.4 Application procedure	Application of the test substance by aliquots of an acetonic stock solution. 199 µg test substance (0.776MBq) per test vessel (or 50 g dry weight soil). Resulting concentration 3980 µg/kg (dry weight).	x
3.5 Experimental conditions and	**Test conditions**: 4 soil blends according to OECD 307 and Commission Regulation (EC) No 440/2008, C.23 at 21.7 – 22.0°C in	

monitoring	the dark. **Sampling**: Per soil 2 test vessels with test substance and their adsorption traps were taken for sampling and measurement of analyses on day 0, 1, 2, 3, 10, 23, 57, 85, 97 and 120. **Statistics:** The DT_{50}, DT_{75} and DT_{90} were calculated by SAS-Procedure NLIN from SAS Institute Inc., Cary North Carolina 27511 using a first order kinetic model.	
3.6 Analytical methods	A consolidated balance of the radioactivity in the soils was compiled at any sampling time for the test vessels with test substance as % of the inserted radioactivity. The half life, the DT_{50} and the DT_{75} and DT_{90} (with confidence levels) of the test substance was calculated. The limit of detection (LOD) and the limit of quantification (LOQ) of the method of analysis were determined. The recovery rate during the exposure of the applied radioactivity of the labelled test substance and the accuracy of transformation data by regression analysis of the concentrations of the test substance as a function of time were calculated. ***Measurement of radioactivity:*** The soils were extracted at any sampling time with an extraction solvent. The activity in the extract samples were determined on a liquid scintillation counter (LSC) using duplicate subsamples. ***Measurement of test substance and metabolites in soil extracts (radio-HPLC)*** Amounts of test substance and metabolites in liquid samples in the soil extracts were determined on a radio-HPLC. ***Analysis method for identification of test substance and metabolites in the extracts*** HPLC-MS analyses of metabolite-containing fractions were usually performed on a mass spectrometer using electrospray ionization (ESI), with data processing. The mass spectrometer was hyphenated to an HPLC system and a radioactivity detector. The chromatographic methods used were identical with those applied in the isolation of the respective metabolites. The effluent of the chromatographic column was split for parallel radioactivity detection and mass spectrometry. Moreover biomass and water content control was performed during the study	x
	4 RESULTS	
4.1 Test conditions	***Test temperature***: The temperature was set to 22 ± 2°C. The measured temperatures were 21.7 –22.0°C (Mean value was 21.9°C). ***Test duration***: 120 days ***Illumination:*** *None* ***Test vessels:*** 250 mL Erlenmeyer flask ***Number of test vessels***: 80 test vessels in total. 20 vessels per soil. 2 of them for control and 18 for regular sampling with test substance. ***Way of ventilation***: Bubbling in the water layer with 2-3 bubbles/second **Mass of soil test vessels:** About 50 g dry matter, equivalent to 57 g wet soil	

	Application of the test substance to the test vessels: With an eppendorf research pipette (volume range 200-1000 µL) from the acetonic stock solution **Number of replicates (test item and control) per soil and sampling day**: 2 **Sampling times:** 9 (without day 0) **Extraction method:** 4 times with extraction solution **Absorption liquids**: About 100 mL 0.5 M sodium hydroxide, about 50 g ethylene glycol **Number of soils**: 4	x
4.2 Results	The objective of this study was to determine the dissipation of Cu-HDO in soil systems under aerobic conditions, the determination of the rates of formation and decline of transformation products (metabolites) as well as the determination of corresponding DT_{50}, DT_{75} and DT_{90} values. **Mass Balance and Distribution:** For mass balance the activity in the pooled soil extract, in the soil after combustion and in the absorption liquids were added and compared to the total applied activity (TAR). The mass balance and distribution of the applied radioactivity is summarized in Table 2-5 for each soil and figure 1 for soil 1. **Biodegradation:** The degradation rates of the test substance over the exposure period at the sampling times were calculated by comparison of absorbed activity of the three absorption liquids in the traps to the total applied activity in the test vessel. The degradation rates are expressed as % of total applied activity (%TAR). The degradation rate of C14-Cu-HDO reached 10 % in all soils in the first 20 days of exposure. Then the degradation rate increased constantly to about 50-60 % TAR at the end of exposure. The following biodegradation rates in relation of the used soil types (for both replicates) were determined at the end of exposure on day 120: Soil 1: 54.0 % / 57.1 % related to TAR Soil 2: 48.0 % / 60.4 % related to TAR Soil 3: 49.4 % / 53.4 % related to TAR Soil 4: 59.9 % / 62.3 % related to TAR Overall, the degradation of the test substance in the soils is characterized by a significant degradation from day 10 to day 120 In an amendment to the study report the mineralization rate of Cu-HDO was assessed using first order multi compartment model (FOMC) under consideration of recommendations of the FOCUS kinetics workgroup. The fit of observation was performed by non-linear regression using the software tool ModelMaker. The mineralization rate was 79.1 days for soil 1, 66.4 days for soil 2, 107.2 days for soil 3 and 64 days for soil 4. The arithmetic mean of the mineralization rate is 79.2 days for all four soil types. **Dissipation times:** The dissipation times of the test substance (presented in table 6) were calculated for each soil type assuming first order kinetics. The following data were obtained: Soil 1: DT_{50} (days): 2.3 DT_{75} (days): 4.6 DT_{90} (days): 7.7 Soil 2: DT_{50} (days): 2.2 DT_{75} (days): 4.5 DT_{90} (days): 7.4 Soil 3: DT_{50} (days): 9.5 DT_{75} (days): 19 DT_{90} (days): 31 Soil 4: DT_{50} (days): 11 DT_{75} (days): 21 DT_{90} (days): 35	X x

	Based on the above mentioned dissipation times of the several soils, the following arithmetic mean values were calculated: DT_{50}= 5.9, DT_{75}= 11.8 and DT_{90}= 19.4(days). In an amendment to the study report the dissipation rate of Cu-HDO was assessed using first order multi compartment model (FOMC). The following data were obtained: Soil 1: DT_{50} (days): 2.0 DT_{75} (days): 11.4 DT_{90} (days): 88.3 Soil 2: DT_{50} (days): 2.3 DT_{75} (days): 6.9 DT_{90} (days): 20.9 Soil 3: DT_{50} (days): 1.5 DT_{75} (days): 10.2 DT_{90} (days): 104.3 Soil 4: DT_{50} (days): 4.5 DT_{75} (days): 17.2 DT_{90} (days): 76 Based on the mentioned dissipation times of the several soils the following arithmetic mean values were calculated for ^{14}Cu-HDO: DT_{50}=2.6, DT_{75}=11.4 and DT_{90}=72.4 days. **Identification of formed metabolites** Based on the data of the radio HPLC samples, extracts from soil 1 were assessed to be representative in respect to their metabolic profile and were therefore used for the identification of formed metabolites. The goal was to identify metabolites >10 % TAR. Three metabolites were identified in the samples of day 1: [redacted] (Rt 13.8 min.), [redacted] (Rt 13.3 min.) and [redacted] (Rt 6.5 min.). Based on the complex soil matrix the identification of metabolites was not possible in any further samples. From day 85 of exposure no relevant peaks with a content ≥ 10% TAR could be found in HPLC. Metabolite 3 ([redacted] is a reaction product of C14-Cu-HDO degradation intermediates with the solvent acetonitrile. This metabolite is therefore assessed to be most likely a workup artefact. A possible hint to the degradation product [redacted] is given by the detection of (14C-free) [redacted] (metabolite 4). The radioactive peak is still intact [redacted], which loses, in the ion source, radioactively labelled CO so quickly and efficiently that the intact molecule [redacted] cannot be detected and forms unlabelled [redacted]. The results of metabolite elucidation are presented in table 7. The pathway of degradation is shown in figure 2. The results of the study show that C14-Cu-HDO dissipated from the different soil types by biodegradation and adsorption to the soil. The degradation in the soils is characterized by the fast formation of non-extractable residues, while mineralization to CO_2 is significant in all soil types.	x x
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	OECD 307, Commission Regulation (EC) No 440/2008 C. 23	
5.2 Conclusion	The results of the study showed that the behaviour of C14-Cu-HDO in the soils was characterized by a significant degradation and adsorption in soil. C14-Cu-HDO is transformed to only a few medium polar metabolites and to a distinctive amount of nonextractable residues in the sediment. Beginning mineralization to CO_2 was observed at day 2-3 in soil 1 and 2 and at day 10 in soil 3 and 4. At the end of exposure the degradation grade was 55.6, 54.2 and 51.4% TAR in soil 1, 2 and 3 and 61.1 % TAR in soil 4. It was obvious that the degradation is not completed after 120 days of exposure. Using a first order multi compartment model (FOMC) the arithmetic mean of the mineralization rate is 79.2 days for all four soil types. The microbial biomass measured in the control at the end of exposure showed that the systems were stable and active during the exposure time. In the representative soil there were found four potential metabolites after 1 day. Because of	x

	matrix contamination no more metabolites could be identified via HPLC-MS. From day 85 of exposure no relevant peaks with a content ≥ 10% TAR could be found in HPLC. The distinct amount of formed carbon dioxide showed that the ring system is break down. Three metabolites were identified in the samples of day 1: [redacted] (Rt 13.8 min.), [redacted] (Rt 13.3 min.) and [redacted] (Rt 6.5 min). Based on the abovementioned dissipation times of the several soils the following arithmetic mean values were calculated: DT_{50}= 5.9, DT_{75}= 11.8 and DT_{90}= 19.4(days). Using a first order multi compartment model (FOMC) the following arithmetic mean values were calculated for ^{14}Cu-HDO: DT_{50}=2.6, DT_{75}=11.4 and DT_{90}=72.4 days.	
5.3.1 Reliability	*1*	
5.3.2 Deficiencies	No	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	May 2013 1.1 Study was amended on 8 May 2013 and contained the calculation of DT50 degradation and dissipation values according to FOCUS Guidance and remarks of RMS. 2.3: Soils exceeded the recommended storage time acc. to OECD 307 of up to three months. Study soils were stored for 6 months.	
Materials and Methods	3.1.4 *Amend*: Radiochemical purity was 90.1% in September 2012. 3.6 *Correction and Clarification* of the Study Report (by the applicant on 31 May 2013): LOQ: The chromatographic profile obtained was used for quantification of the extracts. The soil extracts were concentrated before analyses (concentration factors 30-80). The limit of quantification (LOQ HPLC) of radioactive fractions was set at a level at which a distinct peak could be seen. The lowest amount of radioactivity that could be quantified was about 15 cps, and therefore defined as the limit of quantification. The limit of quantification of the samples (LOQ) was calculated by considering the amount of radioactivity injected and the lowest amount of radioactivity which could be quantified (LOQ HPLC). Representatively for the soil extracts, the sample TS 13 was taken. At this 15 cps were found as lowest amount of activity by HPLC analysis. In acceptance that 15 cps is equivalent to 15 Bq the founded activity was 900 dpm. For calculation the test substance concentration in µg/kg it was assumed that 199 µg of the test substance was mixed with theoretical 50 g dry weight soil. The soil was extracted with 356 g extraction solution. The amount of test substance corresponds to 444488000 dpm. Converted to 1 kg soil there were 889760000 dpm in 7120 g extraction solution (correspond to 3980 µg test substance). 900 dpm are equivalent to 0.004 µg/kg. Extracts LOQ_E = LOQ HPLC *C/IRA LOQ_E = Limit of quantification of conc. extract sample [µg/kg] LOQ HPLC = Limit of quantification of HPLC [dpm] C = Concentration of test substance [µg/kg]	

	IRA = applied radioactivity [dpm] Example: Test substance µg/kg (LOQE) = 900 *3980/8976000 = 0.004 µg/kg.
Results and discussion	4.1 *Amendment*: The test substance stock solution was pipetted to the quartz sand in the test vessels. After mixing and solvent evaporation the soil was added and mixed with the quartz sand. 4.2 *Addition*: The mass balances of the four soils exceeded the OECD recommended range of 90% to 110% of applied radioactivity. Ranges: Soil 1: 55-97%, soil 2: 47-92%, soil 3 56 to 93%, soil 4 74-94%, all AR respectively. 4.2 *Clarification*: For the calculations of the dissipation times (1^{st} order) only measurements of day 1 to 3 were used for soils 1 and 2. For soils 3 and 4 the results till day 23 have been used. According to the Arrehnenius relationship at 12°C the arithmetic mean DT50 value is 13.0 day and the corresponding DT90 value is 42.8 day. The presented chromatograms displayed often badly split peaks that interfered with several co-eluates and their retention times were shifted because of matrix effects (please see Table 8 and Table 9). The applicant stated that no further clean up of the soil samples were possible. In addition the extraction solution (phosphate buffer) increased the matrix effects according to the applicant. 4.2 *Addition*: Please see Table 9. According to the Arrhenius relationship at 12°C the geometric mean DT50 value is 5.7 day and the corresponding DT90 value is 136 day. The T1/2 (based on mineralisation) at 12°C is 171.3 days. 4.2 *ad Metabolites*: The applicant stated that no further clean up and analytical methods for identification and quantification for the transformation products were available.
Conclusion	5.3.2: The study failed to gain full information on the amount, nature and rates of formation and decline of transformation products since only day 1 could be analysed. The description of the degradation pathway cannot be considered as complete. No characterisation of bound residues has been performed.
Reliability	2
Acceptability	Acceptable
Remarks	-

Table 1:

Parameter	Soil 1	Soil 2	Soil 3	Soil 4
Soil type	2.2	2.3	2.4	5M
Batch No.	F 2.2 1311	F 2.3 1211	F 2.4 1311	F 5M 1211
Sampling site:	Germany, Hanhofen, Großer Striet, Nr. 585	Germany, Offenbach, Im Bildgarten, Nr. 508/2	Germany, Leimersheim, Hoher Weg, Nr. 3138	Germany, Mechtersheim, In der Speyerer Hohl, Nr. 977
Sampling depth:	Approx. 20 cm	Approx. 20 cm	Approx. 20 cm	Approx. 20 cm
Sampling quantitiy:	Approx. 5 kg	Approx. 180 kg	Approx. 10 kg	Approx. 13 kg
Sampling date:	29 March 2011	22 March 2011	29 March 2011	25 March 2011
Weather conditions on sampling	Sunshine at 7°C	Sunshine at 16°C	Sunshine at 7°C	Sunshine at 10°C
Organic carbon:	1.93 ± 0.20 %	0.99 ± 0.08 %	2.53 ± 0.65 %	1.27 ± 0.50 %
pH-value (0.01M $CaCl_2$):	5.5 ± 0.1	6.7 ± 0.3	7.1 ± 0.2	7.2 ± 0.1
Cation exchange capacity:	10.0 ± 0.8 meq/100 g	10.0 ± 2.0 meq/100 g	29.0 ± 6.2 meq/100 g	15.0 ± 3.0 meq/100 g
Soil type:	Loamy sand (lS) according to German DIN	Silty sand (uS) according to German DIN	Clayey loam (tL) according to German DIN	Loamy sand (lS) according to German DIN
Maximal water holding capacity:	45.2 ± 5.0 g/100g Dw	35.6 ± 3.0 g/100g Dw	44.7 ± 1.4 g/100g Dw	41.5 ± 4.4 g/100g Dw
Particles size >0.063-2.0 mm (sand):	79.8 ± 4.9 %	60.1 ± 5.9 %	26.6 ± 0.6 %	55.0 ± 2.6 %

Table 2: Distribution and mass balance of radioactivity over the exposure period in soil 1

DE	V No.	% TAR					
		soil		volatiles			Mass balance Mv
		Extract	NER	Ethylene glycol	NaOH	total	
1	TS1	65.9	29.0	<0.01	1.8	96.7	91.3
	TS2	63.5	19.9	<0.01	2.4	85.8	
2	TS3	53.7	25.8	<0.01	5.0	84.5	85.2
	TS4	56.7	26.3	<0.01	2.8	85.8	
3	TS5	47.6	31.7	<0.01	7.3	86.6	85.7
	TS6	50.9	27.7	<0.01	6.2	84.8	
10	TS7	30.2	34.4	<0.01	19.7	84.3	87.3
	TS8	32.8	36.7	<0.01	20.6	90.2	
23	TS9	22.7	22.9	<0.01	29.3	74.8	78.9
	TS10	26.1	20.3	<0.01	36.5	82.9	
57	TS11	16.8	15.4	<0.01	39.6	71.8	76.9
	TS12	17.7	12.7	<0.01	51.5	81.9	
85	TS13	10.0	14.1	<0.01	30.5	54.7	67.0
	TS14	11.8	9.8	<0.01	57.6	79.2	
97	TS15	11.7	21.5	<0.01	45.8	79.0	69.0
	TS16	9.8	22.5	<0.01	26.7	59.0	
120	TS17	6.1	23.1	0.4	54.0	83.6	84.6
	TS18	4.6	23.8	<0.01	57.1	85.5	

DE = Day of exposure V No. = Vessel number NER = Non extractable residues
NaOH = Sum of sodium hydroxide solutions (trap)
% TAR = % of total applied radioactivity

Table 3: Distribution and mass balance of radioactivity over the exposure period in soil 2

DE	V No.	% TAR					
		soil		volatiles			Mass balance Mv
		Extract	NER	Ethylene glycol	NaOH	total	
1	TS19	83.2	7.9	<0.01	1.0	92.1	90.3
	TS20	75.4	13.0	<0.01	<0.01	88.4	
2	TS21	66.0	16.0	<0.01	7.6	89.6	89.6
	TS22	62.5	20.2	<0.01	6.9	89.6	
3	TS23	56.5	22.1	<0.01	11.9	90.5	89.6
	TS24	57.9	19.0	<0.01	11.7	88.6	
10	TS25	22.6	23.9	<0.01	17.1	63.5	71.6
	TS26	25.7	22.2	<0.01	31.8	79.7	
23	TS27	11.7	21.9	<0.01	55.0	88.6	86.7
	TS28	11.2	22.7	<0.01	51.0	84.8	
57	TS29	5.2	15.9	<0.01	58.9	80.0	75.5
	TS30	4.8	15.4	<0.01	50.9	71.0	
85	TS31	4.1	11.8	<0.01	57.2	73.2	70.5
	TS32	3.7	12.7	<0.01	51.3	67.7	
97	TS33	3.9	22.2	<0.01	46.6	72.7	59.8
	TS34	3.0	23.4	<0.01	20.5	46.9	
120	TS35	2.4	19.7	<0.01	60.4	82.5	75.6
	TS36	2.5	18.2	<0.01	48.0	68.7	

DE = Day of exposure V No. = Vessel number NER = Non extractable residues
NaOH = Sum of sodium hydroxide solutions (trap)
% TAR = % of total applied radioactivity

Table 4: Distribution and mass ba1ance of radioactivity over the exposure period in soil 3

DE	V No.	% TAR					
		soil		volatiles			Mass balance Mv
		Extract	NER	Ethylene glycol	NaOH	total	
1	TS37	49.8	39.5	<0.01	0.4	89.7	91.5
	TS38	52.7	40.2	<0.01	0.3	93.2	
2	TS39	46.9	36.7	<0.01	1.1	84.6	85.1
	TS40	47.7	37.0	<0.01	0.8	85.5	
3	TS41	48.4	34.0	<0.01	1.1	83.5	81.3
	TS42	39.3	38.5	<0.01	1.3	79.0	
10	TS43	32.2	41.0	<0.01	5.8	79.0	77.5
	TS44	30.6	40.3	<0.01	5.1	76.0	
23	TS45	36.0	27.0	<0.01	19.0	82.0	83.8
	TS46	35.2	32.5	<0.01	17.9	85.6	
57	TS47	40.8	16.4	<0.01	29.9	87.1	85.8
	TS48	26.6	17.3	<0.01	40.6	84.5	
85	TS49	17.0	20.3	<0.01	48.0	85.3	70.6
	TS50	21.3	19.2	<0.01	15.2	55.8	
97	TS51	15.1	28.5	<0.01	42.4	86.0	85.6
	TS52	9.5	23.3	<0.01	52.2	85.1	
120	TS53	5.7	22.3	<0.01	49.3	77.4	78.2
	TS54	4.5	21.0	<0.01	53.4	78.9	

DE = Day of exposure V No. = Vessel number NER = Non extractable residues
NaOH = Sum of sodium hydroxide solutions (trap)
% TAR = % of total applied radioactivity

Table 5: Distribution and mass ba1ance of radioactivity over the exposure period in soil 4

DE	V No.	% TAR					
		soil		volatiles			Mass balance Mv
		Extract	NER	Ethylene glycol	NaOH	total	
1	TS55	71.3	22.5	<0.01	0.6	94.4	93.4
	TS56	67.5	24.4	<0.01	0.5	92.4	
2	TS57	65.6	25.5	<0.01	0.3	91.4	91.5
	TS58	68.2	22.0	<0.01	1.4	91.6	
3	TS59	66.3	21.7	<0.01	2.1	90.1	91.6
	TS60	64.5	28.5	<0.01	<0.01	93.0	
10	TS61	44.2	28.6	<0.01	11.8	84.6	82.6
	TS62	40.6	27.6	<0.01	12.3	80.5	
23	TS63	31.5	18.5	<0.01	33.6	83.6	83.8
	TS64	30.5	20.5	<0.01	32.9	83.9	
57	TS65	12.1	16.4	<0.01	45.7	74.2	76.3
	TS66	10.3	16.8	<0.01	51.3	78.4	
85	TS67	7.2	17.8	<0.01	52.8	77.8	77.4
	TS68	4.6	17.2	<0.01	55.1	77.0	
97	TS69	4.2	18.3	<0.01	63.3	85.7	85.0
	TS70	4.6	21.6	<0.01	58.0	84.2	
120	TS71	4.9	19.2	<0.01	59.9	84.0	85.2
	TS72	4.1	20.0	<0.01	62.3	86.4	

DE = Day of exposure V No. = Vessel number NER = Non extractable residues
NaOH = Sum of sodium hydroxide solutions (trap)
% TAR = % of total applied radioactivity

Table 6: DTx values from Cu-HDO in soil

Dissipation time	C14-Cu-HDO in Soil 1, soil Type 2.2 Batch F 2.2 1311
DT_{50} (days)	2.3
DT_{75} (days)	4.6
DT_{90} (days)	7.7
M_0	94
Confidence limit 95%	77.93/110.3
k	0.2993
Model	First-order

Dissipation time	C14-Cu-HDO in Soil 2, soil Type 2.3 Batch F 2.3 1211
DT_{50} (days)	2.2
DT_{75} (days)	4.5
DT_{90} (days)	7.4
M_0	97
Confidence limit 95%	84.89/108.1
k	0.3103
Model	First-order

Dissipation time	C14-Cu-HDO in Soil 3, soil Type 2.4 Batch F 2.4 1311
DT_{50} (days)	9.5
DT_{75} (days)	19
DT_{90} (days)	31
M_0	68
Confidence limit 95%	49.09/86.53
k	0.0732
Model	First-order

Dissipation time	C14-Cu-HDO in Soil 4, soil Type 5M Batch F 5M 1211
DT_{50} (days)	11
DT_{75} (days)	21
DT_{90} (days)	35
M_0	81
Confidence limit 95%	70.18/90.87
k	0.0650
Model	First-order

Table 7: Metabolite overview for soil 1, (> 10% TAR, found in radio- HPLC).

[redacted]

*Molecule is an artefact. Explanation see chapter 4.2, “identification of metabolites”.

*

Figure 1: Distribution of radioactivity in the test system soil 1, replicate 1, during the exposure

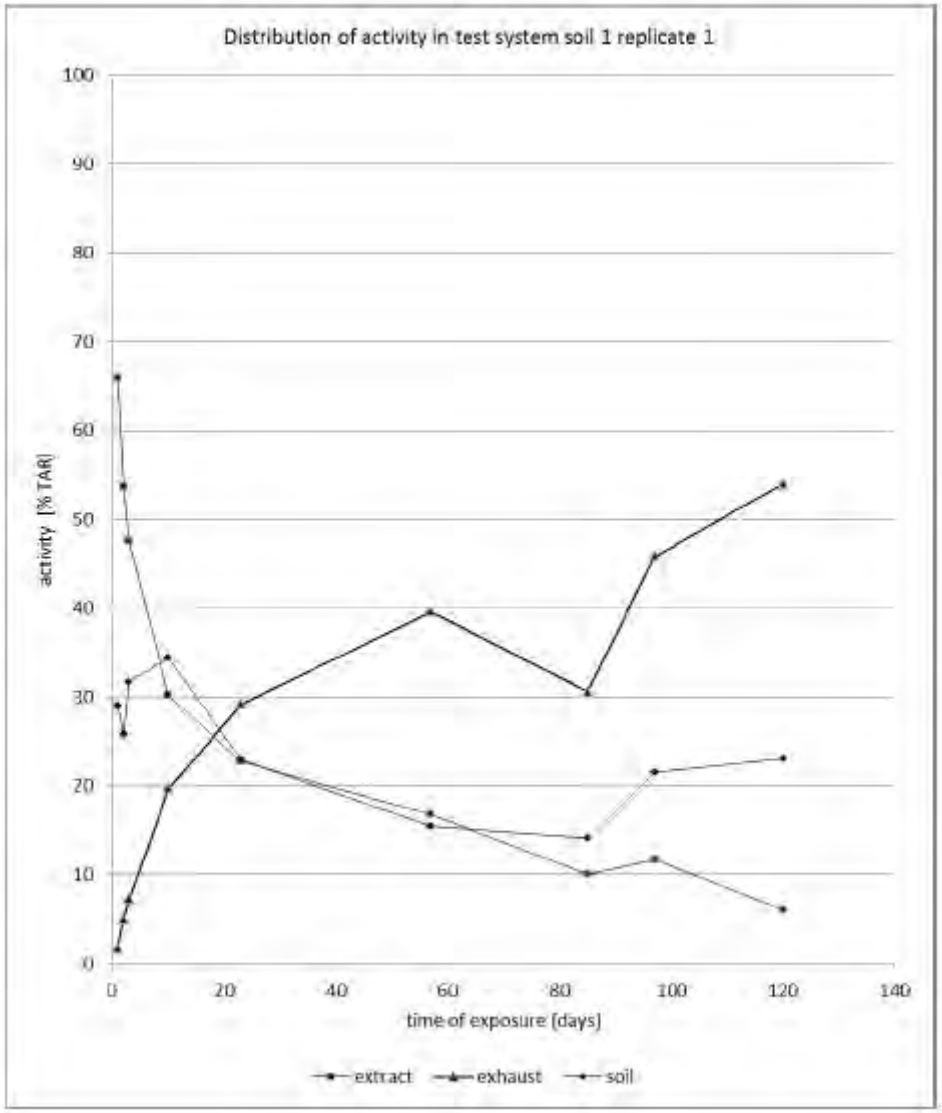


Figure 2: Pathway of degradation of the test substance in soil 1

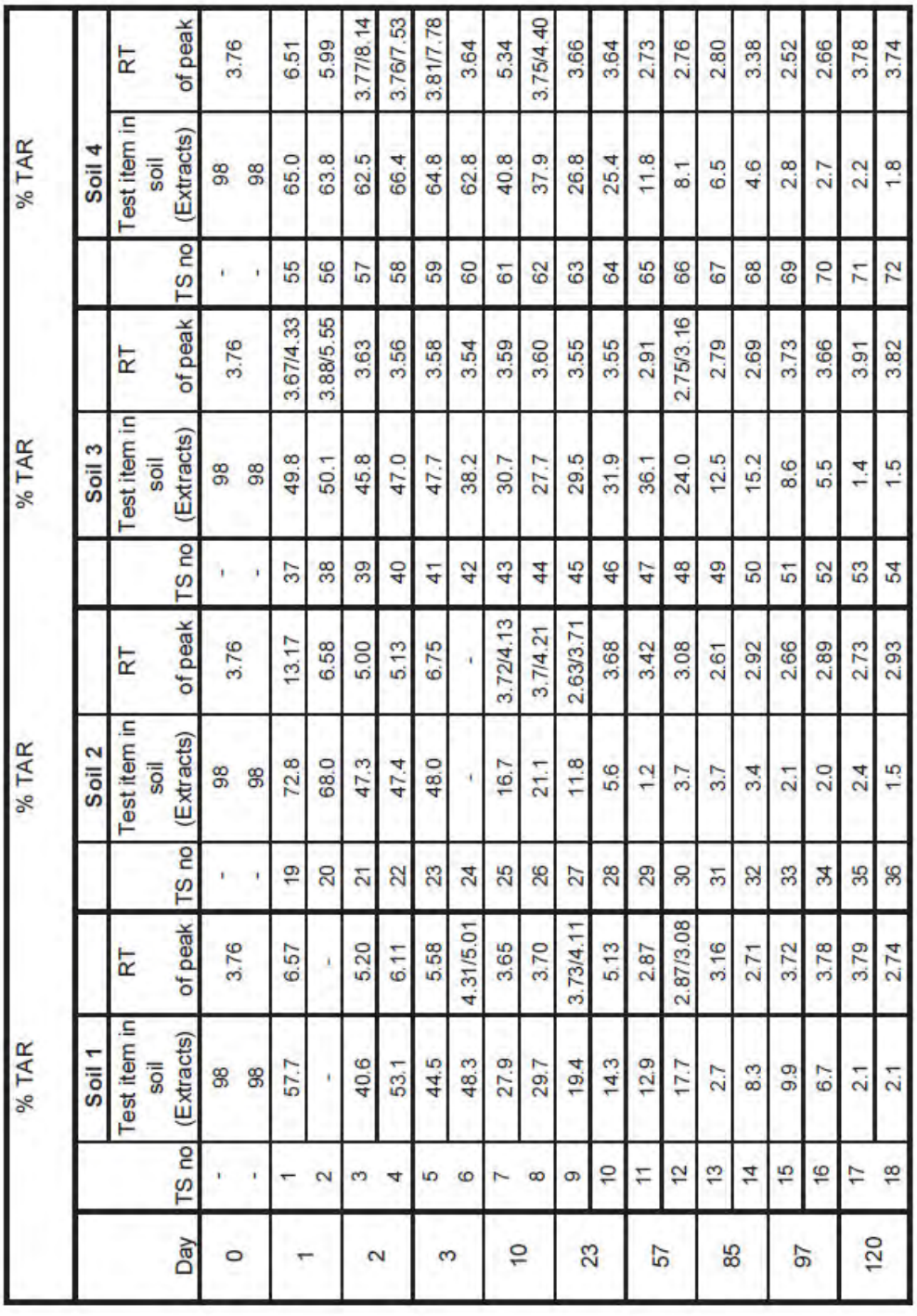
*Molecule is an artefact. Explanation see chapter 4.2, “identification of metabolites”.

*

Table 8 (inserted by RMS AT): Values from HPLC analysis for calculation of the dissipation time (1st order)

	% TAR			% TAR			% TAR			% TAR		
		Soil 1			Soil 2			Soil 3			Soil 4	
Day	TS no	Test item in Sediment (Extracts)	RT of peak	TS no	Test item in Sediment (Extracts)	RT of peak	TS no	Test item in Sediment (Extracts)	RT of peak	TS no	Test item in Sediment (Extracts)	RT of peak
0	-	98	3.76	-	98	3.76	-	98	3.76	-	98	3.76
	-	98		-	98		-	98		-	98	
1	1	57.7	6.57	19	-	-	37	49.8	3.67/4.33	55	65.0	6.51
	2	-	-	20	68.0	6.58	38	50.1	3,88/5,55	56	63.8	5.99
2	3	40.6	5.20	21	47.3	5.00	39	45.8	3.63	57	62.5	3.77/8.14
	4	53.1	6.11	22	47.4	5.13	40	47.0	3.56	58	66.4	3.76/7.53
3	5	44.5	5.58	23	48.0	6.75	41	47.7	3.58	59	64.8	3.81/7.78
	6	48.3	4.31/5.01	24	-	-	42	38.2	3.54	60	62.8	3.64
10							43	30.7	3.59	61	40.8	5.34
							44	27.7	3.60	62	37.9	3.75/4.40
23							45	29.5	3.55	63	26.8	3.66
							46	31.9	3.55	64	25.4	3.64

Table 9 (inserted by RMS At): Values from HPLC analysis for calculation of the dissipation time (FOMC)



		% TAR			% TAR			% TAR			% TAR		
		Soil 1			Soil 2			Soil 3			Soil 4		
Day	TS no	Test item in soil (Extracts)	RT of peak	TS no	Test item in soil (Extracts)	RT of peak	TS no	Test item in soil (Extracts)	RT of peak	TS no	Test item in soil (Extracts)	RT of peak
0	-	98	3.76	-	98	3.76	-	98	3.76	-	98	3.76
	-	98		-	98		-	98		-	98	
1	1	57.7	6.57	19	72.8	13.17	37	49.8	3.67/4.33	55	65.0	6.51
	2	-	-	20	68.0	6.58	38	50.1	3.88/5.55	56	63.8	5.99
2	3	40.6	5.20	21	47.3	5.00	39	45.8	3.63	57	62.5	3.77/8.14
	4	53.1	6.11	22	47.4	5.13	40	47.0	3.56	58	66.4	3.76/7.53
3	5	44.5	5.58	23	48.0	6.75	41	47.7	3.58	59	64.8	3.81/7.78
	6	48.3	4.31/5.01	24	-	-	42	38.2	3.54	60	62.8	3.64
10	7	27.9	3.65	25	16.7	3.72/4.13	43	30.7	3.59	61	40.8	5.34
	8	29.7	3.70	26	21.1	3.7/4.21	44	27.7	3.60	62	37.9	3.75/4.40
23	9	19.4	3.73/4.11	27	11.8	2.63/3.71	45	29.5	3.55	63	26.8	3.66
	10	14.3	5.13	28	5.6	3.68	46	31.9	3.55	64	25.4	3.64
57	11	12.9	2.87	29	1.2	3.42	47	36.1	2.91	65	11.8	2.73
	12	17.7	2.87/3.08	30	3.7	3.08	48	24.0	2.75/3.16	66	8.1	2.76
85	13	2.7	3.16	31	3.7	2.61	49	12.5	2.79	67	6.5	2.80
	14	8.3	2.71	32	3.4	2.92	50	15.2	2.69	68	4.6	3.38
97	15	9.9	3.72	33	2.1	2.66	51	8.6	3.73	69	2.8	2.52
	16	6.7	3.78	34	2.0	2.89	52	5.5	3.66	70	2.7	2.66
120	17	2.1	3.79	35	2.4	2.73	53	1.4	3.91	71	2.2	3.78
	18	2.1	2.74	36	1.5	2.93	54	1.5	3.82	72	1.8	3.74

Section A 7.3.1 Annex Point IIIA, XII.3 Phototransformation in air (estimation method), including identification of breakdown products

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X

The specific first-order degradation rate constant of Cu-HDO with OH-radicals (k_{OH} in cm^3x $molecule^{-1}$ x s^{-1}) has been estimated with an Atmospheric Oxidation Program (see below):

k_{OH} (Cu-HDO) = 68.7220 x $10^{-12}$$cm^3$/molecule x sec

By relating k_{OH} to the average OH-radical concentration in the atmosphere, the pseudo-first order rate constant in air is determined by the following equation:

$kdeg_{air} = k_{OH} \cdot OHconc_{air} \cdot 24 \cdot 3600$

$kdeg_{air}$ (Cu-HDO) = 2.97 d^{-1}

Explanation of symbols

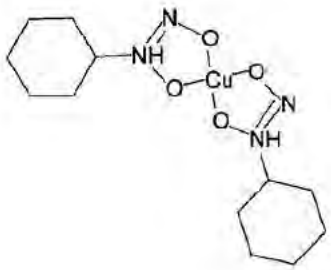
k_{OH}	specific degradation rate constant with OH-radicals	[cm^3 x $molec.^{-1}$ x s^{-1}]	
$OHCONC_{air}$	concentration of OH-radicals in atmosphere	[molec. x cm^{-3}]	$5x10^5$*
$kdeg_{air}$	pseudo first order rate constant for degradation in air	[d^{-1}]	

*The global annual average OH-radical concentration can be assumed to be 5 x 10^5 molecules x cm^{-3} (BUA, 1992).

The half-life of Cu-HDO has been estimated to be 1.868 hours.

Because Cu-HDO is free of Cl, Br or F an effect of Cu-HDO on stratospheric ozone can be excluded.

In addition, there is a very limited possibility that Cu-HDO reaches the atmosphere because of the very low vapour pressure (<0.0000001 hPa at 20°C).



```
SMILES : C1CCC(N4=NO[Cu]3(O4)ON=N(C2CCCCC2)O3)CC1
CHEM   :
MOL FOR: C12 H22 N4 O4 Cu1
MOL WT : 349.88
------------------- SUMMARY (AOP v1.90): HYDROXYL RADICALS -------------------
Hydrogen Abstraction       =  68.7220 E-12 cm3/molecule-sec
**Reaction with N, S and -OH =   0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds   =   0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =   0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =   0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =   0.0000 E-12 cm3/molecule-sec

   OVERALL OH Rate Constant =  68.7220 E-12 cm3/molecule-sec
   HALF-LIFE =     0.156 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =     1.868 Hrs
.......................  ** Designates Estimation(s) Using ASSUMED Value(s)
------------------- SUMMARY (AOP v1.90): OZONE REACTION ----------------------

               ******  NO OZONE REACTION ESTIMATION ******
               (ONLY Olefins and Acetylenes are Estimated)

Experimental Database:  NO Structure Matches

-------------------------------------------------------------------------------
```

	Evaluation by Competent Authorities **EVALUATION BY RAPPORTEUR MEMBER STATE**
Date	December 2005
Materials and Methods	The atmospheric oxidation program is called AopWin and is a part of the estimation software EPISUITE which is available from the Syracuse Research Corporation.
Results and discussion	The vapour pressure is < 0.000001 hPa at 20 °C. $kdeg_{air}$ (Cu-HDO) = 2.97 d^{-1} with c_{OH} = 5*10^5 molecules/cm^{-3} acc. to TGD $kdeg_{air}$ (Cu-HDO) = 8.91 d^{-1} with c_{OH} = 1.5*10^6 molecules/cm^{-3} acc. to AOP Win $T_{1/2}$ = 5.6h with c_{OH} = 5*10^5 molecules/cm^{-3} acc. to TGD $T_{1/2}$ = 1.87h with c_{OH} = 1.5*10^6 molecules/cm^{-3} acc. to AOP Win
Conclusion	Agree with applicant's version
Reliability	n.a.
Acceptability	Acceptable
Remarks	None

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.4.1.1 [redacted] (1993), STUDY REPORT Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) of Bis-(N-Cyclohexyldiazeniumdioxy)-kupfer in a static system (96 hours); OECD 203, Project Number 12 F0141/925032, [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	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" EEC directive 84/449 C.1, updated version of No. 1989	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cu-HDO	
3.1.1	Lot/Batch number	REU E 7360 B	
3.1.2	Specification	Solid	
3.1.3	Purity	99%	
3.1.4	Composition of Product	Not appropriate	
3.1.5	Further relevant properties	Not appropriate	
3.1.6	Method of analysis	Determination of copper by graphite furnace atomic absorption spectrometry	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not appropriate	
3.3	**Reference substance**	Not mentioned in the study report	
3.3.1	Method of analysis for reference substance	Not appropriate	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

3.4	**Testing procedure**	Non-entry field	
3.4.1	Dilution water	See table A7_4_1_1-2	
3.4.2	Test organisms	Fish (rainbow trout), see A7_4_1_1-3	
3.4.3	Test system	Static system, see A7_4_1_1-4	
3.4.4	Test conditions	See A7_4_1_1-5	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality and symptoms	
3.4.7	Sampling	Mortality and symptoms have been determined after 1, 4, 14, 48, 72 and 96 hours	x
3.4.8	Monitoring of TS concentration	Monitoring of TS concentration after 1, 4, 14, 48, 72 and 96 hours	x
3.4.9	Statistics	Probit analysis	
		4 RESULTS	
4.1	**Limit Test**	A range finding study has been performed before start of the acute toxicity test. The LC_{50} after 96 hours of the range finding test has been determined to be between 0.1 and 1.0 mg/L	
4.1.1	Concentration	Not mentioned in the report	
4.1.2	Number/ percentage of animals showing adverse effects	Not mentioned in the report	
4.1.3	Nature of adverse effects	Not mentioned in the report	
4.2	**Results test substance**	Non-entry field	
4.2.1	Initial concentrations of test substance	0.046 / 0.1 / 0.215 / 0.464 / 1.00 / 2.15mg/L	
4.2.2	Actual concentrations of test substance		

Nominal conc. (mg/L)	Analyt. Conc. (mg/L) 96 h	Analyt. Conc. (mg/L) 48 h	Analyt. Conc. (mg/L) 1 h
0.046	0.038	0.038	0.027
0.100	0.066	0.060	0.027
0.215	0.140	0.120	0.027
0.464	0.240	0.200	0.044

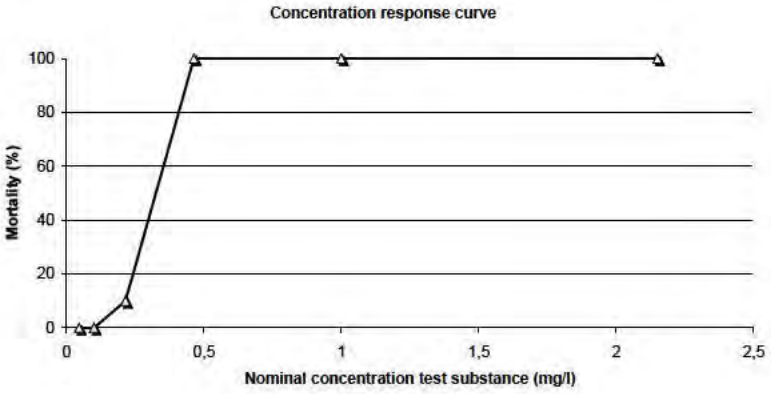
Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

1.000		0.360	0.170
2.150		0.600	0.300

4.2.3 Effect data (Mortality) — see table A7_4_1_1-6 and A7_4_1_1-7

4.2.4 Concentration / response curve



4.2.5 Other effects

Nominal conc. (mg/L)	Symptoms 1 h	Symptoms 4 h	Symptoms 24 h	Symptoms 48 h	Symptoms 72 h	Symptoms 96 h
0.046						
0.100						
0.215						
0.464				(A)T		
1.000			(A)			
2.150		A				

A = Apathy

T = Tumbling

() = slight to very slight

4.3 Results of controls

4.3.1 Number/ percentage of animals showing adverse effects — See A7_4_1_1-6

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

4.3.2	Nature of adverse effects	Apathy. tumbling
4.4	**Test with reference substance**	Not mentioned in the study report
4.4.1	Concentrations	Not appropriate
4.4.2	Results	Not appropriate

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

- OECD Guideline 203 "Fish. Acute Toxicity Test"
- EEC directive 84/449 C.1. updated version of No. 1989

TEST ORGANISMS

- Strain: rainbow trout (Oncorhynchus mykiss WALBAUM 1792)
- Supplier: Forellenhof Fredelsloh. D-83413 Moringen. FRG
- Size: 4.09cm (range: 3.8-4.5cm)
- Weight: 0.597g (range: 0.5-0.81g)
- Age: 2.5 months
- Diet: breeding feet. withdrawal of wood one day before and during exposure

TEST WATER:

- Municipal water of the city of Frankenthal. not chlorinated and passed through a carbon filter
- Total hardness: about 2.5mmol/L
- Acid capacity: about 5.5mmol/L
- pH: about 7.5

TEST SYSTEM

- Test type: static test
- Test concentrations [mg/L]: 0.0464/0.1/0.215/0.464/1/2.15
- Water: drinking water
- Exposure vessel type: glass aquarium. (80cm x 35 cm x 46cm). 100 L volume
- Number of animals per test concentration and vessel: 10
- Test temperature: 11°C

DURATION OF THE TEST: 96h

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

5.2 Results and discussion

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes

Nominal conc. (mg/L)	Analytical conc. (mg/L)	Mortality after 96 h
0.046	0.038	0
0.100	0.066	0
0.215	0.140	1
0.464	0.240	10
1.000	—	10
2.150	—	10

NOEC:	0.100mg/L (nominal)
Maximum concentration tested causing no mortality:	0.100mg/L (nominal)
Maximum concentration tested causing 100 % mortality:	0.464mg/L (nominal)

5.2.1 LC_0

0.100mg/L (nominal)
0.066mg/L (analysed)

5.2.2 LC_{50}(96 h)

LC_{50}(96 h) nominal concentrations

GREATER	0.22	(mg/L)	5% significance level
LOWER	0.46	(mg/L)	1% significance level

LC_{50}(96 h) analytical detected concentrations:

GREATER	0.14	(mg/L)	5% significance level
LOWER	0.24	(mg/L)	1% significance level

5.2.3 LC_{100}

0.46mg/L (nominal)
0.240mg/L (analysed)

5.3 Conclusion

5.3.1 Other Conclusions

5.3.2 Reliability: 1

5.3.3 Deficiencies: No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
DATE	December 2006
MATERIALS AND METHODS	**3.4.7 Sampling** Typing error: 24 hours instead of 14 h **3.4.8 Monitoring of TS concentration** Typing error: 24 hours instead of 14 h
RESULTS AND DISCUSSION	Agree with the applicant's version
CONCLUSION	Agree with the applicant's version
RELIABILITY	1
ACCEPTABILITY	Acceptable
REMARKS	-

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	municipal water of the city of Frankenthal not chlorinated and passed through an active carbon filter
Alkalinity	Acid capacity: about 5.5mmol/L
Hardness	~ 2.5 mmol/L (= ~ 250mg/L $CaCO_3$)
pH	~ 7.5
Oxygen content	>60% of maximum saturation
Conductance	Not reported
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	Forellenhof Fredelsloh D-W3413 Moringen. FRG
Wild caught	No
Age/size	Body length: 4.09cm (range: 3.8 – 4.5) Body weight: 0.597g (Range 0.50 – 0.81) Age: ~ 2.5 months
Kind of food	Breeding feed. On workdays in addition live and frozen artemia
Amount of food	ad libitum
Feeding frequency	n.a., fish were not fed during test
Pretreatment	Adaptation Medical treatment: a few days after arrival prophylactical treatment twice with 0.05mg/L malachite green chloride and once with 10mg/L tetracyline hydrochloride
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static system
Renewal of test solution	Not reported
Volume of test vessels	100L
Volume/animal	10L
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	12 – 18 centigrade
Dissolved oxygen	>60% of maximum saturation
pH	About 8.0
Adjustment of pH	No
Aeration of dilution water	Aerated with oil-free air
Intensity of irradiation	Not reported
Photoperiod	16 hours light. 8 hours darkness

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration		Mortality							
		Number				Percentage			
nominal [mg/L]	Analytical [mg/L]	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0		0	0	0	0	0	0	0	0
0.046	0.038	0	0	0	0	0	0	0	0
0.100	0.066	0	0	0	0	0	0	0	0
0.215	0.140	0	0	0	1	0	0	0	10
0.464	0.240	0	4	10	10	0	40	100	100
1.000	—	0	10	10	10	0	100	100	100
2.150	—	10	10	10	10	100	100	100	100
Temperature [°C]		10 - 12	11	11	11				
pH		8.7 – 8.8	8.7 – 8.8	8.7 – 8.8	8.7 – 8.8				
Oxygen [mg/L]		11.1 – 11.5	11.1 – 11.4	11.3 – 11.6	11.0 – 11.5				

Table A7_4_1_1-7: Effect data

	48h [mg/L]	95% c l.	96h [mg/L]	95% c.l.
LC_0	0.215 (nominal) 0.120 (analytical)	Not reported	0.100 (nominal) 0.066 (analysed)	Not reported
LC_{50}	~ 0.464 (nominal) ~ 0.200 (analytical)	Not reported	>0.22 (nominal) - <0.46 (nominal) >0.14 (analytical)- <0.24 (analytical)	5% significance level 1% significance level 5% significance level 1% significance level
LC_{100}	1.000 (nominal) 0.360 (analytical)	Not reported	0.46mg/L (nominal) 0.240mg/L (analysed)	Not reported

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

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

Criteria for poorly soluble test substances	**n.a.**	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

Official use only

		1 REFERENCE
1.1	**Reference**	A 7.4.1.2 [redacted] (1992) Determination of the acute toxicity of Bis-(N-Cyclohexyldiazeniumdioxy)-kupfer (Cu-HDO) to the water flea Daphnia magna Strauss: Report 92/1699/50/1, [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	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 EG 79/831/EWG, version 1989
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Cu-HDO
3.1.1	Lot/Batch number	Reu-E 7360 B
3.1.2	Specification	Solid
3.1.3	Purity	99%
3.1.4	Composition of Product	Not appropriate
3.1.5	Further relevant properties	Not appropriate
3.1.6	Method of analysis	Not appropriate
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	n.a.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	Non-entry field
3.4.1	Dilution water	see table A7_4_1_2-2
3.4.2	Test organisms	Daphnia magna Strauss from Institute National Recherche Chimique Appliquée, France. see also table A7_4_1_2-3

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 ***Daphnia magna***

3.4.3	Test system	see table A7_4_1_2-4
3.4.4	Test conditions	see table A7_4_1_2-5
3.4.5	Duration of the test	48 h
3.4.6	Test parameter	Immobilisation after 48 h
3.4.7	Sampling	Visual recording of the swimming ability after 0, 3, 6, 24 and 48 hours
3.4.8	Monitoring of TS concentration	Yes, analysis of the test concentration at start (0 hour) and end of the test (48 hours)
3.4.9	Statistics	For the statistical evaluation of the EC_{50} the moving average method was used.
	4 RESULTS	
4.1	**Limit Test**	No
4.1.1	Concentration	Not applicable
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
4.1.3	Nature of adverse effects	Not applicable
4.2	**Results test substance**	Non-entry field
4.2.1	Initial concentrations of test substance	3, 1.5, 0.75, 0.38, 0.19, 0.094, 0.047, 0.023, 0.012mg/L
4.2.2	Actual concentrations of test substance	Between min. 100.0 and max. 100.6% of the nominal concentrations

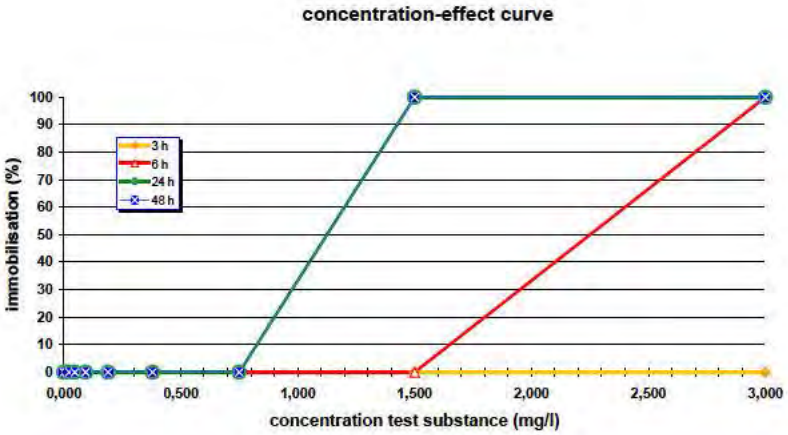
Nominal concentrations	Actual concentrations
3	3
0.38	0.4
0.094	0.1
0	--

4.2.3	Effect data (Immobilisation)	See table A7_4_1_2-6 and A7_4_1_2-7

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

4.2.4 Concentration / response curve



4.2.5 Other effects: Not appropriate; the only test parameter tested was the swimming ability of the test organisms

4.3 Results of controls: All test animals of the control (20 animals) were alive and mobile over the whole test period (see Table A7_4_1_2-6).

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 study was carried out in accordance with EEC Directive 79/831/EEC, Annex V, Part C: Methods for the determination of ecotoxicity, C2. Acute toxicity for Daphnia; Updating Nov. 1989.

TEST ORGANISMS

- Strain: Daphnia magna Strauss from Institute National Recherche Chimique Appliquée, France. Animals are bred in the BASF AG lab since 1978.
- Age: 2-24h
- Feeding: none
- Control group: yes, 20 animals

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- Procedure: a saturated solution of the substance (6mg/L) was filtered and the test concentrations was prepared by dilution
- Vehicle, solvent: water

DILUTION WATER

- Source: M4 water, special test water

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

- Hardness: 2.7mmol/L
- Ca/Mg ratio: 4:1
- pH: 8
- Oxygen content: saturated
- Conductance: 600-700µS/cm

TEST SYSTEM

- Test type: floatability of animals
- Exposure vessel type: special test tube, 20ml volume
- Number of replicates, individuals per replicate: 20
- Test temperature: 20.5° C
- Dissolved oxygen: 8.4mg/L
- pH: 8
- Intensity of irradiation: 5-6µE/(m²s) at 400-700nm
- Photoperiod: day-night: 16:8
- DURATION OF THE TEST: 48 h

TEST PARAMETER: mobility

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes

5.2 Results and discussion

RESULTS: EXPOSED

- Nominal concentration [mg/L]:
- 3/1.5/0.75/0.38/0.19/0.094/0.047/0.023/0.012
- Immobilisation: >1.5mg/L
- test substance solubility: ca. 6mg/L
- Other effects: no data available

RESULTS CONTROL: control group was okay

5.2.1	EC_0	0.75 (mg/L)
5.2.2	EC_{50} (48 h)	1.1mg/L
5.2.3	EC_{100} (48 h)	1.5mg/L
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	December 2006
Materials and Methods	Acceptable
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	-

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	M4 water, special test water
Alkalinity	K_S until pH 4.3: 0.90±0.10mmol/L $\Rightarrow K_B = 10^{-14} / K_S = 1.11^{-14}$ L/mol $\Rightarrow pK_B = 13.95$ L/mol
Hardness	2.7mmol/L
pH	8
Ca / Mg ratio	4:1
Na / K ratio	Not mentioned in the report
Oxygen content	saturated
Conductance	600-700µS/cm
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	Daphnia magna Strauss
Source	Institute National Recherche Chimique Appliquée, France.
Age	2 – 24 hours at the start of the test
Breeding method	According to guideline
Kind of food	No feeding during test period
Amount of food	None
Feeding frequency	No feeding during test period
Pretreatment	Not mentioned in the study report
Feeding of animals during test	No

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	No
Volume of test vessels	20ml
Volume/animal	2ml per animal
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	19.6 – 21.1
Dissolved oxygen	8.3 – 8.6
pH	7.8 – 8.2
Adjustment of pH	No
Aeration of dilution water	The test water has been aerated until saturation and then stored for 24 hours for stabilisation.
Quality/Intensity of irradiation	Artificial light About 5 – 6µE /m² s in the range of 400 – 700nm
Photoperiod	Day : night - rhythm 16 : 8 hours

Table A7_4_1_2-6: Immobilisation data

Test-Substance Concentration (nominal) [mg/l]	Immobile *Daphnia*										
	Number				Percentage				Oxygen [mg/l]	pH	Temperature [°C]
	3h	6h	24h	48h	3h	6h	24h	48h	48h	48h	48h
0	0	0	0	0	0	0	0	0	8.4	8.0	21.1
3	0	20	20	20	0	100	100	100	8.3	8.0	21.1
1.5	0	0	20	20	0	0	100	100	8.3	8.0	21.1
0.75	0	0	0	0	0	0	0	0	8.3	8.0	21.1
0.38	0	0	0	0	0	0	0	0	8.3	8.0	21.1
0.19	0	0	0	0	0	0	0	0	8.4	8.0	21.1
0.094	0	0	0	0	0	0	0	0	8.4	8.0	21.1
0.047	0	0	0	0	0	0	0	0	8.5	8.0	21.1
0.023	0	0	0	0	0	0	0	0	8.3	8.0	21.1
0.012	0	0	0	0	0	0	0	0	8.3	7.8	21.1

Table A7_4_1_2-7: Effect data

	EC_{50}[1]	95% c.l.	EC_0[1]	EC_{100}[1]
3h	>3 (n)		≥3 (n)	>3 (n)
6h	2.1 (n)		1.5 (n)	3 (n)
24h [mg/l]	1.1 (n)		0.75 (n)	1.5 (n)
48h [mg/l]	1.1 (n)		0.75 (n)	1.5 (n)

[1] effect data are based on nominal (n) concentrations

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

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

Criteria for poorly soluble test substances	Not applicable	

Section A7.4.1.4 Inhibition to microbiological activity (aquatic)

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.4.1.4 [redacted] (2001) Title: Determination of the Inhibition of Oxygen Consumption by Activated Sludge in the Activated Sludge Respiration Inhibition test: Report 00/0801/08/2, [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Criteria for data protection	Data submitted to the MS before 14 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 209 "Activated Sludge, Respiration Inhibition Test"	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cu-HDO, p.a.	
3.1.1	Lot/Batch number	W-86	
3.1.2	Specification	Solid blue crystals	
3.1.3	Purity	99%	
3.1.4	Composition of Product		
3.1.5	Further relevant properties		
3.1.6	Method of analysis		
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	n.a.	x
3.3	**Reference substance**	Yes 3,5-dichlorophenol	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**	Non-entry field	
3.4.1	Culture medium	100 x concentrated OECD medium	
3.4.2	Inoculum / test organism	see table A7_4_1_4-2	x
3.4.3	Test system	see table A7_4_1_4-3	

Section A7.4.1.4 Inhibition to microbiological activity (aquatic)

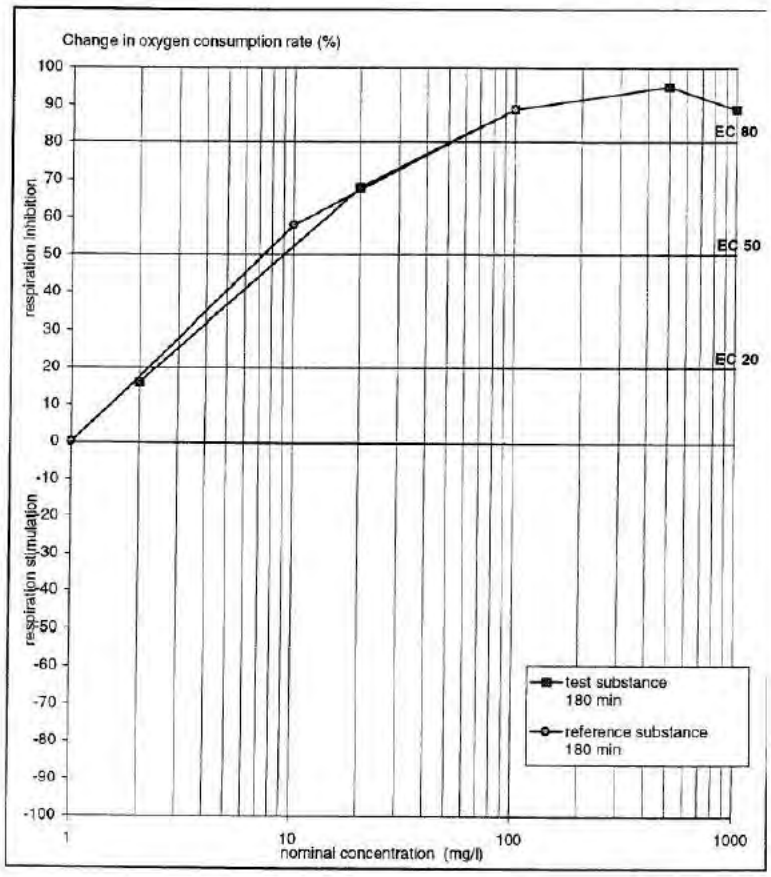
Annex Point IIA7.4

3.4.4	Test conditions	see table A7_4_1_4-4
3.4.5	Duration of the test	180 min
3.4.6	Test parameter	Inhibition of oxygen consumption rate of aerobic microorganisms (activated sludge)
3.4.7	Analytical parameter	oxygen measurement
3.4.8	Sampling	
3.4.9	Monitoring of TS concentration	
3.4.10	Controls	Yes
3.4.11	Statistics	
		4 RESULTS
4.1	**Preliminary test**	performed
4.1.1	Concentration	1000mg/l
4.1.2	Effect data	Respiration inhibition of bacteria of 89% at a concentration of the test substance of 1000mg/l
4.2	**Results test substance**	Non-entry field
4.2.1	Initial concentrations of test substance	1mg/ml, 0.5mg/ml, 0.1mg/ml, 0.02mg/ml, 0.002mg/ml
4.2.2	Actual concentrations of test substance	
4.2.3	Growth curves	
4.2.4	Cell concentration data	

Section A7.4.1.4 Inhibition to microbiological activity (aquatic)

Annex Point IIA7.4

4.2.5 Concentration/ response curve



4.2.6 Effect data

EC_{20} (180 min): ca 2.5mg/l (nominal)

E_{50} (180 min): ca 9.0mg/l (nominal)

EC_{80} (180 min): ca 50mg/l (nominal)

4.2.7 Other observed effects

—

4.3 Results of controls x

4.4 Test with reference substance

Performed

3,5-dichlorophenol

4.4.1 Concentrations

4.4.2 Results

EC_{20} (mg/l)	EC_{50} (mg/l)	EC_{80} (mg/l)	highest concentration tested (mg/l)
Ca 2.2	ca 7.5	ca. 50	100

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods x

Activated sludge respiration inhibition test, annex to EEC directive 88/302/EEC 18 Nov. 1987

This method corresponds to:

- *OECD guidelines for testing of chemicals*
  Activated sludge, respiration inhibition test 209; Paris 1993

Section A7.4.1.4 Inhibition to microbiological activity (aquatic)

Annex Point IIA7.4

		– *International standards ISO 8192-1986 (E) (Method B) Water quality –test for inhibition of oxygen consumption by activated sludge*	
5.2	**Results and discussion**		x
5.2.1	EC_{20}	ca. 2.5mg/l	
5.2.2	EC_{50}	ca. 9mg/l	
5.2.3	EC_{80}	ca. 50mg/l	
5.3	**Conclusion**	The EC_{20} value in the activated sludge respiration inhibition test is <100mg/l. depending on local conditions and existing concentrations, disturbances in the biodegradation process of activated sludge are possible.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	January 2006
Materials and Methods	**3.2 Preparation of TS solution for poorly soluble or volatile test substances** The test substance was added in the required amount to the test vessel with about 180 ml deionised water. The mixture was stirred for 22 +/- 2 hours to obtain an optimal solution. **3.4.2 Inoculum/test organism** Table A 7_4_1_4-2: Preparation of the inoculum for exposure: 50 ml were added to a total volume of **250 ml** to obtain a concentration of 1 g/L dry substance in the test.
Results and discussion	**4.3 Results of controls** The respiration rates of the blank controls are within 15% of each other.
Conclusion	**5.1 Materials and methods** EC guideline C.11 **5.2 Results and discussion** Given values are nominal values.
Reliability	1
Acceptability	Acceptable
Remarks	-

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
Nature	activated sludge
Species	
Strain	
Source	laboratory wastewater plant treating municipal sewage
Sampling site	
Laboratory culture	
Method of cultivation	
Preparation of inoculum for exposure	The inoculum was washed, brought to a concentration of 5g/l dry substance, and aerated during the night. 50 ml were added to a total volume of 150ml to obtain a concentration of 1g/l dry substance in the test.
Pretreatment	
Initial cell concentration	

Table A7_4_1_4-3: Test system

Criteria	Details
Culturing apparatus	Erlenmeyer flasks (250ml volume)
Number of culture flasks/concentration	1/1
Aeration device	
Measuring equipment	pH-electrode, O_2-electrode
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_4-4: Test conditions

Criteria		Details
5.3.3	Test temperature	20±2°C
5.3.4	pH	7.0 – 7.5
5.3.5	Aeration of dilution water	
5.3.6	Suspended solids concentration	

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

			Official use only
		1 REFERENCE	
1.1	**Reference**	—	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dr. Wolman GmbH	
1.2.2	Criteria for data protection	Data submitted to the MS before 14 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**	n. a.	
2.2	**GLP**		
2.3	**Deviations**		
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number		
3.1.2	Specification		
3.1.3	Purity		
3.1.4	Further relevant properties		
3.1.5	Radio-labelling		
3.1.6	Method of analysis		
3.2	**Reference substance**		
3.2.1	Method of analysis for reference substance		
3.3	**Testing/estimation procedure**	Non-entry field	
3.3.1	Test system/ performance		
3.3.2	Estimation of bioconcentration	The log BCF-value can be calculated using the log POW values from chapter partition coefficient.	
		4 RESULTS	
4.1	**Experimental data**		
4.1.1	Mortality/behaviour		
4.1.2	Lipid content		
4.1.3	Concentrations of test material during test		
4.1.4	Bioconcentration factor (BCF)	1.39-1.51	

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

4.1.5	Uptake and depuration rate constants		
4.1.6	Depuration time		
4.1.7	Metabolites		
4.1.8	Other Observations		
4.2	**Estimation of bioconcentration**		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Estimation of bioconcentration The log BCF-value can be calculated using the log POW values from chapter partition coefficient. log BCF =0.85 x log Pow - 0.7	x
5.2	**Results and discussion**	Therefore, the calculated value is in the range of 1.39-1.51. The $BCF_{(fish)}$ is 32.36.	x
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	December 2006
Materials and Methods	Acceptable
Results and discussion	Agree with the applicant's version
Conclusion	**5.1 Materials and methods** The relevant log Pow is 2.6 **5.2 Results and discussion** The calculated log BCF based on a log Pow of 2.6 is 1.51. The BCF_{fish} is 32.36.
Reliability	1
Acceptability	Acceptable
Remarks	-

Section 7.4.3.2 Effects on reproduction and growth rate of fish

Annex Point IIIA XIII 2.2

Justification for non-submission of data	Official use only

Other existing data [☒] Technically not feasible [☐] Scientifically unjustified [☐]

Limited exposure [☒] Other justification [☐]

Detailed justification: x

- The toxicity of Cu-HDO is mainly based on the copper part of the substance.
- A comparison of the ecotoxicity data available for Cu-HDO, K-HDO, other copper compounds and copper in general show that the ecotoxicity of Cu-HDO is predominantly determined by the copper part of the molecule. Data are summarised in the following table:

	Fish LC_{50} mg/l	Daphnia EC_{50} mg/l	Algae LC_{50} mg/l
Cu-HDO	0,22 – 0,46	1,1	0,08
K-HDO	51,3	> 30	15,6
Copper	0,03 – 2,2	0,02 – 0,07	0,005 – 0,05
$CuCl_2$	0,01	0,03	0,1 – 0,18

- Due to the log POW<3 a bioaccumulation of Cu-HDO is not to be expected.
- Effects on reproduction and growth rate can therefore be deduced from existing Copper toxicity studies. A summary of reproduction and early life-stage studies on fish is presented below (Sources: Environmental Health criteria 200 – Copper):

Effects on reproduction and early life-stages (WHO 1998)

Stouthart et al. 1996 (Ref A 7.4.3.2/01) exposed newly fertilized common carp *(Cyprinus carpio)* eggs to copper (19.1 and 50.8µg/litre; 0.3 and 0.8µmol/litre) at pH 6.3 and 7.6. No significant effect of copper on egg mortality, larval heart rate and tail movement or whole-body potassium and magnesium content was observed at pH 7.6. However, whole-body sodium and calcium were significantly decreased and larval mortality and larval deformation were significantly increased at the higher copper exposure. At pH 6.3, exposure to 50.8µg Cu/litre (0.8µmol/litre) significantly increased egg mortality and decreased heart rate and tail movements; premature hatching and a concentration-dependent increase in larval mortality and larval deformation were also observed. The whole-body content of potassium, sodium, magnesium, and calcium were all significantly decreased by both copper exposures at pH 6.3.

Anderson et al. 1991 (Ref A 7.4.3.2/02) carried out both fertilization tests and embryo development tests on topsmelt *(Atherinops affinis)*. In fertilization tests percentage fertilization was measured following exposure of sperm to copper. The NOEC values for four fertilization tests ranged from 32 to>90µg Cu/litre; EC50s ranged from 24 to 163µg Cu/litre. In embryo tests embryos were checked for up to 12 days for viability, abnormalities, mortality and hatching success. The NOEC for embryo abnormalities ranged from 55 to 123 µg Cu/litre; EC50s ranged from 115 to 180µg Cu/litre. The NOEC for larval hatching success and for larval abnormalities ranged from 55 to 123µg Cu/litre, and from 55 to 68µg Cu/litre for the two parameters, respectively; EC50s ranged from 108 to 182µg Cu/litre, and from 75 to 190µg Cu/litre.

Pickering et al. 1977 (Ref A 7.4.3.2/03) exposed fathead minnow *(Pimephales promelas)* to copper (8-100 µg Cu/litre) at 6, 3 and 0 months prior to spawning. The prespawning exposure time had no significant effect on reproduction. However, egg production was significantly lower at concentrations of 37µg Cu/litre or more. The maximum acceptable toxicant concentration (MATC) was estimated to be 32µg Cu/litre.

Scudder et al. 1988 (Ref A 7.4.3.2/04) exposed embryos of fathead minnow *(Pimephales promelas)* to total copper concentrations ranging from 0.6 to 621µg Cu/litre from 5 to 10 h post-fertilization to 2 days post-

Section 7.4.3.2 Effects on reproduction and growth rate of fish

Annex Point IIIA XIII 2.2

hatch. Significant declines in percentage survival and percentage total hatch were observed at 621 µg Cu/litre but not at 338 Cu/litre. A significant increase in the number of embryos with abnormalities was observed at>338µg Cu/litre. Larval fish were exposed to copper at the same concentrations for 28 days post-hatch. Fish growth was significantly reduced and percentage abnormalities increased at the lowest treatment concentration (61µg Cu/litre) and effects increased with increasing concentration. Percent survival was significantly reduced at concentrations of 113µg Cu/litre and above and the 28-day LC_{50} was estimated to be 128µg Cu/litre.

Mount, 1968 (Ref A 7.4.3.2/05) conducted an 11-month, full-life-cycle exposure of fathead minnows to copper in a hard water (hardness 200mg $CaCO_3$/litre, alkalinity 160mg $CaCO_3$/litre, pH 7.9). Growth and survival were significantly reduced at 95µg Cu/litre and reproduction was completely suppressed at 32-34µg Cu/litre, but unaffected at 14-15µg Cu/litre.

In a softer water (hardness 31mg $CaCO_3$/litre, alkalinity 30mg $CaCO_3$/litre, pH 7.0), Mount & Stephen 1969 (Ref A 7.4.3.2/06) reported survival, growth, and reproduction to be significantly affected at 18.4µg Cu/litre, but not at 10.6µg Cu/litre.

McKim et al. 1978 (Ref A 7.4.3.2/07) tested the effects of copper on the growth and survival of embryos and larvae of eight fish species. The standing crop of fish after 30-70 days post-hatch was significantly reduced at exposure concentrations of 32µg Cu/litre for rainbow trout, 34µg Cu/litre for white sucker, 44µg Cu/litre for brook trout, 42µg Cu/litre for lake trout, 46µg Cu/litre for brown trout, 103µg Cu/litre for lake herring, 104µg Cu/litre for northern pike, and 104µg Cu/litre for smallmouth bass.

Horning & Neiheisel 1979 (Ref A 7.4.3.2/08) exposed bluntnose minnow *(Pimephales notatus)* to copper concentrations ranging from 4.3 (control) to 119.4µg Cu/litre. Minnows exposed to 119.4µg Cu/litre for 60 days were significantly smaller than the other groups. Survival of parental bluntnose minnows was not affected by any copper concentrations during the 60-day exposure. Copper concentrations>18µg Cu/litre significantly reduced the number of spawnings, the total number of eggs produced and the number of eggs per female. Therefore, the MATC based on reproductive impairment was between 4.3 and 18µg Cu/litre. Minnows held in "clean" water for 9 months ceased to spawn on exposure to 119.4µg Cu/litre. Fish exposed to 119.4µg Cu/litre for the same 9-month period began to spawn 60 days after being transferred to "clean" water.

McKim & Benoit 1971 (Ref A 7.4.3.2/09) exposed brook trout *(Salvelinus fontinalis)* to copper(II) concentrations ranging from 1.9 to 32.5µg Cu/litre for 22 months. The highest concentration decreased survival and growth in adult fish, and reduced the number of viable eggs produced and hatchability. No effects on adult survival, growth or reproduction were observed at copper concentrations of 17.4µg/litre or less. Concentrations of 17.4 and 32.5µg Cu/litre had marked adverse effects on survival and growth of alevins and juvenile fish. Therefore, the MATC for brook trout exposed to copper (hardness 45mg CaCO3/litre; pH 7.5) was between 9.5 and 17.4µg Cu/litre.

Benoit 1975 (Ref A 7.4.3.2/10) exposed bluegills *(Lepomis macrochirus)* to copper concentrations ranging from 12 to 162µg Cu/litre for a period of 22 months. Adult bluegill survival and reproduction were significantly affected only at the highest copper concentration of 162µg Cu/litre. A 90-day exposure of larvae transferred at hatch revealed a significant reduction in survival at>40µg Cu/litre; larval growth was not significantly reduced at 77µg Cu/litre and below.

In accordance with the rapporteur member state the lowest NOEC value of 20µg/l of the Benoit study is used for the risk assessment.

Cu-HDO contains 18.16% copper. The NOEC for Cu-HDO will therefore be:

NOEC (Cu-HDO) = 0.11mg/l

- In order to confirm that the toxicity to fish of the HDO part is insignificant, a fish juvenile growth test according to the OECD 215 guideline has been performed with K-HDO.

Undertaking of intended data submission [☐]

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

December 2006

Evaluation of applicant's justification

Introductorily, no long term test with fish was carried out with Cu-HDO. A study carried out with K-HDO is available. The justification of the applicant should be modified and the essential arguments for the read across strategy are the following:

The HDO^- anion derived from Cu-HDO and from K-HDO is structurally identical.

In acute tests, K-HDO shows lower toxicity than Cu-HDO. (This can be related to the different effects of the Cu^{2+} and K^+ ions.) In long term studies, the toxicity of K-HDO increased to similar toxicity values as determined for Cu-HDO. For Cu-HDO there are no relevant differences between acute and chronic toxicity.

Comparison of the long term ecotoxicity data available for Cu-HDO, K-HDO:

	Fish	Daphnia	Algae
	EC_{50} mg/L	EC_{50} mg/L	EC_{r50} mg/L
Cu-HDO	0.14 -0.24	1.1	0.19
	NOEC mg/L	NOEC mg/L	NOE_rC mg/L
Cu-HDO	0.074*	0.75	0.0562
K-HDO	0.29	0.47	3.75

* NOEC of Cu-HDO calculated from the most reliable literature data of Cu (Cu-HDO contains 18.16% copper).

Supposing that Cu^{2+} is of higher toxicity to fish than K^+, long term effects of Cu^{2+} from literature data were evaluated. The literature data of Cu are of varying quality and significance. The purity of the test-substances is not specified, the endpoints reported are different and the guidelines used are not comparable and not according to a method which is recommended in the TGD. Therefore the data can only be used to indicate the toxicity of the Cu-compound in Cu-HDO.
To give an indication of the long term toxicity of Cu-HDO, the lowest concentration determined for Cu in literature which was acceptable to fish (parameters according to the relevant guidelines 210, 212 and 215) was used. The estimated long term toxicity of Cu-HDO, based on this sensitive value and calculated for the copper-content in Cu-HDO (18.16%) can be seen as very conservative. However, the toxicity of Cu-HDO derived from the literature studies and the toxicity of K-HDO in juvenile fish gives comparable values. So, the long term study conducted according to OECD guideline 215 and GLP with K-HDO can be used to read across to Cu-HDO. This endpoint is not used for deriving the PNEC and for risk assessment because fish is not the most sensitive species for Cu-HDO.

Conclusion

Overall we agree with the proposal to read across the long-term study in fish from K-HDO to Cu-HDO, based on toxicity profile of Cu-ion and the comparison of the toxicity profiles of K-HDO and Cu-HDO.

Remarks

-

COMMENTS FROM …

Date

Evaluation of applicant's justification

Conclusion

Remarks

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.4.3.2/11 [redacted], 2005, N-Cyclohexyldiazeniumdioxy-potassium – juvenile growth test in the zebra fish (Danio rerio) in a flow through system (28 days), [redacted], Report No. 44F0069/015137, 25 July 2005, unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	Criteria for data protection	Data submitted to the MS before 14 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 215	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	N-Cyclohexyldiazeniumdioxy-potassium (K-HDO)	
3.1.1	Lot/Batch number	W-87	
3.1.2	Specification	Solid, crystalline, white	
3.1.3	Purity	100%	
3.1.4	Composition of Product		
3.1.5	Further relevant properties	Substance stability: the stability of the test substance in water was verified by weekly concentration control analysis. Vapour pressure: Water solubility:	
3.1.6	Method of analysis	HPLC	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**		
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**	Non-entry field	
3.4.1	Dilution water	The test water was analysed before the start of the exposure period. The test water was free of heavy metals and impurities and was considered to be acceptable for the purpose of this study. The water hardness of the dilution water and the hardness in the control and the highest concentration group was constant during the whole exposure and was 1.41 – 1.42mmol/l. This corresponds to approximately 141 – 142mg/l as $CaCO_3$. The pH was nearly constant during the whole exposure period and was 7.8 – 7.9	

		See also table A7_4_3_2-2
3.4.2	Test organisms	see table A7_4_3_2-3
3.4.3	Handling of embryos and larvae (OECD 210/212)	Not applicable (test performed according to OECD 215)
3.4.4	Test system	see table A7_4_3_2-4
3.4.5	Test conditions	see table A7_4_3_2-5
3.4.6	Duration of the test	28 days
3.4.7	Test parameter(s)	Measured and / or determined biological parameters were the mortality of the juvenile fish, toxic signs (symptoms), the weight (wet weight) after 14 days* of exposure and the weight (wet weight) as well as the total length (from tip of the snout to the end of the caudal fin) of surviving fish at the end of exposure. * For technical reasons the intermediate weighing was performed already after 13 days. This change has no effect on the evaluation of the parameter and the results of the study.
3.4.8	Examination / Sampling	Calibration phase: samples of one test vessel per concentration group were taken one day before start of exposure and it was confirmed that the concentrations were within a range of±20% of the nominal concentrations. The analyses were not performed under GLP and are not reported in detail. Exposure period: samples were collected on day zero from each test vessel and subsequently at weekly intervals alternating from one test vessel per concentration group before the replacement of the stock solutions. These samples were analysed for the content of the test substance. Samples were taken from the middle of the test vessel using a beaker and were transported to the analytical laboratory in glass bottles. Retained samples were frozen and sent later to the analytical laboratory if necessary.
3.4.9	Monitoring of TS concentration	Yes
3.4.10	Statistics	Body weight, length, R3: A comparison of each group with the control group was performed using DUNNETTS's test (two-sided) for the hypothesis of equal means. Mortality: A comparison of each group with the control group was performed using Fisher's exact test (one-sided)
		4 RESULTS
4.1	**Range finding test**	Performed The test concentrations were selected on the basis of preliminary tests, which indicated mortality at 10.0mg/l within a test duration of 4 days.
4.1.1	Concentrations	
4.1.2	Number/ percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	Non-entry field
4.2.1	Initial concentrations of test substance	0.0 (dilution water control) / 0.033 / 0.11 / 0.33 / 1.1 and 3.3mg/l

4.2.2 Actual concentrations of test substance

Nominal concentration (mg/l)	Mean analytical determined concentration ± SD (mg/l)	Recovery, mean (%)	Recovery, range (%)
	Mean		
0	n.d.		
0.033	0.033±0.0040	100.8	90.0–124.5[1)]
0.11	0.0978±0.0036	88.9	83.6–92-7
0.33	0.3202±0.0176	97.0	86.4 – 101.2
1.1	0.8170±0.1293	74.3	61.5–87.2[2)]
3.1	1.757±0.0424	53.2	52.3–54.2[3)]

x

Discussion of deviations of>20% of nominal:

[1)] The value measured at start of exposure exceeded the range of±20% of the nominal concentration. The value of the sample taken two days later was in good agreement with the nominal concentration (97%). Furthermore, the concentration was markedly below the No Observed Effect Concentration. Therefore, the deviation did not affect the validity of the study.

[2)] The value of the retain sample of the 11 May 2005 was used, since in the result of the first analysis differed markedly from the value of the sample taken before and after this date. Especially at start of exposure several values were measured which were markedly below the theoretical value. No explanation was found for this finding. The function of technical system was not disturbed. Possibly the losses occurred after sampling. However, all values determined were in a range of 82.7–117.4% of the mean value provided in the table above.

[3)] The measured values deviated markedly from the nominal concentrations, but they were constant over the exposure period, which was only one day for this test group, because of complete mortality within one day.

4.2.3 Effect data

Survival rate				
Test group	Nominal concentration (mg/l)	Mean Analytical concentration (mg/l)	Survivors at end of study	Percentage of survivors related to individuals at study start
0	0 (control)	n.d.	20	100%
1	0.033	0.0411	20	100%
2	0.11	0.100	20	100%
3	0.33	0.334	20	100%
4	1.1	0.683	6	30%
5	3.3	1.787	0	0%

Mean body lengths of the concentration groups and the control group at the end of the study were:			
Test group	Nominal concentration (mg/l)	Mean Analytical concentration (mg/l)	Mean body length of individual fish (mm)
0	0 (control)	n.d.	30.4
1	0.033	0.0411	30.4
2	0.11	0.100	30.0
3	0.33	0.334	30.0
4	1.1	0.683	25.3
5	3.3	1.787	-

- all individuals dead

Mean wet weights of the groups and the control group at the end of exposure were:			
Test group	Nominal concentration (mg/l)	Mean Analytical concentration (mg/l)	Mean weight of individual fish (mg)
0	0 (control)	n.d.	218.5
1	0.033	0.0411	220.4
2	0.11	0.100	216.4
3	0.33	0.334	217.1
4	1.1	0.683	180.2 ($p<0.05$)
5	3.3	1.787	-

- all individuals dead

Tank / "Pseudo"-specific growth rates of the exposure groups in comparison to the control group at day 13 and at the end of exposure were:					
Test group	Nominal concen-tration	Mean Analy-tical	Mean of "pseudo"-specific growth rate R3[1)]	% of control	Statisti cal signifi-

	(mg/l)	concentration (mg/l)	Days 0-13	Days 13-28	Days 0-28	Days 0-13	Days 13-28	Days 0-28	cance
0	0 (control)	n.d.	4.695	3.955	4.298	100	100	100	
1	0.033	0.0411	4.629	4.098	4.345	98.6	103.6	101.1	None
2	0.11	0.100	4.604	4.131	4.350	98.1	104.5	101.2	None
3	0.33	0.334	4.854	3.669	4.219	103.4	92.8	98.2	None
4	1.1	0.683	1.890 [2)]	4.963 [2)]	3.536 [2)]	40.3 [2)]	125.5 [2)]	82.3 [2)]	Yes
5	3.3	1.787	-	-	-	-	-	-	-

[1)] The mean values of R3 are similar to the R2-values (tank-average specific growth rate)

[2)] Mortality rate in this test group>10%

Toxic signs and abnormalities:

Over the exposure period, no toxic signs and no abnormalities in the control and in the surviving fish of the concentration groups were observed.

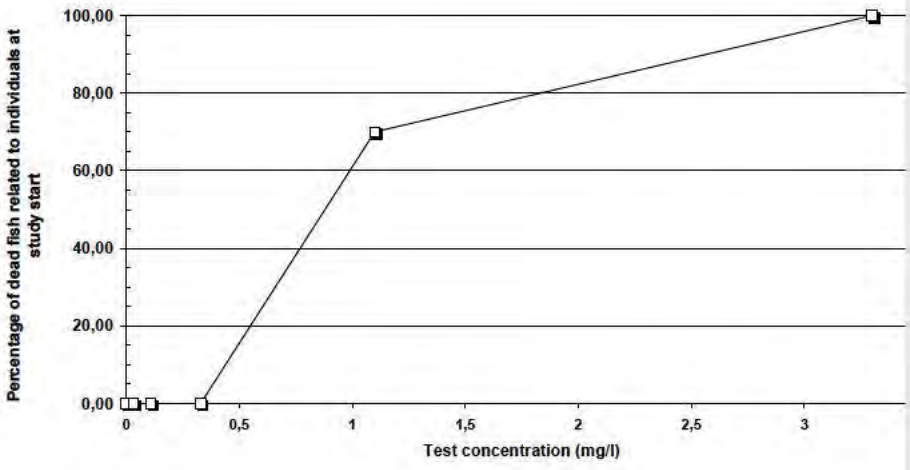
NOEC and LOEC:

The NOEC for survival is 0.33mg/l (nominal concentrations) and 0.29mg/l based on the mean analytically determined concentrations. The LOEC is 1.1mg/l (nominal concentration) and 0.74mg/l based on the mean analytically determined concentrations.

The NOEC for the impairment of the growth rate was 0.33mg/l (nominal concentration) and 0.29mg/l (based on the mean analytically determined concentrations). The LOEC for the impairment of the growth was 1.1mg/l (nominal concentration) and 0.74mg/l (based on the mean analytically determined concentrations).

4.2.4 Concentration / response curve

Graph of the concentration-mortality curve



4.2.5 Other effects

No other effects observed

4.3	**Results of controls**	The survival in the control group was above the minimum value of 90% which is required to meet the validity criteria of the test guideline
4.3.1	Number/ percentage of animals showing adverse effects	0
4.3.2	Nature of adverse effects	No symptoms
4.4	**Test with reference substance**	Not reported in the study
4.4.1	Concentrations	
4.4.2	Results	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

A test on effects on growth of N-Cyclohexyldiazeniumdioxy-potassium on juvenile zebra fish (Danio rerio) was conducted by the [redacted], Germany for a period of 28 days following the OECD guideline for Testing of Chemicals No. 215, adopted January 2000 "Fish, Juvenile Growth Test".

The study was performed under flow-through conditions with 5 concentrations of the test substance and a dilution water control. The temperature was maintained generally at 24°C. the dilution water was none-chlorinated drinking water obtained from the municipal water works mixed with deionised water to achieve a hardness of 1.4mmol (l (149mg/l $CaCO_3$)

The test concentrations were selected on the basis of preliminary tests, which indicated mortality at 10.0mg/l within a test duration of 4 days. The following concentrations spaced by a factor of √10 were selected for this study: 0.0 (dilution water control) / 0.033/0.11/0.33/1.1 and 3.3mg/l. Since the purity of the test substance is 100%, the concentrations based on the test substance and on active ingredient are the same.

5.2 Results and discussion

Survival:

In the control and the concentration groups up to 0.33mg/l all fish survived until sacrifice. In the highest concentration group (3.3mg/l), all fish died during the first day of exposure. In the concentration group 1.1mg/l the survival rate was 30%. Mortalities were observed from days 1 – 14 after start of exposure.

In conclusion, the NOEC for survival is 0.33mg/l (nominal concentrations) and 0.29mg/l based on the mean analytically determined concentrations. The LOEC is 1.1mg/l (nominal concentration) and 0.74mg/l based on the mean analytically determined concentrations.

External abnormalities and abnormal behaviour:

Over the exposure period, no toxic signs and no abnormalities in the control and in the surviving fish of the concentration groups were observed.

Growth rate:

In comparison to the control group the growth rate was statistically significantly reduced in the surviving animals of the concentration group 1.1mg/l after 14* days. No effects on the growth rate were detected in the lower concentration groups 0.033, 0.11 and 0.33mg/l.

For technical reasons the intermediate weighing was performed already after 13 days. This change has no effect on the evaluation of the parameter and the results of the study.

Thus, the NOEC for the impairment of the growth rate was 0.33mg/l (nominal concentration) and 0.29mg/l (based on the mean analytically determined concentrations). The LOEC for the impairment of the growth was 1.1mg/l (nominal concentration) and 0.74mg/l (based on the mean analytically determined

		concentrations). In conclusion, under the conditions of this study, the overall NOEC (no observed effect concentration) was 0.33mg/l (nominal concentration) and 0.29mg/l (based on the mean analytically determined concentrations) and the lowest concentration with effects (LOEC) was 1.1mg/l (nominal concentration) and 0.74mg/l (based on the mean analytically determined concentrations).
5.2.1	NOEC	0.33mg/l (nominal concentration) 0.29mg/l (based on the mean analytically determined concentrations)
5.2.2	LOEC	1.1mg/l (nominal concentration) 0.74mg/l (based on the mean analytically determined concentrations).
5.3	**Conclusion**	- the mortality in the control group was 0% - the mean body weight increase in the control group was>50% of the initial value - the dissolved oxygen content was>60% of the air saturation value throughout the test - the water temperature in all test vessels did not differ by more than 1°C In conclusion, the study was considered to be valid.
5.3.1	Other Conclusions	
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	December 2006
Materials and Methods	Agree with the applicant's version
Results and discussion	**4.2.2 Actual concentrations of test substance** In the two highest concentrations the measured concentrations were lower than expected. At the end of the exposure, the values of the higher concentration ranged from 82.2–87.2%. The toxicity endpoints are given in mean analytically determined concentrations.
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	-

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

Criteria	Details
Dispersion	-
Vehicle	-
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	-

Table A7_4_3_2-2: Dilution water

Criteria	Details
Source	The dilution water was non-chlorinated drinking water obtained from the municipal water works of the city of Frankenthal (hardness approx. 250mg/l $CaCO_3$) purified through a charcoal filter and diluted with deionised water to achieve a hardness of approx. 140 mg/l $CaCO_3$.
Salinity	if relevant
Hardness	1.42mmol/l (1mmol corresponds to approx. 100mg $CaCO_3$/l)
pH	7.9
Oxygen content	8.3mg/l
Conductance	334µSi
Total suspended solids	9mg/l
Total organic carbon	1.45mg/l
Holding water different from dilution water	Hardness was adjusted to approx. 140mg/l $CaCO_3$

Table A7_4_3_2-3: Test organisms

Criteria	Details
Species/strain	Zebrafish (Danio rerio)
Source	The test organism were hatched in the testing facility
Wild caught	No
Age/size	Juvenile
Kind of food	Hatched brine shrimp larvae (Artemia naupli)
Amount of food	The quantity of feeding was adjusted to 4% of the mean body weight of the fish at start of exposure for the first two weeks and to the bodyweight of the intermediate weighing from day 13 on.
Feeding frequency	The daily ration was divided into two equal portions and given to the fish in two portions per day, separated by at least 5 hours. On weekends and holidays, the interval between the two feedings was reduced. Shorter intervals were used also on the day on which the exposure was started for technical reasons and on the day before end of exposure since the feeding should be ended one day before sacrifice. No food was applied for approx. 24 hours before the termination of exposure on day 28.
Post-hatch transfer time	Not applicable
Time to first feeding	Not applicable
Feeding of animals during test	Yes
Treatment for disease within 2 weeks preceding test	The fish were not treated with any medication

Table A7_4_3_2-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	Flow-rate: The mixture of dilution water and a stock solution of the test substance was flowing continuously into the test aquaria at a rate of approx. 5.2L/hour. Taking into account the water volume of the aquaria, the theoretical exchange rate of the water contents was approx. 5 fold per 24 hours. The flow rates were calibrated (maximum deviation less than 10 5) before the exposure was started.
Volume of test vessels	25L
Volume/animal	1.25L
Number of animals/vessel	20
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2-5: Test conditions

Criteria	**Details**

Test temperature

Day of study	Test group (nominal concentration of active ingredient)					
	0 (control) 0mg/l	1 0.03mg/l	2 0.11mg/l	3 0.33mg/l	4 1.1mg/l	5 3.3mg/l
0	24	24	24	24	24	24
1	24	24	24	24	24	24
2	24	24	24	24	24	—
3	24	24	24	24	24	—
4	24	24	24	24	24	—
5	24	24	24	24	24	—
6	24	24	24	24	24	—
7	24	24	24	24	24	—
8	24	24	24	24	24	—
9	24	24	24	24	24	—
10	24	24	24	24	24	—
11	24	24	24	24	24	—
12	24	24	24	24	24	—
13	24	24	24	24	24	—
14	24	24	24	24	24	—
15	24	24	24	24	24	—
16	24	24	24	24	24	—
17	24	24	24	24	24	—
18	24	24	24	24	24	—
19	24	24	24	24	24	—
20	24	24	24	24	24	—
21	24	24	24	24	24	—
22	24	24	24	24	24	—
23	24	24	24	24	24	—
24	24	24	24	24	24	—
25	24	24	24	24	24	—
26	24	24	24	24	24	—
27	24	24	24	24	24	—
28	24	24	24	24	24	—

— no measurement (all fish dead)

<table>
<tr><th>Criteria</th><th>Details</th></tr>
<tr><td></td><td>
Results of the hourly (°C) measurements performed in test vessel = (control):
<table>
<tr><td>Study days</td><td>Minimum</td><td>Maximum</td><td>Mean value</td><td>Standard deviation</td></tr>
<tr><td>0-28</td><td>23.6</td><td>23.8</td><td>23.7</td><td>0.1</td></tr>
</table>
</td></tr>
<tr><td>Dissolved oxygen</td><td>
Dissolved oxygen in the test vessels
<table>
<tr><td>Day of study</td><td>0 (control)
0mg/l</td><td>1
0.03mg/l</td><td>2
0.11mg/l</td><td>3
0.33mg/l</td><td>4
1.1mg/l</td><td>5
3.3mg/l</td></tr>
<tr><td>0</td><td>8.5</td><td>8.5</td><td>8.4</td><td>8.4</td><td>8.5</td><td>8.5</td></tr>
<tr><td>3</td><td>8.5</td><td>8.5</td><td>8.5</td><td>8.4</td><td>8.4</td><td>—</td></tr>
<tr><td>7</td><td>8.4</td><td>8.5</td><td>8.4</td><td>8.5</td><td>8.5</td><td>—</td></tr>
<tr><td>10</td><td>8.3</td><td>8.4</td><td>8.4</td><td>8.5</td><td>8.5</td><td>—</td></tr>
<tr><td>14</td><td>8.3</td><td>8.6</td><td>8.3</td><td>8.4</td><td>8.6</td><td>—</td></tr>
<tr><td>17</td><td>8.4</td><td>8.5</td><td>8.3</td><td>8.2</td><td>8.5</td><td>—</td></tr>
<tr><td>21</td><td>8.4</td><td>8.4</td><td>8.4</td><td>8.3</td><td>8.5</td><td>—</td></tr>
<tr><td>24</td><td>8.3</td><td>8.5</td><td>8.3</td><td>8.4</td><td>8.6</td><td>—</td></tr>
<tr><td>28</td><td>8.3</td><td>8.4</td><td>8.3</td><td>8.4</td><td>8.5</td><td>—</td></tr>
</table>
— no measurement (all fish dead)
</td></tr>
<tr><td>pH</td><td>
pH values in the test vessels
<table>
<tr><td>Day of study</td><td>0 (control)
0mg/l</td><td>1
0.03mg/l</td><td>2
0.11mg/l</td><td>3
0.33mg/l</td><td>4
1.1mg/l</td><td>5
3.3mg/l</td></tr>
<tr><td>0</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td></tr>
<tr><td>3</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td><td>—</td></tr>
<tr><td>7</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td><td>—</td></tr>
<tr><td>10</td><td>7.8</td><td>7.8</td><td>7.9</td><td>7.8</td><td>7.8</td><td>—</td></tr>
<tr><td>14</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.9</td><td>7.8</td><td>—</td></tr>
<tr><td>17</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td><td>—</td></tr>
<tr><td>21</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td><td>—</td></tr>
<tr><td>24</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.9</td><td>7.8</td><td>—</td></tr>
<tr><td>28</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td><td>7.8</td><td>—</td></tr>
</table>
— no measurement (all fish dead)
</td></tr>
<tr><td>Adjustment of pH</td><td>No</td></tr>
<tr><td>Aeration of dilution water</td><td>No</td></tr>
<tr><td>Intensity of irradiation</td><td></td></tr>
<tr><td>Photoperiod</td><td>The aquaria were exposed to dim light at a light cycle of 16 hours light, which was automatically maintained.</td></tr>
</table>

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

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

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

	fulfilled	Not fulfilled
Concentration of dissolved oxygen in all test vessels>60% saturation	**Yes**	
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	**Yes**	
Mortality of control animals<10%	**Yes**	
Increase of fish weight sufficient for detection of the minimum variation of growth rate considered as significant	**Yes**	
Criteria for poorly soluble test substances		

Section A7.5.1.1/01 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.5.1.1/01 [redacted] (2004) Effects of Cu-HDO on the activity of soil microflora. Nitrogen transformation, Report No. 04 10 35 2001 N, [redacted]	
1.2	**Data protection**	Yes	
1.2.1	**Data owner**	Dr. Wolman GmbH	
1.2.2	**Criteria for data protection**	Data submitted to the MS before 14 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 216	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cu-HDO p.a.	
3.1.1	**Lot/Batch number**	U 9598	
3.1.2	**Specification**	Solid blue crystals	
3.1.3	**Purity**	>99%	
3.1.4	**Composition of Product**	Not applicable, investigation has been performed with the pure active substance	
3.1.5	**Further relevant properties**	Substance stability: test substance is stable for at least 2 years at ambient temperatures Volatility: none volatile Water solubility: The water solubility of Cu-HDO depends on the pH of the aqueous sample. The water solubility at 23 °C is given by: pH=4: 34.6mg/L Cu-HDO pH=7: 6.1mg/L Cu-HDO pH=9: 8.6mg/L Cu-HDO Nitrogen content: Cu-HDO contains 4 nitrogen atoms	
3.1.6	**Method of analysis**		x
3.2	**Reference substance**	Dinoseb acetate	
3.2.1	**Method of analysis for reference substance**	Not reported	

Section A7.5.1.1/01 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

3.3	**Testing procedure**	Non-entry field	
3.3.1	**Soil sample / inoculum / test organism**	Soil enriched with lucerne meal	x
3.3.2	**Test system**	see table A7_5_1_1-3	
3.3.3	**Application of TS**	Mixing and application of the test item	

	2 mg Cu-HDO/kg soil	20 mg Cu-HDO/kg soil	Control
Target conc. (mg/kg dry soil)	2	20	0
Amount product weighed in (mg)	100	100	0
Final volume of quartz meal mixture (g)	50	50	0
Concentration in stock application mixture (mg/g)	2.0	2.0	0
Concentration of application mixture[1] (mg/g)	2.0	2.0	0
Added amount of application mixture (g)	0.15	1.50	0
Added amount of quartz meal (g)	1.35	-	1.5
Added volume of water (mL)	8.3	8.3	8.3
Amount of wet soil (g)	169.4	169.4	169.4
Amount of dry soil (g)	150	150	150

[1] The stock mixture was not diluted

3.3.4	**Test conditions**	Soil moisture: approx. 45% of its water holding capacity, Soil samples were incubated at 20°C±2°C while stored in new plastic vessels	
3.3.5	**Test parameter**	Effect on NO_3-nitrogen production after 28 days of exposure	
3.3.6	**Analytical parameter**	NH_4-N; NO_3-N and NO_2-N content were determined	
3.3.7	**Duration of the test**	28 days	
3.3.8	**Sampling**	Soil samples (10 g d.m. soil per replicate) were taken at intervals of 3 hours, 7, 14, and 28 days after application and the NH_4-N, NO_3-N and NO_2-N content were determined	
3.3.9	**Monitoring of TS concentration**	Not reported	
3.3.10	**Controls**	Non-treated soil	
3.3.11	**Statistics**	Calculation of mean values per treatment, standard deviations and coefficient of variation. A t-test has been performed and compared with the table value for p = 0.05 (95%) and n = 3 (f = 2). The calculation is based on the algorithm of Kromidas. The calculations have been performed for all sampling.	

4 RESULTS

4.1	**Range finding test**	Preliminary tests were performed to determine if the soil shows a definitive measurable microbial activity	
4.1.1	**Concentration**		
4.1.2	**Effect data**	Soil is biologically active	
4.2	**Results test substance**	Non-entry field	

Section A7.5.1.1/01 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

4.2.1 Initial concentrations of test substance

0mg/kg d.m., 2mg/kg d.m., 20mg/kg d.m.

4.2.2 Actual concentrations of test substance

Not reported

4.2.3 Growth curves

Not reported

4.2.4 Cell concentration data

Not reported

4.2.5 Concentration/ response curve

Not reported

4.2.6 Effect data

Results of N-transformation

(The calculations were performed with unrounded values.)

NO_3-N contents

Sampling Date [DAA]	Treatment	Repl	Measured values (mg NO_3-N/ 100 g d.m.)	Mean value	SD	CV [%]	mg NO_3-N/ kg d.m.	mg NO_3-N/ kg d.m./ day	Deviation from control [%]
0	Control	1	2.50						
		2	2.59						
		3	2.39	2.49	0.10	4.0	24.9	-	-
	2 mg Cu-HDO/kg	1	2.18						
		2	2.51						
		3	2.50	2.40	0.19	7.8	24.0	-	- 3.9
	20 mg Cu-HDO/kg	1	2.72						
		2	2.58						
		3	2.53	2.61	0.10	3.8	26.1	-	+ 4.7
7	Control	1	6.16						
		2	6.54						
		3	6.59	6.43	0.24	3.7	64.3	9.2	-
	2 mg Cu-HDO/kg	1	6.71						
		2	6.53						
		3	6.53	6.59	0.10	1.6	65.9	9.4	+ 2.5
	20 mg Cu-HDO/kg	1	6.65						
		2	6.32						
		3	6.51	6.49	0.17	2.6	64.9	9.3	+ 1.0
14	Control	1	7.01						
		2	7.56						
		3	7.79	7.45	0.40	5.4	74.5	5.3	-
	2 mg Cu-HDO/kg	1	7.77						
		2	7.63						
		3	8.02	7.81	0.20	2.5	78.1	5.6	+ 4.7
	20 mg Cu-HDO/kg	1	8.06						
		2	7.99						
		3	7.20	7.75	0.48	6.2	77.5	5.5	+ 4.0
28	Control	1	8.76						
		2	8.72						
		3	8.80	8.76	0.04	0.5	87.6	3.1	-
	2 mg Cu-HDO/kg	1	8.75						
		2	8.43						
		3	8.44	8.54	0.18	2.1	85.4	3.1	- 2.5
	20 mg Cu-HDO/kg	1	8.62						
		2	8.65						
		3	8.87	8.71	0.14	1.6	87.1	3.1	- 0.5

Limit of quantification (=LOQ): 0.06 mg/100 g d.m.
DAA = Days After Application
SD = Standard deviation
CV (%) = Coefficient of Variation
Repl = Replicate

Section A7.5.1.1/01 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

NH_4-N contents

DAA	Repl.	0	7	14	28
		mg NH_4-N/100 g dry soil	mg NH_4-N/100 g dry soil	mg NH_4-N/100 g dry soil	mg NH_4-N/100g dry soil
Control	1	2.24	0.10	0.18	<LOQ
	2	2.25	0.14	<LOQ	0.13
	3	2.11	0.14	<LOQ	<LOQ
2 mg Cu-HDO/ kg dry soil	1	1.94	0.10	<LOQ	<LOQ
	2	2.14	0.11	<LOQ	0.10
	3	2.17	0.20	0.10	<LOQ
20 mg Cu-HDO/ kg dry soil	1	2.15	0.17	<LOQ	<LOQ
	2	2.06	0.11	<LOQ	<LOQ
	3	2.20	0.12	<LOQ	0.10

Limit of Quantification (=LOQ): 0.1 mg/100 g dry soil
DAA = Days After Application
Repl. = Replicate

Influence of the test item Cu-HDO on nitrification in the soil (mg N/100 g d.m. soil)

Treatment	Sampling date (days after application)							
Initial content of NO_3-N = 2.33 mg/100g	0		7		14		28	
	NO_3-N (mg/100 g)	I (%)	NO_3-N (mg/100 g)	I (%)	NO_3-N (mg/100 g)	I (%)	NO_3-N (mg/100 g)	I (%)
control	2.49	-	6.43	-	7.45	-	8.76	-
2 mg Cu-HDO/kg	2.40	56	6.59	-4	7.81	-7	8.54	3
20 mg Cu-HDO/kg	2.61	-75	6.49	-1	7.75	-6	8.71	1

Calculations with rounded values

I = Inhibition of nitrification
A – negative inhibition means an increase relative to the control

The inhibition (I) of the nitrification is calculated according to the following equation:

$$I\,(\%) = \frac{a - b}{a - c} \cdot 100$$

a = content of NO_3-N in the control group
b = content of NO_3-N in the treated group
c = initial content of NO_3-N

Effects of the test item Cu-HDO on the nitrogen transformation (mg NO_3-N/kg d.m. soil)

Days after application	Control		2 mg Cu-HDO/ kg dry soil			20 mg Cu-HDO/ kg dry soil		
	NO_3-N (mg/kg d.m. soil)	cv (%)	NO_3-N (mg/kg d.m. soil)	cv (%)	Deviation (%)[1]	NO_3-N (mg/kg d.m. soil)	cv (%)	Deviation (%)[1]
0 (3 hours)	24.9	4.0	24.0	7.8	-3.9	26.1	3.8	+4.7
7	64.3	3.7	65.9	1.6	+2.5	64.9	2.6	+1.0
14	74.5	5.4	78.1	2.5	+4.7	77.5	6.2	+4.0
28	87.6	0.5	85.4	2.1	-2.5	87.1	1.6	-0.5

The calculations were performed with unrounded values
cv = coefficient of variation
[1] + = % stimulation; - = % inhibition

4.2.7 Other observed effects None reported

4.3 Results of controls See above

Section A7.5.1.1/01 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

4.4 Test with reference substance Performed

4.4.1 Concentrations 8.67mg/kg

4.4.2 Results

Effects of the reference item dinoseb acetate on the nitrogen transformation (study code R 04 10 35 N1)

after application	28 days			
Treatment	mg NO_3-N/kg d.m.	SD	CV (%) (n=3)	D (%)[1)]
Control	87.6	0.4	0.5	-
Dinoseb acetate 8.67 mg/kg	111.9	3.2	2.9	+27.7

The calculations were performed with unrounded values.
D (%) = Deviation to control
SD = Standard deviation
CV (%) = coefficient of variation
[1)] + = % stimulation; - = % inhibition

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

EXPOSED

- Nominal concentrations: 0, 2, 20mg/kg
- Number/ of replicates: 3

TEST SOIL:

- Source: Wassergut Canitz
- pH: 5.6
- C_{org} (%): 1.37
- Humus content (%):2.36
- Microbial biomass (mg C/100g d.m.): 27.8=2.03% compared to C_{org}
- N_{min} (mg/100 g d.m.): 2.08
- Total-N (%): 0.14

Particle size distribution (%):

- sand (2-0.063mm) 51
- silt (0.063 - 0.002mm) 39
- clay (<0.002mm) 10

Water holding capacity: WHC (g/100g d.m.) 40.62
Water content (g/100g d m.) 12.95
Cation exchange capacity ($cmol^+/kg$): 7.8

TEST SYSTEM

150g dry matter (d m.) soil per test vessel was weighed; one additional soil sample was used for determination of the initial NO_3-N-content.

The soil was mixed with 0.5% (i.e. 0.75g/150g d.m.) lucerne meal by

Section A7.5.1.1/01 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

		means of a hand-stirrer. The C and N contents of the lucerne meal were determined by BioChem agrar GmbH. The test item was dissolved in water and mixed with the soil by means of a hand-stirrer. Sampling scheme: 0, 7, 14 and 28 days ENDPOINTS ASSESSED: Calculation of the average N-content in mg per kg per day dry soil (separately for NO_3-N	x
5.2	**Results and discussion**	Based on the results of this study Cu-HDO caused no short-term and long-term effects (OECD 216) on the soil nitrogen transformation in a field soil tested up to a concentration of 20 mg Cu-HDO per kg dry soil. Result of t-test: For the N-transformation test no significant differences compared to the control have been found.	x
5.2.1	**NOEC**	> 20mg/kg	x
5.2.2	**EC_{10}**	Not determined	
5.2.3	**EC_{50}**	Not determined	
5.3	**Conclusion**	The coefficients of variation in control (NO_3-N) were within the demanded range (≤15%). In the most recent test, dated 14.01.04 – 11.02.04, the toxic standard dinsoseb acetate caused an increase of the nitrogen transformation of 27.7% on day 28 and thus demonstrates the sensitivity of the test system. The test is therefore considered as valid.	
5.3.1	**Reliability**	1	x
5.3.2	**Deficiencies**	No	x

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	January 2006
Materials and Methods	**3.1.6 Method of analysis** No information in the test report. **3.3.1 Soil sample** Enriched with 0.5% Lucerne meal (C/N = 15/1);
Results and discussion	**5.1 Materials and methods** Calculation of the average N-content in mg per kg per day dry soil (separately for NO_3-N), and if appropriate for NO,-N and NH_4-N for each treatment rate and assessment date. **5.2 Results and discussion** The test design meets the needs for agricultural purposes, but not for biocides. Therefore no NOEC, EC50 and concentration-response relationship were determined. **5.2.1 NOEC** Taking a pragmatic approach the highest concentration tested (20 mg/kg dry soil) causing 0.5% inhibition after 28 days is used as a NOEC value. NOEC $\geq$ 20 mg/kg dry soil.
Conclusion	**5.3.2 Deficiencies** The test design meets the needs for agricultural purposes (only 2 concentrations tested, no determination of a concentration-response relationship, EC_{50} or NOEC), but not for biocides.
Reliability	2
Acceptability	Acceptable
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>Wassergut Canitz, Germany, Sachsen</td></tr>
<tr><td>Geographical reference on the sampling site</td><td></td></tr>
<tr><td>Data on the history of the site</td><td>No treatment with fertilizers</td></tr>
<tr><td>Use pattern</td><td>Agricultural soil</td></tr>
<tr><td>Depth of sampling [cm]</td><td>After uprooting the vegetation cover, the soil was removed to a depth of 20 cm as mixed samples</td></tr>
<tr><td>Sand / Silt / Clay content [% dry weight]</td><td>Particle size distribution (%):
- sand (2-0.063mm) 51
- silt (0.063-0.002mm) 39
- clay (<0.002mm) 10</td></tr>
<tr><td>pH</td><td>pH values during test
<table>
<tr><th>treatment</th><th>before application</th><th>28 days</th></tr>
<tr><td>Control</td><td>5.6</td><td>5.7</td></tr>
<tr><td>Cu-HDO
2 mg/kg d.m.</td><td>5.6</td><td>5.7</td></tr>
<tr><td>Cu-HDO
20 mg/kg d.m.</td><td>5.6</td><td>5.7</td></tr>
</table></td></tr>
<tr><td>Organic carbon content [% dry weight]</td><td>1.37%</td></tr>
<tr><td>Nitrogen content [% dry weight]</td><td>0.14%</td></tr>
<tr><td>Cation exchange capacity [cmol+/kg]</td><td>7.8</td></tr>
<tr><td>Initial microbial biomass</td><td>27.8mg C/100g d m. = 2.03% compared to C_{org}.</td></tr>
<tr><td>Reference of methods</td><td></td></tr>
<tr><td>Collection / storage of samples</td><td>The soil was stored at a temperature of 4°C and approx. 30% of the maximum water holding capacity (WHC) in containers under aerobic conditions in the dark</td></tr>
<tr><td>Preparation of inoculum for exposure</td><td></td></tr>
<tr><td>Pretreatment</td><td></td></tr>
</table>

Table A7_5_1_1-3: Test system

Criteria	Details
Culturing apparatus	Plastic vessels
Number of vessels / concentration	The soil of each treatment was incubated as a series of 3 replicates.
Aeration device	The tops of the vessels used permitted an air exchange with negligible moisture leakage (<1% loss)
Measuring equipment	Mettler-balance AG204 Mettler-balance PB1502 Sartorius-balance LC220S Autoanalyzer II (BRAN+LUEBBE) Digital pH-meter MV-870 Data logger Testo 175 Drying oven
Test performed in closed vessels	No

Table A7_5_1_1-4: Application of test substance

Criteria	Details
Application procedure	The test item was dissolved in water and mixed with the soil by means of a hand-stirrer
Carrier	
Concentration of liquid carrier [% v/v]	
Liquid carrier control	
Other procedures	—

Table A7_5_1_1-5: Test conditions

Criteria	Details
Organic substrate	0.5% Lucerne meal
Incubation temperature	20±2°C in a climatic room
Soil moisture	Water contents during the test (g/100 g d.m.)
Method of soil incubation	
Aeration	

Water contents during the test (g/100 g d.m.)

treatment	Days after application			
	7	14	21	28
Control	18.00	17.96	17.93	17.98
	17.93	17.88	17.87	17.89
	18.05	18.01	17.99	17.99
Cu-HDO 2 mg/kg d.m.	18.00	17.95	17.92	17.96
	17.96	17.91	17.88	17.92
	17.87	17.91	17.86	17.91
Cu-HDO 20 mg/kg d.m.	17.73	17.90	17.84	17.90
	17.91	17.87	17.83	17.88
	17.85	17.86	17.83	17.88

Section A7.5.1.1/02 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.5.1.1/02 [redacted] (2004) Effects of Cu-HDO on the activity of soil microflora (Carbon transformation test), Report No. 04 10 35 2001 N, [redacted]	x
1.2	**Data protection**	Yes	
1.2.1	Data owner	Dr. Wolman GmbH	
1.2.2	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 217	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Cu-HDO	
3.1.1	Lot/Batch number	U 9598	
3.1.2	Specification	Solid blue crystals	
3.1.3	Purity	99%	
3.1.4	Composition of Product	Not applicable, investigation has been performed with the pure active substance	
3.1.5	Further relevant properties	Substance stability: test substance is stable for at least 2 years at ambient temperatures Volatility: none volatile Water solubility: The water solubility of Cu-HDO depends on the pH of the aqueous sample. The water solubility at 23°C is given by: pH=4: 34.6mg/L Cu-HDO pH=7: 6.1mg/L Cu-HDO pH=9: 8.6mg/L Cu-HDO Nitrogen content: Cu-HDO contains 4 nitrogen atoms	
3.1.6	Method of analysis	Not reported	
3.2	**Reference substance**	Dinoseb acetate	
3.2.1	Method of analysis for reference substance	Not reported	
3.3	**Testing procedure**	Non-entry field	
3.3.1	Soil sample / inoculum / test organism	Biologically active agricultural soil: sandy loam soil	
3.3.2	Test system	see table A7_5_1_1-3	

Section A7.5.1.1/02 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

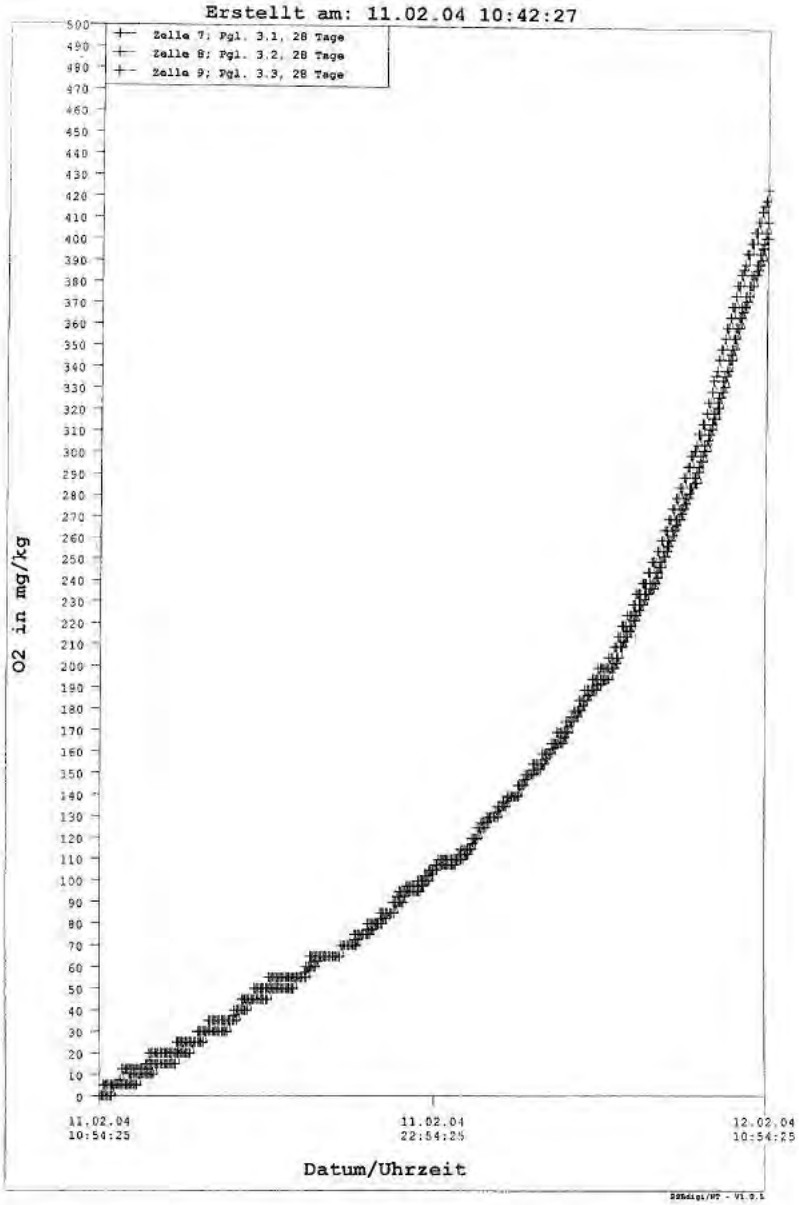
3.3.3	Application of TS	see table A7_5_1_1-4	
3.3.4	Test conditions	Soil moisture: approx. 45% of its maximum water holding capacity. Soil samples were incubated at 20°C±2°C while stored in new plastic vessels	
3.3.5	Test parameter	Inhibition of microbial carbon transformation	
3.3.6	Analytical parameter	Effects on O_2 consumption after 28 days of exposure	
3.3.7	Duration of the test	28 days	
3.3.8	Sampling	Soil samples (100g d.m.) are taken at intervals of 3 hours, 7, 14 and 28 days after application of the test item. Before the beginning of the test, the optimal glucose concentration was determined	
3.3.9	Monitoring of TS concentration	Not reported	
3.3.10	Controls	Non-treated soil	
3.3.11	Statistics	Calculation of mean values per treatment, standard deviation and coefficients of variation	x
		4 RESULTS	
4.1	**Range finding test**	Not performed	x
4.1.1	Concentration		
4.1.2	Effect data		
4.2	**Results test substance**	Non-entry field	
4.2.1	Initial concentrations of test substance	0mg/kg d.m., 2mg/kg d.m., 20mg/kg d.m.	
4.2.2	Actual concentrations of test substance	0mg/kg d.m., 2mg/kg d.m., 20mg/kg d.m.	
4.2.3	Growth curves	Not reported	
4.2.4	Cell concentration data	Not reported	

Section A7.5.1.1/02 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

4.2.5 Concentration/ response curve

Respiration curve 20 mg Cu-HDO /kg (28 days)



4.2.6 Effect data

Effect on carbon transformation in the soil 28 days after treatment with Cu-HDO

Soil	% Deviation from the control [1]	
	2 mg Cu-HDO per kg dry soil	20 mg Cu-HDO per kg dry soil
sandy loam	-0.8	-10.3

[1] based of O_2 consumption
- = inhibition; + = stimulation

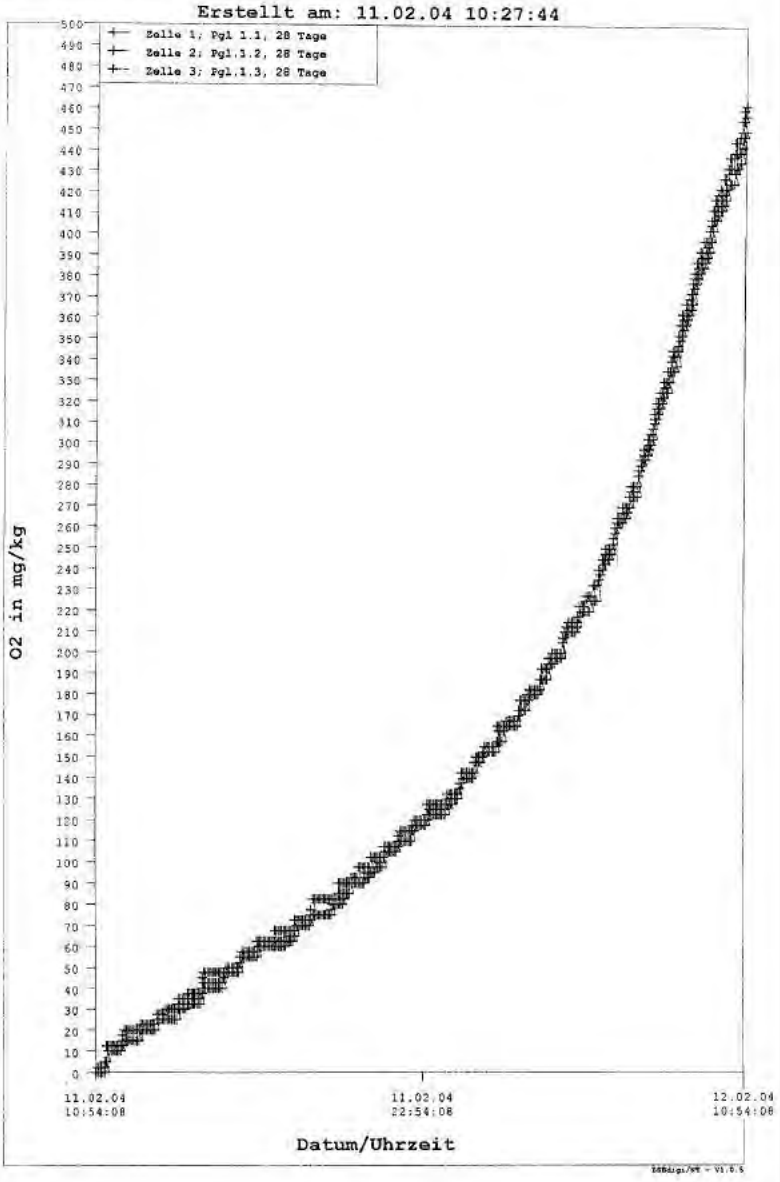
4.2.7 Other observed effects

None reported

Section A7.5.1.1/02 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

4.3 Results of controls Respiration curve control (28 days)



Section A7.5.1.1/02 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

4.4 Test with reference substance — Performed

4.4.1 Concentrations — 8.67mg/kg d.m.

4.4.2 Results

Effects of the reference item dinoseb acetate on the carbon transformation (Study code: R 04 10 35 C1)

after application	28 days			
treatment	mg O_2/kg d.m./h	SD	CV (%) (n=3)	D (%)[1)]
control	9.36	0.07	0.76	-
dinoseb acetate 8.67 mg/kg d.m.	6.98	0.21	3.01	-25.4

D (%) = Deviation to control
SD = Standard deviation
CV (%) = coefficient of variation
1) = % inhibition

The reference item produced in the soil the expected level of effect (25.4% inhibition after 28 days)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The method is based on the initial respiratory response of microbial populations to which a carbon and energy source has been added

EXPOSED
- Nominal concentrations: 0, 2, 20mg/kg
- Number/ of replicates: 3

Reference substance: Dinoseb acetate

TEST SOIL
- Source: Wassergut Canitz
- pH: 5.6
- Corg (%): 1.37
- Humus content (%): 2.36
- Microbial biomass (mg C/100g d.m.): 27.8 = 2.03% compared to Corg.
- Nmin (mg/100g d.m.): 2.08
- Total-N (%): 0.14

Particle size distribution (%):
- sand (2-0.063mm) 51
- silt (0.063-0.002mm) 39
- clay (<0.002mm) 10

Water holding capacity:
- WHC (g/100g d m.) 40.62

Water content (g/100g d m.) 12.95

Cation exchange capacity ($cmol^+/kg$): 7.8

Section A7.5.1.1/02 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

		TEST SYSTEM Determination of carbon transformation in soil after addition of glucose. Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration. A respirometer system was used to determine the O2-consumption over a period of maximum 24 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days ENDPOINTS ASSESSED Calculation of the cumulative O_2 consumption	
5.2	**Results and discussion**	Based on the results of this study Cu-HDO caused no short-term and long-term effects (OECD 217) on the carbon transformation in the field soil tested up to a concentration of 20mg Cu-HDO per kg dry soil For the C-transformation test, significant differences compared to the control have been found for the 20mg/kg test concentration. The highest deviation in comparison to the control is 10.3%. However, an inhibition in the sense of the OECD guideline 217 is given only when the deviation from the control is greater than 25%. Therefore, the value of 20mg/kg can be used as NOEC for the risk assessment.	x
5.2.1	NOEC	20mg/kg	x
5.2.2	EC_{10}	Not determined	
5.2.3	EC_{50}	Not determined	
5.3	**Conclusion**	The coefficients of variation in control were maximum 0.94% and thus fulfilled the demand≤15%. In the most recent test, dated 14.01.-12.02.2004, the toxic standard Dinoseb acetate caused a reduction of the O_2-consumption of 25.4% after 28 days and thus demonstrated the sensitivity of the test system.	
5.3.1	Reliability	1	x
5.3.2	Deficiencies	No	x

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	January 2006
Materials and Methods	**1.1 Reference** A 7.5.1.1/02 [redacted] (2004) Effects of Cu-HDO on the activity of soil microflora (Carbon transformation test), Report No. 04 10 35 2001 **C**, [redacted] **3.3.11 Statistics** Calculation of mean values per treatment, standard deviation and coefficients of variation; a t-test has been performed and compared with the table value for p = 0.05 (95%) and n = 3 (f = 2). The calculation is based on the algorithm of Kromidas. The calculations have been performed for all sampling.
Results and discussion	**4.1 Range finding test** A preliminary test was performed to determine if the soil shows a definitive measurable microbial activity and to determine the optimal glucose concentration.
Conclusion	**5.2 Results and discussion** The threshold value of $\leq$ 25% is not valid for regulatory purposes for biocides. The test design meets the needs for agricultural purposes, but not for biocides. Therefore no NOEC, EC50 or concentration-response relationship was determined. **5.2.1 NOEC** Taking a pragmatic approach the highest concentration tested (20 mg/kg dry soil) causing 10.3% inhibition after 28 days is set equal to the NOEC. NOEC = 20 mg/kg dry soil **5.3.2 Deficiencies** The test design meets the needs for agricultural purposes, but not for biocides.
Reliability	2
Acceptability	Acceptable
Remarks	-

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

Criteria	**Details**
Nature	soil sample
Sampling site:	Country: Germany Federal state: Sachsen Municipality: Canitz Field name: Schlag 34/3 Land owner: Wassergut Canitz
Geographical reference on the sampling site	
Data on the history of the site	Cultivation: At soil removal (2003): fallow Pre-cultivation (2002): peas Application of fertilizers: Organic fertilizer: none Inorganic fertilizers: none Last application of plant protection products: none
Use pattern	agricultural soil
Depth of sampling [cm]	20cm
Sand / Silt / Clay content [% dry weight]	Particle size distribution (%): - sand (2-0.063mm) 51 - silt (0.063-0.002mm) 39 - clay (<0.002mm) 10
pH	5.6 pH values during test: Treatment / before application / 28 days Control: 5.6 / 5.7 Cu-HDO 2 mg/kg d.m.: 5.6 / 5.7 Cu-HDO 20 mg/kg d.m.: 5.6 / 5.7
Organic carbon content [% dry weight]	1.37
Nitrogen content [% dry weight]	N_{min} (mg/100g d.m.): 2.08 Total-N (%): 0.14
Cation exchange capacity [$cmol^+/kg$]	7.8
Initial microbial biomass	27.8mg C/100g d.m.=2.03% compared to C_{org}
Reference of methods	

Criteria	Details
Collection / storage of samples	After uprooting the vegetation cover, the soil was removed to a depth of 20cm as mixed samples. Because the soil was wet, it was carefully dried at room temperature. Afterwards the soil was passed through a 2mm mesh sieve. The soil was stored at a temperature of 4°C and approx. 30% of the maximum water holding capacity (WHC) in containers under aerobic conditions in the dark
Preparation of inoculum for exposure	1100g dry matter (d.m.) soil per vessel was weighed in the mixing vessel of a mixing machine. A homogenous mixture of the test item with quartz meal was added and mixed with the soil in the mixing machine. Water was added to the soil to achieve a moisture of 45% of WHC. The incubation of the prepared soil was carried out in new plastic vessels (4L). The water contents were determined weekly. The determined water contents in the proved test vessels ranged from 17.22g to 17.82g/100g d.m., hence the difference was not greater than 5% from the start value. Water losses were compensated when necessary.
Pretreatment	

Table A7_5_1_1-3: Test system

Criteria	Details
Culturing apparatus	500ml reaction flasks
Number of vessels / concentration	3
Aeration device	
Measuring equipment	Mettler-balance AG204 Mettler-balance PB1502 Sartorius-balance LC220S Mixing machine “Kitchen aid” Respirometer BSB-digi (Selutec) Digital pH-meter MV-870 Data logger Testo 175 Drying oven
Test performed in closed vessels	The assay is based on the determination of O_2 consumption of soil samples after glucose-induced respiration in a closed system for at least 24 hours.

Table A7_5_1_1-4: Application of test substance

Criteria	Details
Application procedure	mixed directly to soil
Carrier	quartz meal
Concentration of liquid carrier [% v/v]	
Liquid carrier control	
Other procedures	

Table A7_5_1_1-5: Test conditions

Criteria	Details
Organic substrate	0.4% Glucose
Incubation temperature	20±2°C
Soil moisture	45% of WHC
Method of soil incubation	
Aeration	

Section A7.5.1.2 Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.5.1.2 [redacted] (1992) title: Effect of Cu-HDO on the mortality of the earthworm Eisenia foetida: Report P92-E106, [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF AG	
1.2.2	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 207 "Earthworm, Acute Toxicity Test"	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Cu-HDO	
3.1.1	Lot/Batch number	Reu-E 7360 B	
3.1.2	Specification	Solid blue crystals	
3.1.3	Purity	99%	
3.1.4	Further relevant properties		X
3.1.5	Method of analysis		X
3.2	**Reference substance**	Yes Chloroacetamide	
3.2.1	Method of analysis for reference substance		X
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	Test substance was suspended in water and mixed into moist artificial soil	X
3.3.2	Application of the test substance	The appropriate amounts of Cu-HDO were ground finely with 10 g of quartz sand and mixed thoroughly into moist artificial soil.	
3.3.3	Test organisms	see table A7_5_1_2-1	X
3.3.4	Test system	see table A7_5_1_2-2	
3.3.5	Test conditions	see table A7_5_1_2-3	
3.3.6	Test duration	14 days	
3.3.7	Test parameter	Mortality, Biomass	
3.3.8	Examination	After 7 days worm mortality was assessed by emptying test medium onto suitable trays, sorting worms from the medium and - necessary – testing their reaction to a mechanical stimulus. After the assessment,	

Section A7.5.1.2 Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

		worms and medium were replaced in the test containers. After 14 days – at the end of the experiment- the same mortality assessment was done and additionally the live eight of the worms determined after gentle washing and drying. The water content of the test medium at the end of the experiment was determined to be 36% (of soil dry weight).	
3.3.9	Monitoring of test substance concentration		X
3.3.10	Statistics	Probit analysis, Dunnetts test	X
		4 RESULTS	
4.1	**Filter paper test**	Not performed	
4.1.1	Concentration		
4.1.2	Number/ percentage of animals showing adverse effects		
4.1.3	Nature of adverse effects		
4.2	**Soil test**		
4.2.1	Initial concentrations of test substance	100, 250, 500, 750 and 1000 [mg/kg dry weight artificial soil]	X
4.2.2	Effect data (Mortality)	see table A7_5_1_2-4 and table A7_5_1_2-5	
4.2.3	Concentration / effect curve		
4.2.4	Other effects		X

Conc. (mg/kg)	Replicates				Average	Standard deviation
	a	b	C	d		
Control	-1.4%	-4.7%	-3.7%	3.8%	-1.5%	3.78%
100	7.0%	8.5%	12.1%	8.8%	9.1%	2.16%
250	- 0.9%	- 6.7%	-11.5%	5.7%	-3.4%	7.43%
500	-15.7%	- 25.4%	-18.8%	-33.2%	-23.3%	7.74%
750	*	- 40.3%	-26.2%	*	-33.2%	0.10%
1000	*	*	*	*	*	*

* no data available, all worms dead

4.3	**Results of controls**		
4.3.1	Mortality	None of the control animals died during the test	
4.3.2	Number/ percentage of earthworms showing adverse effects		
4.3.3	Nature of adverse effects		
4.4	**Test with**	Performed	

Section A7.5.1.2 Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

	reference substance		
4.4.1	Concentrations		
4.4.2	Results	The LC_{50} of Chloroacetamide was determined by Probit analysis to be 21.1mg/kg. The confidence limits are 20.2–22.2mg/kg. The NOEC with respect to mortality was 15mg/kg. The LC_{100} was 30mg/kg.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

TEST ORGANISMS: EARTHWORM
- Strain: Eisenia foetida andrei
- Supplier: C.A.M. von Gestel, RIVM, NL-3720 Bilthoven
- Age/size/weight: less than one year/>250mg
- Feeding: horse manure
- Controls: yes

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- Dispersion: mixed into moist artificial soil
- Reference substance: chloroacetamide

TEST SYSTEM
- Test type: glass container
- Concentrations: 0/100/250/500/750/1000mg/kg

DURATION OF THE TEST: 14 days

ENDPOINTS ASSESSED: mortality

5.2 Results and discussion

EXPOSED
- Nominal concentrations: 0, 100, 250, 500, 750, 1000mg/kg
- Effect data:

[mg/kg]:	100	250	500	750	1000
mortality %:	0	0	5	87.5	100

- Effect concentration
  - LC_0: 250mg/kg
  - LC_{10}: 527mg/kg
  - LC_{50}: 636mg/kg
  - LC_{100}: 1000mg/kg
- Number/percentage of animals showing adverse effects: no particular observations on physical or behavioural changes were made. x

5.2.1	LC_0	LC_0: 250mg/kg	
5.2.2	LC_{50}	LC_{50}: 636mg/kg	
5.2.3	LC_{100}	LC_{100}: 1000mg/kg	
5.3	**Conclusion**		x
5.3.1	Other Conclusions		
5.3.2	Reliability	1	
5.3.3	Deficiencies	No	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	January 2006
Materials and Methods	**3.1.4 Further relevant properties, 3.1.5 Method of analysis, 3.2.1 Method of analysis for the reference substance** No information was provided in the original test report. **3.3.1 Preparation of the test substance** No test solution was prepared according to the original study. **3.3.3 Test organisms** The weight of the earthworms should be 300 to 600 mg/kg (instead of a minimum weight of 250 mg). The deviation from the guideline will not significantly influence the result (mortality) und is acceptable. **3.3.9 Monitoring of test substance concentration** Not performed **3.3.10 Statistics** For the determination of mortality probit analysis was used. For worm biomass (only concentrations considered at which mortality < 30%, $p \leq 0.05$) the Dunett Test was used. **4.2.1 Initial concentrations of test substance** The test concentrations exceeded the factor of 1.8 according to the recommendation in the test guideline (EC, OECD). The deviation is acceptable. **4.2.4 Other effects** The table refers to weight changes of the worms exposed to different concentrations of Cu-HDO.
Results and discussion	**5.2 Results and discussion** In addition to mortal effects a significant reduction in worm weight was determined (see also 4.2.4)
Conclusion	**5.3 Conclusion** LC_{50}: 636 mg ai/kg
Reliability	1
Acceptability	Acceptable
Remarks	-

Table A7_5_1_1-1: Test organisms

Criteria	Details
Species/strain	Eisenia foetida andrei
Source of the initial stock	The test organism was used from an own culture. The animals were originally obtained from a culture from C:A:M: von Gestel, RIYM, NL-3720 Bilthoven.
Culturing techniques	Before being used in the test, the animals were kept in boxes and fed with horse manure
Age/weight	The animals selected for the test were adult; they had a clitellum (minimum weight of 250mg) and were less than 1 year old.
Pre-treatment	Earthworms have been conditioned in an artificial soil for about 24 hours

Table A7_5_1_1-2: Test system

Criteria	Details
Artificial soil test substrate	10% sphagnum peat (Hochmoortorf, schwach-mittel zersetzt, Compo Naturgarten Gärtnertorf, DIN 11540-175) 20% Kaolin (kaolinits content>30%) 1% CaCO3 (merck 2089) 69% quartz sand (0421/ISSO-trocken), Fa. Gebr. Willersinn, particle size:>80% 0.063–0.2mm) The dry constituents were blended and mixed thoroughly with some water. Then additional water was mixed into the artificial soil resulting in a final water content (after the test substance suspended in water was added) of 37% of the dry weight. The pH was determined to be 6.4
Test mixture	The appropriate amounts of artificial soil were ground finely with 10 g of quartz sand and mixed thoroughly into moist artificial soil.
Size, volume and material of test container	Glass container
Amount of artificial soil (kg)/ container	750g wet weight from the test medium
Nominal levels of test concentrations	0, 100, 250, 500, 750, 1000mg/kg dry weight of the artificial soil)
Number of replicates/concentration	4
Number of earthworms/test concentration	10
Number of earthworms/container	10
Light source	Continuous illumination
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7_5_1_2-3: Test conditions

Criteria	Details
Test temperature	20±2°C
Moisture content	37%
pH	6.4
Adjustment of pH	-
Light intensity / photoperiod	Continuous illumination
Relevant degradation products	-

Table A7_5_1_2-4: Mortality data

Test Substance Concentration (nominal) [mg/kg artificial soil]	Mortality			
	Number		Percentage	
	7 d	14 d	7 d	14 d
0	0	0	0	0
100	0	0	0	0
250	0	0	0	0
500	0	2	0	5
750	2	31	5	87.5
1000	20	40	50	100

Table A7_5_1_2-5: Effect data

	14 d [mg/kg soil][1]	95% c.l.
LC_0	250mg/kg	
LC_{50}	636mg/kg	
LC_{100}	1000mg/kg	

[1] data are based on nominal (n) concentrations

Table A7_5_1_2-6: Validity criteria for acute earthworm test according to OECD 207

	fulfilled	Not fulfilled
Mortality of control animals<10%	X	

Section 7.5.1.3 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.5.1.3/02 [redacted], 2006, Cu-HDO - Determination of the effect of chemicals on the emergence and growth of higher plants (oilseed rape (Brassica napus), oats (Avena sativa) and vetch (Vicia sativa)), Experimental Toxicology and Ecology, [redacted], Project No.: 65E0801/003018, unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany	
1.2.2	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 for Testing of Chemicals. No. 208: Terrestrial Plants, Growth Test International Standard; ISO 11269-2: Soil Quality – Determination of the Effects of Pollutants on Soil Flora – Part 2: Effects of Chemicals on the Emergence and Growth of Higher Plants	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Cu-HDO	
3.1.1	Lot/Batch number	Test substance No.: 00/0801-1 Batch-Identification: W-86	
3.1.2	Specification	Solid (crystalline)/blue	
3.1.3	Purity	99g/100g	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Water solubility: 6mg/L	
3.1.6	Method of analysis	Not mentioned in the study report	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Due to the low water solubility of the test substance a homogenous dispersion of the test substance in the soil was guaranteed by mixing a mixture of quartz sand and test substance with the soil: The test substance was grind in a mortar. Then the required amount of the test substance was given to about 13 g of quartz sand and mixed well. This mixture was blended with about 1316 g of dry test substrate (corresponds to about 1500g moist soil with a water content of 40% WHC(max)). Afterwards the soil mixture was portioned in each pot.	

Section 7.5.1.3 Terrestrial plant toxicity
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3.3	**Reference substance**	No reference substances are recommended for this test.	x
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**	Non-entry field	
3.4.1	Dilution water	see table A7_5_1_3-2	
3.4.2	Test plants	see table A7_5_1_3-3	
3.4.3	Test system	see table A7_5_1_3-4	x
3.4.4	Test conditions	see table A7_5_1_3-5	
3.4.5	Test duration	Duration of exposure: 15 days	
3.4.6	Test parameter	Emergence and growth (emergence rate, dry matter, fresh matter, shoot length)	
3.4.7	Sampling	The test substance was incorporated into the soil with quartz sand at various concentrations and seeds were sown. The number of seedlings that emerge is recorded up to the end of the exposure. At least two weeks after 50 per cent of the seedlings have emerged in all control pots, the germs were cut of at soil surface and the shoot length of each scion is recorded. The fresh weight and the dry weight after weight constancy of all shoots of each pot were detected.	
3.4.8	Method of analysis of the plant material	The plants were harvested, weight and the shoot length was recorded.	
3.4.9	Quality control	The Quality Assurance Unit (QAU) inspected the study and reported any inspection results to the Study Director and to Management.	
3.4.10	Statistics	The calculation of the NOEC/LOEC was carried out with Dunnett's (one-sided, $p \leq 0.01$ and $p \leq 0.05$) test except the emergence rate (WILCOXON-test, one-sided, $p \leq 0.01$ and $p \leq 0.05$)	
		4 RESULTS	
4.1	**Results test substance**	Non-entry field	
4.1.1	Applied initial concentration	0, 1000, 500, 250, 125, 62.5mg/kg based on technical test substance	
4.1.2	Phytotoxicity rating	Not appropriate for OECD guideline 209	
4.1.3	Plant height	See tables A7_5_1_3-6a - A7_5_1_3-6c for sprout length	
4.1.4	Plant dry weights	See tables A7_5_1_3-6a - A7_5_1_3-6c	
4.1.5	Root dry weights	Not determined	
4.1.6	Root length	Not determined	
4.1.7	Number of dead plants	One plant of Brassica napus exposed to 62.5mg/kg test substance did not continuing growing	

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4.1.8 Effect data — see table A7_5_1_3-6a –c

4.1.9 Concentration / response curve

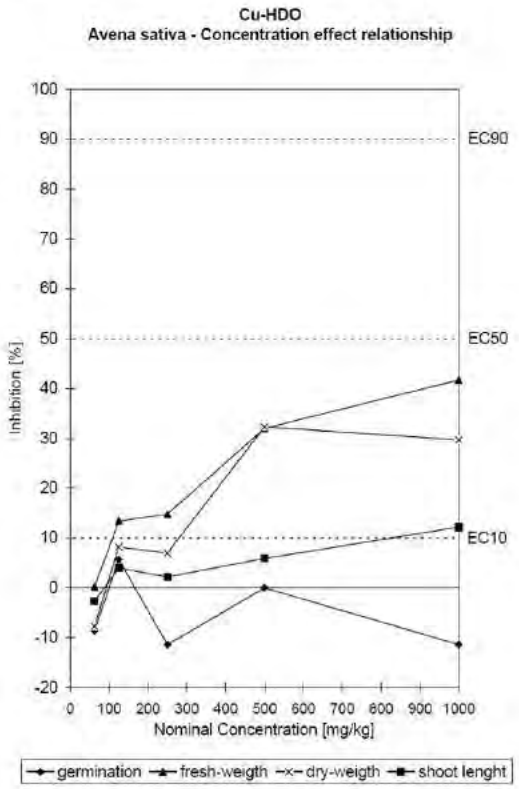
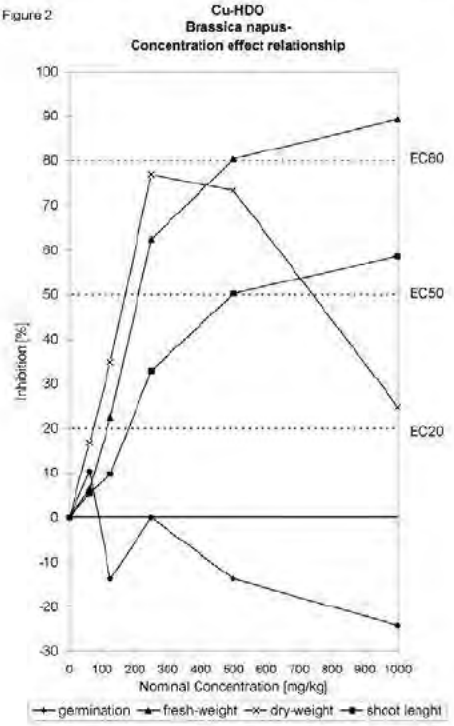


Figure 2



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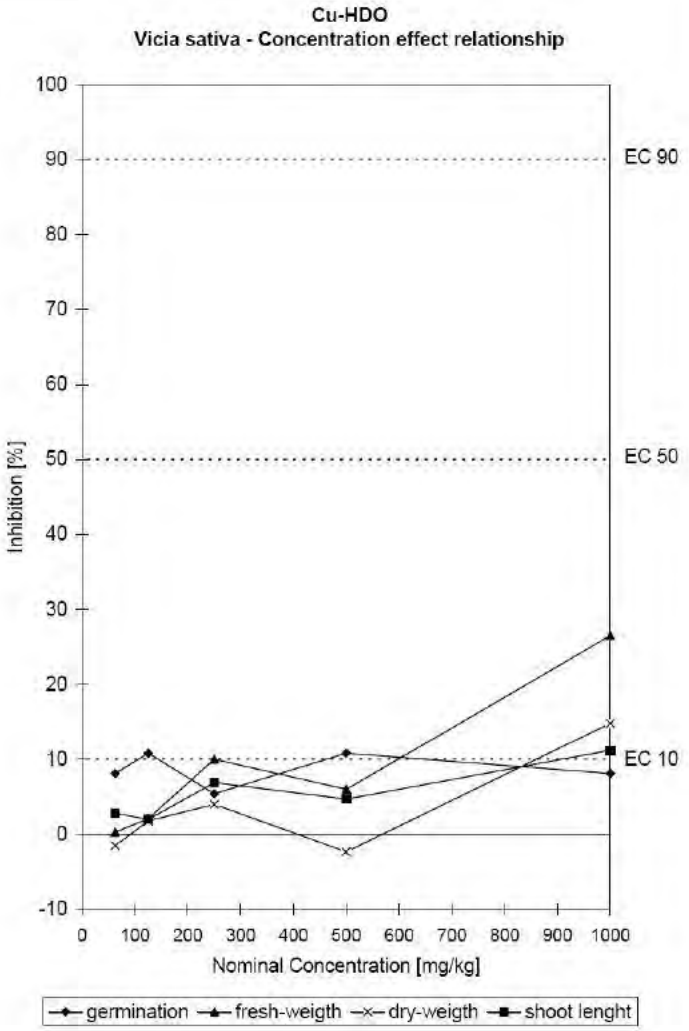

4.1.10 Other effects

Observations during the exposure:

The following observations and changes could be observed:

3 Dec 06:	Pot 39 one plant with yellow leaves
4 Dec 06:	Differences in plant length with increasing test concentrations in the pots with oilseed rape
8 Dec 06:	Pot 46 one plat was atrophied; pot 34 one plant was atrophied but still visible
9 Dec 06:	Pot 37 plants showed partly yellow leaves, oilseed rape showed a dependency of the growth from increasing test concentrations
14 Dec 06:	In the pots of test concentrations 250-1000 mg/kg the leaves showed brown tops
16 Dec 06:	Pot 35 one plant was cut

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Annex Point IIIA XIII 3.4

4.2 Results of controls

4.2.1 Number/ percentage of plants showing adverse effects: 0

4.2.2 Nature of adverse effects: Not appropriate

4.3 Test with reference substance

No reference substances are recommended for this test.

However preliminary investigations (NON-GLP) concerning the emergence rate were performed.

4.3.1 Concentrations: 1000mg/kg DM

4.3.2 Results

Emergence rate of oats (Avena sativa):	80% after 7 days.
Emergence rate of oilseed rape (Brassica napus)	90% after 7 days.
Emergence rate of vetch (Vicia sativa)	80% after 7 days.

The emergence test was carried out from 14 Oct 2005-31 Oct 2005 (NON-GLP).

Determination of the effect of the test substance on the emergence and growth of vetch (Vicia sativa):

There were no visible effects at the test concentration 1000 mg/kg DM to the emergence, length, and fresh matter of vetch. For these preliminary investigations no statistical evaluation was performed. The duration of the exposure was 18 days.

The preliminary investigations were carried out from the 01 Sep 2005-19 Sep 2005 in the Laboratory for Experimental Toxicology and Ecology, Ludwigshafen, Germany.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

OECD Guideline for Testing of Chemicals. No. 208: Terrestrial Plants, Growth Test

International Standard: ISO 11269-2: Soil Quality – Determination of the Effects of Pollutants on Soil Flora – Part 2: Effects of Chemicals on the Emergence and Growth of Higher Plants

The test substance was incorporated into the soil with quartz sand at various concentrations and seeds were sown. The number of seedlings that emerge is recorded up to the end of the exposure. At least two weeks after 50 per cent of the seedlings have emerged in all control pots, the germs were cut at soil surface and the shoot length of each scion is recorded. The fresh weight and the dry weight after weight constancy of all shoots of each pot were detected.

The test was carried out in a growth chamber.

Two Dicotyledonae (Brassica napus and Vicia sativa) were used as test plants and one Monocotyledonae (Avena sativa).

5.2 Results and discussion

Morphological observations:

Visual observed effects like yellow or brown leaves of some

Section 7.5.1.3 Terrestrial plant toxicity
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plants or two atrophied plants have no influence on the result of this study.

Concentration control analyses:

Concentration control analyses in the soil matrix were not carried out on account of the poor water-solubility of the test substance. A homogenous dispersion of the test substance in the soil was guaranteed by mixing a mixture of quartz sand and test substance with the soil. The stability of the test substance in the soil depends on the possibility of degradation processes and chemical and physical procedures. Therefore no prediction concerning the stability of the test substance in the soil could be made.

5.2.1 EC_{20}

TEST RESULTS EC_{20} nominal [mg/kg]:

(related to the dry mass of the soil)

	Avena sativa	Brassica napus	Vicia sativa
Emergence rate:	>1000	>1000	>1000
Dry matter:	357	71	>1000
Fresh matter:	309	113	>803
Shoot length:	>1000	170	> 1000

5.2.2 EC_{50}

TEST RESULTS EC_{50} nominal [mg/kg]:

(related to the dry mass of the soil)

	Avena sativa	Brassica napus	Vicia sativa
Emergence rate:	>1000	>1000	>1000
Dry matter:	> 1000	161	>1000
Fresh matter:	>1000	202	>1000
Shoot length:	>1000	496	>1000

5.2.3 EC_{80}

TEST RESULTS EC_{80} nominal [mg/kg]:

(related to the dry mass of the soil)

	Avena sativa	Brassica napus	Vicia sativa
Emergence rate:	>1000	>1000	>1000
Dry matter:	> 1000	>250*	>1000
Fresh matter:	>1000	493	>1000
Shoot length:	>1000	>1000	>1000

*500 and 1000mg/kg showed an increase of the dry matter

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5.2.4 NOEC/LOEC

TEST RESULTS NOEC/LOEC nominal [mg/kg]:			
	Avena sativa NOEC/LOEC	Brassica napus NOEC/LOEC	Vicia sativa NOEC/LOEC
Emergence rate:	≥1000/>1000	≥1000/>1000	≥1000/>1000
Dry matter:	250/500	125/250	500/1000
Fresh matter:	62.5/125	62.5/125	125/250
Shoot length:	250/500	125/250	125/250

5.3 Conclusion In the controls the germinability was≥5 healthy plants. The test is valid x

5.3.1 Reliability 1

5.3.2 Deficiencies No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2006
Materials and Methods	**3.3 Reference substance** According to ISO 11269-2 a reference substance (Socium trichloracetate) is recommended. In OECD 208 there is no such recommendation. No reference substance has been investigated. **3.4.3 Test system** Table A7_5_1_3-4: Point 5.3.22, Seed germination potential: Avena sativa: 80% after 7 days Brassica napus: 90% after 7 days Vicia sativa: 80% after 7 days
Results and discussion	Agree with applicant's version.
Conclusion	**5.3 Conclusion** In the control the germination was ≥ 80%. NOEC = 62.5 mg/kg EC_{50} = 161 mg/kg (only Brassica napus showed effects ≥ 50%)
Reliability	1
Acceptability	Acceptable
Remarks	-

Table A7_5_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances	
Criteria	**Details**
Dispersion	Yes
Vehicle	Quartz sand
Concentration of vehicle	The required amount of the test substance was given to about 13 g of quartz sand and mixed well. This mixture was blended with about 1316g of dry test substrate (corresponds to about 1500g moist soil with a water content of 40% WHC(max)).
Vehicle control performed	No
Other procedures	No

Table A7_5_1_3-2: Dilution water	
Criteria	**Details**
5.3.1 Source	Not applicable, test substance was not diluted in water
5.3.2 Alkalinity / Salinity	Not applicable
5.3.3 Hardness	Not applicable
5.3.4 pH	Not applicable
5.3.5 Oxygen content	Not applicable
5.3.6 Conductance	Not applicable
5.3.7 Holding water different from dilution water	Not applicable

Table A7_5_1_3-3: Test plants

	Family	Species	Common name	Source (seed/plant)
Dicotyledonae	5.3.8 Brassicaceae	5.3.9 Brassica napus	5.3.10 oilseed rape	5.3.11 10
	5.3.12 Fabaceae	5.3.13 Vicia sativa	5.3.14 vetch	5.3.15 10
Monocotyledonae	5.3.16 Poaceae	5.3.17 Avena sativa	5.3.18 oats	5.3.19 10

Table A7_5_1_3-4: Test system

Criteria	Details
5.3.20 Test type	Growth chamber test
5.3.21 Container type	PVC plant pots with an upper internal diameter of 85mm, covered by plastic Petri-dishes until the beginning of emergence
5.3.22 Seed germination potential	In the controls the germinability was ≥ 5 healthy plants
5.3.23 Identification of the plant species	Not reported
5.3.24 Number of replicates	4
5.3.25 Numbers of plants per replicate per dose	40 seeds per concentration
5.3.26 Date of planting	29. Nov. 2005 (start of exposure of the seed)
5.3.27 Plant density	10 dry seeds per plant and plant pot, after germination of at least 5 plants in each pot of the control, the seedlings in all pots were reduced to five uniform distributed plants.
5.3.28 Date of test substance application	29. Nov. 2005 (start of exposure of the seed)
5.3.29 High of plants at application	Not applicable, the test substance was incorporated into the soil with quartz sand at various concentrations and seeds were sown
5.3.30 Date of phytotoxicity rating or harvest	Measurement of emergence: Daily, beginning with the emergence of the first seedlings and ending after 17 days. Measurement of plant length, fresh weight and dry weight: At the end of the exposure period after 15 days when 5 of the seedlings have emerged in all controls
5.3.31 Dates of analysis	Concentration control analyses in the soil matrix were not carried out.

Table A7_5_1_3-5: Test conditions

<table>
<tr><th colspan="2">Criteria</th><th>Details</th></tr>
<tr><td>5.3.32</td><td>Test type</td><td>Emergence rate and growth inhibition test with higher plants</td></tr>
<tr><td>5.3.33</td><td>Method of application</td><td>The required amount of the test substance was given to about 13g of quartz sand and mixed well. This mixture was blended with about 1316g of dry test substrate (corresponds to about 1500g moist soil with a water content of 40% WHC(max)).</td></tr>
<tr><td>5.3.34</td><td>Application levels</td><td>0, 1000, 500, 250, 125, 62.5mg/kg based on technical test substance</td></tr>
<tr><td>5.3.35</td><td>Dose rates</td><td>0, 1000, 500, 250, 125, 62.5mg/kg based on technical test substance</td></tr>
<tr><td>5.3.36</td><td>Substrate charac-teristics</td><td>Field soil type 2.3; the soil was unsterile and sieved to 5mm before using in the test.
<table>
<tr><td>Max. water holding capacity (WHCmax)</td><td>35.0±3.0g/100g dry weight</td></tr>
<tr><td>pH value</td><td>5.8±1.8 (calcium chloride method)</td></tr>
<tr><td>Organic carbon</td><td>1.02±0.16%</td></tr>
<tr><td>Particle sizes<20µm</td><td>20.4±2.2%</td></tr>
<tr><td>Soil typ (according to USDA)</td><td>loamy sand</td></tr>
<tr><td>Soil typ (according to German DIN)</td><td>loamy sand (IS)</td></tr>
<tr><td>Water content</td><td>10.7g/100g DM</td></tr>
</table>
The soil was prepared at the 10 Nov 2005. 27kg of the delivered soil with a water content of 10.7g/100g DM were mixed with 805g demineralised water in a 60L barrel. The barrel was closed with a cap. After that the soil was incubated until use at room temperature.</td></tr>
<tr><td>5.3.37</td><td>Watering of the plants</td><td>Daily pouring with de-ionized water, beginning with the emergence of the first seedlings. Using de-ionized water with an conductivity of<0.5µS/cm.</td></tr>
<tr><td>5.3.38</td><td>Tempera-ture</td><td>20±2°C</td></tr>
<tr><td>5.3.39</td><td>Thermo-period</td><td>Not appropriate</td></tr>
<tr><td>5.3.40</td><td>Light regime</td><td>White light source, light intensity: Mv 7000±500 Lux, measured on a level with the plant pots, measured at the beginning of the exposure, Light rhythm: day/night (on/off): 16/8 hours</td></tr>
<tr><td>5.3.41</td><td>Relative humidity</td><td>Relative Humidity: 60-80%
Soil humidity in the exposure phase: 45% (of maximum water holding capacity)</td></tr>
<tr><td>5.3.42</td><td>Wind volatility</td><td>Not appropriate</td></tr>
<tr><td>5.3.43</td><td>Observation periods and duration of test</td><td>
<table>
<tr><td>Measurement of emergence:</td><td>Daily, beginning with the emergence of the first seedlings and ending after 17 days.</td></tr>
<tr><td>Measurement of plant length, fresh weight and dry weight:</td><td>At the end of the exposure period after 15 days when 5 of the seedlings have emerged in all controls</td></tr>
<tr><td>Test termination date:</td><td>10.02.2006</td></tr>
</table>
</td></tr>
<tr><td>5.3.44</td><td>Pest control</td><td>Not appropriate</td></tr>
<tr><td>5.3.45</td><td>Any other treatments and procedures</td><td>Daily pouring with de-ionized water, beginning with the emergence of the first seedlings. Using de-ionized water with an conductivity of<0.5µS/cm.
Sowing depth: oats approx. 15mm
oilseed rape approx. 5mm
vetch approx. 10mm</td></tr>
</table>

Table A7_5_1_3-6a: Effective phytotoxicity after test termination - Avena sativa

Test Substance Concentration (nominal) [mg/kg]	Absolute Numbers				Per cent relative to control			
	Sprout length	Plant dry weight	Plant fresh weight	Germination	Sprout length	Plant dry weight	Plant fresh weight	Germination
0	320	0.1899	2.4743	8.8	100.00	100.00	100.00	100.00
62.5	328	0.2044	2.4687	9.5	102.50	107.64	99.77	107.95
125	307	0.1743	2.1436	8.3	95.94	91.79	86.63	94.32
250	312	0.1765	2.1094	9.8	97.50	92.94	85.25	111.36
500	301	0.1285	1.6827	8.8	94.06	67.67	68.01	100.00
1000	281	0.1335	1.4417	9.8	87.81	70.30	58.27	111.36
Temperature [°C]	20 ± 2	20 ± 2	20 ± 2	20 ± 2				
Relative humidity [%]	60 - 80	60 - 80	60 - 80	60 - 80				

Table A7_5_1_3-6b: Effective phytotoxicity after test termination - Brassica napus

Test Substance Concentration (nominal) [mg/kg]	Absolute Numbers				Per cent relative to control			
	Sprout length	Plant dry weight	Plant fresh weight	Germination	Sprout length	Plant dry weight	Plant fresh weight	Germination
0	100	0.1023	2.0483	7.3	100	100.00	100.00	100.00
62.5	94	0.0851	1.9152	6.5	94	83.19	93.50	89.04
125	90	0.0667	1.5925	8.3	90	65.20	77.75	113.70
250	67	0.0235	0.7707	7.3	67	22.97	37.63	100.00
500	50	0.0273	0.4025	8.3	50	26.69	19.65	113.70
1000	41	0.0770	0.2194	9.0	41	75.27	10.71	123.29
Temperature [°C]	20 ± 2	20 ± 2	20 ± 2	20 ± 2				
Relative humidity [%]	60 - 80	60 - 80	60 - 80	60 - 80				

Table A7_5_1_3-6c: Effective phytotoxicity after test termination - Vicia sativa

Test Substance Concentration (nominal) [mg/kg]	Absolute Numbers				Per cent relative to control			
	Sprout length	Plant dry weight	Plant fresh weight	Germination	Sprout length	Plant dry weight	Plant fresh weight	Germination
0	444	0.2827	2.6713	9.3	100.00	100.00	100.00	100.00
62.5	432	0.2870	2.6632	8.5	97.30	101.52	99.70	91.40
125	435	0.2776	2.6143	8.3	97.97	98.20	97.87	89.25
250	413	0.2713	2.4051	8.8	93.02	95.97	90.03	94.62
500	423	0.2893	2.5114	8.3	95.27	102.33	94.01	89.25
1000	394	0.2407	1.9632	8.5	88.74	85.14	73.49	91.40
Temperature [°C]	20 ± 2	20 ± 2	20 ± 2	20 ± 2				
Relative humidity [%]	60 - 80	60 - 80	60 - 80	60 - 80				

Table A7_5_1_3-7: Validity criteria for terrestrial plant toxicity according to OECD Guideline for Testing of Chemicals. No. 208: Terrestrial Plants, Growth Test

	Fulfilled	Not fulfilled
A minimum of 80 per cent of the control seeds produced healthy seedlings	**X**	
The control seedlings exhibited normal growth throughout the test	**X**	**X**

Section A7.5.5 Bioconcentration, terrestrial

Annex Point IIA, VII. 7.5

Official use only

$BCF_{earthworm}$ can be calculated according to the following formula:

$BCF_{earthworm} = (0.84+0.012\ Kow) / RHO_{earthworm}$

Kow is the partition coefficient of Cu-HDO and is equal to 398.1. $RHO_{earthworm}$ is the bulk density of earthworm. According to the TGD on risk Assessment a default value of 1 can be assumed.

$BCF_{earthworm} = (0.84+0.012 *398.1)/1=5.62$

EVALUATION BY COMPETENT AUTHORITIES	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	December 2006
Materials and Methods	Acceptable
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	n.a.
Acceptability	Acceptable
Remarks	-

Section A8 Measures necessary to protect man, animals and the environment

Subsection (Annex Point)			Official use only
8.1		**Recommended methods and precautions concerning handling, use, storage, transport or fire (IIA8.1)**	
8.1.0	**Methods and precautions concerning placing on the market**		
8.1.1	**Methods and precautions concerning production, handling and use of the active substance and its formulations**	Wear personal protection according to the risk classification and the safety recommendations given in the safety data sheet when handling Cu-HDO.	
8.1.2	**Methods and precautions concerning storage of the active substance and its formulations**	Store in original container, tightly closed in a dry and well-ventilated place. Avoid temperatures above 40°C. Do not store with food or feeding stuff. Keep out of reach of unauthorised persons.	X
8.1.3	**Methods and precautions concerning transport of the active substance and its formulations**	The formulation is transported with the precautionary measures usual for dangerous goods. Transport information for the formulation are given in the table below:	X

Land transport ADR/RID and GGVS/GGVE (international / national):	
Class:	8
UN-number:	1760
Description of the good:	CORROSIVE LIQUID, N.O.S. (ALKYLAMINE)
Packaging group:	II
Sea transport IMDG/GGVSee:	
Class:	8
UN-number:	1760
Proper shipping name:	CORROSIVE LIQUID, N.O.S. (ALKYLAMINE)
Packaging group:	II
EMS-number:	F-A, S-B
MFAG:	760
Air transport ICAO-TI and IATA-DGR:	
Class:	8
UN-number:	1760
Proper shipping name:	CORROSIVE LIQUID, N.O.S. (ALKYLAMINE)
Packaging group:	II

Section A8 Measures necessary to protect man, animals and the environment

8.1.4	**Methods and precautions concerning fire of the active substance and its formulations**	Sprayed water, foam, CO_2, extinguishing powder or sand are suitable extinguishing media. Fire-fighters shall wear full protection including self-contained breathing apparatus.	
8.2		**In case of fire, nature of reaction products, combustion gases, etc. (IIA8.2)** In the case of combustion, CO_2/CO, H_2O and N_2/NO_x will be generated.	
8.3		**Emergency measures in case of an accident (IIA8.3)**	
8.3.1	**Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment if available**	On contact with eye, wash affected eye immediately for at least 15 minutes under running water with eyelids help open. On ingestion, rinse mouth immediately and then drink plenty of water, get medical attention. On skin contact, wash thoroughly with soap and water. If inhaled, keep patient calm, move to fresh air, summon medical help.	X
8.3.2	**Emergency measures to protect the environment**	Do not discharge into drains or into the soil.	
8.4		**Possibility of destruction or decontamination following release in or on the following: (a) Air; (b) Water, including drinking water; (c) Soil (IIA8.4)** Contaminated fluid product shall be incinerated.	
8.4.1	**Possibility of destruction or decontamination following release in the air**	Cu-HDO is non-volatile; a release into the air is therefore not to be expected	
8.4.2	**Possibility of destruction or decontamination following release in water, including drinking water**	In the case of water, the undissolved amount of the product is to be separated by appropriate measures (e.g. phase separation or solvent extraction and to be incinerated. The treated water is to be introduced into a public sewer leading to a public owned water treatment works.	
8.4.3	**Possibility of destruction or decontamination following release in or on soil**	For large amounts, dike spillage, pump off product. For small amounts, pick up with suitable absorbent material (e.g. sand, sawdust, general-purpose binder).	

Section A8 Measures necessary to protect man, animals and the environment

8.5	**Procedures for waste management of the active substance for industry or professional users e.g. possibility of re-use or recycling, neutralisation, conditions for controlled discharge, and incineration (IIA8.5)**	Combustion in a licensed incinerator is the only disposal recommended if Cu-HDO or Cu-HDO treated wood cannot be used according to its purpose.	X
8.5.1	**Possibility of re-use or recycling**	—	
8.5.2	**Possibility of neutralisation of effects**	—	
8.5.3	**Conditions for controlled discharge including leachate qualities on disposal**	—	
8.5.4	**Conditions for controlled incineration**	Cu-HDO does not contain any halogens. Approx. 1100°c are advised as incineration temperature. Expected combustion products are CO_2/CO, H_2O and N_2/NO_x	
8.6	**Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms (IIA8.6)**	No undesirable or unintended effects could be observed on beneficial and other non-target organisms.	
8.7	**Identification of any substances falling within the scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of groundwater against pollution caused by certain dangerous substances (IIA8.7)**	No substances identified.	X

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

June 2005

Materials and Methods

8.1.2 Methods and precautions concerning storage of the active substance and its formulations and 8.1.3 Methods and precautions concerning transport of the active substance and its formulations:

The active substance Cu-HDO is not stored or transported as an isolated solid.

8.1.3 Methods and precautions concerning transport of the active substance and its formulations

The company indicated transport information for the formulation. This information was not reviewed, as under this point only "… transport must take into account any surface which could directly or indirectly come in contact with the product" is requested. Appropriate container material: PE.

8.3.1. Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment if available

General advice:	Remove contaminated clothing
If inhaled:	If difficulties occur after inhalation: fresh air, summon physician
On skin contact:	Wash thoroughly with soap and water or if immediately available with polyethylenglycol
On contact with eyes:	In case contact lenses are in the eye remove them immediately; wash for 10 to 15 minutes under running (no pressure) and warm water with eyelids held open or preferably if available with an eye washing bottle, consult an eye specialist.
On ingestion:	Rinse mouth immediately with water and drink some water, summon medical aid.

8.5 Procedures for waste management of the active substance ….

Incineration facilities must comply with the requirements according to the Waste Incineration Directive 2000/76/EC.

8.7 Identification of any substances falling within the scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of groundwater against pollution caused by certain dangerous substances (IIA8.7)

Since Cu-HDO doesn't fulfil the criteria for List I, it is classed in List II, because it is a biocide. There are no additives or impurities in the active substance as manufactured which fall within the scope of the Lists.

Results and discussion -

Conclusion -

Reliability -

Acceptability -

Remarks -

COMMENTS FROM ...
Date
Materials and Methods
Results and discussion
Conclusion
Reliability
Acceptability
Remarks

Section A 9 Classification and labelling

Annex Point IIA, IX

Section A9	Proposals including Justification for the Proposals for the Classification and Labelling of the Active Substance according to Council Directive 67/548/EEC	

Proposed classification and labelling of the active substance

Table 1.: Proposed classification and labelling of the active substance by RMS according to (EC) No 1272/2008, Annex VI, Table 3.2

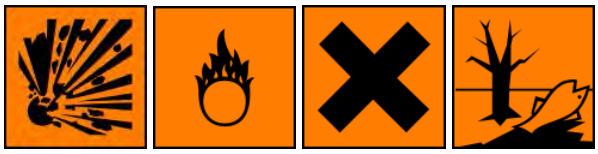
Hazard symbol	
Indication of danger	E explosive O oxidising Xn harmful N dangerous for the environment
R phrases	R2: Risk of explosion by shock, friction, fire or other sources of ignition R8: Contact with combustible material may cause fire R22: Harmful if swallowed R41: Risk of severe damage to eyes R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S phrases	S20/21: When using do not eat, drink or smoke S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice S36/37/39: Wear suitable protective clothing, gloves and eye/face protection S46: If swallowed, seek medical advice immediately and show this container or label S60: This material and its container must be disposed of as hazardous waste. S61: Avoid release to the environment. Refer to special instructions/safety data sheets.
Classification	E; R2 O; R8 Xn; R 22-41 N; R 50-53 SCL: N; R50-53 = $C_n \geq 25\%$; N; R51-53 = $2.5\% \leq C_n < 25\%$; R52-53 = $0.25\% \leq C_n < 2.5\%$;
Labelling	E; O; Xn; N; R: 2-8-22-41-50/53 S: 20/21-26-36/37/39-46-60-61

Table 2: Proposed classification and labelling of the active substance RMS according to Reg. (EC) No 1272/2008[1], Annex VI, Table 3.1 and Reg. (EU) No 286/2011

Classification and Labelling			Justification
GHS Pictograms		 GHS 02/05/07/08/09	
Signal words		Danger, Warning (classification/not labelling)	
Classification		Flam Sol 1 Eye Dam 1 Acute Tox. 4 STOT RE 2 Aquatic Acute 1 (M=1) Aquatic Chronic 1 (M=1)	Aquatic Acute 1 (M=1): Lowest EC_{50} values for fish and algae in the range of 0.1 – 1 mg/L. Aquatic Chronic 1 (M=1): not rapidly degradable and lowest chronic NOE_rC value from algae =0.056 mg/L.
Hazard statements		H228: Flammable Solid	UN-Test N.1
		H318 - Causes serious eye damage	In vivo eye irritation test
		H302 - Harmful if swallowed	Acute gavage test
		H373 – Causes damage to organs (gastrointestinal tract, liver, kidney) through prolonged or repeated exposure	WoE analysis shows toxicological significant effects below guidance value of 100 mg/kg bw day in sub-chronic studies, which is also supported by results from chronic studies.
		H400 - Very toxic to aquatic life (classification) H410 - Very toxic to aquatic life with long lasting effects (classification and labelling)	
Precautionary statement	**Prevention**	P210 Keep away from heat/sparks/open flames/hotsurfaces. — No smoking: P240 Ground/bond container and receiving equipment. P241 Use explosion-proof electrical/ventilating/lighting/…/equipment. P280 - Wear protective gloves/protective clothing/eye protection/face protection. P264 - Wash thoroughly after handling. P270 - Do not eat, drink or smoke when using this product. P273 – Avoid release to the environment	
	Response	P305 + P351 + P338: IF IN EYES: Rinse cautiously with	

		water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P301 + P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P314: Get medical advice/attention if you feel unwell. P330: Rinse mouth P391 – Collect spillage	
	Storage		
	Disposal	P501: Dispose of contents/container in accordance with local/regional/ national/international regulation (to be specified).	