3 MATERIALS AND METHODS

3.1 Test material

[^{14}C]-brodifacoum, radiolabelled in the phenyl ring of the 4-hydroxy coumarin moiety.

Unlabelled brodifacoum was used to establish reference markers on TLC (thin layer chromatography) plates and in co-chromatography.

3.1.1 Lot/Batch number

Cis- and *trans*- [^{14}C]-brodifacoum ref. RFB B2248/120. The isomers were individually allotted the radiochemical code numbers: SM82 (*cis*-isomer) and SM83 (*trans*-isomer).

Sample of the *Cis*- and *trans*- [^{14}C]-brodifacoum were mixed to produce a 64:36 isomer ratio without further purification.

Unlabelled brodifacoum ref no: [redacted] (analytically pure sample), with *cis:trans* ratio of [redacted]

3.1.2 Specification

As given in section 2.

3.1.3 Purity

[redacted] Both isomers had a specific activity of 14.75 mCi/mM.

Unlabelled brodifacoum (analytically pure sample): 100% w/w.

3.1.4 Further relevant properties

1) Water solubility study: brodifacoum is insoluble/sparingly soluble in water with the following solubility values determined using EPA CG-1510 Guideline:

pH 5.2: 0.0038 mg/l

pH 7.4: 0.24 mg/l

pH 9.3: 10 mg/l

(Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [redacted]

2) Aqueous hydrolysis study:

- [^{14}C]-brodifacoum hydrolysis was insignificant with DT_{50} values estimated by extrapolation where possible: 173 days at pH5, 300 days at pH 7 (DT_{50} estimation not possible at pH9). (Reference: Mathis SMG, Benner JP and Skidmore MW (1995). Brodifacoum: Aqueous Hydrolysis in pH 5, pH 7 and pH 9 Solutions at 25°C. Zeneca Agrochemicals Report Number RJ1927B [redacted]).
- [^{14}C]-brodifacoum was determined to be hydrolytically stable. (Reference: Jackson R, Priestley I, Hall BE (1991). The Determination of the Hydrolytic Stability of [^{14}C]-Brodifacoum. Inveresk Research International Report Number 8330 [redacted]).

3) Brodifacoum is stable under normal storage conditions.

4) Vapour pressure is $<<10^{-9}$kPa ($<<10^{-8}$mmHg or $<<10^{-11}$atm) and is therefore negligable. (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [redacted]).

3.1.5 Method of analysis

Brodifacoum was extracted with organic solvents (dichloromethane for the supernatant aqueous solutions and hexane:acetone 1:1 for the soil). The extracts were evaporated to dryness at room temperature and the

		residue dissolved in acetone, applied to silica TLC plates, eluted and autoradiographs developed. Liquid scintillation counting (LSC) was used to determined radioactive content. Any residual compounds remaining in the aqueous solutions after extraction with organic solvents, were analysed using similar autoradiographic techniques following evaporation at room temperature.
3.2	**Degradation products**	Degradation products tested: 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**	Three soils were used, identified with the following names and soil type: 1) Peartree 7 (sandy clay loam); 2) Gore (calcareous sandy loam); 3) Lilyfield (coarse sand). See Table A7_1_3-1 below.
3.5	**Testing procedure**	
3.5.1	Test system and Test conditions	Soil samples were taken and passes through a 2mm sieve. The soil samples (2 g) were then air dried and equilibrated with aqueous calcium chloride solution (20 ml, 0.01 M). Dispersed slurries were formed by shaking the components together briefly in glass stoppered centrifuge tubes (35 ml). Overnight contact was allowed without further agitation (> 16 hours) to re-establish equilibrium wetting and microbial activity. The [^{14}C]-brodifacoum was added to the equilibrated soil-water slurries and each tube then immediately stoppered, re-weighed and the slurry re-dispersed with agitation using a Vortex shaker for *ca.* 1 min. Each sample was then transferred to an end-over-end shaker (at 21 – 22 °C) and mixing continued in the dark at 4 r.p.m. for 16 – 20 hours. At the end of this period, slurries were centrifuged at 2000 r.p.m. on a fixed-head Super Magnum Centrifuge for 15 mins. Aliquots of supernatant (1 ml) were withdrawn taking care to avoid removal of soil particles, and the radioactivity determined by LSC. From the slurries remaining after withdrawal of the supernatant liquid for analysis, further portions of supernatant (2 ml) were removed. Then fresh aqueous calcium chloride colution (3 ml, 0.01 M) was added to restore each slurry to its initial volume. As in the adsorption equilibration, each slurry was re-dispersed using the Vibro-shaker and re-equilibrated by end-over-end shaking for 16 to 20 hours. At the end of the re-distribution period, the residual brodifacoum concentration remaining in the soil solution was again determined from the radioactivity associated with aliquots (1 ml) of supernatant. Four consecutive desorption equilibria were examined after removing increasing volumes of brodifacouom solution, each time restoring the slurry to its original weight with fresh aqueous calcium chloride solution. All experimental procedures involving the test substance were carried out avoiding direct sunlight exposure.

3.5.2	Test solution	[^{14}C]-brodifacoum was applied to the soil slurries as a solution in acetone at rates of 0.9, 1.8, 2.7, 3.6 and 4.5 ppm in acetone (100 µl). These rates were equivalent to a total [^{14}C]-brodifacoum application of 18.06, 36.12. 54.18, 72.24 and 90.3 µg to the soil slurries.
3.6	**Test performance**	
3.6.1	Preliminary test	According to (a)"OECD 106": No, but the analytical method used for determining test substance content was applicable to the radiolabelled brodifacoum. Brodifacoum identity was established by comparing retention indices (R_f) values with the standard pure unlabelled compound. Three methods were used to establish R_f values from chemical mobility data obtained through: a) visualisation by fluorescence quenching under u.v. irradiation (wavelength 254 nm) directly on the plates; b) autoradiographs produced by exposure of Industrex 'C' X-ray film directly onto the plates; c) scanning the plates with an LB 2723 Berthold radiation scanner. Quantitative data was established through c) and by removing labelled silica bands, combusting and scintillation counting the radioactivity from $^{14}CO_2$ evolved in the toluene scintillator. Therefore, even though the water solubility of brodifacoum is very low (see section 3.1.4 above), the use of radiolabelled test substance ensures the sensitvity and applicability of the test method. Also, in this study, the solubility of brodifacoum in the test system aqueous phase was estimated: Aliquots of labelled brodifacoum acetone solution (0.1-10 µg) were measured into a series of duplicate tubes. Solvent was evaporated off in an air stream at room temperature. Aqueous calcium chloride solution (0.01 M, 20 ml) was added, the tubes stoppered, sealed and agitated for 24 hours in a cooled sonicator. At the period end, attempts were made to remove small volumes of saturated aqueous solution (1 ml) before counting radioactive contents by LSC. Counts measured for each 'solution' from each separate brodifacoum addition were plotted against the weight of brodifacoum initially introduced and counts in the initial acetone volume used. For true solutions, a linear correlation with unit slopes should result from when appropriate volume corrections are applied. Break points in linear relationships indicative of incomplete solubility were sought.
3.6.2	Screening test: Adsorption	According to (a)"OECD 106": Yes, although this study predated OECD 106, it was performed in accordance with the principles of these guidelines. See section 3.5 above for details on test method.
3.6.3	Screening test: Desorption	According to (a)"OECD 106": Performed. Although this study predated OECD 106, it was performed in accordance with the principles of these guidelines. See section 3.5 for details on test method.
3.6.4	HPLC-method	According to (a)" OECD-HPLC-method"[1]: No, this was not conducted as part of this study, but was performed as a separate test – please refer to Dossier Section No/Ref No: 7.1.3/01 Hogg A (2002).

[1] OECD (1999) OECD-Guidelines for the Testing of Chemicals. Proposal for a new guideline 121: Estimation of the adsorption coefficient (K_{OC}) on soil and on sewage sludge using High Performance Liquid Chromatography (HPLC), Draft Document (August 1999).

3.6.5	Other test	In addition to the desorption phase of the test, the identity of the radioactivity adsorbed onto the soils was examined. When the desorption phase was complete, soils from the high rate slurry treatments were centrifuged and freed from supernatant. Each soil pellet was extracted with hexane:acetone (1:1, 20 ml), for two periods of 18 hours. Samples were shaken at 20 – 21 °C on a Stuart 8-arm flask shaker. After each 18 hour period the extractions were centrifuged and the supernatant removed as completely as possible each time and the soil debris washed with fresh solvent and re-centrifuged. Extracts and washings were combined, then allowed to evaporate to dryness at room temperature in unsealed containers. The residue was dissolved in acetone, applied to silica TLC plates, eluted and autoradiographs developed. Also, the identification of the radioactivity in the final slurry solutions was attempted, by extracting into dichloromethane, evaporated and the resultant residues dissolved in acetone and analysed using autoradiography.
4	**RESULTS**	
4.1	**Preliminary test**	For the water solubility estimation, a plot of radioactivity in aqueous calcium chloride solution (0.01 M) after sonication, against radioactivity added to each tube for equilibration gave a non-linear curve. For complete solution a linear relation would be expected. Examination of aliquots of the solution, as sampled for counting, revealed the presence of undissolved crystals. These dissolved in the organic solvent of the scintillation medium and lead to exaggerated solubility estimates. Steps taken to remove these crystals had some success and gave a calculated value of brodifacoum solubility in the test system aqueous phase of <105 µg/l.
4.2	**Screening test: Adsorption**	Brodifacoum was found to be strongly bound to each of the soils tested. The amounts of radioactivity bound on the soils were calculated by difference between the quantities added initially and that retained in solution at equilibrium. See Table A7_1_3-2 below.
4.3	**Screening test: Desorption**	During the desorption phase, brodifacoum solution concentrations following the first desorption sequence fell more than expected in each of the soil-solution interactions (30% rather than a 15% decrease as expected from the quantity of solution removed (3 ml)). The exaggerated decrease in solution concentration may have resulted from incomplete brodifacoum adsorption in the first 24 hours with completion in the first desorption phase. It was postulated that more probably, a small increased adsorption onto new sites produced by interparticle abrasion during agitation. However, in view of the very limited water solubility this still represents rapid adsorption equilibration. Hysteresis loops were produced from these data, indicating poorly reversible adsorption to the soil particles. Very weak desorption was indicated from the final brodifacoum solution concentrations determined. See Table A7_1_3-3 below.

4.4 Calculations

Adsorption coefficient = Ka (denoted as Kd in the study report)

Desorption coefficient = Kd

4.4.1 Ka , Kd

Soil	Adsorption coefficient (Ka) *
Lily field / coarse sand	635, 337, 263, 252, 301
Peartree / sandy clay loam	1495, 811, 1280, 1379, 1358
Gore Hill / calcareous sandy loam	1280, 1194, 1119, 1194, 842

*Please note :

- in the study report the adsorption coefficient is denoted as Kd ;
- the desorption coefficient is not given in the study report ;
- see also Table A7_1_3-2 below.

4.4.2 Ka_{oc} , Kd_{oc}

Not given

4.5 Degradation product(s)

Analysis of the soil bound radioactivity indicated that both *cis*- and *trans*-isomer forms appear to undergo substantial rearrangement in the coarse sand soil slurries to produce isomer mixtures. In contrast, chemical forms remained stable in the sandy clay loam and calcareous sandy loam soils.

In the analysis of the final slurry solutions, no new radioactive bands were observed on TLC/autoradiographs which tended to confirm that hydrolysis did not occur. *Cis*- and *trans*-isomer configuration was retained in solution, so that isomerism reported for adsorbed material probably occurred during the process of binding. Radioactive impurities with R_f values larger than brodifacoum tended to accumulate in soil solution. Materials remaining in aqueous solutions were isolated by evaporation. Residues were chromatographed on silica and autoradiographs developed. Almost all of the material remained at the point of application. Since the total radioactivity unextracted from the aqueous phases is considerably less than 1% of the amount introduced into the slurries, no further identification was attempted.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test substance: [^{14}C]-brodifacoum, radiolabelled in the phenyl ring of the 4-hydroxy coumarin moiety;

Batch no: *Cis*- and *trans*- [^{14}C]-brodifacoum ref. RFB B2248/120. The isomers were individually allotted the radiochemical code numbers: SM82 (*cis*-isomer) and SM83 (*trans*-isomer). Sample of the *Cis*- and *trans*- [^{14}C]-brodifacoum were mixed to produce a 64:36 isomer ratio without further purification.

Purity: *cis*- [^{14}C]-brodifacoum: [redacted]; *trans*- [^{14}C]-brodifacoum: [redacted]%; both isomers had a specific activity of 14.75 mCi/mM.

Guidelines: EPA Registration of Pesticides in the United States, Proposed Guidelines. US Federal Register vol. 43 No. 132 pg 29722, 1978. This test method used is broadly in accordance with the later published guideline: OECD 106.

The adsorption and desorption of [^{14}C]-brodifacoum was studied using three soils (a sandy clay loam, a calcareous sandy loam and a coarse sand). Soils were slurried with calcium chloride solution, shaken and equilibrated for >16 hours. The [^{14}C]-brodifacoum was added to the soil slurries as a solution in acetone and the mixture agitated for 16 – 20

		hours in the dark at 21 –22 °C, and then centrifuged and aliquots of supernatant taken for analysis by LSC and TLC/autoradiography (following extraction with organic solvent). Fresh aqueous calcium chloride solution was added to the original volume, and agitated for 16 – 20 hours. At the end of the desorption period, the residual radioactivity remaining in solution was determined as before. Four consecutive desorption equilibria were examined after removing increasing volumes of the aqueous phase, each time restoring the slurry to its original weight with fresh aqueous calcium chloride solution. All experimental procedures involving the test substance were carried out avoiding direct sunlight exposure.
5.2	**Results and discussion**	The adsorption coefficients were all high (>1000 for the sandy clay loam and calcareous sandy loam soil, and >600 for the coarse sand), indicating that brodifacoum is very strongly adsorbed to soils. The adsorption of brodifacoum was the lowest to the soil having the lowest organic carbon content (the coarse sand). With the large water: soil ratios used, adsorption equilibria were established quite rapidly despite very low brodifacoum water solubility. Strong adsorption was followed by a small adsorption increase, possibly due to physical abrasion between the soil particles opening up fresh surfaces, and then very slow desorption but much less than required for a reversible reaction. Brodifacoum is non-ionic, highly lipophillic and can be desorbed into various organic solvents, so that interaction with soil organic matter may be responsible for binding. Both *cis*- and *trans*-isomers were converted to mixtures during adsorption-desorption periods with the coarse sand, but not the sandy clay loam soil. No chemical decomposition was found to occur in aqueous soil slurries over the 7 day period of contact. The low water solubility of brodifacoum, as well as strong adsorption, limited the chemical concentration in soil solutions at equilibrium.
5.2.1	Adsorbed a.s. [%]	The mean % of a.s. adsorbed, as calculated from the initial quantities of [^{14}C]-brodifacoum applied to the soil slurries and the measured amounts of total radioactivity in the solution following the adsorption period (see Table A7_1_3-2 below), are 97.0% for the coarse sand, 99.2% for the sandy clay loam, and 99.1% for the calcareous sandy loam.
5.2.2	K_a (Adsorption Coefficient)	635, 337, 263, 252, 301 for the Lily field / coarse sand soil. 1495, 811, 1280, 1379, 1358 for the Peartree / sandy clay loam soil. 1280, 1194, 1119, 1194, 842 for the Gore Hill / calcareous sandy loam soil. See Table A7_1_3-2 below.
5.2.3	K_d (Desorption Coefficient)	
5.2.4	Ka_{oc}	
5.2.5	Ka/Kd	
5.2.6	Degradation products (% of a.s.)	
5.3	**Conclusion**	The conclusion from this study is that brodifacoum is very strongly bound by soils of widely varying types. This is not surprising given the physico-chemical properties of the molecule (see section 3.1.4 above). The mobility of brodifacoum in soil has been studied separately.

		reference: Jackson R and Hall BE (1992). Aged Soil Leaching of [^{14}C]-Brodifacoum. Inveresk Research International Report No: 8879 (unpublished), [F3.2/02]. The results from this study showed that brodifacoum would not be expected to leach in soil and that no mobile degradation products are produced.
5.3.1	Reliability	2
5.3.2	Deficiencies	No.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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

	Soil 1	Soil 2	Soil 3
Soil name	Peartree	Gore	Lilyfield
Classification	Sandy clay loam	Calcareous sandy loam	Coarse sand
Location	Jealott's Hill Farm, Bracknell, Berkshire, England.	Manor Farm, Newbury, Berkshire, England	Wishanger Farm, Churt, nr Frensham, Surrey, England
Date collected	31st October 1978	31st October 1978	31st October 1978
Particle size analysis:			
Coarse sand [%]	38.0	7.7	67.5
Fine Sand [%]	22.0	41.7	22.5
Silt [%]	17.0	31.6	2.9
Clay [%]	23.0	19.0	7.1
Organic carbon [%]	6.3	11.4	1.2
pH	7.1	7.6	7.6
Cation exchange capacity (MEQ/100 g)	16.5	30.0	4.5
Moisture holding capacity (MHC) % :			
Zero suction	84.1	154.2	30.2
1/3 bar	29.6	43.7	5.4
10 bar	20.7	34.0	2.2
Extractable cations (ppm):			
P	>101	28	38
K	336	90	<37
Mg	138	108	10

Table A7_1 _3-2: Results of screening test – adsorption

Soil Name / Type	Initial application of test substance (µg)	Total test substance in solution (µg)	Concentration of test substance in solution (µg/ml)	Concentration of test substance in soil (µg/g)	Adsorption coefficient
Lilyfied / coarse sand	18.06	0.28	0.014	8.89	635
	36.12	1.04	0.052	17.54	337
	54.18	1.98	0.099	26.10	263
	72.24	2.76	0.138	34.74	252
	90.30	2.90	0.145	43.70	301
Peartree / sandy clay loam	18.06	0.12	0.006	8.97	1495
	36.12	0.44	0.022	17.84	811
	54.18	0.42	0.021	26.88	1280
	72.24	0.52	0.026	35.86	1379
	90.3	0.66	0.033	44.82	1358
Gore Hill / calcareous sandy loam	18.06	0.14	0.007	8.96	1280
	36.12	0.30	0.015	17.91	1194
	54.18	0.48	0.024	26.85	1119
	72.24	0.60	0.030	35.82	1194
	90.3	1.06	0.053	44.62	842

Table A7_1 _3-3: Results of screening test - desorption

Soil Name/ Type	Desorption Cycle A			Desorption Cycle B			Desorption Cycle C			Desorption Cycle D		
	Initial (µg)	Soln. (µg/ml)	Soil (µg/g)	Initial (µg)	Soln. (µg/ml)	Soil (µg/g)	Initial (µg)	Soln. (µg/ml)	Soil (µg/g)	Initial (µg)	Soln. (µg/ml)	Soil (µg/g)
Lilyfied / coarse sand	18.02	0.008	8.93	17.98	0.011	8.88	17.91	0.005	8.90	17.87	0.005	8.89
	35.96	0.032	17.66	35.80	0.019	17.71	35.69	0.011	17.74	35.62	0.015	17.66
	53.88	0.050	26.44	53.63	0.039	26.43	53.42	0.040	26.31	53.14	0.032	26.25
	71.83	0.086	35.05	71.40	0.065	35.05	71.02	0.052	34.99	70.66	0.095	34.38
	89.86	0.102	43.91	89.34	0.108	43.59	88.74	0.082	43.55	88.21	0.074	43.38
Peartree / sandy clay loam	18.04	0.004	8.98	18.02	0.003	8.98	18.01	0.003	8.97	17.98	0.003	8.96
	36.05	0.008	17.95	36.02	0.006	17.95	35.98	0.008	17.91	35.93	0.006	17.90
	54.12	0.013	26.93	54.05	0.010	26.92	54.00	0.008	26.92	53.94	0.009	26.88
	72.16	0.017	35.91	72.08	0.013	35.91	72.01	0.012	35.88	71.93	0.010	35.86
	90.20	0.023	44.87	90.08	0.018	44.86	89.98	0.015	44.84	89.88	0.015	44.79
Gore Hill / calcareous sandy loam	18.04	0.006	8.96	18.01	0.005	8.95	17.98	0.006	8.93	17.94	0.005	8.92
	36.07	0.014	17.90	36.01	0.011	17.89	35.94	0.010	17.87	35.88	0.010	17.84
	54.11	0.016	26.89	54.03	0.017	26.84	53.93	0.018	26.79	53.82	0.014	26.77
	72.15	0.022	35.85	72.04	0.025	35.77	71.91	0.020	35.75	71.78	0.020	35.69
	90.14	0.033	44.74	89.98	0.027	44.72	89.83	0.025	44.67	89.65	0.024	44.58

Doc. IIIA / Section No. 7.2.3.2 Mobility in soil

BPD Data Set IIIA / Annex Point XII.1.3

Official use only

1	**REFERENCE**	
1.1	**Reference**	Jackson R and Hall BE (1992). Aged Soil Leaching of [^{14}C]-Brodifacoum. Inveresk Research International Report No: 8879 (unpublished).
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
2	**GUIDELINES AND QUALITY ASSURANCE**	
2.1	**Guideline study**	Yes, EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 163-1.
2.2	**GLP**	Yes.
2.3	**Deviations**	Yes. There were several minor deviations to the protocol that did not affect the validity of the study.

3 MATERIALS AND METHODS

3.1 Test material Brodifacoum.

3.1.1 Lot/Batch number [^{14}C]-Brodifacoum: Batch no. ICIA0581; Ref No. 91J13.

Non-radiolabelled Brodifacoum reference no: ASY403.

3.1.2 Specification As given in section 2.

3.1.3 Purity [^{14}C]-Brodifacoum: radiochemical purity of 96% and specific activity 925 MBq/mmol.

Non-radiolabelled brodifacoum: 97.7%.

3.1.4 Radiolabelling Brodifacoum [^{14}C]-labelled uniformly in the benzene ring of the hydroxy coumarin moiety.

3.1.5 Further relevant properties 1) Water solubility study: brodifacoum is insoluble/sparingly soluble in water with the following solubility values determined using EPA CG-1510 Guideline:

pH 5.2: 0.0038 mg/l

pH 7.4: 0.24 mg/l

pH 9.3: 10 mg/l

(Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]).

2) Aqueous hydrolysis study:

- [^{14}C]-brodifacoum hydrolysis was insignificant with DT_{50} values estimated by extrapolation where possible: 173 days at pH5, 300 days at pH 7 (DT_{50} estimation not possible at pH9). (Reference: Mathis SMG, Benner JP and Skidmore MW (1995). Brodifacoum: Aqueous Hydrolysis in pH 5, pH 7 and pH 9 Solutions at 25°C. Zeneca Agrochemicals Report Number RJ1927B [F4.1/01]).
- [^{14}C]-brodifacoum was determined to be hydrolytically stable. (Reference: Jackson R, Priestley I, Hall BE (1991). The Determination of the Hydrolytic Stability of [^{14}C]-Brodifacoum. Inveresk Research International Report Number 8330 [F4.1/03]).

3) Brodifacoum is stable under normal storage conditions.

4) Vapour pressure is $<<10^{-9}$kPa ($<<10^{-8}$mmHg or $<< 10^{-11}$atm) and is therefore negligable. (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]).

3.2 Soil types Four soils were used, identified with the following names and soil type (see Table A7_2_1-1 below):

1) 18 Acres (sandy clay loam)

2) Wisborough Green (a silty clay)

3) Pickett Piece (clay)

4) Barassie (sand)

3.3 Testing procedure

3.3.1 Test system for All soils were maintained at *ca.* 75% moisture holding capacity (at 0.33 bar) for a minimum period of 7 days prior to application of the test

	ageing procedure	material. Six samples (2 for aged leaching, 2 for mass balance and 2 for determining CO_2 evolution) of each soil type (50 g dry weight equivalent) were transferred to Erlenmeyer flasks (250 ml capacity). After application of the test substance, a continuous stream of moist, carbon dioxide free air was drawn over the surface of each soil sample. The effluent air from each flask was passed through 2 traps containing ethanolamine (*ca.* 50 ml) to trap evolved $^{14}CO_2$. Ethanolamine traps were replenished and analysed by liquid scintillation counting after 7 days (8 for sand), 14 days (15 for sand), 21 days (22 for sand) and 30 days. The flasks were incubated in the dark at 21 +/- 2 °C for 30 days. The moisture loss from each soil sample was determined gravimetrically at regular intervals and, if necessary, distilled water was added to maintain the moisture content at *ca.* 75% of moisture holding capacity (at 0.33 bar). Two samples of each soil type were incubated for a further 2 days (to cover the leaching period) and ethanolamine traps from these samples were analysed 32 days after application of test substance.
3.3.2	Test system for column leaching	Leaching was conducted using glass cylindrical columns (length = 42 cm, internal diameter = 5 cm) each constructed from 6 x 5 cm long segments and a top segment of 12 cm. A plug of glass wool was placed in the bottom of each column to prevent soil from passing through the glass mesh base. Soil columns were covered with aluminium foil to exclude light. Duplicate columns of soil were prepared for each soil type. The columns were filled with air-dried soil (2 mm sieved) to a height of 30 cm. The columns were agitated gently during packing and then slowly saturated from the bottom of the column with 0.01 M aqueous calcium chloride solution. Where necessary air-dried soil was added to the column to compensate for compaction of the soil during the saturation process. At the end of the 30 day ageing period, duplicate samples of each soil type were transferred to the top of the soil columns containing the corresponding saturated soil type.
3.3.3	Flow rate for soil columns	Each column was leached with *ca.* 1000 ml of 0.01 M calcium chloride solution over *ca.* 48 hours.
3.3.4	Temperature	21 +/- 2 °C
3.3.5	Method of preparation of test solution	Dose solution for the sandy clay loam, silty clay and clay soils: [^{14}C]-brodifacoum in acetonitrile was prepared at a concentration of 196 µg/ml. Dose solution for the sand soil: [^{14}C]-brodifacoum in acetonitrile was prepared at a concentration of 191 µg/ml.
3.3.6	Application of test substance to soil	Aliquots of the dose solution were applied evenly to the surface of the soil in each flask: - sandy clay loam, silty clay and clay soils: 105 µl; - sand soil: 114 µl. The application rate of the test substance to the soil for the ageing procedure was calculated to be 20.5 µg per 50 dry weight of soil, or 0.41 mg/kg (for sandy clay loam, silty clay and clay soils), and 21.8 µg per 50 g dry weight of soil, or 0.44 mg/kg (for the sand soil).

3.3.7	Duration of test	The soil samples were aged for 30 days. The duration of the soil column leaching of the aged brodifacoum treated soil samples was 48 hours.
3.3.8	Number of replicates	2.
3.3.9	Sampling	Ageing procedure: at the end of the ageing period (30 days), 2 samples of each soil type were taken for analysis. Leaching procedure: the leachate from each column was collected as 4 approximately equal fractions in amberlite glass containers. At the end of the leaching period, to reduce the level of soil saturation, the columns were drained for *ca.* 1 hour (*ca.* 3 hours for the sand soil) and the leachate collected as part of the final leachate fraction.
3.3.10	Method of analysis	Ageing procedure: at the end of the ageing period (30 days), 2 samples of each soil type were taken for analysis. extracted with 2 x 100 ml of dichloromethane:methanol (4:1 v/v). For all soils (with the exception of the sand soil), an aqueous layer was obtained following the first extraction which was separated from the organic extract. The radioactivity in each extract was determined by liquid scintillation counting. Soil residues were subject to combustion analysis. After removal of soil, all Erlenmeyer flasks were soaked in acetone. The radioactivity in the acetone washings was determined by liquid scintillation counting. Leaching procedure: the radioactivity in each leachate fraction was determined by liquid scintillation counting. The top soil segments of each soil column (and also the second segment of the sand column) was extracted with 2 x *ca.* 150 ml of dichloromethane:methanol (4:1 v/v). The aqueous layer obtained after the first extraction was analysed separately. The radioactivity in the extracts was determined by liquid scintillation counting and soil residues were subjected to combustion analysis. After removal of soil, all soil columns segments were soaked in acetone. The radioactivity in the acetone washings was determined by liquid scintillation counting. The organic extracts from each soil sample (aged soil samples and leached soil extracts) were analysed by thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and liquid scintillation counting (LSC).
3.3.11	Degradation products	Not identified.
4	**RESULTS**	
4.1	**Ageing period**	Evolution of $^{14}CO_2$, as determined by analysis of ethanolamine traps, was highest for the silty clay soil (5.38% of applied radioactivity), lower for the clay and sandy clay loam soil (3.05% and 2.94% respectively), and lowest for the sand soil (0.90% of applied radioactivity). Most of the applied radioactivity was recovered in the soil extracts (91.66% from the sand, 85.92% from the sandy clay loam, 83.50% from the clay and 77.69% from the silty clay soil). Non-extractable radioactivity accounted for 11.76% of applied radioactivity in silty clay, 8.57% in clay, 7.38% in sandy clay loam and 3.16% in sand soil. TLC analysis of aged soil extracts indicated that the *cis* and *trans* isomers of brodifacoum were the only radiolabelled components in sand

		and the major components in the other three soils. Brodifacoum accounted for 88.79 – 96.88% of extractable radioactivity in the aged soil samples. HPLC analysis confirmed that the isomers of [^{14}C]-brodifacoum were virtually the only radioactive componenets present in the aged soil extracts. The proportion of extractable radioactivity attributable to brodifacoum were 98.82 – 100.00% in aged soil samples. The results of the TLC and HPLC radioanalyses of the aged soil samples are given below in Tables A7_2_1-2 and A7_2_1-3 respectively.
4.2	**Leaching profile**	The rate of $^{14}CO_2$ evolution from those flasks incubated for 32 days was similar, by soil type, to that for the 30 day ageing flasks. For all soil types, the extent of $^{14}CO_2$ evolution during the 2 day leaching period was insignificant. No detectable levels of radioactivity were found in leachates. Based on a limit of reliable determination (LRD) of 30 d.p.m. above background, the proportion of applied radioactivity in the combined leachates was less than 0.36% of the LRD per leachate sample. The majority of applied radioactivity was recovered in the top soil column segment from all columns (85.87% from the sandy clay loam, 82.45% from the silty clay, 81.42% from the clay, and 87.17% from the sand soil). The proportion of non-extractable radioactivity in top soil column segments was 38.43% in sandy clay loam, 50.49% in silty clay, 25.47% in clay and 2.46% in sand soil. With the exception of the sand soil, levels of radioactivity in the bottom 30 cm of all soil columns were below the LRD (0.65% per segment). Low levels of radioactivity (3.37% of applied) were recovered in the second 5 cm segment of the sand columns. Levels in the bottom 25 cm of the sand columns were below the LRD (0.61% per segment). In the TLC analysis, [^{14}C]-brodifacoum accounted for 86.12 – 92.45% of extractable radioactivity in the top soil column segments. HPLC analysis confirmed that the isomers of [^{14}C]-brodifacoum were virtually the only radioactive components present in the top soil column segment extracts. The proportion of extractable radioactivity attributable to brodifacoum were 97.41 – 100.00% in the leached soil samples. The results of the TLC and HPLC radioanalyses of the soil extracts from the top column segments are given below in Tables A7_2_1-4 and A7_2_1-5 respectively.
4.3	**Mass balance**	See Tables A7_2_1-6 and A7_2_1-7 below.
4.4	**Degradation product(s)**	Up to three minor degradation products were detected by TLC in all soils except the sand soil. One such component (designated B) remained on the origin and accounted for 2.99 – 4.76% of extractable radioactivity in aged soils and 1.58% in leached soils; the chromatographic properties of this component were similar to those of 4-hydroxycoumarin. A second minor degradation product (designated A) accounted for 0.98 – 2.61% of extractable radioactivity in aged and leached soils. A third minor degradation product (designated C) was observed only in aged soils (1.15 – 1.36% of extractable radioactivity). See Tables A7_2_1-2 and A7_2_1-4 below.
5	**APPLICANT'S SUMMARY AND CONCLUSION**	
5.1	**Materials and**	Test substance: [^{14}C]-Brodifacoum radiolabelled uniformly in the

	methods	benzene ring of the hydroxy coumarin moiety, Batch no. ICIA0581; Ref No. 91J13; Non-radiolabelled Brodifacoum reference no: ASY403. Purity: [^{14}C]-Brodifacoum: radiochemical purity of 96% and specific activity 925 MBq/mmol; Non-radiolabelled brodifacoum: 97.7%. Guidelines: EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 163-1. The leaching characteristics of aged soil residues of [^{14}C]-Brodifacoum were investigated in four soil types (sandy clay loam, silty clay, clay and sand). [^{14}C]-Brodifacoum was applied to soil (50 g dry weight) at a nominal application rate of 0.4 µg/g and incubated under aerobic conditions at *ca.* 75% of moisture holding capacity (at 0.33 bar) and 21 +/- 2 °C for 30 days. Evolved [$^{14}CO_2$] was trapped and quantified throughout the ageing period. At the end of ageing period, 2 samples of each soil type containing aged [^{14}C]-Brodifacoum were extracted with dichloromethane:methanol (4:1, v/v). The extracts were analysed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Two samples of each aged soil were transferred to the top of cylindrical glass column containing the corresponding saturated soil type. The columns of soil were 30 cm deep with a diameter of 5 cm. Each column was leached with 0.01 M aqueous calcium chloride (*ca.* 1000 ml; equivalent to *ca.* 51 cm depth) over a 2 day period. The radioactivity in leachates and soil columns (5 cm sections) was quantified.
5.2	**Results and discussion**	Total recoveries of radioactivity from aged soils were 94.85 – 96.25% of radioactivity applied to soils. Evolution of $^{14}CO_2$ during the 30 day ageing period was low (*ca.* 1 – 5% of applied radioactivity). Chromatographic analysis of aged soil extracts indicated that [^{14}C]-Brodifacoum was the only major component in all soils. After leaching of the aged residues, most of the radioactivity applied to soils (*ca.* 81 – 87%) was recovered in the top segment of each column. This segment consisted of soil which was transferred to the top of the column at the end of the ageing period. A small proportion (*ca.* 3%) of the applied radioactivity was recovered from the second 5 cm segment of the sand column. No detectable levels of ^{14}C residues were found in leachates, the bottom 25 cm of the sand soil columns, or in the soil columns from the other three soil types, indicating that [^{14}C]-Brodifacoum was effectively immobile in all soils. Total recoveries of applied radioactivity from leached soils, excluding any evolved $^{14}CO_2$, were 86.17 – 94.46%. The top soil segment from each soil column was extracted and chromatographed as described for the aged soils. The chromatographic profiles were very similar to those obtained for aged soil extracts, with brodifacoum being the only major component. With the exception of the sand soil, extractability from leached soils was lower than from aged soils, possibly due to greater adsorption of [^{14}C]-Brodifacoum to soil under saturated conditions.
5.2.1	Degradation products (% of a.s.)	Up to three minor degradation products (*ca.* 1 – 5% of extractable radioactivity) were detected by TLC and these were not identified.
5.3	**Conclusion**	The results from this study indicated that [^{14}C]-Brodifacoum would not be expected to leach in soil and that no mobile degradation products were produced.
5.3.1	Reliability	1
5.3.2	Deficiencies	No.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_2_1-1: Classification and physico-chemical properties of soils

	Soil 1	Soil 2	Soil 3	Soil 4
Soil name	18 Acres	Wisborough Green	Pickett Piece	Barassie
Supplier and date	ICI Agrochemicals, 16 January 1992	ICI Agrochemicals, 16 January 1992	ICI Agrochemicals, 16 January 1992	Scottish Agricultural College, Auchincruvie, Scotland, 21 February 1992
pH (water)	6.7	5.8	6.0	5.2
% Organic matter	4.1	5.5	5.1	0.54
Cation exchange capacity (me/100 g)	10.11	10.92	20.24	1.75
% Moisture at 0.33 Bar	23.23	39.84	36.11	4.10
% Sand	63.7	11.5	40.0	97.4
% Silt	15.8	48.0	19.3	0.6
Classification*	Sandy Clay Loam	Silty Clay	Clay	Sand

*** Classification according to:**

- Sand = 2 – 0.050 mm
- Silt = 0.050 – 0.002 mm
- Clay = <0.002 mm

A7_2_1-2: TLC Radioanalysis of Aged Soil Samples

Soil Name /Type	Mean % Radioactivity On The TLC Plate						
	Brodifacoum			Degradation Products			
	Cis	*Trans*	Total	A	B	C	Other*
18 Acres / Sandy Clay Loam	46.96	44.76	91.72	1.26	3.66	1.35	2.03
Wisborough Green / Silty Clay	49.77	39.02	88.79	2.61	4.76	1.36	2.49
Pickett Piece / Clay	45.78	45.65	91.43	2.00	2.99	1.15	2.45
Barassie / Sand	41.37	55.51	96.88	ND	ND	ND	3.13

*** Background radioactivity on chromatogram**

ND = Not detected

A7_2_1-3: HPLC Radioanalysis of Aged Soil Samples

Soil Name /Type	Mean % Radioactivity in HPLC Peak			
	Brodifacoum			Other*
	Cis	*Trans*	Total	
18 Acres / Sandy Clay Loam	51.14	48.13	99.27	0.74
Wisborough Green / Silty Clay	51.18	47.64	98.82	1.19
Pickett Piece / Clay	51.43	48.58	100.00	0.00
Barassie / Sand	42.88	56.01	98.89	1.11

*** Background radioactivity on chromatogram**

ND = Not detected

Tables A7_2_1-4: TLC Radioanalysis of Soil Extracts From Top Soil Column Segments

Soil Name /Type	Mean % Radioactivity On The TLC Plate						
	Brodifacoum			Degradation Products			
	Cis	*Trans*	Total	A	B	C	Other*
18 Acres / Sandy Clay Loam	50.66	39.42	90.08	2.15	3.89	ND	3.89
Wisborough Green / Silty Clay	47.87	38.25	86.12	2.53	5.21	ND	6.16
Pickett Piece / Clay	44.36	47.00	91.36	0.98	1.58	ND	6.09
Barassie / Sand	41.06	51.39	92.45	ND	ND	ND	7.56

*** Background radioactivity on chromatogram**

ND = Not detected

A7_2_1-5: HPLC Radioanalysis of Soil Extracts From Top Soil Column Segments

Soil Name /Type	Mean % Radioactivity in HPLC Peak			
	Brodifacoum			Other*
	Cis	*Trans*	Total	
18 Acres / Sandy Clay Loam	50.50	46.92	97.41	2.59
Wisborough Green / Silty Clay	53.74	44.61	98.35	1.66
Pickett Piece / Clay	51.73	48.27	100.00	0.00
Barassie / Sand	49.12	50.10	99.22	0.79

*** Background radioactivity on chromatogram**

ND = Not detected

A7_2_1-6: Recoveries Of Radioactivity From Soils Following Ageing Procedure

Soil Name /Type	Recovery Expressed As Mean % Of Radioactivity Applied To Soils				
	Soil Extract	Soil Residue	Ethanolamine Traps	Flask Wash	Total
18 Acres / Sandy Clay Loam	85.92	7.38	2.94	0.01	96.25
Wisborough Green / Silty Clay	77.69	11.76	5.38	0.02	94.85
Pickett Piece / Clay	83.50	8.57	3.05	0.02	95.14
Barassie / Sand	91.66	3.16	0.90	0.03	95.75

A7_2_1-7: Recoveries Of Radioactivity From Soils Following Leaching of Aged Residues

Soil Name /Type	Recovery Expressed As Mean % Of Radioactivity Applied To Soils					
	Soil Column	Leachates	Ethanol-amine Traps	Flask Wash	Column Wash	Total
18 Acres / Sandy Clay Loam	87.59	0.04	3.63	0.31	0.13	91.70
Wisborough Green / Silty Clay	82.90	0.17	5.25	0.33	0.24	88.87
Pickett Piece / Clay	82.61	0.11	2.72	0.38	0.37	86.17
Barassie / Sand	91.82	0.28	1.10	0.64	0.63	94.46

Doc IIIA/Section 7. BPD Data Set IIIA/Annex Point 7.31	***Phototransformation in air (estimation method), including identification of breakdown products***	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [] **Limited exposure** []	**Technically not feasible** [] **Other justification** [✓] **Scientifically unjustified** []	
Detailed justification:	[redacted]	
Undertaking of intended data submission [[✓]	*We will attempt to conduct such a study-deadline mid 2005*	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[redacted]	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIA / Section A7.4.1.1

BPD Data Set IIA / Annex Point VII.7.1

Acute toxicity to fish

Rainbow trout (*Oncorhynchus mykiss*)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1976). Brodifacoum: Determination of Acute Toxicity of PP581 to Rainbow Trout (*Salmo gairdnerii*). ICI Brixham Laboratory Report Number: BL/B/1758 (unpublished) [redacted].	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, the study pre-dates guidelines. However, the study was conducted in accordance with the general scientific principles of OECD (1992) Guideline 203 and EC (1992) Guideline C1, within the limitations of the OECD Draft Guidance Document on Aquatic Toxicity Testing of Difficult substances (March 1999).	
2.2	**GLP**	No. Study pre-dates the requirement for GLP.	
2.3	**Deviations**	No.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum.	
3.1.1	Lot/Batch number	Information not given in study report.	
3.1.2	Specification	As given in section 2.	X
3.1.3	Purity	As given in section 2 of Doc IIIA.	X
3.1.4	Composition of Product	Not applicable.	
3.1.5	Further relevant properties	1) Water solubility study: brodifacoum is insoluble/sparingly soluble in water with the following solubility values determined using EPA CG-1510 Guideline: pH 5.2: 0.0038 mg/l pH 7.4: 0.24 mg/l pH 9.3: 10 mg/l (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [redacted]	X

		2) Aqueous hydrolysis study: ➢ [^{14}C]-brodifacoum hydrolysis was insignificant with DT_{50} values estimated by extrapolation where possible: 173 days at pH5, 300 days at pH 7 (DT_{50} estimation not possible at pH9). (Reference: Mathis SMG, Benner JP and Skidmore MW (1995). Brodifacoum: Aqueous Hydrolysis in pH 5, pH 7 and pH 9 Solutions at 25°C. Zeneca Agrochemicals Report Number RJ1927B [F4.1/01]). ➢ [^{14}C]-brodifacoum was determined to be hydrolytically stable. (Reference: Jackson R, Priestley I, Hall BE (1991). The Determination of the Hydrolytic Stability of [^{14}C]-Brodifacoum. Inveresk Research International Report Number 8330 [F4.1/03]). 3) Brodifaccoum is stable under normal storage conditions. 4) Vapour pressure is $<<10^{-9}$kPa ($<<10^{-8}$mmHg or $<< 10^{-11}$atm) and is therefore negligable. (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]).	
3.1.6	Method of analysis	A calibration curve for analysis of brodifacoum by fluorimetry was prepared using a range of standard solutions prepared by dilution with distilled water, of a concentrated brodifacoum solution in DMSO. A Perkin Elmer MPF-3 Scanning Spectrofluorimeter equipped with a high pressure xenon lamp was used. The excitation and emmission wavelengths used were 315 nm and 380 nm respectively.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Dimethyl sulphoxide (DMSO) was used to prepare the stock concentrates, using a level of <10 mg/l. It was stated in the study report that at this concentration, DMSO is very unlikely to have any effect on the test organisms. The literature reports a 96 hour LC_{50} value of DMSO to rainbow trout of 33000 mg/l *[Willford WA. 'Toxicity of Dimethyl Sulphoxide (DMSO) to fish.' Investigations in Fish Control No. 20. United States Department of the Interior Resource Publication 37. Washington DC, April 1967].* See table A7_4_1_3-1.	X
3.3	**Reference substance**	No.	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_1-2.	
3.4.2	Test organisms	See table A7_4_1_1-3.	X
3.4.3	Test system	See table A7_4_1_1-4.	X

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Acute toxicity to fish

Rainbow trout (*Oncorhynchus mykiss*)

3.4.4	Test conditions	See table A7_4_1_1-5.	
3.4.5	Duration of the test	96 h.	
3.4.6	Test parameter	Mortality.	
3.4.7	Sampling	The fish were observed at regular intervals up to 96 hours after the start of the test. 96 hours was the planned end of the test, but it was reported that observations were continued at the lower dose levels where there were symptoms of toxicity noted but no deaths. The reason stated for doing this was to observe if recovery from symptoms was possible when only fresh water was supplied.	
3.4.8	Monitoring of TS concentration	Yes, analytical samples were taken at 12 hour intervals after the start of the test to determine the concentration of test material in the test solution. From these analyses, the individual and mean measured concentration of brodifacoum in the test solutions were reported.	X
3.4.9	Statistics	In the study, the 24, 48 and 96 hour LC_{50} values were determined by direct reading of the geometric mean survival period/concentration graph. However, the data from this study was then reprocessed (1985) and reported as a supplement to the original report, to give the LC_{50} values calculated from the mean measured concentrations of the test substance using either the probit method *[Ref 1]* or the binomial test *[Ref 2]*: *Ref 1: Finney DJ (1971). Probit Analysis. Third Edition. Cambridge University Press.* *Ref 2: Stephan CE (1977). Methods for calculating an LC50. In: Aquatic Toxicology and Hazard Evalution. Mayer FL, Hamelink JL, Editors. Proceedings 1st Annual Symposium on Aquatic Toxicology ASTM 634 p65-84, 1977.* The results given here are those from the recalculated values.	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**		

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Acute toxicity to fish

Rainbow trout (*Oncorhynchus mykiss*)

4.2.1	Initial concentrations of test substance	The nominal concentrations of brodifacoum in the test solutions were: 0.010, 0.015, 0.022, 0.033, 0.047, 0.068, 0.10, 0.15 and 0.22 mg/l. The mean actual concentrations of brodifacoum in the test solutions were: 0.0092, 0.0110, 0.0215, 0.023, 0.029, 0.055, 0.103, 0.125 and 0.182 mg/l.	X

4.2.2 Actual concentrations of test substance

Nominal exposure conc^n	**0.010 (mg/l)**	**0.015 (mg/l)**	**0.022 (mg/l)**	**0.033 (mg/l)**	**0.047 (mg/l)**	**0.068 (mg/l)**	**0.10 (mg/l)**	**0.15 (mg/l)**	**0.22 (mg/l)**
Measured Conc^n at 0.5 h	-	-	-	-	-	0.075	0.155	0.13	0.19
Measured Conc^n at 12 h	-	-	-	-	-	0.075	0.100	0.15	0.21
Measured Conc^n at 24 h	0.016	0.010	0.021	0.014	0.039	0.035	0.055	0.095	0.145
Measured Conc^n at 48 h	0.006	0.009	0.022	0.021	0.018	0.035	-	-	-
Measured Conc^n at 72 h	0.012	0.015	0.022	0.029	0.033	-	-	-	-
Measured Conc^n at 96 h	0.003	0.010	0.021	0.026	0.024	-	-	-	-
Mean measured conc^n	0.0092	0.110	0.0215	0.023	0.029	0.055	0.103	0.125	0.182

4.2.3	Effect data (Mortality)	LC_{50} values: 0.13 mg/l at 24 h (95% confidence interval: 0.12-0.15); 0.08 mg/l at 48 h (99% confidence interval: 0.06-0.10); 0.05 mg/l at 72 h (95% confidence interval: 0.03-0.10); 0.04 mg/l at 96 h (99% confidence interval: 0.03-0.06); See table A7_4_1_1-7 below for the mortality results.	X
4.2.4	Concentration / response curve	A graph of the concentration-Geometric Mean Survival Period values using logarithmic scales is given in the study report.	
4.2.5	Other effects		
4.3	**Results of controls**		X

4.3.1	Number/ percentage of animals showing adverse effects	No mortalities were observed throughout the test period.	
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**	The study pre-dates guidelines. However, the study was conducted in accordance with the general scientific principles of OECD (1992) Guideline 203 and EC (1992) Guideline C1, within the limitations of the OECD Draft Guidance Document on Aquatic Toxicity Testing of Difficult substances (March 1999). The test system was a continuous flow through type with groups of ten Rainbow Trout *(Oncorhynchus mykiss)* exposed to brodifacoum at concentrations of 0, 0.01, 0.015, 0.022, 0.033, 0.047, 0.068, 0.10, 0.15 and 0.22 mg/l for up to 96 hours.	X
5.2	**Results and discussion**	There was 100% mortality at concentrations of 0.068, 0.10, 0.15 and 0.22 mg/l. At the lower concentrations, there were no mortalities, but observation of the fish included darkening of colour, lethargy and collecting under the lid of the vessels. There were no symptoms observed at the two lowest concentrations of 0.01 and 0.15 mg/l.	
5.2.1	LC_0	0.047 mg/l	X
5.2.2	LC_{50}	0.13 mg/l at 24 h (95% confidence interval: 0.12-0.15); 0.08 mg/l at 48 h (99% confidence interval: 0.06-0.10); 0.05 mg/l at 72 h (95% confidence interval: 0.03-0.10); 0.04 mg/l at 96 h (99% confidence interval: 0.03-0.06).	X
5.2.3	LC_{100}	0.068 mg/l	X
5.3	**Conclusion**	The validity criteria can be considered as fulfilled (see Table A7_4_1_1-8).	X
5.3.1	Other Conclusions		
5.3.2	Reliability	2	
5.3.3	Deficiencies	No	X

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Acute toxicity to fish

Rainbow trout (*Oncorhynchus mykiss*)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
5.1 Summary and Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Not stated in study report, but presume that stirring would have been used to mix the solution.
Vehicle	Dimethyl sulphoxide (DMSO).
Concentration of vehicle	<10 mg/l of DMSO.
Vehicle control performed	Information not given in study report, but the published 96 hour LC_{50} of DMSO to Rainbow Trout is approximately 33000 mg/l *[ref: Willford WA. 'Toxicity of Dimethyl Sulphoxide (DMSO) to fish.' Investigations in Fish Control No 20. United States Department of the Interior Resource Publication 37, Washington DC, April 1967].*
Other procedures	No.

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Not stated in study report.
Alkalinity	Not stated in study report.
Hardness	53 – 61 mg $CaCO_3$/l.
pH	7.60 - 7.80
Oxygen content	Mean of 89.0 - 97.8%
Conductance	Not stated in study report.
Holding water different from dilution water	Not stated in study report.

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Rainbow Trout *(Oncorhynchus mykiss*, previously known as *Salmo gairdnerii).*
Source	Samaki Trout Farm, Wiltshire, UK.
Wild caught	No.
Age/size	Between 2.9 – 5.7 cm in length.
Kind of food	Not stated in study report.

Amount of food	Data not available in report.
Feeding frequency	Data not available in report.
Pretreatment	5 day acclimation period.
Feeding of animals during test	Not stated in study report.

Table A7_4_1_1-4: Test system

Criteria	**Details**
Test type	Continuous flow-through.
Renewal of test solution	Renewal at a rate of 200 ml/minute, with a complete exchange of the test solution occurring within 3 hours.
Volume of test vessels	20 litre glass vessels.
Volume/animal	2 litres per fish.
Number of animals/vessel	10
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	Information not given in study report.

Table A7_4_1_1-5: Test conditions

Criteria	**Details**
Test temperature	13 °C
Dissolved oxygen	Remained above or at 87% air saturation.
pH	The pH was measured twice daily using an Electronic Instruments Model 23A pH meter.
Adjustment of pH	No.
Aeration of dilution water	Information not given in study report.
Intensity of irradiation	Information not given in study report.
Photoperiod	Information not given in study report.

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (nominal) [mg/l]	**Mortality**							
	Number				**Percentage**			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
0				0				0
0.01				0				0
0.015				0				0
0.022				0				0
0.033				0				0
0.047				0				0
0.068			10				100	
0.10		10				100		
0.15		10				100		
0.22	10				100			
Temperature [°C]	13 °C							
pH	7.60 - 7.80							
Oxygen [mg/l]	Remained above or at 87% air saturation							

Table A7_4_1_1-6 a (version of the Rapporter MS): Mortality data

Test-Substance Conc. (nominal) [mg/l]	**Test-Substance Conc. (measured) [mg/l]**	**Mortality**							
		Number				**Percentage**			
		24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
		Series II				Series II			
0.01	0.0092				0				0
0.015	0.011				0				0
0.022	0.0215				0				0
0.033	0.023				0				0
0.047	0.029				0				0
		Series I				Series I			
0.047	Not provided (problem with pump)	Not available				Not available			
0.068	0.055			6				60	100
0.10	0.103		10				100		
0.15	0.125	4	10			40	100		
0.22	0.182	10				100			

Table A7_4_1_1-7: Effect data

	48 h [mg/l]	95 % c.l.	96 h [mg/l][1]	95 % c.l.
LC_0	0.047	-	0.047	-
LC_{50}	0.08	0.06-0.10 (99% confidence internal)	0.04	0.03-0.06 (99% confidence interval)
LC_{100}	0.10		-	

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

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

Table A7_4_1_1-8a (Revised by the Rapporteur MS) : Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%		**Information lacking**
Concentration of dissolved oxygen in all test vessels > 60% saturation	**Yes**	
Concentration of test substance ≥80% of initial concentration during test	**No, but results based on measured concentrations**	

Criteria for poorly soluble test substances	**Yes, brodifacoum is categorised as a poorly soluble substance**	

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BPD Data Set IIA / Annex Point VII.7.2

Acute toxicity to *Daphnia magna* (48 hour static limit test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	Knight, B. (2000). Brodifacoum: Determination of Acute Toxicity to *Daphnia* (48 h, Static, Limit Test). Inveresk Research Report Number 19032 (unpublished) [redacted]	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted as limit test over a 48 h period, in accordance with OECD (1984) Guideline 202 Part 1 and EC (1992) Guideline C2, within the limitations of the OECD Draft Guidance Document on Aquatic Toxicity Testing of Difficult Substances (March 1999), whereby the test is not conducted at concentrations greater than the solubility of the test material.	
2.2	**GLP**	Yes.	
2.3	**Deviations**	Yes, there was a protocol deviation to the preliminary range finding test, where the addition rate of brodifacoum to the *Daphnia* medium was greater than specified in the protocol (about 1000 times the solubility limit, not 100 times). This protocol deviation was necessary to accurately weigh the small amount of test material required to prepare the test solutions and does not affect the validity of the study.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum	
3.1.1	Lot/Batch number	Batch no: 49.	
3.1.2	Specification	As given in section 2.	
3.1.3	Purity	As given in section 2 of Doc IIIA.	
3.1.4	Composition of Product	Not applicable.	
3.1.5	Further relevant properties	1) Water solubility study: brodifacoum is insoluble/sparingly soluble in water with the following solubility values determined using EPA CG-1510 Guideline: pH 5.2: 0.0038 mg/l	

		pH 7.4: 0.24 mg/l pH 9.3: 10 mg/l (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]). 2) Aqueous hydrolysis study: ➢ [^{14}C]-brodifacoum hydrolysis was insignificant with DT_{50} values estimated by extrapolation where possible: 173 days at pH5, 300 days at pH 7 (DT_{50} estimation not possible at pH9). (Reference: Mathis SMG, Benner JP and Skidmore MW (1995). Brodifacoum: Aqueous Hydrolysis in pH 5, pH 7 and pH 9 Solutions at 25°C. Zeneca Agrochemicals Report Number RJ1927B [F4.1/01]). ➢ [^{14}C]-brodifacoum was determined to be hydrolytically stable. (Reference: Jackson R, Priestley I, Hall BE (1991). The Determination of the Hydrolytic Stability of [^{14}C]-Brodifacoum. Inveresk Research International Report Number 8330 [F4.1/03]). 3) Brodifaccoum is stable under normal storage conditions. 4) Vapour pressure is $<<10^{-9}$kPa ($<<10^{-8}$mmHg or $<<10^{-11}$atm) and is therefore negligable. (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]).	
3.1.6	Method of analysis	Brodifacoum was determined using HPLC with fluorescence detection, with external standardisation for quantitation purposes.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Test solutions were prepared by stirring the test material in the dilution medium (*Daphnia* synthetic medium) in excess for a period of about 48 hours. The method of preparation was selected to maximise the concentration in solution but minimise the excess of undissolved test material which might have a physical effect on *Daphnia* survival. The limit test was conducted with the filtrate of the saturated solution following a range finding study. See Table A7_4_1_2-1.	X
3.3	**Reference substance**	No.	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_2-2.	X
3.4.2	Test organisms	*Daphnia magna:* see table A7_4_1_2-3.	

3.4.3	Test system	See table A7_4_1_2-4.	
3.4.4	Test conditions	See table A7_4_1_2-5.	
3.4.5	Duration of the test	48 hours.	
3.4.6	Test parameter	Immobility.	
3.4.7	Sampling	Samples were taken for analysis of brodifacoum at the beginning and end of the limit test (0 and 48 h). The temperature, pH, conductivity and dissolved oxygen concentration were measured at 0, 24 and 48 h, in 2 of the 4 replicate test vessels.	
3.4.8	Monitoring of TS concentration	Yes, Samples were taken for analysis of brodifacoum at the beginning and end of the limit test (0 and 48 h).	
3.4.9	Statistics		
		4 RESULTS	
4.1	**Limit Test**	Performed.	
4.1.1	Concentration	Saturated solution of brodifacoum (i.e. maximum achievable concentration).	X
4.1.2	Number/ percentage of animals showing adverse effects	24 h: - 0 animals at 0% of filtrate from saturated solution (control); - 0 animals at 100% of filtrate from saturated solution. 48 h: - 0 animals at 0% of filtrate from saturated solution (control); - 0 animals at 100% of filtrate from saturated solution.	
4.1.3	Nature of adverse effects		
4.2	**Results test substance**		X
4.2.1	Initial concentrations of test substance		
4.2.2	Actual concentrations of test substance		
4.2.3	Effect data (Immobilisation)		
4.2.4	Concentration /		

	response curve		
4.2.5	Other effects		
4.3	**Results of controls**	There were no adverse effects on the control organisms. See Section 4.1.2 above in results of limit test.	
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**	The test was conducted as limit test over a 48 h period, in accordance with OECD (1984) Guideline 202 Part 1 and EC (1992) Guideline C2, within the limitations of the OECD Draft Guidance Document on Aquatic Toxicity Testing of Difficult Substances (March 1999), whereby the test is not conducted at concentrations greater than the solubility of the test material. The test system was static without renewal of the test media and used *Dapnia magna* as the test organism.	
5.2	**Results and discussion**	The test substance, brodifacoum, has a low water solubility and so the test was conducted with a saturated solution. No vehicle was used. Throughout the study period all test solutions appeared clear and colourless and no precipitation of the test material was evident. Analysis of samples taken at 0 and 48 h during the test indicated that prior to filtration >10 µg/l of brodifacoum was suspended in the water. After filtration, concentrations of brodifacoum were below the limit of detection (<0.54 µg/l).	
5.2.1	EC_0	48 h : >maximum solubility in water (under conditions of the test).	
5.2.2	EC_{50}	48 h : > maximum solubility in water (under conditions of the test).	
5.2.3	EC_{100}		
5.3	**Conclusion**	The results indicate that brodifacoum is not toxic to *Daphnia magna* at the maximum solubility of brodifacoum in the *Daphnia* medium under the conditions of the test.	X
		The No Observed Effect Concentration (NOEC) was concluded to be equal to the limit of solubility (as prepared using a nominal 2 mg/l suspension) under the conditions of the test.	
		The validity criteria as given in Table A7_4_1_2-8 can be considered as fulfilled.	X

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BPD Data Set IIA / Annex Point VII.7.2

Acute toxicity to *Daphnia magna* (48 hour static limit test)

5.3.1	Reliability	2
5.3.2	Deficiencies	No.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Guidelines and quality assurance	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes, the brodifacoum was added to the dilution medium in excess and stirred on a magnetic stirrer for 48 h.
Vehicle	No.
Concentration of vehicle	
Vehicle control performed	
Other procedures	

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Deionised water.
Alkalinity	Data not given in report.
Hardness	200 mg/l Ca CO_3.
pH	7.8
Ca / Mg ratio	Data not given in report.
Na / K ratio	Data not given in report.
Oxygen content	82 – 86 % dissoved oxygen
Conductance	0.56 – 0.58 mS
Holding water different from dilution water	No.

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	Data not given in report.
Source	The *Daphnia magna* used in the study were bred within the testing laboratory.
Age	Between 6 and 24 h old.
Breeding method	The *Daphnia magna* were bred by acyclical parthenogenesis.
Kind of food	Axenic cultures of *Selenastrum capricornutum.*
Amount of food	Data not given in report.
Feeding frequency	Data not given in report.
Pretreatment	Data not given in report.
Feeding of animals during test	No.

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	No.
Volume of test vessels	200 ml.
Volume/animal	100 ml of test solution per vessel was used (20 ml/*Dapnia*).
Number of animals/vessel	5 animals per test vessel.
Number of vessels/ concentration	4 replicate dishes at each concentration. Nominal brodifacoum concentrations 0 and 100 % of saturated solution.
Test performed in closed vessels due to significant volatility of TS	No, but the glass crystallising dishes used were covered with perspex lids with ventilation holes to prevent dust contamination and evaporation loss.

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	0 h: 20.5 – 20.8 °C; 24 h: 20.8 – 21.0 °C. 48 h: 20.7 – 21.1 °C.
Dissolved oxygen	0 h: 82 – 86 % dissolved oxygen; 24 h: 83 – 85 % dissolved oxygen; 48 h: 83 – 86 % dissolved oxygen.

pH	0 h: 7.9 – 8.0; 24 h: 8.0 – 8.1; 48 h: 7.8 – 7.9.
Adjustment of pH	No.
Aeration of dilution water	Yes, the medium was aerated for > 2 h before use.
Quality/Intensity of irradiation	Illumination was provided by artificial daylight fluorescent tubes.
Photoperiod	16 h.

Table A7_4_1_2-6: Immobilisation data

Brodifacoum Concentration [mg/l]		**Immobile *Daphnia***				**Parameter**		
Nominal	**Measured (mean of 0 h and 48 h values)**	**Number**		**Percentage (%)**		**Oxygen [% air saturation]**	**pH**	**Tempera-ture [°C]**
		24 h	**48 h**	**24 h**	**48 h**	**48 h**	**48 h**	**48 h**
2 mg/l (suspensio n)	<0.54 µg/l	0	0	0	0	83 – 86 % dissolved oxygen.	7.8 – 7.9	20.7 – 21.1

Table A7_4_1_2-7: Effect data

	EC_{50}	**95 % c.l.**	**EC_0**	**EC_{100}**
24 h [mg/l]				
48 h [mg/l]	> maximum solubility in water (under conditions of the test)		> maximum solubility in water (under conditions of the test)	

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

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

Criteria for poorly soluble test substances ergänzen	**Yes, brodifacoum is categorised as a poorly soluble substance**	

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Acute toxicity to *Daphnia magna* (48 hour static limit test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	Knight, B. (2000). Brodifacoum: Determination of Acute Toxicity to *Daphnia* (48 h, Static, Limit Test). Inveresk Research Report Number 19032 (unpublished) [BR-959-0081].	
		[redacted].	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted as limit test over a 48 h period, in accordance with OECD (1984) Guideline 202 Part 1 and EC (1992) Guideline C2, within the limitations of the OECD Draft Guidance Document on Aquatic Toxicity Testing of Difficult Substances (March 1999), whereby the test is not conducted at concentrations greater than the solubility of the test material.	
2.2	**GLP**	Yes.	
2.3	**Deviations**	Yes, there was a protocol deviation to the preliminary range finding test, where the addition rate of brodifacoum to the *Daphnia* medium was greater than specified in the protocol (about 1000 times the solubility limit, not 100 times). This protocol deviation was necessary to accurately weigh the small amount of test material required to prepare the test solutions and does not affect the validity of the study.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum	
3.1.1	Lot/Batch number	Batch no: 49.	
3.1.2	Specification	As given in section 2.	
3.1.3	Purity	As given in section 2 of Doc IIIA.	
3.1.4	Composition of Product	Not applicable.	
3.1.5	Further relevant properties	1) Water solubility study: brodifacoum is insoluble/sparingly soluble in water with the following solubility values determined using EPA CG-1510 Guideline:	

		pH 5.2: 0.0038 mg/l pH 7.4: 0.24 mg/l pH 9.3: 10 mg/l (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]). 2) Aqueous hydrolysis study: ➢ [^{14}C]-brodifacoum hydrolysis was insignificant with DT_{50} values estimated by extrapolation where possible: 173 days at pH5, 300 days at pH 7 (DT_{50} estimation not possible at pH9). (Reference: Mathis SMG, Benner JP and Skidmore MW (1995). Brodifacoum: Aqueous Hydrolysis in pH 5, pH 7 and pH 9 Solutions at 25°C. Zeneca Agrochemicals Report Number RJ1927B [F4.1/01]). ➢ [^{14}C]-brodifacoum was determined to be hydrolytically stable. (Reference: Jackson R, Priestley I, Hall BE (1991). The Determination of the Hydrolytic Stability of [^{14}C]-Brodifacoum. Inveresk Research International Report Number 8330 [F4.1/03]). 3) Brodifaccoum is stable under normal storage conditions. 4) Vapour pressure is $<<10^{-9}$kPa ($<<10^{-8}$mmHg or $<<10^{-11}$atm) and is therefore negligable. (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]).	
3.1.6	Method of analysis	Brodifacoum was determined using HPLC with fluorescence detection, with external standardisation for quantitation purposes.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Test solutions were prepared by stirring the test material in the dilution medium (*Daphnia* synthetic medium) in excess for a period of about 48 hours. The method of preparation was selected to maximise the concentration in solution but minimise the excess of undissolved test material which might have a physical effect on *Daphnia* survival. The limit test was conducted with the filtrate of the saturated solution following a range finding study. See Table A7_4_1_2-1.	X
3.3	**Reference substance**	No.	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_2-2.	X

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Acute toxicity to *Daphnia magna* (48 hour static limit test)

3.4.2	Test organisms	*Daphnia magna:* see table A7_4_1_2-3.	
3.4.3	Test system	See table A7_4_1_2-4.	
3.4.4	Test conditions	See table A7_4_1_2-5.	
3.4.5	Duration of the test	48 hours.	
3.4.6	Test parameter	Immobility.	
3.4.7	Sampling	Samples were taken for analysis of brodifacoum at the beginning and end of the limit test (0 and 48 h). The temperature, pH, conductivity and dissolved oxygen concentration were measured at 0, 24 and 48 h, in 2 of the 4 replicate test vessels.	
3.4.8	Monitoring of TS concentration	Yes, Samples were taken for analysis of brodifacoum at the beginning and end of the limit test (0 and 48 h).	
3.4.9	Statistics		
		4 RESULTS	
4.1	**Limit Test**	Performed.	
4.1.1	Concentration	Saturated solution of brodifacoum (i.e. maximum achievable concentration).	X
4.1.2	Number/ percentage of animals showing adverse effects	24 h: - 0 animals at 0% of filtrate from saturated solution (control); - 0 animals at 100% of filtrate from saturated solution. 48 h: - 0 animals at 0% of filtrate from saturated solution (control); - 0 animals at 100% of filtrate from saturated solution.	
4.1.3	Nature of adverse effects		
4.2	**Results test substance**		X
4.2.1	Initial concentrations of test substance		
4.2.2	Actual concentrations of test substance		
4.2.3	Effect data (Immobilisation)		

4.2.4	Concentration / response curve		
4.2.5	Other effects		
4.3	**Results of controls**	There were no adverse effects on the control organisms. See Section 4.1.2 above in results of limit test.	
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**	The test was conducted as limit test over a 48 h period, in accordance with OECD (1984) Guideline 202 Part 1 and EC (1992) Guideline C2, within the limitations of the OECD Draft Guidance Document on Aquatic Toxicity Testing of Difficult Substances (March 1999), whereby the test is not conducted at concentrations greater than the solubility of the test material. The test system was static without renewal of the test media and used *Dapnia magna* as the test organism.	
5.2	**Results and discussion**	The test substance, brodifacoum, has a low water solubility and so the test was conducted with a saturated solution. No vehicle was used. Throughout the study period all test solutions appeared clear and colourless and no precipitation of the test material was evident. Analysis of samples taken at 0 and 48 h during the test indicated that prior to filtration >10 µg/l of brodifacoum was suspended in the water. After filtration, concentrations of brodifacoum were below the limit of detection (<0.54 µg/l).	
5.2.1	EC_0	48 h : >maximum solubility in water (under conditions of the test).	
5.2.2	EC_{50}	48 h : > maximum solubility in water (under conditions of the test).	
5.2.3	EC_{100}		
5.3	**Conclusion**	The results indicate that brodifacoum is not toxic to *Daphnia magna* at the maximum solubility of brodifacoum in the *Daphnia* medium under the conditions of the test.	X
		The No Observed Effect Concentration (NOEC) was concluded to be equal to the limit of solubility (as prepared using a nominal 2 mg/l suspension) under the conditions of the test.	
		The validity criteria as given in Table A7_4_1_2-8 can be considered as	X

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BPD Data Set IIA / Annex Point VII.7.2

Acute toxicity to *Daphnia magna* (48 hour static limit test)

		fulfilled.
5.3.1	Reliability	2
5.3.2	Deficiencies	No.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Guidelines and quality assurance	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes, the brodifacoum was added to the dilution medium in excess and stirred on a magnetic stirrer for 48 h.
Vehicle	No.
Concentration of vehicle	
Vehicle control performed	
Other procedures	

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Deionised water.
Alkalinity	Data not given in report.
Hardness	200 mg/l Ca CO_3.
pH	7.8
Ca / Mg ratio	Data not given in report.
Na / K ratio	Data not given in report.
Oxygen content	82 – 86 % dissoved oxygen
Conductance	0.56 – 0.58 mS
Holding water different from dilution water	No.

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	Data not given in report.
Source	The *Daphnia magna* used in the study were bred within the testing laboratory.
Age	Between 6 and 24 h old.
Breeding method	The *Daphnia magna* were bred by acyclical parthenogenesis.
Kind of food	Axenic cultures of *Selenastrum capricornutum.*
Amount of food	Data not given in report.
Feeding frequency	Data not given in report.
Pretreatment	Data not given in report.
Feeding of animals during test	No.

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	No.
Volume of test vessels	200 ml.
Volume/animal	100 ml of test solution per vessel was used (20 ml/*Dapnia*).
Number of animals/vessel	5 animals per test vessel.
Number of vessels/ concentration	4 replicate dishes at each concentration. Nominal brodifacoum concentrations 0 and 100 % of saturated solution.
Test performed in closed vessels due to significant volatility of TS	No, but the glass crystallising dishes used were covered with perspex lids with ventilation holes to prevent dust contamination and evaporation loss.

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	0 h: 20.5 – 20.8 °C; 24 h: 20.8 – 21.0 °C. 48 h: 20.7 – 21.1 °C.
Dissolved oxygen	0 h: 82 – 86 % dissolved oxygen; 24 h: 83 – 85 % dissolved oxygen; 48 h: 83 – 86 % dissolved oxygen.

pH	0 h: 7.9 – 8.0; 24 h: 8.0 – 8.1; 48 h: 7.8 – 7.9.
Adjustment of pH	No.
Aeration of dilution water	Yes, the medium was aerated for > 2 h before use.
Quality/Intensity of irradiation	Illumination was provided by artificial daylight fluorescent tubes.
Photoperiod	16 h.

Table A7_4_1_2-6: Immobilisation data

Brodifacoum Concentration [mg/l]		**Immobile *Daphnia***				**Parameter**		
Nominal	**Measured (mean of 0 h and 48 h values)**	**Number**		**Percentage (%)**		**Oxygen [% air saturation]**	**pH**	**Tempera-ture [°C]**
		24 h	**48 h**	**24 h**	**48 h**	**48 h**	**48 h**	**48 h**
2 mg/l (suspensio n)	<0.54 µg/l	0	0	0	0	83 – 86 % dissolved oxygen.	7.8 – 7.9	20.7 – 21.1

Table A7_4_1_2-7: Effect data

	EC_{50}	**95 % c.l.**	**EC_0**	**EC_{100}**
24 h [mg/l]				
48 h [mg/l]	> maximum solubility in water (under conditions of the test)		> maximum solubility in water (under conditions of the test)	

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

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

Criteria for poorly soluble test substances ergänzen	**Yes, brodifacoum is categorised as a poorly soluble substance**	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 Acute toxicity to *Daphnia magna*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Report: The Toxicity to *Daphnia magna* of BRODIFACOUM Technical. W J Craig - March 2003. Chemex Environmental International Ltd report - ENV5802/120140 Acute toxicity study of test substance brodifacoum technical.	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with Access to data	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 202	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	X
3.1.1	Lot/Batch number	ECO120140	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	Minimum 99 % brodifacoum	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	HPLC	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_2-1)	
3.3	**Reference substance**	Yes	
3.3.1	Method of analysis for reference substance	Aquatic toxicity: 48 hour EC_{50} of potassium dichromate on *Daphnia magna*. Concentrations: 0.18, 0.32, 0.56, 1.0, 1.8 and 3.2 mg/	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_2-2	
3.4.2	Test organisms	See table A7_4_1_2-3	
3.4.3	Test system	See table A7_4_1_2-4	
3.4.4	Test conditions	See table A7_4_1_2-5	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 Acute toxicity to *Daphnia magna*

3.4.5	Duration of the test	48 hours	
3.4.6	Test parameter	Immobility	
3.4.7	Sampling	Test substance analysis of each concentration carried out as soon as possible after sampling at 0, 24 and 48 hours with samples being frozen until analysis	
3.4.8	Monitoring of TS concentration	Yes Start of study, before and after renewal of solutions at 24 hours, and at the end of the 48 hour exposure period.	
3.4.9	Statistics	EC_{50} determined graphically with 95% confidence limits according to the method of ToxCalc™ Version 5.0 NOEC and LOEC determined by Fisher's Exact test	X

4 RESULTS

4.1	**Limit Test**	Not performed	
4.1.1	Concentration	Not applicable	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0 (control), DMF(control),0.13, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/l	

4.2.2 Actual concentrations of test substance

mg/l Nom	**0**	**0.13**	**0.25**	**0.5**	**1.0**	**2.0**	**4.0**
0 hrs fresh	0	0.10	0.12	0.33	0.73	0.73	1.80
24 hrs aged	0	0.04	0.10	0.26	0.51	0.82	2.70
24 hrs renewed	0	0.09	0.17	0.30	0.83	1.34	-
48 hrs aged	0	0.06	0.10	0.22	0.43	0.79	-
Mean measured	*0*	*0.07*	*0.12*	*0.28*	*0.63*	*0.92*	

4.2.3	Effect data (Immobilisation)	Immobility: See table A7_4_1_2-6; EC_{50} and 95% confidence limits: See table A7_4_1_2-7	X
4.2.4	Concentration / response curve	Slope: 2.9 with 95% confidence limits 2.0 – 3.8.	
4.2.5	Other effects	None stated	
4.3	**Results of controls**	No effects	
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	Potassium dichromate at nominal concentrations 0(control), 0.18, 0.32, 0.56, 1.0, 1.8 and 3.2 mg /l	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 Acute toxicity to *Daphnia magna*

4.4.2	Results	24 hour EC_{50} = 1.3 mg/l (1.1 – 1.6 mg/l; 95% confidence limits) 48 hour EC_{50} = 0.9 mg/l (0.8 – 1.1 mg/l; 95% confidence limits) 48 hour NOEC = 0.56 mg/l 48 hour 100% mortality = 3.2 mg/l	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	OECD 202	
5.2	**Results and discussion**	Test substance is extremely insoluble in water (c.0.03 ppm), of very low vapour pressure, and is subject to rapid photolysis (half-life c.7.5 hrs)	
5.2.1	EC_0	-	
5.2.2	EC_{50}	24 hour EC_{50} = 0.78 mg/l (0.71 – 0.85 mg/l; 95% confidence limits) 48 hour EC_{50} = 0.25 mg/l (0.20 – 0.31 mg/l; 95% confidence limits)	
5.2.3	EC_{100}	-	
5.3	**Conclusion**	R51 /R53 Toxic to aquatic organisms applies. All validity criteria can be considered as fulfilled. Clear dose-response relationship shown (see table A7_4_1_2-8) LC50 is greater than the water solubility.	
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	X

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 Acute toxicity to *Daphnia magna*

Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	DMF
Concentration of vehicle	*1ml/l
Vehicle control performed	Yes
Other procedures	None

*The raw data for this study shows that the final concentration of DMF was 1ml/l in dilution water. It should be noted that this is 10 fold higher than the recommended concentration for solubilising agents for use with 'difficult substances'. Preliminary investigations showed that in order to fully dissolve the required amount of Brodifacoum a higher DMF to test material ratio was necessary. Subsequent dilution of the fully solubilised stock enabled a stable dispersion of the test material to be produced in water. These investigations were conducted prior to commencing the study and were not formally documented. The sample record documents the amounts of the test material used and the actual concentration of Brodifacoum in DMF and water used. The DMF controls for the Daphnia study provide evidence that this higher level of the solubilising agent had no adverse effects on the exposed daphnids. It was considered that the deviation from the protocol was justifiable, as it appeared to be the only way of presenting the test material in a stable and homogenous form.

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	De-chlorinated mains tap water
Alkalinity	214 mg/l
Hardness	240 mg/l $CaCO_3$
pH	*7.0 – 8.0
Ca / Mg ratio	18.6 : 1
Na / K ratio	5.2 : 1
Oxygen content	Minimum of 60% air saturation
Conductance	766µS/cm
Holding water different from dilution water	No

* The adjustment of pH refers to the dilution water used for the Daphnia study. Adjustment was made to approximately pH 7.8 prior to addition of the test material. This pH has been shown to drop by about 0.2 of a unit by the time tests are initiated and water qualities are recorded

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	Shell Research Laboratories
Age	Less than 24 hours
Breeding method	Left under gooseberry bushes by storks
Kind of food	A suspension of *Chlorella vulgaris*
Amount of food	1 mg organic carbon per litre of culture water
Feeding frequency	Daily
Pretreatment	None

Feeding of animals during test	No

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	Renewed after 24 hours
Volume of test vessels	25 ml of solution in 50 ml vessel
Volume/animal	5 ml
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	20 ± 1 °C
Dissolved oxygen	0 hr = 100% ASV; 24 hr (before renewal) = 98% ASV 24 hr (after renewal) = 99% ASV; 48 hr = 98% ASV
pH	0 hr = 7.6 – 7.7; 24 hr (before renewal) = 7.8; 24 hr (after renewal) = 7.8; 48 hr = 7.8.
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Light intensity = 350 lux
Photoperiod	16 hours light and 8 hours dark

Table A7_4_1_2-6: Immobilisation data

Test-Substance Concentration (nominal/effective) [1] [mg/l]	Immobile *Daphnia*						
	Number		Percentage		Oxygen [%ASV]	pH	Tempera-ture [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
0	0	0	0	0	98	7.8	20
DMF control	0	0	0	0	98	7.8	20
0.13	0	0	0	0	98	7.8	20
0.25	0	2	0	10	98	7.8	20
0.50	0	13	0	65	98	7.8	20
1.0	2	18	10	90	98	7.8	20
2.0	17	20	85	100	98	7.8	20
4.0	20	20	100	100	98	7.8	20

[1] specify, if TS concentrations were nominal or measured

Table A7_4_1_2-7: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg/l]	0.78 (m)	1.2 – 1.7	1.0 (m)	4.0 (m)
48 h [mg/l]	0.25 (m)	0.36 – 0.56	0.25 (m)	2.0 (m)

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

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

	fulfilled	Not fulfilled
Immobilisation of control animals <10%	**Yes**	
Concentration of dissolved oxygen in all test vessels at end of test >3 mg/l	**Yes**	

Doc IIIA / Section A7.4.1.3

BPD Data Set IIA / Annex Point VII.7.3

Growth inhibition test on algae (Green alga *Selenastrum capricornutum)*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Knight B. (2000). Brodifacoum: Alga, Growth Inhibition Test (72 , Limit Test). Inveresk Research Laboratory Report Number: 19002 (unpublished) [redacted]	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted in accordance with the following guideline: - OECD (1984) Guideline 201 and EC (1992) Guideline C3. The study was conducted within the limitations of the OECD Draft Guidance Document on Aquatic Toxicity Testing of Difficult Substances (March 1999), whereby the test is not conducted at concentrations greater than the solubility of the test material.	
2.2	**GLP**	Yes.	
2.3	**Deviations**	No.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum	
3.1.1	Lot/Batch number	Batch number: 49.	
3.1.2	Specification	As given in section 2.	
3.1.3	Purity	As given in section 2 of Doc IIIA.	
3.1.4	Composition of Product	Not applicable.	
3.1.5	Further relevant properties	1) Water solubility study: brodifacoum is insoluble/sparingly soluble in water with the following solubility values determined using EPA CG-1510 Guideline: pH 5.2: 0.0038 mg/l pH 7.4: 0.24 mg/l pH 9.3: 10 mg/l	

		(Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]). 2) Aqueous hydrolysis study: ➢ [^{14}C]-brodifacoum hydrolysis was insignificant with DT_{50} values estimated by extrapolation where possible: 173 days at pH5, 300 days at pH 7 (DT_{50} estimation not possible at pH9). (Reference: Mathis SMG, Benner JP and Skidmore MW (1995). Brodifacoum: Aqueous Hydrolysis in pH 5, pH 7 and pH 9 Solutions at 25°C. Zeneca Agrochemicals Report Number RJ1927B [F4.1/01]). ➢ [^{14}C]-brodifacoum was determined to be hydrolytically stable. (Reference: Jackson R, Priestley I, Hall BE (1991). The Determination of the Hydrolytic Stability of [^{14}C]-Brodifacoum. Inveresk Research International Report Number 8330 [F4.1/03]). 3) Brodifaccoum is stable under normal storage conditions. 4) Vapour pressure is $<<10^{-9}$kPa ($<<10^{-8}$mmHg or $<<10^{-11}$atm) and is therefore negligable. (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]).	
3.1.6	Method of analysis	Brodifacoum was determined using High Performance Liquid Chromatography with fluorescence detection. External standardisation was employed for quantitation purposes.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Test solutions were prepared at the maximum achievable solubility. Brodifacoum was added in excess to a volumetric flask which was filled with algal medium and stirred for 48 hours in a temperature controlled room which was maintained at 22 – 23°C. The suspension was then filtered, with the first 50ml of filtrate being discarded to condition the filtrate. The undiluted filtrate was used as the test solution. See table A7_4_1_3-1.	X
3.3	**Reference substance**	No.	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**		
3.4.1	Culture medium	The algal growth medium was prepared as described in OECD Guideline 201.	
3.4.2	Test organisms	See Table A7_4_1_3-2.	
3.4.3	Test system	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	72 hours.	
3.4.6	Test parameter	Cell growth inhibition as measured by algal cell concentrations.	
3.4.7	Sampling	24, 48 and 72 hours.	
3.4.8	Monitoring of TS concentration	Yes, at 0 and 72 hours.	
3.4.9	Statistics	The cell counts at 0, 24, 48 and 72 h were used to determine the average specific growth rate (μ_{ave}) and areas under growth curve (AUC), for each limit test flask, over the 72 h test period. The area under the growth curve (AUC) and growth rate values from 0 to 72 h were analysed separately for homogeneity of variance using Levene's test (see below for reference) at a 1% significance level. The assumption of homogenous variances was satisfied for all the analyses. Reference: Levene H. (1960). 'Robust Test for Equality of Variances' in 'Contributions to Probability and Statistics – Essays in Honour of Harold Hotelling', Olkin *et al* (Eds), Stanford University Press.	
		4 RESULTS	
4.1	**Limit Test**	Performed.	
4.1.1	Concentration	Saturated solution of brodifacoum (i.e. maximum achievable concentration).	X
4.1.2	Number/ percentage of animals showing adverse effects	There was no apparent difference between growth rates and areas under growth curves for the test flasks.	X
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance		
4.2.2	Actual concentrations of test substance		
4.2.3	Growth curves		
4.2.4	Concentration / response curve		
4.2.5	Cell concentration data		

4.2.6	Effect data (cell multiplication inhibition)		
4.2.7	Other observed effects		
4.3	**Results of controls**	There was no apparent difference between growth rates and areas under growth curves for the control flasks.	X
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**	The study was conducted in accordance with OECD (1984) Guideline 201 and EC (1992) Guideline C3 and within the limitations of the OECD Draft Guidance Document on Aquatic Toxicity Testing of Difficult Substances (March 1999), whereby the test is not conducted at concentrations greater than the solubility of the test material. The test system was 72 hours static, shaken with no aeration. The test organism was the green alga, *Selenastrum capricornutum*.	
5.2	**Results and discussion**	The test substance brodifacoum has a low solubility in water and the study was conducted at the limit of solubility. Analysis of 0 and 72 h samples indicated that prior to filtration >10 µg/l of brodifacoum was suspended in the water. Analysis of 0 h filtered solutions indicated that brodifacoum concentration was below the limit of detection of the method (0.54 µg/l). Analysis of 72 h filtered samples indicated the presence of brodifacoum in solution but below quantifiable limits. No adsorption/desorption to algal cells was indicated by analysis of samples without algae at 72 h. There was no apparent difference between the growth rates and areas under the growth curves for the test and control flasks.	
5.2.1	NOE_rC	This was concluded to be equal to the limit of solubility under the conditions of the test (as prepared using a nominal 2 mg/l suspension of brodifacoum).	
5.2.2	E_rC_{50}	> maximum solubility of brodifacoum in algal medium under test conditions.	
5.2.3	E_bC_{50}		
5.3	**Conclusion**	The results indicate that brodifacoum has no effect on algal growth over	X

Doc IIIA / Section A7.4.1.3

BPD Data Set IIA / Annex Point VII.7.3

Growth inhibition test on algae (Green alga *Selenastrum capricornutum)*

		a 72 hour period under the test conditions. The algal cell concentration in control cultures increased at least by a factor of >16 within 3 days, and so the validity criteria for the study can be considered as fulfilled (see Table 3.1).	X
5.3.1	Reliability	2	
5.3.2	Deficiencies	No.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes, stirring for 48 hours.
Vehicle	No.
Concentration of vehicle	
Vehicle control performed	
Other procedures	

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	*Selenastrum capricornutum.*
Strain	278/4.
Source	Culture Collection of Algae and Protozoa (CCAP), Ambleside, Cumbria, UK.
Laboratory culture	Yes.
Method of cultivation	Cultures maintained under axenic conditions.
Pretreatment	
Initial cell concentration	Nominal cell density: 0.06×10^5 cells/ml.

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	250 ml.
Culturing apparatus	The test vessels were Erlenmeyer flasks capped with foil lids maintained within an environmental chamber at a temperature of 23 – 24°C throughout the test period.
Light quality	Artificial daylight fluorescent tubes (400 – 700nm).
Procedure for suspending algae	Orbital rotating (100 r.p.m.) transparent perspex platform.
Number of vessels/ concentration	6.
Test performed in closed vessels due to significant volatility of TS	No.

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	23 – 24°C (for the period 0 – 24 h); 23 – 24°C (for the period 24 – 48 h); 23 – 24°C (for the period 48 – 72 h).
pH	8.2 at 0 h; 8.1 – 8.3 at 72 h.
Aeration of dilution water	No.
Light intensity	8850 lux.
Photoperiod	Continuous.

3. Tables for Applicant's Summary and Conclusion

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

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

Criteria for poorly soluble test substances	**Yes, brodifacoum is categorised as a poorly soluble substance**	

Doc IIIA / Section A7.4.1.3

BPD Data Set IIA / Annex Point VII.7.3

Growth inhibition test on algae (Green alga *Selenastrum capricornutum)*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Knight B. (2000). Brodifacoum: Alga, Growth Inhibition Test (72 , Limit Test). Inveresk Research Laboratory Report Number: 19002 (unpublished) [redacted]	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, the study was conducted in accordance with the following guideline: - OECD (1984) Guideline 201 and EC (1992) Guideline C3. The study was conducted within the limitations of the OECD Draft Guidance Document on Aquatic Toxicity Testing of Difficult Substances (March 1999), whereby the test is not conducted at concentrations greater than the solubility of the test material.	
2.2	**GLP**	Yes.	
2.3	**Deviations**	No.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum	
3.1.1	Lot/Batch number	Batch number: 49.	
3.1.2	Specification	As given in section 2.	
3.1.3	Purity	As given in section 2 of Doc IIIA.	
3.1.4	Composition of Product	Not applicable.	
3.1.5	Further relevant properties	1) Water solubility study: brodifacoum is insoluble/sparingly soluble in water with the following solubility values determined using EPA CG-1510 Guideline: pH 5.2: 0.0038 mg/l pH 7.4: 0.24 mg/l	

		pH 9.3: 10 mg/l (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]). 2) Aqueous hydrolysis study: ➢ [^{14}C]-brodifacoum hydrolysis was insignificant with DT_{50} values estimated by extrapolation where possible: 173 days at pH5, 300 days at pH 7 (DT_{50} estimation not possible at pH9). (Reference: Mathis SMG, Benner JP and Skidmore MW (1995). Brodifacoum: Aqueous Hydrolysis in pH 5, pH 7 and pH 9 Solutions at 25°C. Zeneca Agrochemicals Report Number RJ1927B [F4.1/01]). ➢ [^{14}C]-brodifacoum was determined to be hydrolytically stable. (Reference: Jackson R, Priestley I, Hall BE (1991). The Determination of the Hydrolytic Stability of [^{14}C]-Brodifacoum. Inveresk Research International Report Number 8330 [F4.1/03]). 3) Brodifaccoum is stable under normal storage conditions. 4) Vapour pressure is <<10^{-9}kPa (<<10^{-8}mmHg or << 10^{-11}atm) and is therefore negligable. (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]).	
3.1.6	Method of analysis	Brodifacoum was determined using High Performance Liquid Chromatography with fluorescence detection. External standardisation was employed for quantitation purposes.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Test solutions were prepared at the maximum achievable solubility. Brodifacoum was added in excess to a volumetric flask which was filled with algal medium and stirred for 48 hours in a temperature controlled room which was maintained at 22 – 23°C. The suspension was then filtered, with the first 50ml of filtrate being discarded to condition the filtrate. The undiluted filtrate was used as the test solution. See table A7_4_1_3-1.	X
3.3	**Reference substance**	No.	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**		
3.4.1	Culture medium	The algal growth medium was prepared as described in OECD Guideline 201.	
3.4.2	Test organisms	See Table A7_4_1_3-2.	

3.4.3	Test system	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	72 hours.	
3.4.6	Test parameter	Cell growth inhibition as measured by algal cell concentrations.	
3.4.7	Sampling	24, 48 and 72 hours.	
3.4.8	Monitoring of TS concentration	Yes, at 0 and 72 hours.	
3.4.9	Statistics	The cell counts at 0, 24, 48 and 72 h were used to determine the average specific growth rate (μ_{ave}) and areas under growth curve (AUC), for each limit test flask, over the 72 h test period. The area under the growth curve (AUC) and growth rate values from 0 to 72 h were analysed separately for homogeneity of variance using Levene's test (see below for reference) at a 1% significance level. The assumption of homogenous variances was satisfied for all the analyses. Reference: Levene H. (1960). 'Robust Test for Equality of Variances' in 'Contributions to Probability and Statistics – Essays in Honour of Harold Hotelling', Olkin *et al* (Eds), Stanford University Press.	
		4 RESULTS	
4.1	**Limit Test**	Performed.	
4.1.1	Concentration	Saturated solution of brodifacoum (i.e. maximum achievable concentration).	X
4.1.2	Number/ percentage of animals showing adverse effects	There was no apparent difference between growth rates and areas under growth curves for the test flasks.	X
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance		
4.2.2	Actual concentrations of test substance		
4.2.3	Growth curves		
4.2.4	Concentration / response curve		

4.2.5	Cell concentration data		
4.2.6	Effect data (cell multiplication inhibition)		
4.2.7	Other observed effects		
4.3	**Results of controls**	There was no apparent difference between growth rates and areas under growth curves for the control flasks.	X
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**	The study was conducted in accordance with OECD (1984) Guideline 201 and EC (1992) Guideline C3 and within the limitations of the OECD Draft Guidance Document on Aquatic Toxicity Testing of Difficult Substances (March 1999), whereby the test is not conducted at concentrations greater than the solubility of the test material. The test system was 72 hours static, shaken with no aeration. The test organism was the green alga, *Selenastrum capricornutum*.
5.2	**Results and discussion**	The test substance brodifacoum has a low solubility in water and the study was conducted at the limit of solubility. Analysis of 0 and 72 h samples indicated that prior to filtration >10 µg/l of brodifacoum was suspended in the water. Analysis of 0 h filtered solutions indicated that brodifacoum concentration was below the limit of detection of the method (0.54 µg/l). Analysis of 72 h filtered samples indicated the presence of brodifacoum in solution but below quantifiable limits. No adsorption/desorption to algal cells was indicated by analysis of samples without algae at 72 h. There was no apparent difference between the growth rates and areas under the growth curves for the test and control flasks.
5.2.1	NOE_rC	This was concluded to be equal to the limit of solubility under the conditions of the test (as prepared using a nominal 2 mg/l suspension of brodifacoum).
5.2.2	E_rC_{50}	> maximum solubility of brodifacoum in algal medium under test conditions.

Doc IIIA / Section A7.4.1.3

BPD Data Set IIA / Annex Point VII.7.3

Growth inhibition test on algae

(Green alga *Selenastrum capricornutum)*

5.2.3	E_bC_{50}		
5.3	**Conclusion**	The results indicate that brodifacoum has no effect on algal growth over a 72 hour period under the test conditions.	X
		The algal cell concentration in control cultures increased at least by a factor of >16 within 3 days, and so the validity criteria for the study can be considered as fulfilled (see Table 3.1).	X
5.3.1	Reliability	2	
5.3.2	Deficiencies	No.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes, stirring for 48 hours.
Vehicle	No.
Concentration of vehicle	
Vehicle control performed	
Other procedures	

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	*Selenastrum capricornutum.*
Strain	278/4.
Source	Culture Collection of Algae and Protozoa (CCAP), Ambleside, Cumbria, UK.
Laboratory culture	Yes.
Method of cultivation	Cultures maintained under axenic conditions.
Pretreatment	
Initial cell concentration	Nominal cell density: 0.06 x 10^5 cells/ml.

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	250 ml.
Culturing apparatus	The test vessels were Erlenmeyer flasks capped with foil lids maintained within an environmental chamber at a temperature of 23 – 24°C throughout the test period.
Light quality	Artificial daylight fluorescent tubes (400 – 700nm).
Procedure for suspending algae	Orbital rotating (100 r.p.m.) transparent perspex platform.
Number of vessels/ concentration	6.
Test performed in closed vessels due to significant volatility of TS	No.

Table A7_4_1_3-4: Test conditions

Criteria	**Details**
Test temperature	23 – 24°C (for the period 0 – 24 h); 23 – 24°C (for the period 24 – 48 h); 23 – 24°C (for the period 48 – 72 h).
pH	8.2 at 0 h; 8.1 – 8.3 at 72 h.
Aeration of dilution water	No.
Light intensity	8850 lux.
Photoperiod	Continuous.

3. Tables for Applicant's Summary and Conclusion

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

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

Criteria for poorly soluble test substances	**Yes, brodifacoum is categorised as a poorly soluble substance**	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	Report: The Growth Inhibition of the alga *Selenastrum capricornutum* by BRODIFACOUM Technical. W J Craig - March 2003. Chemex Environmental International Ltd. Report -ENV5801/120140 Toxicity study of test substance brodifacoum technical.	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with Access to data	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 201	X
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	X
3.1.1	Lot/Batch number	ECO120140	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	Minimum 99 % brodifacoum	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	HPLC	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_3-1)	
3.3	**Reference substance**	Yes	
3.3.1	Method of analysis for reference substance	Aquatic toxicity: 72 hour EC_{50} of Potassium dichromate on *Selenastrum capricornutum* with test concentrations of, 0 (control), 0.18, 0.32, 0.56, 1.0 and 1.8 mg/l.	
3.4	**Testing procedure**		

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

3.4.1	Culture medium	*Nutrient*	*Final concentration in culture medium (mg/l)*
		NH_4Cl	15
		$MgCl_2.6H_2O$	12
		$CaCl_2.2H_2O$	18
		$MgSO_4.7H_2O$	15
		KH_2PO_4	1.6
		$FeCl_3.6H_2O$	0.08
		$Na_2EDTA.2H_2O$	0.1
		H_3BO_3	0.185
		$MnCl_2.4H_2O$	0.415
		$ZnCl_2$	0.003
		$CoCl_2.6H_2O$	0.0015
		$CuCl_2.2H_2O$	*0.00001
		$Na_2MoO_4.2H_2O$	0.007
		$NaHCO_3$	50
		**The original figure of 0.0001 was a typographical error, examination by the test laboratory of the raw data for the preparation of the stock solutions for the algal nutrient growth medium showed that the concentration of CuCl2.2H2O used for the study was correct, 0.01µg/l as stated in the guideline. The test laboratory acknowledges that this should have been noted during the preparation of the report and audit.*	
3.4.2	Test organisms	see table A7_4_1_3-2	
3.4.3	Test system	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	72 hours	
3.4.6	Test parameter	Cell multiplication inhibition	
3.4.7	Sampling	Sampling at 0, 24, 48 and 72 hrs	
3.4.8	Monitoring of TS concentration	Yes Start and end of test period	
3.4.9	Statistics	EC_{50} values estimated using a logarithm-linear or logarithm-probit plot of concentration and percent growth inhibition.	

4 RESULTS

4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable

4.2 Results test

substance

4.2.1 Initial concentrations of test substance: 0 (control), DMF, 0.032, 0.056, 0.10, 0.18, 0.32 mg/l

4.2.2 Actual concentrations of test substance

mg/l Nom	**0**	**0.032**	**0.056**	**0.1**	**0.18**	**0.32**
0 hrs fresh	**0**	0.023	0.029	0.047	0.072	0.179
72 hrs aged	**0**	0.004	0.007	0.019	0.031	0.051
Mean measured		*0.014*	*0.018*	*0.033*	*0.051*	*0.115*

4.2.3 Growth curves

4.2.4 Concentration / response curve

Concentration	***Cell density measurements (cells/ml x 10^4)***		
(mg/l)	**24 hours**	**48 hours**	**72 hours**
0 (control)	4.50	26.33	114.89
0 (DMF control)	2.89	21.89	64.44
0.032	6.33	21.56	63.00
0.056	5.11	19.89	43.56
0.1	4.33	13.22	27.67
0.18	3.22	10.33	13.45
0.32	1.89	8.00	9.44

4.2.5 Cell concentration data: see table A7_4_1_3-5

4.2.6 Effect data (cell multiplication inhibition): E_bC_{50} 0 - 72 hrs 0.04 mg/l; E_rC_{50} 0 - 72 hrs 0.12* mg/l; *extrapolated value

4.2.7 Other observed effects: None stated

4.3 Results of controls: No effects

4.4 Test with reference substance: Performed

4.4.1 Concentrations: Potassium dichromate at nominal concentrations 018, 0.32, 0.56, 1.0 and 1.8 mg/l

4.4.2 Results: E_bC_{50} 0 – 48 hrs 0.59 mg/l; E_rC_{50} 0 - 48 hrs 0.86 mg/l; E_bC_{50} 0 - 72 hrs 0.58 mg/l; E_rC_{50} 0 - 72 hrs 0.88 mg/l

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods: OECD 201.

5.2 Results and discussion: Test substance is extremely insoluble in water (c.0.03 ppm), of very low vapour pressure, and is subject to rapid photolysis (half-life c.7.5 hrs)

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

5.2.1	NER_CO	0 – 48 hrs NER_CO < 0.032 mg/l	X
5.2.2	E_rC_{50}	0 – 48 hrs = 0.74 mg/l; 0 – 72 hrs = 0.27 mg/l	X
5.2.3	E_bC_{50}	0 – 48 hrs = 0.15 mg/l; 0 – 72 hrs = 0.06 mg/l	X
5.3	**Conclusion**	R50 /R53 Very toxic to aquatic organisms applies. All validity criteria can be considered as fulfilled. Clear dose-response relationship shown EC50 is greater than the water solubility of the compound.	
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	DMF
Concentration of vehicle	1ml/l
Vehicle control performed	Yes
Other procedures	None

* The raw data for this study shows that the final concentration of DMF was 1ml/l in dilution water. It should be noted that this is 10 fold higher than the recommended concentration for solubilising agents for use with 'difficult substances'. Preliminary investigations showed that in order to fully dissolve the required amount of Brodifacoum a higher DMF to test material ratio was necessary. Subsequent dilution of the fully solubilised stock enabled a stable dispersion of the test material to be produced in water. These investigations were conducted prior to commencing the study and were not formally documented. The sample record documents the amounts of the test material used and the actual concentration of Brodifacoum in DMF and water used. The DMF controls for the Algal study provide evidence that this higher level of the solubilising agent had no adverse effects on the algal growth.
It was considered that the deviation from the protocol was justifiable, as it appeared to be the only way of presenting the test material in a stable and homogenous form

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	*Selenastrum capricornutum*
Strain	CCAP278/4
Source #	Culture Collection of Algae and Protoza Institute of Freshwater Ecology Windermere Laboratory
Laboratory culture	Yes
Method of cultivation	Not stated
Pretreatment	Pre-culture grown in exponential phase. Inoculum level adjusted to give an initial cell density of 1×10^4 cells/ml
Initial cell concentration	Initial cell density – $1x10^4$ cells/ml

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	*200 ml in 250 ml conical flask
Culturing apparatus	Haemocytometer and microscope
Light quality	White light at 6000 – 10000 lux.
Procedure for suspending algae	Shaking at 200 rpm
Number of vessels/ concentration	6 replicates for 0 (control), 3 replicates per test concentration, 3 replicates for DMF (Control)
Test performed in closed vessels due to significant volatility of TS	No

* OECD 201 guideline suggests that '250ml flasks are suitable when the volume of the test solution is 100ml'. This was interpreted as guidance and not a mandatory condition of the experimental design and therefore it is believed that scaling up the medium volume/inoculum ratio has not adversely affected the study.

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	20.0 °C (incubation temperature)
pH	Start of test 7.4 - 7.5; End of test 7.3 - 7.9
Aeration of dilution water	No
Light intensity	White light – 6000-10000 lux
Photoperiod	continuous

Table A7_4_1_3-5: Cell concentration data

Test-Substance Concentration (nominal/effective)[1] [mg/l]	Cell concentrations (mean values) [cells/ml x 10^4]							
	measured				Percent of control			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
0	1	4.50	26.33	114.89	100	100	100	100
0.032	1	6.33	21.56	63.00	100	141	82	55
0.056	1	5.11	19.89	43.56	100	114	76	38
0.10	1	4.33	13.22	27.67	100	96	50	24
0.18	1	3.22	10.33	13.45	100	72	39	12
0.32	1	1.89	8.00	9.44	100	42	31	8
Temperature [°C]	20.0	20.0	20.0	20.0				
pH	7.4 - 7.5			7.3 - 7.9				

[1] specify, if TS concentrations were nominal or measured

3. Tables for Applicant's Summary and Conclusion

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

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

Criteria for poorly soluble test substances	**YES**	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

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1 REFERENCE

1.1	**Reference**	Desmares-Koopmans M.J.E. (2001), Activated Sludge Respiration Inhibition Test with BRODIFACOUM (Contact Time: 30 Minutes), NOTOX B.V., Report No. 328793 (unpublished). [redacted]
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD Guideline No. 209, adopted April 4, 1984 EEC Directive 67/548 amended November 18, 1987 (87/302), Part C, Publication No. L133, adopted May 30, 1988.
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2.
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	1) Water solubility study: brodifacoum is insoluble/sparingly soluble in water with the following solubility values determined using EPA CG-1510 Guideline: pH 5.2: 0.0038 mg/l pH 7.4: 0.24 mg/l pH 9.3: 10 mg/l (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]). 2) Aqueous hydrolysis study: ➢ [^{14}C]-brodifacoum hydrolysis was insignificant with DT_{50} values

		estimated by extrapolation where possible: 173 days at pH5, 300 days at pH 7 (DT_{50} estimation not possible at pH9). (Reference: Mathis SMG, Benner JP and Skidmore MW (1995). Brodifacoum: Aqueous Hydrolysis in pH 5, pH 7 and pH 9 Solutions at 25°C. Zeneca Agrochemicals Report Number RJ1927B [F4.1/01]). ➢ [^{14}C]-brodifacoum was determined to be hydrolytically stable. (Reference: Jackson R, Priestley I, Hall BE (1991). The Determination of the Hydrolytic Stability of [^{14}C]-Brodifacoum. Inveresk Research International Report Number 8330 [F4.1/03]). 3) Brodifaccoum is stable under normal storage conditions. 4) Vapour pressure is $<<10^{-9}$kPa ($<<10^{-8}$mmHg or $<< 10^{-11}$atm) and is therefore negligable. (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]).	
3.1.6	Method of analysis	Nominal concentrations used throughout.	X
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Since BRODIFACOUM was hardly soluble in water, the test substance was quantitatively added to the test medium. During the 30 minutes contact time the test medium was stirred. See also table A7 4 1 4-1	X
3.3	**Reference substance**	Yes, 3,5-dichlorophenol	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**	The synthetic sewage feed (16 ml) and an adequate amount of the test substance were mixed and made up to 300 ml with Milli-RO water. Activated sludge (200 ml) was added and the mixture was aerated in a 1 l bottle during the contact time, using a pipette as an aeration device.	
		Oxygen consumption was measured and recorded for approximately 10 minutes. A well mixed sample of the content was poured into a 300 ml oxygen bottle, and the flask was sealed with an oxygen electrode connected to a recorder, forcing the air out of the vessel. During measurement, the sample was not aerated but continuously stirred on a magnetic stirrer.	X
		The pH was determined in the remaining part of the reaction mixture.	
		This procedure was repeated for the duplicate concentration. In each test series two controls without test substance were tested, one at the start and one at the end of the test.	
		Each batch of activated sludge was checked for sensitivity by testing the reference substance 3,5-dichlorophenol.	
3.4.1	Culture medium	Culturing was not applicable.	

		For storage of sludge and preparation of the test media synthetic sewage feed was used. Synthetic sewage feed (16 g peptone, 11 g meat extract, 3 g urea, 0.7 g NaCl, 0.4 g $CaCl_2.2H_2O$, 0.2 g $MgSO_4 .7H_2O$, 2.8 g K_2HPO_4.) Dissolved in 1 l Milli-Q water[1] and filtered. The pH was 7.5. [1] Milli-Q 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).
3.4.2	Inoculum / test organism	See table A7 4 1 4-2
3.4.3	Test system	See table A7 4 1 4-3
3.4.4	Test conditions	See table A7 4 1 4-4
3.4.5	Duration of the test	Contact time : 30 minutes Measurement/recording of oxygen consumption : approximately 10 minutes
3.4.6	Test parameter	Respiration inhibition
3.4.7	Analytical parameter	Measurement of oxygen concentration (mg O_2/l)
3.4.8	Sampling	After 30 minutes contact time
3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	Two controls without test substance were tested, one at the start and one at the end of the test.
3.4.11	Statistics	The percentage inhibition was plotted against the logarithm of the concentrations and the EC_{50}, and if applicable the EC_{10}, EC_{20} and EC_{80} for the test/reference substance, were determined using linear regression analysis.
		4 RESULTS
4.1	**Preliminary test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Effect data	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	A nominal concentration of 100 mg/l was tested in duplicate.
4.2.2	Actual concentrations of test substance	No measurements conducted during the test.

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

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

	Oxygen consumption mg O2/l/hr	Inhibition %
BRODIFACOUM 100 mg/l (A)	41	-8[1]
BRODIFACOUM 100 mg/l (B)	40	-5[1]

[1] : A negative value indicates stimulation in respiration rate of the sludge. This value is considered as not significant.

4.2.6	Effect data	Not applicable
4.2.7	Other observed effects	Not applicable

4.3	**Results of controls**	

	Oxygen consumption mg O_2/l/hr	Inhibition %
Control (A)	39	-
Control (B)	37	-
Mean C(A) + C(B)	38 (Δ5%)	

4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	3.2, 10 and 32 mg/l
4.4.2	Results	EC50 = 9 mg/l

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Activated Sludge Respiration Inhibition Test with BRODIFACOUM (Contact Time: 30 Minutes), According to: OECD Guideline No. 209, adopted April 4, 1984 EEC Directive 67/548 amended November 18, 1987 (87/302), Part C, Publication No. L133, adopted May 30, 1988. No relevant deviations from test guidelines.	X
5.2	**Results and discussion**	No inhibition in respiration rate of the sludge was recorded at the nominal concentration of 100 mg BRODIFACOUM per litre. Therefore no further testing was needed. Hence, the EC_{50} for BRODIFACOUM exceeded 100 mg/l. The NOEC is concluded to be the nominal brodifacoum concentration of 100 mg/l.	
5.2.1	EC_{20}	Not applicable	
5.2.2	EC_{50}	>100 mg/l	X
5.2.3	EC_{80}	Not applicable	
5.3	**Conclusion**	Under the conditions of this present test, BRODIFACOUM was not toxic to waste water (activated sludge) bacteria at a concentration of 100 mg/l. This concentration was prepared by direct addition of BRODIFACOUM to the test vessels and exceeded the solubility of BRODIFACOUM in water. The NOEC is concluded to be the nominal brodifacoum concentration of 100 mg/l.	
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	X

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

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

Criteria	Details
Dispersion	Yes, in the final test medium
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	Quantitatively added

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
Nature	Micro-organisms in activated sludge
Species	Multi species
Strain	Not applicable
Source	Municipal sewage treatment plant
Sampling site	'Waterschap de Maaskant', 's-Hertogenbosch, the Netherlands
Laboratory culture	No. After the day of sampling the batch of sludge was used on subsequent days (maximum four days).
Method of cultivation	Not applicable
Preparation of inoculum for exposure	The sludge was coarsely sieved and washed with tap-water. A small amount of the sludge was weighed and dried at ca. 105°C to determine the amount of suspended solids (4.0 g/l of sludge, as used for the test). Before use the pH was checked (measured value: 7.5). The batch of sludge was used on subsequent days (maximum four days), therefore 50 ml of synthetic sewage feed was added to each litre of activated sludge at the end of each working day. The sludge was kept aerated at test temperature until use.
Pretreatment	Not applicable
Initial cell concentration	The number of micro-organisms was determined as the amount of Mixed Liquor Suspended Solids (MLSS) per litre test medium (4.0 g/l of sludge, as used for the test).

Table A7_4_1_4-3: Test system

Criteria	Details
Culturing apparatus	Test bottles: 1 l all glass test bottles Measuring bottles: approximately 300 ml all glass oxygen bottles
Number of culture flasks/concentration	Control: in duplicate Reference substance: three concentrations Test substance : in duplicate
Aeration device	A pipette was used as an aeration device.
Measuring equipment	Oxygen electrode (Tri Ox EO 200, WTW, FRG) supplied with a recorder (Kipp BD40)
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_4-4: Test conditions

Criteria	Details
Test temperature	19.5°C
pH	No measurements were performed at the start of pH-values of the reaction mixture after the 30 minutes contact time are given below. Control (A) - 6.8 Control (B) - 6.8 Reference substance 3.2 mg/l 6.9 Reference substance 10 mg/l 6.9 Reference substance 32 mg/l 6.9 BRODIFACOUM 100 mg/l (A) 6.8 BRODIFACOUM 100 mg/l (B) 6.8
Aeration of dilution water	Not applicable Aeration with clear oli-free air was applied during the 30 minutes contact time. The oxygen concentrations at the start of the mesurement of the respiration rate is given below. Control (A) - 6.6 Control (B) - 7.2 Reference substance 3.2 mg/l 7.4 Reference substance 10 mg/l 7.8 Reference substance 32 mg/l 6.9 BRODIFACOUM 100 mg/l (A) 6.3 BRODIFACOUM 100 mg/l (B) 7.3

Suspended solids concentration	See also table A7 4 1 4-2. 4.0 g/l of sludge, as used for the test

Doc IIIA / Section A7.4.1.4

BPD Data Set IIA / Annex Point VII.7.4

Inhibition to microbial activity (aquatic) (Growth inhibition test to *Pseudomonas putida*)

Official use only

1 REFERENCE

1.1 Reference

Mather, J I, and Tapp, J F. (1988). Brodifacoum: Determination of the toxicity to *Pseudomonas putida*. ICI Brixham Laboratory Report Number : BL/B/3447 (unpublished[redacted]).

[redacted].

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No, but method used was based on that originally described by Bringham and Kuhn, which had been modified by Slabbert. The references for these methods are as follows:

- Bringham G, and Kuhn, R (1980). Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in the cell multiplication inhibition test. Water Research 14, 231-241;
- Slabber, J L (1986). Improved bacterial growth test for rapid water toxicity screening. Bulletin of Environmental Contamination Toxicology 37, 565-569.

2.2 GLP

Yes.

2.3 Deviations

No.

3 MATERIALS AND METHODS

3.1 Test material

Brodifacoum.

3.1.1 Lot/Batch number

ICI Reference number: 65415; Brixham reference number: R677.

3.1.2 Specification

As given in section 2.

3.1.3 Purity [redacted]

3.1.4 Composition of Product

Not applicable.

3.1.5 Further relevant properties

1) Water solubility study: brodifacoum is insoluble/sparingly soluble in water with the following solubility values determined using EPA CG-1510 Guideline:

pH 5.2: 0.0038 mg/l

		pH 7.4: 0.24 mg/l pH 9.3: 10 mg/l (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]). 2) Aqueous hydrolysis study: ➢ [^{14}C]-brodifacoum hydrolysis was insignificant with DT_{50} values estimated by extrapolation where possible: 173 days at pH5, 300 days at pH 7 (DT_{50} estimation not possible at pH9). (Reference: Mathis SMG, Benner JP and Skidmore MW (1995). Brodifacoum: Aqueous Hydrolysis in pH 5, pH 7 and pH 9 Solutions at 25°C. Zeneca Agrochemicals Report Number RJ1927B [F4.1/01]). ➢ [^{14}C]-brodifacoum was determined to be hydrolytically stable. (Reference: Jackson R, Priestley I, Hall BE (1991). The Determination of the Hydrolytic Stability of [^{14}C]-Brodifacoum. Inveresk Research International Report Number 8330 [F4.1/03]). 3) Brodifaccoum is stable under normal storage conditions. 4) Vapour pressure is $<<10^{-9}$kPa ($<<10^{-8}$mmHg or $<<10^{-11}$atm) and is therefore negligable. (Reference: Wollerton C and Husband R (1991). Pure Brodifacoum: Physico-Chemical Data File. ICI Agrochemicals Report No: RJ0959B [B2.1/02]).	
3.1.6	Method of analysis	Information not given in study report.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	The test solutions to be used in the test culture flasks were prepared in 0.1 % v/v acetone, to give nominal concentrations of 1, 10, 100 and 1000 mg/l brodifacoum. Each test culture flask contained 4 ml of growth medium concentrate (culture medium), 0.05 ml of the brodifacoum test solution, 1 ml of *pseudomonas putida* test inoculum and deionised water to give a final flask contents volume of 50 ml. See Table A7_4_1_4-1.	X
3.3	**Reference substance**	Yes, 3,5-dicholorophenol was used (1.8 ml of a 500 mg/l nominal concentration stock solution was added to the test culture flask instead of the brodifacoum test solution: see 3.2 above).	
3.3.1	Method of analysis for reference substance	Information not given in study report.	
3.4	**Testing procedure**		
3.4.1	Culture medium	A basal salt solution was prepared by dissolving the following in deionised water and making up to 1 litre with deionised water: - 26.25 g dipotassium hydrogen phosphate	

		- 11.25 g potassium dihydrogen phosphate - 1.175 g trisodium citrate dihydrate - 2.5 g ammonium sulphate - 0.25 g magnesium sulphate heptaydrate This solution was autoclaved at 121°C for 15 minutes. A glucose solution was prepared by dissolving 6.25 g glucose in deionised water and making up to 1 litre with deionised water. This solution was also autoclaved at 121°C for 15 minutes. Equal volumes of the salt solution and the glucose solution were mixed together in a sterile flask to form a growth medium concentrate, which was stored at 4°C until use.
3.4.2	Inoculum / test organism	See table A7_4_1_4-2.
3.4.3	Test system	See table A7_4_1_4-3.
3.4.4	Test conditions	See table A7_4_1_4-4.
3.4.5	Duration of the test	6 hours.
3.4.6	Test parameter	Growth inhibition.
3.4.7	Analytical parameter	Optical density.
3.4.8	Sampling	
3.4.9	Monitoring of TS concentration	No.
3.4.10	Controls	4 types of controls were tested: - a control containing deionised water, growth medium concentrate, and test inoculum (control); - and a vehicle control containing deionised water, growth medium concentrate, test inoculum, and blank vehicle (acetone) solution (sovlent or vehicle control); - an abiotic control containing deionised water and growth medium concentrate only (abiotic blank control); - abiotic controls containing deionised water, growth medium concentrate, and the test solutions of brodifacoum at the four test concentrations (abiotic chemical controls). The abiotic blank and chemical controls were used to compensate for any background colour or turbidity.
3.4.11	Statistics	The degree of inhibition of growth compared to that of the controls was

calculated as described in the method references (see section 2.1 above).

The formula used was:

$$\% \text{ inhibition} = 100 \times \left[1 - \frac{(x - \text{Chemical control value})}{(y - \text{Blank control value})} \right]$$

where x = Mean optical density value of test flask contents,

and y = Mean optical density value of control flask contents.

4 RESULTS

4.1	**Preliminary test**	Not performed.
4.1.1	Concentration	
4.1.2	Effect data	
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	1, 10, 100, 1000 µg/l.
4.2.2	Actual concentrations of test substance	Nominal concentrations were used to calculate the results.
4.2.3	Growth curves	
4.2.4	Cell concentration data	The percentage growth inhibition compared with that of the controls were calculated to be: 4% for 1 µg/l 0% for 10 µg/l 0% for 100 µg/l 0% for 1000 µg/l
4.2.5	Concentration/ response curve	
4.2.6	Effect data	EC_{10}: >1000 µg/l EC_{50}: >1000 µg/l
4.2.7	Other observed effects	
4.3	**Results of controls**	The vehicle (acetone) control gave 0% inhibition of growth.
4.4	**Test with**	Performed, using 3,5-dichlorophenol.

	reference substance		
4.4.1	Concentrations	18 mg/l	
4.4.2	Results	88% inhibition of growth.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test was conducted according to a method based on that originally described by Bringham and Kuhn, which had recently been modified by Slabbert (see section 2.1 for references). This test measures the degree of inhibition of growth of a pure culture of *Pseudomonas putida* during a 6 hour period when the cells are in the logarithmic growth phase.	
5.2	**Results and discussion**	Brodifacoum has a low water solubility and so acetone was used as a vehicle. The properties of brodifacoum give no indications to assume any relevant influences on the test results. The percentage growth inhibition compared with that of the controls were calculated to be: 4% for 1 µg/l, 0% for 10 µg/l, 0% for 100 µg/l, and 0% for 1000 µg/l.	
5.2.1	EC_{20}	EC_{10}: >1000 µg/l	X
5.2.2	EC_{50}	EC_{50}: >1000 µg/l	X
5.2.3	EC_{80}		
5.3	**Conclusion**	The validity criteria can be considered as fulfilled as the vehicle control gave 0% inhibition of growth, and the reference substance (3,5-dichlorophenol), gave 88% inhibition of growth indicating that the *Pseudomonas putida* culture was responding normally to this know toxicant. The results with brodifacoum revealed a very low toxicity to *Pseudomonas putida.*	
5.3.1	Reliability	2	X
5.3.2	Deficiencies	No.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*June 2005*

Doc IIIA / Section A7.4.1.4 **BPD Data Set IIA / Annex Point VII.7.4**	**Inhibition to microbial activity (aquatic)** **(Growth inhibition test to *Pseudomonas putida*)**

Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	TGD recommends to use the cell multiplication inhibition test with *P.putida* described in Bringham G, and Kuhn, R (1980) only as last option if no other tests are available.

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

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

Criteria	Details
Dispersion	No.
Vehicle	Yes, acetone was used to dissolve the brodifacoum.
Concentration of vehicle	0.1% v/v.
Vehicle control performed	Yes, a 0.1 % v/v solution of acetone was added to the test culture flask instead of the brodifacoum test solutions.
Other procedures	

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
Nature	Bacterium
Species	*Pseudomonas putida*
Strain	NCIB9494
Source	National Collections of Industrial and Marine Bacteria Ltd, Aberdeen, UK.
Sampling site	
Laboratory culture	Yes, obtained as a freeze-dried culture.
Method of cultivation	The freeze-dried culture was rehydrated in 0.5 ml of nutrient broth. A loop of this suspension was streaked onto a nutrient agar slope in a universal bottle. This was incubated at 25°C for 24 hours, and then stored at 4°C until use as the stock culture.
Preparation of inoculum for exposure	18-20 hours before the start of the test, a loop of the *Pseudomonas putida* stock culture was added to a sterile conical flask containing 4 ml of the test medium concentrate and 46 ml of deionised water. This mixture of inoculum and growth medium solution was incubated overnight at 25°C on an orbital shaker (150 rpm). After this period, the cells were diluted by addition of fresh growth medium solution at 25°C to an optical density which gave an adsorption of 0.8 (+/- 0.05) at 600 nm (4 cm cells) on a Uvikon 860 spectrophotometer. This was used as the test inoculum.
Pretreatment	
Initial cell concentration	Cell concentration measured as optical density (see above).

Table A7_4_1_4-3: Test system

Criteria	Details
Culturing apparatus	Conical flasks.
Number of culture flasks/concentration	2 flasks per concentration.
Aeration device	The test was performed by shaking at 150 rpm for 6 hours at 25°C on an incubator shaker.
Measuring equipment	The optical density of each flask content was measured at 600 nm on a Uvikon 860 spectrophotometer. 4 cm quartz optical cells were used with 8% v/v growth medium solution in the reference cell.
Test performed in closed vessels due to significant volatility of TS	No.

Table A7_4_1_4-4: Test conditions

Criteria	Details
Test temperature	Measurements not given in report, but test was conducted at a controlled temperature of 25°C.
pH	
Aeration of dilution water	No.
Suspended solids concentration	

Doc IIIA/Section 7. BPD Data Set IIIA/Annex Point 7.4.2	**Bioconcentration**	x
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [✓] **Limited exposure** []	**Technically not feasible** [] **Scientifically unjustified** [] **Other justification** [✓]	
Detailed justification:	[redacted]	X
Undertaking of intended data submission [[ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action* June 2005	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA/Section 7. BPD Data Set IIIA/Annex Point 7.4.2	**Bioconcentration**	x
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [✓]	**Technically not feasible** [] **Scientifically unjustified** []	
Limited exposure []	**Other justification** [✓]	
Undertaking of intended data submission [[ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
	[redacted]	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA/Section 7.4.3.3.1 **BPD Data Set IIIA/Annex Point XIII.2.3**	**Bioaccumulation in an appropriate species of fish**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [✓] **Limited exposure** [✓]	**Technically not feasible** [✓] **Other justification** [ ]	**Scientifically unjustified** [✓]
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*July 2005*	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	

Doc IIIA/Section 7.4.3.3.1 **BPD Data Set IIIA/Annex Point XIII.2.3**	**Bioaccumulation in an appropriate species of fish**
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc IIIA/Section 7.4.3.3.1 **BPD Data Set IIIA/Annex Point XIII.2.3**	**Bioaccumulation in an appropriate species of fish**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [✓] **Limited exposure** [✓]	**Technically not feasible** [✓] **Scientifically unjustified** [✓] **Other justification** [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*July 2005*	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

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

			Official use only
		1 REFERENCE	
1.1	**Reference**	Staniland, J (2005) The toxitiy to *Eisenia foetida foetida* of Brodifacoum. Chemex Environmental International Ltd. Ref:ENV7010/120140	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with access to data	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Static test conditions according to SOP E260 based on OECD 207	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	As given in section 2	X
3.1.1	Lot/Batch number	Chemex reference: ECO120140	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	100% (w/w)	
3.1.4	Composition of Product	N/A	
3.1.5	Further relevant properties	Insoluble, must be kept in cool, dry place.	
3.1.6	Method of analysis	N/A	
3.2	**Reference substance**	2-Chloracetamide (98%)	
3.2.1	Method of analysis for reference substance	N/A	
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	(see table A7_5_1_2-1)	
3.3.2	Application of the test substance	Test substance added with fine sand and mixed.	
3.3.3	Test organisms	(see table A7_5_1_2-2)	
3.3.4	Test system	(see table A7_5_1_2-3)	
3.3.5	Test conditions	(see table A7_5_1_2-4)	
3.3.6	Test duration	14 days	
3.3.7	Test parameter	Mortality	
3.3.8	Examination	Number of alive animals was obsereved after 7 days	
3.3.9	Monitoring of test	No	

	substance concentration	
3.3.10	Statistics	ToxCalc version 5.0 "Comprehensive Toxicity Data Analysis and Database Software" was used to calculate LD_{50} and confidence limits.
		4 RESULTS
		If appropriate, include tables. Sample tables are given below
4.1	**Filter paper test**	Not performed
4.1.1	Concentration	N/A
4.1.2	Number/ percentage of animals showing adverse effects	N/A
4.1.3	Nature of adverse effects	N/A
4.2	**Soil test**	*Non-entry field*
4.2.1	Initial concentrations of test substance	0, 318, 556 and 994 mg/kg
4.2.2	Effect data (Mortality)	(see table A7_5_1_2-5 and table A7_5_1_2-6)
4.2.3	Concentration / effect curve	N/A
4.2.4	Other effects	N/A
4.3	**Results of controls**	
4.3.1	Mortality	No mortality seen
4.3.2	Number/ percentage of earthworms showing adverse effects	N/A
4.3.3	Nature of adverse effects	N/A
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	32, 56, 99, 178, 316 mg/kg
4.4.2	Results	LC_{50} 194 mg/kg dry weight
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Static test conditions according to SOP E260 based on OECD 207

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

5.2	**Results and discussion**	No animals were killed by the test substance at any concentration tested (<994 mg/kg). 0 out of 40 control organisms died during the study and this represents an acceptable level of health of the test organism maintained under test conditions.
5.2.1	LC_0	n/a
5.2.2	LC_{50}	>994 mg/kg* *The OECD 207 guideline "Earthworm Acute Toxicity Tests" specifies concentrations up to 1000 mg/kg. Any minor deviations in test sample concentrations are derived from calculations based on actual sample mass weighed in the preparation of the test substrate. This difference between rangefinder and definitive test concentrations (1mg/kg) has little significance at a level of 1000mg/kg
5.2.3	LC_{100}	n/a
5.3	**Conclusion**	(see validity criteria summarized in table A7_5_1_2-7).
5.3.1	Other Conclusions	n/a
5.3.2	Reliability	1
5.3.3	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	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_5_1_2-1: Preparation of TS solution

Criteria	Details
Type and source of dilution water	Deionised water
Alkalinity / Salinity	N/A
Hardness	N/A
Oxygen content	N/A
Conductance	N/A
Holding water different from dilution water	N/A
In case of the use of an organic solvent	
Dispersion	At low concentrations, the test solution was prepared in organic solvent carrier.
Vehicle	Acetone
Concentration of vehicle	N/A
Vehicle control performed	No
Other procedures	The test substrate was homogenized

Table A7_5_1_1-2: Test organisms

Criteria	Details
Species/strain	Eisenia foetida andrei
Source of the initial stock	Blades Biological Ltd, Kent
Culturing techniques	Maintained in the laboratory under static conditions in a mixture of cattle dung and the artificial basic substrate.
Age/weight	Adult (at least 2 months old with clitellum) weighing approximately 300-600mg.
Pre-treatment	Holding temperature 19.0 to 20.0°C

Table A7_5_1_1-3: Test system

Criteria	Details
Artificial soil test substrate	Percentages are in terms of dry weight) 10% sphagnum peat (as close to pH 5.5-6.0 as possible, with no visible plant remains, finely ground, dried to a measured moisture content. 20% Kaolin clay. 60% industrial fine sand (find sand should be dominant with more than 50% of the particle between 50 and 200 microns) 10% B&Q Organic Peat Free Multipurpose Compost About 1% calcium carbonate, pulverised, added to bring the pH to between 6.0 and 6.5. The constituents were blended together in the correct proportions and the moisture content determined ar 105°C. Deionised water was added to give a resultant moisture content of approximately 40%
Test mixture	Test material and fine sand
Size, volume and material of test container	Square plastic containers (2 litres).
Amount of artificial soil (kg)/ container	750g of test substrate
Nominal levels of test concentrations	0, 318, 556, 994 mg/kg
Number of replicates/concentration	4
Number of earthworms/test concentration	N/A
Number of earthworms/container	10 animals per container
Light source	Constant light (400-800 lux)
Test performed in closed vessels due to significant volatility of test substrate	N/A

Table A7_5_1_2-4: Test conditions

Criteria	Details
Test temperature	20±2°C
Moisture content	45.6 %w/w at the start of the test and 43.0 % w/w at the end.
pH	6.4
Adjustment of pH	No
Light intensity / photoperiod	400-800 fux
Relevant degradation products	N/A

Table A7_5_1_2-5: Mortality data

Test Substance Concentration (nominal/measured)[1] [mg/kg artificial soil]	Mortality			
	Number		Percentage	
	7 d	14 d	7 d	14 d
Control	0	0	0	0
318	0	0	0	0
556	0	0	0	0
994	0	0	0	0
Temperature [°C]	20	20		
pH	6.4			
Moisture content	43-45.6 %w/w	43-45.6 %w/w		

[1] specify, if TS concentrations were nominal or measured

Table A7_5_1_2-6: Effect data

	14 d [mg/kg soil][1]	95 % c.l.
LC_0		
LC_{50}	>994	N/A
LC_{100}		

[1] indicate if effect data are based on nominal (n) or measured (m) concentrations

Table A7_5_1_2-7: Validity criteria for acute earthworm test according to OECD 207

	fulfilled	Not fulfilled
Mortality of control animals < 10%	Yes	

Doc IIIA / Section 7.5.3.1.1/01 BPD Data Set IIIA / Annex Point XIII.3.4

Acute oral toxicity on birds

Acute oral toxicity to Mallard Duck

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1980). The Acute Oral Toxicity (LD_{50}) of Brodifacoum to the Mallard Duck. Huntingdon Research Centre Report No: ICI 308 WL/791275, July 1980 (unpublished) [redacted].

1.2 Data protection

[redacted]

1.2.1 Data owner

[redacted]

1.2.2 Companies with letters of access

[redacted]

1.2.3 Criteria for data protection

[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

Yes, protocol based on the USA EPA Proposed Guidelines 163.71-1 Avian single oral LD_{50}, published in the The Federal Register Vol. 43, No. 132 on 10 July 1978 Part II, page 29726.

2.2 GLP

Yes, according to GLP regulations as set out in 'Title 21of the US Code of Federal Regulations, Part 58'.

2.3 Deviations

Yes, the post-treatment observation period was extended to ensure that all delayed mortalities were recorded.

3 METHOD

3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	Y0052/002/004
3.1.2	Specification	Please refer to Section 2 of Doc IIIA.
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	Not applicable.
3.1.5	Further relevant properties	
3.1.6	Method of analysis in the diet	Not applicable.
3.2	**Administration of the test substance**	As a 0.05% w/v suspension in corn oil See table A7_5_3_1_2-1 below.
3.3	**Reference substance**	No.
3.3.1	Method of analysis for reference substance	Not applicable.
3.4	**Testing procedure**	
3.4.1	Test organisms	Mallard Duck (*Anas platyrhynchos).* See table A7_5_3_1_1-2 below.
3.4.2	Test system	See table A7_5_3_1_1-3 below.
3.4.3	Diet	Huntingdon Research Centre chick diet in meal form, specially formulated to contain no added synthetic Vitamin K.
3.4.4	Test conditions	See table A7_5_3_1_1-4 below.
3.4.5	Duration of the test	28 days (single oral dose followed by 28 day observation period).
3.4.6	Test parameter	Clinical observations and mortalities.
3.4.7	Examination / Observation	Clinical examinations and observations: Symptoms of toxicity and mortality were recorded daily throughout the study. Gross macroscopic examinations: *Post-mortem* examinations were carried out during the study on early decedents, or at termination at the end of the study.
3.4.8	Statistics	LD_{50} value calculated using method of Litchfield and Wilcoxon *[ref: Litchfield JT and Wilcoxon F (1949) J. Pharm. exp. Ther., 96, 99].*