RESULTS FOR EYE IRRITATION TO RABBIT WITH BRODIFACOUM (Washed eyes)				
	Cornea (Opacity)	**Iris**	**Conjunctiva**	
			Redness	**Chemosis**
Score (average of animals investigated)	**0 to 4**	**0 to 2**	**0 to 3**	**0 to 4**
1 - 2 h	Not assessed	1	1	0
24 h	0	0	0	0
48 h	0	0	0	0
72 h	0	0	0	0
Average 24 h, 48 h, 72 h	0	0	0	0
Area affected	-	Information not available		
Mean total score (max 110)**	1 - 2 h: 6 24 h: 2 48 h: 1 72 h: 0			
Reversibility*	-	c	c	c
Average time for reversion	-	24 h	24 h	-

***c:** **completely reversible**
nc: **not completely reversible**
n: **not reversible**

** **Scoring system for mean total score described in section 3.3.2.1 above.**

4.3 Other findings

Unwashed eyes

Instillation of the test substance caused moderate initial pain. This was followed by iritis in three animals. Other symptoms were slight redness of the conjunctivae with slight chemosis and some discharge. All eyes appeared normal at the end of the seven day observation period.

Washed eyes

Instillation of the test substance caused slight redness and chemosis of the conjunctivae in all three animals. One animal also had slight iritis. All eyes appeared normal after 72 hours.

5 APPLICANT'S SUMMARY AND CONLCUSION

5.1 Materials and Methods

Test material brodifacoum (PP581); Purity 92.5 %;
Nine New Zealand White male rabbits each received 100mg brodifacoum into the conjunctival sac of the left eye. Three of the animals had the treated eye irrigated for one minute with clean lukewarm tap water 30 seconds after instillation of the test substance. The eyes were observed at 1-2, 24, 48 and 72 hours and 4 and 7 days after instillation.

5.2 Reliablity

Reliability indicator: 2.

5.3 Findings

In the unwashed eye group, instillation of the test substance caused moderate initial pain, the eye being held shut and occasionally rubbed with the paws (class 3 on a 0 - 5 point scale). In three of the animals, this was followed by iritis. Other symptoms were slight redness of the conjunctivae, slight chemosis and some discharge. All eyes appeared normal at the end of the seven day observation period.

In the washed eye group, instillation of the test substance caused slight redness and chemosis of the conjunctivae. All eyes appeared normal at the end of the seven day observation period.

SUMMARY TABLE

Species	**Method**	**Result**	**Mean total score (Max 110)**	**Reference**
Rabbit (*Oryctolagus cuniculus*) (New Zealand White)/ 9 animals	100mg brodifacoum in left eye. 6 animals eye unwashed, 3 animals eye washed.	Mild irritation (class 4 on a 1 - 8 point scale) to both washed and unwashed eyes	<u>Unwashed eye</u> 1 - 2 h: 8 24 h: 2 48 h: 1 72 h: 1 <u>Washed eye</u> 1 - 2 h: 6 24 h: 2 48 h: 1 72 h: 1	Parkinson G R, 1978, CTL/P/404 (C2.1/10),

5.4 Conclusion

Brodifacoum was concluded to be a mild irritant to both washed and unwashed rabbit eyes (class 4 on a 1 - 8 point scale).
The mean total score (max 110) for unwashed eyes was 8 (1 - 2 h), 2 (24 h), 1 (48 h) and 1 (72 h).
The mean total score (max 110) for washed eyes was 6 (1 - 2 h), 2 (24 h), 1 (48 h) and 1 (72 h).
All eyes (both washed and unwashed) appeared normal at the end of the seven day observation period.

	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**	

Syngenta Limited	**Brodifacoum**	**July 2000**

Doc IIIA / Section 6.1.4

Acute Dermal Irritation (Dermal Irritation in the Rabbit)

BPD Data Set IIA / Annex Point VI.6.1.4

1 REFERENCE

1.1 Reference

[redacted], 1978, 'Brodifacoum: Skin and Eye Irritation', [redacted], [redacted]/P/404 [redacted], 26th July 1978.

1.2	**Data protection**	Yes.
1.2.1	Data owner	Syngenta Limited.
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

EPA guidelines 5 13 77.

2.2 GLP

No. Study pre-dates the requirement for GLP.

2.3 Deviations and Deficiencies

None stated.

3 MATERIALS AND METHODS

3.1 Test Material

Brodifacoum (PP581).

3.1.1 LOT/BATCH NUMBER

Not specified.

3.1.2 SPECIFICATION

As given in Section 2 of Doc IIIA.

3.1.3 DESCRIPTION

Buff coloured solid.

3.1.4 PURITY

[redacted]

3.1.5 STABILITY

[redacted].

3.2 Test Animals

3.2.1 SPECIES

Oryctolagus cuniculus (rabbit).

3.2.2 STRAIN

New Zealand White (male).

3.2.3 SOURCE

Not specified.

3.2.4 AGE/WEIGHT AT STUDY INITIATION

2 - 2.5 kg.

3.2.5 NUMBER OF ANIMALS PER GROUP (SEX)

One group of 6 male rabbits.

3.3 Study Design and Methods

3.3.1 APPLICATION

3.3.1.1 Preparation of test substance

0.25 ml of a 0.5w/v solution of brodifacoum in polyethylene glycol 300 was used for each application site.

3.3.1.2 Preparation of test site

An area of approximately 150 mm x 130 mm on each rabbit was clipped free of hair on the dorso-lumbar region. 24 hours after clipping one flank of each rabbit was abraded using a scalpet blade sufficiently deep to penetrate the stratum corneum, but not to disturb the derma (ie no bleeding).

3.3.1.3 Occlusion

Occlusive: the treated areas were covered with polythene patches held in position by adhesive polythene tape passed once around the trunk of the animal. A crepe bandage was then wrapped around the body of the rabbit. After the exposure period, the sites were washed with warm tap water.

3.3.1.4 Exposure period

24 hours.

3.3.1.5 Post-treatment observation period

72 hours.

3.3.2 EXAMINATIONS

3.3.2.1 Scoring system

Draize scale (reference 1):

VALUE	SKIN REACTION
Erythema:	
0	No erythema
1	Very slight erythema
2	Well defined erythema
3	Moderate to severe erythema
4	severe erythema (beet redness)
Oedema:	
0	No oedema
1	Very slight erythema
2	Slight oedema
3	Moderate oedema
4	Severe oedema

3.3.2.2 Examination time points

Animals observed at 24 and 72 hours after application.

3.3.2.3 Other investigations

The study included a comparison of abraded and non-abraded skin.

4 RESULTS

4.1 Scores at different time points/
4.2 Reversibility

RESULTS OF SKIN IRRITATION TO THE RABBIT WITH BRODFIFACOUM					
	Time	**Erythema**		**Oedema**	
	(Hours)	**Intact**	**Abraded**	**Intact**	**Abraded**
Draize scores (average)	**24 h**	0.83	1	0.33	0.67
(0 to a maximum 4)	**72 h**	0	0	0	0
Average score	**24 h, 72h**	0.41	0.5	0.17	0.33
Reversibility*		c	c	c	c
Average time for reversibility		72 hours	72 hours	72 hours	72 hours

***c: completely reversible**
nc: not completely reversible
n: not reversible

4.3 Findings at Treated Skin

Signs of irritation were observed at all but one of the application sites at the end of the 24 hour exposure period. All the sites affected showed slight erythema, while two of the intact areas had slight oedema and three of the abraded areas had slight or moderate oedema. Forty-eight hours later (ie 72 hours after application), there were no signs of irritation at any of the application sites.

5 APPLICANT'S SUMMARY AND CONLCUSION

5.1 Materials and Methods

Test material: brodifacoum (PP581); Purity: [redacted]%;
0.25 ml of a 0.5% w/v solution of brodifacoum in polyethylene glycol 300 was applied to two areas of clipped skin (abraded and non-abraded), of 6 New Zealand White male rabbits for 24 hours. The treated areas were covered with polyethene patches held in position by adhesive polythene tape passed once around the trunk of the animal. A crepe bandage was then wrapped around the body of the rabbit. After the exposure period, the sites were washed with warm tap water

5.2 Reliablity

Reliability indicator:2.

5.3 Findings

Signs of irritation were observed at all but one of the application sties at the end of the 24 hour exposure period. All the sites affected showed slight erythema, while two of the intact areas had slight oedema and three of the abraded areas had slight or moderate oedema. Forty-eight hours later (ie 72 hours after application), there were no signs of irritation at any of the application sites.

SUMMARY TABLE

Species	**Method**	**Result**	**Average Score (all time points)**	**Reference**

Rabbit (*Oryctolagus cuniculus*)	24 hour exposure to occluded skin (abraded and non-abraded)	Slight irritation	Erythema (intact skin): 0.41 Erythema (abraded skin) 0.5 Oedema (intact skin): 0.17 Oedema (abraded skin): 0.33	[redacted], 1978, [redacted] /P/404 [redacted]

5.4 Conclusion X

Brodifacoum was concluded to be a slight irritant to rabbit skin.
The mean skin irritation scores (24 and 72 hours) after application of the test substance brodifacoum, were 0.41 and 0.50 for erythema (intact and abraded skin respectively), and 0.17 and 0.33 for oedema (intact and abraded skin respectively).

	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 6.1.5	**Skin Sensitisation (Guinea pig maximisation test of Ritz and Buehler)**
BPD Data Set IIA / Annex Point VI.6.1.5	

1 REFERENCE

1.1 Reference

[redacted], 1996, 'Brodifacoum: Skin Sensitisation to the Guinea Pig', [redacted], [redacted] /P/5105 [redacted]

1.2	**Data protection**	Yes.
1.2.1	Data owner	Syngenta Limited.
1.2.2	Companies with letter of access	[redacted].
1.2.3	Criteria for data protection	[redacted].

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline Study

Yes, the study was conducted in accordance with the following guidelines:

a) OECD guideline reference 406 (1992): skin sensitisation,

b) Annex V to Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, published in the Seventeenth Adaptation, Commission Directive 92/69/EEC, OJEC L1383A, 131 - 136, 1992 (B.6: skin sensitisation),

c) United States Environmental Protection Agency, Pesticide Assessment Guidelines, Subdivision F, Guideline Reference Number 81 - 6, Dermal Sensitisation Study.

2.2 GLP

Yes.

2.3 Deviations and Deficiencies

None.

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 DESCRIPTION

Off-white solid.

3.1.4 PURITY

[redacted]%.

3.1.5 STABILITY

Please refer to Section 2 of Doc IIIA.

3.1.6 PREPARATION OF TEST SUBSTANCE FOR APPLICATION

a) Induction: a 1 % w/v preparation of the test substance in corn oil was used for the first two inductions, and a 0.1 % w/v preparation of the test substance in corn oil for the third induction.

b) Challenge: brodifacoum was applied as a 0.1 % or 0.05 % w/v preparation in corn oil for the challenge.

3.2 Test Animals

3.2.1 SPECIES

Cavia porcellus (guinea pig).

3.2.2 STRAIN

Crl (HA) BR.

3.2.3 SOURCE

[redacted].

3.2.4 SEX

Male.

3.2.5 AGE/WEIGHT AT STUDY INITIATION

Young adults weighing 349 - 463 g.

3.2.6 NUMBER OF ANIMALS PER GROUP

20 in the test group and 10 in the control group.

3.3 Study Type

Non-adjuvant.

3.4 Application

3.4.1 INDUCTION SCHEDULE

Induction 1: day 0.
Induction 2: day 6 - 8.
Induction 3: day 12 - 14.

3.4.2 CHALLENGE SCHEDULE

Day 28 (ie 2 weeks after the final induction exposure).

3.4.3 SCORING SCHEDULE AND METHOD

Approximately 1, 2 and 3 days after the challenge exposure, erythematous reactions were quantified and recorded, using the four point scale shown below:

Scale	Sensitisation Response
0	No reaction
1	Scattered mild redness
2	Moderate diffuse redness
3	Intense redness and swelling

The sensitisation potential was then assessed by subtracting the percentage of animals responding in the control group from the percentage responding in the test group, to give a net percentage response, which was then classified as follows:

% Response	Description
0	Not a sensitiser
1 - 8	Weak
8 - 28	Mild
29 - 64	Moderate
65 - 80	Strong
81 - 100	Extreme

3.4.4 RECHALLENGE

Not carried out.

3.4.5 CONCENTRATIONS USED FOR INDUCTION

A 1 % w/v (10^4 µg/ml) preparation used for the first two inductions, and
a 0.1 % w/v (10^3 µg/ml) preparation used for the third induction.

3.4.6 CONCENTRATION FREUNDS COMPLETE ADJUVANT (FCA)

Not applicable.

3.4.7 WAY OF INDUCTION

Topical application.

3.4.8 CONCENTRATION USED FOR CHALLENGE

Two concentrations of 0.1 % w/v (10^3 µg/ml) or 0.05% w/v (5×10^2 µg/ml).

3.4.9 REMOVAL OF TEST SUBSTANCE

Both the induction and challenge treatments were left in place with the occlusive dressings for 6 hours. On removal of the dressings, the sites were washed with 3 % teepol and washed with deionised water.

3.5 Positive Control Substance

The substance used for the positive control was hexylcinnamaldehyde.

4 RESULTS

4.1 Effects

4.1.1 RESULTS OF PILOT STUDY

A sighting study to select the dose levels for the induction and challenge stages of the main study were carried out with the guinea pig in the same laboratory.
The doses tested in the sighting study were as follows:

Induction concentration (w/v)	Challenge concentration (w/v)
1%	1% and 0.5%
2.5%	5% and 2.5%
5%	10 % and 5%
10%	10% and 5%

No irritation or toxicity was observed using the 1% w/v concentration for the induction, and the 1% and 0.5% w/v concentrations for the challenge, and so these were initially selected for use in the main study. The actual concentrations used were altered as the study progressed.

4.1.2 INDUCTION PHASE / 4.1.3 CHALLENGE PHASE / 4.1.4 OTHER EFFECTS

Table 1: Detailed information of test method and results

Treatment Phase	Concentration of Test Material	Day of Treatment	Application Type	% Response	Observation / Remarks
Induction 1	1 % w/v	0	Topical	-	One test animal showed signs of severe toxicity and extensive bruising following the second induction and was humanely killed. The dose level for the third induction was therefore reduced to 0.1 % w/v. There were no signs of irritation in any of the test or control animals during the induction phase.
Induction 2	1 % w/v	6 - 8	Topical	-	
Induction 3	0.1 % w/v	12 - 14	Topical	-	
Challenge 1	0.1 % w/v	28	Topical	4	Following the challenge with a 0.1 % w/v preparation of brodifacoum, scattered mild redness or moderate and diffuse redness was seen in 8 of the 19 test animals. Scattered mild redness was seen in 3 of the 8 control animals (one doubtful reading excluded).
Challenge 2	0.05 % w/v	28	Topical	39	Following challenge with a 0.05 % w/v preparation of brodifacoum, scattered mild redness was seen in 7 of the 18 test animals (one doubtful reading excluded). There was no erythematous response in any of the control animals.

5 APPLICANT'S SUMMARY AND CONLCUSION

5.1 Materials and Methods

Test material: brodifacoum; Batch: P3 (Y00052/038) Purity: 96.1 %;
The Ritz and Buehler method (reference 2) was used to assess the skin sensitisation potential of brodifacoum to guinea pigs (Crl(HA)BR strain).
For the first induction, a 1 % w/v preparation of brodifacoum in corn oil was applied topically on the scapular region of the animals and occluded for 6 hours with lint patches, adhesive tape and bandages. This was repeated on the same skin site for the second induction after 7 days (+/- 1 day) with a 1 % w/v solution again. For the third induction after another 7 days (+/- 1 day) using the same procedure, a 0.1 % w/v preparation of brodifacoum was used.
The animals were challenged two weeks after the final induction with either a 0.1 or a 0.05 % w/v preparation of brodifacoum applied for 6 hours to the shorn flanks of the guinea pigs and occluded with lint patches, adhesive tape and bandages.
After the challenge applications, the skin sites were examined approximately 1, 2 and 3 days after removal of the dressings.
Hexylcinnamaldehyde was used as the positive control for the study.

5.2 Reliablity

Reliability indicator: 1.

5.3 Findings

During the induction phase with the test substance, one test animal showed signs of severe toxicity and extensive bruising following the second induction and was humanely killed. The dose level for the third induction was therefore reduced to 0.1 % w/v. There were no signs of irritation in any of the test or control animals during the induction phase.
Following the challenge with a 0.1 % w/v preparation of brodifacoum, scattered mild redness or moderate and diffuse redness was seen in 8 of the 19 test animals. Scattered mild redness was seen in 3 of the 8 control animals (one doubtful reading excluded). The net percentage response was calculated to be 4 %.
Following challenge with a 0.05 % w/v preparation of brodifacoum, scattered mild redness was seen in 7 of the 18 test animals (one doubtful reading excluded). There was no erythematous response in any of the control animals. The net percentage response was calculated to be 39 %.

SUMMARY TABLE

Species	Method	Result	Net % Response	Reference
Guinea pig (*Cavia porcellus*)	Maximisation test of Ritz and Buehler (1980)	Moderate skin sensitisation under test conditions.	4 % for the 0.1 % w/v preparation; 39 % for the 0.05 % w/v preparation	[redacted], 1996, [redacted] P/5105 ([redacted]),

5.4 Conclusion X

Brodifacoum was considered to be a moderate skin sensitiser to the guinea pig under the conditions of the test.
The net response was 4 % for the 0.1 % w/v preparation of brodifacoum in corn oil, and 39 % for the 0.05 % w/v preparation of brodifacoum in corn oil.

Comment:

	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**	

Syngenta	Brodifacoum	November 2003

Doc IIIA/Section 6.2 Percutaneous absorption (*in vitro* test)

BPD Data Set IIA/Annex Point VI.6.2 *IIA, data pointVI 6.2 and IIB, data point VI 6.4 IN VITRO ABSORPTION THROUGH HUMAN EPIDERMIS*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 2003. Klerat Pellets: *In Vitro* Absorption Through Human Epidermis. Syngenta [redacted]	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD (2002) test Guideline No. 428, Skin Absorption: *In vitro* method (in press) ENV/JM/TG (2002) **7**, Annex 6.	X
2.2	**GLP**	Yes	
2.3	**Deviations**	There were no deviations from the Protocol or Guideline Standards	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Klerat Pellets containing brodifacoum	X
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Nominal 0.005% w/w brodifacoum [redacted]	
3.1.3	Description	Red cylindrical pellet	
3.1.4	Purity	[redacted]	X
3.1.5	Source	[redacted]	
3.1.6	Stability	[redacted]	
3.1.7	Radiolabelling	None. Material analysed using cold analytical method (LC-MS-MS)	
3.2	**Test animals**		
3.2.1	Species	Human	
3.2.2	Source	Surgery and/or *post mortem*	
3.2.3	Sex	Female	
3.2.4	Skin type	Epidermal membranes from at least two subjects	
3.2.5	Skin preparation	Skin samples were immersed in water at 60°C for 40-45 seconds and the epidermis teased away from the dermis	

3.2.6	Membrane integrity	Membrane integrity was determined by measurement of the electrical resistance across the skin membrane. Membranes with a measured resistance of greater than 10kΩ were considered intact and used on the study
3.2.7	Diffusion cells	Absorption was measured using glass diffusion cells in which the epidermal sheet formed a horizontal membrane and provided an application area of 2.54cm^2. Throughout the experiment the receptor fluid was stirred and the epidermal membranes were maintained at 32 ± 1°C
3.3	**Administration / exposure**	Dermal
3.3.1	Cell selection	Cells were selected so that the application was represented by six intact membranes (plus two untreated controls)
3.3.2	Number of skin samples per group	8 in total, 6 test samples and 2 controls
3.3.3	Controls	Yes. Two untreated membranes
3.3.4	Nominal dose	0.48µg brodifacoum per cm^2
3.3.5	Dose rate	10mg Klerat Pellets per cm^2
3.3.6	Dose preparation	The pellets were first broken into smaller pieces to ensure best contact with membrane
3.3.7	Dose application	The dose was applied, undiluted, to the membranes by weight
3.3.8	Occlusion	Cells were unoccluded throughout the experiment
3.4	**Absorption**	Non-entry field
3.4.1	Receptor fluid	50% ethanol in water
3.4.2	Exposure period	24 hours
3.4.3	Sampling time	Pre-treatment, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours The volume of fluid in the receptor chamber was maintained by the replacement of the volume of fresh receptor fluid, equal to the sample volume, immediately after each sample was taken
3.5	**Mass balance**	Non-entry field
3.5.1	Skin washing	The cell assemblies remained intact during the washing procedure. Loose residues of the pellets were tipped into the skin wash container. The epidermal surface of the skin was decontaminated by filling the donor chamber (and surface of the skin) with 2ml volumes of water. The skin surface was gently agitated to mimic a washing procedure before the washings were tipped into the skin wash container. Ethanol was added to the wash samples to dissolve the brodifacoum. Donor chambers were then removed and soaked in ethanol
3.5.2	Tape stripping	Following washing the skin was dried naturally. A strip of adhesive tape was pressed onto the skin surface and then carefully peeled off to remove the *stratum corneum*. A maximum of 5 strips were used and were sequentially numbered before soaking in ethanol to extract any brodifacoum
3.5.3	Epidermal tissue	The remaining epidermal tissue was carefully removed and any adhering brodifacoum extracted using ethanol
3.6	**Sample analysis**	All sample were analysed for brodifacoum content using LC-MS-MS

3.6.1	Limit of quantitation	0.01µg/ml
3.6	**Calculations**	Receptor fluid results were expressed as amounts of brodifacoum in the receptor fluid (ug/cm^2), rates of absorption (µg/cm^2/h) and 'percent of dose absorbed' Mass balance and distribution determinations were expressed as 'percent of applied dose'
		4 RESULTS AND DISCUSSION
4.1	**Absorption**	Brodifacoum absorption through human skin was below the limit of quantitation (<0.02µg/cm^2 and <3.53% of the applied dose) over the entire 24 hour exposure period.
4.2	**Mass balance**	The mean percentage recovery of applied test material was 108%. Several cells had high recoveries (119 and 126%) that were considered to be a consequence of a multiplication factor resulting from the majority of the dose being present in just one compartment (skin wash). These recoveries are considered not to affect the interpretation of the data. Virtually all of the applied dose (mean of 108%) was readily removed from the surface of the skin by mild skin washing 24 hours after application. Any test material remaining following mild skin washing was below the limit of quantitation. The amount present in *stratum corneum*, was below the limit of quantitation value of 0.04µg/cm^2 (8.20%) as were the amounts in the remaining epidermal tissue (0.01µg/cm^2 and 1.64%).
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	This study was undertaken in accordance with the draft OECD 428 guideline for *in vitro* percutaneous absorption measurement (OECD, 2002). Human skin samples were prepared in house by immersing the skin samples in water at 60°C for 40-45 seconds and the epidermis was isolated from the dermis. A total of 8 membranes (6 test plus 2 control) were prepared and used to assess brodifacoum penetration. Membrane integrity was determined by measurement of the electrical resistance across the skin membrane. Membranes with a measured resistance of greater than 10kΩ were considered intact and used on the study. Absorption was measured using glass diffusion cells in which the epidermal sheet formed a horizontal membrane and provided an application area of 2.54cm^2. Throughout the experiment the receptor fluid was stirred and the epidermal membranes were maintained at 32 ± 1°C. Klerat pellets were applied to the skin at a rate of 10mg of Klerat Pellets per cm^2. Therefore, a nominal amount of 0.48µg of brodifacoum per cm^2 was present on each skin sample. The receptor fluid was sampled at pre-treatment, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours. After the final receptor fluid sample had been taken, the remaining fluid in the receptor chamber was discarded and the chamber rinsed with fresh receptor fluid, which was also discarded.

Loose residues of the pellets were tipped into the skin wash container. The epidermal surface was decontaminated with water and the washings also tipped into the skin wash container. Ethanol was added to the wash samples to dissolve the brodifacoum. Donor chambers were removed and soaked in ethanol. The skin was dried naturally and up to 5 strips of adhesive tape were pressed onto the skin surface and peeled off to remove the *stratum corneum*. The remaining epidermal tissue was removed and ethanol was used to extract any brodifacoum from the epidermal tissue and tape strips.

Analysis was performed using LC-MS-MS and the limit of quantitation was 0.01µg/ml.

Receptor fluid results were expressed as amounts of brodifacoum in the receptor fluid (ug/cm^2), rates of absorption (µg/cm^2/h) and 'percent of dose absorbed'

Mass balance and distribution determinations were expressed as 'percent of applied dose'

5.2 Results and discussion

Brodifacoum absorption through human skin was below the limit of quantitation (<0.02µg/cm^2 and <3.53% of the applied dose) over the entire 24 hour exposure period.

The mean percentage recovery of applied test material was 108%.

Several cells had high recoveries (119 and 126%) that were considered to be a consequence of a multiplication factor resulting from the majority of the dose being present in just one compartment (skin wash). These recoveries are considered not to affect the interpretation of the data.

Virtually all of the applied dose (mean of 108%) was readily removed from the surface of the skin by mild skin washing 24 hours after application.

Any test material remaining following mild skin washing was below the limit of quantitation. The amount present in *stratum corneum*, was below the limit of quantitation value of 0.04µg/cm^2 (8.20%) as were the amounts in the remaining epidermal tissue (0.01µg/cm^2 and 1.64%).

5.3 Conclusion

The results in this study demonstrated that the absorption of brodifacoum from Klerat Pellets, containing brodifacoum at a concentration of [redacted], would be extremely slow through human epidermis when compared with the absorption of other penetrants using this *in vitro* technique (Dugard *et al*, 1984[a]; Dugard *et al*, 1984[b]).

Brodifacoum absorption through human skin was below the limit of quantitation (<0.02µg/cm^2 and <3.53% of the applied dose) over the entire 24 hour exposure period.

The vast majority of brodifacoum that may come into contact with human skin will be removed during normal washing procedures.

These data predict that the human dermal absorption of brodifacoum from potential exposure to Klerat Pellets will be negligible.

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	*Give date of action*
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	
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 A6_2.1. Summary of brodifacoum absorption through human epidermis

Dose application:	Time period (h)	Absorption rate (µg/cm²/h ± SEM)
Klerat Pellets (0.048g brodifacoum/kg) 10mg/cm² (0.48µg ai/cm²) Unoccluded Duration of exposure: 24h n = 6	0-10 0-24	<0.002 <0.001

Time (h)	Amount (µg/cm²)	Percent absorbed
6	<0.02	<3.53
8	<0.02	<3.53
10	<0.02	<3.53
24	<0.02	<3.53

Footnote:
All values were below the analytical LOQ value of 0.01µg/ml. Table 1 has been annotated with the <LOQ values expressed as µg/cm²/h, µg/cm² and % of applied dose.

Table A6_2.2. Distribution of unabsorbed and absorbed brodifacoum through human epidermis

n = 6	µg of brodifacoum per cm². Mean	SEM	% of applied dose Mean	SEM
**Stratum corneum*	*<0.04	-	*<8.20	-
Skin wash	0.52	0.03	108	6.35
Donor chamber	*<0.04	-	*<8.20	-
*Remaining epidermis	*<0.01	-	*<1.64	-
*Absorbed	*<0.02	-	*<3.53	-
TOTAL RECOVERED	0.52	0.03	108	6.35

*Footnote:
Values indicated were below the analytical LOQ value of 0.01µg/ml. Table 2 has been annotated with the <LOQ values expressed as µg/cm² and % of applied dose. These values have not been included in the calculation of the total recovered.

Stratum corneum = amount in tape strips

Remaining epidermis = amount remaining following tape stripping

Absorbed = amount in receptor fluid

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Subchronic toxicity

Subchronic Oral Toxicity In The Rat – **Residues in Rat Livers**

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1985). 'Brodifacoum: Residues in Rat Livers from a 90-Day Feeding Study [redacted] [redacted].
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Guideline not quoted in report, but the study was conducted in accordance with current scientific principles for residues analyses. For the 90-day feeding study, a guideline was not quoted in the report, but the study was conducted in general accordance with the principles of OECD Guideline 408: 'Subchronic Oral Toxicity – Rodent: 90-day Study'.
2.2	**GLP**	Yes.
2.3	**Deviations**	

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.
3.1.2.1	Description	White powder
3.1.2.2	Purity	[redacted]% w/w
3.1.2.3	Stability	Please refer to Section 2 of Doc IIIA.
3.2	**Test Animals**	
3.2.1	Species	*Rattus norvegicus* (Norway rat)
3.2.2	Strain	Wistar derived rats of [redacted] strain
3.2.3	Source	[redacted]
3.2.4	Sex	Male

3.2.5	Age/weight at study initiation	Approximately 21 days old weighing a mean of 180.3 – 185.0g .	
3.2.6	Number of animals per group	5 males and 5 females per treatment group. The treatment groups were as follows:	

Group	Dietary concentration of brodifacoum (ppm)	Number of animals	
		Main study	Satellite Study
1	0	10	5
2	0.02	10	5
3	0.08	10	5

3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	90 days for main dietary study, and 45 days for satellite dietary study to investigate haematological parameters.	
3.3.2	Frequency of exposure	Daily	
3.3.3	Postexposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	In food.	X
3.3.4.2	Concentration	Food: 0.02, 0.08 ppm.	X
3.3.4.3	Vehicle		X
3.3.4.4	Concentration in vehicle		
3.3.4.5	Total volume applied		
3.3.4.6	Controls	Plain diet.	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes, animals were examined once a day for signs of toxicity or abnormal behaviour. Once a week, a more detailed examination of each rat was made (this included the negative findings recorded of no clinical or behavioural abnormalities).	X
3.4.1.2	Mortality	Yes, at same time periods as for clinical signs.	X
3.4.2	Body weight	Yes, the initial measurement was made immediately before study commenced, and thereafter once a week on the same day and	X

		approximately at the same time.	
3.4.3	Food consumption	Yes, food consumption for each cage of rats was recorded weekly throughout the study. The food utilisation value per cage was calculated as the total food consumed divided by the total weight gained by the animals in the cage during that period.	X
3.4.4	Water consumption	No. Water was available ad libitum.	
3.4.5	Ophthalmoscopic examination	No.	
3.4.6	Haematology	Yes, measured on all satellite and main study animals at termination (45 and 90 days). The parameters determined were: haemoglobin (HG), haematocrit (Hct), red cell count (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), total white cell count, kaolin-cephalin time (KCT), prothrombin time (PT) and a platelet count. Femoral bone marrow smears were cytologically examined.	X
3.4.7	Clinical Chemisty	Yes, measured on all main study animals at termination (90 days). The parameters determined were: Plasma alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), plasma cholesterol, plasma albumin, total protein and triglycerides, plasma amylase and plasma calcium.	X
3.4.8	Urinalysis	No.	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes, determined for all main study animals at termination (90 days). The organs weighed were: Adrenals, brain, heart, kidneys, liver, spleen and testes.	
3.5.2	Gross and histopathology	Yes, the main study rats were subjected to a full post mortem examination immediately following termination at 90 days. Samples of the following tissues were removed from animals in the top dose and control groups and processed histologically: liver, kidney, salivary glands, pancreas, heart, lungs, gonads, bone marrow and spleen.	
3.5.3	**Other examinations**	Yes, the livers from five animals from each group (including control group), were analysed for brodifacoum residues after 45 days of feeding. After a further 45 days, the livers from five control animals and ten animals from the higher dosage group (0.08ppm) were also analysed for brodifacoum residues.	X
3.5.4	Statistics	Bodyweights, food consumption and food utilisation were considered by analysis of variance on a cage basis.	X

		Organ weights were considered by analysis of variance and covariance on final bodyweight. Haematological and biochemical paramters were considered by analysis of variance. The haematological parameters obtained at termination of the satellite study and the main study were considered separately. Analysis of haematological and biochemical measurements, and organ weights allowed for both replicate and litter of origin. All other analyses allowed for replicate only. Groups means were adjusted for any missing values before treatment group means were compared to the control groups mean using Student's t-test, two-sided, based on the error mean square in the analysis.	
3.6	**Further remarks**	The original summary of the 90-day feeding study has been included in this summary of the liver residues analyses, apart from section 5 (Applicant's Summary and Conclusion) where only the data from the report on the residues of the test substance in the livers of the rats is described.	X
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	All animals survived until their scheduled termination and were in good clinical condition throughout the study. Abnormalities were noted for one rat receiving 0.02ppm brodifacoum (from week 8) and for one rat receiving 0.08ppm brodifacoum (at week 5). There were non-specific findings (hair loss, scabs and stained coat) which are commonly found in rats of this age and strain, and are not considered to be related to treatment.	X
4.1.2	Mortality	All animals survived until their scheduled termination and were in good clinical condition throughout the study.	X
4.2	**Body weight gain**	Bodyweight gains during week 1 of animals fed 0.08ppm brodifacoum were slightly reduced compared to the controls although this was not statistically significant. There were no differences in bodyweight gain between control and 0.02ppm dosage groups.	X
4.3	**Food consumption and compound intake**	In animals fed diet containing 0.08ppm brodifacoum, there was a statistically significant reduction in food consumption during the first week of the study. Otherwise there was no evidence of any effect on food consumption in either group, and there was no effect on food utilisation. (See section 3.4.3 above).	
4.4	**Ophtalmoscopic examination**		
4.5	**Blood analysis**		
4.5.1	Haematology	There was no evidence of any effects on haematological parameters in the satellite study at the end of 45 days dietary administration of	X

		brodifacoum. After 90 days dietary administration of brodifacoum, there was a statistically significant increase in both kaolin-cephalin times (KCT) and prothrombin times (PT), in rats fed 0.08ppm brodifacoum. There was no evidence of any effect on these parameters in rats fed 0.02ppm brodifacoum. ***See Table A6_3-1 below.*** No other statistically significant differences were noted between the group means of the control and test groups with respect to any of the other parameters measured and the bone marrow smears examined after 90 days treatment appeared normal.	
4.5.2	Clinical chemistry	There was a statistically significant increase in the plasma cholesterol level of animals in the 0.08ppm brodifacoum group. The increase was only small and there was no evidence of any effects on the other parameters measured. ***See Table A6_3-1 below.***	X
4.5.3	Urinalysis		
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	There was no evidence for any effect on organ weight in rats receiving either 0.02 or 0.08ppm brodifacoum.	
4.6.2	Gross and histopathology	The only effects seen were minor histopathological changes. These were found infrequently and were considered to be incidental in origin.	
4.7	**Other**	See Table A6_3-2 below for a summary of the liver residues analyses results.	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	[redacted]Guideline not quoted in report, but the study was conducted in accordance with current scientific principles for residues analyses. [redacted].	
5.2	**Results and discussion**	After 45 days of feeding brodifacoum containing diets, residues in the livers of rats from the 0.02ppm feeding group were in the range 0.32-1.0mg/kg, and those from the 0.08ppm feeding group were in the range 0.64-1.6mg/kg. The livers of the rats in the control feeding group contained no measurable residues. After 90 days of feeding brodifacoum containing diets, residues in the livers of rats in the 0.08ppm feeding group were in the range 1.4-2.2mg/kg. The livers of the rats in the control feeding group contained no measurable residues. The mean brodifacoum residue in the liver of rats fed 0.02ppm brodifacoum containing diets at 45 days was 50% of the mean residue in the liver of rats fed 0.08ppm diets (0.56mg/kg and 1.12mg/kg respectively). The mean brodifacoum residue in the liver of rats fed	

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Subchronic toxicity

Subchronic Oral Toxicity In The Rat – **Residues in Rat Livers**

		0.08ppm brodifacoum containing diets at 45 days was 64% of the mean residue in the liver of rats at 90 days (1.12mg/kg and 1.75mg/kg respectively). Both these observations indicate a non-linear accumulation of brodifacoum in rat livers.	
5.3	**Conclusion**		
5.3.1	LO(A)EL		X
5.3.2	NO(A)EL		X
5.3.3	Other		
5.3.4	Reliability	1	
5.3.5	Deficiencies		

	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]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Subchronic toxicity

Subchronic Oral Toxicity In The Rat – **Residues in Rat Livers**

Materials and Methods [redacted]

Doc IIIA / Section 6.2

Subchronic toxicity

Subchronic Oral Toxicity In The Rat – **Residues in Rat Livers**

BPD Data Set IIA / Annex Point VI.6.2

	[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 A6_3-1. Results of haematology and clinical chemistry parameters following dietary administration with brodifacoum at 0, 0.02 or 0.08ppm for 90 days.

Parameter changed	Unit	Controls (0 ppm)	0.02 ppm	0.08 ppm	Approximate 95% confidence limits (+/-)
Haemaglobin	g/dl	14.94	14.99 (8)	14.80 (9)	0.38
Haematocrit		0.456	0.459 (8)	0.450 (9)	0.019
Red blood cell count	x 10^{12}/l	8.87	8.82 (8)	8.86 (9)	0.38
Mean cell volume	fl	51.4	52.0 (8)	50.8 (9)	1.0
Mean cell haemoglobin	pg	16.90	17.08 (8)	16.72 (9)	0.43
Mean cell haemoglobin concentration	g/dl	32.91	32.66 (8)	32.99 (9)	0.67
White blood cell count	x 10^{9}/l	5.57	5.76 (8)	5.46 (9)	0.81
Prothrombin time	sec	16.63 (7)	16.51 (8)	35.65**(9)	4.14
Kaolin-cephalin time	sec	22.22 (7)	14.89 (8)	70.47**(9)	17.35
Plasma albumin	g/100ml	4.75	4.83	4.88	0.13
Plasma alkaline phosphatase activity	mU/ml	118	127	119	9
Plasma aspartate transaminase activity	mU/ml	71	80 (9)	76	18
Plasma alanine transaminase activity	mU/ml	49	46	55	9
Plasma cholesterol	mg/100ml	82	85	95*	7
Plasma total protein	g/100ml	6.78	6.85	6.94	0.20
Plasma Triglycerides	mg/100ml	154	163	152	22
Plasma calcium	mg/100ml	12.06	12.24	11.94	0.28
Plasma amylase	mU/ml	5953	5983	5697	214

- Mean based on 10 observations per group unless otherwise indicated by a number in brackets.
- Means adjusted for missing values.
- Confidence interval based on mean group size.

*Statistically significantly different from the control group mean at the 5% level, (Student's 't' : two-sided).
**Statistically significantly different from the control group mean at the 1% level, (Student's 't' : two-sided).

Table A6_3-2. Results of individual animal liver residue analyses following dietary administration with brodifacoum at 0, 0.02 or 0.08ppm for 45 and/or 90 days.

Dosage / Feeding Group (ppm)	Feeding Time Period (days)	Brodifacoum residues (mg/kg)
0 (control)	45	<0.02
0 (control)	45	<0.02
0 (control)	45	<0.02
0 (control)	45	<0.02
0 (control)	45	<0.02
0.02	45	1.0
0.02	45	0.32
0.02	45	0.38
0.02	45	0.51
0.02	45	0.57
0.08	45	0.64
0.08	45	0.99
0.08	45	0.96
0.08	45	1.6
0.08	45	1.4
0 (control)	90	<0.05
0 (control)	90	<0.05
0 (control)	90	<0.05
0 (control)	90	<0.05
0 (control)	90	<0.05
0.08	90	1.6
0.08	90	2.2
0.08	90	1.6
0.08	90	1.7
0.08	90	2.2
0.08	90	1.4
0.08	90	1.4
0.08	90	1.6
0.08	90	1.9
0.08	90	1.9

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Subchronic toxicity

Subchronic Oral Toxicity In The Rat – **Residues in Rat Livers**

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1985). 'Brodifacoum: Residues in Rat Livers from a 90-Day Feeding Study [redacted] [redacted].
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Guideline not quoted in report, but the study was conducted in accordance with current scientific principles for residues analyses. For the 90-day feeding study, a guideline was not quoted in the report, but the study was conducted in general accordance with the principles of OECD Guideline 408: 'Subchronic Oral Toxicity – Rodent: 90-day Study'.
2.2	**GLP**	Yes.
2.3	**Deviations**	

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.
3.1.2.1	Description	White powder
3.1.2.2	Purity	[redacted]% w/w
3.1.2.3	Stability	Please refer to Section 2 of Doc IIIA.
3.2	**Test Animals**	
3.2.1	Species	*Rattus norvegicus* (Norway rat)
3.2.2	Strain	Wistar derived rats of [redacted] strain
3.2.3	Source	[redacted]
3.2.4	Sex	Male

3.2.5	Age/weight at study initiation	Approximately 21 days old weighing a mean of 180.3 – 185.0g .	
3.2.6	Number of animals per group	5 males and 5 females per treatment group. The treatment groups were as follows:	

Group	Dietary concentration of brodifacoum (ppm)	Number of animals	
		Main study	Satellite Study
1	0	10	5
2	0.02	10	5
3	0.08	10	5

3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	90 days for main dietary study, and 45 days for satellite dietary study to investigate haematological parameters.	
3.3.2	Frequency of exposure	Daily	
3.3.3	Postexposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	In food.	X
3.3.4.2	Concentration	Food: 0.02, 0.08 ppm.	X
3.3.4.3	Vehicle		X
3.3.4.4	Concentration in vehicle		
3.3.4.5	Total volume applied		
3.3.4.6	Controls	Plain diet.	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes, animals were examined once a day for signs of toxicity or abnormal behaviour. Once a week, a more detailed examination of each rat was made (this included the negative findings recorded of no clinical or behavioural abnormalities).	X
3.4.1.2	Mortality	Yes, at same time periods as for clinical signs.	X
3.4.2	Body weight	Yes, the initial measurement was made immediately before study commenced, and thereafter once a week on the same day and	X

		approximately at the same time.	
3.4.3	Food consumption	Yes, food consumption for each cage of rats was recorded weekly throughout the study. The food utilisation value per cage was calculated as the total food consumed divided by the total weight gained by the animals in the cage during that period.	X
3.4.4	Water consumption	No. Water was available ad libitum.	
3.4.5	Ophthalmoscopic examination	No.	
3.4.6	Haematology	Yes, measured on all satellite and main study animals at termination (45 and 90 days). The parameters determined were: haemoglobin (HG), haematocrit (Hct), red cell count (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), total white cell count, kaolin-cephalin time (KCT), prothrombin time (PT) and a platelet count. Femoral bone marrow smears were cytologically examined.	X
3.4.7	Clinical Chemisty	Yes, measured on all main study animals at termination (90 days). The parameters determined were: Plasma alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), plasma cholesterol, plasma albumin, total protein and triglycerides, plasma amylase and plasma calcium.	X
3.4.8	Urinalysis	No.	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes, determined for all main study animals at termination (90 days). The organs weighed were: Adrenals, brain, heart, kidneys, liver, spleen and testes.	
3.5.2	Gross and histopathology	Yes, the main study rats were subjected to a full post mortem examination immediately following termination at 90 days. Samples of the following tissues were removed from animals in the top dose and control groups and processed histologically: liver, kidney, salivary glands, pancreas, heart, lungs, gonads, bone marrow and spleen.	
3.5.3	**Other examinations**	Yes, the livers from five animals from each group (including control group), were analysed for brodifacoum residues after 45 days of feeding. After a further 45 days, the livers from five control animals and ten animals from the higher dosage group (0.08ppm) were also analysed for brodifacoum residues.	X
3.5.4	Statistics	Bodyweights, food consumption and food utilisation were considered by analysis of variance on a cage basis.	X

		Organ weights were considered by analysis of variance and covariance on final bodyweight. Haematological and biochemical paramters were considered by analysis of variance. The haematological parameters obtained at termination of the satellite study and the main study were considered separately. Analysis of haematological and biochemical measurements, and organ weights allowed for both replicate and litter of origin. All other analyses allowed for replicate only. Groups means were adjusted for any missing values before treatment group means were compared to the control groups mean using Student's t-test, two-sided, based on the error mean square in the analysis.	
3.6	**Further remarks**	The original summary of the 90-day feeding study has been included in this summary of the liver residues analyses, apart from section 5 (Applicant's Summary and Conclusion) where only the data from the report on the residues of the test substance in the livers of the rats is described.	X
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	All animals survived until their scheduled termination and were in good clinical condition throughout the study. Abnormalities were noted for one rat receiving 0.02ppm brodifacoum (from week 8) and for one rat receiving 0.08ppm brodifacoum (at week 5). There were non-specific findings (hair loss, scabs and stained coat) which are commonly found in rats of this age and strain, and are not considered to be related to treatment.	X
4.1.2	Mortality	All animals survived until their scheduled termination and were in good clinical condition throughout the study.	X
4.2	**Body weight gain**	Bodyweight gains during week 1 of animals fed 0.08ppm brodifacoum were slightly reduced compared to the controls although this was not statistically significant. There were no differences in bodyweight gain between control and 0.02ppm dosage groups.	X
4.3	**Food consumption and compound intake**	In animals fed diet containing 0.08ppm brodifacoum, there was a statistically significant reduction in food consumption during the first week of the study. Otherwise there was no evidence of any effect on food consumption in either group, and there was no effect on food utilisation. (See section 3.4.3 above).	
4.4	**Ophtalmoscopic examination**		
4.5	**Blood analysis**		
4.5.1	Haematology	There was no evidence of any effects on haematological parameters in the satellite study at the end of 45 days dietary administration of	X

		brodifacoum. After 90 days dietary administration of brodifacoum, there was a statistically significant increase in both kaolin-cephalin times (KCT) and prothrombin times (PT), in rats fed 0.08ppm brodifacoum. There was no evidence of any effect on these parameters in rats fed 0.02ppm brodifacoum. ***See Table A6_3-1 below.*** No other statistically significant differences were noted between the group means of the control and test groups with respect to any of the other parameters measured and the bone marrow smears examined after 90 days treatment appeared normal.	
4.5.2	Clinical chemistry	There was a statistically significant increase in the plasma cholesterol level of animals in the 0.08ppm brodifacoum group. The increase was only small and there was no evidence of any effects on the other parameters measured. ***See Table A6_3-1 below.***	X
4.5.3	Urinalysis		
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	There was no evidence for any effect on organ weight in rats receiving either 0.02 or 0.08ppm brodifacoum.	
4.6.2	Gross and histopathology	The only effects seen were minor histopathological changes. These were found infrequently and were considered to be incidental in origin.	
4.7	**Other**	See Table A6_3-2 below for a summary of the liver residues analyses results.	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	[redacted]Guideline not quoted in report, but the study was conducted in accordance with current scientific principles for residues analyses. [redacted].	
5.2	**Results and discussion**	After 45 days of feeding brodifacoum containing diets, residues in the livers of rats from the 0.02ppm feeding group were in the range 0.32-1.0mg/kg, and those from the 0.08ppm feeding group were in the range 0.64-1.6mg/kg. The livers of the rats in the control feeding group contained no measurable residues. After 90 days of feeding brodifacoum containing diets, residues in the livers of rats in the 0.08ppm feeding group were in the range 1.4-2.2mg/kg. The livers of the rats in the control feeding group contained no measurable residues. The mean brodifacoum residue in the liver of rats fed 0.02ppm brodifacoum containing diets at 45 days was 50% of the mean residue in the liver of rats fed 0.08ppm diets (0.56mg/kg and 1.12mg/kg respectively). The mean brodifacoum residue in the liver of rats fed	

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Subchronic toxicity

Subchronic Oral Toxicity In The Rat – **Residues in Rat Livers**

		0.08ppm brodifacoum containing diets at 45 days was 64% of the mean residue in the liver of rats at 90 days (1.12mg/kg and 1.75mg/kg respectively). Both these observations indicate a non-linear accumulation of brodifacoum in rat livers.	
5.3	**Conclusion**		
5.3.1	LO(A)EL		X
5.3.2	NO(A)EL		X
5.3.3	Other		
5.3.4	Reliability	1	
5.3.5	Deficiencies		

	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]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Subchronic toxicity

Subchronic Oral Toxicity In The Rat – **Residues in Rat Livers**

Materials and Methods [redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Subchronic toxicity

Subchronic Oral Toxicity In The Rat – **Residues in Rat Livers**

	[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 A6_3-1. Results of haematology and clinical chemistry parameters following dietary administration with brodifacoum at 0, 0.02 or 0.08ppm for 90 days.

Parameter changed	Unit	Controls (0 ppm)	0.02 ppm	0.08 ppm	Approximate 95% confidence limits (+/-)
Haemaglobin	g/dl	14.94	14.99 (8)	14.80 (9)	0.38
Haematocrit		0.456	0.459 (8)	0.450 (9)	0.019
Red blood cell count	x 10^{12}/l	8.87	8.82 (8)	8.86 (9)	0.38
Mean cell volume	fl	51.4	52.0 (8)	50.8 (9)	1.0
Mean cell haemoglobin	pg	16.90	17.08 (8)	16.72 (9)	0.43
Mean cell haemoglobin concentration	g/dl	32.91	32.66 (8)	32.99 (9)	0.67
White blood cell count	x 10^{9}/l	5.57	5.76 (8)	5.46 (9)	0.81
Prothrombin time	sec	16.63 (7)	16.51 (8)	35.65**(9)	4.14
Kaolin-cephalin time	sec	22.22 (7)	14.89 (8)	70.47**(9)	17.35
Plasma albumin	g/100ml	4.75	4.83	4.88	0.13
Plasma alkaline phosphatase activity	mU/ml	118	127	119	9
Plasma aspartate transaminase activity	mU/ml	71	80 (9)	76	18
Plasma alanine transaminase activity	mU/ml	49	46	55	9
Plasma cholesterol	mg/100ml	82	85	95*	7
Plasma total protein	g/100ml	6.78	6.85	6.94	0.20
Plasma Triglycerides	mg/100ml	154	163	152	22
Plasma calcium	mg/100ml	12.06	12.24	11.94	0.28
Plasma amylase	mU/ml	5953	5983	5697	214

- Mean based on 10 observations per group unless otherwise indicated by a number in brackets.
- Means adjusted for missing values.
- Confidence interval based on mean group size.

*Statistically significantly different from the control group mean at the 5% level, (Student's 't' : two-sided).
**Statistically significantly different from the control group mean at the 1% level, (Student's 't' : two-sided).

Table A6_3-2. Results of individual animal liver residue analyses following dietary administration with brodifacoum at 0, 0.02 or 0.08ppm for 45 and/or 90 days.

Dosage / Feeding Group (ppm)	Feeding Time Period (days)	Brodifacoum residues (mg/kg)
0 (control)	45	<0.02
0 (control)	45	<0.02
0 (control)	45	<0.02
0 (control)	45	<0.02
0 (control)	45	<0.02
0.02	45	1.0
0.02	45	0.32
0.02	45	0.38
0.02	45	0.51
0.02	45	0.57
0.08	45	0.64
0.08	45	0.99
0.08	45	0.96
0.08	45	1.6
0.08	45	1.4
0 (control)	90	<0.05
0 (control)	90	<0.05
0 (control)	90	<0.05
0 (control)	90	<0.05
0 (control)	90	<0.05
0.08	90	1.6
0.08	90	2.2
0.08	90	1.6
0.08	90	1.7
0.08	90	2.2
0.08	90	1.4
0.08	90	1.4
0.08	90	1.6
0.08	90	1.9
0.08	90	1.9

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Teratogenicity Study

Blood Kinetics in the Pregnant Rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]Brodifacoum: Blood Kinetics in the Pregnant Rat[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**	Guideline not quoted in report, but study conducted in accordance with the principles of OECD 414.	
2.2	**GLP**	Yes.	
2.3	**Deviations**	No.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum.	
3.1.1	Lot/Batch number	[redacted]	X
3.1.2	Specification	As given in section 2.	
3.1.2.1	Description	Unlabelled test substance: off-white powder	
3.1.2.2	Purity	Unlabelled test substance: [redacted]%w/w	X
		Radiolabelled test substance: [^{3}H]-phenyl labelled brodifacoum of specific activity 1.04GBq/µmol and radiochemical purity >95%	
3.1.2.3	Stability	Brodifacoum is known to be stable based on knowledge and experience..	X
3.2	**Test Animals**		
3.2.1	Species	Rat.	
3.2.2	Strain	Wistar-derived[redacted]	
3.2.3	Source	[redacted]	
3.2.4	Sex	Virgin females.	
3.2.5	Age/weight at study initiation	210-344g	
3.2.6	Number of animals	24 and 15 for the low dose and high dose groups respectively, split into	

	per group	groups of three for termination at specified time points.				
3.2.7	Control animals	No				
3.2.8	Mating period	The female rats were paired overnight with unrelated males of the same strain. The following morning vaginal smears were taken from the females and examined for the presence of sperm. The day when spermatozoa were detected was designated Day 1 of gestation, and on this same day the successfully mated females were delivered to the study laboratory.				
3.3	**Administration/ Exposure**	Oral.				
3.3.1	Duration of exposure	**Animal no:**	**Dose level (mg/kg_{bw}/day)**	**Dosing period (days of gestation)**	**Accumulated dose (mg/kg_{bw})**	X
		1 – 3	0.0125	1	0.0125	
		4 – 6	0.0125	1 – 3	0.0375	
		7 – 9	0.0125	1 – 5	0.0625	
		10 – 12	0.0125	1 – 7	0.0875	
		13 – 15	0.0125	1 – 9	0.1125	
		16 – 18	0.0125	1 – 11	0.1375	
		19 – 21	0.0125	1 – 13	0.1625	
		22 – 24	0.0125	1 – 16	0.2	
		25 – 27	0.02	7	0.02	
		28 – 30	0.02	7 – 9	0.06	
		31 – 33	0.02	7 – 11	0.1	
		34 – 36	0.02	7 – 13	0.14	
		37 – 39	0.02	7 – 16	0.2	
3.3.2	Postexposure period	6 days.				
		Oral				
3.3.3	Type	Gavage.				
3.3.4	Concentration	0.0125, 0.02 mg/kg bw				
3.3.5	Vehicle	Polyethylene glycol (PEG 600)				
3.3.6	Concentration in vehicle					
3.3.7	Total volume applied	2ml/kg, as bodyweight dependent dose.				
3.3.8	Controls					X

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Teratogenicity Study

Blood Kinetics in the Pregnant Rat

3.4	**Examinations**		
3.4.1	Body weight	Yes, daily.	
3.4.2	Food consumption	No.	
3.4.3	Clinical signs	Yes, daily.	
3.4.4	Examination of uterine content	Number of implantations.	
3.4.5	Examination of foetuses	No.	
3.4.5.1	General		
3.4.5.2	Skelet		
3.4.5.3	Soft tissue		
3.5	**Further remarks**	At the end of the specified dosing period (see section 3.3.1 above), the animals were terminated and a sample of blood taken. The concentration of radioactivity in the blood samples were determined by liquid scintillation counting.	X
		4 RESULTS AND DISCUSSION	
4.1	**Maternal toxic Effects**	There were no indications of maternal toxicity.	
4.2	**Teratogenic / embryotoxic effects**		
4.3	**Other Effects**	The daily oral dosing of 0.0125 mg/kg bw [^{3}H]-brodifacoum resulted in a steadily increasing level of radioactivity in the blood, from 0.6 ng equivalents of brodifacoum/g of blood at Day 2 of gestation, to 3.4 ng equivalents of brodifacoum/g at Day 17 of gestation. The daily oral dosing of 0.02 mg/kg bw [^{3}H]-brodifacoum also resulted in a steadily increasing level of radioactivity in the blood, from 0.7 ng equivalents of brodifacoum/g of blood at Day 8 of gestation, to 4.5 ng equivalents of brodifacoum/g at Day 17 of gestation. The results obtained on Day 8 of gestation from the 0.02 mg/kg bw/day dosage group clearly showed significantly lower levels of radioactivity in the blood than the corresponding 0.012 5mg/kg bw/day dosage group results at the 1% level using the Students t-test. Radioactivity levels from Day 10 to Day 17 of gestation were not significantly different for the two dosage groups, when compared using the same statistical method. ***See Table A6_8-1 for a summary of the results.*** The achieved blood levels of brodifacoum using the two dosing regimes were not significantly different from at least Day 10 of gestation onwards. Therefore, the blood levels were equivalent for most of the	X

dosing period from Day 7 to Day 16 of gestation and hence for most of the period of major organogenesis.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

[redacted]Pregnant female rats were each given daily oral doses of [^{3}H-brodifacoum] by gavage, of either 0.0125mg/kg bw during Days 1 – 16 of gestation, or 0.02 mg/kg bw during Days 7 – 16 of gestation. Both dose groups therefore received the same total dose of brodifacoum (0.2mg/kg bw) which represented the highest possible dose which could be given over the respective dosing periods for each dose group without causing toxic effects. The day of confirmation of mating was designated Day 1 of gestation. At specified time points, groups of 3 rats were terminated and a sample of blood taken, and their uteri examined for implantations. The concentration of radioactivity in the blood samples was determined by liquid scintillation counting. Bodyweights and clinical observations were recorded at daily intervals throughout the study.

5.2 Results and discussion

There were no indications of maternal toxicity after the administration of brodifacoum for the duration of the study. X

Daily dosing of 0.0125 mg/kg bw [^{3}H-brodifacoum] between Days 1 and 16 of gestation resulted in a progressive increase of radioactivity in the blood, reaching a maximum of 3.4 ng equivalents/g of blood by Day 17 of gestation.

Daily dosing of 0.02 mg/kg bw [^{3}H-brodifacoum] between Days 7 and 16 of gestation resulted in a greater increase of radioactivity in the blood up to Day 12 of gestation, reaching a maximum of 4.5ng equivalents/g of blood by Day 17 of gestation.

The achieved blood levels of brodifacoum using the two dosing regimes were not significantly different from at least Day 10 of gestation onwards. Therefore, the blood levels were equivalent for most of the dosing period from Day 7 to Day 16 of gestation and hence for most of the period of major organogenesis.

It is know that Days 10 – 12 of gestation are usually the most critical time for the production of structural abnormalities in the rat. The achieved blood levels of brodifacoum in the dams using the two dosing regimens of Days 1 – 16 and Day 7 – 16 of gestation were similar from about Day 10 onwards; and thus were also similar both during this particularly sensitive period and the remainder of the period of major organogenesis. It was concluded that there were no significant differences between the groups in the foetal exposure to brodifacoum between Days 10-16 of gestation.

5.3 Conclusion X

5.3.1 LO(A)EL maternal toxic effects

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Teratogenicity Study

Blood Kinetics in the Pregnant Rat

5.3.2	NO(A)EL maternal toxic effects	
5.3.3	LO(A)EL embryotoxic / teratogenic effects	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	
5.3.5	Reliability	1
5.3.6	Deficiencies	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2005
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 A6_8-1 Table for maternal parameters: mean concentration of radioactivity in maternal blood following dosing with 0.0125mg/kg_{bw} [^{3}H-brodifacoum] or 0.02mg/kg_{bw} [^{3}H]-brodifacoum

Dose level (mg/kg_{bw}/day)	Dosing period (Days of gestation)	Concentration of radioactivity in the blood at termination (ng/equivalents/g of brodifacoum)
0.0125	1	0.560
0.0125	1 – 3	0.924
0.0125	1 – 5	1.556
0.0125	1 – 7	1.809
0.0125	1 – 9	2.015
0.0125	1 – 11	2.795
0.0125	1 – 13	2.168
0.0125	1 – 16	3.396
0.02	7	0.691*
0.02	7 – 9	1.362
0.02	7 – 11	3.087
0.02	7 – 13	2.427
0.02	7 – 16	4.488

* Statistically significant difference at the 1% level Students t-test (two sided) from the 0.0125mg/kg_{bw}/day dose group sampled at the same day of gestation.

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, excretion and tissue retention in male rats following administration of single oral dose

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1979). 'Brodifacoum: Absorption, excretion and tissue retention in the rat.' [redacted]. [redacted].	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	[redacted]	
2.2	**GLP**	[redacted]	
2.3	**Deviations**		
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum	
3.1.1	Lot/Batch number	Not specified in report but the samples of brodifacoum were obtained [redacted].	
3.1.2	Specification	As given in section 2.	
3.1.2.1	Description		
3.1.2.2	Purity	[redacted]	X
3.1.2.3	Stability	Please refer to Section 2 of Doc IIIA.	
3.1.2.4	Radiolabelling	[^{14}C] labelled in the phenyl ring of the coumarin moiety with a specific radioactivity of 14.75 mCi/mM.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted] (Wistar-derived)	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	Adult/190 – 210g for all experiments apart from the whole body autoradiography investigation, where the rats weighed 80 – 90 g.	

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, excretion and tissue retention in male rats following administration of single oral dose

3.2.6 Number of animals per group

The number of animals per group varied according to the experiment being performed, as 7 separate investigations were carried out. In the study, the groups of animals for each experiment were not given a reference or group number. However, the individual rats were assigned numbers which can be used to track the particular group or experiment. The numbers of animals per group, their assigned individual numbers and the endpoint of the investigation is given below:

EXPERIMENT / INDIVIDUAL ASSIGNED ANIMAL NUMBER	NUMBER OF ANIMALS PER GROUP / EXPERIMENT	TOXICOKINETIC ENDPOINT INVESTIGATED
Excretion and Tissue Retention, Experiment 1 / 1, 2, 3	1 group of 3	Excretion (urine and faeces) Distribution (abdominal fat, kidneys, heart, liver carcass)
Excretion and Tissue Retention, Experiment 2 / 50, 51, 52	1 group of 3	Absorption (blood level) Distribution (pancreas, spleen)
Excretion and Tissue Retention, Experiment 3 / 44, 45, 46 / 47, 48, 49	2 groups of 3	Excretion (urine and faeces)
Excretion and Tissue Retention, Experiment 4 / 10	1 group of 1	Excretion (exhaled air)
Excretion and Tissue Retention, Experiment 5 / 4, 5, 6	1 group of 3	Excretion/Metabolism (bile duct monitoring)
Whole Body Autoradiography/ 7, 8, 9	1 group of 3	Distribution (whole body autoradiography)

[redacted]

		Blood Level Study/ 11 – 43 Animals 35 – 37 were assigned to control group: 10 dose groups of 3 and 1 control group of 3; Absorption (blood levels)
3.2.7	Control animals	Only control animals were numbers 35 – 37 for blood level experiment (absorption)
3.3	**Administration/ Exposure**	Oral
3.3.1	Fasting period	No fasting prior to dosing
3.3.2	Metabolic/enzyme inhibitors or inducers	No
3.3.3	Duration of treatment	Single dose
3.3.4	Frequency of exposure	
3.3.5	Postexposure period	The postexposure periods for the separate investigations were as follows:

EXPERIMENT / INDIVIDUAL ASSIGNED ANIMAL NUMBER	POSTEXPOSURE PERIOD	SAMPLING TIMES	TOXICOKINETIC ENDPOINT INVESTIGATED
Excretion and Tissue Retention, Experiment 1 / 1, 2, 3	10 days	Excreta collected at 24 hour intervals	Excretion (urine and faeces); Distribution (abdominal fat, kidneys, heart, liver carcass)
Excretion and Tissue Retention, Experiment 2 / 50, 51, 52	10 days	Blood sample collected at 10 days	Absorption (blood level); Distribution (pancreas, spleen)
Excretion and Tissue Retention, Experiment 3 / 44, 45, 46 / 47, 48, 49	5 days	Excreta collected at 24 hour intervals	Excretion (urine and faeces)

	Excretion and Tissue Retention, Experiment 4 / 10		Expired air monitored for a 28 hour period	Excretion (expired air)
	Excretion and Tissue Retention, Experiment 5 / 4, 5, 6		Bile collected at 24 hour intervals for 48 hours	Excretion/Metabolism (bile duct monitoring)
	Whole Body Autoradiography/ 7, 8, 9	1, 5 and 10 days		Distribution (whole body radiography)
	Blood Level Study/ 11 – 43 Animals 35 – 37 were assigned to control group	0.25, 0.5, 1, 2, 4, 6, 8, 10, 17 and 24 hours	Blood samples taken at sacrifice	Absorption (blood levels)

3.3.6 Oral

3.3.6.1 Type gavage

3.3.6.2 Concentration

EXPERIMENT / INDIVIDUAL ASSIGNED ANIMAL NUMBER	CONCENTRATION (DOSE LEVEL) (mg/kg)	CONCENTRATION IN VEHICLE (mg/ml)
Excretion and Tissue Retention, Experiment 1 / 1, 2, 3	0.25	0.1
Excretion and Tissue Retention, Experiment 2 / 50, 51, 52	0.25	0.1
Excretion and Tissue Retention, Experiment 3 / 44, 45, 46 / 47, 48, 49	0.5 / 1.5	0.2 / 0.6

		Excretion and Tissue Retention, Experiment 4 / 10	0.25	0.1
		Excretion and Tissue Retention, Experiment 5 / 4, 5, 6	0.25	0.1
		Whole Body Autoradiography/ 7, 8, 9	0.25	0.1
		Blood Level Study/ 11 – 43	0.21	0.084
		Animals 35 – 37 were assigned to control group		
3.3.6.3	Vehicle	Polyethylene glycol (PEG 300).		
3.3.6.4	Concentration in vehicle	See section 3.3.6.2 above.		
3.3.6.6	Total volume applied	2.5 ml dosing solution per kg bw.		
3.3.6.7	Controls			
3.4	**Examinations**			
3.4.1	Observations			
3.4.1.1	Clinical signs	Not stated in study report.		
3.4.1.2	Mortality	Yes.		
3.4.2	Body weight	Not stated in study report.		
3.4.3	Body fluids sampled	See section 3.3.5 above.		
3.4.4	Tissues sampled	See section 3.3.5 above.		
3.4.5	Determination of metabolites	Bile, urine, liver and carcass were investigated qualitatively for metabolites. The metabolites were not identified. The techniques used for the investigations were Scintillation Counting, Thin-Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC).		
3.4.6	Excretion routes	See section 3.3.5 above.		

3.4.7 Other examinations

3.4.8 Statistics

3.5 Further remarks

4 RESULTS AND DISCUSSION

4.1 Observations

In the experiments to investigate excretion:

- rat 44 died on day 3
- rats 45 and 48 died on day 4
- rats 46 and 49 died on day 5
- rat 47 was killed on day 5.

These rats had been administered the highest dose levels of 0.5 mg/kg (rats 44, 45 and 46) and 1.5 mg/kg (rats 47, 48 and 49).

4.2 Body weight

4.3 Absorption

The absorption of [^{14}C]-brodifacoum was investigated following a single oral dose of 0.21 mg/kg: X

- The rate of uptake of radioactivity into blood was fairly rapid with a peak level of 16.1 ng of brodifacoum equivalents per ml whole blood attained at 8 hours after dosing. The levels declined to 6.7 ng equivalents per ml at 17 hours after dosing. Low levels of 1.3 ng equivalents per ml were present at 10 days after dosing. Almost all (82.5 %) of the radioactivity present in whole blood was found to be associated with the plasma. See Table A6_2-1 below.
- Brodifacoum accounted for 39.2 % of the radioactivity in plasma with an additional, more polar component, accounting for 51.1 % of the label.

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, excretion and tissue retention in male rats following administration of single oral dose

4.4 Distribution

The distribution in tissues of [^{14}C]-brodifacoum was investigated following a single oral dose of 0.25 mg/kg:

- At 10 days after dosing, 74.6 % of the dose was retained in the tissues of the animals. The proportion of the retained dose in the tissues was highest in the liver (22.8 %), followed by the pancreas (2.3 %), and then the kidney (0.8 %), heart (0.1 %) and spleen (0.2 %). The remainder of the dose (approximately 50 %) was present in the carcass and skin. See Table A6_2-2 below.
- Whole body autoradiography showed that at 24 hours after dosing, the highest concentration of radioactivity was present in the liver, pancreas and salivary glands. Radioactivity was also present in the gastric mucosa, intestinal mucosa, vertebrae, nasal mucosa, kidneys, adrenals, meninges, fat and skin. At 5 and 10 days after dosing, the autoradiographs showed that high levels of radioactivity were still present in these tissues.

4.5 Metabolism

The metabolism of [^{14}C]-brodifacoum was investigated following a single oral dose of 0.25 mg/kg:

- Thin layer chromatography indicated that brodifacoum was present in both urine and bile (24.0 % and 13.3 % of the recovered radioactivity respectively). When chromatographed on reverse phase thin layers, five areas of radioactivity were detected in bile, accounting for 13.3%, 50.2%, 7.0%, 4.3%, and 4.4% . Under the same chromatographic conditions, three areas of radioactivity were detected in urine, accounting for 24%, 12% and 62% of the recovered radioactivity respectively. Brodifacoum was retained on the origin when chromatographed under these conditions. Co-chromatography showed that 4-hydroxy coumarin was not present in bile and urine.
- HPLC and radiochemical analysis showed that 31.3% and 19.6% of the dose was present in the carcass and liver respectively as unchanged brodifacoum together with two other more polar components which were not identified.

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, excretion and tissue retention in male rats following administration of single oral dose

4.6 Elimination and Excretion

The excretion of [^{14}C]-brodifacoum via the urine and faeces was investigated following single oral doses of 0.25, 0.5 and 1.5 mg/kg:

- At the 0.25 mg/kg dose level, 1.3% and 12.3% of the dose was excreted in urine and faeces respectively during the 10-day postexposure period. Elimination of radioactivity was greatest during the 0 – 48 hour period following dosing, with 1.0% being eliminated in urine and 7.1% in the faeces.
- At the 0.50 and 1.50 mg/kg dose level, 2.9% and 2.8% of the dose respectively was excreted in urine during the 4-day postexposure period, and 30.8% and 42.6% of the dose respectively in faeces during the same period time.

See Table A6_2-3 below.

The excretion of [^{14}C]-brodifacoum via bile was investigated following a single oral dose of 0.25 mg/kg:

- 0.6% of the dose was recovered in bile during the 24 hour period after dosing, and a further 0.8% in the 24 – 48 hour postexposure period.

See Table A6_2-3 below.

The excretion of [^{14}C]-brodifacoum via expired air was investigated following a single oral dose of 0.25 mg/kg:

- The expired air contained no radioactivity, showing that no degradation of brodifacoum to give [^{14}C]-CO_2 or any radiolabelled volatile compound occurred.

The rate of elimination of brodifacoum following dosing at 0.25 mg/kg , as given by the biological half-life, was calculated to be 150 – 200 days.

4.7 Recovery of labelled compound

Not given in study report.

5 APPLICANT'S SUMMARY AND CONCLUSION

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, excretion and tissue retention in male rats following administration of single oral dose

5.1	**Materials and methods**	[redacted]A total of 7 experiments were performed to investigate the absorption, distribution, metabolism and excretion of brodifacoum. All experiments were conducted using a single oral dose of [^{14}C]-brodifacoum, administered to male Alderley Park specific pathogen-free (Wistar-derived) weighing 190 – 200g (apart from the whole body autoradiography investigation where the rats weighed 80 – 90g). For the excretion and distribution experiments, groups of mainly 3 rats were dosed with 0.25, 0.5 or 1.5 mg/kg brodifacoum and excreta collected at 24 hour intervals for 5 – 10 days. The tissue distribution was also investigated in these experiments at the lowest dose level, as was the absorption (blood level) at 10 days after dosing. Whole body autoradiography was performed on 3 rats dosed with 0.25 mg/kg at 1, 5, and 10 days after dosing. Metabolism was investigated in some animals at the 0.25 mg/kg dose level. The absorption experiment was conducted using a total of 30 rats administered 0.21 mg/kg and sacrificed in groups of 3 at intervals up to 24 hours after dosing.	
5.2	**Results and discussion**	All 6 rats dosed at the higher dose levels of 0.5 and 1.5 mg/kg in the excretion and distribution experiments died at 3 – 5 days after administration of brodifacoum. The results presented in section 4 above show that when brodifacoum was administered orally at 0.21 mg/kg, it was rapidly absorbed into the blood with peak levels reached at 8 hours after dosing. The levels declined to less than half at 17 hours after dosing, with low levels still present at 10 days after dosing. At the 0.25 mg/kg dose level, a small amount (11 – 14%) of the radioactivity was slowly eliminated in urine and faeces over 10 days. The levels found in bile were similar to those found in urine and indicate that the biliary and renal routes are of equal significance in the elimination of brodifacoum. After 10 days, 74.6% of the dose was retained in the tissues. The proportion of the retained dose was highest in the liver (22.8 %), followed by the pancreas (2.3 %), and then the kidney (0.8 %), heart (0.1 %) and spleen (0.2 %). The remainder of the dose (approximately 50 %) was present in the carcass and skin. Analysis showed that 31.3% and 19.6% of the dose was present in the carcass and liver respectively as unchanged brodifacoum together with two other more polar components which were not identified The rate of elimination of brodifacoum following a single dose of 0.25 mg/kg, as given by the biological half-life, was calculated to be 150 – 200 days.	X
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No.	

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, excretion and tissue retention in male rats following administration of single oral dose

	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	

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, excretion and tissue retention in male rats following administration of single oral dose

Table A6_2-1 Table for Toxicokinetic Studies:

Levels Of Radioactivity In Blood Of Male Rats Following Administration Of Single Oral Dose Of [^{14}C]-Brodifacoum (0.21mg/kg)

Hours after dosing	Mean concentration of radioactivity (ng equivalents/ml blood)	Rat numbers
0.25	2.8	11, 12, 13
0.50	2.2	14, 15, 16
1	4.4	17, 18, 19
2	5.9	20, 21, 22,
4	10.1	23, 24, 25
6	15.4	26, 27, 28
8	16.1	29, 30, 31
10	13.1	41, 42, 43
17	6.7	38, 39, 40
24	5.7	32, 33, 34
240 (10 days)	1.3	50, 51, 52

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, excretion and tissue retention in male rats following administration of single oral dose

Table A6_2-2 Table for Toxicokinetic Studies:

Distribution Of Radioactivity In Male Rats 10 Days After Administration Of Single Oral Dose Of [^{14}C]-Brodifacoum (0.25mg/kg)

Tissue	Abdominal fat	Liver	Kidney	Heart	Carcass plus skin	Pancreas	Spleen	Blood
Mean concentration of radioactivity (as % of dose)	3.29	22.84	0.78	0.10	50.82	2.33	0.16	0.05
Rat number	1, 2, 3	1, 2, 3	1, 2, 3	1, 2, 3	1, 2, 3	50, 51, 52	50, 51, 52	50, 51, 52

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, excretion and tissue retention in male rats following administration of single oral dose

Table A6_2-3 Table for Toxicokinetic Studies:

Mean Level of Radioactivity In Excreta Of Male Rats Following Single Oral Dose Of [^{14}C]-brodifacoum – Expressed As Mean % Of Dose

Time after dosing (days)	Excreta	Dose Level (mg/kg)[1]		
		0.25	**0.50**	**1.5**
1	Urine	0.88	2.05	2.01
	Faeces	2.43	14.97	14.32
	Bile	0.59		
2	Urine	0.14	0.39	0.38
	Faeces	4.70	10.51	16.22
	Bile	0.77		
3	Urine	0.07	0.22	0.26
	Faeces	1.21	4.3	8.25
4	Urine	0.05	0.19	0.13
	Faeces	0.71	1.47	3.78
5	Urine	0.03	0.47	0.06
	Faeces	0.44	No Faeces	No Faeces
6	Urine	0.05		
	Faeces	0.45		
7	Urine	0.03		
	Faeces	0.34		
8	Urine	0.03		
	Faeces	0.74		
9	Urine	0.03		
	Faeces	0.74		
10	Urine	0.02		
	Faeces	0.74		
Total % of dose excreted	Urine	1.32	2.95	2.81
	Faeces	11.01	30.77	42.57

[1] **Rat numbers: 1, 2, 3 (0.25mg/kg dose); 44, 45, 46 (0.5mg/kg dose); 47, 48, 49 (1.5mg/kg dose)**

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Excretion and tissue retention in male rats following administration of single oral dose

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1985). 'Brodifacoum: Excretion and Tissue Distribution in the Rat Following Oral Administration at Several Dose Levels.' [redacted]
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No, but methods used broadly comparable to OECD guideline 417 for Toxicokinetic studies.
2.2	**GLP**	Yes.
2.3	**Deviations**	

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.
3.1.2.1	Description	
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	Please refer to Section 2 of Doc IIIA.
3.1.2.4	Radiolabelling	[^{14}C]-brodifacoum: uniformly labelled in the phenyl ring of the coumarin moiety with a specific activity of 545.75MBq/mmol (1.043MBq/mg). [^{3}H]-brodifacoum: labelled in the 1 position of the tetrahydro naphthalene ring with a specific activity of 61.64GBq/mmol (117.859MBq/mg).
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	[redacted] (Wistar-derived)
3.2.3	Source	[redacted]
3.2.4	Sex	Male

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Excretion and tissue retention in male rats following administration of single oral dose

3.2.5	Age/weight at study initiation	Adult/190 – 250g
3.2.6	Number of animals per group	4
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Fasting period	No fasting prior to dosing
3.3.2	Metabolic/enzyme inhibitors or inducers	No
3.3.3	Duration of treatment	Single dose
3.3.4	Frequency of exposure	
3.3.5	Postexposure period	7 days
3.3.6	**Oral**	
3.3.6.1	Type	gavage
3.3.6.2	Concentration	0.2, 2.0, 20, 200 µg/kg. The dosage groups were divided into 2 phases: Phase 1 was the administration of a mixture of [^{14}C]- and [^{3}H]-brodifacoum to achieve the top dose level of 200 µg total brodifacoum/kg; Phase 2 was the administration of [^{3}H]-brodifacoum only at the 0.2, 2.0 and 20 µg/kg dose level.
3.3.6.3	Vehicle	Polyethylene glycol (PEG 400).
3.3.6.4	Concentration in vehicle	0.032, 0.36, 3.2 µg/g [^{3}H]-brodifacoum and (42 µg/g [^{14}C]-brodifacoum + 0.4 µg/g [^{3}H]-brodifacoum)
3.3.6.6	Total volume applied	5 ml dosing solution per kg bw.
3.3.6.7	Controls	
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	No.
3.4.1.2	Mortality	No.

3.4.2	Body weight	No.	
3.4.3	Body fluids sampled	Yes, blood was sampled at the end of the 7 day post-exposure period.	
3.4.4	Tissues sampled	Yes, liver, kidneys, pancreas, salivary glands and fat were sampled at the end of the 7 day post-exposure period.	
3.4.5	Determination of metabolites	Yes, metabolites in urine (0 – 48 hour samples) and faeces (0 – 48 hour and 48 – 120 hour samples) were investigated qualitativelyand quantitatively. The metabolites were not identified. The techniques used for the investigations were Scintillation Counting, Thin-Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and linear analysis of thin layers.	X
3.4.6	Excretion routes	Yes, urine and faeces were collected at 24 hour intervals after dosing for 7 days.	
3.4.7	Other examinations	No.	
3.4.8	Statistics		
3.5	**Further remarks**		
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.2	**Body weight**		
4.3	**Absorption**		
4.4	**Distribution**	The comparative tissue distribution of [^{14}C]- and [^{3}H]-brodifacoum at the top dose level of 200 µg total brodifacoum/kg, showed that the values for concentration of radioactivity obtained by tritium measurement after 7 days were generally lower than those obtained by ^{14}C measurement. See Table A6_2-1 for a summary of the data. The tissue distribution of [^{3}H]-brodifacoum at several dose levels showed that after 7 days the concentration of radioactivity ranged between 21.9% of the dose and 36.2% of the dose. The level of radioactivity found in all of the tissues except the pancreas reduced by a factor of approximately ten in proportion to the dose administered. In the pancreas the value obtained at the highest dose level was proportionally considerably higher (1.8% of the dose) in comparison with the values obtained at the lower doses (0.3 – 0.4% of the dose). See Table A6_2-2 for a summary of the data.	X

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Excretion and tissue retention in male rats following administration of single oral dose

4.5 Metabolism	Urine: In all dose groups, radioactivity in the bulked 0 – 48 hour samples was slowly and incompletely extracted into solvent. TLC of the extracts showed that 3 areas of radioactivity were present in the extract of urine from animals in Group A (????ug/kg); a similar though less well defined pattern was found for (Group B / 20µg/kg); whilst for Group C (2.0µg/kg) only the radioactivity at the origin could be clearly defined; and no discrete areas of radioactivity could be detected for Group D (0.2µg/kg). TLC of brodifacoum and 4-hydroxycoumarin indicated that neither compound was present in measurable amounts in urine. Quantitation of the relative amounts of radioactivity could not be done accurately for any group apart from Group A because only small amounts of radioactivity were present in urine and the efficiency of determination of tritium is low. Faeces: Radioactivity in all the aqueous bulked faecal extracts was quickly extracted into solvent. For the 0 – 48 hour bulked samples, TLC of the extracts showed that brodifacoum was the major component accounting for 89%, 81%, 69% and 34% of the radioactivity for Groups A – D respectively. In groups C (2.0µg/kg) and D (0.2µg/kg) an additional component (R_F 0.20) accounted for 8% and 20% respectively of the radioactivity in those 0 – 48 hour faecal extracts. A third component (R_F 0.62) was also apparent in Group D (0.2µg/kg) which accounted for 7% of the radioactivity in the extract. In the 48 – 120 hour extracts, brodifacoum was again the major component accounting for 86% and 51% of the radioactivity for Groups A (200µg/kg) and B (20µg/kg) respectively, with the component at R_F 0.20 accounting for 7% and 24% of the radioactivity respectively. The levels of radioactivity were low in the 48 – 120 hour extracts of Groups C (2.0µg/kg) and D (0.2µg/kg) and so analysis was not attempted.	X

Doc IIIA / Section 6.2 **BPD Data Set IIA / Annex Point VI.6.2**	**Toxicokinetics Studies** Excretion and tissue retention in male rats following administration of single oral dose

4.6	**Elimination and Excretion**	The comparative excretion of [^{14}C]- and [^{3}H]-brodifacoum at the top dose level of 200 µg /kg, showed that the amounts of ^{14}C and ^{3}H recovered in urine and faeces over the 7 day collection period were similar: 0.57% of the ^{14}C dose and 0.43% of the ^{3}H dose was excreted via the urine, and 10.70% of the ^{14}C dose and 10.48% of the ^{3}H dose was excreted via the faeces. The quantity of ^{14}C and ^{3}H excreted each day in the urine and faeces was similar, except for day 1 where the quantity of ^{14}C excreted in the urine was three times greater than that of ^{3}H. See Table A6_2-3 for a summary of the data. The mean values obtained for the daily excretion in the urine of radioactivity by rats dosed with several different dose levels of [^{3}H]-brodifacoum, were 0.4%, 0.5%, 0.6% and 3.1% of the dose respectively during the 7 day period for Group A (200µg/kg), Group B (20µg/kg), Group C (2µg/kg) and Group D (0.2µg/kg); the corresponding values determined for the faeces were 10.0%, 6.6%, 12.1% and 16.1% respectively. The total amounts excreted in urine and faeces were higher for animals in the lowest dose group Group D (0.2µg/kg). See Table A6_2-4 for a summary of the data.
4.7	**Recovery of labelled compound**	Not given in study report.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	[redacted]Groups of 4 male rats ([redacted]specific pathogen-free,Wistar-derived) weighing 190 – 250g, were each given a single oral dose of either a mixture of [^{14}C/^{3}H] at 200µg/kg (phase 1), or [^{3}H]-labelled brodifacoum at 0.2, 2.0 and 20 and 200µg/kg (phase 2). Excreta (urine and faeces) were collected daily for up to 7 days after which time the animals were killed and various tissues (liver, kidney, pancreas, salivary gland and fat) and blood taken for analysis. Urine (0 – 48 hour samples), and faeces (0 – 48 hour and 48 – 120 hour samples), were also investigated for metabolites. The analytical techniques used were Scintillation Counting, HPLC (High Performance Liquid Chromatography), TLC (Thin-Layer Chromatography), with quantitation attempted using a linear analyser.

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Excretion and tissue retention in male rats following administration of single oral dose

5.2	**Results and discussion**	In phase 1 of the study, the fate of the two labelled forms of brodifacoum were not completely identical with the values determined by tritium measurement generally lower with the greatest difference (approximately 20%) observed in the liver and the smallest (approximately 10%) observed in fat. The tritiated form however, was considered to be a sufficiently stable indicator of the fate of brodifacoum to be able to be used in phase 2 of the study. In phase 2, the pattern of excretion for the 7 day period was similar in both urine and faeces although the total radioactivity excreted was slightly higher for animals dosed at the lowest dose level. Levels of radioactivity in the tissues of rats at termination were located principally in the liver and ranged from 21.9% of the dose for Group A (200µg/kg), to 36.2% of the dose for Group B (20µg/kg). In most tissues the levels of radioactivity decreased by a factor of ten in proportion to the dose administered. Similar metabolic profiles were obtained with both urine and faeces from rats in Groups A and B (200µg/kg and 20µg/kg), but some differences were apparent in the excreta from rats in the other two lower dosage groups (Group C (2µg/kg) and Group D (0.2µg/kg). The differences in excretion, tissue retention and metabolism were in general minor and though they may have been the result of dose dependent changes in kinetics of brodifacoum, particularly at the lowest dose level, the evidence for this was not conclusive.	X
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No.	X

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

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Excretion and tissue retention in male rats following administration of single oral dose

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 A6_2-1 Table for Toxicokinetic Studies:

Distribution Of Radioactivity In Tissues Of Male Rats 7 Days After Administration Of Single Oral Dose Of [^{3}H]- and [^{14}C]-Labelled Brodifacoum (200µg/kg$_{bw}$)

Concentration of radioactivity as ng equivalents of brodifacoum						
Tissue	Liver	Kidney	Pancreas	Salivary Glans	Fat	Blood
[^{14}C]	1079	211	1393	412	41	5
[^{3}H]	847	163	1152	365	36	10
Concentration of radioactivity as % of dose						
[^{14}C]	27.96	0.81	2.07	0.34	-	-
[^{3}H]	21.90	0.63	1.78	0.31	-	-

Table A6_2-2 Table for Toxicokinetic Studies:

Distribution Of Radioactivity In Tissues Of Male Rats 7 Days After Administration Of Single Oral Doses Of [^{3}H]-Brodifacoum

Concentration of radioactivity as ng equivalents of brodifacoum

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Excretion and tissue retention in male rats following administration of single oral dose

Group (Dose Level)	Tissue					
	Liver	Kidney	Pancreas	Salivary Gland	Fat	Blood
A (200µg/kg)	847	163	1152	365	36	10
B (20µg/kg)	111	13	19	13	2	0.3
C (2µg/kg)	10	1	2	1	0.3	0.1
D (0.2µg/kg)	0.9	0.2	0.2	0.2	0.1	0.03
Concentration of radioactivity as % of dose						
A (200µg/kg)	21.90	0.62	1.78	0.31	-	-
B (20µg/kg)	36.19	0.70	0.33	0.14	-	-
C (2µg/kg)	29.02	0.61	0.26	0.09	-	-
D (0.2µg/kg)	29.43	0.79	0.35	0.14	-	-

Table A6_2-3 Table for Toxicokinetic Studies:

Mean Daily Level of Radioactivity In Urine and Faeces Of Male Rats Following Single Oral Dose Of [^{3}H]- and [^{14}C]-Labelled Brodifacoum (200µg/kg$_{bw}$)

Radiolabel	Route of excretion	% of dose excreted							
		1	2	3	4	5	6	7	Total excretion
^{14}C	Urine	0.36	0.07	0.04	0.03	0.03	0.03	0.02	0.57
	Faeces	3.70	3.13	1.68	0.68	0.61	0.49	0.41	10.70
^{3}H	Urine	0.12	0.09	0.07	0.05	0.05	0.04	0.02	0.43
	Faeces	3.61	3.15	1.68	0.62	0.56	0.46	0.39	10.48

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Excretion and tissue retention in male rats following administration of single oral dose

Table A6_2-4 Table for Toxicokinetic Studies:

Mean Daily Level of Radioactivity In Urine and Faeces Of Male Rats Following Single Oral Doses Of [^{3}H]-Brodifacoum

Group (Dose Level)	Route of excretion	% of dose excreted							
		1	2	3	4	5	6	7	Total excretion
A (200µg/kg)	Urine	0.12	0.09	0.07	0.05	0.05	0.04	0.02	0.43
	Faeces	3.61	3.15	1.68	0.62	0.56	0.46	0.39	10.48
B (20µg/kg)	Urine	0.09	0.10	0.10	0.07	0.07	0.04	0.04	0.50
	Faeces	1.61	2.49	1.09	0.47	0.43	0.25	0.22	6.56
C (2.0µg/kg)	Urine	0.14	0.11	0.09	0.08	0.06	0.06	0.04	0.59
	Faeces	2.79	4.17	2.35	1.24	0.60	0.50	0.44	12.09
D (0.2µ/kg)	Urine	0.64	0.51	0.43	0.39	0.41	0.41	0.35	3.12
	Faeces	3.10	2.20	3.22	2.61	2.09	1.28	1.59	16.08

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Half-life and residues in rat liver following administration of single oral dose

Official use only

1 REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No, but methods used broadly comparable to OECD guideline 417 for Toxicokinetic studies. The purpose of this study was to determine and compare the residues of three anticoagulant rodenticides and their respective half-lives in the liver of rats given a single oral dose of 0.2mg/kg_{bw}.
2.2	**GLP**	Yes.
2.3	**Deviations**	

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum, bromodiolone and flocoumafen.
3.1.1	Lot/Batch number	Brodifacoum: [redacted]; Bromadiolone[redacted]; Flocoumafen: [redacted].
3.1.2	Specification	As given in section 2.

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Half-life and residues in rat liver following administration of single oral dose

3.1.2.1	Description	Brodifacoum: not given in report Bromadiolone: off-white powder Flocoumafen: off-white powder
3.1.2.2	Purity	Brodifacoum: [redacted] Bromadiolone: [redacted]% Flocoumafen: [redacted]%
3.1.2.3	Stability	Please refer to Section 2 of Doc IIIA.
3.1.2.4	Radiolabelling	
3.1.2.5	Method of analysis	Assay procedures were developed whereby all three compounds could be analysed under similar conditions. Whole liver samples from the animals were homogenised with an organic solvent (chloroform/acetone, 1:1, v/v) and the resultant extracts were subjected to a solid-phase column clean-up procedure prior to high-performance liquid chromatography (HPLC) with fluorescence detection.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	CD rats, Sprague-Dawley derived
3.2.3	Source	[redacted]
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	6 – 7 weeks old with an approximate bodyweight of 200g
3.2.6	Number of animals per group	3 groups of 33 animals (for each of the 3 test substances).
3.2.7	Control animals	Yes, 1 control group of 9 animals.
3.3	**Administration/ Exposure**	Oral
3.3.1	Fasting period	No
3.3.2	Metabolic/enzyme inhibitors or inducers	No
3.3.3	Duration of treatment	Single dose
3.3.4	Frequency of exposure	

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Half-life and residues in rat liver following administration of single oral dose

3.3.5	Postexposure period	Up to 200 days.
3.3.6	**Oral**	
3.3.6.1	Type	gavage
3.3.6.2	Concentration	0.2 mg/kg_{bw} for each test substance/group.
3.3.6.3	Vehicle	Polyethylene glycol 300 (PEG 300)
3.3.6.4	Concentration in vehicle	16mg test substance per 100ml PEG 300.
3.3.6.6	Total volume applied	$1.25 \times 10^{-3} ml/g_{bw}$. Total dose volume adjusted according to individual body weight of animal, in order to achieve dose of $0.2mg/kg_{bw}$.
3.3.6.7	Controls	Control animals not dosed with anything (test substance or vehicle).
3.4	**Examinations**	
3.4.1	Observations	No
3.4.1.1	Clinical signs	No
3.4.1.2	Mortality	No
3.4.2	Body weight	No
3.4.3	Body fluids sampled	No
3.4.4	Tissues sampled	Yes, 3 animals in each test group were sacrificed on Days 1, 3, 7, 14, 28, 50, 100, 150 and 200 after dosing. An additional 3 animals in each test group were sacrificed on Day 28 for liver storage stability measurements. Control animals were sacrificed at the end of the study. The livers were removed after sacrifice for analysis to determine the residue and half-life of the test substance. Animal carcasses were discarded. After extraction, the liver samples were analysed in duplicate for each test substance. Samples were analysed in two batches. Batch One consisted of the Day 1 – 28 samples; Batch Two consisted of the Day 50 – 200 samples together with the Day 28 stability samples.
3.4.5	Determination of metabolites	No
3.4.6	Excretion routes	No
3.4.7	Other examinations	No

3.4.8 Statistics

The duplicate analyses results for each animal were meaned and the statistical analyses were based on these means.

For each test substance, mono-, bi-, and tri-exponential curves were fitted to the concentration-time profiles, and the fits of the various models were compared by constructing analyses of variance. These analyses show the improvement of fit obtained by including each additional exponential term, and the F ratio tested this improvement against the residual variation within the time points.

The differences between curves were tested by comparing the residual mean squeares for the following curve fit:

i) individual curves for each test substance

ii) a common (pooled) curve for all three compounds

iii) curves contstrained to have the same non-linear parameters (ie rate constants) but with different coefficients.

A comparison of i) with ii) gave a test for differences between the rate constants, while a comparison of iii) with ii) gave a test for vertical displacement of the curves (ie difference between coefficients). Estimates of the terminal half-lives were made by taking the slope of the fitted curve at the penultimate time point.

3.5 Further remarks

The **analytical parameters** measured were:

- precision and recovery of measurement from liver,
- linearity of detection,
- limit of detection,
- limit of quantitation,
- stability in stored liver samples,
- specificity.

4 RESULTS AND DISCUSSION

4.1 Observations

4.2 Body weight

4.3 Absorption

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Half-life and residues in rat liver following administration of single oral dose

4.4 Distribution

Brodifacoum: the mean liver concentration was 1.107µ/g at 24 hours after dosing, 1.051µg/g at 28 days and 0.539µg/g at 200 days after dosing.

Bromadiolone: the mean liver concentration was 0.983µ/g at 24 hours after dosing, 0.578µg/g at 28 days and 0.282µg/g at 200 days after dosing.

Flocoumafen: the mean liver concentration was 1.295µ/g at 24 hours after dosing, 0.861µg/g at 28 days and 0.410µg/g at 200 days after dosing.

(See Table A6_2-1 below for summary of results).

Throughout the study period, liver concentrations were statistically significantly higher in the order brodifacoum > flocoumafen > bromadiolone.

4.5 Metabolism

4.6 Elimination and Excretion

For each of the test substances, the elimination of radioactivity was more rapid up to 28 days after dosing than during the subsequent period. During the period of up to 28 days after dosing, the concentrations of the test substances declined in the liver with the following half-lives:

Brodifacoum: (0-28 days) $T_{1/2}$ = 63 days

Bromadiolone: (0-28 days) $T_{1/2}$ = 17 days

Flocoumafen: (0-28 days) $T_{1/2}$ = 6 days

It should be noted that there was a large standard error associated with these estimates and therefore there was no significant difference in these half-lives.

Estimates of the terminal half-lives from the slopes of the fitted bi-exponential curves gave the following results:

Brodifacoum: (0-200 days) $T_{1/2}$ = 282 days

Bromadiolone: (0-200 days) $T_{1/2}$ = 318 days

Flocoumafen: (0-200 days) $T_{1/2}$ = 159 days

Again, it should be noted that there was a large standard error associated with these estimates and therefore there was no significant difference in these half-lives.

All three test substances exist as pairs of diastereo-isomers. The chromatographic conditions used provided separation of these diastereo-isomers. There was no change in the isomer ratio for brodifacoum and flocoumafen in liver samples taken up to 200 days after dosing. However, there was some change in the corresponding isomer ratio for bromadiolone indicating some preferential elimination of one of the diastereo-isomers. ***See Table A6_2-2 below for summary of results.***

4.7 Analytical parameters

Precision and recovery of measurement from liver:

Intra- (within-day) precision measurements of the assay as indicated by the coefficient of variation (CV) of the measured concentration of replicate (n=5) spiked liver samples, actual mean measured concentrations and recovery measurements are given in ***Table A6_2-3 below.***

Linearity of detection:

The relationship between peak areas and concentration was linear in the range 0.02 to 2µg/g for all three test substances.

Limit of quantitation:

The limit of quantitation was set at 0.05µg/g, the lowest spiked recovery standard for all three test substances. At this level, the intra-precision (as given by the coefficient of variation, CV) ranged from 5.7% to 10.8% and the recovery was greater than 70% for all three test substances. ***See Table A6_2-3 below.***

Stability:

All three test substances were found to be stable in livers stored at -20°C for up to 170 days.

Specificity:

Control rat livers taken through the described procedures showed no interfering peaks in their chromatograms at retention times corresponding to the respective test substances.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

[redacted] Methods used were broadly comparable to OECD guideline 417 for Toxicokinetic studies.

The purpose of this study was to determine and compare the residues of three anticoagulant rodenticides and their respective half-lives in the liver of rats given a single oral dose of 0.2mg/kg_{bw}.

Three groups of 33 male rats (CD, Sprague-Dawley derived) 6 – 7 weeks old weighing approximately 200g, were given a single oral dose of 0.2mg/kg_{bw} brodifacoum, bromadiolone or flocoumafen. There was one control group of 9 animals. Three animals in each test group were sacrificed at various time points up to 200 days after dosing, with an additional 3 animals in each test group sacrificed on Day 28 for liver storage stability measurements. After sacrifice the livers were removed for analysis to determine the residue and half-life of the test substance. Assay procedures were developed whereby all three test substances could be analysed under similar conditions.

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Half-life and residues in rat liver following administration of single oral dose

5.2	**Results and discussion**	Mean liver concentrations of 1.107µg/g, 0.983µg/g and 1.295µg/g were obtained 24 hours after dosing for brodifacoum, bromadiolone and flocoumafen, respectively. Over the 200 day study period, liver concentrations were highest after administration of brodifacoum followed by flocoumafen and bromadiolone respectively. Concentrations of brodifacoum, bromadiolone and flocoumafen declined in an apparent bi-exponential manner. During the initial 28 days the half-lives of elimination were 63, 17 and 6 days respectively. However, there was a large standard error associated with these estimates and therefore there was no significant difference in these half-lives. Estimates of terminal half-lives indicated that there was no statistically significant difference between the three test substances. Calculation of the half-lives using the best approximation gave values of 282, 318 and 159 for brodifacoum, bromadiolone and flocoumafen respectively. Mean proportions of the separated diastereo-isomers of bromadiolone showed that the isomer ratio found in test liver samples was different from that obtained for the administered test compound and the ratio of these isomers also changed during the 200 day study period, presumably due to differential elimination. Separation of diastereo-isomers of brodifacoum and flocoumafen was also achieved but there was no change in the ratio of these isomers in any liver samples analysed.
5.3	**Conclusion**	
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	[redacted]

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Half-life and residues in rat liver following administration of single oral dose

	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	

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Half-life and residues in rat liver following administration of single oral dose

Table A6_2-1 Table For Toxicokinetic Studies:

Mean Liver Concentrations In Male Rats Of Brodifacoum, Bromadiolone And Flocoumafen Following A Single Oral Dose Of 0.2 mg/kg_{bw}

Time After Dosing (Days)	Brodifacoum			Bromadiolone			Flocoumafen		
	Mean * (µg/g)	SD (+/-)	CV (%)	Mean * (µg/g)	SD (+/-)	CV (%)	Mean * (µg/g)	SD (+/-)	CV (%)
1	1.107	0.038	3.5	0.983	0.049	5.0	1.295	0.117	9.0
3	1.193	0.099	8.3	0.811	0.054	6.6	1.220	0.105	8.6
7	1.078	0.088	8.2	0.844	0.051	6.0	1.089	0.040	3.7
14	1.121	0.077	6.9	0.727	0.098	13.5	0.927	0.067	7.2
28	1.051	0.126	12.0	0.578	0.033	5.6	0.861	0.141	16.3
50	0.838	0.075	9.0	0.440	0.042	9.6	0.762	0.068	8.9
100	0.679	0.061	9.0	0.366	0.026	7.0	0.537	0.057	10.6
150	0.681	0.055	8.1	0.314	0.056	18.0	0.493	0.018	3.6
200	0.539	0.028	5.2	0.282	0.041	14.4	0.410	0.033	8.2

** Liver concentrations not corrected for recovery*

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Half-life and residues in rat liver following administration of single oral dose

Table A6_2-2 Table For Toxicokinetic Studies:

Mean Proportions Of Each Isomer As A Percentage Of Total Concentration Measured In Rat Livers For Brodifacoum, Bromadiolone And Flocoumafen Following A Single Oral Dose Of 0.2 mg/kg$_{bw}$

Time After Dosing (Days)	Brodifacoum		Bromadiolone		Flocoumafen	
	% Isomer 1	% Isomer 2	% Isomer 1	% Isomer 2	% Isomer 1	% Isomer 2
1	70.0	30.0	63.5	36.5	70.3	29.7
3	70.7	29.3	63.5	36.5	69.3	30.7
7	71.4	28.6	64.9	35.1	70.5	29.5
14	71.1	28.9	70.6	29.4	71.4	28.6
28	72.5	27.5	73.3	26.7	71.4	28.6
28 (stability sample)	73.5	26.5	77.1	22.9	71.4	28.6
50	73.5	26.5	76.1	23.9	72.0	28.0
100	74.8	25.2	83.1	16.9	70.2	29.8
150	74.3	25.7	86.9	13.1	68.8	31.2
200	73.5	26.3	88.2	11.8	68.2	31.8
Liver Spike	71.2	28.8	76.1	23.9	70.0	30.0
Liver Standard	70.4	29.6	78.6	21.4	69.4	30.6

Table A6_2-3 Table For Toxicokinetic Studies:

Mean Intra-precision And Recovery Measurements Of Spiked Rat Liver Samples

Replicate No.	Brodifacoum Conc^n (µg/g)			Bromadiolone Conc^n (µg/g)			Flocoumafen Conc^n (µg/g)		
(Liver Spike Level)	(0.05)	(0.20)	(1.00)	(0.05)	(0.20)	(1.00)	(0.05)	(0.20)	(1.00)
1	0.040	0.179	0.923	0.037	0.163	0.864	0.047	0.181	0.881
2	0.036	0.195	0.943	0.045	0.166	0.874	0.042	0.170	0.795
3	0.037	0.177	0.915	0.035	0.182	0.909	0.044	0.162	0.840
4	0.035	0.164	0.944	0.044	0.174	0.903	0.047	0.180	0.887
5	0.035	0.174	0.912	0.042	0.185	0.927	0.041	0.166	0.871
Mean	0.037	0.178	0.927	0.041	0.174	0.895	0.044	0.172	0.855
SD (+/-)	0.002	0.011	0.015	0.004	0.010	0.026	0.003	0.008	0.038

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Half-life and residues in rat liver following administration of single oral dose

CV (%)	5.7	6.3	1.6	10.8	5.5	2.9	6.3	4.9	4.4
Recovery (%)	73.2	88.9	92.7	81.2	87.0	89.5	88.4	85.9	85.5

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1987). 'Brodifacoum: Elimination from the tissues of rats following administration of single oral doses. [redacted]	
1.2	**Data protection**	Yes.	
1.2.1	Data owner	Syngenta Limited.	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, but methods used broadly comparable to OECD guideline 417 for Toxicokinetic studies.	
2.2	**GLP**	Yes.	
2.3	**Deviations**		
		3 MATERIALS AND METHODS	
3.1	**Test material**	Brodifacoum	
3.1.1	Lot/Batch number	Unlabelled brodifacoum: [redacted] [^{14}C]-brodifacoum: [redacted] (used for Groups 2 and 3) and [redacted] (used for Group 4)	
3.1.2	Specification	As given in section 2.	
3.1.2.1	Description		
3.1.2.2	Purity	Unlabelled brodifacoum: 96% (with *cis:trans* ratio of 60:40) [^{14}C]-brodifacoum (Y00052/010/004): radiochemical purity 96.1% (with *cis:trans* ratio of 59:41) [^{14}C]-brodifacoum (Y00052/028/002): radiochemical purity 95.3% (with *cis:trans* ratio of 61:39)	X
3.1.2.3	Stability	Please refer to Section 2 of Doc IIIA.	
3.1.2.4	Radiolabelling	[^{14}C]-brodifacoum: uniformly labelled in the phenyl ring of the coumarin moiety with specific activities of 546MBq/m mole (100052/010/004) and 1961MBq/m mole (Y00052/028/002).	
3.2	**Test Animals**		

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

3.2.1	Species	Rat
3.2.2	Strain	Alpk:AP
3.2.3	Source	[redacted]
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Adult (approximately 7 weeks old) weighing 174 – 231g
3.2.6	Number of animals per group	24 (Group 2); 36 (Group 3); 39 (Group 4)
3.2.7	Control animals	Yes: 21 animals (Group 1)
3.3	**Administration/ Exposure**	Oral
3.3.1	Fasting period	No fasting prior to dosing
3.3.2	Metabolic/enzyme inhibitors or inducers	No
3.3.3	Duration of treatment	Single dose
3.3.4	Frequency of exposure	
3.3.5	Postexposure period	Up to 2 years
3.3.6	**Oral**	
3.3.6.1	Type	gavage
3.3.6.2	Concentration	0, 0.02, 0.15, 0.35 mg/kg bw (Groups 1, 2, 3, and 4 respectively).
3.3.6.3	Vehicle	Polyethylene glycol (PEG 400).
3.3.6.4	Concentration in vehicle	3.73 µg/g (Group 2); 25.60 µg/kg (Group 3); 79.23 µg/kg (Group 4)
3.3.6.6	Total volume applied	5 ml dosing solution per kg bw (Groups 2 and 3) 4 ml dosing solution per kg bw (Group 4)
3.3.6.7	Controls	5 ml PEG 400 per kg bw
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, but time points for observations not specified in report (see section 4.1 below).

[redacted]

3.4.1.2	Mortality	Yes, but time points for observations not specified in report (see section 4.1 below).
3.4.2	Body weight	Yes, the bodyweights of each rat in Groups 1 to 3 were recorded weekly for the first 10 weeks and then every 2 weeks for the remainder of the study. The bodyweights of rats in Groups 2 to 4 were also recorded when killed for dissection.
3.4.3	Body fluids sampled	Yes, in the three dose groups, samples of blood were taken at termination and were taken at various time points up to 2 years after dosing. Levels of radioactivity were determined and the prothrombin time (PT) and kaolin-cephalin time (KCT) measured.
3.4.4	Tissues sampled	Yes, liver, kidneys, pancreas and salivary glands – abdominal fat was also sampled in the highest (0.35 mg/kg) dosage group. Samples were taken at various time points up to 2 years after dosing.
3.4.5	Determination of metabolites	Yes, at the 0.15 mg/kg dose level (Group 3) at 4, 39 and 104 weeks after dosing; and at the 0.35 mg/kg dose level (Group 4) at 1 and 1 days after dosing, the liver was investigated qualitatively for metabolites and quantitation was attempted. The metabolites were not identified. The techniques used for the investigations were Scintillation Counting, Thin-Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and quantitation of thin layers using an Isomess 3000 linear analyser.
3.4.6	Excretion routes	Yes, for the two lower dosage groups (0.02 and 0.15 mg/kg), urine and faeces were collected for the 24 hour period prior to termination of animals at time points up to 65 weeks after dosing.
3.4.7	Other examinations	No.
3.4.8	Statistics	
3.5	**Further remarks**	

4 RESULTS AND DISCUSSION

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

4.1	**Observations**	The most frequently recorded clinical observations for the rats in groups 1-3 were scabs, hair loss, piloerection and hunching. The observations for the rats in group 4 included subdued behaviour, hunching and pale ears and tails. None of the rats in groups 1 and 2 died during the first year of the study and only two deaths occurred with animals in groups 3. During the second year a number of the animals in all these three groups died but the numbers were not significantly different between the test and control groups and there was no evidence that any of the deaths were related to the administration of the test substance. None of the animals in groups 2 and 3 showed signs of internal haemorrhaging when dissected at the kill times. None of the rats In group 4 died but those which show toxic effects were killed humanely. These and some of the other rats in the group showed signs of internal haemorrhaging when dissected. The surviving animals were all killed at the end of the experimental phase of the study.	
4.2	**Body weight**	The rats in group 2 showed a statistically significant increase in bodyweights during the first two weeks after dosing when compared to the rats in group 1 (given the dose vehicle only). The rats in group 3 showed statistically significant increases in bodyweight during week 2 after dosing, and statistically significant reduced bodyweight gains for weeks 10 and 24 after dosing. Some of the rats in group 4 showed reduced bodyweight gains at sacrifice when compared with other animals in the group.	
4.3	**Absorption**	The concentration of the test substance in the blood at various time intervals after dosing are given in Table A6_2-1. For dose groups 2 and 3, the concentration was found to be <0.01 nmole equivalents per g blood at all time points sampled after dosing. For dose group 4 the concentration in the blood was found to be 0.08 nmole equivalents per g blood at 6 h after dosing, which rose to 0.16 at 18 h, and then declined thereafter to <0.01 at 14 days after dosing. In groups 2 and 3, the clotting times were unaffected throughout the study and were within the normal range usually observed for rats in this laboratory (approximately 14-24 sec for KCT and approximately 12-15 sec for PT). The effect on coagulation was significant for rats in group 4.Herethe PT reached a maximum of 148 seconds at 28 h after dosing and was outside the normal range between 12 and 96 h after dosing. After this the valued were within the range for normal animals. See Table A6_2-2 for a summary of the results.	X

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

4.4 Distribution

The mean values for the distribution of radioactivity in tissues, expressed as both nanomole equivalents per g (nmol equiv/g) of tissue, and as a percentage of the dose, for animals in groups 2-4 are shown in Tables A6_2-3, A6_2-4, A6_2-5, A6_2-6, A6_2-7 and A6_2-8. X

Irrespective of the dose administered the highest concentration of radioactivity in the liver and kidney was found 24 hours after dosing. In the salivary glands the highest concentration was found at 24 hours after dosing except at the lowest dose level. In the pancreas the highest concentrations were found later than 24 hours after dosing.

At all three dose levels, the concentration of radioactivity in the liver was higher than in the kidney and salivary glands at all times and was initially also higher than in the pancreas. At the two lower dose levels (0.02 and 0.15 mg/kg) the concentration in the pancreas was higher than in the liver at 4 weeks after dosing and remained so throughout the rest of the study. At the highest dose level (0.35 mg/kg) the concentrations in the pancreas were higher than in the liver except during the first 24 hours after dosing.

At all three dose levels, the liver retained the largest percentage of the administered dose. The proportion retained in the liver at 12 weeks after dosing at the highest level was 21.2%, which was slightly less than the corresponding values obtained at the two lower dose levels.

4.5 Metabolism

The proportion of brodifacoum in the extracts of livers from Group 3 rats declined slowly from 94% at week 4, to 78% at week 104, and during this time the *cis:trans* ratio altered from 59:41 to 66:34. The proportion of brodifacoum in the extracts of livers from Group 4 rats was relatively unchanged between day 1 and day 14, accounting for 86% and 89% respectively of the radioactivity, and the isomer ratio was not significantly altered.

A more polar component was present in the livers of Group 4 rats which could not be detected in the livers of Group 3 rats and accounted for 11% and 9% of the radioactivity in the day 1 and day 14 extracts respectively. Two additional minor components were also found (at <1%). Data for rats in Group 2 were not obtained.

These data showed that at either dose level and irrespective of the time after dosing brodifacoum was the major component found in the liver and the *cis:trans* isomer ratio of the substance was not significantly altered.

See Table A6_2-9 for a summary of the proportion of brodifacoum in rat liver extracts.

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

4.6	**Elimination and Excretion**	The levels of radioactivity in the urine and faeces excreted during the 24 hour period prior to killing animals given 0.02 and 0.15 mg/kg brodifacoum (Groups 2 and 3), are summarised below in Table A6_2-10. Excretion in both dose groups was similar, and was highest during the 24 hour period after dosing with the most radioactivity found in the faeces. The amounts of radioactivity excreted in urine after the first 24 hour period were below the limits of detection, whereas small amounts were excreted in faeces, suggesting that the principal rout of elimination was via the bile. The rate of excretion was generally consistent with slow elimination from the tissues.
4.7	**Recovery of labelled compound**	Not given in study report.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test material: Brodifacoum; [redacted]Methods used broadly comparable to OECD guideline 417 for Toxicokinetic Studies. Groups of 24, 36 and 39 male rats (Alpk:Ap) weighing 174 – 231 g, were each given a single oral dose of either 0.02, 0.15 or 0.35 mg/kg_{bw} respectively of [^{14}C]-brodifacoum (Groups 2, 3 and 4). The animals were killed in groups of 3 at specific time intervals up to two years after dosing, with blood and selected tissues taken for analysis The analytical techniques used were Scintillation Counting, HPLC (High Performance Liquid Chromatography), TLC (Thin-Layer Chromatography), with quantitation attempted using a linear analyser.

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

5.2	**Results and discussion**	The effects on bodyweight, mortalities and clinical observations could only be attributed to the administration of brodifacoum at the highest dose (0.35 mg/kg). At all three dose levels, the concentration of radioactivity in the liver was higher than in the kidney and salivary glands at all times and was initially also higher than in the pancreas. At the two lower dose levels (0.02 and 0.15 mg/kg), the concentration in the pancreas was higher than in the liver at 4 weeks after dosing and remained so throughout the study. At the highest dose level (0.35 mg/kg), concentrations in the pancreas were also higher than in the liver except during the first 24 hours after dosing. At all three dose levels, the liver retained the largest percentage of the administered dose. At the highest dose (0.35 mg/kg) the proportion retained in the liver at day 84 was 21.2%, which was slightly less than the corresponding values obtained for the two lower dose groups (0.02 and 0.15 mg/kg). The elimination of radioactivity from the liver at the highest dose of brodifacoum was biphasic. There was a rapid phase which also corresponded to a reduction in clotting factor synthesis followed by a slower terminal phase during which blood clotting function was normal. The half-life of elimination from the liver during the rapid phase (days 1-4) was approximately 4 days, and for the slower phase (days 28-84) was 128 days. At the two lower dose levels, clotting factor synthesis was unaffected and the results showed that probably only the slow elimination phase was present in the liver for which the half-life was 350 days. Irrespective of the dose level and the time after dosing, brodifacoum was the major component present in the liver and the *cis:trans* isomer ratio was not substantially altered. The elimination of radioactivity from the kidney followed similar kinetics to that observed in the liver. At the highest dose level, elimination was biphasic with fast initial and slow terminal phase, whilst at the two lower dose levels, probably only the slow elimination phase was apparent. Elimination from salivary glands was slow at all dose levels. At the two lower dose levels, there was an increase in the concentration of radioactivity in the pancreas during the first during the first 13 weeks after dosing, probably as a result of redistribution from other tissues, followed by a slow elimination of radioactivity. At the highest dose level, the concentration of radioactivity in the pancreas increased until day 4 after dosing, but thereafter declined slowly. At the two lower dose levels, radioactivity was below the limit of detection detectionin blood , and undetectableafter day 8 at the highest dose level.	X

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No.

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

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

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

Results and discussion

Include revised version

4.3 Absorption

The concentration of the test substance in the blood at various time intervals after dosing are given in Table A6_2-1. For dose groups 2 and 3, the concentration was found to be <0.01 nmole equivalents per g blood at all time points sampled after dosing. For dose group 4 the concentration in the blood was found to be 0.08 nmole equivalents per g blood at 6 h after dosing, which rose to 0.16 at 18 h, and then declined thereafter to <0.01 at 14 days after dosing.

In groups 2 and 3, the clotting times were unaffected throughout the study and were within the normal historical range usually observed for rats in the laboratory (approximately 14-24 sec for KCT and approximately 12-15 sec for PT). The effect on coagulation was significant for rats in group 4: the PT reached a maximum of 148 seconds at 28 h after dosing and was outside the normal range between 12 and 96 h after dosing. After this the values were within the range for normal animals. See Table A6_2-2 for a summary of the results.

4.4 Distribution *(insert at the end of the paragraph the following)*

After 104 weeks post-dosing, the 11.78% of the lowest dose is still present in the liver, whereas the pancreas contained about 1% . Similar residues were measured for the mid-dose group, while the high-dose group attained about 20 and 2 % in the liver and pancreas, respectively.

5.2 Results and discussion

The effects on bodyweight, mortalities and clinical observations could only be attributed to the administration of brodifacoum at the highest dose (0.35 mg/kg).

At all three dose levels, the concentration of radioactivity in the liver was higher than in the kidney and salivary glands at all times and was initially also higher than in the pancreas. At the two lower dose levels (0.02 and 0.15 mg/kg), the concentration in the pancreas was higher than in the liver at 4 weeks after dosing and remained so throughout the study. At the highest dose level (0.35 mg/kg), concentrations in the pancreas were also higher than in the liver except during the first 24 hours after dosing. Significant amounts of test substance were measured in the liver and pancreas of animals from the three treatment groups (10-20% and 1-2% of the administered dose, respectively), even after 104 weeks after dosing.

At all three dose levels, the liver retained the largest percentage of the administered dose. At the highest dose (0.35 mg/kg) the proportion retained in the liver at day 84 was 21.2%, which was slightly less than the corresponding values obtained for the two lower dose groups (0.02 and 0.15 mg/kg).

The elimination of radioactivity from the liver at the highest dose of brodifacoum was biphasic. There was a rapid phase which also corresponded to a reduction in clotting factor synthesis followed by a slower terminal phase during which blood clotting function was normal. The half-life of elimination from the liver during the rapid phase (days 1-4) was approximately 4 days, and for the slower phase (days 28-84) was 128 days. At the two lower dose levels, clotting factor synthesis was unaffected and the results showed that probably only the slow elimination phase was present in the liver for which the half-life was 350 days. Irrespective of the dose level and the time after dosing, brodifacoum was the major component present in the live[redacted]*ns* isomer ratio was not substantially altered.

The elimination of radioactivity from the kidney followed similar kinetics to that observed in the liver. At the highest dose level, elimination was biphasic with fast initial and slow terminal phase, whilst at the two lower dose levels, probably only the slow elimination phase was apparent.

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

Conclusion	
	Include revised version The test substance is very slowly eliminated from the body since tissues such as the liver and pancreas retained significant amounts of residues (10-20% and 1-2%, respectively) even two years after a single non toxic dose.
Reliability	The reliability indicator is *appropriate*
Acceptability	*Acceptable*
Remarks	Reference to a study, comparing the major TK parameters between the two isomers of the active substance is cited, considering it definitely relevant. Indeed the a.s. is here used as a mixture of the two isomers in a ratio which may be different in different studies. Although the applicant provided the related report, the study has been considered as a non-key study and therefore the summary has not been submitted.
	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	

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

Table A6_2-1 Table for Toxicokinetic Studies:

Mean Concentration Of Radioactivity In The Blood Of Male Rats At Various Timepoints Following A Single Oral Dose Of [^{14}C]-Labelled Brodifacoum (0.02, 0.15 And 0.35 mg/kg$_{bw}$)

Time After Dosing	Concentration Of Radioactivity As nmole Equivalents Per g Blood		
	Group 2 (0.02 mg/kg$_{bw}$)	Group 3 (0.15 mg/kg$_{bw}$)	Group 4 (0.35 mg/kg$_{bw}$)
6 hours			0.08
12 hours			0.14
18 hours			0.16
1 day	<0.01	<0.01	0.15
2 days			0.08
3 days			0.04
4 days			0.02
8 days			0.02
2 weeks		<0.01	<0.01
4 weeks	<0.01	<0.01	<0.01
8 weeks		<0.01	<0.01
12 weeks			<0.01
13 weeks	<0.01	<0.01	
26 weeks		<0.01	
39 weeks	<0.01	<0.01	
52 weeks		<0.01	
65 weeks	<0.01	<0.01	
78 weeks		<0.01	
91 weeks	<0.01	<0.01	
104 weeks	<0.01	<0.01	

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

Table A6_2-2 Table for Toxicokinetic Studies:

Prothrombin Time (PT) And Kaolin Cephalin Time (KCT) In Male Rats At Various Time Points Following Single Oral Dose Of [^{14}C]-Labelled Brodifacoum (0.02, 0.15 And 0.35 mg/kg$_{bw}$)

Time After Dosing	Group 2 (0.02 mg/kg$_{bw}$)		Group 3 (0.15 mg/kg$_{bw}$)		Group 4 (0.35 mg/kg$_{bw}$)	
	Clotting Times (Seconds)		Clotting Times (Seconds)		Clotting Times (Seconds)	
	KCT	PT	KCT	PT	KCT	PT
6 hours					ND	14.3
12 hours					ND	20.7
18 hours					43.7	37.2
1 day	14.9	13.0	15.8	13.0	58.9	95.5
2 days					113.7	147.6
3 days					92.8	39.7
4 days					32.3	18.8
8 days					21.3	15.8
2 weeks			14.0	14.3	15.4	17.4
4 weeks	14.9	12.7	21.3	13.6	20.2	13.4
8 weeks			16.2	12.7	19.6	13.3
12 weeks					17.2	12.5
13 weeks	14.1	15.4	16.5	13.8		
26 weeks			12.3	16.1		
39 weeks	16.6	13.5	15.0	13.8		
52 weeks			15.6	12.7		
65 weeks	16.7	13.5	18.0	13.2		
78 weeks			18.6	12.8		
91 weeks	16.8	14.6	19.8	15.1		
104 weeks	14.7	11.1	13.2	10.9		

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

Table A6_2-3 Table for Toxicokinetic Studies:

Mean Concentration Of Radioactivity (as nanomole equivalents per g tissue) In The Tissues Of Male Rats At Various Timepoints Following A Single Oral Dose Of [^{14}C]-Labelled Brodifacoum at 0.35 mg/kg$_{bw}$ (Group 4)

Time After Dosing	Tissue Concentration Of Radioactivity (nmole equiv/g tissue)					
	Liver	Kidney	Salivary Gland	Pancreas	Carcass	Fat
6 hours	2.91	0.57	0.46	0.80		0.14
12 hours	4.03	0.68	0.88	2.32		0.19
18 hours	4.29	0.73	0.98	4.25		0.29
1 day	4.40	0.75	1.03	4.38		0.27
2 days	3.50	0.65	1.03	4.27		0.17
3 days	2.91	0.54	0.98	4.49		0.15
4 days	2.61	0.51	0.92	4.50		0.14
8 days	2.51	0.47	0.96	4.06		0.13
2 weeks	2.04	0.36	0.73	3.87		0.09
4 weeks	1.83	0.31	0.69	3.46		0.05
8 weeks	1.57	0.28	0.61	3.22		0.05
12 weeks	1.35	0.23	0.50	3.08		0.04

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

Table A6_2-4 Table for Toxicokinetic Studies:

Mean Concentration Of Radioactivity (as a percentage of the dose) In The Tissues Of Male Rats At Various Timepoints Following A Single Oral Dose Of [^{14}C]-Labelled Brodifacoum at 0.35 mg/kg$_{bw}$ (Group 4)

Time After Dosing	Tissue Concentration Of Radioactivity (% of dose)					
	Liver	Kidney	Salivary Gland	Pancreas	Carcass	Fat
6 hours	19.62	0.71	0.12	0.27		
12 hours	24.07	0.84	0.24	0.75		
18 hours	28.04	0.89	0.24	1.55		
1 day	28.92	0.95	0.27	1.70		
2 days	26.47	0.82	0.26	1.73		
3 days	25.11	0.73	0.28	1.72		
4 days	25.05	0.73	0.26	1.97		
8 days	22.52	0.68	0.29	1.82		
2 weeks	23.89	0.61	0.24	1.85		
4 weeks	23.47	0.59	0.26	1.73		
8 weeks	23.00	0.59	0.26	2.00		
12 weeks	21.24	0.55	0.25	2.02		

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

Table A6_2-5 Table for Toxicokinetic Studies:

Mean Concentration Of Radioactivity (as nanomole equivalents per g tissue) In The Tissues Of Male Rats At Various Timepoints Following A Single Oral Dose Of [^{14}C]-Labelled Brodifacoum at 0.15 mg/kg$_{bw}$ (Group 3)

Time After Dosing	Tissue Concentration Of Radioactivity (nmole equiv/g tissue)					
	Liver	Kidney	Salivary Gland	Pancreas	Carcass	Fat
1 day	1.60	0.61	0.56	0.73		
2 weeks	1.39	0.23	0.33	0.92		
4 weeks	1.19	0.21	0.38	1.23	0.08	
8 weeks	0.99	0.20	0.37	1.36		
13 weeks	0.97	0.15	0.29	1.40	0.05	
26 weeks	0.60	0.11	0.27	1.32		
39 weeks	0.55	0.08	0.17	1.04	0.03	
52 weeks	0.56	0.07	0.15	1.00	0.02	
65 weeks	0.49	0.07	0.12	0.91		
78 weeks	0.39	0.05	0.11	0.72		
91 weeks	0.31	0.04	0.09	0.56		
104 weeks	0.30	0.03	0.07	0.55		

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

Table A6_2-6 Table for Toxicokinetic Studies:

Mean Concentration Of Radioactivity (as percentage of dose) In The Tissues Of Male Rats At Various Timepoints Following A Single Oral Dose Of [^{14}C]-Labelled Brodifacoum at 0.15 mg/kg$_{bw}$ (Group 3)

Time After Dosing	Tissue Concentration Of Radioactivity (% of dose)					
	Liver	Kidney	Salivary Gland	Pancreas	Carcass	Fat
1 day	29.71	1.97	0.35	0.73		
2 weeks	37.31	0.96	0.35	1.52		
4 weeks	37.07	0.99	0.40	1.67	46.85	
8 weeks	30.86	0.97	0.40	2.42		
13 weeks	31.74	0.82	0.37	2.28	38.24	
26 weeks	21.66	0.61	0.31	2.11		
39 weeks	22.02	0.54	0.21	1.69	29.48	
52 weeks	20.26	0.45	0.20	1.86	23.73	
65 weeks	15.36	0.38	0.18	1.30		
78 weeks	13.01	0.34	0.15	1.05		
91 weeks	12.39	0.19	0.11	1.02		
104 weeks	11.74	0.22	0.09	1.15		

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

Table A6_2-7 Table for Toxicokinetic Studies:

Mean Concentration Of Radioactivity (as nanomole equivalents per g tissue) In The Tissues Of Male Rats At Various Timepoints Following A Single Oral Dose Of [^{14}C]-Labelled Brodifacoum at 0.02 mg/kg$_{bw}$ (Group 2)

Time After Dosing	Tissue Concentration Of Radioactivity (nmole equiv/g tissue)					
	Liver	Kidney	Salivary Gland	Pancreas	Carcass	Fat
1 day	0.40	0.05	0.02	0.03		
4 weeks	0.19	0.03	0.04	0.12		
13 weeks	0.13	0.04	0.04	0.17		
39 weeks	0.09	0.02	0.03	0.15		
65 weeks	0.06	<0.01	0.02	0.11		
91 weeks	0.04	<0.01	<0.01	0.08		
104 weeks	0.05	<0.01	<0.01	0.08		

Table A6_2-8 Table for Toxicokinetic Studies:

Mean Concentration Of Radioactivity (as percentage of dose) In The Tissues Of Male Rats At Various Timepoints Following A Single Oral Dose Of [^{14}C]-Labelled Brodifacoum at 0.02 mg/kg$_{bw}$ (Group 2)

Time After Dosing	Tissue Concentration Of Radioactivity (% of dose)					
	Liver	Kidney	Salivary Gland	Pancreas	Carcass	Fat
1 day	47.33	1.08	0.11	0.21		
4 weeks	39.16	0.90	0.28	0.99		
13 weeks	34.01	1.39	0.35	1.77		
39 weeks	20.33	0.65	0.25	1.63		
65 weeks	15.97	0.38	0.14	1.23		
91 weeks	10.57	0.32	0.13	1.12		
104 weeks	11.78	<0.01	0.10	1.01		

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

Table A6_2-9 Table for Toxicokinetic Studies:

Proportion Of Brodifacoum Found In Rat Liver At Various Timepoints Following A Single Oral Dose Of [^{14}C]-Labelled Brodifacoum at 0.15 mg/kg$_{bw}$ (Group 3) and 0.35 mg/kg$_{bw}$ (Group 4)

Dose Group and Time After Dosing	Proportion Of Brodifacoum In Liver (as % of radioactivity in liver)	Isomer Ratio (*cis:trans*)
Group 3		Isomer ratio of test substance used for dosing Group 3 animals: 59:41
4 weeks	93.9	59:41
39 weeks	89.7	64:36
104 weeks	78.3	66:34
Group 4		Isomer ratio of test substance used for dosing Group 4 animals: 61:39
1 day	85.9	64:36
14 days	88.8	67:33

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

Table A6_2-10 Table for Toxicokinetic Studies:

Excretion Of Radioactivity In the Urine And Faeces Of Male Rats At Various Timepoints Following A Single Oral Dose Of [^{14}C]-Labelled Brodifacoum at 0.02 mg/kg$_{bw}$ (Group 2) and 0.15 mg/kg$_{bw}$ (Group 3)

Time After Dosing	% Of Dose Excreted			
	Group 2 (0.02 mg/kg)		Group 3 (0.15 mg/kg)	
	Urine	Faeces	Urine	Faeces
1 day	<0.48	5.22	0.37	6.57
2 weeks			<0.06	0.30
4 weeks	<0.46	0.31	<0.06	0.27
8 weeks			<0.06	0.26
13 weeks	<0.46	1.41	<0.06	0.31
26 weeks			<0.06	0.15
39 weeks	<0.46	<0.30	<0.06	0.11
52 weeks			<0.06	<0.04
65 weeks	<0.46	<0.28	<0.06	<0.04

[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1987). 'Brodifacoum: Elimination from the tissues of rats following administration of single oral doses.' [redacted], Report No: CTL/P/1559 [C2.7/05] (unpublished). Experimental work carried out between [redacted] and [redacted].
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No, but methods used broadly comparable to OECD guideline 417 for Toxicokinetic studies.
2.2	**GLP**	Yes.
2.3	**Deviations**	

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	Unlabelled brodifacoum: [redacted] [^{14}C]-brodifacoum: [redacted] (used for Groups 2 and 3) and [redacted] (used for Group 4)
3.1.2	Specification	[redacted]

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

3.1.2.1	Description		
3.1.2.2	Purity	Unlabelled brodifacoum: [redacted]% (with *cis:trans* ratio of [redacted]) [^{14}C]-brodifacoum ([redacted]): radiochemical purity [redacted] % (with *cis:trans* ratio of [redacted]) [^{14}C]-brodifacoum ([redacted]): radiochemical purity [redacted] % (with *cis:trans* ratio of [redacted])	X
3.1.2.3	Stability	[redacted]	
3.1.2.4	Radiolabelling	[^{14}C]-brodifacoum: uniformly labelled in the phenyl ring of the coumarin moiety with specific activities of 546MBq/m mole (100052/010/004) and 1961MBq/m mole (Y00052/028/002).	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Alpk:AP	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	Adult (approximately 7 weeks old) weighing 174 – 231g	
3.2.6	Number of animals per group	24 (Group 2); 36 (Group 3); 39 (Group 4)	
3.2.7	Control animals	Yes: 21 animals (Group 1)	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Fasting period	No fasting prior to dosing	
3.3.2	Metabolic/enzyme inhibitors or inducers	No	
3.3.3	Duration of treatment	Single dose	
3.3.4	Frequency of exposure		
3.3.5	Postexposure period	Up to 2 years	
3.3.6	**Oral**		
3.3.6.1	Type	gavage	
3.3.6.2	Concentration	0, 0.02, 0.15, 0.35 mg/kg bw (Groups 1, 2, 3, and 4 respectively).	

Doc IIIA / Section 6.2

BPD Data Set IIA / Annex Point VI.6.2

Toxicokinetics Studies

Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose

3.3.6.3	Vehicle	Polyethylene glycol (PEG 400).
3.3.6.4	Concentration in vehicle	3.73 µg/g (Group 2); 25.60 µg/kg (Group 3); 79.23 µg/kg (Group 4)
3.3.6.6	Total volume applied	5 ml dosing solution per kg bw (Groups 2 and 3) 4 ml dosing solution per kg bw (Group 4)
3.3.6.7	Controls	5 ml PEG 400 per kg bw
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, but time points for observations not specified in report (see section 4.1 below).
3.4.1.2	Mortality	Yes, but time points for observations not specified in report (see section 4.1 below).
3.4.2	Body weight	Yes, the bodyweights of each rat in Groups 1 to 3 were recorded weekly for the first 10 weeks and then every 2 weeks for the remainder of the study. The bodyweights of rats in Groups 2 to 4 were also recorded when killed for dissection.
3.4.3	Body fluids sampled	Yes, in the three dose groups, samples of blood were taken at termination and were taken at various time points up to 2 years after dosing. Levels of radioactivity were determined and the prothrombin time (PT) and kaolin-cephalin time (KCT) measured.
3.4.4	Tissues sampled	Yes, liver, kidneys, pancreas and salivary glands – abdominal fat was also sampled in the highest (0.35 mg/kg) dosage group. Samples were taken at various time points up to 2 years after dosing.
3.4.5	Determination of metabolites	Yes, at the 0.15 mg/kg dose level (Group 3) at 4, 39 and 104 weeks after dosing; and at the 0.35 mg/kg dose level (Group 4) at 1 and 1 days after dosing, the liver was investigated qualitatively for metabolites and quantitation was attempted. The metabolites were not identified. The techniques used for the investigations were Scintillation Counting, Thin-Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and quantitation of thin layers using an Isomess 3000 linear analyser.
3.4.6	Excretion routes	Yes, for the two lower dosage groups (0.02 and 0.15 mg/kg), urine and faeces were collected for the 24 hour period prior to termination of animals at time points up to 65 weeks after dosing.
3.4.7	Other examinations	No.
3.4.8	Statistics	
3.5	**Further remarks**	

4 RESULTS AND DISCUSSION

4.1 Observations

The most frequently recorded clinical observations for the rats in groups 1-3 were scabs, hair loss, piloerection and hunching. The observations for the rats in group 4 included subdued behaviour, hunching and pale ears and tails.

None of the rats in groups 1 and 2 died during the first year of the study and only two deaths occurred with animals in groups 3. During the second year a number of the animals in all these three groups died but the numbers were not significantly different between the test and control groups and there was no evidence that any of the deaths were related to the administration of the test substance. None of the animals in groups 2 and 3 showed signs of internal haemorrhaging when dissected at the kill times.

None of the rats In group 4 died but those which show toxic effects were killed humanely. These and some of the other rats in the group showed signs of internal haemorrhaging when dissected. The surviving animals were all killed at the end of the experimental phase of the study.

4.2 Body weight

The rats in group 2 showed a statistically significant increase in bodyweights during the first two weeks after dosing when compared to the rats in group 1 (given the dose vehicle only).

The rats in group 3 showed statistically significant increases in bodyweight during week 2 after dosing, and statistically significant reduced bodyweight gains for weeks 10 and 24 after dosing.

Some of the rats in group 4 showed reduced bodyweight gains at sacrifice when compared with other animals in the group.

4.3 Absorption

The concentration of the test substance in the blood at various time intervals after dosing are given in Table A6_2-1. For dose groups 2 and 3, the concentration was found to be <0.01 nmole equivalents per g blood at all time points sampled after dosing. For dose group 4 the concentration in the blood was found to be 0.08 nmole equivalents per g blood at 6 h after dosing, which rose to 0.16 at 18 h, and then declined thereafter to <0.01 at 14 days after dosing. X

In groups 2 and 3, the clotting times were unaffected throughout the study and were within the normal range usually observed for rats in this laboratory (approximately 14-24 sec for KCT and approximately 12-15 sec for PT). The effect on coagulation was significant for rats in group 4.Herethe PT reached a maximum of 148 seconds at 28 h after dosing and was outside the normal range between 12 and 96 h after dosing. After this the valued were within the range for normal animals. See Table A6_2-2 for a summary of the results.

Doc IIIA / Section 6.2 **BPD Data Set IIA / Annex Point VI.6.2**	**Toxicokinetics Studies** Absorption, tissue distribution, metabolism and excretion in male rats following administration of single oral dose	
4.4 Distribution	The mean values for the distribution of radioactivity in tissues, expressed as both nanomole equivalents per g (nmol equiv/g) of tissue, and as a percentage of the dose, for animals in groups 2-4 are shown in Tables A6_2-3, A6_2-4, A6_2-5, A6_2-6, A6_2-7 and A6_2-8. Irrespective of the dose administered the highest concentration of radioactivity in the liver and kidney was found 24 hours after dosing. In the salivary glands the highest concentration was found at 24 hours after dosing except at the lowest dose level. In the pancreas the highest concentrations were found later than 24 hours after dosing. At all three dose levels, the concentration of radioactivity in the liver was higher than in the kidney and salivary glands at all times and was initially also higher than in the pancreas. At the two lower dose levels (0.02 and 0.15 mg/kg) the concentration in the pancreas was higher than in the liver at 4 weeks after dosing and remained so throughout the rest of the study. At the highest dose level (0.35 mg/kg) the concentrations in the pancreas were higher than in the liver except during the first 24 hours after dosing. At all three dose levels, the liver retained the largest percentage of the administered dose. The proportion retained in the liver at 12 weeks after dosing at the highest level was 21.2%, which was slightly less than the corresponding values obtained at the two lower dose levels.	X
4.5 Metabolism	The proportion of brodifacoum in the extracts of livers from Group 3 rats declined slowly from 94% at week 4, to 78% at week 104, and during this time the *cis:trans* ratio altered from 59:41 to 66:34. The proportion of brodifacoum in the extracts of livers from Group 4 rats was relatively unchanged between day 1 and day 14, accounting for 86% and 89% respectively of the radioactivity, and the isomer ratio was not significantly altered. A more polar component was present in the livers of Group 4 rats which could not be detected in the livers of Group 3 rats and accounted for 11% and 9% of the radioactivity in the day 1 and day 14 extracts respectively. Two additional minor components were also found (at <1%). Data for rats in Group 2 were not obtained. These data showed that at either dose level and irrespective of the time after dosing brodifacoum was the major component found in the liver and the *cis:trans* isomer ratio of the substance was not significantly altered. See Table A6_2-9 for a summary of the proportion of brodifacoum in rat liver extracts.	