Doc IIIA/Section 6.11	**Studies on other routes of administration (parenteral routes)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified** [✓] **Other existing data** [✓] **Limited exposure** [✓]	
Detailed justification:	[redacted]	
	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 IIIA / Section 6.12.1

BPD Data Set IIA / Annex Point VI.6.9.1

Medical surveillance data on manufacturing plant personnel if available

Official use only

1 REFERENCE

1.1 Reference

[redacted] 2004, Biological Monitoring of Rodenticide Workers at [redacted] and [redacted]. Report prepared for [redacted], [redacted], unpublished. [BR-959-0136]

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

Refer To Section 4 Below

4 RESULTS

Appropriate text extracted here:

[redacted]

[redacted].

Routine monitoring.

[redacted] had successfully produced these anticoagulants and formulated them into baits without any incidents until 1981. Between June 1981 and September 1982 three cases of poisoning occurred, two at [redacted] and one at [redacted]. These are reported below. Before and after this period, routine monitoring of workers at both companies was routinely undertaken at both sites. Up until the early 1980s, Normotest was used to measure the prothrombin time and since then, the 'one-time prothrombin time test of Quick' has been utilised. Around 1990 efforts were made to develop and utilise a more sensitive routine monitoring technique, PIVKA II analysis, but this test was ultimately considered not to be sufficiently robust as an every-day monitoring technique. With the exception of the cases elaborated here, routine monitoring, using Normotest or the 'one-time prothrombin time test of Quick', has shown no clinical effects in any workers involved in the synthesis of the active substances or the formulation of rodenticidal baits despite the synthesis of tonnes of the active substances and thousands of tonnes of rodenticidal baits. A typical example of such routine analysis is shown in Appendix I.

During this time, there has been no evidence of allergenicity, sensitisation or any other abnormal effects induced by repeated and continual exposure to these anticoagulant rodenticides.

THREE CASES

Case 1

Male 26.

Single.

No previous medical history.

Between 1-2 weeks after leaving rodenticide synthesis, developed:

Tiredness,

Anorexia,

Bruising -one large left elbow,

-one large right arm,

Purpura -legs and soft palate,

Back pain for 6 days, ? renal,

Haematuria.

On admission to hospital, PTT 100 seconds (probably 2 weeks after last possible exposure).

This level of anticoagulation was corrected using vitamin K I and other appropriate treatments.

Case 2

Male 26.

Divorced.

No previous medical history.

Regular rodenticide worker.

September 1982- reported to the Medical Centre: (Appendix II)

Tired, gums bleeding, bruising to right wrist, chest wall, left arm, right hip.

PTT 61 seconds -admitted to hospital.

This level of anticoagulation was corrected using vitamin K 1 and other appropriate treatments.

Return to work November 1982. Returned to rodenticide work September 1983.

Case 3

Male 24.

Married.

No previous medical history.

April 1980-1982

Frequent abnormal Normotest results (Appendix III)

Several readings between 30% and 60%.

June 1982- developed the following symptoms:- Severe right loin pain, gums bleeding, minor sores delayed healing with surrounding bruising.

Medical Centre: Urine positive for occult blood, PTT 82 seconds. Admitted to hospital.

This level of anticoagulation was corrected using vitamin K 1 and other

appropriate treatments.

Following discharge from hospital: October 1982, 4 months after incident before PTT became normal.

Return to work -kept away from technical materials. Only employed on packing dilute materials along with other works.

July 1983 (13 months after initial incident): PTT 35 seconds -admitted.

By time of admission PTT 66 seconds.

This level of anticoagulation was corrected using vitamin K 1 and other appropriate treatments.

It was 2-3 years before tests showed no evidence of effects of anticoagulation.

COMMENTS

Case 1

Up to two weeks after possible exposure, PTT still I 00 seconds.

Case 2

Monitoring PTT at regular intervals gives appropriate warning of exposure

Case 3

No hypersensitivity in patient or family members.

13 months after original exposure. PTT again became elevated at 66 seconds. Why?

2-3 years before effects eliminated.

The mode of action and known effects of coumarin rodenticides is anticoagulation. Effects are determined by measuring prothrombin time. Difenacoum has been produced since 1975, brodifacoum since 1976 and flocoumafen since 1985.

During the early years of production and formulation. it was believed that the effects of these compounds would be at a maximum within 24-48 hours and diminished after 2-4 days.

However, following our experience of poisoning, further studies show that the half-life of these compounds is in the region of months rather than days, as was previously thought.

This was due to the assumption that the excretion curve would continue to fall on the same gradient as the upper part of the descending curve, similar to warfarin. In fact, later studies show that excretion is bi-phasic and that the second phase of excretion can continue for several months. Current data suggests what warfarin is exceptional in having a mono-phasic elimination. Bi-phasic elimination has been shown in a range of

other anticoagulants.

This prolonged elimination sheds light on how patients may present with signs and symptoms several days after last exposure and effects continue for several months. At the present time we are still using PTT measurement as our main monitoring method. Since our three cases in 1981/2, knowledge of the compounds has increased, working practices and containment tightened up, and biological monitoring by PTT has become much more structured. There have been no further incidents since this period.

5 APPLICANT'S SUMMARY AND CONCLUSION

Refer to section 4 above

	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]
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*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*
Remarks	

Appendix I

APPENDIX I

EXAMPLE OF ROUTINE RESULTS

Date	Employee N°	Prothrombin time, s	Date	Employee N°	Prothrombin time, s
23.04.03	6	12.1	25.04.03	11	11.6
	8	12.3		16	11.3
	7	12.8			
	28	12.1	05.06.03	18	12.4
	14	12.3		13	12.3
	27	12.7		2	11.1
	31	11.7		19	12.1
	15	11.6		3	12.1
	9	11.8		41	12.1
	19	11.6		5	11.8
	38	11.1		11	11.6
	36	12.3		12	12.3
	41	12.0		4	11.7
	26	11.7			
	16	10.8	27.06.03	18	12.1
	12	11.6		3	11.8
	20	13.0			
	21	11.7	28.06.03	4	11.9
				11	11.1
			03.07.03	9	11.9

Appendix II

APPENDIX II

CASE 2

Date	Hb.	W.B.C.	Poly.	Lymph	Mono.	Normotest
03.02.82	16.0	07.4	74	23	3	100
19.05.82	15.3	04.9	70	28	2	100
07.07.82	16.6	05.3	62	37	1	100
04.08.82	16.5	06.0	63	36	1	100
30.09.82	Admitted to hospital PTT – 61 seconds					
01.12.82	14.8	03.5	67	31	2	100
12.01.83	14.7	03.9	80	19	1	100
17.02.83						100
02.03.83	14.9	06.0	77	20	3	100
31.03.83						100
06.04.83	15.0	05.5	76	22	2	100
04.05.84	14.8	04.1	73	24	3	100
05.02.86	15.4	04.3	64	34	2	100

Appendix III

APPENDIX III

CASE 3

Date	Hb.	W.B.C.	Poly.	Lymph.	Mono.	Eeo.	Normotest
10.04.82	15.6	07.2	69	30	1		59
03.03.82	15.4	08.6	60	37	3		>100
03.03.82	15.5	08.1	64	35	1		75
06.01.82	16.0	07.1	62	26	2		>100
14.12.81							56
02.12.81	15.9	05.8	65	31	4	2	70
18.11.81	16.1	05.1					>100
07.10.81	15.9	07.2			2	1	100
15.09.81							70
02.09.81	15.0	07.8	71	28	1		>100
19.08.81							20
05.08.81							-
01.07.81							100
06.05.81	15.5	06.6	64	33	3		>100
18.04.81							>100
01.04.81	15.9	8.5	66	30	4		64
Mar. 81	15.7	5.9		30	3		100
Feb. 81	15.5	7.9		30	3		100
Jan. 81	16.2	7.2	68	31	1		90
Dec. 80	16.0	7.3	66	31	1	2	32
Nov. 80	15.9	6.3	66	33	1		100
Oct. 80	16.9	7.4	73	24	2	1	>100
Sep. 80	15.6	6.5	67	30	3		>100
Aug. 80	16.2	6.4	74	20	4	2	>100
Jul. 80	16.7	6.3					?
Jun. 80	16.0	5.6	64	31	3	2	32
May. 80	15.9	6.1	68	31	1		100
Apr. 80	15.9	7.9	78	21	1		110

Doc IIIA / Section 6.12.2

Direct observation, e.g. clinical cases, poisoning incidents if available

BPD Data Set IIA / Annex Point VI.6.9.2

		Official use only
	1 REFERENCE	
1.1 Reference	WHO (World Health Organisation publication), 1995, Environmental Health Criteria 175 - Anticoagulant Rodenticides. International Programme on Chemical Safety, Pages 67 to 72. Not GLP, published [BR-952-0141]	x
	2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)	

Doc IIIA / Section 6.12.2

Direct observation, e.g. clinical cases, poisoning incidents if available

BPD Data Set IIA / Annex Point VI.6.9.2

3 MATERIALS AND METHODS

Refer To Section 4 Below

4 RESULTS

Appropriate text extracted here:
Incidents of human exposures to rodenticides are reported to poison control centres in countries where such facilities exist. In 1988, for example, the American Association of Poison Control Centers (AAPCC) received accounts of 10 626 cases of human exposures to rodenticides. These incidents represented 17% of reported exposures involving pesticides and 0.8% of the total number of cases reported in the AAPCC system. The rodenticide incidents included 4190 cases involving "anticoagulants" (principally warfarin) and 5133 involving "long-acting anticoagulants" (second-generation anticoagulants plus the indandione compounds). More than 95% of the rodenticide cases were classified as "accidental". Most of the remainder were classified as "intentional" and included attempted suicides. Of the 10 540 rodenticide incidents for which the ages of victims were reported, 9406 (89%) involved children under 6 years of age (Litovitz et al., 1994).

Victims in nearly 32% of the rodenticide exposure incidents reported to the AAPCC in 1988 were treated in health care facilities. However, the medical outcome "none" was reported in more than 93% of the 5708 incidents for which information regarding outcomes was reported. The remaining 380 cases included 333 with "minor" medical effects, 41 with "moderate" effects, 4 with "major" effects, and two deaths (Litovitz et al., 1994).

In 1993, the Swedish Poison Information Centre received 338 enquiries concerning exposures to anticoagulant rodenticides. This number represented 0.6% of all enquiries to the centre and 37% of the enquiries concerning pesticides. Of the anticoagulant rodenticide enquiries, 202 pertained to warfarin and 136 to "superwarfarin" compounds (Persson, 1994).
Human exposure to second-generation and indandione anticoagulants produces symptoms consistent with anticoagulation effects (e.g., haematomas, haematemesis, haematuria, easy bruisability). Treatment of cases of exposure, particularly of substantial and repeated exposure, may require vitamin K1 therapy and monitoring of prothrombin times for periods of many months (Rauch et al., 1994).
Suicide and/or unintentional poisonings with anticoagulant rodenticides have occurred in many countries. Thus, Ungvary (1994) reported 70 cases, mostly involving children, that occurred in Hungary between 1988 and 1993.

Doc IIIA / Section 6.12.2 BPD Data Set IIA / Annex Point VI.6.9.2	**Direct observation, e.g. clinical cases, poisoning incidents if available**

Warfarin is widely used as a therapeutic and preventive agent in the treatment of thromboembolic disease. Patients have been maintained for years on this treatment with control of the prothrombin level, which should be kept between 10 and 30% of normal. Diphacinone has also been used as a drug because of its long-lasting action (the half-life in humans is 15-20 days). It ceased to be listed in the American Medical Association Drug Evaluations, (AMA, 1980) because of its structural relation to phenindion, which had been reported to have adverse effects.

Acute poisoning

Typical features of poisoning result from increased bleeding tendency and include:

* minor poisoning: coagulation disturbance detected only by laboratory analyses;
* moderate poisoning: coagulation disturbance resulting in haematomata, haematuria, blood in faeces or excessive bleeding from minor cuts or abrasions, gum bleeding;
* severe poisoning: retroperitoneal haemorrhage, severe gastrointestinal bleeding, cerebrovascular accidents, massive haemorrhage (internal bleeding) resulting in shock.

If anaemia or liver disease is present then the above features may be more severe and persistent and the poisoning may be more difficult to control (Anonymous, 1988).

The onset of the signs of poisoning may not be evident until a few days after ingestion.

Poisoning incidents

Cases of human poisoning with "superwarfarins" were reviewed by Katona & Wason (1989). Fourteen members of a family in the Republic of Korea were poisoned by eating warfarin-containing maize meal. The first symptoms appeared 7-10 days after the beginning of exposure and were followed by massive bruises or haematomata on the buttocks in all cases (Lange & Terveer, 1954).

Pribilla (1966) reported a total dose of about 1000 mg of warfarin to be fatal after 13 days of consumption.

Out of a total of 741 infants, 177 died after the use of warfarin-contaminated talc in Viet Nam. The concentrations of warfarin in the powder varied from 1.7 to 6.5% (Martin-Bouyer et al., 1983).

A 73-year-old woman suffered from recurrent episodes of hypoprothrombinaemia. Clotting tests and further investigation showed that this was due to a warfarin rodenticide intentionally mixed in the woman's cough syrup by her daughter-in-law. As the patient had as many as seven relapses, it was possible to compare different types of therapy. Menadione had no effect (Nilsson, 1957).

Doc IIIA / Section 6.12.2	**Direct observation, e.g. clinical cases, poisoning incidents if available**
BPD Data Set IIA / Annex Point VI.6.9.2	

Several suicidal attempts with chlorophacinone have been reported. Murdoch (1983) reported a case of ingestion of 625 mg chlorophacinone (250 ml of a 0.25% concentrate formulation) by a 37-year-old woman. The prolonged anticoagulant action of chlorophacinone persisted for at least 45 days even though treatment was given. It was found that menadiol, the synthetic analogue of vitamin K1, was ineffective. The natural form, phytomenadione, was effective only when given at high dosage (20 mg daily) 30 days after the ingestion of chlorophacinone.

In a case reported by Dusein et al. (1984), the amount of ingested chlorophacinone was unknown. After adequate therapy, the prothrombin level became normal within 4 weeks.

Vogel et al. (1988) reported the case of an 18-year-old woman hospitalized 3 days after ingesting approximately 100 mg chlorophacinone. Under high-dose vitamin K1 therapy (160 mg) the prothrombin time was normalized, but it increased again following withdrawal of vitamin K1. After prolonged vitamin K1 administration, the prothrombin time finally became normal after 7 weeks.

Brodifacoum poisoning has occurred in South Sumatra, Indonesia. Some of the villagers used a 0.005% brodifacoum rice grain bait as a food source even though they knew it was poisonous and unfit for human consumption. They attempted to remove the rodenticide by repeated washing, rinsing and cooking before eating the rice. Because of the delay in the appearance of poisoning symptoms it appeared that they had been successful, thus encouraging further attempts to purify the rice baits. As a result, deaths occurred before appropriate remedial treatment could be initiated (Anonymous, 1985).

Jones et al. (1984) reported the first case of human brodifacoum poisoning in a 17-year-old boy who attempted suicide by ingesting approximately 7.5 mg (0.12 mg/kg) of brodifacoum in Canada. He was initially seen with gross haematuria, followed by epistaxis and gum bleeding. The prothrombin time and the activated partial thromboplastin time were notably prolonged. He was treated for 56 days with either parenteral or oral vitamin K1 and either fresh or stored plasma until coagulation values remained normal and stable.

Lipton & Klass (1984) reported a similar case in a 31-year-old mentally disturbed woman who ingested over a 2-day period approximately thirty 50-g packages of Talon-G (approximately 75 mg of brodifacoum). Prothrombin time and activated partial thromboplastin time were considerably prolonged (respectively 6-fold and 4-fold above normal values). After 4 days of therapy with high doses of vitamin K1 (up to 125 mg/day), partial correction in the prothrombin time occurred. Vitamin K1 therapy continued with interruptions for 8 months until normal prothrombin time levels were found.

Chong et al. (1986) reported a case of suicidal poisoning after ingestion of 10 mg brodifacoum (as 0.05% Klerat). The coagulation test became normal after large doses and prolonged use of vitamin K1 over 6 months.

Doc IIIA / Section 6.12.2 BPD Data Set IIA / Annex Point VI.6.9.2	**Direct observation, e.g. clinical cases, poisoning incidents if available**

A case of intentional ingestion of brodifacoum (200 g of Talon G, 0.005% brodifacoum) was reported by Hoffman et al. (1988). A profound decrease in the levels of factors II, VII, IX and X, lasting 43 days after ingestion, was observed. Treatment with subcutaneous vitamin K1 in doses up to 100 mg per day was effective.

Weitzel et al. (1990) described three patients with severe bleeding disorders due to deficiency of the vitamin K-dependent blood clotting proteins after ingestion of an anticoagulant. Although the patients denied any ingestion, brodifacoum was detected in their serum at concentrations of 7.6 nmol/litre, 270.7 nmol/litre and 2759 nmol/litre, respectively. The anticoagulant effect was found to persist long after brodifacoum was no longer detectable in the serum. A half-life of approximately 16-36 days was determined for brodifacoum in the plasma.

Kruse & Carlson (1992) reported the case of a 25-year-old man who attempted suicide by consuming a brodifacoum rodenticide. He developed a severe coagulopathy that was treated with vitamin K1 and fresh frozen plasma and he was discharged from hospital with oral phytomenadione. Fifteen weeks later the man presented again with a history of further brodifacoum ingestion. He suddenly became comatose and computer tomography revealed a subarachnoid haemorrhage that led to brain death 24 h later.

Wallace et al. (1990) described the clinical course of a patient poisoned with brodifacoum in a suicide attempt. He developed microhaematuria and melaena. His clotting factors were depressed and were poorly responsive to vitamin K treatment.

Barlow et al. (1982) reported a case of attempted suicide with 25 mg of difenacoum (500 g of rat bait) followed several months later by 1800 g of rat bait. The patient was treated with vitamin K1 (phytomenadione) for 48 and 42 days, respectively, until the pharmacological effect of difenacoum ceased.

Nighoghossian et al. (1990) reported an unusual coagulopathy after accidental exposure to a diphenacoum rodenticide. A 59-year-old man developed subacute tetraparesis following severe sudden neck pain, which on clinical examination was shown to be due to a subdural cervical haematoma. Prothrombin complex activity was low and diphenacoum was present in the plasma. Specific medical management led to a complete recovery.

Greeff et al. (1987) reported accidental bromadiolone poisoning in two children, resulting in prolonged anticoagulation. Descarboxyprothrombin levels were increased in both cases by 27% and 29.9%, respectively (normal, non-detectable level). The first child rapidly recovered after treatment with high-dose intravenous factor IX-prothrombin complex and vitamin K1. The clotting profile became normal on the third day after admission. The second child gave a poor response to 10 mg intravenous vitamin K1 and the dose was increased to 20 mg.

Doc IIIA / Section 6.12.2 **BPD Data Set IIA / Annex Point VI.6.9.2**	**Direct observation, e.g. clinical cases, poisoning incidents if available**

Controlled human studies
Single oral doses of 60, 70, 80 or 120 mg warfarin decreased the prothrombin concentrations in volunteers to zero by the third day. After the administration of 50 mg vitamin K1, the prothrombin concentrations returned by the sixth day to 60, 70, 55 and 63%, respectively, of the normal value (Anonymous, 1965).
When a single oral dose of 20 mg chlorophacinone was given to three volunteers, the lowest prothrombin times were 35, 34 and 38% of the pretreatment value on days 2, 4 and 2, respectively. Eight days after administration without any treatment the values were 80, 100 and 90%, respectively (Anonymous, 1965).

Refer also to 6.12.1

5 APPLICANT'S SUMMARY AND CONCLUSION

Refer to section 4 above

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]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*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*
Remarks	

Doc IIIA / Section 6.13

Acute Oral Toxicity (Oral LD_{50} in Guinea Pig)

BPD Data Set IIIA / Annex Point VI.2

1 REFERENCE

1.1 Reference

[redacted], 1975, 'Acute Oral Toxicity of WBA 8119 to Female Guinea Pig', [redacted], September 1978.

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 guideline study given, but study conducted in accordance with the scientific principles accepted at the time.

2.2 GLP

No. Study pre-dates the requirement for GLP.

2.3 Deviations and Deficiencies

Not applicable.

3 MATERIALS AND METHODS

3.1 Test Material

WBA 8119 (brodifacoum).

3.1.1 LOT/BATCH NUMBER

Not specified.

3.1.2 SPECIFICATION

As given in Section 2 of Doc. IIIA.

3.1.3 DESCRIPTION

Powder.

3.1.4 PURITY

> [redacted]%.

3.1.5 STABILITY

[redacted].

3.2 Test Animals

3.2.1 SPECIES

Cavia porcellus (Guinea pig).

3.2.2 STRAIN

Duncan Hartley.

3.2.3 SOURCE

Hyline Ltd.

3.2.4 SEX

Female.

3.2.5 AGE/WEIGHT AT STUDY INITIATION

450 - 520 g.

3.2.6 NUMBER OF ANIMALS PER GROUP (SEX)

3 or 4 (female).

3.3 Administration/Exposure

Oral.

3.3.1 POSTEXPOSURE PERIOD

Observed to 28 days after treatment.

3.3.2 TYPE

Gavage.

3.3.3 CONCENTRATION

0.20, 0.50,1.00, 2.15 4.64, 5.00, and 10.00 mg/kg.

3.3.4 VEHICLE

Polyethylene glycol (PEG 300).

3.3.5 CONCENTRATION IN VEHICLE

0.20, 0.50, 1.00, 2.15 4.64, 5.00, and 10.00 g/l.

3.3.6 TOTAL VOLUME APPLIED

0.45 - 0.52 ml (1 ml/kg).

3.4 Examinations

The guinea pigs were observed for up to 28 days after treatment. Clinical signs and mortalities were noted. Macroscopic examinations were performed at autopsy.

3.4 Method of Determination of LD_{50}

The LD_{50} was determined by the method of Horn.

4 RESULTS

4.1 LD_{50}

2.78 mg/kg to female guinea pig.

4.2 Effects /4.3 Reversibility/4.4 Findings of Hispathological Examinations

ACUTE ORAL TOXICITY OF WBA 8119 (BRODIFACOUM) TO FEMALE GUINEA PIG			
Dose (mg/kg)	**n Dead/ n Investigated**	**Time of Death (Range)**	**Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations**
0.20	0/3	-	There were no deaths of any animal dosed with 1 mg/kg or less. All deaths occurred between days 7 - 15.

0.50	0/3	-	During the first seven days all guinea pigs appeared subdued and lethargic. No abnormalities were noted from day 7 onwards in the animals surviving the study. Macroscopic examination of survivors at sacrifice on day 28 revealed liver enlargement in all cases from the dose groups >1.00 mg/kg. Signs of minor regressed haemorrhages were noted in survivors of the two highest dosages. The single survivor in the highest (10 mg/kg) dose group had a grossly enlarged and granulated liver. Autopsy of the animals which died during the study showed that death was due to massive internal haemorrhage.
1.00	0/3	-	
1.00	0/4	-	
2.15	2/4	Days 7 -13	
4.64	3/4	Days 7 - 12	
5.00	3/4	Days 5 - 15	
10.00	3/4	Days 7 - 8	
LD_{50}: 2.78 mg/kg			

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and Methods

Test material WBA 8119; Purity: > [redacted] %. The test material as a solution in PEG 300, was administered to groups of 3 - 4 female guinea pigs by oral gavage at dose levels of 0.20, 0.50, 1.00, 2.15, 4.64, 5.00, and 10.00 mg/kg. Animals were observed daily for a period of 28 days, then sacrificed and autopsied.

5.2 Reliability

Reliability indicator: 3.

5.3 Findings

No mortalities were observed in the dose groups of 0.20, 0.50, 1.00, and mg/kg. The mortalities in the top four dose groups (2.15, 4.64 , 5.00, and 10.00 mg/kg) occurred between days 5 - 15. The post-mortem examination showed massive internal haemorrhages in all the guinea pigs which died. Macroscopic abnormatlities among survivors at sacrifice on day 28 included liver enlargement and signs of minor regressed haemorrhages.

SUMMARY TABLE

Route	Method/ Guideline	Species/ Strain/ Sex/ No. Animals per Group	Dose Levels (mg/kg)	Duration of Exposure	Endpoint	Value (mg/kg)/ Remarks	Reference
Oral	-	Guinea pig (*Cavia porcellus*)/	0.20, 0.50, 1.00,	Single dose with 28 day observation	Guinea pig (female)	2.78	M R Hadler, 1975 RIC0558

		Duncan Hartley/ Female/ 3 - 4 per group	2.15, 4.64, 5.00, 10.00	period	oral LD_{50}		(C2.1/03)

5.4 Conclusion

The oral LD_{50} of WBA 8119 (brodifacoum) in the female guinea pig was determined to be 2.78 mg/kg. The results of the post-mortem examinations indicate that the mortalities were due to the anticoagulant action of brodifacoum.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
Date **Materials and Methods** **Results and discussion** **Conclusion** **Reliability** **Acceptability** **Remarks**	**EVALUATION BY RAPPORTEUR MEMBER STATE** [redacted]
Date **Materials and Methods** **Results and discussion** **Conclusion** **Reliability** **Acceptability** **Remarks**	**COMMENTS FROM ...**

Doc IIIA / Section 6.13	**Acute Oral Toxicity (Oral LD_{50} in the Cat and Dog)**
BPD Data Set IIIA / Annex Point VI.2	

1 REFERENCE

1.1 Reference

[redacted], 'WBA 8119: Acute Oral Toxicity', [redacted], January 1976.

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 guideline study given, but study conducted in accordance with the scientific principles accepted at the time.

2.2 GLP

No. Study pre-dates the requirement for GLP.

2.3 Deviations and Deficiencies

Not applicable.

3 MATERIALS AND METHODS

3.1 Test Material

WBA 8119 (brodifacoum).

3.1.1 LOT/BATCH NUMBER

Not available.

3.1.2 SPECIFICATION

As given in Section 2 of Doc. IIIA.

3.1.3 DESCRIPTION

Off-white powder.

3.1.4 PURITY

>91 %.

3.1.5 STABILITY

Please refer to Section 2 of Doc IIIA.

3.2 Test Animals

3.2.1 SPECIES

Canis familiaris (Domestic dog); *Felis sylvestris cattus* (Domestic cat).

3.2.2 STRAIN

Dog: beagle.
Cat: information not given.

3.2.3 SOURCE

Information not given.

3.2.4 SEX

Dog: female
Cat: male and female.

3.2.5 AGE/WEIGHT AT STUDY INITIATION

Dog: 8 - 12 kg.
Cat: 2 - 3 kg.

3.2.6 NUMBER OF ANIMALS PER GROUP (SEX)

Dog: 2 female.
Cat: 2 male and/or female.

3.3 Administration/Exposure

Oral.

3.3.1 POSTEXPOSURE PERIOD

Observed to 28 days after treatment.

3.3.2 TYPE

Gavage.

3.3.3 CONCENTRATION

Dog: 0.25, 1.00, 2.50 and 5.00 mg/kg.

Cat: 5, 10, 25 and 50 mg/kg.

3.3.4 VEHICLE

Polyethylene glycol (PEG 300).

3.3.5 CONCENTRATION IN VEHICLE

Information not given in report.

3.3.6 TOTAL VOLUME APPLIED

Information not given in report.

3.4 Examinations

The animals were observed for up to 28 days after treatment. Clinical signs and mortalities were noted. Macroscopic examinations were performed at autopsy.

3.5 Method of Determination of LD_{50}

Estimated from the mortality data.

4 RESULTS

4.1 LD_{50}

0.25 - 1.00 mg/kg to the female dog.
25mg/kg to the cat.

4.2 Effects /4.3 Reversibility/4.4 Findings of Hispathological Examinations

ACUTE ORAL TOXICITY OF WBA 8119 (BRODIFACOUM) TO THE DOG			
Dose (mg/kg)	**n Dead/ n Investigated**	**Time of Death (range)**	**Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations**
0.25	0/2	-	Dogs dosed with 0.25 mg/kg appeared normal throughout the study. All dogs in the higher dosage groups died within 12 days of dosing and all deaths occurred between days 6 - 12. After approximately 6 days, these animals exhibited subdued behaviour, loss of appetite, pallor, respiratory difficulties, hypothermia, blood in faeces and minor external haemorrhages. Post-mortem examination of the dogs killed *in extremis* revealed a number of haemorrhages, particularly in the neck and thorax. The dogs killed at the end of the 28 days observation period showed no significant abnormalities.
1.00	2/2	Days 6 - 10	
2.50	2/2	Day 12	
5.00	2/2	Days 8 - 12	
LD_{50}: 0.25 - 1.00 mg/kg to the female dog			

ACUTE ORAL TOXICITY OF WBA 8119 TO THE CAT			
Dose (mg/kg)	**n Dead/ n Investigated**	**Time of Death (range)**	**Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations**
5	0/2	-	The only mortality in the study was one cat in the 25 mg/kg dosage group at day 8. This cat became subdued and showed rapid respiration prior to death. The animal was not subjected to post-mortem examination as it died overnight. The animals that received 50 mg/kg showed subdued behaviour and loss of appetite about 2 weeks after dosing, but had fully recovered by the end of the observation period. All other cats appeared normal throughout the study. Post-mortem examination of the surviving animal at the end of the study did not find any significant abnormalities.
10	0/2	-	
25	1/2	Day 8	
50	0/2	-	

LD_{50}: 25mg/kg to the cat

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and Methods

Test material WBA 8119; Purity: >91 %. The test material as a solution in PEG 300, was administered to groups of 2 female dogs and 2 cats (male and/or female) by oral gavage at dose levels of 0.25, 1.00 2.50, 5.00 mg/kg for the dog, and 5, 10, 25 and 50 mg/kg for the cat Animals were observed daily for a period of 28 days, then killed *in extremis* or sacrificed, and autopsied.

5.2 Reliability

Reliability indicator: 3.

5.3 Findings

Dog

Dogs dosed with 0.25 mg/kg appeared normal throughout the study. All dogs in the higher dosage groups died within 12 days of dosing and all deaths occurred between days 6 - 12. After approximately 6 days, these animals exhibited subdued behaviour, loss of appetite, pallor, respiratory difficulties, hypothermia, blood in faeces and minor external haemorrhages.
Post-mortem examination of the dogs killed *in extremis* revealed a number of haemorrhages, particularly in the neck and thorax. The dogs killed at the end of the 28 days observation period showed no significant abnormalities.

Cat

The only mortality in the study was one cat in the 25 mg/kg dosage group at day 8. This cat became subdued and showed rapid respiration prior to death. The animal was not subjected to post-mortem examination as it died overnight.
The animals that received 50 mg/kg showed subdued behaviour and loss of appetite about 2 weeks after dosing, but had fully recovered by the end of the observation period. All other cats appeared normal throughout the study. Post-mortem examination of the surviving animal at the end of the study did not find any significant abnormalities.

SUMMARY TABLE

Route	Method/ Guideline	Species/ Strain/ Sex/ No. Animals per Group	Dose Levels (mg/kg)	Duration of Exposure	Endpoint	Value (mg/kg)/ Remarks	Reference
Oral	-	Domestic dog (*Canis familiaris*)/ Beagle/ Female/ 2 per group	0.25, 1.00, 2.50, 5.00	Single dose with 28 day observation period	Dog (female) oral LD_{50}	0.25 - 1.00	[redacted]
Oral	-	Domestic cat (*Felis sylvestris cattus*)/ Male and/or Female/ 2 per group	5, 10, 25, 50	Single dose with 28 day observation period	Cat oral LD_{50}	25	[redacted]

5.4 Conclusion

The oral LD_{50} of WBA 8119 (brodifacoum) was estimated to be 0.25 - 1.00 mg/kg to the female dog and 25mg/kg to the cat.

In the dog, the autopsies showed numerous haemorrhages indicating that death was consistent with an indirect anticoagulant. No evaluation of possible toxic effects to cats was possible.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
Date **Materials and Methods** **Results and discussion** **Conclusion** **Reliability** **Acceptability** **Remarks**	**EVALUATION BY RAPPORTEUR MEMBER STATE** [redacted]
Date **Materials and Methods** **Results and discussion** **Conclusion** **Reliability**	**COMMENTS FROM ...**

Acceptability **Remarks**

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

Acute oral toxicity on birds

Acute oral toxicity to chickens

Official use only

1 REFERENCE

1.1 Reference [redacted] (1977). The Acute Oral Toxicity (LD_{50}) to the Chicken. Huntingdon Research Centre Report No: [redacted], November 1977 (unpublished) [redacted]

[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, according to US EPA Guidelines (June 1975).

2.2 GLP No. Study pre-dates the requirement for GLP.

2.3 Deviations None reported.

3 METHOD

3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	2,3,4,5RI
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 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	Chicken. 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.
3.4.4	Test conditions	See table A7_5_3_1_1-4 below.
3.4.5	Duration of the test	14 days (single oral dose followed by 14 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 at during the study, 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].*

4 RESULTS

4.1 Limit Test / Range finding test Performed.

4.1.1 Concentration: Dose levels not given in study report.

4.1.2 Number/ percentage of animals showing adverse effects: Mortalities were observed but details not given in study report.

4.1.3 Nature of adverse effects: Mortalities.

4.2 Results test substance

4.2.1 Applied concentrations: Dose levels (mg/kg_{bw}):

0 (control group dosed with corn oil vehicle only), 4.5, 9.0, 18.0, 36.0 and 72.0.

4.2.2 Effect data (Mortality): See Table A7_5_3_1_1-5 for a summary of the mortalities.

4.2.3 Body weight

Group/ Dose level (mg/kg_{bw})	Mean Bodyweight (g)				
	Day 0	Day 3	Day 7	Day 14	Mean Body weight change (Day 0-Day 14)
1/ 0 (control)	2811	2782	2759	2732	-79
2/4.5	2809	2779	2930	3248	+439
3/ 9.0	2784	2749	2723	2388	-396
4/18.0	2700	2764	2477	3045	+345
5/36.0	2813	2941	2490	-	-
6/ 72.0	2694	2715	1950	-	-

The majority of individual bodyweight changes were considered to be within normal limits. the increase in mean bodyweight apparent in Group 2 at Day 14 was due to the fact that four of the five heavier males survived to Day 14 whereas only one of the lighter females was alive at this time. Similarly, the deaths of some of the heavier birds in Group 3 resulted in an apparent bodyweight decrease in this group at Day 14.

4.2.4 Feed consumption

Group	Mean food consumption (g/bird/day) over stated period of Days		
	Days 0-7	Days 7-14	Days 0-14
1/ 0 (control)	223	161	192
2/4.5	163	145	156
3/ 9.0	224	97	161
4/18.0	133	107	120
5/36.0	161	100	131
6/ 72.0	89	-	-

The food consumption in all groups was lower during the second half of the experimental period. There was some indication that food consumption was depressed in groups treated with brodifacoum, and that the severity of this depression was greater in groups treated with high levels of brodifacoum.

4.2.5	Concentration / response curve	Not given in study report, but the 95% confidence limits from the statistical analysis were reported as 1.8 - 11.6 mg/kg_{bw}.
4.2.6	Other effects	Clinical observations: All birds were subdued for up to 1 hour after dosing. From the second day after dosing, fresh and digested blood was observed in the faeces in pens of birds treated with brodifacoum. Many of these birds showed severe and extensive bruising and subcutaneous haemorrhage, and excessive, prolonged bleeding from damaged feathers or small wounds in the skin of the face, comb and wattles. Most of the birds which died were found with blood around the beak and nostrils. Necropsy: All birds which died were found to have extensive haemorrhage of the lungs, and the majority showed intramuscular and intestinal haemorrhage and blood in the abdominal cavity. Of the birds surviving to termination of the study, many still showed extensive bruising, but there was little evidence of damage to the lungs or other internal organs.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	There were no mortalities in the control group. No abnormalities were found in any of the control birds at post-mortem examination.
4.3.2	Nature of adverse effects	See section 4.3.1 above.
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

Test substance: brodifacoum; Batch no [redacted]; Guidelines: US EPA Guidelines (June 1975); Test species: Chicken.

60 birds were randomly allocated to 6 groups, with each group comprising 10 animals (5 male and 5 females) and were given a single oral dose by gavage in corn oil followed by a 14 day observation period. There was 1 control group and 5 treatment groups at dose levels of 0, 4.5, 9.0, 18.0, 36.0 and 72.0 mg/kg_{bw} brodifacoum.

Symptoms of toxicity and mortality were recorded daily throughout the study. Bodyweight and food consumption were measure frequently throughout the study. Gross post-mortem examinations were performed on all birds that died during the test period and then on all surviving birds at termination.

5.2 Results and discussion

Mortality rates in the treatment groups were 50-100 %, with deaths occurring between Days 2 and 14 of the test period.

5.2.1 LD_{50}

4.5 mg/kg_{bw}

5.3 Conclusion

The validity criteria were fulfilled.

5.3.1 Reliability

[redacted]

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 ... *(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_3_1_2-1: Method of administration of the test substance

Carrier / Vehicle	Details
Water	No
Organic carrier	Yes, corn oil as the dose vehicle.
Concentration of the carrier [% v/v]	The concentration of the test substance in the carrier was 2.0% w/v, with the dose volume (ml/bird) adjusted according to the dose level required and the weight of the bird.
Other vehicle	
Function of the carrier / vehicle	To administer the test substance by gavage.

Table A7_5_3_1_2-2: Test animals

Criteria	Details			
Species/strain	Chicken			
Source	Information not given in study report.			
Age (in weeks), sex and initial body weight (bw)	**Group**	**Age (weeks)**	**Sex**	**Mean initial bodyweight**
	1	13	5 male and 5 female per treatment group	223 g
	2	13		163 g
	3	13		224 g
	4	13		133 g
	5	13		161 g
	6	13		89 g
Breeding population	Information not given in study report.			
Amount of food	Food was available *ad libitum*.			
Age at time of first dosing	13 weeks.			
Health condition / medication	Birds used for study assumed to be healthy as no adverse observations noted.			

Table A7_5_3_1_2-3: Test system				
Criteria	**Details**			
Test location	Indoors in holding pens.			
Holding pens	Floor pens measuring 2 x 1.5 m.			
Number of animals	60			
Number of animals per pen [cm²/bird]	10 birds per pen.			
Number of animals per dose	10 per dose level with 1 control group.			
Pre-treatment / acclimation	Not stated in study report but presumed to have been 14 days at least as initial range finding study carried out.			
Diet during test	HRC chick diet.			
Dosage levels (of test substance)	**Group**	**Dose frequency**	**Mean dose volume (ml/bird)**	**Dose level (mg/kg_{bw})**
	1	Single	10.0	0
	2	Single	0.63	4.5
	3	Single	1.25	9.0
	4	Single	2.43	18.0
	5	Single	5.06	36.0
	6	Single	9.70	72.0
Replicate/dosage level	10 birds per dosage level.			
Dosing method	Gavage			
Dosing volume per application	See above under 'dosage levels (of test substance)'.			
Frequency, duration and method of animal monitoring after dosing	Symptoms of toxicity and mortality were recorded daily throughout the study.			
Time and intervals of body weight determination	Bodyweights were recorded on Days 0, 7 and 14.			

Table A7_5_3_1_2-4: Test conditions (housing)

Criteria	Details
Test temperature	16°C.
Shielding of the animals	The birds were housed indoors by group in purpose built pens.
Ventilation	Ventilation was provided by fans adjusted to allow approximately 15 air changes per hour.
Relative humidity	Not given in study report.
Photoperiod and lighting	Not stated in study report.

Table A7_5_3_1_2-5: Mortality data

Test substance dosage level [mg/kg$_{bw}$]	**Mortality data**	
	Total number per dose level (Day)	**Percentage per dose level**
0 (controls)	0/10	0
4.5	5/10	50
9.0	7/10	70
18.0	9/10	90
36.0	10/10	100
72.0	10/10	100
LD_{50}	4.5 mg/kg$_{bw}$	
Temperature [°C]	16°C	
Relative humidity	Not stated in study report	

Table A7_5_3_1_1-7: Validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100

	Fulfilled	**Not fulfilled**
Mortality of control animals <10%	Yes	

Doc IIIA / Section 6.13

Acute Oral Toxicity (Oral LD_{50} in the Rabbit)

BPD Data Set IIIA / Annex Point VI.2

1 REFERENCE

1.1 Reference

[redacted], 1981, 'The Oral Toxicity of Brodifacoum to Rabbits', New Zealand Journal of Experimental Agriculture 9, 23 - 25, RIC0585 [redacted]

1.2 Data protection No.

1.2.1 Data owner

1.2.2 Companies with letter of access

1.2.3 Criteria for data protection

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline Study

No guideline study given, but study conducted in accordance with the scientific principles accepted at the time.

2.2 GLP

No. Study pre-dates the requirement for GLP.

2.3 Deviations and Deficiencies

Not applicable.

3 MATERIALS AND METHODS

3.1 Test Material

Brodifacoum.

3.1.1 LOT/BATCH NUMBER

Not specified.

3.1.2 SPECIFICATION

As given in Section 2.

3.1.3 DESCRIPTION

Solid.

3.1.4 PURITY

[redacted]

3.1.5 STABILITY

Please refer to Section 2 of Doc IIIA.

3.2 Test Animals

3.2.1 SPECIES

Oryctolagus cuniculus (Rabbit).

3.2.2 STRAIN

Wild New Zealand rabbit.

3.2.3 SOURCE

The rabbits used in the trials were trapped locally.

3.2.4 SEX

Male and female.

3.2.5 AGE/WEIGHT AT STUDY INITIATION

0.75 - 2.15 kg.

3.2.6 NUMBER OF ANIMALS PER GROUP (SEX)

6 per group.

3.3 Administration/Exposure

Oral.

3.3.1 POSTEXPOSURE PERIOD

Observed to one month after treatment.

3.3.2 TYPE

The doses were administered orally by drenching.

3.3.3 CONCENTRATION

0.125, 0.250, 0.375, 0.500 and 0.625 mg/kg.

3.3.4 VEHICLE

Triethanolamine:Polyethylene glycol 200:propane-1,2-diol in a ratio of 3:3:94.

3.3.5 CONCENTRATION IN VEHICLE

0.025, 0.05, 0.075, 0.100 and 0.125 g/l.

3.3.6 TOTAL VOLUME APPLIED

3.75 - 10.75 ml (5 ml/kg).

3.4 Examinations

The rabbits were observed for up to a month after treatment for signs of systemic toxicity and mortality.

3.5 Method of Determination of LD_{50}

Calculated from the mortality data using the method of Alvey *et al.*

4 RESULTS

4.1 LD_{50}

0.205 mg/kg.

4.2 Effects /4.3 Reversibility/4.4 Findings of Hispathological Examinations

ACUTE ORAL TOXICITY OF Brodifacoum TO THE RABBIT

Dose (mg/kg)	n Dead/ n Investigated	Time of Death (range)	Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations
0.125	1/6	Day 8	All rabbits dosed with 0.625 and 0.500 mg/kg died within 6 and 14 days of treatment, respectively. Five rabbits dosed with 0.375 mg/kg died within 4 -18 days
0.250	4/6	Days 4 - 9	
0.375	5/6	Days 4 - 18	

0.500	6/6	Days 4 - 14	of treatment. One rabbit treated with 0.125 mg/kg died 8 days after treatment. The remaining rabbits from each dose group survived. The most common symptoms in the dosed animals, which were observed just before death, were lassitude and anorexia. All surviving animals appeared to be fully recovered within a month of dosing.
0.625	6/6	Days 2 - 6	
LD_{50}: 0.205 mg/kg			

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and Methods

Test material brodifacoum; Purity: >91 %. The test material as a solution in triethanolamine:polyethylene glycol (PEG 200):propane-1,2-diol in a ratio of 3:3:94, was administered orally to groups of 6 New Zealand wild rabbits by drenching, at dose levels of 0.125, 0.250, 0.375, 0.500 and 0.625 mg/kg . Animals were observed for up to one month for clinical signs of toxicity and mortalities.

5.2 Reliability

Reliability indicator: 3.

5.3 Findings

All rabbits dosed with 0.625 and 0.500 mg/kg died within 6 and 14 days of treatment, respectively. Five rabbits dosed with 0.375 mg/kg died within 4 -18 days of treatment. One rabbit treated with 0.125 mg/kg died 8 days after treatment. The remaining rabbits from each dose group survived. The most common symptoms in the dosed animals, which were observed just before death, were lassitude and anorexia. All surviving animals appeared to be fully recovered within a month of dosing.

SUMMARY TABLE

Route	Method/ Guideline	Species/ Strain/ Sex/ No. Animals per Group	Dose Levels (mg/kg)	Duration of Exposure	Endpoint	Value (mg/kg)/ Remarks	Reference
Oral		Rabbit *(Oryctolagus cuniculus)* / New Zealand wild rabbit/ Male and female/ 6 per group	0.125, 0.250, 0.375, 0.500, 0.625	Single dose with 28 day observation period	Rabbit oral LD_{50}	0.205	Godrey M E R *et al*, RIC05865 (C2.1/22)

5.4 Conclusion

The acute oral LD_{50} to the New Zealand wild rabbit was determined to be 0.205 mg/kg.
The time to death did not appear to be dose dependent.

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

Reliability indicator	1

Doc IIIA / Section 6.13	**Acute Oral Toxicity (Oral LD_{50} in the Domestic Pig)**
BPD Data Set IIIA / Annex Point VI.2	

1 REFERENCE

1.1 Reference

[redacted], 'The Oral Toxicity of WB 8119 to the Domestic Pig', [redacted] 27th January 1976.

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 guideline study given, but study conducted in accordance with the scientific principles accepted at the time.

2.2 GLP

No. Study pre-dates the requirement for GLP.

2.3 Deviations and Deficiencies

Not applicable.

3 MATERIALS AND METHODS

3.1 Test Material

WBA 8119 (brodifacoum).

3.1.1 LOT/BATCH NUMBER

Not specified.

3.1.2 SPECIFICATION

As given in Section 2 of Doc. IIIA.

3.1.3 DESCRIPTION

Solid.

3.1.4 PURITY

>[redacted]%.

3.1.5 STABILITY

Brodifacoum is known to be stable based on knowledge and experience.

3.2 Test Animals

3.2.1 SPECIES

Sus scrofa (pig).

3.2.2 STRAIN

Large white domestic.

3.2.3 SOURCE

Reported as local pig breeder.

3.2.4 SEX

Male and female.

3.2.5 AGE/WEIGHT AT STUDY INITIATION

18.1 - 31.5 kg.

3.2.6 NUMBER OF ANIMALS PER GROUP (SEX)

2 per dose group (1 male and 1 female).

3.3 Administration/Exposure

Oral.

3.3.1 POSTEXPOSURE PERIOD

Observed to 21 days after treatment.

3.3.2 TYPE

Gavage.

3.3.3 CONCENTRATION

0.5, 1.0, 2.0, 5.0 and 10 mg/kg.

3.3.4 VEHICLE

Polyethylene glycol (PEG 300).

3.3.5 CONCENTRATION IN VEHICLE

1.35 - 2.70 g/l.

3.3.6 TOTAL VOLUME APPLIED

Approximately 10 ml per animal.

3.4 Examinations

The animals were observed daily for clinical signs of toxicity and mortalities up to 21 days after treatment. Bodyweight were recorded prior to treatment and at days 7, 14 and 21 after dosing. Post-mortem examination were performed at either death or termination.

3.5 Method of Determination of LD_{50}

Estimated from the mortality data.

4 RESULTS

4.1 LD_{50}

Estimated as 0.5 - 2.0 mg/kg.

4.2 Effects /4.3 Reversibility/4.4 Findings of Hispathological Examinations

ACUTE ORAL TOXICITY OF WBA 8119 (BRODIFACOUM) TO THE DOMESTIC PIG			
Dose (mg/kg)	**n Dead/ n Investigated**	**Time of Death (range)**	**Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations**

0.5	1/2	Day 18	The majority of animals died between days 10 - 14 after dosing. The only overt signs of toxicity observed were a loss of muscle function associated with mild convulsions. Immediately prior to death bleeding was observed from the nose, ears and rectum. The three animals that survived showed no signs of toxicity. All the animals bodyweight decreased after dosing. Post-mortem examinations of the pigs in the two top dosage groups (5 and 10 mg/kg) showed various haemorrhages and other changes such as blood stained fluid in the intestines. Macroscopic examination of the pigs in the lower three dosage groups (0.5, 1.0 and 2.0 mg/kg) either at death or termination revealed no obvious changes with the exception of congestion of the mucosa.
1.0	0/2	-	
2.0	2/2	Day 6	
5.0	2/2	Days 3 - 4	
10.0	2/2	Days 7 - 8	
LD_{50}: Estimated as 0.5 - 2.0 mg/kg			

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and Methods

Test material WBA 8119 (brodifacoum); Purity >91 %.
The test material as a solution in PEG 300, was administered to groups of 2 domestic pigs (1 male and 1 female) by oral gavage at dose levels of 0.5, 1.0, 2.0, 5.0 and 10.0 mg/kg. The animals were observed daily for clinical signs of toxicity and mortalities up to 21 days after treatment. Bodyweight were recorded prior to treatment and at days 7, 14 and 21 after dosing. Post-mortem examination were performed at either death or termination.

5.2 Reliability

Reliability indicator: 3.

5.3 Findings

The majority of animals died between days 10 - 14 after dosing. The only overt signs of toxicity observed were a loss of muscle function associated with mild convulsions (typical of pigs). Immediately prior to death bleeding was observed from the nose, ears and rectum. The three animals that survived showed no signs of toxicity. All the animals bodyweight decreased after dosing. Post-mortem examinations of the pigs in the two top dosage groups (5 and 10 mg/kg) showed various haemorrhages and other changes such as blood stained fluid in the intestines. Macroscopic examination of the pigs in the lower three dosage groups (0.5, 1.0 and 2.0 mg/kg) either at death or termination revealed no obvious changes with the exception of congestion of the mucosa.

SUMMARY TABLE

Route	Method/ Guideline	Species/ Strain/ Sex/ No. Animals per Group	Dose Levels (mg/kg)	Duration of Exposure	Endpoint	Value (mg/kg)/ Remarks	Reference
Oral	-	Pig (*Sus scrofa*)/ Large White Domestic/ Male and Female/ 2 per group (1 Male and 1 Female)	0.5, 1.0, 2.0, 5.0, 10.0	Single dose	Pig oral LD_{50}	Estimated as 0.5 - 2.0	[redacted]

5.4 Conclusion

The oral LD_{50} in the pig was estimated as 0.5 - 2.0 mg/kg.

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

Doc IIIA / Section 6.13

Acute Oral Toxicity (Oral LD_{50} in the Sheep)

BPD Data Set IIIA / Annex Point VI.2

1 REFERENCE

1.1 Reference

[redacted] 1985, 'Acute toxicity of brodifacoum to sheep', New Zealand Journal of Experimental Agriculture 13, 23 - 25, RIC0615 [redacted]

1.2 Data protection No.

1.2.1 Data owner

1.2.2 Companies with letter of access

1.2.3 Criteria for data protection

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline Study

No guideline study given, but study conducted in accordance with the scientific principles accepted at the time.

2.2 GLP

No. Study pre-dates the requirement for GLP.

2.3 Deviations and Deficiencies

Not applicable.

3 MATERIALS AND METHODS

3.1 Test Material

Brodifacoum.

3.1.1 LOT/BATCH NUMBER

Not specified.

3.1.2 SPECIFICATION

As given in Section 2.

3.1.3 DESCRIPTION

Solid.

3.1.4 PURITY

[redacted]

3.1.5 STABILITY

Please refer to Section 2 of Doc IIIA.

3.2 Test Animals

3.2.1 SPECIES

Ovis aries (sheep).

3.2.2 STRAIN

Not specified.

3.2.3 SOURCE

Not specified.

3.2.4 SEX

Female (ewes).

3.2.5 AGE/WEIGHT AT STUDY INITIATION

Mature animals weighing 31 - 61 kg (41 kg average weight).

3.2.6 NUMBER OF ANIMALS PER GROUP (SEX)

8 per group (one sheep in the lowest dose group removed from the study because of unrelated health problems).

3.3 Administration/Exposure

Oral.

3.3.1 POSTEXPOSURE PERIOD

Observed to 113 days after treatment.

3.3.2 TYPE

Gavage.

3.3.3 CONCENTRATION

1.56, 3.13, 6.25, 12.5 and 25.0 mg/kg.

3.3.4 VEHICLE

Triethanolamine:Polyethylene glycol:propane-1,2-diol in a ratio of 3:3:94.

3.3.5 CONCENTRATION IN VEHICLE

1.56, 3.13, 6.25, 12.5 and 25 g/l.

3.3.6 TOTAL VOLUME APPLIED

Average of 41 ml (1 ml/kg).

3.4 Examinations

Following dosing the animals were observed for signs of systemic toxicity regularly over the next 113 days. Any animal dying during the study were examined to establish the cause of death, and liver samples were taken for analysis of brodifacoum by high pressure liquid chromatography.
Sheep that survived dosing were sacrificed at monthly intervals, starting one month after the last death, for analysis of brodifacoum levels in the liver.

3.5 Method of Determination of LD_{50}

Calculated from the mortality data using probit analysis.

4 RESULTS

4.1 LD_{50}

11 mg/kg.

4.2 Effects /4.3 Reversibility/4.4 Findings of Hispathological Examinations

ACUTE ORAL TOXICITY OF BRODIFACOUM TO THE SHEEP			
Dose (mg/kg)	**n Dead/ n Investigated**	**Time of Death (range)**	**Observations/Reversibility/ Results of Necropsy/Results of Histopathological Examinations**
1.56	-	-	There were no mortalities in the lowest dose group

3.13	1/8	Days 24	(1.56 mg/kg). An unexpectedly low number of animals treated with 25 mg/kg (top dosage group) died during the study. This was attributed to the observed precipitation of brodifacoum from the treatment solution on contact with the saliva of the sheep. Analysis of the livers found the mean brodifacoum level to be 1.44 mg/kg. There was no correlation between liver brodifacoum levels and the dose level, or the time from dosing to death.
6.25	3/8	Days 17 - 22	
12.5	4/8	Days 22 - 36	
25.0	2/8	Days 18 - 20	
LD_{50}: 11 mg/kg			

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and Methods

Test material brodifacoum; Purity >91 %. The test material as a solution in triethanolamine:polyethylene glycol:propane-1,2-diol in a ratio of 3:3:94, was administered orally to groups of female sheep at dose levels of 1.56, 3.13, 6.25, 12.5 and 25.0 mg/kg. Animals were observed for up to 113 days after treatment for signs of toxicity and mortalities. Liver samples were taken for analysis of brodifacoum levels.

5.2 Reliability

Reliability indicator: 3.

5.3 Findings

There were no mortalities in the lowest dose group (1.56 mg/kg). An unexpectedly low number of animals treated with 25 mg/kg (top dosage group) died during the study. This was attributed to the observed precipitation of brodifacoum from the treatment solution on contact with the saliva of the sheep.
Analysis of the livers found the mean brodifacoum level to be 1.44 mg/kg. There was no correlation between liver brodifacoum levels and the dose level, or the time from dosing to death.

SUMMARY TABLE

Route	Method/ Guideline	Species/ Strain/ Sex/ No. Animals per Group	Dose Levels (mg/kg)	Duration of Exposure	Endpoint	Value (mg/kg)/ Remarks	Reference
Oral	-	Sheep *(Ovis aries)*/ Female/ 8 per group	1.56, 3.13, 6.25, 12.5, 25.0	Single dose with 113 day observation period	Sheep (female) oral LD_{50}	11	Godfrey M E R, Laas F J, and Rammell C G, 1985, RIC0615 (C2.1/26)

5.4 Conclusion

The oral LD_{50} of brodifacoum in the sheep was determined to be 11 mg/kg.

Evaluation by Competent Authorities

	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date **Materials and Methods** **Results and discussion** **Conclusion** **Reliability** **Acceptability** **Remarks**	[redacted]
	COMMENTS FROM ...
Date **Materials and Methods** **Results and discussion** **Conclusion** **Reliability** **Acceptability** **Remarks**	

Doc IIIA/Section 6.14	Other tests related to exposure of humans	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified** [✓] **Other justification** [✓] **Limited exposure** []	
Detailed justification:	[redacted]	
	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		
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 6.15 **BPD Data Set IIIA/Annex Point 6.15**	**Food and feedingstuffs**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified** [] **Other justification** [✓]	
Detailed justification:	[redacted]	
	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*	

Doc IIIA/Section 6.16 **Annex Point IIA, 6.16**	**Any other tests related to the exposure of the active substance to humans, in its proposed biocidal products**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified** [] **Other justification** [✓] **Limited exposure** []	
Detailed justification:	[redacted].	
	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	[redacted]	
	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 6.17 **Annex Point IIA, 6.17**	**If the active substance is to be used for action against plants then tests to assess toxic effects of metabolites from treated plants, if any, where different from those identified in animals shall be required.**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified** [] **Other justification** [✓] **Limited exposure** []	
Detailed justification:	[redacted]	
	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	[redacted]	
	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 A7.1.1.1.1

Hydrolysis as a function of pH and identification of breakdown products

BPD Data Set IIA / Annex Point VII.7.6.2.1

Official use only

1 REFERENCE

1.1 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] (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 performed to EPA guidelines (June 1985).

2.2 GLP

Yes.

2.3 Deviations

No.

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum.
3.1.1	Lot/Batch number	^{14}C-labelled brodifacoum Batch No: 94-J40.
3.1.2	Specification	As given in Section 2.
3.1.3	Purity	97.9%
3.1.4	Further relevant properties	Brodifacoum is insoluble/sparingly soluble in water with the following solubility values determined in two separate studies: OECD Guideline 105 (Inveresk, 2000) pH 4: <0.005 mg/l pH 7:<0.005 mg/l pH 9: <0.04 mg/l EPA Guideline CG-1510 (Jealott's Hill, 1991) pH 5.2: 0.0038 mg/l pH 7.4: 0.24 mg/l pH 9.3: 10 mg/l
3.2	**Reference substance**	No.
3.2.1	Initial concentration of reference substance	
3.3	**Test solution**	See Tables A7_1_1_1_1-1 and A7_1_1_1_1-2 for details.
3.4	**Testing procedure**	
3.4.1	Test system	See Tables A7_1_1_1_1-2 and A7_1_1_1_1-3 for details.
3.4.2	Temperature	25°C

3.4.3 pH

Nominal pH	**pH at beginning of study (Day 0)**	**pH at end of study (Day 30)**
5	5.1	5.2
	5.1	5.2
7	7.3	7.4
	7.2	7.2
9	9.2	9.3
	9.2	9.3

3.4.4	Duration of the test	30 days.
3.4.5	Number of replicates	2 for each pH value and each timepoint.
3.4.6	Sampling	0, 2, 8, 14, 21 and 30 days.
3.4.7	Analytical methods	At each sampling time, samples were immediately concentrated by column chromatography. The samples (about 100ml) were applied to

the top of the column using a reservoir. The aqueous eluent was made up to 100ml using water. Brodifacoum was eluted off the column using first methanol (about 6ml) and then dichloromethane (about 3ml). The organic eluents were combined and made up to 10ml with methanol. This column method was carried out at room temperature for pH7 and pH9 samples and at 25°C for the pH5 samples. This was to ensure that the brodifacoum remained in solution whilst being applied to the column. The pH5 methanol/dichloromethane samples were further concentrated by evaporating to dryness under a stream of nitrogen and then redissolved in 1ml dichloromethane.

The organic samples from the column chromatography were analysed by normal phase TLC. All plates were eluted under saturated vapour conditions using the following solvent systems:

Solvent System 1: cyclohexane:dichloromethane:methanol:acetic acid (60:30:10:1 v/v).

Solvent System 2: n-hexane:ethyl acetate (2:1 v/v)

Solvent System 3: chloroform. In some instances, to improve the chromatography in this solvent system, 1M acetic acid was applied to the baseline before sample application.

The parent reference marker (detected by short wavelength ultraviolet light) was compared with the radioactive components of the hydrolyses by co-chromatography. A bio-image was prepared for all TLC plate to give a two-dimensional representation of the radioactive areas on the plate. Autoradiographic images and quantitative data were generated by processing the image files.

Quantification of the amounts of radioactivity in the samples generated from the column chromatography was carried out using LSC.

Please see study report for further details of the analytical methods used.

3.5 Preliminary test

Yes. An initial test was carried out at 50°C using pH4, pH7 and pH9 buffer solutions to assess the degree of hydrolysis of brodifacoum. This work was designed to give a preliminary indication of the hydrolytic stability of brodifacoum so that the number of sampling dates and the intervals between sampling could be selected for the main study. Results of this phase showed that hydrolysis, if any, occurred slowly and therefore 6 sampling dates were chosen, spread at intervals throughout the study. The vials were placed in a pre-heated water bath maintained at 25.2°C for 30 days.

4	**RESULTS**	
4.1	**Concentration and hydrolysis values**	See Tables A7_1_1_1_1-4a and A7_1_1_1_1-4b for a summary of the hydrolysis results.
4.2	**Hydrolysis rate constant (k_h)**	
4.3	**Dissipation time**	DT_{50} values: At pH5 estimated by extrapolation to be approximately 173 days; At pH7 estimated by extrapolation to be approximately 300 days; At pH9 stable to hydrolysis.
4.4	**Concentration – time data**	
4.5	**Specification of the transformation products**	At pH5 the TLC profiles of the organic fraction indicated the presence of several minor breakdown products, with the Day 30 extracts containing at least 3 unknown compounds, although none represented >3.4% of the total extract. Even allowing for the worst case situation where the aqueous fraction at Day 30 comprises a single degradation product that was also present in the organic fraction, there would still be <10% of the total radioactivity. At all sampling times below 30 days, the total loss of brodifacoum, relative to the time 0 value, was <10%.
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: [redacted]; Purity: [redacted]% Guidelines: EPA guidelines (subdivision N, 161-1). [^{14}C]-brodifacoum was introduced through Tuf-Bond septa into amber glass vials containing sterile buffer solutions. The intended final concentration of brodifacoum in the vials was approximately 4 x 10^{-3}µg/ml for pH5 and approximately 40 x 10^{-3}µg/ml for pH7 and pH9. This tenfold difference in concentration was to allow for the very low water solubility of brodifacoum at low pHs. The vials were incubated in the dark. Hydrolysis vials were removed from the incubator for analysis on Days 0, 2, 8, 14, 21 and 30. The incubator maintained a temperature of 25.2°C +/-0.6°C throughout the hydrolysis period. The hydrolysis solutions were concentrated by column chromatography and analysed by thin layer chromatography. Samples of the buffer solutions containing the radiochemical were incubated in the same way as the test solutions and examined for microbial contamination before and after the hydrolysis period.
5.2	**Results and discussion**	Analysis of the hydrolysis solutions showed that brodifacoum degraded slowly at pH5 and pH7. At pH5 just over 10% of the brodifacoum degraded during the 30 day test period. At least 3 degradates were observed, but none accounted for greater than 10% of the total radioactivity. The half-life was estimated by extrapolation to be approximately 173 days. At pH7 less than 10% of the brodifacoum degraded over the 30 day period but degradation was sufficient to estimate the half life by extrapolation as approximately 300 days. At pH9 no significant degradation was observed under the experimental

		conditions of the study.
5.2.1	k_H	
5.2.2	DT_{50}	PH 5: 173 days (estimated by extrapolation); PH 7: 300 days (estimated by extrapolation); PH9: no significant degradation.
5.2.3	r^2	
5.3	**Conclusion**	Very slow degradation at pH5 and pH7. Hydrolytically stable at pH9.
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	

[^{14}C]-Brodifacoum: Hydrolysis Study

Table A7_1_1_1_1-1: Type and composition of buffer solutions (specify kind of water if necessary) and solubility of [^{14}C]-brodifacoum

pH	Type of buffer	Comments
5	Acetate buffer: 14.8ml 0.01M acetic acid and 35.2ml 0.01M sodium acetate	The buffer solutions were made up using chemicals obtained from BDH, AnalaR grade (>99% pure) and Millipore purified water.
7	Acetate buffer: 399ml 0.01M sodium acetate trihydrate and 1ml 0.01M acetic acid	
9	Borate buffer: 0.01M sodium tetraborate	

Table A7_1_1_1_1-2: Description of test solution

Criteria	Details
Purity of water	Millipore purified water: produces reagent grade (ultra pure) quality water (contains no inorganic impurities, suspended solids, microorganisms and <20ppb organics).

Preparation of test medium	99.2ml of each buffer solution was transferred using a burette into 125ml Hypovials. Each vial was sealed using a Tuf-bond septum and the top was crimped with an aluminium cap. The radiolabelled stock solution was prepared by diluting the radiochemical in methanol, to give a concentration of 5.11µg/ml. The required amount of stock solution (0.08ml for pH5, and 0.8ml for pH7 and pH9) was added to the vials by injecting directly through the septa with a sterilised glass syringe (100µl or 1ml). This was to give and approximate final concentration of 4×10^{-3}µg/ml for pH7 and pH9. A further 0.72ml of methanol (which was previousy filtered through a sterile bacterial filter into a 30ml Hypo-vial) was added to the pH5 vials after addition of the radiochemical to bring the co-solvent concentration to 0.8%v.v.
Test concentrations	<u>Nominal concentrations</u> PH5: 4×10^{-3}µg/ml PH7: 40×10^{-3}µg/ml PH9: 40×10^{-3}µg/ml <u>Measured concentrations:</u> PH5: 4.1×10^{-3}µg/ml PH7: 40.6×10^{-3}µg/ml PH9: 40.6×10^{-3}µg/ml The intended final concentration of 4×10^{-3}µg/ml for the pH5 test solutions slightly exceeded the quoted water solubility at 20°C (see section 3.1.4 above). The hydrolysis study was however carried out at 25°C rather than at 20°C and in addition , 0.8% organic solvent (methanol) was added to the test system. Both these factors served to increase slightly the solubility of brodifacoum. Preliminary experiments confirmed that homogeneous solutions of $>4 \times 10^{-3}$µg/ml could be achieved at 25°C. The concentration of [^{14}C]-brodifacoum in the test solutions was determined by Liquid Scintillation Counting (LSC)
Temperature (°C)	25°C
Controls	No
Identity and concentration of co-solvent	Methanol: 0.8%v/v for pH5 test solutions and 0.08%v/v for pH7 and pH9 test solutions.
Replicates	19 in total: 12 for analysis, 4 for sterility checks at beginning and end of hydrolysis test, and 3 spare in case of breakage

	on sterilisation.

Table A7_1_1_1_1-3: Description of test system

Glassware	125ml amber glass Hypo-vials sealed with Fuf-Bond septa and crimped with an aluminium cap, for the test solutions. The vials were incubated in a Grant water bath, fitted with a lid to further exclude light.
Other equipment	Thin Layer Chromtograph (TLC) Merck silica gel 60 F-254 plates. Whatman pHA 220 pH meter. Column chromatography using 'analytical mega bond elut' columns (C18, 1g, 6cc size; Varian). The columns were conditioned using 1 volume of methanol, and rinsed with 2 volumes of water. Jencons electronic thermometer (model 2003). Autoradiographic system (Fuji BAS 2000 Bio-image Analyser).
Method of sterilization	Autoclaving at 120°C for 20 minutes.

Table A7_1_1_1_1-4a: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 5

Compound	**Sampling times (*days*)**							
	0	**2**	**8**	**14**	**21**	**30**		
[^{14}C]-brodifacoum determined as % of radioactivity recovered from test solutions (mean of 2 replicates at each timepoint)	93.8	93.7	89.3	87.7	87.1	82.9		
Transformation product 1 :								
Transformation product 2:								
Transformation product 3 :								
Reference compound								
Volatiles *(if measured)*								
Total % recovery of radioactivity (mean of 2 samples)	99.35	92.15	98.1	90.95	96.05	93.5		

Table A7_1_1_1_1-4b: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 7

Compound	**Sampling times (*days*)**							
	0	**2**	**8**	**14**	**21**	**30**		
[^{14}C]-brodifacoum determined as % of radioactivity recovered from test solutions (mean of 2 replicates at each timepoint)	94.7	94.0	93.3	93.1	90.9	88.0		
Transformation product 1 :								
Transformation product 2:								
Transformation product 3 :								
Reference compound								
Volatiles *(if measured)*								
Total % recovery of radioactivity (mean of 2 samples)	98.6	100.65	96.45	99.5	95.2	93.9		

Table A7_1_1_1_1-4c: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 9

Compound	**Sampling times (*days*)**							
	0	**2**	**8**	**14**	**21**	**30**		
[^{14}C]-brodifacoum determined as % of radioactivity recovered from test solutions (mean of 2 replicates at each timepoint)	92.7	95.2	96.0	95.1	93.6	92.7		
Transformation product 1 :								
Transformation product 2:								
Transformation product 3 :								
Reference compound								
Volatiles *(if measured)*								
Total % recovery of radioactivity (mean of 2 samples)	100.6	101.9	101.15	99.1	97.7	98.95		

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

	pH 5		pH 7		pH 9	
	DT_{50}	**DT_{90}**	**DT_{50}**	**DT_{90}**	**DT_{50}**	**DT_{90}**
Parent compound	173 days (estimated by extrapolation)		300 days (estimated by extrapolation)		Stable to hydrolysis	
Transformation product 1						
Transformation product 2						
Transformation product n						
Reference compound						

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

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

Doc IIIA / Section A7.1.1.1.1

BPD Data Set IIA / Annex Point VII.7.6.2.1

Hydrolysis as a function of pH and identification of breakdown products

Official use only

1 REFERENCE

1.1 Reference

Jackson R, Priestley I, Hall BE (1991). The Determination of the Hydrolytic Stability of [^{14}C]-Brodifacoum. Inveresk Research International Report Number [redacted].

Experimental work carried out between April 1991 and October 1991.

1.2 Data protection

Yes

1.2.1 Data owner

Syngenta.

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 performed to EPA guidelines (subdivision N, 161-1).

2.2 GLP

Yes.

2.3 Deviations

Yes, there were the following minor deviations from the protocol that do not detract from the scientific integrity of the study:

- During the preliminary work to determine the solubility of the test substance and adsorption to glass, samples assayed by liquid scintillation counting were counted for 1 minute and not 5 minutes as stated in the protocol.
- A total viable bacteria (TVB) determination was not carried out during the course of this study. Sterility of test solutions was determined by adding samples of solution to culture tubes containing 2 different growth media, incubating at 37°C followed by visual observation of the tubes. This was considered to be an adequate procedure to determine whether sterile conditions were maintained during the study. The single positive result obtained after 30 days (pH5) was recorded at the time of observation. Negative results (ie no bacterial growth) were not recorded at the time of observation but were recorded retrospectively. This was considered not to affect the accuracy of the data or the integrity of the study.

3 MATERIALS AND METHODS

3.1 Test material Brodifacoum.

3.1.1 Lot/Batch number ^{14}C-labelled brodifacoum Batch No: ICIA0581, Ref No: 78-53.

Non-radiolabelled brodifacoum Ref No: ASY403.

3.1.2 Specification As given in section 2.

3.1.3 Purity Non-radiolabelled brodifacoum: 97.7%.

Radiolabelled brodifacoum: 97.91% radiochemical purity and was radiolabelled with ^{14}C in the phenyl ring of the 4-hydroxy coumarin moiety.

3.1.4 Further relevant properties Brodifacoum is insoluble/sparingly soluble in water with the following solubility values determined in two separate studies:

OECD Guideline 105 (Inveresk, 2000)

pH 4: <0.005 mg/l

pH 7:<0.005 mg/l

pH 9: <0.04 mg/l

EPA Guideline CG-1510 (Jealott's Hill, 1991)

pH 5.2: 0.0038 mg/l

pH 7.4: 0.24 mg/l

pH 9.3: 10 mg/l

3.2 Reference substance No.

3.2.1 Initial concentration of reference substance

3.3 Test solution See Tables A7_1_1_1_1-1 and A7_1_1_1_1-2 for details.

3.4 Testing procedure

3.4.1 Test system See Tables A7_1_1_1_1-2 and A7_1_1_1_1-3 for details.

3.4.2 Temperature 25°C

3.4.3 pH

Nominal pH	**pH at beginning of study (Day 0)**	**pH at end of study (Day 30)**
5	4.97	5.09
	5.00	5.16
7	6.97	6.92
	6.98	6.95
9	9.00	8.92
	8.99	8.94

3.4.4 Duration of the test 30 days.

3.4.5 Number of 2 for each pH value and each timepoint (42 flasks in total).

	replicates	
3.4.6	Sampling	0 (immediately following preparation), 1, 3, 7, 14, 21 and 30 days.
3.4.7	Analytical methods	The concentration of radioactivity in the test solutions was determined at each sampling time point by liquid scintillation counting. Each test sample was partitioned into dichloromethane and every flask was washed with acetone. The radioactive content of the organic and aqueous fractions, and the acetone flask washings determined by liquid scintillation counting. A fixed proportion of each organic extract and acetone flask washings were pooled and analysed by thin layer chromatography (TLC) using either TLC System 1 (Chloroform: 100%), or TLC System 3 (Dioxan:petroleum ether 30:70%v/v). Non-radiolabelled brodifacoum and 4-hydroxycoumarin were co-chromatographed with each sample extract. Following chromatography, the radioactivity on the TLC plates was quantified. A zero time and Day 30 sample extract at each pH were analysed by high performance liquid chromatography (HPLC). For each sample, the radioactivity in fractions of column eluate was determined by liquid scintillation counting. Brodifacoum and 4-hydroxycoumarin were chromatographed as reference standards. Please see study report for more details of analytical methods (HPLC, TLC and LSC).
3.5	**Preliminary test**	No.

4	**RESULTS**	
4.1	**Concentration and hydrolysis values**	See Tables A7_1_1_1_1-4a, A7_1_1_1_1-4b, and A7_1_1_1_1-4c for a summary of the hydrolysis results.
4.2	**Hydrolysis rate constant (k_h)**	
4.3	**Dissipation time**	DT_{50} values not calculated as [^{14}C]-brodifacoum essentially hydrolytically stable as only very limited degradation.
4.4	**Concentration – time data**	
4.5	**Specification of the transformation products**	
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: ICIA0581, Ref No: 78-53 (radiolabelled brodifacoum) and Ref No: ASY403 (non-radiolabelled brodifacoum); Purity: 97.7% (non-radiolabelled brodifacoum) and 97.91% radiochemical purity (radiolabelled brodifacoum); Guidelines: EPA guidelines (subdivision N, 161-1). The hydrolytic stability of [^{14}C]-brodifacoum was investigated in sterile aqueous buffered solutions at pH 5, pH 7 and pH 9 over a 30 day period. Aqueous solutions containing [^{14}C]-brodifacoum at a nominal concentration of 0.04µg/g and containing acetonitrile (0.8%v/v) as a co-solvent were incubated at 25°C in the dark. Duplicate samples were analysed after 0 (immediately following preparation), 1, 3, 7, 14, 21 and 30 days. Sterility of solutions was confirmed at the beginning and end of the incubation period.
5.2	**Results and discussion**	The concentration of radioactivity in solution (as determined by liquid scintillation counting) remained unaltered at pH 9, decreased by about 10% at pH 7 and decreased by about 60% at pH 5 during the incubation period. The balance of radioactivity was recovered from the test container by washing with acetone. The aqueous solutions were acidified and extracted with dichloromethane. Approximately 10% of the radioactivity was not extracted from the aqueous solution at all pHs from Day 1 to Day 30. Dichloromethane extracts and acetone washings were combined, and anlysed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Radioactivity analysing as [^{14}C]-brodifacoum remained essentially unaltered at all pHs from Day 1 to Day 30. At Day 30 brodifacoum accounted for 75%, 67% and 68% of the total radioactivity at pH5, 7 and 9 respectively. Approximately 10% of radioactivity was polar in nature and remained at the origin of the TLC plate. The hydrolytic half-life of brodifacoum at pH 7 and 9 is therefore much greater than 30 days. It was not possible to calculate the half-life because the degradation seen after one day of incubation did not progress. This indicated that some other mechanism during preparation

of solution or during analysis was complicating the situation.

5.2.1 k_H

5.2.2 DT_{50}

5.2.3 r^2

5.3 Conclusion Hydrolytically stable.

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

	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	

[^{14}C]-Brodifacoum: Hydrolysis Study

Table A7_1_1_1_1-1: Type and composition of buffer solutions (specify kind of water if necessary) and solubility of [^{14}C]-brodifacoum

pH	Type of buffer	Solubility of [^{14}C]-brodifacoum in buffer solutions
5	Sodium citrate buffer (pH 5) was prepared from citric acid (0.01M) and trisodium citrate (0.01M).	The solubility of [^{14}C]-brodifacoum in the 3 buffered solutions was determined as at the start of this study, aqueous solubility data for brodifacoum was not available (see section 3.1.4 above). Aliquots (100µl) of [^{14}C]-brodifacoum in acetonitrile were transferred to 3 flasks. The acetonitrile was evaporated off and a volume of buffer was added to give a target concentration of 0.05µg/ml. After sonication for 5 mins, aliquots (3 x 1.0ml) of solution were taken for liquid scintillation counting to determine the concentration of radioactivity. The proportions of radioactivity in solution at pH 5, 7 and 9 were 30%, 58% and 79% respectively. The effect of acetonitrile as a cosolvent was investigated. At pH 5, at a target concentration of 0.05 µg/ml and in the presence of 0.4% (by volume) actonitrile, 94% of the radioactivity was in solution. At pH 7 and pH 9, complete solubilisation (103%) was obtained. Based on these results, it was decided to conduct the study at a target concentration of 0.04µg/ml with acetonitrile as a cosolvent (0.8% by volume).
7	Tris-maleic acid buffer (pH 7) was prepared from Tris-maleic acid (0.01M0 and sodium hydroxide (0.01M0.	
9	Borate-boric acid buffer (pH 9) was prepared from boric acid (0.01M) and sodium tetraborate (0.01M).	

Table A7_1_1_1_1-2: Description of test solution

Criteria	Details
Purity of water	Distilled water.
Preparation of test medium	Aliquots (100 ml) of each buffer solution were dispensed into 15 preweighed conical flasks (250ml capacity). The flasks were sealed and autoclaved for 15 mins at 15psi and 120°C. The flasks were reweighed and sterile (autoclaved) distilled water was added to compensate for any loss during autoclaving. All other apparatus used during the preparation of the test solutions was similarly autoclaved. [^{14}C]-brodifacoum was dissolved in acetonitrile to give a solution of nominal concentration 5.00µg/ml. An aliquot (0.8ml) of [^{14}C]-brodifacoum solution was added to each flask to give a nominal concentration of 0.04µg/g. The total amount of [^{14}C]-brodifacoum added to each flask was calculated as 4.10µg. Duplicate aliquots (0.8ml) of acetonitrile solution were transferred to volumetric flasks (50ml capacity) at the beginning, middle and end of the application period. The flasks were made up to the mark with acetonitrile and aliquots (2 x 1.0ml) were taken for liquid scintillation counting to determine the amount of radioactivity added to each incubation flask. Application of the test material was carried out using aseptic techniques in a Gelaire BSB 6 laminar flow cabinet. Zero-time test solutions were prepared after preparation of all other solutions to allow immediate analysis to be carried out. The amount of applied radioactivity was determined separately using 2 volumetric flasks (50ml), one at the beginning and one at the end of application, as described previously. The total amount of [^{14}C]-brodifacoum added to zero-time flasks was calculated as 4.59µg.
Test concentrations	pH 5: 0.038 µg/g pH 7: 0.037 µg/g pH 9: 0.040 µg/g
Temperature (°C)	25°C
Controls	
Identity and concentration of co-solvent	Acetonitrile at 0.8%v/v
Replicates	2 for each pH and time point (42 flasks in total).

Table A7_1_1_1_1-3: Description of test system

Glassware	Sealed conical flasks (250ml capacity), volumetric flasks (50ml capacity).
Other equipment	Jencons Model 3010 pH meter, Gelaire BSB laminar flow cabinet; TLC (silica gel plates) using chloroform (100%), or Dioxan:petroleum ether (30:70 by volume); RITA 68000 linear analyser (for quantification of radioactivity on TLC plates); HPLC (Hewlett-Packard 1050 system equipped with an autosampler, solvent programmer and u.v. detector connected ot an Apex Silica 5µm column and a Berthold LB507A radiodetector); liquid scintillation analyser (Philips PW 4700 or Packard 1600 TR) with automatic quench correction by external standard-channels ratio).
Method of sterilization	All apparatus (and buffer solutions) used during the preparation of test solutions were autoclaved. Distilled water used was also autoclaved.

Table A7_1_1_1_1-4a: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 5

Compound	Sampling times (*days*)							
	0	**1**	**3**	**7**	**14**	**21**	**30**	
[^{14}C]-brodifacoum determined as % of radioactivity applied to test solutions (mean of the 2 TLC systems used and 2 samples at each timepoint)	92.87	74.78	74.13	77.85	74.57	71.74	74.53	
Transformation product 1 : **Component A/C (with same R_F values as 4-Hydroxycoumarin : 0.01) (mean of the 2 TLC systems used and 2 samples at each timepoint)**	2.44	9.74	9.24	10.73	8.35	10.34	9.095	
Transformation product 2: **Component B (R_F value : 0.10) (mean of 2 samples at each timepoint for TLC system 1 using Chloroform 100%)**	ND	ND	1.85	2.22	ND	ND	0.92	
Transformation product 3 : **Non-extractable component (mean of the 2 TLC systems used and 2 samples at each timepoint)**	2.19	6.05	6.48	7.47	5.95	7.65	8.18	
Reference compound								
Volatiles *(if measured)*								
Total % recovery of radioactivity (mean of 2 samples)	102.67	98.49	96.30	103.66	93.71	97.85	99.55	

Table A7_1_1_1_1-4b: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 7

Compound	Sampling times (*days*)							
	0	**1**	**3**	**7**	**14**	**21**	**30**	
[^{14}C]-brodifacoum determined as % of radioactivity applied to test solutions (mean of the 2 TLC systems used and 2 samples at each timepoint)	93.90	56.49	63.76	69.03	71.41	70.07	67.13	
Transformation product 1 : **Component A/C (with same R_F values as 4-Hydroxycoumarin : 0.01) (mean of the 2 TLC systems used and 2 samples at each timepoint)**	2.41	14.21	13.61	14.52	12.18	12.50	11.74	
Transformation product 2: **Component B (R_F value : 0.10) (mean of 2 samples at each timepoint for TLC system 1 using Chloroform 100%)**	ND	3.19	2.58	2.23	ND	1.82	1.09	
Transformation product 3 : **Non-extractable component (mean of the 2 TLC systems used and 2 samples at each timepoint)**	5.15	9.80	8.91	12.81	7.86	8.85	12.69	
Reference compound								
Volatiles *(if measured)*								
Total % recovery of radioactivity (mean of 2 samples)	105.61	88.51	96.92	104.87	99.46	100.04	98.02	

Table A7_1_1_1_1-4c: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 9

Compound	Sampling times (*days*)							
	0	**1**	**3**	**7**	**14**	**21**	**30**	
[^{14}C]-brodifacoum determined as % of radioactivity applied to test solutions (mean of the 2 TLC systems used and 2 samples at each timepoint)	83.87	58.62	59.78	53.73	60.96	71.22	67.92	
Transformation product 1 : **Component A/C (mean of the 2 TLC systems used and 2 samples at each timepoint)**	4.58	19.85	17.18	15.45	11.94	13.81	13.27	
Transformation product 2: **Component B (R_F value : 0.10) (mean of 2 samples at each timepoint for TLC system 1 using Chloroform 100%)**	ND	3.86	2.66	2.29	ND	1.32	2.03	
Transformation product 3 : **Non-extractable component (mean of the 2 TLC systems used and 2 samples at each timepoint)**	6.45	10.40	10.41	20.36	8.94	9.86	11.50	
Reference compound								
Volatiles *(if measured)*								
Total % recovery of radioactivity (mean of 2 samples)	98.32	98.15	98.15	98.16	89.77	102.04	100.25	

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

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

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

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

Section A7.1.1.1.2
Annex Point IIA7.6.2.2

Phototransformation in water including identity of transformation products

Determination of the direct photolysis rate in water by sunlight

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1 REFERENCE

1.1	**Reference**	Drake R.M (2004) Determination of the direct photolysis rate in water by sunlight of Brodifacoum. Chemex Environmental Internation Ltd. Reference ENV6768/120140
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 OPPTS 835 2210
2.2	**GLP**	Yes
2.3	**Deviations**	Yes

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	100%
3.1.4	Radiolabelling	N/A
3.1.5	UV/VIS absorption spectra and absorbance value	Brodifacoum showed three absorbance maxima in the region 190 to 340nm only one of which was above 290nm. No absorbance was detected (above the base line) for wavelengths above 340 nm
3.1.6	Further relevant properties	N/A
3.2	**Reference substances**	Methanol was used as a reference substance.
3.3	**Test solution**	Brodifacoum was prepared as a 155 mg/l dosing solution in acetonitrile. 1ml of the dosing solution was added to a 100ml volumetric flask and made to volume with 0.2µm filtered deionised water (1.55mg/l – 0.00000296M).
3.4	**Testing procedure**	
3.4.1	Test system	Ten tubes (2 off pyrex and 8 off quartz) were filled with the above solution. The pyrex tubes were placed in boiling tubes and covered in aluminium foil which formed a light proof jacket (control). The remaining tubes were placed in sunlight inclined at angle of about 30° with the tops facing

Section A7.1.1.1.2
Annex Point IIA7.6.2.2

Phototransformation in water including identity of transformation products
Determination of the direct photolysis rate in water by sunlight

		magnetic north. The test was set up at 12.00 on 31 March 2004. The test site is located at a latitude of 52° north. Two samples were taken from the tubes every hour for 6 hours. The samples were analysed using the HPLC conditions below. All samples were injected in triplicate.
3.4.2	Properties of light source	N/A
3.4.3	Determination of irradiance	Brodifacoum was prepared in the same way as for Tier 2 phase 1 of this test. A stock of PNAP was prepared by making 0.165g to 100 ml in acetonitrile (0.01M). An intermediate stock was prepared by diluting 10ml of this stock to 100ml with distilled water (0.001M). 17.40g of pyridine was weighed into a 100ml volumetric flask and was partially filled with 0.2µm filtered deionised water. 1ml of the intermediate PNAP stock was added and the flask made to volume with further deionised water.
3.4.4	Temperature	N/A
3.4.5	pH	N/A
3.4.6	Duration of the test	Exposure period was 6 hours for the tier 1 test and 5 hours for the tier 2 test.
3.4.7	Number of replicates	Each sample was tested 3 times.
3.4.8	Sampling	N/A
3.4.9	Analytical methods	The samples were analysed using HPLC which were all run in triplicate. The conditions were as follows: Chromotography System: Perkin Elmer Quaternary System Mobile phase: Methanol: distilled water: Aecetic acid Flow rate: 1.5ml/min Injection volume 250µl
3.5	**Transformation products**	No
3.5.1	Method of analysis for transformation products	N/A
		4 RESULTS
4.1	**Screening test**	The maximum absorbance between 290 and 800 nm was at 290nm.

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

Annex Point IIA7.6.2.2 Determination of the direct photolysis rate in water by sunlight

4.2	**Actinometer data**	N/A
4.3	**Controls**	Control loss for Brodifacoum was not considered to be significant.
4.4	**Photolysis data**	
4.4.1	Concentration values	**Fraction of day** **Molar concentration** **Run 1** **Run 2** 0.000 0.00916 0.00945 0.078 0.00567 0.00582 0.155 0.00238 0.00230 0.233 0.00092 0.00088 0.310 - - 0.388 - - 0.465 - -
4.4.2	Mass balance	N/A
4.4.3	k^e_p	10.30 day $^{-1}$ (3 hours exposure)
4.4.4	Kinetic order	N/A
4.4.5	k^e_p / k^a_p	0.481 (first 60 minutes) 1.232 (60 to 180 minutes)
4.4.6	Reaction quantum yield (ϕ^e_E)	1.28 x 10^{-3} (first 60 minutes) 3.29 x 10^{-3} minutes (60 to 180 minutes)
4.4.7	k_{pE}	4.68 day^{-1}.
4.4.8	Half-life ($t_{1/2E}$)	Half life in minutes: ϕ^e_E = 1.28 x 10^{-3} Summer = 60 Winter = 366 Spring = 78 ϕ^e_E = 3.29 x 10^{-3} Summer = 23 Winter = 143 Spring = 30
4.5	**Specification of the transformation products**	N/A

Section A7.1.1.1.2 **Annex Point IIA7.6.2.2**	**Phototransformation in water including identity of transformation products** Determination of the direct photolysis rate in water by sunlight

CONCLUSION

5.1	**Materials and methods**	Test guidelines follwed were OPPTS 835 2210
5.2	**Results and discussion**	
5.2.1	k^c_p	10.30 day $^{-1}$ (3 hours exposure)
5.2.2	K_{pE}	4.68 day^{-1}
5.2.3	ϕ^c_E	1.28 x 10^{-3} (first 60 minutes) 3.29 x 10^{-3} minutes (60 to 180 minutes)
5.2.4	$t_{1/2E}$	Half life in minutes: ϕ^c_E = 1.28 x 10^{-3} Summer = 60 Winter = 366 Spring = 78 ϕ^c_E = 3.29 x 10^{-3} Summer = 23 Winter = 143 Spring = 30
5.3	**Conclusion**	Photolysis of Brodifacoum was fast with 38 % removal in the first hour of exposure. Greater than 89 % photolysis was noted to have occurred by around three hours. Futhermore, whatever the season the half life of Brodifacoum is less than a day. In the laboratory the substance completes photolyses.
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 ...

Section A7.1.1.1.2
Annex Point IIA7.6.2.2

Phototransformation in water including identity of transformation products
Determination of the direct photolysis rate in water by sunlight

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*

Doc IIIA / Section A7.1.1.2.1 Biodegradability (ready/inherent)

BPD Data Set IIA / Annex Point VII.7.6.1.1 Ready biodegradability (Closed Bottle Test)

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1	**REFERENCE**	
1.1	**Reference**	Kelly CR and Clayton MA, 2003. Brodifacoum – Determination of Ready Biodegradability by the Closed Bottle Test. Inveresk Research International, Report No: [redacted]
1.2	**Data protection**	Yes.
1.2.1	Data owner	Syngenta.
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. OECD (1992) Guideline 301D – this test guideline was chosen as the test substance (brodifacoum) has limited solubility. The method of addition of the test substance was devised to ensure accurate addition of the test substance, without affecting the biological oxygen demand (BOD) of the test solution.
2.2	**GLP**	Yes.
2.3	**Deviations**	No.

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum.
3.1.1	Lot/Batch number	Batch No. 41.
3.1.2	Specification	Please refer to Section 2 of Doc IIIA.
3.1.3	Purity	94.4 % 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 [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 [redacted] ➢ [^{14}C]-brodifacoum was determined to be hydrolytically stable. [redacted] 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. [redacted]
3.1.5	Composition of Product	Not applicable.
3.1.6	TS inhibitory to microorganisms	No.
3.1.7	Specific chemical analysis	Yes. Please refer to Section 4.2 of Doc IIIA.
3.2	**Reference substance**	Yes. Sodium Benzoate.
3.2.1	Initial concentration of reference substance	2 mg/l
3.3	**Test ing procedure**	
3.3.1	Inoculum / test species	Please see Table A7_1_1_2-2 below.
3.3.2	Test system	Please see Table A7_1_1_2-3 below.
3.3.3	Test conditions	Please see Table A7_1_1_2-4 below.
3.3.4	Method of preparation of test solution	Brodifacoum is a poorly soluble test substance and so the following procedure was used. Brodifacoum was prepared in a volatile organic solvent (acetone) and an appropriate volume added to pieces of filter paper (as the inert carrier). The solvent was allowed to evaporate before

X

		the addition of the filter paper to each bottle.
3.3.5	Initial TS concentration	2.8 mg/l
3.3.6	Duration of test	28 days
3.3.7	Analytical parameter	Dissolved oxygen concentration (to determine oxygen depletion – this was then related to the Theoretical Oxygen Demand, or ThOD, and the concentration of test substance, and expressed as per cent biodegradation).
3.3.8	Sampling	0, 4, 7, 10, 14, 21 and 28 days.
3.3.9	Intermediates/ degradation products	Not identified (not applicable: see results section below).
3.3.10	Nitrate/nitrite measurement	No (not applicable).
3.3.11	Controls	Control (without test substance), solvent control, reference material (sodium benzoate added at 2 mg/l), and a toxicity control.
3.3.12	Statistics	

4 RESULTS

4.1 Degradation of test substance

4.1.1	Graph	
4.1.2	Degradation	3.50 % biodegradation after 28 days.
4.1.3	Other observations	None.
4.1.4	Degradation of TS in abiotic control	Not determined.
4.1.5	Degradation of reference substance	The table of results of cumulative % Biodegradation for the Reference Material (sodium benzoate) are given below (no graph plotted in report):

Day No.	% Biodegradation of sodium benzoate
4	56.89
7	70.36
10	73.35
14	67.66
21	70.36
28	78.14

4.1.6	Intermediates/ degradation products	Not determined as there was only 3.50 % biodegradation after 28 days.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test substance: brodifacoum; Batch no: 41; Guidelines: OECD (1992) Guideline 301D – this test guideline was chosen as the test substance (brodifacoum) has limited solubility. The method of addition of the test substance was devised to ensure accurate addition of the test substance on an inert carrier, so that the biological oxygen demand (BOD) of the test solution remained unaffected by the carrier.

Brodifacoum was prepared in a volatile organic solvent (acetone) and an appropriate volume added to pieces of filter paper (as the inert carrier). The solvent was allowed to evaporate before the addition of the filter paper to each bottle. The addition rate of brodifacoum on the filter paper to each bottle was equivalent to 2.8 mg/l.

The reference material (sodium benzoate) was added at 2 mg/l. Duplicate bottles for control, solvent control, test substance and reference material were prepared using a sewage sludge inoculum and were sampled for dissolved oxygen concentration on days 0, 4, 7, 10, 14, 21 and 28 to determine oxygen depletion. This was then related to Theoretical Oxygen Demand (ThOD) and concentration of test item or reference material and expressed as per cent biodegradation.

As a determination of the potential toxicity of the test substance to the inoculum, 4 bottles containing the test substance and 4 bottles containing the reference material (sodium benzoate) were prepared and

		sampled on Days 0 and 4. The test substance was considered to be readily biodegradable if a transition from 10 to 60% biodegradation is observed in a 10-day window during the 28 day test period.
5.2	**Results and discussion**	Brodifacoum was not readily biodegradable under the conditions of the test. The reference material was readily biodegradable under the conditions of the test. Brodifacoum did not appear to exhibit any inhibitory effects on the microbial inoculum.
5.3	**Conclusion**	Brodifacoum as a poorly soluble substance, was not readily biodegradable under the conditions of the test. The test met all the validity criteria.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

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_1_1_2-1: Guidline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
DOC Die-Away-Test	C.4-A	301A	ready
CO_2 Evolution-Test (Modified Sturm Test)	C.4-C	301B	ready
Modified OECD-Screening-Test	C.4-B	301E	ready
Manometric Respirometry	C.4-D	301F	ready
MITI-I-Test	C.4-F	301C	ready
Closed-Bottle-Test	C.4-E	301D	ready
Zahn-Wellens-test	C.9	302B	Inherent
Modified MITI-Test (II)	-	302C	Inherent
Modified SCAS-Test	C.12	302A	Inherent
Simulation Test with activated Sewage (Coupled Units-Test)	C.10	302A	Simulation Test[1)]

[1)] Test for the determination of the ultimate degradation of test material under conditions which simulate the treatment in an activated sludge plant

Table A7_1_1_2-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	
Strain	
Source	A sewage processing plant treating predominantly domestic sewage.
Sampling site	Haddington Municipal Sewage Treatment Works (a local sewage processing plant), sampled on 17 July 2002.
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	The activated sludge as received from the sewage processing plant was well mixed prior to sampling for solids content determination. The solids content of the sludge was determined as 3.4 g/l. After solids determination the sludge was allowed to settle for at least 30 minutes prior to a sample of the supernatent being withdrawn for use as the test microbial incoculum.
Pretreatment	None
Initial cell concentration	The solids were allowed to separate by settling for at least 30 minutes prior to taking a sample of the supernatent for use as the test microbial inoculum. This inoculum was used in the test vessel at a concentration of 3 mg/l.

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	4 litre preparation vessels were used, which after filling with the appropriate mixture, were used to fill groups of 14 bottles using a slow flowing siphon. When filling the bottles, care was taken to ensure no air bubbles were trapped. Glass stoppers with silicone grease were used to give an air-tight seal in each bottle.
Number of culture flasks/concentration	14
Aeration device	Closed bottle test and therefore not applicable (although mineral medium was strongly aerated for 20 minutes before standing at room temperature for about 23 hours, before addition to the preparation vessels).
Measuring equipment	The pH of each preparation vessel was recorded using a Jenway 370 pH meter.
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	Phosphate Buffer A KH_2PO_4: 8.50 g/l K_2HPO_4: 21.75 g/l $Na_2HPO_4.2H_2O$: 33.40 g/l NH_4Cl: 0.50 g/l
	Calcium Chloride B $CaCl_2.2H_2O$: 36.40 g/l
	Magnesium Sulphate C $MgSO_4.7H_2O$: 22.50 g/l
	Ferric Chloride D $FeCl_3.6H_2O$: 0.25 g/l (with 1 drop of concentrated HCl added to stabilise)
	The above mineral salts stock solutions were made up to volume with deionised water. The mineral media was prepared by adding 30 ml of mineral stock A, B, C and D to deionised water and making up to 30 litres with deionised water.
Additional substrate	No
Test temperature	20.32-23.02°C (water bath) in the dark or diffuse light.
pH	The pH of the preparation vessels were measured (see Table A7_1_1_2-3 above) and determined as follows. Control: pH 7.25 Solvent control: pH 7.23 Test solution: pH 7.24 Reference material: pH 7.25 Toxicity control: pH 7.23
Aeration of dilution water	No, but the mineral medium was strongly aerated for 15 minutes before standing at room temperature for about 23 hours.
Suspended solids concentration	Supernatant only of the activated sludge was used (after being allowed to settle for at least 30 minutes).
Other relevant citeria	See Table A7_1_1_2-3 above.

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

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$		
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		
Criteria for validity		

Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%		
Percentage of removal of reference substance reaches pass level by day 14		

5.3.2.1 Criteria for poorly soluble test substances	5.3.2.2	5.3.2.3
5.3.2.4	**5.3.2.5**	**5.3.2.6**
5.3.2.7	**5.3.2.8**	**5.3.2.9**

Table A7_1_1_2-6: Pass levels and validity criteria for inherent biodegradability tests

	fulfilled	**not fulfilled**
Pass levels		
20% removal (DOC or COD);		✓
Pass values reached within 10-d window (within 28-d test period)		✓
Removal of reference substance (DOC or COD) > 70 % within 14 d	✓	
Criteria for validity		
Percentage of DOC/COD-removal of reference compound ≥ 70 % within 14 days (OECD 302 B)	Not applicable (OECD 301D used)	
Percentage of DOC-removal of reference compound ≥ 40 % within 7 days and ≥ 65 % within 14 days Average residual amount of test compound in blank tests ≥ 40 % (OECD 302 C)	Not applicable (OECD 301D used)	
Removal curve of DOC or COD in the test suspension indicative for biodegradation (gradual elimination over days/weeks)		✓

Criteria for poorly soluble test substances	5.3.2.10	5.3.2.11
	5.3.2.12	**5.3.2.13**
	5.3.2.14	**5.3.2.15**

Doc III-A / Section 7.1.1.2.2 **BPD Annex Point / VII.7.6.1.2**	**Biodegradability (inherent)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [√] **Scientifically unjustified** [√] **Other existing data** [√] **Limited exposure** [√]	
Detailed justification:	[redacted]	
	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	[redacted]	
	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 7.1.2.1.2 **BPD Data Set IIA / Annex Point VII.7.6.2.2**	**Anaerobic degradation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:		
Undertaking of intended data submission [✓]	[redacted] Not submitted	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	*May 2005*	
Evaluation of applicant's justification	[redacted]	
Conclusion		
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		

AstraZeneca

QUOTATION

Client: Sorex Limited

Address: St Michael's Industrial Estate
Widnes
Cheshire WA8 8TJ

Client Agent: Roger Sharples, Regulatory Affairs Manager

Our ref: SOREX/AJP/2004/01

Date: 7 January 2004

Project:	Price:
Brodifacoum: determination of anaerobic biodegradability to ISO 11734	£4360
All work to be carried out to full GLP and a fully audited report will be provided. Study to be completed during the period March to May 2004, with full report available by 31 August 2004	
To provide BPD summary for above	£500
Total	£4860
All costs are exclusive of V.A.T.	
Valid until: 31 March 2004	

Terms of payment: 50% on completion of in-life phase, 50% on production of final reports

Accepted on behalf of the Client	Signed by AstraZeneca UK Limited, for and on behalf of Brixham Environmental Laboratory
Name: R SHARPLES	Name: N SHILLABEER
Position: Regulatory Affairs Manager	Position: Business Manager
Signature:	Signature:
Date: 8th January 2004	Date: 7 January 2004

THIS QUOTATION IS SUBJECT TO THE TERMS AND CONDITIONS PRINTED OVERLEAF

To confirm acceptance of this quotation please sign and return one copy to Brixham Environmental Laboratory for the attention of the Business Development Manager.

Brixham Environmental Laboratory
Freshwater Quarry
Brixham
Devon TQ5 8BA

Tel 01803 882682
Fax 01803 882974

AstraZeneca

QUOTATION

Client: Sorex Limited

Address: St Michael's Industrial Estate
Widnes
Cheshire WA8 8TJ

Client Agent: Roger Sharples, Regulatory Affairs Manager

Our ref: SOREX/AJP/2003/04

Date: 20 October 2003

Project:	Price:
Brodifacoum Photolysis Trial – details as per attached document agreed during meeting at Brixham Laboratory 30th September 2003	
(a) If short (BLS) report required	£4000
(b) If full report required	£6000
Total	£4000 - £6000
All costs are exclusive of V.A.T.	
Valid until: 20 January 2004	

Terms of payment: 50% on completion of in-life phase, 50% on production of final reports

Accepted on behalf of the Client	Signed by AstraZeneca UK Limited, for and on behalf of Brixham Environmental Laboratory
Name: R. SHARPLES	Name: N SHILLABEER
Position: Regulatory Affairs Manager	Position: Business Manager
Signature: R Sharples	Signature: N. Shillabeer
Date: 23rd Oct 2003	Date: 30 October 2003

THIS QUOTATION IS SUBJECT TO THE TERMS AND CONDITIONS PRINTED OVERLEAF

To confirm acceptance of this quotation please sign and return one copy to Brixham Environmental Laboratory for the attention of the Business Development Manager.

Brixham Environmental Laboratory
Freshwater Quarry
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Tel 01803 882882
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Doc IIIA / Section A7.1.3

BPD Data Set IIA / Annex Point VII.7.7

Adsorption / Desorption screening test

Estimation of adsorption with HPLC

Official use only

1 REFERENCE

1.1 Reference

Hogg A (2002). Brodifacoum: Physico-Chemical Testing with Brodifacoum: Estimation of Adsorption Coefficient. Inveresk Research Report No: 21676 (unpublished) [BR-959-0116].

1.2 Data protection

1.2.1 Data owner

1.2.2 Companies with letter of access

1.2.3 Criteria for data protection

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

Yes, OECD guideline 121: 'Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC),' January 2001.

2.2 GLP

Yes.

2.3 Deviations

Yes

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	Batch no: 41.
3.1.2	Specification	Please refer to Section 2 of Doc IIIA.
3.1.3	Purity	94.40 %
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 [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]).
3.1.5	Method of analysis	Brodifacoum was assayed using high performance liquid chromatography (HPLC) with u.v. detection. Three different mobile phases were (for the three tests): Test 1: methanol:0.01M (pH 7.0) phosphate buffer; 65:35, v/v Test 2: methanol:0.01M (pH 2.5) citrate buffer; 65:35, v/v Test 3: methanol:0.01M (pH 3.6) citrate buffer; 65:35, v/v The column used was Zorbax SB CN, 25 cm x 4.6 mm, with column temperature ambient. The flow rate was 1.0 ml/min and the u.v. detection was 225 nm, the injection volume was 150 µl, the run time was 30 min for Test 1 and 40 min for Tests 2 and 3. The data collection used was LabSystems VAX Multichrom 2 version 2.30b. Please also refer to Section 4.2 of Doc IIIA.
3.2	**Degradation products**	Degradation products tested: No

3.2.1	Method of analysis for degradation products	Not applicable.
3.3	**Reference substance**	Yes, Formamide, Acetanilide, Methyl Benzoate, Naphthalene, 1,2,3-Trichlorobenzene, Phenanthrene and DDT. All seven of these reference standards were used for each of the three tests.
3.3.1	Method of analysis for reference substance	The same HPLC system as for brodifacoum was used in each of the three tests. Please see section 3.1.5 above.
3.4	**Soil types**	Not applicable – HPLC estimation method used.
3.5	**Testing procedure**	
3.5.1	Test system	Equipment used: HPLC, LCM 1, serial no. LSM 1000365, supplied by Waters. Balance, BP 210D, serial no. 070407092, supplied by Sartorius. Calibrated thermometer, instrument no. 1364, certificate no. 12337, supplied by Fisher Scientific. Digital maximum/minimum thermometer, supplied by Fisher Scientific. Sonic bath, serial no. (AL)2385, supplied by Kerry. General laboratory glassware, supplied by Fisher Scientific. Volumetric glassware, supplied by Fisher Scientific. pH/ion analyser, model MA 235, serial no. 033209, supplied by Mettler Toledo.
3.5.2	Test solution and Test conditions	Test 1: Duplicate aliquotes (*ca* 10mg) of brodifacoum were accurately weighed into separate 100 ml volumetric flasks and made up to volume using methanol:0.01M (pH 7.0) phosphate buffer; 65:35, v/v. The samples were sonicated to aid dissolution. The stock solutions were then individually diluted by adding 1 ml to separate 10 ml volumetric flasks and made up to volume using methanol:0.01M buffer (pH 7.0) phosphate buffer; 65:35, v/v to give *ca* 10 mg/l solutions. The pH of each *ca* 10 mg/lg solution and the mobile phase was measured in duplicate, using a pH meter. The *ca* 10 mg/l solutions were analysed in duplicate according to the chromatographic conditions given above in section 3.1.5. Test 2: Duplicate aliquotes (*ca* 10mg) of brodifacoum were accurately weighed into separate 200 ml volumetric flasks and made up to volume using methanol:0.01M (pH 2.5) citrate buffer; 65:35, v/v. The samples were sonicated to aid dissolution. The stock solutions were then individually diluted by adding 2 ml to separate 10 ml volumetric flasks and made up to volume using methanol:0.01M (pH 2.5) citrate buffer; 65:35, v/v to give *ca* 10 mg/l solutions. The pH of each *ca* 10 mg/l solution and the mobile phase was measured in duplicate, using a pH meter. The *ca* 10 mg/l solutions were analysed in duplicate according to the chromatographic conditions given above in section 3.1.5. Test 3: Duplicate aliquotes (*ca* 10mg) of brodifacoum were accurately weighed into separate 200 ml volumetric flasks and made up to volume using methanol:0.01M (pH 3.6) citrate buffer; 65:35, v/v. The samples were sonicated to aid dissolution. The stock solutions were then individually diluted by adding 2 ml to separate 10 ml volumetric flasks and made up

		to volume using methanol:0.01M (pH 3.6) citrate buffer; 65:35, v/v to give *ca* 10 mg/l solutions. The pH of each *ca* 10 mg/lg solution and the mobile phase was measured in duplicate, using a pH meter. The *ca* 10 mg/l solutions were analysed in duplicate according to the chromatographic conditions given above in section 3.1.5.
3.6	**Test performance**	
3.6.1	Preliminary test	According to (a)"OECD 106": No
3.6.2	Screening test: Adsorption	According to (a)"OECD 106": No
3.6.3	Screening test: Desorption	According to (a)"OECD 106": Not performed
3.6.4	HPLC-method	According to (a)" OECD-HPLC-method"[1]: Yes Ka_{oc} (adsorption coefficient normalised to the organic carbon content) is deduced from the capacity factor (k') using a calibration plot of log k' versus log Ka_{oc} of the selected reference compounds, using the relationship: $k' = \frac{t_R - t_O}{t_O}$ where, t_R = HPLC retention time of test and reference substance (minutes), and t_O = HPLC dead time (minutes).
3.6.5	Other test	Not applicable.
4	**RESULTS**	
4.1	**Preliminary test**	Not applicable.
4.2	**Screening test: Adsorption**	Not applicable.
4.3	**Screening test: Desorption**	Not applicable.
4.4	**Calculations**	
4.4.1	Ka , Kd	Not determined using OECD 121.
4.4.2	Ka_{oc} , Kd_{oc}	Log Ka_{oc} estimated to be <1.25 in Test 1 (pH 8.46 mobile phase). Log Ka_{oc} estimated to be >5.63 in Test 2 (pH 3.29 mobile phase). Log Ka_{oc} estimated to be >5.63 in Test 3 (pH 4.43 mobile phase).
4.5	**Degradation product(s)**	Not determined using OECD 121.

[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).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test substance: brodifacoum; Batch no: 44; Guidelines: OECD guideline 121: 'Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC),' January 2001.

The principle of this test method is that while passing through the HPLC column along with the mobile phase, the test substance interacts with the stationary phase. As a result of partitioning between mobile and stationary phases the test substance is retarded. The dual composition of the stationary phase having polar and non-polar sites allow for interaction of polar and non-polar groups of a molecule in a similar way as is the case for organic matter in soil or sewage sludge matrices. This enables the relationship between the retention time on the column and the adsorption coefficient on organic matter to be established. Since only the relationship between the retention on the HPLC column and the adsorption coefficient is used in this test, only the determination of the retention time is necessary. Therefore, using a suitable set of reference substances the adsorption coefficient can be estimated.

Please see sections 3.1.5, 1.3 and 1.5 above for the reference substances used and a description of the specific method used for brodifacoum.

5.2 Results and discussion

Test 1 (pH 8.46 mobile phase): Brodifacoum was found not to be retained on the analytical column and was therefore estimated to have a log Ka_{oc} value of <1.25, the valued for acetanilide (the reference standard with the lowest log Ka_{oc} value quoted in OECD guideline 121). Please see Table Table A7_1 _3-1 for a summary of the results.

Test 2 (pH 3.29 mobile phase): Brodifacoum eluted as two peaks which were both eluted after DDT, the reference standard with the largest log Ka_{oc} value quoted in OECD guideline 121. Values of log Ka_{oc} 6.51 and 7.27 for peaks 1 and 2 respectively, were calculated by extrapolation of the appropriate reference standard plot. However, these values are calculated by extrapolation and as such the accuracy of these results are cannot be determined. Therefore, the log Ka_{oc} value of >5.63 is considered a more appropriate estimate. Please see Table Table A7_1 _3-2 for a summary of the results. The two elution peaks observed in Test 2 at the lowest pH used for the mobile phase (pH 3.29) were the resolved diasterioisomeric pairs of brodifacoum (*cis* and *trans*).

Test 3 (pH 4.43 mobile phase): Brodifacoum was found to be eluted after DDT, the reference standard with the largest log Ka_{oc} quoted in OECD guideline 121. Brodifacoum was therefore estimated to have a log Ka_{oc} value of >5.63, the value for DDT. A value of log Ka_{oc} 6.31 was calculated by extrapolation of the appropriate reference standard plot. However, this value was calculated by extrapolation and as such the accuracy of the result cannot be determined. Therefore, the log Ka_{oc} value of >5.63 is considered a more appropriate estimate.
Please see Table Table A7_1 _3-3 for a summary of the results.

Under the conditions of the tests, the stationary phase (Zorbax SB CN column) is acting as a Reverse Phase column, thus facilitating the fast elution of ionised molecules. These results indicate that brodifacoum is in an ionised form at the higher pH which is not retained on the stationary phase.

		Therefore, these results show that under conditions of high pH, brodifacoum is rapidly eluted, whereas under conditions of low pH, brodifacoum is retained on the column. Conversely, at low pH, brodifacoum is in its normal hydrogenated or non-ionised form and is retained on the column.
5.2.1	Adsorbed a.s. [%]	
5.2.2	K_a	
5.2.3	K_d	
5.2.4	Ka_{oc}	Log Ka_{oc} estimated to be <1.25 in Test 1 (pH 8.46 mobile phase). Log Ka_{oc} estimated to be >5.63 in Test 2 (pH 3.29 mobile phase). Log Ka_{oc} estimated to be >5.63 in Test 3 (pH 4.43 mobile phase).
5.2.5	Ka/Kd	
5.2.6	Degradation products (% of a.s.)	
5.3	**Conclusion**	Under basic conditions (high pH), brodifacoum is not likely to be adsorbed onto soils or sewage sludge due to the ionisation of the molecule; whereas under acidic conditions (low pH), brodifacoum is likely to be adsorbed onto soils or sewage sludge as the molecule is in its neutral or non-ionised form. The pH of the mobile phases used in this study were 8.46 (Test 1), 3.29 (Test 2) and 4.43 (Test 3). As stated in the study guideline used (OECD 121), the pH of the aqueous phase has a significant influence on the behaviour of substances. Also, the pH of soils or tanks of sewerage normally varies between pH 5.5 and 7.5. Therefore, brodifacoum is unlikely to behave at the two extremes observed in this study (completely retained at low pH or rapidly eluted at high pH). It is much more likely that in the soil/sediment environmental compartment, most of the brodifacoum would be adsorbed onto the soil or sediment and would not be leached. This has been shown to be the case in a study to investigate the mobility of brodifacoum in soil, where the amount detected in the leachate was below the limit of detection – please refer to Section 7.2.3.2 of Doc IIIA.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*03 Jun. 05*
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: Test 1: Log Ka_{oc} estimation for brodifacoum

Standard Identity	Mean retention time (min)		Mean k' (3)		Log k'		(5)Quoted Log Ka_{oc}		(4)Calculated Log Ka_{oc}	
Replicate	1	2	1	2	1	2	1	2	1	2
Formamide	2.94	2.94	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Acetanilide	3.49	3.50	0.19	0.19	-0.72	-0.72	1.25	1.25	0.89	0.88
Methyl benzoate	3.95	3.96	0.34	0.35	-0.47	-0.46	1.80	1.80	1.86	1.89
Naphthalene	4.78	4.80	0.63	0.63	-0.20	-0.20	2.75	2.75	2.91	2.89
1, 2, 3 - Trichlorobenzene	5.44	5.47	0.85	0.86	-0.07	-0.97	3.16	3.16	3.42	3.40
Phenanthrene	7.00	7.06	1.38	1.40	0.14	0.15	4.09	4.09	4.24	4.25
DDT	10.86	11.00	2.69	2.74	0.43	0.44	5.63	5.63	5.36	5.37
Brodifacoum	2.28	2.29	-0.22	-0.22	N/C	N/C	N/A	N/A	<1.25	<1.25

(1) N/A = Not Applicable

(2) N/C = Not Calculated

(3) $k' = \frac{t_R - t_0}{t_0}$ where t_R is the retention time of the reference standard or test substance and t_R is the retention time of formamide.

(4) Refers to the log Ka_{oc} value calculated using the equation of the line from the appropriate reference standard plot and is used to determine the accuracy of the assay.

(5) Log Ka_{oc} values for reference standards were taken from the OECD guideline 121.

CONCLUSION: The overall mean log Ka_{oc} for brodifacoum in methanol:0.01M phosphate buffer (pH 7.0); 65:35 v/v was estimated to be <1.25.

TABLE A7_1 _3-2: Test 2: Log Ka_{oc} estimation for brodifacoum

Standard Identity	Mean retention time (min)		Mean k' (3)		Log k'		(5)Quoted Log Ka_{oc}		(4)Calculated Log Ka_{oc}	
Replicate	1	2	1	2	1	2	1	2	1	2
Formamide	2.83	2.83	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Acetanilide	3.46	3.46	0.22	0.22	-0.66	-0.66	1.25	1.25	0.93	0.93
Methyl benzoate	3.93	3.93	0.39	0.39	-0.41	-0.41	1.80	1.80	1.86	1.86
Naphthalene	4.91	4.91	0.73	0.73	-0.14	-0.14	2.75	2.75	2.86	2.86
1, 2, 3 - Trichlorobenzene	5.68	5.68	1.01	1.01	0.00	0.00	3.16	3.16	3.38	3.38
Phenanthrene	7.62	7.62	1.69	1.69	0.23	0.23	4.09	4.09	4.24	4.24
DDT	12.63	12.65	3.46	3.47	0.54	0.54	5.63	5.63	5.39	5.39
Brodifacoum (peak 1)	22.19	22.44	6.84	6.93	0.84	0.84	N/A	N/A	6.51	6.50
Difenacom (peak 2)	34.17	34.55	11.07	11.21	1.04	1.05	N/A	N/A	7.25	7.28

(1) N/A = Not Applicable

(2) N/C = Not Calculated

(3) $k' = \frac{t_R - t_0}{t_0}$ where t_R is the retention time of the reference standard or test substance and t_R is the retention time of formamide.

(4) Refers to the log Ka_{oc} value calculated using the equation of the line from the appropriate reference standard plot and is used to determine the accuracy of the assay.

(5) Log Ka_{oc} values for reference standards were taken from the OECD guideline 121.

CONCLUSION: The overall mean log Ka_{oc} for brodifacoum in methanol:0.01M citrate buffer (pH 2.5); 65:35 v/v was calculated to be 6.51 and 7.27 for peaks 1 and 2 respectively.

TABLE A7_1 _3-3: Test 3: Log Ka_{oc} estimation for brodifacoum

Standard Identity	Mean retention time (min)		Mean k' (3)		Log k'		(5)Quoted Log Ka_{oc}		(4)Calculated Log Ka_{oc}	
Replicate	1	2	1	2	1	2	1	2	1	2
Formamide	2.91	2.92	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Acetanilide	3.64	3.66	0.25	0.25	-0.60	-0.60	1.25	1.25	0.99	0.99
Methyl benzoate	4.18	4.20	0.44	0.44	-0.36	-0.36	1.80	1.80	1.84	1.84
Naphthalene	5.36	5.39	0.84	0.85	-0.08	-0.07	2.75	2.75	2.84	2.86
1, 2, 3 - Trichlorobenzene	6.32	6.37	1.17	1.18	0.07	0.07	3.16	3.16	3.37	3.36
Phenanthrene	8.81	8.92	2.03	2.05	0.31	0.31	4.09	4.09	4.22	4.20
DDT	15.80	16.08	4.43	4.51	0.65	0.65	5.63	5.63	5.43	5.41
Difenacom	25.70	26.44	7.83	8.05	0.89	0.91	N/A	N/A	6.29	6.33

(1) N/A = Not Applicable

(2) N/C = Not Calculated

(3) $k' = \frac{t_R - t_0}{t_0}$ where t_R is the retention time of the reference standard or test substance and t_R is the retention time of formamide.

(4) Refers to the log Ka_{oc} value calculated using the equation of the line from the appropriate reference standard plot and is used to determine the accuracy of the assay.

(5) Log Ka_{oc} values for reference standards were taken from the OECD guideline 121.

CONCLUSION: The overall mean log Ka_{oc} for brodifacoum in methanol:0.01M citrate buffer (pH 3.6); 65:35 v/v was calculated to be 6.31.

Doc IIIA / Section A7.2.1

Aerobic degradation in soil, initial test

BPD Data Set IIIA / Annex Point VII.4 and XII.1.1

Official use only

1 REFERENCE

1.1 Reference Hall BE and Priestley I (1992). Brodifacoum: Metabolism in Soil Under Aerobic Conditions. Inveresk Research International Report No: 8795 (unpublished). [F3.1/01]

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 162-1 (October 1982).

2.2 GLP Yes.

2.3 Deviations Yes. There were several minor amendments 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 ref no: ASY403
3.1.2	Specification	As given in section 2.
3.1.3	Purity	[^{14}C]-Brodifacoum radiochemical purity: 96% with a specific activity of 925 MBq.mmol^{-1}. Non-radiolabelled brodifacoum purity: 97.7% with a *cis-trans* isomer ratio of 60:40.
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

Sandy loam/sandy clay loam (although classified under the USDA system as a sandy clay loam, the solid was within 0.4% on clay/silt content of being classified as a sandy loam), (see Table A7_2_1-1 below).

3.3 Testing procedure

3.3.1	Test system	Thirty samples of soil (50g oven dry weight equivalent) were weighed into individual Erlenmeyer flasks (250ml capacity). The soil moisture content in each flask was adjusted (as appropriate) gravimetrically using deionised water to *ca.* 75% of that determined at 0.33bar. The soils were

		maintained at this level during a 5 day acclimatisation period and during incubation (in the dark). Following application to the soil samples, all flasks (with the exception of the two zero-time samples) were connected to a continuous air-flow system with a stream of moist carbon dioxide-free air drawn over the soil surface. The gas mixture leaving the flasks was combined and then passed through a safety trap, a polyurethane plug (expanded volume *ca.* 50ml compressed into an in-line cartridge, volume *ca.* 10ml), ethanediol, sulphuric acid (0.05M) and ethanolamine. The trap contents and polyurethane plugs were removed for analysis and replenished at weekly intervals up to 8 weeks post-application, then at 2 weekly intervals up to 49 weeks and also at the time of removal of a pair of incubation flasks for sample analysis. Final collections were carried out at 52 weeks post-application to the soil samples.
3.3.2	Temperature	19.0 – 22.5°C
3.3.3	Method of preparation of test solution	Radiolabelled brodifacoum: dose solution prepared by dissolving the brodifacoum into acetonitrile at a concentration of 195µg/ml. The radioactive concentration of the dose solution was 8.93µCi/ml. Unlabelled brodifacoum: dose solution prepared by dissolving brodifacoum into acetonitrile at a concentration of 228µg/ml.
3.3.4	Application of test substance to soil	Both radiolabelled and unlabelled brodifacoum: The [^{14}C]-brodifacoum dose solution (100µl) was applied to the surface of 26 soil samples, and the contents of the flasks gently tumbled to effect a uniform distribution. The contents of 2 further flasks were similarly treated with non-radiolabelled brodifacoum dose solution. Two flasks remained untreated. Before, during and after the application of the radiolabelled solution, aliquots (100µl) of solution were transferred to duplicate volumetric flasks (25ml capacity). The volumetric flasks were made up to the mark with acetonitrile and aliquots (2 x 100µl) were assayed by liquid scintillation counting (LSC) to determine the amount of radioactivity applied to the soil samples. The application rate was calculated as 18.96µg brodifacoum/ 50g dry weight equivalent of soil (0.38µg/g or 0.38mg/kg).
3.3.5	Duration of test	52 weeks
3.3.6	Number of replicates	2
3.3.7	Sampling	1, 3, 7, 14, 28 days and 8, 13, 17, 26, 39, 52 weeks
3.3.8	Method of analysis	Each soil sample was extracted overnight with dichloromethane:methanol (4:1 by volume; 100ml) using an orbital shaker. Following separation by centrifugation the soil pellet was further extracted with fresh extractant (50ml) for 2 – 3 hours using a wrist action shaker. The radioactivity present in each extract from samples up to and including Day 7 was quantified by LSC. For Day 14 samples onwards, the two extracts were pooled prior to LSC. After extraction each soil residue was assayed for radioactivity by combustion and LSC. Residues from samples up to and including Day 28 were allowed to dry prior to analysis. Soil residues from Week 8, 13 and 17 samples were further extracted using methanol (1 x 50ml) and methanol:water (80:20 by volume, 2 x 50ml). Extractions were carried out overnight using a wrist action shaker. The radioactivity present in each extract was quantified by LSC. The flasks were soaked in acetone to recover any radioactivity

associated with the apparatus. Aliquots of each apparatus wash were analysed by liquid scintillation counting.

Each polyurethane plug was extracted with acetonitrile (30 – 45g). The radioactivity in the acetonitrile extract was determined by LSC.

The dichloromethane:methanol extracts from each soil sample were pooled. In preparation for thin layer chromatography (TLC), an aliquot (10ml) of each pooled extract was evaporated to dryness at ambient temperature under nitrogen and the residue reconstituted in acetone (1ml). Aliquots of the acetone solution were examined by TLC and a combination of solvent systems:

Solvent System 1: chloroform (100%),

Solvent System 2: dioxan: petroleum ether (30:70 by volume),

Solvent System 3: toluene: propan-2-ol: acetic acid (9:1:1 by volume).

Solvent System 1 was used for all samples, Solvent System 2 for all samples up to and including Week 17 and Solvent System 3 for Day 28 samples and all samples from Week 17 onwards. Authentic brodifacoum was co-chromatographed with each sample. The putative degradation product 4-hydroxycoumarin was also co-chromatographed with all samples except for Week 52 extracts. The position of brodifacoum and 4-hydroxycoumarin (where applicable) on each TLC plate was determined by irradiation with uv light (wavelength 254nm). Radioactivity on each TLC plate was quantified using a RITA radio-TLC analyser.

3.3.9	Degradation products	Not identified

4 RESULTS

4.1	**Degradation of test substance**	The amount of extractable radioactivity decreased with increasing incubation time. The decline in extractable radioactivity coincided with an increase in $^{14}CO_2$ evolution and non-extractable radioactivity. [^{14}C]-Brodifacoum (combined *cis* and *trans* isomers) was the major radiolabelled component in the soil extracts at all time points up to 52 weeks and degraded with a half-life of 157 days. The chromatographic analysis of the soil extracts are given in Tables A7_2_1-2, A7_2_1-3 and A7_2_1-4 below.
4.2	**Mass balance**	The mean total recoveries of applied radioactivity were in the range 97.66 – 107.00% (see Table A7_2_1-5 below). Evolution of $^{14}CO_2$ increased with incubation time, a mean total of 35.80% of the applied radioactivity being recovered as $^{14}CO_2$ at 52 weeks. The levels of radioactivity accounted for by volatiles other than $^{14}CO_2$ were less than 2% over the study period.
4.3	**DT_{50}**	157 days

4.4	**Degradation product(s)**	Up to 5 minor radiolabelled components, none exceeding 10% of the applied radioactivity at any time point, were present in the soil extracts. In addition, an unidentified radiolabelled component, which was less polar than brodifacoum, was also present in the soil extracts; this radioactive component gradually increased throughout the study, and accounted for around 15% of the applied radioactivity at Weeks 39 and 52, at which time points brodifacoum accounted for *c.a.* 23% and 17% respectively.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test substance: [^{14}C]-brodifacoum radiolabelled uniformly in the 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%; Non-radiolabelled brodifacoum: 97.7%. Guidelines: EPA Pesticide Assessment Guidelines, Subdivision N, Paragraph 162-1 (October 1982). [^{14}C]-Brodifacoum was applied to soil (50g oven dry weight equivalent) at a nominal application rate of 0.38mg/kg and incubated under aerobic conditions at *c.a.* 75% of moisture holding capacity (at 0.33bar) and 21 +/- 2°C for up to 52 weeks. Soil samples were extracted with dichloromethane:methanol (4:1 by volume). Evolved $^{14}CO_2$ together with any neutral or basic radiolabelled volatiles were trapped and quantified throughout the incubation period. Profiles of extractable radiolabelled degradates were also examined and a degradation half-life for brodifacoum estimated.
5.2	**Results and discussion**	The amount of extractable radioactivity decreased with increasing incubation time. The decline in extractable radioactivity coincided with an increase in $^{14}CO_2$ evolution and non-extractable radioactivity. Chromatographic analysis of the soil extracts indicated that [^{14}C]-brodifacoum was degraded with a half-life of 157 days. The principal degradation product was an unidentified component which became prominent in soil extracts from 26 week samples. By 52 weeks post-application, the mean contribution of this component was similar to that of parent compound. Up to 5 other unidentified radiolabelled components were present but their overall contribution was relatively minor; 4-hydroxycoumarin did not appear to be present in soil extracts.
5.2.1	DT_{50}	157 days
5.2.2	Degradation products (% of a.s.)	Up to 5 minor radiolabelled components, none exceeding 10% of the applied radioactivity at any time point, were present in the soil extracts. In addition, an unidentified radiolabelled component, which was less polar than brodifacoum, was also present in the soil extracts; this radioactive component gradually increased throughout the study, and accounted for around 15% of the applied radioactivity at Weeks 39 and 52, at which time points brodifacoum accounted for *ca.* 23% and 17% respectively.
5.3	**Conclusion**	
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

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*
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 soil

	Soil 1	Soil 2	Soil 3
Soil type (classification)	Sandy clay loam		
Location and date	Soil (identified as 18 Acres) received on 23 May 1991 and supplied by ICI Agrochemicals.		
pH (water)	7.1		
Cation Exchange Capacity (me. $100g^{-1}$)	13.56		
% Moisture at 0.33bar	75%		
% Sand (0.050 – 2.000mm)	63.1		
% Silt (0.002 – 0.050mm)	16.5		
% Clay (<0.002mm)	20.4		

Table A7_2_1-2: TLC analysis of soil extracts using Solvent System 1 (chloroform 100%)

Time Point	TLC Component (Expressed As Mean % Radioactivity Applied To Soil)							
	Cis-Brodifacoum	*Trans*-Brodifacoum	Origin	A	B	C	D	E
Day 0	41.29	51.84	1.98	NC	NC	NC	NC	NC
Day 1	41.43	45.77	4.29	1.99	NC	NC	NC	NC
Day 3	37.31	42.55	4.47	3.73	1.31	NC	3.49	NC
Day 7	32.86	41.13	3.40	3.12	1.66	1.17	6.01	NC
Day 14	33.83	38.71	2.77	NC	1.75	1.39	3.43	1.33
Day 28	35.03	37.78	1.60	NC	1.66	1.29	NC	0.55
Week 8	35.46	29.46	1.87	0.98	1.31	1.72	1.98	2.62
Week 13	34.84	26.10	3.09	0.63	0.68	1.51	1.64	3.19
Week 17	34.09	21.17	5.88	NC	NC	1.89	2.87	3.90
Week 26	26.25	13.17	9.33	NC	NC	1.66	1.15	6.10
Week 39	15.85	7.20	6.40	NC	1.34	NC	0.78	14.16
Week 52	12.21	4.73	3.49	NC	NC	NC	NC	15.50

NC = No corresponding radiolabelled component

Table A7_2_1-3: TLC analysis of soil extracts using Solvent System 2 (dioxan: petroleum ether 30:70 by volume)

Time Point	TLC Component (Expressed As Mean % Radioactivity Applied To Soil)							
	Cis-Brodifacoum	*Trans*-Brodifacoum	Origin	F	G	H	I	
Day 0	38.64	57.24	1.32	NC	NC	NC	NC	
Day 1	35.95	52.46	2.10	NC	0.42	1.96	2.09	
Day 3	36.63	51.13	4.49	NC	NC	NC	2.99	
Day 7	36.22	43.35	4.84	4.19	1.70	0.26	2.27	
Day 14	37.14	42.59	4.18	NC	1.79	NC	NC	
Day 28	36.02	33.16	1.73	3.96	3.00	0.29	0.83	
Week 8	34.16	33.85	1.64	1.15	3.38	0.61	2.57	
Week 13	33.03	29.24	2.11	2.11	3.41	1.09	2.99	
Week 17	32.03	29.99	3.09	NC	5.84	1.91	2.31	

NC = No corresponding radiolabelled component

Table A7_2_1-4: TLC analysis of soil extracts using Solvent System 3 (toluene: propan-2-ol; acetic acid 9:1:1 by volume)

Time Point	TLC Component (Expressed As Mean % Radioactivity Applied To Soil)							
	Cis-Brodifacoum	*Trans*-Brodifacoum	Origin	J	K			
Day 28	36.89	37.15	NC	2.31	1.48			
Week 17	35.15	25.19	0.61	2.54	5.51			
Week 26	26.63	18.08	0.46	3.34	7.73			
Week 39	17.19	10.10	1.30	1.48	15.89			
Week 52	12.36	8.12	1.02	3.29	14.00			

NC = No corresponding radiolabelled component

Table A7_2_1-5: Mean total % recovery of applied radioactivity

Time Point	Mean Total Recovery (Expressed as Mean % radioactivity applied to soil)
Day 0	102.33
Day 1	101.39
Day 3	103.47
Day 7	101.82
Day 14	98.75
Day 28	97.66
Week8	102.41
Week 13	102.24
Week 17	107.00
Week 26	101.46
Week 39	106.00
Week 52	102.60

Doc. IIIA / Section No. A7.2.3.1

Adsorption / Desorption in soil

BPD Data Set IIIA / Annex Point XII.1.2

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1 REFERENCE

1.1 Reference

Newby SE and White BG (1979). Brodifacoum: Adsorption and Desorption in soils measured under laboratory conditions. ICI Plant Protection Division Report No. TMJ 1764 B (unpublished). [redacted]

1.2 Data protection

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 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.

2.2 GLP

No, study conducted pre-GLP implementation.

2.3 Deviations

No, but additional adsorption-desorption studies were conducted. These were undertaken to investigate brodifacoum stability in soil slurries and the opportunity was taken to compare behaviour of the *cis: trans* mixture with each isomer separately. Also, the identity of the radioactivity adsorbed onto the soils was determined.