SECTION A7.1.1.1.2 **PHOTOTRANSFORMATION IN WATER INCLUDING IDENTITY OF TRANSFORMATION PRODUCTS**

Annex Point IIA7.6.2.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	R. Wilmes, 1983, Orientating Light Stability , Bayer AG (unpublished), 1982-05-21, MO-03-003648	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active ingredient for the purpose of its entry into Annex I/IA	
		3 GUIDELINES AND QUALITY ASSURANCE	
3.1	**Guideline study**	No	
3.2	**GLP**	No, GLP was not compulsory at the time the study was performed	
3.3	**Deviations**	-	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Racumin (coumatetralyl)	
3.1.1	Lot/Batch number	No data	X
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	No data	X
3.1.4	Radiolabelling	No	
3.1.5	UV/VIS absorption spectra and absorbance value	Absorption spectrum in methanol: Maxima: UV region: 209 nm (ε = 45720), 312 nm (ε = 14280) visible region: none The UV spectrum of the compound shows a relatively long-wave and intense maximum at 312 nm, which in aqua bidest. is shifted to 310 nm. The slope of the curve extends to about 350 nm. Interactions with sunlight in the environment (λ > 290 nm) are therefore quite feasible.	
3.1.6	Further relevant properties		

SECTION A7.1.1.1.2 **PHOTOTRANSFORMATION IN WATER INCLUDING IDENTITY OF TRANSFORMATION PRODUCTS**

Annex Point IIA7.6.2.2

3.2 Reference substances	Reference substances not used	
3.3 Test solution	Irradiation in solid state: amounts of 0.1 µg Racumin were spotted on silica gel 60 HPTLC plates and irradiated with sunlight-simulating inflorescent tubes (examination after 7 days) or left covered (= sample-plates held in darkness) for 4 weeks. Irradiation in solution: Saturated solutions of Racumin in aqua bidest., containing 1.4 mg/l of the test compound, were irradiated by immersion lamp or by a rotary irradiation apparatus.	
3.4 Testing procedure		
3.4.1 Test system	Irradiation in solid state: amounts of 0.1 µg Racumin were spotted on silica gel 60 HPTLC plates and irradiated with sunlight-simulating inflorescent tubes (examination after 7 days) or left covered (= sample-plates held in darkness) for 4 weeks. Irradiation in solution: Saturated solutions of Racumin in aqua bidest, containing 1.4 mg/l of the test compound, were irradiated with TQ 150 high-pressure mercury vapour lamps by direct irradiation with the immersion lamp or using the lamp as a water-cooled light source in a rotary irradiation apparatus.	
3.4.2 Properties of light source	Irradiation in solid state: TRUE LITE sunlight- simulating inflorescent tubes. Irradiation in solution: TQ 150 high-pressure mercury vapour lamps with Duran 50 (borosilicate glass) filter. Spectral data of lamps is given in Table A7.1.1.1.2-1:	
3.4.3 Determination of irradiance	n.a.	X
3.4.4 Temperature	n.a.	X
3.4.5 pH	n.a.	X
3.4.6 Duration of the test	n.a.	X
3.4.7 Number of replicates	n.a.	X
3.4.8 Sampling	n.a.	X
3.4.8 Analytical methods	Transformation products were isolated by thin-layer chromatography and identified by mass spectroscopy.	

SECTION A7.1.1.1.2 **PHOTOTRANSFORMATION IN WATER INCLUDING IDENTITY OF TRANSFORMATION PRODUCTS**

Annex Point IIA7.6.2.2

3.5	**Transformation products**	Yes	
3.5.1	Method of analysis for transformation products	Solutions of 100 mg Racumin/l in aqua bidest./acetonitrile (2:1) were irradiated for 3 hours. Photoproducts were isolated by thin-layer chromatography and identified by mass spectroscopy.	
		4 RESULTS	
4.1	**Screening test**	Irradiation in the solid state: after 7 days, the content of the test compound in the irradiated samples had decreased to 8 % of the initial concentration. The experimentally determined half-life was 1 day (48 % degradation). Absorption spectrum in methanol: Maxima: UV region: 209 nm (ε = 45720), 312 nm (ε = 14280) Visible region: none The UV spectrum of the compound shows a relatively long-wave and intense maximum at 312 nm, which in aqua bidest. is shifted to 310 nm. The slope of the curve extends to about 350 nm. Interactions with sunlight in the environment (λ > 290 nm) are therefore quite feasible.	
4.2	**Actinometer data**	n.a.	
4.3	**Controls**	Irradiation in solid state: in the control treatment (= sample-plates held in darkness) a degradation of 14 % was observed after 4 weeks.	
4.4	**Photolysis data**		
4.4.1	Concentration values	n.a.	X
4.4.2	Mass balance	n.a.	X
4.4.3	kcp	n.a.	X
4.4.4	Kinetic order	n.a.	X
4.4.5	kcp / kap	n.a.	X
4.4.6	Reaction quantum yield (ϕcE)	n.a.	X
4.4.7	kpE		X
4.4.8	Half-life (t1/2E)	Irradiation in the solid state: after 7 days, the content of the test compound in the irradiated samples had decreased to 8 % of the initial concentration. The experimentally determined half-life was 1 day (48 % degradation).	

Irradiation in solution: the half-life for direct irradiation of the solution with the immersion lamp was determined to be 34 minutes and the half-life using the lamp as a water-cooled light source in a rotary irradiation apparatus was 68 minutes.

In a range-finding experiment in sunlight (April; 10 a.m. – 3 p.m. Central European Time; clear sky), the extrapolated half-life was 6.6. hours.

4.5 Specification of the transformation products

Solutions of 100 mg Racumin/l in aqua bidest./acetonitrile (2:1) were irradiated for 3 hours. Photoproducts were isolated by thin-layer chromatography (TLC) and identified by mass spectroscopy.

A number of photoproducts were formed, of which salicylic acid (1) was identified as a major product. Further compound zones isolated by thin-layer chromatography disclosed indications of dehydrogenated products (2-4) which however may have been produced also by thermal reaction, e.g. by oxidation during the TLC analysis. (Chemical structures are given in Table A7.1.1.1.2-2).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The stability of Racumin (coumatetralyl) was investigated in aqueous solution and in solid state:

Irradiation in solid state: amounts of 0.1 µg Racumin were spotted on silica gel 60 HPTLC plates and irradiated with sunlight-simulating fluorescent tubes (examination after 7 days) or left covered (= sample-plates held in darkness) for 4 weeks.

Irradiation in solution: Saturated solutions of Racumin in aqua bidest, containing 1.4 mg/l of the test compound, were irradiated with TQ 150 high-pressure mercury vapour lamps by direct irradiation with the immersion lamp or using the lamp as a water-cooled light source in a rotary irradiation apparatus.

5.2 Results and discussion

Irradiation in solid state: in the control treatment (= sample-plates held in darkness) a degradation of 14 % was observed after 4 weeks.

5.2.1 kcp

5.2.2 KpE

5.2.3 ϕcE

5.2.4 t1/2E

Irradiation in the solid state on surface:

The experimentally determined half-life was 1 day (48 % degradation).

SECTION A7.1.1.1.2
Annex Point IIA7.6.2.2

PHOTOTRANSFORMATION IN WATER INCLUDING IDENTITY OF TRANSFORMATION PRODUCTS

		Irradiation in solution: the half-life for direct irradiation of the solution with the immersion lamp was determined to be 34 minutes and the half-life using the lamp as a water-cooled light source in a rotary irradiation apparatus was 68 minutes. In a range-finding experiment in sunlight (April; 10 a.m. – 3 p.m. Central European Time; clear sky), the extrapolated half-life was 6.6. hours.	
5.3	**Conclusion**	Racumin is degraded rapidly by light to a number of products, of which salicylic acid was identified as a major product. Photodegradation in aqueous solution and in an adsorbed state may occur rapidly also in environmental conditions.	
5.3.1	Reliability	3	
5.3.2	Deficiences	Yes, lot/batch number and purity of the test material not mentioned; details about experimental conditions not mentioned (determination of irradiance, temperature, pH, duration of test, number of replicates, sampling, analytical methods);	X

	EVALUATION BY COMPETENT AUTHORITIES
SECTION A7.1.1.1.2 Annex Point IIA7.6.2.2	PHOTOTRANSFORMATION IN WATER INCLUDING IDENTITY OF TRANSFORMATION PRODUCTS
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2004.08.16
Materials and Methods	**Comment:** The lack of information regarding most experimental parameters is serious (**see e.g. 3.1.1, 3.1.3, 3.4.3-3.4.8**). This is mentioned by the applicant but no conclusion is drawn.
Results and discussion	**Comment:** The parameters in **section 4.4** are not available either.
Conclusion	Alternative: Under the conditions of the experiment, the test substance (coumatetralyl or Racumin) was degraded rapidly by light to a number of degradation products, of which salicylic acid was identified as a major product. Due to lack of information, no general conclusion regarding possible degradation rate (half life) can be drawn. However, the results indicate that we have a short half life (about a few hours – 1one day)
Reliability	4
Acceptability	Due to the large information gaps, the applicability of the data cannot be assessed. Therefore, the study is not acceptable.
Remarks	Due to the large information gaps, the applicability of the data cannot be assessed. Therefore, the study is not acceptable. However, if the risk assessment based on no phototransformation (worst case) still give no concern, then we accept that no new study should be performed

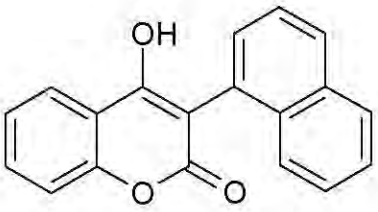
Table A7.1.1.1.2-1: Spectral energy distribution

Criteria	Details		
Test apparatus	TQ 150 high-pressure mercury vapour lamps (OriginalHanau) immersion lamps with Duran 5O filter tube		
Nature of light source	mercury vapour lamps		
Emission wavelenght spectrum	λ (nm)	Radiant flux Φ (W)	Mole quantum per hrx10^{-3}
	238/240	1.0	8
	248	0.7	5
	254	4.0	30
	265	1.4	11
	270	0.6	5
	275	0.3	2
	280	0.7	6
	289	0.5	4
	297	1.0	9
	302	1.8	17
	313	4.3	41
	334	0.5	5
	366	6.4	71
	390	0.1	1
	405/08	3.2	39
	436	4.2	55
	492	0.1	1
	546	5.1	84
	577/79	4.7	82
	Radiant flux Φ of 200-600 nm: 47 W		
Filters	Duran 50 filter tube		
	λ (nm)	Radiant flux Φ (W)	Mole quantum per hrx10^{-3}
	238/240	--	--
	248	--	--
	254	--	--
	265	--	--
	270	--	--
	275	--	--
	280	--	--
	289	--	--
	297	0.1	1
	302	0.5	4
	313	2.5	23
	334	0.4	4
	366	5.8	64
	390	0.1	1
	405/08	2.9	35
	436	3.6	50
	492	0.1	1
	546	4.6	76
	577/79	4.2	74

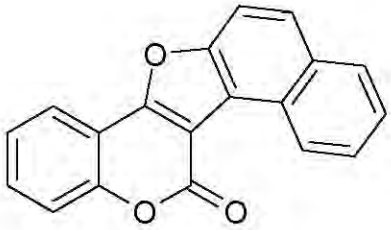
Table A7.1.1.1.2-2: Photoproducts structure

(1)

(2)



(3)



(4)

SECTION A7.1.1.2.1 **BIODEGRADABILITY (READY)**

Annex Point IIA7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	M.J.E. Desmares-Koopmans, 2001, Ready Biodegradability: Closed Bottle Test with coumatetralyl, NOTOX B.V., `s-Hertogenbosch, the Netherlands, Project No. 311873 (unpublished), 2001-05-15, MO-03-003122	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active ingredient for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, Directive 92/69/EEC, method C.4-E and OECD guideline No. 301 D (Closed Bottle Test)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Coumatetralyl	
3.1.1	Lot/Batch number	Batch No.: 0800	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	Not indicated by the sponsor (treated as 100 % pure)	X
3.1.4	Further relevant properties	Solubility in water (at 20 °C): 4 mg/l at pH 4.2; 20 mg/l at pH 5; 425 mg/l at pH 7; 100 – 200 g/l at pH 9; Stability in water: at least 96 h	X
3.1.5	Composition of Product		
3.1.6	TS inhibitory to micro-organisms	EC_{50} = 4210 mg/l (result of the respiration inhibition test with activated sludge; study number 283 A/91, 1991-07-12)	
3.1.7	Specific chemical	No	

	analysis	
3.2	**Reference substance**	Yes, sodium acetate was used as positive control
3.2.1	Initial concentration of reference substance	2 mg/l
3.3	**Testing procedure**	
3.3.1	Inoculum / test species	The source of the test organism was secondary effluent freshly obtained from a municipal sewage treatment plant: `Waterschap de Maaskant`, `s-Hertogenbosch, the Netherlands. The secondary effluent was filtered through a coarse filter paper, the first 200 ml were discarded. The filtrate was kept aerated until inoculation. 4 ml filtrate of the secondary effluent per litre of final volume were used for inoculation.
3.3.2	Test system	250 – 300 ml BOD bottles with glass stoppers were used as test bottles. Parallel groups of BOD bottles were prepared to allow duplicate measurements of oxygen consumption at the test intervals and in the controls (blank control, positive control, toxicity control): Test suspensions: Flask 3A and 3B: mineral medium, inoculum, coumatetralyl (2 mg/l); Flask 4A and 4B: mineral medium, inoculum, coumatetralyl (5 mg/l); Positive control: Flask 2A and 2B: mineral medium, inoculum, Sodium acetate (2mg/l); (blank control and toxicity control see point 3.3.11). A dissolved oxygen meter (WTW: OXI 530, TriOxmatic EO 200 oxygen electrode, electrolyte type ELY/N) was used for the oxygen measurements.
3.3.3	Test conditions	See table A7.1.1.2.1-1
3.3.4	Method of preparation of test solution	A stock solution of 100 mg/l was prepared by adding 100.2 mg of the test substance quantitatively to 1000 ml mineral medium (composition see table A7.1.1.2.1-1). Thorough mixing (15 minutes) and ultra sonication (40 minutes) were used to accelerate dissolving. The pH was increased with 1 N NaOH from 6.5 to 7.7, followed by ultra sonication (10 minutes). This results in a clear and colourless stock solution. Amounts of the

		stock solution corresponding to the test concentrations were then added to the test medium.
3.3.5	Initial TS concentration	2 mg coumatetralyl/l (low) and 5 mg coumatetralyl/l (high)
3.3.6	Duration of test	28 days
3.3.7	Analytical parameter	Measurement of oxygen depletion
3.3.8	Sampling	Measurements of oxygen consumption were performed immediately at the start of the experiment (day 0) and at day 7, 14, 21 and 28 in duplicate. The pH was measured at the start of the test and the test temperature was recorded daily in a vessel with water in the same room.
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	No
3.3.11	Controls	Blank control (mineral medium and inoculum (flask 1A and 1B)) and toxicity control (mineral medium, inoculum, coumatetralyl (2 mg/l) and Sodium acetate (2 mg/l) (flask 5A and 5B)).
3.3.12	Statistics	The biochemical oxygen demand (BOD [mg O_2/mg test substance]) was calculated by subtracting the mean oxygen depletion (mg O_2/l) of the inoculum blank from that exhibited by the test substance. The corrected depletion was divided by the concentration (mg/l) of the test substance. The percentage biodegradation was calculated by dividing the specific BOD by the specific ThOD which was calculated on the basis of the elemental composition of the test substance. A figure of more than 10 % degradation was considered as significant.

4 RESULTS

4.1 Degradation of test substance

4.1.1 Graph	Degradation curve of test substance is given in Table A7.1.1.2.1-2
4.1.2 Degradation	The relative biodegradation values calculated from the O_2 measurements performed during the test period of 28 days revealed no significant degradation of coumatetralyl at both concentrations. Test substance (low): 1 % biodegradation after 7 days; 4 % biodegradation after 14 days; 0 % biodegradation after 21 days; 1 % biodegradation after 28 days; Test substance (high): 0 % biodegradation after 7, 14, 21 and 28 days
4.1.3 Other observations	No inhibition of microbial activity in the toxicity control
4.1.4 Degradation of TS in abiotic control	No abiotic control
4.1.5 Degradation of reference substance	A degradation of 79 % was achieved for sodium acetate after 7days, 80 % after 14 days, 73 % after 21 days and 89 % after 28 days (degradation curve is given in Table A7.1.1.2.1-2).
4.1.6 Intermediates/ degradation products	n.a.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Coumatetralyl was tested for its ready biodegradability in an aerobic, aqueous medium with microbial activity introduced by inoculation of secondary effluent during a test period of 28 days using the closed bottle test according to the Directive 92/69/EEC, method C.4-E and OECD guideline No. 301 D. The concentrations tested were 2 mg/l and 5 mg/l. During the test period the biodegradation of the test substance is determined by registrating the oxygen depletion.

The study shows no significant deviations from the Directive 92/69/EEC, method C.4-E and OECD guideline No. 301 D.

SECTION A7.1.1.2.1 BIODEGRADABILITY (READY)

Annex Point IIA7.6.1.1

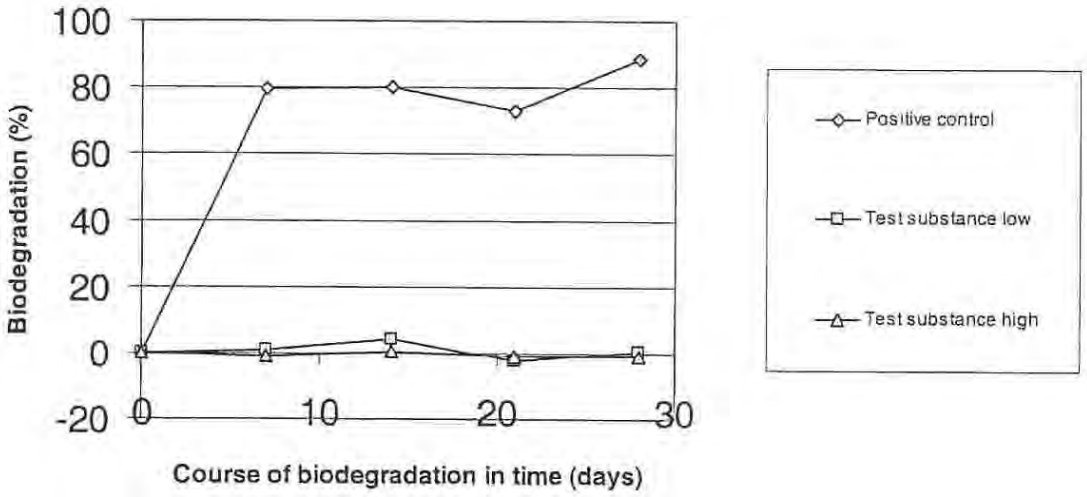
5.2 Results and discussion	The relative biodegradation values of coumatetralyl calculated from the O_2 measurements performed during the test period of 28 days revealed no significant degradation of the test substance at both concentrations. Test substance (low): 1 % biodegradation after 7 days; 4 % biodegradation after 14 days; 0 % biodegradation after 21 days; 1 % biodegradation after 28 days; Test substance (high): 0 % biodegradation after 7, 14, 21 and 28 days A degradation of 80 % was achieved for sodium acetate after 14 days, 89 % after 28 days. The Theoretical Oxygen Demand (ThOD) of coumatetralyl was calculated to be 2.35 mg O_2 per mg. The ThOD of sodium acetate (positive control) was calculated to be 0.78 mg O_2/mg. In the toxicity control coumatetralyl was found to be not inhibitory on microbial activity (the oxygen depletion in the toxicity control was always more than 75 % of the sum of the oxygen depletion of the positive control and the test substance (low)).
5.3 Conclusion	For an acceptable biodegradability test where: • Oxygen depletion in the inoculum blank did not exceed 1.5 mg dissolved oxygen/l after 28 days, • The residual concentration of oxygen in the test bottles did not fall below 0.5mg/l at any time, • The difference of duplicate values expressed as mg O_2/l was less than 20 % throughout the incubation period of 28 days, • The percentage degradation of the reference substance reached the level for ready biodegradability (ca. 60 %) by 14 days, Coumatetralyl was not readily biodegradable under the conditions in the closed bottle test.
5.3.1 Reliability	1
5.3.2 Deficiencies	No

	EVALUATION BY COMPETENT AUTHORITIES
SECTION A7.1.1.2.1 **Annex Point IIA7.6.1.1**	**BIODEGRADABILITY (READY)**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2004-08-16
Materials and Methods	The purity cannot be assessed. The information on water solubility (**see section 3.1.4**) is not in accordance with that in section **A3 (3.5)**.
Results and discussion	Adopted
Conclusion	Adopted
Reliability	1
Acceptability	Acceptable
Remarks	The deficiencies in the materials section are not considered to be decisive for the results.

Table A7.1.1.2.1-1: Test conditions

Criteria	Details
Composition of medium	Stock solutions of mineral components: A) 8.50 g KH_2PO_4, 21.75 g K_2HPO_4, 67.20 g Na_2HPO_4 x 12 H_2O, 0.50 g NH_4Cl dissolved in 1 l Milli-Q water, pH 7.4 ± 0.2 B) 22.50 g $MgSO_4$ x 7 H_2O dissolved in 1 l Milli-Q water C) 36.40 g $CaCl_2$ x $2H_2O$ dissolved in 1 l Milli-Q water D) 0.25 g $FeCl_3$ x 6 H_2O dissolved in 1 l Milli-Q water Preparation of mineral medium: 1 ml of solution (A) to (D) was mixed and made up to 1 l with Milli-RO water. Milli-Q and Milli-RO water: Tap-water purified by reverse osmosis (Milli-RO) and subsequently passed over activated carbon and ion-exchange cartridges (Milli-Q) (Millipore Corp., Bedford, Mass., USA); The concentration of dissolved oxygen was measured for control purposes. The mineral medium was left at test temperature to obtain a saturated solution at the start of the test.
Additional substrate	No
Test temperature	varied between 21 and 22.5 °C
pH	7.3 – 7.4
Aeration of dilution water	No
Suspended solids concentration	4 ml filtrate of the secondary effluent per litre of final volume were used for inoculation.
Other relevant criteria	The test bottles were excluded from light

Table A7.1.1.2.1-2: Time courses of degradation for different test media



SECTION A7.1.1.2.2 **BIODEGRADABILITY (INHERENT)**

Annex Point IIA7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	M.J.E. Desmares-Koopmans, 2001, Inherent Biodegradability: 'Zahn-Wellens / EMPA Test' with coumatetralyl, NOTOX B.V., s'-Hertogenbosch, the Netherlands, Project No. 311884 (unpublished), 2001-06-19, MO-03-003141	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active ingredient for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, Directive 67/548/EEC, method C.9 and OECD guideline No. 302 B	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Coumatetralyl	
3.1.1	Lot/Batch number	Batch No.: 0800	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	Not indicated by the sponsor (treated as 100 % pure)	X
3.1.4	Further relevant properties	Solubility in water (at 20 °C): 425 mg/l at pH 7; 100 – 200 g/l at pH 9; Stability in water: at least 96 h	X
3.1.5	Composition of Product		
3.1.6	TS inhibitory to micro-organisms	EC_{50} = 4210 mg/l (result of the respiration inhibition test with activated sludge; study number 283 A/91, 1991-07-12)	
3.1.7	Specific chemical analysis	No	

SECTION A7.1.1.2.2 BIODEGRADABILITY (INHERENT)

Annex Point IIA7.6.1.1

3.2	**Reference substance**	Yes, aniline
3.2.1	Initial concentration of reference substance	115.4 mg/l
3.3	**Testing procedure**	
3.3.1	Inoculum / test species	Test system: micro-organisms in activated sludge. The source of the test organisms was activated sludge freshly obtained from a municipal sewage treatment plant: `Waterschap de Maaskant`, `s-Hertogenbosch, the Netherlands. The sludge was coarsely sieved and washed with mineral medium. The batch of sludge was used within 6 hours of sampling and aerated until required. A small amount of the sludge was weighed and dried at ca. 105 °C. From this result the amount of suspended solids (ss) in the final test medium was calculated (2.6 g/l). 195 ml activated sludge were used for preparing the mixtures of test substance, blank control and reference substance (0.25 g/l dry matter in the final mixture).
3.3.2	Test system	Test vessels: 3 litre glass vessels <u>Preparation of test vessels:</u> Test substance mixture (1 vessel): 195 ml activated sludge (0.25 g/l dry matter in the final mixture). 200.9 mg coumatetralyl. This amount was added by adding 1566 ml of a stock solution prepared by dissolving 320.8 mg coumatetralyl in 2500 ml mineral medium. Made up to 2 l with mineral medium. DOC (t = 0 h) = 79.78 mg/l. The ratio of inoculum and coumatetralyl (as DOC) was 3.1 : 1. Blank control mixture (1 vessel): 500 ml mineral medium. 195 ml activated sludge (0.25 g/l dry matter in the final mixture). Made up to 2 l with mineral medium. Reference substance mixture (1 vessel): 500 ml mineral medium. 195 ml activated sludge (0.25 g/l dry matter in the final mixture). 230.8 mg aniline. This amount was added by adding 62 ml of a stock solution prepared by dissolving 372.2 mg aniline in 100 ml mineral medium. Made up to 2 l with mineral medium. DOC (t = 0 h) = 106.4 mg/l. The ratio of inoculum and aniline (as DOC) was 2.3 : 1.

		DOC analysis: DOC analysis was performed in triplicate using a DC-190 High Temperature Total Organic Carbon Analyzer (Rosemount Analytical Inc., Dohrmann[R] Division, USA). Analysis set-up: Autosampler; Calibration solution: potassium hydrogen phthalate solution, [C] = 100 mg/l; Injection volume: 100 µl
3.3.3	Test conditions	See table A7.1.1.2.2-1
3.3.4	Method of preparation of test solution	Pre-Test: The DOC concentration of a solution containing ca. 0.5 g/l coumatetralyl, prepared by adding 252.0 mg test substance to 500 ml mineral medium (composition see table A7.1.1.2.2-1), was measured to determine the concentration for the main study. After thoroughly mixing and ultrasonic dispersion, some drops 1 M NaOH (in total 13) were added to increase the pH (ca. 9) and thus the solubility of coumatetralyl in water. Thereafter thorough mixing was used again to accelerate dissolving and to ensure homogeneity. The resulting solution was still very slightly turbid. The DOC concentration of the resulting solution was determined. To avoid solubility problems in the main study, and thus addition of NaOH, a lower test concentration was selected for the main study. The concentration for the main study was based on the mean value (83 %) of the measured DOC concentration (88 %) and the calculated TOC concentration (78 %). Solution for the main test see point 3.3.2
3.3.5	Initial TS concentration	100.5 mg coumatetralyl/l
3.3.6	Duration of test	28 days
3.3.7	Analytical parameter	DOC removal
Sampling		Prior to sampling the volume of the vessels was checked for evaporation and if necessary Milli-RO water was added. The medium was mixed thoroughly. A sample was passed through a rough paper filter (S&S 604) and thereafter through a 0.45 µm filter (S&S FP 030/2). At least the first 5 ml were discarded. If not analysed on the day of sampling, samples were stored in a deep-freezer until analysis. Frequency of sampling: DOC analysis was performed at the start of the test (0 h) and 3

		h after the addition of the test substance in order to estimate any adsorption of coumatetralyl by the activated sludge. In addition samples were taken at day 1, 2, 5, 9, 12, 15, 18, 23 and 28. The sample volume was at least 10 ml. Samples taken at the start of the test (0 h) and 3 h after the addition of the test substance were analysed on the day of sampling. For all other sampling days samples were frozen. Frozen samples were defrosted at room temperature. DOC analysis of the frozen samples was performed twice. The pH and the oxygen concentration were measured at least twice a week. The test temperature was recorded daily in a vessel with water in the same room.
3.3.8	Intermediates/ degradation products	Not identified
3.3.9	Nitrate/nitrite measurement	No
3.3.10	Controls	Blank control (composition see point 3.3.2)
3.3.11	Statistics	The percentage biodegradation was calculated by subtracting the mean DOC at time t of the inoculum blank from that exhibited by the test substance at the same time. The corrected value was divided by the mean DOC at time t = 3 h of the inoculum blank subtracted from that exhibited by the test substance at time t = 3 h. This value was subtracted from 1 and subsequently multiplied with 100. A figure of more than 10 % degradation was considered as significant.
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.2	Graph	Degradation curve of the test substance is given in Table A7.1.1.2.2-3
4.1.2	Degradation	Degradation values see table A7.1.1.2.2-2
4.1.3	Other observations	None
4.1.4	Degradation of TS in abiotic control	No abiotic control
4.1.5	Degradation of reference substance	Degradation values see table A7.1.1.2.2-2. The degradation curve is given in Table A7.1.1.2.2-3
4.1.6	Intermediates/	n.a.

degradation products

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Coumatetralyl was tested for its inherent biodegradability using the `Zahn-Wellens /EMPA Test´ according to the Directive 67/548/EEC, method C.9 and OECD guideline No. 302 B. The degree of biodegradation was investigated by following the decrease of DOC.

The study shows no significant deviations from the Directive 67/548/EEC, method C.9 and OECD guideline No. 302 B.

5.2 Results and discussion

Pre-Test:
The Dissolved organic carbon (DOC) concentration of the solution containing 504.0 mg coumatetralyl/l was determined to be 442.6 mg/l (88 %).

The concentration for the main study was based on the mean value (83 %) of the measured DOC concentration (88 %) and the calculated Total Organic Carbon (TOC) concentration (78 %).

At the start of the main study the DOC of the test solution containing coumatetralyl (100.5 mg/l) was 79.78 mg/l and the DOC concentration of the reference solution containing aniline (115.4 mg/l) was 106.4 mg/l.

No significant DOC removal was observed in the first 3 hours of incubation. The degradation values calculated from the DOC measurements performed during the 28 days test period revealed no degradation of coumatetralyl.

The degradation values for the test and reference substance are given in table A7.1.1.2.2-2.

5.3 Conclusion

For an acceptable biodegradability test where the reference substance aniline showed a removal by at least 70 % within 14 days, no degradation of coumatetralyl was observed within the study duration of 28 days.

Thus, coumatetralyl was not inherently biodegradable under the conditions of the `Zahn-Wellens /EMPA Test´.

5.3.1 Reliability 1

5.3.2 Deficiencies No

	EVALUATION BY COMPETENT AUTHORITIES
SECTION A7.1.1.2.2 Annex Point IIA7.6.1.1	BIODEGRADABILITY (INHERENT)
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2004-08-16
Materials and Methods	The purity cannot be assessed. The information on water solubility (**see section 3.1.4**) is not in accordance with that in section **A3 (3.5)**.
Results and discussion	Adopted
Conclusion	Adopted
Reliability	1
Acceptability	Acceptable
Remarks	The deficiencies in the materials section are not considered to be decisive for the results.

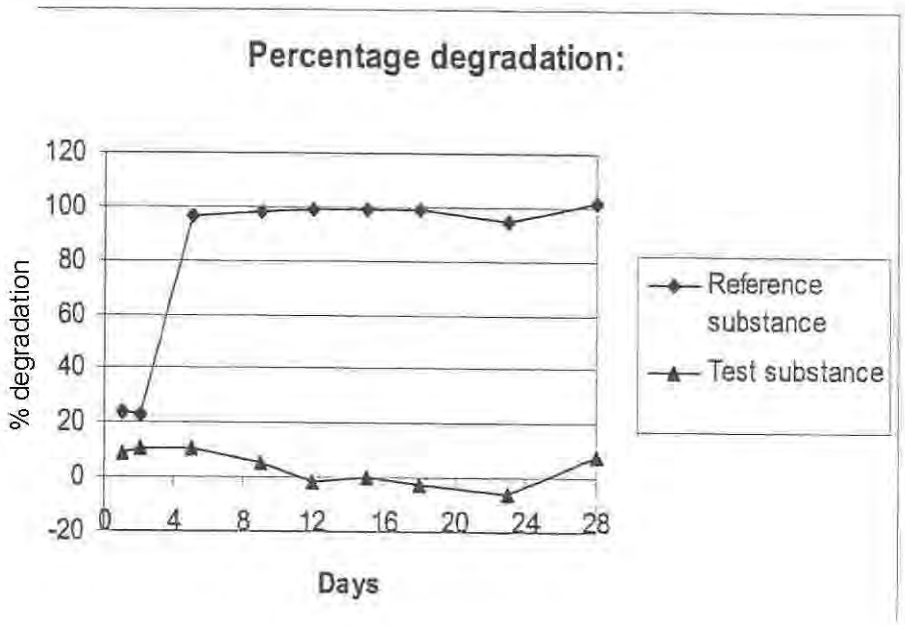
Table A7.1.1.2.2-1: Test conditions

Criteria	Details
Composition of medium	Stock solutions of mineral components: E) 8.50 g KH_2PO_4, 21.75 g K_2HPO_4, 67.20 g Na_2HPO_4 x 12 H_2O, 0.50 g NH_4Cl dissolved in 1 l Milli-Q water, pH 7.4 ± 0.2 F) 22.50 g $MgSO_4$ x 7 H_2O dissolved in 1 l Milli-Q water G) 36.40 g $CaCl_2$ x $2H_2O$ dissolved in 1 l Milli-Q water H) 0.25 g $FeCl_3$ x 6 H_2O dissolved in 1 l Milli-Q water 1 l mineral medium contains: 10 ml of solution (A), 1 ml of solutions (B) to (D) and Milli-RO water. Milli-Q and Milli-RO water: Tap-water purified by reverse osmosis (Milli-RO) and subsequently passed over activated carbon and ion-exchange cartridges (Milli-Q) (Millipore Corp., Bedford, Mass., USA).
Additional substrate	No
Test temperature	varied between 21 and 22.5 °C
pH	7.7 (start, day 0); 7.1 (end, day 28); 7.0 – 7.7 (range during the test)
Oxygen concentration [mg/l]	8.4 (start, day 0); 8.6 (end, day 28); 7.0 – 8.7 (range during the test)
Aeration of dilution water	Yes, continuously aerated with humidified air, in order to ensure that the oxygen concentration did not fall below 2 mg/l.
Suspended solids concentration	195 ml activated sludge were used for preparing the mixtures of test substance, blank control and reference substance (0.25 g/l dry matter in the final mixture).
Other relevant criteria	Test was conducted in the dark, except during sampling and checking of the test system. The vessels were continuously stirred, in such a way that the sludge did not settle.

Table A7.1.1.2.2-2 Percentage degradation

Test vessel	% degradation after x days								
	1	2	5	9	12	15	18	23	28
Reference substance	23	22	96	99	99	99	99	95	102
Test substance	9	10	10	6	-2	0	-3	-6	8

Table A7.1.1.2.2-3 : Biodegradation of COUMATETRALYL in the 'Zahn-Wellens / EMPA Test 1 :



SECTION A7.1.3 **ADSORPTION / DESCRIPTION SCREENING TEST**

Annex Point IIA7.7

			Official use only
		1 REFERENCE	
1.1	**Reference**	P.J. Slangen, 2002, Development and Validation of an Analytical Method for coumatetralyl and Soil Adsorption/Desorption of coumatetralyl on five Soils (Screening Test), NOTOX B.V., s`Hertogenbosch, the Netherlands, Project No. 333473 (unpublished), 2002-03-29, MO-03-003147	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD guideline No. 106	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Coumatetralyl	
3.1.1	Lot/Batch number	Batch No.: 0800 (used for analytical method development) Batch No.: 920804ELB01 (used for analytical method validation and the adsorption/desorption test)	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	99.96 % (used for analytical method development) 99.9 % (used for analytical method validation and the adsorption/desorption test)	
3.1.4	Further relevant properties	Stability in water: at least 96 hours	
3.1.5	Method of analysis	A developed and validated HPLC method was used for analysis of adsorption/desorption samples. Analytical conditions: Column: LiChrospher 100 RP-18, 125 x 4 mm, d_p = 5 µm (Merck); Mobile phase: 75/25 (v/v) methanol/Milli-Q water (see point 3.5.2) pH 2.3 (with ortho-phosphoric acid); Flow: 1.0 ml/min;	

SECTION A7.1.3 Annex Point IIA7.7	ADSORPTION / DESCRIPTION SCREENING TEST
	Detection wavelength: 307 nm; Injection volume 100 µl. Analysis of samples: Samples were removed from the freezer and slowly thawed at room temperature upon the day of analysis. For calibration, ten solutions were prepared in 0.01 M aqueous $CaCl_2$ solution with test substance concentrations in the range of 0.00614 – 2.96 mg/l. Analysis was performed as single measurement of each replicate.
3.2 Degradation products	No
3.2.1 Method of analysis for degradation products	--
3.3 Reference substance	No
3.3.1 Method of analysis for reference substance	--
3.4 Soil types	See table A7.1.3-1
3.5 Testing procedure	
3.5.1 Test system	50 ml polypropylene centrifuge tubes were used as test system. Other equipment used during the test: shaker, centrifuge, vortex mixer
3.5.2 Test solution and test conditions	A stock solution containing 1836 mg/l coumatetralyl in methanol was prepared. A spike solution (Spike 1) was prepared by diluting 272 µl of the stock solution with 0.01 M aqueous $CaCl_2$ solution (2.94 g of $CaCl_2$ x 2 H_2O in 2 l Milli-Q water) to 50 ml. Spike 1 thus had a nominal test substance concentration of 9.99 mg/l and contained 0.54 % of organic co-solvent (methanol). The pH of Spike 1 was 5.46. (Milli-Q water: tap water purified by reversed osmosis and subsequently passed over activated carbon and ion-exchange cartridges -Millipore Corp., Bedford, USA). All experiments were performed at a soil : solution ratio of 1:2 and were carried out at 20 °C ± 1°C.

SECTION A7.1.3 **ADSORPTION / DESCRIPTION SCREENING TEST**

Annex Point IIA7.7

3.6 Test performance	
3.6.1 Preliminary test	Not performed
3.6.2 Screening test: Adsorption	According to "OECD 106": Yes
3.6.3 Screening test: Desorption	Prior to the start of the adsorption/desorption experiment, the appropriate amount of soil was weighed out in 50 ml polypropylene centrifuge tubes and a known amount of 22.5 ml 0.01 M aqueous $CaCl_2$ solution was added. The centrifuge tubes were sealed and placed on a shaker for 4 days for pre-equilibration. Soils were equilibrated at 20 °C ± 1 °C. The amount of soil and the initial concentration of the test substance are given in table A7.1.3-2. Both the adsorption and desorption were conducted with duplicate samples for each soil. For each soil, a blank was included using approximately 25 ml of 0.01 M aqueous $CaCl_2$ solution only and no test chemical. Two controls were also included in the test, each containing known amounts of approximately 22.5 ml of 0.01 M aqueous $CaCl_2$ solution and approximately 2.5 ml of Spike 1 (thus, approximately 1 µg/ml coumatetralyl) but no soil to evaluate adsorption to the container walls. The adsorption/desorption kinetics experiment was initiated by adding an exactly known volume of ca. 2.5 ml of Spike 1 to the pre-equilibrated soil slurries. Hence, the initial concentration of coumatetralyl in the soil solution was approximately 1 µg/ml (exact values see table A7.1.3-2) and in each soil the amount of organic co-solvent (methanol) was < 0.06 %. The centrifuge tubes were sealed and placed in a shaker. At the sampling times (adsorption: 4, 6.5, 22.75 and 48 hours), the soil slurries were centrifuged at 3000 g for 5 minutes at 20 °C and 150 µl of the supernatant was transferred to an amber vial with insert. These samples were stored in the freezer until analysis. The controls and blanks were also sampled. After sampling, the soil slurries were mixed well with a vortex mixer and placed back on the shaker until the next sampling event. At the end of the adsorption phase, the supernatant was removed and replaced by an approximately equal, known volume of fresh 0.01 M aqueous $CaCl_2$ solution. The centrifuge tubes were sealed and placed on the shaker.

	At the desorption sampling times (4, 6.5, 24 and 30.5 hours) samples were taken in the same way as the samples of the adsorption phase and stored also in the freezer until analysis. Fresh 0.01 M aqueous $CaCl_2$ solution (150 µl) was added to the centrifuge tubes and the soil slurries were well mixed with a vortex mixer and placed back on the shaker until the next sampling event.
3.6.4 HPLC-method	OECD guideline; proposal for a new guideline 121: not performed
3.6.5 Other test	Not performed
	4 RESULTS
4.1 Preliminary test	Not performed
4.2 Screening test: Adsorption	The kinetics of adsorption and desorption showed that adsorption equilibrium and desorption equilibrium were reached within 24 hours in the soil types Speyer 2.1, Cranfield 164 and Cranfield 243, but not in Cranfield 115 and Speyer 6S.
4.3 Screening test: Desorption	Adsorption and desorption kinetics see table A7.1.3-3 and table A7.1.3-4. The amount of test substance adsorbed to soil after 48 hours ranged from approximately 50 % (Cranfield 164 silt loam) to 80 % (Cranfield 243 sandy loam). The amount of material desorbed from the soil after 30.5 hours ranged from approximately 1 % (Cranfield 115 clay loam) to approximately 23 % (Cranfield 164 silt loam and Speyer 2.1 sand).
4.4 Calculations	All calculations were based on formulas given in the OECD guideline. Adsorption and desorption coefficients were determined.
4.4.1 Kd, Kdes	Adsorption and desorption parameters see table A7.1.3-5
4.4.2 Koc, Kom	Adsorption and desorption parameters see table A7.1.3-5
4.5 Degradation product(s)	No degradation products tested
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	The adsorption/desorption behaviour of coumatetralyl on soil was studied in five soils which represent major agricultural areas in Europe and North America, using the batch equilibrium method according to the OECD guideline No. 106. The experimental procedure of the test measures the decrease in concentration when aqueous solutions of a chemical are in contact under laboratory conditions with five different soils.

SECTION A7.1.3 Annex Point IIA7.7	ADSORPTION / DESCRIPTION SCREENING TEST
	Adsorption and desorption kinetics were determined at an initial test concentration of approximately 1 µg/ml. The adsorption/desorption experiments were carried out at 20 °C ± 1 °C.
5.2 Results and discussion	The HPLC chromatograms of the adsorption/desorption samples showed no additional peaks that could indicate the presence of compounds other than coumatetralyl. Hence, it was concluded that coumatetralyl was stable during the whole experimental period. The (mean) concentration of coumatetralyl in the control samples ranged from 95.8 % to 97.4 % of the nominal concentration during the adsorption stage of the test. Hence, adsorption of coumatetralyl on the container walls was low, but was nevertheless corrected for in the calculations. The correlation coefficient of the calibration curve used for the determination of the test substance concentration in the adsorption/desorption samples was determined to be 0.9999. The agreement between results for the two replicates of each soil was good. Water-extractable soil matter did not interfere with the analysis. The kinetics of adsorption and desorption showed that adsorption equilibrium and desorption equilibrium were reached within 24 hours in the soil types Speyer 2.1, Cranfield 164 and Cranfield 243, but not in Cranfield 115 and Speyer 6S. Adsorption and desorption kinetics see table A7.1.3-3 and table A7.1.3-4. The amount of test substance adsorbed to soil after 48 hours ranged from approximately 50 % (Cranfield 164 silt loam) to 80 % (Cranfield 243 sandy loam). The amount of material desorbed from the soil after 30.5 hours ranged from approximately 1 % (Cranfield 115 clay loam) to approximately 23 % (Cranfield 164 silt loam and Speyer 2.1 sand). The calculated adsorption and desorption parameters are given in table A7.1.3-5. Mean K_{om} values were determined to be 234 cm^3/g (Speyer 2.1 sand), 107 cm^3/g (Cranfield 115 clay loam), 41 cm^3/g (Cranfield 164 silt loam), 426 cm^3/g (Cranfield 243 sandy loam) and 67 cm^3/g (Speyer 6S clay). Adsorption-desorption hysteresis was observed in all five soils as indicated by the higher desorption equilibrium constants compared to the adsorption equilibrium constants.

SECTION A7.1.3	ADSORPTION / DESCRIPTION SCREENING TEST	
Annex Point IIA7.7		
5.2.1 Adsorbed a.s. [%]	The amount of test substance adsorbed to soil after 48 hours ranged from approximately 50 % (Cranfield 164 silt loam) to 80 % (Cranfield 243 sandy loam).	
5.2.2 Kd	See table A7.1.3-5	
5.2.3 Kdes	See table A7.1.3-5	
5.2.4 Koc	See table A7.1.3-5	
5.2.5 Kd/Kdes	See table A7.1.3-5	
5.2.6 Degradation products (% of a.s.)	No degradation products tested	
5.3 Conclusion	Based on the K_{om} values and the mobility classification scheme according to Mensink, coumatetralyl can be considered to be immobile in Speyer 2.1 sand, Cranfield 115 clay loam and Cranfield 243 sandy loam. It is considered to be slightly mobile in Cranfield 164 silt loam and Speyer 6S clay. (Mensink B. et al., Manual for summarising and evaluating the environmental aspects of pesticides, National Institute of Public Health and Environmental Protection, the Netherlands, Report No. 679101022 (1995)).	X
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

	EVALUATION BY COMPETENT AUTHORITIES
SECTION A7.1.3 **Annex Point IIA7.7**	**ADSORPTION / DESORPTION SCREENING TEST**
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	2004.08.16
Materials and Methods	Acceptable
Results and discussion	Adopted
Conclusion	Adopted
Reliability	1
Acceptability	Acceptable
Remarks	In the conclusion (**section 5.3**), the mobility is assessed as follows: "Based on the K_{om} values and the mobility classification scheme according to Mensink, coumatetralyl can be considered to be immobile in Speyer 2.1 sand, Cranfield 115 clay loam and Cranfield 243 sandy loam. It is considered to be slightly mobile in Cranfield 164 silt loam and Speyer 6S clay." **Comment:** Thus, the substance is less mobile in sandy soil than in clay and silt loam soils. The cause of this assessment is that the measured K_d was quite uniform in the different soils. However, as the organic matter varied between soils, e.g. it was low in the sandy soil (om = 0.97%) and high in the silt loam (om = 5.2%), the calculated K_{om} varied. E.g. in Speyer 2.1 (sandy) and Cranfield 164 (silt loam), the K_d-values were 2.27 and 2.14, respectively. Thereby, the K_{om}-values were calculated to be 2.34 and 41 for the two soils, respectively. Therefore, the classification with respect to mobility in soil, based on K_{om} is not appropriate in this case.

Table A7.1.3-1: Classification and physico-chemical properties of soils used as adsorbents

	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
Name	Speyer 2.1	Cranfield 115	Cranfield 164	Cranfield 243	Speyer 6S
Soil order					
Soil series					
Classification	Sand	Clay loam	Silt loam	Sandy loam	Clay
Location	Teufelskanzel, Rheinzabern, Rheinland-Pfalz, Germany	Chapel Farm, Netherton, Evesham, Worcester, UK	Farditch Farm, Chelmorton, Buxton, Derbyshire, UK	RASE, Stoneleigh, Warwickshire, United Kingdom	"In der unteren Hohnert", Nr. 3412, Siebeldingen, Rheinland-Pfalz, Germany
Horizon [cm]	20	0 – 10	15 – 22	5 - 15	0 - 20
Sand [%]	90.2	43.74	15.95	71.93	23.2
Silt [%]	8.2	23.50	72.91	15.97	34.5
Clay [%]	1.7	32.76	11.14	12.10	42.3
Organic carbon [%]	0.56	1.7	3.0	1.1	2.3
Organic matter [%]	0.97	2.9	5.2	1.9	4.0
Carbonate as $CaCO_3$					
insoluble carbonates [%]					
pH (H_2O)	Not determined	7.9	7.1	5.4	Not determined
pH	6.0 ($CaCl_2$)	7.4 (KCl)	6.5 (KCl)	4.3 (KCl)	6.7 ($CaCl_2$)
Cation exchange capacity (MEQ/100 g)	4	19.6	18.1	3.3	18
Water holding capacity [%]	29	55.3	72.8	51.1	45
Moisture at 1/3 bar [%]		30.4	41.2	22.7	
Extractable cations (MEQ/100 g)					
Ca					
Mg					
Na					
K					

	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
Name	Speyer 2.1	Cranfield 115	Cranfield 164	Cranfield 243	Speyer 6S
H					
	Soil 1	**Soil 2**	**Soil 3**	**Soil 4**	**Soil 5**
Special chemical/mineralogical features					
Clay fraction mineralogy					

Table A7.1.3-2 Experimental details of adsorption/desorption test at a soil : solution ratio of 1:2

Soil	Replicate	Dry weight of soil [g]	Initial test substance concentration [µg/ml]
Speyer 2.1	A	12.5207	0.9866
	B	12.5183	0.9924
Cranfield 115	A	12.0743	0.9741
	B	12.0774	0.9716
Cranfield 164	A	12.0051	0.9650
	B	12.0140	0.9726
Cranfield 243	A	12.4510	0.9722
	B	12.4029	0.9821
Speyer 6S	A	11.9601	0.9676
	B	11.9281	0.9596

Table A7.1.3-3 Adsorption kinetics

	Soil 1 Speyer 2.1		Soil 2 Cranfield 115		Soil 3 Cranfield 164	
	A	B	A	B	A	B
Quantity adsorbed after 48 hours [µg]	12.25	12.97	14.19	14.11	11.69	12.12
Test material adsorbed after 48 hours [%]	51.75	54.45	59.60	59.39	49.27	50.95

Table A7.1.3-3 Adsorption kinetics (continued)

	Soil 4 Cranfield 243		Soil 5 Speyer 6S	
	A	B	A	B
Adsorbed test substance at 48 hours [µg]	18.83	19.01	13.22	13.05
Adsorbed test substance at 48 hours [%]	79.71	80.13	55.56	55.18

Table A7.1.3-4 Desorption kinetics

Soil	Test substance desorbed at 30.5 hours [%]		
	A	B	Mean
Speyer 2.1	23.76	21.55	22.65
Cranfield 115	1.02	0.73	0.88
Cranfield 164	22.82	22.92	22.87
Cranfield 243	7.14	6.55	6.85
Speyer 6S	4.93	4.83	4.88

Table A7.1.3-5 Adsorption and desorption parameters

Soil type	Replicates	K_d [cm^3/g](*)	K_{om} [cm^3/g](*)	K_{oc} [cm^3/g](*)	K_{des} [cm^3/g](*)	K_d / K_{des}
Speyer 2.1	A, B	2.27	234	403	6.67	0.34
Cranfield 115	A, B	3.10	107	185	241	0.013
Cranfield 164	A, B	2.14	41	71	7.02	0.30
Cranfield 243	A, B	8.10	426	735	27	0.30
Speyer 6S	A, B	2.67	67	115	41	0.065

(*) mean values for the given replicates; calculations are based on the results of the final sampling event for both adsorption and desorption.

K_d = adsorption coefficient
K_{om} = organic matter normalised adsorption coefficient
K_{oc} = organic carbon normalised adsorption coefficient
K_{des} = desorption coefficient

Section A7.2.1 and A7.2.2.4

Annex Point IIIA VII.4, XII 1.1

Aerobic and anaerobic degradation in soil

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1 REFERENCE

1.4	**Reference**	Scholtz, K, 1987, Metabolism of [^{14}C] Coumatetralyl ® (Racumin) in soil under aerobic and anaerobic conditions, Bayer AG Institute für Metabolismusforschung, PF-Nr.: 2832, Monheim, June 5, 1987.
1.5	**Data protection**	Yes
1.5.0	Data owner	Bayer Chemicals AG
1.5.1	Companies with letter of access	Bayer Chemicals AG
1.5.2	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**	No
2.2	**GLP**	No
2.3	**Deviations**	Not relevant

3 MATERIALS AND METHODS

3.1	**Test material**	Radiolabelled Coumatetralyl ([benzo-UL-^{14}C-pyran-2-one] (Specific radioactivity 1306,1 kBq/mg) and unlabelled Coumatetralyl
3.1.0	Lot/Batch number	
3.1.1	Specification	CAS No. 5836-29-3
3.1.2	Purity	Radiochemical purity >99% (radiolabelled material) Chemical purity >99% (unlabelled material)
3.1.3	Further relevant properties	None
3.1.4	Method of analysis	The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). $^{14}CO_2$ was trapped in soda lime. The soda lime was dissolved in

Section A7.2.1 and A7.2.2.4
Annex Point IIIA VII.4, XII 1.1
Aerobic and anaerobic degradation in soil

hydrochloric acid, and $^{14}CO_2$ was subsequently liberated into a scintillation cocktail (ß-phenylethylamine/Butyl-PBD cocktail) and analysed by LSC.

Volatile compounds other than $^{14}CO_2$ were trapped in an oil coated quartz wool plug, and extracted with ethylacetate. A subsample of the extract was used for LSC.

Soil samples were extracted with methanol-H_2O, methanol, ethylacetate and shaken with dichloromethane. The radioactivity was determined by LSC. The organic phase in the extracts was analysed by thin-layer chromatography (TLC).

Solid soil samples remaining after extraction were incinerated in an intertechnique Oxymat oxidiser.

3.2 Reference substance

No reference substance was used

3.2.0 Method of analysis for reference substance

Not relevant

3.3 Soil types

BBA (Federal German Biological Research Agency) standard soil 2.2. See table A7_2_1-1.

3.4 Testing procedure

3.4.0 Test system

The metabolism of coumatetralyl was examined in three experiments under: 1) aerobic 2) aerobic/anaerobic conditions and 3) anaerobic conditions. Radiolabelled and unlabelled coumatetralyl were applied to the soil in a concentration corresponding to a final concentration of 0.8 mg active ingredient per 100 g soil dry weight. In all experiments, the active ingredient was applied to the total amount of soil, which was then divided into individual batches of 100 g dry weight after thorough mixing. The tests were conducted in 300 ml conical glass flasks.

Experiment 1, aerobic conditions: The test vessels were fitted with a device for trapping volatile compounds. The moisture content in the test vessels was adjusted to 40% of the maximum water holding capacity. Three replicates were used. At day 7, 42 and 81, one replicate was processed for analysis. Before removal of the soil, any volatile substances present in the headspace were driven

into the trapping device by introduction of air.

Experiment 2, aerobic/anaerobic conditions: The test vessels were fitted with a device for trapping volatile compounds as in the aerobic experiment. After 30 days of incubation, the vessels were set up for anaerobic conditions by flooding the soil with 80 ml of distilled water and the vessels were purged with nitrogen and sealed. Two replicates were used. The replicates were sampled on day 30/60 and 30/92, respectively. Before removal of the soil, any volatile substances present in the headspace were driven into a series of wash bottles by purging with nitrogen.

Experiment 3, anaerobic conditions: The test vessels were maintained under anaerobic conditions for the entire incubation period of 60 days. The test vessels were kept anaerobic by flooding the soil with 80 ml of distilled water, and the vessels were purged with nitrogen and sealed. Two replicates were used. The replicates were processed for analysis at day 30 and 60, respectively. Before removal of the soil, any volatile substances present in the headspace were driven into a series of wash bottles by purging with nitrogen.

A separate batch of soil was set up for analysis of metabolite production. 100 mg of active ingredient dissolved in methanol was added to 1 kg soil (dry weight) and stored under conditions corresponding to the aerobic experiment. The metabolite production was analysed on day 43 and 119.

3.4.1 Test solution and Test conditions

Both radiolabelled and unlabelled coumatetralyl were dissolved in methanol.

The test vessels were incubated in darkness at 22° C ± 3° C and a relative humidity of 60-80%.

4 RESULTS

4.1 Aerobic soil metabolism

See table A7_2_1-2

Coumatetralyl was mineralised in soil under aerobic conditions only. In the strictly aerobic conditions, the amount of $^{14}CO_2$ formed after a 81 day test period corresponded to 51% of the initially applied radioactivity. A large number of radioactive metabolites were found in the fraction extracted from the soil, each in concentrations below 2% of the initially applied radioactivity. Unchanged parent compound accounted for 4% out of the 11% radioactivity extracted after 81 days. A rather large fraction of the

radioactivity (34%) remained in the soil after extraction.

In experiment 2, coumatetralyl was likewise mineralised to $^{14}CO_2$ during the aerobic incubation period (41-43% mineralisation after 30 days). After 30 days of incubation, 18-19% of the radioactivity was extracted from the soil, unchanged parent compound accounting for the majority (13-14%) hereof. Different metabolites were found in concentrations ≤ 1%.

4.2 Anaerobic soil metabolism

See table A7_2_1-2

Under anaerobic conditions in experiment 2 (from day 30) and 3, no mineralisation of coumatetralyl was observed as less than 1% was recovered as $^{14}CO_2$ during the anaerobic incubation periods. In experiment 2, the mineralisation stopped when anaerobic conditions were introduced at day 30. In experiment 3, the majority of the radioactivity was recovered as unchanged parent compound, 68-70% was extracted from the soil, whereas 30-32% was recovered in the surface water. No metabolites were identified.

The amount of active ingredient in the surface water in the flooded test systems depended on the timing of the flooding of the soil. When the soil was flooded 30 days after initiation of the test, only 2% of the initial added radioactivity was recovered in the surface water. In experiment 3, which was flooded at the initiation of the test, 30-32% of the initially added radioactivity was recovered in the surface water.

5 SUMMARY AND CONCLUSION

5.1 Materials and methods

The degradation of radiolabelled coumatetralyl was examined in soil under 1) aerobic conditions, 2) aerobic/anaerobic conditions and 3) anaerobic conditions. Both radiolabelled and unlabelled coumatetralyl was applied to the soil, corresponding to a final concentration of 0.8 mg/kg.

In experiment 1, the test vessels were incubated for 81 days with measurements of the radioactivity and metabolites at day 7, 42 and 81. In experiment 2, measurements were made on day 30 and 60 or day 30 and 92, the conditions switching from aerobic to anaerobic on day 30. In experiment 3, measurements were made on day 30 and 60. The degradation was quantified as percent $^{14}CO_2$ of the initial added radioactivity. The radioactivity remaining in the soil was quantified by LSC upon extraction and incineration. Metabolites were analysed by thin-layer chromatography (TLC).

Section A7.2.1 and A7.2.2.4 **Aerobic and anaerobic degradation in soil**

Annex Point IIIA VII.4, XII 1.1

5.2 Results and discussion

5.2.0	Degradation	Coumatetralyl was mineralised under aerobic conditions in soil as 51% of the initial added radioactivity was minealised after 81 days. No mineralisation occurred under anaerobic conditions.
5.2.1	Degradation products	A large number (≥10) of metabolites were found in the extracted soil from the aerobic test systems, all in low concentrations (≤ 2%) and less than 7% in total after 81 days. Four metabolites were selected for further analysis (MI-MIV). Structures were suggested for metabolite I-III while metabolite MIV was identified as salicylic acid. A degradation pathway was discussed, and it was suggested that coumatetralyl is oxidised leading to the formation of salicylic acid. See figure A7_2_1-2
5.2.2	Unextracted radioactivity	The proportion of unextracted radioactivity, i.e. the bound residues, stabilised at a level of 34% under aerobic conditions. Due to the relatively few measurements, the study gives no clear indication of the proportion of bound residues in relation to time.

5.3 Conclusion Coumatetralyl is mineralised under aerobic conditions in soil. Hovewer, 34% of the activity remains unextracted as bound residues in soil after 81days of incubation. Under anaerobic conditions no mineralisation occurred and the majority of the radioactivity initially added was recovered either in soil extracts (68-70%) or in the surface water covering the soil (30-32%).

5.3.0	Reliability	2
5.3.1	Deficiencies	Test material not identified by batch or lot no. Low number of replicates used. Some reporting deficiencies compared to the large data material. No reference substance was used.

Section A7.2.1 Aerobic degradation in soil

Annex Point IIIA VII.4, XII 1.1

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*14/2 05*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	*2*
Acceptability	*Rapporteur has made this study summary; therefore no comments*
Remarks	

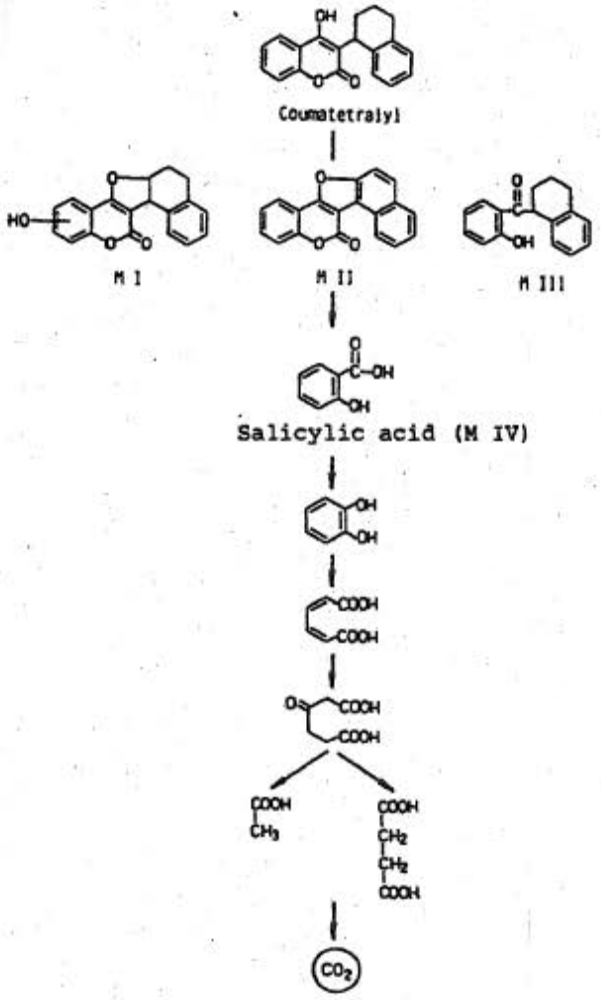
Table A7_2_1-1: Characteristics of the soils used

	Soil 2.2 Standard soil of the Federal Biological Institute
Organically bound carbon	2.6%
Elutriable fraction (<0.02 mm)	14.5%
pH value	6.0
Max. water holding capacity	27.5% 38 g H_2O/100g soil
Biomass at the start of the test in microbial C/kg dry soil	103

Table A7_2_1-2 Metabolism of coumatetralyl in soil under aerobic and anaerobic conditions. Results are presented as % of the initially applied radioactivity.

Experiment	1 Aerobic			2 Aerobic/anaerobic		3 Anaerobic	
Day	**7**	**43**	**81**	**30/30**	**30/62**	**30**	**60**
1.Soil extracted	65	17	11	19	18	70	68
Active ingredient	*57*	*6*	*4*	*14*	*13*	*70*	*68*
Metabolite I	*2*	*<1*	*<1*	*<1*	*<1*	-	-
Metabolite II	*<1*	*2*	*2*	*1*	*1*	-	-
Metabolite III	*<1*	*2*	*2*	*<1*	*<1*	-	-
Metabolite IV (salicylic acid)	*2.0*	*0.6*	*0.1*	*<1*	*<1*	*<1*	*<1*
Not identified	*4*	*6*	*3*	*4*	*4*	-	-
2. Soil after extraction	14	34	34	34	32	4	5
3. Surface water	-	-	-	2	2	30	32
Active ingredient	-	-	-	*2*	*2*	*30*	*32*
Not identified	-	-	-	*<1*	*<1*	*<1*	*<1*
4. $^{14}CO_2$							
Aerobic	11	41	51	43	41	-	-
Anaerobic	-	-	-	<1	<1	<1	<1
5. Volatile organic compounds							
Aerobic	<0.01	<0.01	<0.01	<0.01	<0.01	-	-
Anaerobic	-	-	-	<0.01	<0.01	<0.01	<0.01
Total, ∑ 1. – 5. in %	90	92	96	98	93	104	105

Figure A7_2_1-1 Suggested structures of metabolites MI-III and degradation pathway of coumatetralyl under aerobic conditions.



Section A7.2.1 Aerobic degradation in soil

Annex Point IIIA VII.4, XII 1.1

			Official use only
		6 REFERENCE	
6.1	**Reference**	Scholtz, K, 1987, Degradation of [^{14}C] Coumatetralyl ® (Racumin) in Soils under Aerobic Conditions, [^{14}C]CO_2-Study, Bayer AG Institute for Metabolism Research, PF-No.: 2741, Monheim, February 4, 1987.	
6.2	**Data protection**	Yes	
6.2.0	Data owner	Bayer Chemicals AG	
6.2.1	Companies with letter of access	Bayer Chemicals AG	
6.2.2	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	
		7 GUIDELINES AND QUALITY ASSURANCE	
7.1	**Guideline study**	No	
7.2	**GLP**	No	
7.3	**Deviations**	Not relevant	
		8 MATERIALS AND METHODS	
8.1	**Test material**	Radiolabelled Coumatetralyl ([benzo-UL-^{14}C-pyran-2-one] (Specific radioactivity 1306,1 kBq/mg) and unlabelled Coumatetralyl	
8.1.0	Lot/Batch number		
8.1.1	Specification	CAS No. 5836-29-3	
8.1.2	Purity	Radiochemical purity >99% (radiolabelled material) Chemical purity >99% (unlabelled material)	
8.1.3	Further relevant properties	None	
8.1.4	Method of analysis	The radioactivity in liquid samples was determined by liquid scintillation counting (LSC). $^{14}CO_2$ was trapped in soda lime. The soda lime was dissolved in hydrochloric acid, and $^{14}CO_2$ was subsequently liberated into a scintillation cocktail (ß-phenylethylamine/Butyl-PBD cocktail) and	

Section A7.2.1 **Aerobic degradation in soil**

Annex Point IIIA VII.4, XII 1.1

analysed by LSC.

Volatile compounds other than $^{14}CO_2$ were trapped in an oil coated quartz wool plug, and extracted with ethylacetate. A subsample of the extract was used for LSC.

Soil samples were extracted with methanol-H_2O, methanol, ethylacetate. The concentrated methanol-H_2O extract was partitionned with dichloro-methane and the dichloromethane was combined with the other organic extracts. The radioactivity was determined by LSC. The combined organic extracts were analysed by thin-layer chromatography (TLC).

Soil samples were extracted with methanol-H_2O, methanol, ethylacetate and shaken with dichloromethane The radioactivity was determined by LSC. The organic phase in the extracts was analysed by thin-layer chromatography (TLC).

Solid soil samples remaining after extraction were incinerated in an Oxymat (Intertechnique).

8.2 Reference substance No reference substance was used

8.2.0 Method of analysis for reference substance Not relevant

8.3 Soil types Two soil types were used, a standard soil from Federal Biological Institute and soil from Höfchen Experimental Farm, new field. See table A7_2_1-1

8.4 Testing procedure The testing procedure is described by Anderson, J.P.E.(1983): Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. Agronomy Monograph No. 9 (2nd Edition), 831-871

8.4.0 Test system Radiolabelled and unlabelled coumatetralyl were applied to the two soils in a concentration corresponding to 1 mg active ingredient per 100 g soil. Three experimental set ups were applied using A) standard soil (15 replicates), B) Höfchen soil (15 replicates) and C) sterilised standard soil (6 replicates). In experiments A and B, the active ingredient was applied to the total amount of soil, which was then divided into individual batches of 100 g dry weight after thorough mixing. In experiment C the active ingredient was applied directly to each individual test batch. 250 ml. Erlenmeyer flasks with devices for trapping volatile

Section A7.2.1 **Aerobic degradation in soil**

Annex Point IIIA VII.4, XII 1.1

compounds were used as test vessels. In experiments A and B, three replicates were used for determination of the microbial biomass at day 0, after one month and after 12 months. At day 0, 30, 42, 90, 180 and 365, two replicates were processed for measurement of ^{14}C. In experiment C, two replicates were used for determination of the microbial biomass at day 30 and 365. One replicate was processed at day 30, 90, 180 and 365. Before removal of the soil for analysis, any volatile substances present in the headspace were driven into the trapping device by introduction of air.

8.4.1 Test solution and Test conditions

Both radiolabelled and unlabelled coumatetralyl were dissolved in methanol.

The moisture content in the test vessels was adjusted to 40% of the maximum water holding capacity. The test vessels were incubated in darkness under aerobic conditions at 22° C ± 3° C and a relative humidity of 60-80%.

9 RESULTS

9.1 Aerobic soil metabolism

See table A7_2_1-2 to A7_2_1-4

The results showed that coumatetralyl was mineralised in both soils. After one month, the amount of $^{14}CO_2$ formed corresponded to 38% and 44% of the original applied radioactivity in the standard soil and Höfchen soil, respectively. After six months, 51% and 52% of the initial added radioactivity was recovered as $^{14}CO_2$ in the two soils.

The extraction of radioactivity from the two soils showed that only a minor part of the radioactivity was identified as parent compound after six months (2-4% if the initial added radioactivity). Different metabolites were identified in the extracts, accounting for 3-4%of the initial added radioactivity (day 180), the number of metabolites increasing with time. Measurements of the microbial biomass showed that the soils remained active during the entire incubation period.

The experiment with sterile soil showed that no mineralisation occurred as long as the soil was kept sterile. Towards the end of the experiment, the soil was contaminated with microorganisms, and 3% of the radioactivity was recovered as $^{14}CO_2$ after one year.

Section A7.2.1 **Aerobic degradation in soil**

Annex Point IIIA VII.4, XII 1.1

10 SUMMARY AND CONCLUSION

10.1 Materials and methods

The degradation of radiolabelled coumatetralyl was examined in two soils under aerobic conditions. Both radiolabelled and unlabelled coumatetralyl was applied to the soil, corresponding to a final concentration of 10 mg/kg soil dry weight.

The incubation period was one year, and measurements of the radioactivity were conducted at day 0, 30, 42, 90, 180 and 365. The degradation was quantified as percent $^{14}CO_2$ of the initial added radioactivity. The radioactivity remaining in the soil was quantified by LSC upon extraction and incineration.

10.2 Results and discussion

10.2.0 Degradation

Coumatetralyl was mineralised in both soil types as 38-44% was minealised after one month and 60-61% was mineralised after one year. The degradation potential in the two soils was thus similar although a much larger microbial biomass was measured in the Höfchen soil.

10.2.1 Degradation products (% of a.s.)

The number of metabolites was found to increase with time, whereas the total amount of metabolites remained at a constant level during the incubation period (6-4% in standard soil and 4-3% in Höfchen soil). The nature of the degradation products was not further examined.

10.2.2 Unextracted radioactivity

The proportion of unextracted radioactivity, i.e. the bound residues, were at a similar level at the end of the incubation period at 24% and 29% in the standard soil and Höfchen soil, respectively. Generally, the level of bound residues decreased during the incubation period while the level of $^{14}CO_2$ increased. This showed that the bound residues were not resistant to degradation.

10.3 Conclusion

Coumatetralyl is mineralised under aerobic conditions in soil. The extent of mineralisation was similar in the two soil types used. The level of bound residues decreased over time, suggesting that this fraction was not resistant to degradation.

10.3.0 Reliability

2

10.3.1 Deficiencies

Test material not identified by batch or lot no. Low number of replicates used. Some reporting deficiencies compared to the large data material. No reference substance was used.

Section A7.2.1 Aerobic degradation in soil

Annex Point IIIA VII.4, XII 1.1

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*12. February 05*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	*2*
Acceptability	*OK The rapporteur has made this study summary, therefore no comments*
Remarks	

Table A7_2_1-1: Characteristics of the soils used

	Höfchen **Soil from Höfchen Experimental Farm, new field**	**Soil 2.2** **Standard soil of the Federal Biological Institute**
Organically bound carbon	1.75%	2.6%
Percentage small particles (<0.02 mm)	34.5%	14.5%
pH value	5.4	6.0
Max. water holding capacity	37.0% 58.98 g H_2O/100g soil	27.5% 38 g H_2O/100g soil
Biomass at the start of the test in mg microbial C/kg dry soil	608	103

Table A7_2_1-2 Degradation of coumatetralyl in soil. Results are presented as % of the initially applied radioactivity.

	Standard soil											
Day	0		30		42		90		180		365	
Test	A	B	A	B	A	B	A	B	A	B	A	B
1. Extracted	99	97	26	26	20	20	11	11	8	8	5	5
active ingredient	*99*	*97*	*20*	*20*	*16*	*16*	*7*	*7*	*4*	*4*	*1*	*1*
2. Unextracted	2	2	31	31	34	32	35	37	29	31	24	24
3. $^{14}CO_2$	-	-	38	38	43	41	46	42	53	49	61	59
4. Other volatile compounds	-	-	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05
∑ 1. - 4. in %	101	99	95	95	97	93	92	90	90	88	90	88
Biomass in mg microbial C/kg dry soil	103		107		-		-		-		108	

Table A7_2_1-3 Degradation of coumatetralyl in soil. Results are presented as % of the initially applied radioactivity.

	Höfchen soil											
Day	0		30		42		90		180		365	
Test	A	B	A	B	A	B	A	B	A	B	A	B
1. Extracted	96	98	11	11	8	8	6	6	5	5	4	4
active ingredient	*96*	*98*	*7*	*7*	*5*	*5*	*3*	*3*	*2*	*1*	*0.2*	*0.2*
2. Unextracted	3	3	42	42	41	41	38	38	35	33	27	31
3. $^{14}CO_2$	-	-	43	45	44	44	47	47	53	51	60	62
4. Other volatile compounds	-	-	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05
∑ 1. - 4. in %	99	101	96	98	93	93	91	91	93	89	91	97
Biomass in mg microbial C/kg dry soil	608		674		-		-		-		233	

Table A7_2_1-4 Degradation of coumatetralyl in soil. Results are presented as % of the initially applied radioactivity.

	Sterile standard soil			
Day	30	90	180	365
1. Extracted	101	97	97	81
active ingredient	*101*	*97*	*97*	*71*
2. Unextracted	8	12	11	15
3. $^{14}CO_2$	<0.2	<0.2	<0.2	3
4. Other volatile compounds	<0.02	<0.01	<0.01	<0.05
∑ 1. - 4. in %	109	109	108	99
Biomass in mg microbial C/kg dry soil	0			>1

Section A7.3.1
Annex Point IIIA7.5

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

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11 REFERENCE

11.1 Reference	E. Hellpointner. Calculation of the chemical lifetime of Coumatetralyl in the troposphere. Bayer CropScience AG , Development Metabolism / Environmental Fate,Monheim, Germany, BCS Report MEF-04/233, 2004-05-26
11.2 Data protection	Yes
11.2.0 Data owner	Bayer CropScience AG
11.2.1	
11.2.2 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

12 GUIDELINES AND QUALITY ASSURANCE

12.1 Guideline study	No, as no guidelines are available for this modelling
12.2 GLP	No, as this is not applicable for this modelling report
12.3 Deviations	No

13 MATERIALS AND METHODS

13.1 Test material	coumatetralyl, 4-hydroxy-3-(1,2,3,4-tetrahydro-1-naphthalenyl)-2H-1-benzopyran-2-one
13.2 Structural Formula	 MolWt: 292.34 C19 H16 O3 005836-29-3 Coumatetralyl
13.3 Calculation program	The calculation was performed using AOPWIN (Atmospheric Oxidation Program) Version 1.91. The program is an adoption of the estimation methodology from Atkinson developed by Syracus Research Corporation.
13.4 Regarded mechanisms	OH radical reaction in the troposphere
13.5 Other mechanisms	Ozone reaction in the troposphere (at 7 E+11 mol/cm^3)

Section A7.3.1 **Phototransformation in air (estimation method) including identification of breakdown products**

Annex Point IIIA7.5

13.6	**Mean daily OH concentration in air**	12-hrs day; 1.5 E+6 OH/cm³
13.7	**Transformation products**	formation of various primary radicals leading to secondary oxidation products

14 RESULTS

14.1	**Overall OH-reactivity rate constant**	52.7 E-12 cm³/molecules-sec, then corrected by the author for some assessment uncertainties (see 5.2.1)
	Overall ozone-reactivity rate constant	13.65 E-17 cm³/molecules-sec, then corrected by the author for some assessment uncertainties (see 5.2.3)
14.2	**Overall tropospheric half–life**	2.4 hrs (OH radical reaction) 2.0 hrs (ozone reaction)]
14.3	**Specification of the transformation products**	The chemical stability of coumatetralyl in air is not determined by an attack at one single site, but at different parts of the molecule. This should result in the formation of various primary radicals leading to secondary oxidation products, which can be eliminated from the air by wet and/or dry deposition. In addition to reactions with OH radicals, ozone can contribute to the degradation of coumatetralyl in the air to a high extent.

15 APPLICANT'S SUMMARY AND CONCLUSION

15.1	**Materials and methods**	Calculation using AOPWIN (Atmospheric Oxidation Program) Version 1.91. The program is an adoption of the estimation methodology from Atkinson developed by Syracus Research Corporation.
15.2	**Results and discussion**	A chemical lifetime of coumatetralyl in the air of utmost 3 hours (corresponding to a halflife of utmost 2 hrs) it is to be expected.
15.2.0	k_{OH}	36.8 E-12 cm³/molecules-sec
15.2.1	$t_{1/2air\ OH}$	3.5 hrs
15.2.2	k_{Ozone}	9.1 E-17 cm³/molecules-sec
15.2.3	$t_{1/2air\ ozone}$	3.0 hrs
15.3	**Conclusion**	On account of an estimated chemical lifetime of coumatetralyl in the air of at the most 3 hours it is to be expected that the active ingredient can not be transported in gaseous phase over large distances and can not accumulate in the air. Thus, no difference in the behaviour between coumatetralyl and other organic

Section A7.3.1 Annex Point IIIA7.5	Phototransformation in air (estimation method) including identification of breakdown products
	substances emitted into the air from natural sources (e.g. from plants, soil) is indicated.
15.3.0 Reliability	Reliability indicator = 1
15.3.1 Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*2004.11.09*
Materials and Methods	*Applicants version is acceptable*
Results and discussion	*Adopt applicant's version*
Conclusion	*Adopt applicant's version*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	

SECTION A7.4.1.1.1.1 **ACUTE TOXICITY TO FISH**

Annex Point IIA VII.7.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] J., 2003, Coumatetralyl (technical) : Acute toxicity to Rainbow trout (*Oncorhynchus mykiss*), [redacted] Report N°1392/052 ,05 December 2003, MO-04-001275	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data on existing active substance submitted for the first time for entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD guideline No. 203 Method C1 of commission directive 92/69/EEC	
2.2	**GLP**	Yes	
2.3	**Deviations**	The temperature was outside the range of 14±1°C up to 0.5°C during the test ; this is considered to have no overall effect on the result of the test given that no sublethal effects were observed in the control.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Coumatetralyl (Technical), Racumin Techn S	
3.1.1	Lot/Batch number	0302	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	99.7%	
3.1.4	Composition of Product	Not relevant	
3.1.5	Further relevant properties		
3.1.6	Method of analysis	The test material concentration in test sample was determined by HPLC with a UV/Visible detector using an external standard	
3.2	**Preparation of TS solution for poorly soluble or volatile test**	The adequate amount of test substance were dispersed in dechlorinated tap water (pH adjusted to 9) and stirred at approximately 14°C for a period of 48 to 72 hours to obtain a test concentration of 100 mg/l. Then the pH was adjusted to the pH	

substances	of control water (pH 7.6). This 100 mg/l test concentration sample was further diluted to prepare the remainder of test series of 10, 18, 32 and 56 mg/l.	
3.3 Reference substance	No	
3.3.1 Method of analysis for reference substance	Not relevant	
3.4 Testing procedure		
3.4.1 Dilution water	Laboratory tap water, see table A7.4.1.1-1	
3.4.2 Test organisms	Rainbow trout (*Oncorhynchus mykiss*), see table A7.4.1.1-2	
3.4.3 Test system	Static, see table A7.4.1.1-3	
3.4.4 Test conditions	see table A7.4.1.1-4	
3.4.5 Duration of the test	96 hours	
3.4.6 Test parameter	Mortality and sub-lethal effect	
3.4.7 Sampling	Any mortalities and sub-lethal effect were recorded at 3, 6, 24, 48, 72 and 96 hours after the start of exposure. The water temperature, pH and dissolved oxygen were recorded daily throughout the test.	
3.4.8 Monitoring of TS concentration	Yes, Water sample were taken from control and all test groups at 0 and 96 hours for quantitative analysis	
3.4.9 Statistics	The LC50 value and associated confidence limits at 24, 48, 72 and 96 hours was calculated by trimmed Spearman-Karber method (Hamilton et al 1977) using the tox Calc computer software package (ToxCalc 1999).	X

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 nitial concentrations of test substance	Nominal concentrations: 10, 18, 32, 56, 100 mg/l
4.2.2 Actual concentrations of test substance	100 to 105% at 0 Hours and 108 to 114% at 96 Hours, given in table A.7.4.1.1-5
4.2.3 Effect data (Mortality)	see table A7.4.1.1-6 and table A7.4.1.1-7
4.2.4 Concentration / response curve	See graph A.7.4.1.1-8
4.2.5 Other effects	Sub-lethal effect of exposure was observed at the test concentration of 100 mg/l. These responses were loss of equilibrium and presence of moribund fish. See table A.7.4.1.1-9
4.3 Results of controls	
4.3.1 Number/ percentage of animals showing adverse effects	No mortality occurred and no abnormalities were detected in the 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	Following a preliminary range-finding test, juvenile fish were exposed in groups of ten, to an aqueous solution of

	coumatetralyl over a range of concentrations of 10, 18, 32, 56 and 100 mg/l for a period of 96 hours at a temperature of 12,5°C to 14,0°C under static conditions. The number of mortalities and sub-lethal effects of exposure in test and control vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the test until 96 hours.	
5.2 Results and discussion	The 96-hour LC_{50} value based on nominal test concentration was 53 mg/l with 95% confidence limits of 45-63 mg/l. The No Observed Effect Concentration was 32 mg/l. Analysis of the test preparations at 0 and 96 hours showed measured test concentrations to range from 100 to 114% of nominal and so the results are based on nominal test concentration only.	X
5..2.1 96h-LC_0	32 mg/l	
5.2.2 96h-LC_{50}	53 mg/l (45-63 mg/l)	
5.2.3 96h-LC_{100}	100 mg/l	
5.3 Conclusion	Coumatetralyl is slightly toxic to fish (acute toxicity)	
5.3.1 Other Conclusions	The validity criteria are summarised in table A7.4.1.2-7.	
5.3.2 Reliability	1	
5.3.3 Deficiencies	No	

	EVALUATION BY COMPETENT AUTHORITIES
SECTION A7.4.1.1.1.1 **Annex Point IIA VII.7.1**	**ACUTE TOXICITY TO FISH**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2004.08.17
Materials and Methods	Accepted
Results and discussion	The 96-hour LC_{50} is given with 95% confidence limits (**see 5.2**). **Comment**: Inspection of the "mortality data" (Table A7.4.1.1-6) shows that at 96 hours there is only one observation between zero and 100% mortality.
Conclusion	Adopted
Reliability	2
Acceptability	Accepted
Remarks	None

Table A7.4.1.1-1: Dilution water

Criteria	Details
Source	Laboratory tap water was dechlorinated by passage through an activated carbon filter 5 (Purite Series 500) and partly softened (Elga Nimbus 1248D Duplex Water Softener) giving water with a total hardness of approximately 100 mg $CaCO_3$/l
Alkalinity	--
Hardness	100 mg $CaCO_3$/l
pH	7.7-7.96
Oxygen content	9.0 mg O_2/l
Conductance	324-489 □S/cm
Holding water different from dilution water	No

Table A7.4.1.1-2: Test organisms

Criteria	Details
Species/strain	Rainbow trout (*Oncorhynchus mykiss*)
Source	Test fish were obtained from [redacted].
Wild caught	No
Age/size	Juvenile, standard length of 4.5 cm (sd = 0.3); mean weight of 1.34 g (sd = 0.22)
Kind of food	Commercial trout pellets
Amount of food	-
Feeding frequency	-
Pretreatment	Acclimatisation 8 to 20 October 2003
Feeding of animals during test	No

Table A7.4.1.1-3: Test system

Criteria	Details
Test type	Static test regime
Renewal of test solution	No
Volume of test vessels	20 l
Volume/animal	2 l
Number of animals/vessel	10 (0.67 g fish/l)
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.1-4: Test conditions

Criteria	Details
Test temperature	12.5-14.0℃
Dissolved oxygen	8.2-9.1 mg O_2/l
pH	7.6-8.2
Adjustment of pH	No
Aeration of dilution water	Yes, The test vessels were aerated via narrow glass bore.
Intensity of irradiation	No data
Photoperiod	16 hours light and 8 hours darkness and 20 minutes dawn and dusk transition period

Table A7.4.1.1-5: Actual concentrations of test substance

Sample	Nominal concentration (mg/l)	Concentration found (mgl)	Expressed as percent of the nominal concentration (%)
0 hours	Control	<LOQ	-
	10	9.97	**100**
	18	18.8	**104**
	32	33.5	**105**
	56	58.0	**104**
	100	102	**102**
96 hours	Control	<LOQ	-
	10	10.8	**108**
	18	20.4	**114**
	32	36.4	**114**
	56	63.1	**113**
	100	111	**111**

Table A7.4.1.1-6: Mortality data

Test Substance Nominal Concentration [mg/l]	Mortality						
	Number						Percentage
	3 hours	6hours	24 hours	48 hours	72 hours	96 hours	96 hours
control	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0
56	0	0	6	6	6	6	60
100	0	3*	10**	10	10	10	100

*) Three out ten fish were observed to be moribund after 4 hours. Due to animal welfare implications (Animal scientific procedure Act 1986) these fish were killed and classed as mortalities for the 6-hours time point.

**) in addition after 5.5 hours, four out seven fish were observed to be moribund. These fish were killed for raison as above and classed as mortalities for 24 hours time point

Table A7.4.1.1-7: Effect data

	48 h [mg/l][1]	95 % c.l.	96 h [mg/l][1]	95 % c.l.
LC_0 *)	32	-	32	-
LC_{50} **)	53	45-63	53	45-63
LC_{100} *)	100	-	100	-

[1] effect data are based on nominal concentrations

*) measured; **) calculated

Graph A.7.4.1.1-8 : Concentration-Mortality Curve.

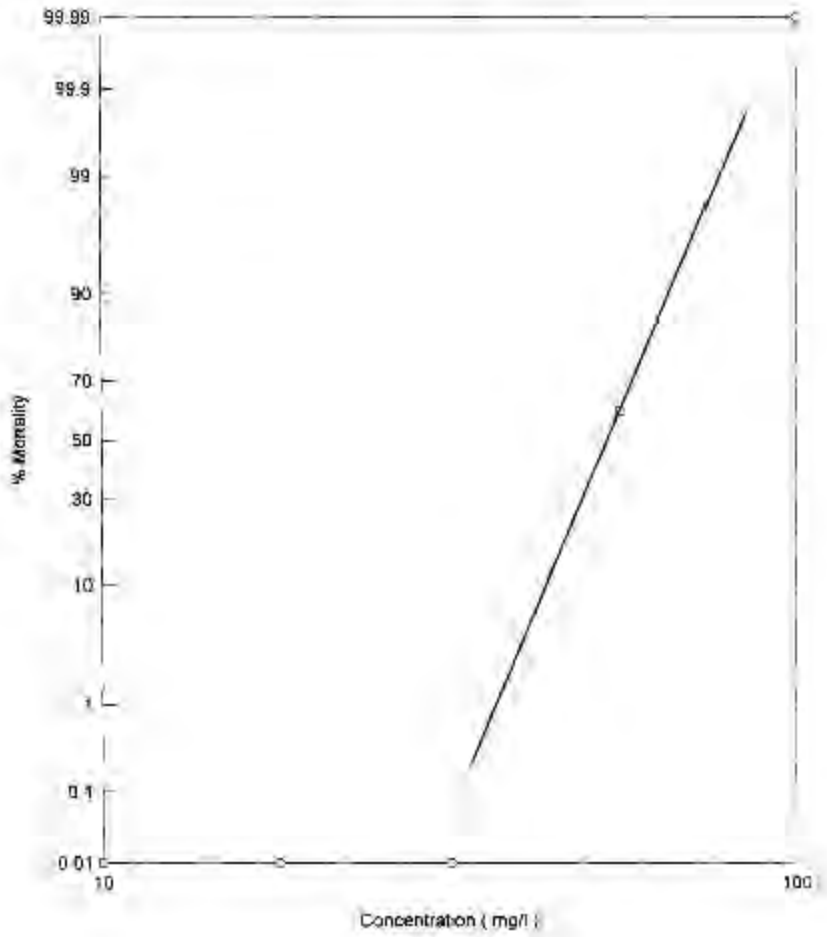

Table A.7.4.1.1-9 : Sub-lethal effects of exposure in the definitive test.

Nominal concentration (mg/l)	Sub-lethal effects	Time (hours)					
		3	6	24	48	72	96
Control	No abnormalities detected						
10	No abnormalities detected						
18	No abnormalities detected						
32	No abnormalities detected						
56	No abnormalities detected						
100	Loss of equilibrium moribund	1/10	1/7* 4/7	All fish dead			

*) Three out ten fish were observed to be moribund after 4 hours. Due to animal welfare implications (Animal scientific procedure Act 1986) these fish were killed and classed as mortalities for the 6-hours time point. In addition after 5.5 hours, four out seven fish were observed to be moribund. These fish were killed for raison as above and classed as mortalities for 24 hours time point

Table A7.4.1.1-10: Validity criteria for acute fish test according to OECD Guideline 203

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

Criteria for poorly soluble test substances		

SECTION A7.4.1.2 **ACUTE TOXICITY TO INVERTEBRATES**

Annex Point IIA VII.7.2 *Daphnia magna*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Heimbach F., 1991, Acute toxicity of coumatetralyl (tech.) to waterfleas (*Daphnia magna*), Bayer AG, [redacted], Report No. HBF/Dm 107 (unpublished), 12 December, 1991, MO-03-003177	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active ingredient for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD guideline No. 202 Part 1 (1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Coumatetralyl (tech.)	
3.1.1	Lot/Batch number	Batch No.: 19/1991	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	99.8 %	
3.1.4	Composition of Product	--	
3.1.5	Further relevant properties	--	
3.1.6	Method of analysis	Determination of coumatetralyl in water by HPLC, RA-652/91, Method 00261	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Coumatetralyl (tech.) was dissolved in Dimethylformamid (DMF) (see table A7.4.1.2-1).	

3.3 Reference substance	Yes, a test was carried out (Report No. HBF/Dm 102, 1991-05-16) under the same conditions using the reference substance potassium dichromate.
3.3.1 Method of analysis for reference substance	No data
3.4 Testing procedure	
3.4.1 Dilution water	Reconstituted deionized water, see table A7.4.1.2-2
3.4.2 Test organisms	*Daphnia magna* first instars (6-24 hours old), see table A7.4.1.2-3
3.4.3 Test system	Static, see table A7.4.1.2-4
3.4.4 Test conditions	see table A7.4.1.2-5
3.4.5 Duration of the test	48 hours
3.4.6 Test parameter	Immobility of animals and symptoms
3.4.5 Sampling	Immobility of animals and symptoms were determined after 24 and 48 hours in the control, solvent control and the test concentrations. At the start of the test pH and dissolved oxygen concentrations were determined. At the end of the test pH and dissolved oxygen were measured in one control beaker, one beaker of the solvent control and in one test beaker of the highest and lowest test concentration. The test temperature was measured.
3.4.6 Monitoring of TS concentration	Yes, At the beginning of the test, all the test concentrations were analysed. At the end of the exposure period, test solutions of 1.0, 5.6 and 18 mg a.i./l were also analysed to confirm stability of the concentrations..
3.4.7 Statistics	No data
	4 RESULTS
4.1 imit Test	Not Performed
4.1.1 Concentration	-
4.1.2 Number/ percentage of animals showing adverse effects	-
4.1.3 Nature of adverse effects	-

SECTION A7.4.1.2 **ACUTE TOXICITY TO INVERTEBRATES**

Annex Point IIA VII.7.2 *Daphnia magna*

4.2 Results test substance	
4.2.1 Initial concentrations of test substance	Nominal concentrations: 1.0, 1.8, 3.2, 5.6, 10.0 and 18 mg/l
4.2.2 Actual concentrations of test substance	Mean measured starting concentrations: 0.75, 1.5, 2.6, 4.5, 8.0 and 14 mg/l Concentrations in 1.0, 5.6 and 18.0 mg/l treatment levels remained stable during the exposure period.
4.2.3 Effect data (Immobilisation)	No observed immobilization in all treatment levels, see table A7.4.1.2-6 and table A7.4.1.2-7
4.2.4 Concentration / response curve	No graph is given in the report
4.2.5 Other effects	No Abnormalities were observed at any of the tested concentrations
4.3 Results of controls	No immobility or symptoms occurred in any of the two the control groups
4.4 Test with reference substance	Performed (Report No. HBF/Dm 102, 1991-05-16)
4.4.1 Concentrations	0.75, 1.00, 1.33, 1.78, 2.37 and 3.16 mg/l
4.4.2 Results	The 24-hour EC_{50} was determined to be 1.71 mg/l with 95 % confidence limits: 1.57 – 1.87 mg/l.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods	Coumatetralyl (tech.) was tested for acute toxicity to water fleas (*Daphnia magna*) according to OECD guideline No. 202 (1984). A total of 240 young daphnid (6-24 hours old) were exposed to six concentrations, one solvent control and one negative control (10 x 3 replicates per treatment group). Duration of the exposure was 48 hours in static conditions. Test concentrations were verified by chemical analysis. An organic solvent (DMF – 0.1 ml/l) was used
5.2 Results and discussion	Based on mean measured concentrations, an EC_{50} value of > 14 mg/l at 24 and 48 hours was estimated. The NOEC was determined to be 14 mg/l, the LOEC > 14 mg/l. No effects on mobility or other symptoms were observed at any concentration tested.
5.2.1 EC_0	$\geq$ 14 mg/l after 24 h and 48 h
5.2.2 EC_{50}	> 14 mg/l after 24 h and 48 h

SECTION A7.4.1.2	**ACUTE TOXICITY TO INVERTEBRATES**
Annex Point IIA VII.7.2	*Daphnia magna*

5.2.3	EC_{100}	> 14 mg/l after 24 h and 48 h
5.3	**Conclusion**	Under the conditions of the test (48 hours, static) no immobilisation, nor sublethal effects were observed among daphnids in any of the test groups. Based on mean measured test concentrations, the 48 hour-EC50 of coumatetralyl to *Daphnia magna* was estimated to be > 14 mg a.i./l (highest tested concentration) and the 48h NOEC value was 14 mg a.i./l.
5.3.1	Reliability	2
5.3.2	Deficiencies	Main relevant information are provided but: Test concentrations were not verified in all test groups at the end of the exposure period; Environmental parameters (pH, O_2, Temperature) were not measured in each group at the start of the study and at study termination

	EVALUATION BY COMPETENT AUTHORITIES
SECTION A7.4.1.2	**ACUTE TOXICITY TO INVERTEBRATES**
Annex Point IIA VII.7.2	*Daphnia magna*
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2004.08.17
Materials and Methods	The vehicle DMF is used for preparation of test solutions. **Comment:** The water solubility given in section A3 (**3.5**) implies that a vehicle is not needed. However, the results of the vehicle controls did not deviate from the water controls, so it does not affect the reliability of the test. In addition the notifier cleims that at the time where the study was conducted, the estimation of aqueous solubility into the dilution water (specific to the test) was not enough reliable to prepare solutions with confidence. Therefore an organic solvent (DMF) has been used.
Results and discussion	At the highest concentration tested, no effects were observed. Concentrations higher than 14 mg/L could have been tested.
Conclusion	Adopted
Reliability	2
Acceptability	Acceptable.
Remarks	Even though the study did not result in an EC_{50}-value, the worst case estimation of EC50 > 14 mg a.i./L could be used in the risk assessment. For the environmental risk assessment, a specific measure of the acute toxicity to *Daphnia ma*gna is not needed, because for the ERA, the results of the algae growth inhibition test (72 hours) and the reproduction study with Daphnia magna are available.

Table A7.4.1.2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes, dimethylformamide (DMF – 0.1 ml/l) 360.7 mg coumatetralyl (tech.) was dissolved in 2 ml DMF. From this solution 0.556, 0.311, 0.178, 0.10 and 0.056 ml were made up to 1 ml with DMF. 0.1 ml of the original solution was made up to 1000 ml test water to prepare the test concentration of 18 mg/l. Likewise, 0.1 ml of each of the other DMF-solutions were made up to 1000 ml with test water to prepare the test concentrations of 10, 5.6, 3.2, 1.8 and 0.1 mg/l. Each concentration was treated in an ultrasonic bath for 30 minutes and stirred for 20 minutes.
Concentration of vehicle	0.1 ml/l
Vehicle control performed	Yes,
Other procedures	-

Table A7.4.1.2-2: Dilution water

Criteria	Details
Source	Dilution water was prepared according to DIN 38412 L11, using deionised water by adding salts to yield the following concentrations: $CaCl_2$ x 2 H_2O: 0.002 mole/l; $MgSO_4$ x 7 H_2O: 0.0005 mole/l; $NaHCO_3$: 0.00075 mole/l; KCl: 0.000075 mole/l
Alkalinity	-
Hardness	250 mg $CaCO_3$/l
pH	8.0 (at the start of the test)
Ca / Mg ratio	See composition of the reconstituted dilution water
Na / K ratio	See composition of the reconstituted dilution water
Oxygen content	8.4 mg/l (at the start of the test)
Conductance	-
Holding water different from dilution water	No

Table A7.4.1.2-3: Test organisms

Criteria	Details
Strain	Daphnia magna
Source	Breeding kept internally, original source of the strain was [redacted], [redacted]
Age (at start of the study)	6 – 24 hours old
Breeding method	The animals are maintained in the laboratory in 2 litre glass containers; the water is changed weekly (dilution water according to DIN 38412 L11, 20 ± 1 °C, 16:8 hour light-dark cycle)
Kind of food	Single cell green algae *Scenedesmus subspicatus* and occasionally an aqueous suspension of commercial ornamental fish food (trade name: TetraMin®).
Amount of food	-
Feeding frequency	-
Pretreatment	Breeding kept under the same environmental conditions as for the test.
Feeding of animals during test	No

Table A7.4.1.2-4: Test system

Criteria	Details
Renewal of test solution	No - static conditions
Volume of test vessels	50 ml test solution in a 100 ml beaker
Volume/animal	5 ml
Number of animals/vessel	10
Number of vessels/ concentration	3 beakers
Test performed in closed vessels due to significant volatility of TS	Beakers were covered with watch glass

Table A7.4.1.2-5: Test conditions

Criteria	Details
Test temperature	20.1 °C (beakers were placed in a climatic chamber at 20 ± 1 °C)
Dissolved oxygen	8.2 – 8.6 mg/l
pH	7.85 – 8.0
Adjustment of pH	No
Aeration of dilution water	No aeration during the exposure period
Quality/Intensity of irradiation	Not relevant
Photoperiod	16:8 light-dark cycle

Table A7.4.1.2-6: Immobilisation data

Test Substance Concentration [1] (measured) [mg/l]	Immobile Daphnia						
	Number		Percentage		Oxygen [mg/l]	pH	Temperature [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
Control	0	0	0	0	8.2	7.85	20.1
Solvent control	0	0	0	0	8.5	7.89	20.1
0.75	0	0	0	0	8.5	7.88	20.1
1.5	0	0	0	0			20.1
2.6	0	0	0	0			20.1
4.5	0	0	0	0			20.1
8.0	0	0	0	0			20.1
14.0	0	0	0	0	8.6	7.88	20.1

Table A7.4.1.2-7: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg/l]	> 14	-	≥ 14	> 14
48 h [mg/l]	> 14	-	≥ 14	> 14

[1] effect data are based on mean measured concentrations

LOEC > 14 mg/l, NOEC ≥ 14 mg/l

Table A7.4.1.2-8: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202

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

Criteria for poorly soluble test substances		

SECTION A7.4.1.3 **GROWTH INHIBITION TEST ON ALGAE**

Annex Point IIA VII.7.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	Heimbach F., 1991, Growth Inhibition of Green Algae (Scenedesmus subspicatus) by coumatetralyl (tech.), [redacted], Report No. HBF/A1 96 (unpublished), 1991-12-09, MO-03-003167	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active ingredient for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD guideline No. 201 (1984) and ISO guideline ISO 8692 (1989)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No deviations according to OECD guideline No. 201	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Coumatetralyl (tech.)	
3.1.1	Lot/Batch number	Batch No.: 19/1991	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	99.8 %	
3.1.4	Composition of Product		
3.1.5	Further relevant properties	Solubility in water: 425 mg/l (pH 7), 100 – 200 g/l (pH 9)	X
3.1.6	Method of analysis	HPLC	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	The test substance was dissolved in Dimethylformamid (DMF) (see table A7.4.1.3-1).	X
3.3	**Reference**	Yes,	

substance	a test was carried out (Report No. HBF/A1 93, 1991-11-12) under the same conditions using the reference substance potassium dichromate ($K_2Cr_2O_7$).
3.3.1 Method of analysis for reference substance	No data
3.4 Testing procedure	
3.4.1 Culture medium	The algae of the stock culture were cultivated in a nutrient solution (see table A7.4.1.3-2, for pH see table A7.4.1.3-4). In the test phase, the test suspensions contain the following(based on 1 l): according to OECD guideline No. 201: 15 mg NH_4Cl, 12 mg $MgCl_2$ x 6 H_2O, 18 mg $CaCl_2$ x 2 H_2O, 15 mg $MgSO_4$ x 7 H_2O, 1.6 mg KH_2PO_4, 80 µg $FeCl_3$ x 6 H_2O, 100 µg Na_2EDTA x 2 H_2O, 185 µg H_3BO_3, 415 µg $MnCl_2$ x 4 H_2O, 3 µg $ZnCl_2$, 1.5 µg $CoCl_2$ x 6 H_2O, 0.01 µg $CuCl_2$ x 2 H_2O, 7 µg Na_2MoO_4 x 2 H_2O and 50 mg $NaHCO_3$
3.4.2 Test organisms	Freshwater green algae (*Scenedesmus subspicatus*), See table A7.4.1.3-2
3.4.3 Test system	Static, See table A7.4.1.3-3
3.4.4 Test conditions	See table A7.4.1.3-4
3.4.5 Duration of the test	96 hours
3.4.6 Test parameter	Inhibition of growth of biomass Inhibition of growth rate
3.4.7 Sampling	After 24, 48, 72 and 96 hours, the cell counts were determined in the control, the solvent control and the test concentrations. pH values were controlled at the beginning of the test and after 24, 48, 72 and 96 hours in the control, the solvent control and the test concentrations. Temperatures were determined at the end of the test in the control, the solvent control and the test concentrations.
3.4.8 Monitoring of TS concentration	Yes, at the beginning of the test, concentrations tested were analysed after preparing the test solutions.

3.4.9 Statistics	The EC50 for the growth of the biomass (EbC50) and for the algal growth rate (ErC50) were calculated using a probit analysis by the method of "maximum likelihood". For mathematical reasons, the calculation of a 95 % confidence interval was not practical for this test species and for its corresponding evaluation. The NOEC was estimated by an analysis of variance (Dunett`s-Test).	
	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	Nominal concentrations: 1.0, 1.8, 3.2, 5.6, 10 and 18 mg/l	X
4.2.2 Actual concentrations of test substance	Measured starting concentrations: 0.94, 1.7, 3.0, 5.2, 9.2 and 14 mg a.i./l See table A7.4.1.3-5	
4.2.3 Growth curves	Growth curves of the algal cells at the different test concentrations are given in the report (figure 1, page 12).	
4.2.4 Concentration / response curve	A concentration-effect curve of coumatetralyl for the growth of the biomass is given in the report (figure 2, page 14). The regression line has a slope of s = 2.40 (LITCHFIELD & WILCOXON method, 96 hour exposure).	
4.2.5 Cell concentration data	The photometrically determined cell counts of the main test are presented in table A7_4_1_3-6.	
4.2.6 Effect data (cell multiplication inhibition)	The growth of biomass (E_bC_{50}) was found to be 15.2 mg/l after 72 h and 14.8 mg/l after 96 h. The algae growth rate (E_rC_{50}) was found to be > 18 mg/l after 72 h and 96 h. The LOEC was determined to be 10 mg/l, the NOEC 5.6 mg/l.	
4.2.7 Other observed effects	No abnormalities, e.g. morphological changes, were observed.	
4.3 Results of controls	Cell counts of the control and the solvent control in the main test are presented in table A7.4.1.3-6.	

SECTION A7.4.1.3 **GROWTH INHIBITION TEST ON ALGAE**

Annex Point IIA VII.7.3

4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	0.18, 0.32, 0.56, 1.0 and 1.8 mg/l
4.4.2	Results	The 72-hour EC_{50} value determined for the growth of the biomass (E_bC_{50}) was found to be 0.82 mg/l. The EC_{50} value for the algal growth rate was determined to be 1.32 mg/l after 72 hours.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods	Coumatetralyl (tech.) was tested for its inhibition potential effect to the growth of green algae (*Scenedesmus subspicatus*) in accordance with the OECD guideline No. 201 (1984) and the ISO guideline ISO 8692 (1989). Algal cultures were exposed under static conditions to a negative control (6 replicates), a solvent control (6 replicates) and six test substance concentrations (3 replicates each). After 24, 48, 72 and 96 hours, the cell counts were determined. The test shows no significant deviations from the OECD guideline No. 201.
5.2 Results and discussion	Based on nominal concentrations, the inhibition of growth of biomass (E_bC_{50}) for coumatetralyl (tech.) was found to be 15.2 mg/l after 72 h and 14.8 mg/l after 96 h. The algae growth rate (E_rC_{50}) was found to be > 18 mg/l after 72 h and 96 h. The LOEC was determined to be 10 mg/l, the NOEC 5.6 mg/l. No abnormalities, e.g. morphological changes, were observed. The staring measured concentrations ranged from 78 to 94 % of expected nominal concentrations (for an average: 90.8 % allowing to base the results on nominal concentrations). Increasing algal cell numbers during the test may have resulted in an increasing adsorption of the test substance or incorporation and/or metabolism by algae. For this reason, the exposure concentrations were not determined analytically at the end of the exposition period of 96 hours. Coumatetralyl could not be detected in the control samples.
5.2.1 NOEC	5.6 mg/l
5.2.2 ErC50	> 18 mg/l after 72 h and 96 h
5.2.3 EbC50	15.2 mg/l after 72 h and 14.8 mg/l after 96 h.

SECTION A7.4.1.3 GROWTH INHIBITION TEST ON ALGAE

Annex Point IIA VII.7.3

5.3	**Conclusion**	Based on nominal concentrations and under the static conditions of the test, the inhibition of growth of biomass (E_bC_{50}) for coumatetralyl (tech.) was found to be 15.2 mg/l after 72 h and 14.8 mg/l after 96 h. The algae growth rate (E_rC_{50}) was found to be > 18 mg/l after 72 h and 96 h (maximum tested concentration). The LOEC was determined to be 10 mg/l, the NOEC 5.6 mg/l.
5.3.1	Reliability	2
5.3.2	Deficiencies	Main relevant information is provided. No deviations to the guideline were recorded. Test concentrations were not verified at the end of the exposure period but starting concentrations were measured and similar to nominal values Furthermore, the test substance is known to be hydrolitically stable.

	EVALUATION BY COMPETENT AUTHORITIES
SECTION A7.4.1.3 **Annex Point IIA VII.7.3**	**GROWTH INHIBITION TEST ON ALGAE**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2004.08.17
Materials and Methods	The vehicle DMF is used for preparation of test solutions. **Comment:** The water solubility (**see 3.1.5**) stated is not in accordance with that given in section A3 (**3.5**). Any of the water solubility values imply that a vehicle is not needed. However, the results of the vehicle controls did not deviate from the water controls, so it does not affect the reliability of the test. Furtheremore at the time when the study was conducted, the estimation of aqueous solubility into the dilution water (specific to the test) was not enough reliable to prepare stock solutions with confidence. Therefore, as recommended by the guideline, an organic solvent has been used
Results and discussion	Adopted
Conclusion	Adopted
Reliability	1-2
Acceptability	Acceptable
Remarks	E_rC_{50} is to be preferred for E_bC_{50} and effect concentrations recorded after 72 hours exposure for those recorded after 96 hours. Chemical analysis verified that exposure concentrations were about 90% of nominal. Therefore, the results of this test would be based on nominal concentrations and on records after 72 hours: E_rC_{50} > 18 mg/L and NOEC = 5.6 mg/L.

Table A7.4.1.3-1: Preparation of test solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	See below (ultrasonic-bath and magnetic stirrer)
Vehicle	Yes, Organic solvent: dimethylforfamide (0.1 ml/l)
Concentration of vehicle	Highest concentration: 0.1 ml/l
Vehicle control performed	Yes, observation of the number of algal cells and abnormalities was performed in a solvent control (control with 0.1 ml/ l DMF)
Other procedures	-

Table A7.4.1.3-2: Test organisms

Criteria	Details
Species	Freshwater green algal *Scenedesmus subspicatus*
Strain	SAG 86/81
Source	No data
Laboratory culture	Yes
Method of cultivation	The stock culture of the green algal species is maintained under 16 hours of light per day and at 20 °C in an autoclaved nutrient solution in accordance with Bringmann & Kuehn (1980, Water Research 14: 231-241). The stock cultures are transferred into fresh nutrient solution once a week. All nutrient solutions are prepared using deionized filter-sterilised water. The inoculation and transfer of algae suspensions is done under sterile conditions (sterile bench).
Pretreatment	The pre-cultures are inoculated with 1 x 10^4 cells/ml. For the test, the algae from the exponentially-growing pre-culture are exposed to the various test concentrations 3 days later at an initial concentration of 1 x 10^4 cells/ml.
Initial cell concentration	Each test culture started with a cell concentration of 1 x 10^4 cells/ml

Table A7.4.1.3-3: Test system

Criteria	Details
Volume of culture flasks	300 ml labelled Erlenmeyer flasks filled with each 150 ml test suspension (including algal cells and test compound).
Culturing apparatus	The Erlenmeyer flasks were exposed in an incubator at 23 ± 2 °C and 8000 lux continuous light (spherical, ± 20 %, measured with a three-dimensional quantum radiometer photometer).
Light quality	Illumination was provided by 2 x 4 fluorescent lamps (Osram L 140 W/20 S) that were attached on the sides of the incubator. Moreover, the inner walls of the incubator were covered with reflective material, so that a uniform illumination at all points of the interior was assured.
Procedure for suspending algae	By intermittent turning of the support frame in the incubator (6.5 thrusts per revolution, 3 revolutions per minute), sedimentation of the algal cells was prevented and the light exposure of the individual flasks was made even more uniform.
Number of vessels/ concentration	3 vessels / concentration; control and solvent control: in each case 6 vessels
Test performed in closed vessels due to significant volatility of TS	Yes, Test flasks were sealed with sterile cotton-wool balls

Table A7.4.1.3-4: Test conditions

Criteria	Details
Test temperature	22.0 – 23.2 °C
pH	At the start of the test, pH values of 8.28 (main test) was determined in the 10x nutrient solution (*). pH of the different test concentrations and controls at the individual test times (lower and upper value): 7.75 – 8.21 (main test)
Aeration of dilution water	No aeration was provided during the test
Light intensity	8000 lux; the illumination intensity of the lamps was adjusted by means of two dimmers to maintain it at the level required by the guideline.
Photoperiod	Continuous light

(*) nutrient solution was given at 10 times final concentration (10x) from 3 stock solutions (in accordance with the ISO guideline) in the test flasks for preparing the test suspensions

Table A7.4.1.3-5: Analysis of test water

Nominal concentration [mg a.i./l]	Actual concentration [mg a.i./l]			% of nominal concentration
	1. Detection	2. Detection	Mean value	
1.0	0.94	0.93	0.94	94
1.8	1.7	1.7	1.7	94
3.2	3.0	3.0	3.0	94
5.6	5.2	5.2	5.2	93
10	9.2	9.1	9.2	92
18	14	14	14	78

Coumatetralyl could not be detected in the control samples

Table A7.4.1.3-6: Cell concentration data

Test-Substance Concentration (nominal) [mg/l]	Percentage inhibition compared to solvent control							
	Biomass				Growth rate			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	94.9	94.8	97.2	98.2	97.8	98.3	99.8	100
Solvent control	100	100	100	100	100	100	100	100
1.0	113.3	109.3	111.2	111.9	*105.4*	102	103.1	102.3
1.8	11.2	113.6	112.4	109.3	*104.6*	104.5	102.5	101.2
3.2	95.9	93.993.9	94.7	94.7	98.2	97.5	99.0	98.8
5.6	112.3	103.1	102.2	100.7	105.0	99.0	100.8	96.6
10	81.6	66.2	61.0	62.9	91.4	81.6	87.7	91.4
18	82.6	59.2	46.0	42.8	92	73.9	78.0	81.9
Temperature [°C]	*	*	*	*				
pH **(mean value)	7.92	7.93	8.12	7.96				

* Test temperature was 22.0 – 23.2 °C

** mean value of pH at 0 hours = 7.78

Table A7.4.1.3-7: Validity criteria for algal growth inhibition test according to OECD Guideline 201

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

Criteria for poorly soluble test substances		

SECTION A7.4.1.4 **INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)**

Annex Point IIA VII.7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	Mueller G., 1991, Investigation of the ecological behaviour of Racumin S, [redacted], Study number 283 A/91 (unpublished), 1991-07-12, MO-03-003165	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active ingredient for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, ISO 8192, "Test for inhibition of oxygen consumption by activated sludge" (1986)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No deviation to the ISO guideline comparison with 67/548/EC, method C.11 and/or OECD guideline No. 209 shows...	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Racumin S (= coumatetralyl)	
3.1.1	Lot/Batch number	Not reported, 238008729 (raw data)	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	99.7 %	
3.1.4	Composition of Product		
3.1.5	Further relevant properties		
3.1.6	Method of analysis	No analytical verification	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	direct weighing	

3.3 Reference substance	Yes, 3, 5-Dichlorophenol
3.3.1 Method of analysis for reference substance	Not required
3.4 Testing procedure	
3.4.1 Culture medium	Synthetic medium
3.4.2 Inoculum / test organism	Activated sludge, see table A7.4.1.4-1
3.4.3 Test system	Not detailed in the report, See SOP 3.005 Sewage sludge exposed in 300 ml test vessels with 250 ml test solution made from 8 ml synthetic sewage feed, 25 ml sludge and deionised water. Synthetic feed as in guidelines. Test substance was weighed directly in the test vessels. Exposure time: 3 hours. Measurement of oxygen consumption for 10 minutes in BOD flasks.
3.4.4 Test conditions	Static conditions Suspended solid concentration: concentration of inoculation material: 6 g/l TS;
3.4.5 Duration of the test	Period of the study: from 24 June to 25 June 1991
3.4.6 Test parameter	Inhibition of respiratory rate
3.4.7 Analytical parameter	Oxygen measurement
3.4.8 Sampling	No sampling intervals. Except for the control for which, respiratory rate was measured once at the beginning of all measurements and a second time at the end
3.4.9 Monitoring of TS concentration	No
3.4.10 Controls	The respiratory rate of a defined quantity of activated sludge in synthetic medium was determined and compared to those measured in mixtures with different test substance concentrations; Concentration for autoxidation: 10,000 mg/l.
3.4.11 Statistics	No specification about calculation procedure

4 RESULTS

SECTION A7.4.1.4 **INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)**

Annex Point IIA VII.7.4

4.1 Preliminary test	Yes on June 25, 1991
4.1.1 Concentration	100 – 1000 and 10000 mg/L (test substance) 1 – 20 mg/L (reference substance) 10000 (autooxidation)
4.1.2 Effect data	-Compared to the control: 0%, 22.4, 74.1% reduction of respiration rate for the three test substance concentrations, respectively 0% and 65.5% reduction for the reference conditions.
4.2 Results test substance	
4.2.1 Initial concentration of test substance	Nominal concentrations: 560, 1000, 1800, 3200 and 5600 mg/l
4.2.2 Actual concentrations of test substance	No chemical analysis requested
4.2.3 Growth curves	No graph is given in the report but given in raw data
4.2.4 Cell concentration data	Not relevant
4.2.5 Concentration/ response curve	A concentration/response curve (inhibition vs. concentration) is see graph 7.4.1.4-2
4.2.7 Effect data	EC_{50} = 4210 mg/l, EC_{25} = 538 mg/l, EC_{75} = 32,900 mg/l
4.2.8 Other observed effects	-
4.3 Results of controls	Mean value of the two measurements was 31.5 mg 0_2/L/hour (raw data)
4.4 Test with reference substance	Performed with 3,5-Dichlorophenol
4.4.1 Concentrations	Two concentrations: 1 and 20 mg/L
4.4.2 Results	Respectively 14.3% and 76.2% reduction compared to control giving a EC50 value between 5 and 30 mg/L
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	To assess the toxicity of Racumin S (= coumatetralyl) to bacteria a test was investigated according to the ISO 8192, "Test for inhibition of oxygen consumption by activated sludge" (1986). A defined quantity of activated sludge (inoculum = 6 g/l) was exposed five concentrations of Racumin S (560, 1000, 1800,

		3200 and 5600 mg/l). The respiratory rate of each mixture was determined and compared to that determined in a mixture without test substance. 3,5-Dichlorophenol as reference substance was tested (1 and 20 mg/L)	
5.2	**Results and discussion**	The EC_{50} value of Racumin S was determined to be 4210 mg/l. At nominal test concentrations of 560 – 5600 mg/l, inhibition of respiratory rate was observed between 23.8 % and 55.6 % (see table A7.4.1.4-3). Results from the reference substance indicated a EC50 between 5 and 30 mg/L. The two measurements done with the control indicated very similar respiratory rates.	X
5.2.1	EC20		
5.2.2	C50	4210 mg/l	
5.2.3	EC80		
5.3	**Conclusion**	Based on test conditions, the EC50 of coumatetralyl to aquatic microbial activity is defined as 4210 mg/l. Due to the low toxicity to bacteria the self cleaning potential of surface waters is not endangered. Validity criteria were not reported but the test indicated a clear dose-response relation ship and a low level of toxicity. The raw data indicate that the validity criteria are met.	
5.3.1	Reliability	2 Some reporting deficiencies but completed by the attached raw data and SOPs	
5.3.2	Deficiencies	Yes, Some reporting deficiencies but completed by the raw data and SOPs attached to the report	

	EVALUATION BY COMPETENT AUTHORITIES
SECTION A7.4.1.4 **Annex Point IIA VII.7.4**	**INHIBITION TO MICROBIAL ACTIVITY (AQUATIC)**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2004-08-17
Materials and Methods	**Section 2.3,** The study was conducted according to the ISO guideline 8192. However, according to OECD TG 209 the reference substance should have been tested in three concentrations but only two were used. This is not considered critical because the results were within the validity criteria.
Results and discussion	Adopted. The parameters listed in **Section 5.2** demonstrate that the validity criteria (of OECD TG 209) were fulfilled.
Conclusion	Adopted
Reliability	2
Acceptability	Acceptable
Remarks	

Table A7.4.1.4-1 Inoculum / Test organism

Criteria	Details
Nature	activated sludge
Species	-
Strain	-
Source	Laboratory sewage treatment plant
Sampling site	Laboratory sewage treatment plant
Laboratory culture	Yes
Method of cultivation	-
Preparation of inoculum for exposure	No data
Pretreatment	No pretreatment was performed
Initial cell concentration	Concentration of inoculation material: 6 g/l TS

Gragh A7.4.1.4.2: Concentration/response curve (inhibition vs. concentration)

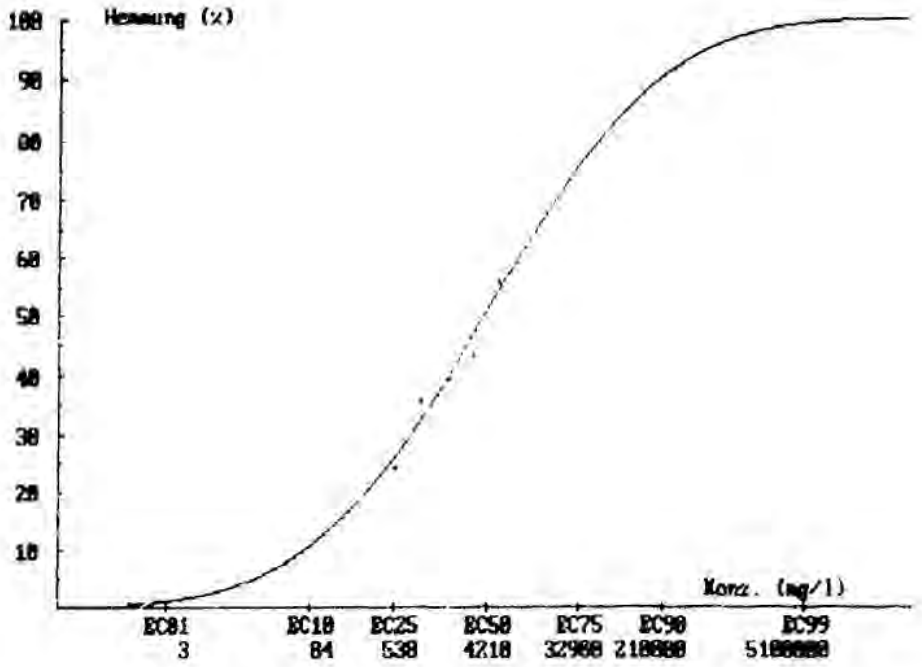

Table A7.4.1.4.3: Test results of test substance

Test Compound [mg/l] (*)	Respiratory Rate [mg /l · h]	Inhibition [%]
560	24.0	*23.8*
1000	20.4	*35.2*
1800	19.2	*39.0*
3200	18.0	*42.9*
5600	14.0	*55.6*

(*) nominal concentrations

SECTION A7.4.2 **BIOCONCENTRATION IN AQUATIC ORGANISMS (FISH)**

Annex Point IIA, VII.7.5

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1992, Coumatetralyl: Bioconcentration in fish, [redacted], Report No. BF-008 (unpublished), 1992-05-07, MO-03-003252	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active ingredient for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes,OECD guideline No. 305 E (1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No, with regard to the OECD guideline 305E (1984)	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Phenyl-U-^{14}C – coumatetralyl (specific radioactivity: 37.74 kBq/mg (1.02 µCi/mg)) (radioactive material) Coumatetralyl (unlabelled material)	
3.1.1	lot/Batch number	Batch No.: 126 (radioactive material) Batch No.: 19/1991 (unlabelled material)	
3.1.2	Specification	no data (radioactive material) as given in section 2 of dossier (unlabelled material)	
3.1.3	Purity	radiochemical purity: ~ 99 % (radioactive material); chemical purity: > 99 % (radioactive material), 99.8 % (unlabelled material)	
3.3.4	Further relevant properties	The following properties are given in the report: water solubility = 4 mg/l in distilled water at 20 °C; the hydrolytic half-life in buffers at pH 4, 7 and 9 is determined to be > 1 year, log P_{ow} = 3.46; acute toxicity to fish (rainbow trout): LD_{50} = 48 mg/l;	X
3.3.5	Radiolabelling	Phenyl-U-^{14}C – coumatetralyl	
3.3.6	Method of analysis	<u>Processing of fish samples for radioassay</u> Fish samples were collected and dissected into edible (body, muscle, skin, skeleton, fins) and non-edible (head, internal organs) portions. After determining the wet weight of the samples	

	they were frozen, lyophilised re-weighed and homogenised. Aliquotal parts were taken for radioactivity measurement. Radioactivity measurement Normally triplicate subsamples were analysed due to inhomogeneities in the freeze-dried tissues caused mainly by the scales. The radioactivity of extracts and solutions was determined by means of liquid scintillation measurement (LS-measurement); solid samples were combusted before the LS-measurement.
3.4 Reference substance	No
3.4.1 Method of analysis for reference substance	-
3.5 Testing/ estimation procedure	
3.5.1 Test system/ performance	Test animals The bluegill sunfish (*Lepomis macrochirus*) used in this 456-hour dynamic study were obtained from [redacted] All test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 hours prior to testing. No mortality was noted 14 hours prior to the test initiation and all unsuitable fish (i.e. injured, deformed etc.) were eliminated from inclusion in the test prior to assignment to test groups. During the acclimation and test periods, the fish received frozen chironomid larvae once daily ad libitum. Test system A dosing system comprising a Hamilton ®Microlab MT dispenser with a 250 µl-syringe for each aquarium controlled by an EPSON HX20 computer (for dosing of stock solution) and flow-meters (for water flow control) was used for the introduction of [^{14}C]-coumatetralyl and diluent water into the 100 litre test aquaria. Aerated reconstituted water was delivered to the glass aquaria at an average rate of approx. 25 l per hour per aquarium during the exposure period (120 hours), an amount sufficient to replace the approximately 100 litre test volume about 6 times in a 24-hour period and stock solution ([^{14}C]-coumatetralyl in acetone) was dosed at a rate of 50 µl every 72 seconds (= 2.5 ml/h). Water (continuously 25 l/h) and aliquots of [^{14}C]-coumatetralyl stock solution (515 mg/l, 0.05 ml every 72 sec) were delivered to a 2000 ml-mixing cell to yield a nominal exposure concentration of 51.5 µg/l. The mixture was running continuously from the mixing vessel

into the respective aquarium.

The control aquarium also received an amount of acetone solvent (0.1 ml/l) as the exposure aquarium.

The exposure system consisted of one 51.5 µg/l nominal concentration aquarium and one control aquarium. The test aquaria arranged in a lab room were kept at 22°C (± 1) by adding diluent water electronically thermostated to that temperature. The diluter system was calibrated by volumetric measurements of syringe dispenser aliquots and flow-rate of flow meters.

Preparation of the test substance

The stock solutions for the treatment of the fish were prepared as follows: The radioactive material (ca. 38.85 MBq, 377.4 kBq/mg delivered in a 2 l bottle) was diluted in 2 l acetone p.a. by adding the solvent to the tracer. Thus the concentration in the stock solution was 515 mg/l with a specific radioactivity of 37.74 kBq/mg or 2264 dpm/µg (equivalent to 850 dpm per 7 ml-sample of test medium).

Test procedure

Uptake phase

The uptake phase was initiated by transferring groups of 54 randomly selected and previously acclimated fish (length 7.77 ± 1.25 cm, body weight 7.98 ± 4.85 g) to each of the control and test chambers. The initial loading (4.3 g fish/l and 0.72 g fish/l/day) was in accordance with the OECD guideline No. 305 E. The fish were observed initially and every 24 hours on working days thereafter during the exposure period of 120 hours for mortality and/or adverse behaviour. At the same intervals pH and dissolved oxygen were measured in all aquaria. The temperature was recorded hourly in the control tank. Water and fish were sampled throughout the uptake period at 0, 2, 4, 8, 24, 32, 48, 120 hours after the start of the study. The water and tissue samples were radioassayed following the procedure described in point 3.1.6 of this study summary.

Depuration phase

After 120 hours of the exposure period, the addition of the [^{14}C]-coumatetralyl test material ceased. At the beginning of the depuration phase, the aquaria were cleaned mechanically, emptied by suction to a water height of ca. 5 cm, and filled with uncontaminated diluent water (22°C). During that procedure the fish remained in the aquaria. The fish were then exposed to flowing uncontaminated diluent water (22°C) for 336 hours. During the depuration period, water and fish were sampled at 128, 144, 192, 288 and 456 hours after the start of the study and were

radioassayed following the procedure in point 3.1.6 of this study summary. The fish were observed initially and every 24 hours on working days during the depuration period of 336 hours for mortality and/or adverse behaviour. At the same intervals pH and dissolved oxygen were measured in all aquaria. The temperature was recorded hourly in the control tank.

Sampling

Fish

Fish were sampled at 0, 2, 4, 8, 24, 32, 48, 120, 128, 144, 192, 288 and 456 hours after the start of the study. On these dates, four fish from each chamber were collected at and processed individually. The fish were dissected into edible and viscera/non-edible parts. Samples were treated and measured as described in point 3.1.6 of this study summary. Fresh and dry weight of the fish portions were determined.

Water

On each sampling day, 3 samples of 7 ml of water were removed from each aquarium. The concentrations of ^{14}C calculated as [^{14}C]-coumatetralyl in water were calculated by liquid scintillation counting of triplicate 7 ml-samples pipetted directly from each control and test tank. To each sample 7 ml scintillation cocktail (United Technologies Packard Instant Scint. Gel) were added.

Chemical and physical test parameters

Water quality parameters of dissolved oxygen and pH were measured initially and throughout the study at least on working days in the control and exposure chambers. The temperature was recorded hourly in the control tank. The test chambers were not aerated throughout the test. Dissolved oxygen levels remained at or above 96 % saturation.

Due to bacterial growth in the test aquaria test tanks had to be cleaned on hours 120 and 360 of study.

3.5.2 Estimation of bioconcentration

Calculation of results. In evaluating the data obtained from the bioconcentration study, a steady-state approach was used. This consists of a two compartment model (water and fish) which is used to describe the movement of the test material in and out of the test fish. This approach is used to determine the steady-state bioconcentration factor (BCF), the uptake rate constant and the depuration rate constant. The results were calculated using an electronic spreadsheet (™Lotus 1-2-3).

The water concentration of coumatetralyl was calculated by the formula:

Net dpm/ml = Activity of test water – Activity of control water

(dpm/ml)

$$\text{Water concentration } \mu g/l = \frac{(\text{net dpm/ml}) * 1000}{\text{specific activity of parent compound (dpm/}\mu g)}$$

The tissue concentration of coumatetralyl was calculated by the formula:

Net dpm/g = Activity of test tissue – Activity of control tissue (dpm/g)

$$\text{Tissue concentration mg/kg} = \frac{(\text{net dpm/g}) * 1000}{\text{specific activity of parent compound (dpm/mg)}}$$

Bioconcentration factors for fish portions were determined during the uptake period by dividing the [^{14}C]-tissue radioactivity by the mean [^{14}C]-water radioactivity up to and including that day.

dpm/ml (water) = (dpm (treated) - dpm (control))/sample volume

Sample volume in all water radioactivity measurements was 7 ml.

$$\text{dpm/g (fish portion)} = \frac{(\text{dpm/g dry weight (treated)} - \text{dpm/g dry weight (control)})}{(\text{sample weight (fresh)} / \text{sample weight (dry)})}$$

$$\text{Bioconcentration factor} = \frac{\text{dpm/g (fish portion)}}{\text{dpm/ml (water)}}$$

Bioconcentration factor for whole fish were determined by the following calculation:

$$\text{BCF (T)} = \frac{(\text{BCF (E)} * \text{fresh weight (E)}) + (\text{BCF (V)} * \text{fresh weight (V)})}{(\text{fresh weight (E)} + \text{fresh weight (V)})}$$

(T) = whole fish
(E) = edible portion
(V) = non-edible portion

Statistics

The uptake rate constant and depuration rate constant were determined by the Dow BIOFAC computer program. The BCF at steady state, the time to reach 90% of steady state and the time to reach 1/2 of test compound clearance (depuration) were also

calculated from the estimated rate constants. Measures of the variability of the estimated parameters were provided by the standard deviation of each estimate.

The measured bioconcentration factors in the fish samples were multiplied with the mean exposure concentration of 52 µg/l.

4 RESULTS

4.1 Experimental data

4.1.1	Mortality/behaviour	Neither deaths nor abnormal behaviour were observed among the fish
4.1.2	Lipid content	Lipid content was not determined
4.1.3	Concentrations of test material during test	Water concentrations ranged from 49 µg/l to 53 µg/l through 120 hours of the bioconcentration (uptake) phase. The average water concentration (using the mean value for each sample) during the uptake phase was 52 (±1.4) µg/l. These concentrations compared very well with the expected nominal concentration of 51.5 µg/l of [^{14}C]-coumatetralyl. The results of Radioanalysis (individual and mean values) of [^{14}C]-coumatetralyl in edible tissue and non-edible tissue during 120 hours of constant exposure to [^{14}C]-coumatetralyl and 14 days of depuration in clean water are summarised in table 9 to 14 of the report. Graphs showing the uptake and depuration of the test substance in the various tissue types are given in figure 1 to 3 of the report. The mean tissue residues at steady state were calculated to be 0.17 mg/kg for edible tissue, 1.12 mg/kg for viscera and 0.59 mg/kg for whole fish. The depuration half-lives see table A7.4.2-1
4.1.4	Bioconcentration factor (BCF)	The bioconcentration factors of the whole fish and the edible parts are 11.4 (±2.8) and 3.32 (±1.20), respectively. The BIOFAC calculated BCF values for edible parts and whole fish corresponded well with the respective observed bio-concentration factors of 3.0 X and 10.4 X for [^{14}C]-coumatetralyl at 120 hours. Time to reach 90% of steady-state in the whole fish was 46.8 hours.
4.1.5	Uptake and depuration rate constants	See table A7.4.2-1
4.1.6	Depuration time	See table A7.4.2-1
4.1.7	Metabolites	No metabolites identified

4.1.8 Other Observations -

4.2 Estimation of bioconcentration

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

A dynamic 456-hour study (120 hours of exposure, 336 hours for the depuration part) was conducted according to OECD guideline No. 305 E (1984) to evaluate the bioconcentration of [^{14}C]-coumatetralyl by bluegill sunfish (*Lepomis macrochirus*). The dosing system allowed approximately 6 renewals of each 100 l aquaria during the test. . A total of 112 fish were exposed to one solvent control and one treated group (nominal concentration 51.5 µg/l). Acetone (0.1 ml/l) was used as solvent.

Radioanalysis (^{14}C-CO_2) of edible and non-edible portions of individual fish were performed at different time points throughout the exposure and depuration period. At the same time points radioactivity in water samples was measured. Bioconcentration factors (BCFs) were calculated for total [^{14}C]-residues. BCFs for whole fish were calculated from BCFs of edible and non-edible portions. The kinetic data were calculated using a computer program (BIOFAC) which models a two compartment system.

5.2 Results and discussion

During the study, neither deaths nor abnormal behaviour were observed among the fish.

Temperature remained at 22 °C; the dissolved oxygen concentrations ranged between 8.5 and 10.3 mg/l, corresponding to 98 – 116 % saturation at the respective temperature and were considered adequate for testing; the pH values of the treated chamber were consistent with the control throughout the study and ranged from 7.4 to 7.9.

Results of the study (calculated with BIOFAC) are given in table A7.4.2-1.

The bioconcentration factors of the whole fish and the edible parts are 11.4 (±2.8) and 3.32 (±1.20), respectively. The BIOFAC calculated BCF values for edible parts and whole fish corresponded well with the respective observed bio-concentration factors of 3.0 X and 10.4 X for [^{14}C]-coumatetralyl at 120 hours.

Time to reach 90% of steady-state in the whole fish was 46.8 hours.24 hours after cessation of exposure 63, 69 and 67 % of the maximum measured plateau residues were depurated from edible portions, non-edible portions and whole fish, respectively. After 14 days in uncontaminated water more than 99 % of the maximum plateau radioactivity was depurated from edible portions, non-edible portions and whole fish, respectively. The half life for clearance from whole fish was 14.1 hours.

5.3 Conclusion

The validity criteria according to the OECD guideline No. 305 (1996) can be considered as fulfilled.

Coumatetralyl is accumulated very rapidly by bluegill sunfish with a total residue bioconcentration factor of 11.4 for whole fish. When exposure ceases, the residues are depurated quickly with a half-life of approximately 14.5 hours. Accumulation in edible parts is less (3 X) than in whole fish (11 X).Time to reach 90% of steady state during the exposure phase was 46.8 hours.

The bioconcentration factor for the test substance may be overestimated in this study because all calculations refer to radioactivity (sum of parent compound, metabolites and mineralization products).

According to the results an accumulation of coumatetralyl is not to be expected.

SECTION A7.4.2 **BIOCONCENTRATION IN AQUATIC ORGANISMS (FISH)**

Annex Point IIA, VII.7.5

5.3.1	Reliability	1-2	
5.3.2	Deficiencies	No, with regard to OECD guideline 305E (1984) but: no data about specification of radioactive material; study was performed with one concentration of the test substance instead of at least two; no TOC measurement; no replicates	X

	EVALUATION BY COMPETENT AUTHORITIES
SECTION A7.4.2 Annex Point IIA, VII.7.5	BIOCONCENTRATION IN AQUATIC ORGANISMS (FISH)
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2004.08.17
Materials and Methods	In **3.3.4**, Log P_{ow} = 3.46 (no information on pH). However, in Section A3 (**3.9**), log P_{ow} = 1.5 at pH=7, whereas it is 2.9 at pH 5.8. Acetone is used as a carrier for preparation of test solutions (**see 3.5.1**). **Comment:** The water solubility (**see 3.1.5**) is stated to be much lower than that given in Section A3 (**3.5**). The solubility stated in A3 is so high that a vehicle is not needed. Deviations from OECD 305E and other deficiencies as listed in **5.3.2**.
Results and discussion	Adopted
Conclusion	Adopted
Reliability	2
Acceptability	Acceptable
Remarks	The pH dependence of log P_{ow} indicates that at lower pH values than in the test, the BCF may be higher. At the pH during the test (pH = 7.4-7.9), log P_{ow} is expected to be about 1, while at e.g. pH = 5 Log P_{ow} is 3.4. Even though that the lack of a second test concentration is considered to weaken the conclusion, the low BCF values recorded at the one concentration tested and the log P_{ow} (approx. 1.5-3) indicate that this substance is not bioaccumulative.

Table A7.4.2-1 Results of the study (calculated with BIOFAC)

	edible	whole fish	viscera
Bioconcentration factor (BCF)	3.32 (±1.20)	11.4 (±2.8)	20.8 (±6.0)
Time to reach 90% of Steady-State, [hours]	29.3 (±8.3)	46.8 (±9.1)	46.9 (±10.5)
t(1/2) for clearance, [hours]	8.81 (±2.49)	14.1 (±2.7)	14.1 (±3.2)
Uptake rate constant, [1/hour]	0.26 (±0.06)	0.56 (±0.08)	1.02 (±0.19)
Clearance rate constant, [1/hour]	0.08 (±0.02)	0.05 (±0.01)	0.05 (±0.01)

SECTION A7.4.3.1 **PROLONGED TOXICITY TO AN APPROPRIATE SPECIES OF FISH**

Annex Point IIIA XIII.2.1

		Official use only
	1 REFERENCE	
1.1 Reference	[redacted] 1992, Coumatetralyl (technical grade): Prolonged Toxicity (21 days) to Rainbow Trout in a semi-static test, [redacted], Report No. FF – 318 (unpublished), 1992-06-05, MO-03-003331	
1.2 Data protection	Yes	
1.2.1 Data owner	Bayer CropScience AG	
1.2.2 Companies with letter of access		
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active ingredient for the purpose of its entry into Annex I/IA	
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes, OECD guideline No. 204 (1984)	
2.2 GLP	Yes	
2.3 Deviations	No	
	MATERIALS AND METHODS	
3.1 Test material	Racumin S techn.	
3.1.1 Lot/Batch number	Mixed batch No.: 19/1991	
3.1.2 Specification	As given in section 2 of dossier	
3.1.3 Purity	99.8 %	
3.1.4 Composition of Product	Only if investigation with biocidal product, give percentage of any ingredients	
3.1.5 Further relevant properties		
3.1.6 Method of analysis	HPLC with UV-detection	
3.2 Preparation of TS solution for poorly soluble or volatile test substances	Static renewal system: In the test several volumes of a stock solution of the test substance in acetone p.a. were dosed into the respective aquaria of each concentration to be tested. The stock solutions were prepared immediately before the start of the test as well as before the changes of test media.	
3.3	see table A7.4.3.1-1	
3.4 Reference substance	No	

3.4.1	Method of analysis for reference substance	-
3.5	**Testing procedure**	
3.5.1	Dilution water	Reconstituted water, see table A7.4.3.1-2
3.5.2	Test organisms	Rainbow trout (*Oncorhynchus mykiss*), see table A7.4.3.1-3
3.5.3	Test system	Semi-static, see table A7.4.3.1-4
3.5.4	Test conditions	see table A7.4.3.1-5
3.5.5	Duration of the test	21 days
3.5.6	Test parameter	Mortality and symptoms of intoxication (sublethal effects)
3.5.7	Sampling	During the test, fish were examined each working day for the mortalities and symptoms of intoxication. Dissolved oxygen and pH values were determined each working day in each aquarium; water temperature was measured and recorded hourly. The behaviour of the test substance in the aquaria was observed daily. Body weight and length data were determined during the test.
3.5.6	Monitoring of TS concentration	Yes, immediately before the start of the test, water samples were taken from the centre of the aquaria for the analytical determination of the active ingredient concentrations. Analytical determinations of the active ingredient concentrations were made in the test media at the beginning of the test as well as before the first changing of the test media and at test termination. Concentrations below the NOEC were not analysed.
3.5.7	Statistics	A 21-day LC_{50} value was not calculated, because in no group 100 % mortality occurred during the test. Analysis of variance was performed on the body weight and length data and on the condition factor calculated thereof. Groups being statistically significant different to the control group were identified by means of Duncan´s test (Statgraphics Users Guide, Version 5.0, Statistical Graphics Corporation, Inc., Rockville, maryland, USA, 1989).
4	**RESULTS**	
4.1	**Limit Test**	Not performed
4.1.1	Concentration	-

SECTION A7.4.3.1 **PROLONGED TOXICITY TO AN APPROPRIATE SPECIES OF FISH**

Annex Point IIIA XIII.2.1

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	The test was conducted in two parts. In the first part four groups of ten fish were exposed to nominal concentrations of 15.8, 50.0, 158.0 and 500.0 µg/l and in the second part four groups of ten fish were exposed to nominal concentrations of 0.158, 0.500, 1.58 and 5.00 µg/l.
4.2.2	concentrations of test substance	Results of the water samples coumatetralyl see table A7.4.3.1-9. Coumatetralyl could not be detected in the control samples. The lowest concentration of coumatetralyl which could be detected in the water was 0.005 mg/l.
4.2.3	data (Mortality)	see table A7.4.3.1-6 and table A7.4.3.1-7
4.2.4	concentration / response curve	No graph is given in the report as there was no clear dose – mortality relationship.
4.2.5	Other effects	Some fish escaped from the aquaria of different concentrations (1 at control, 1 at 0.158 µg/l, 2 at 5.0 µg/l, 1 at 158 µg/l and 1 at 500 µg/l). Observable symptoms were noted among the fish in the 15.8 (exopthalmus, day20) and 500.0 µg/l (fish mainly at the bottom and swimming behaviour slightly irregular (slight symptom), day 21) test levels.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	Neither mortalities nor symptoms of intoxication occurred in the control and the solvent control groups.
4.3.2	Nature of adverse effects	-
4.4	**Test with reference substance**	Not performed

SECTION A7.4.3.1 **PROLONGED TOXICITY TO AN APPROPRIATE SPECIES OF FISH**

Annex Point IIIA XIII.2.1

4.4.1 Concentrations	-	
4.4.2 Results	-	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods	According to OECD guideline 304 (1984), trouts were exposed 21 days to a negative control, a solvent control (acetone 0.1 ml/l) and 4 test substance concentrations in two parts. One part consisted in the test concentrations of 15.8, 50, 158 and 500 µg/l; the second part in test concentrations of 0.158, 0.500, 1.58 and 5.00 µg/l. Test solutions were renewed after 7 day-exposure periods. No deviation to the guideline was observed.	
5.2 Results and discussion	A 21-day LC_{50} value was not calculated, because in no group 100 % mortality occurred during the test and there was no clear dose-mortality relation ship. The lowest lethal concentration (LLC) was 15.8 µg/l. Neither mortalities nor symptoms of intoxication occurred in the control and the solvent control groups. There were no statistically significant difference in body weight, length and condition factor between the control and the treated groups. The analytical results showed that the concentrations were in accordance with the nominal values and that the test substance was stable for the duration of the test. For these reasons the reported results are related to the nominal concentrations. No changing of the behaviour of the test substance was observed in the aquaria.	X

SECTION A7.4.3.1 **PROLONGED TOXICITY TO AN APPROPRIATE SPECIES OF FISH**

Annex Point IIIA XIII.2.1

5.2.1 21d-LC50	Not calculated
5.2.2 NOEC	5.0 µg/l
5.2.3 LOEC	15.8 µg/l
5.3 Conclusion	Validity criteria are fulfilled, see table A7.4.3.1-8. There was no clear Dose - response relationship for mortality. There were no statistically significant difference in body weight, length and condition factor between the control and the treated groups.
5.3.1 Other Conclusions	-
5.3.2 Reliability	1
5.3.3. Deficiencies	No

	EVALUATION BY COMPETENT AUTHORITIES
SECTION A7.4.3.1 **Annex Point IIIA XIII.2.1**	**PROLONGED TOXICITY TO AN APPROPRIATE SPECIES OF FISH**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2004.08.17
Materials and Methods	Acetone is used as a carrier for preparation of test solutions (**seeTable A7.4.3.1-1**). **Comment:** The water solubility is stated to be >500 mg/L at pH=8 in section A3 (**3.5**). Therefore, a vehicle may not be needed to make solutions in the µg/L range. However, the results of the vehicle controls did not deviate from the water controls, so it does not affect the reliability of the test. Furtheremore, it is common to use organic solvent for the preparation of stock solutions.
Results and discussion	The lack of a concentration related response is mentioned (**see 5.2**). **Comment:** This causes not only a lack of LC_{50}-calculations but reduces the reliability of the study.
Conclusion	Adopted
Reliability	2
Acceptability	Acceptable
Remarks	The NOEC of 5 µg/L is considered to be sufficiently reliable for use for the ERA.

Table A7.4.3.1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes, organic solvent: acetone
Concentration of vehicle	0.1 ml/l
Vehicle control performed	Yes, in both parts of the test, groups ten fish were similarly exposed to 0.1 ml acetone p. a./l as solvent control.
Other procedures	-

Table A7.4.3.1-2: Dilution water

Criteria	Details
Source	Reconstituted water prepared by adding salt stock solutions to demineralised water to yield the following ionic concentrations (according to ISO) was used: Ca^{2+} = 0.384 mmol/l; Mg^{2+} = 0.096 mmol/l; Na^{+} = 0.148 mmol/l; K^{+} = 0.015 mmol/l; Cl^{-} = 0.783 mmol/l; HCO_3^{-} = 0.148 mmol/l; SO_4^{2-} = 0.096 mmol/l
Alkalinity	No data
Hardness	40- 60 mg of $CaCO_3$/l
pH	8.1 (Control at day 0 of the test, for both parts)
Oxygen content	11.8 mg/l (Control at day 0 of the test, first part); 10.9 mg/l (Control at day 0 of the test, second part)
Conductance	< 0.2 µmho/cm
Holding water different from dilution water	No

Table A7.4.3.1-3: Test organisms

Criteria	Details
Species/strain	Rainbow trout (*Oncorhynchus mykiss*)
Source	Test fish were obtained from [redacted]
Wild caught	No
Age/size	In the first part mean body weight of the fish at the beginning of the test was 3.3 ± 0.8 g, mean body length was 6.8 ± 0.5 cm. In the second part mean body weight of the fish at the beginning of the test was 4.3 ± 1.0 g, mean body length was 7.4 ± 0.7 cm.
Kind of food	During the pre-test period and during the test the fish were fed a commercial fish diet (Brutfutter FB 50, Kronen Fischkraftfutter, Wesel, Germany).
Amount of food	During the pre-test period: 1-3 % of the body-weight; During the test the fish were fed at a rate of 2.2 % (fresh weight) of the estimated initial body-weight.
Feeding frequency	Quantity of food was delivered daily
Pretreatment	All fish were held in culture tanks and observed for at least 14 days prior to testing. Less than 3 % mortality was noted in the lot prior to the test initiation and all unsuitable (e.g. injured, deformed etc.) fish were eliminated from inclusion in the test prior to assignment to test groups.
Feeding of animals during test	Yes, see information above

Table A7.4.3.1-4: Test system

Criteria	Details
Test type	Semi-static
Renewal of test solution	The test media were renewed at day 7 and at day 14 of the test.
Volume of test vessels	40 l
Volume/animal	4 l, (in the first part the loading was 0.8 g fish/l test medium and in the second part the loading was 1.1 g fish/l test medium).
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.3.1-5: Test conditions

Criteria	Details
Test temperature	Water temperature: 12 ± 1 °C
Dissolved oxygen	8.2 – 11.8 mg/l
pH	6.9 – 8.1
Adjustment of pH	No
Aeration of dilution water	Yes, dilution water was aerated to oxygen saturation with air. No aeration provided in the test solutions in tanks
Intensity of irradiation	No data
Photoperiod	16 hours light/8 hours dark

Table A7.4.3.1-6: Mortality data

Day No.	Mortality (number of fish) / Mortality (percent)							
	Test substance: nominal concentration [µg/l]							
	0.158	0.500	1.58	5.00	15.8	50.0	158.0	500.0
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
13	0	0	0	0	1 / 10	0	0	0
14	0	0	0	0	1 / 10	2 / 20	1 / 10	0
15	0	0	0	0	2 / 20	3 / 30	3 /30	0
16	0	0	0	0	2 / 20	3 / 30	3 / 30	0
17	0	0	0	0	2 / 20	3 / 30	5 / 50	0
20	0	0	0	0	6 / 60	5 / 50	7 / 70	3 / 30
21	0	0	0	0	7 / 70	6 / 60	7 / 70	4 / 40
Temperature [°C]	12 ± 1							
pH	6.9 – 8.1							
Oxygen [mg/l]	8.2 – 11.8							

In the controls as well as in the solvent controls neither symptoms nor mortalities occurred.