Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.1.1.1.1 [redacted] (2004) Hydrolysis of K-HDO as function of pH, report 01/2002, Laboratory project ID: 001/2004, [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 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 111, 79/831/EWG, OPPTS 835.2110	x
2.2	**GLP**	No	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	K-HDO hydrate	x
3.1.1	Lot/Batch number	E7350/1	
3.1.2	Specification	Solid	
3.1.3	Purity	100 %	x
3.1.4	Further relevant properties		
3.2	**Reference substance**	No	
3.2.1	Initial concentration of reference substance	--	
3.3	**Test solution**	K-HDO in aqueous solution	
3.4	**Testing procedure**	Non-entry field	
3.4.1	Test system	Quantification of K-HDO with the UV/VIS spectroscopy after complexation with Fe, HPLC for the detection of degradation products	
3.4.2	Temperature	Pre-test: 50°C Main-test. 30, 42 and 50°C (Temperature-control see paragraph 4.4)	

Section A7.1.1.1.1
Annex Point IIA7.6.2.1

Hydrolysis as a function of pH and identification of breakdown products

3.4.3 pH

Pre-test: pH 4, pH 7, pH 9

Main-test: pH 4

PH has been measured at the beginning and at the end of the test. The respective values are presented in the following table:

Puffer	pH Start	pH End
4,0	4,22	4,14
7,0	7,00	7,02
9,0	9,03	9,07

3.4.4 Duration of the test

Up to 7 days

x

3.4.5 Number of replicates

One hydrolysis test; repeating determination of each sample at every analysis day

3.4.6 Sampling

Sampling intervals: at 0,1,2,3,4,7 days (see 4.4)

At the end of the each testing interval the sample were thermostated at 20°C. Then the aliquots for the analysis were taken out and the samples were replaced at once in the thermostated ovens (at testing temperature).

3.4.7 Analytical methods

Quantification of K-HDO with the UV/VIS spectroscopy after complexation with Fe (Ref. A 7.1.1.1.1/02 "Validation of a Photometer method for the determination of K-HDO in water").

Validation data:

LOQ = 8,7 mg K-HDO/l

LOD = 1,6 mg K-HDO/l

Correlation coefficient: 0,99998

Recovery:

Sample	Recovery (%)
Level 1	100,5
Level 2	99,0
Level 3	101,0
Level 4	96,5

Accuracy

Sample	Content sample (mg K-HDO/l)
Level 1	
1	150,1
2	150,7
3	150,3
Level 2	
1	60,7
2	58,1
3	59,0
Level 3	
1	15,4
2	15,7
3	15,6
Level 4	
1	5,53
2	5,77
3	6,21
4	5,68
5	5,47
6	6,02

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

For the detection of degradation products HPLC was used.

3.5 Preliminary test Yes

4 RESULTS

4.1 Concentration and hydrolysis values

PH value (buffer / measured pH)	T/°C	Conc. of test substance / mg/100ml	Stability check
4 (monopotassium citrate/ 4,14)	50	0 day: 99,2 5. day: 9,2	Hydrolytically not stable
7 (monopotassium phosphate/ 7,02)	50	0 day: 83,39 5. day: 81,38	Sufficient hydrolytically stable
9 (boric acid/ 9,07)	50	0 day: 94,03 5. day: 93,03	Sufficient hydrolytically stable

4.2 Hydrolysis rate constant (k_h) See below

4.3 Dissipation time

4.4 Concentration – time data

At pH 4

Temperature t /°C	Temperature control of measurement day	Retention time	Conc. of test substance C /mg/100 ml	Velocity constant K /d^{-1} a)	Half-life value $t_{0,5}$ /d b)
30	30	0	100,28		
	31	1	55,13	0,598	1,16
	31	2	17,75	0,866	0,80
	31	3	12,22	0.702	0,988
	31	4	10,88	0,555	1,248
	31	7	11,85	0,305	2,271
				Mean	**1,293**
42	42	0	99,71		
	42	1	36,10	1,016	0,682
	41,5	2	10,93	1,106	0,627
	41,5	3	8,94	0,804	0,862
	41,3	4	10,36	0,566	1,224
	41,5	7	11,04	0,314	2,204
				Mean	**1,12**
50	50	0	99,71		
	50	1	16,18	1,819	0,381
	50	2	9,08	1,198	0,578
	50	3	10,02	0,766	0,905
	50	4	10,29	0,568	1,220

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

	50	7	8,44	0,353	1,963
				Mean	**1,01**

4.5 Specification of the transformation products

The degradation of K-HDO leads to the formation of cyclohexanone oxime, which was checked with HPLC in combination with a diode-array detector.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

TEST TYPE: hydrolysis study – OECD-guideline 111 and see table A7_1_1_1_1-1, A7_1_1_1_1-2 and A 7_1_1_1-3

- Test medium: Bi-distilled, sterile water
- Test system: quantification of K-HDO in batches stored at different temperatures and pH

DURATION:

5 and 7 days

5.2 Results and discussion

- Stable at pH=7 and pH=9.
- Half life $t_{1/2}$: 1,26 days at 25°C and pH=4
- Rate constant: 0,5485 d-1 at 25 °C

The reaction did not follow a first order reaction, which is expected for hydrolysis. The reaction order was not determined which is in accordance with the OECD 111 guideline. However based on the averaged velocity constants at 30°C and 42 °C the constants A (A=249.47 d^{-1}) and B (B=15170,7 J/mol) of the Arrhenius equation are estimated. For 25 ° C the Arrhenius equation yields to a half-life of 1,26 d.

BREAKDOWN PRODUCTS:

The only breakdown product identified was Cyclohexanone oxime; no quantification of degradation products has been performed.

5.2.1 k_H See above

5.2.2 DT_{50} See above

5.2.3 r^2 The hydrolysis reaction did not follow a first order kinetic. Therefore the r^2 was not calculated.

5.3 Conclusion

At pH 7 and pH 9 K-HDO is considered to be hydrolytically stable. At pH 4 a degradation of K-HDO was observed. The reaction did not follow a first order kinetic. A half life of 1,26 d is estimated based on the velocity constants measured at 30 and 42°C. The only breakdown product identified was Cyclohexanone oxime; no quantification of degradation products has been performed

5.3.1 Reliability 1 X

5.3.2 Deficiencies No

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2005
Materials and Methods	**2.1 Guideline study** 79/831/EWG is not a test guideline, but an adaptation to technical progress. The guideline is EC C.7. **3.1 Test material** K-HDO monohydrate (includes 1 molecule of water of crystallisation) **3.1.3 Purity** Corresponds to 91.1% when 1 molecule of water of crystallisation is disregarded. **3.4.4 Duration of the test** Pre-test: 5 days Main-test: 7 days
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	2
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7_1_1_1_1-1: Type and composition of buffer solutions (specify kind of water if necessary)

pH	Type of buffer (final molarity)	Composition
4	monopotassium citrate see OECD-guideline 111	9.0 mL 0.1 M NaOH + 50 mL 0.1 M monopotassium citrate made up to 100 mL with bi-distilled water (at 18°C)
7	monopotassium phosphate see OECD-guideline 111	29.63 mL 0.1 M NaOH + 50 mL 0.1 M monopotassium phosphate made up to 100 mL with bi-distilled water (at 20°C)
9	boric acid see OECD-guideline 111	21.30 mL 0.1 M NaOH + 50 mL 0.1 M boric acid (in 0.1 M KCl) made up to 100 mL with bi-distilled water (at 20°C)

Table A7_1_1_1_1-2: Description of test solution

Criteria	Details
Purity of water	Bi-distilled, sterile water
Preparation of test medium	Buffer preparation according OECD-guideline 111. The buffers were thermostated at 20°C. After pH-control nitrogen was dumped in the bottles for 5 minutes. Then the weighed K(HDO) was transferred into the testing bottle and the bottle was shaken.
Test concentrations (mg a.i./L)	(1) 100.28 mg/100 ml (2) 99,71 mg/100 ml (3) 99,71 mg/100 ml
Temperature (°C)	(1) 30 °C (2) 42 °C (3) 50 °C
Controls	
Identity and concentration of co-solvent	none
Replicates	--

Table A7_1_1_1_1-3: Description of test system

Glassware	100 ml brown glass bottle, DURAN with new Polypropylene - screw caps; all sterile
Other equipment	Water bath : Lauda DLK 15 pH meter : Knick pH-Meter 765 Calimetric
Method of sterilization	temperature sterilisation

Table A7_1_1_1_1-4: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 4*)*

Compound	Sampling times (*days*)							
	0	***1***	***2***	***3***	***4***	***7***	t_6	t_n
Parent compound [mg/100ml]	100,28	55,13	17,75	12,22	10,88	11,85		
Transformation product 1								
Transformation product 2								
Transformation product n								
Reference compound								
Volatiles *(if measured)*								
Total % recovery								

Table A7_1_1_1_1-5: Dissipation times of parent compound, transformation products and reference compound at pH 5, pH 7 and pH 9

	pH 5		pH 7		pH 9	
	DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}
Parent compound						
Transformation product 1						
Transformation product 2						
Transformation product n						
Reference compound						

Table A7_1_1_1_1-6: Specification and amount of transformation products *(adjust table size as required)*

CAS-Number	*CAS and/or IUPAC* Chemical Name*(s)*	Amount [%] of parent compound measured at		
		pH 5	pH 7	pH 9

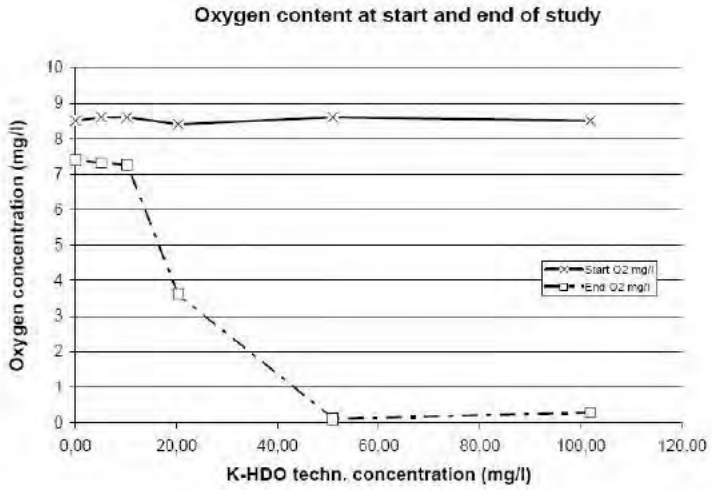
Section A7.1.1.2.1 **Biodegradability (ready)**
Annex Point IIA7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.1.1.2.1 [redacted] (1996) Prüfung der biologischen Abbaubarkeit von K-HDO, techn. 30 % ig im Verdünnungs BSB-test nach 30 Tagen [redacted] (Determination of the Biodegradability and the Elimination of K-HDO, techn. 30%, respectively from water in the modified static Zahn-Wellens Test), Report 95/0179/04/2, [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 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 DIN 38409 part 51, annex to guideline 92/69/EEC biochemical oxygen demand C.5	
2.2	**GLP**	No	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	K-HDO	
3.1.1	Lot/Batch number	74N9702	
3.1.2	Specification	K-HDO, 30,4 % in water	
3.1.3	Purity	99 %, 30,4 % K-HDO in aqueous solution	x
3.1.4	Further relevant properties	Substance stability: stable for at least 3 years Vapour pressure: approx. 23 mbar at 20 °C (water) Water solubility: miscible with water in each ratio Adsorption potential (log Pow): -0,2. at 25 °C (pH: 7,2)	
3.1.5	Composition of Product	30,4 % K-HDO, 69,6 % water	
3.1.6	TS inhibitory to microorganisms	Yes, EC_{20} = 4,8 mg/l	x
3.1.7	Specific chemical analysis	No analytical control of the test substance according to guideline	
3.2	**Reference substance**	No testing of a reference substance, this BSB dilution method were carried out to confirm the biodegradation in project 95/0179/10/2	x
3.2.1	Initial concentration of reference substance	No testing of a reference substance	
3.3	**Testing procedure**	Non-entry field	

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

3.3.1	Inoculum / test species	Activated sludge	
3.3.2	Test system	bottle test	
3.3.3	Test conditions	see table A7_1_1_2-4	
3.3.4	Method of preparation of test solution	Different via a dilution series produced concentration of the test substance were mixed with defined dilution water and incubated in the darkness for 30 days. The dilution water has been pre-incubated for 7 days.	
3.3.5	Initial TS concentration	20,4 mg/l	x
3.3.6	Duration of test	30 days	
3.3.7	Analytical parameter	Biochemical oxygen demand	
3.3.8	Sampling	The oxygen content has been measured at start and end of the test.	
3.3.9	Intermediates/ degradation products	Not identified	
3.3.10	Nitrate/nitrite measurement	No	
3.3.11	Controls	Control without test substance	
3.3.12	Statistics	No	

4 RESULTS

4.1	**Degradation of test substance**	*Non-entry field*	
4.1.1	Graph		x
4.1.2	Degradation	Biodegradation degree (BOD/THOD) after 30 days: approx. 60 %	
4.1.3	Other observations	No	
4.1.4	Degradation of TS in abiotic control	Not relevant according to the guideline	
4.1.5	Degradation of	No testing of a reference substance, this BSB dilution method was	

	reference substance	carried out to confirm the biodegradation in project 95/0179/10/2	
4.1.6	Intermediates/ degradation products	Degradation products were not determined	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	DIN 38409 part 51, annex to guideline 92/69/EEC biochemical oxygen demand C.5 Different, via a dilution series produced concentration of the test substance were mixed with defined dilution water and incubated in the darkness for 30 days. The dilution water has been pre-incubated for 7 days. At the start and the end of the test the dissolved oxygen has been measured with an oxygen electrode and the BOD value has been calculated in g per g test substance. A blind value without test substance has also been tested. A short term respiration test has been performed prior to the BOD test according to ISO 8192. Inoculum: filtrate from adaptation run For adaptation purposes 10 mg/l K-HDO, techn. 30 % were incubated in the modified test according to Zahn-Wellens OECD 302 B with activated sludge from laboratory sewage plants, which are running with communal and synthetic sewage. The dry substance content was 1 g/l. On day 29 for the first time and then weekly 200 mg/l yeast extract were fed. On day 68 once again 10 mg/l K-HDO, techn. 30 % was added. At the following day a sufficient test quantity was taken, filtrated through a paper filter and used for the inoculation of the dilution water.	
5.2	**Results and discussion**	"The BOD method C.5 is a guideline study and the determination of the BOD removal is general use for evaluation of the biodegradability of a substance. There is scientific no doubt that the analytical determination of the BOD is a valid method. Indeed due to the preadaptation of the inoculum the assessment of ready biodegradability cannot be derived from this result. But K-HDO is biodegradable and as stated clearly in the comments the test would be performed under a test concentration that could be expected inhibitory influences of the biodegradation process. This concentration was necessary for analytical reasons. As a consequence of these circumstances the biodegradation degree of about 60 % BOD removal represents a worst case result."	x
5.3	**Conclusion**	The evaluation of the test bottles was only possible in the cases there the test substance was diluted 1 : 50 (20,36 mg/l K-HDO techn. 30%). With other dilutions the evaluation of the bottles was not possible because either the oxygen consumption was too high (> 7 mg/l) or too low (< 3 mg/l). In the first case the test concentration is too high and the complete oxygen consumption does not enable the calculation of the BOD value. In the second case the measuring of a sufficient oxygen demand is not possible because the test concentration is too low. This procedure to use different concentration is applied for the BOD-determination of sewage. Not only the measured BOD value but also the observed oxygen consumption of the different dilutions is an unambiguous indicator of the biological degradability of K-HDO at low, non-toxic concentrations.	x
5.3.1	Reliability	1	x

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

5.3.2	Deficiencies	No	x

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

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2005
Materials and Methods	**3.1.3 Purity** K-HDO as 30.4 % aqueous solution; purity of the solution: 99% **3.1.6 TS inhibitory to micro-organisms** Yes, EC20 = 4.8 mg 30% K-HDO in water / L **3.2. Reference substance** The BOD test was carried out using the same inoculum as in the Zahn-Wellens Test (Doc III A7.1.1.2.2.). In the BOD test no reference substance was tested. In the Zahn-Wellens test the reference substance Diethylene glycole was tested proving the high quality of the inoculum with 100% degradation in 10 days. **3.3.5 Initial TS concentration** The concentration of 20.4 mg/L refers to 30% K-HDO/L, this corresponds to 6.1 mg/L K-HDO.
Results and discussion	**4.1.1 Graph** The data mentioned under this heading is not mentioned in Doc IV.
Conclusion	**5.2 Results and discussion** The substance has been tested at inhibitory concentrations. The inoculum has been adopted for 69 days. **5.3 Conclusion** Although the test was run at inhibitory concentrations the biodegradation degree of about 60% BOD removal after 30 days does not represent a worst case result, since the inoculum has been pre-adapted for 69 day. The result can be quoted as a qualitative hint for a potential biodegradation of K-HDO. There is no proof for a ready biodegradation of K-HDO. **5.3.2 Deficiencies** There are two major deficiencies in the test report: - The inoculum has been adapted to K-HDO for 69 days. - K-HDO has been tested at inhibitory concentrations.
Reliability	2
Acceptability	acceptable
Remarks	-

COMMENTS FROM ...
Date
Materials and Methods
Results and discussion
Conclusion
Reliability
Acceptability
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	Not applicable
Strain	Not applicable
Source	Activated sludge from a laboratory waste water plant fed with municipal sewage
Sampling site	Laboratory plants with municipal waste water
Laboratory culture	Cultured in the laboratory waste water plant
Method of cultivation	DIN 38409 part 51, annex to guideline 92/69/EEC biochemical oxygen demand C.5 Different, via a dilution series produced concentration of the test substance were mixed with defined dilution water and incubated in the darkness for 30 days. The dilution water has been pre-incubated for 7 days. At the start and the end of the test the dissolved oxygen has been measured with an oxygen electrode and the BOD value has been calculated in g per g test substance. A blind value without test substance has also been tested.
Preparation of inoculum for exposure	The inoculum was washed with drinking water
Pretreatment	Adaptation
Initial cell concentration	1 g/l dry weight

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	Respirometer
Number of culture flasks/concentration	2
Aeration device	According to guideline
Measuring equipment	WTW oxygen electrode
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	Activated sludge
Additional substrate	Yes, yeast extract
Test temperature	20 +/- 1 °C
pH	6 - 9 according to the guideline
Aeration of dilution water	According to the guideline
Suspended solids concentration	Supernatant from the study 95/0179/10/2
Other relevant criteria	no further 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 $ThCO_2$	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	Not applicable to BSB dilution method	
Criteria for validity		
Deviation of the degradation degree of the test substance in the plateau phase > 20 %	Not applicable	
Degradation degree of the reference substance > 60 % after 14 days		Not applicable Reference substance was not tested
Oxygen demand at the end of the test, blank control < 1.5 mg/l	X	
Oxygen concentration at the end of the test, test substance > 0.5 mg/l	X	
Degradation degree in the inhibition control > 25 % after 14 days	Not applicable to BSB dilution method	

The test is valid: Yes, Reliability 2 (restrictions are non GLP and no reference substance was proved)

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		

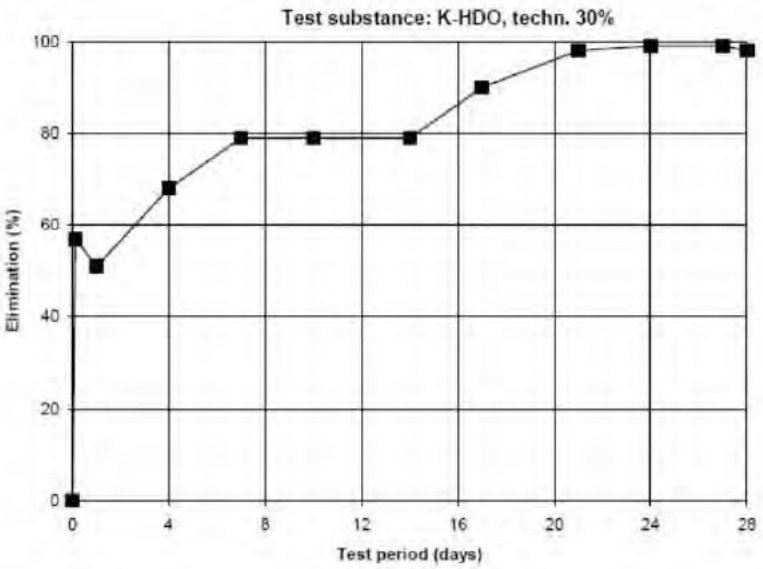
Section A7.1.1.2.2 **Biodegradability (inherent)**
Annex Point IIA7.6.1.2

				Official use only
		1 REFERENCE		
1.1	**Reference**	A 7.1.1.2.2 [redacted] (1995) Determination of the biodegradability or the Elimination of K-HDO, techn. 30 % in the modified static Zahn-Wellens-Test: [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 According to OECD Guideline 302 B; Directive 88/302/EWG and ISO 9888		x
2.2	**GLP**	Yes		
2.3	**Deviations**	No		
		3 MATERIALS AND METHODS		
3.1	**Test material**	K-HDO as 30 % aqueous solution (Xyligen 30 F)		
3.1.1	Lot/Batch number	74 N 9702		
3.1.2	Specification	Liquid		x
3.1.3	Purity	99%		
3.1.4	Further relevant properties	Substance stability:	stable for at least 3 years	
		Vapour pressure: approx.	23 mbar at 20 °C (water)	
		Water solubility:	miscible with water in each ratio	
		Adsorption potential (log Pow):	-0,2. at 25 °C pH: (7,2)	
3.1.5	Composition of Product	30 % K-HDO, 70 % water		
3.1.6	TS inhibitory to microorganisms	Yes, EC_{20} (mg/l): 4.8		x
3.1.7	Specific chemical analysis	Yes, Thermal Energy Analyzer (TEA) as described in A4.2/02		x
3.2	**Reference substance**	Yes Diethylene glycol		
3.2.1	Initial concentration of reference substance	202 mg/l DOC		
3.3	**Testing procedure**	Non-entry field		
3.3.1	Inoculum / test species	Activated sludge from laboratory plants with municipal waste water, no adaptation		

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

3.3.2	Test system	Modified static test according to Zahn-Wellens (substance); method for testing the biological degradation of substances by measuring the decrease of the dissolved organic carbon (DOC)	
3.3.3	Test conditions	The test substance, a defined inorganic medium and as inoculum activated sludge from a municipal sewage plant or laboratory plant were mixed and aerated at room temperature up to 28 days. Samples are taken in regular intervals and were analysed in this case with substance specific analysis. In addition to the samples with test substance, an assay to determine the blank value (no test substance) and a test with a reference substance was performed.	
3.3.4	Method of preparation of test solution	Test substance was used as delivered (K-HDO 30 % in water)	
3.3.5	Initial TS concentration	2 mg/l	x
3.3.6	Duration of test	Test duration (days) : 28 Duration of adaptation phase (days) : <1 Duration of degradation phase (days) : 21	
3.3.7	Analytical parameter	DOC concentration	
3.3.8	Sampling	The samples of the test substance measurements were analyzed with substance specific chemical analysis by ZAX/PG.	
3.3.9	Intermediates/ degradation products	Intermediates or degradation products have not been determined	
3.3.10	Nitrate/nitrite measurement	No	
3.3.11	Controls	Yes	
3.3.12	Statistics	No	
		4 RESULTS	
4.1	**Degradation of test substance**	Non-entry field	

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

4.1.1	Graph		
4.1.2	Degradation	98 % after 28 day(s)	x
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 reference substance	97 % after 7 days	
4.1.6	Intermediates/ degradation products	Not determined	

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

Decrease of the test substance (substance specific analysis) after 28 days: 98 %

Decrease of the test substance (substance specific analysis) after 3 hours: 57 %

The test substance is well eliminated from water

5.3 Conclusion

The elimination of K-HDO from water by absorption has been verified with this test; it is assumed that this will normally also take place in sewage plants and the environment.

x

Criteria for validity:

Reference substance:	diethylene glycol
Degree of degradation of the reference substance (%DOC):	97 after 7 days
Degradation of the reference substance:	
after 14 days > 80%:	☒ yes ☐ no
Test is valid:	☒ yes ☐ no

5.3.1 Reliability: 1

5.3.2 Deficiencies: No

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

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2005
Materials and Methods	**2.1 Guideline study**
	According to OECD 302B; EC guideline C.9 and ISO 9888
	3.1.2 Specification
	colourless liquid with substance specific odour
	3.1.6 TS inhibitory to micro-organisms
	Yes, EC20 = 4.8 mg 30% K-HDO in water / L
	3.1.7 Specific chemical analysis
	As the study is no key study, no study summary of this method is available.
	3.3.5 Initial TS concentration
	2 mg / L K-HDO (active substance)
Results and discussion	**4.1.2 Degradation**
	98% of K-HDO were eliminated from the water phase after 28 days. 57% of this elimination process took place during the first three hours, which indicates elimination by adsorption.
Conclusion	**5.1 Material and methods**
	EC C.9
	OECD 302 B
	ISO 9888
	5.3. Conclusion
	K-HDO is eliminated from the water phase by 98% after 28 days. 57% of this elimination takes place within the first 3 hours and is due to adsoption. Therefore K-HDO can not be regarded as being inherently and/or ultimately biodegradable.
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
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	not applicable
Strain	not applicable
Source	Activated sludge from laboratory plants with municipal waste water
Sampling site	Laboratory plants with municipal waste water
Laboratory culture	Cultured in the laboratory wastewater plant
Method of cultivation	The test substance, a defined inorganic medium and as inoculum activated sludge from a municipal sewage plant or laboratory plant were mixed and aerated at room temperature up to 28 days.
Preparation of inoculum for exposure	The inoculum was washed with drinking water
Pretreatment	No adaptation
Initial cell concentration	1 g/l dry weight

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	DOC analyser
Number of culture flasks/concentration	1
Aeration device	Yes
Measuring equipment	DOC-analyser
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2-4: Test conditions

<table>
<tr><th>Criteria</th><th>Details</th></tr>
<tr><td>Composition of medium</td><td>According to guideline OECD 302 B:
Stock solutions:
(a) Potassium dihydrogen orthophosphate, KH_2PO_4: 8.5 g
Dipotassium hydrogen orthophosphate, K_2HPO_4: 21.75 g
Disodium hydrogen orthophosphate dihydrate, $Na_2HPO_4.2H_20$: 33.4 g
Ammonium chloride, NH4Cl: 0.5 g
Water: ad. 1 litre.
PH:7.4.
(b) Calcium chloride, anhydrous, $CaCl_2$: 27.5 g
or Calcium chloride dihydrate, $CaCl_2.2H_20$: 36.4 g
water: ad 1 litre.
(c) Magnesium sulphate heptahydrate, $MgS0_4.7H_20$: 22.5 g
water: ad 1 litre.
(d) Iron (III) chloride hexahydrate, $FeCl_3.6H_2O$: 0.25 g
water: ad. 1 litre.
Preparation of mineral medium:
10 ml of solution (a)
800 ml water
1 ml of solutions (b), (c) and (d)
water: ad. 1 litre</td></tr>
<tr><td>Additional substrate</td><td>No addititional substrate</td></tr>
<tr><td>Test temperature</td><td>20-25 °C</td></tr>
<tr><td>pH</td><td>
<table>
<tr><td>Date</td><td>Day</td><td>BW1</td><td>KS1</td><td>PS1</td></tr>
<tr><td></td><td></td><td>pH</td><td>pH</td><td>pH</td></tr>
<tr><td>26.6.95</td><td>0</td><td>7.0</td><td>6.9</td><td>7.1</td></tr>
<tr><td>26.6.95</td><td>0.125</td><td>7.8</td><td>7.9</td><td>7.8</td></tr>
<tr><td>27.6.95</td><td>1</td><td>7.2</td><td>7.2</td><td>7.3</td></tr>
<tr><td>30.6.95</td><td>4</td><td>7.0</td><td>5.3</td><td>7.2</td></tr>
<tr><td>3.7.95</td><td>7</td><td>6.7</td><td>8.1</td><td>6.8</td></tr>
<tr><td>5.7.95</td><td>9</td><td>6.9</td><td>7.2</td><td>nv</td></tr>
<tr><td>6.7.95</td><td>10</td><td>7.1</td><td>7.1</td><td>7.2</td></tr>
<tr><td>10.7.95</td><td>14</td><td>7.0</td><td>7.1</td><td>7.0</td></tr>
<tr><td>13.7.95</td><td>17</td><td></td><td></td><td>6.8</td></tr>
<tr><td>17.7.95</td><td>21</td><td></td><td></td><td>6.8</td></tr>
<tr><td>20.7.95</td><td>24</td><td></td><td></td><td>6.9</td></tr>
<tr><td>23.7.95</td><td>27</td><td></td><td></td><td>7.0</td></tr>
<tr><td>24.7.95</td><td>28</td><td></td><td></td><td>7.1</td></tr>
</table>
</td></tr>
<tr><td>Aeration of dilution water</td><td>According to the guideline</td></tr>
<tr><td>Suspended solids concentration</td><td>1g/l</td></tr>
</table>

Other relevant criteria	No other information

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		
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);		not relevant, specific analysis
Pass values reached within 10-d window (within 28-d test period)		not relevant, inherent method
Removal of reference substance (DOC or COD) > 70 % within 14 d	X	
Criteria for validity		
Percentage of DOC/COD-removal of reference compound ≥ 70 % within 14 days (OECD 302 B)	X	
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)	X	
Removal curve of DOC or COD in the test suspension indicative for biodegradation (gradual elimination over days/weeks)	X, with specific analysis	

Criteria for poorly soluble test substances		

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], 2006, Adsorption/desorption study with K-HDO according to OECD 106, [redacted], Report no. 05 10 35 2029, 2006, unpublished, Ref. A 7.1.3/03	
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**	(N-cyclohexyldiazenium-dioxy)-potassium	
3.1.1	Lot/Batch number	W-87	
3.1.2	Specification	White crystalline powder:	
3.1.3	Purity	100 % as (N-Cyclohexyldiazeniumdioxy)-potassium monohydrate 91,1 % (N-Cyclohexyldiazeniumdioxy)-potassium	
3.1.4	Further relevant properties	vapor pressure: $1x10^{-6}$ hPa water solubility: 452 g/L	x
3.1.5	Method of analysis	Determination of the K-HDO content was performed out by analysis of K-HDO using HPLC and UV/Vis detection. The method was calibrated and validated in the range from 0.1-130 µg/mL K-HDO.	
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.	x
3.2.1	Method of analysis for degradation products	Not necessary	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Soil types**	see table A7_1 _3-1	x

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

3.5 Testing procedure

3.5.1 Test system

The adsorption/desorption behaviour of K-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.

3.5.2 Test solution and Test conditions

Solution	Preparation
Blank solution	1.831 g CaCl2 Tetra hydrate (Merck, Suprapur), 1 L volumetric flask, filled up with ultra pure water
Stock Standard solution and Standard solutions	Aliquots of the K-HDO-Standard solution dissolved with eluent in 100 mL volumetric flasks
0.01 n CaCl2-solution	3.661 g CaCl2 Tetra hydrate (Merck, Suprapur) in 2 L ultra pure water
Test item solution	solution of the test item in 0.01 n $CaCl_2$- solution, concentration approximately 1000 mg/L
Eluent	550 mL of 0.05 n KH_2PO_4 (6.804 g in 1 L ultra pure water) and 450 mL Methanol , after mixing the pH-value will adjust with o-phosphoric acid to pH 2.5, Degassing by ultrasonication for 15 min
Extraction solution	Methanol/Eluent 20/80 (v/v) Degassing by ultrasonication for 15 min

3.6 Test performance

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 K-HDO analysis was checked for the $CaCl_2$ soluble K-HDO blank, the Methanol/Eluent extract soluble K-HDO 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.

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

The five soils described were used. The test parameters were based on the results of the preliminary studies. One control sample without soil and one blank sample without test item solution were subjected to the same procedure. All experiments were performed in duplicate. The distribution coefficient K_d at equilibrium as well as the organic carbon normalized adsorption coefficient K_{OC} was calculated.

In a tier 3 approach the adsorption isotherms were determined. The five soils with six test concentrations covering two orders of magnitude were used.

3.6.3 Screening test: Desorption

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

The determination of desorption kinetics was carried out with all soil systems. For the determination of desorption the serial proceeding was used. After the adsorption test, the aqueous phase was separated as much as possible and replaced with 49.5 mL 0.01 n $CaCl_2$ solution. The extraction bottle was shaked for 2, 4, 8, 24 and maximum 72 h. After each period the bottle was centrifuged and an aliquot of 500 µL was discharged and analysed. After sampling an equivalent volume (500 µL) of 0.01 N $CaCl_2$ solution was added and the extraction procedure was continued as described. The K_{des} values at equilibrium were calculated.

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

		Moreover desorption isotherms were determined (calculated from the analysed concentration at equilibrium time (8 h).	
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 Summary: The time dependant adsorption at different soil/test item ratios for two soils was investigated. The adsorption of both of the soils reached more than 25 %. The equilibration time was fixed to 24 h.	x
4.2	**Screening test: Adsorption**	see table A7_1_3-3	
4.3	**Screening test: Desorption**	Desorption isotherms and Desorption kinetics were measured. Results see table A7_1 _3-4	
4.4	**Calculations**		
4.4.1	Ka , Kd	The Freundlich adsorption coefficients are in the range from 20.5 to 223.8. The averaged Freundlich adsorption coefficient amounts to 85.7. See details given in table A7_1 _3-3 The Freundlich desorption coefficients are in the range from 27 to 343.8. The averaged Freundlich desorption coefficient amounts to 124.3. See details given in table A7_1 _3-3	
4.4.2	Ka_{oc} , Kd_{oc}	See table A7_1_3-3 and A7_1_3-4	
4.5	**Degradation product(s)**	No significant amount of degradation products was measured.	

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The adsorption/desorption behaviour of K-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 adsorbtion in all soils exceeded 25 %. 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 20.5 to 223.8. The averaged Freundlich adsorption coefficient amounts to 85.7. See details given in table A7_1 _3-3	x
5.2.3	K_d	The Freundlich desorption coefficients are in the range from 27 to 343.8. The averaged Freundlich desorption coefficient amounts to 124.3. The organic carbon normalized Freundlich desorption coefficients are in the range from 1064 to 16293. The averaged organic carbon normalized Freundlich desorption coefficient amounts to 8832. See details given in table A7_1 _3-3	x
5.2.4	Ka_{oc}	The organic carbon normalized Freundlich adsorption coefficients are in the range from 805 to 10606. The averaged organic carbon normalized Freundlich adsorption coefficient amounts to 6007. See details given in table A7_1 _3-3.	x
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 K-HDO, adsorption exceeds 25 % for all soils. The organic carbon normalized Freundlich adsorption coefficient values are considerable and in the range from 805 to 10606. (Mean value amounts to 6007). The resulting organic carbon normalized Freundlich desorption coefficient value are in the range from 1064 to 16394 (Mean value amounts to 8832) and therefore 1.4 fold higher than the corresponding adsorption values. The test item K-HDO is practically irreversibly adsorbed on the soils.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

February 2006

Materials and Methods

3.1.4 Further relevant properties

vapor pressure < $1x10^{-6}$ hPa

3.2 Degradation products

loss during filtration, adsorption to container walls: average recovery rate: 98% mean recovery rate (soil LUFA 2.1 and LUFA 6S, 92 hours agitation): 90.2%; the test item is considered to be stable according to OECD 106.

3.4 soil types, table A7_1_3-1

Bruch West: specified as loamy sand (soil type 5); deviations: organic carbon content 2.54% (default: <0.5-1.5%), pH 7.2 (default: <4-6)
LUFA 2.1: specified as sand; According to OECD: specification as loamy sand (soil type 5) is more approbriate due to the features
LUFA 2.2: specified as loamy sand (soil type 5) deviation: organic carbon content 2.11% (default: <0.5-1.5%)
LUFA 2.3: specified as sandy loam. According to OECD: specification as loamy sand (soil type 5)
LUFA 6 S: specified as clay loam/clay (soil type 6) deviations: organic carbon content 1.83% (default: <0.5-1.0%), pH 6.8 (default: >7)
Deviation from OECD 106: no information on geographical reference of the site, data of sampling, use pattern, depth of sampling and other information relating to the collection and storage of the soil samples.

Results and discussion

4.1 Preliminary test, Table A7_1_3-2

Volume of CaCl2 solution: 50 mL (final volume)

5.2 Results and discussion

for Ratio soil/test item solution = 2:

[mL/g]	*Bruch West*	*LUFA 2.1*	*LUFA 2.2*	*LUFA 2.3*	*LUFA 6S*
Distribution coefficient at adsorption equilibrium Kd	12.2	47.7	189.2	22.3	42.0
Organic carbon normalized adsorption coefficient	479.3	7566.3	8967.3	2190.0	2296.5

Agree with the applicant's version with the amendments given above

Conclusion

Agree with the applicant's version

Reliability

1

Acceptability

acceptable

Remarks

The test is considered sufficient to meet the demands of the data requirements, particularly with regard to the fact that no environmental exposure by the representative product is expected. However, for use within the risk assessment for other products or product applications this study might be reviewed again.

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	K-HDO	
Sample purity	100 % as (N-Cyclohexyldiazeniumdioxy)-potassium monohydrate	
Weighed soil	2 g soil	
Volume of $CaCl_2$ solution	49-50 mL $CaCl_2$ extraction solution	X
Nominal concentration of a.s. final solution	Analytically verified concentrations used	
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 K-HDO content was performed by analysis of K-HDO using HPLC and UV/VIS detection. The method was calibrated in the range from 0.1-130 µg/mL K-HDO and validated.	
Method	HPLC-UV/VIS	
Recovery rate ($CaCl_2$-soluble K-HDO recovery rate)	95.6 % at 0.1 mg/L level 100.8 % at the 10 mg/L level	
Detection limit	0.05 mg/L $CaCl_2$ extract	

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

	Bruch West	LUFA 2.1	LUFA 2.2	LUFA 2.3	LUFA 6S	
Concentration of test material [mg/L]	0.8-201	0.8-201	0.8-201	0.8-201	0.8-201	
After contact of 24 hours with soil	0.387-175.8	0.176-126	0.079-35	0.28-146.9	0.142-124.6	
Correction for blank with soil	0	0	0	0	0	
Correction for blank without soil	0	0	0	0	0	
Final corrected concentration [mg/L]	0.387-175.8	0.176-126	0.079-35	0.28-146.9	0.142-124.6	
Initial concentration of test solution [mg/L]	0.8-201	0.8-201	0.8-201	0.8-201	0.8-201	
Decrease in concentration [mg/L]	0.413-26.2	0.624-75	0.72-166	0.52-54.1	0.658-76.4	
Quantity adsorbed [µg]	20.8-1259.6	31.4-3750.8	36.2-8300	26-2705	33.1-3819	
Quantity of soil [g of oven-dried equivalent]	2	2	2	2	2	
Quantity adsorbed [µg] per gram of soil	10.4-628.5	15.7-1872.8	18.1-4142.5	13-1351	16.5-1909	
Test material adsorbed [%]	51.8-12.5	78.1-37.1	90.1-82.6	64.8-26.9	82.3-38	
Temperature [°C]	22.9	22.9	22.9	22.9	22.9	
Distribution coefficient at adsorption equilibrium Kd	26.88-3.58	89.4-14.9	289-118.2	45.9-9.2	149-15.3	X
Organic carbon normalized adsorption coefficient	1058-140.7	14186-2358.7	10796-5602	4499-902	6339-822	X
Freundlich adsorption coefficient	20.5	66.3	223.8	38.1	79.8	
Organic carbon normalized Freundlich adsorption coefficient	805	10518	10606	3739	4360	
PH-value of the aqueous phase at adsorption equilibrium	7.2-7.4	5.9-6.7	5.5-6.3	6.75-7	6.9-7.2	

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

	Bruch West	LUFA 2.1	LUFA 2.2	LUFA 2.3	LUFA 6S	
Temperature [°C]	22.1	22.1	22.1	22.1	22.1	
Evaluated concentration range [µg/g]	20-5015	20-5015	20-5015	20-5015	20-5015	
Freundlich desorption coefficient	27	103.3	343.8	53.7	93.5	
Organic carbon normalized Freundlich desorption coefficient	1064	15472	16293	5261	5112	
[%] of desorbed test material	34-55	17-24	4-9	23-39	14-35	
Correlation coefficient R^2	0.964	0.998	0.976	0.972	0.985	

Section 7.3.1 Annex Point IIIA, VII.5 — Phototransformation in air (estimation method), including identification of breakdown products

Official use only

The degradation rate constant of K-HDO with OH-radicals (k_{OH} in cm^3x $molecule^{-1}$ x s^{-1}) has been estimated with an Atmospheric Oxidation Program (AOP 1.91):

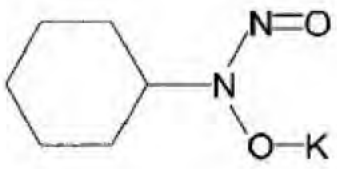
k_{OH} (K-HDO) = 34.3610 x 10^{-12} cm^3/(molecule x sec)

The half-life of K-HDO has been estimated to be 3.735 hours.

Because of this short lifetime in the atmosphere and because K-HDO is free of Cl, Br or F an effect of K-HDO on stratospheric ozone can be excluded.

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

Calculation report:




```
SMILES : C1CCCCC1N(N(=O)O)OK
CHEM   :
MOL FOR: C6 H11 N2 O2 K1
MOL WT : 182.26
------------------- SUMMARY (AOP v1.91): HYDROXYL RADICALS -------------------
Hydrogen Abstraction       =  34.3610 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 =  34.3610 E-12 cm3/molecule-sec
   HALF-LIFE =     0.311 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =     3.735 Hrs
------------------- SUMMARY (AOP v1.91): OZONE REACTION ----------------------

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

Experimental Database:  NO Structure Matches

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

Section 7.3.1 Annex Point IIIA, VII.5 — Phototransformation in air (estimation method), including identification of breakdown products

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Oct. 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). k_{OH} (K-HDO) is the specific first-order degradation rate constant of K-HDO with OH-radicals. The OH radical concentration used for the estimation of the half-life (3.735 hours) is $1.5 \cdot 10^6$ OH / cm^3. Estimation applying an OH radical concentration of $0.5 \cdot 10^6$ OH / cm^3 according to EU TGD, Part II, Chapter 2.3.6.3: Half-life time is 11.2 hours.
Conclusion	agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	none
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

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] (1980) Report on the test of the acute toxicity of Xyligen 30F in fish (golden orfe - Leuciscus idus L.) [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 DIN 38412, test procedure with water organisms (group L) general notes on the design, performance and evaluation of biological test procedures (L1) and determination of the effect of constituents of water on fishes - fish test (L15) draft, January 1979.	
2.2	**GLP**	No	x
2.3	**Deviations**	Prior to the implementation of GLP	x
		3 MATERIALS AND METHODS	
3.1	**Test material**	K-HDO as 30 % aqueous solution (Xyligen 30F)	
3.1.1	Lot/Batch number		x
3.1.2	Specification	Liquid	
3.1.3	Purity		x
3.1.4	Composition of Product		
3.1.5	Further relevant properties		x
3.1.6	Method of analysis		x
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	—	
3.3	**Reference substance**	Chloroacetamide	
3.3.1	Method of analysis for reference substance		x
3.4	**Testing procedure**	Non-entry field	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

3.4.1	Dilution water		x
3.4.2	Test organisms	Fish (golden orfe - Leuciscus idus L.)	x
3.4.3	Test system	Static system	x
3.4.4	Test conditions	Test organisms: Golden orfe (Leuciscus idus), supplied by P.Eggers, Hohenwestedt, Germany. Body weight: 1.9 g Body length: 6.4 cm Test temperature: 20°C Adaption time: 3 days Investigations: Determination of the mortality after 4, 24, 48, 72 and 96 hours. Determination or calculation of the median lethal concentration (LD_{50}), the LC_5 and the LC_{95} by probit analysis (Finney, D. J., Probit Analysis, Cambridge University Press, 3 rd ed. 1971).	x
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality and symptoms	
3.4.7	Sampling		x
3.4.8	Monitoring of TS concentration	Yes	x
3.4.9	Statistics	Probit analysis	
		4 RESULTS	
4.1	**Limit Test**		
4.1.1	Concentration		
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	46, 68, 100, 147, 215, 316, 464, 681 mg/l	
4.2.2	Actual concentrations of test substance		x
4.2.3	Effect data (Mortality)	LC 50 after: 4 h greater than 680 mg/l (10 % level of significance) 24 h about 680 mg/l 48 h about 340 mg/l (figure from intrapolation) 96 h equal to 171 mg/l (slope factor = 1.19	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

4.2.4	Concentration / response curve		x
4.2.5	Other effects	Tumbling of animals after 4 h and 48 h in the 215 mg/l and 316 mg/l group	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects		x
4.3.2	Nature of adverse effects		
4.4	**Test with reference substance**		
4.4.1	Concentrations		x
4.4.2	Results		x

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines:

- DIN 38412, test procedure with water organisms (group L)

DURATION OF THE TEST: 96 h

5.2 Results and discussion

RESULTS: EXPOSED

Summary and assessment:
LC 50 after:

4 h	greater than	680 mg/l (10 % level of significance)
24 h	about	680 mg/l
48 h	about	340 mg/l (figure from intrapolation)
96 h	equal to	171 mg/l (slope factor = 1.19)

No effect level: 100mg/l
Highest concentration tested without mortality: 100 mg/l

5.2.1	LC_0	100 mg/l	x
5.2.2	LC_{50}(96 h)	171 mg/l	x
5.2.3	LC_{100}		x
5.3	**Conclusion**		
5.3.1	Other Conclusions		
5.3.2	Reliability	1	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

5.3.3	Deficiencies	No

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2005
Materials and Methods	2.2. GLP: Prior to the implementation of GLP 2.3 Deviations: Yes (OECD guideline 203 originally adopted in 1981) 3.1 Test material 3.1.1 Lot/Batch Nr.: Test substance Nr.: 79/571 3.1.3 Purity: K-HDO as 30 % aqueous solution (Xyligen 30F) 3.1.5 Further relevant properties: not mentioned in the report 3.1.6 Method of analysis: Test substance was not analysed 3.3. Reference substance 3.3.1 Method of analysis for reference substance: Reference substance was not analysed 3.4.1 Dilution water: See table A7_4_1_1-2 3.4.2 Test organisms: See table A7_4_1_1-3 Kind of food: Hostenstolz ad libitum 3.4.3.Test system: See table A7_4_1_1-4 No renewal of test solution, 10 L test vessels 3.4.4 Test conditions: See table A7_4_1_1-5 No details given for adjustment of pH, aeration of dilution water, Inntensity of irridation and illumination 3.4.7 Sampling: No datails given in the report 3.4.8 Monitoring of the test substance: no monitoring
Results and discussion	4.2.2 Actual concentration of test substance: only initial concentrations available 4.2.4 Concentration/response curve: No graph is given in the report 4.3 Results of control 4.3.1 Number/percentage of animals showing adverse effect: 0 4.4. Reference Substance: 4.4.1 Concentrations: not given in the report 4.4.2 Results: LC_{50}: 27 mg/L 5.2 Results and discussion 5.2.1 LC_0: 100 mg/L corresponds to 30 mg/L (100% w/w K-HDO) 5.2.2 LC_{50}: 171 mg/L corresponds to 51.30 mg/L (100% w/w K-HDO) 5.2.3 LC_{100}: 316 mg/L corresponds to 94.81 mg/L (100% w/w K-HDO)
Conclusion	Very poor report and evaluation table. No measurement of the test substance. As K-HDO is hydrolytically stable at pH 7 and 9, the test can be accepted. Furthermore, in all other aquatic tests the nominal concentrations were confirmed within analytical measurements.

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

Reliability	*2*
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Prepared from demineralized water
Alkalinity	
Hardness	~ 2.6 mmol/l
pH	~ 8.0
Oxygen content	> 8 mg/l
Conductance	
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Leuciscus idus
Source	P. Eggers, Hohenwestedt, Germany
Wild caught	No
Age/size	Body length: 6,4 cm Body weight: 1,9 g
Kind of food	
Amount of food	
Feeding frequency	
Pretreatment	adaptation
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	
Volume of test vessels	
Volume/animal	
Number of animals/vessel	10
Number of vessels/ concentration	
Test performed in closed vessels due to significant volatility of TS	

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	20°C
Dissolved oxygen	> 6 mg/l
pH	About 8.0
Adjustment of pH	
Aeration of dilution water	
Intensity of irradiation	
Photoperiod	

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (nominal) [mg/l]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0	0	0	0	0				
46,4	0	0	0	0				
68,1	0	0	0	0				
100	0	0	0	0				
147	0	2	2	2				
215	0	1	5	9				
316	0	3	9	10				
464	3	10	10	10				
681	2	10	10	10				
Temperature [°C]				20				
pH				7.7-7.9				
Oxygen [mg/l]				7.8-8.3				

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	95 % c l.	96 h [mg/l][1]	95 % c.l.
LC_0	*>100*		>100	
LC_{50}	340		171	
LC_{100}				

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

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

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

Criteria for poorly soluble test substances	**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 7.4.1.2/01 [redacted] (2002) Xyligen K 30 F – Determination of the acute effect on the swimming ability of the water flea Daphnia magna Straus, Report 01/0069/50/2, [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 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 202	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	K-HDO, 31,4 % aqueous solution	
3.1.1	Lot/Batch number	U 8456	
3.1.2	Specification	Liquid	
3.1.3	Purity		x
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.	
3.3	**Reference substance**	Yes	
3.3.1	Method of analysis for reference substance		
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	x
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	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

3.4.6	Test parameter	Immobilisation after 48 h	
3.4.7	Sampling		x
3.4.8	Monitoring of TS concentration	Yes	x
3.4.9	Statistics	No statistical evaluation was required.	x
		4 RESULTS	
4.1	**Limit Test**	No	
4.1.1	Concentration		
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	100 - 6,25 mg/l, dilution factor was 2	x
4.2.2	Actual concentrations of test substance	Analytical recovery: Min. 80 % of the nominal concentrations	x
4.2.3	Effect data (Immobilisation)	See table A7_4_1_2-6	
4.2.4	Concentration / response curve		x
4.2.5	Other effects		x
4.3	**Results of controls**	Immobilisation in the control was ≤ 10 %	
4.4	**Test with reference substance**	Reference substance was tested.	
4.4.1	Concentrations	Potassium-dichromate was tested	
4.4.2	Results	EC_{50} (24 h)= 1,07 mg/l	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The study was carried out in accordance with EEC Directive 92/32/EEC, Annex V, Part C: Methods for the determination of ecotoxicity, C2. Acute toxicity for Daphnia., OECD 202 and US-EPA OPPTS 850.1010 TEST ORGANISMS - Strain: Daphnia magna Strauss from Institute National Recherche Chimique Appliquée, France. Animals are bred in the BASF AG lab since 1978. - Age: 2-24 h - Feeding: none	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

- Control group: yes, 20 animals

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- Procedure: a stock solution of the substance (100 mg/L) was prepared and the test concentrations was prepared by dilution
- Vehicle, solvent: water

DILUTION WATER

- Source: M4 water, special test water
- Hardness: 2,2-3,2 mmol/L
- Ca/Mg ratio: 4:1
- pH: 8
- Oxygen content: saturated
- Conductance: 550-650 µS/cm

TEST SYSTEM

- Test type: Swimming ability of animals
- Exposure vessel type: special test tube,
- Number of replicates, individuals per replicate: 20
- Test temperature: 18-22 ° C
- Dissolved oxygen: > 3 mg/L
- pH: 8
- Intensity of irradiation: 1-8 µE/(m²s) at 400-700 nm
- Photoperiod: day-night: 16:8
- DURATION OF THE TEST: 48 h

TEST PARAMETER: Swimming ability

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes

5.2 Results and discussion

RESULTS: EXPOSED

- Nominal concentration [mg/l]:

RESULTS CONTROL: control group was okay

5.2.1	EC_0	>100 (mg/l)	x
5.2.2	EC_{50} (48 h)	> 100 mg/l	x
5.2.3	EC_{100} (48 h)	> 100 mg/l	x

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

5.3 Conclusion

5.3.1 Reliability 1

5.3.2 Deficiencies No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2005
Materials and Methods	3.1 Test material 3.1.3 Purity: 31.4 % K-HDO 3.4.2 Test organisms: See table A7_4_1_2-3 3.4.7 Sampling: (0h, 24h, 48 h) 3.4.8 Monitoring of TS concentrations: Intervals: at the beginning and the end 3.4.9 Statistics: Not available because of the lack of effects.
Results and discussion	Agree in general with applicant's version 4.2.1 Initial concentrations of test substance: 6.25 mg/L, 12.5 mg/L, 25 mg/L, 50 mg/L, 100 mg/L 4.2.2 Actual concentrations of test substance: 6.25 mg/L: 6.28 and 6.25 mg/L 100 mg/L: 102 and 98 mg/L 4.2.5 Concentrations/response curve: no mortality seen in the test 4.2.5 Other effects: not reported 5.2.1 EC_0: 100 mg/L corresponds to 30 mg/L (100% w/w K-HDO) 5.2.2 EC_{50}: >100 mg/L corresponds to >30 mg/L (100% w/w K-HDO) 5.2.3 EC_{100}: >100 mg/L corresponds to >30 mg/L (100% w/w K-HDO)
Conclusion	agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

Remarks

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	M4 water, special test water
Alkalinity	
Hardness	2.7 mmol/L
pH	8
Ca / Mg ratio	4:1
Na / K ratio	
Oxygen content	saturated
Conductance	550-650 µS/cm
Holding water different from dilution water	

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	
Kind of food	—
Amount of food	None
Feeding frequency	—
Pretreatment	e.g. acclimation
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	10 ml
Volume/animal	0,5 ml per animal
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	18-22°C
Dissolved oxygen	> 3
pH	8
Adjustment of pH	No
Aeration of dilution water	
Quality/Intensity of irradiation	Artificial light About 1 – 8 µE /m² s) in the range of 400 – 700 nm
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]
	3 h	6 h	24 h	48 h	3 h	6 h	24 h	48 h	48 h	48 h	48 h
100	0		1	2	0		5	10	8.8	8	19.5-20.4
50	0		1	1	0		5	5	8.8	8	19.5-20.4
25	0		0	0	0		0	0	8.8	8	19.5-20.4
12.5	0		0	0	0		0	0	8.8	8.1	19.5-20.4
6.25	0		0	0	0		0	0	8.8	8.1	19.5-20.4

Table A7_4_1_2-7: Effect data

	EC_{50}[1]	95 % c l.	EC_0[1]	EC_{100}[1]
3 h	>100 mg/l(n)		≥ 100 mg/l (n)	> 100 mg/l (n)
6 h	>100 mg/l (n)		≥ 100 mg/l (n)	> 100 mg/l (n)
24 h [mg/l]	> 100 mg/l (n)		≥ 100 mg/l (n)	> 100 mg/l (n)
48 h [mg/l]	>100 mg/l (n)		≥ 100 mg/l (n)	> 100 mg/l (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 >3 mg/l	**X**	
Concentration of test substance ≥80% of initial concentration during test	**X**	

Criteria for poorly soluble test substances		

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.4.1.3 [redacted] (2002), N-cyclohexyl-diazenium-dioxy-potassium - Determination of the inhibitory effect on the cell multiplication of unicellular green algae, Report 01/0069/60/1, [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 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 EC directive 92/69/EEC, Annex V, part C 3, OECD 201, OPPTS 850.5400	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	K-HDO	
3.1.1	Lot/Batch number	U 8456	
3.1.2	Specification	Liquid	
3.1.3	Purity	31.4 % K-HDO in water	
3.1.4	Composition of Product	31,4 % K-HDO, 69,6 % water	
3.1.5	Further relevant properties	Substance stability: stable for at least 3 years Vapour pressure: approx. 23 mbar at 20 °C (water) Water solubility: miscible with water in each ratio	
3.1.6	Method of analysis	Photometry. Evaluation was carried out using a calibration graph set up with known concentrations of the test item. Apparatus: Spectrophotometer (reference is made to A 4.1)	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	Yes, Potassium dichromate	
3.3.1	Method of analysis for reference substance	Not reported	
3.4	**Testing procedure**	Non-entry field	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

3.4.1 Culture medium

OECD medium

The culture medium is prepared according to EC-guideline 92/69/EC, Appendix V, Part C3, which correspond to OECD 201.

Reagents	Formula	Conc in Test assay
Ammonium chloride	NH_4Cl	15 mg/l
Magnesium chloride hexahydrate	$MgCl_2*6H_2O$	12 mg/l
Calcium chloride dihydrate	$CaCl_2*2H_2O$	18 mg/l
Magnesium sulphate-heptahydrate	$MgSO_4*7H_2O$	15 mg/l
Potassium dihydrogen phosphate	KH_2PO_4	1,6 mg/l
Ferric (III) chloride-hexahydrate	$FeCl_3*6H_2O$	0,08 mg/l
Disodium dihydrogen ethylenediamintetraacetat	$Na_2EDTA*2H_2O$	0,1 mg/l
Boric acid	H_3BO_3	185 mg/l
Mangan (II) chloride-tetrahydrate	$MnCl_2*4H_2O$	415 mg/l
Zinc chloride	$ZnCl_2$	3 µg/l
Cobalt chloride-hexahydrate	$CoCl_2*6H_2O$	1,5 µg/l
Copper (II) chloride-dihydrate	$CuCl_2*2H_2O$	0,01 µg/l
Sodium molybdate-dihydrate	$Na_2MoO_4*2H_2O$	7 µg/l
Sodium hydrogen-carbonate	$NaHCO_3$	50 mg/l

3.4.2 Test organisms

Desmodesmus subspicatus CHODAT SAG 86.81

3.4.3 Test system

72 h static test

3.4.4 Test conditions

TEST ORGANISMS

- Strain: Desmodesmus subspicatus Chodat SAG 86.81
- Source/supplier: SAG (collection of algal cultures, Göttingen)
- Method of cultivation: liquid culture in BASF AG, seed culture was taken to inoculate a pre-culture
- Control group: yes
- Initial cell concentration: 10000 cell/ml
- Number of Replicates: 3

STOCK AND TEST SOLUTION AND THEIR PREPARATION

Procedure: test substance was stirred in demin. water for 20 min. The nominal concentration of the stock solution was 125 mg/l. By diluting the stock solution the nominal concentrations were prepared

GROWTH/TEST MEDIUM CHEMISTRY

- Water was prepared according the EEC 92/69/EEC and the OECD guideline.
- pH: 8

TEST SYSTEM

- Test type: Determination of the mean fluorescence after 0, 24, 48, 72 h,
- Number of replicates: 3
- Test temperature: 23 ± 2 °C
- pH: 8
- Intensity of irradiation: 60-120 µEinstein/m²s, permanent illumination, white light source (400-700 nm)

3.4.5 Duration of the test

72 hours

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

3.4.6	Test parameter	Measurement of the in vivo chlorophyll-a-fluorescence (pulsed excitation at 435 nm)
3.4.7	Sampling	Determination of the mean fluorescence after 0, 24, 48, 72 h.
3.4.8	Monitoring of TS concentration	Yes, the analytical verifications of the test substance were investigated at different concentrations in OECD-medium.
3.4.9	Statistics	Determination of the mean fluorescence after 0, 24, 48, 72 h. Calculation of the growth rate over the total duration of the study for each concentration level and comparison of values of treated samples in relation to untreated samples. The EC values are calculated (linear regression analysis) from the concentration-response relationship. The LOEC is determined by comparing the means of the biomass or growth rate of the various concentrations levels with the control. The Duncan multiple range test is carried out at a 95 % significance level. Every higher tested concentration must have at least the same or a stronger effect then the LOEC. The NOEC is the tested concentration immediately below the LOEC.

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	
4.1.2	Number/ percentage of animals showing adverse effects	
4.2	**Results test substance**	Non-entry field
4.2.1	Initial concentrations of test substance	0, 0,39, 0,78, 1,56, 3,13, 6,25, 12,5, 25, 50, 100 ml/l
4.2.2	Actual concentrations of test substance	Nominal concentrations were confirmed within analytical measurements:

Sample no.	Date of sample preparation	Date of sampling	Expected concentration	Value found *)
01/0069/60/1/b/h0/K	Jan 29, 2002	Jan 29, 2002	0 mg/l	< 0.1 mg/l
01/0069/60/1/b/h0/100	Jan 29, 2002	Jan 29, 2002	100 mg/l	101 mg/l
01/0069/60/1/b/h0/6,25	Jan 29, 2002	Jan 29, 2002	6.25 mg/l	6.52 mg/l
01/0069/60/1/b/h0/0,39	Jan 29, 2002	Jan 29, 2002	0.39 mg/l	0.42 mg/l
01/0069/60/1/b/h72/K	Jan 29, 2002	Feb 01, 2002	0 mg/l	< 0.1 mg/l
01/0069/60/1/b/h72/100	Jan 29, 2002	Feb 01, 2002	100 mg/l	100 mg/l
01/0069/60/1/b/h72/6,25	Jan 29, 2002	Feb 01, 2002	6.25 mg/l	6.20 mg/l
01/0069/60/1/b/h72/0,39	Jan 29, 2002	Feb 01, 2002	0.39 mg/l	0.44 mg/l

*) mean value of two determinations

The analytical verifications of the test substance were investigated at different concentrations in OECD-medium. The analytical results

x

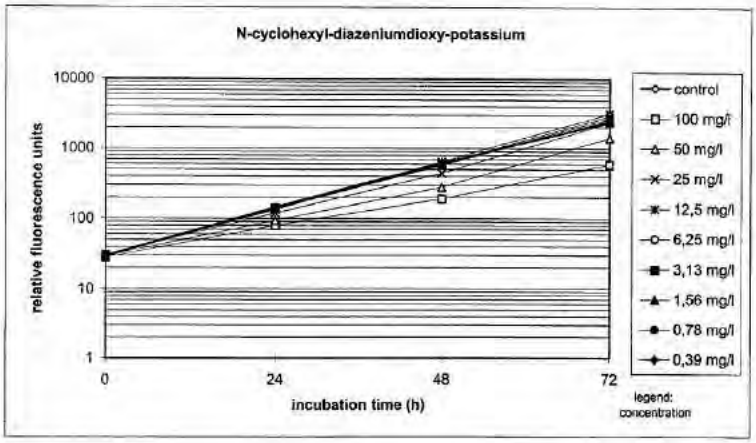
Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

yielded 80 % or higher recoveries; they varied between 101 and 108 % of the nominal concentrations at test initiation and between 99 % to 113 % at test termination. Therefore all biological results are related to the nominal concentrations of the test item.

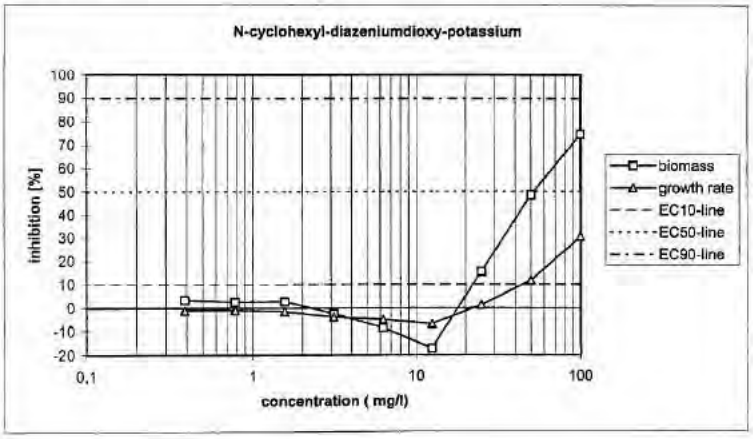
4.2.3 Growth curves

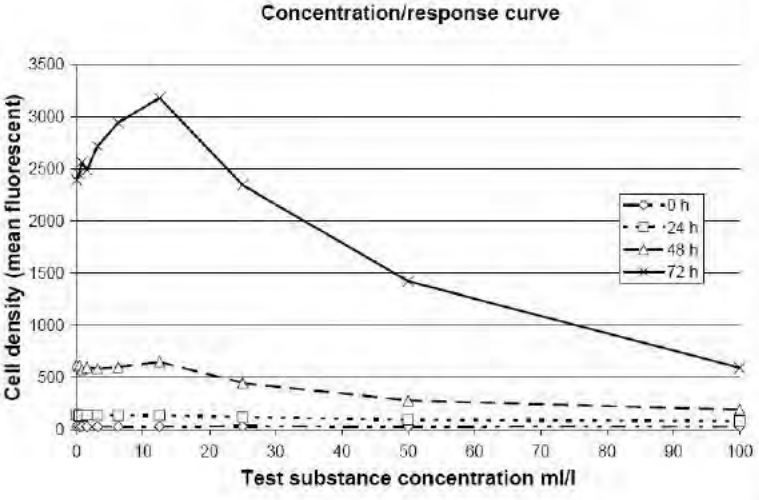
Growth curves of Desmodesmus *subspicatus* at different test substance concentrations (relative fluorescence units)



4.2.4 Concentration / response curve

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





Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

4.2.5 Cell concentration data — see table A7_4_1_3-5

4.2.6 Effect data (cell multiplication inhibition)

Inhibition of the algal biomass and growth rates after 72 h

concentration (mg/l)	biomass relative	biomass Inhibition (% of the control)	growth rate relative	growth rate inhibition (% of the control)
0 (control)	1967	0,0	0,061	0,0
100	497	74,7	0,042	30,6
50	1012	48,5	0,054	11,8
25	1661	15,6	0,060	1,3
12,5	2306	-17,2	0,065	-6,8
6,25	2134	-8,5	0,064	-4,8
3,13	2012	-2,3	0,063	-3,8
1,56	1910	2,9	0,062	-1,3
0,78	1915	2,6	0,062	-1,0
0,39	1900	3,4	0,062	-1,0

Results give the concentrations (related to weighed test sample), which bring about an inhibition of growth by 10%, 50%, and 90%, respectively, as compared to the control, after 72 hours.

TEST RESULT

Effect on the development of biomass:

E_bC_{10} (72 h) = 22,2 mg/l
E_bC_{50} (72 h) = 52,0 mg/l
E_bC_{90} (72 h) > 100 mg/l

Effect on growth rate:

E_rC_{10} (72 h) = 44,5 mg/l
E_rC_{50} (72 h) > 100 mg/l
E_rC_{90} (72 h) > 100 mg/l

No observed effect concentration (95% significance level)
NOEC (72 h) = 12,5 mg/l

Lowest observed effect concentration (95% significance level)
LOEC (72 h) = 25,0 mg/l

4.2.7 Other observed effects — None reported

4.3 Results of controls — The cell multiplication factor in the untreated control was after 72 hours: 90-fold

4.4 Test with reference substance — Potassium dichromate

4.4.1 Concentrations — Not reported

4.4.2 Results — E_bC_{50}=0,46 mg/l

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods — The test was performed according to EEC directive 92/69/EEC, appendix V, C, Algae.

TEST ORGANISMS

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

		- Strain: Desmodesmus subspicatus Chodat SAG 86.61 - Control group: yes STOCK AND TEST SOLUTION AND THEIR PREPARATION - procedure: prepared from 125 mg/L stock solution - solvent/dispersant: none - tested range of concentrations: see above GROWTH/TEST MEDIUM CHEMISTRY - Hardness: no data - pH: 8 - Dissolved oxygen: TEST SYSTEM - Test type: Determination of the mean fluorescence after 0, 24, 48, 72 h, calculation of the integral of biomass growth - Number of replicates: 3 - Test temperature: 23°C - pH: 8 - Intensity of irradiation: 60 - 120 µEinstein/m²s, permanent illumination, white light source TEST PARAMETER: MONITORING OF TEST SUBSTANCE CONCENTRATION: yes	
5.2	**Results and discussion**	RESULTS: EXPOSED - Nominal concentrations: see above - Cell density data: - Growth curves: yes	
5.2.1	NOEC (72 h)	12,5 mg/l	x
5.2.2	LOEC (72 h)	25 mg/l	x
5.2.3	E_rC_{50}	> 100 mg/l	x
5.2.4	E_bC_{50}	52.0 ml/l	x
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2005
Materials and Methods	acceptable
Results and discussion	Results: 4.2.1 Initial concentrations of test substance: The unit is mg/L 5.2.1 NOEC: 12.5 mg/L, corresponds to 3.75 mg/L (100% w/w K-HDO) 5.2.2 LOEC: 25 mg/L corresponds to 7.5 mg/L (100% w/w K-HDO) 5.2.3 E_rC_{50}: >100 mg/L corresponds to >30 mg/L (100% w/w K-HDO) 5.2.4 E_bC_{50}: The unit is mg/L, the value 52mg/L correspons to 15.6 mg/L (100% w/w K-HDO)
Conclusion	agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_3-1: 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_1_3-2: Test organisms

Criteria	Details
Species	Desmodesmus subspicatus
Strain	Chodat SAG 86.61
Source	SAG (collection of algal cultures, Göttingen)
Laboratory culture	Yes
Method of cultivation	Exponentially growing algae are cultured under defined conditions for several generations, and multiplication of cells is determined under the influence of test substance in relation to an untreated control. Test temperature was 23 °C. Test duration was 72 hours.
Pre-treatment	Seed-culture: A seed culture was incubated for 7 days at 23°C ± 2 °C. Final cell density: 343 x 10^4 cells/ml Pre-culture: Seed-culture was taken to inoculate a pre-culture (initial cell density: 1 x 10^4 cells/ml). The pre-culture was incubated for 3 days at 23 °C ± 2 °C. final cell density: 31 x 10^4 cells/ml.
Initial cell concentration	approx. 10^4 cells/ml

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	250 ml
Culturing apparatus	Erlenmeyer-flask
Light quality	Permanent illumination, Colour of light is universal white, (Osram)
Procedure for suspending algae	Shaking
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No, Erlenmeyer flasks were plugged with gas-permeable silicon-sponge caps

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	23 ± 2 °C
pH	pH-values at the start (t = 0 h) and at the end (t = 72 h) of the test
Aeration of dilution water	No
Light intensity	60-120 µE/m²s] Colour of light is universal white, L 25(Osram)
Photoperiod	Permanent illumination

pH-values at the start (t = 0 h) and at the end (t = 72 h) of the test

concentration (mg/l)	uninoculated 0 h	uninoculated 72 h	inoculated 72 h
0 (control)	8,0	8,1	7,9
100	8,0	8,0	7,9
50	8,0	8,0	8,0
25	8,0	8,0	8,0
12,5	8,0	8,0	8,0
6,25	8,1	8,0	8,1
3,13	8,1	8,1	8,1
1,56	8,1	8,1	8,1
0,78	8,1	8,1	8,1
0,39	8,1	8,1	8,1

Table A7_4_1_3-5: Cell concentration data

Test-Substance Concentration (nominal) [ml/l]	Cell density (parameter fluorescent, mean values) Measured				Percent of control			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
0	30	146	621	2393	100	100	100	100
0,39	29	134	612	2452	98	92	99	102
0,78	30	136	572	2565	102	93	92	107
1,56	29	141	597	2488	98	97	96	104
3,13	28	140	583	2720	96	96	94	114
6,25	29	136	598	2945	99	93	96	123
12,5	29	138	649	3184	98	94	104	133
25	31	120	445	2346	103	82	72	98
50	29	95	280	1422	99	65	45	59
100	28	80	191	592	94	55	31	25

3. Tables for Applicant's Summary and Conclusion

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

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

Criteria for poorly soluble test substances		

Section A7.4.1.4/01 Inhibition to microbiological activity (aquatic)

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.4.1.4/01 [redacted] (1995) Prüfung der Atmungshemmung von Belebtschlamm durch K-HDO techn. 30 %ig im Kurzzeitatmungstest, Report 95/0179/08/1, [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 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**	No	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	K-HDO, 30 % aqueous solution.	
3.1.1	Lot/Batch number	74 N 9702	
3.1.2	Specification	Liquid	x
3.1.3	Purity	30 %	
3.1.4	Composition of Product	30 % K-HDO, 70 % water	
3.1.5	Further relevant properties	Substance stability: stable for at least 3 years Vapour pressure: approx. 23 mbar at 20 °C (water) Water solubility: miscible with water in each ratio	
3.1.6	Method of analysis	Thermal Energy Analyser (TEA) as described in A 4.2/02	x
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	Yes 3,5-dichlorophenol	
3.3.1	Method of analysis for reference substance	Not reported	
3.4	**Testing procedure**	Non-entry field	
3.4.1	Culture medium	100 x concentrated OECD medium	
3.4.2	Inoculum / test organism	Activated sludge	
3.4.3	Test system	see table A7_4_1_4-3	

Section A7.4.1.4/01 Inhibition to microbiological activity (aquatic)

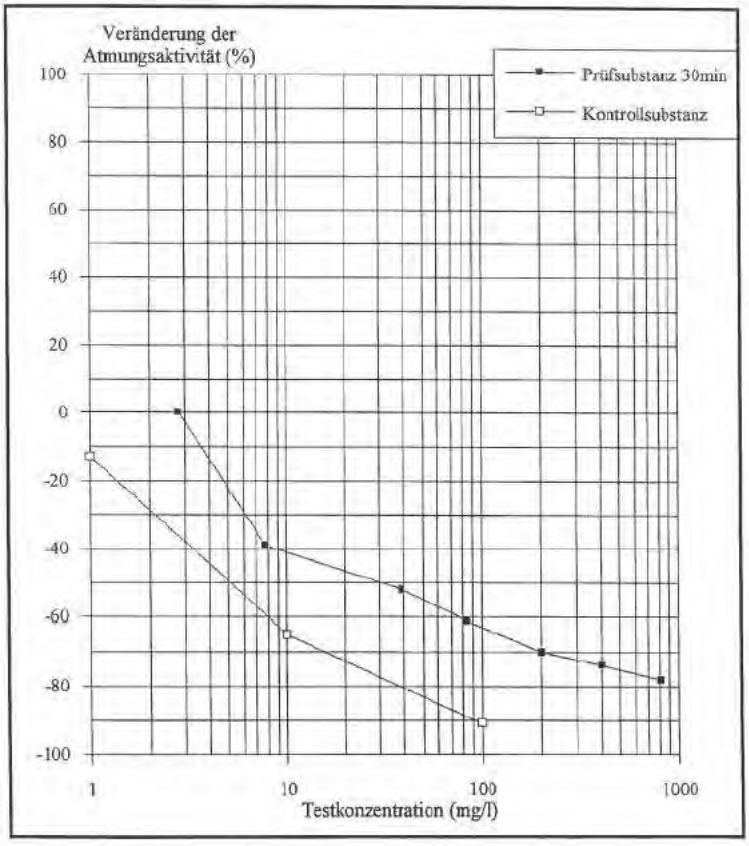
Annex Point IIA7.4

3.4.4	Test conditions	see table A7_4_1_4-4	
3.4.5	Duration of the test	30 - 180 min	x
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	In the short-term respiration test the effect of the test substance on the respiration of aerobe microorganisms is measured after a short exposure time of 30 and 180 minutes.	
3.4.9	Monitoring of TS concentration	Not reported	
3.4.10	Controls	Yes, sample without test substance and control substance (3,5-Dichlorphenol)	
3.4.11	Statistics	No, not relevant according to the guideline	
		4 RESULTS	
4.1	**Preliminary test**	No preliminary tests were carried out	
4.1.1	Concentration		
4.1.2	Effect data		
4.2	**Results test substance**	Non-entry field	x
4.2.1	Initial concentrations of test substance	2,8; 7,8; 39; 84; 202; 403; 812 mg/l	
4.2.2	Actual concentrations of test substance	2,8; 7,8; 39; 84; 202; 403; 812 mg/l	x
4.2.3	Growth curves	Respiration curve	
4.2.4	Cell concentration data	Not relevant due to the guideline (dry weight is 1 g/l)	

Section A7.4.1.4/01 Inhibition to microbiological activity (aquatic)

Annex Point IIA7.4

4.2.5 Concentration/ response curve



4.2.6 Effect data

EC_{20} (30 min): ca 4,8 mg/l (nominal)

EC_{50} (30 min): ca 30 mg/l (nominal)

EC_{80} (30 min): The EC_{80} value was not reached in the doses effect relationship (EC80 > 812 mg/l)

4.2.7 Other observed effects

None observed

4.3 Results of controls

Übersicht der Meßwerte - Kontrollsubstanz (KS) und abiot. Sauerstoffverbrauch (PC)

Ansatz-Nr.:	PC	MW BW	1 KS	2 KS	3 KS	4 KS	5 KS	6 KS	7 KS
Substanzkonzentration (mg/l)			1	10	100				
Sauerstoffzehrung ($mgO_2/l \cdot h$) 30 min		23	20	8	2				
Atmungshemmung (%) 30 min	-	-	-13	-65	-91				
Atmungsforderung (%) 30 min	-	-	-	-	-	-	-	-	-

MW = Mittelwert

4.4 Test with reference substance

Performed

3,5-Dichlorophenol

4.4.1 Concentrations

1, 10, 100 mg/l

4.4.2 Results

EC_{20} (mg/l)	EC_{50} (mg/l)	EC_{80} (mg/l)	highest concentration tested (mg/l)

Section A7.4.1.4/01 Inhibition to microbiological activity (aquatic)

Annex Point IIA7.4

		ca 1	ca 5	ca. 38	1000	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	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 – International standards ISO 8192-(1994) Water quality –test for inhibition of oxygen consumption by activated sludge In the short-term respiration test, the effect of a test substance on the respiration of aerobe microorganisms after a short exposure time (30 to 80 minutes) will be tested. The effective concentrations at which the respiration compared to a control value without test substance is inhibited for 20, 50 and 80 % (EC20, EC50, EC80) are the determined test results.	x
5.2	**Results and discussion**		x
5.2.1	EC_{20}	ca. 4,8 mg/l	x
5.2.2	EC_{50}	ca. 30 mg/l	x
5.2.3	EC_{80}	The EC_{80} value was not reached in the doses effect relationship (EC80 > 812 mg/l)	x
5.3	**Conclusion**	No respiration inhibition up to 4,8 mg/l Validity criteria: Deviation of the reference value < 15 %: No EC_{50} of 3,5-Dichlorphenol in the range 5 – 30 mg/l: Yes Test is valid: yes	x
5.3.1	Reliability	1	x
5.3.2	Deficiencies	No	

Section A7.4.1.4/01 Inhibition to microbiological activity (aquatic)

Annex Point IIA7.4

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	23.11.2005
Materials and Methods	**3.1.2 Specification** Colourless liquid with weak odour **3.1.6 Method for analysis** As the study is no key study, no study summary of this method is available. **3.4.5 Duration of the test** 30 min
Results and discussion	**4.2 Results test substance** All TS concentrations and effect data refer to 30% K-HDO in water. **4.2.2 Actual concentration of test substance** Results are given as nominal values.
Conclusion	**5.1 Material and Methods** "Activated sludge respiration inhibition test" EC Test-guideline C.11 **5.2 Results and discussion** All effect data refer to 30% K-HDO in water. The EC_{10} value has been determined on the basis of the concentration/response curve under point 4.2.5: EC_{10}: ca. 3.6 mg 30% K-HDO/L corresponds to ca. 1.1 mg K-HDO/L (nominal) **5.2.1 EC_{20}** EC_{20}: ca. 4.8 mg 30% K-HDO /L corresponds to 1.44 mg K-HDO/L (nominal) **5.2.2 EC_{50}** EC_{50}: ca. 30 mg 30% K-HDO /L corresponds to 9 mg K-HDO/L (nominal) **5.2.3 EC_{80}** At the highest concentration tested (812 mg/L 30% K-HDO in water) an inhibition of 78% was observed. **5.3 Conclusion** The two control respiration rates are within 15% of each other.
Reliability	2
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	

Section A7.4.1.4/01 Inhibition to microbiological activity (aquatic)

Annex Point IIA7.4

Materials and Methods

Results and discussion

Conclusion

Reliability

Acceptability

Remarks

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
Nature	activated sludge
Species	not applicable
Strain	not applicable
Source	laboratory wastewater plant treating municipal sewage
Sampling site	Laboratory wastewater plant
Laboratory culture	Cultured in the laboratory wastewater plant
Method of cultivation	Laboratory wastewater plant
Preparation of inoculum for exposure	The inoculum was washed with drinking water
Pretreatment	Aeration for 24 h
Initial cell concentration	1 g/l dry weight

Table A7_4_1_4-3: Test system

Criteria	Details
Culturing apparatus	Erlenmeyer flasks (250 ml volume)
Number of culture flasks/concentration	1/1
Aeration device	Shaking
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
5.3.1	Test temperature	20 ± 2 °C
5.3.2	pH	7,5 +/- 0,5
5.3.3	Aeration of dilution water	According to guideline
5.3.4	Suspended solids concentration	1 g/l dry weight

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

Annex Point IIIA XIII 2.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.4.3.4 [redacted] **(2002)** title: Influence of Xyligen K 30 F on Survival and Reproduction of Daphnia magna in a semi static test over 21 days. Report 13601221, [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 211, OPPTS 850.1300	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	K-HDO, 30 %	
3.1.1	Lot/Batch number	U 8456	
3.1.2	Specification	Liquid	
3.1.3	Purity	31,4 %	
3.1.4	Composition of Product	Test product is a 31,4 % K-HDO solution in water	
3.1.5	Further relevant properties	Colour: yellowish-brown Solubility: pure = 1 year (expiry date: July 10, 2002) – in water: not indicated Storage: in original container, at room temperature, in the dark	
3.1.6	Method of analysis	Photometric system: Spekol 1200, detection wave length: 244 nm, cuvette: quartz glass 5 cm Samples were quantified by measuring the absorption units AU with reference to the calibration curve. The later was obtained by correlation of AU of the standard solutions to their corresponding concentration in mg/L. The correlation was performed using a linear regression function given by equation y = a*x+b (1) where y = absorption unit, x = concentration of the test item in the samples, a = slope, b = y.axis intercept. The concentration of the test item in the treatment samples and in the control samples were calculated by equation c = x*d (2) where c = concentration in the original samples, x = concentration of the test item found in injected samples, d = dilution factor The recovery of the test item in a sample was calculated by equation % of nominal = $(c/c_{nom})*100$ % (3) where c = concentration of the test item in sample found by equation 2, c_{nom} = nominal concentration of the test item provided by the study director. The limit of detection DL was determined according to DIN 32645. The limit of quantification LOQ was determined as the lowest fortification level at which	

		acceptable recovery (70 to 110 % of nominal) was obtained.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Dilution
3.3	**Reference substance**	Not reported
3.3.1	Method of analysis for reference substance	
3.4	**Testing procedure**	Non-entry field
3.4.1	Dilution water	see table A7_4_3_4-2
3.4.2	Test organisms	see table A7_4_3_4-3
3.4.3	Handling of offspring	
3.4.4	Test system	see table A7_4_3_4-4
3.4.5	Test conditions	see table A7_4_3_4-5
3.4.6	Duration of the test	21 days
3.4.7	Test parameter	Reproduction and survival: the mortality of the test animals and the number of young were recorded each day. Dead animals and offspring were removed at the same times. The pH values and dissolved oxygen concentrations in the control and all test concentrations were measured at the start and end of each treatment period. The water temperature was measured in one control beaker at the start am end of each treatment period. The behaviour of the test item in test water was determined at each test medium renewal period in the freshly prepared and old test media of all test concentrations.
3.4.8	Examination / Sampling	One sample from the freshly prepared stock solutions and duplicate samples from the freshly prepared test media of all test concentrations and the control were taken at the first treatment period (day 0) and at a treatment period in the second and third week (days 10 and 17). For the determination of the stability of the test item under test conditions respectively the maintenance of the test item concentrations during the test period at three dates (day 3, 12 and 19), a sufficient volume from the freshly prepared test media of all concentrations and the control were incubated under the same conditions as the test itself, however without food and daphnia. One of these three stability control treatments last for 72 hours (weekend), two for 48 hours, corresponding to the different test medium renewal periods.
3.4.9	Monitoring of TS concentration	The concentrations of the test item were measured in all duplicate test medium samples from the lowest (0,39 mg/L), a middle (3,13 mg/L) and the highest test concentration (50,0 mg/L). From the control samples only one of the duplicate samples was analysed from each sampling date.

3.4.10 Statistics

The NOEC and the LOEC for the reproduction rate were evaluated by the multivariate Williams-test after analysis of variance (ANOVA). The EC 50 (21 day) of the reproduction rate was determined by Probit analysis.

4 RESULTS

4.1 Range finding test

Not performed

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

50, 25, 12,5, 6,25, 3,13, 1,56, 0,78 and 0,39 mg/l

4.2.2 Actual concentrations of test substance

Determination of the test item in the test samples at test item concentration of nominal 0,39 mg/L (two replicates). X

Age		Concentration measured (mg/L)	% of nominal
Days	Hours		
0	0	0,136 / 0,149	35 / 38
2	48	0,322 / 0,348	83 / 89
10	0	0,440 / 0,361	113 / 93
12	48	0,196 / 0,156	50 / 40
16	0	0,224 / 0,134	57 / 34
19	72	0,261 / 0,326	67 / 84
Mean			65

Determination of the test item in the test samples at test item concentration of nominal 3,13mg/L (two replicates).

Age		Concentration measured (mg/L)	% of nominal
Days	Hours		
0	0	3,12 / 3,15	100 / 101
2	48	3,31 / 3,20	106 / 102
10	0	3,17 / 3,18	101 / 102
12	48	2,89 / 2,97	92 / 95
16	0	2,90 / 2,83	93 / 90

19	72	2,29 / 2,34	73 / 75
Mean			94

Determination of the test item in the test samples at test item concentration of nominal 50,0 mg/L (two replicates).

Age		Concentration measured (mg/L)	% of nominal
Days	Hours		
0	0	51,8 / 53,3	104 / 107
2	48	51,9 / 51,0	104 / 102
10	0	51,3 / 54,0	103 / 108
12	48	52,0 / 51,1	104 / 102
16	0	51,4 / 52,5	103 / 105
19	72	48,2 / 48,8	96 / 98
Mean			103

At the lowest test concentration of 0,39 mg/L, the measured concentration is below the limit of quantification.

Under the test conditions, the test item was sufficiently stable during the test medium renewal periods of 48 and 72 hours.

Since the determined concentrations at the nominal test concentrations of 3,13 and 50,0 mg/L were well within the range of 80 % to 120 %, all reported results are related to nominal concentrations of the test item.

X

4.2.3 Effect data

Number of surviving adult daphnia exposed to test item on day 21

Nominal concentration of Xyligen 30 F (mg/L)								
Control	0,39	0,78	1,56	3,13	6,25	12,5	25,0	50,0
100 %	90 %	80 %	90 %	100 %	100 %	100 %	50 %	10 %

Total number of alive, young daphnia reproduced by all adults (cumulative values) in % of control on day 21

Nominal concentration of Xyligen 30 F (mg/L)								
Control	0,39	0,78	1,56	3,13	6,25	12,5	25,0	50,0
100 %	93,7 %	81,6 %	89,6 %	86,4 %	82,4 %	50,0 %	2,4 %	0,0 %

Number of alive offspring reproduced per surviving adult within 21 days of exposure (mean reproduction rate)

Nominal concentration of Xyligen 30 F (mg/L)								
Control	0,39	0,78	1,56	3,13	6,25	12,5	25,0	50,0
106,0	104,3	96,0	102,1	91,6	87,3	52,0	3,0	0,0

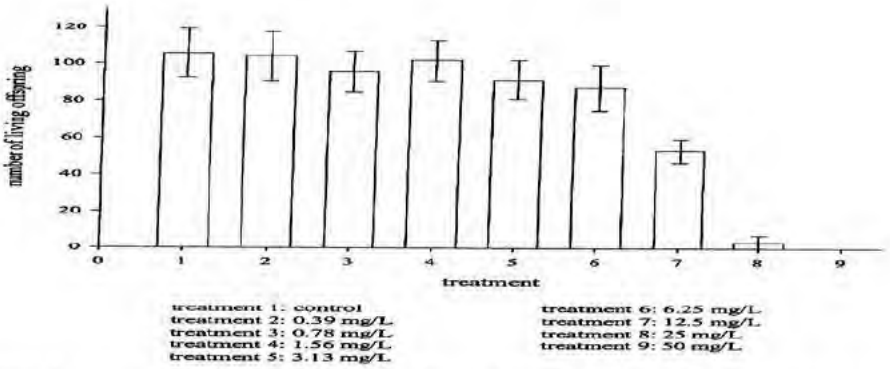
NOEC: 1,56 mg/l

LOEC: 3,13 mg/l

$EC_{50,reprotox}$: 9,7 mg/l

4.2.4 Concentration / response curve

There is no curve available in the test report. The results are presented in table form in § 4.2.3. The total number of alive offspring (mean value + SD) per parent animal alive at the end of the test is given in graphic form in the following:



4.2.5 Other effects

With the exception of the reported mortality and the reduced reproduction rates, no particular signs of intoxication were observed at the test animals during the test.

4.3 Results of controls

In the control all the daphnia survived until the end of the test. The first young daphnia released from their parent animals were recorded in the control group at the observation on day 8. The mean reproduction rate for each individual daphnia,which survived until the end of the test in the control group was 106 ± 13,3 (mean ± SD).

The experiment is valid, since the survival rate of the adult *Daphnia* in the control was at least 80 % at the end of the test and the mean number of alive offspring in the control was higher than 60 per surviving adult *Daphnia* after 21 days.

4.4 Test with reference substance Not reported

4.4.1 Concentrations

4.4.2 Results

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

TEST ORGANISMS

- Strain: Daphnia magna (Strauss), Clone 5, from UBA, Germany.
- Age: 6.5-20.5 h
- Feeding: yes, (with Scenedesmus subspicatus)
- Control group: yes,
- Number of animals: 10 animals/group

STOCK AND TEST SOLUTION AND THEIR PREPARATION

DILUTION WATER

- Source: M4 water, special test water
- Hardness: 2.5 mmol/L
- Ca/Mg ratio: 4:1
- pH: 7.7-8.5
- Oxygen content: > 8.1 mg/l

TEST SYSTEM

- Test type: floatability of animals
- Exposure vessel type: special test tube, 80 ml volume
- Number of replicates, individuals per replicate: 10
- Test temperature: 21 ° C
- Dissolved oxygen: > 8.1 mg/L
- pH: 7.7-8.5
- Intensity of irradiation: 390-450 lux
- Photoperiod: day-night: 16:8

DURATION OF THE TEST: 21 d

TEST PARAMETER: mobility

MONITORING OF TEST SUBSTANCE CONCENTRATION: yes

5.2	**Results and discussion**	RESULTS: EXPOSED - Nominal concentration [mg/L]: - Effect data (NOEC): see above - test substance solubility: soluble - Analytical monitoring of test concentration RESULTS CONTROL: control group was okay	
5.2.1	NOEC	1,56 mg/L	x
5.2.2	LOEC	3,13 mg/L	x
5.2.3	EC_{50} (EC_x)	9,7	x
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2005
Materials and Methods	acceptable
Results and discussion	4.2.2 Actual concentrations of test substance and 4.2.3 Effect data: Only at the lowest testconcentration the measured concentration is lower than 80 % of nominal (reason: below the limit of quantification). At higher concentrations (where effects are reported), the test item was sufficiently stable during the test medium renewal periods and above 80% recovery rate. Therefore it is accepted to give the effect data in nominal concentrations. 5.2.1 NOEC: 1.56 mg/L corresponds to 0.47 mg/L (100% w/w K-HDO) 5.2.2 LOEC: 3.13 mg/L corresponds to 0.94 mg/L (100% w/w K-HDO) 5.2.3 EC_{50}: 9.7 mg/L corresponds to 2.91 mg/L (100% w/w K-HDO)
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_3_4-2: Dilution water

Criteria	Details
Source	The synthetic medium Elendt "M4" is used for the culture and test. The medium is prepared on the basis of an ultrapure, deionised water
Salinity	Reported
Hardness	2.5 mmol/l (=250 mg/L) as $CaCO_3$
PH	8.0 ± 0.5
Ca / Mg ratio	About 4:1
Na / K ratio	No mention in the test report
Oxygen content	Approx. 8,5 mg/L
Conductance	No mention in the test report
TOC	No mention in the test report
Holding water different from dilution water	The cultivation of the parental daphnia is performed in the same kind of test water as used in the test.

Table A7_4_3_4-3: Test organisms

Criteria	Details
Strain / Clone	Daphnia magna STRAUS, CLONE 5
Source	UBA, Germany
Age	6.5 – 20,5 hours at start of the test
Breeding method	Standard laboratory conditions. Similar temperature and light conditions as in the test.
Kind of food	Green algae of the species Scenedesmus subspicatus
Amount of food	0,1 – 0,2 mg/daphnia/day
Feeding frequency	Daphnia are fed at least each working day
Pretreatment	No pre-treatment described in the test report
Feeding of animals during test	The daphnia were fed each working day with green algae. The amount of food was based on the concentration of total organic carbon (TOC) in the food suspension. Amount of TOC/daphnia and day: Days 0 – 3: 0,1 mg TOC/daphnia Days 4 – 6/10 – 12: 0,15 mg TOC/daphnia Days 13/17 – 20: 0,2 mg TOC/daphnia Day 7: 0,3 mg TOC/daphnia Day 14: 0,5 mg TOC/daphnia

Table A7_4_3_4-4: Test system

Criteria	Details
Test type	Semistatic
Renewal of test solution	The test media of all test concentrations and of the control were renewed on days 3,5,7,9,12,14,16 and 19 of the exposure period (every Monday, Wednesday and Friday). By that, a total of 9 treatments were performed.

Volume of test vessels	100 ml glass beakers containing 80 ml test media
Volume/animal	8 ml test media /animal
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No mention in the test report

Table A7_4_3_4-5: Test conditions

Criteria	**Details**
Test temperature	21 °C
Dissolved oxygen	> 8.1 mg/l
pH	7.7 – 8.5
Adjustment of pH	No mention in the test report
Aeration of dilution water	No mention in the test report
Quality/Intensity of irradiation	390-450 lux
Photoperiod	Light regime: 16 h light / 8 h dark

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

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

Criteria for poorly soluble test substances		

Section A7.5.1.1/03 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.5.1.1/03 [redacted] (2005 Effects of Xyligen LP 15684 on the activity of soil microflora. (Carbon transformation test), [redacted]. Report No.: 04 10 35 2026 C, unpublished	
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 (2000)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Xyligen 30 F (tested under the laboratory product number Xyligen LP 15684)	
3.1.1	Lot/Batch number	VM 2012	
3.1.2	Specification	Yellowish liquid	
3.1.3	Purity	30 % (30.41 % analysed)	
3.1.4	Composition of Product	30 % (30.41 % analysed) K-HDO; water ad 100 %	
3.1.5	Further relevant properties	—	
3.1.6	Method of analysis		
3.2	**Reference substance**	Dinoseb acetate (Pestanal)	
3.2.1	Method of analysis for reference substance		
3.3	**Testing procedure**	Non-entry field	
3.3.1	Soil sample / inoculum / test organism	Biologically active agricultural soil: loamy sand soil	
3.3.2	Test system	see table A7_5_1_1-3	
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	

Section A7.5.1.1/03 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

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 (100 g 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		
3.3.10	Controls	Non-treated soil	
3.3.11	Statistics	Calculation of mean values per treatment, standard deviation and coefficients of variation	
		4 RESULTS	
4.1	**Range finding test**	—	
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	20 mg/kg soil d.w., 100 mg/kg soil d.w.	X
4.2.2	Actual concentrations of test substance		
4.2.3	Growth curves		
4.2.4	Cell concentration data		
4.2.5	Concentration/ response curve		
4.2.6	Effect data		

Effects on carbon transformation in soil after treatment with Xyligen 30 F

Days after application	Control		Xyligen 30 F 20 mg/kg soil d.w.			Xyligen 30 F 100 mg/kg soil d.w.		
	O_2 consumption (mg/kg soil d.w./h)	CV (%)	O_2 consumption (mg/kg soil d.w./h)	CV (%)	Deviation from control (%)	O_2 consumption (mg/kg soil d.w./h)	CV (%)	Deviation from control (%)
0	7.99	0.87	8.15	0.41	+ 2.0	7.76	0.96	-2.9
7	8.06	0.67	8.06	0.35	0.0	7.83	0.65	-3.0
14	7.78	0.99	7.83	1.45	+ 0.6	7.61	0.49	-2.1
28	7.31	1.75	7.28	0.51	- 0.3	7.17	2.22	-1.9

4.2.7	Other observed effects		
4.3	**Results of controls**	See 4.2.6. No differences greater than 25 % to the control in O2 consumption were found for any test concentration of Xyligen 30 F at any time interval in	X

Section A7.5.1.1/03 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

		comparison to the respective control.	
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	8.67 mg/kg d.m.	X
4.4.2	Results	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 test was performed in accordance to the OECD guideline 217 (2000). 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 O_2-consumption over a period of maximum 24 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment, aliquots were withdrawn and subjected to the measurement.	
5.2	**Results and discussion**	Based on the results of this study Xyligen 30 F caused no short-term and long-term effects (OECD 217) on the carbon transformation in the field soil tested up to a concentration of 100 mg Xyligen 30 F per kg soil d.w.	X
5.2.1	NOEC		
5.2.2	EC_{10}		
5.2.3	EC_{50}		
5.3	**Conclusion**	Validity criteria: The coefficients of variation in control were maximum 1.75 % and thus fulfilled the demanded range (≤ 15 %). In the most recent test, dated 14.01.04 – 11.02.04, 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.	X
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	X

Section A7.5.1.1/03 Inhibition to microbial activity (terrestrial)

Annex Point IIA7.4

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2005
Materials and Methods	Agree with applicant's version.
Results and discussion	**4.2.1 Initial concentrations of test substance** Concentrations refer to Xyligen 30 F. **4.3 Results of control** For non-agrochemicals it is irrelevant whether differences to the control are < 25%. No differences > 3% to the control in O_2 consumption were found for any test concentration of Xyligen 30 F at any time interval in comparison to the respective control. **4.4.1 Concentrations** Typing error: 8.67 mg/kg dry weight
Conclusion	**5.2 Results and discussion** The applied test design meets the needs for the testing of agrochemicals, therefore no NOEC or ECx-values were determined. After 28 days an inhibition of 1.9% was reached with the highest concentration tested. **5.3 Conclusion** It can be assumed that the NOEC ≥ 100 mg Xyligen 30 F/ kg soil dry weight which corresponds to ≥ 30 mg K-HDO/ kg soil dry weight. **5.3.2 Deficiencies** The study was designed to investigate agrochemicals (only 2 concentrations tested, no dose response curve, no determination of NOEC and ECx).
Reliability	2
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7_5_1_1-1: Microbial sample / Inoculum

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	GPS position: 12.694435960 degrees East 51.403774567 degrees North
Data on the history of the site	Cultivation: At soil removal (2004) fallow ground Pre-cultivation (2003) fallow ground Application of fertilizers: Organic fertilizer: none Inorganic fertilizers: none Last application of plant protection products: none
Use pattern	Agricultural soil
Depth of sampling [cm]	20 cm
Sand / Silt / Clay content [% dry weight]	Particle size distribution (%): - sand (2 - 0.063 mm) 50.2 - silt (0.063 - 0.002 mm) 39.1 - clay (< 0.002 mm) 10.7
pH	6.6
Organic carbon content [% dry weight]	1.46
Nitrogen content [% dry weight]	N_{min} (mg/100 g d m.): 0.99 Total-N (%): 0.14
Cation exchange capacity [$cmol^+/kg$]	12.15
Initial microbial biomass	24.37 mg C/100 g d.m. = 1.67 % compared to C_{org}
Reference of methods	
Collection / storage of samples	The soil was removed to a depth of 20 cm as mixed samples. Because the soil was wet, it was carefully dried at room temperature. Afterwards the soil was passed through a 2 mm mesh sieve. The soil was stored at a temperature of 4°C in containers under aerobic conditions in the dark
Preparation of inoculum for exposure	1200 g soil d.w. per vessel was weighed in the mixing vessel of a mixing machine. The test item was dissolved in water and the test solution was then mixed with the soil in the mixing machine. Water was added to the soil to achieve a moisture of 45 % of WHC. The incubation was carried out in new plastic vessels. The water content of the soil in each test vessel was determined weekly and ranged with values from 16.27 g to 16.62 g/100 g soil d.w. within the

	required range (40 – 50 % of WHC). Water loss was compensated when necessary.
Pretreatment	

Table A7_5_1_1-3: Test system

Criteria	Details
Culturing apparatus	500 ml 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	—
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	The method is base on the initial respiratory response of microbial populations to which a carbon and energy source has been added (substrate-induced respiration, SIR)
Aeration	—

Section A7.5.1.1/04 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.5.1.1/04 [redacted] 2005, Effects of Xyligen LP 15684 on the activity of soil microflora (Nitrogen Transformation Test), [redacted], report No.: 04 10 35 2026 N, unpublished	
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**	Xyligen 30 F (tested under the laboratory product number Xyligen LP 15684)	
3.1.1	*Lot/Batch number*	VM 2012	
3.1.2	*Specification*	Yellowish liquid	
3.1.3	*Purity*	30 % (30.41 % analysed)	
3.1.4	*Composition of Product*	30 % (30.41 % analysed) K-HDO; water ad 100 %	
3.1.5	*Further relevant properties*	—	
3.1.6	*Method of analysis*	Photometric determination (UV/VIS)	
3.2	**Reference substance**	Dinoseb acetate (Pestanal)	
3.2.1	*Method of analysis for reference substance*		
3.3	**Testing procedure**	Non-entry field	
3.3.1	*Soil sample / inoculum / test organism*	Biologically active agricultural soil: loamy sand soil	
3.3.2	*Test system*	see table A7_5_1_1-3	

Section A7.5.1.1/04 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

3.3.3 *Application of TS*

Mixing and application of the test item			
	Xyligen 30 F 20 mg/kg soil d.w.	Xyligen 30 F 100 mg/kg soil d.w.	Control
Amount product weighed in (mg)	625.00	625.00	0.00
Final volume of stock solution (ml)	250.00	250.00	0.00
Concentration in stock solution (mg/ml)	2.50	2.50	0.00
Dilution factor	5.00	—	—
Concentration of application solution (mg/ml)	0.50	2.50	0.00
Added volume of application solution (ml)	6.00	6.00	0.00
Added volume of water (ml)[1]	4.17	4.17	10.17
Amount of wet soil (g)	165.38	165.38	165.38
Amount of dry soil (g)	150.00	150.00	150.00

[1] Additionally to application solution

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*

3.3.10 *Controls* Non-treated soil

3.3.11 *Statistics* Calculation of mean values per treatment, standard deviations and coefficient of variation

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*

Section A7.5.1.1/04 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

4.2 Results test substance Non-entry field

4.2.1 Initial concentrations of test substance 0 mg/kg soil d.w., 20 mg/kg soil d.w., 100 mg/kg soil d.w. x

4.2.2 Actual concentrations of test substance

4.2.3 Growth curves

4.2.4 Cell concentration data

4.2.5 Concentration/ response curve

4.2.6 Effect data

Effects on nitrogen transformation in soil after treatment with Xyligen 30 F

Days after application	Control		Xyligen 30 F 20 mg/kg soil d.w.			Xyligen 30 F 100 mg/kg soil d.w.		
	NO_3-N (mg/kg soil d.w.)	CV (%)	NO_3-N (mg/kg soil d.w.)	CV (%)	Deviation from control	NO_3-N (mg/kg soil d.w.)	CV (%)	Deviation from control
0	12.8	3.2	12.5	0.5	- 2.3	12.6	0.9	- 2.1
7	47.5	3.0	48.3	1.7	+ 1.8	49.7	1.2	+ 4.6
14	55.4	0.7	56.7	1.3	+ 2.3	59.7	1.6	+ 7.8
28	69.6	1.3	69.4	1.8	- 0.4	72.7	1.7	+ 4.4

4.2.7 Other observed effects

4.3 Results of controls

4.4 Test with reference substance Performed

4.4.1 Concentrations 8.67 mg/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 soil d.w.	SD	CV (%) (n=3)	D (%)[1]
Control	87.6	0.4	0.5	—
Dinoseb acetate 8.67 mg/kg soil d.w.	111.9	3.2	2.9	+ 27.7

The calculations were performed with unrounded values

Section A7.5.1.1/04 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

D (%) = deviation to control
SD = standard deviation
CV (%) = coefficient of variation
[1]+ = % stimulation; - = % inhibition

Section A7.5.1.1/04 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test was performed in accordance to the OECD guideline 216 (2000). Determination of the nitrogen transformation (NO_3-nitrogen production) in soil enriched with Lucerne meal (concentration in soil 0.5 %). Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration. NH_4-nitrogen, NO_3- and NO_2-nitrogen was determined by using the Autoanalyzer II (BRAN + LUEBBE).	
5.2	**Results and discussion**	Nitrogen Transformation: The findings are summarized in 4.2.6. No differences greater than 25 % to the control in the nitrogen transformation were found for any concentration of Xyligen 30 F in comparison to the respective control. The coefficients of variation during the experiment were within the demanded limit (control ≤ 15 %). Validity criteria: The coefficients of variation in control (NO_3-N) were maximum 3.2 % and thus fulfilled the demanded range (≤ 15 %) In the most recent test, dated 14.01.04 – 11.02.04, the toxic standard Dinoseb acetate caused an increase of the nitrogen transformation of 27.7 % on day 28 and thus demonstrated the sensitivity of the test system	x
5.2.1	*NOEC*		
5.2.2	*EC_{10}*		
5.2.3	*EC_{50}*		
5.3	**Conclusion**	Based on the results of this study Xyligen 30 F 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 100 mg Xyligen 30 F per kg dry soil.	x
5.3.1	*Reliability*	1	x
5.3.2	*Deficiencies*	No	x

Section A7.5.1.1/04 **Inhibition to microbial activity (terrestrial)**

Annex Point IIA7.4

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2005
Materials and Methods	Agree with applicant's version.
Results and discussion	**4.2.1 Initial concentrations of test substance** Concentrations refer to Xyligen 30F.
Conclusion	**5.2 Results and discussion** For non-agrochemicals it is irrelevant whether differences to the control are < 25%. No differences > 7.8% (increase) to the control were found at any test concentration of Xyligen 30 F at any time interval in comparison to the respective control. The applied test design meets the needs for testing of agrochemicals. No NOEC or ECx-values were determined. After 28 days an increase of 4.4% of the nitrogen production was reached with the highest concentration tested. **5.3 Conclusion** Therefore it can be assumed that the NOEC ≥ 100 mg Xyligen 30 F/ kg soil dry weight, which corresponds to ≥ 30 mg K-HDO /kg soil dry weight. **5.3.2 Deficiencies** The study was designed to investigate agrochemicals. Therefore only 2 concentrations were tested and no NOEC and ECx-values were determined.
Reliability	2
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7_5_1_1-1: Microbial sample / Inoculum

Criteria	Details
Nature	Soil sample
Sampling site:	Wassergut Canitz, Germany, Sachsen
Geographical reference on the sampling site	GPS position: 12.694435960 degrees East 51.403774567 North
Data on the history of the site	No treatment with organic or inorganic fertilizers
Use pattern	Cultivation: At soil removal (2004): fallow ground Pre-cultivation (2003): fallow ground
Depth of sampling [cm]	After uprooting the vegetation cover, the soil was removed to a depth of 20 cm as mixed samples
Sand / Silt / Clay content [% dry weight]	Particle size distribution (%): - sand (2 - 0.063 mm) 50.2 - silt (0.063 - 0.002 mm) 39.1 - clay (< 0.002 mm) 10.7
pH	6.6
Organic carbon content [% dry weight]	1.46 %
Nitrogen content [% dry weight]	N_{min}: 0.99 mg/100 g soil d.w. Total-N: 0.14 %
Cation exchange capacity [cmol+/kg]	12.15
Initial microbial biomass	24.37 mg C/100 g soil d.w. =1.67 % compared to C_{org}.
Reference of methods	
Collection / storage of samples	The soil was removed to a depth of 20 cm as mixed samples. Because the soil was wet, it was carefully dried at room temperature. Afterwards the soil was passed through a 2 mm mesh sieve. Subsequently, the soil was stored at a temperature of 4°C in containers under aerobic conditions in the dark
Preparation of inoculum for exposure	
Pretreatment	Before the start of the test the following parameters were determined: - pH-value - Soil moisture - Maximum water-holding capacity (WHC)

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 was added to the soil to achieve a moisture of 45 % of WHC. The water content of the soil in each test vessel was determined weekly and ranged with values from 16.00 to 16.86 g/100 g soil d.w. within the required range (40 – 50 % of WHC). Water loss was compensated when necessary.
Method of soil incubation	The incubation of the prepared soil was carried out in new plastic vessels (500 ml) under the following conditions: - Temperature: 20 ± 2 °C in a climatic room - Illumination: darkness - Water content of soil: approx. 45 % of WHC - Test duration: 28 days
Aeration	The tops of the vessels used permitted an air exchange with negligible moisture leakage (< 1% loss)

Section A7.5.1.2/02
Annex Point IIIA XIII 3.2

Earthworm, acute toxicity test

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.5.1.2/02 [redacted], Acute toxicity of Xyligen LP 15684 to the earthworm Eisenia fetida in artificial soil, [redacted], Report No.: 04 10 48 098, unpublished	
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 207 "Earthworm, Acute Toxicity Test" 1984	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Xyligen 30 F (tested under the laboratory product number Xyligen LP 15684)	
3.1.1	Lot/Batch number	VM 2012	
3.1.2	Specification	Yellowish liquid	
3.1.3	Purity	Nominal: 30 % K-HDO (measured: 30.41 % K-HDO)	
3.1.4	Composition of Product	30 % K-HDO, water ad 100 %	
3.1.5	Further relevant properties		
3.1.6	Method of analysis	Photometric determination (UV/VIS)	
3.2	**Reference substance**	Yes, 2-chloroacetamide	
3.2.1	Method of analysis for reference substance		
3.3	**Testing procedure**	Non-entry field	
3.3.1	Preparation of the test substance	Test solutions were made by dispersing weighed amounts of the test item in deionised water, immediately prior to application. The test item was dispersed in sufficient deionised water such that the addition of the test solutions to the basic substrate (at 25 % water content) resulted in a final water content of the substrate of 35 %. Treated substrate was thoroughly mixed using a mixing machine immediately before application.	

Section A7.5.1.2/02
Annex Point IIIA XIII 3.2

Earthworm, acute toxicity test

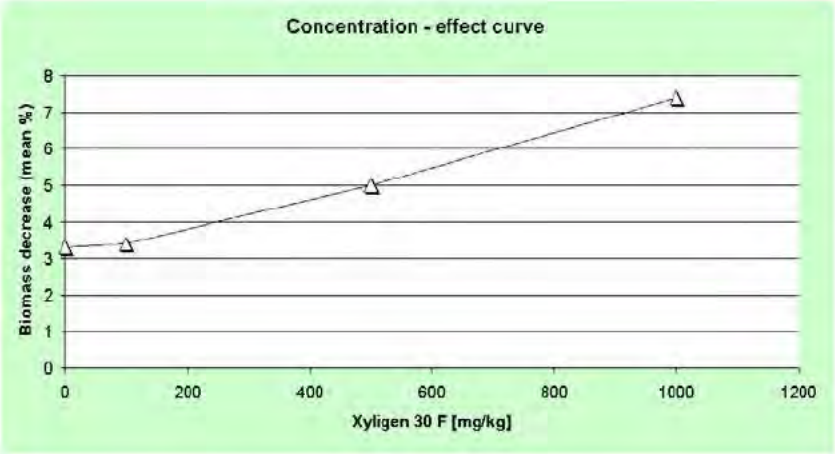
3.3.2	Application of the test substance	The order of application was as follows: - Deionised water (control) - From the lowest to the highest concentration of the test item.	
3.3.3	Test organisms	see table A7_5_1_2-2	
3.3.4	Test system	see table A7_5_1_2-3	
3.3.5	Test conditions	see table A7_5_1_2-4	
3.3.6	Test duration	14 days	
3.3.7	Test parameter	Adult mortality, biomass decrease of surviving adult, morphology and behaviour of the worms	
3.3.8	Examination	Assessments were performed after incubation periods of 7 and 14 days in the climatic chamber	
3.3.9	Monitoring of test substance concentration		
3.3.10	Statistics	Dunnett test	
		4 RESULTS	
4.1	**Filter paper test**	Non-entry field	
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**	Non-entry field	
4.2.1	Initial concentrations of test substance	Control, 100, 500, and 1000 [mg/kg soil dry weight]	x
4.2.2	Effect data (Mortality)		

[mg/kg]:	control	100	500	1000
mortality %:	0	0	0	0

Section A7.5.1.2/02
Annex Point IIIA XIII 3.2

Earthworm, acute toxicity test

4.2.3 Concentration / effect curve



4.2.4 Other effects

[mg/kg]:	control	100	500	1000
Biomass decrease (mean %)	3.3	3.4	5.0	7.4

X

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 — No mortality, abnormal behaviour, or pathological symptoms of the worms were observed in any treatment or control group during the test.

4.3.3 Nature of adverse effects — —

4.4 Test with reference substance — Performed

4.4.1 Concentrations — 14.1, 18.3, 23.8, 31.0 and 40.4 mg a.i./kg soil dry weight

4.4.2 Results — The 14-day LC_{50} of the reference item 2-chloroacetamide applied equivalent to 14.1, 18.3, 23.8, 31.0 and 40.4 mg a.i./kg soil dry weight was calculated as 29.98 mg a.i./kg soil dry weight

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods — The study was performed according to OECD 207 (1984). The artificial soil filled in glass vessels was treated with different concentrations of the test item before earthworms were placed on top of the soil. Four treatment groups (three test item concentrations, one water treated control) with four replicates each and each containing ten adult earthworms. The number of surviving adult earthworms and their weight loss after 14 days as well as behaviour and pathological symptoms were determined.

5.2 Results and discussion — The test item Xyligen 30 F caused no mortality at any tested concentrations. No mortality occurred in the control group. No abnormal behaviour or toxic

Section A7.5.1.2/02
Annex Point IIIA XIII 3.2

Earthworm, acute toxicity test

		symptoms of the worms were observed in any treatment or control group during the test. Mean fresh weights of surviving worms were reduced by 3.3 % in the control and 3.4, 5.0 and 7.4 % for the 100, 500 and 1000 mg Xyligen 30 F/kg soil d.w. treated groups, respectively. The weight reduction was statistically significant ($p \leq 0.05$) relative to the control at the test item concentrations of 500 and 1000 mg Xyligen 30 F /kg soil d.w., respectively. Based on the statistical evaluation of these results, the no-observed-effect-concentration (NOEC) was determined at 100 mg Xyligen 30 F/kg soil dry weight. The LC_{50} could not be calculated but it can be concluded that the LC_{50} is greater than 1000 mg Xyligen 30 F/kg soil dry weight.	
5.2.1	LC_0	LC_0: > 1000 mg Xyligen 30 F / kg soil dry weight	X
5.2.2	LC_{50}	LC_{50}: > 1000 mg Xyligen 30 F / kg soil dry weight	X
5.2.3	LC_{100}	LC_{100}: > 1000 mg Xyligen 30 F / kg soil dry weight	X
5.2.4	NOEC	100 mg Xyligen 30 F / kg soil dry weight	X
5.3	**Conclusion**	The validity criterion for the control group was accomplished: adult mortality: ≤ 10 % (being 0 % after 14 days)	
5.3.1	Other Conclusions		
5.3.2	Reliability	1	
5.3.3	Deficiencies	No	X

Section A7.5.1.2/02
Annex Point IIIA XIII 3.2

Earthworm, acute toxicity test

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2005
Materials and Methods	Agree with applicant's version.
Results and discussion	**4.2.1 Initial concentrations of test substance** TS concentrations refer to Xyligen 30 F. **4.2.4 Other effects** Given concentrations refer to Xyligen 30 F.
Conclusion	**5.2.1 LC_0** LC_0 : ≥ 1000 mg Xyligen 30 F / kg soil dry weight corresponds to ≥ 300 mg K-HDO / kg soil dry weight. **5.2.2 LC_{50}** LC_{50} : > 1000 mg Xyligen 30 F / kg soil dry weight corresponds to > 300 mg K-HDO / kg soil dry weight. **5.2.3 LC_{100}** LC_{100} : > 1000 mg Xyligen 30 F / kg soil dry weight corresponds to > 300 mg K-HDO / kg soil dry weight. **5.2.4 NOEC** NOEC: 100 mg Xyligen 30 F / kg soil dry weight corresponds to 30 mg K-HDO / kg soil dry weight. **5.3.3 Deficiencies** 3 different concentrations were used in the test instead of 5.
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ... *(specify)*
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7_5_1_2-1: Preparation of TS solution

Criteria	Details
Type and source of dilution water	Deionised water
Alkalinity / Salinity	
Hardness	
pH	
Oxygen content	
Conductance	
Holding water different from dilution water	
In case of the use of an organic solvent	
Dispersion	
Vehicle	
Concentration of vehicle	
Vehicle control performed	
Other procedures	

Table A7_5_1_1-2: Test organisms

Criteria	Details
Species/strain	Earthworm *Eisenia foetida* (SAVIGNY) 1826, subspecies *Eisenia fetida andrei* (BOUCHÉ)
Source of the initial stock	The animals were originally purchased from "W. Neudorff GmbH KG"
Culturing techniques	Breeding medium: Mixture of horse manure, straw, peat (1:1:1) Breeding conditions: - Breeding cages (50 cm x 40 cm x 30 cm) - constant diffuse light - Temperature: about 20 °C - Moist soil - pH: about 7
Age/weight	Adult worms (about 6 months old with clitellum) Weight: 330 – 476 mg/worm
Pre-treatment	24 hours acclimatization to test conditions

Table A7_5_1_1-3: Test system

Criteria	Details
Artificial soil test substrate	10 % sphagnum peat 20 % kaolinite clay 0.5 % calcium carbonate 69.5 % industrial quartz sand (more than 50 % of the particles between 0.05 mm and 0.2 mm) deionised water
Test mixture	Mixing time: 2 x 1 minute for each replicate Rotations per minute: 240
Size, volume and material of test container	1 litre glass container
Amount of artificial soil (kg)/ container	About 750 g wet weight corresponding to 556 g dry weight of artificial soil with about 35 % water content.
Nominal levels of test concentrations	0, 100, 500, 1000 mg/kg dry weight of the artificial soil)
Number of replicates/concentration	4
Number of earthworms/test concentration	40
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_2-4: Test conditions

Criteria	Details
Test temperature	18 - 21 °C
Water content	35.1 – 35. 3 % at the beginning and 34.6 – 35.3 % at test termination corresponding to 52.8 – 53.1 % and 52.0 – 53.1 % of water holding capacity
pH	Test initiation: 5.9 – 6.0 Test termination: 5.8 – 5.9
Adjustment of pH	No
Light intensity / photoperiod	Continuous illumination, 600 lx
Relevant degradation products	

Table A7_5_1_2-5: 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
500	0	0	0	0
1000	0	0	0	0
Temperature [°C]	18 - 21 °C			
pH	5.8 – 6.0			
water content	35.1 – 35. 3 % at the beginning and 34.6 – 35.3 % at test termination corresponding to 52.8 – 53.1 % and 52.0 – 53.1 % of water holding capacity			

Table A7_5_1_2-6: Effect data

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

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

Table A7_5_1_2-7: 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

	Justification for non-submission of data	Official use only
Other existing data [☒]	Technically not feasible [☐] Scientifically unjustified [☐]	
Limited exposure [☒]	Other justification [☐]	
Detailed justification:	A terrestrial plant toxicity test with K-HDO is not available. However, a respective test according to OECD Guideline OECD 208 was performed with Cu-HDO (see below).	x
	Potassium (K) is ubiquitous in soil and used as fertiliser. Potassium, is one of the three essential plant macronutrients, and is taken up by crops from soils in relatively large amounts. Potassium increases yields and improves the quality of agricultural produce, and enhances the ability of plants to resist diseases, insect attacks and increases the uptake and use of nitrogen and other nutrients. Vegetal tissues contain an average of 2 to 10 % of K, therefore it is required in large quantities by the growing plant and no negative effect is expected by the potassium ion in the relevant concentration range.	
	Copper (Cu) is an essential micronutrient but is required in small amounts only. 5-20 mg/kg in plant tissue is adequate for normal growth and more than 20 mg/kg is considered toxic (WHO, Environmental Health Criteria 200, Copper, page 182).	
	The low solubility of Cu-HDO does not play an important role, because the test substance was homogenous dispersed in the soil of the mentioned study.	x
	Consequently the study with Cu-HDO can be considered as a worst-case study for K-HDO, because the more toxic effect of Cu is considered in this study.	
Undertaking of intended data submission [☐]		

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	May, 2007
Evaluation of applicant's justification	The result of the plant toxicity test (OECD 208) with Cu-HDO as a test substance shows that plants are the least sensitive of the terrestrial species ($NOEC_{micro\text{-}organisms}$ = 28.8 mg/kg; $NOEC_{earthworm}$ = 85 mg/kg; $NOEC_{plants}$ = 120.6 mg/kg; all converted to artificial soil), when treated with Cu-HDO.
Conclusion	The fact that plants are the least sensitive of the terrestrial species when treated with Cu-HDO and the argumentation given above by the applicant can be accepted as an indication that plants would not be the most sensitive species either, when treated with K-HDO.
Remarks	-

COMMENTS FROM OTHER MEMBER STATE (SPECIFY)

Date	Give date of comments submitted
Evaluation of applicant's justification	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Remarks	

Section 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	99 g/100g	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Water solubility: 6 mg/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 1500 g 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
Annex Point IIIA XIII 3.4

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, 62.5 mg/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 62.5 mg/kg test substance did not continuing growing	
4.1.8	Effect data	see table A7_5_1_3-6a -c	

Section 7.5.1.3 Terrestrial plant toxicity
Annex Point IIIA XIII 3.4

4.1.9 Concentration / response curve

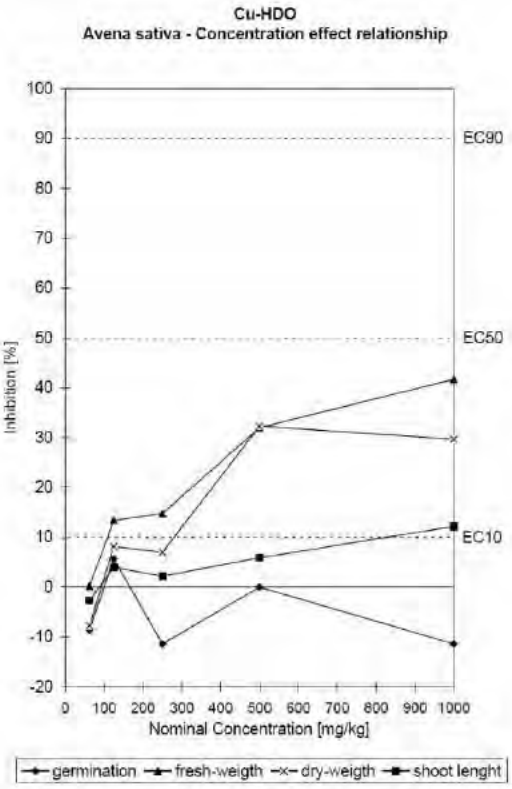
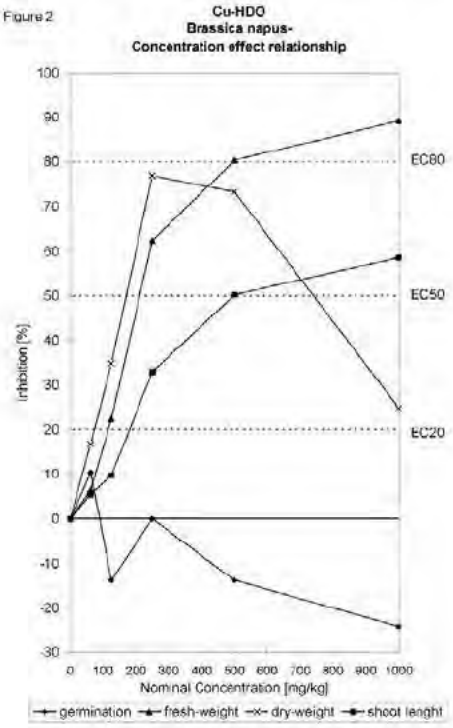
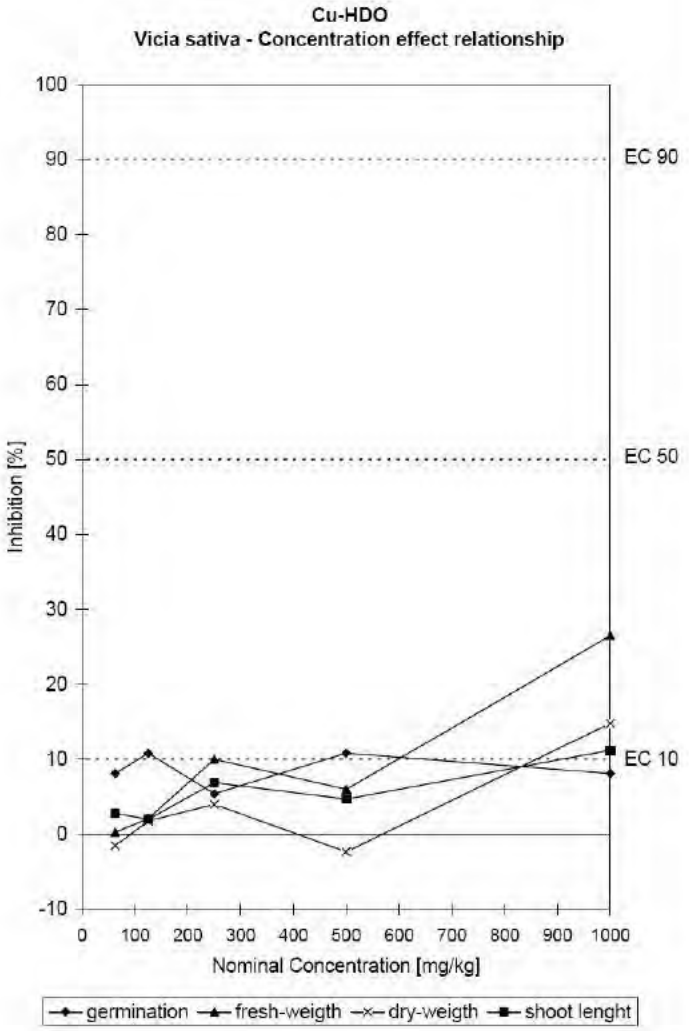


Figure 2



Section 7.5.1.3 **Terrestrial plant toxicity**
Annex Point IIIA XIII 3.4



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

Section 7.5.1.3 **Terrestrial plant toxicity**
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	1000 mg/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 plants or two atrophied plants have no influence on the result of

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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

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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 (Sodium trichloracetate) is recommended. In OECD 208 there is no such recommendation. No reference substance has been investigated. **3.4.3 Test system** Table A 7_5_1_3-4: Point 5.3.24 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	-
	COMMENTS FROM ... *(SPECIFY)*
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table A7_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 1316 g of dry test substrate (corresponds to about 1500 g 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 85 mm, 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

Criteria	Details
5.3.32 Test type	Emergence rate and growth inhibition test with higher plants
5.3.33 Method of application	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 1500 g moist soil with a water content of 40% WHC(max)).
5.3.34 Application levels	0, 1000, 500, 250, 125, 62.5 mg/kg based on technical test substance
5.3.35 Dose rates	0, 1000, 500, 250, 125, 62.5 mg/kg based on technical test substance
5.3.36 Substrate characteristics	Field soil type 2.3; the soil was unsterile and sieved to 5 mm before using in the test. Max. water holding capacity (WHCmax): 35.0 ± 3.0 g/100g dry weight pH value: 5.8 ± 1.8 (calcium chloride method) Organic carbon: 1.02 ± 0.16 % Particle sizes < 20µm: 20.4 ± 2.2 % Soil typ (according to USDA): loamy sand Soil typ (according to German DIN): loamy sand (IS) Water content: 10.7 g/100g DM The soil was prepared at the 10 Nov 2005. 27 kg of the delivered soil with a water content of 10.7 g/100g DM were mixed with 805 g demineralised water in a 60 L barrel. The barrel was closed with a cap. After that the soil was incubated until use at room temperature.
5.3.37 Watering of the plants	Daily pouring with de-ionized water, beginning with the emergence of the first seedlings. Using de-ionized water with an conductivity of < 0.5 µS/cm.
5.3.38 Temperature	20 ± 2 °C
5.3.39 Thermoperiod	Not appropriate
5.3.40 Light regime	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
5.3.41 Relative humidity	Relative Humidity: 60 - 80 % Soil humidity in the exposure phase: 45 % (of maximum water holding capacity)
5.3.42 Wind volatility	Not appropriate
5.3.43 Observation periods and duration of test	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 Test termination date: 10.02.2006
5.3.44 Pest control	Not appropriate
5.3.45 Any other treatments and procedures	Daily pouring with de-ionized water, beginning with the emergence of the first seedlings. Using de-ionized water with an conductivity of < 0.5 µS/cm. Sowing depth: oats approx. 15 mm oilseed rape approx. 5 mm vetch approx. 10 mm

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**	

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 K-HDO.	X
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.	

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)** .	
8.4.1	**Possibility of destruction or decontamination following release in the air**	K-HDO is non-volatile, since it has a low vapour pressure; 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**	Contaminated fluid product shall be incinerated. 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 K-HDO or K-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**	K-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

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

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Jan. 2006
Materials and Methods	n.a.

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

Results and discussion

8.1.1. Application of K-HDO is restricted to industrial fully automatic systems which practically exclude any exposure.

For the case of interventions necessary due to failure of the fully automatic system clean personal protective equipment, including a mask and daily new gloves, is highly essential. This since the product is a skin irritant and may cause severe damage of the eye.

Since the knowledge about the potential human toxicity of the biocidal substance K-HDO will remain limited due to the fact that the absence of alerts for reproductive toxicity was deduced only from repeated dose toxicity and developmental toxicity studies, but no reproductive toxicity study was submitted, the biocidal efficacy is considered to be a sufficient toxicological alert to trigger safety measures for pregnant and lactating women: Pregnant and lactating women should neither work in the K-HDO production plant nor with the application of K-HDO nor with the processing of K-HDO treated wood composites.

For the same reasons and also because the risk assessment for infants exposure chewing K-HDO treated wood composites resulted in an unacceptable risk the use of K-HDO treated wood composites has to be restricted to applications where biocidal treatment is unavoidable which could be construction but definitely excludes indoor living areas applications with the potential of direct human contact. This requirement is also in line with Article 3.7. of BPD 98/8/EC that aims to limit the use of biocide to the minimum necessary.

Since no analytical methods and no toxicological risk assessment for K-HDO contamination in food and feeding stuff was provided the use of K-HDO treated wood composites must exclude applications that may lead to contact with food and feeding stuff or contamination thereof.

8.1.2 Furthermore, the inner lining of the containers should consist of polyethylene (PE). Temperatures should not drop below -12°C, since otherwise crystallisation may occur.

8.3.1.

general advice: Remove contaminated clothing

if inhaled: keep patient calm, move to fresh air, summon medical help.

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.

on skin contact: wash thoroughly with soap and water

8.5. Additionally, according to the European waste list 2001/118/EEC the following waste classification is proposed: The six-digit code for wastes from wood preserving agents should start with 03 02 XX.

8.7. Since K-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.

Conclusion See above

Reliability n.a.

Acceptability acceptable

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

Remarks	-
	COMMENTS FROM ... (SPECIFY)
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A9 Classifiaction and Labelling

Subsection (Annex Point)		**Official use only**
Field of application	Protection of wood composites (e.g. plywood and particle board) against wood-destroying fungi	X
Application methods	Addition to the adhesive line; observe the manufacturer's instruction given in the safety data sheet and the technical leaflet.	
Contains	30 % N-cyclohexyldiazeniumdioxide-potassium	
Proposed classification and labelling:	 Xn – Harmful R 22 Harmful if swallowed R 36/38 Irritating to eyes and skin S 2 Keep out of reach of children S 13 Keep away from food, drink and animal feeding stuffs S 20/21 When using, do not eat, drink or smoke S 46 If swallowed, seek medical advice immediately and show this container or label	X
Packaging:	• 200 L metal-polydrums with PE-inliner • IBC container, 1000 L with PE inside-box, • road tanker; Xyligen 30 F is compatible with the stated packaging material.	
Proposal for safety data sheet:	See Ref. B 9 Safety Data Sheet of Xyligen 30 F (Ref. B 9)	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	JAN 2006
Evaluation of applicant's proposal	**Field of application** With the efficacy data submitted only application with particle boards and not with plywood is supported. See document IIIA.5 and IIIB.5. **Hazard symbol:** Xn **Indication of danger:** harmful if swallowed, irritant **Risk Phrases:** (referring to the active substance, minimum purity 96.5%w/w) **R11,** highly flammable **R25**, toxic if swallowed (LD_{50}, rat, oral: 136 mg/kg bw) **R38**, irritating to skin: The average score is for back and ear after 24 hours at least 2 and for back not reversible. **R41**, risk of serious damage to eyes: 1 and 24 hours after eye exposure bleeding was observed (reversible till day 8) **R48/22**, Danger of serious damage to health by prolonged exposure: Carcinogenicity study: local effects in GI at ~ 34 mg/kg bw with structurally related Cu-HDO (read across) **R52/53**, harmful to aquatic organisms, may case long-term adverse effects in the aquatic environment **Safety phrases:** **S2**, Keep out of reach of children **S9**, Keep container in a well ventilated place **S 13,** Keep away from food, drink and animal feeding stuffs **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 **S45** In case of accident or if you feel unwell seek medical advice immediately (show the label where possible) **S46** If swallowed, seek medical advice immediately and show this container or label **S61** Avoid release to the environment. Refer to special instructions/safety data sheets
Conclusion	See Evaluation of applicant's proposal
Remarks	**For the proposed classification and labeling according to Reg. EC 1272/2008 (CLP) reference is made to Doc IIA.**
	COMMENTS FROM OTHER MEMBER STATE (SPECIFY)
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	