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

Annex Point IIA-VII.7.6.2.2

4.4.4	Kinetic order	First order (samples with recoveries < 75% were not used for the calculation of kinetics)
4.4.5	k^c_p / k^a_p	The used actinometer does not include a determination of the photolysis rate constant, since constant absorption is assumed.
4.4.6	Reaction quantum yield (ϕ^c_E)	The quantum yield of etofenprox was determined to be Φ = 0.248 in buffer solution (pH 7) and Φ = 0.147 in natural pond water.
4.4.7	k_{pE}	The direct photolysis sunlight rate constant of the test substance in water bodies in the environment is not given in the report, but can be easily calculated by assuming 1st order kinetics and using the equation $t_{1/2} = \ln 2 / k_{pE}$

Theoretical photolysis rate constant ($days^{-1}$) at the surface of water *	**Spring**	**Summer**	**Autumn**	**Winter**
Latitude 30° N	- 0.075	- 0.089	- 0.050	- 0.032
Latitude 40° N	- 0.062	- 0.083	- 0.034	- 0.016
Latitude 50° N	- 0.047	- 0.073	- 0.018	- 0.005

* Conditions: pure water close to the surface, longitude 10°, terrestrial type of atmosphere, typical ephemeride and ozone values

4.4.8	Half-life ($t_{1/2E}$)	Estimation of half-lives of etofenprox in an aquatic environment at different latitudes, on the basis of the quantum yield value that was determined in the buffer solution (software used: GCSOLAR v.1.2, EPA)

Theoretical lifetime (days) at the surface of water *	**Spring**	**Summer**	**Autumn**	**Winter**
Latitude 30° N	9.2	7.8	13.8	21.8
Latitude 40° N	11.2	8.4	20.6	44.2
Latitude 50° N	14.9	9.5	38.4	131.0

* Conditions: pure water close to the surface, longitude 10°, terrestrial type of atmosphere, typical ephemeride and ozone values

4.5	**Specification of the transformation products**	see enclosed table A7_1_1_1_2_01-3

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

Annex Point IIA-VII.7.6.2.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test guidelines: - Directives 95/36/EEC and 94/37/EEC
- SETAC Guideline (March 1995)
- OECD Guidance Document (97)21
- EPA OPPTS 835.2210
- Japan MAFF Guideline, 16

x

The photolytic degradation of [2-^{14}C-propyl]-etofenprox (batch no. MRH/MTC 276/31; radiochemical purity 100%; specific activity: 4.20 MBq/mg) and [α-^{14}C-benzyl]-etofenprox (batch no. MRH/MTC 277/20; radiochemical purity: 100%, specific activity: 3.24 MBq/mg) under artificial sunlight and the quantum yield of photodegradation according to the ECETOC method was determined in sterile buffer solution at pH 7 and natural pond water.

The buffer solution (0.01 M phosphate) was prepared using ultra pure water. The natural water was collected from a pond system (Ormalingen BL/Switzerland) at a depth of 10-20 cm below the surface and then, passed through a 0.2 mm sieve. Before use, one liter of buffer solution and pond water were sterilised by autoclaving.

The application solution was prepared in acetonitrile based on a 1+1 mixture of both radiolabelled etofenprox. Aliquots of 22 mL of both aqueous solutions were transferred to each individual test vessel (25 mL). Thereafter, duplicate aliquots of 400 µL of the application solution were added to the test vessels to obtain a concentration of ^{14}C-etofenprox of 0.288 mg/L. Each incubation vessel (cylindrical of 2.26 x 11 cm for inner diameter and height, respectively) consisted of Duran glass covered with quartz glass plates screwed onto the top.

All vessels were maintained at 25 ±1°C using a refrigerated circulator and exposed to continuous light for 15 days. Samples were exposed to artificial sunlight from a 1.8 kW xenon irradiation source (Suntest CPS, Heraeus®), equipped with an UV filter system to remove wavelengths below 290 nm. A mean light intensity of 17.2 W/m^2 within the visual light spectrum (300 nm to 400 nm) was used. Corresponding control samples were maintained under the same conditions but in the dark.

The gas mixture leaving each vessel was passed through a series of traps, for any liberated $^{14}CO_2$ and organic volatile.

Duplicate samples were taken for analysis after 0, 1, 1.8, 4.6, 11.7 and 15 days of continuous irradiation for the buffer solution and after 0, 1, 1.8, 4.6, 6.7, 13.5 and 15 days for the pond water. Dark control samples were taken from both test systems on days 1.8, 4.6, 6.7, 11.7 and 15 of incubation. At each interval, the samples were radiochemically quantified by LSC and analysed by HPLC and TLC. Trapping solutions for CO_2 (2N NaOH) and for organic volatiles (ethylene glycol) were exchanged at each sampling time and the trapped radioactivity was determined.

Additionally, the quantum yield [Φ] of disappearance of ^{14}C-etofenprox was determined with the ECETOC method using the uranyl acetate/ oxalic axid actinometer. These values were used to calculate the environmental lifetimes in pure water at different latitudes and seasons, using the GCSOLAR Vers. 1.20 (1999) computer program.

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

Annex Point IIA-VII.7.6.2.2

5.2 Results and discussion

5.2.1 k^c_p

Measured photolysis rate constant k^c_p. for the test substance. x

Buffer (pH 7): 0.148

Pond water: 0.087

5.2.2 K_{pE}

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

Theoretical photolysis rate constant (days^{-1}) at the surface of water *	**Spring**	**Summer**	**Autumn**	**Winter**
Latitude 30° N	0.075	0.089	0.050	0.032
Latitude 40° N	0.062	0.083	0.034	0.016
Latitude 50° N	0.047	0.073	0.018	0.005

* Conditions: pure water close to the surface, longitude 10°, terrestrial type of atmosphere, typical ephemeride and ozone values

5.2.3 ϕ^c_E

The quantum yield of etofenprox was determined to be

Φ = 0.248 in buffer solution (pH 7) and

Φ = *0.147 in natural pond water.*

5.2.4 $t_{1/2E}$

Estimation o half-lives of etofenprox in an aquatic environment at different latitudes, on the basis of the quantum yield value that was determined in the buffer solution (software used: GCSOLAR v.1.2, EPA)

Theoretical lifetime (days) at the surface of water *	**Spring**	**Summer**	**Autumn**	**Winter**
Latitude 30° N	9.2	7.8	13.8	21.8
Latitude 40° N	11.2	8.4	20.6	44.2
Latitude 50° N	14.9	9.5	38.4	131.0

* Conditions: pure water close to the surface, longitude 10°, terrestrial type of atmosphere, typical ephemeride and ozone values

5.3 Conclusion

Validity criteria can be considered as fulfilled.

Etofenprox is rapidly photodegraded under simulated sunlight in both buffer solution at pH 7 and natural pond water. x

The experimental photolytic half-lives were calculated using first-order reaction kinetics to be 4.7 and 7.9 days for buffer solution at pH 7 and pond water, respectively. The control samples incubated under the same conditions in the dark were shown to be stable.

The quantum yield (Φ) of etofenprox was determined to be 0.248 and 0.147 days for buffer solution at pH 7 and pond water, respectively.

Two metabolites, identified as α-CO and PENA, exceeded 10% of the applied radioactivity during the course of the study, and reached their maximum levels after 15 days of irradiation, in sterile buffer and sterile natural water solutions

5.3.1 Reliability

1

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

Annex Point IIA-VII.7.6.2.2

5.3.2 Deficiencies No

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

Criteria	Details
Purity of water	Buffer solution: Baker buffer solution No. 5656 (phosphate, pH 7), diluted to 0.01 mol/L with ultra pure water (ELGA water purifier unit) Natural water: pond water (pH 8), filtered through 0.2 mm sieve - buffer solution and pond water were sterilised (121°C, 30 min) before use
Preparation of test chemical solution	Stock solutions: - ^{14}C-benzyl label: 6.732 MBq/5 ml acetone - ^{14}C-propyl label: 7.029 MBq/5 ml acetone Application solution: Stock solutions (40 µl of each radiolabel) were combined and solvent (acetone) was evaporated; residue was dissolved in 105 ml acetonitrile. Based on the specific activity of 3.685 MBq/mg the concentration of test substance was calculated to be 0.288 mg/L Test solutions: Aliquots of 22 ml of the aqueous solutions (12 flasks/water type) were treated with 400 µl of the application solution. To minimise the adsorption of test item to the glass surface the final content of acetonitrile was increased to 1.8%.
Test concentrations	5.24 µg a.s./L
Temperature	25 ± 1°C
Preparation of a.s. solution	see "Test solutions" above
Controls	Yes, incubations in the dark (one replicate per sampling interval and test medium:)
Identity and concentration of co-solvent	Acetonitrile, 1.8% (v/v)

Table A7_1_1_1_2_01-2: Description of test system.

Criteria	Details
Laboratory equipment	Test vessels: Cylindrical Duran glass vessels (25 ml; inner diameter x height: 2.26 x 11 cm), covered with quartz glass plates screwed onto the top of each vessel Reaction vessel area: 4.0 cm^2 (vessels were put in the centre of the irradiated area UV-VIS spectrometer: Lamba 2, Perkin Elmer. USA Irradiation was top- down; the quartz windows were transparent for all relevant wavelengths.
Test apparatus	Suntest CPS, Original Hanau (Heraeus, Germany)
Properties of artificial light source:	
Nature of light source	Xenon arc lamp, 1.8 kW
Emission wavelength spectrum	300 – 800 nm
Light intensity	17.2 W/m^2
Filters	UV filter to simulate outdoor sunlight (UV-edge 290 nm)
Properties of natural sunlight:	natural sunlight was not used
Latitude	n.a.
Hours of daylight	n.a.
Time of year	n.a.
Light intensity	n.a.
Solar irradiance (L_λ)	n.a.

n.a. = not applicable

Table A7_1_1_1_2_01-3: Specification and amount of transformation products (expressed as percentage of the applied radioactivity) after 15 day incubation in the light.

CAS-No	Common Name	CAS and/or IUPAC Chemical Name	Amount [%] of parent compound measured after 15 days in	
			Buffer solution	Pond water
	Etofenprox		9.4	32.5
	α-CO	2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzoate	63.6	37.8
	PENA	2-(4-ethoxyphenyl)-2-methylpropyl alcohol	12.0	14.4
	m-PB-acid	3-phenoxybenzoic acid	5.0	3.8
	M1	unknown	1.7	3.3
	M2	unknown	n.d.	2.9
	M3	unknown	4.3	5.3
	M4	unknown	n.d.	n.d.
	M5	unknown	4.0	n.d.

n.d. = not detected

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**3.1 Test material** Information given under this heading refers to the unlabelled reference substance. **3.1.2 Specification** The specification for this batch is given in section 3 (RCC Study No. 751803). **4.3 Controls** The controls showed low recoveries at day 12 and 15; though an extra washing step for the test vessels with ethyl acetate was established, showing reasonable recoveries in the buffer and pond water samples. The justification for these low recoveries due to adsorption to glass is therefore satisfactory. Also no radioactivity was detected in the volatile traps.
Conclusion	**5.1 Materials and methods** Test guidelines: Directives 95/36/EEC and 94/37/EEC cannot be considered as test guidelines. The study design is a mixture of the quoted references. Mass balance: The test concentration was below the water solubility limit. After establishing an extra washing step of the test vessels recoveries for the buffer and the pond water could often be raised by more than 50% coming to an overall range from 75 to 108% AR, which is also quite low compared with the recommendations of the quoted guidelines (mass balance for radioactive material should be between 90 and 110 % AR). **5.2.1.und 5.2.2, rate constants** Typing error, see Section 4.4.3 and 4.4.7 for proper values. **5.3 Conclusion** A categorisation of the degradation results like "rapidly/fairly/… photodegradable" is not accepted.
Reliability	1
Acceptability	Acceptable
Remarks	**2.3 Deviations** The content of acetonitrile was raised to 1.8% in the test vessels and was above the recommended 1%. Justification (high adsorption to glass walls) is acceptable.

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Thus, J.L.G., van der Laan-Straathof, J.M.Th. and Keetelaar-Jansen, W.A.J. (1993): Biodegradation of 14C-Etofenprox in an adapted modified Sturm test. Solvay Duphar B.V.,'s-Graveland, The Netherlands; unpublished report no. C.DNL.62.002 (September 10, 1993) Experimental phase :May 28, 1993 – July 20, 1993	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.	
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	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline No. 301 B (1982), EEC Directive 79/831, Annex V, Part C (see table A7_1_1_2_1-1)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 Etofenprox	x
3.1.1	Lot/Batch number	90S01	
3.1.2	Specification	As given in section 2	x
3.1.3	Purity	> 99 %	
3.1.4	Further relevant properties	Water solubility 22.5 µg/L	
3.1.5	Radiolabelled compound	α-^{14}C-MTI-500 (Trebon^{R}) Specific activity: 1.01 MBq/mg Radiochemical purit:y 96.5 %	x
3.1.6	TS inhibitory to microorganisms	No	
3.1.7	Specific chemical analysis	Liquid scintillation counting and HPLC	
3.2	**Reference substance**	No	
3.2.1	Initial concentration of reference substance	Not applicable	

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

3.3	**Test ing procedure**		
3.3.1	Inoculum / test species	Not adapted activated sludge (see table A7_1_1_2_1-2)	
3.3.2	Test system	The test was performed in three 2-L glass bottles closed with a plastic screw cap from where a 15 mL plastic tube with holes was suspended. A vial with 5 mL 1 M KOH was placed in this tube to absorb the CO_2. (see table A7_1_1_2_1-3)	
3.3.3	Test conditions	In each bottle, 100 µL of [^{14}C]-etofenprox solution was added to 0.7 L mineral salt medium and 8.8 mL inoculum and exposed under aerobic conditions for a period of 28 days. In addition 100 µL of this solution was added in triplicate to 100 mL of a mixture of acetonitrile/water 1:1 v/v (standard solutions). The flasks were made up to 1000 g with mineral salt medium and incubated on an orbital shaking machine (about 50 rpm) in a room at 20±1°C in the dark for 28 (see table A7_1_1_2_1-4)	
3.3.4	Method of preparation of test solution	A stock solution containing 0.10 mg 14C-etofenprox in acetone was prepared. 0.10 mL of this solution was added to the incubation bottles. 1 mg unlabelled etofenprox / mL was diluted 1:10 with acetonitrile / water 1:1 (v/v). this solution was used as HLPC reference.	
3.3.5	Initial TS concentration	0.0108 mg TS/l	x
3.3.6	Duration of test	29 days	
3.3.7	Analytical parameter	CO_2 evolution	
3.3.8	Sampling	The $^{14}CO_2$ absorption vials were replaced by fresh ones after 7, 14, 21 and 28 days. After 28 days of incubation 5 mL of 0.5 M HCl was added to the test flasks for removing CO_2 or carbonate dissolved in the medium and a fresh CO_2 absorption vial was mounted. On day 29 of the study, the medium, the remaining medium and sludge was filtered over a nylon membrane filter (pore size 0.45 µm). The sludge and the filter were extracted twice with 20 mL acetonitrile by intensive mixing on a tube mixer and ultrasonic bath for 10 minutes. The sludge was separated from the extract by centrifugation for 10 minutes at 3000 g. After this extraction, the filter was removed. The two extracts of each sludge sample were pooled and made up to 45 mL.	
3.3.9	Intermediates/ degradation products	Quantified by HPLC but not identified	
3.3.10	Nitrate/nitrite measurement	Not applicable	
3.3.11	Controls	The sample treatment and analysis method was validated by spiking 50 mL aqueous medium in triplicate with 10 µL of the 14C-etofenprox stock solution. The recovery after freeze drying was 109 % and after HPLC/LSC analyis 108% of nominal.	
3.3.12	Statistics	ng TS = (dpm*Vtot)/(Vinj*sa) with TS is testsubstance 14C dpm is decays per minute (radioactivity unit) Vtot is total volume of sample in mL	

Section A7.1.1.2.1 Biodegradability (ready)

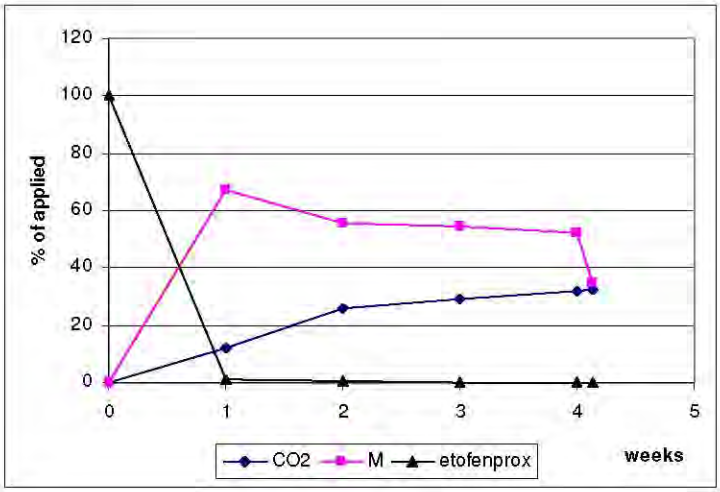
Annex Point IIA-VII.7.6.1.1

Vinj is injection volume (0.201mL)
s.a. is specific activity to convert dpm to µg (60.06 dpm/ng)

4 RESULTS

4.1 Degradation of test substance

4.1.1 Graph



weeks	CO2	M	etofenprox
0	0	0	100
1	11.9	67.1	1.2
2	25.7	55.7	0.6
3	29.2	54.5	0
4	32	52.2	0
4.14	32.3	34.7	0

M = polar metabolite

4.1.2 Degradation: 99 % degradation at plateau

4.1.3 Other observations: polar intermediates are formed that degrade further to CO_2.

4.1.4 Degradation of TS in abiotic control: Not reported in this study

4.1.5 Degradation of reference substance: Not applicable

4.1.6 Intermediates/ degradation products: See 4.1.1

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The ready biodegradability of [α-^{14}C-benzyl]-etofenprox (radiochemical purity: 96.5%) was determined under aerobic conditions by measuring the $^{14}CO_2$ evolution as well as the loss of parent compound. The inoculum (activated sludge) was obtained from an activated sludge plant

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

treating predominantly domestic waste water. The concentration of the inoculum was 30 mg dry weight/L.

The test was performed in three 2-L glass bottles closed with a plastic screw cap from where a 15 mL plastic tube with holes was suspended. A vial with 5 mL 1 M KOH was placed in this tube to absorb the CO_2. In each bottle, 100 µL of [^{14}C]-etofenprox solution was added to 0.7 L mineral salt medium and 8.8 mL inoculum and exposed under aerobic conditions for a period of 28 days. In addition 100 µL of this solution was added in triplicate to 100 mL of a mixture of acetonitrile/water 1:1 v/v (standard solutions). The flasks were made up to 1000 g with mineral salt medium and incubated on an orbital shaking machine (about 50 rpm) in a room at 20±1°C in the dark for 28 days.

The $^{14}CO_2$ absorption vials were replaced by fresh ones after 7, 14, 21 and 28 days. After 28 days of incubation 5 mL of 0.5 M HCl was added to the test flasks for removing CO_2 or carbonate dissolved in the medium and a fresh CO_2 absorption vial was mounted. On day 29 of the study, the medium, the remaining medium and sludge was filtered over a nylon membrane filter (pore size 0.45 µm). The sludge and the filter were extracted twice with 20 mL acetonitrile by intensive mixing on a tube mixer and ultrasonic bath for 10 minutes. The sludge was separated from the extract by centrifugation for 10 minutes at 3000 g. After this extraction, the filter was removed because it looked clean. The two extracts of each sludge sample were pooled and made up to 45 mL.

The pH of the medium was measured at the start and after 28 days of incubation. After 24 hours of incubation, the amount of $^{14}CO_2$ absorbed in the KOH solutions was determined by LSC. At each sampling time 50 mL of the medium of each bottle was taken with a pipette and stored frozen in a sample bottle until analysis. The amount of radioactivity in the medium extracts and in the standard solution was determined by LSC by taking 1 mL aliquots in triplicate. The amount of radioactivity in the extracted sludge samples was determined by combustion analysis.

The amount of parent compound, [^{14}C]-etofenprox, and metabolites in the standard solutions, medium extracts and sludge extracts was determined by HPLC/LSC analysis.

5.2 Results and discussion

The amount of [^{14}C]-etofenprox added to the test bottles was 10.8 µg of which 95.8% consists of etofenprox (10.3 µg). The average pH ranged from 7.5 at the start to 6.6 at the end of the experiment.

Recovery

After 29 days of incubation, the total amount of radioactivity recovered in the CO_2 trap, the medium, in sludge extract and sludge residue was 88.7% of applied radioactivity.

Extractable and non-extractable radioactivity in the sludge

The amount of extractable radioactivity was 6.7% of applied in the sludge extract and 15% of applied in the sludge residue after 29 days of incubation.

Identification of radioactivity

The major metabolites detected were CO_2 (32%) and polar metabolites.

Biodegradation

After one week of incubation, only 1.2% of the added amount of etofenprox could be detected and decreased to 0.6% or less until Day

x

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

		29. The DT_{50} was determined to be less than 2 days, assuming a first order degradation. Therefore, [^{14}C]-etofenprox can be classified as "readily biodegradable".	
5.3	**Conclusion**	Etofenprox can be classified as "readily biodegradable".	x
5.3.1	Reliability	1	x
5.3.2	Deficiencies	No	

Table A7_1_1_2_1-1: Guidline-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_1-2: Inoculum / Test organism.

Criteria	Details
Nature	Secondary (biological activated sludge
Species	not specified
Strain	not specified
Source	Activated sludge plant treating predominantly domestic waste water
Sampling site	RWZI Horstermee in nederhorst den Berg, The Netherlands, on June 2, 1993
Laboratory culture	No
Method of cultivation	not specified
Preparation of inoculum for exposure	No pre-treatment, 3.4 mg/mL dray weight
Pretreatment	no adaptation

Initial cell concentration	30 mg/L based on dry weight

Table A7_1_1_2_1-3: Test system.

Criteria	Details
Culturing apparatus	2-litre glass bottle (Scott Duran. dry sterilised at 175°C, closed with plastic screw cap.
Number of culture flasks/concentration	3
Aeration device	orbital shaking
Measuring equipment	Radioactivity measurement with LSC and HPLC
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2_1-4: Test conditions.

Criteria	Details
Composition of medium	1 mL of solutions A, B, C, D, and E were added to 1 L ultrapure water. A: 0.14 g $FeCl_3$ in 1 L distilled water B: 22.5 g $MgSO_4$*7H_2O in 1 L distilled water C: 27.5 g $CaCl_2$ per 1 L distilled water D: 8.5 g KH_2PO_4 / L 21.75 g K2HPO_4 / L 22.2 g Na_2HPO_4*2H_2O / L 1.7 g NH_4Cl E: 40 g $(NH_4)2SO_4$ per L distilled water
Additional substrate	No
Test temperature	20 ± 1 °C thermostat
pH	Bottle no. start end 1 7.5 6.7 2 7.6 6.6 3 7.5 6.6
Aeration of dilution water	No
Suspended solids concentration	30 mg dry weight / L
Other relevant criteria	orbital shaking, CO2 trapped by 5 mL 1 M KOH in trap inside the incubation bottle

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05. 2005
Materials and methods	**3.1 Test material** Information given under this heading refers to the unlabelled reference substance. **3.1.2 Specification** No detailed specification and reference to section 2 was given in the original test report. **3.1.5 Radiolabelled compound** The test was conducted with [alpha-^{14}C-benzyl]-etofenprox, [2-^{14}C-propyl]-etofenprox was not used, though different metabolites can be formed. **3.3.5 Initial TS concentration** The low test concentration was justified in the test report with the low water solubility of etofenprox.
Conclusion	**5.2 Results and discussion** Recovery The mass balance varied from 79% after 1 week to 84% AR. 20% were lost after 1 week of incubation, which was probably caused by adsorption to the sludge. So after 29 days of incubation, the total amount of radioactivity recovered in the CO_2 trap, the medium, in sludge extract and sludge residue was 89% AR. Still 11% AR were missing, which could be due to the formation of other volatile metabolites. **5.3 Conclusion** Etofenprox can be regarded as not readily biodegradable. The pass levels refer to the ultimate degradation of the test substance (60% $ThCO_2$). The study was conducted with radioactive material. After 4 weeks still 52% AR remained in the medium. This radioactivity was characterised as polar metabolites (etofenprox below detection limit).
Reliability	2
Acceptability	Acceptable
Remarks	The test result is consistent with the outcome of the first test on biodegradability (closed bottle test, 17% degradation within 28 days, key study).

Section A7.1.1.2.1/02 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Thus, J.L.G., van der Laan-Straathof, J.M.Th. (1992): Determination of the biodegradability of etofenprox in a closed bottle test. Solvay Duphar B.V., Environmental research department, Noordereinde 56, 1243 JJ's-Graveland, The Netherlands; unpublished report no. C.DNL.62.002 (February 28, 1992) Dates of experimental phase: no information in the report.	x
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]. Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data submitted to the MS before 14 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline No. 301 D EEC Method C6	
2.2	**GLP**	Yes	
2.3	**Deviations**	Minor deviations: - Ammonium chloride was omitted from the medium to prevent nitrification. - Test duration: 56 days instead of 28 days (but measurements also after 28 days)	x
		3 MATERIALS AND METHODS	
3.1	**Test material**	Etofenprox	
3.1.1	Lot/Batch number	90S01	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	> 99 %	
3.1.4	Further relevant properties	Low volatility and low water solubility	
3.1.5	Radiolabelled compound	No	
3.1.6	TS inhibitory to microorganisms	No	
3.1.7	Specific chemical analysis	No	
3.2	**Reference substance**	Sodium acetate	
3.2.1	Initial concentration of reference	6.7 mg/litre	

Section A7.1.1.2.1/02 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

	substance		
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	Secondary (biological) activated sludge. See table 3.3.1.	
3.3.2	Test system	See table 3.3.2	
3.3.3	Test conditions	The test was performed in 42 BOD-bottles (280 ml volume each). These bottles were filled as follows: A: 7 bottles with mineral salt solution B: 7 bottles with mineral salt solution and inoculum C: 7 bottles with mineral salt solution, inoculum and sodium acetate D: 7 bottles with mineral salt solution, inoculum and silica gel E: 7 bottles with mineral salt solution, inoculum and sodium acetate and etofenprox coated on silica gel F: 7 bottles with mineral salt solution, inoculum and etofenprox coated on silica gel *The silical gel was used as a carrier since etofenprox is nearly insoluble in water (details see point 3.3.4).* Although no additional oxygen consumption was expected from the silica gel, controls with silica gel treated with dichloromethane only were included in the test. This technique was described by Nyholm and Seiero ("Biodegradability testing of poorly soluble compounds by means of manometric respirometry", Chemosphere; 21 (12): 1477-1487; 1990) The bottles were completely filled and closed with glass stoppers. They were then incubated in the dark at 20 ± 1°C.	x
3.3.4	Method of preparation of test solution	Sodium acetate: a stock solution of 1.0 g/litre water was prepared. 1.87 ml of this solution was added to the BOD-bottles, giving an initial concentration of 6.7 mg sodium acetate/litre. Etofenprox: the test compound is practically insoluble in water. For that reason etofenprox was tested in the presence of silica gel to guarantee a reproducible availability of the compound to the microorganisms. The test substance was first dissolved in dichloromethane (1g/litre). Of this solution, 0.56 ml was added to 2g silica gel (100-200 mesh) weighed in a glass petri dish. The solvent was allowed to evaporate by placing the Petri dish in a ventilated hood for 3 hours and the entire contents were then transferred to the BOD bottle. a stock solution of 1.0 g/litre in dichloromethane was prepared. 0.56 ml of this solution was added to 2 g silica gel and the 2 g silica were added to the BOD-bottles, giving an initial concentration of 2.0 mg etofenprox/litre.	
3.3.5	Initial TS concentration	2.0 mg/litre.	
3.3.6	Duration of test	56 days	
3.3.7	Analytical parameter	Oxygen consumption by the micro-organisms	
3.3.8	Sampling	At time zero (immediately after filling the bottles) the oxygen content and the pH were measured in each of the test condition bottles. The oxygen content was measured after 5, 15, 28 and 56 days in two bottles of every test condition (A to F).	

Section A7.1.1.2.1/02 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

		The pH was measured at time 0 and after 28 days.
3.3.9	Intermediates/ degradation products	Not assessed.
3.3.10	Nitrate/nitrite measurement	Not performed.
3.3.11	Controls	Yes, see 3.3.3. Bottles A: control BOD-bottles for the endogenous respiration (comparison to bottles B) Bottles B: control BOD-bottles for the oxygen depletion due to silica gel (comparison to bottles D) and for the biodegradation of the sodium acetate (comparison to bottles C) Bottles C: control BOD-bottles for the toxicity of etofenprox (comparison to bottles E) Bottles D: control BOD-bottles for the biodegradation of etofenprox (comparison to bottles F).
3.3.12	Statistics	Not performed

4 RESULTS

4.1	**Degradation of test substance**	
4.1.1	Graph	Not available
4.1.2	Degradation	17 % within 28 days for TS
4.1.3	Other observations	
4.1.4	Degradation of TS in abiotic control	Not performed
4.1.5	Degradation of reference substance	72 % within 28 days for sodium acetate
4.1.6	Intermediates/ degradation products	Not assessed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Ready biodegradability of etofenprox has been assessed with the closed bottle test in which the biodegradability of organic compounds in an aerobic environment is determined in a closed bottle. The test compound which provides the sole source of carbon and energy was added to an aqueous mineral salt solution and exposed to relatively low numbers of unadapted micro-organisms for a period of 56 days. To follow the course of biodegradation, the oxygen consumption by the micro-organisms was determined. Sodium acetate was used as a positive control. The biodegradation was calculated as the ratio of the biochemical oxygen demand (BOD) to the theoretical oxygen demand (ThOD). The ThOD are estimated to be 2.59 g O_2/g etofenprox and 0.78 g O_2/g sodium acetate.
5.2	**Results and discussion**	Results are given in tables 5.2a and 5.2b. After 56 days of incubation there is no significant difference between the oxygen content of the bottle with the silica gel with and without

Section A7.1.1.2.1/02 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

		etofenprox. Sodium acetate was biodegraded also in the presence of etofenprox. It is thus concluded that etofenprox is not toxic to the microorganisms. From the results it can be concluded that the inoculum activity is good since 55% of the sodium acetate was degraded within 5 days and 72% within 28 days. This is the maximum degradation that could reasonably be expected for sodium acetate. Unexpectedly, some oxygen depletion due to the silica gel was measured (bottles B-D), but this is of the same order of magnitude as the endogenous respiration. This phenomenon does not influence the results of the tests.	
5.3	**Conclusion**	The active ingredient etofenprox does not meet the "ready biodegradability" criteria (measured BOD within 28 days at least 60% of TOD) as defined in the guidelines.	
5.3.1	Reliability	1	x
5.3.2	Deficiencies	No	

Table 3.3.1: Inoculum / Test organism.

Criteria	Details
Nature	secondary (biological) activated sludge
Species	not specified
Strain	not specified
Source	activated sludge plant treating predominantly domestic waste water
Sampling site	RWZI Horstermeer in Nederhorst den Berg, The Netherlands, on August 29, 1991
Laboratory culture	no
Method of cultivation	not applicable
Preparation of inoculum for exposure	not applicable
Pretreatment	The sludge was preconditioned to reduce the endogenous respiration rate. This was done by aerating the sludge (200 mg dry weight (DW)/litre) for one week.
Initial cell concentration	The sludge was diluted to a concentration in the BOD bottles of 2 mg (dry weight)/litre.

Table 3.3.2: Test system.

Criteria	Details
Culturing apparatus	280 ml capacity BOD (biochemical oxygen demand)-bottles (Wertheim, cat. no. 270202) The bottles were completely filled and closed with glass stoppers.
Number of culture flasks/concentration	7
Aeration device	no data
Measuring equipment	The oxygen meter used was a WTW Oxi 96 with WTW EO 96 electrode with an internal magnetic stirrer. The pH meter used was a Philips PW 9420.
Test performed in closed vessels due to significant volatility of TS	no

Table 3.3.3: Test conditions.

Criteria	Details
Composition of medium	1 ml of solutions A, B, C and D were added to 1000 ml distilled water. Solution A contains per 1000 ml distilled water: KH_2PO_4 8.5g K_2HPO_4 21.75g $Na_2HPO_4.2H_2O$ 33.3g The pH was checked and needs to be 7.2 Solution B contains 22.5 g $MgSO_4.7H_2O$ per 1000 ml distilled water. Solution C contains 27.5 g $CaCl_2$ per 1000 ml distilled water. Solution D contains 0.14 g $FeCl_3$ per 1000 ml distilled water.
Additional substrate	The test compound is practically insoluble in water. For that reason etofenprox was tested in the presence of silica gel to guarantee a reproducible availability of the compound to the microorganisms.
Test temperature	20 ± 1.5 °C
pH	6.9-7.0
Aeration of dilution water	Not specified
Suspended solids concentration	No assessed
Other relevant criteria	No

Table 5.2 a: Oxygen depletion (mean values of duplicate bottles, calculated from measured values).

O_2 depletion (mg O_2/l)					
Time (days)	Endogenous (A-B)	Silica gel (B-D)	Sodium acetate (B-C)	Sodium acetate + etofenprox (D-E)	Etofenprox (D-F)
5	0.25	0.60	2.90	3.45	0.75
15	0.45	1.20	3.05	4.15	0.85
28	0.50	1.65	3.75	3.90	0.90
56	0.45	2.60	3.75	2.90	-0.20

Table 5.2 b: Biodegradation.

	Biodegradation (%)			
	5 days	15 days	28 days	56 days
Sodium acetate	55	58	72	72
Etofenprox	14	16	17	-4

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2006
Materials and methods	**1.1 Reference:** The correct report number is C.DNL.62.001 **2.3 Deviations:** In addition to the deviations mentioned the following deviations were applied/ocurred: - Instead of an effluent/extract/mixture, activated sludge was used as an inoculum. The inoculum was taken from an actibated sludge plant. - The test compound is practically insoluble in water. For that reason etofenprox was tested in the presence of silicagel to guarantee a reproducible availability of the compound to the microorganisms. - In the protocol it was stated that the bottles were to be placed in a climate room at 20 ± 1°C. In this experiment, howeber, during short times (one day about five hours) the temperature was 20 ± 1.5°C. **3.3.3 Test conditions:** According to OECD guideline 301D at least 10 bottles per run should have been applied instead of only 7.
Conclusion	Agree with the applicant's version.
Reliability	2
Acceptability	Acceptable
Remarks	The test result is consistent with the outcome of a second test on biodegradability (modified sturm test, 32% degradation within 28 days, non-key study).

Section A7.1.2.1.1 Aerobic Biodegradation

Annex Point XI.-2.1 *Fate in STP*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Völkel, W. (2012), ^{14}C-Etofenprox – Biodegradation in activated sludge under aerobic conditions, IES Ltd, unpublished report No20110163, October 02, 2012. Experimental phase February 28 to September 03, 2012	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data on existing active substance for the purpose of its first entry for Product Type 18 into Annex I of Directive EC 98/8	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guidelines for Testing Chemicals: 314 B, Biodegradation in activated sludge (Adopted on October 03, 2008)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Etofenprox (unlabelled and ^{14}C labelled)	
3.1.1	Lot/Batch number	217196-CHEM-26-1(radiolabelled material). 5H0103 (unlabelled material for co-chromatography)	x
3.1.2	Specification	As given in section 2	
3.1.3	Purity	Radiochemical purity 100% (determined before use)	
3.1.4	Further relevant properties	Water solubility 22.5 µg/L	
3.1.5	Radiolabelled compound	Specific activity: 5.45 MBq/mg	
3.1.6	TS inhibitory to microorganisms	No	
3.1.7	Specific chemical analysis	Liquid scintillation counting, TLC and HPLC	
3.2	**Reference substance**	No. Reference standards were used for identification of metabolites by co-chromatography.	
3.2.1	Initial concentration of reference substance	Not applicable	
3.3	**Test ing procedure**		
3.3.1	Inoculum / test species	Not adapted activated sludge (see table A7_1_1_2_1-2)	
3.3.2	Test system	The test was performed in 1 L all-glass flasks. The sludge was agitated by a magnetic stirrer in order to keep the solids in suspension and the system was continuously ventilated with moistened air. The exhaust air was passed through a trapping system equipped with, in total, two	

Section A7.1.2.1.1 **Aerobic Biodegradation**

Annex Point XI.-2.1 ***Fate in STP***

		absorption traps, one containing ethylene glycol and the other 2 N NaOH (in this sequence) per flask, to trap organic volatiles and $^{14}CO_2$, respectively. In addition, 2 L of activated biotic sludge and abiotic sludge were placed in 5 L all-glass metabolism flasks and incubated together with the smaller flasks to determine the formation of $^{14}CO_2$ and other radioactive volatiles. (see table A7_1_1_2-3)
3.3.3	Test conditions	200 mL of activated biotic sludge (total suspended solids TSS 4.38 g/L in tap water) or abiotic sludge (TSS 4.30 g/L in tap water autoclaved and poisoned with HgCl2) were placed in the 1 L flasks and the pH measured. The flasks were incubated in the dark at a temperature of 20.1 ± 0.1 °C for 30 days. 2 L of activated biotic sludge and abiotic sludge were placed in the 5 L metabolism flasks and incubated together with the smaller flasks. (see table A7_1_1_2-4)
3.3.4	Method of preparation of test solution	A stock solution of ^{14}C-etofenprox was prepared in 5 mL acetone.1.35 mL of the stock solution was diluted in 12 mL acetone to prepare the application solution. The flasks containing 200 mL of biotic or abiotic sludge were treated by adding an aliquot of 200 µL of the application solution. The flasks containing 2 L of biotic or abiotic sludge were treated accordingly by adding 2000 µL of the application solution. The amount of organic solvent applied did not exceed 0.1% of the total aqueous volume.
3.3.5	Initial TS concentration	The target concentration of the test item was 1.5 µg etofenprox/L sludge suspension.
3.3.6	Duration of test	30 days
3.3.7	Analytical parameter	Chromatography, parent compound disappearance, CO_2 evolution
3.3.8	Sampling	Entire samples were taken for processing after 1 and 6 hours and after 1, 2, 7, 14 and 30 days of incubation. After centrifugation of the samples (60 min at approximately 2139 × *g* or 15 min at approximately 3000 × *g*) the supernatants were decanted into approximately 20 mL of acetone and the radioactivity determined in duplicate aliquots of 10 mL by LSC. Thereafter, concentrates were submitted to Thin Layer Chromatography (TLC) analysis. The solid phase was exhaustively extracted at room temperature using 10 mL acetone/water (6/1; v/v) up to 4 times. Each extraction was performed with a shaker at approximately 250 revolutions per minute for 30 minutes. Each extraction step was followed by centrifugation (60 min at 2139 × *g* or 15 min at 3000 × *g*) and filtration of the decanted extracts (Whatman 597 ½, Ø150 nm). The volume of the individual extracts was determined and the radioactivity quantified by LSC. All room temperature extracts containing more than 2% of the radioactivity applied were combined. Aliquots of the pooled extracts were concentrated under a steam of N_2 and the radioactivity in the concentrated extracts were measured by LSC. Selected samples were submitted to TLC analysis. The residual radioactivity remaining in solid phase after the extraction

Section A7.1.2.1.1 **Aerobic Biodegradation**

Annex Point XI.-2.1 ***Fate in STP***

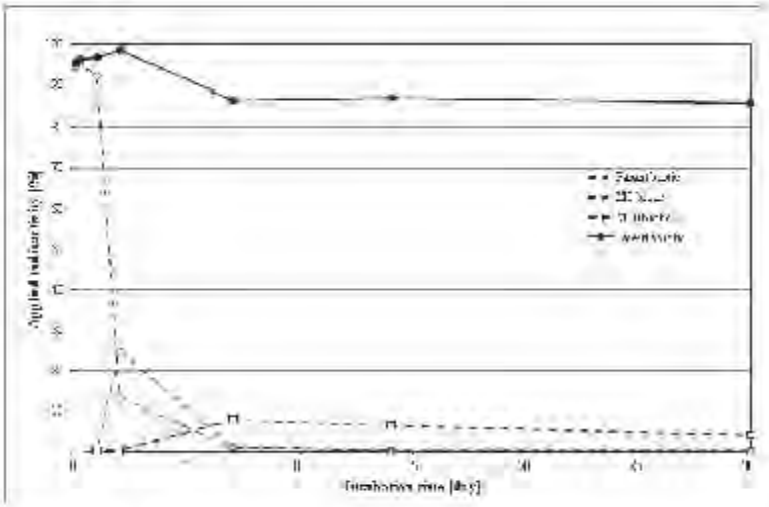
		procedure was quantified by LSC, after combusting an aliquot of the air-dried and homogenised solid phase.
3.3.9	Intermediates/ degradation products	Identified Identified by co-chromatography of reference standards
3.3.10	Nitrate/nitrite measurement	No
3.3.11	Controls	Abiotic controls were run alongside the activated sludge samples
3.3.12	Statistics	The calculations of DT_{50} and DT_{90} values were calculated by using FOCUS Kinetics Guidance on estimating persistence and degradation kinetics from Environmental Fate Studies. Kinetic fits were generated by CAKE version 1.4 (Release).

4 RESULTS

4.1 Degradation of test substance

4.1.1 Graph

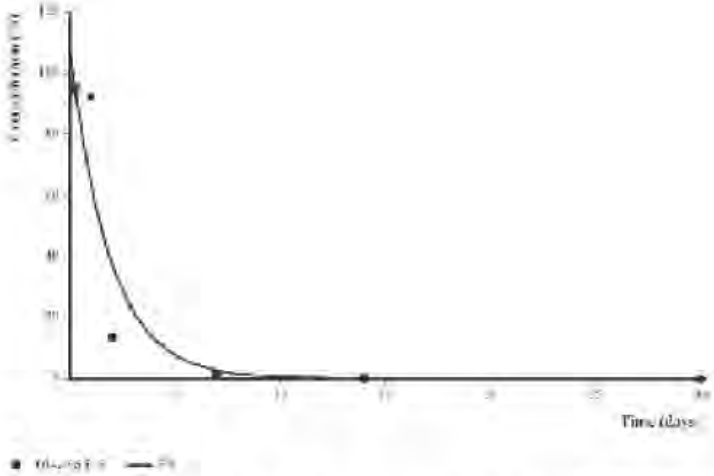
Graphical representation of the degradation of etofenprox in biotic and abiotic sludge



Degradation of etofenprox and fitted degradation kinetics curve (SFO) in biotic sludge

Section A7.1.2.1.1 Aerobic Biodegradation

Annex Point XI.-2.1 ***Fate in STP***



4.1.2 Degradation

No parent substance left after 7 days incubation. A degradation half-life of 1.3 days was calculated.

Pattern of degradation and formation of metabolites in biotic and abiotic sludge total system treated with ^{14}C-etofenprox. Values given in percent of applied radioactivity.

Total activated sludge [% AR]	Replicate	Incubation time [day]						
		0.04	0.25	1	2	7	14	30
Parent	biotic	94.6	95.3	92.2	13.3	0.9	*	*
	abiotic	95.3	96.2	96.8	98.6	86.6	87.2	86.0
M1 (PENA)	biotic	*	*	*	3.9	*	*	*
	abiotic	*	*	*	0.3	1.8	2.0	1.8
M2	biotic	*	*	4.4	2.2	*	*	*
	abiotic	*	*	*	*	*	*	*
M3	biotic	*	*	*	24.8	1.0	0.1	0.1
	abiotic	*	*	*	*	1.3	<0.1	0.2
M4 (4'-OH)	biotic	*	*	*	9.6	1.5	*	*
	abiotic	*	*	*	*	*	*	*
M5	biotic	*	*	*	2.2	2.5	*	*
	abiotic	*	*	*	*	*	*	*
M6	biotic	*	*	*	3.6	*	*	*
	abiotic	*	*	*	*	*	*	*
M7	biotic	*	*	*	2.7	0.3	*	*
	abiotic	*	*	*	*	*	*	6.0
M8 (a-CO)	biotic	*	*	*	*	*	*	*
	abiotic	*	*	*	*	4.0	*	*
M9	biotic	*	*	*	0.2	*	*	*
	abiotic	*	*	*	*	*	*	*
M10	biotic	*	*	*	0.1	7.7	6.3	3.9
	abiotic	*	*	*	*	*	*	*
M11	biotic	*	*	*	*	*	*	*
	abiotic	*	*	*	*	*	4.5	*
Not analysed**	biotic	3.0	3.1	1.6	*	2.3	3.9	4.1
	abiotic	2.2	4.1	1.2	0.6	4.4	2.6	1.8
Non-extractables	biotic	1.4	0.9	1.6	32.2	59.7	54.7	60.5
	abiotic	1.2	0.9	1.6	0.8	0.9	1.2	0.5
$^{14}CO_2$	biotic	n.p.	<0.1	<0.1	0.5	20.7	32.4	35.9
	abiotic	n.p.	<0.1	<0.1	0.4	0.1	<0.1	<0.1
Other volatiles in EG	biotic	<0.1	<0.1	<0.1	1.1	0.1	<0.1	<0.1
	abiotic	<0.1	0.1	<0.1	0.5	<0.1	<0.1	<0.1
TOTAL	abiotic	**99.1**	**99.4**	**99.9**	**96.5**	**96.7**	**97.4**	**104.6**
	biotic	**98.7**	**101.4**	**99.5**	**101.1**	**99.1**	**97.5**	**96.3**

* Not detectable.
** Not analysed due to low amounts of radioactivity in the respective samples.
n.p. Not performed.

Section A7.1.2.1.1 **Aerobic Biodegradation**

Annex Point XI.-2.1 ***Fate in STP***

4.1.3 Other observations

Several metabolites formed, one of which (M3) rose to 24.8% of the applied radioactivity after 2 days but was quickly degraded to 1% by day 7, and a second (M10) which appeared on day 2, reached its maximum of 7.7% at day 7 and degraded to 3.9% at the end of the study. The parent compound shows very strong sorption to the solids.

Recoveries of applied radioactivity in biotic and abiotic sludge treated with ^{14}C-etofenprox. Values given in percent of applied radioactivity.

Activated Sludge [% AR]	Replicate	Incubation time [day]						
		0.04	0.25	1	2	7	14	30
Aqueous Phase	biotic	3.0	3.1	1.6	27.0	10.2	7.2	5.5
	abiotic	2.2	4.1	1.2	1.7	4.4	7.0	7.9
Room temperature extracts	biotic	94.6	95.3	96.6	35.7	6.0	3.0	2.7
	abiotic	95.3	96.2	96.8	97.7	93.7	89.2	87.9
Extracts and aqueous phase	biotic	97.6	98.4	98.2	62.7	16.2	10.3	8.1
	abiotic	97.5	100.3	97.9	99.4	98.1	96.2	95.8
Non-extractables	biotic	1.4	0.9	1.6	32.2	59.7	54.7	60.5
	abiotic	1.3	0.9	1.6	0.8	0.9	1.2	0.5
$^{14}CO_2$	biotic	<0.1	<0.1	<0.1	0.5	20.7	32.4	35.9
	abiotic	<0.1	<0.1	<0.1	0.4	0.1	<0.1	<0.1
Other volatiles in EG	biotic	<0.1	<0.1	<0.1	1.1	0.1	<0.1	<0.1
	abiotic	<0.1	0.1	<0.1	0.5	<0.1	<0.1	<0.1
TOTAL	biotic	**99.1**	**99.4**	**99.9**	**96.5**	**96.7**	**97.4**	**104.6**
	abiotic	**98.7**	**101.4**	**99.5**	**101.1**	**99.1**	**97.5**	**96.3**
MEAN ± SD	biotic				**99.1 ± 2.8**			
	abiotic				**99.1 ± 1.8**			

4.1.4 Degradation of TS in abiotic control

Test substance is practically not degraded in abiotic controls

4.1.5 Degradation of reference substance

Not applicable

4.1.6 Intermediates/ degradation products

See 4.1.1 and 4.1.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The degradation study of etofenprox under aerobic conditions was performed according to the OECD 314B guideline. The inoculum (activated sludge) was obtained from an activated sludge plant treating predominantly domestic waste water and was used at a concentration of about 4.3g/L in 1- or 5-litre glass vessels. The flasks were incubated in the dark at a temperature of 20.1 ± 0.1 °C for 30 days at a concentration of 1.5 µg etofenprox/L sludge suspension.

Entire samples were taken for processing after 1 and 6 hours and after 1, 2, 7, 14 and 30 days of incubation. After centrifugation of the samples (60 min at approximately 2139 × *g* or 15 min at approximately 3000 × *g*) the supernatants were decanted into approximately 20 mL of acetone and the radioactivity determined in duplicate aliquots of 10 mL by LSC. Thereafter, concentrates were submitted to Thin Layer Chromatography (TLC) analysis.

The solid phase was exhaustively extracted at room temperature using 10 mL acetone/water (6/1; v/v) up to 4 times. Each extraction was performed with a shaker at approximately 250 revolutions per minute for 30 minutes.

Each extraction step was followed by centrifugation (60 min at 2139 × *g* or 15 min at 3000 × *g*) and filtration of the decanted extracts (Whatman 597 ½, Ø150 nm). The volume of the individual extracts was

Section A7.1.2.1.1 **Aerobic Biodegradation**

Annex Point XI.-2.1 ***Fate in STP***

		determined and the radioactivity quantified by LSC. All room temperature extracts containing more than 2% of the radioactivity applied were combined. Aliquots of the pooled extracts were concentrated under a steam of N_2 and the radioactivity in the concentrated extracts were measured by LSC. Selected samples were submitted to TLC analysis. The residual radioactivity remaining in solid phase after the extraction procedure was quantified by LSC, after combusting an aliquot of the air-dried and homogenised solid phase. The half life was determined by following the FOCUS kinetics guideline using the CAKE version 1.4 software.	
5.2	**Results and discussion**	The total mean recovery and standard deviation in biotic and abiotic samples was 99.1 ± 2.8% of applied radioactivity (AR) and 99.1 ± 1.8% AR, respectively. One hour after treatment, 3.0% of applied radioactivity was recovered from the aqueous phase of the biotic test. The amount of radioactivity in the aqueous phase increased to a maximum of 27.0% AR at 2 days, followed by a decrease over time, reaching 5.5% AR after 30 days of incubation. The extractable radioactivityat room temperature decreased rapidly from 94.6% after one hour to a minimum of 2.7% AR after 30 days of incubation. Accordingly the amount of non-extractable radioactivity increased from 1.4% AR to a maximum of 60.5% AR after 30 days of incubation. Mineralisation of ^{14}C-etofenprox in biotic sludge reached a maximum of 35.9% AR after 30 days of incubation. Only minor amounts of volatile products, other than $^{14}CO_2$, were detected, reaching a maximum of 1.1% AR. In the abiotic system most of the radioactivity remained as extractable radioactivity from the sludge and was identified as etofenprox.	X
		The pattern of degradation of ^{14}C-etofenprox and formation of the main metabolite are graphically presented in 4.1.1. ^{14}C-Etofenprox rapidly degraded in biotic sludge. The total amount of ^{14}C-etofenprox decreased from 94.6% AR after 1 hour of incubation to 0.9% AR after 7 days of incubation, and was not detectable after 14 and 30 days of incubation, respectively (Table in 4.1.2). In abiotic sludge, the degradation of ^{14}C-etofenprox was slower, with ^{14}C-etofenprox decreasing from 95.3% AR after 1 hour of incubation to 86.0% AR after 30 days of incubation. ^{14}C-Etofenprox degraded into 11 radioactive fractions. In biotic sludge, one radioactive fraction (M3) exceeded 10% AR, and one radioactive fraction (M10) reached 5% AR at two consecutive sampling intervals. While M3 was not detected in biotic sludge incubated for up to 1 day, M3 accounted for 24.8% AR at 2 days of incubation. Thereafter, the amount of M3 decreased to 1.0% AR at 7 days of incubation and 0.1% AR was detected after 14 and 30 days of incubation, respectively.	x
5.3	**Conclusion**	The degradation of ^{14}C-etofenprox was investigated in biotic sludge incubated under aerobic conditions in the dark at a temperature of 20.1 ± 0.1 °C. ^{14}C-Etofenprox rapidly degraded, resulting in a DT_{50} value of 1.3 days following SFO kinetics.	x
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A7.1.2.1.1 Aerobic Biodegradation

Annex Point XI.-2.1 *Fate in STP*

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2012
Materials and Methods	**3.1.1 Lot/Batch number** Typing error: 217196-CHEM**357**-26-1
Results and discussion	**4.1.3 Other observations** Etofenprox degraded into 11 radioactive fractions. **5.2 Results and discussion** In the abiotic system most **(87.9% AR after 30 days)** of the radioactivity remained as extractable radioactivity from the sludge and was identified as etofenprox. Company statement concerning metabolite M3: Characterisation was attempted by co-chromatography with reference standards of the already known metabolites (α-CO, PENA and 4'-OH), but it was none of the available metabolites. From its polarity it was guessed that etofenprox split and this is one part of the molecule. Identification was not possible due to the low concentrations. From the available data a degradation half life of 0.9 days was calculated.
Conclusion	**5.4 Conclusion** DT_{50} = 1.3 days DT_{90} = 4.4 days
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
Simulation Test with activated Sewage	-	314B	Simulation Test[1)]

[1)] Test for the determination of the primary and 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 plant treating predominantly domestic waste water
Sampling site	Basel (ARA Birs, 4127 Birsfelden)
Laboratory culture	No
Method of cultivation	not applicable
Preparation of inoculum for exposure	The sludge was passed through a 2 mm sieve and constantly aerated before use. Dilution with tap water to achieve suspended solids concentration
Pretreatment	no adaptation
Initial cell concentration	Approximately 4.3 g activated sludge/L

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	1- or 5-litre glass bottles. The sludge was agitated by a magnetic stirrer in order to keep the solids in suspension and the system was continuously ventilated with moistened air. The exhaust air was passed through a trapping system equipped with, in total, two absorption traps, one containing ethylene glycol and the other 2 N NaOH (in this sequence) per flask, to trap organic volatiles and $^{14}CO_2$, respectively
Number of culture flasks/concentration	16
Aeration device	Moisture air
Measuring equipment	Radioactivity measurement with LSC, TLC and HPLC
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	Tap water
Additional substrate	No
Test temperature	20.1±0.1°C
pH	-
Aeration of dilution water	Yes
Suspended solids concentration	Approximately 4.3 g activated sludge/L
Other relevant citeria	Agitation with magnetic stirrer

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

	fulfilled	not fulfilled
Pass levels		
Not applicable		
Criteria for validity		
Mass balance 90-110%	×	

Section 7.1.2.2.2/01 Degradation in Water-Sediment Systems

Annex Point IIIA-XII.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Lewis C.J. (2001): (14C)-MTI-500: Degradation and retention in water-sediment systems. Covance Laboratories Ltd., Harrogate, England; unpublished report no. CLE 719/6-D2142 (January 29, 2001) Experimental phase September 15, 1998 to October 23, 2000 Lewis, C.J. (2001): (14C)-MTI-500: Degradation and retention in water-sediment systems. Amended Final Report 1. Covance Laboratories Ltd., Harrogate, England; unpublished report no. CLE 719/6-D2142 (July 22, 2002) This amendment gives an explanation of the consequences of a mis-labelled reference standard supplied as 4'-OH. The degradation product named Unknown 1 in this study, should have been referred as 4'-OH	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes SETAC (1995) and EC Directive 95/36/EC (1995)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	MTI-500, Etofenprox	x
3.1.1	Lot/Batch number	9604	
3.1.2	Specification	As given in section 2	x
3.1.3	Purity	99.99 %	
3.1.4	Further relevant properties	Solubility in water: 22.5 µg/L	
3.1.5	Radiolabelling	A) [α-^{14}C-benzyl]-etofenprox - Batch: MRH/MTC 277/29 - Specific activity: 366.67 MBq/mmol - Radiochemical purity: >99% (from certificate of analysis) (B) [2-^{14}C-propyl]-etofenprox - Batch: MRH/MTC 276/37 - Specific activity: 576.09 MBq/mmol) - Radiochemical purity: >99% (from CoA)	
3.1.6	TS inhibitory to microorganisms	No	
3.2	**Reference substance**	No	

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3.3	**Testing procedure**		
3.3.1	Sediment	Water and sediment from the Mill stream pond (Dorset, UK) and the Emperor Lake (Derbyshire, UK) were sampled on 7 and 1 July 1998, respectively. The water and sediment samples were passed through a 0.2 and 2 mm sieve, respectively, and dispensed into the incubation vessels on the day (Emperor Lake), or the day after (Mill stream pond). The water and sediment characteristics are listed in Table A7_1_2_2_2_01-1)	x
3.3.2	Test system	Details on laboratory equipment etc. in tabular form (see table A7_1_2_2_2_01-2) Each test vessel (glass cylinders of 4.5 cm diameter) contained a 2.5 cm sediment layer (dry weight of 16.5 g for Mill stream pond and 24.8 g for Emperor lake) covered with water to a depth of 6 cm (weight of 97.1 and 99.4 g for Mill stream pond and Emperor lake, respectively). Prior to application, the water-sediment units were pre-incubated for 67 days (Mill stream pond, group A) or 74 days (Emperor Lake, group B) in the dark at 20±2°C until equilibration. Moistened CO_2-free air was drawn over the water surface. An additional incubation group consisted of the Emperor lake water-sediment system (group C) acclimatised under a 12 h fluorescent lighting/12 h dark regime.	x
3.3.3	Test conditions	Relevant test conditions in tabular form (see table A7_1_2_2_2_01-3)	
3.3.4	Method of preparation of test solution	Equal amounts (873 kPq) of each radiolabelled test item were pooled, the solvent removed and the residue reconstituted in acetonitrile to produce a 0.35 mg/mL solution. 3.6.1.3 non-labelled test item ware dissolved in 10 ml acetonitrile (0.26 mg/ml).	
3.3.5	Application of test item	After pre-incubation, [^{14}C]-etofenprox was applied at a rate of 32.6 µg/unit, equivalent to a field rate of approximately 200 g a.s./ha and a surface area of the vessels of 15.9 cm^2. The test substance, in acetonitrile (92 µL) was dispensed drop-wise, onto the surface of the water of eight water-sediment units per incubation group.	
3.3.6	Duration of test	Pre-incubation: 67 days Mill Stream (A), 74 days Emperor lake (B) Post application: 99 days	
3.3.7	Temperature / light	20 ± 2 °C / 12 hours light/dark cycles for group C	
3.3.8	Sampling	Single incubation units from each group were removed for analysis at intervals of 0 (immediately after application), 7, 14, 30, 59 and 99 days after application. At each sampling date, the surface water was carefully removed from the sediment and partitioned twice with dichloromethane. The sediment was extracted 3 times with methanol and then with methanol/HCl (95:5, v/v). Sediments for fractionation into fulvic acid, humic acid and humin were extracted with NaOH, and the radioactivity in the humin fraction was determined by combustion followed by LSC. Radioactivity in the surface water's dichloromethane extracts and in the sediment's methanol and methanol/HCl extracts, containing >5% of applied radioactivity was analysed by HPLC and TLC. The trap reagents were collected when the units to which they were attached were removed, and, for units incubated for longer than a month, additionally at 30 and 59 days after the application. Radioactivity in the trapping solutions was quantified by LSC. Finally, the radioactivity associated with the	

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		apparatus used in the study was determined by LSC.
3.3.9	Intermediates/ degradation products	Identified using HPLC, LC-MS or TLC (see below). The radioactivity, containing >5% of applied radioactivity, was analysed by HPLC for identification of the degradation products.
3.3.10	Analytical methods	HPLC with radioactivity detection, TLC; LC-MS; Liquid scintillation counting. Details see table A7_1_2_2_2_01-4
3.3.11	Statistics	The DT_{50} and DT_{90} values of etofenprox in the two natural water/sediment systems were calculated assuming first order kinetics.
		4 RESULTS.
4.1	**Recovery**	Overall recovery decreased from 96, 98 and 97% of applied radioactivity, initially, to 84, 83 and 93% after 99 days, in incubation groups A, B and C, respectively. Recovery of radioactivity decreased to and remained below 90% in the Mill stream pond system (dark) at 14 days and in the Emperor Lake system (dark) at 99 days. Recovery of radioactivity from the Emperor Lake system, under light/dark cycle, was higher than 90% at all times.
4.2	**Degradation of test substance**	
4.2.1	Mineralisation	After 99 days of incubation, 28, 18 and 19% of the applied radioactivity present in the NaOH traps was shown to be $^{14}CO_2$ in the Mill stream pond, the Emperor Lake incubated in the dark and in the Emperor Lake incubated under a light/dark cycle, respectively. No other volatile products could be detected (≤0.1% of applied radioactivity) in the ethanediol and 2% paraffin in xylene traps.
4.2.2	Test item	The level etofenprox in the surface water amounted initially from 22 to 32% of the applied radioactivity in the three incubated groups. Not more than 1% was detected after 14 days, 30 or 59 days after application in groups A, C and B, respectively. The initial decrease was more rapid in the Mill stream pond system than in the Emperor Lake system and more rapid under a light/dark cycle than in the dark. Levels of etofenprox in the sediment decreased from between 62 and 70% of applied radioactivity, initially, to between 8 and 25% at 99 days, in all groups.
4.2.3	Metabolites	Only one degradate, later identified as 4'-OH (see Amendment no. 1), exceeded 10% of applied radioactivity. It was mainly present in the sediments and reached the maximum levels of 14.4, 16.2 and 21.9% of applied radioactivity, in the whole system, in incubation groups B (day 14), C (day 7) and A (day 7), respectively, and then decreased to ≤10% of applied radioactivity at 30 days. A large number of minor degradation products were present in the water-sediment systems, each accounting for less than 10% of applied radioactivity (structure not identified). Three metabolites were identified as DP, PB-acid (also known as m-PB-acid) and P-acid (also known as EPMP) and reached maximum values of 7.0% (day 30), 2.4% (day 14) and 5.4% (day 30) of the applied radioactivity, respectively, in water and sediment phases of the Emperor Lake system under a light/dark cycle. However, the identity of α-CO (maximum 0.8%) and DE + 4'-OH (maximum 2.5%) was not confirmed by TLC although the metabolic pathway based on the identified

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compounds proposes these metabolites. Their low accumulation indicates that they are quickly degraded into identified or other unidentified compounds in the water sediment systems.

The results are described in more detail in tableA7_1_2_2_2_01-5

4.2.4 Degradation of etofenprox in aquatic systems

Based on the results of the water/sediment study, the degradation rates of etofenprox in the water phases and entire systems were calculated assuming first order kinetic. The DT_{50}- and DT_{90}-values were calculated for the 3 incubation systems. There was a more rapid dissipation of etofenprox under a light/dark cycle (group C) than in the dark (group B). x

System	Mill stream pond dark	Emperor Lake dark	Emperor Lake light/dark
Water phase	DT_{50} = 2.1 days DT_{90} = 7.1 days	DT_{50} = 10.4 days DT_{90} = 34.5 days	DT_{50} = 2.1 days DT_{90} = 7.1 days
Entire system	DT_{50} = 6.5 days DT_{90} = 23.8 days	DT_{50} = 20.1 days DT_{90} = 71.0 days	DT_{50} = 7 or 22 days* DT_{90} = 104 or >99 days*

* computed values using the poor fit suggest a DT_{50} of 22 days and DT_{90} of 104 days.

4.2.5 Degradation of 4'-OH metabolite

The degradation rates of the metabolite 4'-OH were calculated only for the total system, because most of the compound was present in the sediment phase

System	Mill stream pond dark	Emperor Lake dark	Emperor Lake light/dark
Entire system	DT_{50} = 29.7days DT_{90} = 97.9 days	DT_{50} = 21.8 days DT_{90} = 59.8 days	DT_{50} = 27.0 days DT_{90} = 87.1 days

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The degradation and metabolism was investigated in two natural water-sediment systems (Mill stream pond and Emperor Lake) using a mixture (1+1) of [2-^{14}C-propyl]-etofenprox (batch no. MRH/MTC276/37; radiochemical purity: >99%) and [α-^{14}C-benzyl]-etofenprox (batch no. MRH/MTC277/29; radiochemical purity: >99%) in the dark over a period of 99 days. In addition the degradation of [^{14}C]-etofenprox was determined in the Emperor Lake water-sediment system under a 12 h dark/12 h light photoperiod.

Water and sediment from the Mill stream pond (Dorset, UK) and the Emperor Lake (Derbyshire, UK) were sampled on 7 and 1 July 1998, respectively. The water and sediment samples were passed through a 0.2 and 2 mm sieve, respectively, and dispensed into the incubation vessels on the day (Emperor Lake), or the day after (Mill stream pond).

Each test vessel (glass cylinders of 4.5 cm diameter) contained a 2.5 cm sediment layer (dry weight of 16.5 g for Mill stream pond and 24.8 g for Emperor lake) covered with water to a depth of 6 cm (weight of 97.1

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and 99.4 g for Mill stream pond and Emperor lake, respectively). Prior to application, the water-sediment units were pre-incubated for 67 days (Mill stream pond, group A) or 74 days (Emperor Lake, group B) in the dark at 20±2°C until equilibration. Moistened CO_2-free air was drawn over the water surface. An additional incubation group consisted of the Emperor lake water-sediment system (group C) acclimatised under a 12 h fluorescent lighting/12 h dark regime.

After pre-incubation, [^{14}C]-etofenprox was applied at a rate of 32.6 µg/unit, equivalent to a field rate of approximately 200 g a.s./ha and a surface area of the vessels of 15.9 cm^2. The test substance, in acetonitrile (92 µL) was dispensed dropwise, onto the surface of the water of eight water-sediment units per incubation group.

Thereafter, incubation units were maintained in the dark (groups A and B) or under a 12 h light/dark cycle (group C) at 20±2°C over a period of 99 days. During the incubation period, the units were slightly agitated on an orbital shaker and air was drawn over the water surface. The effluent air from the incubation units was passed trough a series of traps to collect polar and non-polar volatiles (ethanediol and 2% paraffin in xylene traps) and to absorb evolved CO_2 (two 0.5M NaOH traps).

At each sampling date, the surface water was carefully removed from the sediment and partitioned twice with dichloromethane. The sediment was extracted 3 times with methanol and then with methanol/HCl (95:5, v/v). Sediments for fractionation into fulvic acid, humic acid and humin were extracted with NaOH, and the radioactivity in the humin fraction was determined by combustion followed by LSC.

During incubation, redox potential of sediment and redox potential, pH and oxygen content of water were determined weekly during acclimatisation and at each sampling timepoint during the study.

The microbial biomass of the sediments was determined by the fumigation-extraction method at the beginning and at the end of the study

Single incubation units from each group were removed for analysis at intervals of 0 (immediately after application), 7, 14, 30, 59 and 99 days after application. Radioactivity in the surface water's dichloromethane extracts and in the sediment's methanol and methanol/HCl extracts, containing >5% of applied radioactivity was analysed by HPLC and TLC. The radioactivity, containing >5% of applied radioactivity, was analysed by HPLC for identification of the degradation products. The trap reagents were collected when the units to which they were attached were removed, and, for units incubated for longer than a month, additionally at 30 and 59 days after the application. Radioactivity in the trapping solutions was quantified by LSC. Finally, the radioactivity associated with the apparatus used in the study was determined by LSC.

The DT_{50} and DT_{90} values of etofenprox in the two natural water/sediment systems were calculated assuming first order kinetics.

5.2 Results and discussion

Recovery

Overall recovery decreased from 96, 98 and 97% of applied radioactivity, initially, to 84, 83 and 93% after 99 days, in incubation groups A, B and C, respectively. Recovery of radioactivity decreased to and remained below 90% in the Mill stream pond system (dark) at 14 days and in the Emperor Lake system (dark) at 99 days. Recovery of

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		radioactivity from the Emperor Lake system, under light/dark cycle, was higher than 90% at all times. Mineralization After 99 days of incubation, 28, 18 and 19% of the applied radioactivity present in the NaOH traps was shown to be $^{14}CO_2$ in the Mill stream pond, the Emperor Lake incubated in the dark and in the Emperor Lake incubated under a light/dark cycle, respectively. No other volatile products could be detected (≤0.1% of applied radioactivity) in the ethanediol and 2% paraffin in xylene traps. Identification of radioactivity The level etofenprox in the surface water amounted initially from 22 to 32% of the applied radioactivity in the three incubated groups. Not more than 1% was detected after 14 days, 30 or 59 days after application in groups A, C and B, respectively. The initial decrease was more rapid in the Mill stream pond system than in the Emperor Lake system and more rapid under a light/dark cycle than in the dark. Levels of etofenprox in the sediment decreased from between 62 and 70% of applied radioactivity, initially, to between 8 and 25% at 99 days, in all groups. Only one metabolite, later identified as 4'-OH (see Amendment no. 1), exceeded 10% of applied radioactivity. It was mainly present in the sediments and reached the maximum levels of 14.4, 16.2 and 21.9% of applied radioactivity, in the whole system, in incubation groups B (day 14), C (day 7) and A (day 7), respectively, and then decreased to ≤10% of applied radioactivity at 30 days. A large number of minor degradation products were present in the water-sediment systems, each accounting for less than 10% of applied radioactivity (structure not identified). Three metabolites were identified as DP, PB-acid (also known as m-PB-acid) and P-acid (also known as EPMP) and reached maximum values of 7.0% (day 30), 2.4% (day 14) and 5.4% (day 30) of the applied radioactivity, respectively, in water and sediment phases of the Emperor Lake system under a light/dark cycle. However, the identity of α-CO (maximum 0.8%) and DE + 4'-OH (maximum 2.5%) was not confirmed by TLC although the metabolic pathway based on the identified compounds proposes these metabolites. Their low accumulation indicates that they are quickly degraded into identified or other unidentified compounds in the water sediment systems.	
5.3	**Conclusion**	The results of this study showed a more rapid dissipation of etofenprox applied at a rate equivalent to 200 g a.i./ha in a water-sediment system, i.e. Emperor Lake, under a light/dark cycle (DT_{50} = 2.1 days) than in the dark (DT_{50} = 10.4 days). A rapid dissipation was also observed in the second water-sediment system, i.e. Mill stream pond, in the dark (DT_{50} = 2.1 days). In the water phase, only the parent compound could be identified in relevant amounts, i.e. >3.5%. Its maximum initial concentration was 22 to 32% of the applied radioactivity, then decreased to<1% on days 14 to 59. One major degradation product, later identified as 4'-OH, was detected mainly in sediment extracts in all incubation groups at the maximum levels of 14.4 to 21.4% of applied radioactivity at 7 or 14 day, and thereafter, decreased to ≤10% of applied radioactivity after 30 days. The dissipation time (DT_{50}) of 4'-OH in the entire system ranged from 22 to 30 days.	x
5.3.1	Reliability	1	x

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5.3.2	Deficiencies	No

Table A7_1_2_2_2_01-1: Characteristics of the surface waters and sediment of the study of Lewis (2001).

System			Mill stream pond	Emperor Lake
Surface water				
Total nitrogen		[mg/L]		
	at start		<0.05	<0.05
	at end		10.5	6.3/4.9*
Total phosphorus		[mg/L]		
	at start		0.5	<0.05
	at end		1.1	0.2/0.3*
Dissolved organic carbon		[mg/L]		
	at start		37.2	32.5
	at end		139.9	42.0/56.0*
Sediment				
Sediment type (UK classification)			clay loam	sandy (clay) loam
Particle size distribution		[%]		
sand	63 – 2000 µm		43.88	62.02
silt	2 – 63 µm		36.70	19.85
clay	< 2 µm		19.42	18.13
Organic carbon		[%]	7.3	5.1
pH	(water)		7.8	6.1
	(1M KCl)		7.6	5.5
CEC		[meq/100 g dry mass]	37.4	26.5
Microbial biomass**		[µg C/g soil]		
	at start		1797.3	1359.3
	at end		131.9	147.1/681.8*
MWHC		[%]	149.0	102.2

MWHC maximum water holding capacity
CEC cation exchange capacity
* Values determined in the soil sample incubated under a 12 h light/12 h dark regime
** Microbial biomass determined by the fumigation-extraction method according to VANCE, BROOKS AND JENKINSON (1987).

Table A7_1_2_2_2_01-2: Incubation system.

Criteria	Details
Apparatus	Borosilicate glass cylinders (4.5 cm diameter), containing sediment to a height of 2.5 cm and 6 cm associated water (kept constant by adding deionised water
Number of replicates/concentration	System A: Mill stream pond system in the dark System B: Emperor lake in the dark System C: 12 hours dark / 12 h fluorescence light system 1 replicate for each system
Air pre-treatment	The incoming air was passed through a soda lime trap, a deionised water trap and a safety trap before entering the incubation vessel.
Trapping system	The exhaust air was passes trough a series of traps: Trap1: ethanediol Trap2: 2% paraffin in xylene Trap3: 0.5 M NaOH Trap 4: 0.5 M NaOH

	Trap 5 deionised water (after outlet manifold)

Table A7_1_2_2_2_01-3: Test conditions.

Criteria	Details		
Oxygen concentration	Determined approx. weekly during acclimatisation and at each sampling point. (Reported as % saturation; Jenway 9070 oxygen meter)		
		Acclimatisation	Test phase
	A:	50 to 90 %	>90 %
	B:	60 to 100 %	70 -95 %
	C:	60 to 100 %	>90 %
pH value in water	Measured in the surface water using a calibrated pH meter and pH electrode.		
	A:	8.0 – 8.8	8.5 – 9.0
	B:	5.6 – 7.5	6.7 – 8.7
	C:	5.5 – 7.5	4.5 – 8.5
pH value in sediment	A:	8.0 – 8.8	not measured
	B:	6.0 - 6.8	not measured
	C:	5.5 – 6.9	not measured
Redox potential in water	Measured using a calibrated electrode attached to a mV meter		
	A:	290 – 420 mV	310 – 410
	B:	400 – 500 mV	350 - 590
	C:	400 – 600 mV	350 – 600
Redox potential in sediment	Measured using a calibrated electrode attached to a mV meter		
	A:	-70 – 20 mV	-70 – 60
	B:	50 – 200 mV	0 -110
	C:	0 – 180 mV	-10 - 100

System A: Mill stream pond / dark
System B: Emperor lake / dark
System C: Emperor lake / 12 hours photoperiod

Table A7_1_2_2_2_01-4: Details of the analytical methods.

Method	Details	
HPLC 1	Column	Inertsil ODS2 (250 x 4.6 mm; 5 µm)
	Mobile phase	A: acetic acid (1%) in water; B: acetic acid (1%) in methanol 0 min 40 % A 30 min 17 % A 45 min – 50 min: 0 % A 55 min – 60 min 40 % A
	Flow rate	1 ml/min
	Detection	UV at 254 nm; radioactivity using Ramona flow through monitor or β-ram (Lablogic) with CaF_2 cell
HPLC 2	Column	Capital ODS-H (150 x 4.6 mm; 3 µm)
	Mobile phase	A: acetic acid (1%) in water; B: acetic acid (1%) in acetonitrile 0 min 70 % A 25 min 0 % A 30 min – 35 min:70 % A
	Flow rate	1 ml/min

	Detection	UV at 234 nm; radioactivity using Ramona flow through monitor or β-ram (Lablogic) with CaF_2 cell

Table A7_1_2_2_2_01-4: Details of the analytical methods (continued).

HPLC 4	Column	Capital ODS-H (150 x 4.6 mm; 3 µm)
	Mobile phase	A: acetic acid (1%) in water; B: acetic acid (1%) in acetonitrile 0 min 70 % A 25 min – 30 min 0 % A 35 min – 40 min :70 % A
	Flow rate	1 ml/min
	Detection	UV at 234 nm; radioactivity using Ramona flow through monitor or β-ram (Lablogic) with CaF_2 cell
TLC 1	Plates	Merck silica gel $60F_{254}$ or Whatman K6F silica gel60 A
	Solvent	Toluene 100 %
TLC 2	Plates	Merck silica gel $60F_{254}$ or Whatman K6F silica gel60 A
	Solvent	Hexane / diethyl ether (8:1 v/v)%
TLC 3	Plates	Merck silica gel $60F_{254}$ or Whatman K6F silica gel60 A
	Solvent	Trimethylpentane / propan-2-ol (19:1 v/v)
TLC 4	Plates	Merck silica gel $60F_{254}$
	Solvent	Hexane / ethyl acetate / ammonia (80:20:1 v/v/v)
TLC 5	Plates	Whatman K6F silica gel60 A
	Solvent	Hexane / toluene (2:1 v/v)
TLC 6	Plates	Whatman K6F silica gel60 A
	Solvent	Chloroform 100%
TLC 7	Plates	Whatman K6F silica gel60 A
	Solvent	Hexane / ethyl acetate (9:1 v/v)
TLC 8	Plates	Whatman K6F silica gel60 A
	Solvent	Toluene / ethyl acetate /acetic acid (90:10:1 v/v/v)
TLC 9	Plates	Whatman K6F silica gel60 A
	Solvent	Chloroform /methanol/ acetic acid (9:1 v/v)
LC/MS System 1	Instrument	Finnigan LCQ Ion Trap Mass Spectrometer with HP1050 quaternary or HP1100 binary HPLC system via API interface
	Column	Capitol ODS H 150 x 4.6 mm
	Mobile phase	A: 1.0 acetic acid in water; B: 1.0 % acetic acid in acetonitrile 0 min 70 % A; 25 min – 30 min 0 % A
	Detection	m/z 100 – 700; ESP-
LC/MS System 2	Instrument	Finnigan LCQ Ion Trap Mass Spectrometer with HP1050 quaternary HPLC system via API interface
	Column	Capitol ODS H 150 x 4.6 mm
	Mobile phase	A: water; B: acetonitrile 0 min70 % A; 25 min – 30 min 0 % A
	Detection	m/z 100 – 700; ESP-, ESP+
LC/MS System 3	Instrument	Finnigan LCQ Ion Trap Mass Spectrometer with HP1100 binary HPLC system via API interface
	Column	Zorbax SIL 250 x 4.6 mm
	Mobile phase	A: Hexane/ethylacetate/ammonia (80:20 v/v); B: Methanol/ammonia (80:20 v/v) 0 min 100 % A; 25 min – 30 min 10 % A
	Detection	m/z 100 – 600; APCI+

Table A7_1_2_2_2_01-5: Degradation of etofenprox and formation of metabolites in water/ sediment systems (values are given as % of applied radioactivity).

Days after application	Mill stream pond in the dark (group A)					
	0	7	14	30	59	99
Water phase	**23.3**	**10.3**	**23.8**	**4.9**	**0.9**	**0.7**
extractable	**22.4**	8.2	19.6	3.0	0.3	0.2
Etofenprox	**22.3**	2.2	0.7	–	–	–
4'-OH	n.d.	**0.6**	0.5	–	–	–
DP	n.d.	0.3	**1.2**	–	–	–
PB-acid	n.d.	**0.3**	n.d.	–	–	–
P-acid*	n.d.	1.5	**1.7**	–	–	–
not extractable	0.9	2.1	**4.2**	1.9	0.6	0.5
Sediment	**72.6**	**80.2**	**62.6**	**64.4**	**54.8**	**55.3**
extractable	72.5	**76.2**	51.7	35.5	31.3	32.7
Etofenprox	**70.1**	42.3	15.1	10.7	7.2	7.8
4'-OH	n.d.	**21.4**	17.1	7.0	7.4	6.1
DP	n.d.	1.8	**4.3**	3.0	3.1	2.4
PB-acid	n.d.	n.d.	n.d.	n.d.	n.d.	**1.0**
P-acid*	n.d.	0.1	1.0	**1.8**	0.3	0.1
not extractable	0.1	4.0	10.9	**28.9**	23.5	22.6
$^{14}CO_2$	**n.a.**	**2.1**	**1.5**	**17.0**	**27.6**	**28.2**
TOTAL	**95.9**	**92.8**	**88.3**	**86.8**	**83.6**	**84.4**

Days after application	Emperor Lake, dark (group B)					
	0	7	14	30	59	99
Water phase	**33.2**	**18.7**	**19.8**	**12.6**	**6.4**	**1.3**
extractable	**32.6**	18.0	19.2	11.6	4.1	0.5
Etofenprox	**32.1**	29.6	12.9	4.1	0.1	–
4'-OH	n.d.	0.8	**2.2**	0.7	n.d.	–
DP	n.d.	0.4	**1.1**	0.6	n.d.	–
PB-acid	n.d.	**0.9**	0.6	0.8	0.2	–
P-acid*	n.d.	1.2	1.0	**1.9**	1.5	–
not extractable	0.6	0.7	0.6	1.0	**2.3**	0.8
Sediment	**64.6**	**60.2**	**72.4**	**82.2**	**70.5**	**62.8**
extractable	64.5	58.8	70.1	**78.3**	47.0	32.0
Etofenprox	**63.1**	45.6	47.3	55.1	13.8	7.6
4'-OH	n.d.	3.9	**12.2**	9.3	2.5	1.5
DP	n.d.	1.1	2.4	1.9	**3.8**	3.8
PB-acid	n.d.	**0.7**	0.5	n.d.	n.d.	n.d.
P-acid*	n.d.	n.d.	0.2	0.5	**1.0**	0.6
not extractable	0.1	1.4	2.3	3.9	23.5	**30.8**
$^{14}CO_2$	**n.a.**	**0.2**	**0.2**	**0.9**	**14.9**	**17.8**
TOTAL	**97.8**	**95.3**	**96.0**	**95.9**	**92.3**	**82.9**

	Emperor Lake, light/dark cycle (group C)					
	0	**7**	**14**	**30**	**59**	**99**
Water phase	**33.1**	**12.9**	**17.2**	**9.0**	**3.9**	**0.7**
extractable	**32.0**	11.9	16.4	7.7	2.1	0.2
Etofenprox	**31.5**	3.2	7.6	0.5	–	–
4'-OH	n.d.	**1.8**	1.7	n.d.	–	–
DP	n.d.	**1.0**	0.6	0.7	–	–
PB-acid	n.d.	1.1	**1.6**	1.0	–	–
P-acid*	n.d.	2.5	**3.5**	3.7	–	–
not extractable	1.1	1.0	0.8	1.3	**1.8**	0.5
Sediment	**64.2**	**83.2**	**75.6**	**82.4**	**81.6**	**72.7**
extractable	64.0	**72.7**	69.9	67.4	66.9	45.4
Etofenprox	**61.9**	45.2	44.0	37.0	46.6	24.9
4'-OH	n.d.	**14.4**	12.3	7.6	4.6	1.9
DP	n.d.	4.1	3.9	**6.3**	2.9	2.1
PB-acid	n.d.	**1.2**	0.9	0.2	0.7	0.9
P-acid*	n.d.	1.1	1.4	**1.7**	0.5	n.d.
not extractable	0.2	10.5	5.7	15.0	14.7	**27.3**
$^{14}CO_2$	**n.a.**	**1.4**	**1.0**	**4.9**	**7.2**	**19.3**
TOTAL	**97.3**	**98.1**	**94.1**	**96.5**	**93.0**	**92.8**

n.a. not applicable
n.d. not detected
– not analysed by chromatography
* runs together with P-alc (PENA/EPMP)

The mean maximum values are in highlighted **bold**

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27. 05. 2005
Materials and methods	**3.1 Test material** Information given under this heading refers to the unlabelled reference. **3.1.2 Specification** No detailed specification and reference to section 2 was given in the test report. **3.3.1 Sediment** Previous pesticide applications: For the Mill stream pond no pesticide has been applied for the last 3 years, for the Emperor Lake it was stated that there was no use of pesticide (no time period given). The C_{org} of the sediments do not meet the recommendations of the OECD guideline 308, since all two sediments show a high to very high content. **3.3.2 Test system** According to the OECD guideline 308 a minimum of 50 g (dry weight basis) of sediment is recommended. Also the use of CO_2 free air might have an influence (increase) on pH of the water and should not be used. The acclimatisation phase (67 and 74 days) was quite long due to variation in pH oxygen consumption and redox potential. In the Emperor lake system (B) still pH and redox potential show variations during the test phase. **4.2.4 Degradation of etofenprox in aquatic systems** For the calculation of the degradation times for the whole system for Mill stream pond only 4 out of 6 samples, for Emperor Lake 5 and for Emperor Lake light/dark also only 4 out of 6 samples were used. The main reasons were, that more Etofenprox was found than in the previous samples and/or it did not fit the general trend. No detailed technical explanations were given. One reason might be the low solubility and adsorption to glass ware. The correlation coefficient was > 0.9 for the dark systems and 0.7 for the light/dark incubation system.
Conclusion	**5.3 Conclusion** The extent of bound residues in sediment increased up to 31% AR after 99 days in the lake system, whereas in the Mill stream pond it reached its maximum of 29% after 30 day and decreased to a constant level of around 23% until the termination of the study.
Reliability	2
Acceptability	Acceptable

Remarks	In an additional study (A 7.1.2.2.2/03) the DT_{50} and DT_{90} values in sediment for etofenprox and the major metabolite 4'-OH in Mill Stream Pond and Emporer Lake in the dark were calculated: Etofenprox: DT_{50} (sediment): 17.9 days (Mill Stream Pond), 32.2 days (Emporer Lake) DT_{90} (sediment): 59.4 days (Mill Stream Pond), 106.9 days (Emporer Lake) 4'-OH: DT_{50} (sediment): 55.8 days (Mill Stream Pond), 26.4 days (Emporer Lake) DT_{90} (sediment): 185.5 days (Mill Stream Pond), 87.8 days (Emporer Lake) $DT_{50\ and\ 90}$ values for the entire system refer to degradation, whereas $DT_{50\ and\ 90}$ values for the water or the sediment phase refer to dissipation throughout the whole Doc. III-A.

Section A7.1.2.2.2/02 Degradation in Water-Sediment Systems
Annex Point IIIA-XII.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Lewis C.J. (2002): (14C)-MTI-500: Recovery of radioactivity, isolation and analysis of a degradation product from a water-sediment system. Covance Laboratories Ltd., Harrogate, England; unpublished report no. CLE 719/14-D2149 (July22,2002) Experimental phase: May 11, 2001 to March 23, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] LKC UK Ltd.	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes SETAC (1995) and EC Directive 95/36/EC (1995)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	MTI-500, Etofenprox	x
3.1.1	Lot/Batch number	9604	
3.1.2	Specification	As given in section 2	x
3.1.3	Purity	99.99 %	
3.1.4	Further relevant properties	Solubility in water: 22.5 µg/L	
3.1.5	Radiolabelling	A) [α-^{14}C-benzyl]-etofenprox - Batch: MRH/MTC 277/29 - Specific activity: 366.67 MBq/mmol - Radiochemical purity: >99% (from certificate of analysis) (B) [2-^{14}C-propyl]-etofenprox - Batch: MRH/MTC 276/37 - Specific activity: 576.09 MBq/mmol) - Radiochemical purity: >99% (from CoA)	
3.1.6	TS inhibitory to microorganisms	No	
3.2	**Reference substance**	No	
3.3	**Testing procedure**		
3.3.1	Sediment	The degradation and metabolism of [^{14}C]-etofenprox, radiolabelled in two positions, was investigated using water and sediment from the Mill stream pond. The water and sediment characteristics are listed in table A7_1_2_2_2_02-1.	
3.3.2	Test system	Details on laboratory equipment etc. in tabular form (see table A7_1_2_2_2_02-2)	x

Section A7.1.2.2.2/02 Degradation in Water-Sediment Systems
Annex Point IIIA-XII.2.1

		Each test vessel (glass cylinders of 4.5 cm diameter) contained a 2.5 cm sediment layer (average dry weight of 12.95 g) covered with water to a depth of 6 cm (average weight of 93.25 g). Moistened CO_2-free air was drawn over the water surface and the units were pre-incubated in the dark at 20±2°C for 67 days until equilibration.
3.3.3	Test conditions	Relevant test conditions in tabular form (see table A7_1_2_2_2_02-3)
3.3.4	Method of preparation of test solution	Equal amounts (873 kPq) of each radiolabelled test item were pooled, the solvent removed and the residue reconstituted in acetonitrile to produce a 0.35 mg/mL solution. 3.6.1.3 non-labelled test item ware dissolved in 10 ml acetonitrile (0.26 mg/ml).
3.3.5	Application of test item	After pre-incubation, an amount corresponding to 200 g a.s./ha of [2-^{14}C-propyl]-etofenprox or [α-^{14}C-benzyl]-etofenprox was applied to separate incubation groups (equivalent to about 33 µg/100 mL). Each radiolabelled compound, in acetonitrile (84 and 86 µL), was dispensed drop-wise, onto the surface of the water of eight water-sediment units.
3.3.6	Duration of test	Post application: 100 days
3.3.7	Temperature / light	20 ± 2 °C / in the dark
3.3.8	Sampling	Single incubation units from each group were removed for analysis at intervals of 0 (immediately after application), 3, 7, 14, 30, 62 and 100 days after application. The effluent air was passed through a polyurethane foam bung trap, and a series of traps to collect volatile material. At each sampling date, the surface water was carefully removed from the sediment, added to NaCl solution (10 mL, 2M) and diluted with acetonitrile (100 mL). Water samples containing >5% of applied radioactivity were mixed with dichloromethane (50 mL) and neutralised with HCl to produce two phases. The aqueous phase was partitioned dichloromethane (50 mL), acidified with concentrated HCl (5 mL) and partitioned with further dichloromethane (2 x 50 mL). The sediment was extracted twice with acetonitrile (100 mL) and then once with methanol/HCl (95:5 v/v, 100 mL).
3.3.9	Intermediates/ degradation products	Identified using HPLC or TLC (see below). Extracts, containing >5% of applied radioactivity, were analysed by HPLC for identification of the degradation products.
3.3.10	Analytical methods	HPLC with radioactivity detection, TLC; LC-MS; Liquid scintillation counting. Details see table A7_1_2_2_2_02-4
3.3.11	Statistics	The recovery of radioactivity and the DT_{50} and DT_{90} values of etofenprox and it major metabolite 4'-OH in a natural water/sediment systems were calculated using a two phase exponential model for the total system and a single phase exponential model for the water phase. two phase: $y = (a * e^{-k_1 t}) + (b * e^{-k_2 t})$ one phase: $Y = Co * e^{-kt}$ Accumulation and single phase decline (for metabolite): $y = (b * e^{-k_1 t}) - (a * e^{-k_a t})$

4 RESULTS

4.1	**Recovery**	During the course of the study, overall recovery ranged from 96 to 101% of applied radioactivity after [2-^{14}C-propyl]-etofenprox and from 91 to 99% after [α-^{14}C-benzyl]-etofenprox. At 100 days, recovery was 98% and 96%, respectively.

Section A7.1.2.2.2/02 Degradation in Water-Sediment Systems
Annex Point IIIA-XII.2.1

4.2	**Degradation of test substance**	Detailed results in Table A7_1_2_2_2_02-5
4.2.1	Mineralisation	Up to a mean of 35% of volatile compounds could be detected after 100 days of incubation in volatile traps. Almost all this radioactivity was present in the NaOH traps and was assumed to be [^{14}C]-carbon dioxide.
4.2.2	Test item	In the water phase, only the parent compound could be detected at a level higher than 10% of applied radioactivity. After treatment with the propyl label, etofenprox amounted to 29.7% on day 0, decreased to 2.9% on day 3 and to 0.2% on day 14. After treatment with the benzyl label, etofenprox amounted to 30.7% on day 0, decreased to 4.0% on day 3 and then to 0.3% on day 7. In the sediment, the amount of etofenprox following propyl label application, decreased from a maximum of 65.7% on day 0 to 19.0% of the applied radioactivity on day 30, and then slightly increased to 21.1% at the end of study. After application with the benzyl label, the amount of etofenprox decreased from 65.9% initially, to 14.5% of applied radioactivity after 100 days.
4.2.3	Metabolites	Water phase: The degradation products 4'-OH, DP and PB-acid, accounted for only small proportions of radioactivity (≤1%) with either radiolabelled form of the test substance. Two unknown compounds were also observed until Day 14, but remained below 3% of the applied radioactivity (Unknown 3 at Day 3 with the propyl label). Sediment: The major degradation product of etofenprox, i.e. 4'-OH, was mainly detected in the sediment. After treatment with the propyl label, 4'-OH increased from 13.5% on Day 3, to the maximum level of 19.3% on Day 7, and decreased to 7.9% at 100 days. After treatment with the benzyl label, 4'-OH increased from 13.9% on Day 3, to the maximum level of 17.7% on Day 7, and decreased to 2.0% at 62 days. After 100 days, its concentration was 6.3% of the applied radioactivity. Metabolites corresponding to DP (max. 0.9% of applied), DE (max. 0.7%), PB-acid (max 0.2%), P-acid (max. 0.3%) and α-CO (max 0.2%) were occasionally detected in the sediment. Two not identified metabolites were also detected in the sediment. However, none of them exceeded 1.5% of the applied radioactivity.
4.2.4	Degradation rates of etofenprox in aquatic systems	Based on these results, the degradation rates of etofenprox in the water phase and the total system were determined:

	DT_{50}	DT_{90}
Etofenprox		
Water phase	1.0 days	3.2 days
Entire system	6.5 days	143 days

4.2.5	Degradation rates of 4'-OH metabolite	The degradation rates of the metabolite 4'-OH were calculated only for the total system:

	DT_{50}	DT_{90}
4'-OH		
Entire system	57 days	185 days

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and**	The degradation and metabolism of [^{14}C]-etofenprox, radiolabelled in two positions, was investigated using water and sediment from the Mill

Section A7.1.2.2.2/02 Degradation in Water-Sediment Systems
Annex Point IIIA-XII.2.1

	methods	stream pond. Each test vessel (glass cylinders of 4.5 cm diameter) contained a 2.5 cm sediment layer (average dry weight of 12.95 g) covered with water to a depth of 6 cm (average weight of 93.25 g). The water and sediment characteristics are listed in Table A7_1_2_2_2_02-1 below. Moistened CO_2-free air was drawn over the water surface and the units were pre-incubated in the dark at 20±2°C for 67 days until equilibration. After pre-incubation, an amount corresponding to 200 g a.s./ha of [2-^{14}C-propyl]-etofenprox (batch no. MRH/MTC276/37; radiochemical purity: >99%) or [α-^{14}C-benzyl]-etofenprox (batch no. MRH/MTC277/29; radiochemical purity: >99%) was applied to separate incubation groups (equivalent to about 33 µg/100 mL). Each radio-labelled compound, in acetonitrile (84 and 86 µL), was dispensed drop-wise, onto the surface of the water of eight water-sediment units. The air drawn over the surface of the units was passed trough a series of traps to collect evolved radio-labelled material. The test systems were incubated in the dark at 20±2°C over a period of 100 days. Single incubation units from each group were removed for analysis at intervals of 0 (immediately after application), 3, 7, 14, 30, 62 and 100 days after application. The trap reagents were collected when the units to which they were attached were removed, and, for units incubated for longer than a month, additionally at 30 and 62 days after the application. Radioactivity in the trapping solutions was quantified by LSC. Surface water extracts containing >5% of applied was analysed by HPLC for identification of the degradation products. Radioactivity in the acetonitrile and methanol sediment extracts was analysed by HPLC and the identity of the major degradation products was confirmed by TLC. Finally, the radioactivity associated with the incubation units was determined by LSC. The DT_{50} and DT_{90} values of etofenprox in the natural water/sediment system were calculated assuming first order kinetics. The redox potential of sediment and water and the pH and oxygen content of water, were determined in two monitoring units at each sampling interval.
5.2	**Results and discussion**	<u>Recovery</u> During the course of the study, overall recovery ranged from 96 to 101% of applied radioactivity after [2-^{14}C-propyl]-etofenprox and from 91 to 99% after [α-^{14}C-benzyl]-etofenprox. At 100 days, recovery was 98% and 96%, respectively. <u>Distribution of the radioactivity</u> In the water phase, the amount of radioactivity decreased very rapidly from a mean of 30% of applied radioactivity on day 0 to 1% at the end of the study, after 100 days, for both labels. A small portion of applied radioactivity (up to 3% in the benzyl label after 3 days) was present in water as dissolved $^{14}CO_2$. In the sediment, a mean of 70% of applied radioactivity was found on day 0. Thereafter, the radioactivity increased to a mean maximum of 90% of applied radioactivity within 3 days and decreased to 60% at 100 days. Radioactivity extracted into acetonitrile decreased from a mean of 66% of applied radioactivity, at day 0, to 28% at 100 days, whereas radioactivity extracted into acidified methanol increased from 3% to 12% over the same period. The amount of radioactivity that was not extracted from the sediment was 1% of the applied radioactivity, initially. After treatment with the propyl label, not extracted

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Annex Point IIIA-XII.2.1

radioactivity increased to 28% at 30 days, and then decreased to 19% at 100 days. After treatment with the benzyl label, not extracted radioactivity increased to 22% at 100 days.

Mineralization

Up to a mean of 35% of volatile compounds could be detected after 100 days of incubation in volatile traps. Almost all this radioactivity was present in the NaOH traps and was assumed to be [^{14}C]-carbon dioxide.

Identification of radioactivity

In the water phase, only the parent compound could be detected at a level higher than 10% of applied radioactivity. After treatment with the propyl label, etofenprox amounted to 29.7% on day 0, decreased to 2.9% on day 3 and to 0.2% on day 14. After treatment with the benzyl label, etofenprox amounted to 30.7% on day 0, decreased to 4.0% on day 3 and then to 0.3% on day 7. The degradation products 4'-OH, DP and PB-acid, accounted for only small proportions of radioactivity (≤1%) with either radiolabelled form of the test substance. Two unknown compounds were also observed until Day 14, but remained below 3% of the applied radioactivity (Unknown 3 at Day 3 with the propyl label).

In the sediment, the amount of etofenprox following propyl label application, decreased from a maximum of 65.7% on day 0 to 19.0% of the applied radioactivity on day 30, and then slightly increased to 21.1% at the end of study. After application with the benzyl label, the amount of etofenprox decreased from 65.9% initially, to 14.5% of applied radioactivity after 100 days. The major degradation product of etofenprox, i.e. 4'-OH, was mainly detected in the sediment. After treatment with the propyl label, 4'-OH increased from 13.5% on Day 3, to the maximum level of 19.3% on Day 7, and decreased to 7.9% at 100 days. After treatment with the benzyl label, 4'-OH increased from 13.9% on Day 3, to the maximum level of 17.7% on Day 7, and decreased to 2.0% at 62 days. After 100 days, its concentration was 6.3% of the applied radioactivity. Metabolites corresponding to DP (max. 0.9% of applied), DE (max. 0.7%), PB-acid (max 0.2%), P-acid (max. 0.3%) and α-CO (max 0.2%) were occasionally detected in the sediment. Two not identified metabolites were also detected in the sediment. However, none of them exceeded 1.5% of the applied radioactivity.

Degradation

Based on these results, the degradation rates of etofenprox in the water phase and the total system were determined using a single phase and a two phase model, respectively. The apparent degradation rate of 4'OH was determined using a single phase formation and single phase degradation model.

5.3 Conclusion

The results obtained in the water/sediment system (Mill stream pond) were similar in terms of degradation products formed and rates of degradation from those obtained in the previous study (CLE 719/6 – D2142). The metabolic pathway is summarised in Figure A7_1_2_2_2_02-1.

x

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	DT_{50}	DT_{90}
Etofenprox		
Water phase	1.0 days	3.2 days
Entire system	6.5 days	143 days
4'-OH		
Entire system	57 days	185 days

The major unidentified metabolite observed in the previous study was clearly identified as 4'-OH.

5.3.1 Reliability 1 x

5.3.2 Deficiencies No

Table A7_1_2_2_2_02-1: Characteristics of the surface waters and sediment of the study of Lewis (2001).

System	Mill stream pond
Surface water	
Total nitrogen [mg/L]	
at start	4.2
at end	5.6
Total phosphorus [mg/L]	
at start	<0.05
at end	1.2
Dissolved organic carbon [mg/L]	
at start	4.7
at end	63.9
Sediment	
Sediment type (UK classification)	clay
Particle size distribution [%]	
sand 63 – 2000 µm	30
silt 2 – 63 µm	31
clay < 2 µm	39
Organic carbon [%]	9.2
pH (water)	7.7
(1M KCl)	7.2
CEC [meq/100 g dry mass]	35.2
Microbial biomass* [µg C/g soil]	
at start	654
at end	615

CEC Cation exchange capacity
* Microbial biomass determined by the fumigation-extraction method according to VANCE, BROOKS AND JENKINSON (1987)

Table A7_1_2_2_2_02-2: Incubation system.

Criteria	Details
Apparatus	Borosilicate glass cylinders (4.5 cm diameter), containing sediment to a height of 2.5 cm and 6 cm associated water (kept constant by adding deionised water
Number of replicates/concentration	1 replicate for each time point
Air pre-treatment	The incoming air was passed through a soda lime trap, a deionised water trap and a safety trap before entering the incubation vessel.
Trapping system	The exhaust air was passes trough a series of traps: Sidearm: polyurethane foam Trap1: ethanediol Trap2: 2% paraffin in xylene Trap3: 2 M NaOH Trap 4: 2 M NaOH

Table A7_1_2_2_2_02-3: Test conditions.

Criteria	Details	
Oxygen concentration	Determined approx. weekly during acclimatisation and at each sampling point. (Reported as % saturation; Jenway 9070 oxygen meter)	
	Acclimatisation	Test phase
A:	50 - 98 %	50 - 95 %
pH value in water	Measured in the surface water using a calibrated pH meter and pH electrode.	
A:	7.8 – 8.5	8.4 – 8.5
pH value in sediment	A: 7.0 – 7.2	not measured
Redox potential in water	Measured using a calibrated electrode attached to a mV meter	
A:	350 – 450 mV	180 – 400
Redox potential in sediment	Measured using a calibrated electrode attached to a mV meter	
A:	-50 – 100 mV	-20 – 10

Table A7_1_2_2_2_02-4: Details of the analytical methods.

Method	Details	
HPLC 2	Column	Waters Spherisorb S5C8 (250 x 4.6 mm; 5 µm)
	Mobile phase	A: acetic acid (1%) in water; B: acetonitrile 0 min 100 % A 10 min 50 % A 40 min: 0 % A 40 – 45 min 0 % A 46 min: 100 % A
	Flow rate	1 ml/min
	Detection	UV at 234 nm; radioactivity using Ramona flow through monitor or β-ram (Lablogic) flow through cell
HPLC 1	Column	Capital ODS-H (150 x 4.6 mm; 3 µm)
	Mobile phase	A: acetic acid (1%) in water; B: acetic acid (1%) in acetonitrile 0 min 70 % A 25 min 0 % A 30 min – 35 min:70 % A
	Flow rate	1 ml/min
	Detection	UV at 234 nm; radioactivity using Ramona flow through monitor or β-ram (Lablogic) with CaF_2 cell
HPLC 3	Column	Capital ODS-H (150 x 4.6 mm; 3 µm)
	Mobile phase	A: water pH 2.5 adjusted with phosphoric acid; B: acetic acid (1%) in acetonitrile 0 min 70 % A 20 min – 30 min 10 % A 30.1 min – 38 min :70 % A
	Flow rate	1 ml/min
	Detection	UV at 234 nm; radioactivity using Ramona flow through monitor β-ram (Lablogic)
TLC 2	Plates	Whatman K6F silica gel60 A
	Solvent	Hexane / diethyl ether (8:1 v/v)%
TLC 8	Plates	Whatman K6F silica gel60 A
	Solvent	Toluene / ethyl acetate /acetic acid (90:10:1 v/v/v)
TLC 9	Plates	Whatman K6F silica gel60 A
	Solvent	Chloroform /methanol/ acetic acid (9:1 v/v)
LC/MS System 1	Instrument	Autospec (Micromass), Magnetic Sector Instrument with inletsystem HP 5890 Series II GC
	Column	Supelco MDN-5 (30 m x 0.25 mm x 0.25 µm film) 80°C for 1 min, 8°C/min to 299 °C
	Detection	electron impact, 70 V ionisation energy; 8000 V accelerating voltage; 250°C
		m/z 100 – 700; ESP-

Table A7_1_2_2_2_02-5: Degradation of etofenprox and formation of metabolites in water/ sediment systems (values are given as % of applied radioactivity).

Days after application	**Mill stream pond**						
	0	**3**	**7**	**14**	**30**	**62**	**100***
Label	**[2-^{14}C-propyl]-etofenprox**						
Water phase	**29.5**	**9.4**	**10.8**	**5.0**	**2.3**	**1.8**	**1.2**
Etofenprox	**29.7**	2.9	1.0	0.2	n.a.	n.a.	n.a.
4'-OH	n.d.	0.6	**1.0**	n.d.	n.a.	n.a.	n.a.
DP	n.d.	0.2	**0.6**	n.d.	n.a.	n.a.	n.a.
DE	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.
P-alc/P-acid	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.
α-CO	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.
Sediment	**69.7**	**91.4**	**87.4**	**83.3**	**73.4**	**69.9**	**61.6**
extractable*	68.8	**85.5**	81.7	65.9	45.8	43.0	42.8
Etofenprox	**65.7**	63.8	53.8	43.7	19.0	20.1	22.4*
4'-OH	n.d.	13.5	**19.3**	11.1	13.5	9.4	8.5*
DP	n.d.	n.d.	0.5	0.4	0.5	0.3	**1.4***
DE	n.d.	n.d.	n.d.	n.d.	0.2	n.d.	**0.7***
P-alc/P-acid	n.d.	n.d.	n.d.	**0.3**	n.d.	n.d.	0.2*
α-CO	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	**0.2***
not extractable	0.9	6.0	5.7	17.4	**27.6**	26.9	18.8
Volatile**	**n.a.**	**0.5**	**0.4**	**8.2**	**19.8**	**25.4**	**34.7**
TOTAL	**99.2**	**101.3**	**98.6**	**96.6**	**95.6**	**97.2**	**97.6**

n.d. not detected

n.a. not applicable

* radioactivity in acetonitrile and in acidified methanol extracts of sediment

** almost all this radioactivity was present in the sodium hydroxide traps, except Day 3 which contained 0.1% in the ethanediol trap, and is assumed to be due to trapped $^{14}CO_2$

The maximum values are highlighted in **bold**

Table A7_1_2_2_2_02-5: Degradation of etofenprox and formation of metabolites in water/ sediment systems (values are given as % of applied radioactivity). - continued -

Days after application	**Mill stream pond**						
	0	**3**	**7**	**14**	**30**	**62**	**100**
Label	**[α-^{14}C-benzyl]-etofenprox**						
Water phase	**29.6**	**8.4**	**7.7**	**3.3**	**0.7**	**2.4**	**0.6**
Etofenprox	**30.7**	4.0	0.3	n.a.	n.a.	n.a.	n.a.
4'-OH	n.d.	**0.4**	0.2	n.a.	n.a.	n.a.	n.a.
DP	n.d.	0.3	**0.4**	n.a.	n.a.	n.a.	n.a.
DE	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.
PB-acid	n.d.	**0.6**	n.d.	n.a.	n.a.	n.a.	n.a.
PB-alc***	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.
α-CO	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.
Sediment	**69.7**	**88.0**	**79.9**	**75.6**	**76.9**	**55.4**	**58.2**
extractable*	69.0	**82.5**	69.0	60.5	63.7	36.3	36.1
Etofenprox	**65.9**	60.5	38.4	43.5	44.6	24.3	15.5*
4'-OH	n.d.	13.9	**17.7**	7.5	9.6	2.0	7.0*
DP	n.d.	n.d.	**0.9**	0.4	n.d.	n.d.	2.1*
DE	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PB-acid	n.d.	n.d.	n.d.	**0.2**	n.d.	n.d.	n.d.
PB-alc***	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-CO	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
not extractable	0.7	5.5	11.0	15.1	13.2	19.1	**22.1**
Volatile**	**n.a.**	**2.0**	**3.4**	**17.4**	**19.6**	**37.9**	**36.8**
TOTAL	**99.3**	**98.4**	**91.2**	**96.3**	**97.4**	**96.0**	**96.2**

n.d. not detected

n.a. not applicable

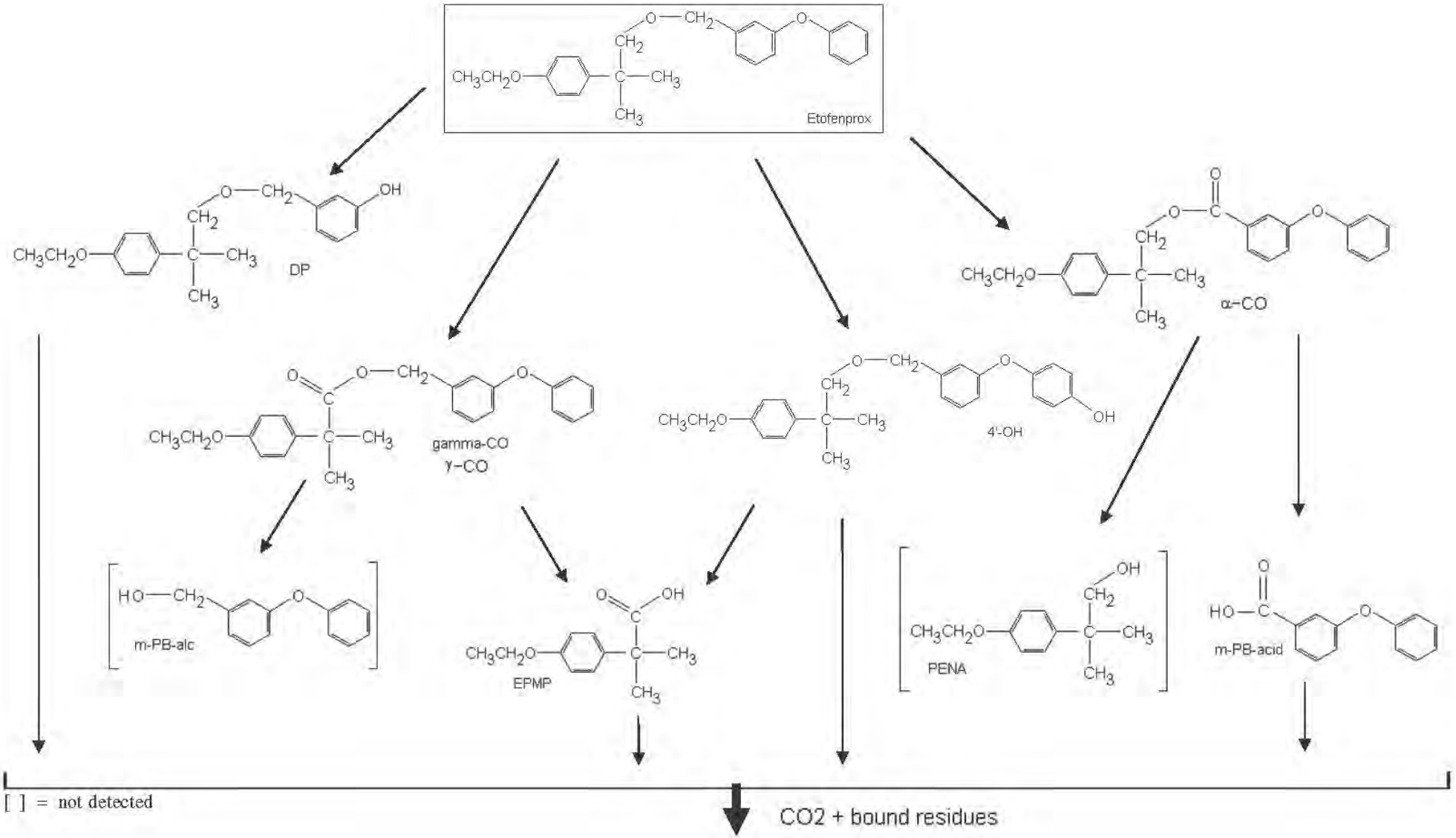
* radioactivity in acetonitrile and in acidified methanol extracts of sediment

** all this radioactivity was present in the sodium hydroxide traps and is assumed to be due to trapped $^{14}CO_2$

*** PB-alc is known as m-PB-alc

The maximum values are highlighted in **bold**

Figure A7_1_2_2_2_02-1: Proposed metabolic pathway of etofenprox in water-sediment systems.



Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27. 05. 2005
Materials and methods	**3.1 Test material** Information given under this heading refers to the unlabelled reference substance. **3.1.2 Specification** No detailed specification and reference to section 2 was given in the original test report. **3.3.2 Test system** According to the OECD guideline 308 a minimum of 50 g (dry weight basis) of sediment is recommended. Also the use of CO_2 free air might have an influence (increase) on pH of the water and should not be used. The acclimatisation phase (67 days) was quite long. **4.2.4 Degradation rates** r^2 for Etofenprox was > 0.9 for the total system and the water phase. For 4'-OH (total system) r^2 was 0.8.
Conclusion	**5.3 Conclusion** Proposed metabolic pathway: The principal route of degradation of Etofenprox is by hydroxylation to 4'-OH and further metabolism to P-acid (also known as EPMP). Etofenprox can also be degraded to α-CO and y-CO and further to PB-acid or P-acid. Another minor path involves the cleavage of the ether linkage between the two benzene rings to give DP. The formation of bound residues and mineralization to CO_2 were also shown.
Reliability	2
Acceptability	Acceptable
Remarks	The aims of the second study were to obtain a better recovery of applied radioactivity by changing the experimental design and to attempt to identify the major degradation products, which were not identified in the first study. In an additional study (A 7.1.2.2.2/03) the DT_{50} and DT_{90} values in sediment for Etofenprox and the major metabolite 4'-OH in the Mill Stream Pond in the dark were calculated: Etofenprox: DT_{50} (sediment): 54.2 days DT_{90} (sediment): 180.0 days 4'-OH: DT_{50} (sediment): 86.2 days DT_{90} (sediment): 286.4 days $DT_{50\ and\ 90}$ values for the entire system refer to degradation, whereas $DT_{50\ and\ 90}$ values for the water or the sediment phase refer to dissipation throughout the whole Doc. III-A.

Section A7.1.3 Adsorption/desorption screening test

Annex Point IIA 7.7

			Official use only
		1 REFERENCE	
1.1	**Reference**	Völkel W. (1999); Adsorption/desorption of MTI-500 (etofenprox) on three soils; RCC Ltd., Environmental Chemistry & Pharmanalytics Division, Itingen, Switzerland; unpublished report no. 663175 (March 9, 1999) Dates of experimental work: February 16, 1998 – July 24, 1999	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data submitted to the MS after May 13, 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes: OECD 106	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes: 10% organic solvent (acetone) was used to keep the test substance completely dissolved in 0.01M $CaCl_2$	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Etofenprox (non labelled test item used as analytical reference substance)	
3.1.1	Lot/Batch number	9604	
3.1.2	Specification	As given in section 2 Deviating from specification given in section 2 as follows	x
3.1.3	Description	Not reported	
3.1.4	Purity	99.99%	
3.1.5	Stability	No information in the report.	
3.1.6	Further relevant properties	Solubility in water: 22.5 µg/L at 20 ± 0.5°C Vapour pressure: 8.13 x 10^{-7} Pa at 25°C Stability in water: hydrolytically stable at pH 4, 7 and 9 n-Octanol/water partition coefficient: log Pow = 6.9	
3.1.7	Radiolabelling	[α-^{14}C-benzyl]-etofenprox - Batch: MRH/MTC 277/29 - Specific activity: 336.7 MBq/mmol - Radiochemical purity: > 99% - Description: liquid at room temperature - Stability: no information provided in the report - Density: 1.157 at 23°C	

Section A7.1.3 Adsorption/desorption screening test

Annex Point IIA 7.7

3.1.8	Method of analysis	Total radioactivity by liquid scintillation counting (LSC) (Packard TRI-CARB 2000 Ca); soil was combusted using an OX 500 Sample Oxidizer (Zinsser Analytic). Analysis of individual compounds by thin layer chromatography (coated TLC plates silica gel 60F 254, Merck, toluene as the solvent) with radioactivity or UV detection at 254 nm. (Berthold TLC Linear Analyser)	
3.2	**Degradation products**	Degradation products tested: Yes (Screening tests for metabolites/degradation products showed, that at the test substance was stable and that no degradation products were present > 10 % of the a.s. added)	
3.2.1	Method of analysis for degradation products	Analysis of individual compounds by thin layer chromatography (coated TLC plates silica gel 60F 254, Merck, toluene as the solvent) with radioactivity or UV detection at 254 nm. (Berthold TLC Linear Analyser)	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	not applicable	
3.4	**Soil types**	I. Sisseln, sandy loam II. Les Barges, silt loam III. Speyer 2.2, loamy sand (see table A7_2_3_1-1 for details)	
3.5	**Testing procedure**		
3.5.1	Test system	The soil I was obtained from the top 30 cm soil layer and the soil II was sampled in the field from the surface layer and kept on the field as a monticule of 2 m height. The top 50 cm were transferred to the laboratory. Soil II was sampled was collected from arable land at the Swiss Research Station Les Barge (VS) and transferred to the laboratory. Soil III was obtained from the LUFA Speyer, Germany In the laboratory soil was stored air dried in closed plastic or glass containers at room temperature in the dark. The soils were air-dried, 2 mm sieved and stored in the dark at room temperature until use. The moisture content of the stored soils was determined by drying at 105°C under reduced pressure until a constant weight was reached (3 to 16 hours).	x

Section A7.1.3 Adsorption/desorption screening test

Annex Point IIA 7.7

3.5.2	Test solution and Test conditions	A volume of 25 mL of a 0.01 M calcium chloride ($CaCl_2$) solution was added to aliquots of 5 and 1 g dry weight soil per tube, to obtain the two soil/solution ratios of 1:5 and 1:25 (w/w). The soil aliquots (5 and 1 g) were conditioned with 0.01 mol $CaCl_2$ per litre solution at a ratio of 2 to 1 of volume to dry soil mass, incubated and shaken at 20±1°C overnight. After equilibration, the aqueous phase was decanted. The application solution was prepared by diluting 0.146 mg etofenprox with 40 mL acetone (10%). 22.5 mL of 0.01 M $CaCl_2$ and 2.5 mL of the application solution were applied to the 5 g and 1 g samples of the three soils. The concentration of the test substance was 0.365 mg a.i./L 0.01 M $CaCl_2$ solution.
3.6	**Test performance**	
3.6.1	Preliminary test	According to (a) "OECD 106": Yes Before the application, the air-dried soils were conditioned with 0.01 M calcium chloride ($CaCl_2$) in a shaker (240 strokes/min), in the dark at 20±1°C. The pre-test showed that 10% organic solvent (acetone) were necessary for dissolving the test substance in the $CaCl_2$ solution.
3.6.2	Screening test: Adsorption	According to (a) "OECD 106": Performed For the adsorption test, the soil/aqueous phase mixtures were shaken at 240 strokes per minute, in the dark at 20±1°C. After one and two hours of agitation, the suspensions were separated by centrifugation at 2400 rpm for 10 minutes. Following centrifugation, sub samples (1 mL) of the aqueous phases were taken after 1 and 2 hours and analysed for radioactivity by LSC. The equilibrium concentration of the test substance (C_e) and its total amount in the aqueous phase was calculated. The tubes were weighed out to determine the total volume of $CaCl_2$ solution exposed to the soil during adsorption. After the adsorption step, the volumes of the supernatants as well as the amounts of $CaCl_2$ solution remaining within the sedimented soil were determined gravimetrically. The remaining soil samples were weighed to determine of the amount of retained water.
3.6.3	Screening test: Desorption	According to (a) "OECD 106": Performed For the investigation of desorption, 25 mL of untreated 0.01 mol $CaCl_2$ per litre solution was added and the mixture was shaken for 16 hours. After shaking, the mixture was centrifuged at about 20°C and the supernatant analysed for the radioactivity by LSC. Two desorption steps were performed. Each soil and concentration was tested in duplicate
3.6.4	HPLC-method	According to (a) "OECD-HPLC-method": No
3.6.5	Other test	Not applied
	4 RESULTS	
4.1	**Preliminary test**	Etofenprox was found to be stable before (94.6%) and after application (95.5%) as well as after two hours of agitation. Results in tabular form (see table A7_2_3_1-2)

Section A7.1.3 Adsorption/desorption screening test

Annex Point IIA 7.7

4.2	**Screening test: Adsorption**	For samples incubated at the soil to aqueous ratio of 1:5, 97.7%, 98.3% and 97.3% of the radioactivity applied was adsorbed to soils I, II and III, respectively, after only 2 hours of agitation. For soil samples incubated at a soil to aqueous ratio of 1:25, 95.3%, 97.0% and 94.5% was adsorbed. Results in tabular form (see table A7_2_3_1-3 and A7_2_3_1-4)
4.3	**Screening test: Desorption**	For soil samples incubated at the soil to aqueous ratio of 1:5, only 1.4%, 0.9% and 2.8% of the radioactivity adsorbed was found in the desorption solutions of soils I, II and III, respectively, after two steps of 16 hours of desorption. For soil samples incubated at a soil to aqueous ratio of 1:25, only 1.7%, 1.3% and 2.4% was desorbed. Results in tabular form (see table A7_2_3_1-5)

Section A7.1.3. Adsorption/desorption screening test

Annex Point IIIA-XII.1.2

4.4 Calculations

4.4.1 Ka , Kd

4.4.2 Ka_{oc} , Kd_{oc}

Soil	ratio	Adsorption coefficients [mL/g]*		
		K'	K'_{OC}	K'_{OM}
Sisseln	1:5	234	14923	8656
Les Barges	1:5	343	9025	5235
Speyer	1:5	196	8548	4958
Sisseln	1:25	519	33067	19181
Les Barges	1:25	836	22009	12766
Speyer	1:25	434	18968	11002

Kd was not separately calculated

4.5 Degradation product(s)

There were no degradation products > 10%

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The adsorption and desorption of [α-^{14}C-benzyl]-etofenprox (batch no. MRH/MTC277/29; radiochemical purity: >99%) was investigated in three European soils, i.e. Sisseln (soil I, sandy loam), Les Barges (soil II, silt loam) and Speyer 2.2 (soil III, loamy sand). The soil I was obtained from the top 30 cm soil layer and the soil II was sampled in the field from the surface layer.

The soils were air-dried, 2 mm sieved and stored in the dark at room temperature until use. The moisture content of the stored soils was determined by drying at 105°C under reduced pressure until a constant weight was reached (3 to 16 hours). Before the application, the air-dried soils were conditioned with 0.01 M calcium chloride ($CaCl_2$) in a shaker (240 strokes/min), in the dark at 20±1°C. In a pre-test, 10% organic solvent (acetone) was determined for dissolving the test substance in the definitive screening test.

A volume of 25 mL of a 0.01 M calcium chloride ($CaCl_2$) solution was added to aliquots of 5 and 1 g dry weight soil per tube, to obtain the two soil/solution ratios of 1:5 and 1:25 (w/w). The soil aliquots (5 and 1 g) were conditioned with 0.01 mol $CaCl_2$ per litre solution at a ratio of 2 to 1 of volume to dry soil mass, incubated and shaken at 20±1°C overnight. After equilibration, the aqueous phase was decanted.

The application solution was prepared by diluting 0.146 mg etofenprox with 40 mL acetone (10%). Thereafter, 22.5 mL of 0.01 M $CaCl_2$ and 2.5 mL of the application solution were applied to the 5 g and 1 g samples of the three soils. The concentration of the test substance was 0.365 mg a.i./L 0.01 M $CaCl_2$ solution.

For the adsorption test, the soil/aqueous phase mixtures were shaken at 240 strokes per minute, in the dark at 20±1°C. After one and two hours of agitation, the suspensions were separated by centrifugation at 2400 rpm for 10 minutes. Following centrifugation, sub samples (1 mL) of the aqueous phases were taken after 1 and 2 hours and analysed for radioactivity by LSC. The equilibrium concentration of the test substance (C_e) and its total amount in the aqueous phase was calculated. The tubes were weighed out to determine the total volume of $CaCl_2$

Section A7.1.3. Adsorption/desorption screening test

Annex Point IIIA-XII.1.2

solution exposed to the soil during adsorption. After the adsorption step, the volumes of the supernatants as well as the amounts of $CaCl_2$ solution remaining within the sedimented soil were determined gravimetrically. The remaining soil samples were weighed out to determine of the amount of water retained.

For the investigation of desorption, 25 mL of untreated 0.01 mol $CaCl_2$ per litre solution was added and the mixture was shaken for 16 hours. After shaking, the mixture was centrifuged at about 20°C and the supernatant analysed for the radioactivity by LSC. Two desorption steps were performed. Each soil and concentration was tested in duplicate.

The quantity of etofenprox was determined after 1 and 2 hours in the adsorption solution as well as after 16 hours in the desorption solution. Radioactivity was determined by LSC. The adsorption coefficients were calculated for the samples after 2 hours of adsorption. The radioactivity remaining in the wet soil samples (mass balance) after desorption was determined by combustion.

5.2 Results and discussion

5.2.1 Adsorbed a.s. [%]

Soil	ratio	Organic carbon [g/100 g dry soil]	Adsorbed a.s. [%]
Sisseln	1:5	1.57	97.7
Les Barges	1:5	3.80	98.3
Speyer	1:5	2.29	97.3
Sisseln	1:25	1.57	95.3
Les Barges	1:25	3.80	97.0
Speyer	1:25	2.29	94.5

For samples incubated at the soil to aqueous ratio of 1:5, 97.7%, 98.3% and 97.3% of the radioactivity applied was adsorbed to soils I, II and III, respectively, after only 2 hours of agitation. For soil samples incubated at a soil to aqueous ratio of 1:25, 95.3%, 97.0% and 94.5% was adsorbed.

5.2.2 K_a

Soil	ratio	Adsorption coefficients [mL/g]*		
		K'	K'_{OC}	K'_{OM}
Sisseln	1:5	234	14923	8656
Les Barges	1:5	343	9025	5235
Speyer	1:5	196	8548	4958
Sisseln	1:25	519	33067	19181
Les Barges	1:25	836	22009	12766
Speyer	1:25	434	18968	11002

Adsorption constants calculated after two hours of adsorption.

5.2.3 K_d

Not calculated. For soil samples incubated at the soil to aqueous ratio of 1:5, only 1.4%, 0.9% and 2.8% of the radioactivity adsorbed was found in the desorption solutions of soils I, II and III, respectively, after two steps of 16 hours of desorption. For soil samples incubated at a soil to aqueous ratio of 1:25, only 1.7%, 1.3% and 2.4% was desorbed.

Section A7.1.3. Adsorption/desorption screening test

Annex Point IIIA-XII.1.2

5.2.4	Ka_{oc}	see 5.2.2	
5.2.5	Ka/Kd	not calculated	
5.2.6	Degradation products (% of a.s.)	No degradation products formed > 10 %. Etofenprox was found to be stable before (94.6%) and after application (95.5%) as well as after two hours of agitation.	
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. The results showed a strong adsorption of etofenprox on soil at a concentration of 0.365 mg/L. According to the K_{oc} values for adsorption (8548 - 14923 mL/g at the ratio 1:5 and 18968 – 33067 mL/g at the ratio 1:25), etofenprox can be classified as immobile in soils.	x
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7_2_3_1-1: Classification and physico-chemical properties of soils used as adsorbents.

Soil	**Sisseln**	**Les Barges**	**Speyer 2.2**
Origin	**Switzerland**	**Switzerland**	**Germany**
Classification (acc. to USDA)	sandy loam	silt loam	loamy sand
Particle size [%]			
Sand (> 0.05 mm)	57.9	11.8	81.9*
Silt (0.002 – 0.05 mm)	26.2	68.8	13.0*
Clay (< 0.002 mm)	15.9	19.4	5.1
pH (KCl)	7.1	6.9	6.0
Organic carbon [g/100 g soil]	1.57	3.80	2.29
$CaCO_3$ [g/100 g soil]	6.11	9.39	0.55
CEC [mEq/100 g soil]	13.8	25.4	9.7
MWHC [%]	52.4	96.6	44.3

CEC cation exchange capacity
MWHC maximal water holding capacity
* values for silt 0.002 – 0.063 mm; sand > 0.063 mm (classification DIN)

Table A7_2_3_1-2: Conditions of test

Test substance	α-^{14}C-benzyl-etofenprox
Sample purity	> 99% (radiochemical purity)
Weighed soil	1 g, 5 g
Volume of $CaCl_2$ solution	25 ml
Nominal concentration of a.s. final solution	not calculated
Analytical concentration of final a.s. solution	0.365 mg/L
Concentration of the test solution (show calculation)	1.054 mg etofenprox in 5 ml acetone; 0.50 ml of this solution diluted with 39.5 ml acetone; LSC measurement of concentration = 0.146 mg in 40 ml = application solution 5 g soil + 22.5 ml of 0.01 $McaCl_2$ solution + 2.5 ml application solution = test solution
Details of the analytical method used:	Measured using LSC
Method	not applicable
Recovery rate	not applicable
Detection limit	not applicable

Table A7_2_3_1-3: Results of screening test – adsorption (soil:aqueous phase ratio = 1:5 (w/w))

Soil-No.	**Soil 1**		**Soil 2**		**Soil 3**	
Sample-No.	1	2	1	2	1	2
Soil to aqueous phase ratio = 1 : 5 (w/w)						
Concentration of test material [mg/l]	0.365	0.365	0.365	0.365	0.365	0.365
After contact of....hours with soil	2.3	2.3	1.8	1.7	2.8	2.8
Correction for blank with soil	-	-	-	-	-	-
Correction for blank without soil	-	-	-	-	-	-
Final corrected concentration [mg/l]	-	-	-	-	-	-
Initial concentration of test solution [mg/l]	0.365	0.365	0.365	0.365	0.365	0.365
Decrease in concentration [mg/l]	0	0	0	0	0	0
Quantity adsorbed [µg]	8.92	8.92	8.97	8.98	8.89	8.88
Quantity of soil [g of oven-dried equivalent]	5.0	5.0	5.0	5.0	5.0	5.0
Quantity adsorbed [µg] per gram of soil	1.78	1.78	1.79	1.79	1.78	1.78
Test material adsorbed [%]	97.7	97.7	98.3	98.4	97.4	97.3
Temperature [°C]	20 ± 1	20 ± 1	20 ± 1	20 ± 1	20 ± 1	20 ± 1
Volume of solution recovered after centrifugation [ml]	25.3	25.3	25.3	25.3	25.3	25.3
Volume of solution not recovered [ml]	2.5	2.4	3.5	4.3	1.5	1.3
Corresponding quantity of test substance [mg]	a)	a)	a)	a)	a)	a)

Table A7_2_3_1-4: Results of screening test – adsorption (soil:aqueous phase ratio = 1:25 (w/w))

Soil-No.	**Soil 1**		**Soil 2**		**Soil 3**	
Sample-No.	1	2	1	2	1	2
Soil to aqueous phase ratio = 1 : 25 (w/w)						
Concentration of test material [mg/l]	0.365	0.365	0.365	0.365	0.365	0.365
After contact of....hours with soil	5.2	4.7	3.2	3.0	7.1	5.5
Correction for blank with soil	-	-	-	-	-	-
Correction for blank without soil	-	-	-	-	-	-
Final corrected concentration [mg/l]	-	-	-	-	-	-
Initial concentration of test solution [mg/l]	0.365	0.365	0.365	0.365	0.365	0.365
Decrease in concentration [mg/l]	0	0	0	0	0	0
Quantity adsorbed [µg]	8.71	8.69	8.85	8.86	8.66	8.59
Quantity of soil [g of oven-dried equivalent]	1.0	1.0	1.0	1.0	1.0	1.0
Quantity adsorbed [µg] per gram of soil	8.71	8.69	8.85	8.86	8.66	8.59
Test material adsorbed [%]	95.5	95.2	97.0	97.1	94.9	94.1
Temperature [°C]	20 ± 1	20 ± 1	20 ± 1	20 ± 1	20 ± 1	20 ± 1
Volume of solution recovered after centrifugation [ml]	25.2	25.3	25.3	25.5	25.2	25.0
Volume of solution not recovered [ml]	0.3	0	0.5	0	0	0
Corresponding quantity of test substance [mg]	a)	a)	a)	a)	a)	a)

a) not given in the report

Table A7_2_3_1-5: Results of screening test - desorption:

Soil-No.	**Soil 1**		**Soil 2**		**Soil 3**	
Sample-No.	1	2	1	2	1	2
Soil to aqueous phase ratio = 1 : 5 (w/w)						
Temperature [°C]	20 ± 1	20 ± 1	20 ± 1	20 ± 1	20 ± 1	20 ± 1
Concentration in combined washings [mg/l]	0.13	0.12	0.08	0.08	0.27	0.24
Corresponding quantity of test material [mg]	a)	a)	a)	a)	a)	a)
Quantity desorbed [µg]	a)	a)	a)	a)	a)	a)
[%] of adsorbed test material, which is desorbed	1.4	1.3	0.9	0.9	2.9	2.7
[%] of adsorbed test material, which is not desorbed	98.6	98.7	99.1	99.1	97.1	98.3
Soil to aqueous phase ratio = 1 : 25 (w/w)						
Temperature [°C]	20 ± 1	20 ± 1	20 ± 1	20 ± 1	20 ± 1	20 ± 1
Concentration in combined washings [mg/l]	0.15	0.15	0.11	0.11	0.24	0.18
Corresponding quantity of test material [mg]	a)	a)	a)	a)	a)	a)
Quantity desorbed [µg]	a)	a)	a)	a)	a)	a)
[%] of adsorbed test material, which is desorbed	1.7	1.8	1.3	1.3	2.8	2.1
[%] of adsorbed test material, which is not desorbed	98.3	98.2	98.8	98.8	97.2	97.9

a) not given in the report

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**3.1.2 Specification** No detailed specification and reference to section 2 was given in the original test report. **3.5.1 Test system** The soil I was obtained from the top 30 cm soil layer in the field and kept on the field as a monticule of 2 m height. The top 50 cm were transferred to the laboratory. Soil II was collected from arable land at the Swiss Research Station Les Barge (VS) and transferred to the laboratory. Soil III was obtained from the LUFA Speyer, Germany In the laboratory soil was stored air dried in closed plastic or glass containers at room temperature in the dark. The air-dried soils were 2 mm sieved before use. The moisture content of the stored soils was determined by drying at 105°C under reduced pressure until a constant weight was reached (3 to 16 hours).
Conclusion	**5.3 Conclusion** Based on the result of the screening test etofenprox showed high adsorption to soil particles. However based on the distribution coefficient alone, the conclusion that etofenprox can be classified as immobile in soils, is not accurate.
Reliability	1
Acceptability	Acceptable
Remarks	The experimental period was before the adoption of the reviewed OECD test guideline 106 in 2000, which proposes a different test design for screening purposes.

Section A7.1.3 Adsorption / Desorption

Annex Point IIA7.7

			Official use only
		1 REFERENCE	
1.1	**Reference**	Völkel, W., 2011, ^{14}C-Etofenprox: Adsorption/Desorption on Soil, Innovative Environmental Services (IES) Ltd., report no. 8180 015, unpublished	x
1.2	**Data protection**	Yes	
1.2.1	Data owner	Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data on existing a.s. to maintain a.s. Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EPA, OPPTS 835.1230	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	2-(4ethoxyphenyl)-2-methyl-[2-14C-propyl] 3-penoxy benzyl ether	
3.1.1	Lot/Batch number	217196-CEM357-26-1	
3.1.2	Specification		
3.1.2.1	Purity	Radiochemical purity: 98.6%; re-determined before use: 97.3%	
3.1.2.2	Further relevant properties	Specific radioactivity: 5.45 MBq/mg	
3.1.3	Method of analysis	The quantity of radioactivity was determined by liquid scintillation counter (LSC). The amount of radioactivity adsorbed to the soil was determined for all samples by combustion of soil. Furthermore, soil samples were extracted and the extracts analysed by TLC in order to take into account the degradation of etofenprox. The limit of quantification (LOQ) for etofenprox was 1.83 ng/L for the aqueous phase, 0.14 µg/kg for the soil extract and 0.056 µg/kg for the non-extractable. The limit of detection (LOD) for etofenprox was 1.22 ng/L for the aqueous phase, 0.09 µg/kg for the soil extract and 0.038 µg/kg for the non-extractable.	
3.2	**Degradation products**	Degradation products tested: No	
3.2.1	Method of analysis for degradation products	Not applicable	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference	Not applicable	

Section A7.1.3 Adsorption / Desorption

Annex Point IIA7.7

	substance	
3.4	**Soil types**	The adsorption/desorption characteristic of 14C-etofenprox was studied in 5 US soils: Gold 18 (soil I, sandy clay loam), RFH Exp. Farm (soil II, loamy sand), Stoneville R&D (soil III, clay), Cantire (soil IV, sandy loam) and Costal AG Research (soil V, sandy loam) using the batch equilibrium method. The soil characteristics are summarized in Table A7_1 _3-1.
3.5	**Testing procedure**	Non-entry field
3.5.1	Test system	In the preliminary test a control experiment was performed to assess potential adsorption of the test item on the surface of the vessel. Different types of tube materials (Teflon, Polyethylene, Polypropylene, Glass and Silanised Glass) were tested at a test item concentration of 0.8 µg/L.
3.5.2	Test solution and Test conditions	After 24 hours of shaking, test solutions were analysed by LSC. Following, a solubility test in 0.01 M CaCl2 solution was performed, using a solution at a concentration of 0.8 µg/L. The radioactivity in solution was determined after 24 hours and 95 hours.
3.6	**Test performance**	Non-entry field
3.6.1	Preliminary test	According to (a)"OECD 106": No The preliminary test was performed with two soils (I and IV) at a test item concentration of 0.00016 µL and three soil-to-solution ratio (1:25; 1:50; 1:100) with an adsorption time of 24 hours. Solutions were analysed by LSC.
3.6.2	Screening test	According to (a)"OECD 106": No The screening test was performed with five soils at soil-to-solution ratio of 1:25 (4 g/100 mL) at a test item concentration of 0.000191 µg/L. After 2, 5, 24 and 48 hours of shaking, duplicate tubes were sampled, and the supernatants measured by LSC. The pH of the aqueous phase was measured before and after (24 hours) contact with the soils, at the soil to aqueous solution ratio of 1:25.
3.6.3	Advanced test	According to (a)"OECD 106": No The advanced test was performed with five soils at a soil to solution ratio of 1:25 (4 g/100 mL) using five initial test item concentrations (0.0016, 0.0008, 0.0004, 0.0002 and 0.0001 µg/mL) and an agitation time of 24 hours for both adsorption and desorption. After 24 hours of shaking, duplicate tubes were sampled and the supernatants measured by LSC. Freundlich desorption isotherms were determined on the soils used during the adsorption isotherms experiment (five concentrations). After the adsorption step, the soil-solution mixture was centrifuged and the aqueous phase removed. The volume of solution was replaced by an equal volume of 0.01 M CaCl2 without test item. The new mixture was agitated further for 24 hours. Thereafter, duplicate tubes were sampled and analysed as for the adsorption step. Additionally, the soil samples were air-dried, removed from their tubes without washing the tube walls and then aliquots of each sample combusted to determine the radioactivity remaining in the soil.
3.6.4	LSC-method	The quantity of radioactivity was determined by liquid scintillation

x

Section A7.1.3 Adsorption / Desorption

Annex Point IIA7.7

		counter (LSC). The amount of radioactivity adsorbed to the soil was determined for all samples by combustion of soil. Furthermore, soil samples were extracted and the extracts analysed by TLC in order to take into account the degradation of etofenprox. The limit of quantification (LOQ) for etofenprox was 1.83 ng/L for the aqueous phase, 0.14 µg/kg for the soil extract and 0.056 µg/kg for the non-extractable. The limit of detection (LOD) for etofenprox was 1.22 ng/L for the aqueous phase, 0.09 µg/kg for the soil extract and 0.038 µg/kg for the non-extractable.
3.6.5	Other test	As above
		4 RESULTS
4.1	**Preliminary test**	After the control experiment Teflon tubes were selected as the vessel of choice to use in the study. The soil-to-solution ratio 1:25 (4g/100mL) showed the best recoveries. It seemed that adsorption to the tube walls could be decreased by increasing the amount of soil. It was therefore, decided to proceed using the ratio 1:25 in the screening and advanced test.
4.2	**Screening test: Adsorption**	The equilibration time for the adsorption of test item to the soils (I to V) was reached after 24 hours, with values of 95.8%, 93.5%, 95.6%, 95.5% and 93.2% of the applied amount respectively. After an equilibration time of 24 hours for desorption radioactivity was determined and expressed in parent equivalents of 0.0000026, 0.0000060, 0.0000027, 0.0000024 and 0.0000069 mg/L for soil I to V, respectively. In the adsorption phase, after addition of 0.0177 µg 14C-etofenprox, 0.0119 µg, 0.0122 µg, 0.0139 µg, 0.0134 µg and 0.0123 µg were adsorbed to soils I to V, respectively. In the desorption phase, after addition of 0.01906 µg 14C-etofenprox and successive replacement of supernatant equilibration solution with CaCl2 0.0182 µg, 0.0172 µg, 0.0180 µg, 0.0182 µg and 0.0171 µg was still adsorbed, to soils I to V, respectively. The amount of radioactivity determined in the aqueous phase and in the soil after 48 hours of shaking (adsorption phase) and after 48 hours of desorption were used to calculate the distribution coefficients. The corresponding Koc and Kd values are shown in Table A7_1_3_2
4.3	**Advanced test**	The adsorption and desorption constants are summarized in A7_1 _3-3. Except for soil I, the calculated Kdes, Foc values were higher than those obtained for the adsorption isotherms, indicating a partially irreversible adsorption process.
4.4	**Calculations**	Non-entry field
4.4.1	K_{FOC}	28524 mL/g
4.4.2	$K_{des, FOC}$	42299 mL/g

Section A7.1.3 Adsorption / Desorption

Annex Point IIA7.7

4.5	**Degradation product(s)**	Not reported

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Guideline: EPA, OPPTS 835.1230 Method: The adsorption/desorption characteristic of 14C-etofenprox was studied at 20°C in the dark in 5 US soils: Gold 18 (soil I, sandy clay loam), RFH Exp. Farm (soil II, loamy sand), Stoneville R&D (soil III, clay), Cantire (soil IV, sandy loam) and Costal AG Research (soil V, sandy loam), using the batch equilibrium method. The soil adsorption coefficients, including the Freundlich adsorption constant KF and KFOC, were determined in each soil. The soil adsorption phase was followed by a single desorption phase to determine the reversibility of adsorption. The study consisted in three parts: preliminary test, screening test and advanced test. Following preliminary and screening tests, an adsorption and desorption equilibration time of 24 hours each was selected for the advanced test.
5.2	**Results and discussion**	The calculated Freundlich coefficient (KF) for adsorption ranged from 23.5 to 871.9, while the 1/n values ranged from 0.81 to 1.16. When corrected for organic matter content of the soil, the resultants KFOC values ranged from 1546 to 100214. These data indicated that 14C-etofeprox was highly adsorbed to the soils studied. Except for soil I, the desorption constant of 14C-etofenprox were higher than the adsorption constant thus demonstrating that adsorption was not fully reversible. The adsorption constant KF were not found to be correlated to the organic carbon content of the soils.
5.2.1	K_{FOC}	28524 mL/g
5.2.2	$K_{des, FOC}$	42299 mL/g
5.3	**Conclusion**	Using the McCall Classification scale to assess a chemical's potential mobility in soil (based on KFOC), 14C-etofenprox can be classified as being immobile for soils I, II, III and V. For soil IV, low potential mobility was observed.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2012
Materials and Methods	**1.1 Reference:**
	Typing error; Report no. **818 01 015**
	3.4 Soli Types:
	Typing error in Table A7_1_3-1: Soil IV has **70% sand** instead of 10%;
Results and discussion	Agree with applicant
Conclusion	Agree with applicant
Reliability	1
Acceptability	Acceptable
Remarks	None
	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	I Gold 18	II RFH Exp. Farm	III Stoneville R&D	IV Cantire	V Coastal AG Research
Origin	**US**	**US**	**US**	**US**	**US**
Soil Type (acc. to USDA)	Sandy clay loam	Loamy sand	Clay	Sandy loam	Sandy loam
Particle size (mm)					
> 0.05 (sand) %	51	80	17	10	54
0.002 – 0.05 (silt) %	22	16	33	23	26
< 0.002 (clay) %	27	4	50	7	20
Particle size (mm)					
pH (0.01M$CaCl_2$)	6.42	6.92	6.90	7.29	7.00
pH (water)	7.1	7.0	7.2	7.7	7.4
Organic carbon (g/100 g soil) %	0.87	0.34	1.04	2.2	0.54
Organic matter* (g/100 g soil) %	1.5	0.58	1.8	3.8	0.93
Cation exchange capacity (mmol/100g soil)	11.6	8.9	27.3	12.7	13.0
Nitrogen content %	0.08	0.02	0.12	0.20	0.04
C/N-ratio*	10.9	17.0	8.7	11.0	13.5

*: %OM and C/N ratio were calculated as follows: %OM = 1.724 * %organic carbon; C/N ratio = % organic carbon / % nitrogen content

Table A7_1 _3-2: Distribution coefficients of 14C-etofenprox in five soils

Parameter	Soil I	Soil II	Soil III	Soil IV	Soil V	Mean values
K_{oc}(mL/g)	46313	61301	40312	24031	36044	41600
K_d (mL/g)	402.9	208.4	419.2	528.7	194.6	350.8
K_{oc}des (mL/g)	200807	184845	99571	60394	101862	129496
$K_d$des (mL/g)	1747	628	1036	1329	550	1058

Table A7_1 _3-3: Adsorption and desorption constants of 14C-etofenprox in five soils

Parameter		Soil I	Soil II	Soil III	Soil IV	Soil V	Mean values
Soil Type		Sandy clay loam	Loamy sand	Clay	Sandy loam	Sandy loam	-
pH ($CaCl_2$)		6.42	6.92	6.90	7.29	7.00	-
% OC		0.87	0.34	1.04	2.2	0.54	-
$K_{ads, F}$	[mL/g]	871.863	23.485	298.650	34.002	28.272	
$K_{ads, Foc}$	[mL/g]	100214	6907	28716	1546	5236	28524
$K_{ads, Fom}$	[mL/g]	58129	4007	16657	896	3037	16545
1/n	-	1.16	0.87	0.99	0.81	0.86	0.94
r^2	-	0.9874	0.9731	0.9742	0.9936	0.9616	
$K_{des, F}$	[mL/g]	218.420	326.499	817.999	80.536	43.433	
$K_{des, Foc}$	[mL/g]	25106	96029	78654	3661	8043	42299
$K_{des, Fom}$	[mL/g]	14563	55701	45623	2123	4665	24535
1/n	-	0.89	1.03	1.01	0.82	0.93	0.94
r^2	-	0.9733	0.9801	0.9831	0.9712	0.9989	

Section 7.2.2.1
Annex Point IIIA-XII.1.1, XII.1.4

Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

			Official use only
		1 REFERENCE	
1.1	**Reference**	Völkl S. (2001): 14C-Etofenprox: Degradation and metabolism in four soils incubated under aerobic conditions. RCC Ltd., Environmental Chemistry & Pharmanalytics Division, Itingen, Switzerland; unpublished report no. 728987 (December 18, 2001) Experimental phase: January 23, 2001 to July 03, 2001 First / Second amendment to report Völkl S. (2003): 14C-Etofenprox: Degradation and metabolism in four soils incubated under aerobic conditions. RCC Ltd., Environmental Chemistry & Pharmanalytics Division, Itingen, Switzerland; unpublished report no. 728987 (June 03, 2003) Experimental phase: July 12, 2002 – August 21, 2002 In this amendment the presence of the metabolites α-CO, 4'-OH, DE and DP in the soil extract was confirmed by GC-MS and the presence of low amounts of the metabolites m-PB-acid and PENA/EPMP was confirmed by TLC analysis.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.	
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 SETAC (1995), OECD Draft Guideline (1999), JMAFF Guideline, 12 Nohsan 8147 and US EPA Subdivision N, 162-1 (1982)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	Etofenprox	x
3.1.1	Lot/Batch number	9604	x
3.1.2	Specification	As given in section 2	x
3.1.3	Purity	99.99 %	x
3.1.4	Further relevant properties	Solubility in water: 22.5 µg/L	
3.1.5	Radiolabelling	A) [α-^{14}C-benzyl]-etofenprox - Batch: MRH/MTC 277/20 - Specific activity: 1220 MBq/mmol - Radiochemical purity: >97.6 % (B) [2-^{14}C-propyl]-etofenprox - Batch: MRH/MTC 276/31 - Specific activity: 1580 MBq/mmol)	

Section 7.2.2.1
Annex Point IIIA-XII.1.1, XII.1.4

Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

		- Radiochemical purity: 100%	
3.1.6	TS inhibitory to microorganisms	No	
3.2	**Reference substance**	No	
3.3	**Testing procedure**		
3.3.1	Soil	The soils Senozan (soil I, St. Et Loire / France), Gartenacker (soil II, Les Barge / Switzerland), Georgia (soil III, Montezumea, Georgia / USA) and Cajon (soil IV, California, USA) were freshly collected in the field from the soil top layer (0-20 cm). All soils were sieved (≤ 2 mm) and acclimated at room temperature for about 2 weeks before treatment. The soil characteristics are listed in Table 7_2_2_1-1.	x
3.3.2	Test system	Details on laboratory equipment etc. in tabular form (see table A7_2_2_1-2) Soil samples (100 g dry weight) were incubated under aerobic conditions in all-glass flasks. The system was ventilated with moist air. Exhaust air was passed through traps to absorb organic volatiles and CO_2. The moisture content was adjusted to about 40% of the maximum water holding capacity (MWHC).	
3.3.3	Test conditions	Relevant test conditions in tabular form (see table A7_2_2_1-3)	
3.3.4	Method of preparation of test solution	The test substance was dissolved in acetone and aliquots of 430 µL from the application solution were added dropwise to each soil sample (equivalent to 100 g dry soil).	
3.3.5	Application of test item	Etofenprox was applied at a concentration of 0.3 mg a.i./kg dry soil, corresponding to a field rate of 0.3 kg a.i./ha (assuming an even distribution in the top 10 cm soil layer and 1.0 g/cm^3 soil density). The soil was then homogeneously mixed allowing the organic solvent to evaporate.	
3.3.6	Duration of test	Post application: 120 days	
3.3.7	Temperature / light	20 ± 2 °C / in the dark	
3.3.8	Sampling	Duplicate soil samples were taken after 0, 7, 14, 21, 28, 55, 92 and 120 days of incubation at 20°C for soils I, II, III and IV. One replicate was taken for soil I incubated at 10°C at the same sampling intervals except for Day 0.	
3.3.9	Intermediates/ degradation products	LSC analyses of the trapping solutions were performed. The soil extractions were performed up to 3 times with acetonitrile/water at room temperature, followed by a soxhlet extraction, for samples from day 7 onwards. Soil samples from day 120 were submitted to a harsh extraction procedure (acetonitrile/0.1M hydrochloric acid). The extracts were analysed by HPLC and TLC.	
3.3.10	Analytical methods	HPLC with radioactivity detection, TLC; Liquid scintillation counting.; mass spectroscopy. Details see table A7_2_2_1-4	
3.3.11	Statistics	The recovery of radioactivity and the DT_{50} and DT_{90} values of etofenprox in 4 different soils systems were calculated using a one compartment model with non-linear curve fitting applying first order kinetics: $Y = C_o * e^{-kt}$	

4 RESULTS

Section 7.2.2.1 Annex Point IIIA-XII.1.1, XII.1.4 — Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

4.1	**Recovery**	During the course of the study, the total mean recoveries were 97.2%, 97.0%, 96.7% and 96.4% of the applied radioactivity in soils I to IV, respectively. The recovery for soil I incubated at 10°C was 97.5% of the applied radioactivity.
4.2	**Degradation of test substance**	
4.2.1	Mineralisation	The mineralization was high in all soils. After 120 days of incubation, the amount of $^{14}CO_2$ reached maximum values of 44.0%, 38.2%, 45.6% and 39.1% of the applied radioactivity in soils I to IV, respectively, and 29.9% in soil I incubated at 10°C. Other volatile products did not exceed 0.9% of applied radioactivity.
4.2.2	Extractability	Immediately after treatment (Day 0), 96.4%, 95.2%, 95.2% and 94.2% of the applied radioactivity could be extracted from soils I to IV. The amount of extractable residues decreased continuously with time in all soils amounting to 27.4%, 42.5%, 57.9%, 76.6% and 54.1% of the applied radioactivity on Day 14 in soils I to IV and soil I incubated at 10°C, respectively. At the end of incubation (Day 120), the extractable radioactivity amounted to 4.5%, 4.6%, 10.9%, 12.1% and 10.8% for soils I to IV and soil I (10°C), respectively. The amount of non-extractable radioactivity increased continuously in all soils amounting to 47.5%, 39.2%, 23.5%, 15.2% and 34.2% of the applied radioactivity after 14 days of incubation in soils I to IV and soil I incubated at 10°C, respectively. It reached maximum amounts of 54.9%, 57.0% (Day 55), 47.9%, 49.9% (Day 92) and 55.8% (Day 55) in soils I to IV and soil I (10°C), respectively. Thereafter, it decreased by further mineralization to $^{14}CO_2$. The major part of the non-extractable radioactivity was bound to immobile humic acids and humin fractions amounting to 38.4%, 44.3%, 31.7%, 37.6% and 44.3% of the applied radioactivity in soils I to IV and soil I (10°C), respectively, at Day 120. The corresponding radioactivity associated with the fulvic acid fraction accounted for 10.0%, 8.4%, 9.2%, 7.4% and 10.4% of the applied radioactivity in soils I to IV and soil I (10°C), respectively.
4.2.3	Metabolites	^{14}C-etofenprox amounted to 95.6%, 94.8%, 94.6% and 93.7% of the radioactivity applied immediately after treatment (Day 0) in soils I to IV, respectively. It degraded rapidly and at the end of incubation, after 120 days, ^{14}C-etofenprox amounted to 2.3%, 2.9%, 6.5%, 8.0% and 6.1% of the applied radioactivity in soils I to IV and soil I (10°C), respectively. Six metabolites were identified as α-CO, 4'-OH, DE, DP, m-PB-acid and PENA/EPMP, but none exceeded 5.3% of the applied radioactivity. The amount of unidentified radioactivity (6 radioactive fractions) did not exceed 2.5% of the applied radioactivity. Ten additional radioactive fractions were detected after harsh extraction, but none of them exceeded an amount of 5.5% of the applied radioactivity. The results obtained are summarised in Table A7_2_2_1-5
4.2.4	Degradation rates of etofenprox in aquatic systems	Based on these results, the degradation rates of etofenprox in the four soils were calculated. The DT50 values for degradation of etofenprox were between 7 and 25 days, the DT90 values between 22 and 84 days. Details are described in Table A7_2_2_1-5
4.2.5	Degradation rates of metabolites	The degradation rates of the metabolites α-CO, 4'-OH, DE, and DP were calculated. Details are described in Table A7_2_2_1-5

Section 7.2.2.1
Annex Point IIIA-XII.1.1, XII.1.4

Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The route and rate of degradation was investigated in 2 European soils (Senozan and Gartenacker) and 2 American soils (Georgia and Cajon) using a mixture (1+1) of [α-^{14}C-benzyl]-etofenprox (batch no. MRH/MTC277/20; radiochemical purity: 97.6%) and [2-^{14}C-propyl]-etofenprox (batch no. MRH/MTC276/31; radiochemical purity: 100%), incubated under aerobic conditions at 20°C and 10°C for up to 120 days. The soils Senozan (soil I), Gartenacker (soil II), Georgia (soil III) and Cajon (soil IV) were freshly collected in the field from the soil top layer (0-20 cm). All soils were sieved (≤ 2 mm) and acclimated at room temperature for about 2 weeks before treatment.

Etofenprox was applied at a concentration of 0.3 mg a.i./kg dry soil, corresponding to a field rate of 0.3 kg a.i./ha (assuming an even distribution in the top 10 cm soil layer and 1.0 g/cm^3 soil density). The test substance was dissolved in acetone and aliquots of 430 µL from the application solution were added dropwise to each soil sample (equivalent to 100 g dry soil). The soil was then homogeneously mixed allowing the organic solvent to evaporate. The soil samples were incubated aerobically in glass metabolism flasks in the dark at 20±2°C and 10±2°C for 120 days. The flasks were connected to an open air-flow–through system adjusted to about 30-50 mL/minute. The exhaust air was passed through a trapping system equipped with absorption traps containing 50 mL of ethylene glycol and 50 mL of 2N NaOH, in order to trap organic volatiles and $^{14}CO_2$, respectively. The moisture content was adjusted to about 40% of the maximum water holding capacity (MWHC).

Duplicate soil samples were taken after 0, 7, 14, 21, 28, 55, 92 and 120 days of incubation at 20°C for soils I, II, III and IV. One replicate was taken for soil I incubated at 10°C at the same sampling intervals except for Day 0. LSC analyses of the trapping solutions were performed. The soil extractions were performed up to 3 times with acetonitrile/water at room temperature, followed by a soxhlet extraction, for samples from day 7 onwards. Soil samples from day 120 were submitted to a harsh extraction procedure (acetonitrile/0.1M hydrochloric acid). The extracts were analysed by HPLC and TLC. The purity and stability of [^{14}C]-etofenprox in the application solution was determined before and after treatment by HPLC. The microbial biomass was determined before the start and at the end of incubation.

5.2 Results and discussion

Recovery

During the course of the study, the total mean recoveries were 97.2%, 97.0%, 96.7% and 96.4% of the applied radioactivity in soils I to IV, respectively. The recovery for soil I incubated at 10°C was 97.5% of the applied radioactivity.

Extractable and non-extractable radioactivity

Immediately after treatment (Day 0), 96.4%, 95.2%, 95.2% and 94.2% of the applied radioactivity could be extracted from soils I to IV. The amount of extractable residues decreased continuously with time in all soils amounting to 27.4%, 42.5%, 57.9%, 76.6% and 54.1% of the applied radioactivity on Day 14 in soils I to IV and soil I incubated at 10°C, respectively. At the end of incubation (Day 120), the extractable radioactivity amounted to 4.5%, 4.6%, 10.9%, 12.1% and 10.8% for soils I to IV and soil I (10°C), respectively.

Section 7.2.2.1
Annex Point IIIA-XII.1.1, XII.1.4

Aerobic degradation in soil (rate and route of degradation, including identification of metabolites and degradation products)

The amount of non-extractable radioactivity increased continuously in all soils amounting to 47.5%, 39.2%, 23.5%, 15.2% and 34.2% of the applied radioactivity after 14 days of incubation in soils I to IV and soil I incubated at 10°C, respectively. It reached maximum amounts of 54.9%, 57.0% (Day 55), 47.9%, 49.9% (Day 92) and 55.8% (Day 55) in soils I to IV and soil I (10°C), respectively. Thereafter, it decreased by further mineralization to $^{14}CO_2$. The major part of the non-extractable radioactivity was bound to immobile humic acids and humin fractions amounting to 38.4%, 44.3%, 31.7%, 37.6% and 44.3% of the applied radioactivity in soils I to IV and soil I (10°C), respectively, at Day 120. The corresponding radioactivity associated with the fulvic acid fraction accounted for 10.0%, 8.4%, 9.2%, 7.4% and 10.4% of the applied radioactivity in soils I to IV and soil I (10°C), respectively.

Mineralization

The mineralization was high in all soils. After 120 days of incubation, the amount of $^{14}CO_2$ reached maximum values of 44.0%, 38.2%, 45.6% and 39.1% of the applied radioactivity in soils I to IV, respectively, and 29.9% in soil I incubated at 10°C. Other volatile products did not exceed 0.9% of applied radioactivity.

Identification of radioactivity

^{14}C-etofenprox amounted to 95.6%, 94.8%, 94.6% and 93.7% of the radioactivity applied immediately after treatment (Day 0) in soils I to IV, respectively. It degraded rapidly and at the end of incubation, after 120 days, ^{14}C-etofenprox amounted to 2.3%, 2.9%, 6.5%, 8.0% and 6.1% of the applied radioactivity in soils I to IV and soil I (10°C), respectively. Six metabolites were identified as α-CO, 4'-OH, DE, DP, m-PB-acid and PENA/EPMP, but none exceeded 5.3% of the applied radioactivity. The amount of unidentified radioactivity (6 radioactive fractions) did not exceed 2.5% of the applied radioactivity. Ten additional radioactive fractions were detected after harsh extraction, but none of them exceeded an amount of 5.5% of the applied radioactivity.

Degradation

Kinetics of degradation for etofenprox and its degradation products α-CO, 4'-OH, DE and DP were calculated by using the first order kinetic model and the corresponding amounts detected in extracts from Day 0 to Day 120. Etofenprox degraded rapidly with half-lifes (DT_{50}) of about 7, 8, 14, 25 and 13 days from soils I to IV and soil I incubated at 10°C, respectively. The DT_{50} values of the metabolites α-CO, 4'-OH, DE and DP ranged from 12-45, 14-56, 32-41 and 17-66 days, respectively in the four soils.

5.3 Conclusion

The results of this study show that the metabolic pathway of etofenprox was very similar in all soils incubated aerobically at 20°C or 10°C. The main degradation product was $^{14}CO_2$, ranging from 38% to 46% of the applied radioactivity in soils I to IV and 30% in soil I at 10°C, within 120 days of incubation. Up to 12 radioactive fractions were detected, but none of them exceeded 5.3% of the applied radioactivity. The main metabolites were characterised as α-CO (max. 3.5%), 4'-OH (max. 5.3%), DE (max. 3.6%) and DP (max. 3.6%). x

5.3.1 Reliability 1

5.3.2 Deficiencies No

Table A7_2_2_1-1: Characteristic of the soils used in the study of Völkl (2001).

Soil **Origin**	**Senozan** **France**	**Gartenacker** **Switzerland**	**Georgia** **USA**	**Cajon** **USA**
Soil type (according to USDA)	silt clay loam	loam	sandy loam	sandy loam
Particle size (according to USDA)				
sand > 50 µm [%]	19.9	47.3	80.5	73.3
silt 2-50 µm [%]	51.0	43.4	10.6	18.7
clay < 2 µm [%]	29.1	9.3	8.9	8.0
pH ($CaCl_2$)	6.7	7.2	6.8	7.4
(KCl)	5.8	n.d.	n.d.	n.d.
Organic carbon [g/100 g soil]	1.2	2.2	0.3	0.6
CEC [meq/100 g soil]	19.33	12.8	4.1	8.9
Microbial biomass* [mg microbial C/100 g soil]				
start of incubation (Day 0)	30	40.7	7.5	11.2
end of incubation (Day 120)	31.2/27.5**	37.7	11.2	12.8
MWHC [g water/100g soil]	55.3	64.6	30.9	35.5
40% MWHC [g water/100g soil]	22.2	25.8	12.4	14.2

CEC cation exchange capacity
MWHC maximum water holding capacity
n.d. not determined
* determined according to ANDERSON AND DOMSCH
** biomass determined in the soil sample incubated at 10°C.

Table A7_2_2_1-2: Incubation system.

Criteria	**Details**
Apparatus	All glass metabolism bottles containing 100g soil (dry weight)
Number of replicates/concentration	1 replicate for each soil
Air pre-treatment	The incoming air was passed through a purified water trap.
Trapping system	The exhaust air was passes trough a series of traps: Trap 1: ethanediol (ethylene glycol) Trap 2: 2M NaOH

Table A7_2_2_1-3: Details of the analytical methods.

Method	Details	
HPLC	Column	YMC ODS-AS (150 x 3 mm; 3 µm) with pre-column LiChrospher (Merck) 100 C-18 4 x 4 mm, 5 µm
	Mobile phase	A: water adjusted to pH 2.5 with phosphoric acid; B: acetonitrile 0 min 70 % A 20 – 30 min 10 % A 31 - 35 min: 70 % A
	Flow rate	1 ml/min
	Detection	UV at 276 nm; radioactivity using Packard Flow Scintillation Analyzer, 500TR, with Flo-Scint QA scintillator
TLC	Plates	Pre-coated 5 x 20 cm, 0.25 mm coating silica gel 60 F_{254} or RP-18 plate; detection by scanning with TLC linear analyzer (Tracemaster, 40, Dr. Berthold, Germany or phosphor imaging with a Fuji BAS 1000 Imager. Non-labelled materials were detected by quenching of uv-light of 254 nm,
	Solvent 1	toluene / ethyl acetate 50/50 v/v
	Solvent 2	chloroform / methanol 95/5 v/v
	Solvent 3	toluene / ethyl acetate 90/10 v/v
	Solvent 4	toluene / ethyl acetate 19/1 v/v
	Solvent 5	toluene
	Solvent 6	hexane / ethyl acetate 90/10 v/v
	Solvent 7	toluene / ethyl acetate / acetic acid 90/10/1 v/v/v
	Solvent 8	methanol (on RP-18)
	Solvent 9	chloroform / methanol / acetic acid 95/5/1 v/v/v
	Solvent 10	methanol / acetonitrile 50/50 v/v (on RP-18)
	Solvent 11	toluene / ethyl acetate / acetic acid 80/20/1 v/v/v
	Solvent 12	chloroform / acetic acid 99/1 v/v
Mass spectrometry 1	Instrument	Thermo Finnigan PolarisQ
	Ionisation	electron Impact, positive
	Scan mode	full scan m/z 50 - 650
Mass spectrometry 2	Instrument	Hewlett Packard MS 5973
	Ionisation	electrospray ionisation, positive
	Scan mode	full scan m/z 50 -650 or single ion monitoring m/z 163, 135, 300, 376, 390, 392

Table A7_2_2_1-4: Recovery of radioactivity and distribution of the active substance and metabolites after application of [^{14}C]-etofenprox to 4 different soils and aerobic incubation at 20°C and 10°C (values given in % of applied radioactivity).

Soil	Days after appli-cation	$^{14}CO_2$	Not extracted radio-activity	Extracted radio-activity	Etofen prox	α-CO	4'-OH	DE	DP	m-PB-acid	PENA/ EPMP	Total
Soil I Senozan silt clay loam	0	n.p.	5.6	**96.4**	95.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**102.0**
	7	12.2	28.1	56.1	46.4	2.5	4.4	n.d.	2.8	n.d.	n.d.	**96.4**
	14	20.0	47.5	27.4	18.1	2.3	3.3	0.2	1.8	0.7.	n.d.	**94.9**
	21	27.0	51.5	17.5	10.6	0.7	1.8	0.6	1.7	0.4	0.7	**96.0**
	28	29.2	53.7	13.6	8.0	0.7	1.6	n.d.	1.5	0.8	n.d.	**96.6**
	55	34.5	54.9	7.7	4.8	0.4	0.6	0.2	0.6	0.3	n.d.	**97.2**
	92	39.7	52.1	5.0	3.7	0.3	0.3	n.d.	0.2	n.d.	n.d.	**96.9**
	120	44.0	49.1	4.5	2.3	0.2	0.3	n.d.	0.4	0.1	n.d.	**97.5**
Soil I Senozan (10°C) silt clay loam	7	4.7	19.2	75.6	65.2	**3.5**	3.1	0.8	2.0	1.0	n.d.	**99.5**
	14	7.8	34.2	54.1	42.0	2.2	2.8	1.0	2.5	1.2	0.6	**96.0**
	21	12.6	45.0	37.7	25.4	2.4	3.3	0.6	2.0	1.2	0.4	**95.3**
	28	16.2	48.8	31.6	21.8	1.7	3.1	0.4	2.1	0.9	0.4	**96.6**
	55	21.2	**55.8**	18.9	11.1	1.2	1.7	0.4	1.6	0.3	0.4	**96.9**
	92	26.2	55.7	11.9	7.9	0.8	1.0	n.d.	0.8	n.d.	n.d.	**93.8**
	120	29.9	54.5	10.8	6.1	0.6	0.8	n.d.	0.9	0.5	n.d.	**95.3**
Soil II Garten-acker loam	0	n.p.	5.1	95.2	94.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**100.3**
	7	6.0	21.6	70.1	55.8	3.4	5.2	0.7	1.6	1.5	1.5	**97.7**
	14	11.9	39.2	42.5	28.0	3.0	4.9	n.d.	2.3	**2.5**	**1.9**	**93.6**
	21	21.9	48.9	25.3	15.3	1.7	3.7	0.2	1.6	1.0	0.6	**96.1**
	28	26.4	55.3	16.1	9.9	0.8	2.1	0.3	1.2	0.6	0.2	**97.9**
	55	32.6	57.0	8.3	4.5	1.3	0.7	0.1	0.5	0.2	0.1	**97.9**
	92	34.2	54.9	6.1	4.6	0.4	0.4	n.d.	n.d.	n.d.	n.d.	**95.2**
	120	38.2	52.8	4.6	2.9	0.3	0.3	n.d.	0.1	n.d.	n.d.	**95.6**
Soil III Georgia sandy loam	0	n.p.	5.1	95.2	94.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**100.3**
	7	1.4	9.5	88.1	79.4	1.9	n.d.	**3.6**	0.9	1.4	0.8	**99.0**
	14	12.9	23.5	57.9	47.7	1.4	2.7	2.0	2.6	n.d.	1.5	**94.4**
	21	17.7	32.4	42.9	28.4	1.3	2.8	3.0	**3.6**	1.7	0.9	**93.0**
	28	25.7	38.1	32.6	21.1	1.1	1.6	2.1	3.1	1.1	0.9	**96.4**
	55	36.5	44.8	16.0	10.5	0.5	0.8	0.9	1.6	0.5	0.4	**97.3**
	92	22.9	47.9	11.9	7.7	0.6	0.6	0.7	1.2	0.3	0.3	**91.3**
	120	**45.6**	42.9	10.9	6.5	0.3	0.6	0.7	1.0	0.4	0.3	**99.4**
Soil IV Cajon sandy loam	0	n.p.	6.3	94.2	93.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**100.5**
	7	1.5	3.5	94.7	90.8	0.9	0.4	0.7	0.2	1.0	0.6	**99.6**
	14	5.3	15.2	76.6	65.4	1.8	**5.3**	1.6	1.3	n.d.	1.1	**97.2**
	21	9.9	19.0	63.5	52.9	2.3	3.6	1.5	1.1	0.5	0.7	**92.5**
	28	15.8	32.2	48.0	39.8	1.5	2.6	1.4	0.9	0.7	0.7	**96.0**
	55	26.1	41.2	29.0	22.4	1.4	2.3	0.7	0.7	0.3	0.3	**96.4**
	92	26.7	49.9	14.6	10.7	0.6	1.3	0.5	0.5	n.d.	0.1	**91.3**
	120	39.1	46.3	12.1	8.0	0.5	1.4	0.3	0.5	n.d.	0.6	**97.5**

n.p. not performed

n.d. not detected or below limit of detection

The mean maximum values of metabolites are highlighted in **bold**

Table A7_2_2_1-5: Kinetics of degradation of etofenprox and its degradation products

Soil	Soil I	Soil I / 10°C	Soil II	Soil III	Soil IV
Etofenprox					
DT_{50} (days)	7	13	8	14	25
DT_{90} (days)	22	41	28	46	84
Kinetic constant k1 (1/day)	0.1069	0.0556	0.0830	0.0502	0.0275
Correlation coefficient (r)	0.9958	0.9887	0.9964	0.9833	0.9885
α-CO					
DT_{50} (days)	12	34	13	37	45
DT_{90} (days)	40	113	44	122	150
Kinetic constant k1 (1/day)	0.0581	0.0205	0.0529	0.0189	0.0153
Correlation coefficient (r)	0.9341	0.9469	0.9622	0.9587	0.9474
4'-OH					
DT_{50} (days)	14	56	19	29	44
DT_{90} (days)	46	186	63	96	145
Kinetic constant k1 (1/day)	0.0499	0.0124	0.0366	0.024	0.0159
Correlation coefficient (r)	0.9754	0.949	0.9817	0.898	0.9022
DE					
DT_{50} (days)	*	*	*	32	41
DT_{90} (days)				105	137
Kinetic constant k1 (1/day)				0.0219	0.0167
Correlation coefficient (r)				0.9711	0.9897
DP					
DT50 (days)	24	63	17	43	66
DT90 (days)	78	209	56	144	219
Kinetic constant k1 (1/day)	0.0291	0.011	0.0414	0.0160	0.0105
Correlation coefficient (r)	0.9762	0.9706	0.9958	0.9745	0.9559

* Calculation of the kinetic is not possible due to the very low amounts detected (<1% of applied radioactivity)

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27. 05. 2005
Materials and methods	**3.1 Test material** Information given under this heading refers to the unlabelled reference substance. **3.1.1 Lot/Batch number** MR-9301 **3.1.2 Specification** No details given in the test report **3.1.3 Purity** 99.7 % **3.3.1 Soils** No information for pesticide usages was given for the soils from US. The recommendation of the OECD guideline 307 concerning the selection of soils which should show a variation in their soil properties was not fulfilled. PHs varied only from 6.7 to 7.4. Also the two soils from US were quite similar in soil texture and C_{org}.
Conclusion	**5.3 Conclusion** The content of bound residues was continuously high in the European soils (up to 57% AR) and decreased very slowly. Though the US soils showed a lower organic carbon content and CEC bound residues also reached approximately 50% AR.
Reliability	1
Acceptability	Acceptable
Remarks	In an additional study on photolysis on soil surface a DT_{50} of 19.3 days was calculated. In the dark control a DT_{50} of 22.2 days was calculated. Up to 10 minor degradation products were detected, six of which were characterised as α-CO, 4'-OH, DE, m-PB-acid, a mixture of PENA and EPMP and DP. Non of the degradation products exceeded 7.7% (α-CO) of the applied radioactivity. Photo-degradation of ^{14}C-MTI-500 will occur in the environment to form the major photo-degradate α-CO. However, more rapid ways of its disappearance from soil will be direct mineralization and formation of bound residues.

Section A7.3.1
Annex Point IIIA-VII.5

Phototransformation in air (estimation method) including identification of breakdown products

Official use only

1 REFERENCE

1.1	**Reference**	Bates, M. (2001): MTI-500: estimation of the photochemical oxidative degradation – amended final report; Covance Laboratories Ltd.; unpublished report No. 719/12-D2141; (January 31, 2001) Dates of experimental work: not applicable; calculation only
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes: Directives 91/414/EEC and 94/37/EEC EPA OPPTS 830 series Japan MAFF Guideline, published in NohSan Number 4200 (28 January 1985
2.2	**GLP**	No Calculations are not subject to GLP requirements
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	MTI-500 (Etofenprox)
3.1.1	Lot/Batch number	54023
3.1.2	Specification	As given in section 2 Deviating from specification given in section 2 as follows
3.1.3	Purity	99.3%
3.2	**Reference substances**	not applicable
3.3	**Calculation method**	The chemical structure of the test substance was translated into "SMILES" format and entered into the computer simulation program "EPWIN v3.05" US EPA version, 12 July 2000. This software includes "AopWin v1.90" which calculates the atmospheric oxidation at 25°C by hydroxyl radical reaction and/or ozone reaction.

4 RESULTS

4.1	**Hydroxyl radicals reaction**	Overall OH rate constant = 61.16 * 10^{-12} cm^3/molecule-sec Half-life = 2.07 hours
4.2	**Ozone reaction**	No ozone reaction estimation

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A7.3.1
Annex Point IIIA-VII.5

Phototransformation in air (estimation method) including identification of breakdown products

5.1	**Materials and methods**	Test guidelines: Directives 91/414/EEC and 94/37/EEC EPA OPPTS 830 series Japan MAFF Guideline, published in NohSan Number 4200 (28 January 1985 The chemical structure of the test substance was translated into SMILES format and entered into the computer simulation program "EPWIN v3.05" US EPA version, 12 July 2000. This software includes "AopWin v1.90" which calculates the atmospheric oxidation at 25°C by hydroxyl radical reaction and/or ozone reaction.
5.2	**Results and discussion**	Overall OH rate constant = 61.16 * 10-12 cm^3/molecule-sec Half-life = 2.07 hours No ozone reaction estimation
5.3	**Conclusion**	Etofenprox is not persistent in air and does not react with ozone
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2011
Materials and methods	3.1: Substitution: Chemical structure of active substance 3.1.1: Substitution: not applicable 3.1.2: Substitution: not applicable 3.1.3: Substitution: not applicable 3.3: Update: The chemical structure of the test substance was translated into "SMILES" format and entered into the computer simulation program "EPWIN v4.0" US EPA version. This software includes "AopWin v1.92" which calculates the atmospheric oxidation at 25°C by hydroxyl radical reaction and/or ozone reaction. 3.3: Addition: The estimation for Etofenprox was carried out with respect to the OH radical reaction, using a 24-hours-day with $5x10^5$ radicals/cm^3. An excerpt from the AOPWIN protocol is presented below: SMILES : C(C)(C)(c3ccc(OCC)cc3)COCc1cc(Oc2ccccc2)ccc1 MOL FOR: C25 H28 O3 MOL WT : 376.50 -- SUMMARY (AOP v1.92): HYDROXYL RADICALS (25 deg C) – Hydrogen Abstraction = 19.2132 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 = 42.9445 E-12 cm3/molecule-sec Addition to Fused Rings = 0.0000 E-12 cm3/molecule-sec OVERALL OH Rate Constant = 62.1576 E-12 cm3/molecule-sec HALF-LIFE = 0.258 Days (24-hr day; 0.5E6 OH/cm3) HALF-LIFE = 6.195 Hrs -- SUMMARY (AOP v1.91): OZONE REACTION (25 deg C) -- ****** NO OZONE REACTION ESTIMATION ****** (ONLY Olefins and Acetylenes are Estimated)

	4.1: Correction and addition: The half-life of Etofenprox in the troposphere is calculated to be 6.195 hours corresponding to 0.258 days (24 h day, $5x10^5$ radicals/cm^3). Based on this value a $kdeg_{air}$-value of 0.84 d^{-1} was derived. $kdeg_{air}$ = 2.68 d^{-1} with k_{OH}: specific degradation rate constant with OH-radicals [cm^3xmolec.$^{-1}$xs^{-1}]- 62.1576 x 10^{-12} cm^3xmolec.$^{-1}$xs^{-1} (see AOPWIN protocol excerpt given above) c_{OH} = $5*10^5$ moleculesxcm^{-3} acc. to TGD $kdeg_{air}$ (pseudo 1st order rate const. for degr. in air) = k_{OH} • c_{OH} • 24 • 3600 [d^{-1}]
Conclusion	5.1: Update: The chemical structure of the test substance was translated into "SMILES" format and entered into the computer simulation program "EPWIN v4.0" US EPA version. This software includes "AopWin v1.92" which calculates the atmospheric oxidation at 25°C by hydroxyl radical reaction and/or ozone reaction. 5.2: Correction: Overall OH rate constant= 62.1576 E-12 cm3/molecule-sec Half-life = 6.195 hrs (24-hr day; 10^5 OH/cm3)
Reliability	1
Acceptability	Acceptable with the amendments above
Remarks	---

Section A7.4.1.1/01 Acute toxicity to fish

Annex Point IIA-VII.7.1 **Rainbow trout (*Oncorhynchus mykiss*)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1995a): Etofenprox technical - acute toxicity to Rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions; [redacted] unpublished report no. 94-12-5625 (March 07, 1995). Dates of experimental work: December 09, 1994 – December 13, 1994	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data submitted to the MS after May 13, 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EPA, Subdivision E, Series 72, § 72-1 (quality criteria of EEC guideline C.1 are met in this study)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, temperature of the test solutions ranged from 9 to 11°C (instead of 12 ± 1°C)	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Etofenprox	
3.1.1	Lot/Batch number	56-003	
3.1.2	Specification	As given in section 2 Deviating from specification given in section 2 as follows	X
3.1.3	Description	Crystalline solid	
3.1.4	Purity	95.6%	X
3.1.5	Stability	No information in the report.	
3.1.6	Further relevant properties	Solubility in water: 22.5 µg/L at 20 ± 0.5°C Vapour pressure: 8.13 x 10^{-7} Pa at 25°C Stability in water: hydrolytically stable at pH 4, 7 and 9	
3.1.7	Method of analysis	Aqueous samples containing the test material were processed by liquid/liquid extraction and analysed on a high performance liquid chromatographic (HPLC) system using ultraviolet (UV) detection	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Stock solutions (primary: 660 µg a.s./mL; secondary: 66 µg a.s./mL) were prepared in acetone. Highest nominal test concentration (6.0 µg a.s./L) was maintained (e.g. flow-through test) by mixing 0.21 ml of the secondary stock solution with 2.3 L dilution water per cycle (148 cycles/day). This maximum test concentration was proportionally diluted (60%) to produce the remaining nominal test concentrations (3.6, 2.2, 1.3 and 0.78 µg a.s./L). See also table A7_4_1_1_01-1	

Section A7.4.1.1/01 Acute toxicity to fish

Annex Point IIA-VII.7.1 **Rainbow trout (*Oncorhynchus mykiss*)**

3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	
3.4	**Testing procedure**	
3.4.1	Dilution water	See enclosed table A7_4_1_1_01-2
3.4.2	Test organisms	See enclosed table A7_4_1_1_01-3
3.4.3	Test system	See enclosed table A7_4_1_1_01-4
3.4.4	Test conditions	See enclosed table A7_4_1_1_01-5
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Mortality (absence of gill movement and reaction to gentle prodding), sublethal effects (erratic swimming behaviour, lethargy), physical characteristics of the test solutions (e.g. presence of precipitate, film on solution's surface)
3.4.7	Sampling	Mortalities were removed at 24, 48, 72, and 96 hours of exposure
3.4.8	Monitoring of TS concentration	Yes, sampling at 0-hour (test initiation) and 96-hour (test termination)
3.4.9	Statistics	probit analysis (for calculation of LC50)

4 RESULTS

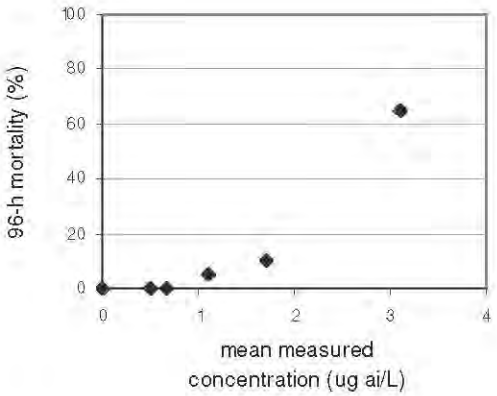
4.1	**Limit Test**	Not performed
4.1.1	Concentration	
4.1.2	Number/ percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0.7, 1.3, 2.2, 3.6 and 6.0 µg a.s./L (nominal concentrations)
4.2.2	Actual concentrations of test substance	

Nominal conc. (µg as/L)	Measured conc. (µg as/L)			Percent of nominal (%)
	0-hour	96-hour	Mean (SD) *	
Control	< 0.20	< 0.21	–	–
Solvent Control	< 0.20	< 0.21	–	–
0.78	0.49	0.51	0.50 (0.048)	64
1.3	0.68	0.64	0.66 (0.086)	51
2.2	1.05	1.15	1.10 (0.082)	50
3.6	1.90	1.40	1.70 (0.33)	46
6.0	3.35	2.90	3.10 (0.30)	52

* based on the mean measured concentrations, the treatment levels tested were defined as 0.50, 0.66, 1.1, 1.7 and 3.1 µg as/L

Section A7.4.1.1/01 Acute toxicity to fish

Annex Point IIA-VII.7.1 **Rainbow trout (*Oncorhynchus mykiss*)**

4.2.3	Effect data (Mortality)	- Mortality data as absolute numbers of immobile fish and as percent of exposed animals: see table A7_4_1_1_01-6 - LC_0, LC_{50} and LC_{100} values (including 95 % c.l.): see table A7_4_1_1_01-7
4.2.4	Concentration / response curve	
4.2.5	Other effects	- darkened pigmentation - lethargy - erratic swimming - partial loss of equilibrium
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects observed in control and solvent control
4.3.2	Nature of adverse effects	
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	
4.4.2	Results	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	EPA, Subdivision E, Series 72, § 72-1 Valid study (quality criteria of EEC guideline C.1 are met in this study) Deviation: Temperature of test solution was 9 – 11°C, instead of 12 ± 1°C according to guideline. This deviation did not affect the results of the study.
5.2	**Results and discussion**	
5.2.1	LC_0	0.66 µg a.s./L (96 h; based on mean measured concentration)
5.2.2	LC_{50}	2.7 µg a.s./L (96 h; based on mean measured concentration)
5.2.3	LC_{100}	> 3.1 µg a.s./L (96 h; based on mean measured concentration)

Section A7.4.1.1/01 Acute toxicity to fish

Annex Point IIA-VII.7.1 **Rainbow trout (*Oncorhynchus mykiss*)**

5.3	**Conclusion**	Validity criteria can be considered as fulfilled. Based on the results of this study (see below) and EPA criteria (1985) etofenprox technical would be classified as very highly toxic to rainbow trout (*Oncorhynchus mykiss*)
5.3.1	Other Conclusions	
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Table A7_4_1_1_01-1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	Yes: acetone
Concentration of vehicle	0.0091% (v/v)
Vehicle control performed	Yes; a flow-through system similar to the one used to deliver the etofenprox stock solutions was used to deliver the solvent stock solution (0.43 ml acetone/ml water). The concentration of solvent contained in the solvent control aquaria (0.091 ml/L) was equivalent to the concentration of acetone in the highest treatment level solutions.
Other procedures	Flow-through test

Table A7_4_1_1_01-2: Dilution water.

Criteria	Details
Source	Water from a 100 m bedrock well supplemented on demand with untreated well water from Town of Wareham (Massachusetts, USA)
Alkalinity	26 – 28 mg/L $CaCO_3$
Hardness	32 – 36 mg/L $CaCO_3$
pH	6.9 – 7.3
Oxygen content	8.8 – 10.6 mg/L (83% – 94% saturation)
Conductance	120 µmhos/cm
Holding water different from dilution water	No

Table A7_4_1_1_01-3: Test organisms.

Criteria	Details
Species/strain	Rainbow trout (*Oncorhynchus mykiss*)
Source	[redacted]
Wild caught	No
Age/size	Mean total length: 41 mm (range: 35 – 48 mm; n = 30) Mean total weight: 0.69 g (range: 0.37 – 1.02 g; n = 30)
Kind of food	Commercial pelleted food (Zeigler Brothers, Inc. Salmon Starter #1)
Amount of food	During acclimatisation: daily, *ad libitum* 48 hour prior to test initiation and during exposure: no feeding
Feeding frequency	Daily (see above)
Pretreatment	14 days acclimatisation in 500 l fibreglass tank under a photoperiod of 16 h light and 8 h dark. Water equivalent to the one used in test, temperature 11 – 12°C.
Feeding of animals during test	No

Table A7_4_1_1_01-4: Test system.

Criteria	Details
Test type	Flow-through
Renewal of test solution	Flow-through system: 50 ml Glenco gas-tight syringe in conjunction with a Sage Syringe pump, calibrated to deliver 0.21 ml/cycle of the 66 µg a.s./ml stock solution into chemical mixing chamber, which received 2.3 L dilution water/cycle. Solution in mixing chamber contained a TS concentration equivalent to the highest nominal test concentration, which was diluted proportionally (60%) to produce the other test concentrations. Flow-rate was 148 cycles/day (→ 6.7 volume replacements/24 hours). System was in proper operation for 5 days prior to test initiation to allow proper equilibration of the test substance.
Volume of test vessels	11 L
Volume/animal	1.1 L
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1_01-5: Test conditions.

Criteria	Details
Test temperature (°C)	See table table A7_4_1_1_01-6
Dissolved oxygen (mg/L)	See table table A7_4_1_1_01-6
pH	See table table A7_4_1_1_01-6
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	20 – 100 footcandles (220 – 1000 lux)
Photoperiod	16 hours light/8 hours dark

Table A7_4_1_1_01-6: Mortality data, based on mean measured concentrations

Test substance concentration (measured) [mg/l]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0	0
0.50	0	0	0	0	0	0	0	0
0.66	0	0	0	0	0	0	0	0
1.10	0	0	1	1	0	0	5	5
1.70	0	0	0	2	0	0	0	10
3.10	0	2	9	13	0	20	45	65
Temperature [°C]	10-11	11	11	11				
pH	7.1	6.9	7.1-7.2	7.3				
Oxygen [mg/l]	9.6-10.6	9.2-10.2	9.7-10.0	8.8-9.8				

Table A7_4_1_1_01-7: Effect data, based on mean measured concentrations.

	48 h [µg/L]	95 % c.l.	96 h [µg/L]	95 % c.l.
LC_0	1.7		0.66	
LC_{50}	> 3.1		2.7	2.2 – 3.6
LC_{100}	> 3.1		> 3.1	
NOEC			0.66	

Table A7_4_1_1_01-8: Validity criteria for acute fish test according to OECD Guideline 203.

Criteria	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 *

* results are therefore based on mean measured concentrations

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*27.05.2005*
Materials and methods	**3.1.2 Specification** According to document A3 the physical state changes from white crystals to amber liquid with decreasing purity from 99,8 % to 99,3%. **3.1.4 Purity:** Within the 5 batch analysis a purity between 97,2 % and 99,6% is indicated. 56-003 contained the same main impurities as later production batches (e.g. 5 batch analysis) at comparable percentages. The concentration of etofenprox is with 95,6% slightly lower than in the 5 batch analysis. Therefore the deviations to the specification are not considered to be ecooxicologically relevant.
Conclusion	Agree with the applicant´s version
Reliability	1
Acceptability	Acceptable
Remarks	-

Section A7.4.1.1/03 Acute toxicity to fish

Annex Point IIA-VII.7.1 **Metabolite α-CO, Rainbow trout (*Oncorhynchus mykiss*)**

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (2002a): Acute toxicity of α-CO to Rainbow trout (*Oncorhynchus mykiss*) in a 96-hour flow-through test; [redacted] unpublished report no. 841573 (August 22, 2002). Dates of experimental work: April 22, 2002 – June 26, 2002
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data submitted to the MS after May 13, 2000 on existing a.s. for the purpose of its entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD 203 (1992) EEC C.1 (1992) EPA OPPTS 850.1075 (Draft, 1996) - considered
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	α-CO
3.1.1	Chemical name (IUPAC)	2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzoate
3.1.2	Lot/Batch number	1000821-4
3.1.3	Description	Solid, white
3.1.4	Purity	99.74%
3.1.5	Stability	No information in the report.
3.1.6	Further relevant properties	Solubility in water: 42.5 µg/L at 20°C/pH 7 Stability in water: hydrolytically stable
3.1.7	Method of analysis	Aqueous samples containing the test material were processed by liquid/liquid extraction and analysed on a high performance liquid chromatographic (HPLC) system using ultraviolet (UV/VIS) detection
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	An application solution (600 µg α-CO/mL) was prepared in DMF (N,N-Dimethylformamide). At start of the test, 4.8 mL of this application solution was mixed up directly into the test media (48 L tap water). Flow-through system: 20 dosages/hour, 60 µl/dosage; test water flow rate: 288 L/24 hours (12 L/hour), e.g. six fold theoretical volume exchange per day. See also table A7_4_1_1_03-1
3.3	**Reference substance**	No

Section A7.4.1.1/03 Acute toxicity to fish

Annex Point IIA-VII.7.1 **Metabolite α-CO, Rainbow trout (*Oncorhynchus mykiss*)**

3.3.1	Method of analysis for reference substance	
3.4	**Testing procedure**	
3.4.1	Dilution water	see table A7_4_1_1_03-2
3.4.2	Test organisms	see table A7_4_1_1_03-3
3.4.3	Test system	see table A7_4_1_1_03-4
3.4.4	Test conditions	see table A7_4_1_1_03-5
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Test fish were observed after approximately 2, 24, 48, 72 and 96 hours test duration for visible abnormalities and mortality
3.4.7	Sampling	Not applicable
3.4.8	Monitoring of TS concentration	Yes, duplicate sampling from the test medium and from the solvent control before the start of the test (0-hour), after 48 hours and after 96-hours (test termination)
3.4.9	Statistics	Not applicable

4 RESULTS

4.1	**Limit Test**	Yes
4.1.1	Concentration	60 µg/L
4.1.2	Number/ percentage of animals showing adverse effects	No mortalities or other visible abnormalities were observed during the test period of 96 hours
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	60 µg α-CO/L (nominal concentration)

4.2.2 Actual concentrations of test substance

Nominal conc. [µg/L]	Age of sample [hours]	α-CO measured [µg/L] *	[% of nominal]
Solvent Control	0-hour	not detected	not applicable
	48-hour	not detected	not applicable
	96-hour	not detected	not applicable
60	0-hour	46.7	78
	48-hour	52.2	87
	96-hour	44.2	74

* the reported biological results are related to the mean measured test item concentration of 48 µg/L

4.2.3 Effect data (Mortality)

- Mortality data as absolute numbers of immobile fish and as percent of exposed animals in tabular form: see table A7_4_1_1_03-6
- LC_0, LC_{50} and LC_{100} values for 48 and 96 h (including 95 % c.l.): see table A7_4_1_1_03-7

Section A7.4.1.1/03 Acute toxicity to fish

Annex Point IIA-VII.7.1 **Metabolite α-CO, Rainbow trout (*Oncorhynchus mykiss*)**

4.2.4	Concentration / response curve	No mortalities or other visible abnormalities were observed during the test period of 96 hours
4.2.5	Other effects	No mortalities or other visible abnormalities were observed
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects observed in control and solvent control
4.3.2	Nature of adverse effects	
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	
4.4.2	Results	
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	OECD 203 (1992) EEC C.1 (1992) EPA OPPTS 850.1075 (Draft, 1996) considered Valid study No deviations to study guideline
5.2	**Results and discussion**	
5.2.1	LC_0	48 µg /L (96 h; based on mean measured concentration)
5.2.2	LC_{50}	> 48 µg a.s./L (96 h; based on mean measured concentration)
5.2.3	LC_{100}	> 48 µg a.s./L (96 h; based on mean measured concentration)
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. At concentrations representing its maximum solubility in water α-CO can be classified as not toxic to rainbow trout (*Oncorhynchus mykiss*)
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A7_4_1_1_03-1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	Yes; DMF (N,N-Dimethylformamide)
Concentration of vehicle	0.01% (v/v)
Vehicle control performed	Yes; same DMF concentration as in treatment, applied/maintained by the same flow-through technique
Other procedures	Flow-through test

Table A7_4_1_1_03-2: Dilution water.

Criteria	Details
Source	Local tap water (dinking water Itingen, Switzerland), mixed with deionised water for reduction of total hardness
Alkalinity	
Hardness	180 mg/L $CaCO_3$
pH	7.6
Oxygen content	8.7 – 9.4 mg/L
Conductance	
Holding water different from dilution water	No

Table A7_4_1_1_03-3: Test organisms.

Criteria	Details
Species/strain	Rainbow trout (*Oncorhynchus mykiss*)
Source	[redacted]
Wild caught	No
Age/size	Mean body length: 51 ± 4 mm (mean ± SD; n = 30) Mean body wet weight: 1.1 ± 0.3 g (mean ± SD; n = 30)
Kind of food	Commercial fish diet (HOKOVIT 502, 1.2 mm; supplied by H.U. Hoffmann AG, Bützberg, Switzerland)
Amount of food	During holding (> 2 weeks prior to test start) and acclimatisation (7 days prior to test start in test water). Feeding was stopped 48 hour prior to test initiation, and fish were not fed during exposure
Feeding frequency	
Pretreatment	Fish were held in [redacted] laboratories for more than two weeks without any medication. Prior to test start, fish were acclimated for 7 days to the test water and temperature. During 4 weeks prior to test start, no fish died in the test fish batch and all fish were healthy.
Feeding of animals during test	No

Table A7_4_1_1_03-4: Test system.

Criteria	Details
Test type	Flow-through, limit test
Renewal of test solution	Application solution (600 µg α-CO/ml DMF) or DMF alone (solvent control) were dosed into mixing vessels (volume about 2 L) using a Hamilton Digital Dispenser (Hamilton, Germany). Dosage: 1.2 ml/hour, 60 µl/dosage Test water continuously flowed into the mixing vessels at a rate of 288 L/24 hours (i.e. 12 L/hour). Flow rate through the aquaria (volume 48 L) corresponded to a six fold theoretical volume exchange/day.
Volume of test vessels	48 L
Volume/animal	6.9 L
Number of animals/vessel	7
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1_03-5: Test conditions.

Criteria	Details
Test temperature	See table table A7_4_1_1_01-6
Dissolved oxygen	See table table A7_4_1_1_01-6
pH	See table table A7_4_1_1_01-6
Adjustment of pH	No
Aeration of dilution water	Yes, slight aeration during test
Intensity of irradiation	50 – 500 Lux
Photoperiod	16 hours light/8 hours dark (30 min transition period)

Table A7_4_1_1_03-6: Mortality data, based on mean measured concentrations

Test substance concentration (measured) [µg a.s./L]	Mortality									
	Number					Percentage				
	2 h	24 h	48 h	72 h	96 h	2 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0	0	0	0	0	0
Solvent control	0	0	0	1 *	1 *	0	0	0	14.3 *	14.3 *
48	0	0	0	0	0	0	0	0	0	0
Temperature [°C]	13	13	13	13	13					
pH	7.6	7.6	7.6	7.6	7.6					
Oxygen [mg/l]	9.3-9.4	9.0-9.1	8.9-9.0	8.8-8.9	8.7-9.0					

* one test fisch jumped out of the aquarium during measurements after 72 hours test duration and died. The remaining 6 test fish in the solvent control showed no abnormalities over the test period.

Table A7_4_1_1_03-7: Effect data, based on mean measured concentrations

	48 h [µg/L]	95 % c.l.	96 h [µg/L]	95 % c.l.
LC_0	48	n.d.	48	n.d.
LC_{50}	> 48	n.d.	> 48	n.d.
LC_{100}	> 48	n.d.	> 48	n.d.
NOEC	48	n.d.	48	n.d.

* n.d. = not determinable

Table A7_4_1_1_03-8: Validity criteria for acute fish test according to OECD Guideline 203.

Criteria	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 *

* results are therefore based on mean measured concentrations

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	Agree with the applicant´s version
Conclusion	Agree with the applicant´s version
Reliability	1
Acceptability	Acceptable
Remarks	-