Section 7.1.2.2.2/01
Annex Point IIIA-XII.2.1

Degradation in Water-Sediment Systems

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 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.

Section 7.1.2.2.2/01 **Degradation in Water-Sediment Systems**
Annex Point IIIA-XII.2.1

		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
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 dissipation 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 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)
	COMMENTS FROM...

Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

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] 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	
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) Each test vessel (glass cylinders of 4.5 cm diameter) contained a 2.5 cm	x

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

		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

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.

Distribution of the radioactivity

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

Section A7.1.2.2.2/02 Degradation in Water-Sediment Systems
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

Section A7.1.2.2.2/02 Degradation in Water-Sediment Systems

Annex Point IIIA-XII.2.1

	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

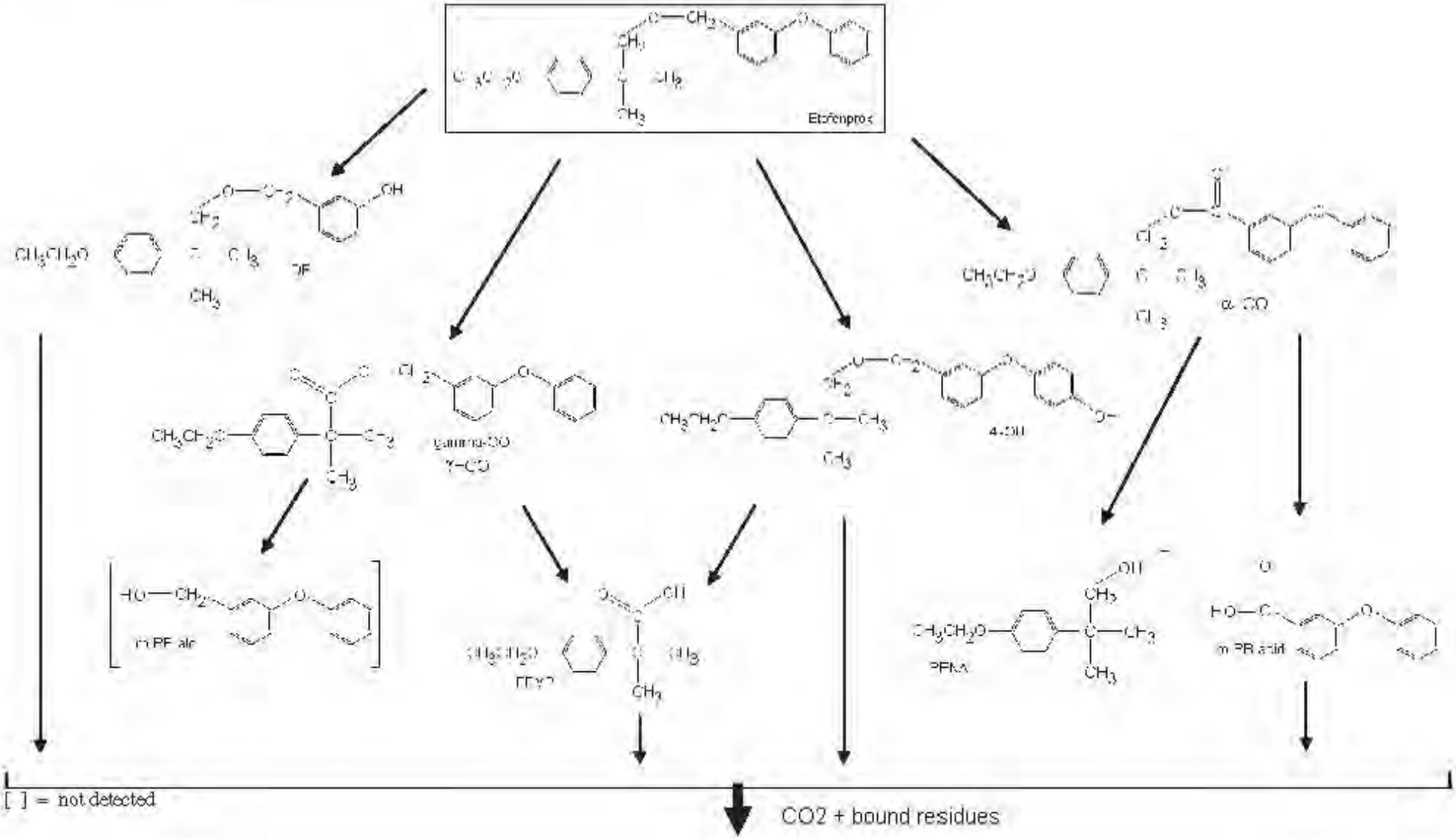
* radioactivity in acetonitrile and in acidified methanol extracts of sediment

** 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 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
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	

Reliability	
Acceptability	
Remarks	

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
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.	

Section A7.1.3 Adsorption/desorption screening test

Annex Point IIA 7.7

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)
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 $CaCl_2$ solution exposed to the soil during adsorption. After the adsorption step, the volumes of the supernatants as well as the amounts of $CaCl_2$

Section A7.1.3. **Adsorption/desorption screening test**

Annex Point IIIA-XII.1.2

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.

5.2.4 Ka_{oc}

see 5.2.2

5.2.5 Ka/Kd

not calculated

Section A7.1.3. Adsorption/desorption screening test

Annex Point IIIA-XII.1.2

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

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) - Radiochemical purity: 100%	

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)**	
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.
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	

Acceptability	
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

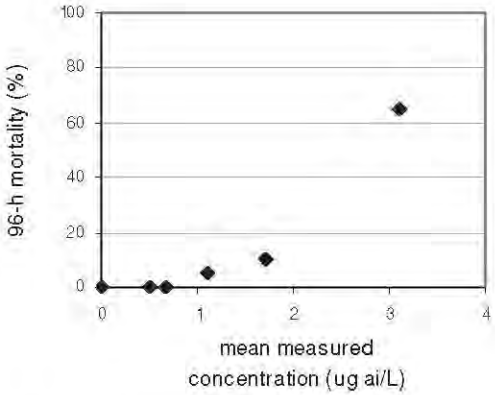
4.1	**Limit Test**	Not performed
4.1.1	Concentration	
4.1.2	Number/ percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0.7, 1.3, 2.2, 3.6 and 6.0 µg a.s./L (nominal concentrations)

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	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

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] *	α-CO measured [% 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] 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	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.1.2/01 Acute toxicity to invertebrates

Annex Point IIA-VII.7.2 *Daphnia magna*

Official use only

1 REFERENCE

1.1	**Reference**	Gries T. (2003): Etofenprox technical: static renewal acute toxicity test with daphnids (*Daphnia magna*); Springborn Smithers Laboratoires (Europe) AG, Horn, Switzerland; unpublished report no. 1045.000.110 (March 12, 2003). Dates of experimental work: February 17, 2003 – February 21, 2003
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 202, Part1 (1984) EEC C.2 (1992)
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Etofenprox technical
3.1.1	Lot/Batch number	87028
3.1.2	Specification	As given in section 2 Deviating from specification given in section 2 as follows
3.1.3	Description	Oily liquid (solid when low temperature)
3.1.4	Purity	98.86%
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 Hydrolytic stability: 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 gas chromatographic (GC) system equipped with a mass spectrometer (MS) detector.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A primary stock solution was pepared in acetone (100 µg a.s./mL acetone) and used to produce secondary stock solutions in the range of 0.4 – 50 µg a.s./mL acetone. 500 µl of the secondary stock solutions (or acetone alone for the solvent control) were applied to 5 L dilution water to produce the test solutions. After 24 hours of exposure the daphnids were transferred into freshly prepared test solutions using wide bore pipettes. See also enclosed table A7_4_1_2_01-1

Section A7.4.1.2/01 Acute toxicity to invertebrates

Annex Point IIA-VII.7.2 *Daphnia magna*

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_2_01-2
3.4.2	Test organisms	See enclosed table A7_4_1_2_01-3
3.4.3	Test system	See enclosed table A7_4_1_2_01-4
3.4.4	Test conditions	See enclosed table A7_4_1_2_01-5
3.4.5	Duration of the test	48 hours
3.4.6	Test parameter	Immobilised daphnids after 0, 24 and 48 hours Observations on the physical characteristics of the test solutions at 0, 24 and 48 hours
3.4.7	Sampling	No sampling of animals (immobilised daphnids were not removed)
3.4.8	Monitoring of TS concentration	Yes Aliquots for analytical dose verification at hour 0 and 24 were taken from the volumetric flasks in which the test solutions were prepared. Additional replicates (n=6) were prepared for dose verification analysis at hour 48. Replicates of each treatment level were combined prior to taking the aliquots used for dose verification analysis.
3.4.9	Statistics	Effect concentrations were calculated using a special EC_{50} program (EC50.bas) EC_{50} values were calculated using linear regression of probit-transformed response versus logarithm-transformed mean measured concentration
	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	0.04, 0.09, 0.2, 0.4, 0.9, 2.0, 5.0 and 10.0 µg/L

Section A7.4.1.2/01 Acute toxicity to invertebrates

Annex Point IIA-VII.7.2 *Daphnia magna*

X

4.2.2 Actual concentrations of test substance

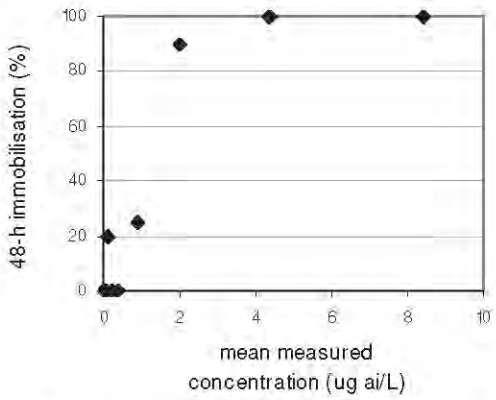
Nominal conc. [µg/L]	Mean recovery [%]	Measured conc. [µg/L]	Percent of nominal [%]
Control	<LOQ	<LOQ	not applicable
Solvent Control	<LOQ	<LOQ	not applicable
0.04	100 *	0.0395 *	see *
0.09	100 *	0.0890 *	see *
0.2	101	0.199	99.5
0.4	91.7	0.363	90.8
0.9	100	0.892	99.1
2.0	101	2.00	100.0
5.0	88.7	4.38	87.6
10.0	85	8.40	84.0

* the 2 lowest concentrations were not analysed since they were below the LOQ (0.1 µg as/L), hence the nominal concentrations were reported. Nominal concentrations were calculated with the purity of the test item (98.86%).

4.2.3 Effect data (Immobilisation)

- Mortality data as absolute numbers of immobile daphnids and as percent of exposed animals: see table A7_4_1_2_01-6
- EC_{50} (including 95 % c.l.), LOEC and NOEC values: see table A7_4_1_2_01-7

4.2.4 Concentration / response curve



Note: immobilisation (20%) at the lowest test item concentration was considered not to be test item related since there was no dose-response relationship at the lower concentrations

4.2.5 Other effects: Lethargic, swimming carrying and floating animals

4.3 Results of controls: No adverse effects observed in control and solvent control

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

Guidelines: OECD 202
ECC C.2

No deviations to guidelines

Section A7.4.1.2/01 Acute toxicity to invertebrates

Annex Point IIA-VII.7.2 *Daphnia magna*

5.2	**Results and discussion**	Results below are given for the 48-hour exposure to etofenprox technical under static renewal conditions	
5.2.1	EC_{50}	1.2 µg a.s./L (C.I. = 0.97 – 1.53 µg a.s./L)	
5.2.2	EC_{100}	4.38 µg a.s./L	
5.2.3	NOEC	0.089 µg a.s./L (based on nominal concentrations)	X
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. (see validity criteria summarized in table A7_4_1_2-8) The 48-h EC50 was calculated to be 1.2 µg ai/L (slope 5.71) with a 95% confidence interval ranging from 0.97 to 1.53 µg ai/L	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7_4_1_2_01-1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	Yes: acetone
Concentration of vehicle	0.01% (v/v)
Vehicle control performed	Yes (equal concentration as in test solutions)
Other procedures	Static renewal test: after 24 hours of exposure the daphnids were transferred into freshly prepared test solutions using wide bore pipettes.

Table A7_4_1_2_01-2: Dilution water.

Criteria	Details
Source	Elendt M4 medium, prepared of town of Horn (Switzerland) well water which was deionised with a Culligan Reverse Osmosis system
Alkalinity	26 – 28 mg/L as $CaCO_3$
Hardness	168 – 172 mg/L as $CaCO_3$
pH	7.66 – 7.74
Ca / Mg ratio	
Na / K ratio	
Oxygen content	8.20 – 8.77 mg/L (94 – 100% saturation)
Conductance	435 – 455 µS/cm
Holding water different from dilution water	No

Table A7_4_1_2_01-3: Test organisms.

Criteria	Details
Species/strain	*Daphnia magna*
Source	In-house laboratory cultures maintained at Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland. The strain originated from the EPA, Ohio, USA.
Age	< 24 hours
Breeding method	Holding/culturing: Elendt M4 medium (800 ml in 1 L Erlenmeyer flasks), 20 ± 2°C, 16h light/8h dark (400 lux), culture water is fully renewed once or twice a week. Young daphnids were obtained by removing immature daphnids from culture vessels (e.g. isolating sexually mature animals), which produce young daphnids.
Kind of food	Green alga *Ankistrodemsmus falcatus* (ANK); solution containing ± 4 x 10^7 cells/ml
Amount of food	0.5 – 2.5 ml/day
Feeding frequency	daily
Pretreatment	Holding and culture in equal dilution water that was used in the test
Feeding of animals during test	No

Table A7_4_1_2_01-4: Test system.

Criteria	Details
Renewal of test solution	Static renewal test: after 24 hours of exposure the daphnids were transferred into freshly prepared test solutions using wide bore pipettes.
Volume of test vessels	250 ml glass beakers (containing 200 ml test solution)
Volume/animal	40 ml
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2_01-5: Test conditions.

Criteria	Details
Test temperature	See table A7_4_1_2_01-6
Dissolved oxygen	See table A7_4_1_2_01-6
pH	See table A7_4_1_2_01-6
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	fluorescent lamps; 315 - 318 lum at water surface
Photoperiod	16 hours light/8 hours dark photoperiod

Table A7_4_1_2_01-6: Immobilisation data.

Test substance concentration (measured) [µg a.s./L]	Immobile *Daphnia*				Oxygen [mg/l]	pH	Temperature [°C]
	Number		Percentage				
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
Control	0	0	0%	0%	8.26	7.62	19.8
Solvent control	0	0	0%	0%	8.20	7.65	19.8
0.0395 [a]	1 [b]	4 [b]	5% [b]	20% [b]	8.24	7.63	19.7
0.0890 [a]	0	0	0%	0%	8.36	7.60	19.7
0.199	0	0	0%	0%	8.28	7.61	19.7
0.363	0	0	0%	0%	8.37	7.56	19.8
0.892	0	5	0%	25%	8.30	7.60	19.7
2.00	0	18	0%	90%	8.24	7.55	19.8
4.38	10	20	50%	100%	8.39	7.59	19.8
8.40	8	20	40%	100%	8.33	7.62	19.8

[a] based on nominal concentrations
[b] considered not to be test item related (no dose response relationship of the immobilisation at the lower concentrations)

Table A7_4_1_2_01-7: Effect data

	24 hours	48 hours
Regression	Probit(Y)=-0.957+4.71*log(X)	Probit(Y)=-4.51+5.71*log(X)
Slope	4.71	5.71
EC_{50} [µg a.s./L] (95 % c.l.)	7.2 ([a])	1.2 (0.97 – 1.53)
EC_{100} [µg a.s./L]	> 8.40	4.38
NOEC[µg a.s./L]	0.199 [c]	0.0890 [b,c]

Y: immobilisation in %
X: mean measured etofenprox concentration in µg a.s./L
[a] no confidence interval could be calculated
[b] based on nominal concentrations
[c] based on visual inspection of the data

Table A7_4_1_2_01-8: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202.

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

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**4.2.2 Actual concentrations of test substance:** Nominal test concentrations 0.2, 0.4, 0.9, 2, 5 and 10 µg a.s./L were analysed at hour 0, hour 24 (aged solution), hour 24 (new solution) and hour 48. From hour 24 to hour 48 the test item concentrations declined on average to 65.5%. For the mean measured test concentration over the whole testing period see the table under point 4.2.2 The test results are given in mean measured concentrations. **5.2.3 Results and discussion NOEC** The NOEC of 0.089 µg a.s./L is based on sublethal effects (lethargic, swimming carrying and floating animals) The 48-h $NOEC_{immobilisation}$ is 0.363 µg a.s./L.
Conclusion	Agree with the applicant´s version
Reliability	1
Acceptability	*Acceptable*
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.1.2/02 Acute toxicity to invertebrates

Annex Point IIA-VII.7.2 **Metabolite α-CO, *Daphnia magna***

			Official use only
		1 REFERENCE	
1.1	**Reference**	Bätscher R. (2002b): Acute toxicity of α-CO to *Daphnia magna* in a 48-hour immobilization test; RCC Ltd, Inc., Environmental Chemistry & Pharmanalytics Division, Itingen, Switzerland, unpublished report no. 841575 (August 22, 2002). Dates of experimental work: April 22, 2002 – May 14, 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 202, Part I (1984) EEC C.2 (1992) EPA OPPTS 850.1010 (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, 300 µl of this application solution was directly mixed up directly into 3000 ml test water to prepare the test medium with a test item concentration of 60 µg/L. See also table A7_4_1_2_02-1	
3.3	**Reference substance**	No	

Section A7.4.1.2/02 Acute toxicity to invertebrates

Annex Point IIA-VII.7.2 **Metabolite α-CO, *Daphnia magna***

3.3.1	Method of analysis for reference substance	
3.4	**Testing procedure**	
3.4.1	Dilution water	See enclosed table A7_4_1_2_02-2
3.4.2	Test organisms	See enclosed table A7_4_1_2_02-3
3.4.3	Test system	See enclosed table A7_4_1_2_02-4
3.4.4	Test conditions	See enclosed table A7_4_1_2_02-5
3.4.5	Duration of the test	48 hours
3.4.6	Test parameter	Immobilised daphnids after 0, 24 and 48 hours
3.4.7	Sampling	No sampling of animals (no effects were observed)
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) and after 48 hours (test termination). One sample of the DMF application solution was analysed prior to test initiation.
3.4.9	Statistics	Not applicable (no adverse effects observed)

4 RESULTS

4.1	**Limit Test**	Yes
4.1.1	Concentration	60 µg/L(nominal concentration)
4.1.2	Number/ percentage of animals showing adverse effects	No mortalities or other visible abnormalities were observed during the test period of 48 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][a]	[% of nominal]
Solvent Control	0-hour	not detected	not applicable
	48-hour	not detected	not applicable
60	0-hour	58.2	97
	48-hour	29.8	72[b]

[a] the reported biological results are related to the mean measured test item concentration of 44 µg/L

[b] based on 1 replicate sample only (the other classified as outlier)

Section A7.4.1.2/02 **Acute toxicity to invertebrates**

Annex Point IIA-VII.7.2 **Metabolite α-CO, *Daphnia magna***

4.2.3	Effect data (Immobilisation)	- Mortality data as absolute numbers of immobile daphnids and as percent of exposed animals: see table A7_4_1_2_02-6 - EC_{50} (including 95 % c.l.) and EC_{100} could not be quantified due to the absence of adverse effects; EC_0 and NOEC values: see table A7_4_1_2_02-7
4.2.4	Concentration / response curve	Not applicable, because there were no adverse effects observed
4.2.5	Other effects	No adverse effects observed in control and solvent control
4.3	**Results of controls**	No adverse effects observed in control and solvent control
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**	Guidelines: OECD 202, Part I (1984) EEC C.2 (1992) EPA OPPTS 850.1010 (Draft, 1996) - considered No deviations to guidelines
5.2	**Results and discussion**	Results below are given for the 48-hour exposure to 60 µg/L α-CO (nominal concentration)
5.2.1	EC_{50}	could not be quantified due to the absence of adverse effects
5.2.2	EC_{100}	could not be quantified due to the absence of adverse effects
5.2.3	NOEC	44 µg/L α-CO (based on measured concentration)
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. (see validity criteria summarized in table A7_4_1_2_02-8) No adverse effects were observed
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A7_4_1_2_02-1: 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 (equal concentration as in test solutions)
Other procedures	

Table A7_4_1_2_02-2: Dilution water.

Criteria	Details
Source	Reconstituted test water according to Dir. 92/69/EEC, C.2. For EPA-requirements, hardness was lowered to one-half of the normal hardness.
Alkalinity	0.4 mmol/L
Hardness	1.25 mmol/L (125 mg/L as $CaCO_3$)
pH	7.4 – 8.0
Ca / Mg ratio	4 : 1
Na / K ratio	10 : 1
Oxygen content	8.6 – 8.8 mg/L
Conductance	
Holding water different from dilution water	No

Table A7_4_1_2_02-3: Test organisms.

Criteria	Details
Species/strain	*Daphnia magna*
Source	In-house laboratory cultures maintained at RCC (Itingen, Switzerland) in reconstituted water. The strain originated from the University of Sheffield (UK) in 1992 (defined from the supplier as clone 5).
Age	6 – 24 hours (not first brood progeny)
Breeding method	Cultivation of parental daphnids is done in reconstituted water of the quality identical to the water quality used in the test regarding pH, main ions, total hardness, as well as temperature and light conditions.
Kind of food	
Amount of food	
Feeding frequency	
Pretreatment	Parental daphnids were maintained in dilution water for at least 48 hours prior to the start of the test
Feeding of animals during test	No

Table A7_4_1_2_02-4: Test system.

Criteria	Details
Renewal of test solution	No (static test)
Volume of test vessels	500 ml glass beakers (containing 250 ml test solution)
Volume/animal	25 ml
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_2_02-5: Test conditions.

Criteria	Details
Test temperature	See table A7_4_1_2_02-6
Dissolved oxygen	See table A7_4_1_2_02-6
pH	See table A7_4_1_2_02-6
Adjustment of pH	No
Aeration of dilution water	No aeration during test (however test water was aerated prior to the start of the study until oxygen saturation was reached)
Quality/Intensity of irradiation	550 - 690 lux
Photoperiod	16 hours light/8 hours dark photoperiod (30 min transition period)

Table A7_4_1_2_02-6: Immobilisation data, based on mean measured concentrations

Test substance concentration (measured) [µg/L]	Immobile *Daphnia*				Oxygen [mg/l]	pH	Temperature [°C]
	Number		Percentage				
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
Control	0	0	0%	0%	8.7	7.5	21
Solvent control	0	0	0%	0%	8.7	7.4	21
0.0395 [a]	0	0	0%	0%	8.6	7.5	21

Table A7_4_1_2_02-7: Effect data, based on mean measured concentrations

	24 hours	48 hours
EC_0 [µg α-CO/L]	> 44	> 44
EC_{50} [µg α-CO/L]	could not be quantified due to the absence of adverse effects	
EC_{100} [µg α-CO/L]	could not be quantified due to the absence of adverse effects	
NOEC[µg α-CO/L]	> 44	> 44

Table A7_4_1_2_02-8: Validity criteria for acute *Daphnia* immobilisation test according to OECD Guideline 202.

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

* 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	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.1.3/01 Growth inhibition test on algae

Annex Point IIA-VII.7.3 *Pseudokirchneriella subcapitata* **(formerly** *Selenastrum capricornutum***)**

Official use only

1 REFERENCE

1.1	**Reference**	Gries T., Purghart V. (2003): Etofenprox technical: static toxicity test with the freshwater algae *Pseudokirchneriella subcapitata*; Springborn Smithers Laboratoires (Europe) AG, Horn, Switzerland; unpublished report no. 1045.000.430 (February 17, 2003). Dates of experimental work: April 23, 2002 – May 08, 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 201 (1984) EEC C.3 (1992)
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Etofenprox technical
3.1.1	Lot/Batch number	87028
3.1.2	Specification	As given in section 2 Deviating from specification given in section 2 as follows
3.1.3	Description	Oily liquid (solid when low temperature)
3.1.4	Purity	98.86%
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 Hydrolytic stability: 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 gas chromatographic (GC) system equipped with a mass spectrometer (MS) detector.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A primary stock solution was prepared in acetone (1.5 mg a.s./mL acetone) and used to produce further stock solutions in the range of 0.03 – 0.68 mg a.s./mL acetone. 100 µl (2 highest concentrations/acetone alone for the solvent control) or 50 µl (4 lowest concentrations) of these stock solutions were applied to 1000 ml or 500 ml dilution water, respectively, to produce the test solutions. See also enclosed table A7_4_1_2_01-1
3.3	**Reference substance**	No

Section A7.4.1.3/01 Growth inhibition test on algae

Annex Point IIA-VII.7.3 *Pseudokirchneriella subcapitata* **(formerly** *Selenastrum capricornutum*)

3.3.1	Method of analysis for reference substance	
3.4	**Testing procedure**	
3.4.1	Culture medium	Composition (minerals, hardness) of algal growth medium according to OECD guideline #201. pH was adjusted to 8.0 ± 0.2 using 0.1N HCl or 0.1N NaOH
3.4.2	Test organisms	See enclosed table A7_4_1_3_01-2
3.4.3	Test system	See enclosed table A7_4_1_3_01-3
3.4.4	Test conditions	See enclosed table A7_4_1_3_01-4
3.4.5	Duration of the test	72-hours
3.4.6	Test parameter	Inhibition of (i) area under the growth curve (AUC) and (ii) specific growth rate (μ)
3.4.7	Sampling	24-hour sampling intervals for determination of cell density
3.4.8	Monitoring of TS concentration	Yes, analytical dose verification at time 0 (samples taken from the volumetric flasks in which test solutions were prepared) and at the end of the exposure (replicates of each treatment level were combined for analysis)
3.4.9	Statistics	Bonferroni t-test and Dunnett's test for calculation of the NOEC

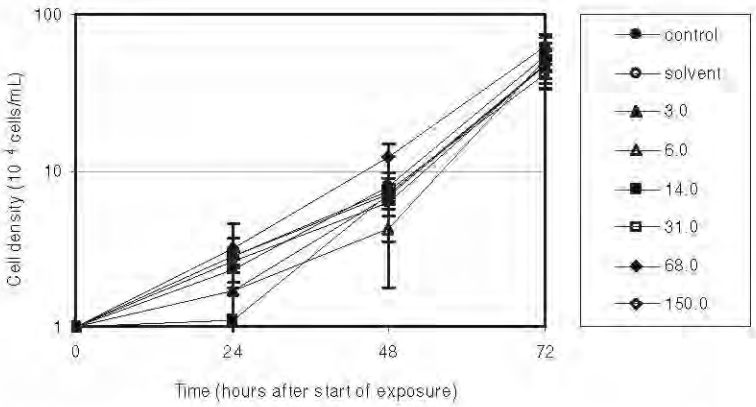
4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	
4.1.2	Number/ percentage of animals showing adverse effects	
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	3.0, 6.0, 14, 31, 68 and 150 µg etofenprox technical/L (nominal concentrations)
4.2.2	Actual concentrations of test substance	X

Nominal conc. [µg a.s./L]	Recovery [%] Hour 0	Hour 72
Control	<LOQ	<LOQ
Solvent Control	<LOQ	<LOQ
3.0	98.4	25.3
6.0	100	19.9
14.0	88.2	16.9
31.0	96.2	<LOQ
68.0	87.6	13.2
150	87.5	12.5

Section A7.4.1.3/01 — Growth inhibition test on algae

Annex Point IIA-VII.7.3 — *Pseudokirchneriella subcapitata* (**formerly** *Selenastrum capricornutum*)

4.2.3	Growth curves		
4.2.4	Concentration / response curve	No inhibitory effects were observed: no dose-response relationship	
4.2.5	Cell concentration data	See enclosed table A7_4_1_3_01-5	
4.2.6	Effect data (cell multiplication inhibition)	E_bC_{50}: > 150 µg a.s./L, NOEC (AUC): 150 µg a.s./L E_rC_{50}: > 150 µg a.s./L NOEC (growth rate): 150 µg a.s./L	X
4.2.7	Other observed effects	No inhibitory effects were observed	
4.3	**Results of controls**	No inhibitory effects were observed, no statistically significant differences between control and solvent control (controls were pooled for further statistical analyses)	
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**	Guidelines: OECD 201 ECC C.3 No deviations to guidelines	
5.2	**Results and discussion**		
5.2.1	NOE_rC	150 µg a.s./L	*
5.2.2	E_{r50}	> 150 µg a.s./L	*
5.2.3	E_bC_{50}	> 150 µg a.s./L	*
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. (see validity criteria summarized in table A7_4_1_2-8)	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7_4_1_3_01-1: Preparation of TS solution for poorly soluble or volatile test substances.

Criteria	Details
Dispersion	No
Vehicle	Yes: acetone
Concentration of vehicle	0.01% (v/v)
Vehicle control performed	Yes: same acetone concentration as in test solutions
Other procedures	

Table A7_4_1_3_01-2: Test organisms.

Criteria	Details
Species	*Pseudokirchneriella subcapitata*
Strain	SAG 61.81
Source	Cultured at Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland; originally obtained from Plant Physiological Institute, University Göttingen, Germany
Laboratory culture	Yes
Method of cultivation	Stock cultures in 100 ml algal medium (according to OECD 201) in 250 ml Erlenmeyer flasks covered with stainless steel caps that permit gas exchange; transfer to fresh medium twice a week. Incubation under similar conditions as during the test: orbital shaker set to 60 rpm, temperature 22.9 – 24.8°C, illumination by cool white fluorescent lamps at 6100 – 7990 lux.
Pretreatment	Inoculum used to initiate test cultures was taken from a stock culture that had been set up 5 days prior to testing
Initial cell concentration	approximately 10^4 cells/ml

Table A7_4_1_3_01-3: Test system.

Criteria	Details
Volume of culture flasks	250 m Erlenmeyer flasks, 100 ml medium/flask
Culturing apparatus	Water bath for temperature regulation
Light quality	Continuous illumination at 6100 – 8400 lux (cool white fluorescent lamps)
Procedure for suspending algae	Orbital shaker (Gerhardt) set at 65 rpm
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No (stainless steel caps that permit gas exchange)

Table A7_4_1_3_01-4: Test conditions.

Criteria	Details
Test temperature	See table A7_4_1_3_01-5
pH	See table A7_4_1_3_01-5
Aeration of dilution water	No
Light intensity	See table A7_4_1_3_01-5
Photoperiod	Continuous illumination

Table A7_4_1_3_01-5: Cell concentration data.

Test substance concentration (nominal) [µg/l]	**Cell concentrations (mean values of 3 replicates) [x 10^4 cells/ml]**					
	measured			**Percent of control**		
	24 h	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
Control	2.3	7.9	54.9	100.0	100.0	100.0
Solvent control	2.6	6.2	48.8	113.0	78.5	88.9
3.0	1.7	6.9	47.3	73.9	87.3	86.2
6.0	1.7	4.3	52.3	73.9	54.4	95.3
14	1.1	7.1	48.6	47.8	89.9	88.5
31	2.8	7.4	41.5	121.7	93.7	75.6
68	3.2	12.3	63.7	139.1	155.7	116.0
150	2.8	7.1	46.8	121.7	89.9	85.2
Temperature [°C]	23.7-24.1 (hour 0-24)	23.7-24.4 (hour 24-48)	23.9-24.2 (hour 48-72)			
pH	8.03-8.74 (hour 0)		7.60-8.48 (hour 72)			
Light intensity (lux)	6400-7900	6100-7700	6200-7600			

Table A7_4_1_3_01-6: Validity criteria for algal growth inhibition test according to OECD Guideline 201.

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

* at hour 0 recoveries ranged from 87.5 to 100% indicating correct dosage of the test solutions. After 72 hours the recoveries ranged from 12.5 to 25.3% in biological samples. The lower recoveries at the end of the exposure period were most likely caused by aggradations and/or adsorption to algal cells. Since the hour 0 measurements verified the correct dosage, the concentrations were reported as nominal concentrations.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**4.2.2 Actual concentrations of test substance and** **4.2.6 Effect data:** The concentration of the test substance shall be maintained within 80 % of the initial concentration throughout a time corresponding to the duration of the test. At nominal 150 µg a.s./L the recovery rate at hour 0 was 87.7% and at hour 72 12.5%, the mean is 37.5%. The explanation , the lower recoveries at the end of the exposure period were most likely caused by aggregations and/or adsorption to algal cells, is not demonstrated in the test (no measurements of the amount incorporated into the algal biomass or no measurements of the concentrations in a test vessel duplicate without algae, respectively). So the results have to be given at mean measured concentrations: The average test concentration at the relevant nominal test concentration of 150 µg a.s./L is 37%. The result have to be corrected according this value.
Conclusion	**5. Results and discussion** 5.2.1: NOE_RC (growth rate) : 56.25 µg a.s./L 5.2.2: E_rC_{50}: > 56.25 µg a.s./L 5.2.3: E_bC_{50}: > 56.25 µg a.s./L 5.2.4: NOE_BC (AUC): 56.25 µg a.s./L
Reliability	2
Acceptability	Acceptable
Remarks	The validity criteria for algal growth inhibition test according to OECD Guideline 201 (concentration of test substance ≥80% of initial concentration during test) are not fulfilled. So the results have to be corrected to the mean measured concentration.
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	

Acceptability	
Remarks	

Section A7.4.1.3/02 Growth inhibition test on algae

Annex Point IIA-VII.7.3

Metabolite α-CO, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)

Official use only

1 REFERENCE

1.1	**Reference**	Bätscher R. (2002c): Acute toxicity of α-CO to *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a 96-hour algal growth inhibition test; RCC Ltd, Inc., Environmental Chemistry & Pharmanalytics Division, Itingen, Switzerland, unpublished report no. 841577 (August 22, 2002). Dates of experimental work: April 19, 2002 – May 10, 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 201 (1984) EEC C.3 (1992) EPA OPPTS 850.5400 (1996, draft) – 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, 250 µl of this application solution was directly mixed up directly into 2500 ml test water to prepare the test medium with a test item concentration of 60 µg/L. See also table A7_4_1_2_02-1
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference	

Section A7.4.1.3/02 **Growth inhibition test on algae**

Annex Point IIA-VII.7.3 **Metabolite α-CO, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)**

	substance	
3.4	**Testing procedure**	
3.4.1	Culture medium	Composition (minerals, hardness) of algal growth medium according to OECD guideline #201. Calculated water hardness of the test water: 0.24 mmol/L (= 24 mg/L) as $CaCO_3$
3.4.2	Test organisms	See enclosed table A7_4_1_3_02-2
3.4.3	Test system	See enclosed table A7_4_1_3_02-3
3.4.4	Test conditions	See enclosed table A7_4_1_3_02-4
3.4.5	Duration of the test	96-hours
3.4.6	Test parameter	Inhibition of (i) area under the growth curve (AUC) and (ii) specific growth rate (μ) Microscopic examination of the shape of the algal cells at end of the exposure period
3.4.7	Sampling	24-hour sampling intervals for determination of cell density
3.4.8	Monitoring of TS concentration	Yes, analytical dose verification at time 0 (samples taken from the DMF application solution, the test medium and the solvent control) and at the end of the exposure (samples taken from the test medium and the solvent control – stability samples)
3.4.9	Statistics	None (no toxic effects observed)
	4 RESULTS	
4.1	**Limit Test**	Yes
4.1.1	Concentration	60 μg α-CO/L(nominal concentration)
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
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	60 μg α-CO/L(nominal concentration)

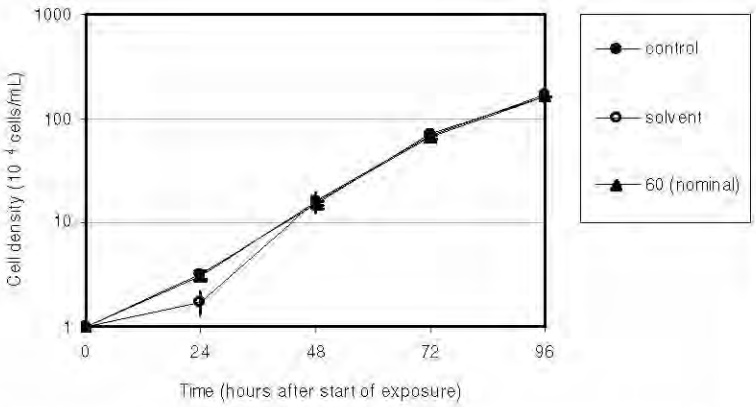
4.2.2 Actual concentrations of test substance

Nominal conc. [μg/L]	Age of sample [hours]	α-CO measured [μg/L] *	[% of nominal]
Solvent Control	0-hour	not detected	not applicable
	96-hour	not detected	not applicable
60	0-hour	57.8	96
	96-hour	47.5	79

* the reported biological results are related to the mean measured test item concentration of 53 μg/L

Section A7.4.1.3/02 **Growth inhibition test on algae**

Annex Point IIA-VII.7.3 **Metabolite α-CO, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)**

4.2.3	Growth curves	
4.2.4	Concentration / response curve	No inhibitory effects were observed: no dose-response relationship
4.2.5	Cell concentration data	See enclosed table A7_4_1_3_02-5
4.2.6	Effect data (cell multiplication inhibition)	E_bC_{50}: > 53 µg α-CO/L, NOEC (AUC): 53 µg α-CO/L E_rC_{50}: > 53 µg α-CO/L NOEC (growth rate): 53 µg α-CO/L
4.2.7	Other observed effects	No inhibitory effects were observed
4.3	**Results of controls**	No inhibitory effects were observed
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**	Guidelines: OECD 201 ECC C.3 No deviations to guidelines
5.2	**Results and discussion**	Results below are given for the 96-hour exposure to 60 µg/L α-CO (nominal concentration).
5.2.1	NOE_rC	53 µg α-CO/L (based on measured concentration)
5.2.2	E_{r50}	> 53 µg α-CO/L (based on measured concentration)
5.2.3	E_bC_{50}	> 53 µg α-CO/L (based on measured concentration)
5.3	**Conclusion**	Validity criteria can be considered as fulfilled. (see validity criteria summarized in table A7_4_1_2-8)
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A7_4_1_3_02-1: 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 (equal concentration as in test solutions)
Other procedures	

Table A7_4_1_3_02-2: Test organisms.

Criteria	Details
Species	*Pseudokirchneriella subcapitata*
Strain	SAG 61.81
Source	Cultured in the RCC laboratories (Itingen, Switzerland) according to the test guidelines; originally obtained from the Sammlung von Algenkulturen Göttingen (SAG, Experimentelle Phykologie und Sammlung von Algenkulturen, Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Universität Göttingen, Göttingen, Germany)
Laboratory culture	Yes
Method of cultivation	Stock cultures in 15 ml algal medium (according to OECD 201) in 50 ml Erlenmeyer flasks covered with glass dishes. Incubation under identical conditions as during the test: temperature 22 – 23°C, illumination by fluorescent tubes (Philips TLD 36W/840) at 7900 lux (mean value), continuous stirring by magnetic stirrers.
Pretreatment	Inoculum used to initiate test cultures was taken from a stock culture that had been set up 3 days prior to testing
Initial cell concentration	approximately 10^4 cells/ml

Table A7_4_1_3_02-3: Test system.

Criteria	Details
Volume of culture flasks	50 ml Erlenmeyer flasks, 15 ml medium/flask
Culturing apparatus	Water bath for temperature regulation
Light quality	Continuous illumination at 7370 – 9240 lux (mean value: 7900 lux; fluorescent tubes Philips TLD 36W/840)
Procedure for suspending algae	Magnetic stirrers
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No (flasks covered with glass dishes)

Table A7_4_1_3_02-4: Test conditions.

Criteria	Details
Test temperature	See table A7_4_1_3_02-5
pH	See table A7_4_1_3_02-5
Aeration of dilution water	No
Light intensity	See table A7_4_1_3_02-5
Photoperiod	Continuous illumination

Table A7_4_1_3_02-5: Cell concentration data.

Test substance concentration (measured) [µg/L]		**Cell concentrations (mean values of 3 replicates) [x 10^4 cells/ml]**							
		measured				**Percent of control**			
		24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
Control	m	3.15	15.13	70.55	166.45	100.0	100.0	100.0	100.0
	s	0.33	1.60	5.69	1.75				
	n	6	6	6	6				
Solvent control	m	1.70	16.00	69.60	167.98	54.0	105.8	98.7	100.9
	s	0.41	3.21	2.78	6.07				
	n	3	3	3	3				
53	m	3.08	15.38	66.37	164.48	97.8	101.7	94.1	98.8
	s	0.28	1.06	2.14	2.11				
	n	3	3	3	3				
Temperature [°C]		22	22	23	23				
pH		7.9 (start)			8.2-8.3 (end)				

Table A7_4_1_3_02-6: Validity criteria for algal growth inhibition test according to OECD Guideline 201.

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

* in the test medium, incubated under the test conditions but without algal cells, a decrease of the test item concentration was determined (79% of nominal concentration recovered). This decrease could be due to a loss by adsorption onto the glass surfaces and/or to a precipitation of the test item due to the dosage above the water solubility limit of α-CO. The biological results are based on the mean measured test item concentration of 53 µg/L.

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	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA-VII.7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Czech P. (2002): Toxicity of etofenprox to activated sludge in a respiration inhibition test; RCC Ltd, Inc., Environmental Chemistry & Pharmanalytics Division, Itingen, Switzerland, unpublished report no. 841615 (June 11, 2002). Dates of experimental work: February 25, 2002 – March 21, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
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 209 (1984) EU Commission Directive 87/302/EEC (1988)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Etofenprox	
3.1.1	Lot/Batch number	21054	
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	99.6%	
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	The respiration rate was determined by measuring the concentration of dissolved oxygen with an oxygen electrode (WTW OXI 539 meter)	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Test item amounts of 3.18, 6.25, 12.59, 24.96 and 50.17 mg were directly weighed (analytical balance) into test flasks; 284 mL tap water were added. The test item was mixed into the tap water by ultrasonic treatment for 15 minutes and intense stirring for 24 hour at room temperature in the dark to dissolve a maximum amount of the test item and/or disperse it as homogenously as possible. See also table A7_4_1_4-1	

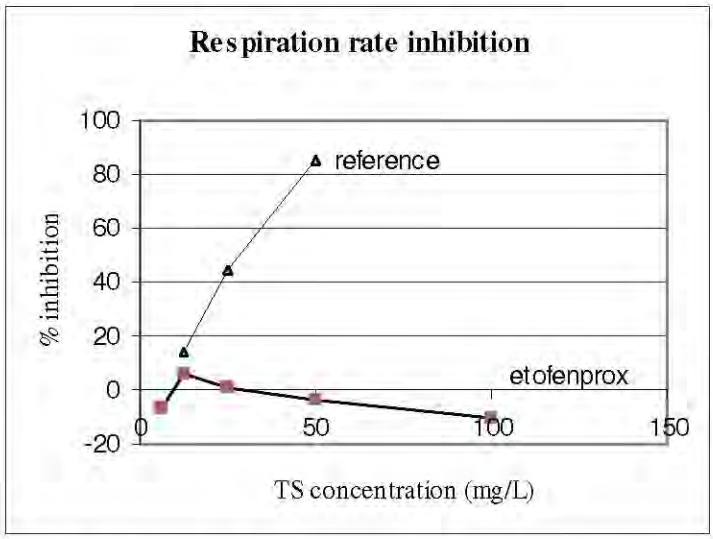
Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA-VII.7.4

3.3	**Reference substance**	Yes: 3,5-dichlorophenol 0.5 g 3,5-dichlorophenol were dissolved in 10 mL 1 M NaOH and diluted to about 30 mL with purified water. Excess NaoH was neutralised with 0.5 M sulphuric acid; the mixture was diluted to 1 L with purified water (conc.: 500 mg/L). Aliquots of this stock solution were mixed with synthetic waste water and tap water, respectively
3.3.1	Method of analysis for reference substance	The respiration rate was determined by measuring the concentration of dissolved oxygen with an oxygen electrode (WTW OXI 539 meter)
3.4	**Testing procedure**	
3.4.1	Culture medium	Synthetic waste water: 16 g Peptone — 11 g meat extract 3.0 g urea — 0.7 g NaCl 0.4 g $CaCl_2*2H_2O$ — 0.2 g $MgSO_4*7H_2O$ 2.8 g K_2HPO_4 — filled to 1 L with deionised water
3.4.2	Inoculum / test organism	Details on inoculum see table A7_4_1_4-2
3.4.3	Test system	1000 mL glass flasks; During the first 3 hours aeration with compressed air at a flow rate of approximately 1 L /minute.
3.4.4	Test conditions	Temperature: 20 -21 °C Conc. of dissolved oxygen: > 2.5 mg/L
3.4.5	Duration of the test	3 hours
3.4.6	Test parameter	Respiration rate inhibition
3.4.7	Analytical parameter	dissolved oxygen measurement
3.4.8	Sampling	At start and after 3 hours
3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	- Inoculum control without test item - Reference substance with inoculum
3.4.11	Statistics	The inhibitory effect is expressed as % of mean respiration rate of two controls. The 3 hour EC50, EC20, and EC80 values of the test item could not be determined due to absence of a toxic effect.
	4 RESULTS	
4.1	**Preliminary test**	Not performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	See 4.2.6
4.2.2	Actual concentrations of test substance	x

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA-VII.7.4

4.2.3	Growth curves	Not determined
4.2.4	Cell concentration data	Not determined
4.2.5	Concentration/ response curve	

4.2.6 Effect data

Test substance Concentration mg/L	Oxygen Consumption mg/L/min	Inhibition TS %	Inhibition Reference %
6.3	0.913	-6.9	
12.5	0.806	5.6	14.4
25	0.849	0.6	44.8
50	0.883	-3.4	84.9
100	0.942	-10.3	

4.2.7	Other observed effects	The test was not dissolved, but was present as a suspension
4.3	**Results of controls**	Control I: 0.891 mg O_2/L/min Control II: 0.816 mg O_2/L/min
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	12.5, 25, 50 mg/L
4.4.2	Results	EC_{50} = 17.1 mg/L

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA-VII.7.4

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The toxicity of etofenprox (batch 21054, purity 99.6 %) to activated sludge was tested in a respiratory inhibition test according the guidelines OECD No. 209 (1984) and EU Directive 87/302/EEC, Part C, (1988). x

Activated sludge from a domestic waste water treatment plant (Ergolz II, Füllinsdorf, Switzerland) at a concentration of 1.2 g dry weight per L test medium. The test substance was weighed directly into the incubation flasks (1 L, glass) and homogenized in 284 mL tap water by ultrasonic treatment. 16 mL synthetic waste water and 200 mL inoculum were added. During the incubation period of 3 hours the flasks were aerated at a rate of 1 L air/min and a temperature of 20 to 21°C. The dissolved oxygen was measured with an oxygen electrode (WTW OXI 539 meter). The inhibition was calculated by comparing the respiration rate of the test item flasks with those of controls without test item.

To verify the validity of the system, a reference substance (3,5-dichlorophenol, batch 02611ES, Aldrich, purity 99.1%) was incubated under the same conditions.

5.2 Results and discussion

Test substance Concentration	Oxygen Consumption	Inhibition TS	Inhibition Reference
mg/L	mg/L/min	%	%
6.3	0.913	-6.9	
12.5	0.806	5.6	14.4
25	0.849	0.6	44.8
50	0.883	-3.4	84.9
100	0.942	-10.3	

There was no inhibition of the respiration of activated sludge up to the highest tested concentration of 100 mg/L etofenprox. This is about 500 time higher than the solubility of etofenprox in water (0.0225 mg/L).

The reference substance inhibited the respiration as expected, indication that the system was valid.

5.2.1 EC_{20} no inhibition

5.2.2 EC_{50} no inhibition

5.2.3 EC_{80} no inhibition

5.3 Conclusion

The test was valid as shown by control and reference substance tests.

Etofenprox did not inhibit the respiration of activated sludge up to the highest test concentration of 100 mg/L.

5.3.1 Reliability 1

5.3.2 Deficiencies No

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

Criteria	Details
Dispersion	Yes
Vehicle	No
Concentration of vehicle	-
Vehicle control performed	No
Other procedures	Ultrasonic treatment, intense stirring

Table A7_4_1_4-2: Inoculum / Test organism.

Criteria	Details
Nature	Activated sludge
Species	not specified
Strain	not specified
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	ARA Ergolz II, Fuellinsdorf, Switzerland
Laboratory culture	No
Method of cultivation	-
Preparation of inoculum for exposure	The sludge was washed with tap water, centrifuged and the supernatant decanted. A homogenised aliquot of the sludge weighed, dried and weighed again to determine the wet to dry weight ratio. Based on this value, an appropriate amount of sludge was used to prepare a stock suspension containing 3 g dry material / L.
Pretreatment	3 days prior to use the sludge was fed daily with 50 mL synthetic waste water. The dry weight was determined and the pH adjusted from 8.7 to 7.1 with diluted sulphuric acid.
Initial cell concentration	1200 mg suspended solids/in test medium based on dry weight.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**3.1.2 Specification** No detailed specification was given in the original test report. Used batch is different from those given in section 2. **4.2.2 Actual concentration of test substance** The following nominal concentrations of Etofenprox were tested: 6.25, 12.5, 25, 50 and 100 mg/l. All test concentrations were far above the water solubility limit of Etofenprox. Thus all test concentrations were slightly turbid inhomogeneous dispersions. The actual concentrations of the dissolved substance were not determined.
Conclusion	**5.1 Materials and methods** The amount of activated sludge was 1.2 g dry weight per L test medium. The OECD guideline 209 recommends a concentration of 1.6 g/L. However other validity criteria are fulfilled and this deviation is considered as acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA-XIII.2.2 **Zebra fish (*Brachydanio rerio*)**

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (2005): Toxic effects of MTI-500 (Etofenprox) to zebra fish (*Brachydanio rerio*) in an early-life stage toxicity test ; [redacted] unpublished report no. 853517 (June xx, 2005). Dates of experimental work: October 07, 2004 – May 19, 2005
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 210, OPPTS 850.1400
2.2	**GLP**	Yes
2.3	**Deviations**	Different strain of zebra fish were used in order to improve the quality of test fish population. The duration of the test was extended for technical reasons.

3 MATERIALS AND METHODS

3.1	**Test material**	Etofenprox technical
3.1.1	Lot/Batch number	87031
3.1.2	Specification	As given in section 2
3.1.3	Description	Oily liquid
3.1.4	Purity	99.0%
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 Hydrolytic stability: hydrolytically stable at pH 4, 7 and 9
3.1.7	Method of analysis	GC/MS analytical method
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Due to the low water solubility of the test item, the organic solvent DMF was used for the dosage of the test system. An application solution with a concentration of 832.5 mg/L (used for the dosage of the highest tests concentration) was prepared by dissolving about 166.5 mg of the test item completely in 200 mL DMF by stirring for 15 minutes. In a series of subsequent dilution steps this application was diluted with DMF to prepare the application solutions for the dosage of the lower test concentrations
3.3	**Reference substance**	No

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA-XIII.2.2 **Zebra fish (*Brachydanio rerio*)**

3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Dilution water	Analytical grade salts were dissolved in deionized water to obtain the nominal concentrations presented in table A7_4_3_2-1
3.4.2	Test organisms	See enclosed table A7_4_3_2-2
3.4.3	Test system	See enclosed table A7_4_3_2-3
3.4.4	Test conditions	See enclosed table A7_4_3_2-4
3.4.5	Duration of the test	5 days post fertilization (Day 0 to 5) plus 35 days post-hatch
3.4.6	Test parameter(s)	- Eggs development and hatching rate: the embryonic development and hatching of larvae was recorded daily from Day 1 to Day 5 - The hatching time and the development rate: the mean hatching time represents the mean time span between the start of the test (Day 0) and the hatching of the experimental cohort of larvae. The developmental rate is the reciprocal of the hatching time (unit: 1/day) and represents that portion of hatching which take place per day -Development and survival of larvae and juvenile fish: larvae were observed for mortality (e.g. absence of respiratory movement and/or lack of reaction to mechanical stimulus) at least each working day. Juvenile fish were observed for mortality and visible abnormalities as abnormal appearance (body shape) and behaviour (e.g. uncoordinated swimming, atypical quiescence and atypical behavior) at least each working day. The number of live fish were counted and recorded at least twice a week. -Fish length and fish weight were determined at the end of the test.
3.4.7	Examination / Sampling	Daily inspections for the test parameters
3.4.8	Monitoring of TS concentration	Yes. The test item concentrations were measured in the application solution samples and test medium samples only from the test concentrations of nominally 25 and 50 µg/L.
3.4.9	Statistics	Survival rates from the different test concentrations were evaluated for significant differences to the control and solvent control by the multivariate Williams-test after a one-way analysis of variance (ANOVA). For all test parameters, the control and the solvent control were compared to each other by a STUDENT t-test.
	4 RESULTS	
4.1	**Range finding test**	Yes (Not GLP)
4.1.1	Concentrations	Not applicable
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
4.1.3	Nature of adverse effects	Not applicable
4.2	**Results test substance**	

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA-XIII.2.2 **Zebra fish (*Brachydanio rerio*)**

4.2.1	Initial concentrations of test substance	3.1, 6.3, 12.5, 25 and 50 µg/L (nominal concentrations)
4.2.2	Actual concentrations of test substance	The mean measured test item concentrations in the test media of nominally 25 and 50 µg/L were analytically determined to be 22 and 51 µg/L (89 and 102% of nominal values)
4.2.3	Effect data	- For hatching rate and the development rate of the larvae, no concentration effect relationship was observed - Sporadic mortality of larvae and fish was observed at all treatments up to and including test concentrations of nominally 25 µg/L. In the highest test concentration of nominally 50 µg/L mortality and visible abnormalities (e.g. apathy, fish lying on the bottom of the test vessel) were observed 3 days after the larvae had hatched. Mortality increased successively during the first week after hatching. However after Day14 post hatch, no further mortality was observed -The mean fish length and body wet weight obtained in the test concentrations up to and including the highest test concentration of nominally 50 µg/L were nearly identical or even slightly higher compared to the control and the solvent control. Effect level concentrations (NOEC, LOEC and MATC) are presented in table A7_4_3_2-
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	The mean survival rate of the fish in the control and the solvent control was 86.6 ± 13.5% and 88.3 ± 4.3%, respectively, demonstrating the suitability of the test conditions
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 210 (1992) Valid study Deviations to study guideline: different strain of zebra fish were used in order to improve the quality of test fish population and the duration of the test was extended for technical reasons
5.2	**Results and discussion**	
5.2.1	NOEC	25 µg/L (mean measured 22 µg/L)
5.2.2	LOEC	50 µg/L (mean measured 51 µg/L)
5.2.3	MATC	35 µg/L (mean measured 33 µg/L)
5.3	**Conclusion**	Validity criteria can be considered as fulfilled.
5.3.1	Other Conclusions	
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Table A7_4_3_2-1: Dilution water

Criteria	Details
$CaCl_2 \times 2H_2O$	2.0 mmol/L (= 294 mg/L)
$MgSO4 \times 7H_2O$	0.5 mmol/L (= 123 mg/L)
$NaHCO_3$	0.75 mmol/L (= 65 mg/L)
KCl	0.075 mmol/L (= 5.8 mg/L)
Hardness	2.5 mmol/L (= 250 mg/L as $CaCO_3$
Alkalinity	0.8 mmol/L
Dissolved oxygen concentration	5.6 mg/L
pH	7.7 to 7.9

Table A7_4_3_2-2: Test organisms

Criteria	Details
Species/strain	Zebra fish (*Brachydanio rerio*)
Source	[redacted]
Wild caught	No
Age/size	Newly fertilized eggs
Pre-treatment	The parental fish were held in an aquarium with local tap water. Two weeks prior to test start, the batch was adapted to the same type of water as used in the test. A 16-hour light to 8 hour dark photoperiod was applied. The fish were fed with a commercial fish diet and brine shrimps. The brood batch was regularly checked for abnormal behaviour, diseases or mortality. No visible abnormalities were observed in the fish during the three months prior to test start and no medication was applied
Feeding of animals during test	Day 0 – Day 2: Embryo-stage and newly hatched larvae: none Day 3 – Day 10: Mixture of dry-food powder and freshly hatched artemia larvae Day 11 – test end: Freshly hatched artemia larvae. Fish were not fed 24 hour prior to the end of the test to allow clearance of the digestive tracts before weighing the fish. Food was given at least in the morning and the afternoon of each working day. On weekends food was given at least once per day

Table A7_4_3_1-3: Test system

Criteria	Details
Test type	Flow through
Flow rate of the test medium	0.45 L/h (= 11 L/day)
Volume of test vessels	- Day 0 to Day 15: Glass vessels with about 200 mL test medium - Larvae to juvenile fish: glass beakers with about 1.1 L test medium
Number of replicates / concentration	4
Number of eggs/ concentration	60
Loading rate of juvenile fish	0.05 g fish wet weight/liter/day

Table A7_4_3_2-4: Test conditions

Criteria	Details
Water temperature	24.2 – 25.8°C (measured)
Light conditions	16-hour light to 8-hour darkness photoperiod daily
Light Intensity	230 to 410 Lux
Aeration of dilution water	Test water was aerated prior to the start of the test until oxygen saturation was reached.

Table A7_4_3_1-5: Effect data, based on nominal concentrations.

Effect	Concentration [µg/L]	
Egg development and hatching rate (NOEC, LOEC)	≥ 50	> 50
Time to hatch / development rate (NOEC, LOEC)	≥ 50	> 50
Survival of larvae and juvenile fish (NOEC, LOEC)	25	50
Fish length and weight at test end (NOEC, LOEC)	≥ 50	> 50
Overall (NOEC, LOEC)	25	50
MATC	35	

Table A7_4_3_1-9: Validity criteria for fish tests according to OECD Guideline 210

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 based on mean measured concentrations of nominally 25 and 50 µg/L

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29.08.05
Materials and methods	Agree with the applicant´s version
Conclusion	Agree with the applicant´s version
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.4.3.3.1 Bioconcentration in an appropriate species of fish

Annex Point IIIA-XIII.2.3 **Bluegill sunfish (*Lepomis macrochirus*)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2002): Bioconcentration: flow-through fish test with MTI-500 (Trebon) in bluegill sunfish; [redacted] unpublished report no. 841573 (May 30, 2002). Dates of experimental work: November 06, 2000 – August 16, 2001	
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 205 (1996) EPA OPPTS 850.1730 (Draft, 1996)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Etofenprox	
3.1.1	Lot/Batch number	MR-9301	
3.1.2	Specification	As given in section 2 Deviating from specification given in section 2 as follows	
3.1.3	Description	solid	
3.1.4	Purity	99.7%	
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 277/20 - Specific activity: 32.94 mCi/mmol (= 87.49 µCi/mg) 1218.8 MBq/mmol (= 3.237 MBq/mg) - Radiochemical purity: > 99% (the purity after re-purification at RCC was 97.7% and 96.0% (TLC in SS8) for the material used in the pre-test and in the main test, respectively) - Stability in stock/application solution: test item was sufficiently stable after stirring at room temperature for 7 days in dimethylformamide (DMF)	

Section A7.4.3.3.1 Bioconcentration in an appropriate species of fish

Annex Point IIIA-XIII.2.3 **Bluegill sunfish (*Lepomis macrochirus*)**

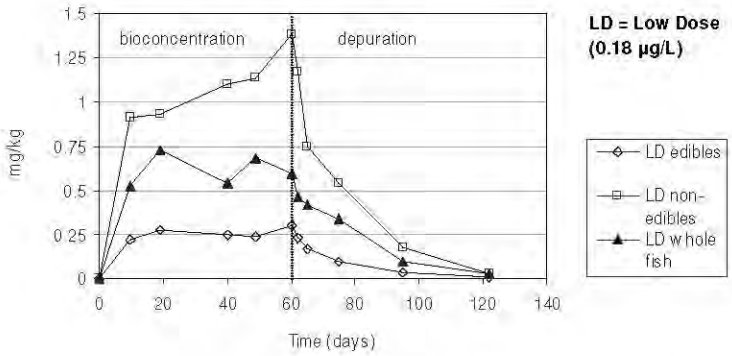
3.1.8	Method of analysis	LSC: Radioactivity was measured with a Packard liquid scintillation counter (TRI-CARB 2500TR or 2000 CA) using INSTA-GEL II Plus or HIONIC-FLUOR scintillation fluid. Tissue was solubilised using Soluene 350 (Packard Instr.) HPLC: was used as primary system for determination of metabolite patterns in exposure water and fish tissue and was performed on a system consisting of a gradient pump (Merck-Hitachi L-6200), an autosampler (Merck-Hitachi AS-2000 A), an UV detector (Merck-Hitachi L-4000), a radiodetector (Packard Radiometric 500TR) and data processing system (Packard FLOW-ONE). TLC: was used to determine the radiochemical purity of the test item and as secondary analytical method for water extracts. For TLC analysis samples were concentrated, TLC with toluene/ethyl acetate or methanol solvent on pre-coated (5 cm x 20 cm, 0.25 mm) silca gel $F_{254}S$ (Merck) using a CAMAG Linomat for application. Detection: Berthold Automatic TLC-Linear Analyser
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Pre-test: a stock solution was prepared in DMF (N,N-Dimethyl-formamide) containing 0.75 mg ^{14}C-etofenprox/ml. Application solutions were prepared from this stock solution by further dilution in DMF to reach concentrations of 2, 10 and 20 µg/ml. Main test: a stock solution was prepared in DMF (N,N-Dimethyl-formamide) containing 1.577 mg ^{14}C-etofenprox/ml. Application solutions were prepared from this stock solution by further dilution in DMF to reach concentrations of 2 and 10 µg/ml. See also table A7_4_3_3_1-1
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	
3.4	**Testing/estimation procedure**	
3.4.1	Dilution water	See table A7_4_3_3_1-2
3.4.2	Test organisms	See table A7_4_3_3_1-3
3.4.3	Test system	See table A7_4_3_3_1-4
3.4.4	Test conditions	See table A7_4_3_3_1-5
3.4.5	Duration of the test	Exposure phase: 60 days Depuration phase: 62 days
3.4.6	Test parameter	Radioactivity levels in fish and water were determined at various sampling intervals during the entire study
3.4.7	Sampling	See table A7_4_3_3_1-6
3.4.8	Monitoring of TS concentration	Yes See table A7_4_3_3_1-6 for sampling scheme
3.4.9	Statistics	Depuration curves of radioactivity, plateau levels in fish and kinetic bioconcentration factors were calculated by the non-linear parameter estimation program ORIGIN (MicroCal Software Inc., Norhthampton, MA 01060, USA)

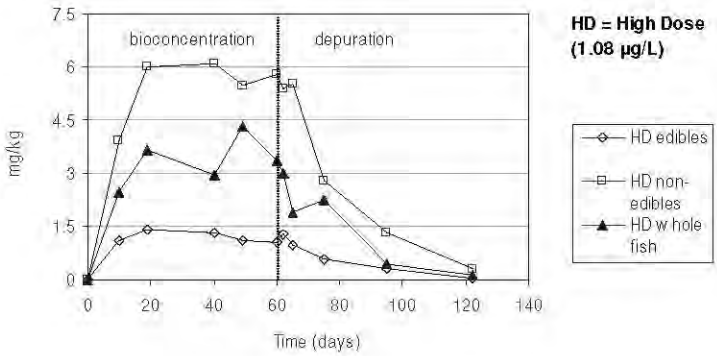
Section A7.4.3.3.1 **Bioconcentration in an appropriate species of fish**
Annex Point IIIA-XIII.2.3 **Bluegill sunfish (*Lepomis macrochirus*)**

4 RESULTS

4.1 Experimental data

4.1.1	Mortality/behaviour	During exposure no mortality or other adverse effects were observed in fish
4.1.2	Lipid content	See table A7_4_3_3_1-7
4.1.3	Concentrations of test material during test	Concentration in water and fish (edible and non-edibles): see table A7_4_3_3_1-8 Concentration in lipid: see table A7_4_3_3_1-7 Depuration half-lives: see table A7_4_3_3_1-9 Graphs showing bioconcentration and depuration in fish of low dose (LD) and high dose (HD) tanks:





4.1.4	Bioconcentration factor (BCF)	Bioconcentration factors based on the total radioactivity in the low-dose and high dose exposure water (0.18 and 1.08 µg/L, respectively):

Tissue	Bioconcentration factor	
	Low dose	High dose
Edibles	1489	1128
Non-edibles	6656	5460
Whole fish	3517	3353
Lipid	47844	43656

Section A7.4.3.3.1 Bioconcentration in an appropriate species of fish

Annex Point IIIA-XIII.2.3 **Bluegill sunfish (*Lepomis macrochirus*)**

4.1.5	Uptake and depuration rate constants	Accumulation and depuration rate constants (day^{-1}) based on best fitting curves. Regression coefficients as measure for the goodness of fit are given in brackets:	X

Tissue	Accumulation		Depuration	
	Low dose	High dose	Low dose	High dose
Edibles	0.182	0.289	0.0774	0.0439
	(0.952)	(0.938)	(0.980)	(0.955)
Non-edibles	0.114	0.130	0.0691	0.0445
	(0.935)	(0.973)	(0.966)	(0.976)
Whole fish	0.211	0.130	0.0434	0.0437
	(0.929)	(0.901)	(0.967)	(0.896)

4.1.6	Depuration time	see table A7_4_3_3_1-9	
4.1.7	Metabolites	Water: no metabolites were detected in amounts > 10 %. Three metabolites (α-CO, DE and m-PB-acid) were found in low (< 5%) amounts. Fish: throughout all fish extracts (edibles, non-edibles at both dose levels) exclusively parent item was found. Hence total radioactivity levels in fish were representative for the bioconcentration of the parent item etofenprox.	
4.1.8	Other Observations	No other unusual observations about the test, no deviations from the guideline.	
4.2	**Estimation of bioconcentration**	Etofenprox has potential for bioconcentration in bluegill sunfish when exposed to 0.18 and 1.08 µg/L. For bioconcentration factors see point 4.1.4.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	OECD 305 (1996) EPA OPPTS 850.1730 (Draft, 1996) Valid study No deviations to study guideline	
5.2	**Results and discussion**		
5.2.1	BCF	Edibles: 1309 ± 255 Non-edibles: 6058 ± 846 Whole fish: 3435 ± 116 Lipid: 43656 – 47844	X
5.2.2	Accumulation	Plateau level reached after 19 days exposure	
5.2.3	Depuration half-lives:	9 – 16 days	

Section A7.4.3.3.1 Bioconcentration in an appropriate species of fish

Annex Point IIIA-XIII.2.3 **Bluegill sunfish (*Lepomis macrochirus*)**

5.3	**Conclusion**	Validity criteria can be considered as fulfilled. Bioconcentration of etofenprox was observed in bluegill sunfish that were exposed to the test item at concentrations of 0.18 and 1.08 µg/L. Plateau levels were reached in general after 19 days of exposure. Based on plateau values, BCF values averaged 1309 ± 255, 6058 ± 846 and 3435 ± 116 for edibles, non-edibles and whole fish, respectively. However, taking into account the depuration half-lives of 9 – 16 days and the plateau levels reached after 19 days, it can be concluded that the uptake of etofenprox in fish is highly revesible.	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7_4_3_3_1-1: 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 (control tank received identical amount of solvent without test item)
Other procedures	Prior to use for preparation of stock- and application solutions, ^{14}C-etofenprox was purified using RP18 reverse phase (Varian 1225-6031) or silica gel (Merk 7734) columns. Radiochemical purity after purification was ≥ 96%.

Table A7_4_3_3_1-2: Dilution water.

Criteria	Details
Source	Tap water
Alkalinity	
Hardness	178.5 mg/L as $CaCO_3$
pH	Accumulation phase: 7.8 – 8.0 Depuration phase: 7.8 – 7.9
Oxygen content	Accumulation phase: 5.6 – 8.1 mg/L Depuration phase: 7.8 – 8.5 mg/L
TOC	Accumulation phase (day 40): 37.8 – 50.5 mg/L Depuration phase (day 95): 3.1 – 6.8 mg/L
Holding water different from dilution water	No

Table A7_4_3_3_1-3: Test organisms.

Criteria	Details
Species/strain	Bluegill sunfish (*Lepomis macrochirus*)
Source	[redacted]
Wild caught	No
Age/size	Mean body wet weight: 1.6 – 2.1 g
Kind of food	Commercial fish diet (HOKOVIT 502, 1.2 mm; supplied by H.U. Hoffmann AG, Bützberg, Switzerland)
Amount of food	Approximately 2% of the average fish body weight during acclimatisation and study, taking into account increasing body weights and the decreasing number of fish per sampling interval
Feeding frequency	Daily
Pretreatment	Fish were allowed to acclimate to the laboratory environment for at least 1 week
Feeding of animals during test	Yes

Table A7_4_3_3_1-4: Test system.

Criteria	Details
Test type	Exposure under flow-through conditions (60 days), followed by a depuration phase (62 days)
Renewal of test solution	Application solutions containing 2 and 10 µg ^{14}C-etofenprox/ml DMF (for the low and high dose, respectively) or DMF alone (control) were dosed into mixing vessels for pre-dilution with tap water, delivered by a Hamilton Digital Dispenser (Hamilton, Germany). Dosage: 35 ml/24 hours (application solutions or DMF alone) Test water continuously flowed into the mixing vessels at a rate of 350 L/24 hours (i.e. 14.58 L/hour). Flow rate through the aquaria (volume 75 L) corresponded approximately to a fivefold theoretical volume exchange/day.
Volume of test vessels	75 L (volume of test substance/control water)
Volume/animal	Control tank: 3 L/animal Treatment tanks: 0.765 L/animal (initial values)
Number of animals/vessel	Control tank: 25 Treatment tanks: 98 (initial values)
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_3_1-5: Test conditions.

Criteria	Details
Test temperature	Accumulation phase: 21 – 23°C Depuration phase: 21 – 23°C
Dissolved oxygen	See table table A7_4_3_3_1-1
pH	See table table A7_4_3_3_1-1
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	about 500 Lux
Photoperiod	12 – 16 hours light daily

Table A7_4_3_3_1-6: Fish and water sampling schedule.

Fish sampling	Number of fish samples from each treated tank			Control fish samples	H2O samp-les[a]	Sample point (days)	Action
	Total radio-activity (LSC)	Parent/ Metabo-lites	Lipid content				
					2	-3	Start test
					2	-2	
					2	-1	
					2	0	Fish added[d]
					2	4	
A[b] 1st	6	–	6	6	2 (+2)[c]	10	
2nd	6	–			2 (+2[c]	19	
3rd	6	–			2 (+2)[c]	40	
4th	6	8			2 (+2)[c]	49	
5th	6	8	6	6	2 (+2)[c]	60	End of uptake period, start of depuration
Subtotal	30	16	12	12			
B[b] 6th	6				2	62	
7th	6				2	65	
8th	6				2	75	
9th	6				2	95	
10th	6			6	2	122	
Subtotal	30	0	–	6			
Reserve fish	10	–	–	7			
Total	70	16	12	25			
Total fish sampled	98			25			

[a] Water samples were taken before feeding of the fish
[b] A = uptake phase of the test; B = depuration phase of the test
[c] Additional sampling of water to be analysed directly
[d] Fish were added after at least 10 tank volumes run through the tanks during the pre-equilibration period

Table A7_4_3_3_1-7: Bioconcentration factors based on lipid content in bluegill sunfish treated with concentrations of 0.18 µg/L (low dose) and 1.08 µg/L (high dose) ^{14}C-etofenprox

Treatment	Sampling interval	Lipid content (mg/g fish)	Concentration			BCF (lipid)
			Fish (µg-eq/kg)	Lipid (µg-eq/kg)	Water (µg/L)	
Low dose	Day 10	77.4	526	6796	0.18	37756
	Day 60	69.5	594	8547	0.18	47483
	At plateau	73.5 [a]	633 [b]	8612 [b]	0.18	47844
High dose	Day 10	89.6	2459	27444	1.08	25411
	Day 60	64.0	3340	52188	1.08	48322
	At plateau	76.8 [a]	3621 [b]	47148 [b]	1.08	43656

[a] Average value for both sampling intervals
[b] Average plateau concentration calculated via best fit (non-linear parameter estimation)

Table A7_4_3_3_1-8: Actual concentrations of total radioactivity from ^{14}C-etofenprox in the water and residues in edible and non-edible parts of fish during accumulation and depuration (values were corrected for background and expressed as radioactivity in µg parent equivalents per kg (fish: fresh weight) based on a specific radioactivity of 87.49 µCi/mg).

Time interval (days)	Water			Fish					
	control	Low dose	High dose	Low dose			High dose		
				Edible	Non-edibles	Whole fish	Edible	Non-edibles	Whole fish
-3	n.d.	0.25	0.93	–	–	–	–	–	–
-2	n.d.	0.18	0.89	–	–	–	–	–	–
-1	n.d.	0.19	1.06	–	–	–	–	–	–
0 [a]	<BG	0.16	0.77	–	–	–	–	–	–
Mean ± SD		0.20 ± 0.04	0.91 ± 0.12						
0 [b]	<BG	0.15	0.95	–	–	–	–	–	–
4	n.d.	0.13	1.00	–	–	–	–	–	–
10	n.d.	0.15	0.96	218 EX	915 EX	526 EX	1125	3922 EX	2459 EX
19	n.d.	0.17	1.17	278	928 EX	727	1390	6012	3676
40	<BG	0.26	1.25	246	1103	541	1331	6102	2976
49	n.d.	0.19	1.37	241	1134	685	1091	5454	4318
60	<BG	0.20	0.83	304	1387	594	1078	5794	3340
Mean ± SD		**0.18 ± 0.04**	**1.08 ± 0.19**	267 ± 29 [c]	1208 ± 156 [c]	637 ± 85 [c]	1203 ± 146 [c]	5841 ± 288 [c]	3578 ± 570 [c]
62	n.d.	0.1	0.07	231	1169	458	1279	5395	3008
65	n.d.	0.1	0.05	172	747	416	967	5527	1891
75	n.d.	<BG	0.02	102	540	337	572	2761	2251
95	<BG	<BG	<BG	32	181	101	293	1333	433
122	<BG	<BG	<BG	5	23	27	48	303	116

[a] Before adding the fish
[b] After adding the fish
[c] Mean value indicates average plateau level
BG Based on a scintillation background of 18 dpm/10 ml, background levels of 0.009 µg-eq/kg at 87.49 µCi/mg
EX Excluded from the calculation of the average plateau level

Table A7_4_3_3_1-9: Depuration characteristics following first order kinetics

	Low dose			High dose		
	Edible	**Non-edibles**	**Whole fish**	**Edible**	**Non-edibles**	**Whole fish**
kdep (day^{-1})	0.0774	0.0691	0.0434	0.0439	0.0445	0.0437
SD (kdep)	0.0121	0.0140	0.0076	0.0091	0.0067	0.0145
R-squared	0.980	0.966	0.967	0.955	0.976	0.896
$t_{0.5}$ (days)	9.0	10.0	16.0	15.8	15.6	15.9
$t_{0.95}$ (days)	38.8	43.4	69.1	68.3	67.4	68.6

kdep Depuration rate constant via best fitting curve
SD Standard deviation
R-squared Regression coefficient as measure for the goodness of fit
$t_{0.5}$ Time point at 50% depuration, i.e. half life in days: ln 2/kdep
$t_{0.95}$ Time point at 50% depuration, i.e. half life in days: 3/kdep

Table A7_4_3_3_1-10: Validity criteria for bioconcentration in fish test according to OECD Guideline 305.

Criteria	**fulfilled**	**not fulfilled**
Temperature variation less than ± 2°C	X	
Concentration of dissolved oxygen in all test vessels > 60% saturation	X	
pH value in a range of ± 0.5 pH units and varied between 7.8 and 8.0	X	
Concentration of the test item in the chambers maintained within of the mean measured values during the uptake phase	X *	
Mortality or other adverse effects in both control and treated fish were <10% at the end of the test	X	
During the uptake phase more than 95% of steady state is reached with a confidence margin of ± 20%	X	

* 22% applied for the low dose (0.2 µg/L) due to a single high value of 0.26 µg/L at day 40

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	Agree with the applicant´s version
Conclusion	**4.1.5 Uptake and depuration rate constants:** values based on the total radioactivity **5.2 Results and discussion, 5.2.1 BCF:** The BCF-values given in this table are based on parent equivalents (total radioactivity) The BCF-values should be corrected for the content of the parent item in exposure water (78.5 -87.7 %) The corresponding BCFs are: Edibles: 1554 ± 171 Non-edibles: 7213 ± 389 Whole fish: 3951 ± 8 **5.3. Conclusion:** Agree with the applicants version, but the remark "However, taking into account the depuration half-lives of 9 – 16 days and the plateau levels reached after 19 days, it can be concluded that the uptake of etofenprox in fish is highly reversible" should be seen under the aspect that 50% depuration for the whole fish will be reached at day 16 (LD and HD), the 95 % depuration at day 69 (LD) and 68.6 (HD), however. (see Table A7_4_3_3_1-9: Depuration characteristics following first order kinetics).
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	